Assessment of Antimicrobial Potential of *Manilkara hexandra* Leaf

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INTRODUCTION

Nature has been a source of medicinal agents for thousands of years since it comprises of compounds that are highly diverse and often provide highly specific biological activities. This follows from the proposition that essentially all natural products have some receptor binding capacity.[1] Herbal medicines have been the basis of treatment for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha.[2] To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have folklore reputation in a more intensified way.[3] Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antimicrobial agents.[4] Some natural products have been approved as new antimicrobial drugs, but there is a continuous and urgent need to screen more and more plant species and discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action to combat new and re-emerging infectious diseases of today's era.[5]

Bacteria and fungi cause some important human diseases, especially in immunocompromised or immunodeficient patients. Despite the existence of potent

ABSTRACT: An increasing reliance on the use of herbal remedies in the industrialized society due to prevalence of multi-drug resistant strains has raised the development of new effective therapeutic agents from plants. This study focuses on the antimicrobial potential of *Manilkara hexandra* (Roxb.) Dubard leaf extracted by Soxhlet extraction method using three solvents with increasing polarity viz., petroleum ether, acetone and methanol. The microbial strains investigated included 9 Gram-positive bacteria, 14 Gram-negative bacteria, 7 yeast and 4 moulds. The antimicrobial activity was done by agar disc diffusion method at two different concentrations viz., 250 and 500 µg/disc. The antimicrobial activity was found to be concentration dependent. All the three extracts showed better activity against bacterial than fungal strains. Maximum antibacterial activity was shown by methanol extract. The results were compared with the zones of inhibition produced by commercially available standard antibiotics. The minimum inhibitory concentration (MIC) of *M. hexandra* was evaluated within a range of concentration from 250–32,000 µg/ml.

Key words: Antimicrobial activity, *Manilkara hexandra*, Sapotaceae, Minimum inhibitory concentration.

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antibacterial and antifungal agents, the past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents [6] that lead to repeated use of antibiotics and insufficient control of diseases,[7] imposing the need for a permanent search and development of new drugs.[8] Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.[9] In an effort to discover new lead compounds, many researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against microbial infections.[8]

Plant based antimicrobials represent a vast untapped source of medicines and there is a need for further exploration of plant antimicrobials. It is estimated that several plant-derived drugs prescribed in the industrialized world were discovered by studying folk knowledge. However, less than one-half of 1 percent of all plant species in the world has been studied for potential pharmacological activity.[10] Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc[11] i.e. any part of the plant may contain active components. Scientific analysis of plant components may give a new source of antimicrobial agents with possibly novel mechanisms of action.[12, 13] World Health Organization has also approved the study of medicinal plants for the development of new drug lead.[14] In recent years, many researchers all over the world have screened plant extracts to detect and utilize secondary metabolites with relevant biological activities as medicinal agents.[15, 16]

This work was aimed at the investigation of antibacterial as well as antifungal property of different extracts of *Manilkara hexandra* (Roxb.) Dubard leaf against an array of human pathogens including Gram-positive and Gram-negative bacteria as well as some fungi like yeast and moulds. The impelling motive behind the present work was to emphasize the possibilities and scientific importance for the use of *M. hexandra* leaf extracts in treating common ailments due to microbial infections encountered worldwide.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *Manilkara hexandra* (Roxb.) Dubard (Sapotaceae) commonly known as Rayan were collected in the month of August 2005, from Anand Agriculture University, Anand. The identity of the plant was confirmed by Dr. Sriram, Research Scientist, Anand Agricultural University, Anand and Dr. P. S. Nagar, former taxonomist, Department of Biosciences, Saurashtra University, Rajkot. A voucher specimen of the plant (No. PSN 428) was deposited at Department of Biosciences, Rajkot. The plant material was thoroughly washed under running tap water, air dried and homogenized to fine powder and stored in airtight bottles for further experiments.

Extraction

Ten grams of dried crude powder of *M. hexandra* leaf was successively extracted by Soxhlet extraction method using three solvents with increasing polarity viz., petroleum ether, acetone and methanol. The solvent was evaporated under reduced pressure and the extract thus obtained was stored in air-tight bottles at 4°C for further experiments. The respective yields (%) were calculated with reference to air dried leaf powder.

Microorganisms and growth conditions

The investigated microbial strains were obtained from the National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India. The microbial strains investigated include 9 Gram-positive bacteria, *Bacillus cereus* ATCC 11778, *Bacillus megaterium* ATCC 9885, *Bacillus subtilis* ATCC 6633, *Corynebacterium rubrum* ATCC 14898, *Micrococcus flavus* ATCC 10240 *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29737, *Staphylococcus epidermides* ATCC 12228, *Staphylococcus subfava* NCIM 2178; 14 Gram-negative bacteria, *Alcaligenes fecalis* ATCC 8750, *Citrobacter freundii* ATCC 10787, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Klebsiella aerogenes* NCTC 418, *Klebsiella pneumoniae* NCIM 2719, *Proteus mirabilis* NCIM 2241, *Proteus morganii* NCIM 2040, *Proteus vulgaris* NCTC 8313, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas pseudoalcaligenes* ATCC 17440, *Pseudomonas putida* ATCC 12842, *Pseudomonas testosteroni* NCIM 5098, *Salmonella typhimurium* ATCC 23564; 7 yeast, *Candida albicans* ATCC 2091, *Candida albicans* ATCC 18804, *Candida glabrata* NCIM 3448, *Candida tropicalis* ATCC 4563, *Cryptococcus luteolus* ATCC 32044, *Cryptococcus neoformans* ATCC 34664, *Trichosporon beigelii* NCIM 3404 and 4 moulds, *Aspergillus candidus* NCIM 883, *Aspergillus flavus* NCIM 538, *Aspergillus niger* ATCC 6275, *Mucor hiemalis wehmer* NCIM 873. Bacterial cultures were grown on nutrient broth (Hi-Media) at 37°C for 24 h and the fungal cultures were grown on Sabouraud dextrose broth (Hi-Media) at 28°C for 48 h. Bacteria, yeast and mould were maintained on nutrient agar, MGYP and PDA (potato dextrose agar) slants respectively at 4°C.

Standard antimicrobials

The investigated microorganisms were tested against standard antimicrobials. The standard antimicrobials used for bacterial strains were piperacillin (100 μg/disc) and gentamicin (10 μg/disc) and that for fungal strains were amphotericin B (100 units/disc) and fluconazole (10 μg/disc).

Antimicrobial assay

Preparation of inoculum: The test bacterial strains were inoculated into nutrient broth and were incubated at 37°C on a rotary shaker whereas test fungal strains were inoculated into Sabouraud dextrose broth and incubated at 28°C on a rotary shaker. The inoculum size was maintained as per the 0.5 McFarland standard (1×10^8 cfu/ml). The activated inoculum was used for antimicrobial assay.

Preparation of test compound: The petroleum ether (MPE), acetone (MAC) and methanol (MME) extracts of *M. hexandra* (Roxb.) Dubard leaves were dissolved in dimethylsulphoxide (DMSO) and the stocks were prepared at concentrations of 25 and 12.5 mg/ml. The antimicrobial activity was evaluated at two different concentrations viz., 500 and 250 μg/disc.

Antimicrobial susceptibility testing

The antimicrobial activity of various extracts of *M. hexandra* leaves was determined by agar disc diffusion method.[17, 18] The molten Mueller Hinton Agar No. 2 media (Hi-Media) for bacteria and Sabouraud dextrose agar media (Hi-Media) for fungi was inoculated with 200 μl of the inoculum (1×10^8 cfu/ml) when the temperature of media reached 40–42°C and then poured into the Petri plate (Hi-Media). Sterile disc (7 mm) (Hi-Media) was saturated with 20 μl of the test compound (MPE, MAC and MME) with the concentrations of 500 and 250 μg/disc and allowed to dry. The disc was then introduced on the layer of the seeded agar plate. For each microbial strain, negative controls were maintained where pure solvent (DMSO) was used instead of the extract since it does not possess any antimicrobial effect,[19] and for positive control the standard antimicrobials with known concentration were used. The bacterial plates were incubated at 37°C for 24 h and the fungal plates at 28°C for 48 h. The results of antimicrobial activity were obtained by measuring the diameter of the zones of inhibition. The values in the result were expressed as mean ± SEM. The values were compared with the standard antimicrobials. The experiment was performed under strict aseptic conditions for three times to minimize error.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined by disc diffusion method.[20, 21] The Mueller Hinton agar plates for bacteria and Sabouraud dextrose agar plates for fungi, containing an inoculum size of 1×10^8 cfu/ml, were used. Two-fold serial dilutions of petroleum ether, acetone and methanol extracts of *M. hexandra* leaf, ranging from 250-32,000 μg/ml, were prepared in DMSO. The prepared dilutions were impregnated on sterile discs and were placed aseptically on seeded agar plates. Appropriate controls were maintained. All the plates were incubated at 37°C for bacteria and at 28°C for fungi. The lowest concentration within the plate, which showed a clear zone around the disc, was considered as MIC.

RESULTS AND DISCUSSION

The leaves of *M. hexandra* were air dried under shade and the powder was extracted with petroleum ether, acetone and methanol successively using Soxhlet extraction method. The yields of the extracts were found to be 5.5, 4.8 and 23% w/w respectively.

Antimicrobial activity

The presence of antibacterial and antifungal substances in higher plants is well established.[22, 23] Plants have provided sources for novel drug compounds as plant derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic systems of medicine or it can be the base for the development of medicine, a natural blueprint for the development of new drugs.[24] Much of the exploration and utilization of natural products as antimicrobial arise from microbial sources. Though soil microorganisms or fungi produce most of the clinically used antibiotics, higher plants can be very good source of antibiotics.

Present study was conducted to investigate the antimicrobial potential of the petroleum ether, acetone and methanol extracts of *M. hexandra* leaf at two different concentrations. The microorganisms used for the antimicrobial studies were Gram-positive and Gram-negative bacteria, yeast and moulds. The results of antimicrobial activity of *M. hexandra* leaf extracts were summarized in Table 1.

The results showed that methanol extract (MME) was better than the other two extracts (MPE and MAC) which implied that antimicrobial activity is

TABLE 1 : *In vitro* **antimicrobial activity of** *Manilkara hexandra* **(Roxb.) Dubard leaf against some medically important microorganisms.**

MICROORGANISMS	INHIBITION ZONE (mm)[a]									
	Manilkara hexandra (ROXB.) DUBARD LEAF EXTRACTS[b] [EXTRACTIVE YIELD IN%[c]]						ANTIMICROBIALS[d]			
	MPE [5.5]		MAC [4.8]		MME [23]					
	CONCENTRATION OF PLANT EXTRACT (µg/DISK)						PC	G	AP	FU
	500	250	500	250	500	250	100 µg/DISC	10 µg/DISC	100 NITS/ DISC	10 µg/DISC
Gram-positve bacteria										
Bacillus cereus ATCC11778	10 ± 0	9 ± 0	13.5 ± 0.31	12.5 ± 0.31	14.5 ± 0.31	12 ± 0	16 ± 2	12 ± 1	-	-
Bacillus megaterium ATCC9885	-	-	9.5 ± 0.31	-	12 ± 0	10.5 ± 0.31	11.5 ± 0.5	20 ± 1	-	-
Bacillus subtilis ATCC6633	-	8.75 ± 0.15	-	-	10 ± 0	9 ± 0	17.5 ± 2.5	14 ± 1	-	-
Corynebacterium rubrum ATCC14898	-	-	9.75 ± 0.15	-	11 ± 0	9 ± 0	23.5 ± 0.5	20.5 ± 0.5	-	-
Micrococcus flavus ATCC10240	-	8 ± 0	11.5 ± 0.31	9 ± 0	11.75 ± 0.47	10.25 ± 0.15	32.5 ± 2.5	36.5 ± 0.5	-	-
Staphylococcus aureus (1) ATCC25923	-	-	9.5 ± 0	-	10 ± 0	8 ± 0	25 ± 3	17.5 ± 0.5	-	-
Staphylococcus aureus (1) ATCC29737	-	-	10.5 ± 0.47	-	11 ± 0	9 ± 0	24 ± 3	14 ± 1	-	-
Staphylococcus epidermides ATCC12228	-	-	10.5 ± 0.31	-	9.25 ± 0.15	-	10 ± 1	20 ± 2	-	-
Staphylococcus subflava NCIM2178	-	-	-	-	10.25 ± 0.15	8.5 ± 0	21.5 ± 0.5	13.5 ± 0.5	-	-
Gram-negative bacteria										
Alcaligenes fecalis ATCC8750	-	-	-	-	9 ± 0	-	-	16 ± 2	-	-
Citrobacter freundii ATCC10787	-	-	-	-	-	-	19 ± 1	10 ± 0	-	-
Enterobacter aerogenes ATCC13048	11 ± 0.63	9.5 ± 0.31	20.5 ± 0.31	17 ± 0	22 ± 0	17 ± 0.63	10 ± 1	15 ± 1	-	-
Escherichia coli ATCC25922	-	-	-	-	9 ± 0	8 ± 0	14 ± 1	21 ± 1	-	-
Klebsiella aerogenes NCTC418	9 ± 0	-	13.75 ± 0.15	12 ± 0	14.75 ± 0	12 ± 0	17 ± 0	11.5 ± 0.5	-	-
Klebsiella pneumoniae NCIM2719	9 ± 0	-	11 ± 0	9 ± 0	11.75 ± 0.15	11 ± 0	25 ± 0	21 ± 1	-	-
Proteus mirabilis NCIM2241	-	8 ± 0	9 ± 0	8 ± 0	10 ± 0	9 ± 0	22.5 ± 2.5	24 ± 1	-	-
Proteus morganii NCIM2040	-	-	11.25 ± 0.15	10.5 ± 0.31	13.75 ± 0.15	12	18.5 ± 0.5	28 ± 0	-	-
Proteus vulgaris NCTC8313	-	-	-	-	10 ± 0	8 ± 0	12.5 ± 2.5	21.5 ± 1.5	-	-
Pseudomonas aeruginosa ATCC27853	-	-	9.5 ± 0.31	9 ± 0	10.5 ± 0.31	9 ± 0	21.5 ± 1.5	19 ± 1	-	-
Pseudomonas putida ATCC12842	9 ± 0	9 ± 0	12 ± 0	9 ± 0	13 ± 0	11.25 ± 0.15	35 ± 0	30.5 ± 0.5	-	-

(Continued…)

MICROORGANISMS	INHIBITION ZONE (mm)[a]									
	Manilkara hexandra (ROXB.) DUBARD LEAF EXTRACTS[b] [EXTRACTIVE YIELD IN%[c]]						ANTIMICROBIALS[d]			
	MPE [5.5]		MAC [4.8]		MME [23]					
	CONCENTRATION OF PLANT EXTRACT (µg/DISK)						PC	G	AP	FU
	500	250	500	250	500	250	100 µg/DISC	10 µg/DISC	100 NITS/ DISC	10 µg/DISC
Pseudomonas pseudoalcaligenes ATCC17440	-	9 ± 0	12.75 ± 0.15	11 ± 0.15	12.5 ± 0.31	12 ± 0	23.5 ± 1.5	25.5 ± 0.5	-	-
Pseudomonas testosterone NCIM5098	-	-	10 ± 0	-	11 ± 0	9 ± 0	-	14.5 ± 0.5	-	-
Salmonella typhimurium ATCC23564	-	-	-	-	10 ± 0	9±0	20 ± 1	21 ± 1	-	-
Yeast										
Candida albicans (1) ATCC2091	-	-	9 ± 0.31	8.5 ± 0	9 ± 0	-	-	-	12.5 ± 0.5	-
Candida albicans (2) ATCC18804	-	-	-	-	-	-	-	-	17± 0	22 ± 1
Candida glabrata NCIM3448	-	-	-	-	-	-	-	-	18.5 ± 0.5	28 ± 1
Candida tropicalis ATCC4563	9 ± 0	10 ± 0	-	-	-	-	-	-	12 ± 1	-
Cryptococcus luteolus ATCC32044	-	-	-	-	-	-	-	-	15.5 ± 0.5	18.5 ± 0.5
Cryptococcus neoformans ATCC34664	-	-	-	-	-	-	-	-	14 ± 1	20 ± 0
Trichosporon beigelii NCIM3404	9.5 ± 0.31	10.75 ± 0.15	8 ± 0	-	8 ± 0	-	-	-	16.5 ± 0.5	24.5 ± 0.5
Mould										
Aspergillus candidus NCIM883	-	-	-	-			-	-	20 ± 0	17.5 ± 0.5
Aspergillus flavus NCIM538	-	-	15.5 ± 0.31	11.5 ± 0.31	16.5 ± 0.31	13 ± 0.63	-	-	18 ± 0	21 ± 1
Aspergillus niger ATCC6275	-	-	-	-			-	-	19 ± 1	14 ± 0
Mucor hiemalis wehmer NCIM873	-	-	9 ± 0	-	12.25 ± 0.15	10.5 ± 0.31	-	-	16.5 ± 0.5	-

-. no activity, Negative controls did not show any activity.

[a] values are mean ± SEM; mean values include the diameter of the paper disc (7 mm).

[b] MPE-petroleum ether extract, MAC-acetone extract, MME-methanol extract.

[c] Percentage extract yield (w/w) was estimated as dry extract weight/dry material weight × 100.

[d] Pc-Piperacillin, G-Gentamicin, Ap- Amphotericin-B, Fu-Fluconazole.

TABLE 2: Minimum Inhibitory Concentration (MIC) of methanol extract of *Manilkara hexandra* (Roxb.) Dubard leaf.

MICROORGANISMS	MIC OF *Manilkara hexandra* LEAF EXTRACTS (µg/ml)		
	MPE	MAC	MME
Gram-positve bacteria			
Bacillus cereus ATCC 11778	2000	2000	1000
Bacillus megaterium ATCC 9885	NA	4000	4000
Bacillus subtilis ATCC 6633	8000	NA	8000
Corynebacterium rubrum ATCC 14898	NA	4000	4000
Micrococcus flavus ATCC 10240	2000	2000	1000
Staphylococcus aureus (1) ATCC 25923	NA	8000	4000
Staphylococcus aureus (2) ATCC 29737	NA	4000	4000
Staphylococcus epidermides ATCC 12228	NA	8000	8000
Staphylococcus subflava NCIM 2178	NA	NA	8000
Gram-negative bacteria			
Alcaligenes fecalis ATCC 8750	NA	NA	2000
Enterobacter aerogenes ATCC 13048	8000	8000	8000
Escherichia coli ATCC 25922	8000	NA	8000
Klebsiella aerogenes NCTC 418	NA	2000	1000
Klebsiella pneumoniae NCIM 2719	16000	< 250	< 250
Proteus mirabilis NCIM 2241	4000	8000	1000
Proteus morganii NCIM 2040	NA	2000	1000
Proteus vulgaris NCTC 8313	NA	NA	4000
Pseudomonas aeruginosa ATCC 27853	NA	2000	4000
Pseudomonas putida ATCC 12842	4000	4000	4000
Pseudomonas pseudoalcaligenes ATCC 17440	8000	2000	2000
Pseudomonas testosteroni NCIM 5098	NA	250	2000
Salmonella typhimurium ATCC 23564	NA	NA	2000
Yeast			
Candida albicans (1) ATCC 2091	NA	16000	8000
Trichosporon beigelii NCIM 3404	NA	4000	2000
Mould			
Aspergillus flavus NCIM 538	8000	4000	2000
Mucor hiemalis wehmer NCIM 873	8000	8000	4000

NA : No activity.
MPE : petroleum ether extract of M. hexandra,
MAC : acetone extract of M. hexandra,
MME : methanol extract of M. hexandra.

better with the polar solvents. Also, the antimicrobial activity observed was concentration dependent for each extract. The mean zones of inhibition produced against the test microorganisms ranged between 8–22 mm. The highest zone of inhibition was obtained at the higher concentration with *Enterobacter aerogenes*. Amongst all the microbial strains investigated, a Gram-negative bacteria- *Citrobacter freundii*, yeasts- *Candida albicans* (2 strains), *Candida glabrata*, *Cryptococcus luteolus* and *Cryptococcus neoformans* and amongst moulds- *Aspergillus candidus* and *Aspergillus niger* were most resistant strains which did not show any activity at all whereas a Gram-positive bacteria–*Bacillus cereus* and Gram-negative bacteria *Enterobacter aerogenes* and *Pseudomonas putida*

were most susceptible bacteria (Table 1). However, all the three extracts were more active against bacterial strains than the fungal strains.

The methanol extract inhibited 76.47% of microbial strains a higher concentration and 64.70% at lower concentration. The methanol extract was totally inactive at the lower concentration (250 μg/disc) against *Staphylococcus epidermides*, *Alcaligenes fecalis*, *Citrobacter freundii* and all the seven yeast *spp*.s tested. In case of acetone extract, 58.82% of microorganisms were inhibited at higher concentration while 35.30% were inhibited at lower concentration. On the other hand the petroleum ether extract did not show any appreciable activity against all the microorganisms used in present investigation; It could inhibit only 20.58% of microorganisms at higher concentration while 26.47% at the lower concentration (Table 1).

The extracts were compared to the standard antibiotics. The standard antibacterial agents used were piperacillin (100 μg/disc) and gentamicin (10 μg/disc) while the standard antifungal agents used were amphotericin B (100 units/disc) and fluconazole (10 μg/disc) (Table 1).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations of the extracts of *M. hexandra* leaf are reported in Table 2. The most active extracts were subjected to the evaluation of MIC. The MIC of *M. hexandra* was evaluated within a range of concentration from 250–32,000 μg/ml. The results of petroleum ether extract (MPE) showed MIC ranging from 2000–16000 μg/ml. The MIC of petroleum ether extract for *Bacillus cereus* and *Micrococcus flavus* was 2000 μg/ml; for *Proteus mirabilis* and *Pseudomonas putida* it was 4000 μg/ml; for *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas pseudoalcaligenes*, *Aspergillus flavus* and *Mucor hiemalis* was observed as 8000 μg/ml and MIC for *Klebsiella pneumoniae* was observed as 16,000 μg/ml (Table 2).

The results of acetone extract (MAC) showed MIC ranging from 250–16,000 μg/ml. The MIC of acetone extract for *Pseudomonas testosteroni* was 250 μg/ml while for *Klebsiella pneumoniae* it was < 250 μg/ml; for *Bacillus cereus*, *Micrococcus flavus, Klebsiella aerogenes, Proteus morganii, Pseudomonas aeruginosa and Pseudomonas pseudoalcaligenes* MIC was 2000 μg/ml; for *Bacillus megaterium*, *Corynebacterium rubrum*, *Staphylococcus aureus* (2), *Pseudomonas putida* and *Trichosporon beigelii* it was 4000 μg/ml; for *Staphylococcus aureus* (1), *Staphylococcus epidermides*, *Enterobacter aerogenes, Proteus mirabilis,* and *Mucor hiemalis* was 8000 μg/ml and the MIC values for Candida albicans (1) was 16,000 μg/ml.

The results of methanol extract (MME) showed MIC ranging from 250–8000 μg/ml. The MIC of methanol extract for *Klebsiella pneumoniae* was < 250 μg/ml; for *Bacillus cereus*, *Micrococcus flavus, Klebsiella aerogenes, Proteus mirabilis and Proteus morganii* was 1000 μg/ml; for *Alcaligenes fecalis, Pseudomonas pseudoalcaligenes, Pseudomonas testosteroni, Salmonella typhimurium, Trichosporon beigelii and Aspergillus flavus* it was 2000 μg/ml; the MIC value for *Bacillus megaterium*, *Corynebacterium rubrum*, *Staphylococcus aureus* (1), *Staphylococcus aureus* (2), *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida* and *Mucor* was 4000 μg/ml and that for *Bacillus subtilis, Staphylococcus epidermides*, *Staphylococcus subfava*, *Enterobacter aerogenes, Escherichia coli, and Candida albicans* (1) it was 8000 μg/ml.

The results showed that methanol extract of *M. hexandra* leaf possessed measurable *in-vitro* antimicrobial activity against many of the microorganisms implicated in the pathogenesis of the human infections. The broad range of inhibition found implied that the extract had a comparable antimicrobial activity. This may be because of presence of tannins which were detected to an appreciable amount in phytochemical analysis. Tannins (non-crystallizable substances) have astringent actions, which form the basis for their therapeutic applications.[25] It has been found that plants which contain tannins possess antimicrobial activity.[26] Hence the significant antibacterial activity of the extract could be ascribed to the tannins. However, further work is needed to isolate the active principle from the active plant extracts.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials represents a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Therefore, continuous further exploration of plant derived antimicrobials is needed today.

CONCLUSIONS

On the basis of the present findings, we conclude that amongst three extracts of *Manilkara hexandra* leaf, methanol extract showed better antimicrobial activity followed by acetone extract. The antimicrobial activity was better at higher concentration i.e. 500 μg/disc. The MIC range was from 250–32,000 μg/ml. From the present exploration the methanol extract might turn a good candidate for searching antibacterial agents.

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REFERENCES

1. Verdine GL. The combinatorial chemistry of nature. *Nature*. 1996; 384: 11–3.
2. Kumar VP, Chauhan NS, Padh H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J Ethnopharmacol*. 2006; 107: 182–8.
3. Awadh Ali NA, Juelich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J Ethnopharmacol*. 2001; 74: 173–9.
4. Recio MC. A review of some antimicrobial compounds isolated from medicinal plants reported in the literature 1978–1988. *Phytother Res.* 1989; 3: 1445–53.
5. Rojas R, Bustamante B, Bauer J, Fernandez I, Alban J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol*. 2003; 88: 199–4.
6. Baquero F. Gram-positive resistance. Challenges for the development of new antibiotics. *J Antimicrob Therap.* 1997; 39: 1–6.
7. National Center for Infectious Diseases (NCID) Campaign to prevent antimicrobial resistance in health care settings. Center for Disease Control and Prevention [2002], Available from: http.//www.cdc.gov/drugresistance/healthcare/problem.htm
8. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm Wiss Technol.* 2004; 37: 263–8.
9. Zakaria M. Isolation and characterization of active compounds from medicinal plants. *Asia Pacific J Pharmacol.* 1991; 6: 15–20.
10. Balick MJ, Cox PA. Plants people and culture. The Science of Ethnobotany. New York. Scientific American Library, 1996.
11. Gordon MC, David JN. Natural product drug discovery in the next millennium. *Pharm Biol.* 2001; 39: 8–17.
12. Motsei ML, Lindsey KL, van Staden J, Jaeger AK. Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *J Ethnopharmacol.* 2003; 86: 235–41.
13. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J Ethnopharmacol.* 2004; 93: 1–7.
14. World Health Organization (WHO), Promoting the Role of Traditional Medicine in Health Systems. A Strategy for the African Region 2001–2010. Harare, World Health Organization, 2000.
15. Balandrin MF, Kjocke JA, Wurtele E, Bollinger SW, Bollinger H. Natural plant chemicals. sources of industrial and medicinal materials. *Science.* 1985; 228: 1154–60.
16. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol.* 2007a; 31: 53–8.
17. Bauer AW, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standardised single method. *Am J Clin Pathol.* 1966; 45: 493–96.
18. Parekh J, Chanda S. *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. *Afr J Biotech.* 2007b; 6: 760–70.
19. Pelczar MJ, Chan ECS, Krieg NR. Microbiology concepts and applications, Mc Graw-Hill Inc, New York. 1993; p.967.
20. NCCLS (National Committee for Clinical Laboratory Standards) Approved Standard M7-A3, 3rd ed., NCCLS, Villanova, PA, 1993.
21. Okigbo RN, Mbajiuka CS, Njoku CO. Antimicrobial potentials of (UDA) *Xylopia aethopica* and *Occimum gratissimum* L. on some pathogens of man. *Int J Mol Med.* 2005; 1: 392–7.
22. Dhar ML, Dhar MM, Dhawan BN, Mehrotra MN, Roy C. Screening of Indian plants for biological activity. *Indian J Exp Biol.* 1968; 6: 232–47.
23. Bhakuni DS, Bitiner M, Marticorena C, Silva M, Weldt E. Screening of Chilean plants for antimicrobial activity. *Lloydia.* 1974; 37: 621–32.
24. Javed S, Ali M. Antimicrobial activity in higher plants. *Hamdard Medicus* 2002; 45: 71–4.
25. Edward PC, Varrd ET, Lynn RB. A Textbook of Pharmacognosy, Henry Kinston, London, 1970.
26. Trease GE, Evans WC. Pharmacognosy. Balleire Tindal, London, 1989.

Phytochemical and Antimicrobial Activity of Leaf Extract of *Asparagus racemosus* Willd.

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INTRODUCTION

Plants, the first medicines of human being, have played a remarkable role in healthcare since the ancient times. Traditional plant-based medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates for a variety of diseases that threaten human health. *Asparagus* is the name of a genus of plants, a member of the family Asparagaceae (formerly placed in the Liliaceae). The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant.[1] *Asparagus* is the Greek word for "stalk" or "shoot". About 300 species of *Asparagus* are known to occur in the world in many countries in both hemispheres and throughout temperate and tropical regions. Some of the European species are *A. officinalis, A. sprengeri and A. acutifolius. A. officinalis* is reported

ABSTRACT: The aim of the present study was to explore the leaf extract of *Asparagus racemosus* Willd. belonging to family Asparagaceae for its antimicrobial activity. The *in vitro* antimicrobial activity of the leaf extract (ethanol-EE) of *Asparagus racemosus* and its fractions (hexane- HE and chloroform-CE) were assayed using the agar plate diffusion and nutrient broth dilution methods. Test microorganisms studied were *Bacillus pumilis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Aspergillus nigerand Candida albicans.* EE (300 mg/ml) inhibited the growth of all the test organisms and the maximum zone of inhibition against gram positive organism is *S. aureus* (14.3 ± 0.2 mm); against gram negative is *E. coli* (14.0 mm); and against fungal organism is *C. albicans* (16.2 ± 0.2 mm). EE and CE showed minimum inhibitory concentration (MIC) of 12.5 and 25 mg/ml respectively against the *B. pumilis, S. aureus, E. coli and C. albicans*. The minimum bactericidal concentration (MBC) of EE was found to be 12.5 mg/ml, against *S. aureus* and *E . coli* where as for CE , the MBC was 50 mg/ml against *S. aureus, E. coli, B. pumilis* and *C. albicans* . The EE exhibited antimicrobial activities followed by CE. HE exhibited least antimicrobial activity. The preliminary phytochemical screening of EE, CE and HE revealed the presence of sterols, flavonoids, tannins and carbohydrates, determined by utilizing standard methods of analysis. Preliminary phytochemical screening as well thin layer chromatography of the EE, CE and HE revealed that the leaves of *A. racemosus* contain flavonoids which might possibly be responsible for the antimicrobial activity of the extracts.

