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Determination of bioactive compounds in methanol leaf extract of *Hyptis lanceolata* Poir. using gas chromatography and mass spectrometry (GC-MS) technique

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Abstract
Hyptis lanceolata Poir. is a medicinal plant wildly distributed in parts of Nigeria. The plant is used in traditional medicine for the cure of skin infections and as an analgesic in Nigeria. The plant is underutilized and documentation of its chemical composition is poor. The leaves of the plant were subjected to sohxlet extraction using methanol as solvent. The extracts were further subjected to Gas Chomatography and Mass Spectrometry (GC-MS) to determine the presence of bioactive compounds. GC-MS analysis of the methanolic extract of the plant showed the presence of eight compounds. The major compounds include α-Linolenic acid (45.86%), dihomo ɣ-linolenic acid (30.50%), squalene (8.27%) and Imidodicarbonimidic diamide, N,N-dimethyl- (6.61%). Previous studies have shown these compounds to be efficient in the treatment of several ailments, hence the presence of these compounds support the use of this plant in traditional medicinal. It is recommended that the methanol extract of the plant be subjected to further pharmacological and phytochemical studies in order to achieve the full potentials of the plant.

Keywords: α-Linolenic acid, dihomo ɣ-linolenic, GC-MS, *Hyptis lanceolata*, squalene

Introduction
Hyptis lanceolata is a wild plant seen growing along road sides, abandoned waste lands and damp areas in parts of Edo State, Nigeria. The plant has also been reported to be distributed in most parts of West Africa in countries such as Cameroun, Benin and Senegal (Johnson, 1997; Akobundu and Agyakwa, 1998) [1, 2]. It is an aromatic herb which belongs to the family Lamiaceae. It has a quadrangular stem with lanceolate leaves which are acute at apex and serrated at margin (Hutchinson *et al*., 1963) [3]. The flowers are white, formed in a dense globose inflorescence.
Plants with medicinal potentials contain bioactive compounds which have been exploited by herbalists in traditional medicine for treating different diseases. *H. lanceolata* is used in traditional medicine by the Ijaw tribe in Nigeria. The leaves are used as analgesic and for the cure of cutaneous and subcutaneous skin infections such as eczema (Burkill, 1997) [4]. The root of the plant is used for the management of pulmonary diseases (Burkill, 1997) [4].
Despite its importance in traditional medicine, *H. lanceolata* is underutilized and documentation of its phytochemical composition is scanty, hence, the need for this research which is aimed at determining the phytochemical composition of the plant using GC-MS technique.

Materials and Methods
Collection and Identification of Plant Materials
Plant materials were collected from an uncultivated farmland in Ewu, Esan Central Local Government Area of Edo State, Nigeria. Herbarium press of the plant was made and was taken to the University of Ilorin, Department of Plant Biology Herbarium where it was identified and given herbarium number (UILH/001/1347).

Preparation of Plant Extract
The leaves of the plant were air-dried for three days and then ground to powder. The dried leaf powder (200gm) was extracted in Soxhlet apparatus in 400 ml of methanol.

The extraction was concentrated by slow evaporation process using a water bath. The extract obtained was kept in moisture free McCartney bottles before use for GC-MS analysis.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The GCMS equipment used is GCMS-QP2010 PLUS SHIMADZU JAPAN fused with a GC column (AOC 2i) coated with polymethyl silicon with a dimension of 0.25mm x 30mm. Experimental conditions of GCMS were as follows: film thickness: 0.25µm, flow rate of mobile phase (carrier gas: He) was set at 1.0ml/min, temperature programme was 40 °C and raised to 290 °C at 5 °C/min and injection volume was 1.0µ. a scan interval of 0.5 seconds with scan range of 40-600m/z. Total GC running time was 20min.

Identification of Compounds: The results were interpreted using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown components was compared with the spectrum of the known components stored in NIST database.

Results: The GC-MS analysis of methanol leaf extract of *H. lanceolata* revealed the presence of eight compounds represented by eight peaks on the chromatogram (Figure 1). The retention time, molecular weight, molecular formular, and the concentrations of the compounds (peak) are presented in Table 1. Major compounds in the extract are α-Linolenic acid (45.86%), dihomo ɣ-linolenic acid (30.50%), squalene (8.27%) and Imidodicarbonimidic diamide, N,N-dimethyl (6.61%).

