

GC-MS Analysis of Volatiles Present in *Pappea Capensis* Extracts

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ABSTRACT

The use of medicinal plants played a crucial role in human survival for many years. *Pappea capensis* is used mostly in the Northern part of South Africa and neighboring countries. The woody part of the tree was collected, shade dried, and powdered. The extraction experiments (ethanolic extract, methanol extract, and water extract) were done at Synexa Life Sciences. For the purpose of identifying the biochemical elements present in the wood portion of *Pappea capensis*, the extracts were concentrated and analyzed using Gas Chromatography-Mass Spectroscopy at Central Analytical Facilities (CAF), University of Stellenbosch, South Africa. GC-MS identified 41 compounds which included 4-ethylbenzaldehyde, 2, 4-di-ter-butyl phenol, acetic acid, and butanoic acid, have a role in antioxidant, antimicrobial, antitumor, and antifungal effects. Regardless of their quantities, seven (7) unidentified phytochemical substances were discovered; their existence may have a favourable effect on therapeutic agents and be a source of the biological activities ascribed to them by conventional healers. Furthermore, it is a holistic plant for use in traditional medicine and aesthetic value among the indigenous communities in Limpopo due to the several varied chemical components that have been found. The three extracts must be fractionated according to bioassay-guidance to identify the pure components and establish which ones are physiologically active. The medicinal plants, being the only sources that traditional healers rely on for the treatment of their patients, have received tremendous attention in drug therapy, discovery, and development. These studies have demonstrated that *Pappea capensis* is a plant with potential for use in phytopharmaceuticals.

Key words: *Pappea capensis*, GC-MS, Medicinal plant, Phytochemical compounds.

INTRODUCTION

Most people have entrusted plants with having qualities of an important source of medicine for decades. The practice of using herbal remedies to cure and heal sick individuals has persisted through families for decades. Traditional medicine is an amalgam of expertise. It is carried out in accordance with varied cultural theories, beliefs, and traditional practices that are employed to uphold health, identify, prevent, and treat diseases.¹ The use of various forms of supplementary or alternative medicine, as well as other forms of traditional medicine, is rising everywhere. The previous study has shown that herbs can provide remedial actions and have historically been used as popular folk medicines.² For generations, *Pappea capensis* has been used by numerous Indian tribes as a primary medical resource to treat illness.^{2,3} No matter where they are in the world, the Chinese take part actively in the import and export of their medical system, which is another admirable cultural tradition.³ It is a sad reality that the current generation is becoming more disconnected from nature, and that because of our unhealthy lives, new and re-emerging diseases are appearing. However, the truth is that every disease, including cancer, has a remedy in our incredibly diverse natural world. The research studies have shown that *in vitro* screening methods could play a critical role in identifying the elect crude plant extracts with potentially valuable properties for additional chemical and pharmacological investigations.⁴ The studies on phytochemistry have grabbed research attention in recent years as a distinct discipline. Studies done on new drug development focused on plant biochemistry and natural product organic

chemistry. With the enormous diversity of organic chemicals that plants produce and accumulate, such a discovery is troubling. It discusses these compounds' molecular structures, production, turnover, and metabolism, as well as their typical dispersal and genetic function.⁵ According to their function in plant metabolism, phytochemicals—the fundamental substances derived from plants—are either classed as primary or secondary ingredients.

Primary elements include the common sugars, purines, and pyrimidines of nucleic acids, amino acids, proteins, chlorophylls, etc. the secondary elements are the remaining plant compounds such as phenolics, alkaloids, and terpenes.⁶ These secondary compounds have been found to protect plants. Still, recent research demonstrates that these compounds isolated from the plant source also provide protection against microorganisms in humans. The factual dietary role of phytochemicals is becoming more possible daily as research reveals more of their extraordinary benefits.⁷ There is a noticeable speedy development in the scientific fields, there were several drastic developments in analytical techniques, including Gas Chromatography-Mass Spectroscopy (GC-MS), NMR, TLC, and UV, which were powerful tools for separation, identification, and structural determination of phytochemicals.⁸

