

Research Article

***In-vitro* Studies on the Herbal Plant *Microdesmis keayana*
Methanol Leaves Extract Phytochemicals by GC-MS
Analysis: A Further Evidence for its Phytochemical
Potentials in Enhancement of Reproductive Functions**

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ABSTRACT

Microdesmis keayana (*Microdesmis*) belongs to the Pandaceae family, and it is called "Apata" in Yoruba South-Western Nigeria. The study is undertaken determining the availability or absence of erectile and pro-fertility compounds of the methanol extract of leaves of *Microdesmis keayana* by mass spectroscopy using gas chromatography (GC-MS). Methodologically, GC-MS analysis was used. The GC-MS analysis revealed six (6) peaks corresponding to six distinct phytoconstituents which include: 9, 12-Octadecadienoic acid (Linoleic acid) (peak area: 22.99%), 11-Phenylundecanoic acid (peak area: 17.66%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (peak area: 21.14%), Squalene (peak area: 14.01%), n-Hexadecanoic acid (peak area: 13.11%), and Stigmasterol (peak area: 11.09%) respectively. Sequel to the findings of this study, methanol extract of *Microdesmis keayana* leaves may be an effective reproductive therapeutic agent, paving the way for the development of several treatment regimens based on compounds derived from this extract.

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INTRODUCTION

Treatment of diseases using natural product of plant origin has gained momentum in human society. In the world health care system of mankind, medicinal plants have been playing important roles. According to scientific literature, only about 2% of all plants on the planet have been subjected to pharmacological testing. The reasons for the use of natural plant products have been largely due to long-term clinical experience with little or no specific data on their efficacy and biosafety (Zhu *et al.*, 2002). Equally importantly, need to validate unscientific usage of natural products of plant origin through a scientific investigation is very imperative based on wider incremental consumption of herbal plants for improving person's health (Sofowora, 1989; Bakari *et al.*, 2015).

In the open scientific literature, information on the bioactive constituents in *Microdesmis keayana* and their structural linkages to bioactivity are not enough. From the foregoing, it is therefore imperative to provide this information for bridging the knowledge gap on bioactive constituents of *M. keayana* leaf extract. The unfolding constituents presented on the studied plant via research study, will ultimately, participate in medicinal utilization of studied botanical, thereby, giving credence to scientific innovation on developmental botanic processes in relation to phytomedicine and phytopharmacology.

Microdesmis keayana (J. Leonard) (synonymous *Microdesmis puberula*) belongs to the family, Pandaceae. The Yoruba (Ilaje) of Western Nigeria refer to it as 'Apata'. *Microdesmis* is the common name for this plant. It is a dioecious shrub that can reach heights of 3-6 m. And it is found throughout western Africa, from Senegal to south-western Nigeria (Burkill, 1997). According to oral communication and information gathered from village physicians, herbalists, herb sellers, and those engaged in

self-medication in local communities area of Ondo State, Nigeria, the root of *M. keayana* in tincture preparation with the leaves is widely used to curtail male sexual dysfunction and other related ailments. The leaves, which are practically alternate, distichous, ovate, with linear and simple stipules, are usually consumed to treat diarrhea, sometimes on conjunction with twig sap. In Liberia, Nigeria, and Sierra Leone, the infusion of the leaves is used to enhance menstrual period in female as well as an abortive agent (Neuwinger, 2000). The leaves decoction, or leafy twigs or ground young leaves, is used as a steam bath or wash in Ghana and Cote d'Ivoire to treat arthritis or painful headache (Cuningham, 1993).

The present study is therefore undertaken determining the availability or absence of erectogenic and spermatogenic compounds of the methanol extract of leaves of *Microdesmis keayana* by mass spectroscopy using gas chromatography (GC-MS).

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Microdesmis keayana* were collected from the wild in the Digbolu camp area of the Ikoya Community in the Okitipupa Local Government Area of Ondo State, Nigeria. Plant leaves were identified by a Taxonomist at the Botany Unit of the Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria.

