PHYTOCHEMICAL CONSTITUENTS OF TICKBERRY (*OSTEOSPEMUM MONILIFERUM* L.) LEAF AND STEM EXTRACTS

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Abstract. Tickberry (*Osteospemum moniliferum*) is renowned for its ethnomedicinal uses. This study aimed to evaluate the chemical composition of the leaves and stems of *O. moniliferum*. The phytochemical constituents were identified by phytochemical screening and gas chromatography-mass spectroscopy (GC-MS) techniques in hexane, and chloroform extracts. Fourier transmission infrared spectroscopy (FTIR) and Energy-dispersive X-ray (EDX) microanalysis were employed to identify the powdered samples' functional groups and elemental composition. The fluorescence of leaves and stem powders was examined using various chemical solvents. Phytochemical analysis of leaves and stems showed positive results for various phytochemical compounds. GC-MS revealed the presence of forty-seven compounds in leaf and stem extracts. FTIR analysis revealed different peak values with various functional groups in powdered samples, confirming the presence of alcohol, carboxylic acid, alkanes, amides, and alkyl halides. EDX microanalysis revealed the presence of carbon (65.07%), oxygen, chlorine, sodium, calcium, potassium, magnesium, and copper. Aluminium and silicon were only found in leaf samples. This study stands as the first report on the phytochemical constituents of this species. The identified phytochemicals indicate the potential therapeutic applications of *O. moniliferum* for a variety of ailments. These findings could pave the way leading to the establishment of potential drugs.
Keywords: *Asteraceae, FTIR, GC-MS, medicinal plants, phytochemicals*

Introduction

Plants have been used in traditional medicine since ancient times in developed and developing countries due to their accessibility and low risk of adverse effects (Amer and Mohammad, 2022). Although modern medicine is available in most of the world, large portions of the population in developing countries continue to rely on traditional doctors and herbal medicines for their basic care (Moeti, 2022). There has been an increase in the demand for medicinal plants due to their ability to be used as an alternative allopathic medicine, as well as their availability, relative efficiency, affordability, and low risk of side effects (Ashis, 2003). Besides providing nutrition, plants contain different phytochemicals such as Alkaloids, phenolic compounds, and flavonoids, also known as secondary metabolites. Plants produce hundreds of chemical compounds for various

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purposes, including defense against herbivorous mammals, fungi, insects, and diseases (Mahomoodally, 2013; Patel and Savjani, 2015). Humans have developed an interest in determining the precise constituents and sources of most healing abilities associated with plant parts (Ahmad and Mehmood, 2010; Shrivastava, 2017). These investigations on various plant species have resulted in the development of "phytochemistry", regarded as the science of the substances found in plant extracts. Analyzing these chemical compounds would help identify various biological processes in plants (Ragavendran et al., 2012). Developing reliable techniques is critical for quality control and searching for novel compounds (Pai et al., 2022). As a result of the extraction and identification of these bioactive compounds, specific drugs with high activity profiles have been delivered (Yadav et al., 2017). Fourier-transformed infrared spectroscopy and gas chromatography coupled with mass spectrometry are widely used to detect the functional groups and identify various plant bioactive compounds (Fan et al., 2018; Satapute et al., 2019). Gas chromatography-mass spectrometry is reliable for accurately identifying numerous chemicals from plant extracts, such as alkaloids, flavonoids, organic acids, and amino acids (Razack et al., 2015).

The Asteraceae, also known as the sunflower family, is one of the world's largest flowering plants, comprising over 1600 genera with 2500 species. Members of the Asteraceae can be found on every continent (except for Antarctica) and in every habitat type due to their widespread distribution and ease of ecological adaptation (Rolnik and Olis, 2021). The plants from this family contain a wide range of phytochemical substances, including polyphenols, phenolic acids, saponins, essential oils, flavonoids, sesquiterpene lactones, and lignans. The biological activity of plants from this family is due to their chemical composition. *Osteospemum moniliferum* is commonly known as African daisy. There is no known literature on pharmacological uses, but according to some traditional healers, it is traditionally utilized in some cultures in treating fever and digestive/ stomach issues. Despite the numerous studies reported on the Asteraceae, the phytoconstituents of *O. moniliferum* have not been reported. This study thus aimed to investigate the phytochemical composition of hexane, chloroform and methanol leaf and stem extracts of *O. moniliferum* using phytochemical screening and GC-MS. Additionally, the plant was characterized in powder form to identify functional groups, elemental composition, and determine its fluorescence using fourier-transformed infrared (FTIR) spectrum, energy-dispersive X-ray (EDX) microanalysis and fluorescence microscopical analysis.

Materials and methods

Plant collection

Leaves and stems of *O. moniliferum* were collected from plants grown at the University of KwaZulu-Natal (UKZN), Westville campus (29°49'7.21" S; 30°56'50.45" E). A voucher specimen was deposited at the Bews Herbarium, UKZN, Pietermaritzburg campus (Accession no. NU0094285).

Sample preparation

The leaf and stem materials were collected in June 2019 and air-dried separately for two months at room temperature. After drying, the plant material was ground into a fine

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powder using a Biovopeak electric laboratory mill (BMLL-2). The powdered samples were stored in an airtight container away from direct sunlight until further use.

Extraction

Subsequently, 10 g of leaf and stem powder were dispersed into two round-bottom flasks containing 100 mL of hexane. The flask was connected to a reflux apparatus and heated for three hours to obtain the crude extracts. The extracts were filtered using a funnel and Whatman® No.1 filter paper. The process was repeated thrice, and the same succession was conducted using chloroform and methanol. The leaf and stem extracts were air-dried in a well-ventilated dark room. Once the solvent had evaporated entirely, the extracts were stored in airtight jars at 4°C for further studies. The percentage yield was determined by using the formula below:

$$\text{Extract yield (\%)} = \text{weight of dried extract (g)/ weight of powdered material (g)} \times 100 \quad \text{(Eq.1)}$$

Qualitative phytochemical screening

Phytochemical screening of the extracts was carried out using standard protocols (Evans, 1997; Harborne, 1998; Pooja and Vidyasaga, 2016), with some modifications according to Ramasar et al. (2022).

Detection of alkaloids

Wagner's test: Wagner's reagent (aqueous iodine solution in potassium iodide) was added to a test tube containing 1 mL of extract. A red/brown precipitate was indicative of the presence of alkaloids, alkaloids present (+) Mayer's test: Two drops of Mayer's reagent (potassium mercuric iodide solution) were added to 1 mL of the extracts. A white creamy precipitate was an indication of the presence of alkaloids.