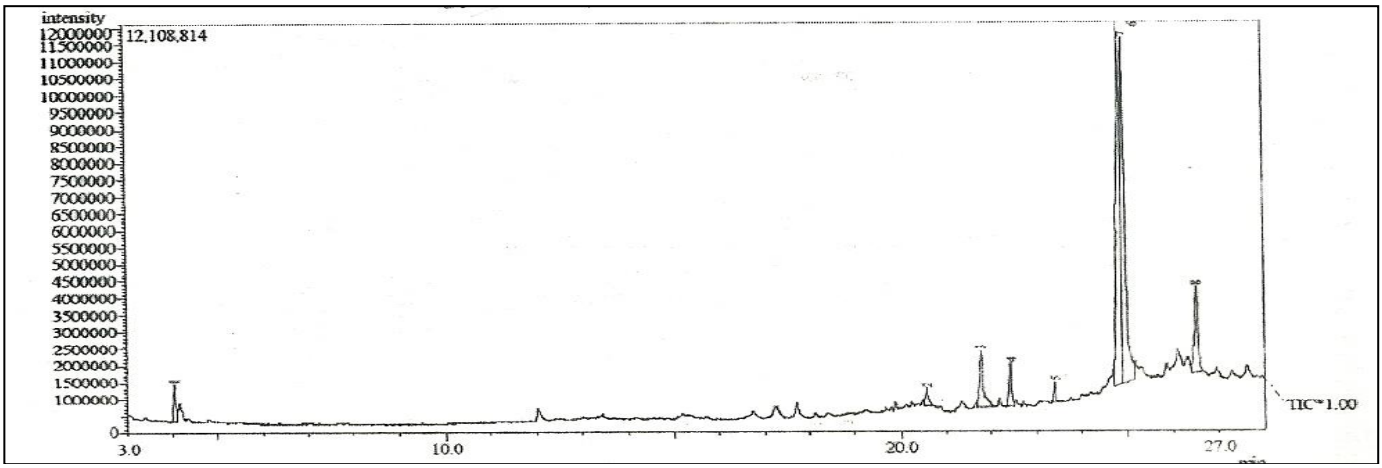

Fig 1: GC-MS chromatogram of methanol leaf extracts of *Hyptis lanceolata*

Table 1: Chemical compounds detected by GC-MS analysis of methanol leaf extracts of *Hyptis lanceolata*

S/N	RT	Compound Name	Nature of compound	Molecular Formular	Molecular Weight	Peak (%)
1	4.05	Pelargonic alcohol	Fatty alcohol	$C_9H_{20}O$	144	2.77
2	20.58	Palmitic acid β-monoglyceride	Fatty acid	$C_{19}H_{38}O_4$	330	1.57
3	21.79	Imidodicarbonimidic diamide, N, N-dimethyl-	Alkaloid	$C_4H_{11}N_5$	129	6.61
4	22.43	Dipalmitin	Fatty acid	$C_{35}H_{68}O_5$	568	3.04
5	23.40	Stearic acid ethyl ester	Fatty acid ester	$C_{20}H_{40}O_2$	312	1.39
6	24.85	α-Linolenic acid	Fatty acid	$C_{18}H_{20}O_2$	278	45.86
7	24.95	Dihomo ɣ-linolenic acid	Fatty acid	$C_{20}H_{34}O_2$	306	30.50
8	26.53	Squalene	Triterpene	$C_{30}H_{50}$	410	8.27