Preparation of plant extract

Microdesmis keayana plant material collected was dried under shade for 10 days before being ground into powder with a mechanical grinder. The plant extract was prepared by basic extraction and fractionation procedures for experimental purposes as previously described and published by Abubakar and Haque (2020). Powdered sample of 35 g was placed in maceration chamber with methanol as solvent and macerated for 72 days. Following the

extraction period, the extract was concentrated in an oven at 35 °C to produce dried extract to be used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Gas chromatography and mass spectrometry (GC-MS) analysis of *Microdesmis keayana*

The phytochemicals derivatives of the extract were identified using GC-MS apparatus as cited by Bakari *et al.* (2015). A HP-5 MS capillary column was used for the GC system. Helium was used as carrier gas at a flow rate of 1 mL/min. The injection volume was 2 µL. Ionization energy EI of 70 eV was used for mass spectroscopy detector. The GC-MS apparatus was carried out by Chem-Station software package (Agilent Technologies). Both the injector and detector temperature were 250 °C. The oven temperature was held at 100 °C for 1 min, increased to 260 °C at a heating rate of 4 °C/min, and held for 10 min. Thereafter, identification of compounds was carried out.

Identification of phytochemicals in *Microdesmis keayana*

GC-MS chromatogram of *Microdesmis keayana* revealed six peaks, indicating the presence of six different compounds. The extract's active ingredients were identified through comparison of their retention parameters, area's peak percentage, and spectra's mass fragmentation design to those saved in the database of the National Institute of Standards and Technology [NIST] (Stein, 1990); as well as published literature (Mc-Lafferty, 1986; Bakari *et al.*, 2015). These library resources were employed matching the recognised plant material active ingredients. Compounds' names, molecular weights, formulas, structures, and bioactivities were determined.

RESULTS AND DISCUSSION

The GC-MS chromatogram of *Microdesmis keayana* leaves methanol extract (Figure 1) revealed six peaks, indicating six phytochemical compounds found present.