Detection of phenolic compounds

Ferric chloride test: One millilitre of 10% iron (III) chloride ($FeCl_3$) was mixed into a test tube containing 1 mL sample. A blue-black or brownish-green colouration confirmed the presence of tannins. Lead acetate test: One millilitre of 10% lead acetate solution was added to 1 mL of extract. A bulky white precipitate confirmed the presence of phenolic compounds. Gelatin test: Three drops of 1% gelatin solution were added to a test tube containing 1 mL of extract. A white precipitate indicated the presence of quinones (Evans, 1997).

Detection of flavonoids

Alkaline reagent test: Two millilitres of 5% sodium hydroxide (NaOH) was added to 2 mL of extract. An intense, yellow-coloured solution that decolourised upon adding 1 mL 50% H_2SO_4 indicated the presence of flavonoids. Acid hydrolysis test: One millilitre of concentrated H_2SO_4 was added to 1 mL extract. Positive results were indicated by the intense yellow solution indicating the presence of flavones and flavanols (Harborne, 1998).

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Detection of proteins

Biuret test: One millilitre of 10% NaOH was added into a test tube containing 2 mL of extract. The content was thoroughly mixed, followed by adding 95% ethanol. After that, 1 mL of 0.5% copper sulphate was added along the side of the test tube. The formation of a purpish violet or pink colouration indicated the presence of proteins (Harborne, 1998).

Detection of carbohydrates

Molisch's test: The extract (3 mL) was diluted with 2 mL of distilled water and then filtered. After that, three drops of Molisch's reagent were added to the filtrate, followed by carefully adding 1 mL of concentrated H_2SO_4 along the side of the test tube without mixing. The solution was allowed to stand for two minutes. The visualisation of a purple ring between the layers of the solvents confirmed the presence of carbohydrates (Evans, 1997).

Detection of gum and mucilage

Ruthenium red test: Two drops of ruthenium red were added to a test tube containing 2 mL of the extract. A pink colour change indicated the presence of mucilage (Evans, 1997). Precipitation test: extract (1.5 mL) was mixed with 2 mL of distilled water. Thereafter, 2 mL of absolute ethanol was added to the solution and continuously stirred. The appearance of a white or cloudy precipitate indicated the presence of gums or mucilage (Evans, 1997).

Detection of saponins

Foam test: In a test tube containing 2.5 mL of extract, 10 mL of distilled water was added and rapidly shaken for ten minutes. A positive result was indicated by the presence of a froth layer (1-2 cm), whereas the results were negative if there was no froth formation [94]. Olive oil test: Two drops of olive oil were added into a test tube containing 2 mL of the extract, and the mixture was rapidly shaken by hand. A soluble emulsion droplet indicated the presence of saponins (Evans, 1997).

Detection of steroids

Salkowski's test: In a test tube containing 1.5 mL of extract, 1 mL of chloroform was added and thoroughly mixed. After that, 1.5 mL of concentrated H_2SO_4 was added along the side of the test tube. A reddish-brown colour indicated the presence of a steroid ring (Harborne, 1998). Liebermann-Burchard test: One mL of chloroform and 1 mL of acetic acid were added to a test tube containing 1 mL of the extract. The solution was allowed to stand in ice for 10 min, and subsequently, 1-2 drops of concentrated H_2SO_4 were added along the side of the test tube. A dark colour and red or reddish-brown rings in the upper layer indicated the presence of steroids.

Detection of resins

Turbidity test: Extract (1 mL) was dissolved in 1 mL of acetone and thoroughly mixed. This was followed by adding 2 mL of distilled water to the mixture. The appearance of a turbid solution indicated the presence of resins (Harborne, 1998).

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Thin layer chromatography

Thin layer chromatography was analysis was performed using a method described by Bantho et al. (2022). Hexane, chloroform, methanol leaves, and stem extracts were prepared for thin-layer chromatography analysis. A baseline was drawn using a pencil on the border of a 10 cm x 10 cm silica gel 60 F254 plate (E. Merck) with a uniform thickness of 0.2 mm. Each extract droplet was added to the plate using glass capillaries. Then, it was placed in a beaker containing a mobile solution consisting of 9 parts toluidine: One-part ethyl acetate (mobile phase). The beaker was covered with foil, and the solution was left to stand on the mobile phase until it reached the line on the top. The plate was then viewed under ultraviolet light at a wavelength of 360 nm. The plate was sprayed with anisaldehyde sulphuric acid reagent and then dried in an oven at 105 ˚C for 5-10 min. The retention factor (Rf) value was calculated using the following formula:

$$Rf = \frac{distance\ travelled\ by\ compound}{distance\ travelled\ by\ the\ solvent} \quad \text{(Eq.2)}$$

Gas chromatography-Mass spectrometry (GC-MS) analysis

The extracts were analysed using GC-MS (QP 2010 plus Shimadzu, Japan) detached on a capillary column (30 m X 0.25 mm ID X 0.25 µm film thickness of 5% phenylmethyl-siloxane). Helium was used as a carrier gas at a 1.2 mL/min flow rate. The instrument was set at an initial temperature of 50°C for 1.5 min and was increased to 200°C at a rate of 4°C/min, with a holding time of ten minutes. This was then increased to 300°C at a 10°C/min rate held for 10 min. The injector temperature was programmed at 240°C, set for split-less mode injection (1 µl). The mass spectral scan range was 40-500 m/z, with a30 min running time. The chemicals compounds were identified by comparing their mass spectra and retention time to those in the National Institute of Standards and Technology (NIST) database.

Fourier-transformed infrared (FTIR) analysis

The FTIR analysis characterised the functional groups of bioactive compounds in the *O. moniliferum* leaf and stem. Leaf and stem powder of *O. moniliferum* were subjected to FTIR spectroscopy on a Perkin Elmer spectrum 100 FTIR spectrometer (Shelton, Connecticut, USA), software version 6.1. The spectra were examined and imaged, ranging from 4000-400 $cm^{-1,}$ with a resolution of 4 cm^{-1}. The functional groups of the phytochemicals present in each sample were identified by comparing the detected peak frequencies to the references in the literature (Coates, 2000; Stuart, 2004).

Fluorescence microscopy

A small quantity of powdered samples (leaf and stem) was placed separately onto a clean microscopic slide, and 1-2 drops of freshly prepared reagent were added and the slide was gently tilted to mix the mixture. This was allowed to stand for 3 to 5 min to allow the solution to be efficiently absorbed by the powder. The slides were viewed under brightfield light and ultraviolet 2A (330/380 nm) light microscope using a Nikon Eclipse 80i compound microscope coupled to a Nikon DS-Fi1camera. The reagents used were acetic acid, acetone, sodium hydroxide (aq), chloroform, diethyl ether, ethanol, hydrochloric acid, hexane, methanol, sulphuric acid, and distilled water.

