African Journal of Environment and Natural Science Research
ISSN: 2689-9434
Volume 3, Issue 5, 2020 (pp. 28-37)
www.abjournals.org

ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL SCREENING OF PEANUT (ARACHIS HYPOGEA) LEAVES

Ahmed Alafy Alex[1], Davuram Francis Dommun[1], Dimas Kubmarawa[1], James Onyeka Okechukwu[1], and Emmanuel Ifeanyi Victor[2]

[1]Department of Chemistry, School of Physical and Applied Sciences, Moddibo Adama University of Technology, Yola, Adamawa State, Nigeria.
[2]Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Chuwuemeka Oduegwu Ojukwu University, Uli, Anambra State, Nigeria

Abstract: *Phytochemical screening and antioxidant activity of the crude ethanolic and essential oil extract of Arachis hypogea was determined using standard methods. Fresh leaves of Arachis hypogea was extracted using modified steam distillation. Phytochemical screening of the ethanolic, n-hexane, ethyl acetate and aqueous crude extracts were carried out and the results revealed the presence of alkaloids, glycosides, flavonoids, saponins, phytosteroids, steroids, phenols, anthraquinones, carbohydrates, and tannins in all the extracts, except for the aqueous extract where glycosides, phytosterols, steroids, phenols and anthraquinones were found to be absent. The antioxidant activity of the essential oil of Arachis hypogea was screened using DPPH method in comparison to ascorbic acid as standard and the IC_{50} values for the plant's crude ethanolic extract was found to be 0.462 for Arachis hypogea and 0.428 for ascorbic acid, while the IC_{50} values obtained for essential oil was 0.428 for ascorbic acid but absent in Arachis hypogea. Arachis hypogea oil was also analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) and 15 compounds were obtained and identified. The main compounds are; 4,7-dimethyl-1H-isoindol-1,3-yl methyl ether (37.14%), tetradecanoic acid (21.98%), cyclohexanol (4.21%), O-phenylaniline (3.69%), amongst others. These results, support the plant's use in the traditional treatment of chronic diarrhoea, fever, diabetes, kidney and bladder diseases, e.t.c. Hence, this suggest its usage in the formulation of new antioxidant and antibacterial drugs.*

Keywords: Arachis Hypogea, Extracts, Phytochemicals, Dpph Method, Antioxidant, Essential Oil

INTRODUCTION

Peanut (*Arachis hypogaea L.*) is one of the world's most popular oil seed crops which is grown as an annual plant but perennial growth is possible in climates which are warm until harvest. Its high oil and protein contents make it an important commodity for both human use and livestock feed. Its oil is aperient and emollient; used as a substitute for olive oil. The oil predominates in mono unsaturated fats, beneficial for cardiac patients, and also prevents heart attacks [1].

Essential oils or aromatic plant essences are volatile and fragrant substances with an oily consistency typically produced by plants. They can be liquid at room temperature though a few of them are solid or resinous, and shows different colours ranging from pale yellow to emerald green and from blue to dark brownish red [2]. They are synthesized by all plant

organs, i.e., buds, flowers, leaves, stems, seeds, fruits, roots, e.t.c. and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes [3].

Furthermore, they are projected to protect the plant by acting as antifungal, antibacterial, insecticidal or antiviral components and are also thought to promote the dispersion of seeds and pollens by attracting insects [4]. Many epidermal cellular structures are capable of producing essential oils and there is a wide variety of chemical constituents [5]. Essential oils are highly complex mixtures of volatile compounds [6], but are basically composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and the shikimic acid pathways respectively. However, the essential oil composition can vary with the developmental stage of the plant [5,7].

Essential oils have been used as antibacterial, antifungal, antioxidant and insecticidal agents [7]. Their extraction from plant materials can be achieved by various methods, of which hydro distillation, and steam distillation are the most common methods of extraction [8,9]. Other methods include solvent extraction, aqueous infusion, cold or hot pressing, effleurage, supercritical fluid extraction and phytonic process [10,11].

