Phytochemical Screening and Pharmacological evaluation of the modified Stem of *Achras sapota* linn.

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ABSTRACT

Achras Sapota Linn belonging of Sapindaceae family and can be widely found in the world. In the present study the phytochemical study is to be carried out on the methanolic extraction of the modified stem of *Achras Sapota Linn* by standard method. The principal constituents of *Achras Sapota Linn* include alkaloid, steroid, flavonoid, saponin, reducing sugar, tannin, amino acid, protein, anthraquinone glycoside, deoxy sugar, phenolic compound. The main biological activity was found as anti-oxidant.

Keywords: Standardization, Quality tests, Traditional medicine, Physicochemical parameters

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INTRODUCTION

Achras Sapota Linn is belonging of Sapindaceae family (1). Generally these plants are cultivated more or less throughout the India; a native of S. America. Traditionally these plants are used (1). On that occasion we have analyzed the primary phytochemical constituencies of these plants. Phytochemicals analyses need fresh plants. The leaves and flower of the Achras Sapota Linn plunged in to boiling alcohol of its collection. The isolation and purification of plant constituents is mainly carried out using thin layer chromatography (TLC) as for nature of the substance. In the plant tissue culture Alkaloid, flavonoide, saponine, tannin, protein, phenolic compound are present in large amount.

MATERIAL AND METHOD

Plant material

The leaves and flower of Achras Sapota Linn ware collected from Sabang - Paschim Midnapore- West Bengal and the collection time is June at the hot and humid climate. The leaves ware dried in shade, so far Homogenize for 5 minutes and passed through 40 mesh sieve to get fine powder.

Preparation of extracts

Coarsely dry powder of leaves and flower of the plant ware extracted with methanol. The whole extract ware collected in the conical flask. Than after evaporate the extract under 40°C as a result get a dry mass. Place the dry mass in vacuum desiccators or do freeze drying to get a complete powder.

Qualitative Phytochemical analysis

The Phytochemical group test of the modified stem of the plants was performed by the standard methods with the methanolic extract.

Nitric oxide scavenging activity

Nitric oxide scavenging activity of the modified stem of *Achras sapota Linn*. was measured by the spectrophotometric method (an in-vitro evaluation). Sodium nitroprusside (5mM) in phosphate-buffered saline

was mixed with a control without the test compound, but with an equivalent amount of methanol. Test solutions at different concentrations (5–150 mcg/ml) were dissolved in methanol and incubated at 25°C for 30 min. After 30 min, 0.5ml of the incubated solution was removed and diluted with 0.5ml Griess reagent. The absorbance of the chromophore formed during the diazotization of the nitrite with sulphanilamide and the subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm.

Free radical scavenging activity

Free radical scavenging activity is to be carried on the modified stem of *Achras sapota Linn.* (an in-vitro evaluation). The free radical scavenging activity of the methanolic extract of the modified stem of *Achras sapota Linn.*was measured by 1,1-diphenyl- 2-picryl- hydrazil (DPPH) using the method of Blois (1958). 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of MEAS solution in water in different concentration (5–250mcg/ml). After 30 minutes, absorbance was measured at 517nm. Lower absorbance of reaction mixture indicated higher free radical scavenging activity.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity generally performed on the modified stem of modified stem of *Achras sapota Linn.* (an in vitro evaluation). The ability of MEAS to scavenge Hydrogen peroxide was determined according to the method of Ruch etal., (1989). A solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer Ph-7.4. Hydrogen peroxide concentration was determined spectrophotometrically measuring absorbance with extinction coefficient for hydrogen peroxide of 81 M(1)cm(-1). Extract 2–10 mcg/ml in distilled water were added to a hydrogen peroxide solution (0.6ml,40Mm). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against blank solution containing the phosphate buffer without hydrogen peroxide.

RESULTS AND DISCUSSION

Macro-morphology

A large handsome tree with rough dark grey modified stem and dense crown. Leaves oblong–lanceolate and elliptic-oblong, 7.5–12.5 cm., obtuse or subacute, shining both sides with numerous very fine inconspicuous secondary nerves and petiole 1.3–2 cm. long. Flowers are long pedicelled. Fruits globose, 3.8–5 cm. diam., usually with 5 large black shinning seeds, pink flesh and brownish epicarp. Fruits when ripe are delicious and are eaten.

Qualitative Phytochemical analysis

Various plant constituents present in the modified stem of *Achras sapota Linn.* are identified by qualitative test. The present study revealed that the presence of the medicinally active constituents in the modified stem of *Achras sapota Linn.* as summarized in the *table 1*. Alkaloid, flavonoid, saponin, tannine, Amino acid, protein and phenolic compounds ware present in the methanol extract (3–6).

Nitric oxide scavenging activity

The scavenging of the nitric oxide by *Achras sapota Linn.* was concentration dependent and the data are given in the *table 2*. There was a moderate inhibition of nitric

Table 1: Qualitative analysis of the modified stem of *Achras sapota*

Chemical constituents	Methanol extract	Methanol-water extract
Alkaloid	Present	Present
Steroid	Absent	Absent
Flavonoid	Present	Present
Saponin	Present	Present
Reducing sugar	Absent	Absent
Tannin	Present	Present
Amino acid	Present	Present
Protein	Present	Present
Anthraquinone glycoside	Absent	Absent
Deoxy sugar	Absent	Absent
Phenolic compound	Present	Present

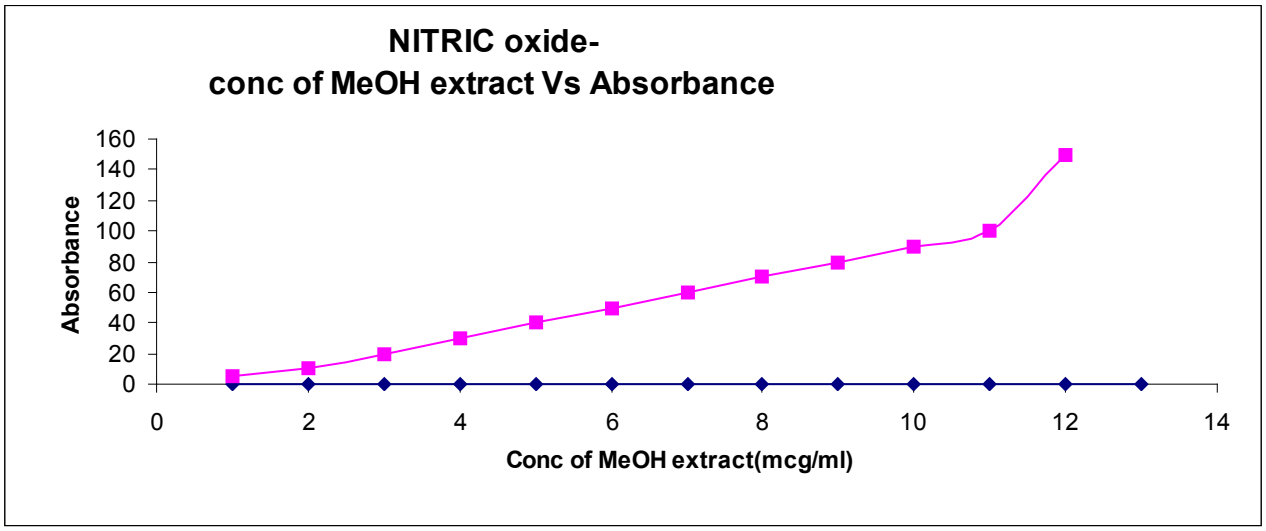

Figure 1. Nitric oxide scavenging activity of modified stem of *Achras sapota* - concentration vs. absorbance response

Table 2: Nitric oxide scavenging activity of modified stem of Achras sapota

Concentration(mcg/ml)	Set 1	Set 2	Set 3	Mean abs	% inhibition
0	0.268	0.271	0.263	0.267333	0
5	0.234	0.245	0.247	0.242	9.486891
10	0.227	0.229	0.224	0.226667	15.22971
20	0.223	0.225	0.22	0.222667	16.72784
30	0.22	0.225	0.219	0.221333	17.22722
40	0.217	0.22	0.217	0.218	18.47566
50	0.216	0.204	0.206	0.208667	21.97129
60	0.193	0.203	0.197	0.197667	26.09114
70	0.175	0.187	0.195	0.185667	30.58552
80	0.158	0.185	0.155	0.166	37.95131
90	0.15	0.161	0.149	0.153333	42.69538
100	0.147	0.158	0.146	0.150333	43.81898
150	0.129	0.133	0.131	0.131	51.05993

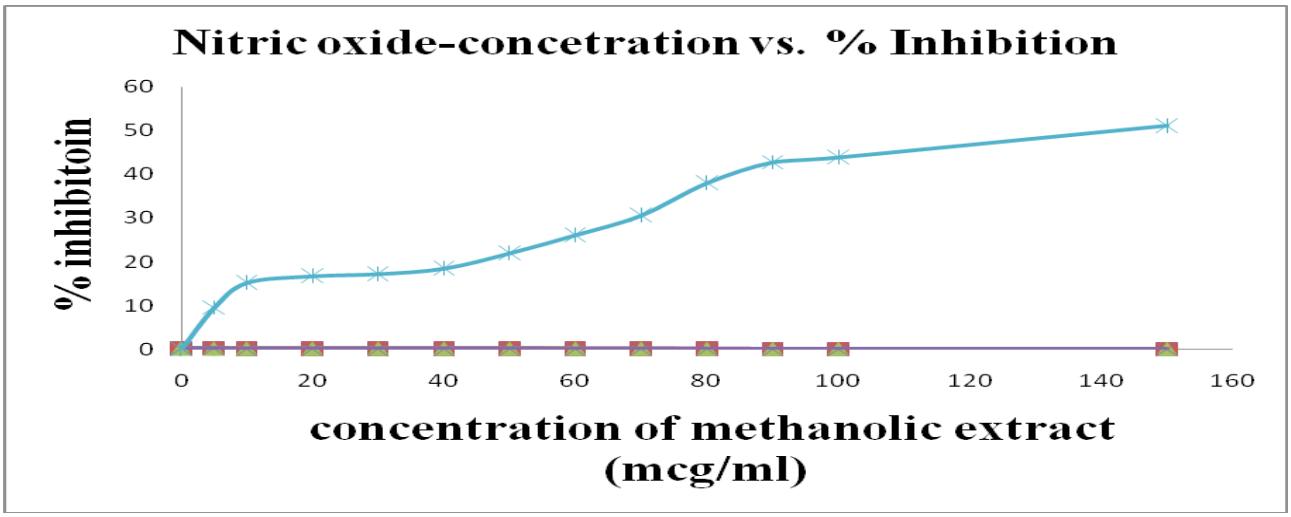

Figure 2. Nitric oxide scavenging activity of modified stem of *Achras sapota* - concentration vs. absorbance response

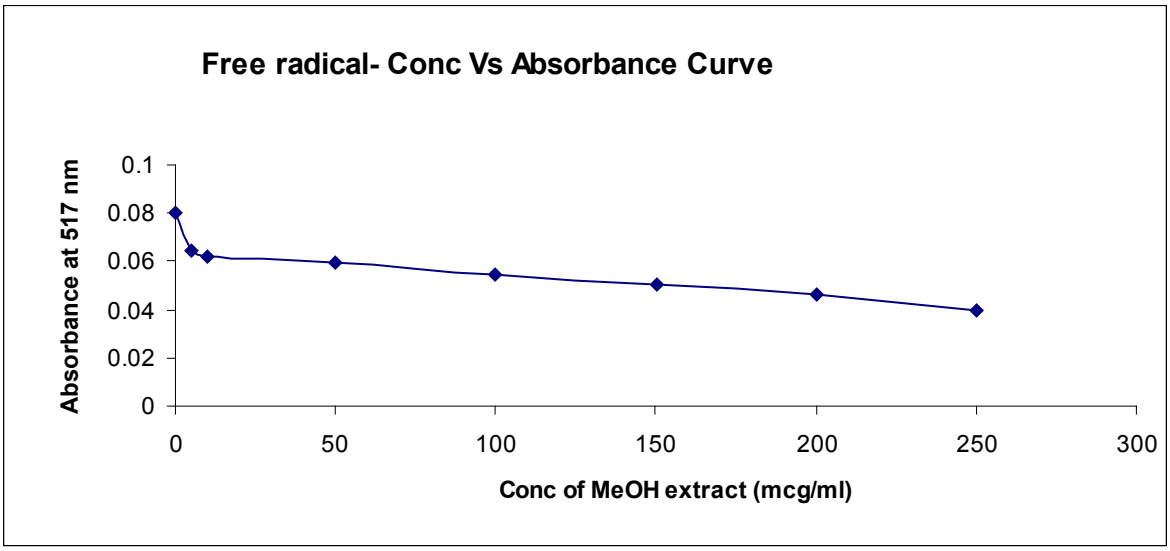

Figure 3. Free radical scavenging activity of the modified stem of *Achras sapota* - absorbance vs. concentration curve

Table 3: Free radical scavenging activity of the modified stem of *Achras sapota*

Concentration (mcg/ml)	1st set	2nd set	3rd set	Mean absorbance	% inhibition
0	0.098	0.068	0.074	0.08	0
5	0.064	0.065	0.064	0.064333	19.58333
10	0.064	0.062	0.061	0.062333	22.08333
50	0.061	0.058	0.06	0.059667	25.41667
100	0.057	0.052	0.055	0.054667	31.66667
150	0.052	0.048	0.051	0.050333	37.08333
200	0.051	0.039	0.05	0.046667	41.66667
250	0.043	0.034	0.041	0.039333	50.83333

oxide formation, with the IC50 at 140mcg/ml (*Figure 1–2*). Nitric oxide was generated from sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with the oxygen, leading to reduce production of nitric oxide. Significant scavenging activity was observed for *Achras sapota Linn.*

Free radical scavenging activity

The scavenging of free radical by the methanolic extract of the modified stem of *Achras sapota Linn.* was concentration dependent and data is given in the *table 3*. Here was moderate inhibition of free radical, with IC50 244mcg/ml (*Figure 3–4*). The present study reveals that the modified stem of *Achras sapota Linn.* has potent antioxidant activity against DPPH induced free radicals.

Reactive oxygen species are continuously formed in cells as consequence of oxidative biochemical reaction and external factors. However, they become harmful when they are produced in excess under certain abnormal conditions such as inflammation, ischemia and in the presence of iron ions. Under these conditions the endogenous antioxidants may be unable to counteract ROS formation. ROS formed may cause cellular damage and these damage may involve in etiology of diverse human diseases. Exogenous antioxidant supplement is helpful to overcome this severe problem of free radicals, which may scavenge these free radicals.

Hydrogen peroxide scavenging activity

The scavenging of the hydrogen peroxide by *Achras sapota Linn.* was Concentration dependent and data are given in the *table 4*. There was a moderate inhibition of hydrogen peroxide formation, with the IC50 at 9mcg/ml

Table 4: Hydrogen peroxide scavenging activity of the modified stem of *Achras sapota*

Concentration (mcg/ml)	1st set	2nd set	3rd set	Mean absorbance	% Inhibition
0	0.045	0.051	0.061	0.052	0
2	0.034	0.039	0.052	0.041667	19.87179
4	0.027	0.024	0.029	0.026667	36
6	0.02	0.012	0.018	0.016667	37.5
8	0.016	0.006	0.01	0.010667	36
10	0.003	0.001	0.005	0.003	71.875

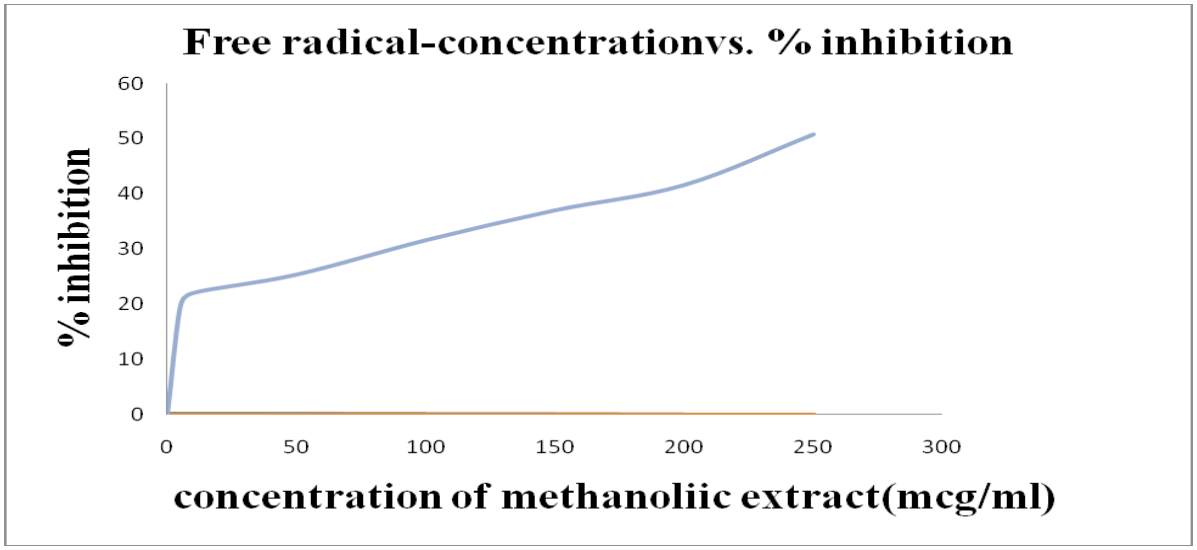

Figure 4. Free radical scavenging activity of the modified stem of *Achras sapota* - % Inhibition vs. concentration curve

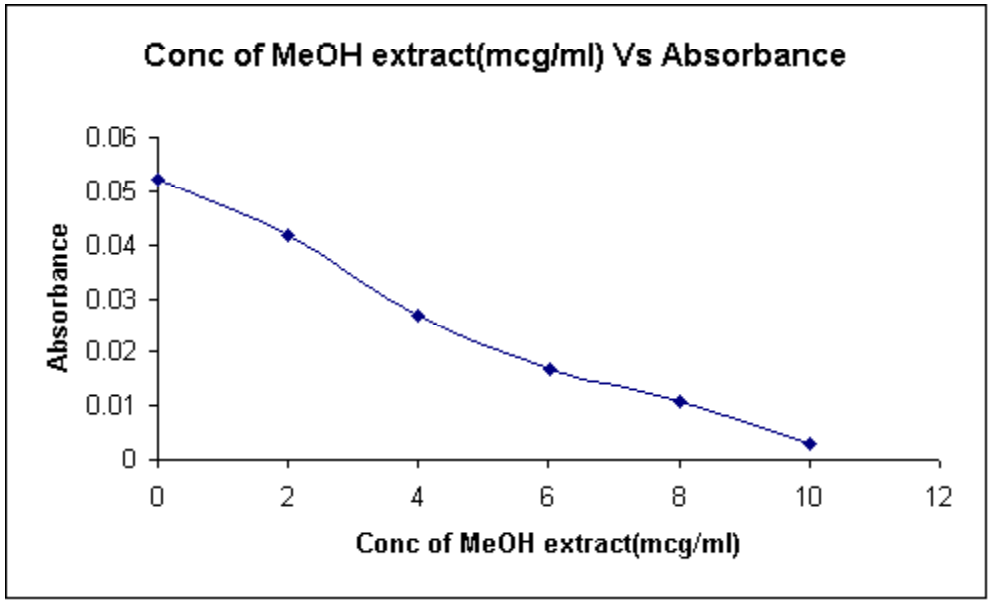

Figure 5. Hydrogen peroxide scavenging activity of the modified stem of *Achras sapota* - Absorbance vs. concentration curve

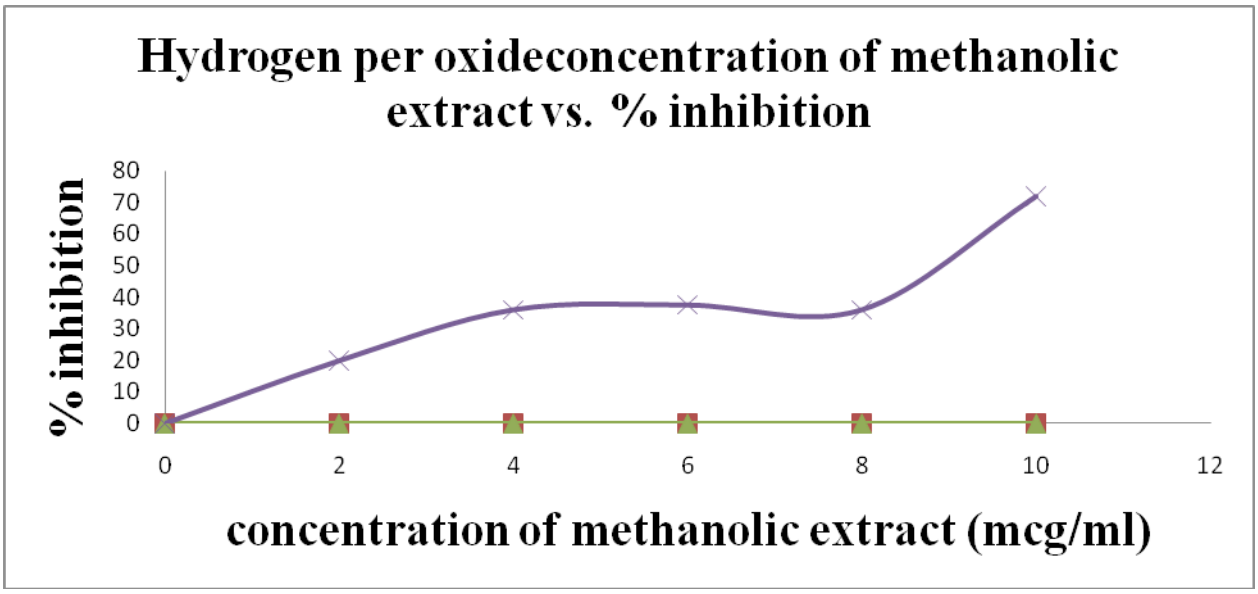

Figure 6. Hydrogen peroxide scavenging activity of the modified stem of *Achras sapota* - % inhibition vs. concentration curve

(*Figure 5–6*). The potentially reactive hydrogen peroxide radicals can cause oxidative damage to DNA, lipids and proteins. The effect of MeOH extract on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron dependent DNA damage assay, which showed significant results.

CONCLUSION

In the present study, preliminary qualitative phytochemical tests revealed the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds saponins, proteins and amino acids in the methanolic extracts of the modified stem of *Achras sapota Linn.*

In vitro experiments have shown that, the methanolic extract of the modified stem of *Achras sapota Linn*. possess NITRIC OXIDE SCAVENGING ACTIVITY, FREE RADICAL SCAVENGING ACTIVITY, HYDROGEN PEROXIDE SCAVENGING ACTIVITY may be of due to the presence of flavonoids and phenolic compounds.

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In Vitro Antioxidant Activity Of Methanolic Extract Of Aerial Parts Of *Salvia splendens* (Scarlet Sage)

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ABSTRACT

In vitro antioxidant activity of methanolic extract of aerial parts of *Salvia splendens* was determined by DPPH free radical scavenging, hydrogen peroxide scavenging and superoxide anion scavenging assays. Ascorbic acid and butylated hydroxyl anisole were used as standard antioxidants for the analysis. All the analysis was made with the use of UV–Visible spectrophotometer (Schimadzu uv-vis 1700). The methanolic extract of aerial parts of *Salvia splendens* had shown very significant DPPH (1, 1, Diphenyl-2-picryl-hydrazyl) radical scavenging, hydrogen peroxide scavenging, and superoxide anion scavenging activity compared to standard antioxidants. The Ic_{50} values of methanolic extract in DPPH radical scavenging, hydrogen peroxide scavenging and superoxide anion scavenging assays are found to be 460 µg/ml, 358 µg/ml and 527µg /ml respectively. The results concluded that the methanolic extract have a potential source of antioxidants of natural origin.

Keywords: Antioxidant, Salvia splendens, DPPH, Hydrogen peroxide, superoxide anion.
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INTRODUCTION

Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radicals, which can form dangerous chain reactions. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves.

Antioxidants are substances when present at low concentrations compared with that of an oxidisable substrate that significantly delays or prevents oxidations of that substrate.

Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive oxygen species, which are linked to a variety of diseases including cancer, diabetes, shock, arthritis and acceleration of the ageing process.

Antioxidants may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non radical compounds, and chain–breaking to prevent continued hydrogen abstraction from substrates.

Several synthetic antioxidants eg, BHA (Butylated hydroxy anisole), BHT (Butylated hydroxy toluene) are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants especially anthocyanins, phenolics and flavonoids are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption.