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Energy-dispersive X-ray (EDX) microanalyses

Mineral elements within *O. moniliferum* were determined by placing approximately 0.2 mg of leaf and stem powdered samples separately on an aluminium stub coated with gold using Quorum Q150 RES gold coater. The samples were viewed using the Zeiss Ultra Plus FEG SEM with EDX equipped with Aztec software (Oxford instrument, UK).

Results and discussion

Phytochemical screening

According to Unuofin et al. (2018), the extraction solvent used will have a significant impact on elucidating the phytochemical composition of medicinal plants. The structural properties of compounds determine their solubility in polar solvents (Zlotek et al., 2016). Therefore, it is crucial to isolate both polar and non-polar molecules using polar and non-polar solvents (Nemudzivhadi and Masoko, 2015). When compared to other solvents, methanol was found to be the best extractant (*Table 1*). The methanol extract yielded the highest percentage, with 16% for the leaf and 12% for the stem extracts. The hexane extracts obtained the lowest yield (5.1% and 4.6%, respectively). The results imply that there are more polar compounds in the leaves of *O. moniliferum* and fewer non-polar compounds in the stem.

Table 1. *The percentage yield of the extracts of O. moniliferum*

Solvents	Leaf extract yield (%)	Stem extract yield (%)
Hexane	5.1	4.6
Chloroform	7.8	6.3
Methanol	16	12

The therapeutic benefit of the plant lies in chemical compounds that have a definite physiological action on the body (Tariq and Reyaz, 2013). The most important plant bioactive compounds are alkaloids, terpenoids, flavonoids, steroids, cardiac glycosides, and protein compounds. The confirmation of a bioactive compound was indicated by a high presence (+++) based on strong resemblance; a moderate presence (++) for medium resemblance; present (+) for weak resemblance; and (-) for no resemblance, appearance, or change of color in the reactive solution. In the present study, alkaloids, phenolic compounds, flavonoids, polyphenols, steroids, tannins, proteins, amino acids, saponins, mucilage, resins, terpenoids and glycosides were all confirmed to be present in hexane, chloroform, and methanol extracts of the leaves and stems (*Table 2*). Alkaloids, flavonoids, proteins and phenolics were detected in all the extracts. These results are based on the work of Waghmare and Suradkar (2020), who tested for phytochemicals of some plants from the family Asteraceae. and only observed tannins in the hexane leaf extract. Resins and steroids were detected in hexane and chloroform extracts, while carbohydrates were present in the hexane leaf and chloroform (leaf and stem) extracts. Mucilage was not detected in the chloroform extract, whereas chloroform stem, methanolic leaves, and stem extracts produced a positive reaction for saponins.

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Table 2. *Qualitative phytochemical screening of extracts of Osteospemum moniliferum*

Phytoconstituent	Test/reagent	Hexane		Chloroform		Methanol	
		leaf	stem	leaf	stem	leaf	Stem
Alkaloids	Mayer's	-	-	+	+	-	-
	Wagner's	+	+	+	+	+	+
Tannins	Ferric chloride	+	-	-	-	-	-
Phenols	Lead acetate	+	+	++	++	++	+++
Tannins (quinones)	Gelatin	+	+	-	-	-	-
Flavonoids	Alkaline reagent	-	-	-	-	+	+
	Acid hydrolysis	+	+	+	+	+	+
Steroids	Salkowski	+	+	+	+	-	-
	Liebermann-Burchard	+	+	++	+	-	-
Saponins	Foam	-	-	-	+	++	+
	Olive oil	-	-	-	+	+	+
carbohydrates	Molisch's	+	-	+	+	-	-
Proteins	Biuret	+++	+++	+++	+++	++	++
	Ninhydrin	+	-	-	-	-	-
Resins	Acetone	+	+	+	+	-	-
Gum and mucilage	Ruthenium red	-	-	-	-	+	+
	Precipitation	+	+	-	+	+	+

(-) Absent, (+) present, (++) moderate presence, (+++) high presence

According to the literature, many important components have higher therapeutic potential when carbohydrates are present (Saha et al., 2020). It is asserted that carbohydrates have a special significance since they are crucial in providing the energy needed for defence and serve as a signal for monitoring the defence genes. Alkaloids are present in many medicinal plants, and it make up an appreciable percentage in many accessible drugs, hence highly essential in disease management (Bhatnager, 2023). Many plants produce alkaloids as secondary metabolites to defend against herbivory, microbial diseases, and invertebrate pests due to their toxicity and extremely bitter flavor. Alkaloids are nitrogen-containing phytometabolites and have played a significant role in traditional medicine (Raks et al., 2018). Alkaloids were first used therapeutically in the 19th century, when compounds were separated primarily for narcotic and analgesic properties (Shang et al., 2018). Plant alkaloids are used as muscle relaxants, local anaesthetics, and analgesics, as well as antihypertensive, anti-arrhythmia, anti-malaria, anticancer, and anti-HIV agents due to their pharmacological properties (Pan et al., 2012; Valadao et al., 2015). Therefore, detecting alkaloids in both leaf and stem extracts of *O. moniliferum* highlights the significance that plants from the Asteraceae family play in the traditional treatment of wounds and sores (Suntar, 2014). Phenolic compounds are major secondary metabolites abundant in medicinal plants (Abeysinghe et al., 2021). Among other biological properties, it has been claimed to have antioxidant, antidiabetic, and antibacterial effects (Kumar and Goel, 2019). They have antioxidant properties that help to protect against the damaging effects of free radical scavenging activity (Tahara, 2007). Also, they are thought to be responsible for the antioxidant activity of most plant species

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(Giorgi et al., 2009). Saponins are a type of bioorganic molecule that is abundant in plants. They are naturally occurring glycosides with soap-like frothing properties that create foams when agitated in aqueous solutions (El-Aziz et al., 2019). These compounds have several functions, including precipitating and coagulating red blood cells and binding cholesterol (Rajkumar and Panambara, 2022).

Among secondary metabolites found in plants, flavonoids are a type of polyphenol that is extensively distributed. These compounds have significant pharmacological significance due to their anticarcinogenic, anti-inflammatory, antiviral, anti-mutagenic, antioxidant, antiulcerogenic and antimicrobial (Valdés et al., 2015), antiallergic, antitumor and antihepatotoxic properties (Gomes et al., 2011). Plants high in flavonoids may be effective as antibacterial agents (Ballard and Marostica, 2020). Furthermore, flavonoids may have cardioprotective properties against ischaemia reperfusion, according to a number of studies (Lecour and Lamont, 2011; Njoku et al., 2017). A study by Kumar Hotta et al. (2021) revealed the presence of flavonoids in *Chrysanthemum indicum* and *Calendula officinalis*. Similarly, Yeasmin et al. (2016) performed a spot phytochemical analysis on three colours of flowers of *Chrysanthemum morifolium*, and the results revealed the presence of a variety of phytochemicals, namely, alkaloids, flavonoids, saponins, tannins, terpenoids, and phenol content.