This research work is centered on the phytochemical screening of the crude leaf extract of *Arachis hypogea*; the isolation and analysis of the essential oils in the plant leaf to evaluate its antioxidant activities using steam distillation and Gas Chromatography-Mass Spectroscopic (GC-MS) methods respectively.

MATERIALS AND METHODS

Materials Used

Incubator at 35^{o}C and 37^{o}C, rotatory evaporator, steam distillation apparatus, gas chromatography coupled to a mass spectrometer, weighing balance, ethanol, n-hexane, ethyl acetate, Molish reagent, Mayer's reagent, HCl, NaOH, conc. H_2SO_4, Fehling's solution A & B, $FeCl_3$, conc. nitric acid, chloroform, benzene, ammonium solution, Wagner's reagent, iodine solution, diethyl ether and ammonium hydroxide.

Collection of Plant Materials

The plant leaves of *Arachis hypogea* were collected from Tunfure community of Akko Local Government Area in Gombe State, Nigeria. The leaves were identified in the Department of Biological Sciences, Gombe State University. The leaves were dried sufficiently under shade at room temperature and was finely powdered using pestle and mortar. The voucher specimen was deposited and kept in good condition for all subsequent analysis.

Preparation of Aqueous Extracts of the Leaves

The powdered leaf sample was extracted using cold maceration exhaustively at room temperature with ethanol for 72 hours. The extract obtained was filtered and concentrated under reduced pressure with a rotary evaporator. The extraction procedures were repeated to obtain aqueous, n-Hexane, and ethyl acetate crude extracts of the sample leaf each. The fractional aqueous and solvent extracts obtained was concentrated to dryness on the rotary evaporator and screened for their phytochemical properties for the ethanolic extract.

Phytochemical Analysis

Phytochemical examination was carried out for all the extracts to determine the presence of alkaloids, glycosides, saponins, flavonoids, phytosterols, steroids, phenols, anthraquinones, tannins and carbohydrates according to standard methods [12,13].

Extraction of Essential Oil

500 g of pulverized form of the fresh leaf sample was placed inside a steam distiller. Steam was generated and passed through the leaf sample in the distiller. The steam-volatile oils volatilized with the steam, condensed and was collected in conical flasks as distillates. The distillation process was carried out for a period of 2-3 hours and the oils obtained settled on top of water and was removed with the aid of a separating funnel [14].

DPPH Free Radical Scavenging Assay

The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant's activities [15]. The essential oil was dissolved in methanol, and various concentrations (5, 10, 25, 50 and 100 µL/ml or µg/ml, as the case may be) were used. The assay mixture contained in a total volume of 1ml was 500 µL of the oil, 125 µL of prepared DPPH (1ml in methanol), and 375 µL solvent (methanol). After about 30 minutes of incubation at 25^0C, the decrease in absorbance was measured at λ = 517nm. The radical scavenging activity was then calculated. The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percent inhibition plotted against their respective concentration.

Gas Chromatography - Mass Spectrometry Analysis

The essential oil was prepared for GC-MS analysis [16]. Its composition was determined by gas chromatography coupled to a mass spectrometer. Trace GC ULTRA gas chromatograph was coupled to a Polaris Q MS ion trap mass spectrometer. The column was VB-5 (methylpolysiloxane, 5% phenyl), 30 m x 0.25 mm x 0.25 µm film thickness with helium as carrier gas. Injection was performed at 220˚C in the split mode and 1µL of sample was injected. GC oven temperature was kept at 40°C for 2 minutes and programmed to 180˚C at a rate of 4˚C/min and increased to 300°C at a rate of 20°C/min then kept constant at 300˚C for 2 min.

The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200°C; resolution, 1000. Mass units were monitored from 30 to 450 m/z.

The components of the oils were identified by comparison of the mass spectra fragmentation patterns with those found in data bases [17].

The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes C_8 - C_{16}. Chemical constituents were identified by comparing their mass spectra peaks.