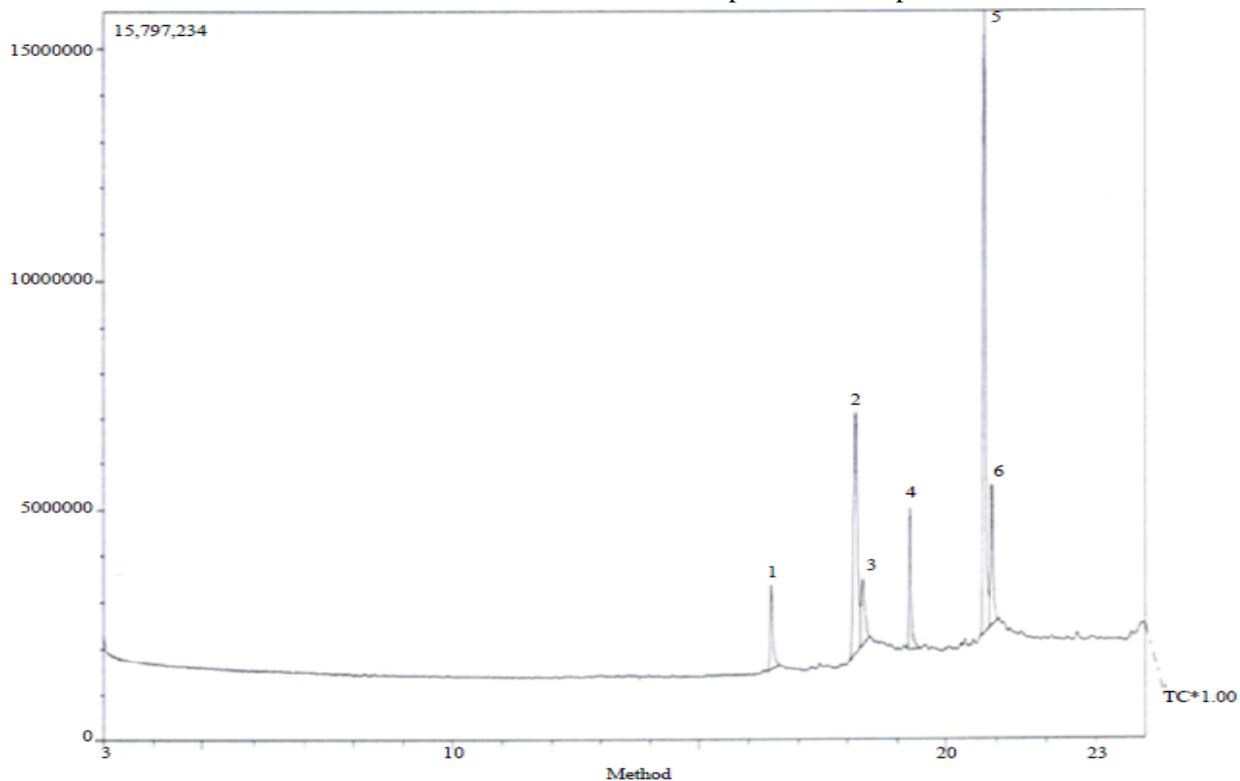


Figure 1: GS-MS chromatogram of *Microdesmis keayana* leaves extract

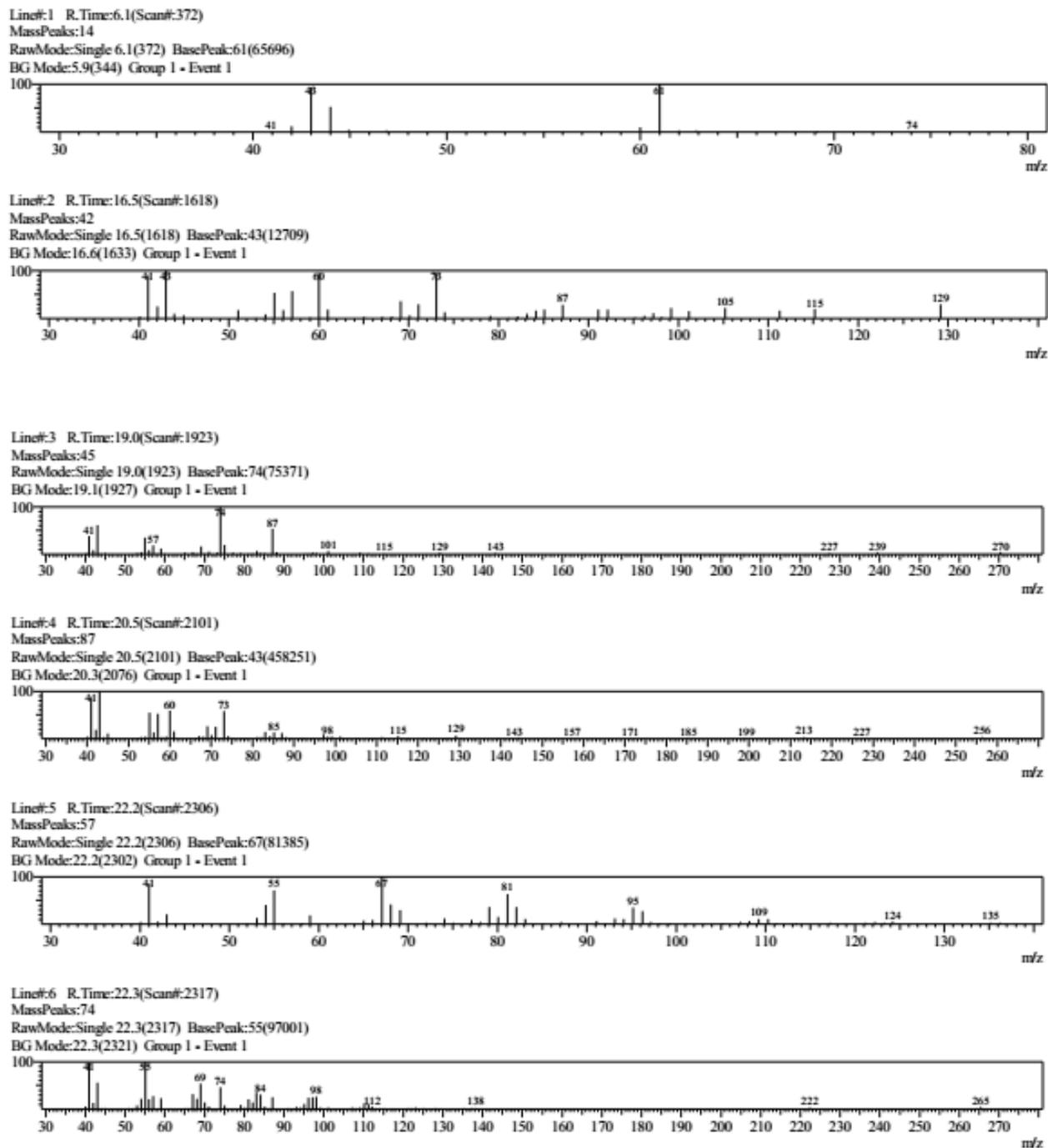
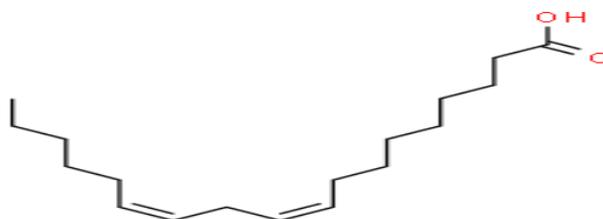
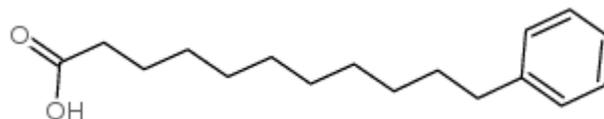