Terpenoids have been useful in treating and preventing various illnesses, including cancer, viral infections, bacterial infections, malaria, and inflammatory infections or diseases (Maharaj et al., 2022). Steroids have been discovered to inhibit cholesterol synthesis (Sarala et al., 2011) and can reduce airway inflammation in asthma patients (Krishnaiah et al., 2009). Plant-derived steroids exhibit cardiotonic effects and antibacterial and insecticidal properties (Iqbal et al., 2015). Steroids are used to stimulate the bone marrow and promote growth. They are known to promote lean body mass and aid in preventing bone loss in older men (Amalraj and Gopi, 2017). Methanol extracts in the present study did not show any presence of steroids. However, a similar study conducted by Guchu et al (2020) revealed the absence of steroids in the methanolic leaf extracts of *Vernonia lasiopus*, a shrub that belong to the Asteraceae family. Plant tannins have been reported to have spasmolytic, antioxidant and free radical scavenger effects. Tannin has an anticancer property due to its growth-inhibiting activity (Zarin et al., 2016). Plants with high phenolic content could be effective anti-tumour (Sangeetha et al., 2014) and antioxidant (Yang et al., 2007) agents. These phytochemicals in the extracts indicate their numerous medicinal properties, such as antibacterial, antioxidant and anti-inflammatory properties.

Thin layer chromatography (TLC)

Thin layer chromatography results This diagram depicts how the compounds are separated (*Figure 1 A and B*). hexane leaf extract showed 3 bands under UV light, while the stem had only one band visible (*Figure 1B*). Chloroform leaf extract revealed the presence of 8 bands (*Figure 1B*). No bands were present in methanol extracts. This could be because of the majority of the molecules on the methanol were most likely somewhat non-polar, which accounts for the absence of properly separated chemicals (Adefuye and Ndip, 2013).

Different solvent solutions produce variable Rf values for certain phytochemicals. This variation in the Rf values of phytochemicals provides an important indication for evaluating their polarity and aids in selecting the best solvent system for pure material separation in column chromatography (Ahlam, 2019). Hexane had the highest Rf values

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of 0.53 and 0.54 for leaf and stem extracts, while chloroform leaf extract had the lowest Rf value of 0.02. Compounds with the highest Rf value travel faster and are less polar than the compounds that travel slowly and mostly found close to the baseline.

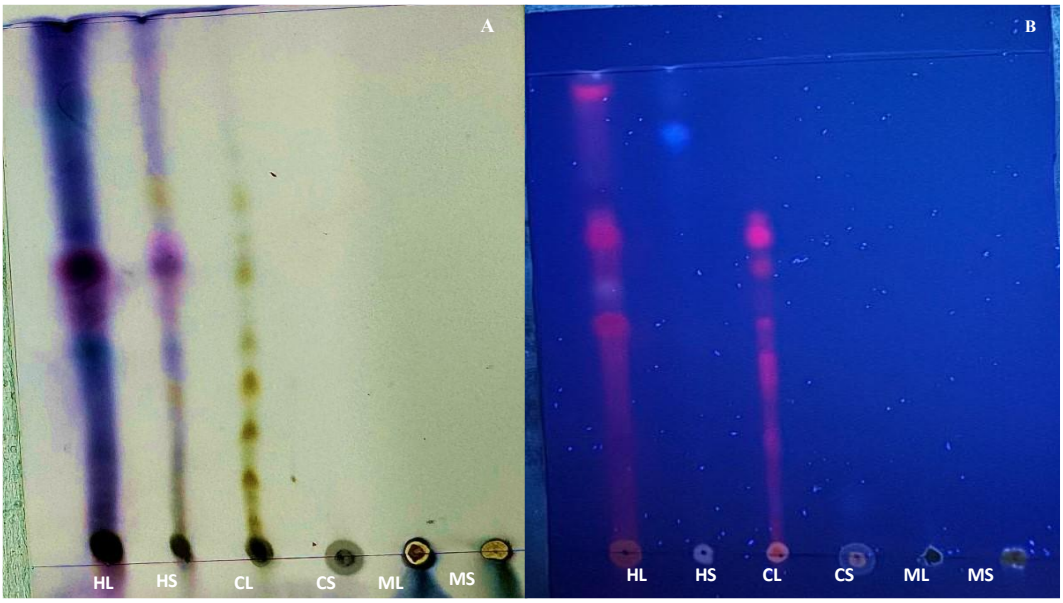

Figure 1. *TLC chromatograms of leaves and stem extracts of Osteospemum moniliferum A) under visible light after spraying with anisaldehyde solution; B) under UV light 360 nm. HL= Hexane leaf; Hs= Hexane stem; CL= Chloroform leaf; CS= Chloroform stem; ML= Methanol leaf; MS= Methanol stem extracts*

GC-MS analysis

The biological potential of various therapeutic plant extracts can be determined by examining their chemical structure and composition. To the best of our knowledge, no research on GC-MS-based plant metabolic characterization has been conducted that has revealed various bioactive components in *O. moniliferum* extracts. As a result, the GC-MS analysis was carried out in a predetermined study. Peak height, retention time, and chemical formulas were used to confirm the identification of the compounds present in the plant samples. The highest number of compounds (17) was observed in chloroform leaf extracts, followed hexane extracts (15), hexane stem (15), and chloroform stem extracts (13) (*Tables 3 and 4*). A total of fifteen soluble compounds were identified in leaf hexane extracts with tridecane, 2 methyl-, hexadecane, pentadecane, heptadecane, and eiscosane,7-hexyl-, tetratetracontane, heneicosane being major compounds detected (*Table 3*). 2-tert-butyl-4,6-bis (3,5-di-tert-butyl-4-hydroxybenzene) phenol is known to have antioxidant, anti-inflammatory and UV stabilizing properties (Ashwathanarayana and Naika, 2017). The major compounds identified in chloroform leaf extracts included sebacic acid, butyl 8-chlorooctyl ester (17.20%), 13-docosenamide (z) (4.34%), nonadecanamide (4.26%), cyclotetracosane (2.60%), 1-Heneicosanol (2.23%), 8-methyl-6-nonenamide (2.08%).