The Lamiaceae (Labiatae) family comprises 200 genera and 3000 species. One of the largest genera of the family, *Salvia* L., is represented by over 900 species and some species of salvia have been cultivated worldwide for use in folk medicines and for culinary purposes. The plants are typically 30–150 cm tall, herbaceous or suffruticose, and perennial, rarely biennial, or annual, with attractive flowers in various colours.

The name *salvia* comes from the Latin word “salvare”, which means to heal. *Salvia* species have been used since ancient times for more than sixty different ailments ranging from aches to epilepsy, and mainly to treat colds,

bronchitis, tuberculosis, hemorrhage, and menstrual disorders.

The main secondary metabolite constituents of *Salvia species* are terpenoids and flavonoids. The aerial parts of these plants contain flavonoids, triterpenoids, and monoterpenes, particularly in the flowers and leaves, while diterpenoids are found mostly in the roots. However some American *Salvia* species contain diterpenoids in the aerial parts, and in certain *Salvia* species, triterpenoids and flavones are present in the roots.

Salvia has always been a greatly esteemed medicinal herb in view of its multifarious curative effects. Many compounds isolated from salvia extracts are associated with antiseptic, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, hypoglycemic, anti spasmodic, Cytotoxic and anti tumor activities.

In the present study DPPH free radicals scavenging activity, hydrogen peroxide scavenging activity and superoxide anion scavenging activity methods have been used to measure the antioxidant activity of the methanolic extract of the *Salvia splendens*.

MATERIALS AND METHODS

Plant material

The plant was collected from the horticulture, Palandu, RanchI and the plant was identified and authenticated by H.J Chowdary, joint director, botanical survey of India, botanical garden, howrah. The plant material was dried under shade, powdered and passed through 40 mesh sieve.

Preparation of the extract

The powdered plant material was extracted with methanol in a soxhlet apparatus for 72 hours. After extraction the solvent will be filtered and then evaporated under reduced pressure. The obtained crude methanolic extract (SSME) is used for phytochemical screening and In vitro antioxidant studies. Phytochemical screening and the preliminary chemical examination of the methanolic extract revealed the presence of anthocyanins, flavonoids, terpenoids, glycosides, reducing sugars.

DPPH (1, 1-Diphenyl-2-picryl- hydrazyl) free radical scavenging activity [5-13]

The free radical scavenging activity of the samples were measured by 1,1 diphenyl-2-picryl–hydrazyl (DPPH) using the method described by Shimada et al.

Briefly 0.1 mM solution of the DPPH in ethanol was prepared; 1ml of the solution was added to 3 ml of sample in methanol at different concentrations (25–500 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes.

Then the absorbance was measured at 517 nm by using a UV-visible Spectrophotometer (Schimadzu UV-Vis 1700). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the fallowing formula:

$$\text{DPPH scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control reaction and A_1 was absorbance in the presence of standard or sample.

SCAVENGING OF HYDROGEN PEROXIDE[5-13]

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of (Ruch et al, 1989). A solution of hydrogen peroxide (2mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorptivity 81 $(mol/l)^{-1}c.m^{-1}$. The samples of extract (25–500 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer with out hydrogen peroxide.

$$\%\text{scavenged } (H_2O_2) = [(A_0-A_1)/A_0] \times 100$$

A_0 = Absorbance of the control.

A_1 = Absorbance of the extract

Super oxide anion scavenging activity [5-13]

The Super oxide anion scavenging activity of extract was performed according to the method of (Nishimiki et al., 1972.) One ml of nitro blue tetrazolium (NBT) solution (156micro grams NBT in 100mM phosphate buffer, pH 7.4), 1 ml NADH solution (468 micro grams in 100mM phosphate buffer, pH 7.4) and 0.1mg of sample solution of extract in methanol were mixed. The reaction started by adding 100 micro grams of phenazine metho sulphate (PMS) solution to the mixture. The reaction mixture was incubated at 25 degree centigrade for 5 minutes and the absorbance at 560 nm was measured against blank samples.

Deceased absorbance of the reaction mixture indicates increased super oxide anion scavenging activity. L-ascorbic acid was used as a positive control.

The percentage inhibition of super oxide anion generation was calculated using the fallowing formula.

$$[(A_0-A_1)/A_0] \times 100$$

A_0= Absorbance of the control.

A_1= Absorbance of the extract

RESULT AND DISCUSSION

As shown in Table 1 the methanolic extract of *Salvia splendens* showed DPPH free radical scavenging activity between 25–500 µg/ml in a dose dependent manner (IC_{50}=460µg/ml). These results indicated that the tested extract had a notable effect on scavenging of DPPH free radical when compared with ascorbic acid and Butylated hydroxy anisole.

As shown in Table 2 the methanolic extract of *Salvia splendens* showed hydrogen peroxide scavenging activity between 25–500 µg/ml in a dose dependent manner (IC_{50}=358µg/ml). These results indicated that the tested extract had a notable effect on scavenging of hydrogen peroxide when compared with ascorbic acid and Butylated hydroxy anisole.

As shown in Table 3 the methanolic extract of *Salvia splendens* showed Superoxide anion scavenging activity between 25–500 µg/ml in a dose dependent manner (IC_{50}=527µg/ml). These results indicated that the tested extract had a notable effect on scavenging of Superoxide anion when compared with ascorbic acid and Butylated hydroxy anisole.

CONCLUSION

The Antioxidant and free radical scavenging activities of Methanolic extract of Salvia splendens might be due to the presence of high amounts of phenolic and flavonoid compounds.

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Table 1: Anti radical activity of *Salvia splendens* extracts observed with DPPH"

Sample	Concentration (µg/ml)	%Inhibition	IC_{50} (µg/ml)
Methanolic extract of Salvia splendens	25	8.38±1.22	
	50	17.88±3.50	
	75	27.15±0.66	
	100	34.43±0.76	
	250	46.35±2.32	
	500	50.99±1.01	460
Ascorbic acid	25	51.71±0.40	
	50	55.34±0.40	
	75	57.77±1.57	
	100	59.90±1.76	
	250	66.42±1.78	
	500	68.26±1.26	148.38
BHA	25	43.97±1.52	
	50	57.57±0.35	
	75	60.62±1.25	
	100	66.25±1.76	
	250	69.74±1.52	
	500	71.24±1.28	121

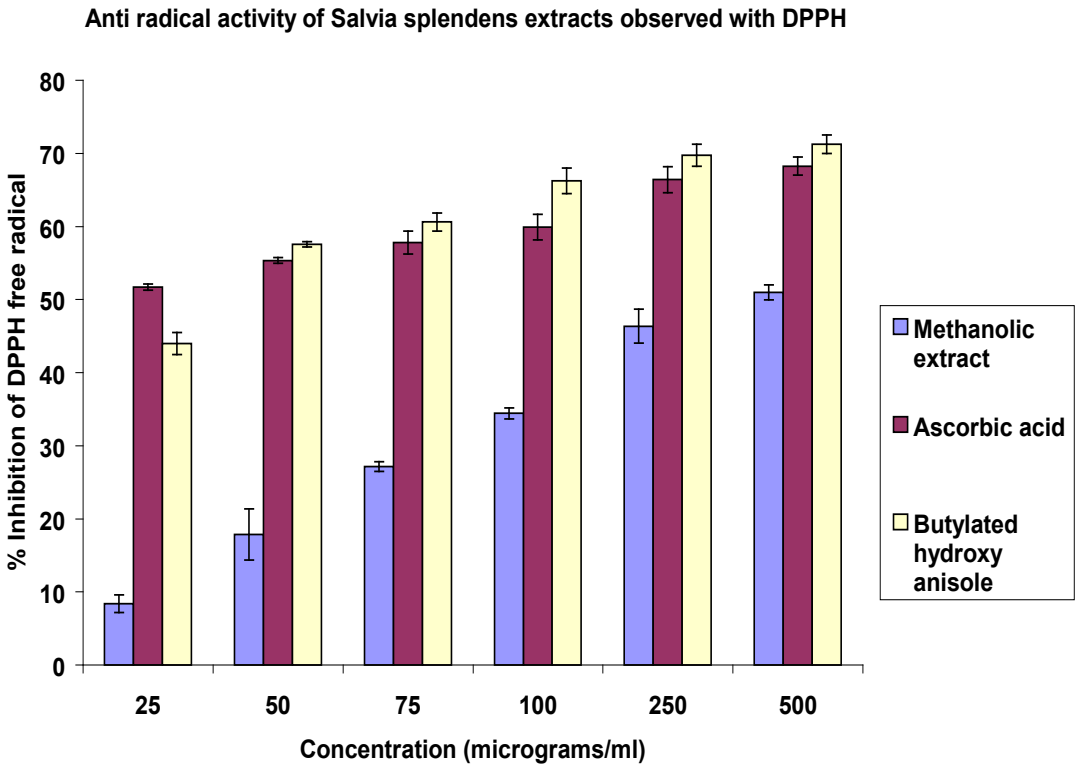

Figure 1: Antiradical activity of salvia splendens extract observed with DPPH

Table 2: Hydrogen peroxide scavenging activity of methanolic extract of Salvia splendens

Sample	Concentration (µg/ml)	%Inhibition	IC_{50}(µg/ml)
Methanolic extract of Salvia splendens	25	7.03±2.05	
	50	15.59±3.78	
	75	31.13±1.27	
	100	38.07±0.80	
	250	47.72±0.52	
	500	57.15±1.54	358
Ascorbic acid	25	13.78±0.06	
	50	24.42±0.69	
	75	35.96±0.18	
	100	49.16±0.18	
	250	56.42±0.87	
	500	58.84±0.37	299
BHA	25	15.24±2.24	
	50	18.28±0.48	
	75	37.68±0.38	
	100	48.64±0.26	
	250	58.72±0.77	
	500	62.64±0.27	278.42

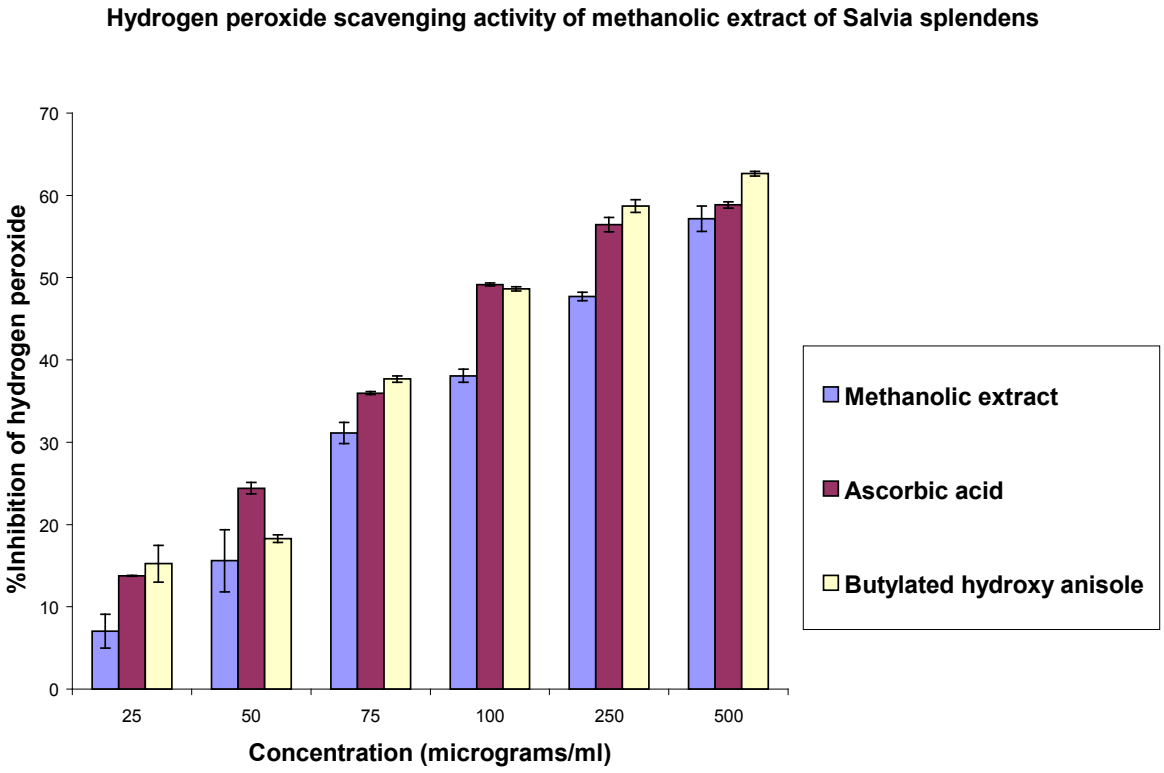

Figure 2: Hydrogen peroxide scavenging activity of methanolic extract of Salvia splendens

Table 3: Superoxide anion scavenging activity of methanolic extract of *Salvia splendens*.

Sample	Concentration (µg/ml)	%Inhibition	IC_{50}(µg/ml)
Methanolic extract of Saliva splendens	25	0.34±0.05	
	50	2.55±0.64	
	75	12.56±1.81	
	100	16.28±1.60	
	250	29.37±2.52	
	500	45.43±4.85	527
Ascorbic acid	25	10.24±1.24	
	50	15.82±0.68	
	75	18.26±1.78	
	100	28.48±0.25	
	250	70.16±2.34	
	500	78.82±0.29	258
BHA	25	15.62±0.42	
	50	19.84±0.86	
	75	26.42±1.28	
	100	52.58±1.89	
	250	82.20±0.28	
	500	88.68±1.42	192

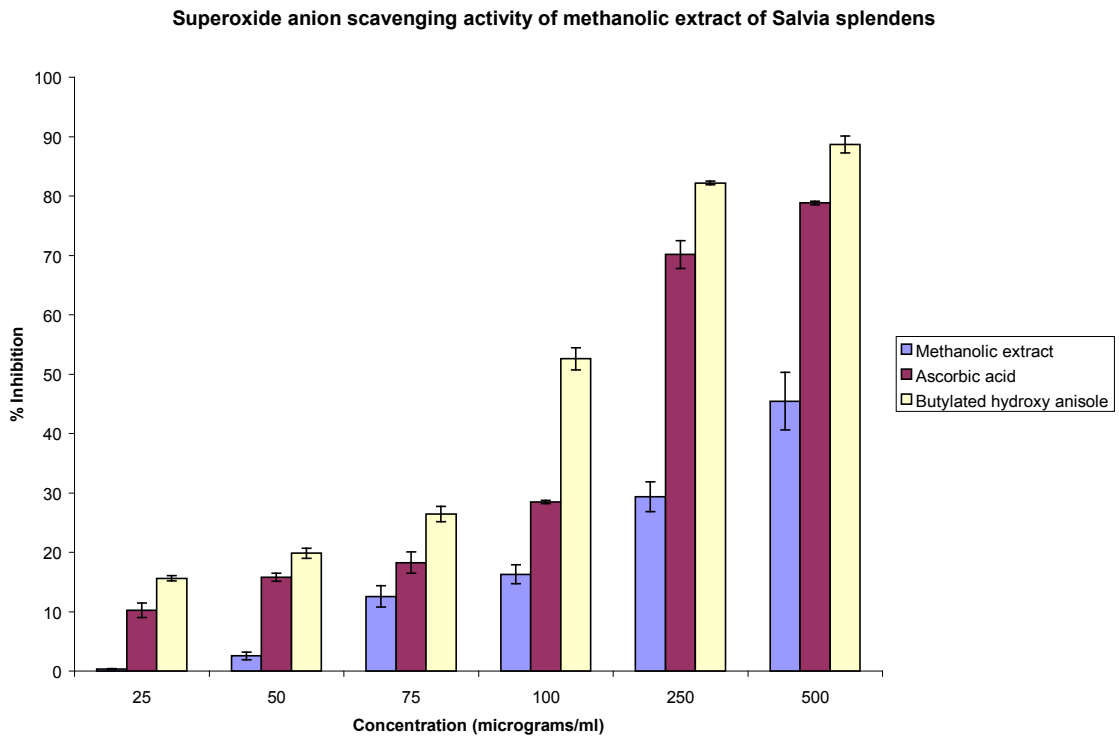

Figure 3: Superoxide anion scavenging activity of methanolic extract of Salvia splendens

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Pharmacognostical Standardisation of aerial parts of *Salvadora Persica*

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ABSTRACT

Salvadora persica L. (Salvadoraceae) is a subtropical tree, of medicinal interest, native to the Arabian Peninsula Egypt, and India. The stems and roots are widely used among Muslims as a toothbrush and to detoxify and strengthen the weakened gums. In the present study pharmacognostic studies of aerial parts of *Salvadora persica* were done. Physicochemical and preliminary phytochemical studies of aerial parts were also carried out.

Keywords: *Salvadora persica*, leaf, stem, petiole.
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INTRODUCTION

The toothbrush tree, *Salvadora persica* (other names *Galenia asiatica, Salvadora indica*), is a small more than one foot in diameter, bark scabrous and cracked, whitish with pendulous extremities. The root bark of the tree is light brown and the inner surfaces are white, odour is like cress and taste is warm and pungent. Its fibrous branches have been used as toothbrushes by Islamic communities–miswaks. It has been scientifically proven to be very useful in the prevention of tooth decay even when used without any other tooth cleaning means. *Salvadora persica* is composed of Trimethylamine, an alkaloid which may be salvadorine, chlorides, high amounts of fluoride and silica, sulphur, vitamin C, small amounts of tannins, saponins, flavonoids and sterols. (1–3)

MACROSCOPICAL CHARACTERS (5–6)

The plant grows in plains, especially abundant along the costal regions. It is a tree with pendulous branches. The leaves are simple, alternate, elliptic oblong, 3–5 cm long, 1.5–2.5 cm wide, thick and coriaceous, petiole upto 2cm wide.

Inflorescence–terminal or axially panicle calyx–four lobed, copular, corolla, - 4 petals gamopetalous, lobes recurved. Stamens- 4 epipetalous, filaments filiform ovary–4 lobed, unilocular, one ovuled. Fruit is a one–seeded drupe.

MATERIALS AND METHODS FOR ANATOMICAL STUDIES (7)

Collection of specimens

The plant specimens for the proposed study were collected from Madurai. It was identified and authenticated by Taxonomist Dr. Stephen, Madurai. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Aceticacid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary–butyl alcohol (8). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58–60 C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the of Rotary Microtome. The thickness of the sections was 10–12 um. Dewaxing of the sections was done by customary procedure (9). The sections were stained with

Figure 1. The Plant *Salvadora persica*

Synonyms (1–3):

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Brassicales
Family	:	Salvadoraceae
Genus	:	Salvadora
Species	:	*S. Persica* *S. wightiana Planchon ex* *S. indica Wight Thwaites* *S. Koenigii Arn*

Vernacular Names:

Common name	:	Miswak, Toothbrush tree
Hindi	:	Jhak, Kharjal
Telugu	:	Ghuma, Varagogu
Karnataka	:	Goni–Mara
Tamil	:	Kalawa, Karkol, Perungoli, Ughaiputtai

Ethnomedical Information (4)

Stem and Leaf	:	Antidote to poisons of all sorts.
Stem bark	:	Febrifuge, Emmenogogue.
Leaf	:	Antiscorbutic, Astringent, Antiasthmatic, Piles, In painful tumors.
Fruit	:	Lithnotriptic, Stomachic.
Seed	:	Purgative, Diuretic.
Root	:	Pain due to spleen troubles.
Leaf and fruit	:	Carminative, externally in rheumatism.

Toluidine blue (10) as per the method published by O'Brien et al. (1964)). The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and fast-green and IKI (for Starch)

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid were prepared (8).

MICROSCOPIC FEATURES OF LEAF

The leaf has fairly prominent midrib and thick, smooth and even lamina (Fig 2) Midrib is slightly raised on the adaxial side and projects into a shallow hump on the abaxial side. The midrib, region is 300cm thick. It has a single prominent vascular bundle surrounded by

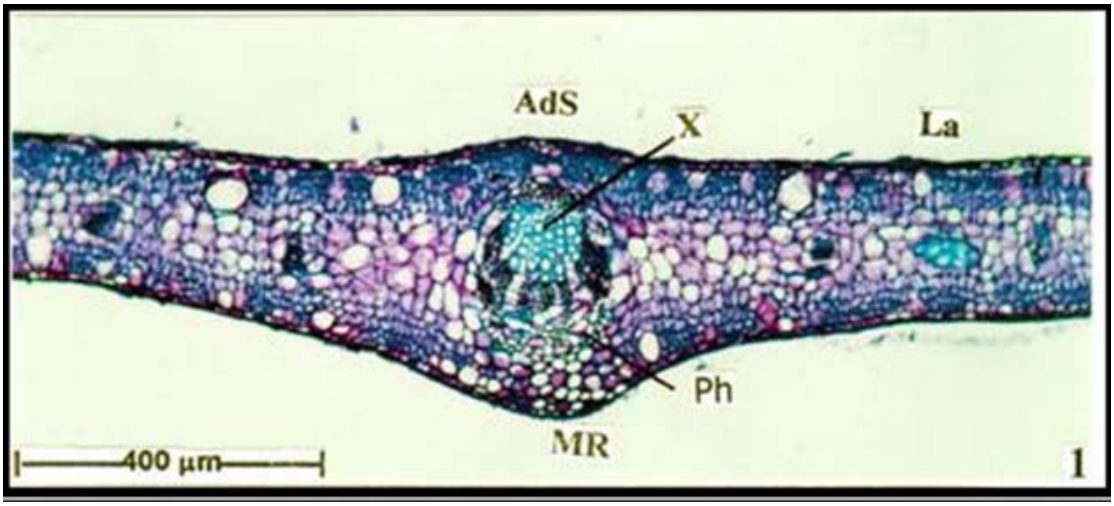

Figure 2. Anatomy of the leaf
[Ads–Adaxialside, X–Xylem, La–Lamina, Ph–Phloem, MR–Midrib)

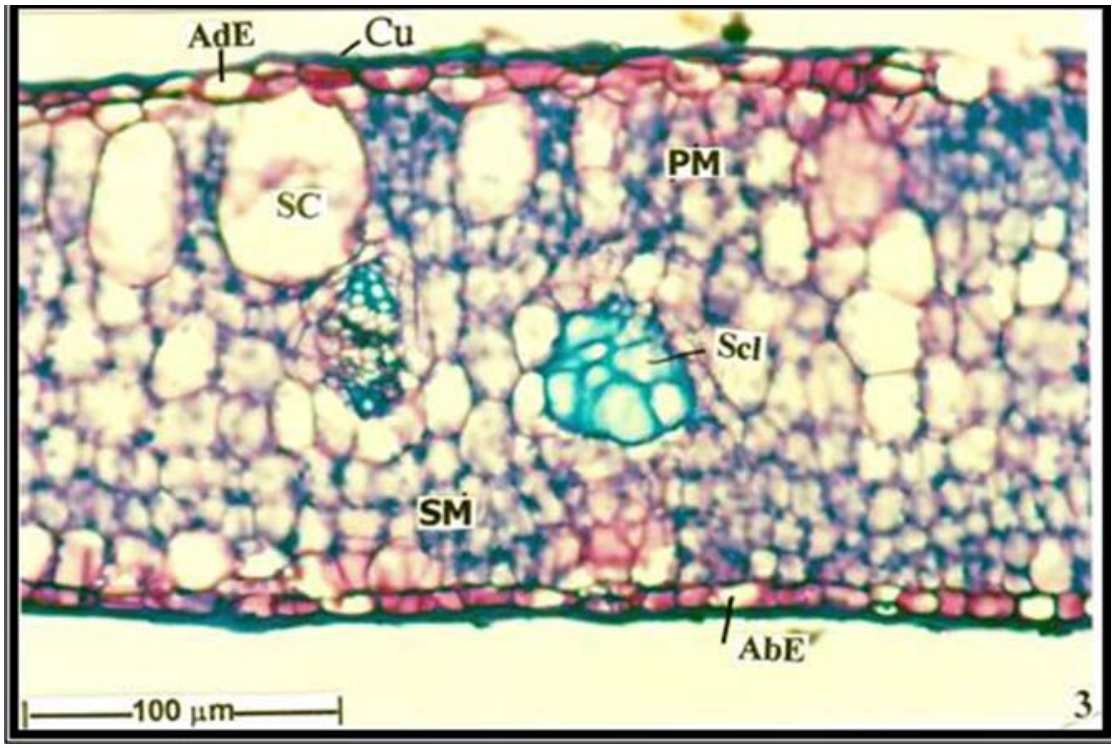

Figure 3. Anatomy of the lamina
[AdE–Adaxial epidermis, Cu–Cuticle, SC–Secretory Cavity, PM–Palisade mesophyll, Scl–Scleroid, SM–Spongy mesophyll, AbE–Abaxial epidermis]

parenchymatous ground tissue. The vascular bundle has about six radial parallel files of xylem elements and broad pad of phloem elements.