Figure 2: Depicts spectra's mass of six compounds recognized through GC-MS assay in *Microdesmis keayana* leaves

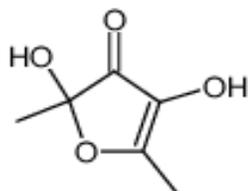
Table 1: Depicts names of compounds, molecular formula, molecular weight, retention time, peak area percentage and bioactivity of recognized compounds in *Microdesmis keayana* leaves by GC-MS.

S/No.	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Retention Time/Period (mins)	Peak area (%)	Bioactivity
1.	9,12-Octadecadienoic acid (Linoleic acid)	C ₁₈ H ₃₂ O ₂	280.44	6.10	22.99	Lipid metabolism regulation, Antiarteriosclerotic, Anticoronary, and antioxidant activities (Yuan <i>et al.</i> , 2014)
2.	11-Phenylundecanoic acid	C ₁₇ H ₂₆ O ₂	262.38	16.5	17.66	Androgen receptor (Igwe <i>et al.</i> , 2016)
3.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144.12	19.0	21.14	Dietary, Flavoring ingredient of food grade (Padmashree <i>et al.</i> , 2018)
4.	Squalene	C ₂₉ H ₄₈ O	412.68	20.5	14.01	Antioxidant, Antitumor, Cancer-Preventive, Cardio-protective, Immunostimulant (Ezealisiji and Nwoke, 2020)
5.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	22.2	13.11	Antioxidant, and anti-atherosclerotic activity (Cho, 2015)
6.	Stigmasterol	C ₂₉ H ₄₈ O	412.69	22.3	11.09	Reduction of Atherosclerosis, Cardioprotective, Cholesterolytic agent (Ezealisiji and Nwoke, 2020)

(1) **9, 12- Octadecadienoic acid**



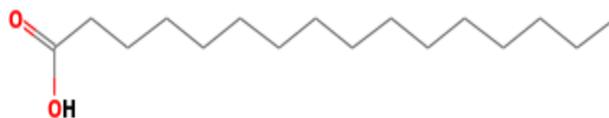
(2) **11- Phenylundecanoic acid**



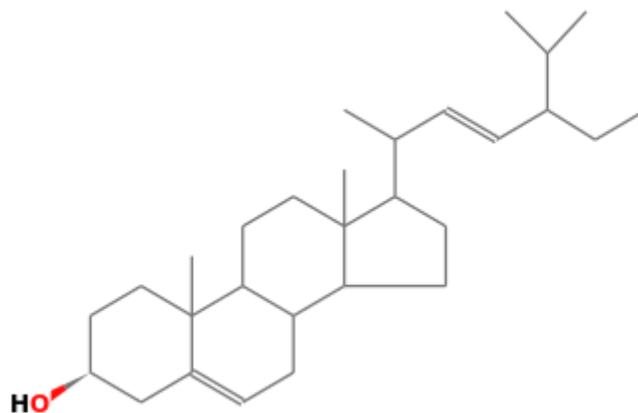
(3) **2,4-Dihydroxy-2,5-dimethyl-3(2H)-furans-3-one**



(4) **squalene**



(5) **n – Hexadecanoic acid**



(6) Stigmasterol

Figure 3 (1-6): Molecular structure of identified compounds exhibiting reproductive functions in *Microdesmis keayana* leaves by GC-MS analysis.