The compounds identified in the stem extracts were 20, and 17 for hexane, and chloroform, respectively (*Table 4*). Pentadecane, 2, 6, 10 -trimethyl- (5.85%), hexadecane (5.82%), tridecane, 2- methyl (4.01%), were major compounds identified in the hexane

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extract. E-15 heptadecenal (8.60%), trichloro acetic acid, hexadecyl ester (7.54%), 1-tricosane (5.48%), cycloeicosane (5.31%), heptadecyl heptafluorobutyrate (3.32%), Pentadecane, 2,6,10-trimethyl (3.05%), and 2-tert-butyl-4,4-bis(3,5-ditert-butyl-4-hydroxybenzyl) phenol (2.26%) were some of the compounds found in the chloroform extracts. These bioactive compounds are responsible for various pharmacological actions like antimicrobial, anti-inflammatory, anticancer, hepatoprotective, diuretic and anti-malaria activities (Choudhary et al., 2019; Bantho et al., 2022).

Table 3. *Bioactive compounds identified from the leaf extracts of Osteospemum moniliferum by gas chromatography-mass spectrometry analysis*

Compound name	Formula	RT	Peak (%)	Solvent
Decane, 2,5,6-trimethyl-	$C_{13}H_{28}$	3.410	2.17	Hexane
Benzene, 1,2,4-trimethyl-	C_9H_{12}	4.530	1.22	Hexane
Undecane	$C_{11}H_{24}$	5.325	1.79	Hexane
Tridecane, 2-methyl-	$C_{14}H_{3O}$	15.845	6.37	Hexane
Hexadecane	$C_{16}H_{34}$	18.355	6.31	Hexane
Pentadecane	$C_{15}H_{32}$	20.740 29.100	6.02 1.62	Hexane Chloroform
Tridecane, 5-propyl-	$C_{16}H_{34}$	25.088	2.08	Hexane
Heptadecane, 2,6,10,15-tetramethyl	$C_{21}H_{44}$	29.115 25.067	1.04 1.41	Hexane Chloroform
Nonadecane, 2-methyl-	$C_{20}H_{42}$	32.825	2.13	Hexane
Eicosane, 7-hexyl-	$C_{26}H_{54}$	34.565	2.54	Hexane
Heneicosane	$C_{21}H_{44}$	34.950	1.13	Hexane
Heptadecane	$C_{17}H_{36}$	37.850	3.31	Hexane
Tetratetracontane	$C_{44}H_{90}$	38.325 42.296	1.11 1.25	Hexane Chloroform
2-methyloctacosane	$C_{29}H_6O$	42.310 32.810	1.80 2.29	Hexane Chloroform
Tritetracontane	$C_{43}H_{88}$	43.705	1.95	Hexane
3-Eicosene, (E)-	$C_{20}H_{40}$	24.925	1.87	Chloroform
Cyclotetracosane	$C_{24}H_{48}$	28.982	2.60	Chloroform
Octadecanamide	$C_{18}H_{37}NO$	32.459	3.61	Chloroform
Cyclotetracosane	$C_{24}H_{48}$	32.711	2.64	Chloroform
13-Docosenamide, (Z)	$C_{22}H_{43}NO$	35.561	4.34	Chloroform
Tridecanoic acid, thiophen-2-ylmethylenehydrazide	$C_{18}H_{30}N_2OS$	35.610	2.72	Chloroform
Bis(dodecanamido)methane	$C_{45}H_{90}N_2O_2$	35.696	3.15	Chloroform
Nonadecanamide	$C_{19}H_{39}NO$	36.005	4.26	Chloroform
1-Heneicosanol	$C_{21}H_{44}O$	36.120	2.23	Chloroform
8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	38.854	2.08	Chloroform
Ethyl pentadecyl ether	$C_{17}H_{36}O$	39.276	2.04	Chloroform
Tetracosane	$C_{24}H_50$	39.350	1.46	Chloroform
Eicosane	$C_{20}H_{42}$	42.296	1.25	Chloroform

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Table 4. *Bioactive compounds identified from the stem extracts of Osteospemum moniliferum by gas chromatography-mass spectrometry analysis.*

Compound name	Formula	RT	Peak (%)	Solvent
Dodecane	$C_{12}H_{26}$	15.885	5.65	Hexane
Hexadecane	$C_{16}H_{34}$	18.210	5.82	Hexane
Phenol,3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	18.406 18.587	1.22 1.80	Hexane Chloroform
Pentadecane,2,6,10-trimethyl-	$C_{18}H_{38}$	20.794	5.85	Hexane
Eicosane	$C_{20}H_{42}$	22.854	1.68	Hexane
Benzestrol	$C_{20}H_{26}O_2$	23.305	5.35	Hexane
Tridecane, 2-methyl-	$C_{14}H_{3O}$	25.142	4.01	Hexane
Cycloeicosane	$C_{20}H_{40}$	29.015 24.955	1.57 5.31	Hexane Chloroform
Nonadecane, 2-methyl-	$C_{20}H_{42}$	31.297	1.69	Hexane
Tetratetracontane	$C_{44}H_{90}$	32.323	3.67	Hexane
Cyclooctacosane	$C_{28}H_{56}$	32.862 29.134	3.31 1.79	Hexane Chloroform
Eicosane, 2-methyl-	$C_{21}H_{44}$	34.600	3.42	Hexane
Eicosane, 7-hexyl-	$C_{26}H_{54}$	34.979	1.62	Hexane
2-methyloctacosane	$C_{29}H_{60}$	36.021	1.63	Hexane
Tetracosane, 11-decyl-	$C_{34}H_{70}C_{34}H_7$	43.725	1.96	Hexane
Hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	22.938	3.05	Chloroform
Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	27.168	1.17	Chloroform
E-15-Heptadecenal	$C_{17}H_{32}O$	32.730	7.54	Chloroform
Heptadecane	$C_{17}H_{36}$	32.825	1.38	Chloroform
Trichloroacetic acid, hexadecyl ester,	$C_{18}H_{33}Cl_3O_2$	36.147	5.48	Chloroform
1-Tricosene	$C_{23}H_{46}$	39.307	3.32	Chloroform
Heptadecyl heptafluorobutyrate	$C_{21}H_{35}F_7O_2$	41.964	1.43	Chloroform
Bis(dodecanamido)methane	$C_{45}H_{90}N_2O_2$	42.252	1.98	Chloroform
Tricosyl trifluoroacetate	$C_{25}H_{47}F_3O_2$	45.006	1.04	Chloroform