The three *Pappea capensis* extracts will be subjected to GC-MS analysis in this study. GC-MS is the most popular technique for identification and quantification. The identification of these unidentified chemical components in the *Pappea capensis* wood extracts will be made by interpreting the spectra and comparing them to reference spectra, as was done in earlier studies.⁹ This is a qualitative analysis study, there is no need to use standards because unknown

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compounds will be identified by comparing them using the Wiley Library and National Institute of Standards and Technologies Library (NIST) a match factor >900, which gives an excellent match.¹⁰

The plant, *Pappea capensis*, contained similar constituents like alkaloids, flavonoids, triterpenoids, and sterols, as in other studies.¹¹

With the help of the GC-MS technique, the objective of this work was to qualitatively identify the chemical components found in the *Pappea capensis* wood extracts, which may offer insight into their usage in traditional medicine.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC/MS)

Method and material

Collection of sample

On the 23.5-kilometer route R101 between Mokopane and Naboomspruit in the Limpopo region, the tree's woody pieces were gathered. Both the scientists at the National Botanical Gardens in Pretoria, South Africa, and the Bloemfontein Museum verified the tree's legitimacy. The specimen was given the specimen voucher numbers PC Zietsman & A Makhoahle 5448 and kept in the herbarium of the National Museum, Bloemfontein (NMB). These gathered wood samples were room-temperature dried, then mechanically ground and kept at room temperature pending examination.

Preparation of the extract

Three 360g samples of wood each received 1080ml of ethanol, water, or methanol for extraction. Depending on their consistency, the various sample volumes were modified. A total volume of 1080 ml was created by adding the remaining solvent, and the solutions were let to leak out for 24 hours.

The particulates were removed from each solution by filtering using a Millipore funnel with medium filter paper (Bright sign nr102) attached to a Millipore vacuum pump after the samples had been left unfiltered for 24 hours. In cases where more filtration was required based on the final extract, a sample was centrifuged once more in 50ml conical tubes.

For the removal of solvents from the extracts, both aqueous and organic solvents were removed using freeze-drying processes with a Virtis Freeze drier and a Rotary evaporate (55°C). These freeze-drying and vacuum evaporation steps were repeated on all the samples followed by weighing to determine their yield. The extractions methods and procedures were performed at Synexa Life Sciences. These dried crude extracts were then subjected to the GC-MS examination and analysis.

GC-MS analysis

The phytochemical investigation of ethanol, water, and methanol extracts of *Pappea capensis* was evaluated using the GC-MS system of Central Analytical Facilities (CAF), University of Stellenbosch, South Africa.

500 mg of methanolic, aqueous, and ethanolic wood extracts were sonicated overnight with one milliliter of dichloromethane. Then one microlitre of the sonicated mixture was injected onto the GC-MS system in splitless mode. On a Shimadzu 2010 QB gas chromatograph with an MSD detector outfitted with an HP-5 fused silica capillary Column (30m x 0.25 mm x 25m film thickness), the GC-MS analysis was performed. An all-glass injector operating in split mode and using Helium as the carrier gas at a flow rate of 1 mL min⁻¹ was then used to inject the plant aqueous extract. The injector temperature, ion source temperature, and interphase temperature for the oven were all set to 200 degrees Celsius. The column temperature was increased to 45°C (3 min hold at 45°C, 4°C min), then gradually increased to 150°C (3 min

hold at 150°C, 4°C min), then raised to 250°C and a 15 min hold. The split ratio of 1:5 was used during the sample run.¹² Their results were evaluated by using the Wiley275 mass Spectral library search program.

RESULTS

Identification of chemical constituents

Identification of the unknown constituents was done through the interpretation of the mass spectrum of the GC-MS obtained using NIST mass spectral library (NIST95). The identification of the mass spectrum of the unknown constituents was achieved by comparing them with the spectrum of the known components in the NIST library. The Wiley mass spectral Search program was used to assist in constituents' identification. The results obtained are regarded as tentative because this was a qualitative study, there was no need to run standards that are done during quantitative studies.