Natural substances of plant origin have been used for centuries to treat a wide range of homo sapiens diseases. Plant materials such as leaves, stems, bark, and roots, among others, are used to prevent, alleviate, and restore abnormalities to normal. It is thought that the use of an alcoholic solvent in the extraction, preservation, and column chromatography procedures affects the amount of phytoconstituents extracted. Therefore, phytochemicals with diverse biological activities were discovered in *Microdesmis keayana* methanol leaves extract. Figure 1 shows the GC-MS profile of the compounds identified in the total ion chromatogram (TIC) of methanol leaves extract of *Microdesmis keayana*. Six peaks were detected in the GC-MS chromatogram of the plant material's methanol extract, indicating the presence of six phytochemicals. Figure 2 depicts the mass spectra as well as the retention time of the identified compounds, whereas Table 1 displays the compounds' names, molecular formula, molecular weight, retention time, and bioactivity. Figure 3 depicts the molecular structure of the identified compounds in

Microdesmis keayana leaves that exhibit reproductive functions and other bioactivity.

The compound 9,12-Octadecadienoic acid (Linoleic acid) had lipid metabolism regulation, antiarteriosclerotic, and antioxidant activities relaying retention period of 6.10 and percentage peak area of 22.99 percent (Yuan *et al.*, 2014). Lipid metabolism regulation is important in balancing normal triglyceride hepatocyte deposition, which can lead to non-alcoholic fatty liver disease (NAFLD). The four main mechanisms that regulate hepatic lipid metabolism are fatty acid uptake, de novo lipogenesis, lipolysis, and fatty acid oxidation. Controlling transcription factor expression at the molecular level is critical in the treatment of NAFLD (Zakaria *et al.*, 2021). Atherosclerosis is a multifactorial chronic disease characterized primarily by changes in the blood lipid profile and inflammation of the vessel wall. Atherosclerosis-related cardiovascular disease is now the leading cause of death in developed countries. As a result, early detection and treatment of atherosclerosis can reduce the likelihood of clinical manifestations developing. Medicinal plants' anti-atherosclerotic activity is

primarily manifested through their anti-inflammatory, antioxidant, antiatherogenic, hypotensive, lipid-lowering, and anti-thrombotic properties. Furthermore, the majority of medicinal plants have pleiotropic anti-atherosclerotic activity. Equally important, pharmacological substances and/or compounds derived from medicinal plants are distinguished by their relative safety and lack of side effects, allowing them to be considered as potential anti-atherosclerotic effective agents. The direct anti-atherosclerotic effect of some medicinal plants was confirmed in clinical trials of carotid intima-media thickness (IMT) progression during long-term medication with medicinal plants (Kirichenko *et al.*, 2020). Antioxidants are compounds that prevent or reduce the effects of reactive oxygen or reactive nitrogen species. In animals, they are used as dietary supplements to help with cancer treatment, improve immune function, and reduce treatment toxicity (Studdert, 2012).

The compound 11- Phenylundecanoic acid has been implicated as an androgen receptor with a retention time of 16.5 and a peak area percentage of 17.66 percent (Igwe *et al.*, 2016). This compound generates the alkaloid keayanadine, a 2-adrenergic receptor antagonist used to reverse sedation caused by 2-adrenogenic receptor agonists like xylazine (authors' unpublished data). An alkaloid is a small organic compound found in plants that is derived primarily from amino acids and contains nitrogen. They have a high level of physiological activity, are water soluble, and usually have a bitter taste (Studdert, 2012). Nutrition, especially nutrition derived from natural plant products, has been shown to increase the sensitivity and number of androgen receptors in cells. This plant-based nutritional supplement helps to increase androgen receptors and make the most of circulating testosterone. Testosterone is an anabolic hormone found primarily in men. This powerful hormone is

present in all bodies to varying degrees. It regulates key anabolic (growth) functions such as regulating reproductive functions, stimulating sexual arousal and libido, increasing resting metabolic rate, promoting muscle mass and strength, increasing bone density and strength, stimulating bone growth and maturation, and promoting body hair and facial hair growth. When testosterone binds to an androgen receptor and enters a target cell, it has a mildly androgenic effect. Before testosterone can have any effect on the body, it must first bind to an androgen receptor inside a cell (Anonymous, 2022).