Keywords : *Asparagus racemosus*; Asparagaceae; Antimicrobial; MIC; MBC; Flavonoids.

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TABLE 1: Preliminary phytochemical screening.

S. NO	NAME OF THE TEST	ETHANOL EXTRACT	HEXANE FRACTION	CHLOROFORM FRACTION	RESIDUAL ETHANOLIC FRACTION
1	Leibermann-Burchard test (Sterols)	+ ve	+ ve	+ ve	- ve
2.	Shinoda test (Flavonoids)	+ ve	+ ve	+ ve	- ve
3.	Foam test (Saponins)	+ ve	+ ve	+ ve	+ ve
4.	Neutral FeCl3 test (Phenolic compounds)	+ ve	- ve	+ ve	- ve
5.	Mayer's, Dragendorff's test (Alkaloids)	- ve	- ve	- ve	- ve
6.	Molisch's test (Carbohydrates)	+ ve	- ve	- ve	+ ve

to be a popular vegetable consumed in many parts of the world.[2] Out of several species of *'Asparagus'* grown in India, *A. racemosus*, *A. gonaclades* and *A. adsendens* are most commonly used in indigenous medicine.[3] *A. racemosus* is commonly mentioned as a rasayana in the Ayurveda. Rasayanas are those plant drugs which promote general well being of an individual by increasing cellular vitality or resistance.

A study of literature survey claimed some therapeutic attributes for the leaves of *A. racemosus* (Sanskrit:- Shatavari) and has been used in treatment of boils[4], as antiseptic[4] and pesticide.[4] *A. racemosus* leaves are also used for various traditional uses in India like tonic[4], heel cracks[5], scabies[6], stomachache[7], urinary disorders[7] and high blood pressure[8], as galactogogue and aphrodisiac.[9] However no scientific proof, justifying all the above uses of the leaves of *A. racemosus* is available so far. Based on the traditional uses of *A. racemosus*, the present study was undertaken to evaluate antimicrobial effect of the ethanol leaf extract.

MATERIALS AND METHODS

Plant material

The plant material *(A. racemosus)* was collected from Chikkala Village, near Nidadavolu, West Godavari Dist., A.P., India in September 2002. The identification of the plant sample was carried out by Dr. M.Venkaiah, Associate Professor, Department of Botany, A.U., Visakhapatnam. The specimen (voucher No. BMK/BGR-10/2003) was kept in the Herbarium of the Phytochemistry and Pharmacognosy specialization, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Chemicals

All solvents (Hexane, chloroform and ethanol) were procured from Ranbaxy (India). Test reagents?? Media ingredients?? TLC solvent, sorbent, plate etc.??

Preparation of the extracts

The plant material of *A. racemosus* was dried under shade, ground mechanically to fine powder in a grinder and weighed accurately as 450.86 g. The powdered material was subjected to successive solvent extraction with ethanol using soxhlet apparatus. The extract was concentrated under vacuum (50°C), dried completely and weighed (76 g). Ethanol extract was then fractionated with hexane and then with chloroform until the solvent used for extraction becomes colorless. The hexane, chloroform and residual ethanolic fractions were concentrated under vacuum (50°C), dried completely and weighed. Their weights were : hexane fraction (25 g), chloroform fraction (20 g) and residual ethanolic fraction (16 g).

Phytochemical screening

The ethanol extract (EE) and its fractions (HE , CE and residual ethanolic fraction) were tested by the Libermann Burchard, Shinoda , Foam, Ferric chloride, Mayer's and Dragendorff's, and Molisch's tests to determine the presence of sterols, flavonoids, saponins, phenolic compounds, alkaloids and carbohydrates respectively.

the extracts (EE, CE and HE) were subjected to TLC analysis for the presence of flavonoids. Silica gel G, (mesh size 100–200, Acme) absorbent was used for plate preparation. The spots were made with capillary tube, and n-butanol, acetic acid and water (4: 1 : 5, upper layer v/v) was used as solvent system. The visualization of spots was carried out by spraying with $FeCl_3$: K_3FeCN_6 (1% aq.sol 1:1)[10] and Rf values of the extract were calculated.[11,12] The spots were also identified by observation under UV light and by exposure to ammonium vapour.

Organisms

The test microorganisms used for the antimicrobial activity screening were *Bacillus pumilis (B. pumilis),*

Bacillus subtilis (B. subtilis), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Proteus vulgaris (P. vulgaris), Staphylococcus aureus (S. aureus), Aspergillus niger (A. niger), Candida albicans (C. albicans). All the organisms were obtained from Department of Biotechnology, University college of Pharmaceutical Sciences, Andhra University, India.

Antimicrobial Tests

Antimicrobial activity of the EE, HE and CE was determined using agar plate diffusion technique.[13,14] The dried plant extract and fractions were dissolved in 1% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100 and 300 mg/ml. The microorganisms were maintained on agar slants. The inocula were prepared by inoculating the test organisms i.e. bacteria in nutrient broth and incubating them for 24 hours at 37°C while for fungi, sabouraud dextrose broth was used which was incubated for 48 hours. The final inoculum size was adjusted to 1 x 10^6 cfu/ml for bacteria or 1 x 10^8 cfu/ml for fungi. One milliliter of the diluted cultures was inoculated into sterile molten nutrient agar (48°C) and poured into sterile petri dishes. Similarly, 1 ml of the diluted fungal suspensions was poured into sterile sabourand dextrose agar plates. These were gently swirled and allowed to solidify. Afterwards, 6 mm wells were bored into the solidified and inoculated agar plates using sterile borer. The wells were filled with 100 µl of 100 and 300 mg/ml of each extract/fraction. penicillin G (10 U), ampicillin (25 µg), ciprofloxacin (5 µg), Gentamycin (10 µg) and nystatin (10 µg) standard discs were placed on the agar plate. Plates were left for 2 hours in a refrigerator for the extract to diffuse into the agar. Plates were then incubated overnight at 25°C and 37°C for fungi and bacterial strains respectively. At the end of the incubation period, inhibition zones were recorded in millimetres as the diameter of growth free zones around the bored holes using a transparent metre rule. Each extract and standard antibiotics were independently tested in triplicate.

Minimum Inhibitory Concentration

MIC was determined using the broth dilution technique.[15,16] The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extracts/fractions under study (EE, HE and CE). The microorganisms were prepared as described earlier. A two-fold serial dilution of each extracts was made to a concentration ranging from 0.098–100 mg/ml using nutrient broth. Each dilution was seeded with 200 µl of test micro-organisms to the standard concentration (1 x 10^6 cfu/ml). MIC is defined as the lowest concentration where no visible turbidity was observed in the test tubes.

TABLE 2: TLC analysis of *A. racemosus* leaf extracts.

EXTRACT	DISTANCE RUN BY SOLVENT (cm)	DISTANCE RUN BY SOLUTE (cm)	R_f VALUE
EE	5.6	2.9	0.52
HE	5.4	2.6	0.48
CE	5.7	2.9	0.51

EE–Ethanol extract, HE–Hexane extract, CE–chloroform extract.

Minimum bactericidal concentration (MBC)

MBC were determined by using the broth dilution technique[16] by assaying the test tubes resulting from MIC determinations. A 1oopful of the content of each test tube was independently inoculated by streaking on a solidified nutrient agar plate incubated at 37°C for 24 hours and then observed for bacterial growth. The lowest concentration of the subculture with no growth was considered the minimum bactericidal concentration.

Determination of MIC index value

MIC index value is calculated using the following mathematical equation Provide reference? Use equation option to insert equation

RESULTS AND DISCUSSION

The results of the preliminary phytochemical screening of ethanol extracts and it fractions of *A. racemosus* are presented in Table 1. The preliminary phytochemical screening of the ethanol extract (EE) and its fractions (HE and CE) revealed the presence of sterols, flavonoids, saponins, phenolic compounds and carbohydrates. The results of the thin layer chromatography (TLC) of EE, HE and CE of *A. racemosus* are presented in Table 2. TLC plates when placed in a chamber saturated with ammonia vapours, showed yellow color. The plates when placed under UV light showed fluorescent spots. The developed plates when sprayed with $FeCl_3$: K_3FeCN_6 (1% aq.sol 1:1) developed an orange color spot. Rf values of the ethanol extract (EE) and its fractions (HE and CE) were as 0.52, 0.48, 0.51 respectively. The results of TLC revealed the presence of flavonoids in the extracts.

The antimicrobial screenings are recorded in Table 3 expressing the zones of inhibition of bacterial and fungal growth. The extracts showed considerable amount of

TABLE 3 : Susceptibility study of the extracts of *Asparagus racemosus* against test microorganisms.

EXTRACTS/ STANDARDS	CONC. (mg/ml)	UNITS	INHIBITION ZONE (mm)																									
			B.P			B.S			S.A			E.C			P.V			P.A			A.N			C.A					
EE	300	mg/ml	13.8	±	0.2	14.0	±	0.0	14.3	±	0.2	14.0	±	0.0	12.0	±	0.0	12.2	±	0.2	15.8	±	0.2	16.2	±	0.2
	100	mg/ml	12.0	±	0.0	12.0	±	0.0	10.2	±	0.2	10.0	±	0.0	10.0	±	0.3	10.0	±	0.0	13.8	±	0.2	14.0	±	0.0
HE	300	mg/ml	10.2	±	0.2	10.2	±	0.2	10.0	±	0.0	10.0	±	0.0	9.3	±	0.2	10.0	±	0.0	10.0	±	0.0	12.2	±	0.2
	100	mg/ml	8.0	±	0.0	9.0	±	0.0	8.0	±	0.0	8.0	±	0.0	8.0	±	0.0	8.0	±	0.0	8.0	±	0.0	9.2	±	0.2
CE	300	mg/ml	11.2	±	0.4	10.3	±	0.2	12.3	±	0.3	11.3	±	0.7	10.2	±	0.2	10.7	±	0.2	12.0	±	0.0	13.0	±	0.0
	100	mg/ml	8.0	±	0.0	8.0	±	0.0	10.0	±	0.0	10.2	±	0.2	8.0	±	0.0	9.0	±	0.0	9.0	±	0.0	10.2	±	0.2
PB	10	µg/disc	17.2	±	0.2	16.2	±	0.2	13.3	±	0.3	16.5	±	0.3	16.0	±	0.0	8.0	±	0.0	-			-		
AM	25	µg/disc	14.0	±	0.0	14.2	±	0.2	10.3	±	0.3	14.0	±	0.0	14.0	±	0.0	13.2	±	0.2	-			-		
CP	5	µg/disc	22.0	±	0.0	23.7	±	0.3	23.0	±	0.0	22.0	±	0.0	ND			22.0	±	0.0	-			-		
GM	10	µg/disc	18.2	±	0.2	21.0	±	0.6	22.0	±	0.0	14.0	±	0.0	ND			14.0	±	0.0	-			-		
NY	10	µg/disc	-			-			-			-			-			-			19.5	±	0.3	20.2	±	0.2
DMSO	100	%	-			-			-			-			-			-			-			-		-

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, PB = Benzyl Pencillin, AM = Ampicillin, CP = Ciprofloxacin, GM = Gentamycin, NY = Nystatin, DMSO = Dimethylsulfoxide, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,ND = No disc, - = no inhibition. Values are expressed in Mean ± S.E.M

TABLE 4 : Minimum inhibitory concentrations (MIC) of *A. racemosus* leaf extracts.

EXTRACTS	ORGANISMS	CONCENTRATION (mg/ml)										
		0.098	0.195	0.391	0.781	1.563	3.125	6.250	12.500	25.000	50.000	100.000
EE	B.P	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	B.S	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	S.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	E.C	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	P.V	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	P.A	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	C.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	–*	–	–
EE	A.N	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	–*	–
CE		+	+	+	+	+	+	+	+	+	–*	–

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, PB = Benzyl Pencillin, AM = Ampicillin, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,+ = growth, – = no growth. – = MIC.*

inhibition against *B. pumilis*, *B. subtilis, S. aureus, E. coli*, *P. vulgaris, P. aeuroginosa*, *A. niger* and *C. albicans.* EE (300 mg/ml) inhibited the growth of all the test organisms and the maximum zone of inhibition was found to be against gram positive organism *S. aureus* (14.3 ± 0.2 mm); gram negative organism *E. coli* (14.0 mm); and fungal organism *C. albicans* (16.2 ± 0.2 mm). The standard antibiotic discs used in this study inhibited the growth of the test bacterial and fungal organisms. The zone of inhibition produced by penicillin G disc against *P. aeuroginosa* was found to be smaller than those produced by some extracts especially EE, HE and CE (300 mg/ml).

From the results of the MIC and MBC presented in Tables 4 and 5 respectively, it can be surmise that the active constituents responsible for the widest activities in this plant were residing in the CE fraction of the ethanol extract compared with HE; this was basically because the EE and CE fraction notably exhibited minimal inhibitory concentration of 12.5 and 25 mg/ml respectively against the *B. pumilis*, *S. aureus, E. coli* and *C. albicans*. HE fraction had an MIC of 50 mg/ml against *B. pumilis*, *B. subtilis, S. aureus, E. coli*, *P. vulgaris* and *P. aeuroginosa*.

The minimum bactericidal concentration of EE was found to be 12.5 mg/ml against *S. aureus* and

TABLE 5: Minimum bactericidal concentrations (MBC) of *A. racemosus* leaf extracts.

EXTRACTS	ORGANISMS	CONCENTRATION (mg/ml)										
		0.098	0.195	0.391	0.781	1.563	3.125	6.250	12.500	25.000	50.000	100.000
EE	B.P	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	B.S	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	S.A	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	E.C	+	+	+	+	+	+	+	–*	–	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	P.V	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	P.A	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*
EE	C.A	+	+	+	+	+	+	+	+	–*	–	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	–*	–
EE	A.N	+	+	+	+	+	+	+	+	+	–*	–
HE		+	+	+	+	+	+	+	+	+	+	–*
CE		+	+	+	+	+	+	+	+	+	+	–*

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger, C.A = Candida albicans,+ = growth, – = no growth. – = MBC.*

E. coli. For CE fraction, the MBC was 50 mg/ml against *S. aureus* , *E. coli*, *B. pumilis* and *C. albicans* and for HE fraction; the MBC was 100 mg/ml towards *B. pumilis*, *B. subtilis, S. aureus, E. coli, P. vulgaris* and *P. aeuroginosa*.

From the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) presented; it was observed that the broadest activity of the extract was against *E. coli* and *S. aureus.* MIC index results are presented in Table 6.

The EE exhibited antimicrobial activities followed by CE fraction. HE fraction exhibited least antimicrobial activity. Ethanol extract (EE) and its fractions (CE and HE) exhibited appreciable activity against *S. aureus,* a pyogenic bacterium known to play a significant role in invasive skin diseases including superficial and deep follicular lesion.[17] It also showed appreciable activity against *E. coli*. The broad-spectrum antibacterial activity exhibited by the EE and CE could be related with the concentrations of sterols, flavonoids, saponins, phenolic compounds and carbohydrates in these extracts. These classes of compounds are known to show curative activity against several pathogens[18] and may explain some of its antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been documented.[19-21]

The literature survey of plant reported the presence of flavonoids[22] and preliminary phytochemical screening as well as thin layer chromatography of the

TABLE 6: MIC index of ***A. racemosus*** leaf extracts.

EXTRACTS	ORGANISMS	MIC (mg/ml)	MBC (mg/ml)	MIC INDEX
EE	B.P	12.500	25.000	2
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	B.S	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	S.A	12.500	12.500	1
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	E.C	12.500	12.500	1
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	P.V	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	P.A	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2
EE	C.A	12.500	25.000	2
HE		50.000	100.000	2
CE		25.000	50.000	2
EE	A.N	25.000	50.000	2
HE		50.000	100.000	2
CE		50.000	100.000	2

Key: EE = Ethanolic extract, HE = Hexane extract, CE = Chloroform extract, B.P = Bacillus pumilus, B.S = Bacillus subtilis, S.A = Staphylococcus aureus, E.C = Escherichia coli, P.V = Proteus vulgaris, P.A = Pseudomonas aeuroginosa, A.N = Aspergillus niger,C.A = Candida albicans.

extracts revealed that the leaves of *A. racemosus* contain flavonoids. Several flavonoids isolated from the medicinal plants have been discovered to possess significant antimicrobial activity.[21,23]

This study confirmed that the ethanol extract (EE) and its hexane (HE) and chloroform (CE) fractions of leaves of *A. racemosus* exhibit antimicrobial activity and the effects observed are attributable due to the presence of flavonoids in the plant.

In conclusion, the fact that the extracts (EE and CE) produced inhibitory activities but less when compared to reference drugs against almost all the test bacteria and fungi provides some scientific basis for some of the uses in traditional medicine like treatment of boils and scabies and as antiseptic. We therefore, suggest the isolation and possible characterization of the active constituent(s) from the extracts of this plant species as possible antibacterial agents.

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REFERENCES

1. Oketch Rabah HA. Phytochemical Constituents of the Genus *Asparagus* and their biological activities. *Hamdard*. 1998; 41**:** 33–43.
2. Shao YU, Poobsasert O, Kennelly EJ, Chin CK, Ho CT, Huang MT, et al. Steroidal saponins from *Asparagus officinalis* and their cytotoxic activity. *Planta Medica*. 1997; 63**:** 258–62.
3. Rao SB. Saponins (Sapogenins) from Indian Medicinal Plants:- Part I Sapogenins from *Asparagus*. *Indian J Pharmacy*. 1952; 14**:** 131–132.
4. Sharma GK. Medical ethnobotany in the Shivalik Range of the Himalayas. *Journal of the Tennessee Academy of Science*. 2004; 79**:** 67.
5. Ignacimuthu S, Ayyanar M , Sivaraman KS. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). *Journal of Ethnobiology and Ethnomedicine*. 2006; 2**:** 25.
6. Yesodharan K, Sujana KA. Status of ethnomedicinal plants in the parambikulam wildlife sanctuary, kerala, south india. *Ann. For.* 2007; 15**:** 322–334.
7. Sajem AL, Gosai K. Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. *Journal of Ethnobiology and Ethnomedicine*. 2006; 2**:** 33.
8. Bhutani KK. *Herbal Wealth of North-East India, Database and Appraisal,* Azad Hind Stores, Chandigarh, 2008.
9. Sumeet D, Shefali K. Ethnomedicinal uses of some plant species by ethnic and rural peoples of indore district of madhya pradesh, India. *Pharm. Review*. 2008; 6**:** 2.
10. Markham K. *Isolation Techniques for Flavonoids.* Academic press, New York, 1975.
11. Harbone JB. *Phytochemical Methods,* Reinhold publication, London, 1973.
12. Bobitt J M. *Thin Layer Chromatography*, Reinhold publication, London, 1966.
13. Odama IE, Shok M, Olurinola PF. *Ceiba Petandra* (Silk Cotton Tree). *The State of Medicinal Plant Research in Nigeria*. Ile-Ife, Univ. Press, Nigeria, 1986.
14. Chung KT, Thomson WR, Wu-Yan CD. Growth Inhibition of Selected Food-Borne Bacteria by Plant Extracts. *J. Appl. Microbiol*. 1990; 71**:** 398–401.
15. Sidney MF, William JM, Elvyn GS. *Bailey and Scott's Diagnostic Microbiology*. C.V. Mosby, St. Louis, 1978.
16. Volleková A, Kòst'àlovà D, Sochorovà R. Isoquinoline Alkaloids from *Mahonia aquifolium* Stem bark is active against Malassezia spp. *Folia Microbiol*. 2001; 46**:** 107–110.
17. Srinivasan D, Nathan S, Suresh T, Perumalsamy PZ. Antimicrobial Activity of Certain India Medicinal Plant used in Folkloric Medicine. *J. Ethnopharmacol*. 2001; 74**:** 217–220.
18. Eloff JN. Quantifying the bioactivity of plant extracts during screening and Bio-assay guided fractionation. *Phytomedicine*. 2004; 11 **:** 370–377.
19. Pereira JA, Pereira APG, Ferreira ICFR, Valentão P, Andrade PB, Seabra R, et al. Table olives from Portugal: phenolic compounds, antioxidant potential and antimicrobial activity. *J. Agric. Food Chem*. 2006; 54**:** 8425–8431.
20. Proestos C, Chorianopoulos N, Nychas GJE, Komaitis M. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem*. 2005; 53**:** 1190–1195.
21. Rauha J P, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Food Microbiol*. 2000; 56**:** 3–12.
22. Velavan S, Nagulendran KR, Mahesh R, Hazeena Begum V. The Chemistry, Pharmacological and Therapeutic Applications of *Asparagus racemosus*- A Review. *Phcog Rev.* 2007; 1**:** 350–360.
23. Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother. Res.* 2001; 15**:** 39–43.

HPTLC Finger Print Profile of Dried Fruit of *Physalis alkekengi* Linn.

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INTRODUCTION

The dried fruits of *Physalis alkekengi* Linn (Fam. Solanaceae) are known differently in different languages such as Urdu: Kaknaj, Papotan; English: Winter cherry, Bladder cherry; Hindi: Kakanaja and it occurs in Southern Europe through China to Japan but does not occur in India, even though fruits are available in the Indian markets, in the name of Kaknaj.[1,2,3] A diffuse perennial, with glabrous or slightly pubescent stems, whitish flowers and reddish fruits, 4–12 cm long, with blood red, inflated calyx, often grown as an ornamental plant.

The berries are very juicy and have an acidulous bitter taste. The fruits as well as leaves contain an amorphous bitter principle.[4-6] The fruits also contain vitamin C, a carotenoid pigment (physalein) and probably an alkaloid.[7] They are reported to be diuretic, febrifuge, hydragogue and vermifuge.[8,9] Berries contain malic and citric acids, a volatile matter, sugar, mucilage, pectin, woody fibre and water. They act on liver and are diuretic, alterative, anthelmintic and laxative, useful in kidney and urinary diseases and also in skin diseases.[10]

Castellani and Browing tried the use of an ethereal extract of berries in 5 grain doses, 3–4 times a day in cases of typical sprue in conjunction with the usual milk diet and alkaline treatment and found improvement in general condition of patient.

ABSTRACT: Dried fruit of *Physalis alkekengi* Linn. (Fam. Solanaceae) is called as Kaknaj in the Unani system of medicine and used as diuretic, antiseptic, corrective of liver and sedative. Standardization of this drug is the key factor in regulating the therapeutic efficacy. Organoleptic parameters are not enough in establishing the standards of herbal drugs. Instrumental analysis of herbal drugs, which gives a more concrete picture regarding the qualitative and quantitative aspects of bioactive molecules, is widely accepted in the quality assessment of herbal drugs. However, such work related to traditional herbal medicines is lacking or in infantile stage. In the present study, morphological and physicochemical parameters and HPTLC finger print studies of *Physalis alkekengi* have been carried out and the results provide referential information for standardization.

Keywords: Fluorescence analysis, HPTLC analysis, Physicochemical parameters.

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Berries are remedy for anemia and rheumatism as they contain salicylic salts and rich in alkaline and mineral salts, viz., lime and phosphates.[11]

However no scientific standard parameters are available to determine the quality and genuineness of the drug. In the present study an attempt was made to standardize the drug with respect to HPTLC technique to provide beneficial information in regarding the standardization according to WHO guidelines.

MATERIALS AND METHODS

Collection of Material

Genuine *Physalis alkekengi* Linn was collected from the authorized agent in the local market of Hyderabad in Andhra Pradesh state of India. After confirmation of its botanical identity with the help of botanist, the fruits were subjected for morphological, physicochemical and HPTLC finger print studies.

Morphological studies: Routine procedures were followed for studying the external morphology of the drug including macroscopic and microscopic studies.

Physicochemical parameters: Physicochemical parameters such as ash and extractive values were determined according to the methods described in Unani Pharmacopoeia of India, 2008.[1]

Fluorescence analysis: Fluorescence analysis was carried out as per the method described by Trease and Evans.[12]

HPTLC Apparatus

HPTLC system composed of an automatic TLC applicator, basic marathon autosampler, densitometer CD 60 of DESAGA Sarstedt Gruppe system and UV-Vis Cabinet for recognition of spots. The chromatographic and the integrated data were recorded using computer based software DESAGA ProQuant 1.6 Version.

Preparation of extract of the drug sample for HPTLC:

The dried fruit was crushed with mortar and pestle to form coarse powder. Five grams powder of drug was macerated in 100 ml of methanol in a stoppered conical flask and was kept for 2 hours with gentle shaking in regular intervals. Later the contents were filtered through Whatman no. 41 filter paper and the filtrate was evaporated to get 20 ml of solution. The solution thus obtained was used as sample for the determination of components.

Development of HPTLC technique:

Development and determination of the solvent system:

Sample applied	: Sample drug solution of *Physalis alkekengi* about 10 µl.
Solvent system	: Toluene: ethyl acetate: methanol (7: 2: 1)
Migration distance	: 95 mm
Scanning wavelength	: 366 nm

The sample was spotted with the help of automatic TLC applicator system of the DESAGA Sarstedt Gruppe on precoated aluminum sheets of silica gel 60 F_{254} (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the twin-trough glass TLC chamber to the

TABLE 1 : Peak area and R_f values of the components of the drug.

PEAK NO.	NAME	MIGRATION DISTANCE (mm)	AREA PERCENTAGE (%)	HEIGHT (mm)	R_f VALUE
1	Component 1	15.0	81.4	1351.32	0.06
2	Component 2	26.3	1.4	81.70	0.20
3	Component 3	36.4	0.3	8.31	0.31
4	Component 4	44.5	2.0	53.25	0.41
5	Component 5	51.2	0.4	9.91	0.48
6	Component 6	55.6	0.2	9.62	0.54
7	Component 7	71.1	4.5	110.09	0.72
8	Component 8	76.7	1.1	96.25	0.78
9	Component 9	84.2	2.4	73.43	0.87
10	Component 10	90.1	6.4	163.95	0.94

maximum height of the plate so that the components are separated on the polar phase of silica gel and mobile phase of solvent system.

After developing, the TLC plate was dried completely and the sports were observed with UV cabinet system for detection of spots at 366 nm. Further it was scanned with densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366 nm for maximum number of components. A corresponding densitogram was obtained in which peaks were appeared for the spots corresponding to R_f values of each component.

RESULTS AND DISCUSSION

Organoleptic parameters

The fruit of *Physalis alkekengi* Linn is reddish brown in colour, bitter in taste with characteristic odour.

Morphology of Physalis alkekengi Linn-fruit

***Macroscopic*:** Red coloured berry, globose, about 1 to 1.5 cm in diameter, outer surface wrinkled, with dried flesh; unilocular, completely packed with seeds, overlapping, centrally oriented, insignificant placenta present; seeds 1.8 to 2.2 mm, numerous, flat, with curved embryo, hilum in the concavity; fruit sweet and sour in taste (Fig. 1).

***Microscopic*:** Cuticle present; fruit wall not distinguishable as epicarp, mesocarp and endocarp clearly; the outer layer consists of a single layer of non lignified, thin walled cell with brown contents; below this are a few layers of horizontally oriented cells with orange contents and loosely arranged layers of parenchyma, with mucilage cells; inner layers of the fruit wall and the placentae proliferate into the locule packed with minute seeds.

***Powder*:** The powder is brownish-orange in colour; shows sclereids, parenchymatous cells, endospermic parenchymatous cells rich in oil and aleurone grains.

Physicochemical Characters

The physicochemical parameters of the drug such as total ash, water-soluble ash, acid insoluble ash, alcohol soluble matter and water-soluble matter (% w/w) were tabulated in table-2.

Fluorescence analysis of powdered drug

The flourescence analysis of the powdered drug upon treatment with different reagents and observation in UV short and long wavelength regions and also in visible light showed corresponding colours in the solution as described in table-3.

FIGURE 1: Macroscopic feature of dried fruit of *Physalis alkekengi* Linn.

Fluorescence analysis of powdered drug extracts in different solvents

The fluorescence analysis of powdered drug extracts carried out in different solvents and observed in UV short and long wavelength regions and also in visible light shown corresponding colours in the solution as described in the table-4.

Multi-wavelength scan

Multi-wavelength scan was carried out from 250 nm to 400 nm in the steps of 4 nm as shown in figure 2 from which it shows clearly that at UV 366 nm, more number of peaks were obtained and also found better intense peaks as shown in figure 4.

HPTLC ANALYSIS

It is evident from table-1 that there are ten spots with R_f values 0.06 (dark brown), 0.20 (blue), 0.31

TABLE 2 : The physicochemical parameter of the drug.

ASH VALUES (% w/w)	
Total ash	Not more than 6 percent
Water soluble ash	Not more than 5 percent
Acid insoluble ash	Not more than 1 percent
SOLVENT SOLUBLE MATTER (% w/w)	
Alcohol soluble matter	Not less than 10 percent
Water soluble matter	Not less than 22 percent
Foreign matter	Not more than 2 percent

TABLE 3 : Flourescence analysis of powdered drug upon treatment with different reagents.

S. NO	REAGENTS	UV LIGHT		VISIBLE LIGHT
		SHORT 254 nm	LONG 366 nm	
1.	Powder as such	Blackish	Light yellow	Brownish orange
2.	Powder treated with 1N NaOH in methanol	Dark green	Light Blue	Light yellow
3.	Powder treated with 1N NaOH in water	Black	Pale yellow	Brown
4.	Powder treated with 1N HCl	Dark black	Light green	Brown
5.	Powder treated with 50% HNO_3 aqueous	Grey	Pale yellow	Light brown
6.	Powder treated with 50% H_2SO_4 aqueous	Dark black	Light blue	Brown

TABLE 4 : Fluorescence analysis of powdered drug extracts with different solvents.