Percentage yield

From the three batches of approximately 500 mg of sonicated powdered wood, mean percentage yields of 8.2% of methanolic extract, 4.6% aqueous extract, and 4.23% ethanoic extracts were obtained. The methanol extracts gave the highest percentage yield. Nearly all the compounds were polar. Major components were assigned using NIST and Wiley 275 from programmed libraries. The relative percentage amount of each peak was determined by comparing its average peak area to the total areas on each extract.

Bioactive compounds present in the extracts

The bioactive compounds in methanol, aqueous, and ethanol extracts obtained from *Pappea capensis* are shown in Tables 1-3. The compounds' identification and classification were based on their elution order in an HP-5 fused silica capillary column (30m x 0.25 mm x 25m film thickness), the elution time, library identification name, and the amounts at which these bioactive compounds were also presented. A very large quantity of the top three major compounds present in the methanolic extracts was 2-propenoic acid, dodecyl ester (24.48%), 4-ethylbenzaldehyde (18.33%), and 1,3-Di-tert-butylbenzene (10.62%).

The aqueous extracts contained (R or S)-2, 3-butanediol (15.05%), followed by 3-Di-tert-butylbenzene (9.06%) and 4-ethylbenzaldehyde (8.21%). The results of the ethanoic extract had 4-hydroxy-4-methyl-2-pentanone (20.36%), hexadecanoic acid (12.95%), and 5-(hydroxymethyl)-furfural (4.39%) as the three major compounds. The GC-MS results of the three extracts presented in Figures 1-3 showed the retention time in the column and the detected peaks, which correspond to bioactive compounds present in the extracts.

DISCUSSION

Plants have almost unlimited ability to produce a lot of constituents. The different plant extracts from the wood of *Pappea capensis* through chronological extraction with solvents of increasing polarity ethanol, methanol, and water. The GC-MS analysis of the ethanol, water, and methanol extracts revealed the presence of different bioactive compounds. The three extracts of *Pappea capensis* yielded a total of 41 compounds, 7 of which were unknown. The following compounds dodecane, pentadecane, tetradecane, docosane, and 2-tetradecyloxyethanol were found in both the methanolic and aqueous extracts in various quantities. The GC-MS discovered five and two unidentified compounds from the aqueous and ethanolic extracts, respectively. The three bioactive compounds (1, 3-Di-tert-butylbenzene, 4-ethylbenzaldehyde, and 2, 4-Di-tert-butylphenol) were present in all extracts (ethanol, methanol, and water) but in different quantities. Overall results of the three extracts didn't reveal a common major compound in them.