Phenolic compounds are organic compounds found throughout the plant kingdom. They are known for their hepatoprotective, anti-inflammatory, antioxidant, antimicrobial and anticancer properties (Amoo et al., 2011; Saibabu et al., 2015). Phenol,3,5-bis (1,1-dimethyl ethyl)-, and 2-tert-Butyl-4,6-bis(3,5-ditert-butyl-4-hydroxybenzyl) phenol- are phenol compounds that were present in the study. Extracts contained alkanes such as dodecane, hexadecane, pentadecane, tridecane,2-methyl, eicosane, and heptadecane. Undecane is used as an antifungal agent, transducer for immunosensor and its production method, carcinogen, and enzyme inhibitor solvent (Jamuna and Paulsamy, 2013). Eicosane is known for its use in the lubricant, cosmetic, and petroleum industries (Bantho et al., 2022). Additionally, it is used in ethnomedicine as an antioxidant and antimicrobial agent (Ahsan et al., 2017). Hexadecane has been reported to possess antimicrobial, antioxidant, antidiabetic, anti-inflammatory, antidiarrhoeal, cytotoxicity, and anthelmintic activities (Gnanavel and Saral, 2013; Banakar and Jayaraj, 2018). Tetracosane was only found in hexane extracts. This compound is cytotoxic against cancer cells and has anti-diarrhoea, antibacterial, cardiomyopathy, anti-inflammatory,

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antioxidant, and anticorrosive (Majeed et al., 2013; Dandekar et al., 2015). Tetratetracontane was found to have cytoprotective and antioxidant activities (Kuppuswamy et al., 2013). 13-decosenamide(z) is a fatty amide with antioxidant and antimicrobial activity (Thorat, 2018). E-15-heptadecenal, one of the major compounds observed in chloroform extracts (8.60%), is an aldehyde and has been reported for its antibacterial activity (Kumar et al., 2011). 4-ethylbenzoic acid is a phenolic compound reported to have anti-inflammatory, antibacterial, antifungal, analgesic, and antioxidant (Choudhary et al., 2019). Most of the identified compounds were shown to have significant biological activities (Ralte et al., 2022), and they all belonged to different chemical groups. Furthermore, numerous compounds have yet to be properly described. Additional research on the extraction and characterization of chemical compounds from plant extracts is needed to confirm their diverse pharmacological importance.

FTIR analysis

Functional groups can be employed in various pharmaceutical products, including anticancer, antiulcer, anti-inflammatory, antioxidants, and antimicrobials (Maobe et al., 2013). Fourier Transform Infrared is an advanced technology used to identify pharmacologically active molecules in natural products based on the peak values of the FTIR spectrum. The FTIR spectrum serves as a chemical fingerprint and offers a distinct qualitative characterization of antimicrobial activity since no two bioactive compounds have the same FTIR spectrum (Easmin et al., 2017). Fourier-transformed infrared spectroscopy was employed to identify the functional groups of bioactive compounds present in *O. moniliferum*. Both leaf and stem have displayed the existence of different functional groups. Multiple peaks were seen, indicating that both leaf and stem contained a variety of functional metabolite groups. The spectra revealed strong and medium absorption frequency peaks at 3278.59, 2916.42, 1618.26, 1032.25, and 531.99 cm^{-1} for the powdered leaf sample (*Figure 2A*). The powdered stem showed significant peaks at 3311.70, 1624.89, 1370.95, 1240.04, 1032.15, and 591.52 cm^{-1} (*Figure 2B*). The spectrum of the leaf showed a strong O-H stretching peak that corresponded to the functional group of alcohols at 3279.59 cm^{-1} and at 3311.70 cm^{-1} in the stem spectra (*Table 5*). A medium peak detected at 2916.42 cm^{-1} was assigned to C-H stretching functional groups of alkanes and O-H stretching of carboxylic acids in the leaf.

A strong peak at 1618.26 and 1624.89 cm^{-1} in the leaf and stem corresponded to the N-H bending groups of amines. The occurrence of the medium peak at 1370.95 cm^{-1} by C-H bending was indicative of alkanes, and the strong peak at 1240.04 cm^{-1} corresponded to ether functional groups for the stem spectra. The spectral absorption produced a medium peak at wavelengths of 1032.25 and 1032.15 cm^{-1} of leaf and stem, indicative of primary amines. Alkyl halides, also known as halo compounds, were discovered in both samples. The spectra of the leaves and stem showed strong peaks at 591.52 and 531.99 cm^{-1}, corresponding to the C-l (stretching) functional group of alkyl halides (Coates, 2000).

The FTIR spectra of the leaf confirmed the presence of alkyl halides (531.99). Alkyl halides are known to have strong antibacterial properties (Vanitha et al., 2019). The wavelengths 1032.52 and 1032.12 represent the primary amines. This is related to alkaloids, which typically include one or more heterocyclic rings with nitrogen atoms (Ziegler and Facchini, 2008). Amines and alkanes were considered the major functional groups of bioactive compounds (Pratheeba et al., 2015). A study by Bashir et al. (2020) on *Vernonia amygdalina* also confirmed the presence of alkanes, alcohols, and amines.

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The presence of alcohol, alkanes, which is a component in plant wax (Dove and Mayes, 1991), alkyl halides (Kamble and Gaikwad, 2016), carboxylic acids, ethers and amines detected by FTIR analysis may be the reason for possible medicinal properties of the plant (Mariswamy et al., 2012). The OH group plays a crucial role in antidiabetic, antioxidant, and antibacterial activities (Sabandar et al., 2017). The functional groups discovered in the leaves and stems of *O. moniliferum* supported the findings of the qualitative phytochemical screening, which revealed comparable phytochemical contents in the leaves and stems of the plant.