RESULTS AND DISCUSSIONS

As shown in table 1, the phytochemical analyses revealed that most of the bioactive compounds tested for, were present in the ethanolic, n-hexane, and ethyl-acetate leaf extracts of *Arachis hypogea*, except for the aqueous extract of which glycosides, phytosterols, steroids, phenols and anthraquinones were all found to be absent.

Table 1: Phytochemical Evaluation of *Arachis hypogea* Leaf Extracts

Phytochemicals Constituents	Ethanolic Extract	n-Hexane Extract	Ethylacetate Extract	Aqueous Extract
Alkaloids	+	+	+	+
Glycosides	+	+	+	–
Flavonoid	–	+	+	+
Saponins	+	+	+	+
Phytosterols	+	+	+	–
Steroids	+	+	+	–
Phenols	+	+	+	–
Anthraquinones	+	+	+	–
Carbohydrates	+	+	+	+
Tannins	+	+	+	+

Key: Present (+); Absent (-)

These results are in line with some research where most of the phytochemicals screened were also found to be present [18,19]. However, there is a variation in these results as compared with some other research. This variation could be due to the part of the plant used, age of the plant, percentage humidity, climatic condition, soil condition, geographical location, time of harvesting, method of extraction e.t.c. [20].

The chemical constituents present in these extracts have some therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties. Flavonoids have antifungal, antibacterial and antiinflammatory activities. Terpenes and alkaloid are known to have antimicrobial and bactericidal properties against several organisms. Saponins, flavonoids, and tannins have antibacterial activities which help act as plant protectants against pathogens in the wild, whereas, phenols, flavonoids, and tannins have anticarcinogenic and antioxidant activities [21].

From the information obtained in table 1, the plant's ethanolic, n-hexane, ethylacetate as well as aqueous crude extracts contained phenolic compounds as well as tannins in high quantity. Based on previous findings, there is a high correlation between antioxidant activities and phenolic compounds [22]. This implies that compounds that have tannins in nature are expected to exhibit antioxidant activities even though other phenolic compounds like flavonoid also possess antioxidant activity and they are known to be in synergistic relationship with tannins in plants [23].

Furthermore, both the crude ethanolic extract and essential oil extract from the leaves of *Arachis hypogea* showed a reasonable zone of inhibition as seen in tables 2 and 3 respectively.

Crude ethanolic extracts and essential oil extracts have shown the scavenging effects of the leaves of *Arachis hypogea* on DPPH. The results obtained for the crude ethanolic extract and the essential oil extract from the spectrophotometer were expressed as inhibition in percentage as they were compared with the standard antioxidant (ascorbic acid) as shown in tables 4 and 5 respectively. The lowest concentration of the essential oil (5µl/ml) and crude ethanolic extract (5µg/ml) showed the lowest inhibition values of 3.870% and 21.440% respectively. This is very far from the standard (ascorbic acid) which is 68.180% in both cases.

Table 2: Antioxidant Activity of Crude Ethanolic Extract of *Arachis hypogea*

Concentration (µg/ml)	*Arachis Hypogea*	Ascorbic Acid
5	1.422	0.576
10	0.938	0.457
25	0.765	0.415
50	0.385	0.385
100	0.296	0.266

Table 3: Antioxidant Activity of the Essential Oil Extract of *Arachis hypogea* using Ascorbic Acid as Standard Blank Solution 1.810

Concentration (µl/ml)	*Arachis Hypogea*	Ascorbic Acid
5	1.740	0.576
10	1.622	0.457
25	1.542	0.415
50	1.320	0.385
100	0.987	0.266

Table 4: Antioxidant Activity Inhibition of *Arachis hypogea* Expressed in Percentage (%) of Crude Ethanolic Extract using Ascorbic Acid as Standard

Concentration (µg/ml)	*Arachis Hypogea* %	Ascorbic Acid %
5	21.440	68.180
10	48.200	74.750
25	57.740	77.100
50	78.730	78.730
100	83.650	85.300

Table 5: Antioxidant Activity Inhibition of *Arachis hypogea* Expressed in Percentage (%) of Essential Oil Extract using Ascorbic Acid as Standard