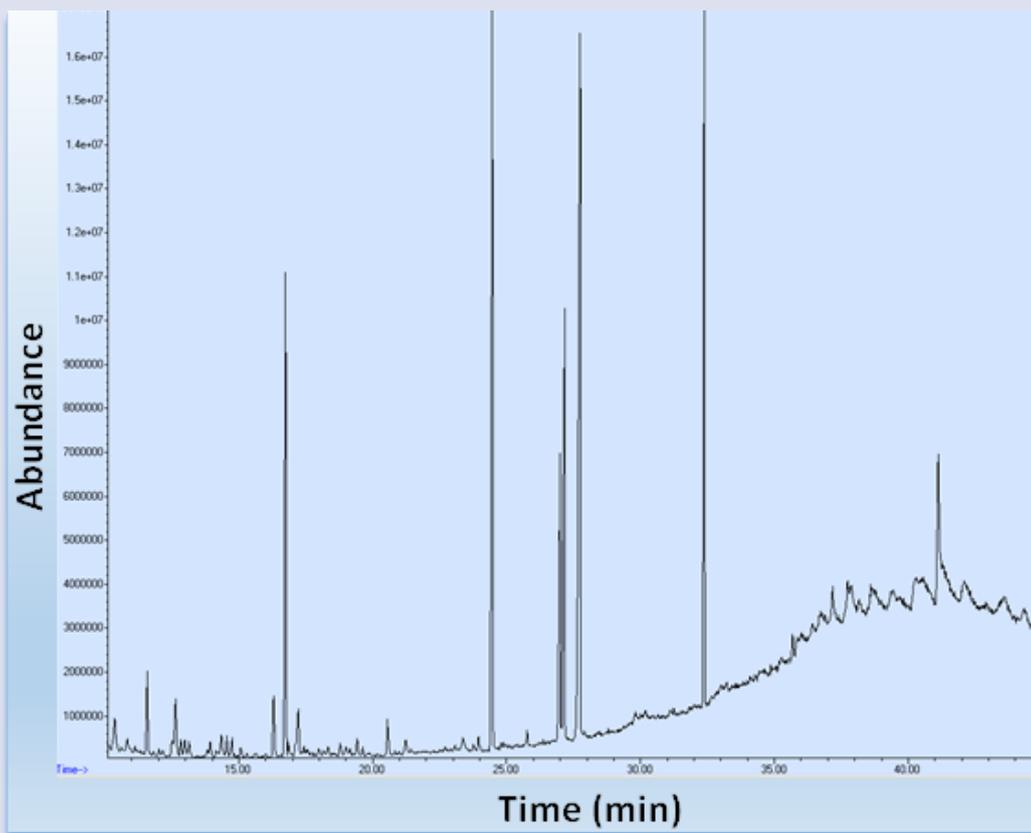


Figure 1: A typical chromatogram of a 500 mg methanolic wood extract.