S.NO	EXTRACTION SOLVENT	UV LIGHT		VISIBLE LIGHT
		254 nm	366 nm	
1.	Petroleum ether	Yellow	Black	Dark brown
2.	Chloroform	Black	Pale yellow	Dark brown
3.	Ethyl acetate	Black	Pale yellow	Brown
4.	Alcohol	Black	Blue	Yellow
5.	Acetone	Black	Pale yellow	Yellow

(brown), 0.41 (blue), 0.48 (light blue), 0.54 (blue), 0.72 (blue), 0.78 (light brown), 0.87 (light blue) and 0.94 (light green) as shown in figure 3 indicating the occurrence of atleast ten different components in the methanolic extract It is also clear from table-1 and the chromatogram as shown in figure-2, that out of ten components, the component with R_f value 0.06 (dark brown) and component at R_f value 0.94 (light green) were found to be more predominant as the percentage area is more with 81.4% and 6.4% respectively. And remaining components were found to be very less in quantity as the percentage area for all the spots was less than 4.5%. Thus the developed chromatogram will be specific

FIGURE 2 : Multiwavelength scan carried out in the range of 250 nm to 400 nm showing variation in number of peaks at different wavelengths.

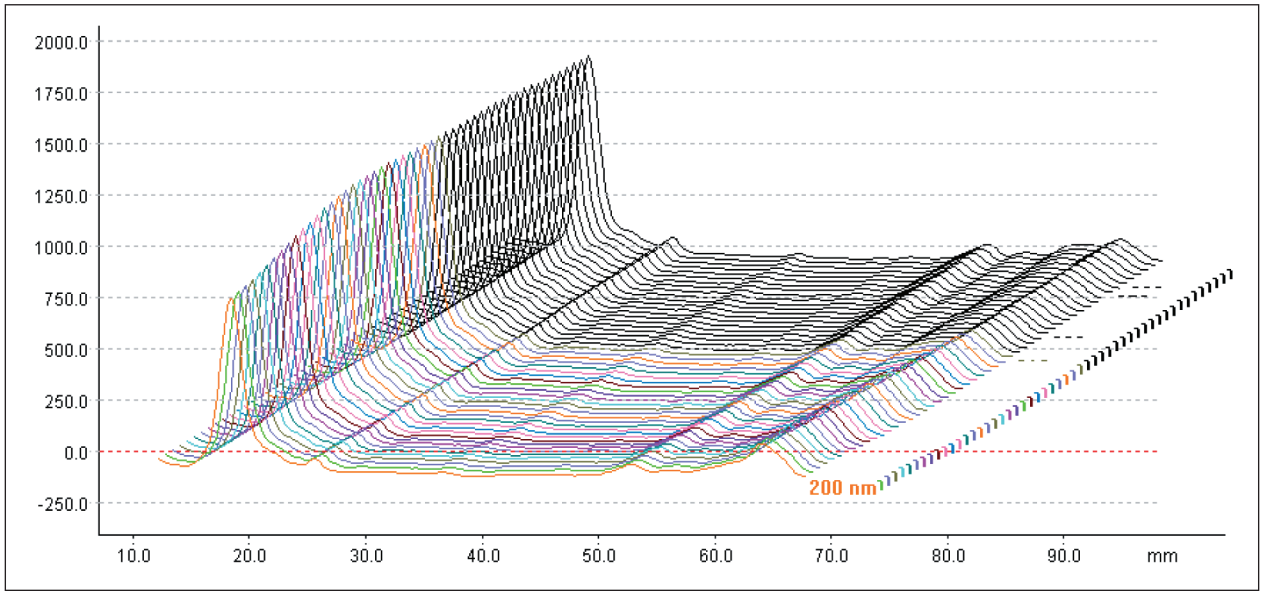
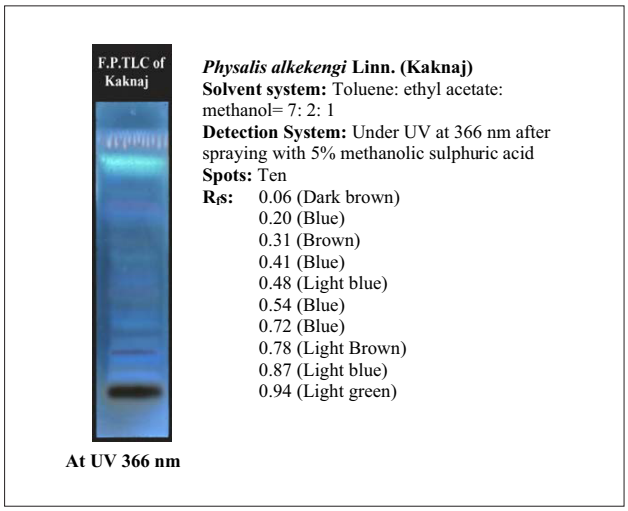


FIGURE 3 : Methanolic extract: Finger print TLC plate of *Physalis alkekengi* Linn (Kaknaj)



with selected solvent system and constant R_f values, and serve the better tool for standardization of the drug.

Chemical compounds, some of which are having therapeutic activities, are species specific and vary from species to species. These compounds can be visualized by developing chromatograms. Characteristic TLC/ HPTLC finger printing of a particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species, Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

CONCLUSION

In addition to organoleptic parameters, chromatographic finger printing of herbal medicines will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of dried *Physalis alkekengi* Linn. can provide standard finger prints and can be used as a reference for the identification and quality control of the drug.

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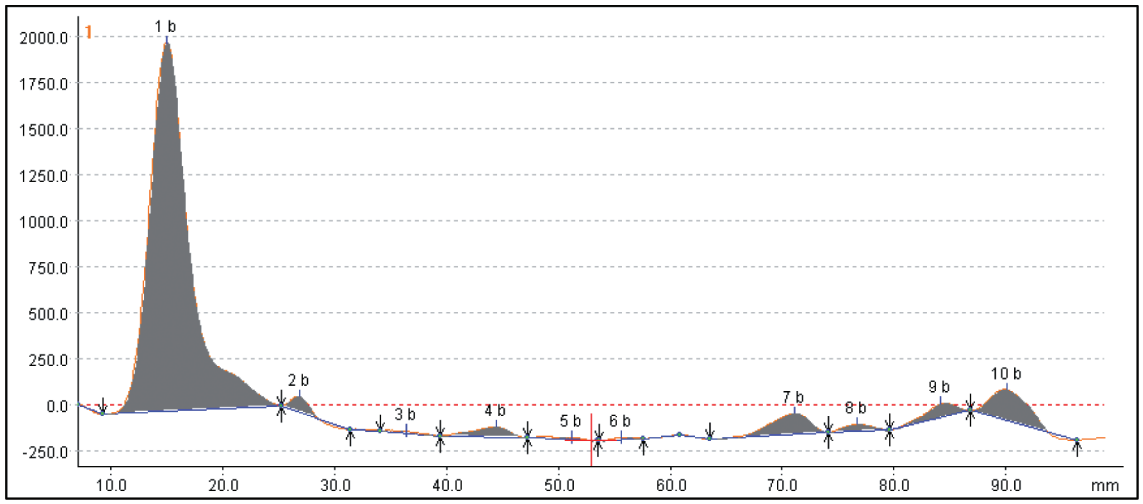
REFERENCES

1. Anonymous. The Unani Pharmacopoeia of India, Department of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi. 2008; p.40.
2. Anonymous. National Formulary of Unani Medicine, Part-II, Vol. I, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi. 2007.
3. Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants (Reprinted edition). CSIR, New Delhi, 1992; p.191.
4. Matsuura T, Kawai M, Nakashima R, Butsugan Y. Bitter Principles of *Physalis Alkekengi var Francheti* : Structure of Physalin A. *Tetrahedron Letters*. 1969; 10:(20): p.2.

FIGURE 4 : Methanolic extract, Finger print densitogram of *Physalis alkekengi* Linn (Kaknaj) showing peaks of the corresponding spots obtained on the chromatogram.



5. Kawai M, Matsuura T. The structure of physalin C: A bitter principle of *Physalis Alkekengi var. Francheti Tetrahedron*. 1970; 2:(7): p.1743–1745.
6. Reiko Sunayama, Masanori Kuroyanagi, Kaoru Umehara and Akira Ueno. Physalin and neophysalins from *Physalis alkekengi* var. *francheti* and their differentiation inducing activity. *Phytochemistry*. 1993; 34:(2): p. 529–533.
7. Basey K and Wooley JG. Alkaloids of physalis alkekengi. *Phytochemistry*. 1973; Vol.12: p. 2557–59.
8. Anonymous. The Wealth of India, Raw Materials, Ph-Re. Publications and Information Directorate, New Delhi; CSIR. 1969; 8: p. 37.
9. Ambasta SP (Ed.). The useful plants of India. Publications and Information Directorate, (CSIR, New Delhi, India. 1992) p. 452.
10. Ali Safiuddin, Adviya Muffarida. Tarakki Urdu Bureau Unani, New Delhi, 1986.
11. Nadkarni KM. Indian Materia Medica, Popular Prakashan, Bombay, Vol. I, 1976; p. 750
12. Trease GE and Evans WC. *Pharmacognosy*, 13th edition, (Bailliere Tuidall, London, 1989) p. 799–803.

Determination of Total Phenols, Free Radical Scavenging and Antibacterial Activities of *Mentha longifolia* Linn. Hudson from the Cold Desert, Ladakh, India.

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INTRODUCTION

Oxidative stress is involved in the pathogenesis of various chronic diseases such as atherosclerosis, cancer, inflammation, and neurodegenerative disorders like Parkinson's and Alzheimer's diseases.[1,2] Antioxidants protect against free radicals and they are essential in obtaining and preserving good health. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods and to extend their shelf-life.[3] Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and/or activators of antioxidative defense enzyme systems to suppress the radical damages in biological systems.[4,5] Hence, in recent days much attention is being focused on the use of antioxidants to protect the cells from biological damages likefree radicals.[6]

Most of the antioxidants in use commercially [e.g., butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)] are synthetic and some of them have been suspected of causing or promoting negative health effects therefore, some

ABSTRACT: The aim of the study was to evaluate the antioxidant and free radical scavenging properties, total phenolic contents and antibacterial activities of the methanol extract of *Mentha longifolia* Linn. Hudson. growing in the high altitude cold desert region of Ladakh, Jammu and Kashmir, India. The antioxidant activities of the extracts were investigated with four different methods such as 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), Reducing power assay and Ferric reducing antioxidant property (FRAP). *M . longifolia* exhibited the strongest activity as ABTS scavenger compared to DPPH and FRAP. All the assays carried out found that the plant has antioxidant potential. The total polyphenol content (TPC) and total flavonoid content of the extract was determined as 107.208 ± 34.2 mg GAE/g dry wt. and 42.47 ± 7.97 mg QE/g dry wt. respectively where 39.61% of TPC were flavonoids. A high correlation was found between the DPPH and ABTS free radical scavenging potency (R^2 = 0.869). The study has validated the medicinal potential of *Mentha longifolia* Linn. Hudson and revealed that the consumption of the plant could be beneficial as it possesses the desirable quality of antioxidant activity.

Key words: Antioxidant activity, Antibacterial activity, *Mentha longifolia* L., Polyphenols.

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restrictions are placed on their applications and there is a trend to substitute them with naturally occurring antioxidants.[1,7] Plant originated polyphenols and flavonoids have powerful anti-oxidant activities. These phytochemicals are able to scavenge a wide range of reactive oxygen species, including hydroxyl radicals, peroxyl radicals, hypochlorous radicals and superoxide radicals inhibiting lipid peroxidation in human tissues.[8–10] Polyphenols in plants include simple phenols, phenolic acids (both benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins.[11]

Mentha longifolia Linn. Hudson is an erect aromatic perennial herb belonging to the family Laminaceae is one of the important members of the family and the habitat pertains to moist places and water channels. The plant is commonly known as '*Jangli Pudina'* in Hindi and commonly used as herbal tea, flavoring agent and medicinal plant. The plant is extensively used in the study area, Ladakh, Jammu and Kashmir, India as antidysenteric, carminative, antiseptic and stimulant in the Amchi system of medicine. The leaves of this plant are also used to make a local dish '*Phololing Chamyk*' which is taken with roti while the dried leaves are used to treat abdominal pains, as stimulant, diureticand relieve headache and rheumatism.[12,13] Considering the importance of the phytochemicals responsible for the medicinal properties of *M. longifolia* L. the present investigation was carried out to analyse antioxidative activity, antibacterial activity and quantify the phenols and flavonoids.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonicacid (ABTS), 2,4,6-tri (2-pyridyl)-s-triazines, potassium ferricyanide, potassium persulfate, trichloroacetic acid, gallic acid and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $FeCl_3$, ascorbic acid and butylated hydroxy toluene (BHT) were procured from Himedia Laboratories Pvt. Ltd. (Germany). Folin-Ciocalteu phenol reagent and other chemicals were obtained from Merck Chemical Supplies (Damstadt, Germany).

Collection and extraction of plant sample

The plant material of *Mentha longifolia* Linn. Hudson was collected from natural habitats and the herbal garden, DIHAR of Ladakh during May 2009. The plant samples were air-dried and ground into uniform powder using pestle and mortar. The methanol extract was prepared by dissolving 5 g of dried powder in 85% methanol. The solvent was concentrated using rotary vacuum evaporator.

Quantification of polyphenols

Total phenolic content in the extract was determined by the modified Folin-Ciocalteu method.[14] An aliquot of the extract was mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm using UV-VIS spectrophotometer. The amount of total polyphenols in the extract was calculated from the calibration curve in terms of gallic acid equivalents ($y = 0.002x + 0.039$, $R^2 = 0.982$).

Quantification of flavonoids

Total flavonoid contents were determined by the method of Ordon et al.[15] A volume of 0.5 ml of 2% $AlCl_3$ ethanolic solution was added to 0.5 ml of the sample solution. After incubation for one hour at room temperature, the absorbance was measured at 420 nm. Flavonoid concentration of the extract was expressed as mg/g equivalent of quercetin ($y = 0.009x + 0.145$, $R^2 = 0.987$).

DETERMINATION OF ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity

The hydrogen atom- or electron donating ability of the corresponding extract and some pure compounds were measured from the bleaching of the purple coloured methanol solution of 2, 2'- diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay used stable radical DPPH as a reagent.[16] 5 ml of various concentrations such as 20, 40, 60, 80 and 100 ppm of the extracts in methanol were added to 5 ml of 0.002% DPPH solution. After 30 minutes incubation at room temperature in dark, the absorbance was read against blank at 517 nm. The blank used was methanol and the control was 5 ml methanol and 5 ml DPPH solution (0.002%). Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100.$$

Where A_{blank} is the absorbance of the control reaction (containing all the reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition was calculated using the graph by plotting inhibition

percentage against extract concentration. Ascorbic acid (AA) and the synthetic antioxidant reagent butylated hydroxy toluene (BHT) were used as positive controls and all the tests were replicated thrice.

ABTS radical scavenging activity

For ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical) assay, the method of Re et al[17] was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS+ solution with 60 ml of methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using ABTS+ solution was freshly prepared for each assay. Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS+ solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of butylated hydroxy toluene (BHT). The percentage inhibition [%] was calculated:

$$I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100.$$

where A_{blank} is the absorbance of ABTS radical + methanol; A_{sample} is the absorbance of ABTS radical + sample extract/standard. All the tests were replicated thrice and the extract concentration providing 50% inhibition was calculated using the graph by plotting inhibition percentage against extract concentration.

Reducing power assay

Plant extracts (200-1000 ppm) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%), and the mixture was incubated at 50°C for 30 min. 2.5 ml of trichloroacetic acid (10%) was then added to the mixture and centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.[18]

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain[19] was adopted for the FRAP (Ferric reducing antioxidant property) assay. The stock solutions included 300 mM acetate buffer (3.1 g of CH_3COONa and 16 ml of CH_3OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before the assay. Plant extract (150 µL) was allowed to react with 2850 µl of the FRAP solution for 30 min in dark. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results are expressed in µM Fe (II)/g dry mass using the following equation based on calibration curve $y = 0.773x - 0.072$, $R^2 = 0.991$ and compared with that of butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and ascorbic acid.

Determination of antibacterial activity

Antibacterial activity of *M. longifolia* Linn. was studied against four bacterial strains viz. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial strains were grown on Muller Hinton (MH) agar plates and suspended in MH broth. Minimum inhibition concentration (MIC) values were determined using the broth dilution method.[20] The bacterial suspensions were aerobically incubated for 24 h at 37°C. MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. Negative control cultures contained only sterile physiological tris-buffer, while the disc soaked in standard broad spectrum antibiotics i.e. streptomycin and amoxicillin were used as positive controls.

Statistical analysis

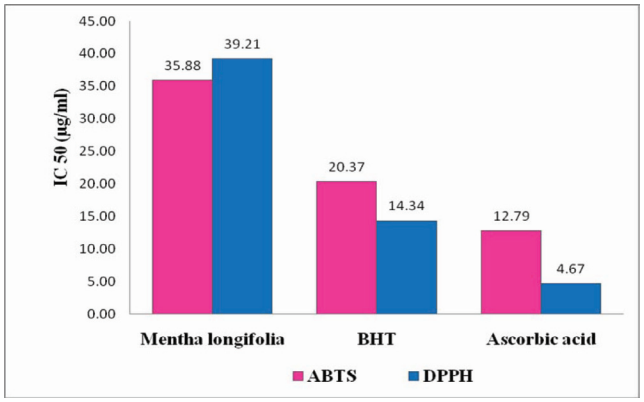
The experimental results were expressed as average ± standard deviation of average of three replicates. Decolorisation was plotted against the sample extract concentration and a linear regression curve was established in order to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the free radical by 50%. Statistical analysis were performed using SPSS 11.5.

RESULTS AND DISCUSSION

Total polyphenolic content

Phenolic compounds are a class of antioxidant agents which act as free radical terminators[21] and are considered as a major group of compounds that contribute to the antioxidant activities of plant materials because of their scavenging ability on free radicals due to their hydroxyl groups.[3] Flavonoids are a group of polyphenolic compounds with known properties of free radical scavenging, antibacterial and anti-inflammatory action.[22] Total polyphenol content observed in *M. longifolia* L. was 107.208 ± 34.2 mg GAE/g dry wt.

FIGURE 1: IC 50 µg/ml (50% free radical scavenging) of *Mentha longifolia* Linn. Extracts, BHT and AA determined by DPPH and ABTS method.



and total flavonoid content was 42.47 ± 7.97 mg QE/g dry wt, however it is 39.61% of total polyphenolic content. It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores.[23]

TABLE 1: Ascorbic acid Equivalent Antioxidant Capacity (AEAC) determined by DPPH, ABTS and RPA (mg g -1 dry wt.) for *Mentha longifolia* Linn.

PLANT EXTRACT	DPPH	ABTS	RPA
Mentha longifolia	15.30 ± 0.41	74.38 ± 4.47	8.35 ± 7.22

Antioxidant activity

The antioxidant activity of *M. longifolia* L. was observed in the present study by *in vitro* assays such as DPPH, ABTS, FRAP and reducing power assays to evaluate the free radical scavenging activity and antioxidant capacity of the extract (Table 1). The antioxidant activity of methanol extract of *M. longifolia* L. was examined by comparing with the activity of known antioxidants such as ascorbic acid and BHT with inhibition of DPPH radical. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity.[24] The antioxidant activity of the extracts increased with an increasing amount of extract. The methanol extract was able to reduce the stable free radical DPPH to the yellow coloured diphenylpicrylhydrazine with an IC_{50} of 39.21 µg/ml, while AA was 4.67 µg/ml and BHT was 14.34 µg/ml. The results of DPPH free radical scavenging activity were showed in Fig.1. The antioxidant activity (IC_{50}) of *Mentha longifolia* L. was previously reported as 24.07 µg/ml.[25] However, these contradictory results are most likely due to differences in methodology and experimental conditions used in the different studies.

The result of IC_{50} values of ABTS of synthetic antioxidants like BHT and AA were higher than DPPH assay results. However, *M. longifolia* L. showed lower IC_{50} values of ABTS assay (Fig. 1). Earlier reports on honey and potato samples also showed that the average AOA values based on AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) determined by ABTS assay were two to three times higher as compared to DPPH assay and the present study also showed five or six times higher average AOA values for ABTS in comparison with DPPH (Table 1). In the present study there is a significant correlation (R^2 = 0.869) between ABTS and DPPH and the higher correlation suggested that both the methods have similar predictive capacity for free radical scavenging for *M. longifolia* L. (Fig. 3). In previous studies[26] higher correlation was reported between ABTS and DPPH for fruit extracts (R^2 = 0.90) and sweet potato (R^2 = 0.822).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability of a compound generally depends on the presence of reductones, which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom.[27] The results of reducing power assay were also followed the same way as the other antioxidant activity assays like DPPH and ABTS. The reducing power of *M. longifolia* L. increased

FIGURE 2: Reducing power of various concentrations of methanolic extract of *Mentha longifolia* Linn. , Ascorbic acid and β-Carotene.

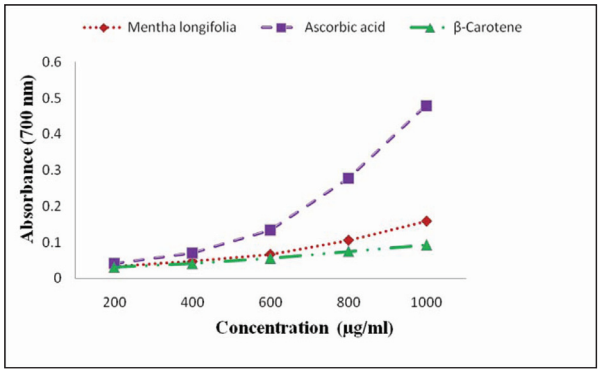


TABLE 2: Ferric Reducing antioxidant property (FRAP) (µM Fe (II) g -1 dry wt.)of the *Mentha longifolia* Linn. Extracts, AA, BHT and BHA.

S. NO	SAMPLE	FRAP
1.	Mentha longifolia	233.51 ± 0.68
2.	Ascorbic Acid (AA)	4412.44 ± 402.87
3.	Butylated Hydroxy Toluene (BHT)	3918.11 ± 496.63
4.	Butylated Hydroxy Anisole (BHA)	4118.11 ± 706.07

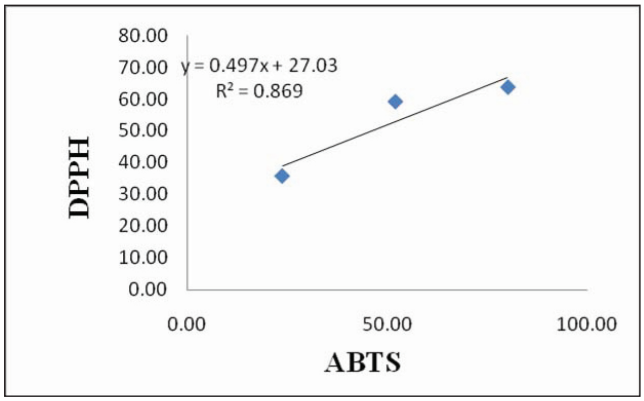
with increasing concentrations of the sample. The results suggested that the reducing power was dependent on concentration. Reducing power of *M. longifolia* L. and standard compounds exhibited the following order: ascorbic acid > methanol extract > β-carotene. With increasing the concentration there was increased absorbance of the reaction mixture which indicates increased reducing power. Hence the reducing power of extract is increased as amount of extract was increased (Fig. 2).

The antioxidant potential i.e. ferric reducing antioxidant power (FRAP) assay of the methanolic extract of *M .longifolia* L. was determined against a standard curve of ferrous sulphate and the antioxidant capacity was showed as 233.51 ± 0.68 µM equivalents of Fe (II) $g^{=1}$ dry wt. The present study gave the FRAP values of *M. longifolia* L. and standard compounds in the following order: ascorbic acid > BHA > BHT > methanol extract (Table 2).

Antibacterial activity

Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics. In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives.[28] The antibacterial activity results were shown in Table. 3. Present findings showed that the methanol extract of *M. longifolia* against *Bacillus subtilis* and *Staphylococcus aureus* showed similar minimum inhibitory concentrations (16 µg/ ml).

FIGURE 3: Linear correlation between percentage scavenging activity of ABTS and DPPH.



CONCLUSION

The results of this study showed that *M. longifolia* has strong radical scavenging and reducing activity. Traditionally local people of Ladakh were using *M. longifolia* since long time and it may serve as a good antioxidant source for this region to prevent skin cancer as the intensity of UV radiation is higher and other high altitude maladies due to hypoxic conditions. The preliminary phytochemical investigation and total polyphenols and flavonoids determination indicates the presence of polyphenols and flavonoids in the plant. Hence, the antioxidant potential of the plant may be attributed to the presence of the polyphenols. By comparison of three AOA assays – ABTS, RPA and DPPH – with different ability of using stable radicals to react with antioxidants, ABTS assay was found to be more sensitive for the determination of the antioxidant activity for *M. longifolia* L. Total antioxidant (FRAP) assay also showed high Fe^{3+} to Fe^{2+} reducing ability of the plant extracts. Further studies will be needed to evaluate its potential in various animal models and the isolation and identification of the potent antioxidant principles.

ACKNOWLEDGEMENTS

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REFERENCES

1. Galvez M, Martin-Cordero C, Houghton PJ and Ayuso MJ. Antioxidant activity of methanol extracts obtained from *Plantago* species. *J. Agric. Food Chem*. 2005; 53: 1927–1933.

TABLE 3: Minimum inhibitory concentrations (MICs) in µg/ml of antibacterial activity.

BACTERIAL STRAINS	MINIMUM INHIBITORY CONCENTRATIONS (µg/ml)
Bacillus subtilis	16.00
Staphylococcus aureus	16.00
Escherichia coli	64.00
Pseudomonas aeruginosa	32.00

2. Kukic J, Petrovic S and Niketic M Antioxidant activity of four endemic *Stachys* taxa. *Biol. Pharm. Bull.* 2006; 29: 725–729.
3. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P and Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 2006; 97: 654–660.
4. Yu L, Perret J, Davy B, Wilson J and Melby CL. Antioxidant properties of cereal products. *Food Chem. Toxicol.* 2002; 67: 2600–2603.
5. Prior R L, Wu X and Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* 2005; 53: 4290–4302.
6. Kiselova Y, Ivanova D, Chervenkov T, Gerova D, Galunska B and Yankova T. Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytother. Res.* 2006; 20: 961–965.
7. Nakiboglu M, Urek RO, Kayali HA and Tarhan L. Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chem.* 2007; 104: 630–635.
8. Rice-Evans CA, Miller NJ and Paganga G. Antioxidants properties of phenolic compounds. *Trends Plant Sci.* 2007; 2:152–60.
9. Marja PK, Anu IH, Heikki JV, Jussi-Pekka R, Kalevi P, Tytti SK and Marina H .Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 1999; 47: 3954–62.
10. Sugihara N, Arakawa T, Ohnishi M and Furuno K. Anti- and pro-oxidative effects of flavonoids on metal induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes located with α-linolenic acid. Free Radic. *Biol. Med.* 1999; 27: 1313–23.
11. Dixon RA and Paiva NL. Stress-induced phenylpropanoid metabolism. *Plant Cell.* 1995; 7: 1085–97.
12. Kumar GP, Gupta S, Murugan MP and Singh SB. Ethnobotanical studies of Nubra valley - A cold arid zone of Himalaya, Ethnobotanical Leaflets. 2009; 13: 752–65.
13. Murugan MP, Kumar GP, Raj XJ, Gupta S and Singh SB. Phyto foods of Nubra valley, Ladakh –The Cold Desert. *J. Traditional Knowledge. (In press)*, 2010.
14. Wolfe K, Wu X and Liu RH. Antioxidant activity of apple peels. *J. Agri. Food Chem.* 2003; 51: 609–614.
15. Ordon Ez AAL, Gomez JD, Vattuone MA and Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chem.* 2006; 97: 452–458.
16. Cuendet M, Hostettmann K and Potterat O. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv. Chim. Acta.* 1997; 80(8): 1144–1152.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.* 1999; 26: 1231–1237.
18. Yen GH and Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agri. Food Chem.* 1995; 43: 27–32.
19. Benzie IFF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 1996; 239: 70–76.
20. Ericcson HM and Sherris JC. Antibiotic sensitivity testing: report of an international collaborative study. *Acta Pathol. Microbiol. Scand.* 1971; 217: 1.
21. Shahidi F and Wanasundara PKJPD. Phenolic antioxidants. Crit. *Rev. Food Sci. Nutr.* 1992; 32: 67–103.
22. Balasundram N, Ai TY, Sambanthamurthi R, Sundram K and Samman S. Antioxidant properties of palm fruit extracts. *Asian Pac. J. Clin. Nut.* 2005; 4: 319–324.
23. Vaya J, Belinky PA and Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. *Free Radical Biol. Med.* 1997; 23(2): 302–313.
24. Koleva II, Van Beek TA, Linssen JPH, De Groot A and Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.* 2002; 13:8–17.
25. Nickavar B, Azadeh Alinaghi and Mohammad Kamalinejad. Evaluation of the antioxidant properties of five *Mentha* species. *Iranian J. Pharm. Res.* 2008; 7(3): 203–209.
26. Teow C, Truong V, McFeeters RF, Thompson RL, Pecota KV, Yencho GC. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours, *Food Chem.* 2007; 103 829–838.
27. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Comp. Anal.* 2006; 19: 669–675.
28. Penso G. The role of WHO in the selection and characterisation of medicinal plants. *J. Ethnopharmacol.* 1980; 2: 183–188.

Comparative Phytochemical and Biological Evaluation of Different Extracts Obtained from the Leaves of *Saraca asoka*

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INTRODUCTION

The Asoka tree (literally meaning "sorrow-less") (*Saraca asoka* (Roxb.) Wilde, or *Saraca indica* L.) is a plant belonging to the Caesalpiniaceae subfamily of the legume family. It is an important tree in the cultural traditions of the Indian subcontinent and adjacent areas. In Sanskrit it is known as Sita-Ashok, Anganapriya, Ashopalava, Asoka, Ashok, Asupala, Apashaka, Ashoka, Hemapushpa, Kankeli, Madhupushpa, Pindapushpa, Pindipushpa, Vanjula, Vishoka, Vichitra. In Bengali it is known as asoke. The Asoka is a rain-forest tree. Its original distribution was in the central areas of the Deccan plateau, as well as the middle section of the Western Ghats in the western coastal zone of the Indian subcontinent .The Asoka is prized for its beautiful foliage and fragrant flowers. It is a very handsome, small, erect evergreen tree, with deep green leaves growing in dense clusters. Its flowering season is around February to April. The Asoka flowers come in heavy, lush bunches. They are bright orange-yellow in color, turning red before wilting. As a wild tree, the Asoka is a vulnerable species. It is becoming rarer in its natural habitat, but isolated wild Asoka trees are still to be found in the foothills of central and eastern Himalayas, in scattered locations of the northern plains of India as well as on the west coast of the subcontinent near Mumbai. There are a few varieties of the Asoka tree. One variety is larger and highly spreading. The columnar varieties are common in cultivation. The bark of the herb is strongly astringent and uterine sedative. It acts directly on the muscular fibers of the uterus. It has a stimulating effect on the endometrium and the ovarian tissue.