Lamina - (Fig 3)

The lamina was 160µm thick. It had thin epidermal layers of spindle shaped or tabular, thin walled cells with thick cuticle. The epidermal cells were less than 10µm thick. The mesophyll was not well differentiated into palisade and spongy parenchyma. It consists of several layers of vertically oblong, compact cells. A few adaxial layers of cells had dense chloroplasts and resemble the palisade cells. Along upper part of the mesophyll tissue, there were highly dilated, wide oblong cells. They did not contain any specific cell inclusions. (Fig 3) There were massive circular clusters of foliar scleroids distributed in the

median part of the mesophyll zone. The vascular bundles of the lateral veins are also placed in the median zone of the lamina. The vascular bundles are collateral and do not project beyond the leaf surfaces. They also do not possess the bundle sheath cells.

Venation (Fig 4)

The lateral veins and vein–branches are fairly thick and straight. They form wide and distinct vein–islets of polygonal outline. The islets are random in orientation. The vein–terminations are also distinct. They are long and slender or short and thick, simple or branched. The striking character of the veins is the presence of clusters of brachysclereids at the tips of the vein terminations. The terminal scleroids are polygonal, thick walled and wide–lumened. Some of the scleroids are also elongated and lobed.

Petiole (Fig 5)

The petiole is circular in cross–sectioned outline and measures 450µm thick at the base and 750µm at the apex. It has thin epidermal layer of small, thick walled and cuticularized epidermal cells. The ground tissue has 8–10 layers of circular to angular, thick walled, compact parenchyma cells. The vascular strand has deep are of xylem and phloem with a narrow, adaxial gap (Fig 5), or almost closed, excentric ring (Fig 5), xylem consists of circular thick walled, radial chains of elements. Phloem occurs in wide sheaths around the xylem. The vascular strand has discrete nests of sclerenchyma cells around its periphery.

Stem (Fig 6a)

Fairly old stem of 1.75mm thick exhibits thick secondary xylem cylinder with an anomalous feature which is known as included phloem or interxylany phloem. The stem has narrow, less prominent epidermal layer with thick cuticle. Cortical zone is wide having several layers of tangentially elongated parenchyma cells and thick, irregular, scattered masses sclerenchyma cells. Phloem zone is narrow and uniformly encircles the xylem cylinder. Secondary xylem is thick and continuous cylinder of 350µm width. It consists of circular, thin walled, radial multiples of two or three vessels, thick walled fibres, wide, straight xylem rays and tangentially stretched or circular masses of included or interxylanyphloem (Fig 6a). Primary Xylem is found around the inner boundary of the secondary xylem. Primary xylem consists of numerous radial files of metaxylem elements and obliterated protoxylem elements. Adjoining the primary xylem, there are wide

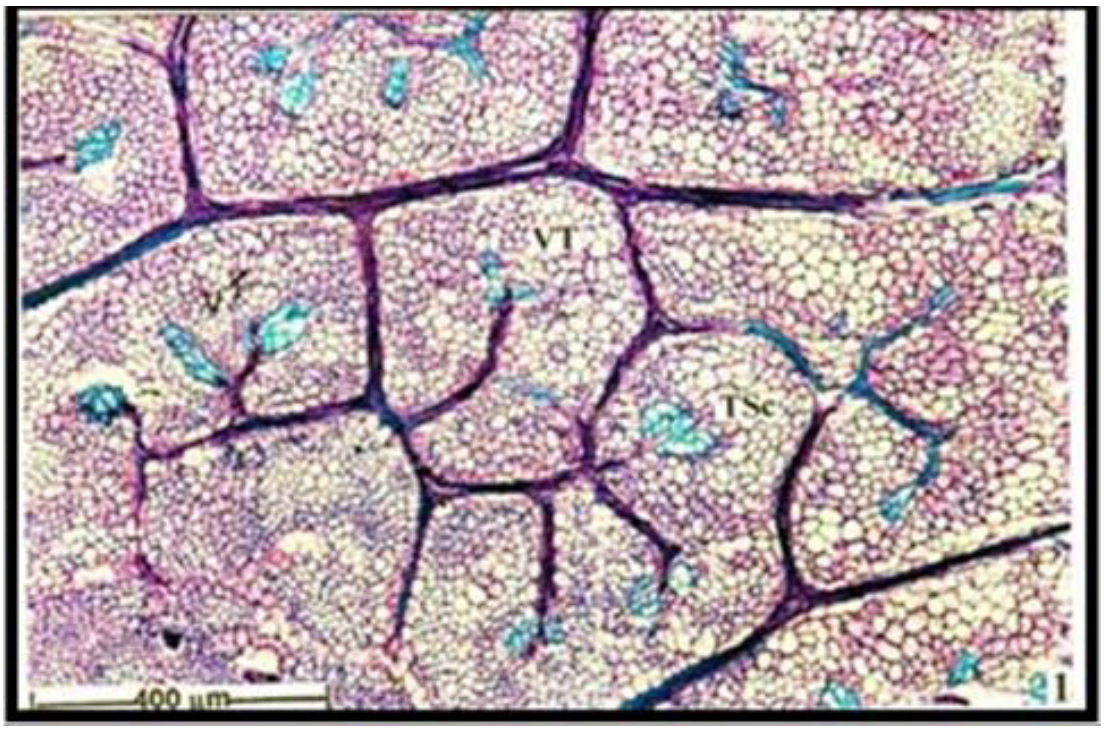

Figure 4. Venation
[VT–Vein–termination, TSc–Terminal Sclereid]

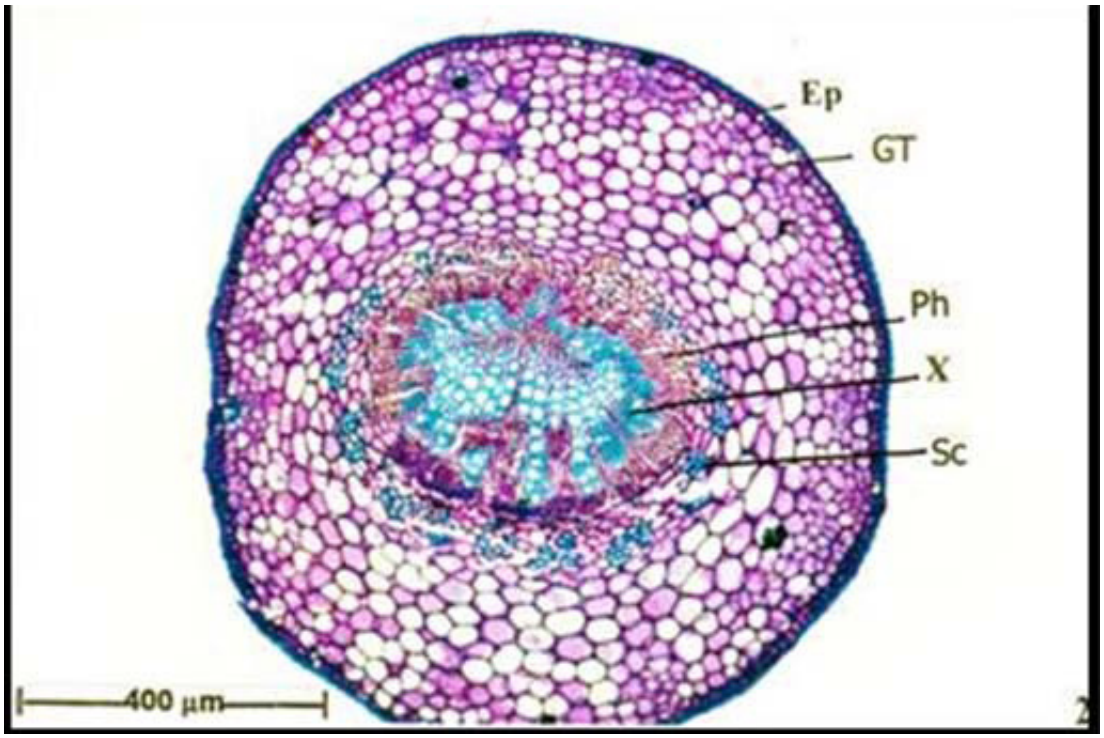

Figure 5. Anatomy of the petiole
[Ep–Epidermis, GT–Ground Tissue, Ph–Phloem, X–Xylem, Sc–Sclerenchyma]

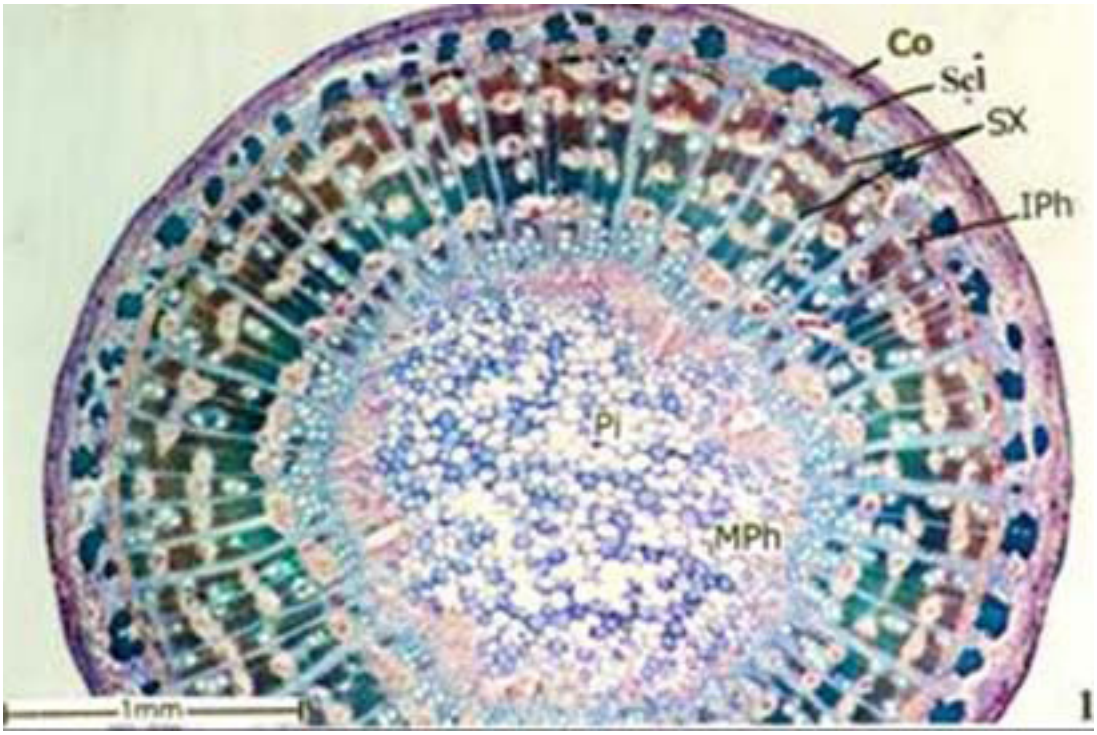

Figure 6a. Anatomy of the stem
Co–Cortex, Scl–Sclereid, SX–Secondary Xylem, IPh–Included Phloem, MPh–Medullary Phloem, Pi–Pith]

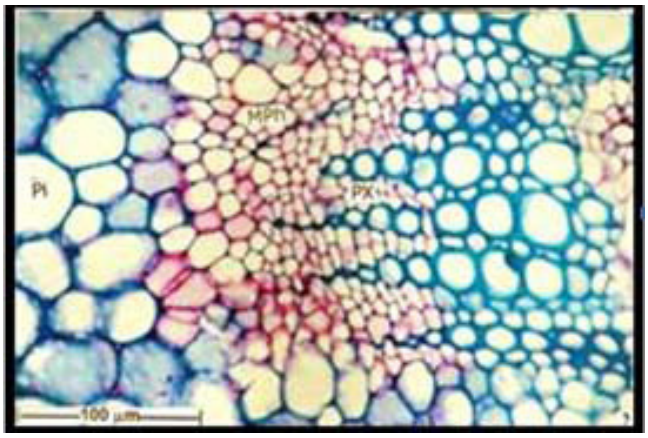

Figure 6b. Structure of the phloem
[MPh–Medullary Phloem, PX–Primary Xylem, Pi–Pith]

nests of pith–phloem or medullary phloem. The pith is wide and homogenous with uniform type of circular, compact, parenchyma cells (Fig 6b).

POWDER MICROSCOPIC OBSERVATIONS

The powder of aerial parts of *Salvadora persica* were macerated in jeffreys maceration fluid. (10%chromic acid+10%HNO_3 in equal volumes.). In the powdered preparation of, the following elements were observed.

Leaf powder
1. Epidermis

In the leaf powder small piece of abaxial and adaxial epidermis of the leaf were observed. Abaxial epidermis (Fig 7) was found to be densely stomatiferous. The stomata were cyclocytic type. A stoma was surrounded by two polar and two lateral subsidiary cells or five subsidiary cells surrounding all around.

The adaxial epidermis had sparse stomata which were also cyclocytic type (Fig 8). The epidermal cells were polygonal, random in orientation and had straight anticlinal walls.

Veins

The leaf powder also showed broken pieces of veins which were branched. At the tip of each branch there was a spherical cluster of brachyscleroids or stone–cells (Fig 9). The scleroids were also found to be scattered and detached from the veins. The scleroid-clusters were 100 × 60 µm in size. The individual scleroids were 20 × 30 µm in size.

Trichomes

Spherical subsessile glandular trichomes were other type of inclusion in the powder. The trichome (Fig. 10) as seen in surface view were circular, comprising of a few

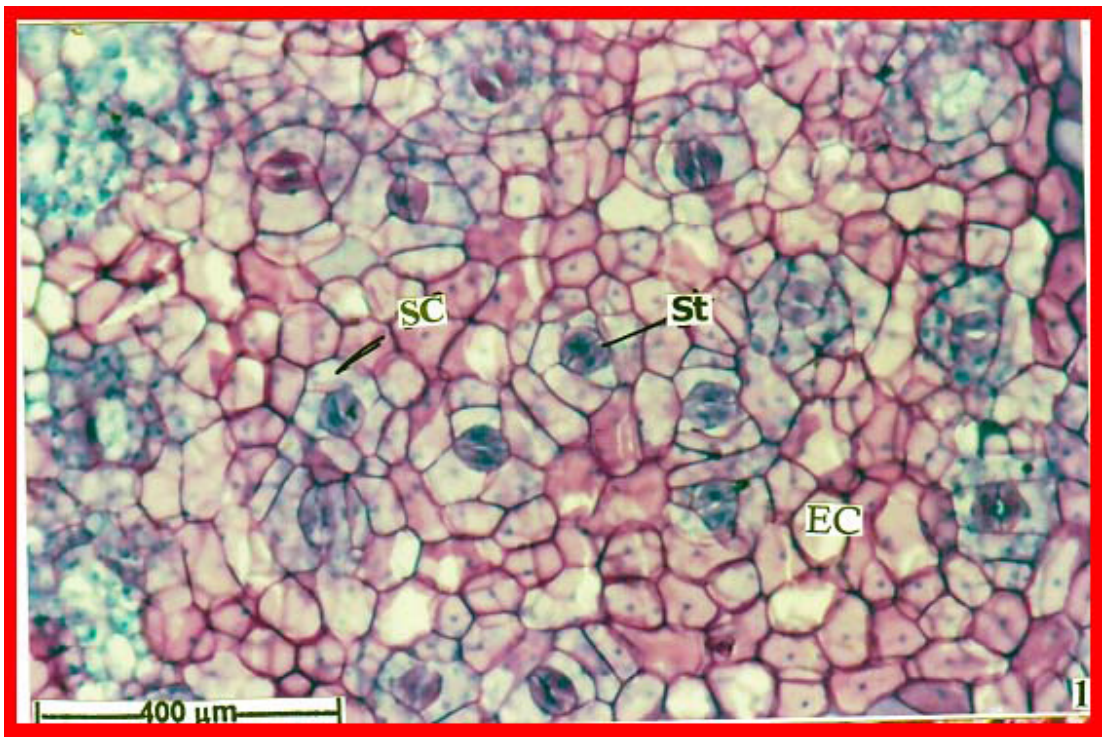

Figure.7. Abaxial epidermis.
SC-Subsidiary cells. St-Stomata. EC-Epidermal cells.

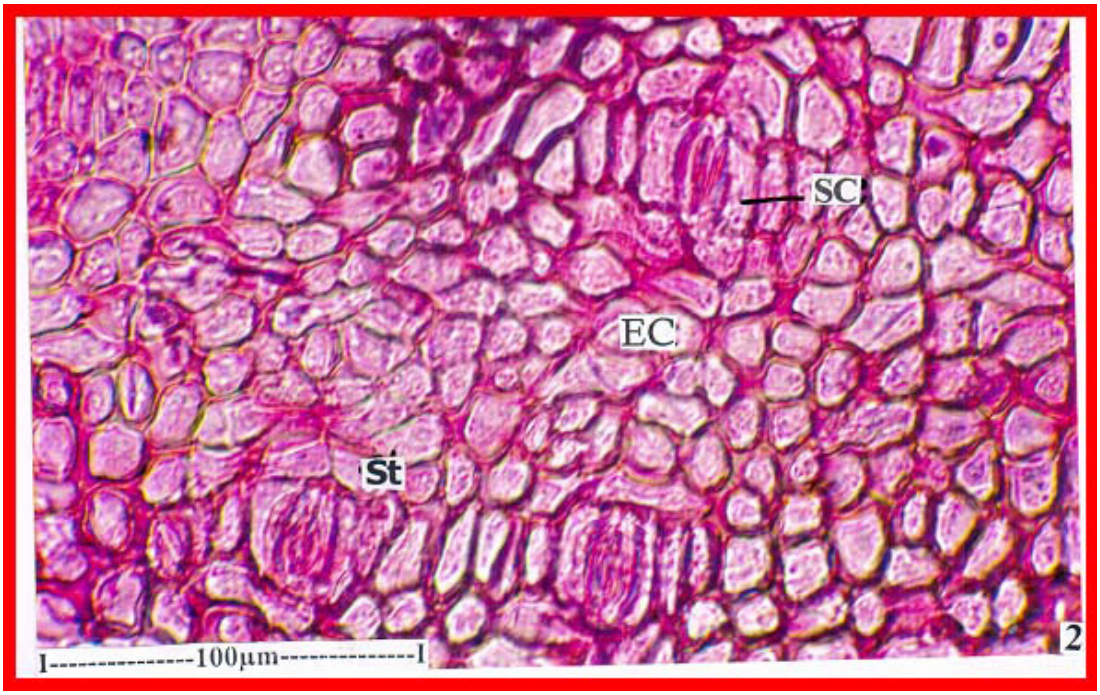

Figure.8. Adaxial epidermis.
St-Stomata. SC-Subsidiary cell EC-Epidermal cells.

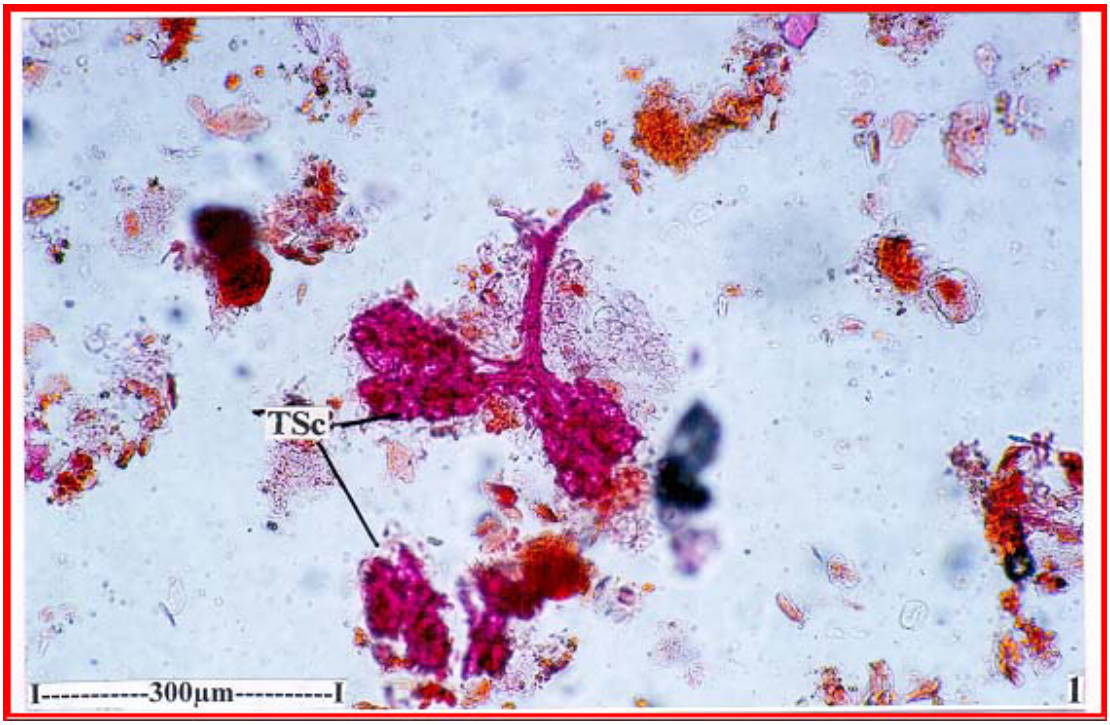

Figure.9. Broken veins. TSc-Terminal Scleroids.

Figure 10. Trichomes.
GTr-Glandular trichomes.

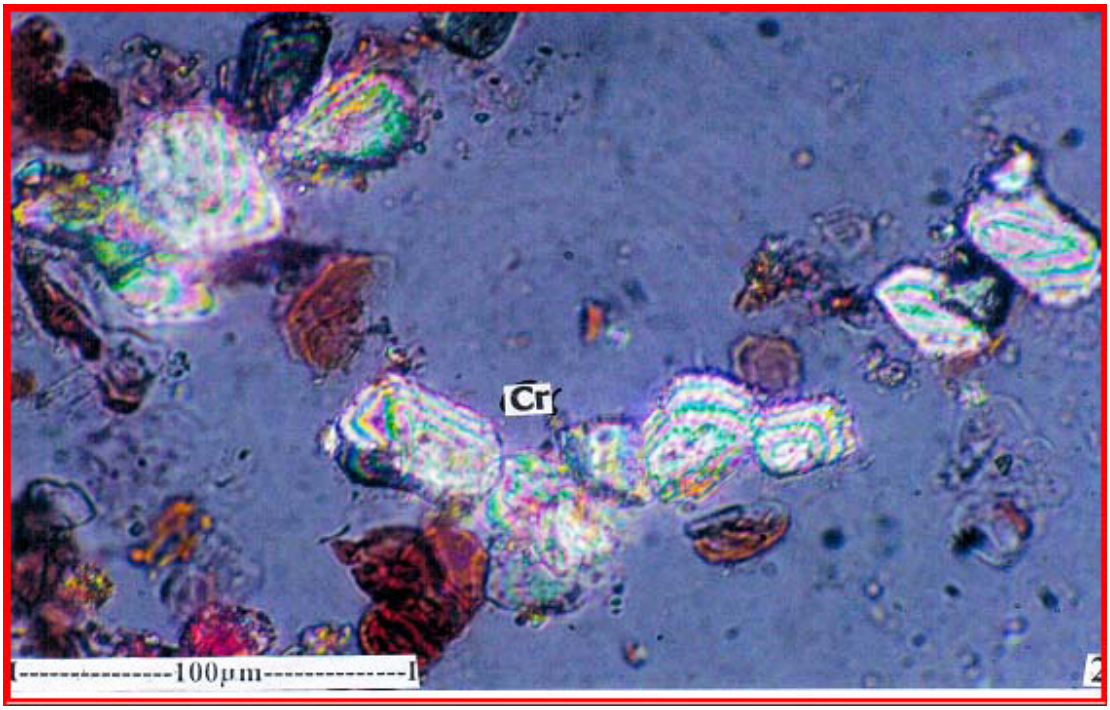

Figure 11. Calcium oxalate crystals. Cr-Crystals.

triangular, densely staining cells. The gland was 60–70 µm wide.

Crystals

When the powder was viewed under the polarized light microscope, thick prismatic crystals of calcium oxalate were seen as bright pieces. (Fig 11) The prismatic crystals were 20 × 40 µm in size.

Scleroids

Foliar scleroids were another characteristic feature of the powder. Short, thick and lobed scleroids or long, thin, unbranched filiform scleroids were observed in the powder (Fig 12.)

Stem Powder

The stem maceration showed different types of xylem elements. These elements include xylem fibres, xylem parenchyma and vessel elements.

Xylem fibres

Xylem fibers were long, narrow, thick walled and narrow lumened. (Fig 12.) They had arrow of slit-like pits. Their walls were lignified. They were 70–90µm long and 10µm wide.

XylemParenchyma

Xylem Parenchyma cells which were rectangular to squarish with thick walls were also seen in the powder. They were either solitary or in groups (Fig 13). They had simple pits.

Vessel Elements

They were cylinderical wide and thick walled (Fig 13). They had simple, wide, circular horizontal perforation plate or oblique perforation plate. The lateral pits were circular and dense. The vessel elements were 100–160µm long and 50µm wide.