Concentration (µl/ml)	*Arachis Hypogea* %	Ascorbic Acid %
5	3.870	68.180
10	10.390	74.750
25	14.810	77.100
50	27.100	78.730
100	45.500	85.300

However, the highest concentration of the essential oil (100µl/ml) showed appreciably higher inhibition value of 45.500% while the highest concentration for the crude ethanolic extract (100µg/ml) showed a higher inhibition value of 83.650%. This is very close to the standard (ascorbic acid) with a value of 85.300%. This implies that there was a distinguishing increase in inhibition as the essential oils and crude extract concentration increased [24]

The IC_{50} values obtained for the crude ethanolic extract and essential oil extract as compared with ascorbic acid is shown in table 6.

Table 6: IC_{50} values of the crude ethanolic and the essential oil extracts of *Arachis hypogea* and ascorbic acid

	IC_{50} Value of Ethanolic extract	**IC_{50} value of Essential oil extract**
Arachis Hypogea	0.462	Nil
Ascorbic acid	0.428	0.279

The GC-MS analysis showed that the essential oil contained 15 components as presented in table 7, with their respective retention time and area percentage.

Table 7: GC-MS analysis of Essential Oil of *Arachis hypogea*

Constituents	**Retention Time (minutes)**	**Area Percentage (%)**
4,7- dimethyl-1H-isoindol-1,3-yl methyl ether	13.522	37.140
o-Biphenylamine, o-phenylaniline	14.179	3.690
1,4-Benzenediol	14.611	1.880
Octadecanoic acid	17.698	2.910
Tetradecanoic acid	19.422	21.980
Cyclohexanol	19.460	4.210
Stearic acid	19.623	4.000
Tritetracontane	20.270	4.760
2-methyltetracosane	20.805	2.140
5,5-dibutylnonane	21.128	2.510
Hexadecanoic, (3-bromoprop-2-ynyl) ester	22.260	2.720
p-Hetylbenzonitrile	22.405	4.590
Tetrapentacontane-1,54- dibromo	22.733	2.650
Bisoflex	22.758	3.100
Fumaric acid, 10-chlorodecyl pentyl ester	23.758	2.030

The fragmentation pattern of some selected most abundant compounds obtained from the GC - MS analysis of *Arachis hypogea* is shown in the figures below. While figure 1 shows the fragmentation pattern of 2,3-dimethyl-4-methoxyphenylacetonitrile, figure 2 shows that of cis-10-heptadecanoic acid.

African Journal of Environment and Natural Science Research
ISSN: 2689-9434
Volume 3, Issue 5, 2020 (pp. 28-37)
www.abjournals.org