ABSTRACT: In the present study, evaluated the analgesic and anti-inflammatory activity of the extracts of *Saraca asoka* in Swiss albino mice and Wister albino rats respectively. Dried leaves of *Saraca asoka* were extracted by petroleum ether, chloroform and methanol successively by percolation. The comparative TLC study was also done with these extracts. The extracts had been screened at the dose of 200 mg/kg body weight orally and exhibited significant analgesic and anti-inflammatory properties.

Keywords: *Saraca asoka* (Roxb.) Wilde; petroleum ether; chloroform; methanol; analgesic activity; anti-inflammatory activity.

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TABLE 1:

NAME OF EXTRACT	SOLVENT SYSTEM	R_f VALUES
Petroleum ether extract	Benzene: Chloroform: Ethyl acetate (2:4:4)	(A) R_f 1 = 4/4.65 = 0.86
,,	,,	(B) R_f 2 = 3.3/4.65 = 0.70

Asoka is a very valuable medicinal plant. Some ethnopharmacological uses of this plant are known. Its bark was used in metrohhagia, menorrhagia, chronic lymphadenitis and inflammation.[1] Some modern research has explored another useful activity of *Saraca asoka*. Chemoprevention of skin cancer by the flavonoid fraction of *Saraca asoka* flower has been found.[2] Potential anticancer activity of *Saraca asoka* extracts towards transplantable tumours in mice has also been reported by some other researchers.[3] *In vitro* examination of bark extraction from *Saraca asoka* has shown oxytocic acvity.[4] Molluscicidal activity of *Saraca asoka* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminate* has also been reported.[5] But till now no work has been reported on the leaves of *Saraca asoka*.

Present work is based on the phytochemical and biological evaluation of the different extracts obtained from the leaves of *Saraca asoka*. The TLC profile is reported for petroleum ether, chloroform and methanol extract of the leaves of *Saraca asoka*. The analgesic and anti-inflammatory effect of these extracts are also found and the details of all those data have been described here.

FIGURE 1, 2 & 3: TLC Profile of Pet-ether extract, Chloroform extract and Methanol extract.

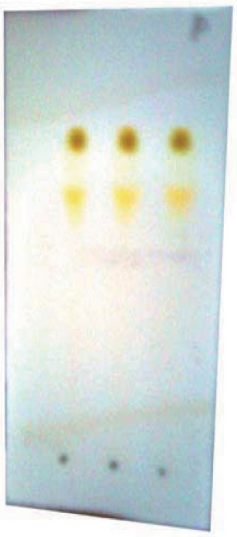


FIG. 1

FIG. 2

FIG. 3

TABLE 2:

NAME OF EXTRACT	SOLVENT SYSTEM	R_f VALUES
Chloroform extract	Benzene: Chloroform: Ethyl acetate (2:4:4)	(A) R_f 1 = 4.1/4.75 = 0.86
,,	,,	(B)R_f 2 = 3.3/4.65 = 0.70

MATERIALS AND METHODS

Plant material

The leaves of *Saraca asoka* were collected from Nadia, West Bengal in the month of January 2010 and were authenticated at Indian Botanical Garden, Howrah, West Bengal, India. A voucher specimen (voucher no. CNH/I-I/5/2010/Tech.II/182) has been preserved in our research laboratory for future reference.

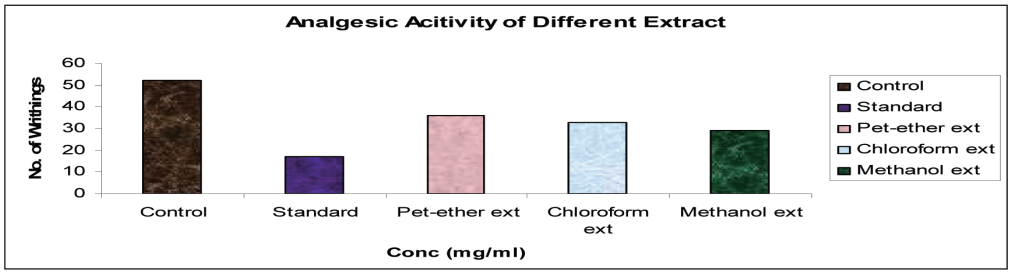
Extraction

The shade dried crushed leaves of *Saraca asoka* (1.76 kg) were subjected to percolation by petroleum

TABLE 3:

NAME OF EXTRACT	SOLVENT SYSTEM	R_f VALUES
Methanol Extract	Ethyl acetate :Methanol(7:3)	(A) R_f 1 = 5.15/5.8 = 0.88
,,	,,	(B) R_f 2 = 4.7/5.8 = 0.81
,,	,,	(C) R_f 3 = 4.3/5.8 = 0.74
,,	,,	(D) R_f 4 = 2.8/5.8 = 0.48

FIGURE 4: Graphical representation of Analgesic Activity.



ether, chloroform and methanol successively with each solvent for two times (1.4 L × 2). The three extracts thus obtained were subjected to solvent evaporation. The extractive values were calculated for the extracts.

General procedure

The petroleum ether, chloroform and methanol extracts were subjected to a battery of chemicals tests for detection of steroids, terpenoids, tannins, glycosides, carbohydrates and saponins. TLC was carried out on silica Gel $60F_{254}$. No spraying reagent is used for visualization of the spot.

AnalgesicActivity: The pharmacological screening of the petroleum ether, chloroform and methanol extracts of the leaves of *Saraca asoka* was carried out using standard protocols to determine the analgesic activity.[10] The crude extract was suspended in 2% dimethyl sulfoxide (DMSO) for administration to Swiss albino mice.[12]

Acetic acid induced writhing reflex: Thirty Swiss albino mice were divided into five groups of six mice each (n = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min. Group II, III, IV andV received aspirin and the extracts (each separately) at the dose of (200 mg/kg b.w., p.o.) respectively. 30 min after aspirin and extracts the writhing reflex was noted for the period of 15 min.

Anti-inflamatory activity: The pharmacological screening of the petroleum ether, chloroform and methanol extracts obtained from the *Saraca asoka* was carried out using a standard protocols for anti-inflammatory activity.[10] The crude extract was suspended 2% DMSO for administration to albino rats.

Carrageenan induced rat paw edema: Thirty rats were divided into five groups containing six rats in each group (n = 6). The concentration of carrageenan was selected as 0.1ml of 1% and was injected subcutaneously 30minutes after administration of the extracts (200 mg/kg p.o.) into the planter region of right hind paw to induce edema. The paw volume was measured initially and at 1, 2, 3 and 4 hr after injection using plethysmometer. Indomethacin 10 mg/kg was injected through i.p. route as standard drug. Percentage inhibition of edema was calculated by the formula:

$$\text{Inhibition} = (1 - vt/vc) \times 100$$

Where vt and vc indicates mean relative changes in paw volume of the test and control respectively.

RESULTS

Phytochemical screening

The phytochemical screening of petroleum ether extract has shown positive results for both terpenoids

TABLE 4: Analgesic effect of extract on acetic-acid induced writhing in mice. (n = 6)

TREATMENT	DOSE	MEAN NO. OF WRITHINGS ± SEM	% INHIBITION
Control (Acetic acid)	10 ml/kg	52.83 ± 1.4	-
Standard (Aspirin)	300 mg/kg	17.65 ± 1.66**	66.59
Pet-ether extract	200 mg/kg	36.21 ± 1.22**	31.46
Chloroform extract	200 mg/kg	33.34 ± 1.35**	36.89
Methanol extract	200 mg/kg	29.76 ± 1.54**	43.67

Values are mean ± S.E.M. One way ANOVA with Tukey-Kramer multiple comparison post test.

***P<0.001 when compared to control.*

TABLE 5: Anti-inflammatory effect of different extracts on carrageenan induced paw edema in rats. (n = 6)

TREATMENT	DOSE	1 HR	2 HR	3 HR	4 HR	% INHIBITION
Control (Carrageenan)	200 mg/kg	0.74 ± 0.08	1.50 ± 0.57	1.7 ± 0.57	2.09 ± 0.81	-
Standard (Indomethacin)	200 mg/kg	0.21 ± 0.05*	0.52 ± 0.05**	0.34 ± 0.03**	0.22 ± 0.03**	89.48
Pet-ether extract	200 mg/kg	0.31 ± 0.05*	0.55 ± 0.03**	0.42 ± 0.05**	0.34 ± 0.03**	83.74
Chloroform extract	200 mg/kg	0.29 ± 0.04**	0.51 ± 0.05**	0.39 ± 0.07**	0.31 ± 0.06**	85.17
Methanol extract	200 mg/kg	0.25 ± 0.05**	0.49 ± 0.08**	0.37 ± 0.04**	0.29 ± 0.05**	86.13

Values are mean ± S.E.M. One way ANOVA with Tukey-Kramer multiple comparison post test.
***P<0.001 when compared to control. *P<0.01 when compared to control.*

and steroids. Its chloroform extract has shown positive response for steroids, terpenoids and carbohydrates. The methanol extract has shown positive response for condensed tannins, saponins, carbohydrates, steroids and glycosides.

TLC PROFILE

Comparative TLC Study of Saraca asoka

The TLC profile study of the different extracts of the leaf of *Saraca asoka* with details has been described below:

(1)TLC study of petroleum ether extract of the leaves of *Saraca asoka* has been illustrated on Table 1 and the picture of the TLC plate has shown in Figure 1.

(2)TLC study of chloroform extract of the leaves of *Saraca asoka* is illustrated on Table 2 and the picture of the TLC plate is shown on Figure 2.

(3)TLC study of methanol extract of the leaves of *Saraca asoka* is illustrated on Table 3 and the picture of the TLC plate is shown on Figure 3.

The analgesic activity of the petroleum ether, chloroform and methanol extracts of *Saraca asoka* leaves were shown in the Table IV and Fig 4. The anti-inflammatory activities of the petroleum ether, chloroform and methanol extracts of *Saraca asoka* leaves were shown in the Table V and Fig 5.

DISCUSSION

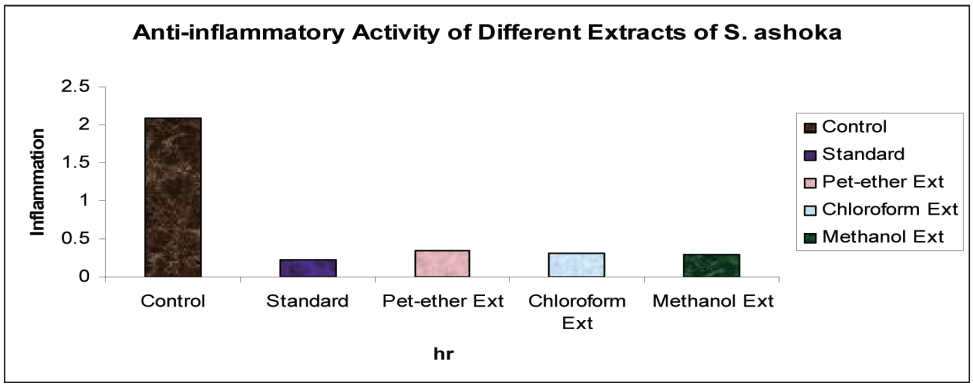
The acetic acid induced writhing is normally used to evaluate the peripheral analgesic effect of drugs. The method is thought to be mediated through peritoneal mast cell, acid sensing ion channel and the prostaglandin pathway. Therefore it can be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway.

The petroleum ether, chloroform and methanol extracts obtained from the leaves of *Saraca asoka* showed analgesic activity, which ease found to be statistically significant at higher concentration (200 mg/kg ,i.p) in acute acetic acid induced writhing. Among the three extracts, the methanol extract had found to be most potent. However, the activity was less potent as compared to aspirin.

Inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular disease. A number of natural products are used in various traditional medicine systems to relieve symptoms from inflammation. The extracts obtained from the percolation of *Saraca asoka* leaves by petroleum ether, chloroform and methanol has shown significant anti-inflammatory activity in carrageenan induced inflammation model. Carrrageenan induced paw edema model is known to sensitive to cyclooxygenase inhibitors. It has been used to evaluate the effect of NSAIDS, which primarily inhibits the cyclooxygenase involved in prostaglandin synthesis. There are two phases in inflammatory reaction in carrageenan-induced paw edema model in rats: first phase and second phase. The first phase, which occurs between 0 to 2.5 h after injection, has been attributed to the release of histamine or serotonin. The second phase of inflammatory reaction which is measured after 3 h is caused by the release of bradykinin, protease, prostaglandin and lysosome.

The extracts had shown a significant inhibitory effect towards anti-inflammatory activity. The petroleum ether extract had inhibited 83.74%, the chloroform extract had inhibited 85.17% the methanol extract had inhibited 86.13% which is less than standard drug (89.48%). But it is very close and comparable with standard indomethacin. Therefore, it can be inferred that the inhibitory effect of those three extracts on carrageenan-induced inflammation could be due

FIGURE 5: Graphical representation of Anti-inflammatory Activity.



to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Thus the results presented in the current study indicate that the petroleum ether, chloroform and methanol extract, extracted from the leaves of *Saraca asoka* have potent and significant anti-inflammatory activity. However a more detail study is required to identify the exact mechanisms of action.

CONCLUSION

The petroleum ether, chloroform and methanol extracts of the leaves of *Saraca asoka* has shown analgesic activity in Swiss albino mice, and anti-inflammatory activity in albino rats.

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REFERENCES

1. Khare CP. Indian Medicinal Plants. Springer-Verlag Berlin Heidelberg, Springer, LLC., 233 Spring street, Newyork, NY 10013,USA, ISBN-978-0-387-70637-5, 2007; 583.
2. Cibin TR, Gayathri Devi D, Annie Abraham. Research Article on Chemoprevention of skin cancer by the flavonoid fraction of *Saraca asoka*. *Phytotherapy research*. *PTR*, (2010 May); 24; 666–672.
3. Varghese CD, Satish, Nair C, Panikkar KR. Potential anticancer activity of *saraca asoca* extracts towards transplantable tumours in mice. *Indian Journal of Pharmaceutical Sciences*. (1992 Jan–Feb); 54(1): 37–40.
4. Teunis B, Middelkoop and Rudi P, Labadie. In vitro Examination of Bark Extracts from *Saraca asoca* (Roxb.) de Wilde and Rhododendron Arboreum Sm. for Oxytocic Activity. *Pharmaceutical Biology.* (1 March 1986); 24: 41– 44.
5. Arundhati Singh and Singh VK. Molluscicidal activity of *Saraca asoca* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminate*. *Veterinary Parasitology.* (14 October 2009); 164: 2–4, 206–210.
6. Moulisha Biswas K, Biswas AK, Ghosh PK, Haldar A. Pentacyclic triterpinoid possessing analgesic and anti-inflammatory activities from the fruits of *Dregea volubilis*. *Oriental Pharmacy and experimental Medicine.* (2009); 9(4): 315–319.
7. Laurence M. Harwood, Christopher J. Moody. Experimental organic chemistry: Principles and Practice (Illustrated on 2nd edition). ISBN 9780632020171, 1987; 159–173.
8. Vogel AI, Tatchell AR, Furnis BS, Hannaford AJ, Smith PWG. Vogel's Textbook of Practical Organic Chemistry (Hardcover) by Pearson Education Limited,(5th Edition),1989.
9. Fair JD, Kormos CMJ. Chromatogr. A, (doi:10.1016/j.chroma.2008.09.085), 2008; 1211(1–2): 49–54. Available from: http://en.wikipedia.org/wiki/Chromatography.
10. Mukherjee PK. Quality control of herbal drugs, Business Horizons Pharmaceutical Publishers, New Delhi, 2nd edition. 2007; 554–555 .
12. Parkes MW, pickens JT. Conditions influencing the inhibition of analgesic drugs of the response to intraperitonial of phenylbenzoquine in mice. *Brit. J. Pharmacol.* 1965; 25: 81–87.
11. Sutradhar RK, Rahman AM, Ahmad M, Bachar SC, Saha A, Guha SK. Bioactive alkaloid from *Sida cordifolia* Linn. With analgesic and anti inflammatory activities. *Iran. J. Phamacol. T.* (2006); 5: 175–178.
13. Bhatt KR, Mehata RK, Srivastava PN. A simple method for recording anti-inflammatory effect in rat paw edema. *Indian J. Physio. Pharm.* (1977); 21: 399–400.
14. Moulisha Biswas K, Biswas AK, Ghosh PK, Haldar A. Pentacyclic triterpinoid possessing anti-inflammatory activity from the fruits of *Dregea volubilis*- *Pharmacognosy Magazine[phcog mag].* (2009); 5(19): 64–68.
15. Moulisha Biswas K, Biswas AK, Ghosh PK, Haldar A. Pentacyclic triterpinoid possessing analgesic activity from the fruits of *Dregea volubilis*- Pharmacognosy *Magazine[phcog mag].* (2009); 5(19): 90–92.

The Study of *in vitro* Antimicrobial Activity and Phytochemical Analysis of Some Medicinal Plants in Chamoli Garhwal Region

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INTRODUCTION

Infectious diseases are the leading cause of death world wide. Antibiotic resistance has become a global concern.[1] The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens.[2] Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.[3] Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.[4] Chamoli Garhwal is a varietals emporium of medicinal plants and is one of the richest districts in the Uttrakhand in regard to genetic resources of medicinal plants. The aim of this study was to evaluate the activity of extracts from 11 plants against several Gram positive and Gram negative bacterial strains *in vitro*.

ABSTRACT: The antibacterial effect of some selected medicinal plants at Chamoli Garhwal Region were evaluated by *in-vitro* antimicrobial study against the strains *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. The solvents used for the extraction of plants were water and methanol. The *in vitro* antibacterial activity was performed by agar disc diffusion and agar well diffusion method. The significant antibacterial activity of active extracts was compared with the standard antibiotics, piperacillin (100 µg/disc) and gentamicin (10 µg/disc). The results obtained in the present study suggest that *Eupatorium adenophorum* Spreng can be used against the test organisms.

Key Words: Antibacterial activity, Aqueous extract, Methanol extract.

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MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant/plant parts were collected randomly from the semi arid region of Chamoli, Uttrakhand, India. The plants and the parts screened, together with their families and vernacular names, are given in Table 1. The taxonomic identities of these plants were confirmed by Dr. Sanskrita Bisht, Botanist, Department of Botany, Govt. P.G. College Gopeshwar Chamoli (H.N.B. Garhwal University, Srinagar Garhwal) and the voucher specimens of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of the crude powder of the 11 plants collected was determined as follows. Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue black precipitate indicated the presence of tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 mg filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayer's reagent/ Wagner's reagent/ Dragendroff's reagent, creamish precipitate/ brownish-red precipitate/ orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated the presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + H_2SO_4); green blue colour indicated the presence of cardiac glycosides. Steroids (Liebermann- Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4, blue green ring indicated the presence of steroids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + HCl + magnesium ribbon, pink- tomato red colour indicated the presence of flavonoids.[5]

Extraction of Plant Material

Aqueous extraction: 10 g of air dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume to one fourth of the original volume. It was then autoclaved at 121°C and at 15 lbs pressure and stored at 4°C.[6]

Methanol extraction: 10 g of air dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept at ambient temperature for one week. Later on supernatant was collected and the solvent was evaporated to make the final volume to one-fourth of the original volume and stored at 4°C in airtight bottles.[6]

TABLE 1: Ethno botanical information of some traditionally used medicinal plant species selected for antibacterial activity.

PLANT SPECIES	FAMILY	COMMON NAME	PART USED	THERAPEUTIC USE
Abrus precatorius L.	Fabaceae	Chanothi	Leaf/Stem	Purgative, Emetic, Aphrodisiac
Caesalpinia pulcherrima	Caesalpiniaceae	Galtoro	Aerial Parts	Reclamation Plant
Cardiosperm halicacabum L.	Sapindaceae	Kagdonia	Aerial Parts	Rheumatism, Fever
Casurina equisetifolia L.	Casuarinacea	Sharu	Leaf/Stem/Fruit	Diarrhoea, Dropsy
Ficus benghalenis L.	Moraceae	Vad	Branching Root	Diarrhoea, Dysentery
Bauhinia variegates L.	Caesalpiniacea	Kachnar	Bark	Fever, Antitumor
Betula utilis	Betulaceae	Birch	Leaf/Stem	Antiseptic
Aconitum heterophyllum Wall	Rananculaceae	Atis	Leaf/ Root	Skin disorder, Antitumor
Eupatorium adenophorum Spreng	Asteriaceae	Basya	Leaf/Flower	Anticoagulant, Wound healing
Delonix regia L	Fabaceae	Gulmohar	Pod	Skin disease
Santalum album L.	Santalaceae	Shwet chandan	Leaf/ Stem	Gastric irritability, Dysentery, Bronchial disorders

TABLE 2: Preliminary phytochemical analysis of screened medicinal plant species.

PLANT SPECIES	TANNINS	SAPONINS	FLAVONOIDS	STEROIDS	CARDIAC GLYCOSIDES	ALKALOIDS		
						MT	WT	DT
Abrus precatorius L.	-	+	-	-	-	-	++	-
Caesalpinia pulcherrcma	+	+	-	-	-	-	+	-
Cardiosperm halicacabum L.	+	+	+++	-	-	-	+	-
Casurina equisetifolia L.	+	++	+++	-	-	-	+	-
Ficus benghalenis L.	-	+	+	-	-	-	+	-
Bauhinia variegates L.	++	+	+++	++	+++	+	++	++
Betula utilis	-	-	-	+++	-	+	++	++
Aconitum heterophyllum Wall	+	+	-	+	-	+	++	+
Eupatorium adenophorum Spreng	++	+	++	-	-	++	++	+++
Delonix regia L	+	+	+	+	-	-	+	-
Santalum album L.	+	+	-	+	+	-	-	-

MT= Mayor's Test, WT= Wagner's Test, DT= Dragendorff's Test
- Not present, + sparingly present, ++ Present, +++ highly present.

Bacterial Strains

In vitro antimicrobial activity was examined for aqueous and methanol extracts from 11 medicinal plants used by traditional healers. Amongst four microorganisms investigated, two Gram-positive bacteria were *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923, while two Gram-negative bacteria were *Pseudomonas aeruginosa* ATCC 13048, *Escherichia coli* ATCC 25922 and all the microorganisms were maintained at 4°C on nutrient agar slants.

Media Preparation and Antibacterial Activity

The antimicrobial assay was performed by two methods viz. agar disc diffusion method[7] for aqueous extract and agar well diffusion method[8] for solvent extract. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculums (1 x 10^8 cfu/ml) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85 cm). 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37^{0}C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiments were done three times and then mean values are presented.

RESULTS AND DISCUSSION

The antimicrobial assay was performed by two methods viz. agar disc diffusion method and agar well diffusion method. Table 3 summarizes the microbial growth inhibition of both aqueous and methanol extracts of the screened plant species. The aqueous extract of only three plants showed antibacterial activity (*Caesalpinia pulcherrima*, *Casuarina equisetifolia* and *Eupatorium adenophorum* Spreng); the other extracts did not show antibacterial activity. On the other hand methanol extract of almost all the plants show antibacterial activity towards bacterial strains. The maximum antibacterial activity was shown by *Eupatorium adenophorum* Spreng followed by *Caesalpinia pulcherrima* and *Casuarina equisetifolia* respectively. The methanol extracts of the investigated plants showed maximum antibacterial activity against Gram-negative *K. pneumoniae*. Similar results were also reported by Venkatesan et al.[9], Prescott et al.[10] and Stainer et al.[11] who reported disease such as pneumonia, urinary and respiratory tract infection caused by *Klebsiella* species. The studied plants were most active against Gram- positive *B. cereus*. The significant antibacterial activity of active extracts was compared with the standard antibiotics,

TABLE 3: Antibacterial activity of aqueous and methanol extracts of screened medicinal plant.

PLANT NAME	EXTRACT	ZONE OF INHIBITION (mm)			
		B. cereus	*S. aureus*	*P. aeruginosa*	*E. coli*
Abrus precatorius L	Aqueous	-	a-	-	-
	Alcoholic	11	-	10	-
Caesalpinia pulcherrima	Aqueous	12	9	-	-
	Alcoholic	20	18	15	14
Cardiosperm halicacabum L	Aqueous	-	-	-	-
	Alcoholic	-	11	-	-
Casuarina equistifolia L	Aqueous	13	11	-	-
	Alcoholic	19	17	12	12
Ficus benghalenis L	Aqueous	-	-	-	-
	Alcoholic	16	12	-	-
Bauhinia variegates L.	Aqueous	-	-	-	6
	Alcoholic	12	10	-	13
Betula utilis	Aqueous	-	10	-	-
	Alcoholic	13	16	10	-
Aconitum heterophyllm Wall	Aqueous	-	-	-	9
	Alcoholic	10	19	14	-
Eupatorium adenophorum Spreng	Aqueous	12	15	10	14
	Alcoholic	19	24	16	18
Delonix regia L.	Aqueous	10	-	-	-
	Alcoholic	17	15	-	-
Santalum album L.	Aqueous	-	-	-	-
	Alcoholic	14	18	11	-
Piperacillin		18	25	15	20
Gentamicin		14	17	20	22

piperacillin (100 µg/disc) and gentamicin (10 µg/disc). Preliminary phytochemical analysis revealed the presence of alkaloids (+ve test for Wagner's - Table 2) and saponins. The other secondary metabolites like tannins, flavonoids, steroids, cardiac glycosides, etc. were present in trace amounts in some of the plants (Table 2). It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective.

However, the present study of *in vitro* antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies. In conclusion, *Eupatorium adenophorum* Spreng extracts possess a broad spectrum activity against the bacterium responsible for causing disease.

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REFERENCES

1. Westh H, Zinn CS, Rosdahl VT et al. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microb Drug Resist*. 2004; 10: 169–176.
2. Bandow JE, Brotz H, Leichert LIO et al. Proteomic approach to understanding antibiotic action. *Antimicrob Agents Chemother*. 2003; 47: 948–955.
3. Rojas R, Bustamante B, Bauer J et al. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol*. 2003; 88: 199–204.
4. Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm-Wiss u-Technol*. 2004; 37: 263–268.

5. Oguyemi AO. In: Sofowora A. ed. Proceedings of a Conference on African Medicinal Plants. *Ife-Ife: Univ Ife;* 1979; 20–22.
6. Harbone JB. Phytochemical Methods. London: Chapman and Hill; 1973.
7. Parekh J, Nair R, Chanda S. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J Pharmacol.* 2005; 37: 408–409.
8. Bauer AW, Kirby WMM, Sherris JC et al. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966; 45: 493–496.
9. Venkatesan M, Vishwanathan MB, Ramesh N et al. Antibacterial potential from Indian *Suregada angustifolia*. *J Ethnopharmacol.* 2005; 99: 349–352.
10. Prescott LM, Harley JP, Klein DA. Microbiology 4th ed. Boston: The McGraw-Hill Companies Inc.; 1999; 685.
11. Stainer RY, Ingraham JL, Wheelis ML et al. General Microbiology, 5th ed. London: The MacMillan Press Ltd. 1986.

Proximate Composition, Antibacterial and Anthelmintic Activity of *Capsicum frutescens* (L.) Var. *Longa* (Solanaceae) Leaves

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INTRODUCTION

Chilies are high in vitamin A and C, but low in calories and sodium. Chilies contain potassium, magnesium and folic acid. Capsaicinoids are responsible for pungency and are considered as active compounds. Capsaicin accounts for about 50 to 70% of the total capsaicinoids. Other components are dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin. When eaten, capsaicin stimulates the release of endorphins, which give a pleasurable feeling. Moreover, chilies are believed to increase blood circulation, relieve rheumatic pain, treat mouth sores and infected wounds, reduce blood clots and aid digestion by stimulating saliva

ABSTRACT

Introduction: *Capsicum frutescens* (L.) var. *longum* (Solanaceae) is a well used tropical and subtropical medicinal plant. The present study was undertaken to investigate proximate composition, antibacterial and anthelmintic activity of *C. frutescens* leaves.

Methods: The leaf material was tested for proximate parameters namely carbohydrates, proteins, crude fibre, crude fat, ash and moisture. The powdered leaf material was extracted using methanol. Antibacterial activity of the methanolic extract was tested by agar well diffusion method. Anthelmintic activity of the methanolic extract was studied using Indian earthworm.

Results: The protein, ash, crude fat and crude fibre contents were not appreciably high. The carbohydrate content was comparatively higher. Among microelements, iron was found to be in high concentration. Potassium content was found to be high than phosphorus. The methanolic extract showed dose dependent antibacterial and anthelmintic activity. Among the bacteria studied, *S. aureus* was found to be more susceptible to the methanolic extract followed by *K. pneumoniae* and *P. aeruginosa*. The anthelmintic effect of all concentrations was lesser when compared to standard. Phytochemical analysis revealed saponins, tannins, alkaloids, glycosides and steroids in methanol extract.

Conclusion: The leaves may be used as a good source of carbohydrates and other nutrients. The methanolic extract could be used in the treatment of bacterial and helmintic infections. The presence of various phytochemicals might be responsible for the biological potential of the extract. Further studies on isolation of constituents from the extract and their biological activities are under investigation.

Key words: *Capsicum frutescens, longum*, Nutritive composition, Agar well diffusion, Anthelmintic, *Pheretima pasthuma*.