With a retention time of 19.0 and a peak area percentage of 21.14 percent, the compound 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one has been implicated as a dietary, food-grade flavour ingredient (Padmashree *et al.*, 2018). This finding supports the report's assertion that "diet is one piece of the fertility puzzle, and it happens to be a piece that someone can control, along with alcohol consumption, smoking habits, and stress levels". "Diet alone will not unclog a fallopian tube or cure a lack of sperm," says the author. Rather, nutrition is important for overall health and has been shown to influence fertility (authors' unpublished data).

With a retention time of 20.5 and a peak area percentage of 14.01 percent, squalene demonstrated antioxidant activity (Ezealisiji and Nwoke, 2020). According to the findings of this study (Table 1), the plant contains antioxidative compounds (Yuan *et al.*, 2014; Ezealisiji and Nwoke, 2020) that could play an important role in increasing human fertility; and according to Meena and Sreenivasula (2014), successful fertility is dependent on a variety of factors, including antioxidative status. This study's phytochemicals are a type of naturally occurring compound found in plants (Meena and Sreenivasula, 2014). They may have anti-cancer, anti-cardiovascular disease (as shown in Table 1), anti-osteoporosis, and anti-menopausal

effects when consumed by mammals. Both animals and humans consume phytochemicals on a regular basis. Regular dietary intake or nutritional supplements containing phytochemicals provide exposure (Meena and Sreenivasula, 2014).

With a retention time of 22.2 and a peak area percentage of 13.11 percent, the compound n-Hexadecanoic acid methylester, also known as palmitic acid, demonstrated antioxidant and anti-atherosclerotic activity (Cho, 2015). Atherosclerosis is a risk factor for hypertension that can result in coronary heart disease, heart failure, stroke, peripheral artery disease, and even death (Kaplan, 2015). Obesity, smoking, and high cholesterol levels all have a significant impact on the risk of coronary artery disease and stroke (Kaplan, 2015).

Stigmasterol, a phytochemical (plant sterol) with a retention time of 22.3 and a peak area percentage of 11.09 percent, was discovered to reduce atherosclerosis and act as a cholesterol-lowering and cardioprotective agent (Ezealisiji and Nwoke, 2020). It is one of the most abundant plant sterols, and it plays a critical role in the structure and physiology of cell membranes (Ezealisiji and Nwoke, 2020). In the European Union, it is classified as a food additive and may be used in food production to increase phytochemical content, potentially lowering low density lipoprotein (LDL) cholesterol levels (Capel, 2017). A fascinating aspect of sperm production is the dynamic change in membrane lipid composition that occurs during spermatogenesis. The amount of cholesterol and its intermediates in the male reproductive system and nongonadal tissues

COMPETING INTERESTS

None

varies greatly. Cholesterol precursors such as testis meiosis-activating sterol and desmosterol accumulate in testes and spermatozoa from several mammalian species. Furthermore, cholesterol biosynthetic genes, particularly the meiosis-activating sterol-producing enzyme cytochrome, show stage-specific expression patterns during spermatogenesis. Disparities in gene expression patterns suggest that sterol compounds are regulated in a complex temporal and cell-type-specific manner during spermatogenesis, which also includes dynamic interactions between germ and Sertoli cells. Sterol composition modulation in spermatozoal membranes during epididymal transit and in the female reproductive tract, which is required for successful fertilization, adds to the functional importance of sterol compounds in sperm production (Keber *et al.*, 2013).

CONCLUSION

The present study has presented the GC-MS analysis of the methanol leaves extract of *Microdesmis keayana* by furthering its evidence in the enhancement of reproductive functions, thereby revealing presence of six peaks representing six phytochemicals of pronounced reproductive functions such as 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with retention time/period of 19.0, 11-Phenylundecanoic acid with retention time of 16.5, stigmasterol with retention time of 22.3, and others. These phytochemical compounds have pharmacological properties that are beneficial to humans. As a result, more research can be done on the extract to isolate and purify the various compounds, which could be useful for drug development and formulation.

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