RT=Retention time

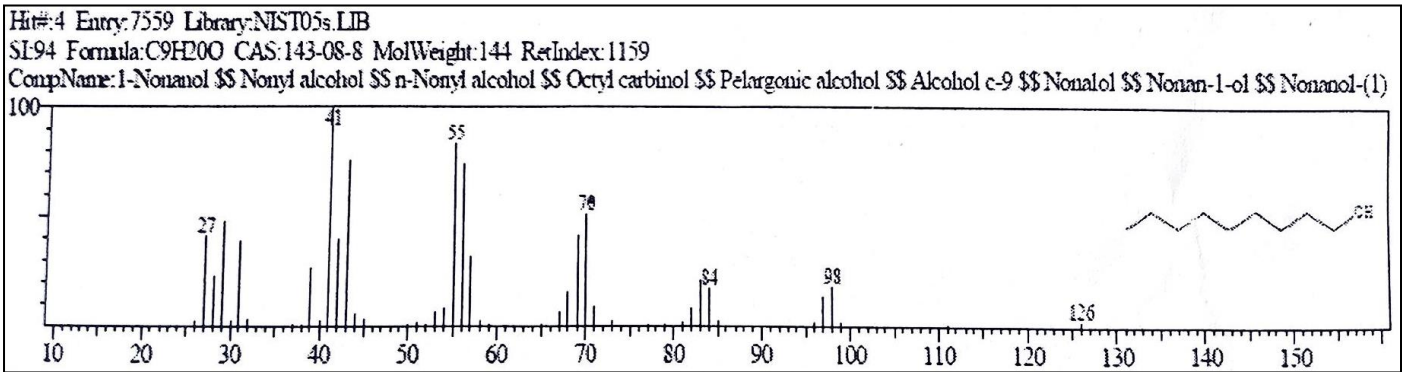

Fig 2: Pelargonic acid

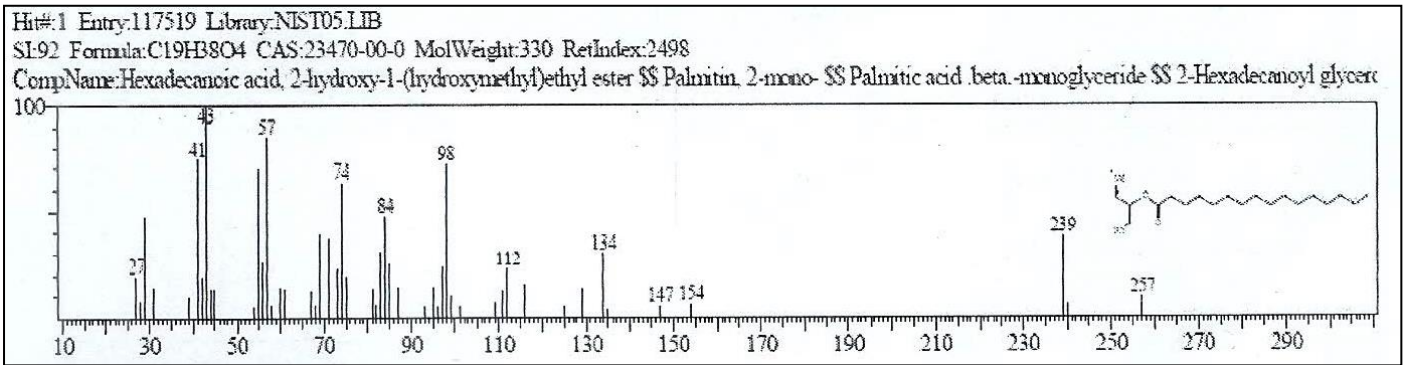

Fig 3: Palmitic acid β-monoglyceride

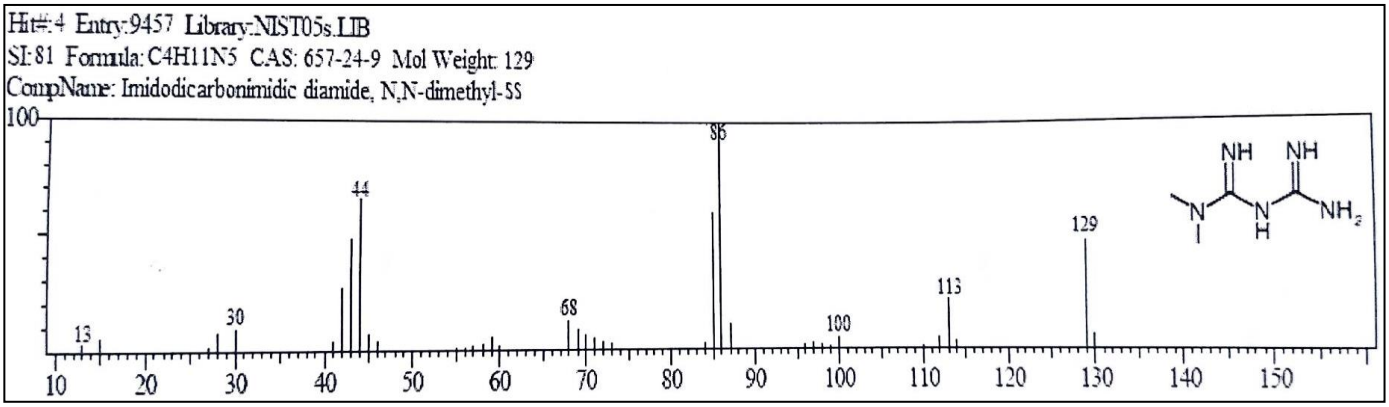

Fig 4: Imidodicarbonimidic diamide, N,N-dimethyl-

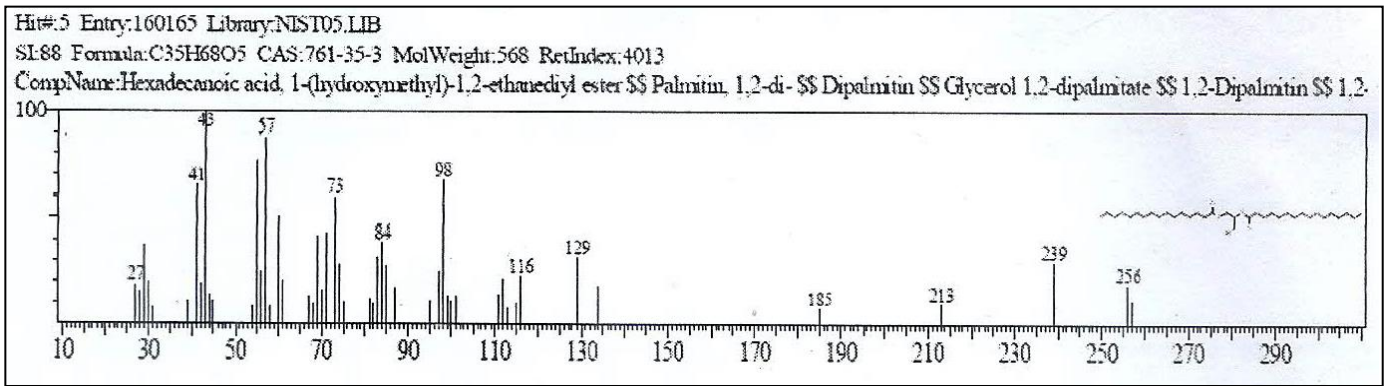

Fig 5: Dipalmitin

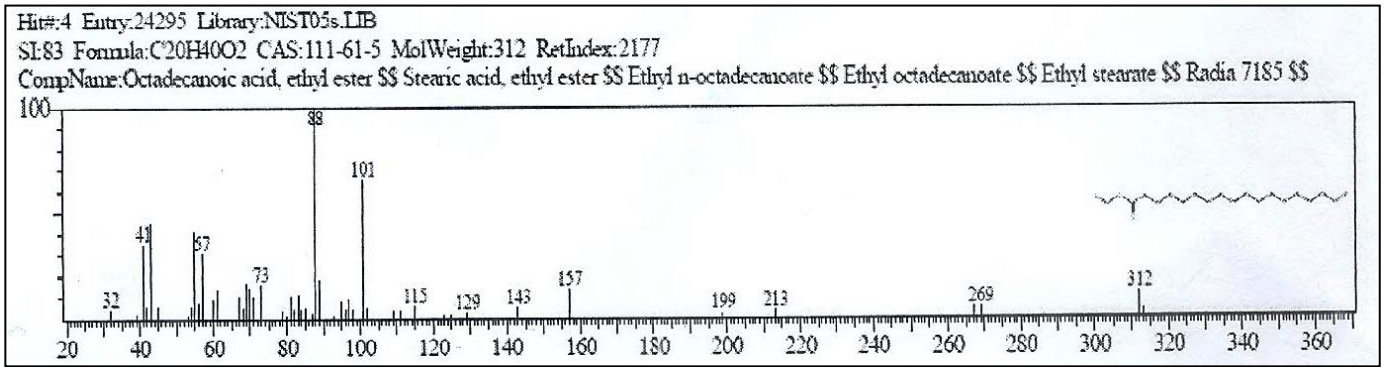

Fig 6: Stearic acid ester

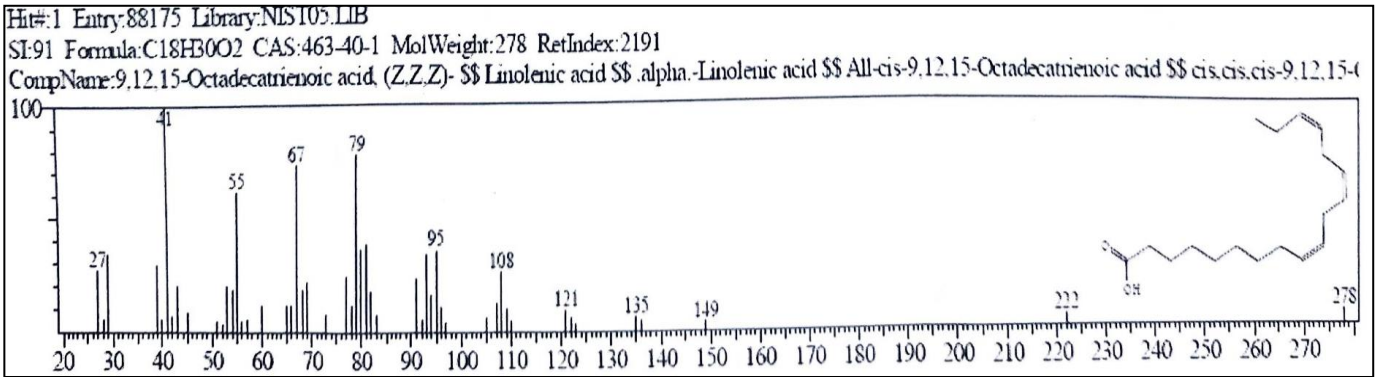