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and gastric juice flow.[1] Capsaicin has been tested for its effects on experimental carcinogenesis and mutagenesis. There is no solid evidence showing that chili and capsaicin are carcinogenic in humans. In contrast, many studies reveal substantial antioxidant, antigenotoxic and anticarcinogenic effects of chili extracts and capsaicin.[2–3] Therefore, capsaicin is suggested as an important dietary phytochemical with antioxidant and chemopreventive activities. *Capsicum frutescens* (L.) var. *longum* (Solanaceae) is a well used tropical and subtropical medicinal plant. It is known as Bird chilli in English, Kutaviraa in Ayurveda and Surkh mirch in Unani. It is cultivated as a condiment crop. It is more woody and taller with small pod like berries and innumerable small flat seeds. The crimson or orange red fruits are elongate conical some what flattened and very pungent. The pungent principles are present in the flesh rind and seeds as well. It acts as stimulant, accelerates oxygenation of cells, encourages adrenal glands to produce corticosteroids, and increases gastrointestinal secretion. It is also carminative, antispasmodic and antiseptic. It is applied externally, in painful muscle spasms in areas of shoulder, arm and spine; for treating arthritis, rheumatism, neuralgia, lumbago and chilbains. The British Pharmacopoeia reported rubefacient and vasostimulant action. The plant contains hydroxybenzoic acid, hydroxycinnamic acid and ascorbic acid. Fruits contain up to 1% capsaicin.[4–5] The literature survey revealed scanty information on biological activities of *C. frutescens* leaves. The present study was undertaken to investigate the proximate composition, antibacterial and anthelmintic activity of *C. frutescens* leaves.

MATERIALS AND METHODS

Collection and Identification

The plant material was collected during April 2010 from Hosanagara Taluk of Shivamogga district, Karnataka, India. The plant sample was identified by Dr. K.G Bhat, MGM College, Udupi, Karnataka. A voucher specimen (Voucher No: KU/AB/KA-2036) was deposited in the University Herbaria, PG Department of Studies and Research in Applied Botany, Shankaraghatta-577451 for future reference.

Solvent Extraction and Phytochemical Analysis

The leaves were separated from the plants, washed 2–3 times with tap water and once with sterile water, shade dried and powdered. For extraction, a known amount of powder (100 gm) was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extract was filtered, concentrated in vacuum under reduced pressure using rotary flash evaporator and dried. The extract was subjected to phytochemical screening to detect the presence of secondary metabolites.[6–7]

TABLE 1 : Proximate composition of *C. frutescens* leaves.

PARAMETER	CONTENT
Moisture (%)	26.9
Ash (%)	1.52
Crude fibre content (%)	2.68
Total carbohydrates (%)	49.01
Total protein (%)	2.68
Crude Fat (%)	1.52
Nutritive value (cal/100 g)	290.6

Determination of Nutritive Composition

The nutritive composition of powdered leaves was carried out to determine proximate and mineral composition using various techniques. The moisture content was determined by drying powder in oven at 100°C until constant weight, ash by incineration in a muffle furnace at 550°C for 48 h, proteins by nitrogen determination using Kjeldahl method and conversion of nitrogen to proteins by the factor 6.25. Fat was determined by Bligh dyer technique and crude fiber by successive digestion of the defatted sample with 0.26 N sulphuric acid and 0.23 N potassium hydroxide solutions. Percentage carbohydrate was calculated using the formula: 100—(percentage of ash + percentage of moisture + percentage of fat + percentage of protein). Nutritive value was determined by: Nutritive value = 4 x̣ percentage of protein + 9 x̣ percentage of fat + 4 x̣ percentage of carbohydrate.[8]

Screening for Antibacterial Activity

The antibacterial efficacy of the methanol extract was tested against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by agar well diffusion method.[9] Briefly, wells of 6 mm were bored in Muller-Hinton agar plates inoculated with 24 hours old broth cultures of test bacteria. The extracts (10, 25, 50 and 100 mg/ml of 10% dimethylsulfoxide (DMSO)), standard (Rifampicin, 1mg/ml) and control (10% DMSO) were added into the labeled wells. The plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded. Experiment was carried in triplicate and the average reading was noted.

TABLE 2 : Antibacterial activity of methanol extract of *C. frutescens* leaves.

TREATMENT	CONCENTRATION (mg/ml)	INHIBITION ZONE IN cm		
		S. aureus	*K. pneumoniae*	*P. aeruginosa*
Control	10%	0.0	0.0	0.0
Standard	1	3.8 ± 0.31	3.8 ± 0.19	3.9 ± 0.29
Methanol extract	10	0.0	0.0	0.0
	25	0.8 ± 0.13	0.8 ± 0.06	0.0
	50	1.0 ± 0.09	0.9 ± 0.10	0.8 ± 0.13
	100	1.3 ± 0.14	1.1 ± 0.08	1.0 ± 0.07

Screening for Anthelmintic Activity

The anthelmintic assay was performed on adult Indian earthworm Pheretima pasthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Standard drug (Piperazine citrate, 1%) and different concentrations of methanol extract (10, 25, 50 and 100 mg/ml of DMSO) were poured into labeled petriplates containing saline. A saline control was kept. Six worms of nearly equal size were introduced into each of the plates. Observations were made for the time taken for paralysis and death of worms. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed by dipping the worms in slightly warm water.[10,11]

RESULTS

Preliminary phytochemical analysis of *C. frutescens* leaves showed the presence of phytoconstituents namely saponins, tannins, alkaloids, glycosides and steroids in methanol extract.

Nutritive composition of *C. frutescens* leaves was represented in the Table 1. The carbohydrate content of leaf was found to be 49.01%. The protein content (2.68%) was less. The ash, crude fat and crude fibre contents were not appreciably high. The nutritive value was 290.6 cal/100g.

The result of antibacterial activity of methanol extract is shown in Table 2. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it was reported as positive and absence of zone as negative. In this study, the extract has shown inhibition of test bacteria in a concentration dependent manner. Among bacteria, *S. aureus* was found to be more susceptible to extract followed by *K. pneumoniae* and *P. aeruginosa* as revealed by wider inhibition zones. Standard antibiotic caused more inhibitory activity than methanol extract. No inhibition of test bacteria was observed in case of control and 10 mg/ml concentration of methanol extract. It appears that overall the bacteria were found to be sensitive to extract. The reasons for this could be that the components from the plant were active against microorganisms and are most often obtained through solvent extraction.

TABLE 3 : Anthelmintic activity of methanol extract of *C. frutescens* leaves.

EXTRACT/ DRUG	CONCENTRATION	PARALYSIS TIME (IN MIN)	DEATH TIME (IN MIN)
Saline	0.85%	-	-
Methanol extract	10 mg/ml	198.35 ± 13.09	256.23 ± 29.83
	25 mg/ml	173.21 ± 08.73	221.39 ± 24.12
	50 mg/ml	131.10 ± 29.18	167.16 ± 13.64
	100 mg/ml	110.42 ± 16.93	151.09 ± 18.50
Standard	1%	85.15 ± 10.24	114.28 ± 08.91

The result of anthelmintic activity of methanol extract of *C. frutescens* leaves is shown in Table 3. The extract exhibited marked anthelmintic activity by causing paralysis and death of worms and the effect was found to be dose dependent. The anthelmintic effect of all the concentrations of extract tested was lesser when compared to standard drug (1% piperazine citrate).

DISCUSSION

Medicinal plants, as a group, comprise approximately 8000 species and account for about 50% of all the higher flowering plant species of India. The traditional healers have used this resource since time immemorial for the benefit of mankind. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural plant origin and natural products play an important role in drug development programs in the pharmaceutical industry. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness.[12-15] In this study, the preliminary phytochemical analysis of methanol extract of *C. frutescens* leaves showed the presence of phytoconstituents namely saponins, tannins, alkaloids, glycosides and steroids.

Relevant to human existence and living is good nutrition. All human beings require a number of complex organic compounds as added caloric requirements to meet the need for their muscular activities. Carbohydrates, fats and proteins form the major portion of the diet, while minerals and vitamins form comparatively a smaller part. The increasing populations of the world food demands have overwhelmed the available land resources. It has been reported that protein-calories malnutrition deficiencies is a major factor responsible in nutritional pathology. The dietary fibre plays an important role in decreasing the risks of many disorders such as constipation, diabetes, cardiovascular diseases, obesity etc. The carbohydrates are main source and store of energy. They are the starting substances for biological synthesis of many compounds.[8,16,17]

Antibiotics provide the main basis for the therapy of infections. Over use of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains. Bacteria have the ability to transmit and acquire resistance. Multi-drug resistant strains of pathogens are widely distributed in hospitals and are increasingly being isolated.[12, 18-20] Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance.[21] Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics.[22] Antimicrobial activities of tannins[23], flavonoids[24], saponins[25], terpenoids[26] and alkaloids[27] have been documented. The methanol extract of *C. frutescens* leaves was found to possess most of these phytoconstituents. The antibacterial activity of extract in this study could be chiefly due to the presence of these phytoconstituents and is suggestive of the possible use of the plant in treatment of bacterial infections as most strains have already developed resistance to most of the currently used antibiotics.

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature.[28] During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health.[29] The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of natural compounds for their proclaimed anthelmintic activity. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. [11] Indigenous system of medicine reports a number of natural sources for their anthelmintic efficacy. However, their scientific evaluation as compared to commercial anthelmintics is limited. Many plants have proven to possess anthelmintic activity *in vitro* and *in vivo*. Tannins were found to possess anthelmintic activities. Reported anthelmintic effect of tannins is that they can bind to free proteins in

the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death.[30-31] Preliminary phytochemical analysis revealed the presence of tannins in the methanol extract which might be responsible for the anthelmintic effect of extract. The result of the present study is suggestive that the extract could be used in the control of round worm infections such as Ascariasis, hookworm infections etc as the worms used in the study are in anatomical and physiological resemblance with the intestinal round worms.

CONCLUSION

The results of the present study revealed that the leaves contain considerable quantity of carbohydrates. The plant may be used as a source of carbohydrates and other nutrients. The extract could be used in the treatment of bacterial and helmintic infections. The presence of various phytochemicals might be responsible for the biological potential of the extract. Further studies on the isolation of constituents from extract and their biological activities are under investigation.

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REFERENCES

1. Uhl SR. Spices, Seasonings & Flavorings. Technomic Publishing Company. Lancaster. 2000; pp 329.
2. Surh YJ, Lee E, Lee JM. Chemoprotective properties of some pungent ingredients present in red pepper and ginger. *Mutat Res.* 1998; 402(1–2): 259–267.
3. Prasad NS, Raghavendra R, Lokesh BR, Naidu KA. Spice phenolics inhibit human PMNL 5-lipoxygenase. *Prostag Leukotr Ess.* 2004; 70: 521–528.
4. Hill AF, Sharma OP. Hills Economic Botany. 2nd edn. Tata McGraw-Hill Publishing Company Ltd, New Delhi. 1996; pp 588–590.
5. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Springer Verlag, Berlin 2007; pp 120.
6. Manjunatha BK, Patil HSR, Vidya SM, Kekuda TRP, Mukunda S and Divakar R. Studies on the antibacterial activity of *Mucuna monosperma* DC. *Indian Drugs.* 2006; 43: 150–152.
7. Mathad P, Mety SS. Phytochemical and Antimicrobial activity of *Digera Muricata* (L.) Mart. *E-Jour of Chem.* 2010; 7(1): 275–280.
8. Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Curr Sci*. 2005; 89: 1252–1255.
9. Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). *Food Chem*. 2004; 84(4): 519–525.
10. Grime AS, Bhalke RD, Ghogare PB, Tambe VD, Jadhav RS and Nirmal SA. Comparative *in vitro* anthelmintic activity of *Mentha piperita* and *Lantana camara* from Western India. Dhaka Univ. *J Pharm Sci*. 2006; 5 (1–2): 5–7.
11. Temjenmongla, Yadav AK. Anticestodal efficacy of folklore medicinal plants of Naga tribes in Northeast India. *Afr J Trad CAM*. 2005; 2(2): 129–133.
12. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A, Bora U. Indian medicinal herbs as sources of antioxidants. *Food Res Int*. 2008; 41: 1–15.
13. Adwan G, Abu-Shanab B, Adwan K, Abu-Shanab F. Antibacterial effects of nutraceutical plants growing in Palestine on *Pseudomonas aeruginosa*. *Turk J Biol*. 2006; 30: 239–242.
14. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotech*. 2005; 4: 685–688.
15. Nair R, Kalariya T, Chanda S. Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol*. 2005; 29: 41–47.
16. Roger P, Elie F, Rose L, Martin F, Jacop S, Mercy AB, Felicite MT. Methods of preparation and nutritional evaluation of Dishes consumed in a malaria endemic zone in Cameroon (Ngali II). *Afr J Biotech*. 2005; 4: 273–278.
17. Spiller GA. Dietary fiber in prevention and treatment of disease. In: Spiller GA (Eds). CRC handbook of dietary fiber in human nutrition, CRC Press LLC, Washington, 2001; pp 363–431.
18. Harbottle H, Thakur S, Zhao S, White DG. Genetics of Antimicrobial Resistance. *Anim Biotechnol*. 2006; 17: 111–124.
19. Akram M, Shahid M, Khan AU. Etiology and Antibiotics Resistance Pattern of Community Acquired Urinary Infections in J N M C Hospital Aligarh India. *Ann Clin Microbiol Antimicrob.* 2007; 6: 4.
20. Khan AU, Musharraf A. Plasmid Mediated Multiple Antibiotic Resistances in *Proteus mirabilis* Isolated from Patients with Urinary Tract Infection. *Med Sci Mont*. 2004; 10: 598–602.
21. Okeke IN, Laxminarayan R and Bhutta ZA. Antimicrobial Resistance in developing countries. Part 1: recent trends and current status. *Lancet Infect Disease*. 2005; 5: 481–493.
22. Abu-Shanab B, Adwan G, Abu-Safiya D. Antibacterial activities of some plant extracts used in Palestine in popular medicine. *Turk J Biol*. 2004; 28: 99–102.
23. Doss A, Mubarack HM, Dhanabalan R. Pharmacological importance of *Solanum trilobatum*. *Indian J Sci Tech*. 2009; 2(2): 41–43.
24. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *J Appl Microbiol*. 2007; 103(6): 2056–2064.
25. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta, M. Antimicrobial activity of saponins from *Medicago* sp.: structure-activity relationship. *Phytotherapy Res.* 2006; 20(6): 454—457.
26. Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y. Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiol Immunol*. 2004; 48(4): 251–261.

27. Navarro V, Delgado G. Two antimicrobial alkaloids from *Bocconia arborea*. *J Ethnopharmacol*. 1999; 66(2): 223–6.
28. Dewanjee S, Maiti A, Kundu M, Mandal SC. Evaluation of Anthelmintic activity of crude extracts of *Diospyros peregrine, Coccinia grandis* and *Schima wallichii*. *Dhaka Univ J Pharm Sci*. 2007; 6(2): 121–123.
29. Nunomura RCS, Dasilva ECC, Oliverira DF, Garcia AM, Boeloni JN, Nunomura SM, Pohlit AM. *In vitro* studies of the anthelmintic activity of Picrolemma sprucei Hook.f. (Simaroubaceae). *Acta Amazonica*. 2006; 36(3): 327–330.
30. Athnasiadou S, Kyriazakis F, Jackson RL, Coop. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *In vivo* studies. *Vet Parasitol*. 2001; 99: 19.
31. Thompson DP, Geary TG. The structure and fuction of helminth surfaces. In: Marr JJ, Editor. Biochemistry and Molecular biology of Parasites. 1st Edn. New York. Academic press 1995; pp 203–232.

Pharmacognostical Standardization of Leaves of *Xanthium strumarium* Linn.

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INTRODUCTION

Plants have played an important role as a source of useful compounds to mankind. *Xanthium strumarium* belongs to the family Compositae and is found in Korea, Japan, Europe, India and the United states. Its dried seeds and leaves are used as a raw material in Chinese medicine for fever, diaphoresis, pain, paralysis, ulcerative skin disease, neuralgia, small-pox and malignant tumor. It is also known to have antibacterial effects against typhoid bacillus, dysentery bacillus and yellow staphylococcus. The roots are used to induce robustness, for hypothyroidism and as an anti-tumor agent; and the leaves are reported to reduce blood sugar levels.[1–5] In previous chemical studies on *X. strumarium* carboxyatractyloside, caffeyolqunic acid, xanthol, isoxanthol, hydroquinone, thiazinedione, a dimeric xanthanolide, 11α,13-dihydroxyxanthatin and β-sitosterol-D-glucoside were identified.[6–9] The present investigation of *Xanthium strumarium* Linn leaves is taken up to establish pharmacognostic profile which will help in crude drug identification as well as standardization of the quality and purity of the drug in crude form.[10–11]

ABSTRACT: *Xanthium strumarium* L. (Compositae) (Hindi: Chota-gokhru), is a gregarious weed found abundantly throughout India. The whole plant is used as a diaphoretic, sedative, sudorific, diuretic and sialagogue. The ethanolic extract of the leaves exhibits significant anti-inflammatory, analgesic, antitrypanosomal and anti-microbial effects. The present study deals with the pharmacognostical evaluation of leaves of *Xanthium strumarium* Linn. Macromorphology and microscopy (transverse section, powder microscopy and quantitative microscopy) were studies to establish the salient diagnostic features. The preliminary phytochemical analysis and thin layer chromatography has also been performed. The results of this study could be useful in setting some diagnostic indices for identification and preparation of the monograph of the plant.

Keywords: *Xanthium strumarium*, pharmacognostical studies, physicochemical analysis, quantitative microscopy, TLC finger printing.

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MATERIALS AND METHODS

Collection and authentication of plant material

X. strumarium plants were collected in October 2009 from the campus of IFTM, Moradabad, U.P., India and botanical identification was done by Dr. Alok Lehri, Scientist, National Botanical Research Institute (NBRI), Lucknow, India (Ref. No. NBRI/CIF/115/2009). A voucher specimen has been preserved in the department for further verification.

Morphological and microscopical studies

The macromorphology of the leaves were studied according to standard methods.[12–13] Hand section of leaf was taken, stained and mounted following usual micro techniques[14] and representative diagrams were taken with help of inverted microscope for photo documentation (Leitz, Japan). The different powder characteristics were studied according to standard methods. (15–16)Separate slides were prepared for observation of lignified tissue (pholoroglucinol + HCl), non lignified characters and starch (iodine solution).

Quantitative Microscopy

The stomatal number, stomatal index, vein-islet number, veinlet termination number, average length of trichomes per mg of leaf powder and average epidermal area per gm of leaf powder were determined.(13,17)

Determination of physicochemical parameters

Loss on drying value indicates that where the drug is safe regarding any growth of bacteria, fungi and yeast; percentage of ash value (indicative of the purity of the drug) and extractive values (represent the presence of polar and non polar compounds) were performed according the official methods.(18–19) Fluorescence analysis was carried out following reported methods.(20–21)

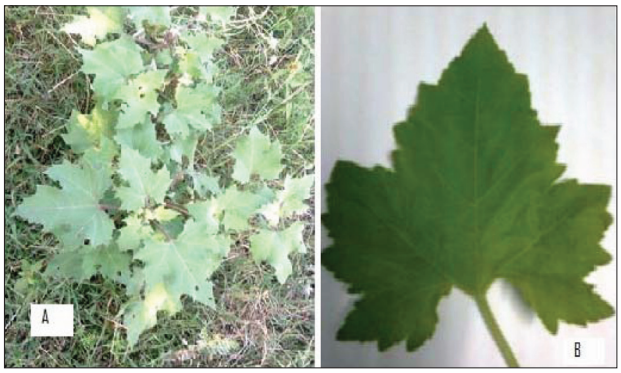
Preliminary phytochemical screening

The shade dried and coarsely powdered leaves were extracted successively with petroleum ether, chloroform and alcohol by using soxhlet apparatus and finally water extract was prepared by decoction. Different extracts were screened for the presence of various groups of phytoconstituents using different chemical tests.(22–25)

TLC fingerprint profile

The thin layer chromatography profiles of petroleum ether, chloroform, ethanol and water extracts were studied(26–27) and R_f values were determined.

FIGURE 1: A Young plant of *X. strumarium*, B-Leaf of *X. strumarium*.



RESULTS

Macroscopic characters

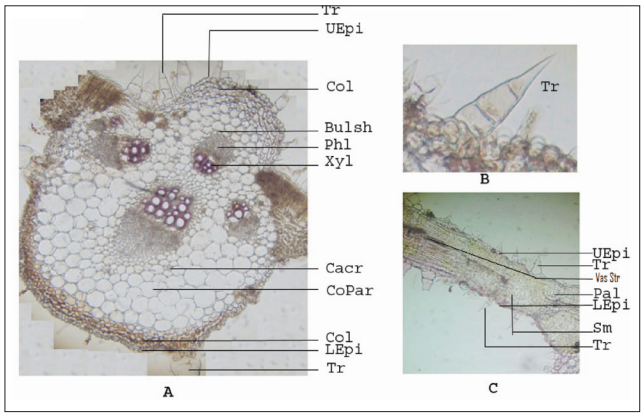
Leaves are dark green, numerous, 5–7.5 cm long and almost as broad as long, broadly triangular–ovate or sub orbicular, acute, often 3-lobed, rough with appressed hairs on both sides, irregularly inciso-serrate, somewhat cordate and shortly cuneate at the base; petioles 2.5–7.5 cm long, hairy. Leaves occur on long petioles and also have three prominent veins on the upper surface on the leaf that arises from the same point. All subsequent leaves are alternate (Figure 1).

Microscopical characteristics

Midrib: Transverse section of the midrib shows concavo-convex outline in the basal and middle region which becomes plano-convex in the apical region. Upper and lower epidermis consists of single layer of cells covered with thick cuticle. 2–5 celled covering trichomes are present on both surfaces. The upper and lower epidermis is followed by three layers of collenchymatous cells. Collenchyma on both dorsal and ventral side is followed by a zone of parenchyma consisting round to oval thin walled parenchymatous cells showing small to distinct intracellular spaces. There are four collateral type vascular bundles out of which one is large than the other three. These are present in scattered form and composed of small strands of xylem and phloem. Calcium oxalate crystals are present in the parenchymatous cells (Figure 2A).

Lamina: Lamina has a structure of dorsiventral type of leaf. The lamina shows upper epidermis, mesophyll and lower epidermis. Upper epidermis is composed of single layer of rectangular cells covered with cuticle. It also shows presence of 2–5 cells with covering trichomes on both the sides. Vascular strand

FIGURE 2: Transverse section of leaf of *Xanthium strumarium*.



A: Tranverse section through midrib, B: Trichome, C: Transverse section through lamina.

Abbreviations: Tr, trichome; UEpi, upper epidermis; Col, collenchymas; Bulsh, bundle sheath; Phl, phloem; Xyl, xylem; Cacr, calcium oxalate crystal; CoPar, cortical parenchyma; LEpi, lower epidermis; Sm, spongy mesophyll; Pal, palisade parenchyma; Vas Str, Vascular Strand.

and prism shaped calcium oxalate crystals were present in mesophyll region. Loosely arranged round to oval shaped, spongy parenchymatous cells were present in the lamina (Figure 2C).

Petiole: Transverse section of petiole shows two prominent grooves towards upper side where as lower side is round. The epidermis is composed of single layer of cells. Few trichomes are observed on epidermal cell which are identical with that of leaf. Two to three layers of collenchymatous cells are found around the epidermal cell. Nine to ten collateral vascular bundles are present in the ground tissue. The xylem is found towards upper side and phloem lies towards lower side. The remaining portion of ground tissue is composed of parenchymatous cells (Figure 3).

Powder characters

For the powder microscopy separate slides were prepared with glycerin, phlorogluinol + HCl and iodine. The powder was green in colour and contains anisocytic stomata, covering trichomes, epidermal cell, spongy parenchyma with vein let, annular lignified xylem vessel and prism shaped calcium oxalate crystals (Figure 4).

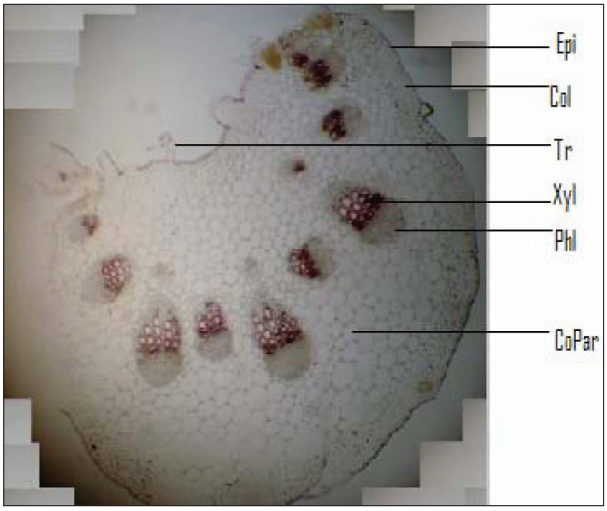
Quantitative microscopy

The results of quantitative microscopy were furnished in Table 1.

Physicochemical parameters

Physicochemical parameters like percentage of moisture content, total ash, acid insoluble ash, water

FIGURE 3: Transvers section of petiole of *X. strumarium* L.

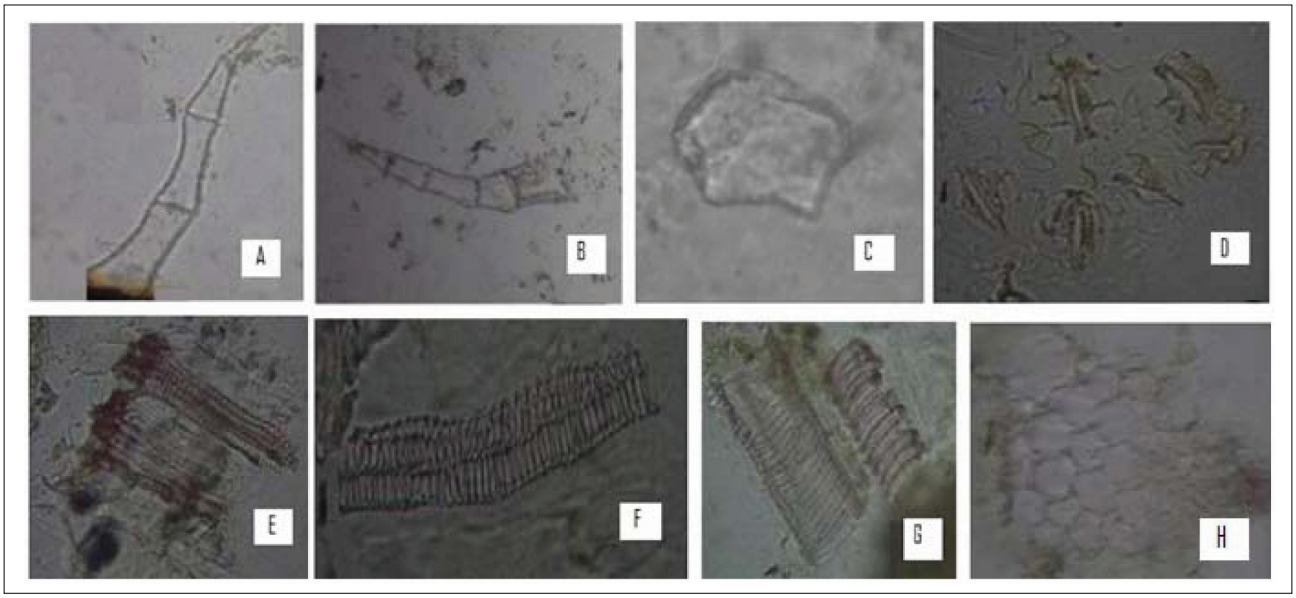


Abbreviations: Epi, Epidermis; Col, Collenchymas; Tr, trichome; Phl, phloem; Xyl, xylem; Co Par, cortical parenchyma.

TABLE 1: Leaf constants of *Xanthium strumarium*.

LEAF CONSTANT	RANGE	AVERAGE
Stomatal number (upper surface)	108–120	114.8
Stomatal number (lower surface)	192–216	204.6
Stomatal index (upper surface)	11.76–18.75	15.40
Stomatal index (lower surface)	16.21–25	21.45
Vein–islet number	17–21	19.2
Veinlet termination number	6–8	6.3
Average length of trichome per mg of leaf powder	85.6 cm	
Average epidermal area per g of leaf powder	297.47 cm^2	

FIGURE 4: Powder characteristics of *Xanthium strumarium* leaf.



A-Three cells covering trichome; B-Five cells covering trichome; C-prismatic form calcium oxalate crystal; D-Epidermal cells with anisocytic stomata; E-scalariform vessel with spongy mesophill; F-Scalariform xylem vessel; G-Annular xylem vessel; H-cortical parenchyma.

soluble ash, petroleum ether soluble extractive, chloroform soluble extractive, ethanol soluble extractive and water soluble extractive were determined and depicted in Table 2. The results of fluorescence analysis of the powder drug are presented in Table 3.

Preliminary phytochemical screening

Petroleum ether extract, chloroform extract and ethanolic extract were prepared by soxhlet apparatus by successive extraction method and water extract was prepared through decoction. Preliminary phytochemical screening revealed the presence of carbohydrates, steroids, cardiac glycosides, oils and fats in petroleum ether extract, anthracene glycosides in chloroform extract and carbohydrates, amino acids, cardiac glycosides, saponin glycosides and tannins in the ethanolic extract. Water extract contains amino acids, saponin glycosides and tannins (Table 4.)

TLC finger print profile

Thin layer chromatography of the petroleum ether, chloroform, alcohol and water extracts was carried out using hexane:diethyl ether:acetic acid, toluene:ethyl acetate:pyridine, toluene:ethyl acetate:pyridine, and ethanol:pyridine:ethyl acetate as a mobile phase respectively and R_f value were calculated (Table 5).

TABLE 2: Physicochemical parameters of *Xanthium strumarium* leaf.

PARAMETER	%w/w*	S.D.
Total ash	12.65	0.1
Acid insoluble ash	1.89	0.44
Water soluble ash	7.24	0.46
Petroleum ether soluble extractive	3.23	0.60
Chloroform soluble extractive	6.88	0.18
Alcohol soluble extractive	16.21	0.72
Water soluble extractive	26.92	0.73
Moisture content	9.75	0.28

**average of three readings*

TABLE 3: Fluorescenceanalysis of *Xanthium strumarium* leaf powder.