PHYSICOCHEMICAL CONSTANTS

The physicochemical parameters are mainly used in judging the purity and quality of the drug. Ash values of a drug give an idea of the earthy matter or inorganic composition or other impurities present along with the drug. The ash values of the powdered leaves revealed a high percentage of sulphated ash. Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drug. The results suggest that the powdered drug have high percentage of ethanol soluble extractive value. The loss on drying reveals the percentage of moisture present in the drug. The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant materials and presented in Tables I, II and III.

PRELIMINARY PHYTOCHEMICAL SCREENING

The behavior of powder with various chemical reagents and preliminary chemical tests for extracts were also carried out according to the standard procedures described by Kokate and Horborne. The results are delineated in Table IV.

Table 1 Physicochemical constants of aerial parts of *Salvadora persica*

S.No.	Ash values	Percentage (% w/w)
1.	Total ash	9.8
2.	Water soluble ash	1.22
3.	Water insoluble ash	1.08
4.	Acid soluble ash	1.3
5.	Acid insoluble ash	0.6
6.	Sulphated ash	6.1
	Loss on drying	0.29

Table 2 Quantitative Microscopy of leaves of *Salvadora persica*.

Parameters		
Vein islet number		13.6
Vein terminal number		16.8
Stomatal number		
	Upper epidermis	41.9
	Lower epidermis	62
Stomatal index		
	Upper epidermis	21.79
	Lower epidermis	32.1
Palisade ratio		4.03

Table 3

Extractive values	
Ethanol soluble extractive	34%w/w
Water soluble extractive	22%w/w
Ether insoluble volatile	13%w/w
Ether insoluble non-volatile	30%w/w

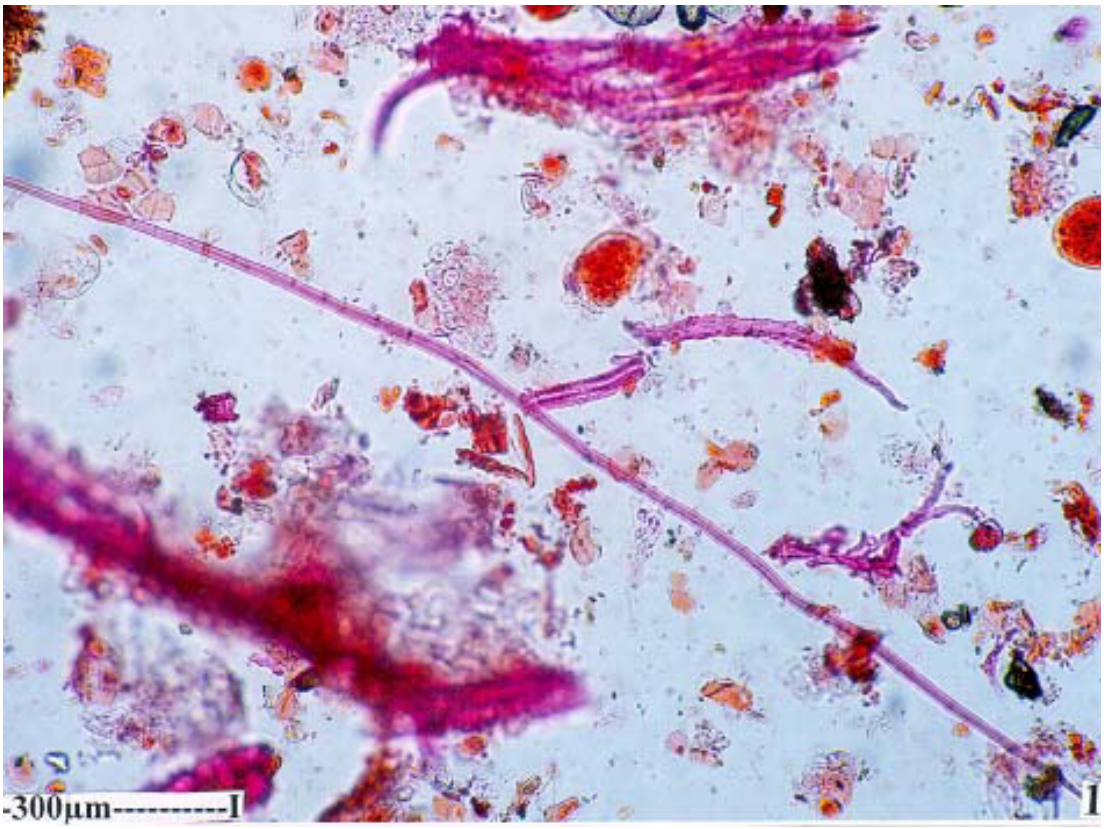

Figure.12. Foliar scleroids.

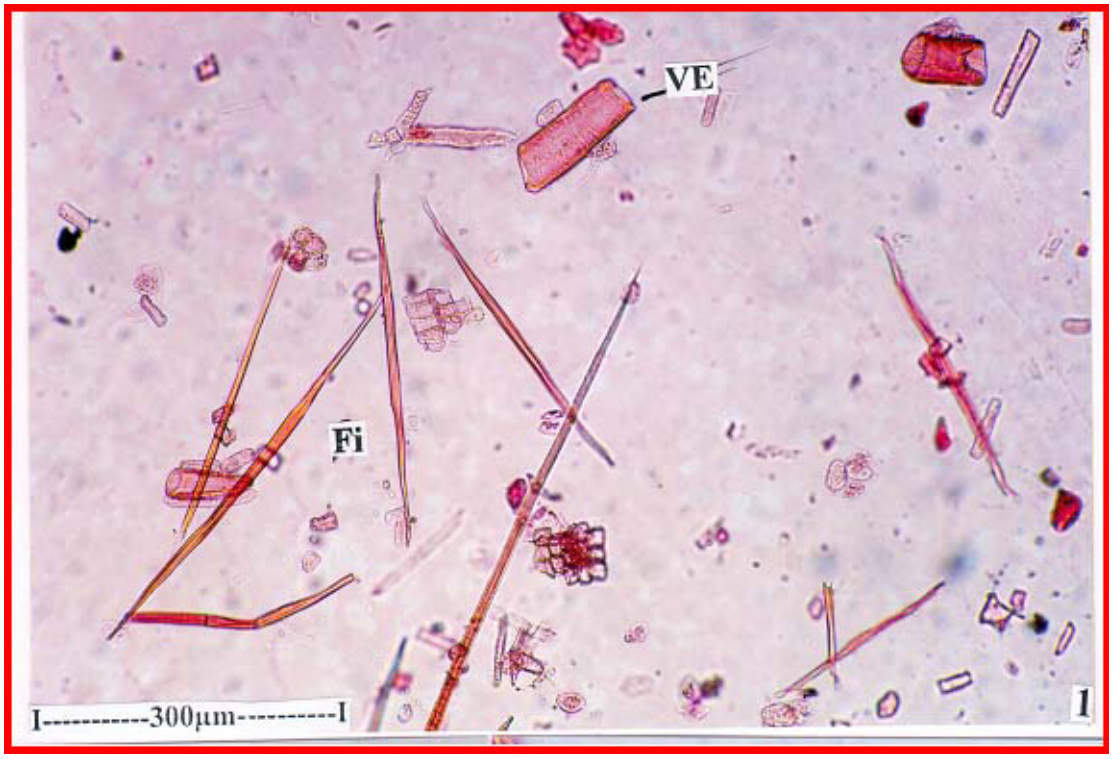

Figure 13. Xylem fibres, Xylem parenchyma and Vessel elements.
Fi-fibres VE-Vessel.

Table 4 Preliminary phytochemical test for different extracts of aerial parts of *Salvadora Persica* obtained by successive solvent extraction.

Phytocontituents	Pet.ether extract	Hexane extract	Chloroform extract	Alcohol extract	Aqueous extract	Dry powder
Alkaloids	–	–	+	+	–	+
Glycosides	–	–	–	+	+	+
Terpenoids	–	–	–	+	+	+
Saponins	–	–	–	+	+	+
Flavonoids	–	–	–	+	+	+
Proteins	–	–	–	–	–	–
Carbohydrates	–	–	–	+	+	+
Mucilage	–	–	–	–	–	–
Sterols	+	+	–	–	–	+

+ indicates positive results.
– indicates negative results.

RESULTS AND DISCUSSION

Pharmacognostical studies of leaves of *Salvadora persica* Linn has brought to light microscope features as well as preliminary phytochemical data of diagnostic values. Anatomy of the plant sometimes proves helpful for identification of fragmentary samples.

The midrib region of leaf of *S. persica* has single prominent vascular bundle surrounded by parenchymatous ground tissue (Fig 2). In the lamina the mesophyll is not well differentiated into palisade and spongy parenchyma (Fig 3). The striking character of the veins is the presence of clusters of brachysclereids at the tips of vein–terminations (Fig 4). The petiole has vascular strand which has deep are of xylem and phloem with a narrow adaxial gap (Fig 5). The stem has thick secondary xylem cylinder with an anomalous feature which is known as included phloem or intraxylanyphloem (Fig 6).

In the powdered preparation of the leaves the following elements were observed. Densely Stomatiferous abaxial epidermis (Fig 7) with cyclocytic type stomata, the adaxial epidermis (Fig 8) with sparse stomata (cyclocytic) and broken pieces of veins (Fig 9). A spherical cluster of brachyscleroids or stone cells are present at the tip of each branch of vein. Spherical subsessile glandular trichomes (Fig 10) prismatic crystals of calcium oxalate (Fig 11). and short thick, and lobed scleroids (Fig 12) are characteristic features of the leaf powder of *S. persica*.

Physicochemical constants such as solubility, extractive values, and other parameters of the drugs are collaborative evidences in drug standardization. The drug powder exhibits specific colour reaction when mixed with different reagent, thereby indicating the presence and absence of different compounds in the drug. As shown in Table IV the drug contains alkaloid, glycoside, flavonoid, terpenoid, carbohydrate and steroid. Thus anatomical characters coupled with preliminary phytochemical results are specific for the leaves of *Salvadora persica*.

CONCLUSION

Establishing standards is an integral part establishing the correct identity and quality of a crude drug. Before any drug can be included in the Pharmacopoeia these standards must be established. The majority of the information on the identify, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physico-chemical parameters. As there is no record on pharmacognostical work on leaves of *Salvadora persica*, the present work was undertaken to produce some pharmacognotical standards. The above studies provide information in respect of their identification, chemical constituents and physico-chemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic Pharmacopoeia. It will also determine therapeutic diagnostic tools for the scientists who are keen and sincere to evaluate the herbal medicine of indigenous resources.

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Bougainvillea glabra – A Natural Indicator

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ABSTRACT

The present study was designed to evaluate the juice extracted from flower petals (bracts) of climbing woody vine *Bougainvillea glabra* Choisy (Nyctaginaceae) as a titration indicator. A comparative study of Bougainvillea juice, a natural indicator, with synthetic indicator viz. phenolphthalein, methyl orange and neutral red-methylene blue were carried out to evaluate the accuracy of Bougainvillea juice as an acid-base indicator. The results indicated that juice obtained from flowers of *Bougainvillea glabra* can be used as acid base indicator in titration of strong acid with strong base because similar results were obtained by phenolphthalein and the rationale behind using Bougainvillea juice in preference to phenolphthalein is its easy availability, inertness, ease of preparation and cost-effectiveness and also the indicator papers prepared using Bougainvillea juice can be used to differentiate solutions in pH range of 7–10 because it showed different colors at each pH from 7–10.

Keywords: *Bougainvillea glabra*, Indicator, Phenolphthalein, Methyl orange, Neutral red-Methylene blue.

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INTRODUCTION

Indicators are substances whose solutions change color due to changes in pH. These are called acid-base indicators. They are usually weak acids or bases, but their conjugate base or acid forms have different colors due to differences in their absorption spectra. They are also known as neutralization indicator (1).

Indicators can be classified into two main classes according to their source.

1. Synthetic indicators: it includes all the synthetically prepared acid base indicators.
2. Natural indicators: it includes natural flowers, fruits, and other plant parts as acid base indicators (2).

Bougainvillea is named after French Navigator Louis de Bougainvillea who first discovered these plants and its flowers available in red, yellow, pink, white, purple, scarlet and lavender. *Bougainvillea glabra* is native of Brazil and a deciduous (leaf losing), climbing woody vine. Its tiny white flowers usually appear in clusters surrounded by colorful papery bracts, hence the name paper flower. It is a popular plants of California, South Taxes, Florida, the Caribbean, South Africa, Malaysia, the Mediterranean and other areas with warm climates and having medicinal uses like anti-inflammatory, insecticidal (3), decoction of its leaves used in diarrhea (4), cough and sore throat and also used as dye color and fragrance in cosmetics (5). The main parts used in the plants are leaves, flower and stem. The main constituents are alkaloids, flavonoids, steroid and D-pinitol a major carbohydrate is isolated i.e. betacynin in bark (3). In this work, an attempt has been made to compare natural indicator with synthetic one. Here bracts associated with white flowers of *Bougainvillea glabra* were used as source of acid-base indicator.

MATERIAL AND METHODS

Collection of Flowers

The flowers of *Bougainvillea glabra* Choisy were collected from local area of Mandsaur (Madhya Pradesh) and petals (bracts) were separated from the flowers. Then petals were washed with water to remove the dust.

Apparatus and Chemicals

Burette, Mortar pestle; Ammonia, Acetic acid, Hydrochloric acid, Sodium hydroxide, Oxalic acid, Methyl orange and Phenolphthalein of analytical grade were used.

Preparation of Reagents

0.1M each of ammonia solution, acetic acid, hydrochloric acid and sodium hydroxide; 0.1N oxalic acid, methyl orange and phenolphthalein solutions were prepared as per Indian Pharmacopoeia (I.P. 1996) (6).

Preparation of Juice from Petals

1 gm of fresh petals of *Bougainvillea glabra* were cut into small pieces and triturated in mortar and pestle by adding 10 ml of water. After triturating, the petals were strained by using muslin cloth and juice was collected in beaker.

Experimental Procedure

The diluted juice of Bougainvillea flowers was filled in a dish and then properly sized strips of whatman filter paper were dipped in it for 5–6 hours and finally dried it at 30–40°C. Then these papers were dipped in acid solution and in basic solution and color changed were observed. It showed pink color in acidic and yellow color in basic solution. These observations helped us to proceed further (7).

The calibration of apparatus like burettes, pipettes, and other required instruments and standardization of acids and bases were done as per procedures given in Indian Pharmacopoeia (I. P. 1996).

10 ml of titrant with two drops of indicator *Bougainvillea glabra* (B. G.) was titrated against titrates and the color changes for the indicator is listed in Table 1.

The results of screening for strong acid–strong base (HCl–NaOH), strong acid–weak base (HCl–NH_4OH), weak acid–strong base (CH_3COOH–NaOH) and weak acid–weak base (CH_3COOH–NH_4OH) are listed in Table 2 & 3. Each

Table 1: Bougainvillea glabra (B.G.)

Titrant	Indicator	Color (at initial point)	Titrate	Color (at end point)
HCl	B.G.	Pink	NaOH	Yellow
CH_3COOH	B.G.	Pink	NaOH	Yellow
HCl	B.G.	Pink	NH_4OH	Yellow
CH_3COOH	B.G.	Pink	NH_4OH	Yellow

Table 2: Volume of Titrate with Standard Indicator

Chemicals		Volume of titrate required for equivalent point with titrant (10 ml) with indicator	
Titrant (0.1N)	Titrate (0.1N)	Standard Indicator	B.G. Ind.
HCl	NaOH	7.5 ± 0.02 (Ph. Ind.)	8.2 ± 0.04
CH_3COOH	NaOH	9.7 ± 0.02 (Ph. Ind.)	10.1 ± 0.07
HCl	NH_4OH	19.3 ± 0.48 (M.O. Ind.)	22.7 ± 0.48
CH_3COOH	NH_4OH	25.1 ± 0.24 (N.R.-M.B. Ind.)	27.7 ± 0.48

B.G. Ind. - Bougainvillea glabra indicator; Ph. Ind. - Phenolphthalein indicator;
M.O. Ind. - Methyl orange indicator; N.R.-M.B. Ind. – Neutral red-methylene blue indicator

Table 3: pH of the Solution at the End Point of Titration

Solutions	pH
HCl and NaOH with Ph. Ind.	7-8
HCl and NaOH with B.G. Ind.	7-8
CH_3COOH and NaOH with Ph. Ind.	8-9
CH_3COOH and NaOH with B.G. Ind.	7-8
HCl and NH_4OH with M.O. Ind.	2-4
HCl and NH_4OH with B.G. Ind.	7-8
CH_3COOH and NH_4OH with N.R.-M.B. Ind.	6-7
CH_3COOH and NH_4OH with B.G. Ind.	7-8

B.G. Ind. - Bougainvillea glabra indicator; Ph. Ind. - Phenolphthalein indicator;
M.O. Ind. - Methyl orange indicator; N.R.-M.B. Ind. – Neutral red-methylene blue indicator

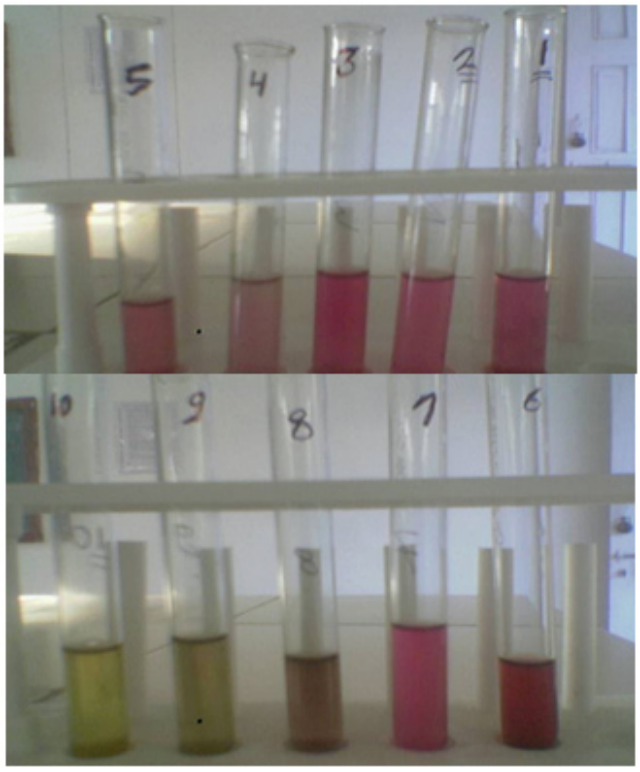

Figure 1 Color Change of Different pH Solution with B.G. Juice

titration is carried out five times by using 0.1N strength of acid and alkali and results were recorded as mean ± SEM.

Color Change Interval of the Indicator at Different pH (1–10)

To locate the exact color change interval of the indicator, we prepared solutions having pH ranging from 1–10. To prepare it we used distill water, 0.1N NaOH, 0.1N HCl and pH meter. After preparing the solutions two drops of bougainvillea juice is added to each test tube having solutions with pH ranging from 1–10. Color change was observed in each case and results are shown in Fig. 1.

RESULTS AND DISCUSSION

By using strong acid, strong base, weak acid and weak base in different combinations and along with synthetic & natural indicator result can be concluded in terms of difference in volume consumed of a particular acid or base in given set of combination.

In case of strong acid and strong base, volume consumed with phenolphthalein and bougainvillea juice are almost similar, differing only by 0.7 ml and pH at end point was same in both the cases i.e. 7–8. Since difference in volume is not significant and pH at the end point is also same, so we can say that bougainvillea juice can be used in place of phenolphthalein.

In case of weak acid and strong base, volume consumed with phenolphthalein and bougainvillea juice are almost similar, differing only by 0.5 ml but pH at end point was different in both the cases i.e. 8–9 with phenolphthalein and 7–8 with bougainvillea juice. It is not advisable to use bougainvillea juice in such titrations because pH at end point is different.

In case of weak base and strong acid, volume consumed with methyl orange and bougainvillea juice are not similar, differing by 5.0 ml but pH at end point was different in both the cases i.e. 2–4 with methyl orange and 7–8 with bougainvillea juice. It is not advisable to use bougainvillea juice in such titrations because pH at end point is different and volume difference is also large.

In case of weak acid and weak base, volume consumed with neutral red-methylene blue and bougainvillea juice are not similar, differing by 3.5 ml but pH at end point was different in both the cases i.e. 6–7 with neutral red-methylene blue and 7–8 with bougainvillea juice. It is not advisable to use bougainvillea juice in such titrations because pH at end point is different and volume difference is also large.

The color change interval of bougainvillea juice was also determined by preparing solutions having pH from range of 1–10 and adding two drops of bougainvillea juice to each test tube. It showed that from pH 1–7 bougainvillea juice impart pink color to the solution but at pH 8 it imparts brown (chocolaty) color, at pH 9 it shows greenish color and at pH 10 it imparts yellow color to the solution.

CONCLUSION

Natural indicators are the results of ever increasing demand of mankind towards better alternatives, which not only reduces cost but also healthful to the user and on the all results obtained by natural indicator is similar to that of available synthetic indicator.

Juice obtained from flowers of *Bougainvillea glabra* can be used as acid base indicator in titration of strong acid with strong base with similar results as obtained by phenolphthalein. The rationale behind using bougainvillea juice in preference to phenolphthalein is easy availability, inertness, non carcinogenicity, low cost and ease of preparation.

pH papers prepared by juice of bougainvillea can also be used to differentiate the solutions having pH in range of 7–10 because it showed different colors at pH 7, 8, 9 and 10.

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Pharmacognostical Studies on *Momordica tuberosa* Cogn.

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ABSTRACT

The study was aimed at establishing pharmacognostical profile for the leaves of plant *Momordica tuberosa*. Morphoanatomy of leaves of this plant was studied in order to establish its complete profile to aid in its identification and avoid confusion in taxanomic species. These were established using light microscopy, WHO recommended physicochemical and phytochemical procedures. The parameters presented here like morphoanatomy may be used to establish the authenticity of leaves of this plant as this plant has been used traditionally in India and also to differentiate between closely related Momordica species.

Keywords: Anatomy, Leaves, Pharmacognosy, Physico chemical, Transverse section.
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INTRODUCTION

The plant *Momordica tuberosa* belongs to family Cucurbitaceae, originating in tropical regions of India and South East Asia. *M. tuberosa* Cogn. (Cucurbitaceae) is commonly known as Karchikai (Kannada) or Athalkai (Tamil) and is traditionally used as abortifacient in India (1). The plant is a climbing annual or perennial herb with slender, scandent, branched, striate stem. The leaves are orbicular – reniform in outline, deeply cordate at the base, obtusely lobed with 5–7 lobes. Fruits are pyrifrom or broadly fusiform, fleshy dark green and 8-ribbed, sparsely hairy. The roots are woody, tuberous and perennial (2). There are reports suggesting its antihyperglycemic(3), anti-implantation and antiovulatory (4), anti diarrhoeal (5) and anticonvulsant activities (6). We have earlier reported (7) the in vitro and in vivo antioxidant activity of its tubers which was used as basis to evaluate its hepatoprotective property in CCl_4 model. Cucurbitacins, bitter substances, have been reported from the fruits of this plant (8). Fruits are reported to contain citric acid, maleic acid and Vitamin C (9).

However, there are no reports on the pharmacognostical features of the plant. Hence, the present investigation is an attempt in this direction and includes morphological and anatomical evaluation, determination of physico-chemical constants and preliminary phytochemical screening of different extracts of *M. tuberosa*.

MATERIALS AND METHODS

Plant material

The leaves of *M. tuberosa* were collected from the suburban fields of Raichur during January and were identified and authenticated by Prof. Srivatsa, Retired Professor of Botany, L.V.D. College, Raichur. A Herbarium specimen (VLCP-02/05) was deposited in the Department of Pharmacognosy, V.L. College of Pharmacy, Raichur.

Chemicals and Instruments

Rotary Microtome was used to take sections of leaves. Compound microscope, glass slides, cover slips, watch glass and other common glasswares were used in this experiment. Photographs were taken with Nikon Labphot2 Microscopic Unit. Various solvents used like ethanol (95%), petroleum ether, chloroform and reagents used for staining different sections like Toluidine blue,

safranin, Fast-green, iodine and KI were procured from S.D. Fine chemicals, Mumbai, India.

Macroscopic and microscopic analysis

The macroscopic and microscopic features of the leaves were studied according to Easu (10). For microscopic studies cross section were prepared according to procedure of Jonansen (11). The sections were stained with toluidine blue as per the method published by O'Brien et al (12). Where ever necessary sections were also stained with safranin and Fast-green and IKI (for starch). Glycerin mounted temporary preparations were made for macerated/ cleared material. Powdered materials of fruits and leaves were cleared with NaOH, stained with phloroglucinol and Conc. HCl and mounted in glycerin medium. Different cell components were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard books (10).