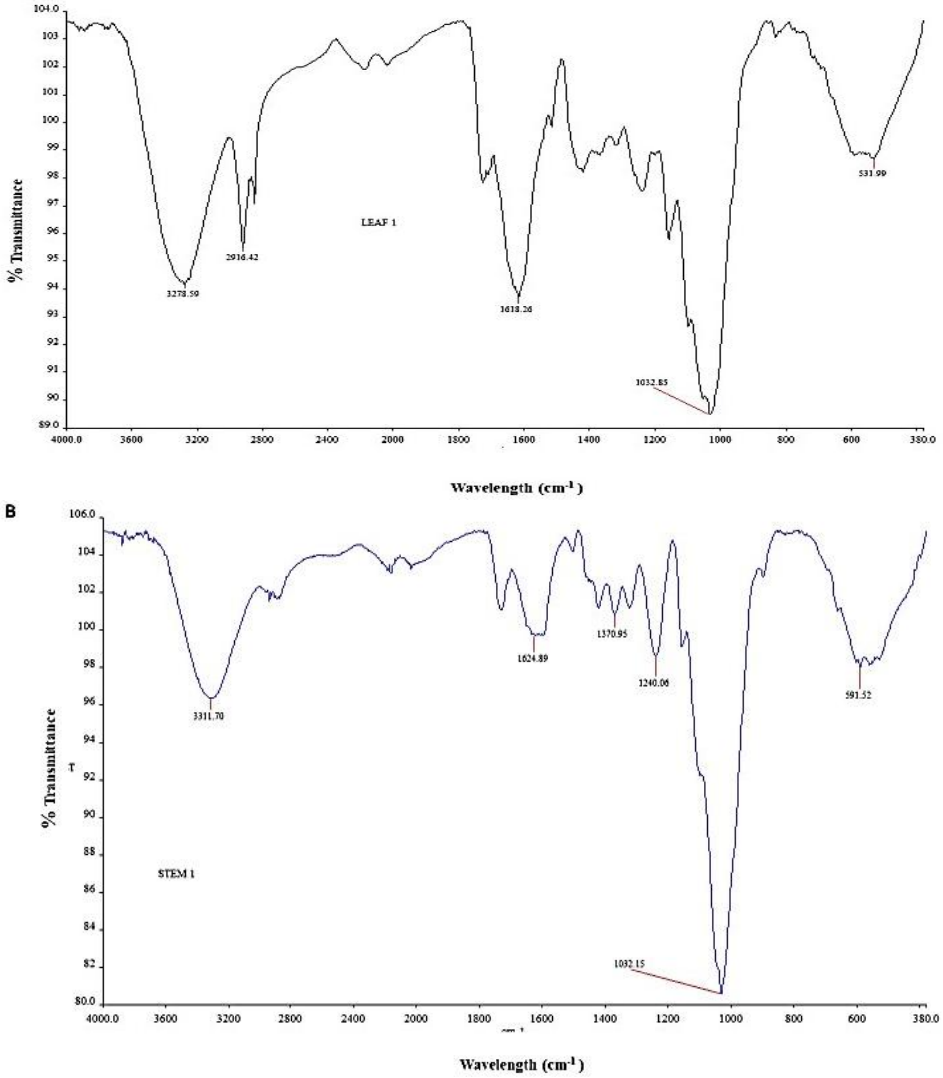

Figure 2. *Fourier-transform infrared spectroscopy analysis of leaf and stem powder of Osteospemum moniliferum (a) leaf powder; (b) stem powder*

Fluorescence analysis

A variety of chemical components in plant material exhibit fluorescence. Each chemical compound has its fluorescent colour. Plant material portrays different colours when exposed to different chemicals. Many organic substances, such as alkaloids like berberine, do not glow in the daylight but fluorescence when exposed to ultraviolet light

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(Bantho et al., 2022). The fluorescence results of dried leaf and stem powder treated with various chemical agents are shown in table 6. Under visible light and UV light, the powdered samples were treated with different solvents. The assay revealed distinct and noticeable colour changes for different samples (*Table 6*). The fluorescence analysis of powdered samples plays a vital role in the determination of the quality and purity of the drug. When treated with 50% H_2SO_4, phytosterols show green fluorescence under UV light.

Table 5. *Fourier Transform Infrared spectrum analysis of Osteospemum moniliferum powder*

Sample	Absorption frequency (cm^{-1})	Intensity	Functional group assignment	Predicted compounds
Leaf	3279.59	Strong broad	O-H stretch	Alcohol
	2916.42	Medium	C-H stretch, O-H stretch	Alkanes, carboxylic acids
	1618.26	Strong	N-H bend	Secondary amines
	1032.25	Medium	C-N stretch	Primary amines
	531.99	Strong	C-I stretch	Alkyl halides
Stem	3311.70	Strong broad	O-H stretch	Alcohol
	1624.89	Strong	N-H bend	Amines
	1370.95	Strong	C-H bending	Alkanes
	1240.04	Strong	C-O stretch	Ethers
	1032.15	Medium	C-N stretch	Primary amines
	591.52	Strong	C-I stretch	Alkyl halides

Table 6. *Fluorescence analysis of dried leaf and stem powder of Osteospemum moniliferum*

Organic solvent	Leaf powder		Stem powder	
	Brightfield	Fluorescence in UV (330/380i)	Brightfield	Fluorescence in UV (330/380i)
Powder only	Blackish brown	Green, blue, orange	Brown	Green, blue
Water	Greenish brown	Blue, orange, brown, pinkish	Brown, black	Dark brown
Acetic acid	Brown	Blue, orange	Brown	Blue, orange
Hexane	Brown	Green, blue	Brown, light green	Blue, green
Ethyl acetate	Brown	Blue, pinkish	Brown	Blue
Chloroform	Greenish brown	Blue	Brown	Blue
Acetone	Brown	Blue, orange	Brown	Orange, blue
Ethanol	Brownish black	Blue, orange	Brown	Blue
Methanol	Brown, light green	Blue, orange, green	Brown	Blue, green, reddish
HCl	Green	Dark green, orange, blue	Green	Dark green
Aqueous NaOH	buddy Brown	Orange, green, blue	Muddy brown	Blue, orange, pink
Diethyl ether	Brown	Orange, blue	Brown, black	Blue
50% H_2SO_4	Dark brown	Blue	Brownish black	Green, blue

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Elemental analysis using EDX