TREATMENT	DAY LIGHT	UNDER UV LIGHT	
		254 nm	365 nm
Dry powder	Green	Light green	Dark green
Powder + conc. HNO_3	Brown	Dark green	Reddish brown
Powder + conc. HCL	Dark green	Dark green	Blackish green
Powder + glacial acetic acid	Brownish green	Dark green	Blackish green
Powder + 40% NaOH	Dark green	Dark green	Blackish green
Powder + 5% $AgNO_3$	Green	Green	Dark green
Powder + 5% $CuSO_4$	Green	Green	Dark green
Powder + Mayer's reagent	Green	Green	Blackish green
Powder + Wagner's reagent	Brownish	Dark green	Blackish green
Powder +10% Picric acid	Green	Green	Black
Powder + Fehling's solution A	Green	Dark green	Black
Powder + 2% Resorcinol	Green	Green	Dark green
Powder + Conc. H_2SO_4	Dark green	Dark brown	Brownish green
Powder + Bromine water	Green	Dark green	Black

DISCUSSION

Leaves of the *X. strumarium* are dark green in colour, odourless and triangular-ovate in shape. The transverse section of the leaf shows 2–5 cellular covering trichomes, collateral scattered vascular bundle, prism shape calcium oxalate crystal, collenchymas below the upper epidermis and above the lower epidermis. In the lamina region upper epidermis is followed by 1–2 layers of palisade parenchyma and in the extreme end of lamina, palisade cells are replaced by spongy mesophyll, vascular stand and prism shape calcium oxalate crystal are also present in lamina region. Petiole of the leaf contains scattered ten collateral vascular bundles. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude

TABLE 4: Preliminary phytochemical screening of different extracts of *Xanthium strumarium* L. leaves.

GROUP OF PHYTOCONSTITUENT	EXTRACTS			
	PE	CE	AE	WE
Carbohydrates	–	-	+	-
Gums and mucilages	-	-	-	-
Proteins	-	-	-	-
Amino acids	-	-	+	+
Fats and oils	+	-	-	-
Steroids	+	-	-	-
Cardic glycosides	+	-	+	-
Anthraquinone glycoside	-	+	-	-
Saponin glycoside	-	-	+	+
Flavonoids	-	-	-	-
Alkaloids	-	-	-	-
Tannins	-	-	+	+

PE=Petroleum ether extract, CE =Chloroform extract, AE = Alcoholic extract, WE =water extract + indicates present, - indicates absent.

TABLE 5: Thin layer chromatography of different extracts of *Xanthium strumarium* Leaf.

EXTRACT	MOBILE PHASE	NUMBER OF SPOTS AND THEIR R_f VALUE
Petroleum ether	Hexane: diethyl ether: acetic acid (2:0.5:0.5)	Four spots, R_f values: 0.18, 0.47, 0.56 and 0.78
Chloroform	Toluene: ethyl acetate: pyridine (3.5:0.5:0.5)	Eight spots, R_f values: 0.13, 0.18, 0.22, 0.42, 0.45, 0.50, 0.57 and 0.68
Alcoholic	Toluene: ethyl acetate: pyridine (3.5: 0.5:0.5)	Eight spots R_f values: 0.21, 0.49, 0 .56, 0.61, 0.69, 0.74, 0.80 and 0.87
Aqueous	Ethanol: pyridine: ethyl acetate (1.5: 1: 2.2)	Two spots, R_f values: 0.62 and 0.70

drug. Hence various physicochemical parameters like moisture content, ash values, extractive values, TLC and phytochemical screening of various extracts were established to substantiate the standardization data on *X. strumarium*.[26, 27] The present study on pharmacognostical characteristics of *Xanthium strumarium* L. leaves will provide useful information for its correct identification and may enable those who handle this plant to maintain its quality control. Further, the authentic plant material can be explored for its pharmacological and phytochemical potential.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, 3rd edition (International Book Distributors, Dehradun, 1995); 1356–1357.
2. The Wealth of India-A Dictionary of Indian Raw Material and Industrial Products, Ist Supplement Series (Raw Materials), Vol. 7 (NISCOM, CSIR, New Delhi,1966); 1–4 .
3. Nadkarni AK. Indian Materia Medica,Vol.1 (Popular Prakashan, Mumbai, 2007); 1297–1298.
4. Chopra RN, Chopra IC. Glossary of Indian Medicinal Plants (CSIR, New Delhi, 1986); 259.
5. Ambasta SP.The Useful Plants of India (NISCAIR, New Delhi) 692.
6. Bisht NPS, Singh Ranbir. Chemical investigation of the leaves of (*X. strumarium* L., *JICS* 4) 1978; 707–708.
7. Ahmed A, Mahmoud, Ali A. El-Gamal. Axanthanolide and a dimeric xanthanolide from *Xanthium* species, *Planta Medica*. 1999; 65: 470–472.
8. Luping Qin, Ting Han, Huiliang Li, Qiaoyan Zhang, Hanchen Zheng.A new thiazinedione from *Xanthium strumarium*. *Fitoterpia* 2006; 77: 245–246.
9. Brain KR, Turner TD. The Practical Evaluation of Phyto-pharmaceuticals (Wright – Scientechnica, Bristol) 1975; 7–9.
10. Patra A, Jha S, Narasimba MP. Pharmacognostical standardization of leaves of *Hhgrophilla spinosa* T. Anders. *Phcog J.* 2009; 1(2): 82–87.
11. Patil AG, Joshi KA, Patil DA, Phathak AV, Naresh C. Pharmacognostic and physio-chemical studies on the leaves of *Cardiospermum hilicacabum* L. *Phcog J.* 2009; 1(4): 267–272.
12. Mukherjee PK. Quality Control of Herbal Drugs (Business Horizons Pharmaceutical Publisher, New Delhi) 2002; 138–141.
13. Evans WC. Trease and Evans Pharmacognosy, 15th edition (W.B. Saunders, Edinburgh London New York Philadelphia St Louis Sydney Toronto) 2000; 519–520, 545–647.
14. Johansen DA. Plant microtechniques ,1st edition (Mc Graw-Hill Book Company, New York and London) 1940; 182–203.
15. Kokate CK. Practical Pharmacognosy,1st edition, (Vallabh Prakashan, New Delhi, 1986) 15–30.
16. Agarwal SS, Paridhavi M. Herbal Drug Technology, (University Press (India) Private Limited, Himayatnagar, Hyderabad, 2007) 219–222.
17. Wallis TE. Practical Pharmacognosy, 6thedition, (J.&A. Churchill Ltd, London, 1953) 115–116, 179–182.
18. Indian Pharmacopoeia, 4th edition, Vol. II, (Government of India, Ministry of Health and Family Welfare, Controller of Publications, New Delhi) 1996; A53 –A54.
19. WHO/PHARM/92.559/rev.1. Quality Control Method of Medicinal Plant Materials (Oraganisation Mondaile De La santé, Geneva). 1992; 22–34.
20. Chase CR, and Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development a system of identification. *J. Am. Pharmacol. Assoc.* 1949; 38: 32 .
21. Kokoski CJ, Kokoski RJ, Salma FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J. Am. Pharmacol. Assoc.* 1958; 47–715.
22. Harborne JB. Method of extraction and isolation In. Phytochemical Methods. Chapman & Hall, London. 1998; 60–66.
23. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 20th edition (Nirali Prakashan, Pune 2002); 108–109.
24. Ali M.Text Book of Pharmacognosy, 2th edition (CBS Publishers and Distributers, New Delhi, 2003); 64–65, 96–97, 139–140, 272, 283.
25. Khandelwal KR. Practical Pharmacognosy, 14th edition (Nirali Prakashan, Pune 2002); 146–156.
26. Stahl E. Thin Layer Chromatography, a laboratory handbook (Springer Verlag Berlin Heidelberg, New York 1969).
27. Wagner H, Bladt S, Zgainski EM. Plant drug analysis (Springer VErlag Berlin Heidelberg, New York, 11983).

Pharmacognostic Evaluation of Roots of *Cocos nucifera* linn.

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INTRODUCTION

Cocos nucifera, the coconut tree, belonging to the family Arecaceae is a tropical tall branchless palm extensively cultivated in south India, West Bengal, coastal Maharashtra, Orissa, Lakshadweep and Andaman Islands. The tree can be identified with stout annulate often curved trunk with swollen base bearing huge pinnate lanceolate acuminate leaves and woody spadix embedded in the oblong spathe embedding numerous spikes bearing female flowers at its base and the male towards the apex, of them former develop into large obovoid angular and fibrous drupes. All parts of the palm are economically important and when medicinal value is focused, parts like root, leaf, flower, spadix, fruit and shell are used in skin disorders, eczema, dysuria, dysentery, eye disorders, diseases of head, menorrhagia and leucoderma in Siddha system of medicine; and the above parts are used to cure gastritis, hemorrhage, pain in bladder, diseases of pitta, fever, polyuria and leucorrhoea in Ayurveda. As far as the medicinal value of root is concerned roots are astringent, diuretic, anthelmintic, and useful in curing pharyngodynia, uterine disorders, blenorrhagia, bronchitis, hepatopathy, strangury and helminthiasis. A decoction of root is astringent and useful as mouth wash and gargle. The roots are roasted, grounded and used as dentifrice.[1] It is anthelmintic and diuretic, used as an astringent for gargle in sore throat, it is highly useful in uterine diseases. The decoction is prescribed in blenorrhagia, bronchitis and liver complaints with or without jaundice.[2]

ABSTRACT: Root of *Cocos nucifera* Linn. (Arecaceae) is astringent, diuretic, anthelmintic and useful in curing pharyngodynia, uterine disorders, blenorrhagia, bronchitis, hepatopathy, strangury and helminthiasis. It is an important commodity in folklore medicines of south India for urinary troubles. So far there is no record on detailed pharmacognostic evaluation of this crude drug, hence this communication throws light on parameters essential to fix standards for this medicinal material.

Keywords: *Cocos nucifera* (Linn.), Arecaceae, Coconut root, Pharmacopoeial standards.

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FIGURE 1: **Diagrammatic ts of *C. Nucifera* root.**

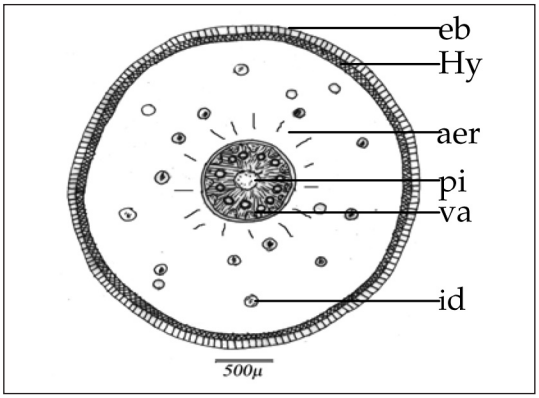
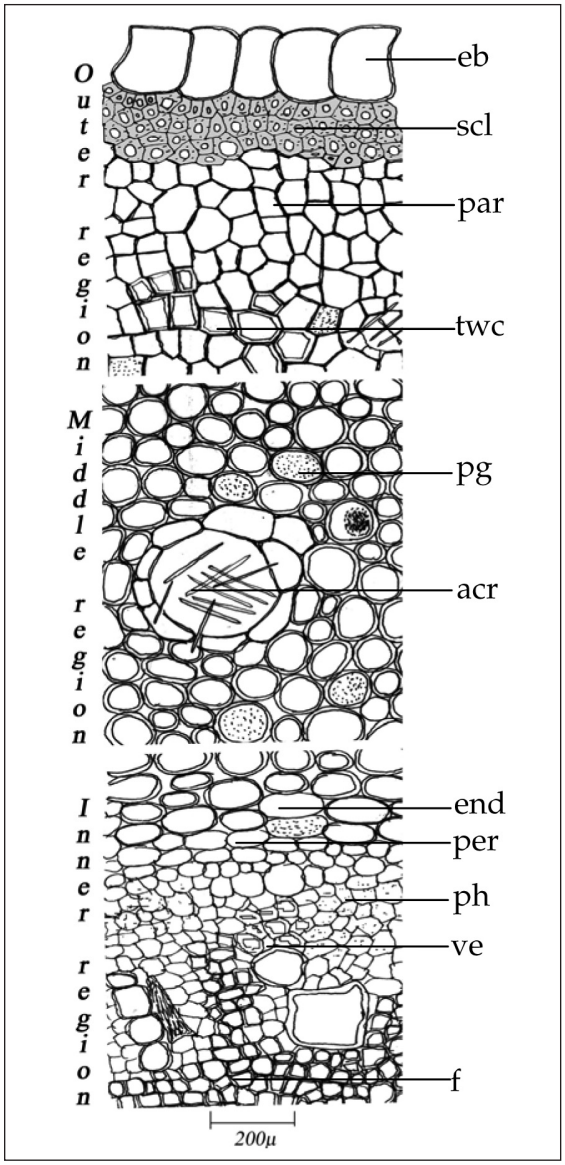


FIGURE 2: **Detailed ts of *C. Nucifera* root.**



MATERIAL

The roots of the plant were collected from the Chengalpet, Tamil Nadu in the month of October 2009, authenticated with the help of regional flora.[3]

METHODS

Few fresh roots were fixed using FAA for studying the microscopic characters; histochemical tests were performed as per the standard methods.[4] Washed roots were air dried, coarsely powdered, used for physico-chemical analysis.[5] The coarsely powdered plant material was successively extracted with *n*-hexane, chloroform, ethyl acetate and 90% ethanol using Soxhlet apparatus and used for preliminary phytochemical[6] and TLC[7] studies.

RESULTS

Macroscopy

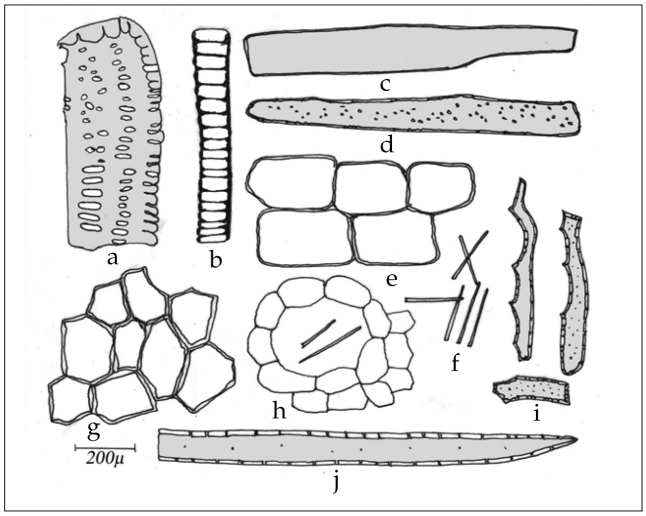
The aerial roots are finger like, smooth surfaced, capped with a dark brown root cap at their tip, variable in length, 0.5 to 1 cm in diameter, reddish brown or pink to faintly yellowish in color; fracture short in the bark, hard and fibrous in the wood; odor not characteristic, and taste astringent.

Microscopy

Diagrammatic transection of the root shows epiblema having a narrow zone of sclerenchymatous hypodermis; outer bark is embedded with idioblasts containing acicular crystals of calcium oxalate. A small and hard core of stele formed by xylem and phloem placed in the centre (Fig. 1).

Detailed transection shows an outer epiblema formed by thin-walled rectangular cells, followed by 2 to 3 rows of hypodermis formed by small sclerenchymatous cells which usually develops pits on maturity. Cortex consists of thick-walled parenchyma and is differentiated into outer compactly arranged elongated polygonal cells and inner rounded to oval cells with intercellular spaces which forms air spaces in fully matured roots; some cells of the parenchyma in the outer cortex forms idioblasts loaded with acicular crystals of calcium oxalate and few cells look like fibers due to thickening of the wall. Distinct endoderm and a narrow pericycle demark the cortex from inner dense core of stele formed by small sized patches of phloem and xylem composed of few vessels and lignified fibers surrounding it. The central region consists of small

FIGURE 3: Powder characters of *C. Nucifera* root.



a, scalariform vessel; b, annular vessel; c, elongated parenchyma; d, pitted parenchyma; e, group of cortical parenchyma; f, acicular crystals of calcium oxalate; g, epiblema in surface view; h, idioblast; i, sclereids from hypodermal region; j, fragment of fibre.

pith-like cells which often develops pits on their walls. Brown content is found throughout the parenchymatous cells of the cortex (Fig. 2).

Powder microscopy

Powder shows fragments of epiblema in surface view exhibiting comparatively large sized, hexagonal shaped cells; thin-walled, elongated sclereids from the hypodermal region of the root, elongated parenchyma from the cortical region, few pitted parenchyma from the pith region, scalariform to annularly thickened vessels and trachieds, acicular crystals of calcium oxalate are scattered throughout the powder (Fig. 3).

TABLE 1: Physico chemical studies.

PARAMETER	MEAN VALUE ± SD (N = 3)
Total Ash (% w/w)	5.549 ± 0.203
Water-soluble ash (% w/w)	2.33 ± 0.041
Acid-insoluble ash (% w/w)	0.540 ± 0.012
Alkalinity (cc of 0.1N HCl/g)	0.23 ± 0.004
Loss on drying at 105° (% w/w)	7.79 ± 0.029
Alcohol soluble extractive (% w/w)	15.05 ± 0.550
Water soluble extractive (% w/w)	11.948 ± 0.085

DISCUSSION

The physico-chemical data obtained on analysis of roots are shown in Table 1. Ash content was 5.7% indicating lesser amount of inorganic components present. Most of the mineral compounds present are water soluble in nature as indicated by 2.33% of water soluble ash. The acid insoluble ash of 0.5% was revealing the siliceous content is very less. Alcohol soluble extractive and water soluble extractive of 15 and 12% were suggestive of presence of more polar secondary metabolites like glycosides, steroids, triterpenoids, flavonoids, coumarins, phenols and tannins. 3.69% and 0.69% of hexane and chloroform (successive) extracts showed the presence of lesser amount of low polar compounds. Results of TLC and preliminary phytochemical studies are detailed in the Table 2 & 3 respectively.

TABLE 3: Preliminary phytochemical study.

QUALITATIVE TEST	*N*-HEXANE EXTRACT (3.69%)	CHLOROFORM EXTRACT (0.69%)	ETHYL ACETATE EXTRACT (0.33%)	ETHANOL EXTRACT (13.504%)
Alkaloid	-	-	-	-
Quinone	-	-	-	-
Coumarin	-	-	-	-
Flavonoid	-	-	+	+
Steroid	+	+	+	+
Phenol	-	+	+	+
Tannin	-	-	+	+
Glycoside/Sugar	-	-	+	+
Iridoid	-	-	-	-
Terpenoid	-	-	-	-

TABLE 2: Thin layer chromatographic (Tlc) study.

SOLVENT SYSTEMS	R_f OF SPOTS AFTER DERIVATISATION WITH VANILLIN SULPHURIC ACID SPRAY REAGENT
Toluene : Ethyl acetate (3:2)	0.18 (pale pink, 0.25 (blue), 0.65 (brown), 0.66 (pale blue), 0.90 (pale blue), 0.97 (dark blue)
Chloroform: Methanol (4:6)	0.49 (green), 0.79 (reddish brown), 0.90 (blue), 0.94 (blue)
Chloroform : Ethyl acetate (1:1)	0.28 (green), 0.77 (brown), 0.82 (reddish brown), 0.96 (dark blue)

CONCLUSION

Standards such as macro-, microscopic, physico-chemical, preliminary phytochemical and TLC were derived and described which are of diagnostic importance in authentication and quality control of the roots of *C. nucifera* Linn.

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REFERENCES

1. Anonymous. The Wealth of India, Raw Materials. New Delhi: Publication and Information Directorate, CSIR. Vol. IIC; 1950; 281.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants. Varanasi: Chaukambha Orientalia. 1935;Vol. 4: 2581–2583.
3. Gamble JS. Flora of the Presidency of Madras. Calcutta: Botanical Survey of India. 1967;Volume 3: 1086–1087.
4. Johanson DA. Plant microtechnique. London: Mac Graw Hill Book Company. 1940; 2581–2583.
5. Anonymous. Quallity Control Methods of Medicinal Plant Materials. Geneva: World Health Organization (WHO). 1998.
6. Harborne JB. Phytochemical Methods. London: Chapman and Hall. 1973; 70.
7. Stahl I. Thin Layer Chromatography. A Laboratory Hand Book (Student Edition). Berlin: Springer-Verlag. 1969; 52-86; 127–128; 900.

Analysis of nutritional components of *Taraxacum mongolicum* and its antibacterial activity

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INTRODUCTION

Taraxacum mongolicum Hand-Mazz. is a herbaceous perennial plant of the family Asteraceae, which is widely distributed in Asia and Europe. As a traditional Chinese medicine, *it* has been widely used in clinic for treating abscesses, reducing eye inflammation, viral infectious diseases and provoking diuresis.[1–5] Furthermore, *T. mongolicum*, especially its leaves and flowers, has been used as edible vegetables by local people in Northern China. Moreover, nutritional components of *T. mongolicum* are slightly different in different areas. At present, it is mainly obtained from the market as a cultivated vegetable, which replaced a wild dandelion. However, the main nutritional components in China and its antibacterial activity have not yet been reported. Thus, in this study, we measured the nutritional components and evaluated its antibacterial activity.

MATERIALS AND METHODS

Plant materials

The plants of *Taraxacum mongolicum* Hand. –Mazz. were obtained from the suburb of Jinan, China, in April 2010. The biological identification of the plant

ABSTRACT: *Taraxacum mongolicum* Hand-Mazz. has been used as a Chinese traditional medicine for a long history. In this study, its nutritional components were analyzed, including main nutritional components and micronutrients. At the same time, antibacterial test was evaluated. The results showed that nutritional components and mineral elements were very rich. Among of which, moisture, carbohydrate and protein are the major part of nutrients and the content of calcium, potassium, magnesium and phosphorus accounted for over 6.0% of the total minerals. The activity test indicated that only ethanol extracts of *T. mongolicum* exhibited antibacterial activity against some bacteria, such as *Staphylococcus aureus* and its isolated strain from air, *Escherichia coli* and *Pseudomonas aeruginosa*. The results support the clinical use of the plant in the treatment of inflammation in north China.

Keywords: *Taraxacum mongolicum*, nutritional components, antibacterial activity.
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TABLE 1: The content of main nutritional components in *Taraxacum mongolicum* (g/100 g).

NUTRITIONAL COMPONENTS	CONCENTRATION	NUTRITIONAL COMPONENTS	CONCENTRATION
Protein	4.15 ± 0.672	Fat	1.08 ± 0.073
Moisture	84.02 ± 6.965	Crude fibre	2.01 ± 0.367
Carbohydrate	5.03 ± 0.834	Crude ash	3.22 ± 0.483

was done by author, where the voucher specimen is conserved in the herbarium of Shandong University of TCM, China.

Nutritional components

The crude proteins were determined using Kjeldahl technique.[6] Determination of vitamin and physiochemical parameters like moisture, total ash, total fat and fiber contents were performed using the method reported by the Association of Official Analytical Chemists.[7] The mineral elements such as phosphorus (P), potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) were determined with atomic absorption spectrometry.

Preparation of the aqueous and ethanol extraction

The plants of *T. mongolicum* were air-dried, powdered, and macerated in water for 2h. The aqueous solution was boiled twice, each 1.5 h and then concentrated to final concentration of 1g/ml. In the same, the powder (5.0 kg) was macerated in ethanol (70%, v/v) and mixed well, in water bath for 3 h at 50˚C. The solvent was filtered and concentrated to final concentration of 1g/ml.

Antibacterial assay

Selected test microorganisms were *Escherichia coli*, *Staphylococcus aureus* and its isolated strain from air, *Shigella fiexneri*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Antibacterial tests were carried out by the disc diffusion method.[8] Sterile paper discs (6 mm in diameter) prepared from Whatman were impregnated with aqueous and ethanol solution placed on the inoculated agar. Negative control and positive control was prepared with ethanol and erythromycin respectively. The inoculated plates were incubated at appropriate temperature for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

MIC assay

The minimum inhibitory concentration was studied for the microorganisms that were determined as sensitive in the disc diffusion method. The aqueous extract and ethanol extract were first diluted to 500 µg/ml, and then serial two-fold dilutions were made with nutrient broth in the concentration rang from 10 to 500 µg/ ml. The last tube containing 5 ml of nutrient broth without extract and 1 ml of the inocula was used as a negative control.

RESULTS

Nutritional components

The content of main nutritional components in the *Taraxacum mongolicum* were shown in Table 1, which indicated that moisture, carbohydrate and protein are the major part of nutrients in the dandelion and its high protein (4.15 ± 0.672%) showed its better nutritional value in the leaf vegetables.

TABLE 2: The content of main micronutrients in *Taraxacum mongolicum* (mg/g).

MICRONUTRIENT	CONCENTRATION	MICRONUTRIENT	CONCENTRATION
P	3.87 ± 0.517	Zn	0.04 ± 0.006
K	40.03 ± 3.859	Cu	0.01 ± 0.006
Ca	12.15 ± 7.611	Mn	0.04 ± 0.008
Na	0.29 ± 0.017	V_{B1}	0.03 ± 0.005
Mg	4.24 ± 0.521	V_{B2}	0.38 ± 0.006
Fe	0.23 ± 0.076	V_C	0.05 ± 0.004

TABLE 3: Antibacterial activity of *Taraxacum mongolicum*.

EXTRACTS (μg/ml)	INHIBITION ZONE DIAMETERS (mm)					
	Staphylococcus aureus	*Staphylococcus aureus* (ISOLATED)	*Escherichia coli*	*Pseudomonas aeruginosa*	*Shigella fiexneri*	*Proteus vulgaris*
Aqueous extract	-	-	-	-	-	-
Ethanol extract	12.67 ± 0.583	17.34 ± 0.586	13.33 ± 1.024	10.05 ± 0.957	-	-
MIC	50	50	50	100	-	-
Positive control	33 ± 1.218	28 ± 1.563	27 ± 2.074	26 ± 1.815	-	-
Negative control	-	-	-	-	-	-

Mineral and vitamin

Taraxacum mongolicum contains minerals like Ca, Mg, K and Na, etc (Table 2). All of micronutrients, the content of calcium, potassium, magnesium and phosphorus accounted for over 6.0% of the total minerals, especially its calcium and potassium, which was considered one of the most abundant mineral elements in the vegetables. The experiment also displayed that dandelion is naturally rich in vitamins and V_{B2} is the highest content.

Antibacterial activity

The antibacterial activity of aqueous and ethanol extract of *Taraxacum mongolicum* against six bacterial species was summarized in Table 3. The zone of inhibition above 7 mm in diameter was taken as positive result. The results revealed that only ethanol extract showed antibacterial activity with varying magnitudes only in four strains, while aqueous extract did not. The MIC were 50 μg/ml for *Staphylococcus aureus*, *Staphylococcus aureus* (isolated) and *Escherichia coli* and 100 μg/ml for *Pseudomonas aeruginosa*.

DISCUSSION

T. mongolicum has a long history for green leafy vegetable for more than 1000 years. Our study indicated that the minerals, especially the calcium, potassium, phosphorus and magnesium are very rich. Of all the minerals, potassium and calcium (5.218%) were carefully matched with regular vegetables, such as legumes,[9] potato[10] and spinach,[11] etc. This means that, by dry weight, up to 5% of dandelion is potassium and calcium. So *T. mongolicum* is called as an ideal source of potassium and calcium. This may suggest that many Chinese people go to the fields or suburbs for dandelion in spring.

It was reported that ethanol extracts of the dried aerial parts of *T. mongolicum* have been shown to have anti-inflammatory and anticancer effects, which was agreed with the antibacterial effects in this experiment. It was further inferred that phenylpropanoids and sesquiterpene lactones in dandelion might be key to this activity.[12–14] As the above compounds are insoluble in water, so no antibacterial activity of aqueous extract was shown.[15–16]

In summary, our study showed that *T. mongolicum* has a higher nutritional value, better antimicrobial effects and edible plant.

REFERENCES

1. Bensky D, Clavey S, Stöger E, Gamble A. Chinese Herbal Medicine Materia Medica. 3rd ed. Seattle, WA: Eastland Press, 2004.
2. Kuusi T, Pyysalo H, Autio K. The bitterness properties of dandelion II. Chemical investigations. *LWT-Food Science and Technology*. 1985; 18: 347–349.
3. Shi SY, Zhou CX, Xu Y, Tao QF, Bai H, Lu FS, Lin WY, Chen HY, Zheng W, Wang LW, Wu YH, Zeng S, Huang KX, Zhao Y, Li XK, Qu J. Studies on chemical constituents from herbs of Taraxacum mongolicum. *Zhongguo Zhong Yao Za Zhi*. 2008; 33: 1147– 1157.
4. Zhu WY, Pang ZL, Liang MY, Zhang JH. The effect of taraxacum mongo to inhibit the mutation in the bone marrow lymphocyte of mice induced by cyclophosphamide. *Cancer. Aberrations. Mutation*. 2003; 15: 164–167.
5. Takasaki M, Konoshima T, Tokuda H, Masuda K, Arai Y, Shiojima K, Ageta H. Anti-carcinogenic activity of *Taraxacum* plant I. *Biological & Pharmaceutical Bulletin*. 1999; 22: 602–605.
6. Kjeldahl JZ. A new method for the determination of nitrogen in organic bodies. *Analytical Chemistry*. 1983; 22: 366–382.

7. AOAC (Association of Official Analytical Chemists) Official Methods of Analysis (OMA), 15th Ed. AOAC, Arlington, Virginia, USA, 1990.
8. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of Clinical Microbiology. 6th edition. Washington, USA, 1995.
9. Grusak MA. Genetic diversity for seed mineral composition in the wild legume *Teramnus labialis*. *Plant Foods for Human Nutrition*. 2008; 63: 105–109.
10. Camire ME, Kubow S, Donnelly DJ. Potatoes and human health. *Critical Reviews in Food Science and Nutrition*. 2009; 49: 823–840.
11. Citak S, Sonmez S. Mineral contents of organically and conventionally grown spinach (Spinacea oleracea L.) during two successive seasons. *Journal of Agricultural and Food Chemistry*. 2009; 57: 7892–7898.
12. Hu C, Kitts DD. Dandelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. *Phytomedicine*. 2005; 12: 588–597.
13. Yasukawa K, Akihisa T, Inoue Y, Tamura T, Yamanouchi S, Takido M. Inhibitory effect of the methanol extracts from compositae plants on 12-O-tetradecanoylphorbol-13-acetate-induced ear oedema in mice. *Phytotherapy Research*. 1998; 12: 484–487.
14. Jeon HJ, Kang HJ, Jung HJ, Kang YS, Lim CJ, Kim YM, Park EH. Anti-inflammatory activity of *Taraxacum officinale*. *Journal of Ethnopharmacology*. 2008; 115: 82–88.
15. Schütz K, Carle R, Schieber A. Taraxacum-a review on its phytochemical and pharmacological profile. *Journal of Ethnopharmacology*. 2006; 107: 313–323.
16. Schütz K, Muks E, Carle R, Schieber A. Separation and quantification of inulin in selected artichoke (*Cynara scolymus* L.) cultivars and dandelion (*Taraxacum officinale* WEB. ex WIGG.) roots by high-performance anion exchange chromatography with pulsed amperometric detection. *Biomedical Chromatography*. 2006; 20: 1295–1303.