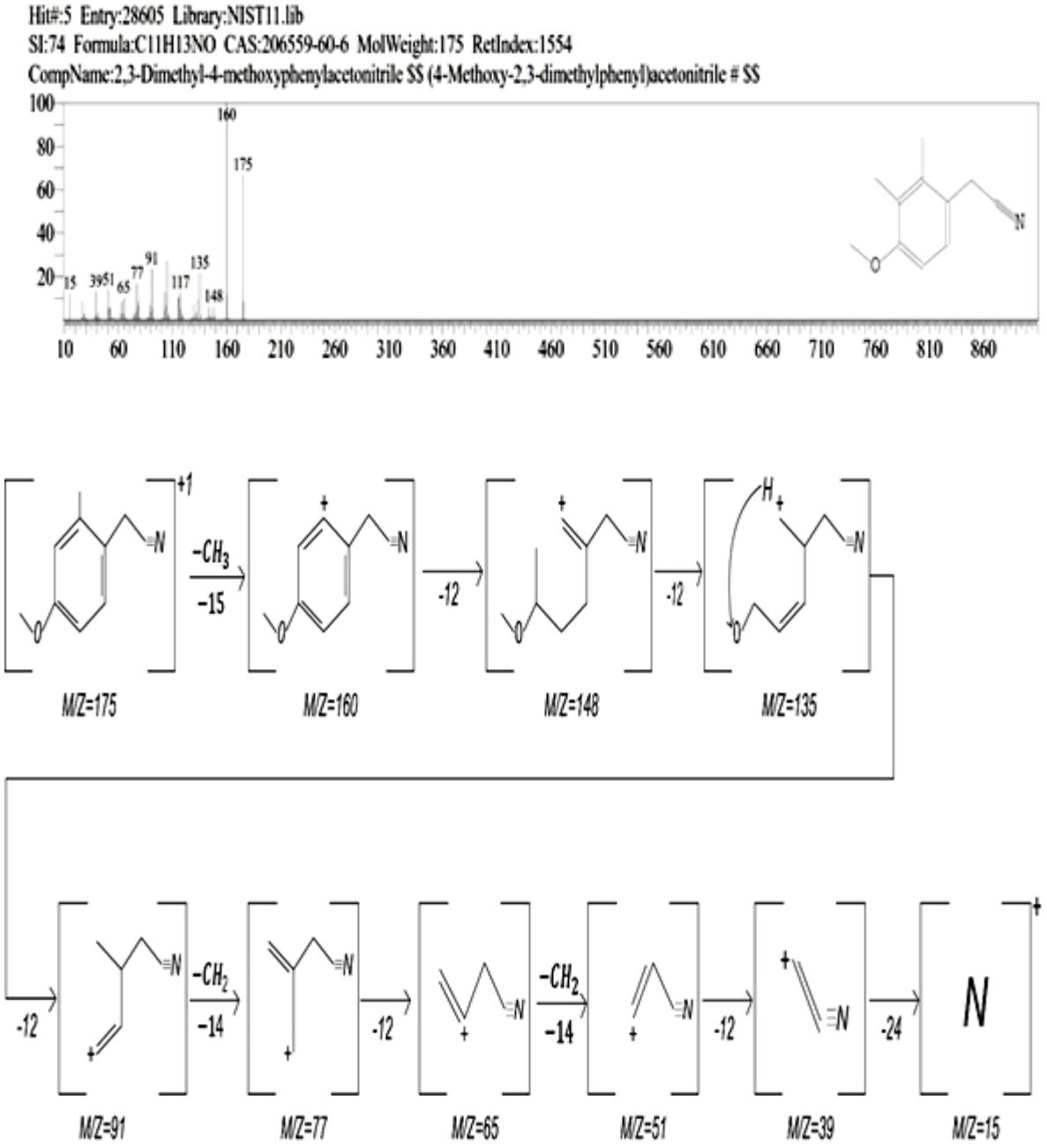

Figure 1: Fragmentation Pattern of 2,3-Dimethyl-4-Methoxyphenylacetonitrile.

African Journal of Environment and Natural Science Research
ISSN: 2689-9434
Volume 3, Issue 5, 2020 (pp. 28-37)
www.abjournals.org