Physico-chemical analysis

The ash values of leaves were determined as per the WHO guidelines (13) and crude fiber content by Dutch process (14). The behavior of the powdered leaves with different chemical reagents and fluorescence characters were studied according to Kokoski et al (15) and Brain (16). Angle of repose for leaves powder (Funnel method) was also determined. For the determination of bulk density, Tapping method was followed (17). The quantitative microscopical determinations were performed according to WHO guidelines (13).

Preliminary Phytochemical screening

This was carried out by using methods of Kokate (18) and Khandelwal (19).

RESULTS AND DISCUSSION

Macroscopic characters

The leaves are orbicular rein outline 2–4.5 to 2–5.5 cm, glabrous, or with scattered hairs, punctuate (but not scabrid) on both surfaces, deeply cordate at the base, obtusely but not deeply 5–7 lobed, the lobes short and acute or obtuse. The petiole is 1.3–1.8 cm long, striate and pubescent.

Microscopic characters

Transverse section of leaf

The leaf is dorsiventral with prominent midrib and the lamina. The midrib has single stranded vascular system (fig 1). The vascular strand is top shaped, bicollateral and prominent, placed within projecting abaxial part of the midrib. The vascular strand consists of a massive cluster of wide thick walled angular xylem elements measuring maximum of 40 µm in diameter. These were two phloem strands, one on the metaxylem side and another smaller strand on the protoxylem side (fig 1).

Lamina

The leaf blade or the lamina (fig 2) is 230 µm thick. It is fairly thick abaxial epidermis comprising of cylindrical cells with prominent cuticle. The cells are 30 µm thick. The abaxial epidermis is thin and delicate and consists of narrow rectangular cells. The mesophyll tissue consists of a wide zone of palisade cells and equally wide region of spongy parenchyma. The palisade cells are two layered with upper layer of cells being higher and lower row of

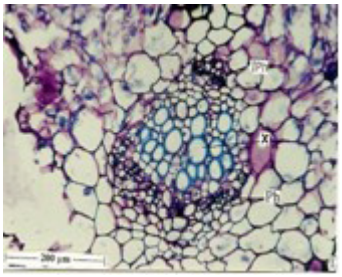

Figure 1: T S of Leaf midrib enlarged (IPh- Inner phloem; Ph-Phloem; X Xylem)

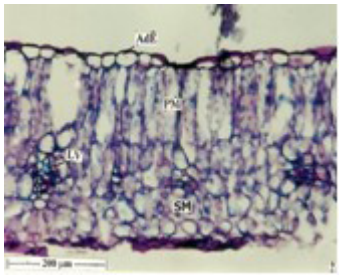

Figure 2: T S of lamina through lateral vein (AdE- Adaxial epidermis; LV- Lateral vein; PM–Palisade mesophyll; SM- Spongy mesophyll)

cells shorter. The two rows are 170 µm in height. The spongy mesophyll shows four or five layers of spherical or lobed parenchyma cells, loosely arranged forming intercellular spaces. The vascular strands of the lateral veins are located in the median part of the lamina and do not project above the level of the epidermis. The lateral vein vascular strands are vertically oriented with a small group of phloem and four or five cells of xylem. A layer of bundle sheath cells are often seen around the vascular strand.

Cystoliths

Calcium carbonate cystoliths bodies are frequently seen in the mesophyll tissue of the leaf. In sectional view, the cystoliths appear as a circular body located in widened cells which are called lithocysts. The cystoliths are 110 µm in diameter. The cystolith is mostly subepidermal in position (fig 3).

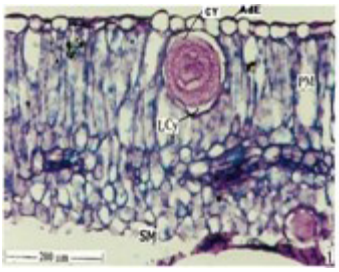

Figure 3: T S of lamina showing cystolith
(Cy-Cystoliths; LCy- Lithocyst)

Petiole

The petiole has wide concave adaxial side and a triangular abaxial part with undulate outline. It is 1.4 mm vertically and 1.8 mm horizontally. The ground tissue consists of about three layers of collenchymatous outer zone, remaining portions having large, uniwalled, compact parenchyma cells (fig 4).

The vascular system consists of five discrete vascular bundles, arranged in a ring with wide gap in between

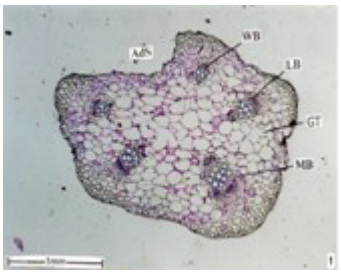

Figure 4: T S of petiole entire view. GT-Ground tissue LB- Lateral bundle; MB-Median bundle; WB- Wing bundle)

of the five bundles, one towards the lower part is the largest and represents the median bundle, two smaller bundles found on the lateral part, are the lateral bundles, two bundles occurring in the adaxial wing like part are the wing bundles. The vascular bundles are bicollateral having phloem both on the inner and outer parts of the vascular bundles (fig 4).

Venation

Secondary and tertiary veins of the lateral veins are present forming wide reticulations. The vein islets are distinct and wide, rectangular and random in orientation. The vein terminations are well developed and mostly branched once or twice forming a dendroid configuration. The terminal part of the vein termination becomes slightly thick.

Powder characters

Leaf powder shows presence of multicellular covering trichomes and prisms of calcium oxalates (fig 5). The cleared powder exhibits the cystoliths that are usually in pairs. They are attached to a common wall of two lithocysts. Occasionally more than three cystoliths are also seen in the lamina (fig 5). Anomocytic stomata are present.

Preliminary Phytochemical screening

The screening for phytochemicals revealed the presence of sterols, saponins, triterpenoids alkaloids, flavonoids, bitters and carbohydrates in leaves (table 1).

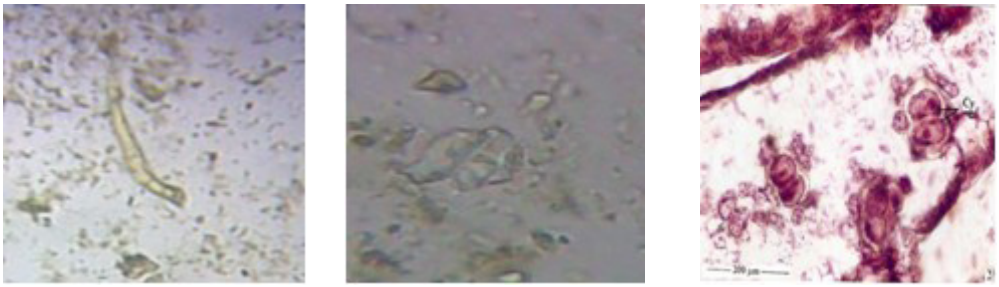

Figure 5: Leaf powder characters

Table 1: Preliminary phytochemical screening of leaves of *M. tuberosa*

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	–	+	–	–
Carbohydrates	–	–	++	++
Flavonoids	–	–	++	++
Saponins	–	–	–	+
Cardiac glycosides	–	–	–	–
Anthraquinones	–	–	–	–
Tannins	–	–	–	–
Proteins	–	–	–	–
Steroids	++	++	++	–
Triterpenes	++	++	++	–

Physico-chemical constants

Ash values signify the amount of inorganic impurities, resistant materials like sand, soil and stone particles in crude drugs. The percentages of total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying and extractive values in different solvents are given in table 2. The physical properties and nature of different extracts prepared by successive extraction method are given in table 3. To determine powder characters of 40- mesh size, the angle of repose and bulk density were also calculated. To determine powder characters of 40-mesh size, the angle of repose and bulk density were also calculated. The leaves showed an angle of repose of 37°10′ and 0.65 g/c.c. of bulk density. The results of quantitative microscopy are as shown in table 4. Results of behavior of the leaf powder with different chemical reagents in visible light and UV light are given in table 5.

Table 2 Physicochemical parameters of leaves of *M. tuberosa*

Parameters	Values (% (w/w)
Total ash	3.86
Acid insoluble ash	1.26
Water soluble ash	2.90
Sulphated ash	1.26
PEEV	1.10
CEV	1.40
EEV	4.80
WEV	19.0
LOD	8.10

PEEV-Pet ether extractive value, CEV-Chloroform extractive value, EEV-Ethanol extractive value, WEV-Water extractive value, LOD-Loss on drying

Table 3: Colour and consistency of leaves extracts of *M. tuberosa*.

Extracts	Colour	Consistency
Pet ether (60 – 80°c)	Pale yellowish	Waxy
Chloroform	Light Brown	Sticky
Ethanol (95%)	Dark brown	Semi solid
Water	Dark brown	Sticky paste

Table 4: Quantitative microscopical results of leaves of *M. tuberosa*

Parameters	Values
Stomatal number	288.83 - Upper
	355.55 - Lower
Stomatal index	19.11 - Upper
	20.78 - Lower
Vein islet number	2.5
Veinlet termination no	1.75
Palisade ratio	2.81
Length of trichomes	146.3 – 532 µ

CONCLUSION

The present study of Pharmacognostical evaluation of leaves of *M. tuberosa* will provide most needed information on its identification. To authenticate and substantiate the leaves, morphological and microscopical studies will be of great help which could even be used to distinguish it from other species of *Momordica*.

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Table 5: Fluorescence analysis of leaves of *M. tuberosa*

Treatment	Fluorescence	
	Day light	UV light
Powder as such	Green	Brownish green
Powder + 1N NaOH (Aq)	Greenish	Deep red
Powder + 1N NaOH (MeOH)	Greenish	Red
Powder + 1N HCl (Aq)	Greenish	Deep red
Powder + Iodine N/50	Green	Greenish red
Powder + 50% H_2SO_4	Greenish brown	Brownish red
Powder + 50% HNO_3	Green	Brownish red
Powder + 1N NaOH (MeOH) C	Green	Red
Powder + 1N HCl (MeOH) C	Green	Dark red
Powder +1N NaOH (Aq) C	Green	Dark red

C- with Nitrocellulose, MeoH-Methanolic, Aq-Aqueous.

Pharmacy, Raichur and S.C.S. College of Pharmacy, Harapanahalli for providing necessary research facilities.

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Phytochemical investigation and antimicrobial studies on the leaf extracts of *Psoralea corylifolia* Linn.

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ABSTRACT

Aqueous and alcoholic extracts from *Psoralea corylifolia* leaves were screened for the presence of chemically active compounds by standard methods and evaluated for their antimicrobial activity *in vitro* by disc diffusion method. The results revealed the presence of saponins, tannins, flavonoids, glycosides, carbohydrates, tannins and phenolic compounds, gums and mucilages, fixed oils and fats. Alkaloids were not detected from any of the leaves extract under study. Aqueous and alcoholic extracts exhibited broad-spectrum antibacterial and antifungal activity against *Eschericia coli, Pseudomonas aeruginosa, Staphylococcus pyogenes* and *Candida albicans*. Alcoholic extract is better than that of aqueous extract of *P. corylifolia* leaves in respect to their antimicrobial activity and the broad spectrum of activity makes it a promising indigenous drug.

Keywords: Alcoholic extract, Antimicrobial activity, Aqueous extract, Leaves, *Psoralea Corylifolia*.

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INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (1).

The genus *psoralea* Linn. contains approximately 120 species distributed worldwide. These plants are mainly found in South Africa, North Africa, Australia, South America, North America, Asia and the Mediterranean region. Among the different species of *Psoralea* plants known worldwide *Psoralea corylifolia* (known as *Cullen corylifolia)* is a rare and endangered herbaceous medicinal plant, native to India. In India, it is distributed in Tamil Nadu, Uttar Pradesh, Rajasthan, Bihar, Gujarat and Andhra Pradesh (2).

Psoralea corylifolia Linn. (family: Fabaceae; Papilionaceae) is an erect, annual plant, upto 30–180 cm high and found throughout India. Leaves are stalked, simple, 1–3 inches long, 1–2 inches broad, firm in texture, covered with numerous black dots, hairs few, stalks up to 1 inch long hairy gland dotted. Flowers are dense, 10–30 in a bunch arising in the axils of leaves; fruits are small, subglobose, without hairs, slightly compressed. Single seeded pods are kidney shaped, 2–5 mm long, 2–3 mm broad and 1–1.5 mm thick, consisting of a sticky oily pericarp, a hard seed coat and kernel.

Various parts of this plant have been used in the folk, Siddha and Ayurvedic systems of medicine. In Ayurvedic medicine, the plant is described as stomachic, deobstruent, anthelmintic, diuretic, diaphoretic and aphrodisiac (3). Earlier studies have shown significant antibacterial activity of aqueous, alcohol, petroleum ether extracts and essential oil obtained from the seeds of *P. corylifolia* (4). The essential oil of *P. corylifolia* has shown moderate antifungal activity (5). Alcohol extracts of both leaves and seeds of *P. corylifolia* exhibited potential antifilarial activity on cattle filarial parasite *Setaria cervi* (6). No attempt has been made to study the antimicrobial activity of *P. corylifolia* extracts. Hence the present work was aimed to study the phytochemical properties and antimicrobial activity of different leaf extracts of *Psoralea corylifolia* Linn.

MATERIALS AND METHODS

Plant materials

The plant material of *P. corylifolia* was collected from the survey of medicinal plant unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India. The plant was authenticated by Dr Athar Ali Khan, Department of Botany, A.M.U., Aligarh, where the voucher specimen has been deposited (Voucher No. 1122).

Preparation of the plant extract

Dried and powdered leaves of *P. corylifolia* were extracted with ethanol and water, separately. The crude ethanol and aqueous extracts were dried and dissolved in 95% ethanol and distilled water before use (6).

Phytochemical screening

The leaves extract of *P. corylifolia* were analyzed for the presence of alkaloid, saponin, tannins and phenolic compounds, glycosides, flavonoids, lignins, carbohydrates, gums and mucilages, phytosterols, fixed oil and fat, proteins and free amino acids according to standard methods (7, 8).

Screening for alkaloids

A small portion of solvent free alcohol extract and aqueous extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as Mayer's reagent, Dragendroff's reagent, Hager's reagent and Wagner's reagent. Precipitation in any of the 4 test indicates the presence of alkaloids.

Screening for saponin

About 0.5 g of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taken as a preliminary evidence for the presence of saponin.

Screening for tannins and phenolic compounds

Extract of the sample was treated with 5% ferric chloride test solution. The resultant color was noted. A violet color indicated the presence of hydrolysable tannin. Or into 1% solution of gelatin containing 10% sodium chloride in a beaker, 0.5 g of the extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannin. Or into 10% lead acetate solution, 0.5 g of the extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins and phenolic compounds.

Screening for phytosterol (Libermann Burchard test)

1 g of extract was dissolved in few drops of dry acetic acid and 3 ml of acetic anhydride was added followed by few drops of concentrated sulphuric acid. A bluish green color indicates the presence of phytosterol.

Screening for flavonoid: Shinoda's test

Extract is dissolved in alcohol. To that piece of magnesium followed by concentrated hydrochloric acid is added. Red color indicates the presence of flavonoids. Or 5 ml of 20% sodium hydroxide was added to equal volume of the sample extract. A yellow solution indicates the presence of flavonoid.

Screening for glycosides (Borntrager's test):

Extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. A layer of pink, red or violet color indicates the presence of glycosides.

Screening for carbohydrates:

Minimum amount of extracts were dissolved in 5 ml distilled water and filtered. The filtrate was subjected to test for carbohydrates.

a. *Molisch's test:* The filtrate was treated with 2–3 drops of 1% alcoholic α-naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. A purple color indicates the presence of carbohydrates.
b. *Fehling's test:* Filtrate was treated with 1 ml Fehling's solution and heated. An orange-red precipitate indicates the presence of carbohydrates.
c. *Legal's test:* Extract was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple color in ammoniacal layer indicates the presence of carbohydrates.

Screening for fixed oil and fat:

Small quantity of various extracts was separately pressed between two filters. Appearance of oil stain on the paper indicates presence of fixed oil.

Few drops of 0.5 N alcoholic potassium hydroxide were added to small quantity of various extracts along

with phenolphathlein. The mixture was heated on a water bath for 1–2 h. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Screening for proteins

Dissolve small quantities of various extracts in few ml of water and treated with

a. *Millon's reagent:* Appearance of red color indicates presence of proteins.
b. *Ninhydrin's reagent:* Appearance of purple color indicates presence of proteins.
c. *Biuret test:* Equal volume of 5% solution of sodium hydroxide and 15% solution of copper sulphate were added. Pink color indicates presence of proteins.

Screening for lignins:

Extract was treated with alcohol followed by addition of phloroglucinol and hydrochloric acid. Appearance of red color indicates presence of lignins.

Screening for gums and mucilages:

About 10 ml of various extracts were treated with absolute alcohol and filtered. Occurrence of precipitation indicates the presence of gums and mucilages.

Antimicrobial activity on various extracts of Psoralea corylifolia leaves

Microorganism used: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus pyogenes and *Candida albicans* are the microorganisms were used for present *in vitro* antimicrobial assay. All the organisms were obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India.

Determination of antimicrobial activity

Disc diffusion assay method was used to screen the extracts for antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus pyogenes* and *Candida albicans*. The organisms to be tested were inoculated on Muller hinton agar. After an incubation period of 24 h at 37°C, three or four colonies were isolated from these media and inoculated into 4 ml of Muller hinton agar broth. After an incubation period of 2 h at 37°C, the cultures were adjusted with sterile solution to obtain turbidity comparable to that of Me Farland No. 0.5 standard. Muller hinton agar plates were swabbed with the respective broth culture of the organisms and incubated over night at 37°C. The diameters of the inhibition zone were measured in millimeter.

Activity Index:

The zone of inhibition in extract and the standard antimicrobial agent were used to calculate the activity index.

$$AI = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard antimicrobial agents}}$$

Proportion Index:

Number of positive results obtained for aqueous and alcoholic extract of plant part was against all the

Table 1: Phytochemical screening of aqueous and alcoholic extracts of *P. corylifolia* leaves

Sl. No.	Phytochemical constituents	Results	
		Aqueous extract	Alcoholic extract
1.	Alkaloids	–	–
2.	Carbohydrates	+	+
3.	Fixed oils and fats	+	+
4.	Saponins	+	+
5.	Tannins and phenolic compounds	+	+
6.	Proteins and amino acid	–	–
7.	Gums and mucilage	+	+
8.	Flavonoids	+	+
9.	Lignin	–	–
10.	Phytosterol	–	–
11.	Glycosides	+	+

+ = Present, – = Absent

Table 2: Antimicrobial activity of *P. corylifolia* extracts

Sl. No.	Plant extract	Zone of inhibition (mm) (± SEM)				Proportion
		E. coli	*P.aeruginosa*	*S. pyogenes*	*C. albicans*	Index (%)
1	SAA	17.3 ± 0.2	28.0 ± 0.1	19.2 ± 0.4	14.1 ± 0.5	-----
2	Aqueous extract	----	14.2 ± 0.3	11.3 ± 0.1	8.0 ± 0.4	75
3	Alcoholic extract	10.1 ± 0.2	16.0 ± 0.1	14.2 ± 0.3	9.4 ± 0.2	100

SAA- Standard antimicrobial agent (Riboflavin)

microbials and total number tests carried out were used to evaluate the proportion index.

$$\text{Proportion index (\%)} = \frac{\text{No. of positive results}}{\text{Total number of tests}} \text{x100}$$

RESULTS AND DISCUSSION

The study on *P. corylifolia* leaves extract revealed the presence of saponins, tannins, flavonoids, glycosides, carbohydrates, tannins and phenolic compounds, gums and mucilages, fixed oils and fats. The leaves did not show the presence of alkaloids in any of the extracts that were tested for its presence (Table 1).

The results of the antimicrobial activity presented in table 2 shows that the alcoholic extract exhibited appreciable antimicrobial property by inhibiting the growth of all microorganisms, whereas the aqueous extract inhibited the growth of all microorganisms except *E. coli*. Generally, proportion index of antimicrobial activities of *P. corylifolia* extracts shows the highest activity in alcoholic extract as presented in table 2.

The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. There are records that show the benefits of these compounds detected from *P. corylifolia* for example:

Saponin is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchloles-trolaemia, hyperglycaemia, antioxidant, anticancer, antiinflammatory, weight loss and antifungal etc. It is also known to have antimicrobial properties.

Tannin is reported to exhibit antiviral, antibacterial, antitumor and antimicrobial activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic plant tannin have been recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars (9).

Flavonoid have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. They show antiallergic, anti-inflammatory, antimicrobial and anticancer activity.

CONCLUSION

Our observations confirm that the alcoholic extract is better than that of aqueous extract of *P. corylifolia* leaf in respect to their antimicrobial activity. Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, glycosides, carbohydrates, tannins and phenolic compounds, gums and mucilages, fixed oils and fats. We recommend further research on this plant leaves for possible isolation and characterization of the various chemical active substances.

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IN VITRO AND *IN VIVO* ANTICOAGULANT ACTIVITY OF *IMPERATA CYLINDRICA* A NOVEL ANTICOAGULANT LEAD FROM NATURAL ORIGIN.

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ABSTRACT:
Anticoagulant monotherapy has been the basis of treatment for patients with atrial fibrillation, left ventricular thrombus, as well as for prevention or treatment of deep venous thrombosis and pulmonary embolism. Considering the adverse effects with existing anticoagulant therapy, alternative drugs from natural origin can help to get the new molecular lead as a hope towards the better efficacious and safe anticoagulant. The present investigation was planned to evaluate the in vitro and in vivo anticoagulant activity of *Imperata cylindrica* (IC). Methanolic extract of IC has demonstrated presence of tannins and polyphenols. It can be concluded from the present investigation that IC has exhibited significant anticoagulant activity in vivo and in vitro. Oral administration of IC after 1, 2 and 3rd hr have exhibited 4, 6 and 9 fold increase in prothrombin time when compared to base value and 6, 7 and 9 fold increase in prothrombin time at 10th, 30 and 60th min after i.v. administration when compared to base value. IC may be acting on the extrinsic cascade of clotting probably by binding with the antithrombin. IC could be a hope towards development of a novel anticoagulant with optimized efficacy and reduced side effects.

Keywords: anticoagulant, *Imperata cylindrica*, prothrombin time, ventricular thrombus

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INTRODUCTION

Anticoagulant monotherapy has been the basis of treatment for patients with atrial fibrillation, prosthetic heart valves, markedly reduced left ventricular function or left ventricular thrombus, as well as for prevention or treatment of deep venous thrombosis and pulmonary embolism (1).

Antiplatelet therapy is the cornerstone for both primary and secondary prevention therapies for ischemic events resulting from coronary atherosclerotic disease. Dual antiplatelet therapy (aspirin plus a thienopyridine, usually clopidogrel) has assumed a central role in the treatment of acute coronary syndromes and after coronary stent deployment. In addition to antiplatelet therapy, anticoagulant therapy might be indicated for stroke prevention in a variety of conditions that include atrial fibrillation, profound left ventricular dysfunction and after mechanical prosthetic heart valve replacement. For this reason, the use of triple antithrombotic therapy (a dual antiplatelet regimen plus anticoagulant) is expected to become more prominent, given an aging patient population (2).

The clinical use of glycosaminoglycans and particularly heparin is associated with various side effects such as bleeding tendency and induced thrombocytopenia. The extensive use of oral anticoagulant therapy has provided more in-depth knowledge of the associated adverse reactions and the potential risks (3).

However, non-haemorrhagic adverse reactions may also play a considerable role in both temporary and continuative therapy (4). Non haemorrhagic side effects include four principal types of adverse reactions (5, 6):

(a) Ecchymosis and purpura appearing in subjects with skin fragility (i.e. elderly or subjectsunder long-term

steroid therapy), or by an excessive anticoagulant effect.

(b) Maculo-papular, vescicolous, or urticate rashes which are extremely itchy.

(c) 'Purple toes syndrome', a rare syndrome.

(d) Skin necrosis, the most serious non-haemorrhagic side effects.

Insulin resistance (IR), which occurs in type 2diabetes, appears to be a common precursor of both diabetes and macro vascular disease. Metabolic disturbances that commonly occur in patients with IR are atherogenic dyslipidemia, hypertension, glucose intolerance and a prothrombotic state. The prothrombotic state is characterized by increased fibrinogen levels, increased plasminogen activator inhibitor (PAI)-1 and different abnormalities in platelet function. Thrombosis thus gets promoted and thrombolysis is being retarded (7).

Considering the place of the anticoagulant therapy in the cardiovascular disorders and the adverse effects with existing anticoagulant therapy, alternative drugs from natural origin can help to get the new molecular lead as a hope towards the better efficacious and safe anticoagulant agent.