Review on *Ruellia tuberosa* (Cracker plant)

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INTODUCTION

Ruellia tuberosa Linn. belongs to family Acanthaceae, a native of Central America, introduced into Indian garden as ornament.[1] It is used medicinally in West Indies, Central America, Guiana and Peru.[2] *Ruellia tuberosa* has tuberous root to tide over dry season and an ingenious seed dispersal system that helps it spread. The ripe seed capsules of *Ruellia tuberosa* breaks open with force when drops of water fall on the capsule and seeds are dispersed away from the plant. This explosive behavior gave the plant local name in English **'Cracker plant'**.

Taxonomical Profile

Kingdom	:	Planata
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Scrophulariales
Family	:	Acanthaceae
Genus	:	Ruellia
Species	:	*Ruellia tuberosa*[3,4]

Synonyms: *Ruellia picta, Ruellia clandestina*

Common name: Bluebell, Daniel's great gun, large bell-flower, Minnie root, popping pod, sheep potato, snapdragon root, Duppy gun.[5]

ABSTRACT: *Ruellia tuberosa* Linn. (Acanthaceae), known as cracker plant is traditionally used as diuretic, anti-pyretic, analgesic, anti-hypertensive, anthelmentic, abortifacient, emetic, in bladder disease, kidney disorder, bronchitis, gonorrhoea and syphilis. Many phytoconstituents have been identified. It has been experimentally proved which possess anti-oxidant, anti-microbial, anti-cancer and gastroprotective activity and antinociceptive and anti-inflammatory activity. Present review summarizes the traditional claims, phytochemistry and pharmacology of *Ruellia tuberosa* reported in scientific literature.

Keywords: Cracker plant, *Ruellia tuberosa*.
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FIGURE 1: *Ruellia tuberosa* Linn.

Vernacular names

Hindi	:	Chatakani Phallis
Gujrati	:	Bandukadi[6]
Tamil	:	Pottakanchi
French	:	Patate chandelier[7]
English	:	Cracker plant

Ruellia tuberosa is an erect, sub-erect or diffuse perennial herb up to 60–70 cm tall herb with stout, 4-angled stems, swollen and purplish at the nodes, tender parts sparsely hairy. Roots are slender, elongated, fusiform tuberous and off white in colour. Leaves with up to 2 cm long petiole; lamina; oblong-obovate to oblanceolate, 5–9 x 2–4 cm, shining, basally cuneate to attenuate, entire to undulate, obtuse to somewhat acute. Flowers shortly pedicellate, blue-violet, showy, 5–5.5 cm long, solitary or in 1–3-flowered, terminal or axillary cymes; bracts and bracteoles linear-lanceolate, 5 mm long. Calyx lobes equal, linear, 1.3–2 cm long, ciliate, acute. Corolla pubescent outside, tube; 3.5 cm long, abruptly narrowed at base, limb 5–lobed, lobes equal, ovate, c. 1.5 cm long, Stamens with oblong-sagittate, c. 4 mm long, puberulous anthers. Ovary oblong, c. 4 mm long, glabrous; style 2.3–2.5 cm long. Capsule is subcylindrical, brownish black, 1.5 cm long, 24–28-seeded, minutely beaked at tip, seeds are flatten, orbicular; 2–2.5 mm in diameter. The plants are propagated by cutting tubes or through seeds produce in brownish black capsules explode on ripening and the seed disperse all around *Ruellia tuberosa* originates from tropical America, but is naturalized in Southeast Asia (Thailand, Peninsular Malaysia, Java) and elsewhere in the tropics (India, Sri Lanka, Africa), as an escape from cultivation and as an ornament.[8–13]

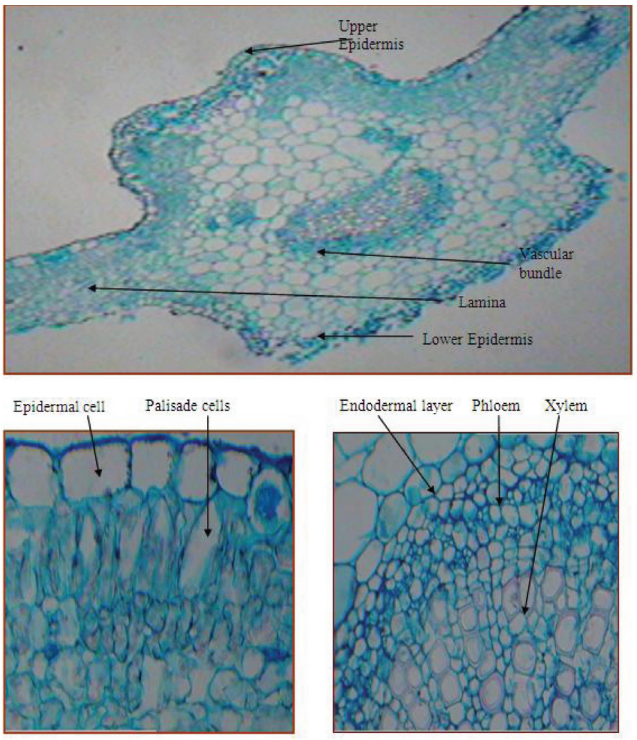
Microscopical identification

Microscopy of Leaf: It is a dorsiventral leaf.

Lamina: Upper epidermis and lower epidermis is single layered with polygonal cells covered outside with a thin walled cuticle having diacytic stomata, covering trichomes. Mesophyll is a differentiated into palisade and spongy parenchyma. Palisade tissue cells in two layers. Upper layered palisade cell larger than lower rows of palisade cell. Spongy parenchyma is a thin, 2 to 4 layers loosely arranged with intercellular space. Vascular strands are seen.

Midrib: Epidermal layers of lamina are in continuity with that of midrib. The dorsal surface of the midrib having central shallow depression and ventral surface is flat. A 2 to 4 layered collenchyma can be seen below the upper epidermis and above the lower epidermis. The rest of midrib is occupied by the cortical parenchyma with the arc shaped collateral vascular bundle embedded in the middle.

FIGURE 2: Microscopy of leaf.



Microscopy of root

Epidermis is a thin layer having thick walled epidermal cells and bearing dense root hairs. Hypodermis; Single layered immediately below the epidermis with thin walled cells. Cortex is wide, homogeneous with characteristic pattern of parenchyma cells, many layers having thick walled parenchymatous cells; they become smaller toward the periphery and smaller intercellular spaces. Brownish coloring matter present in cortex region. Cambium is formed external to the xylem mass. Vascular tissue; Phloem occupies larger area than xylem. Phloem seen around the xylem vessels. Xylem consists of vessels and lignified parenchyma. Pith consists of large parenchyma with intercellular spaces. Wide pith, within the vascular cylinder.

Microscopy of stem

T.S. of the stem is more or less circular. Following are the important tissues from the periphery to the centre.

Epidermis is single layered consisting of rectangular cells and shows the presence of thick cuticle. Cortex is heterogeneous having outer layers of collechyma cells and inner layers of parenchyma cells wider than outer zone. Parenchyma cells are compactly arranged. A continuous zone of cambium occurs in between xylem and phloem. Vascular bundles; phloem elements are seen in small isolated groups around the xylem vessels. Xylem forms a continuous layer surround the pith, well developed, consists of tracheids and xylem parenchyma. Pith is large and is made of thin walled, big polygonal parenchyma with intercellular spaces.

FIGURE 3: Microscopy of root.

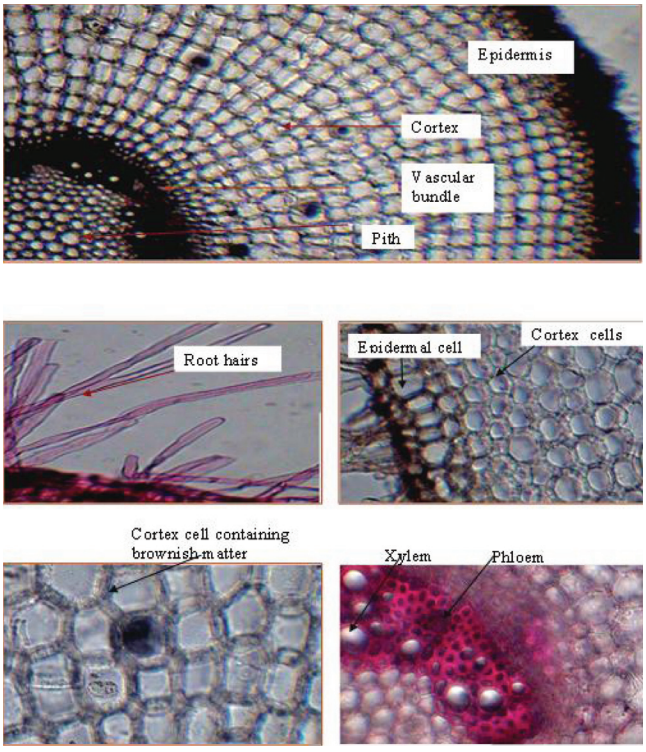
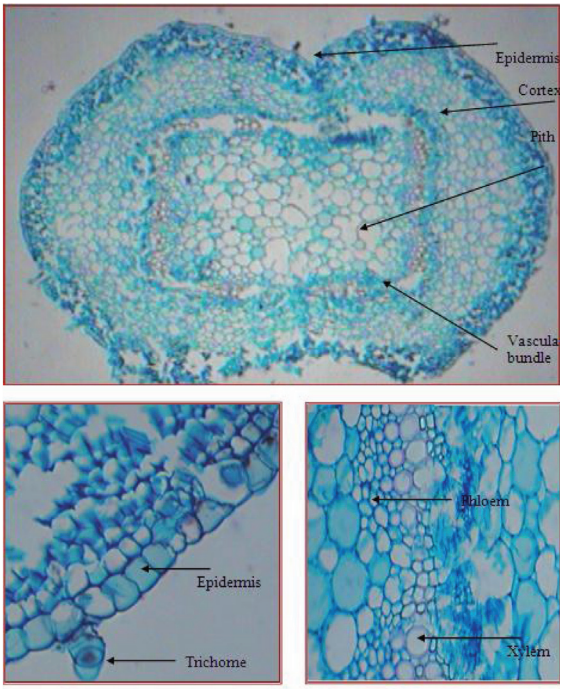


FIGURE 4: Microscopy of stem.



Physical constants of Ruellia tuberosa

TABLE: 1 Proximate analysis of leaf, root and stem of *Ruellia tuberose.*

PARAMETER	*AVERAGE VALUES %w/w		
	LEAF	ROOT	STEM
Water soluble extractive value	28.0	19.0	17.0
Alcohol soluble extractive value	13.5	33.0	0.9
Total ash value	24.77	8.33	10.55
Water soluble ash value	8.0	3.7	5.4
Acid insoluble ash value	11.5	5.3	0.48
Loss on drying	3	5	3.5
Foaming index	less than 100	less than 100	less than 100

** The values given here are expressed as percentage of air dried material. Each value is average of three determinations.*

Ethnobotanical uses

- In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases.[14] *Ruellia tuberosa* used in stomach cancer.[15–16]
- Dried and ground root in dose of two ounces cause abortion, also used in sore eyes.[17]
- The herb also exhibits emetic activity and employed substitute of ipecac, also used in bladder stones and decoction of leaves used in treatment of Bronchitis.[18]
- In Suriname's traditional medicine system, it is used as anthelmentic and also in management of joint pain and strained muscles. In folk medicine, it has been used as diuretic, anti-pyretic, anti-diabetic, antidotal, thirst-quenching agent, analgesic and anti-hypertensive activity.[19–20]
- Paste of leaves applied on skin diseases, wounds, boils etc. Seeds employed in sexual debility, spermatorrhoea, leucorrhoea etc.[21]
- Roots used for oestrus induction and as an anthelmintic.[22]
- *R. tuberosa* used as cooling in urinary problem, used in treatment of uterine fibroids.[23–24]
- It has recently been incorporated as a component in an herbal drink in Taiwan.[25]
- Whole plant is used to treat bladder diseases and frequent micturition; decoction with *Petiveria alliacea* is drunk to "clean out" uterine tract (dilation and curettage) or as an abortifacient. Root infusion is used for kidney diseases; in a syrup for whooping cough; infusion or decoction for a diabetes remedy**;** tubers in a tea used for cleansing the blood. Root and leaf used in form of tea alleviates retention of urine and it is suggested as a remedy to weakness.[26]
- Tuber powder (5–10gm) is given with milk for checking abdominal pain after delivery and stomach ache.[27–28]

Phytochemistry

Flavonoids, steroids and triterpenoids: Chwan-Fwu Lin et. al, isolated five flavonoids cirsimaritin (yellow prism; m.p. 255–256°C), cirsimarin (white prism; m.p. 158–160°C) , cirsiliol 4′-glucoside (yellow prisms; m.p. 214–216°C) sorbifolin (yellow solid; m.p. 274–275°C;), and pedalitin (yellow solid) along with betulin (white prisms; m.p. 255–256°C), vanillic acid (colorless solid; m.p. 212–213°C), and indole-3-carboxaldehyde (yellow solid) from the ethyl acetate extracts of *Ruellia tuberosa*. Compounds cirsimaritin and cirsiliol 4′-glucoside showed cytotoxicity against KB cell line.[29]

The leaves contained only traces of apigenin and luteolin, while flowers malvidin -3, 5- diglucoside in appreciable quantity. The flowers buds contained the maximum proportion of flavonoids (3% apigenin -7-O-glucoronide and the other flavones were identified as apigenin 7-O-glucoside, apigenin -7-O-rutinoside and luteolin -7- O-glucoside.[30] Seed oil yields myristic, capric and lauric acids.[31]

The aerial part of *Ruellia tuberosa* is reported to contain apigenin 7-β-D glucuronide[32] and maividin 3, 5-diglucoside [33] and some other natural products-includes n-alkenes (C_{11} –C_{37}), esters and sterols- stigmasterol, B-sitosterol, campesterol, cholesterol.

A triterpenoid, 21-methyldammer-22-en-3β, 18, 27, triol reported from the aerial part of *Ruellia tuberosa* (white crystal, m.p. 184–185°C).[34]

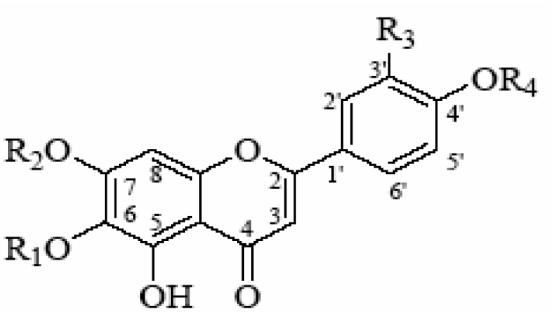
The tubers of the plant are reported to contain n-alkanes, triterpenoids and phytosterols, lupeol. In which n-alkanes (C23-C33) with maximum occurrence of n-nonacosane (C29: 44.83%) and n-hentriacotane (26.52%), Sterol- stigmasterol, B-sitosterol, campesterol.[35]

Alkaloid: Arun et al., isolated of Tylocrebrine, a phenanthrene alkaloid from aerial part of *Ruellia tuberosa* through bioassay directed column chromatography and elucidating its anti-cancer and anti-inflammatory potential.[36]

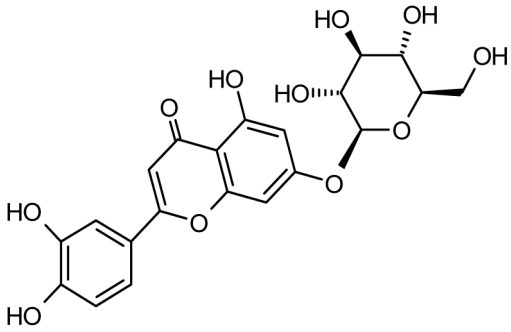
Pharmacological Activity

Antioxidant: The antioxidant activity of different extracts of stem of *Ruellia tuberosa* L. was investigated by various *in Vitro* methods like 2, 2-diphenyl-1- Picrylhydrazyl (DPPH) free radical-scavenging assays and the hydrogen peroxide induced luminol chemiluminescence assay. The methanolic extract (ME) and its four fractions of water (WtF), ethyl acetate (EaF), chloroform (CfF), and n-hexane (HxF) were evaluated for antioxidant activity. The results of revealed that *R. tuberosa* possesses potent antioxidant activity. The antioxidant activities of the different fractions tested decreased in the order of EaF > CfF > ME > WtF > HxF according to the hydrogen peroxide-induced luminol chemiluminescence assay, and results were the same with the exception of the rank order of HxF and WtF according to the DPPH free radical-scavenging assay. The results provide useful information on the pharmacological activities associated with free radicals of this traditional folk remedy.[37]

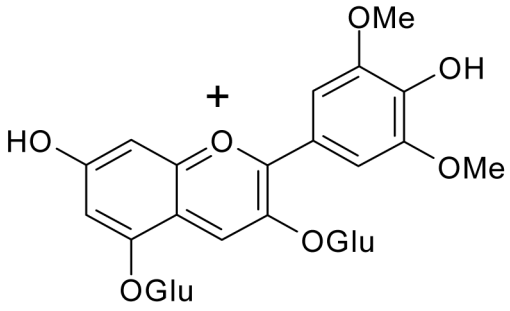
Gastroprotective activity: Aqueous extract of *R. tuberosa* roots showed a strong and dose-dependent gastro protective activity in alcohol induced gastric lesion model of rats (in terms of reduction in length



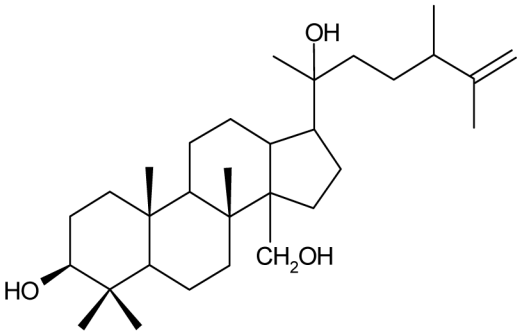
NAME OF FLAVANOIDS	R1	R2	R3	R4
cirsimaritin	CH_3	CH_3	H	H
cirsimarin	CH_3	CH_3	H	Glc
cirsiliol 4′-glucoside	CH_3	CH_3	OH	Glc
sorbifolin	H	CH_3	H	H
pedalitin	H	CH_3	OH	H



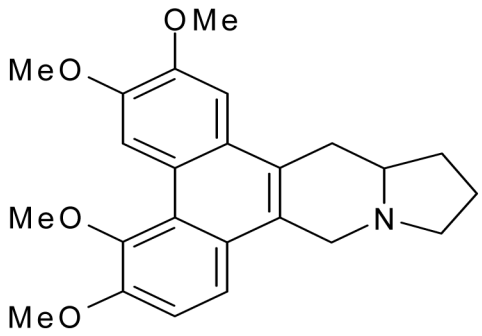
Luteolin - 7 - O – glucoside



Malvidin -3, 5- diglucoside



21–Methyldammar-22-en-3 β, 18, 27-triol



Tylocrebrine

FIGURE 5: Chemical constituents of *R. tuberose*.

of haemorrhagic gastric lesions). The extract also had a mild erythropoietic and moderate analgesic activity and was well tolerated even with subchronic treatment.[38]

Antimicrobial activity: The antibacterial activities of hexane, dichloromethane, ethyl acetate and methanol extracted fractions of *R. tuberosa* were investigated against Gram positive and Gram negative bacteria. The ethyl acetate and methanol fractions exhibited the highest rates of antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[39]

Anticancer activity: Methanol extract of aerial part of herb *R. tuberose* possessed cytotoxicity. The minimum inhibitory concentration (IC_{50}) for methanol extract was found to be 3.5 and 1.9 µg/m l in H460 and MDA-MB231 cancer cells respectively. Tylocrebrine was isolated from *Ruellia tuberosa* through bioassay directed column chromatography and elucidating its anti-cancer and anti-inflammatory potential.[40]

Antinociceptive and anti-inflammatory activity: The ethanol extract of *R. tuberosa* L. was evaluated for

its antinociceptive and anti-inflammatory properties in experimental mice and/or rat models. In the hot-plate test, the group that received a dose of 300 mg/kg showed maximum time needed for the response against thermal stimuli and maximum possible analgesia was similar to that of diclofenac sodium. The extract at 500 and 250 mg/kg doses showed significant reduction in acetic acid-induced writhing in mice, which was similar to diclofenac sodium. The extract also demonstrated significant inhibition in serotonin and egg albumin-induced hind paw edema in rats at the doses 100, 200 and 300 mg/kg. The anti-inflammatory properties exhibited by the extract were comparable to that of indomethacin at a dose of 5 mg/kg.[41]

CONCLUSION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. *Ruellia tuberosa* has been used traditionally in the treatment of various ailments. The literature review showed that the presence of alkaloid, flavanoids and phenolic compounds in various part of the plant. The pharmacological review suggested that *Ruellia tuberosa* (cracker plant) has reported antioxidant, gastroprotective, antinociceptive, anti-inflammatory, antimicrobial, and anticancer. Further pharmacogical and phytochemical exploration is required for systemic investigation of this plant.

REFERENCES

1. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary, Springer. 2007; p.561.
2. Kritikar KR and Basu BD. Indian medicinal plants.1935; 3:1866–67.
3. United States Department of Agriculture (USDA), Agricultural Research Service, Beltsville Area (ARS), National Genetic Resources Program. Germplasm Resources Information Network-(GRIN). Available at http://www.ars-grin.gov/cgi-bin/npgs/html/taxon, Accessed 2007.
4. Encyclopedia of life. Available at http://www.eol.org/pages/590262, Accessed oct 25, 2008.
5. http://www.arubaplaza.com/index.php.
6. Pandey CN. Medicinal plants of Gujarat, (Gujarat Ecological Education and Research Foundation, Gujarat (India). 2005; p. 387.
7. The wealth of India Vol. IX, (Council of Industrial and Scientific Research, New Delhi, 1972; p. 90.
8. Pandey CN. Medicinal plants of Gujarat, (Gujarat Ecological Education and Research Foundation, Gujarat (India) 2005; p. 387.
9. The wealth of India Vol. IX, (Council of Industrial and Scientific Research, New Delhi, 1972; p. 90.
10. Howard, Richard A. Flora of the Lesser Antilles: Leeward and Windward Islands. Vol. 6: Dicotyledoneae-Part 3 Arnold Arboretum, Harvard University. 1989; P. 658.
11. Shah GL. Flora of Gujarat State, Sardar Patel University. 1978 ;1: pp, 20, 552.
12. Shaukat Ali Chaudhary. Flora in Lyallpur and the adjacent canal-colony districts, West Pakistan Agricultural University. 1969; page no. 30, 152.
13. http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=220011777.
14. Suseela L and Prema S. Pharmacognostic study on *Ruellia tuberosa*. *Journal of Medicinal and aromatic Plant sciences*. 2007; 29: 117–122.
15. Reddy MB, Reddy KR, Reddy MN. Ethnobotany of Cuddapah district, Andhra Pradesh, India. *International Journal of Pharmacognosy.* 1991; 29: 273–280.
16. Graham JG. et al.: Review; Plants used against cancer – an extension of the work of Jonathan Hartwell Journal of Ethno pharmacology. 2000; 73: 347–377.
17. Kritikar KR and Basu BD. *Indian medicinal plants.* 1935; Vol. iii: 1866–67.
18. The wealth of India Vol. IX, (Council of Industrial and Scientific Research, New Delhi). 1972; p. 90.
19. Chiu NY, Chang KH. The illustrated medicinal plants of Taiwan. *Mingtong Medical J.* 1995; 226: 1.
20. Chen FA, Wu AB, Shieh P, Kuo DH, Hsieh CY. Evaluation of the antioxidant activity of *Ruellia tuberosa*. *Food Chem*. 2006; 94: 14–18.
21. Selvam ABD. Inventory of Vegetable Crude Drug samples housed in Botanical Survey of India, Howrah Pharmacognosy Reviews. 2008;Vol 2(3): 36.
22. Lans CA. Creole Remedies. Case studies of ethnoveterinary medicine in Trinidad and Tobago. Wageningen University dissertation no. 2992.
23. Cheryl A Lans. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *Journal of Ethnobiology and Ethnomedicine*. 2006; 2: 45.
24. Michael J Balick. Medicinal Plants Used By Latino Healers for Women's Health Conditions In New York City. *Economic Botany.* 2000; 54(3): 344–357.
25. Wagner H, Danninger H, Iyengar MA, Subramanian SS, Nair AGR et.al. Synthesis of glucuronides in the flavonoid-series. Isolation of apigenin-7-D-glucuronide from *Ruellia tuberosa* L. and its synthesis. *Chem. Ber.* 1971; 104: 2681–2687.
26. Howard, R. A Medicinal Plants of the Guiana's (Guyana, Surinam, French Guiana). 1983; 314.
27. Swarnkar S, and Katewa SS. Ethnobotanical Observation on Tuberous Plants from Tribal Area of Rajasthan (India). *Ethnobotanical Leaflets.* 2008; 12: 647–66.
28. Choudhary K, Singh M and Pillai U. Ethnobotanical Survey of Rajasthan - An Update, American-Eurasian Journal of Botany. 2008; 1 (2): 38–45.
29. Chwan-Fwu Lin et. al. Bioactive flavonoid from *Ruellia tuberosa*. *J Chin Med*. 2006; 17(3): 103–109.
30. Subramanian SS, Nair AGR. "Apigenin glycoside from Thunbergia fragrans and *Ruellia tuberosa*. *Current science*. 1974; p.480.
31. Howard R. A Medicinal Plants of the Guiana's (Guyana, Surinam, French Guiana). 1983; 314.

32. Wagner H, Danniger H, iyengar NA, Seligurau O, Farkas L, subramannian SS and Nair AGR. *Chem. Berr.* 1971; 104: 2681.
33. Subramanian SS, Nair AGR. "Apigenin glycoside from Thunbergia fragrans and *Ruellia tuberosa*. *Current science*. 1974; p.480.
34. Singh RS, Pandey HS and Singh BK, Pandey RP. A new triterpenoid from *Ruellia tuberosa* Linn., *Indian Journal of Chemistry.* 2002; 41B: 1754–56 .
35. Andhiwal CK. Chandra Haas Varshney RP. Phytochemical investigation of *Ruellia tuberosa* L. *Indian drugs.* 1985; 23: 49.
36. Arun S, Giridharan P, Suthar A, Kulkarni-Almeida A, Naik V, Velmurugan R, Ram V, et. al. Isolation of Tylocrebrine from *Ruellia tuberosa* through bioassay directed column chromatography and elucidating its anti-cancer and anti-inflammatory potential. Book of Abstracts, 7th Joint Meeting of GA, AFERP, ASP, PSI & SIF, Athens, Greece. 2008; 25.
37. Chen FA, Wu AB, Shieh P, Kuo DH, Hsieh CY. Evaluation of the antioxidant activity of *Ruellia tuberosa*. *Food Chem*. 2006; 94: 14–18.
38. Arambewela LSR, Thambugala R and Ratnasooriya WD. Gastroprotective activity of *Ruellia tuberosa* root extract in rats. *Journal of tropical medicinal plants,* 2003; 4(2): 191–194.
39. Wiart C, Hannah M, Yassim M, Hamimah H, Sulaiman M. Anti-microbial activity of *Ruellia tuberosa* L. *American Journal of Chinese Medicine.* 2005; 33(4): 683–685.
40. Arun S, Giridharan P, Suthar A, Kulkarni-Almeida A, Naik V, Velmurugan R, Ram V, et. al., Isolation of Tylocrebrine from *Ruellia tuberosa* through bioassay directed column chromatography and elucidating its anti-cancer and anti-inflammatory potential. book of Abstracts, 7th Joint Meeting of GA, AFERP, ASP, PSI & SIF, Athens, Greece. 2008; p.25.
41. Ashraful Alam M, Nusrat Subhan et. al Antinociceptive and anti-inflammatory properties of *Ruellia tuberosa*. *Pharmaceutical Biology.* 2009; 47(3): 209–214.

Phytochemical and Pharmacological Investigations on *Adhatoda zeylanica* (Medic.): A Review

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INTRODUCTION

Nature is still mankind's greatest chemist and many compounds that remain undiscovered in plants are beyond the imagination of even our best scientists. From time immemorial man has been interested in trying to control diseases. As the world's population is nearing 5 billion, with this rate of growth, ¾th of the world's population can't afford the products of western pharmaceutical industries. Therefore, they have to rely upon the traditional medicine, which are derived from plants. One third of all pharmaceuticals are of plant origin. Though considerable advances are made in the pharmaceutical sciences, especially in synthetic chemistry, but plants and their derivatives continue to maintain their significance in medicines. In fact, modern people are now showing increased interest in natural drugs then synthetic once, primarily because of a high degree of adverse side effects caused by the latter. It is evident from the present scenario, that herbal cure is gaining world wide acceptance and has emphasized on modern scientific exploration, extraction and evaluation of foil medicines

ABSTRACT: Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. The plant *Adhatoda zeylanica* Medic. syn. *A. vasica* Nees commonly known as vasaka, family Acanthaceae, is extensively used traditionally for treating cold, cough, whooping cough, chronic bronchitis, asthma and also used as a sedative, expectorant, antispasmodic. In recent years the interest in this plant has increased considerably with substantial progress on its chemical and pharmacological properties. *Adhatoda zeylanica* is an important source of phytoconstituents like quinazoline alkaloid vasicine, vasicinone, vasicinol, vasicinine and vasicoline. The alkaloids vasicine and vasicinone shows bronchodilatory action. Hence in view of immense medicinal importance of the plant this review is therefore an effort to compile all the information reported on its phytochemical and pharmacological activities, these information will be helpful to create interest towards the plant and may be useful in developing new formulations, which are more effective and have more therapeutic values.

Keywords: *Adhatoda zeylanica*, Acanthaceae, Vasicine, Vasicinone, Bronchodilator.