Fig 7: α-Linolenic acid

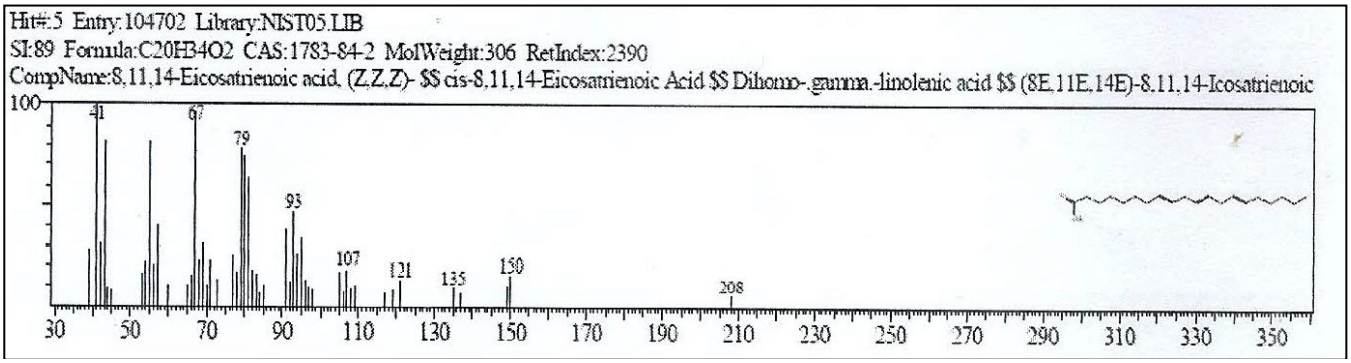

Fig 8: Dihomo γ-linolenic acid

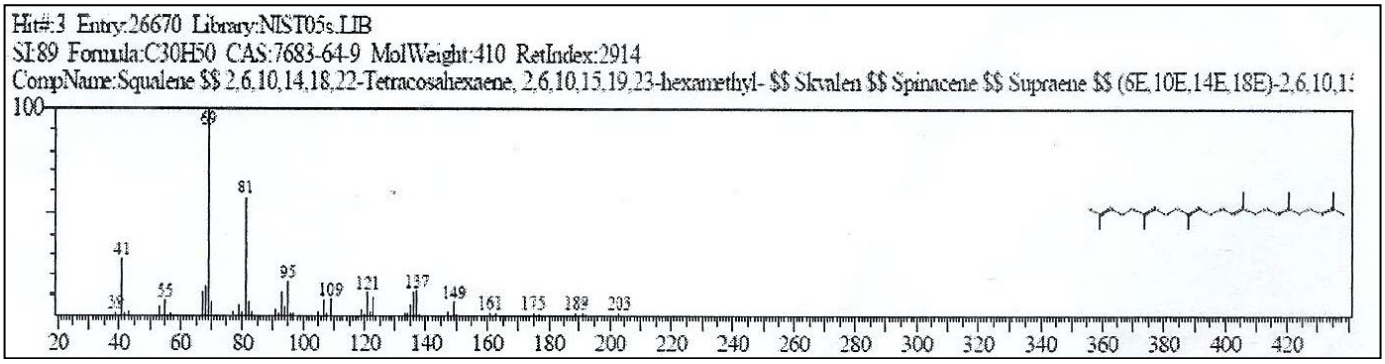

Fig 9: Squalene

Discussion

The compound Imidodicarbonimidic diamide, N,N-dimethyl- (Figure 4) also known as methformin in the pharmaceutical industries was found in the leaf extract of *H. lanceolata*. It is the first line drug in the treatment of type II diabetes and also the treatment of polycystic ovary syndrome (Maruthur *et al*., 2016) [5]. The compound works by suppressing the production of glucose by the liver (Okenwa, 2014) [6]. It was first reported in the ethanol leaf extract of *H. lanceolata* by Okenwa (2014) [6] with a concentration of 5.37%. However, present study reports this compound in methanol extract in a slightly higher concentration of 6.61%.