Charak Sidhisthan in Charak Samhita mentions the reference of *Imperata cylindrica* (IC) commonly called as *"Darbha"* in 6th chapter and Shloka no. 83. The shloka states that, IC is being used while administering the blood enema which is administration of whole blood through rectum. The drug is mixed with the blood prior to administration explains the anticoagulant properties of the drug. The drug may have potential anticoagulant activity in vitro as well as in vivo. The present investigation was planned to evaluate the in vitro and in vivo anticoagulant activity of *Imperata cylindrical* (IC).

MATERIAL

Plant Material

The whole plant is collected from Rajgurunagar, Pune. The crude material was shade dried and powdered in pulveriser.

METHODS

Peparation of methanolic extract of imperata cylindrica (mic)

The whole plant of *Imperata cylindrica* was shade-dried and powdered. The powdered material (100 g) was subjected to soxhlet extractor using methanol as a solvent. The extract was suspended in 1% CMC and used for pharmacological studies.

Acute oral toxicity studies

Adult albino mice of either sex were subjected to acute toxicity studies as per guideline (425) suggested by Organization for Economic Co-operation and Development (8). The mice were observed for 2 h for behavioral, neurological and autonomic profiles and for any lethality during next 48 h.

Phytochemical screening

Phytochemical screening of IC was performed for assessing the presence of tannins, flavonoids, saponins etc. as a qualitative testing (9).

Total phenolic compound analysis (10, 11)

The amount of total phenolics in MIC was determined with the Folin-Ciocalteu reagent. To 50 ml of each sample (three replicates), 2.5 ml 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765. Results were expressed as percentage of gallic acid.

Total flavonoid content (12)

The total flavonoid content was determined using the Dowd method. 5 mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption readings at 415 nm after 10 minutes against a blank sample consisting of a 5 mL MIC solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (0–100 mg/L) as the standard. Total flavonoid content is expressed as percentage of quercetin equivalents (QE).

HTPLC fingerprinting study

The test sample of IC was spotted in the form of band of width 6 mm with CAMAG µL syringe on precoated silica gel aluminium plate $60F_{254}$ (10 cm × 10 cm with 0.2 mm thickness E. Merck, Germany) using CAMAG Linomat 5 applicator (Switzerland) fitted with a 100 µL syringe. The Linear ascending development was carried out in solvent system (20 mL) toluene: ethyl acetate: formic acid (5:4:0.5 v/v/v/v) in a glass twin through chamber (10×10 cm) previously saturated with mobile phase for 30 min. The HPTLC plate was allowed to run up to 80 mm from the point of application. HPTLC plate was dried in hot air oven at 60°C. Densitometric scanning was performed using CAMAG TLC scanner 3 in the absorbance mode at 280 nm and operated by winCATS software (V 1.4.3.6336).

The slit dimension was 5×0.45 mm with the scanning speed of 20 mm s^{-1}. Evaluation was done via peak area with linear regression.

Pharmacological screening (13)

In vitro anticoagulant activity

IC was tested for anticoagulant activity in human plasma. Blood was placed in 3.8% sodium citrate. Different concentrations of MIC were prepared in normal saline and Prothrombin time (PT) is being measured. 1–10 IU of heparin per 100 µL of human citrated plasma were employed as standard.

In vivo anticoagulant activity

Oral route of administration

Albino rats of either sex weighing 150–200g were used for the study. MIC was administered in doses of 100, 200 and 400 mg/ kg, p.o.

Intravenous route of administration

Albino rats of either sex weighing 150–200g were used for the study. Animals were anaesthetized and MIC in normal saline is introduced in femoral vein and the jugular vein is canulated to get the blood withdrawn. MIC was administered intravenously in the dose of 3, 10 and 30 mg/ kg. The blood was withdrawn at different intervals and PT was measured.

Table 1: Retention factor of peaks from fingerprinting of IC

Sr. No.	Peak No.	Retention factor
1	1	0.20
2	2	0.27
3	3	0.37
4	4	0.50
5	5	0.57
6	6	0.62
7	7	0.66
8	8	0.72

RESULTS

Peparation of methanolic extract of Imperata cylindrica (IC)

The yield of the yellowish green colored extract was found to be 3% w/w.

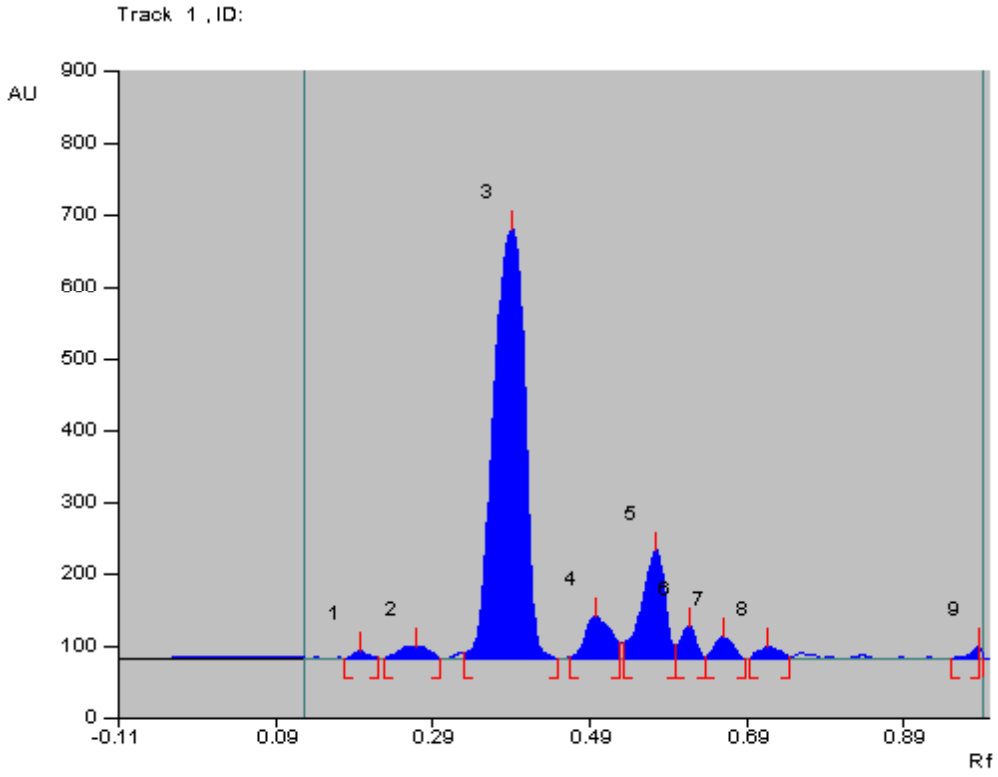

Figure 1: *HPTLC Figure printing of methanolic extract of IC*

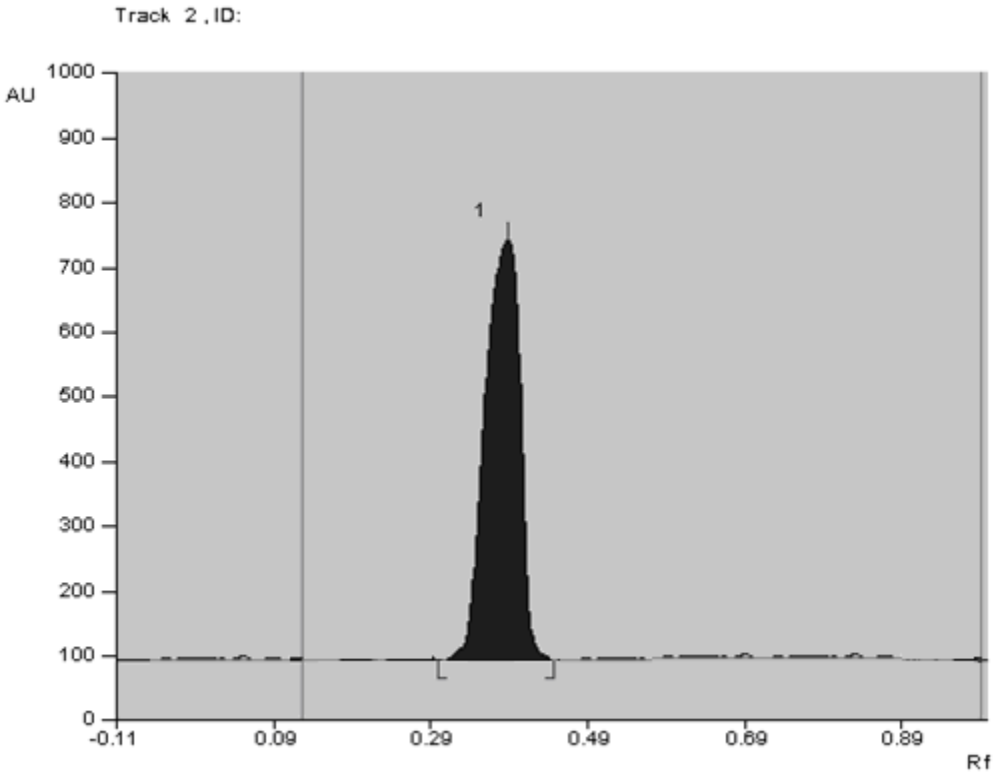

Figure 2: *HPTLC Figure printing of gallic acid*

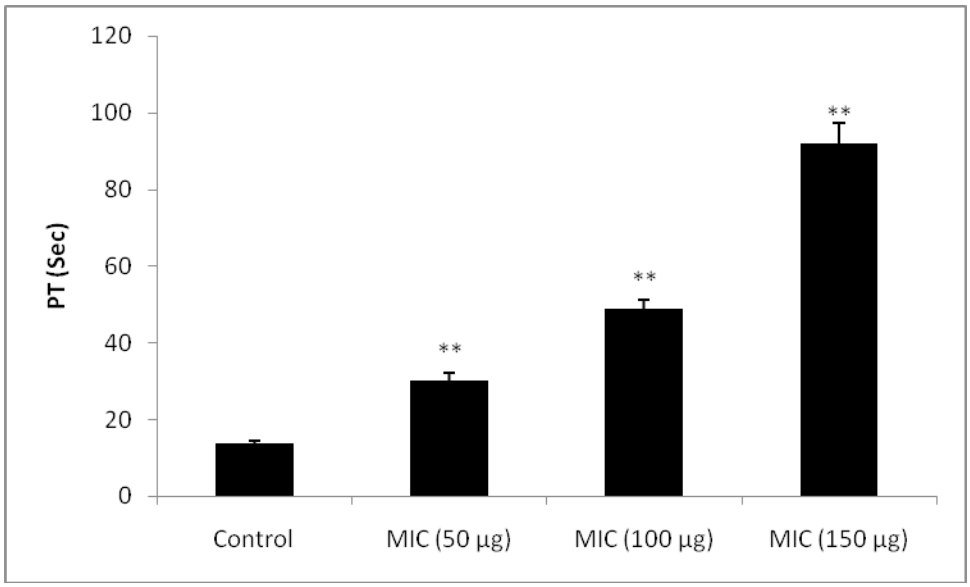

Figure 3: *In vitro anticoagulant activity of IC*
Values are expressed as Mean, analysed by ANOVA followed by Dunnett test, *p<0.05, **p<0.001

Phytochemical Screening:

The preliminary phytochemical investigation of MIC demonstrated the presence of tannins and flavonoids.

Total phenolic content of MIC was found to be 30 % GAE w/w.

Total flavonoidal content of MIC was found to be 0.7 % QE w/w

HTPLC fingerprinting study

The HPTLC fingerprints of MIC showed 8 well resolved peaks (Fig. 1)along with gallic acid (Fig. 2) having R_F 0.37 (Table 1).

Pharmacological screening
In vitro anticoagulant activity

A concentration dependent increase in prothrombin time (PT) was observed after addition of MIC to citrated human plasma. Different concentrations of MIC 50, 100 and 150 µg of MIC were able to produce significant ($p< 0.05$) prolongation in the prothrombin time as 30.21, 49.05 and 92 sec. respectively when compared to basic value as 13.87 sec (Fig. 3).

To determine the effect of IC on coagulation of blood after oral administration, the baseline blood was collected. Animals (n=5) were divided into different groups of 100, 200 and 400 mg/ kg of MIC orally. Blood samples were collected from 1, 2 and 3rd hr of drug administration and PT was calculated. The results indicated that the onset of action of MIC was before 1st hr of drug treatment which produced 4 fold increase in PT compared to base reading. MIC had significantly ($p< 0.05$) prolonged the PT at 1, 2 and 3rd hr and exhibited 6 and 9 fold increase in PT when compared to base value. It can be concluded from the results that the duration of the action of MIC would be greater that 3 hr after oral administration (Table 2).

Intra venous route of administration

MIC has demonstrated significant anticoagulant activity when given intravenously in the dose of 3, 10 and 30 mg/ kg, i.v. MIC exhibited 6 fold increase in PT when compared to base value at 10th min of drug administration. There was 7 and 9 fold increase in PT compared to base value at 30th and 60th min after administration of the drug which shows analogy with the 2nd and 3rd hr results after oral administration of MIC (Table 3).

DISCUSSION:

Chromatographic fingerprinting has been suggested to check the authenticity or provide quality control of herbal medicine. Chromatography has the advantages

Table 2: *In vivo anticoagulant activity of IC* Oral route of administration

Groups	Treatment	Prothrombin Time (sec.)		
		1st hr.	2nd hr.	3rd hr.
Control	1% CMC 1ml/kg, p.o.	13.29 ± 0.61	15.28± 0.52	13.87±0.81
I	MIC 100 mg/ kg, p.o.	41.33± 2.18**	60.34± 4.18**	96.10±7.11**
II	MIC 200 mg/ kg, p.o.	50.37± 3.91**	60.60± 4.11**	135.31± 9.01**
III	MIC 400 mg/ kg, p.o.	60.23± 3.10**	95.16±5.10**	140.12±11.09**

Values are expressed as Mean, analysed by ANOVA followed by Dunnett test,

* p<0.05,

** p<0.001

Table 3 Intra venous route of administration

Groups	Treatment	Prothrombin Time (Sec.)		
		10 min	30 min	60 min
Control	Saline 1ml/kg, i.v.	15.20 ± 1.01	15.88 ± 0.92	14.20 ± 0.88
I	MIC 3 mg/kg, i.v.	53.11 ± 1.98**	94.00 ± 5.19**	83.00 ± 6.10**
II	MIC 10 mg/kg, i.v.	82.19 ± 5.14**	98.12± 4.99**	121.19 ± 10.22**
III	MIC 30 mg/ kg, i.v.	97.19 ± 2.66**	109.36 ± 8.14**	135.02 ± 9.91**

Values are expressed as Mean, analysed by ANOVA followed by Dunnett test,

* p<0.05,

** p<0.001

of separating a complicated system into relatively simple sub-system and then presenting the chemical pattern of herbal medicine in the form of a chromatogram. HPTLC fingerprinting is the best way for chemical standardization and it also helps to provides the comprehensive idea about the class of compound present in the plant. A simple and accurate fingerprinting method using CAMAG HPTLC Instrument has been developed for quality control of the IC. For the first time, HPTLC fingerprint was investigated and used for evaluation of IC collected from Rajgurunagar, Pune. Recent researches indicate that the polyphenols, being secondary metabolites, are present in rich amount in several plants. Many of them possess antioxidant, anti-inflammatory and several others therapeutic properties. Therefore this study also established HPTLC fingerprint for the methanol extract of IC.

In the present investigation of in vitro anticoagulant activity, prolongation in PT exhibited by MIC was found to be 65% when compared to prolongation produced by 4IU of heparin per 100 µL of human citrated plasma.

Prolongation of PT after treatment of IC could explain its role in the extrinsic pathway of coagulation. Increased PT probably directs the mechanism of MIC towards inhibition of factors such as I, II, V, VII, X in the extrinsic cascade for coagulation.

It can be concluded from the in vivo anticoagulant activity that IC has significant anticoagulant activity when administered orally. The results indicated that the onset of action of MIC was before 1st hr of drug treatment which produced 4 fold increase in PT compared to base reading and the duration of the action of MIC would be greater that 3 hr after the oral administration.

When administered intravenously, the onset of action of MIC was found to be 10 min after the drug treatment and the duration of the action of MIC would be greater that 1 hr after the intravenous administration of MIC.

It can be concluded from the present investigation that IC has exhibited significant anticoagulant activity both in vivo after oral and intra venous administration and in vitro. IC may be acting on the extrinsic cascade of clotting probably binds with the antithrombin. The complex formed may be inhibiting the conversion of prothrombin to thrombin and finally inhibiting the conversion of the soluble fibrinogen to insoluble fibrin clot.

There is a great need of developing and optimizing the anticoagulant therapy using oral or long acting anticoagulants which will be for who must be treated lifelong and should be devoid of "off target" effects, such as liver dysfunction and other non haemorrhagic side effects. Challenges for the new anticoagulants remain their higher costs relative to heparins and vitamin K antagonists, and lack of appropriate antidotes. In such a situation IC could be the hope towards development of a novel anticoagulant with optimized efficacy and reduced side effects.

The potential of IC in the procoagulant stage in diabetes particularly type II and related micro and macro vascular complications needs to be screened.

In accordance with the fact that the drug having an anticoagulant as well as antiplatelet activity could reduce the side effects of the triple therapy. Further the antiplatelet activity of IC needs to be explored.

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Pharmacognostic and Physio-Chemical Studies on the Leaves of *Cardiospermum halicacabum* L.

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ABSTRACT:

Cardiospermum halicacabum L. (Sapindaceae) commonly known as 'Balloon vine' is a dioecious, hairy, climbing vine with balloon like clusters of white flowers framed by finely dissected, delicate foliage. The leaf has a bitter taste; the entire plant is used as anti-inflammatory, antibiotic against many bacteria, antiparasitic, antipyretic and as an analgesic. The ethanolic extract of leaves exhibits significant anti-arthritic effect. The present study was therefore carried out to provide requisite pharmacognostic details about the leaf. Pharmacognostic investigation of the leaf and leaf powder of *Cardiospermum halicacabum* L. was carried out to determine its morphological, anatomical and phytochemical diagnostic features. The preliminary phytochemical analysis and Thin Layer Chromatography has been performed. The leaf was also characterized for its physico-chemical properties. The presence of covering trichomes and anomocytic type of stomata are the characteristic features observed in the microscopy of leaf. Preliminary phytochemical analysis indicated presence of tannins, saponins, flavonoids, glycosides and cardiac glycosides. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: Cardiospermum halicacabum L., Pharmacognosy, Physicochemical analysis..

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INTRODUCTION

Traditional knowledge of plants is responsible for most of the medicine and food used in modern society. The exploration of traditional knowledge for cures to common diseases is attractive, but also overwhelming. *Cardiospermum halicacabum* L., commonly known as Balloon vine, is an important medicinal herb belonging to family Sapindaceae. The plant is a dioecious, hairy, climbing vine with clusters of white flowers, finely dissected, delicate foliage and balloon like fruits. The root of the plant is considered as diaphoretic, diuretic and aperient. It is also administered in fever. The whole plant is applied to reduce swellings and hardened tumors (1). There is a claim that it is used by some locals to treat rheumatoid arthritis in Asian and African communities. This claim is substantiated by the researchers (2-3).

The whole plant has been used as anti-inflammatory (4-5), as an antibiotic against many bacteria such as *Escherichia coli, Salmonella typhi* etc. (4-6), as an antipyretic (4-7), antiparasitic (8), as an effective non toxic antifertility herb (9) and as analgesic (4). Eswar Kumar *et al.* (10) reported that the ethanolic extract of leaves exhibits significant anti-arthritic effect.

In the global market, balloon vine has been utilized in several products, 'Love in a puff', 'Balloon Vine' and 'Heartseed'. It is also one of the ingredients in "Allergy Relief Liquid ™" and "Bioforce Pollinoson® Tabs" marketed by Bioforce USA as a natural relief for hay fever, allergies, sneezing, watery eyes, and allergic reactions. Another US based company, Boericke and Tafel produces "Florasone Cardiospermum Cream" for skin ailments such as swelling, scaling, blisters/vesicles, burning and pain. These products are supported by the various claims concerning the many medicinal properties of balloon vine (11).

Therefore the present investigation of *Cardiospermum halicacabum* L. leaves is taken up to establish pharmacognostic profile of the leaves which will help in crude drug identification as well as in standardization of the quality and purity.

MATERIALS AND METHODS

Fresh leaves of *Cardiospermum halicacabum* were collected from Haji Malang (Kalyan, M.S., India), washed under running tap water and blotted dry for further studies. Herbarium of *Cardiospermum halicacabum* was prepared

and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The leaves were dried in preset oven at 40 ± 2°C for about two weeks, ground into powder and used for further analysis. Physicochemical constants such as total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described by Mukherjee (12). Preliminary phytochemical analysis of powdered leaf was performed as described by Khandelwal (13) and Kokate (14). Phytochemical analysis was carried out using Thin Layer Chromatography as described by Wagner and Bladt (15). Fluorescence analysis was conducted using methods of Kokoski (16) and Chase and Pratt (17).

RESULTS

Macroscopic Characters

(Plate 1 A, 1 B)
Color: Green.

Size and Shape: Petiole: 1.7–3.4 cm long, ultimate segments of the leaves 2.7–4.0 cm in length, lanceolate.

Texture: Sparsely pubescent.

Extra features: Leaves alternate and compound, deltoid, 2-ternate, serrate, very acute at the apex and narrowed at the base.

Microscopy

Surface preparation of leaves revealed presence of covering unicellular trichomes and anomocytic type of stomata (Plate 1 G, 1 H).

Transverse section of leaf

It is dorsiventral leaf. Following tissues are present in midrib and lamina.

Midrib

The midrib is broadly hemispherical on the abaxial side with short lump on the adaxial side. Covering unicellular trichomes are present on either sides of midrib. Inverted vascular bundle (i.e., xylem towards the dorsal surface and phloem towards the ventral surface) is the characteristic of the leaf (Plate 1 C).

Lamina

The lamina of the leaf 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 covering unicellular trichomes. Mesophyll is differentiated into 1–2 layers of palisade followed by 3–4 layered spongy parenchyma. Lower epidermis consists of single layer of rectangular cells (Plate 1 D).

Transverse section of petiole

Transverse section of petiole shows two prominent grooves towards upper side whereas the lower side is round. The epidermis is composed of single layer of cells. Few trichomes are observed on epidermal cells, which are identical with that of leaf. Chlorenchymatous hypodermis is present below grooves. 4–6 vascular bundles are present in the ground tissue. Each vascular bundle is collateral. The xylem is found towards upper side and the phloem lies towards lower side. The remaining portion of the ground tissue is composed of parenchyma (Plate 1 E).

Transverse section of rachis

Transverse section of rachis is polygonal in outline with two winged projections at the upper side. Epidermis is single layered and is composed of horizontally flattened cells. The cells are compactly arranged and showed presence of cuticle. Covering unicellular trichomes are present on epidermal cells. The peripheral layers in the ground tissue are composed of collenchymatous and chlorenchymatous cells. These cells occur as bands and they alternate with each other thus forming a continuous layer next to epidermis. This forms the hypodermis. A continuous ring of pericyclic fibers is present in the ground tissue. Vascular bundles are arranged in a ring in the ground tissue and are collateral. The rest of ground tissue is parenchymatous (Plate 1 F).

Powder characteristics
Preliminary examination of powder

Leaf powder is green in color with characteristic odor and smooth texture.

Microscopic examination of powder

The various diagnostic characteristics of powdered leaf are shown in Table -1.

Table 1: Microscopical features of the powdered leaves of Cardiospermum halicacabum L.