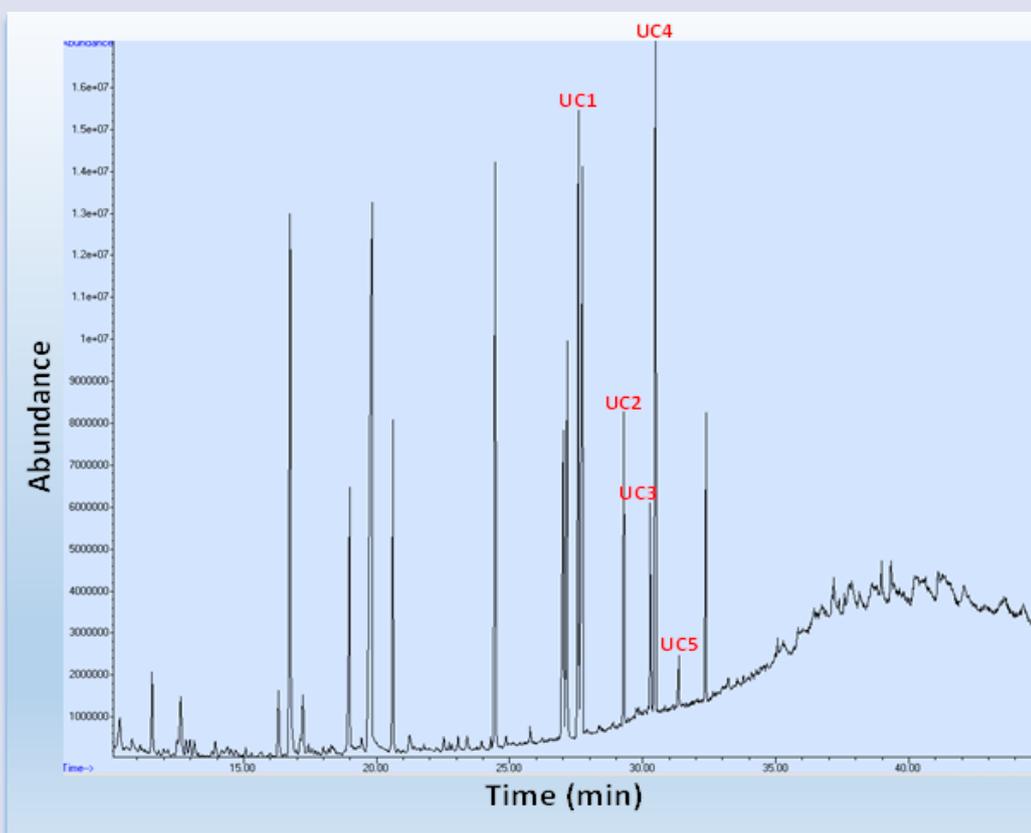
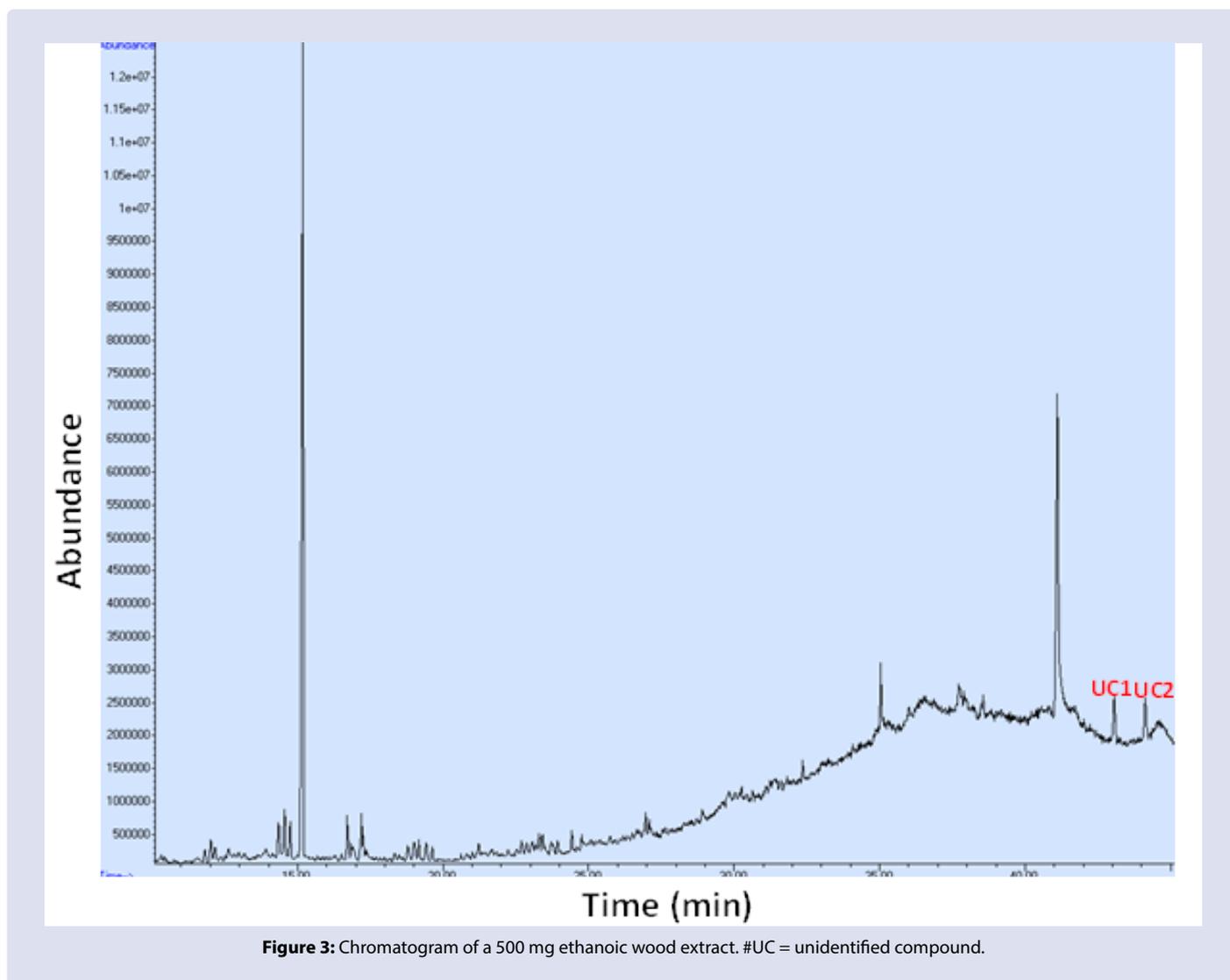


Figure 2: Chromatogram of a 500 mg aqueous wood extract. UC = unidentified compound.