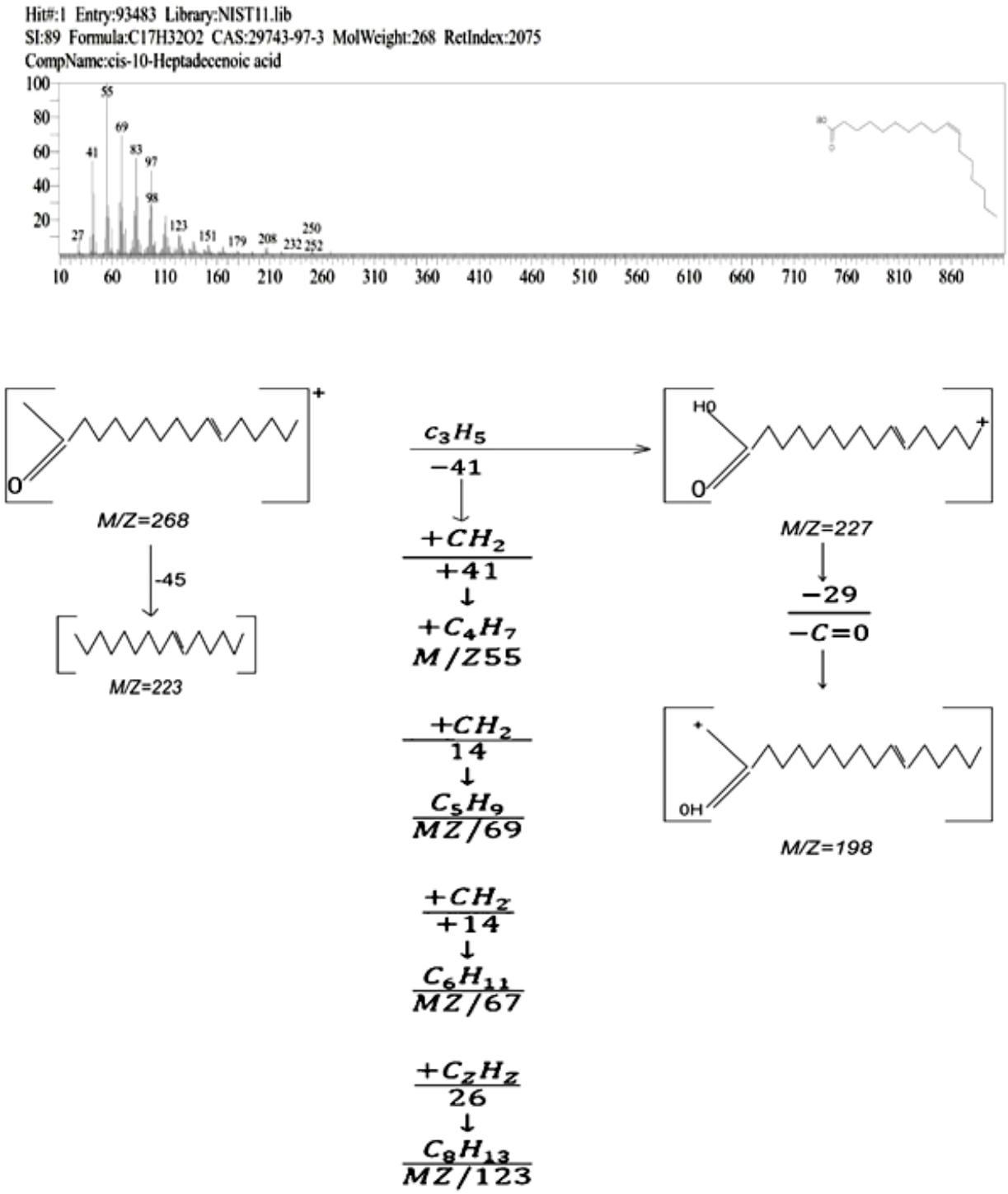

Figure 2: Fragmentation Pattern of cis-10-Heptadecanoic Acid.

CONCLUSION

Phytochemical screening of the leaf extracts from *Arachis hypogea* revealed and justified its use medicinally and as food because of the richness in secondary class metabolites. Its use in traditional medicine is validated by presence of these phytochemicals. The antioxidant activities of the essential oil and ethanolic extracts against standard ascorbic acid also indicated the potential and potency of this plant as an antioxidant and as a therapeutic agent. Hence, there is a need for more active research on this plant as it can be used as possible raw materials for the formulation of new drugs.

Acknowledgment

The authors acknowledge the supervisory role of Prof. Dimas Kubmarawa of the Department of Chemistry, Modibbo Adama University of Technology, Yola, Adamawa State and the Department of Chemistry, Gombe State University, Gombe State, Nigeria for providing the facilities used in carrying out this research.