No.	Observations	Plate 1
1.	Covering unicellular trichomes.	I
2.	Spiral thickening	J
3.	Leaf surface showing stomata	K
4.	Fibers	L

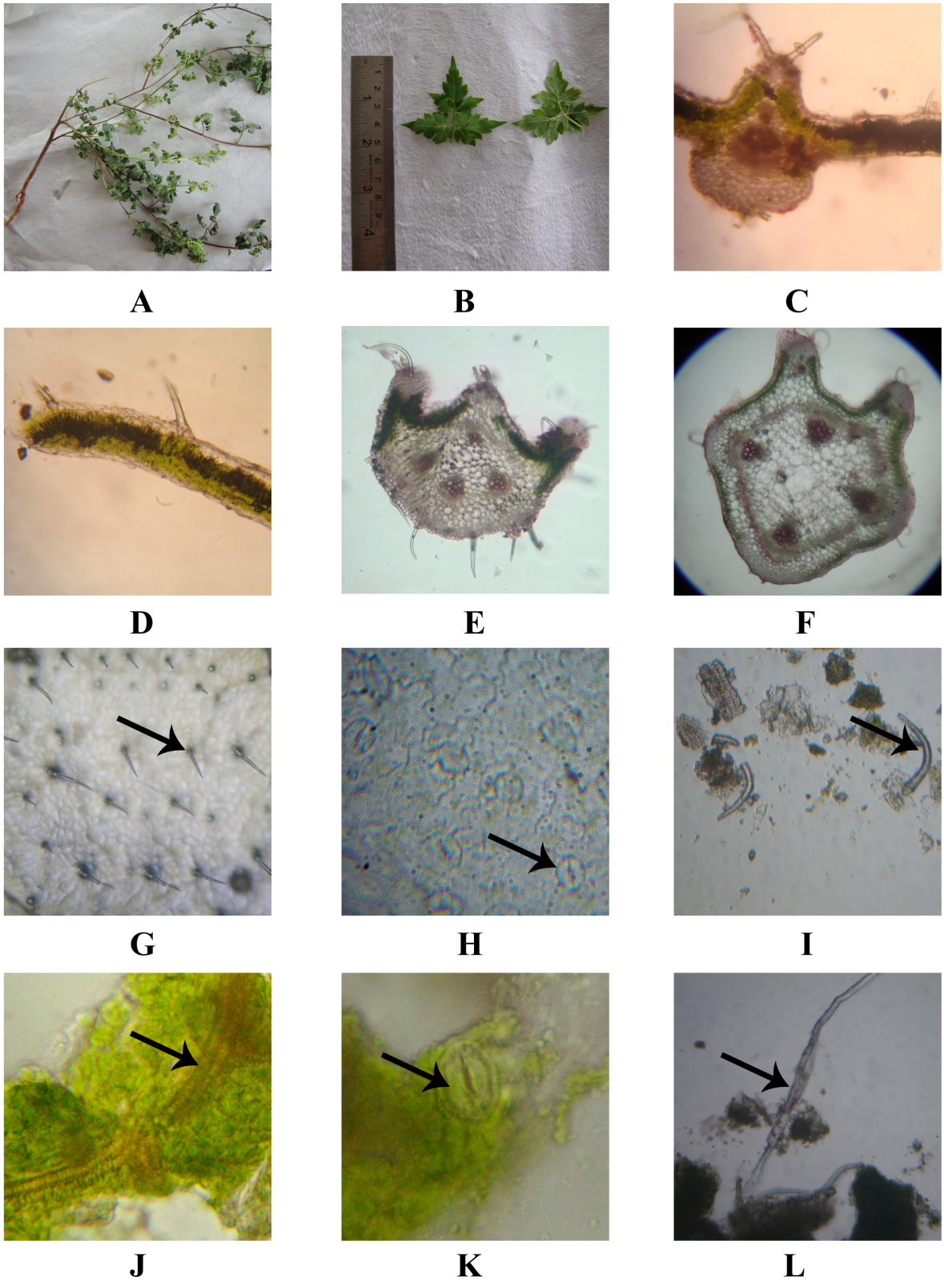

Plate No.1: Macroscopic, microscopic and powder characteristics of *Cardiospermum halicacabum* Linn.

Table 2: Quantitative leaf microscopy of Cardiospermum halicacabum L.

No.	Parameter	Value
1.	Stomatal index	33.6
2.	Number of Stomata	20
3.	Vein islet number	17
	Stomatal size	
4.	Length (µm)	15.6 – 20.8
	Breadth (µm)	7.8 – 13

Table 3: Physico-chemical studies of Cardiospermum halicacabum L. leaves

No.	Parameter	Observation
1.	***Ash values***	
	a. Total ash content (%)	12.9
	b. Acid insoluble ash (%)	1.5
	c. Water soluble ash (%)	4.3
2.	***Extractive values***	
	a. Water soluble extractive values (%)	18.08
	b. Alcohol soluble extractive values (%)	19.84
3.	***Loss on drying (%)***	56.18

Quantitative determination

The number of stomata, vein islet number, measurement of stomatal index and size of stomata were done with the help of calibrated ocular micrometer. Values are tabulated in Table-2.

Table 4: Preliminary phytochemical screening of Cardiospermum halicacabum L. leaves

No.	Tests for Phytoconstituents	WE	AE	CE
1.	Carbohydrate	+	+	+
2.	Proteins	+	–	–
3.	Amino acid	+	–	–
4.	Saponins	+	–	–
5.	Tannins	+	+	+
6.	Hydrolysable Tannins	–	–	+
7.	Flavanoid	+	–	–
8.	Steroid	–	+	+
9.	Glycosides	+	–	–
10.	Cardiac glycosides	+	+	+
11.	Anthraquinone	–	–	–
12.	Volatile oil	–	–	–

WE: Water Extract, AE: Alcohol Extract, CE: Chloroform Extract, + : Present, – : Absent

Physico-chemical Parameters

The data on ash values are indicative of the purity of drug, extractive values are representative of the presence of polar or non-polar compounds and loss on drying value also indicates that where the drug is safe regarding any growth of bacteria, fungi and yeast (18). Loss on drying, percentage of total ash, acid insoluble ash, water soluble ash and different extractive values are tabulated in Table-3.

Phytochemical Evaluation

Preliminary phytochemical screening is tabulated in Table-4.

Table 5: Chromatographic result of Cardiospermum halicacabum L. leaf extract:

No.	Compound	Extract	Number of Spots	Rf value
1.	Arbutin	Methanolic	4	0.05, 0.13, 0.44, 0.50
		Aqueous	2	0.05, 0.11
2.	Cardiac glycoside	Methanolic	5	0.11, 0.26, 0.62, 0.75, 0.91
		Aqueous	1	0.15
3.	Essential oil	Methanolic	9	0.15, 0.26, 0.29, 0.39, 0.42, 0.53, 0.57, 0.89, 0.94
		Aqueous	–	–
4.	Bitter principle	Methanolic	5	0.16, 0.30, 0.42, 0.68, 0.77
		Aqueous	2	0.21, 0.47
5.	Pungent principle	Methanolic	5	0.52, 0.63, 0.76, 0.84, 0.90
		Aqueous	–	–
6.	Anthracene	Methanolic	4	0.25, 0.53, 0.58, 0.93
		Aqueous	1	0.28
7.	Saponin	Methanolic	3	0.29, 0.48, 0.85
		Aqueous	1	0.57

Table 6: Fluorescence analysis of Cardiospermum halicacabum L. leaves

No.	Treatment	Observation under		
		Ordinary light	UV light	
			254 nm	366 nm
1.	Powder as such	Green	Green	Green
2.	Powder + Nitrocellulose	Green	Green	Green
3.	Powder + 1N NaOH in methanol	Green	Green	Green
4.	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Green	Dark brown	Brown
5.	Powder + 1N HCl	Brownish green	Black	Dark brown
6.	Powder + 1N HCl + Nitrocellulose in amyl acetate	Green	Black	Reddish brown
7.	Powder + 1N NaOH in water	Green	Black	Brownish green
8.	Powder + 1N NaOH in water, dried and mounted in Nitrocellulose in amyl acetate	Dark green	Black	Brownish green
9.	Powder + HNO_3 (1:1)	Brownish green	Brown	Brown
10.	Powder + H_2SO_4 (1:1)	Dark green	Dark brown	Brownish green

Results for TLC are tabulated in Table-5.

Color reaction of powdered drug with different reagents and their fluorescence analysis were studied and recorded in Table-6.

DISCUSSION

The diagnostic microscopic features of leaf are presence of covering unicellular trichomes and anomocytic stomata. The powder microscopy of leaf also showed presence of unicellular trichomes, stomata, spiral thickening and fibers. Presences of covering unicellular trichomes, collenchyma, chlorenchyma, collateral vascular bundle are few of the important characteristics of the petiole and rachis.

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs i.e. presence or absence of foreign inorganic matter such as metallic salts and / or silica (19).

In present study, the stomatal index and number of stomata was found to be 33.6 and 20 respectively. Vein islet number was found to be 17. The total moisture content was found to be 56.18%, along with total ash 12.9 %, of which, 1.5 % is acid insoluble ash, and 4.3 % is water soluble ash. The extractive values were found to be 18.08% and 19.84% for water and alcohol respectively.

Thin Layer Chromatography revealed that methanol gives better extraction of the phytochemicals than water since the methanolic extract resolved into maximum number of bands as compare to aqueous extract. The pharmacognostical study is one of the major criteria for identification of plant. The present study on the pharamcognostical characteristics of *Cardiospermum halicacabum* L. leaves will provide useful information for its correct identity and may enable those who handle this plant to maintain its quality control. In addition the results of the present study could be useful for preparation of a monograph of the plant.

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Standardization of Vaisvanara Churna: A Poly-herbal Formulation

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ABSTRACT

Standardization of herbal formulations is essential in order to asses the quality of drugs for therapeutic value. The World Health Organization (WHO) in 1999, has given a detail protocol for the standardization of herbal drugs comprising of a single content, but very little literature is available for the standardization of poly-herbal drugs. We have developed a simple scheme for the standardization and authentification of Vaisvanara Churna comprising of four botanical ingredients. Two samples from different manufactures were procured and subjected to various physicochemical analyses, HPTLC fingerprinting and botanical characterization along with in-house formulation using authentic ingredients as controls. The set parameters were found to be sufficient to evaluate the Vaisvanara Churna and can be used as reference standards for the quality control/quality assurance study.

Keywords: Standardization, Quality tests, Traditional medicine, Physicochemical parameters

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INTRODUCTION

Quality assurance of herbal products may be ensured by proper quality control of the herbal ingredients and by means of good manufacturing practice. Some of herbal products have many herbal ingredients with small amount of individual herb being present. Assuring the quality of Ayurvedic medicines was traditionally the responsibility(1) of the physician who prepared the medicine himself and maintained a fiduciary relationship with the patient. Even though the Drugs and Cosmetics Act brought Ayurveda under its purview in 1964, individual physicians require no license to prepare medicines and administer them to patients even today. The Act included 56 classical texts and gave approval to medicines prepared according to their directions besides specifying the provisions of GMP for Ayurveda. Subsequently three volumes of Ayurvedic Formulary were brought out by an official committee of the Ministry of Health, which listed 635 formulations of which 431 had pharmacopoeial standards specified. Between 1999–2001 the Ayurvedic Pharmacopoeia of India was published in three volumes, which gave the botanical identity of plants, composition, analytical procedures, etc. In spite of the efforts made for the standardization of Ayurvedic medicines, major problems remain because the Formulary lists only 635 whereas the herbal medicines in actual use are believed to be at least 1000 with many regional variations. The absence of post-market surveillance and the paucity of test laboratory facilities also make the quality control of Ayurvedic medicines exceedingly difficult at this time(2).

Therefore, an attempt has been made to standardize Vaisvanara Churna, an Ayurvedic compound formulation as prescribed in Ayurvedic Formulary(3), used in flatulence, Rheumatism and Heart disease.

The individual plant powders of the formulation were subjected to various pharmacognostical parameters. Three formulation, one in-house preparation and two samples from different manufactures were procured and subjected to various physicochemical analysis, TLC and HPTLC fingerprinting and botanical characterization using authenticate ingredients as controls.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used in the experiment were of analytical grade. Menthol, Cavacol, Zingiberine and Galic acid were

purchased from Sigma Aldrich, USA. All the solvents used in the experiment were procured from Merck Specialities Pvt. Ltd, Mumbai, India.

Apparatus

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttenz, Swizerland)

Syringe: 100µL Hamilton (Bonadug, Swizerland)

TLC chamber: Glass twin trough chamber (20 × 10 × 4).

Densitometer: TLC scanner 3 with CATS software; CAMAG

HPTLC Plate: 20 × 10cm, 0.2 precoted with silica gel 60F_{254}; Merck

pH meter: Elico Ltd., Hyderabad, India.

Flame Photometer: Digital Biomed Flame Photometer, Hyderabad.

Muffle furnace: Dolphin Industries Ltd., Mumbai.

Plant mateials

Vaisvanara Churna consists of *Terminaila chebula (Pericarp), Trachyspermum ammi (fruit), Apium leptophyllum (fruit), Zingiber officinalie (Rhizome) and*, Rock salts. All these ingredients were procured from the local market of Jeypore, Koraput, Odissa, India and all the plant material were authenticated by Mr. S.R. Dash H.O.D Dept of Botany Vikram Dev College Jeypore, Koraput Odisa. Voucher specimens of the same have been deposited in the museum of Dept. of Pharmacognosy, Jeypore College of Pharmaceutical Sciences for future reference.

Preparation of Vaisvanara Churna

In-house formulation of Vaisvanara churna was prepared as per Ayurvedic Formulary of India. All ingredients are taken and roasted in a stainless steel pan at a low temperature till it becomes free from moisture. The ingredients are powdered individually in a pulverizer and pass through 80# sieve. Each ingredients *Terminaila chebula* (174.9gm), *Zingiber officinalie* (72.91gm), Rock Salt (29.16gm), *Apium leptophyllum* (43.74gm), *Trachyspermum ammi* (29.16gm) were weight separately, mixed together to obtain a homogeneous blend.

Marketed samples

The marketed samples of various brands of Vaisvanara Churna i.e. Dindayal (D) and Srinivas(S) and the in-house preparation(I) were standardized based on their oganoleptic charecters, physical characteristics and physicochemical properties.

Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. The organoleptic characters of the samples were carried out based on the method described by Siddique et. al(4).

Microscopic Study

Individual microscopic analysis(5) of each ingredients of the formulation along with in-house formulation (I) and the marketed formulations were carried out to by classical pharmacognostical methods. The authenticity of the individual ingredients was confirmed by comparison of their power characteristics with those given in the literature.

Physicochemical Investigation
Loss on drying

Loss on dying is the loss of mass expressed as percent w/w. About 10g of dug samples of each formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105°C for 5hrs. Percentage was calculated with reference to initial weight.

Determination of pH

The pH of different formulations(6) in 1% w/V and 10% w/V of water soluble portions was determined using standard glass electrode at 24°C according to the prescribed standard method in Indian Pharmacopoeia.

Determination of total ash(7)

Ashing involves an oxidation of the components of the products. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the formulation for marketing. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and nonphysiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material was placed in a suitable tared crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.

Acid insoluble ash

The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter

was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Determination of solvent Extractive values(7)
Alcohol soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent.25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Water soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Fluorescence analysis(4)

The power samples were exposed to ultraviolet light at wavelength of 254nm and 366nm after being treated with different reagents. One mg of powdered drugs of each formulation were placed on a micro slide and observed under UV 366, UV 254 and in daylight to observe the fluorescent characteristics of the powder, if any. One mg of the powdered drugs of each formulations were placed on a micro slide and treated with 1N HCl and observed under UV 366, UV 254 and in daylight while wet. One mg of the powdered drugs of each formulation were placed on a micro slide and treated with 1N NaOH and 1 N NaOH in methanol each and observed after a few minutes in daylight, under UV 254 and UV 366. One mg of the powdered drugs of each formulation were placed on a micro slide and treated with 50% KOH and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drugs of each formulation were placed on a micro slide and treated with 50% H_2SO_4 and conc. H_2SO_4 each and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drugs of each formulation were placed on a micro slide and treated with 50% HNO_3 and conc. HNO_3 each and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drugs of each formulation were placed on a micro slide and treated with iodine water and observed under UV 366 and UV 254 and in daylight while still wet.

Determination of physical Characteristics(8,9)
Bulk density and Tap density

The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (D_b) is:

$$D_b = M/V_b$$

Where M is the mass of the particles and V_b is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle of repose

Angle of Repose has been used as an indirect method of quantifying powder flowability; because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel.

Tan α = H/R or α = arc tan H/R

Where α is the angle of repose, R being the radius of the conical pile.

Hausner ratio

It is related to interparticle friction and as such can be used to predict the powder flow properties. Powders with

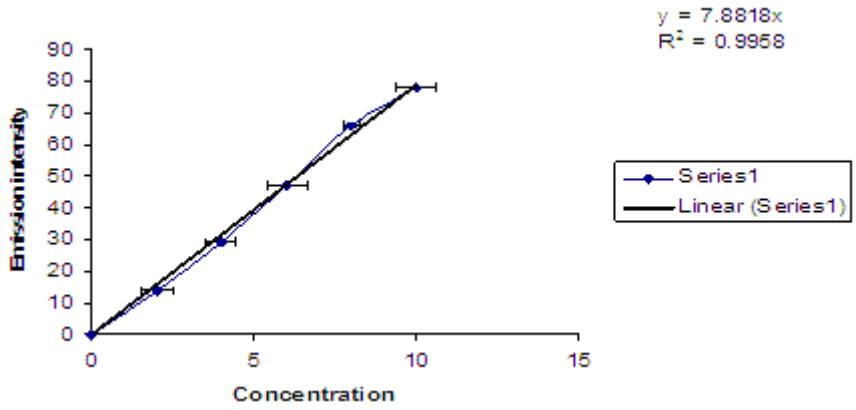

Figure 2: *The standard graph for sodium*

low interparticle friction such as coarse spheres, have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner ratio greater than 1.6. The equation for measuring the Hausner ratio is: Df / Do, where Df = Tapped density and Do = Bulk density.

Carr's index

Another indirect method of measuring the powder flow from bulk density is Carr's index. The equation for measuring Carr's index is: % compressibility = (Df-Do/Do) × 100 where Df = Tapped density and Do = Bulk density

Estimation of sodium contents(10,11)

Sodium content was estimated by flame photometry by using a flame photometer. A stock solution of NaCl 100µg/ml was prepared in distilled water and further dilutions were made to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml respectively for preparing the standard graph (Figure 2). Sodium contents of the formulations were estimated by flame photometric method based on the measurement of emission intensity. The method was validated for linearity, precision and accuracy. The method obeyed Beer's law in the concentration range 1–10 µg/ml. 10 g of the powdered sample was shaken with 100 ml water in a mechanical shaker for 20 min, filtered and used for determination of the unknown concentrations in the different samples.

HPTLC finger printing profile

HPTLC study(12) of methanolic extracts of the individual ingredients, in-house formulation and marketed formulations were carried out along with

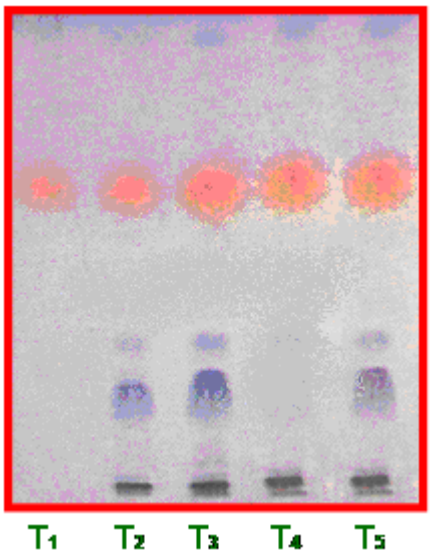

Figure 3a: *TLC finger pint profile of Trachyspermum ammi and Vaisvanara Churnas* Track 1: Thymol; Track 2: Vaisvanara Churna (Formulation I); Track 3: Vaisvanara Churna (Formulation D); Track 4: *Trachyspermum ammi*; Track 5: Vaisvanara Churna (Formulation S); Solvent System: Toluene: Ethyl acetate(9.3:0.7)

the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. For HPTLC, 2gm of each sample (Formulation-I, D and S) was extracted with 25ml of methanol on boiling water bath for 25minutes consecutively three times using fresh potion of 25ml methanol, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre coated silica gel aluminium plate 60F–254 (10cm× 10cm with 250µm thickness) using Camag

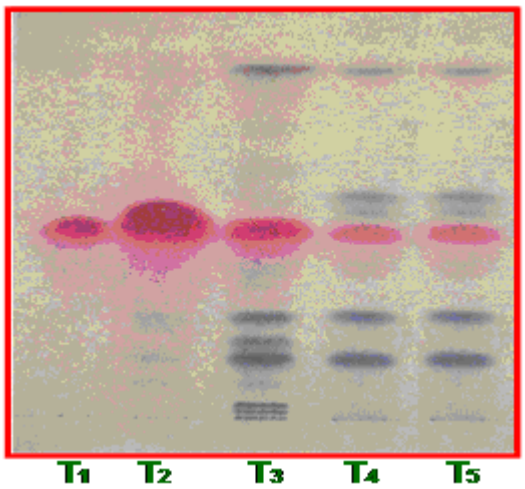

Figure 3b: *TLC finger print profile of Apium leptophyllum and Vaisvanara Churnas*
Track 1: Carvacrol; Track 2: *Apium leptophyllum*; Track 3: Vaisvanara Churna (Formulation I); Track 4: Vaisvanara Churna (Formulation D); Track 5: Vaisvanara Churna (Formulation S); Solvent System: Toluene: Ethyl acetate(9.3:0.7)

Figure 3d: *TLC finger print profile of Terminalia Chebula and Vaisvanara Churnas*
Track 1: Gallic acid; Track 2: Vaisvanara Churna (Formulation I); Track 3: Vaisvanara Churna (Formulation D); Track 4: Vaisvanara Churna (Formulation S); Track 5: *Terminalia Chebula*; Solvent System: Chloroform: Ethyl acetate: Formic acid (2.5:2:0.8)

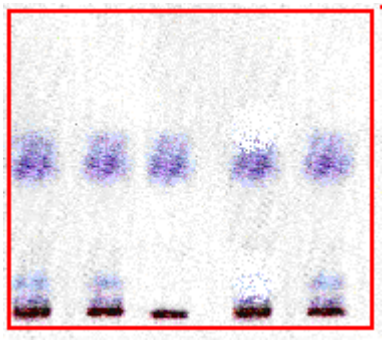

Figure 3c: *TLC finger pint pofile of Zingiber officinale and Vaisvanara Churnas*
Track 1: Vaisvanara Churna (Formulation I); Track 2: Vaisvanara Churna (Formulation D); Track 3: Zingiberine; Track 4: *Zingiber officinale*; Track 5: Vaisvanara Churna (Formulation S); Solvent System: Toluene: Ethyl acetate (9.3:0.7)

Linomat IV sample applicator and 100µl Hamilton syringe. The samples, in the form of bands of length 5mm, were spotted 15mm from the bottom, 10mm apart, at a constant application rate of 15nl/s using nitrogen aspirator. Plates were developed using mobile phase consisting of toluene-ethyl acetate and chloroform-ethyl acetate-formic acid. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/reflectance mode at 254nm and 365nm. The TLC finger print profiles are reported in Fig 3a–e. The HPTLC finger print profiles of the formulations are presented in Fig 4a–c.

RESULT AND DISCUSSION

In house formulation was prepared in accordance with the Ayurvedic Fomulay of India. As part of standardization procedure, the finished product Vaisvanara Churna was tested for relevant physical and chemical parameters along with samples from two different manufacturers, D and S for a comparative study.

All the samples were brown in color, smooth powder, having characteristic odor, possessing pungent/salty taste. The organoleptic properties of the marketed formulations and the in-house formulations were found to be comparable (Table 1) and variation was insignificant.