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from plants. These are either used directly as a plant extract or modified through further synthesis.[1] Now a days natural medicines are gaining prominence, because they are economical, easily available and relatively free from side effects. The increased global demand for poly-herbal formulations is a reflection of positive impact of consolidated efforts aimed at reviving science of phytop-harmacy.[2] *Adhatoda zeylanica* Medic. family Acanthaceae is an evergreen, gregarious, stiff, perennial shrub, 1–6m in height, distributed throughout India, upto an altitude of 1300 m. Leaves elliptic-lanceolate or ovate-lanceolate, entire, 5–30cm long, hairy, light green in colour. Shrub grows on waste lands and in variety of habitats and soil. There is considerable demand for this plant within the country for its beneficial effects, particularly in bronchitis. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping-cough and chronic bronchitis and asthma, as sedative-expectorant, antispas-modic and as anthelmintic.[3] Hence in view of immense medicinal importance of the plant this review is therefore an effort to compile all the information reported on its phytochemical and pharmacological activities, these infor-mation will be helpful to create interest towards the plant and may be useful in developing new formulations, which are more effective and have more therapeutic values.

PLANT PROFILE

Common vernacular name[3]

Arusa	(Hindi)
Adusoge	(Kannad)
Basak	(Bengali)
Aradusi	(Gujrati)
Adhatodai	(Tamil)
Bansa	(Panjabi)
Arusa	(Urdu)
Basongo	(Uriya)
Bangra	(Garhwal)
Addasaramu	(Telugu)
Bahekar	(Kashmir)

Taxonomic Classification[4]

Kingdom	-	Plantae
Subkingdom	-	Tracheobionta
Superdivision	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Asteridae
Order	-	Scrophulariales
Family	**-**	**Acanthaceae**
Genus	-	*Adhatoda*
Species	-	*Adhatoda zeylanica*

MORPHOLOGICAL CHARACTERS

Plant is a dense shrub 1–6 m high with many long opposite ascending branches. Stem with yellowish bark. Leaves-12.5–20 by 3.8–6.3 cm, elliptic-lanceolate, acuminate, minutely puberulous when young, glabrous when mature, entire, dark green above paler beneath, base tapering, main nerves 10–12 pairs with reticulate venation between petioles 1.3–2.5 cm long. Flower short dense axillary pedunculate spikes 2.5–7.5 cm long, towards the ends of the branches, peduncles 3.8–10 cm, stout, shorter than the leaves, bracts reaching 2.2 by 1.3 cm. Calyx rather less than 1.3 cm. long glabrous or slightly pubescent, divided to within 2 mm. of the base, segments imbricate, oblong-lanceolate, acute, 3- nerved, reticulately veined. Corolla white, with a few irregular rose-coloured bars in the throat, 3.2 cm long, long pubes-cent outside, tube 1.3 cm long the lower half cylindrical, 4 mm. diameter upper half much laterally flatted. Fila-ments hairy at the very base, long, stout, curved lower anther-cells minutely apiculate, (not white spurred) at the base. Ovary pubescent, subacute shortly and bluntly pointed, pubescent, solid stalk flattened, 1 cm long, seeds 6 by 5 mm, orbicular-oblong.[5]

PHYTOCHEMICAL INVESTIGATION

Plant contains chief active principal quinazoline alkaloids such as vasicine, adhatodine, vasicinone, deoxy-vasicinone.[5] A novel alkaloid and galactoside isolated from the roots have been characterized as 9 - acetoam-ido-3, 4 - dihydro pyrido - (3, 4-*b*)–indole and O–ethyl - α - D galactoside respectively. In addition to sitosterol β-D - glucoside, D - galactose and deoxyvasicinone have also been isolated from the roots of this plant.[6] Vasicol a new alkaloid is also isolated from the plant.[7] High - performance thin layer chromatographic method has been developed for the simultaneous determination of pharmacologically important quinazoline alkaloids vasi-cine and vasicinone in *Adhatoda zeylanica*.[8] The alka-loid vasicine has been found to occur in higher plants. Minor alkaloids which include vasicinol, vasicinone, deoxyvasicinone and deoxyvasicine have been reported in lower plants.[9] Two new aliphatic hydroxyketones, isolated from the aerial part have been characterized as 37- hydroxy hexatetracont - 1- en- 15 - one and 37-hydroxy hentetracontan -19- one.[10,11]

^{1}H NMR spectroscopy of *l*- vasicine by the use of Moher's method using MTPA (α–methoxy–α - (trif-luoromethyl) phenyl acetic acid) esters for establishing the absolute configuration was reported.[12] Further a non nitrogenous neutral principle, vasakin,[13] (+) -

vasicinone[14] and two new quinazoline alkaloids, one of which named as adhavasinone have been isolated[15] and two new pyrroloquinazoline alkaloids, desmethoxyaniflorine and 7- methoxyvasicinone were identified from the ethanolic extract of the leaves.[16] A reverse phase HPLC method of estimating vasicine in the leaves was developed. Using this technique, vasicine was estimated in two polyherbal drug formulations as well as from the leaves and stem of the plant.[17] In another study, a rapid and accurate method using HPLC was developed for detection, monitoring and quantification of vasicine in the plant as well as herbal preparations containing plant.[18] Synthesis of analogues of vasicine, vasicinone and deoxyvasicinone were reported.[19] Leaves of vasaka were subjected to authentication and the marketed formulations containing the leaf extract were obtained from local pharmacies to estimate vasicine and vasicinone contents using HPLC method. The vasicine content of various formulations varied from 22.8 μg to 86.4 μg/10 ml of the marketed formulations. At the operative chromatographic conditions vasicinine was not detected in any of the formulation analyzed. The HPLC technique now allows routine analysis of vasicine containing complex traditional formulation.[20] The flavonoids in the leaves were identified as kaempferol, quercetin, vitexin and isovitexin while the phenolic acids were *p*-hydroxybenzoic acid, syringic acid and *p*-coumaric acid.[21] The leaves were found to contain free vitamin C and carotene.[22] Both leaves and flowers contained the flavones luteolin.[23] The mineral elements of the leaves were identified as calcium, magnesium, potassium, sodium and iron.[24] The petroleum ether extract yielded an aliphatic alcohol characterized as 29-methyltriacontan-1-ol.[25] The stalk, which is non woody, was found to contain the lignins composed of guaiacyl-, syringyl- and *p*- hydroxyphenyl propane building units similar to hardwood lignins.[26] The petroleum ether extract of the flowers contained a number of non-nitrogenous compounds *viz*., triatricontane, β-sitosterol, α-amyrin and β-sitosterol-D-glucoside; the ether extract yielded kaempferol and quercetin, the ethyl acetate and *n*-butanol extracts afforded kaempferol-3-β-D-glucoside and kaempferol-3-sophoroside.[27] The young inflorescence yielded (±) - vasicinone.[28,29] Flowers in addition yielded a fat containing traces of tridecanoic acid, pentadecanoic acid and a new glucoside identified as 2', 4-dihyroxychalcone-4-glucoside.[30] The fixed oil obtained from the seeds contained arachidic(3.1 percent), behenic (11.2 percent), lignoceric (10.7 percent), cerotic (5.0 percent), oleic (49.9 percent) and linoleic (12.3 percent) acids. The unsaponifiable matter yielded β-sitosterol.[31] The structure of vasicinol isolated from the roots was established as 6-hydroxypeganine.[32] The mass fragmentation of the alkaloids vasicine, vasicinol as well as its methyl ether was also studied.[33] The other alkaloids isolated were 9-acetamido-3,4-dihydropyrido-(3,4-b)-indole, *O*-ethyl-α-D-galactoside,1,2,3,9-tetrahydropyrrolo(2,1-b) quinazolin-9 (1H) –one, sitosterol-β-D-glucoside, D-galactose and deoxyvasicinone;[34] vasicol (1,2,3,4,9, 11-hexahydropyrrolo(2,1-b) quinazolin-3,11-diol, vasicinone, adhatonine and vasicinolone.[35] Vasicinolone was reported as the oxidative product of vasicinol.[36] The total and reducing sugars present in the root and the bark were estimated.[37]

PHARMACOLOGICAL INVESTIGATION

Although many pharmacological studies have been performed on the basis of chemical constituents present, a lot more are still to be exploited, explored and utilized. Important pharmacological findings are summarized below:

Bronchodilator activity

Vasicinone isolated from the leaves has a bronchodilator action on the normal lungs and powerful bronchodilator action against the histamine-induced bronchoconstriction in guinea pig's lungs and tracheal chain. *l* –Vasicinone was, however, stronger in action than *dl*-form.[38] Vasicine showed bronchodilator activity in both *in vivo* and *in vitro* experimental studies, its activity being comparable to theophylline.[39]

Antitussive activity

The antitussive activity of *Adhatoda zeylanica* extract was evaluated in anaesthetized guinea pigs and rabbits and in unanaesthetized guinea pigs. On oral administration to the guinea pigs, the antitussive activity of the extract was similar to codeine against coughing induced by irritant aerosols.[40]

Hypoglycemic activity

The non-nitrogenous principle obtained from the leaves as suspension administrated orally (25 mg/kg) to fasting male rabbits lowered the blood sugar of rabbits for a short period of two hours. The average fall over a period of four hours was 7.5 percent which was far less than the fall due to similar dosage of tolbutamide.[41] The ethanolic extract of the leaves exhibited hypoglycemic activity in rats.[42] The effect of the two unani drugs, Arusa (leaves of *Adhatoda zeylanica*) and Kalongi (seeds of *Nigella sativa*) was studied in induced diabetic rabbits. Both the extracts were compared for hypoglycemic effect with a standard drug glibenclamide. Diabetes was observed in

rabbits (fasting blood glucose level ranged from 200–250 mg/100 ml) within 24 hrs after injection of alloxan and divided into four groups i.e. diabetic control (distilled water), diabetic standard (glibenclamide), diabetic test (Arusa) and diabetic test (Kalongi). The test drugs were administered to the treated group, while the vehicle was administered to the animals of control group, orally. Blood glucose was estimated by the end point *o*-toluidine. The study revealed that the aqueous extract of Arusa and Kalongi in the dose of 100 mg/kg and 200 mg/kg, respectively given orally reduced the blood glucose level in induced diabetic rabbits. The significant reduction (P less than 0.05) in blood glucose level started after 3 hrs which continued for 6 hrs in both the groups.[43]

Platelet activity

On repeated oral and intramuscular administration of vasicine hydrochloride resulted in a dose related increased in platelet count in normal rats, mice,rabbits and dogs. This increase in platelets was also associated with significant hyperplasia of megakaryocytes in the bone marrow. The findings revealed that vasicine could hold promise for control of capillary haemorrhages and for correction of drug- induced bone marrow depression.[44]

Antitumour activity

The alcoholic extract of the leaves did not show antitumour activity against various transplantable tumours.[45]

Wound healing activity

The activity of alcoholic extract of leaves was studies for its wound healing effect in buffalo calves. A significant increase in rate of healing, breaking strength, tensile strength, energy absorption and extensibility was observed. The treated wounds revealed significantly higher values of these parameters in comparison to control.[46,47]

Enzyme activity

The decoction of the leaves of the plant activated the trypsin enzyme in *in vitro* studies, which in turn stimulated the digestive process.[48]

Antimicrobial activity

The alcoholic extracts of the leaves and root showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The water extract of the leaves also showed activity against *Staphylococcus aureus.*[49–52] Aqueous and ethanolic extracts from various parts of seven plants were screened against three gram - positive and five gram - negative bacteria. Of the twenty - four extracts assayed by agar well diffusion method, ethanolic extract from the shoots of *Adhatoda zeylanica* was the most active, exhibiting greatest inhibitory activity against multi- drug resistant *E. coli*.[53]

Antiviral activity

The crude extract of the leaf and bark showed 20–39.9 percent inhibition of potato virus X and 85 percent inhibition against bean common mosaic virus.[54,55]

Insecticidal activity

The powder of the leaves was a non-poisonous indigenous insecticide and recommended for controlling the insect pests for stored seeds.[56]

Antifeedant and toxic activity

The effect of crude methanolic extracts of *Adhatoda zeylanica* leaves on the feeding and performance of *spodoptera littoralis* larvae was investigated in the laboratory. The extract exhibited strong antifeedant and toxic activity against the larvae when applied either on leaf discs or incorporated into artificial diet.[57]

Radiomodulatory influence

The radiomodulatory influence of ethanolic extract of *Adhatoda zeylanica* leaf extract against radiation - induced hematological alterations in peripheral blood of Swiss albino mice was studied at various post - irradiation intervals between 6 h to 30 days.[58]

Immunostimulant activity

Vasaka exhibited marginal increase in the WBC count to the extent of 16%. Vasaka showed statistically significant protective effect against cyclophosphamide induced myelosuppression to an extent of 80%.[59] Effect of alcoholic extract of plant leaf (500 mg/kg, p .o.) on haematological profile, splenic lymphocytes and peritoneal macrophages was studied 5, 10, 15 and 20 days after treatment in swiss albino mice. Its effect on macrophages phagocytic index, *E.coli* induced abdominal peritonitis and SRBC induced delayed type hypersensitivity was also evaluated. Plant showed significant increase in total WBC, blood lymphocytes, splenic lymphocytes and peritoneal macrophages. It also showed significant protection against *E. coli* induced abdominal peritonitis.[60]

Uterine activity

Vasicine showed uterotonic activity in human myometrium strip and abortifacient activity in guinea pigs.[61]

Effect on Chikungunya

The symptoms and signs of *Chikungunya* were studied among 500 patients and the methods to fight the disease with traditional Siddha medicines are described. The patients were administered doses of decoction prepared with the powder of various plants including decoction prepared with *Adhatoda zeylanica* leaves. In the first 12 hrs, pain was reduced but fever persisted. In the next three hrs, pain was reduced further and fever came down by 2°C. In next 6 hrs normality was attained. Out of 500 patients treated, 450 got cured of the diseases.[62]

Traditional Uses

The plant is pungent, bitter, acrid, cooling, useful in bronchitis, leprosy, blood impurities, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, constipation, jaundice, tumors, and diseases of the mouth.[5] The root is diurectic, useful in bronchitis, asthma, bilious vomiting, sore eyes, fevers, gonorrhoea. The leaves are emmenagogues, useful in gonorrhea. The flowers improve the circulation of the blood. The fruit is useful in bronchitis.[63] The leaves and the roots of this plant are considered a very efficacious remedy for all sorts of coughs, being administered along with ginger.[64] The leaves are also used for rheumatism. The flower, leaves, and root but especially the first, are supposed to possess antispasmodic qualities. The flowers and the fruit are bitter, aromatic and antispasmodic.[65] The fresh flowers are used in ophthalmia.[66] The powdered root is used in Mysore by native doctors in cases of malarial fever. It has expectorant and antispasmodic properties and its use has been recommended in the treatment of cold, cough, asthma and even diphtheria.[67] Fresh juice of leaves with honey relieves the irritable cough by its soothing action on the nerves and by liquefying the sputum which makes expectoration easier.[68] Both the decoction and powder form constitutes of many Ayurvedic preparations for infection of the respiratory tract.[69] Plant is one of the ingredients of the preparations known as Vasavaleha (Dabur), Kasamrit Herbal (Baidyanath) and Vasaka capsule (Himalaya Drug Company). The plant is recommended for snake bite. Daily application of massage of leaf extracts effectively reduces the inflammatory and bleeding condition of gums.[70]

TOXICOLOGICAL STUDIES

The allergenic pollen grains of the plant were common during the months of October and November in Pondicherry.[71] A pilot survey of air borne pollen grains as well as clinical survey of pollen allergy conducted at Kolhapur during monsoon season revealed that only two patients with asthma/rhinitis gave a significant positive reaction on skin testing.[72] In another survey at Nagpur, 7 of the 50 patients treated at the allergy and chest clinic, Jasleen Hospital, Nagpur showed allergy due to the pollens of *Adhatoda zeylanica*.[73]

ETHNOBOTANICAL STUDIES

An ethnobotanical exploration was carried out during 2001–2002 in village Barali Kass and its allied areas, revealed that 47 species were used as folk medicine for curing of several diseases. The data was collected from the local peoples including reliable hakims, physicians and old villagers. Plant species with their botanical names, local names, status and folk medicinal uses have been collected and enlisted. Some of the medicinal plants used from the study area includes *Adhatoda vasica, Phoenix sylvestris, Plumbago zeylanica, Clematis grata, Adiantum caudatum*.[74]

CONCLUSION

Adhatoda zeylanica is highly regarded as an universal panacea in the Ayurvedic medicine because it is extensively used for treating cold, cough, whooping-cough and chronic bronchitis and asthma, as sedative-expectorant, antispasmodic and as anthelmintic. However, well controlled double-blind clinical trials are lacking. It is one of the most versatile plants having a wide spectrum of medicinal activities. This medicinal plant is the unique source of various type of compounds chief principle is a quinazoline alkaloid, vasicine accompanied by vasicinone, vasicinol, vasicinine, vasicoline and adhatodine. Very little work has been done on the medicinal applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from *Adhatoda zeylanica*. Although crude extracts from leaves of *Adhatoda zeylanica* have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity, proper standardization and well controlled double-blinding clinical trials. As the global scenario is changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from *Adhatoda zeylanica* should be emphasized for the control of various diseases. Therefore it is necessary to exploit its maximum potential in the field of medicinal and pharmaceutical science for novel and fruitful application.

REFERENCES

1. Khan IA and Khanum A. *Medicinal and aromatic plants of India*, Ukaaz publications, first edition, 2005; p. 9.
2. Sarin YK. *Illustrated manual of herbal drugs used in Ayurveda. Council of Scientific and Industrial Research and Indian Council of Medical Research*. 1996; p. 178–179.
3. Anonymous, *The wealth of India*-Raw Materials, Vol-I, Publication and Information Directorate, CSIR, New Delhi, 2002; p. 76–79.
4. Trease and Evans. *Pharmacognosy*, Saunders Elsevier, 16th edition, 2002; p. 36.
5. Kirtikar KR and Basu BD. *Indian Medicinal Plants*, International Book Distributors, Book seller and Publisher. 2005; Vol-III: p.1899.
6. Jain MP and Sharma VK. Phytochemical investigation of roots of *Adhatoda vasica*. *J. Med. Plant. Res*. 1982; 46: 250–255.
7. Dhar KL, Jain NP, Kaul SK and Atal CK, Vasicol. A new alkaloid from *Adhatoda vasica*. *Phytochemistry*. 1981; 20: 319–321.
8. Chaudhary A, Poi R, Chaitaly Das, Sanyal N, Biswas J and Bhattacharya A. '*Modern techniques for analysis of the chemical actives and quality parameters of some promising medicinal herbs to interface the prospects of some commercial cultivation in different agroclimatic regions of West Bengal and possible exploration for value addition*. 2005; 24(1): 95.
9. Sharma DN, Khosa RL and Joshi VK. Content and seasonal variation of Alkaloids of Indian vasaka from Varanasi. *Indian Drugs*. 1989; 25(5): 328.
10. Bahrtiya HP and Gupta PC. A chalcone glycoside from the flowers of *Adhatoda vasica*. *Phytochemistry*. 1982; 21(1): 247.
11. Singh RS, Mishra TN, Pandey HS and Singh BP. Aliphatic hydroxyl ketones from *Adhatoda vasica*. *Phytochemistry*. 1991; 30(11): p. 3799–3801.
12. Joshi BS, Newton MG, Lee DW, Barber AD and Pelletie SW. *Reversal of absolute stereochemistry of pyrolo quinazoline alkaloids vasicine, vasicinone, vasicinol and vasicinolone*. Elsevier science Ltd. 1996; 7(1): p. 25–28.
13. Inamdar MC, Khorana ML, and Rajarama Rao MR. Pharmacology of a non nitrogenous neutral principle isolated from the leaves of *Adhatoda vasica*. *Planta Med*. 1996; 13: 194–199.
14. Poi R and Adityachaudhary N. Occurrence of (+)-vasicinone in *Adhatoda vasica* Nees. *J Indian Chem. Soc.* 1988; 65: 814.
15. Chowdhary BK and Bhattacharyya. A further quinazoline alkaloid from *Adhatoda vasica*. *Phytochemistry.* 24: 1985; 3080–3082.
16. Thappa RK, Agarwal SG, Dhar KL, Gupta VK, and Goswami KN. Two pyrroloquinazolines from *Adhatoda vasica*. *Phytochemistry.* 1996; 42**:** 1485–1488.
17. Sharma A, Lal K and Handa SS. Herbal drug standardization: HPLC determination of vasicine in polyherbal formulations. *Int J Pharmacog.* 1992; 30: 205–208.
18. Narayana DBA, Agarwal S, Luthra SK and Srinivas NS. A HPTLC method for the quantitative analysis of vasicine in *Adhatoda vasica*. *Indian Drugs.* 1995; 32: 583–586.
19. Sharma RL, Gupta RK, Chowdhary BK, Dhar KL and Atal CK. Synthesis of some vasicine analogues. *Indian J Chem*. 1979 18B: 449–450.
20. Siddiqui AR, Khan MI, Khan MJ. Estimation of vasicine and vasicinone in Ayurvedic formulations containing *Adhatoda vasica* Nees leaf extract using HPLC technique. *Hamard Medicus.* 2008; 51(2): 116 –119.
21. Daniel M and Sabnis SD. Chemosystematics of some Indian members of the Acanthaceae. *Proc Indian Acad Sci (Plant Sci)*. 1987; 97: 315–323.
22. Basu NM, Ray GK and De NK. Vitamin-C and carotene content of several herbs and flowers used in Ayurvedic system of medicine. *J Indian Chem Soc.* 1947; 24: 358–360.
23. Nair AGR, Nagarajan S and Sankara Subramanian S. Luteolin as a characteristic flavone of Acanthaceae. *Curr Sci*. 1965; 34: 79–80.
24. Patel MR, Agarwal YK and Raj KPS. Varying content of metals in four leafy drugs. *Sci Cult*. 1980; 46: 302–303.
25. Singh RS, Misra TN, Pandey HS and Singh BP. A new aliphatic alcohol from *Adhatoda vasica*. *Fitoterapia*. 1992; 63: 262–263.
26. Agarwal AK, Bansal AK, Upadhyaya JS, Ansari MN and Jain MC. Lignins of some non wood fibrous plants- Spectroscopic studies. *Chimica Acta Turcica*. 1993; 21: 297–301.
27. Rangaswami S and Seshadri R. Crystalline chemical components of the flower of *Adhatoda vasica*. *Curr Sci*. 1971; 60: 84–85.
28. Choudhary MK and Chakrabati P. Chemical investigation of *Adhatoda vasica* Nees. *Indian Agric.* 1977; 21: 225–226.
29. Choudhary MK. Chemical investigation of *Adhatoda vasica* Nees. *Naturwissenschaften*. 1979; 66: 205.
30. Bhartiya HP and Gupta PC. A chalcone glycoside from the flowers of *Adhatoda vasica*. *Phytochemistry.* 1982; 21: 247.
31. Handa KL, Ishwar Chandra and Vasudev. Examination of the fixed oil of *Adhatoda vasica* seeds. *J Sci Ind Res.* 1956; 15B: 612–613.
32. Bhatnagar AK, Bhattacharji S and Popli SP. On the identity of vasicicol. *Indian J Chem*. 1965; 3: 525.
33. Bhatnagar AK and Popli SP. Mass fragmentation of the alkaloids of *Adhatoda vasica* Nees. *Indian J Chem*. 1966; 4: 291–292.
34. Jain MP, Kaul SK, Dhar KL and Atal CK. Novel nor-harmal alkaloid from *Adhatoda vasica*. *Phytochemistry.* 1980; 19: 1880–1882.
35. Dhar KL, Jain MP, Kaul SK and Atal CK. Vasicol, a new alkaloid from *Adhatoda vasica*. *Phytochemistry.* 1981; 20: 319–321.
36. Jain MP and Sharma VK. Phytochemical investigation of roots of *Adhatoda vasica*. *Planta Med*. 1982; 46: 250.
37. Saraf S, Tyagi S, Ojha AC and Rawat GS. Phytochemical study of some medicinal plant. *Asian J Chem*. 1995; 7: 229–231.
38. Amin AH, Mehta DR and Samarth SS. *In: Proceedings, First International Pharmacological Meeting.* Stockholm, Pergamon Press Ltd., Oxford. 1963; 7: 377.
39. Gupta OP, Sharma ML, Ghatak BL and Atal CK. Pharmacological investigation of vasicine and vasicinone–the alkaloids of *Adhatoda vasica*, *Indian J Med Res,* 1977; 66: 680–691.
40. Dhuley JN. Antitussive effect of *Adhatoda vasica* extract on mechanical or chemical stimulation induced coughing in animals. *J Ethnopharmacol,* 1999; 67: 361–365.
41. Modak AT and Rajaramarao MR. Hypoglycemic activity of non–nitrogenous principle from the leaves of *Adhatoda vasica* Ness. *Indian J Pharm*. 1966; 28: 105–106.
42. Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN and Ray C. Screening of Indian Plants for biological activity Part I. *Indian J Exp Bio.* 1968; 6: 232–247.
43. Zainab K, Rehman SZ, Latif TA, Jahan N. Hypoglycemic activity of Barg-e-Arusa and Tukhm-e-Kalonji on alloxan-induced diabetes mellitus. *Hamdard Medicus*. 2008; 51(1): 77-81.

44. Atal CK, Sharma ML, Khajuria A and Kaul A. Thrombopoietic activity of vasicine hydrochloride. *Indian J Exp Bio.* 1982; 20: 704–709.
45. Indap MA, Ambaye RY and Bhat ML. Antitumour evaluation of some indigenous plants. *Indian Drugs.* 1986; 23: 477.
46. Bhargava ML, Singh H and Kumar A. Evaluation of *Adhatoda vasica* as a wound healing agent in buffaloes–clinical, mechanical and biochemical studies. *Indian Vet J.* 1988; 65: 33–38.
47. Zama MS, Singh HP and Kumar A. Comparative study on *Adhatoda vasica* and pancreatic tissue extract on wound healing in buffaloes. *Indian Vet J.* 1991; 68: 864– 866.
48. Vijaya S and Vasudevan TN. The effect of some medicinal plants on activity of digestive enzymes, *Indian Drugs,* 1994; 31: 215–217.
49. George ML, Venkataraman PR and Pandalai KM. Investigations on plant antibiotics. Part II. A search for antibiotic substances in some Indian medicinal plants. *J Sci Ind Res* 1947; 76 (Suppl): 107–114.
50. Kurup PA. Studies on plant antibiotics. Screening of some medicinal plants. *J Sci Ind Res.* 1956; 15C: 153–154.
51. Joshi CG and Magar NG. Antibiotic activity of some Indian medicinal plants. *J Sci Ind Res.* 1952; 11B: 261–263.
52. Thomas E, Shanmugam J and Rafi M. *In vitro* antibacterial activity of certain medicinal plants of Kerala. *Biomedicine.* 1999; 19: 185–190.
53. Aneja KR and Gupta KK. Therapeutic evaluation of certain Indian drug plants. *Hamdard medicus*. 2007; 50(2): 145–155.
54. Singh R and Singh R. Screening of some extracts for antiviral properties. *Technology* (Sindri). 1972; 9: 415–416.
55. Tripathi RN, Tripathi RKR and Pandey DK. Assay of antiviral activity in the crude leaf sap of some plants. *Environ Indian*. 1981; 4: 86–87.
56. Srivastava AS, Saxena HP and Singh DR. *Adhatoda vasica,* a promising insecticide against pests of storage. *Labdeb J Sci Technol*. 1965; 3: 138–139.
57. Sadek MM. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against spodopetra littaralis (Lep., Noctuidae). *J Appl Ent*. 2005; 127: 396–404.
58. Kumar A, Ram J, Samarth RM and Kumar M. Modulatory influence of *Adhatoda vasica* Nees leaf extract against gamma irradiation in Swiss albino mice. *Phytomed*. 2005; 12: 20.
59. Jinyavarghese K, Karpe ST and Kulkarni SR. Immunostimulant activity of *Adhatoda vasica*. *Indian Drugs.* 2005; 42(6): 345–352.
60. Thakur SR. Immunomodulatory potential of *Adhatoda vasica, Asian journal of Microbiology, Biotechnology and Environmental Sciences*. 2007; 9(3): 553–557.
61. Gupta OP, Wakhloo RL, Sharma ML and Atal CK. Vasicine–a potent uterine stimulant. Studies on human myometrium. *J Obstet Gyeaecol India*. 1979; 29: 935–938.
62. Viswanathan MV, Raja DK, Khanna SD. Siddha way to cure Chikungunya, *Indian Journal of Traditional Knowledge*. 2008; 7(2): 345–346.
63. Jarald EE and Jarald SE. *Textbook of Pharmacognosy and Phytochemistry*, CBS Publishers and Distributors. 2009, p. 29.
64. Chopra RN, Nayar SL and Chopra IC. *Glossary of Indian Medicinal Plants*, National Institute of Science communication (Council of Science and Industrial Research New Delhi). 1999; p. 7.
65. Heinrich M, Barnes J, Gibbons S and Williamson EM. *Fundamentals of Pharmacognosy and Phytochemistry*, Churchill Livingstone, 2008, p.49.
66. Nandkarni KM and Nandkarni AK. *Indian Materia Medica*, Vol-I, Bombay Popular Parkashan. 2000; p.40.
67. Shah B and Seth AK. *Textbook of Pharmacognosy and Phytochemistry*, Elsevire, 2010: p. 525.
68. Ansari SH. *Essential of Pharmacognosy*, Birla Publication. 2006; p.726.
69. *Indian Herbal Pharmacopoeia*, Indian Drug Manufacturers Association, Mumbai. 2002; p. 29.
70. Khare CP. *Indian Medicinal Plants*, An Illustrated Dictionary, Springer International. 2007; p. 18.
71. Saha JC and Kalyanasundaram S. Studies on pollen allergy in Pondicherry. *Indian J Med Res.* 1965; 50: 193–198.
72. Chaubal PD and Gadve SB. Study of pollen allergy in Kolhapur during monsoon. *Indian J Chest Dis Allied Sci*. 1984; 26: 38–40.
73. Arbat A and Patil GV. Common allergenic pollen and spores causing respiratory allergy from capital. *J Soc Pure Appl Nat Sci*. 1985; 1: 5–7.
74. Rehman EU. Valuable medicinal plants of village Barali Kass and its adjacent areas, Kotli Azad Kashmir Pakistan. *J Phytopharmcothera Nat Pro.* 2008; 1(1): 73–78.