**Table 1: Tentative identification and area percentage (%) of volatiles in the methanolic wood extract.**

The concentration of the compounds is reported in terms of area percent

500 mg ethanoic methanol extract

Retention time	Library identification compound name	Area percent (%)	Molecular formula	Molecular weight	Biological activity
11.58	Dodecane	1.98	$\text{CH}_{10}\text{CH}_3$	170	Antibacterial activity and antifungal activity ¹³
12.64	Pentadecane	1.79	$\text{C}_{15}\text{H}_{32}$	212	Antimicrobial activity ¹⁴
16.30	Tetradecane	1.69	$\text{C}_{14}\text{H}_{30}$	198	Antioxidant, antifungal ^{15,16}
16.74	1,3-Di-tert-butylbenzene	10.62	$\text{C}_{14}\text{H}_{22}$	190	Inked to the inborn metabolic disorder celiac disease ¹⁷
17.22	Docosane	1.79	$\text{C}_{22}\text{H}_{46}$	436	Antimicrobial activity ¹⁸
20.57	Hexadecane	1.02	$\text{C}_{16}\text{H}_{34}$	226	Antibacterial, antioxidant activities ¹⁹
24.47	4-Ethylbenzaldehyde	18.33	$\text{C}_9\text{H}_{10}\text{O}$	137	Nematocidal activity and antifungal activity ²⁰
27.01	2-Tetradecyloxyethanol	8.64	$\text{C}_{16}\text{H}_{34}\text{O}_2$	258	Nematocidal activity ²¹
27.15	1-Tridecene	10.39	$\text{C}_{13}\text{H}_{26}$	182	Antimicrobial activity ²²
27.75	2-Propenoic acid, dodecyl ester	24.48	$\text{C}_9\text{H}_8\text{O}_3, \text{C}_{22}\text{H}_{44}\text{O}_2$	72, 340	anticancer and antibacterial activities ²³
32.39	2,4-Di-tert-butylphenol	13.98	$\text{C}_{14}\text{H}_{22}\text{O}$	206	Antibacterial, anti-inflammatory, cytotoxicity, antiviral, insecticidal, and nematocidal activities ²⁴
41.12	Hexadecanoic acid	3.53	$\text{C}_{16}\text{H}_{32}\text{O}_2$	256	Antioxidant, anti-inflammatory, hypocholesterolemic, and cancer prevention activities ²⁵

NB: Take note that all the compounds are tentatively reported as we had to rely solely on the NIST 95 and WILEY275 libraries for compound matches as we did not run standards to confirm during this qualitative study.

Table 2: Tentative identification and area percentage (%) of volatiles present in the aqueous wood extract.

The concentration of the compounds is reported in terms of area percent					
500 mg aqueous extract					
Retention Time	Library identification	Area Percent (%)	Molecular formula	Molecular weight	Biological activity
11.56	Dodecane	1.33	CH ₁₀ CH ₃	170	antibacterial activity and antifungal activity ¹³
12.64	Pentadecane	1.30	C ₁₅ H ₃₂	212	antimicrobial activity ¹⁴
16.31	Tetradecane	1.23	C ₁₄ H ₃₀	198	Antioxidant, antifungal ^{15,16}
16.75	1,3-Ditertiarybutylbenzene	9.06	C ₁₄ H ₂₂	190	linked to the inborn metabolic disorder celiac disease ¹⁷
17.24	Docosane	1.32	C ₂₂ H ₄₆	436	antimicrobial activity ¹⁸
18.98	(R or S)-2,3-Butanediol	4.01	C ₄ H ₁₀ O ₂	90	enhances virulence of certain microorganisms ²⁶
19.82	(R or S)-2,3-Butanediol	15.05	C ₄ H ₁₀ O ₂	90	enhances virulence of certain microorganisms ²⁶
20.61	Butanoic acid	3.67	C ₄ H ₈ O ₂	88	antitumor activity and anticancer activity, activating the mitochondrial pathway of apoptosis ²⁷
21.24	HENEICOSANE	0.42	C ₂₁ H ₄₄	296	Antibacterial activity ²⁸
24.46	4-Ethylbenzaldehyde	8.21	C ₉ H ₁₀ O	137	nematocidal activity and antifungal activity ²⁰
25.79	LAURYL ACETATE	0.37	C ₁₄ H ₂₈ O ₂	228	weak anticancer activity and antibacterial activity ²⁹
27.02	2-Tetradecyloxyethanol	5.96	C ₁₆ H ₃₄ O ₂	258	nematocidal activity ²¹
27.15	1-DODECANOL	6.69	C ₁₂ H ₂₆ O	186	Antibacterial activity. ³⁰
27.59	unidentified compound 1	9.01			
27.73	2-Propenoic acid, dodecyl ester	10.14	C ₉ H ₈ O ₃ , C ₂₂ H ₄₄ O ₂	72, 340	Anticancer and antibacterial activities ²³
29.29	unidentified compound 2	4.02			
30.30	unidentified compound 3	2.91			
30.49	unidentified compound 4	10.21			
31.35	unidentified compound 5	0.81			
32.37	2,4-Di-tert-butylphenol	3.41	C ₁₄ H ₂₂ O	206	antibacterial, anti-inflammatory, cytotoxicity, antiviral, insecticidal, and nematocidal activities ²⁴