Microscopic examination was carried out for individual ingredients present in the formulation along with different Vaisvanara Churna to see the presence of *Trachyspermum ammi, Apium leptophyllum, Terminalia chebula, Zingiber officinal*, in formulation of churna (Formulation I) (fig.1). The Glandular hairs, trichomes and elongated cell containing aleurone grain and oil droplets indicated the presence of *Apium leptophyllum*, Vessels and vessel with parenchymatous cells indicated the presence of *Zingiber oficinale*, Criss cross fibers and fibers with peg like out growth indicated the presence of *Terminalia chebula*,

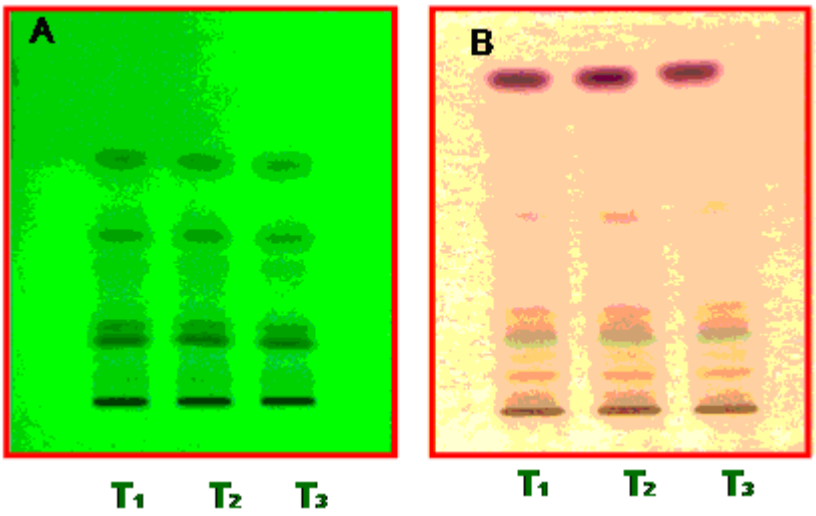

Figure 3e: *TLC finger pint profile of different Vaisvanara Churnas (A-365nm and B254-nm)*
Track 1: Vaisvanara Churna (Formulation I); Track 2: Vaisvanara Churna (Formulation D); Track 3: Vaisvanara Churna (Formulation S); Solvent System: Toluene: Ethyl acetate (5:1)

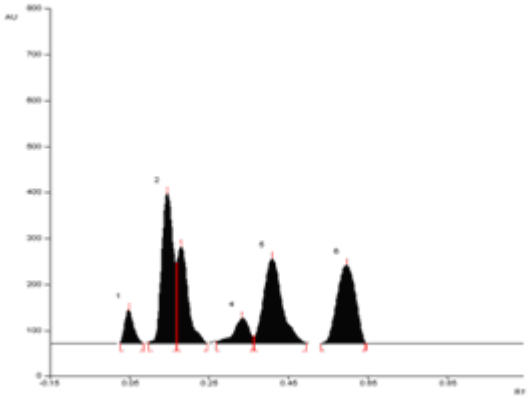

Figure 4a: *HPTLC fingerprinting of Vaisvanara Churna (Fomulation I)*

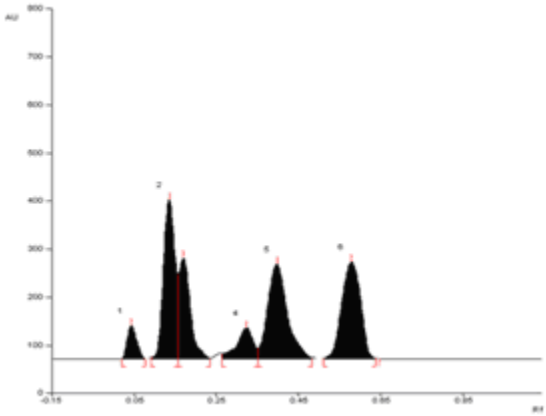

Figure 4c: *HPTLC fingerprinting of Vaisvanara Churna (Fomulation S)*

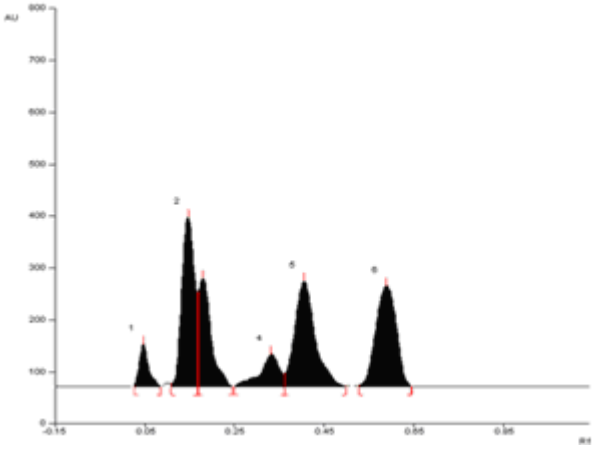

Figure 4b: *HPTLC fingerprinting of Vaisvanara Churna (Fomulation D)*

Unicellular warty trichomes and vittae indicated presence of *Trachyspermum ammi*. The microscopic examination of marketed formulations also represents same microscopic characters as that of in-house formulation therefore not represented.

Quality tests for different Vaisvanara Churna and its individual ingredients were performed for moisture content, ash content, water soluble extractive, methanol soluble extractive, acid insoluble ash and water insoluble ash, and were found to be within standard ranges(13). Water soluble and alcohol soluble extractive values of individual ingredients of churna are given in table 2. The ash values(14) of the samples were carried out based on

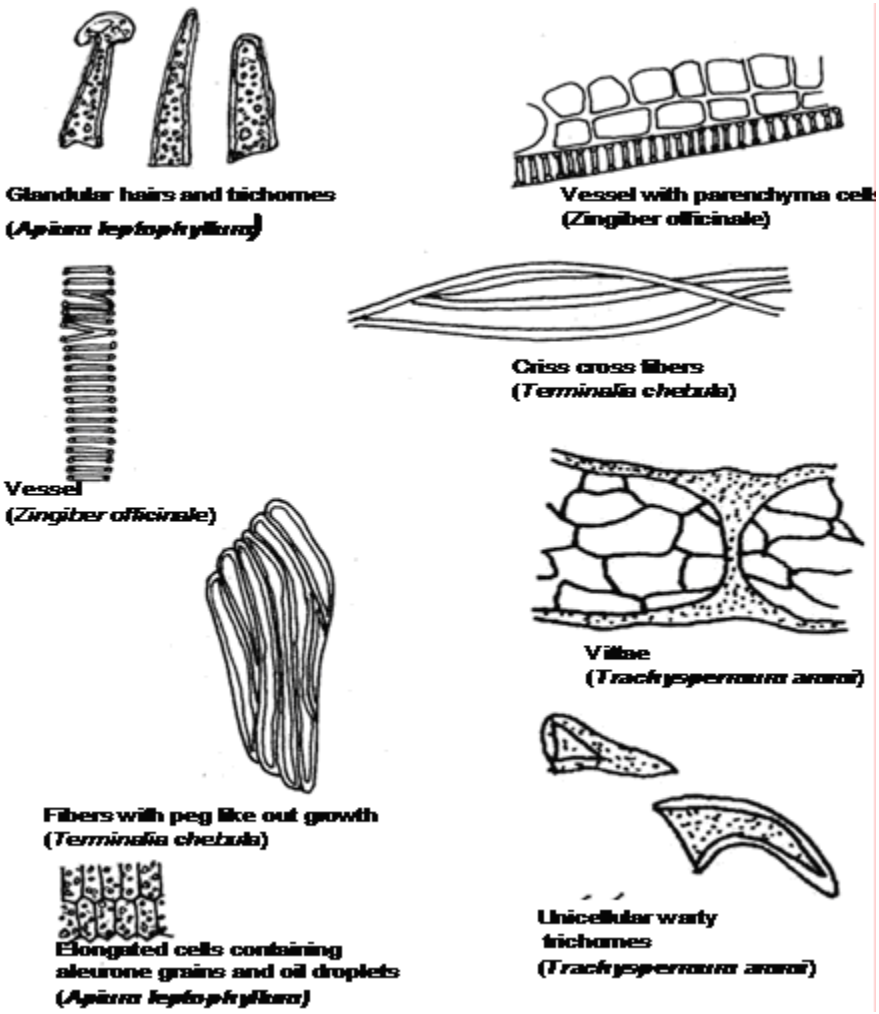

Figure 1: *Microscopic study of Vaisvanara Churna*

Table 1: Organoleptic properties of different Vaisvanara Churna formulation

Different Formulation	Appearance	Color	Taste	Odor
In-House(I)	Powder	Light brown	Astringent/Pungent/Salty	Characteristic
Shrinivas(S)	Powder	Brown	Pungent/Salty	Characteristic
Dindayal(D)	powder	Creamish brown	Pungent/Salty	Characteristic

Table 2: Physicochemical analysis of individual ingredients present in Vaisvanara Churna

Parameters (In %)	*Trachyspermum ammi*	*Apium leptophyllum*	*Terminalia chebula*	*Zingiber officinale*
Water soluble extractive	42±0.326599	8±0.22509	26±0.716705	20±0.33466
Alcohol soluble extractive	17.9±0.8024	24±0.700393	37.9±1.07269	16.2±0.7966
Total ash values	8.62±0.29	8.14±0.76	4.66±0.20	5.57±0.26
Acid insoluble ash	0.49±0.02	10±0.38	4.43±0.24	1.41±0.18
Water soluble ash	9.2±0.12	15.67±0.88	18.29±1.54	24.35±1.71

Table 3: Physicochemical analysis of Vaisvanara Churna formulations

Parameters (In %)	In-house formulation(I) Mean(n=6)±SD	Formulation S Mean(n=6)±SD	Formulation D Mean(n=6)±SD
Water soluble extractive	44±0.320936	38±0.76528	50±0.312517
Alcohol soluble extractive	27.9±0.7250	14±0.41087	14.01±0.6346
Total ash values	14.9±0.35	13.4±0.33	16±0.19
Acid insoluble ash	1.6±0.13	1.2±0.22	1.49±0.19
Water soluble ash	8.28±1.23	7.68±0.67	8.89±0.76
pH of 1% w/v solution	7.12±0.34	6.67±0.11	7.04±0.17
pH of 10% w/v solution	7.08±0.67	6.59±0.21	7.13±0.21
Loss on drying (w/w)	4.34±1.09	3.41±0.76	2.86±0.92

Table 4: Powder fluorescence test of different Vaisvanara Churna formulations

Material	I			S			D		
	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm
Powder as such	LY	BR	FY	PY	BR	PY	P.Y	BR	PY
In NaOH(1N) in H_2O	Y	GY	Y	Y	GY	Y	O	LY	Y
P + In HCl (1N)	YB	GY	G	YB	Y	GB	PY	GY	G
P + In NaOH (1N) in MeOH	YG	BR	PG	YG	GY	PG	YG	GY	PG
P + 50% KOH	YB	GY	FB	Y	GY	FB	YB	Y	FB
P + 50% H_2SO_4	YB	Y	LG	LB	GY	BG	PY	Y	G
P + 50% HNO_3	ReB	Y	FB	ReB	GY	FB	ReY	GY	FB
P + Conc. HNO_3	ReB	Y	FB	Y	GY	BR	Re	GY	FB
P + Conc. H_2SO_4	YB.	GY	G	YB	GY	FB	YB	GY	LG
P + Iodine in H_2O	BL	BR	FB	BL	BR.	FB	BL	BL.	BL

BL: Black, BR: brown, PY: Pale yellow, Y: yellow, G: Green, LG: Light green, LY: Light yellow, GY: Greyish yellow, FY: Fluorescencent yellow. F.B.: Fluorescencent blue, Re: red, GB:Greenish brown, LB:Light brown, , YB:Yellowish brown, BG:Brownish green, ReB:Reddish brown, ReY:Reddish yellow, YG: Yellowish green, PG:Pale green. P: Powder.

Table 5: Physical characteristic of different Vaisvanara Churna formulations

Parameters	Formulation-I Mean(n=6)±SD	Formulation-S Mean(n=6)±SD	Formulation-D Mean(n=6)±SD
Tap density	0.5±0.02	0.526±0.01	0.625±0.002
Bulk density	0.32±0.03	0.408±0.01	0.416±0.01
Angle of repose	47±0.26	48.2±0.28	46.33±0.08
Hausner ratio	1.42±0.09	1.25±0.02	1.4±0.05
Carr's index	15.62±0.61	12±1.8	13±0.73

Table 6: Estimation of sodium in sample

Different formulations	Sodium content (%)
In house formulation(I)	4.69
Shrinivas(S)	4.82
Dindayal(D)	5.45

the method as described by world health organization (WHO) guidelines for medicinal plant materials.

The physicochemical comparisons between in house formulation and marketed formulations are given in Table 3. Variations were observed in most of the physicochemical parameters studied. The total ash value of formulation I was found to be higher than that for S and D. Similarly, the acid insoluble ash values were also relatively higher in case of I. Acid insoluble ash value for in house formulation(I) was found to be 1.6±0.13 and in case of marketed formulation S and D this was found to be 1.2±0.22 and 1.49±0.19 respectively. On the contrary, water soluble ash percentage of I and D were comparable except for S, which was comparatively low. The extractive values of formulations in water were found to be much higher than alcohol extractive values. However values of I (27.9±0.72) in alcohol is more when compared to S (14±0.41) and D (14.01±0.63). The extractive values in water was found to be different for I, S and D. These variations may be due to variation in the quality of raw materials used, their season of collection and storage time. Loss on drying at (105°C) and pH of 1% w/v and 10% w/v aqueous solution are also presented in Table 3. pH of 1% and 10%w/v solution revealed that the formulations are nearly neutral. In Table 4 the fluorescent analysis of different Vaisvanara Churna formulation have been reported.

The physical characteristics of the in house formulation (I) and two market formulations (average value along with standard deviation) are shown in Table 5. The results of the market formulations and in house formulation were found to be comparable. The flowability of the formulation was found to be poor in both market formulation and in house formulation, which was further confirmed by high values of Hausner ratio and Carr's index. For the estimation of sodium by flame photometer the emission intensity of different concentrations are presented in table 6. The sodium content was found to be less in in-house formulation (4.69%) and highest in Dindayal formulation (5.45%).

In fig 3a a band (R_f 0.69) corresponding to thymol (Tack 1) was observed for *Trachyspermum ammi* and different Vaisvanara Churna indicating the presence of *Trachyspermum ammi* in all formulations In fig 3b the band (R_f 0.5) corresponding to carvacrol which is the active constituent of *Apium leptophyllum* indicate the presence of *Apium leptophyllum* in the formulations. Similarly bands at 0.9 (zingiberin) and 0.56 (gallic acid) indicates the presence of *Zingiber oficinale* and *Terminalia chebula* in the formulations. When the TLC plates of the formulations were observed at 254 and 365nm, uniformity in the preparation of the formulations was observed. HPTLC fingerprint profile of the Vaisvanara Churna formulations

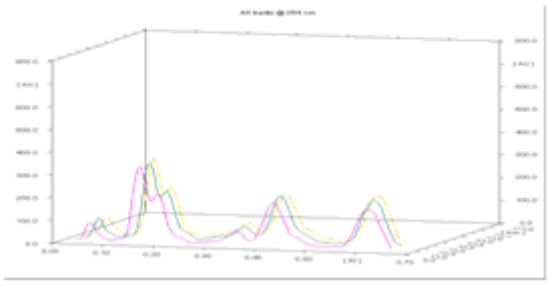

Figure 5: *3D view of the components of Vaisvanara Churna*

are depicted in figure 4a–c indicates the presence of all the ingredients in proportional quantity in the formulations. The 3D view of the samples of Vaisvanara Churna (I) represented in fig 5 also proves the above fact.

CONCLUSION

Ayurvedic medicine Vaisvanara Churna has been standardized by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. Pharmacognostic characters established for the raw materials could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis. Purity and potency of the materials and formulations following the procedure given could be performed in QC/QA laboratory of pharmaceutical house.

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Standardization of "Chopchiniyadi Churna": An Ayurvedic polyherbal formulation

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Abstract

Standardization of herbal formulations is essential in order to assess the quality, purity, safety and efficacy of drugs based on the amounts of their active principles. The present research work is an attempt to standardize "Chopchiniyadi Churna" an ayurvedic polyherbal formulation used in the treatment of rheumatism, epilepsy, anthelmintic, malignant ulcers and some diseases of the central nervous system. Three marketed and one in-house formulations were used for the study. All the formulations were standardized on the basis of organoleptic characters, physical characteristics and physico-chemical properties. The set parameters were found to be sufficient to evaluate the churna and can be used as reference standards for the quality control/quality assurance purposes.

Keywords: Chopchiniyadi churna (CC), Polyherbal formulation, Quality Standards.

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INTRODUCTION

Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles. (1). It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical locations. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of herbal or Ayurvedic medicines so as to ensure quality control and quality assurance. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.(2)

In the present research work, an attempt was made to standardize *Chopchiniyadi churna* a polyherbal formulation made up of ten herbs (Table 1) used in the treatment of rheumatism, diaphoretic, anthelmintic, stimulant, syphilis, gout, seminal-weakness, epilepsy, skin diseases, joint pain, malignant ulcers and nervous system disorders as per WHO guidelines.(3)

MATERIALS AND METHODS

Collection of herbs and preparation of formulations.

The crude drugs used in preparation of chopchiniyadi churna were collected from local market, Indore and identified in Department of Botany, Government Agriculture College, Indore. One in house formulations was prepared, as per the procedure mentioned in Ayurvedic text "Ayurveda Sar Sangrah" and three marketed formulations of different manufacturers were procured from the market. A voucher specimen, SCOPE/Phcog/07-09/07(a-i) has been retained in the museum of our department for further references. All plant parts were then dried in shade, powdered and passed through sieve no. 85 # and lastly packed in a well closed container to protect them from moisture

Table 1: Ingredients of *Chopchiniyadi chuna*

Botanical name	Common Name	Family	Part Used
Smilax china	Chopchini	Liliaceae	Roots
Piper longum	Pippali	Piperaceae	Fruits
Piper longum	Pippali	Piperaceae	Roots
Syzygium aromaticum	Laung	Myrtaceae	Flower buds
Piper nigrum	Black Pepper	Piperaceae	Fruits
Anacyclus pyrethrum	Aakaarkarabha	Compositae	Roots
Zingiber officinale	Sunthi	Zingibaraceae	Rhizomes
Emblica ribes	Vidanaga	Myrsinaceae	Fruits
Hyoscyamus niger	Khurasani Ajwain	Solanaceae	Seeds
Cinnamomum zeylanicum	Dalchini	Lauraceae	Bark

The coding of the marketed and in-house formulations was done as follows:

Coding of formulations.

CC 1 - Dabur Pvt. Ltd

CC 2 - Baidyanath Pvt. Ltd

CC 3 - Vyas Pharmaceuticals

CC 4 - In-house formulation

Botanical Parameters (4)

Organoleptic evaluation was carried out to assess the color, odor and taste of the marketed and in-house formulations.

Physico-chemical investigations (5, 6)

Physico chemical investigations of the formulations carried out including determination of extractive values, ash values and loss on drying by I R moisture balance.

Determination of pH (7)

The pH of different formulations in 1% w/v and 10% w/v of water soluble portions were determined using pH paper (Range 3.5–6) and (6.5–14) with standard glass electrode.

Estimation of crude fibre content (8)

2 gm of drug was taken in a beaker and 50ml of 10% nitric acid was added. It was heated to boil with stirring (30 sec.). This was strained through fine cloth on a buchner funnel. The residue was washed with boiling water and transfered to a beaker. 50ml of 2.5% v/v sodium hydroxide solution was added. It was strained and washed with hot water. The residue was transferred in a clean and dried crucible. The residue was weighed and the crude fibre content was determined.

Determination of physical characteristics of powder formulation (9, 10)

Physical characteristics like bulk density, tap density, angle of repose, Hausner ratio and Carr's index were determined for different formulations.

Phytochemical screening (11)

Active phytochemical constituents like glycosides, flavonoids, alkaloids, tannins, steroids and carbohydrates were identified in aqueous extracts of all formulations.

Quantitative estimation of tannins (12)

1 gm of powdered drug was refluxed in 100 ml of 70% aqueous acetone for 2 hours followed by filtration. The filterate was concentrated and partitioned with solvent ether (3 times) and then with n- butanol previously saturated with water. The n- butanol soluble portion was dried over water bath until constant weight. Total tannin content was calculated by formula:

$$\%\ \text{w/w total tannin content} = \frac{\text{Weight of n- butanol fraction in gm}}{\text{Weight of sample in gm}} \times 100$$

Quantitative estimation of Flavonoids (13)

1gm of powdered drug was boiled in 100 ml methanol for 1 hour followed by filtration. 1ml of filterate was placed in 10ml volumetric flask. 3ml methanol and 0.3 ml $NaNO_2$ were added in the flask. 3ml of $AlCl_3$ was added after 5min. 2ml of 1M NaOH was added and the net volume was made to 10 ml with methanol and absorbance was measured against a blank at 510nm. The total flavonoids content was calculated using following equation.

A = 0.01069c - 0.001163

A = absorbance, c = flavonoid content µg/g.

Microbial contamination (14)

The samples were tested for the presence of microorganisms like *E. coli*, *Salmonella, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Heavy metal analysis (15)

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid were added and boiled for 10 minutes. The mixture was cooled down and volume made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, copper, arsenic and mercury.

RESULT AND DISCUSSIONS

All the formulations of chopchiniyadi churna were evaluated as per WHO guidelines. In-house formulation was prepared as per procedure mentioned in Ayurved Sar Sangrah. Botanical parameters revealed that the formulations were brown to yellowish brown in color, with pleasant odor and pungent taste (Table II). The physicochemical comparisons between in-house formulation and marketed formulations are given in Table III. The results obtained with the market formulations and the in-house formulations were found to be comparable and variation was insignificant. The physical characteristics of the in-house formulation and three market formulations (average values along with standard deviation) (n = 3) are shown in Table IV. The results of the market formulations and in-house formulation were found to be comparable. The flowability of the formulation was found to be poor in both market formulations and in-house formulation, which was further confirmed by high values of Hausner ratio and Carr's index.

The results obtained for phytochemical screening reveals that Phytoconstituents like glycosides, carbohydrates, tannins and flavonoids were present in all sample (Table V and Table VI).

Various microorganisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* contaminate herbal drugs and cause serious health hazards (16). For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table VII) revealed the absence of these microorganisms thereby confirming the non toxic nature of the formulations.

Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations. However, heavy metals have been associated

Table 2: Botanical parameters of various formulations of chopchiniyadi churna

Code → Organoleptic Characters ↓	CC1	CC2	CC3	CC4
Color	Yellow Brown	Brown	Brown	Yellow Brown
Odor	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Pungent	Pungent	Pungent	Pungent

Table 3: Physical and chemical evaluation of samples of *Chopchiniyadi churna*

Parameters	CC1	CC2	CC3	CC4
Total ash value (%w/w)	1.997 ± 0.19	3.483 ± 0.07	2.617 ± 0.15	3.350 ± 0.01
Acid insoluble ash value (%w/w)	0.210 ± 0.01	0.467 ± 0.03	0.220 ± 0.01	0.403 ± 0.02
Water soluble ash value (%w/w)	0.962 ± 0.26	1.340 ± 0.21	1.330 ± 0.17	1.343 ± 0.20
Alcohol soluble extractive value (%w/w)	16.539 ± 0.170	12.703 ± 0.080	14.367 ± 0.166	12.427 ± 0.146
Water soluble extractive value (%w/w)	24.510 ± 0.100	24.733 ± 0.154	28.343 ± 0.100	26.140 ± 0.145
Loss on drying (%w/w)	0.867 ± 0.06	0.667 ± 0.06	0.667 ± 0.06	1.133 ± 0.06
pH 1 % solution (%w/v)	5.87 ± 0.03	5.77 ± 0.03	6.10 ± 0.05	5.83 ± 0.06
pH 10% Solution (%w/v)	5.27 ± 0.06	4.83 ± 0.06	4.87 ± 0.03	5.37 ± 0.03
Crude fiber (gm)	0.27 ± 0.08	0.13 ± 0.04	0.13 ± 0.04	0.37 ± 0.12

Values are expressed as Mean ± SEM (n = 3)

Table 4: Physical charaterstics of samples of *Chopchiniyadi Churna*

Code → / Parameters↓	CC1	CC2	CC3	CC4
True density gm/cm^3	0.564 ± 0.010	0.946 ± 0.010	0.650 ± 0.018	0.525 ± 0.019
Bulk densitygm/cm^3	0.392 ± 0.018	0.488 ± 0.019	0.435 ± 0.014	0.402 ± 0.021
Porosity %	88.1 ± 0.012	91.0 ± 0.026	89.3 ± 0.028	87.4 ± 0.020
Angle of Repose	42° ± 0.24	41° ± 0.31	41° ±0.28	42° ±0.37
Fluff density	0.392 ± 0.010	0.488 ± 0.014	0.435 ± 0.010	0.402 ± 0.024
Tapped density	0.577 ± 0.007	0.697 ± 0.001	0.605 ± 0.008	0.558 ± 0.007
Hausner's ratio	1.471 ± 0.005	1.428 ± 0.006	1.390 ± 0.001	1.388 ± 0.003

Table 5: Phytochemical screening of *Chopchiniyadi churna*

Parameters	CC1	CC2	CC3	CC4
Alkaloids	–	–	–	–
Glycosides	+	+	+	+
Tannins	+	+	+	+
Flavonoid	+	+	+	+
Steroids	–	–	–	–
Carbohydrates	+	+	+	+

+ indicates presence
– indicates absence

TABLE 6: Quantitative estimation Of Tannins And Flavonoids in *Chopchiniyadi churna*

Parameter	CC1	CC2	CC3	CC4
Total Flavonoid content (µg/g)	4.199 ± 0.078	1.555 ± 0.105	8.739 ± 0.126	4.283 ± 0.062
Total Tannin content (%w/w)	4.393 ± 0.141	6.330 ± 0.081	2.867 ± 0.066	4.687 ± 0.092

TABLE 8: Heavy metal analysis of marketed and in-house formulations of Chopchiniyadi Churna

Formulation code	CC1	CC2	CC3	CC4
Arsenic	–	–	–	–
Lead	–	–	–	–
Mercury	–	–	–	–
Copper	–	–	–	–

Table 7: Screening for micro-organisms in marketed and in-house formulations of Chopchiniyadi Churna

Formulation Code → / Name of Microbes↓	CC1	CC2	CC3	CC4
E.coli	–	–	–	–
Salmonella typhi	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–
Staphylococcus aureus	–	–	–	–

with various adverse effects (17) including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs (18). Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table VIII), thereby further confirming the non toxic nature of the preparation. Hence, Chopchiniyadi is a safe polyherbal formulation and is free from any toxic materials.

The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards.

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