REFERENCES

[1] Adams, R.P. (2007). Identification of essential oil components by gas chromatograph/quadrupole mass spectroscopy. 4th ed., Carol Stream: Allured Publishing, pp. 54-50.
[2] Balz, R. (1999). The Healing Power of Essential Oils. 1st ed., Twin Lakes: Lotus Press, pp. 27– 80
[3] Charles, D.J., & and Simon, J.E. (2016). Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. Journal of the American Society for Horticultural Science, 115, 458-462.
[4] Cox, F.R., Adams, F., & Tucker, B.B. (1982). Liming, fertilization and mineral nutrituon- peanut science and technology. American Peanut Research and Education Society Inc., 189-194.
[5] Da Porto, C., Decorti, D., & Kikic, I. (2009). Flavour compounds of Lavandula Angustifolia L. use in food manufacturing: comparison of three different extraction methods. Food Chemistry, 112, 1072-1078.
[6] Duke, J.A. (2008). Duke's Handbook of Medicinal Plants of Latin America. Vol. 1, CRC press, pp. 6366.
[7] Harbone, J.B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall Ltd., pp. 279.
[8] Kubmarawa, D., Ajoku, G.A., Enwerem, N.M., & Okorie, D.A. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr. J. Biotechnol., 6, 1690-1696.
[9] Kubmarawa, D., Khan, M.E., Mtswenem, M., Uwemedimo, A., Timi, P.G., Hassan, M., & Atiko, R. (2008). The phytochemistry and chemotherapeutic effects of some African medicinal plants. Book of Proceedings, Chemical Society of Nigeria, 6(1), 154-159.
[10] Kubmarawa, D., Kidah, M.I., & Shagal, M.H. (2016). Antimicrobial activities of essential oils from some medicinal and aromatic plants. British Biotechnology Journal, 14(3), 1-6.

[11] Margaris, N., Koedam, A., & Vokou, D. (1982). Aromatic Plants: basic and applied aspects, vol. 1, London: Martinus Nijhoff Publishers, pp 45-78.
[12] Miguel, M.G. (2010). Antioxidant activity and anti-inflamatory activity of essential oils in propolis from Algarve. Advances in Environmental Biology, 5(2), 345-350.
[13] Odabasoglu, F., Aslan, A., & Cakir, A. (2004). Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res, 18, 938-941.
[14] Pourmortazavi, S.M., & Hajimirsadeghi, S.S. (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. Journal of Chromatography, 1163, p. 224.
[15] Prabasheela, B., Venkateshwari, R., Nivetha, S., Priya, P., & Karthik, K. (2015). Phytochemical analysis and antioxidant activity of the ethanolic extract of three different forms of Arachis hypogea. International Journal of Food Science and Nutrition, 3(2), 100-121.
[16] Rice-Evans, C., Miller, N., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. Trends in Plant Science, 2, 152-159.
[17] Sangwan, N.S. (2016). Regulation of essential oil production in plants. Plant Growth Regulation, 34, 3-21.
[18] Shagal, M.H., Kubmarawa, D., & Alim, H. (2012). Preliminary phytochemical investigation and antimicrobial evaluation of roots, stem-bark and leaves extracts of Diospyros mespiliformis. International Research Journal of Biochemistry and Bioinformatics (ISSN-2250-9941), 2(1), 011-015.
[19] Singh, S., Handa, T., Narayanam, M., Sahu, A., Junwal, M., & Shah, R.P. (2010). A critical review on the use of modern sophisticated hyphenated tools in the characterization of impurities and degradation products. Journal of Pharmaceutical and Biomedical Analysis, 1(2), 31-35.
[20] Sofowora, A. (1986). The state of medicinal plants research in Nigeria. 1st ed., Ibadan: University Press, pp 235-239.
[21] Surburg, H., & Panten, J. (2006). Common fragrance and flavour materials: preparation, properties and uses, 5th ed, Weinheim: WILEY-VCH.
[22] Usman, H., Abdulrahman, F.I., & Ladan, A.A. (2007). Phytochemical and antimicrobial evaluation of tribulus terrestris. L Zygophylaceae growing in Nigeria. J. of BIOSC. Medwel, 2, 244-247.
[23] Zagga, A.L., Abduljabbar, I.A., Garko, M.R., Tsoho, B., & Gbande, S. (2018). Phytochemical composition of Adansonia digitata leaf extracts. Procedings of 6th Biodiversity Conference, UniUyo, pp. 300-304.