NB: Take note that all the compounds are tentatively reported as we had to rely solely on the NIST 95 and WILEY275 libraries for compound matches as we did not run standards to confirm during this qualitative study.

Table 3: Tentative identification and area percentage (%) of volatiles present in the ethanoic wood extract.

The concentration of the compounds is reported in terms of area percent					
500 mg ethanoic wood extract					
Retention time	Library identification compound name	Area percent (%)	Molecular formula	Molecular weight	Biological activity
15.18	4-HYDROXY-4-METHYL-2-PENTANONE	20.36	(CH ₃) ₂ C(OH)CH ₂ COCH ₃	116	Antibacterial activity ^{30,31}
16.73	1,3-Di-tert-butylbenzene	1.21	C ₁₄ H ₂₂	190	Inked to the inborn metabolic disorder celiac disease ¹⁷
16.85	Acetic acid	0.31	CH ₃ COOH	60	Antibacterial activity ³²
17.21	Furfural	1.12	C ₅ H ₄ O ₂	96	Potential biological activities such as antimicrobial, antiviral ³³ , antioxidant ³⁴ , antitumor, antihistaminic and fungicides ³⁵
24.43	4-Ethylbenzaldehyde	0.61	C ₉ H ₁₀ O	137	Nematocidal activity and antifungal activity ²⁰
26.97	Benzothiazole	0.83	C ₇ H ₅ NS	135	anticancer, antimicrobial, antidiabetic, anti-inflammatory, antiviral ³⁶
32.37	2,4-Di-tert-butylphenol	1.58	C ₁₄ H ₂₂ O	206	Antibacterial, anti-inflammatory, cytotoxicity, antiviral, insecticidal, and nematocidal activities ²⁴
35.05	5-(Hydroxymethyl)furfural	4.39	C ₆ H ₆ O ₃	126	Genotoxic, an indirect mutagen, causes DNA damage and is a carcinogen ³⁷
41.11	Hexadecanoic acid	12.95	C ₁₆ H ₃₂ O ₂	256	Antioxidant, anti-inflammatory, hypocholesterolemic, and cancer prevention activities ²⁵
43.07	Unidentified compound 1	1.55			
44.13	Unidentified compound 2	1.57			

NB: Take note that all the compounds are tentatively reported as we had to rely solely on the NIST 95 and WILEY275 libraries for compound matches as we did not run standards to confirm during this qualitative study.