Energy Dispersive X-ray has been used to analyse the composition of elements in the plant. The elemental analysis of the leaf and stem revealed the presence of calcium (Ca), sodium (Na), sulphur (S), copper (Cu), magnesium (Mg), potassium (K), oxygen (O), aluminium (Al), carbon (C), silicon (Si), phosphorus(P), chlorine (Cl) and molybdenum (Mo) (*Table 7 and Figure 3*). Aluminium, S and Si were present in the leaf and absent in the stem, while Mo was absent in the leaf and detected in the stem. Overall, the leaf sample contained more elements than the stem sample. Most of the elements observed play a vital role in physiological processes and plant nutrition. Carbon showed the highest weight of 65.07% and 51.10%, respectively, followed by oxygen (19.15% for leaves and a higher amount of 40.49% for the stem), while other elements were present in trace quantities. The nutritional and medicinal functions of a plant may depend on the type of mineral element present (Prasad, 1993). Each element serves a variety of roles in the body. Silicon is important in preventing artery and vein hardening (Ragavendran et al., 2012), whereas phosphorus is essential for enzymatic phosphorylation activities. Additionally, Mg is a crucial component in the conversion of trace elements, also known as essential elements, which support several metabolic processes, overall health, and the treatment of diseases by working as co-factors to numerous enzymes, blood glucose into energy and regulates the muscle activity of the heartbeat and has been reported to be beneficial in the fight against stroke and cell repair, whereas calcium is required for strong bones, teeth, blood, and the regulation of skeletal, heart, and tissue muscles (Afolayan and Otunola, 2014; Sakuntala et al., 2019). It is also a central atom in the chlorophyll molecules and is crucial for photosynthesis (Adegbaju et al., 2019). Calcium is involved in the prevention and management of illnesses (Aliyu et al., 2008). The presence of calcium could be explained by the existence of calcium crystals, which are frequent in species of medicinal plants (Anitha and Sandiya, 2014). These crystals have not yet been observed in *O. moniliferum*, but they have been observed in a few Asteraceae species (Raeski et al., 2023). Oxalate crystals are effective for identifying adulterants due to changes in their morphological structure (Anitha and Sandiya, 2014). Sulphur treats various skin conditions and irritations, including eczema, psoriasis, acne, and antibacterial agents (Gupta and Nicol, 2004). An element like potassium enhances the synthesis and translocation of carbohydrates, which causes the thickening of plant cell walls, allowing the plant to remain upright. Potassium is an essential element in the human body. Potassium is essential for the movement of nutrients. Nutrients are necessary for entering cells to prevent cell death (Ragavendran et al., 2012). An abundant amount of potassium was detected in the stem (2.75%). Copper, a trace dietary mineral, is required by all living things. It is mostly found in the human liver, muscle, and bone. Copper compounds are fungicides, bacteriostatic agents, and wood preservatives (Fry et al., 2012). One of the crucial roles of copper in the human body is that it aids in producing red and white blood cells (Sakuntala et al., 2019). Chlorine was found in leaves (9.14%) compared to the stem (3.24%). Chlorine participates in the Photosystem II Hill reaction and the stomatal control mechanism in plant leaves to fulfil several metabolic and physiological tasks (Chen et al., 2010); this explains why the leaves contain more Cl than the stem.

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Table 7. *Elemental composition of the powdered leaf and stem of Osteospermum moniliferum*

Element	Composition (%)	
	Leaf	**Stem**
C	65.07	51.10
O	19.15	40.49
Na	2.42	0.47
Mg	0.64	0.33
Al	0.07	-
Si	0.06	-
P	0.12	0.18
S	0.31	-
Cl	9.14	3.24
K	1.16	2.75
Ca	1.48	0.31
Cu	0.37	0.58
Mo	-	0.55

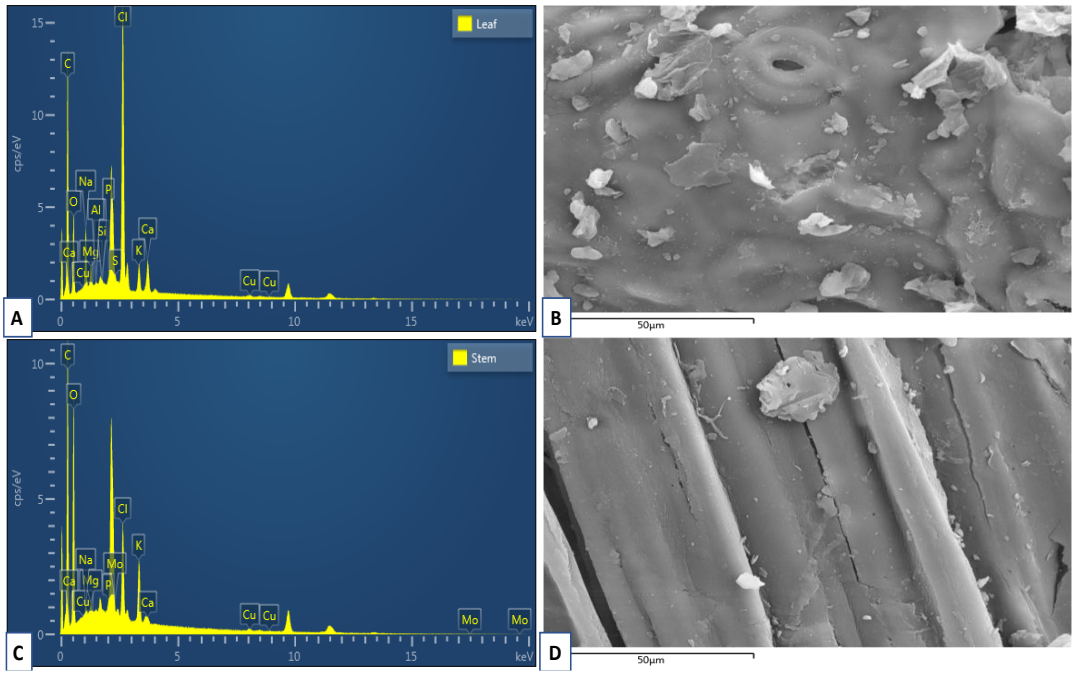

Figure 3. *Scanning electron micrographs of the leaf and stem of Osteospemum moniliferum. (A) EDX spectra of powdered leaf, (B) micrograph of powdered leaf used for EDX microanalysis, EDX spectrum of powdered stem material, (D) SEM micrograph of powdered stem particles used for EDX analysis*

Conclusions

Phytochemical analysis of *O. moniliferum* leaf and stem extracts revealed the presence of numerous bioactive chemicals in varying amounts. Elements such as carbon, oxygen, chlorine, magnesium, silicon, aluminium, phosphorus and sulphur were identified by elemental analysis and EDX. These components have a significant impact on the formation of secondary metabolites which affect the quality of the herbal raw material. Furthermore, no harmful heavy metals were discovered during the analysis. The GC-MS

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analysis showed the presence of several phytocompounds that contribute to antioxidant, antimicrobial, anticancer, anti-inflammatory and other properties. The various functional groups identified by FTIR indicated the presence of alcohol, amines, alcohol, alkanes and carboxylic acids. The presence of the OH group indicates the higher potential of the plant towards inhibitory activity against microorganisms. Therefore, findings of the qualitative tests, TLC, fluorescence microscopy and GC-MS analysis clearly indicate that *O. moniliform* leaves and stems contain phytochemical compounds that are potential sources of natural antioxidants that could serve various therapeutic purposes. Additionally, the powdered samples contained several functional groups that are related to these phytocompounds and also contained essential components required for the proper functioning of medicinal plants. Further studies should be conducted on the isolation, identification and characterization of the bioactive compounds in *O. moniliferum* that may be responsible for its bioactivity. Further investigation is needed to potentially develop novel drugs using some of the bioactive compounds found in *O. moniliferum*. Furthermore, additional investigations on the plant's bioactivity, toxicity profile and clinical trials are essential for broad-spectrum drug discovery.

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