Extract of *H. lanceolata* also yielded two important omega 3 fatty acid compounds, α-linolenic acid and dihomo γ-linolenic acid (Figure 7 and Figure 8), at a high concentration of 45.86% and 30.50% respectively. A-linolelic acid had been reported in several plants such as the bulb of *Allium cepa* (Ustunes *et al*., 1985) [7], seeds of *Anacardium occidentale*, fruit of *Ananas comosus*, fruit of *Anethum graveolens* and the shoot of *Asparagus officinalis* (Duke, 1992) [8]. However, this is the first time the compound is reported in methanol leaf extract of *H. lanceolata*. Omega 3 fatty acids are unsaturated lipids that are very important to health of humans (Sujatha, 2014) [9]. The compound exhibits bioactivities such as immunostimulant, hypotensive (Cunane and Thompson, 1995) [10], prostaglandin synthesis inhibitor (Ferretti and Flanagan, 1996) [11], antihypertensive, anti-inflammatory, antileukotriene, propecic, vasodilator and prostaglandin synthesis inhibitor (Calder, 2013; Katalin and Ioana-Daria, 2017) [12, 13].

Several studies carried out on dihomo γ-linolenic acid have shown the compound to possess important health potentials. Depending on the type of cell dihomo γ-linolenic acid can be broken down by enzymes to produce prostaglandins of the 1 series and/or be metabolized into 15-(S)-hydroxy-8,11,13-eicosantrienoic acid. It has been found that these two products of oxidation is efficient in treating several conditions like vasodilation and lowering of blood pressure, arresting cancer cell growth, proliferation of muscle cells related to development of antherosclerotic plaque and suppression of severe inflammation (Tabolacci *et al*., 2010; Skuladottir *et al*., 2011; Williams *et al*., 2011) [14, 15, 16]. Several researchers have also suggested that dihomo γ-linolenic acid is special among the polyunsaturated fatty acid family in its ability to inhibit tumor growth and metastasis (wang *et al*., 2012) [17]. Experiments has revealed that the compound can retard both

motility as well as invasiveness of colon cancer cells in humans by elevating E-cadhedrin expression, a cell to cell adhesion molecule which function as an inhibitor to metastasis (Jiang *et al*., 1998; Watkins *et al*., 2005) [18, 19].

Also reported for the first time in the methanol extract of *H. lanceolata* is the triterpene, squalene (Figure 9). It serves as a precursor for the production of secondary metabolites like vitamins, hormones and sterols (Lozano-Grande *et al*., 2018) [20]. It is a source of carbon for microorganisms in aerobic and anaerobic fermentation (Ghimire *et al*., 2016) [21]. It had been reported in previous studies to be present in several plant parts such as seed oil of *Carica papaya*, leaf of *Bidens pilosa*, seed oil of *Glycine max*, seed oil of *Cocos nucifera*, olive oil, rice, grape seed oil, peanut, corn and amaranth (Duke, 1992; Lozano-Grande *et al*., 2018) [8, 20]. It has therapeutic potentials such as antibacterial, antifungal, antitumor, immunostimulant, cardioprotector, antioxidant, chemopreventive and lipoxygenase, anticancer as well as detoxifying (Smith, 2000; Liu *et al*., 2009; Farvin *et al*. 2006; Lozano-Grande *et al*., 2018) [22, 23, 24, 20]. It also has relevance in the cosmetic industries for the production of emollients and moisturizers (Kelly, 1999; Lozano-Grande *et al*., 2018) [25, 20].

Pelargonic alcohol (Figure 2) is a fatty alcohol having a straight chain. The bioactivity of the compound is little known, and has been reported to be used in the cosmetic industry primarily for the production of artificial lemon oil (Opdyke, 1973) [26].

Conclusion

This study provides information on the chemical composition of *Hyptis lanceolata* thereby improving on the poor data available on the phytochemical composition of the plant. Findings indicated that the methanolic extract of the plant can be a good source of compounds relevant in both cosmetic and pharmaceutical industry for production of important products like creams, perfumes as well as drugs for ailments such as diabetes, cancer, microbial skin infections and cardiovascular ailments. Further research is recommended to be carried out on the plant to ascertain more of its pharmacological potentials.

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