However, GC-MS revealed some of the volatiles, which is biologically active compounds. In the previous studies, this volatile was reported to be responsible for the pharmacological activities which may be associated with the healing effect of this plant by traditional healers. The compound 2, 4di-tert-butyl phenol from the leaves of *Pereskia bleo* (Kunth) was proven to exhibit antifungal and antioxidant activity and had cytotoxicity against the human cancer cell lines; KB, MCF-

7, CasKi, HCT 116, A549; normal human cell line MRC-5, HeLa and H9c2 cell lines.³⁸⁻⁴⁰ These leaves of *Pereskia bleo* are traditionally used in Malaysia for the treatment of cancer.⁴⁰ The hexadecanoic acid extracted from the marine algal was found to possess selective cytotoxic activity in human leukemia cells and no cytotoxicity activity in normal human dermal fibroblast (DF) cells.⁴¹ Additionally, hexadecanoic acid in the same study induced apoptosis in the human leukemic cell line.⁴¹

In addition, 4-ethylbenzaldehyde was found to have antibacterial activity against gram-positive bacteria *Staphylococcus aureus*. These studies have also proven that alkanes and aldehydes are carcinogenic, and even have activation pathways that lead to the formation of cancer. Other studies showed that butanoic acid possesses anticancer properties by inducing apoptosis.⁴² This study also found surfactants, detergents, agricultural by-products, and flavorings, as indicated in table 1-3 above from the extracts.

In vitro, the antioxidant activity of the three extracts indicated the existence of antioxidants and phenols from *Pappea capensis* (data not published). Those results indicated the need to test further any possible compounds associated with this clear trapping of these radicals by the three extracts of *Pappea capensis*.

These results were in collaboration with the previous studies, which used the same plant and had shown that the leaf and stem bark carried medicinal benefits such as curative effects associated with hypoglycaemic activity due to phytochemicals.^{43,44} These antimicrobial screenings of this plant indicated that microorganisms killing was owed to the compounds eluted per extraction methods, as each could have isolated different active compounds.⁴⁶ The aqueous extract was found to be the only extraction technique that is mostly used by traditional healers to make medicinal mixtures for their patients. In the previous study, Makhoahle et al. reported that *Pappea capensis* ethanolic and methanolic extracts results correlated with other studies whereby they have more antimicrobial activity than water extract.⁴⁵⁻⁴⁷ These active antimicrobial compounds in this study need further investigation with purified extracts to identify the active compounds associated with antimicrobial activity observed in the three extracts.

The previous study using the same extracts has shown that they have very less cytotoxic and genotoxic activities.⁴⁸ The identified bioactive compounds such as hexadenoic acid correlated with the results of Harada, et al., which indicated that hexadenoic acid was also selectively cytotoxic to human leukemic cells but not cytotoxic to normal HDF cells in vitro.⁴¹

The GC-MS analysis also identified alkanes and aldehydes, which are poisonous. That indicates agreement with the results of the confirmatory tests performed on *Pappea capensis* water extract performed in the other study, which suggested that there may be a toxin from the water extract that gave false pro-inflammatory activity caused by endotoxin contamination of the extract that could be attributed by the plant.^{49,50} There is also confirmed and correlates with the results in Table 2 that shows the water extracts eluted the majority of compounds, of which most are alkanes and aldehydes as part of the plant.

CONCLUSION

The GC-MS identification of seven (7) unknown bioactive chemical compounds, regardless of their amounts, may perhaps be significant as therapeutic agents and a source of the biological activities claimed by traditional healers. It is a holistic plant for use in traditional medicine and for aesthetic value among the local communities in Limpopo since many different chemical compounds have been detected in it, and some others remain unidentified. More instrumental analysis is required to carry out bioassay-guided fractionation of the three extracts to determine the purified compounds and identify biologically active ones.

Due to the recognition of the value of medicinal plants as potential sources of novel compounds with therapeutic significance and a new direction for the discovery of bioactive compounds that will pave the way in drug development, medicinal plants, which are the cornerstone of traditional medicine, have recently been the subject of very intense pharmacological studies. Therefore, GC-MS analysis was

used to identify the bioactive compound in *Pappea capensis*, which revealed the existence of 41 components. Among the discovered substances, butanoic acid, acetic acid, 2, 4-di-ter-butyl phenol, and 4-ethylbenzaldehyde play a part in the antioxidant, antibacterial, anticancer, and antifungal actions. The *Pappea capensis* may serve as a new potential source of remedies due to the presence of various phytochemicals, bioactive compounds, and biological activities, according to previous investigations and the results of the current study.

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