GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Extraction and characterization of essential oils from synthesized food spice (*Lippia multiflora*, ginger & turmeric)

Olukayode Adediran Okunade [1,*] and Abiodun Olaniyi Fatoye [2]

[1] *Department of Food Technology, Federal Polytechnic, Ado Ekiti, Nigeria.*
[2] *Department of Science Technology, Federal Polytechnic, Ado Ekiti, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2024, 26(03), 103–109

Publication history: Received on 30 January 2024; revised on 10 March 2024; accepted on 13 March 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.26.3.0087

Abstract

Spices are used globally, and are of great importance in indigenous culinary and traditional medicine applications, they are the product of dried fruits, seed root, bark or vegetative substances. This study synthesized food spice from *Lippia multiflora*, ginger and turmeric. Some anti-nutritional factors and the chemical composition of the extracted essential oils were evaluated.

The chemical composition of the extracted essential oils revealed the presence of over ten (10) compounds, with 2-Pyrrolidinone, 1 methyl (35.21 %), Cyclo-hexane-carboxylic acid (24.00 %), Diethyl Phthalate (15.46 %) and 2-Pyrimidinamine,4,6-dimethyl (5.60 %) being the predominant compounds in the oil.

The phytochemical screening of the food spice indicated the respective phytate, oxalate and tannin values for the food spice as; 0.71 mg/100 g, 1.2 mg/100 g and 0.51 mg/100 g respectively.

These results are in agreement with previous studies where essential oils were shown to contain complex mixtures of compounds with varying chemical compositions.

Keywords: Essential oils; Bioactive compounds; Flavour; Nutrition; Health

1. Introduction

Spices are of great importance in indigenous culinary and traditional medicine globally. Ginger, garlic, turmeric etc are used globally as spices which in addition to contributing taste and aroma to foods, also contain a variety of significant bioactive substances, which are of considerable application pharmacologically, and for food science and technology. The bioactive compounds in these spices may be single or in combination, and some act synergistically to control spoilage of foods, facilitating their use as bio-preservatives [1, 2]. Spices have been used for centuries to flavor food while killing selective bacteria. For example, spices used in making sausages will inhibit bacteria that cause spoilage while not harming the bacteria that add flavor to the sausage.

Spices depending on the origin and active principle present are classified as pungent spices –pepper, ginger, chillies and mustard; aromatic fruits – cardamom, nutmeg, mace, fenugreek, aniseed, caraway, dill, celery, cumin, coriander, etc.; aromatic barks – cinnamon and cassia; phenolic spices containing eugenol – clove and pimento; and coloured spices – paprika, saffron and turmeric. Leaves and/or branches of aromatic plants; all or part of the plant can be used. Examples include basil, bay leaf, parsley, rosemary, tarragon and thyme, oregano and chervil, ripened fruits or seeds of plants. Examples include dill, fennel, coriander, fenugreek, berberis, mustard and black pepper, roots or bulbs of certain plants. Examples include garlic, onion, celery and ginger [3].

* Corresponding author: Olukayode Adediran Okunade

Spices contain essential oils. An essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetheroleum, or simply, as the oil of clove. An essential oil is "essential" in the sense that it contains the "essence of" the plant's fragrance, the characteristics fragrance of the plant from which it is derived. Chemically, an essential oil is composed of various small molecules, including predominantly, mono and sesqui-terpenes, simple aromatic compounds and various other aliphatic compounds. They are generally extracted by distillation and often by using steam. Other processes include expression, solvent extraction, absolute oil extraction, resin tapping, wax embedding, and cold pressing [4, 5, 6, 7].

Essential oils are often used for aromatherapy, a form of alternative medicine, however, improper use of essential oils may cause harm including allergic reactions, inflammation and skin irritation, Children may be more susceptible to the toxic effects of its improper use. Essential oils are valuable natural products used as raw materials in many fields, including perfumes cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides [7, 8]. Inhalation of essential oils or their individual volatile terpenes has been reported to play a significant role in controlling the central nervous system [5, 6, 7].

Many oils show antibacterial, fungicidal, relaxant, stimulating, anti-depressant effect and can be very effective therapeutic agents. Essential oils are known for their therapeutic properties hence, they are used in the treatment of various infections caused by both pathogenic and non-pathogenic organisms [7].

2. Materials and methods

2.1. Sample sourcing

Leaves with seeds of *Lippia multiflora* and matured, blemish free corms of ginger and turmeric were procured from local farms, near Ekiti State A.D.P farms, close to Aba Erinfun, Ikare Road, Ado Ekiti, Ekiti State, Nigeria. These plant materials were identified at the Department of Botany (Herbarium unit), Ekiti State University, Ado Ekiti with a further confirmation at the Herbarium unit of Afe Babalola University, Ado Ekiti (ABUAD).

2.1.1. Sample preparation

The leaves with seeds of *L. multiflora* and corms of ginger and turmeric was sorted to remove extraneous matters, and cleaned thoroughly. The sorted leaves, and corms were air dried, milled, and sieved individually.

The spice was then prepared from the raw materials in the following proportion:

- 60 g of *L. multiflora*
- 20 g of *Z. officinale*
- 20 g of Turmeric

The mix was thoroughly homogenized and packaged in air tight plastic containers and polyethylene bags.

2.2. Extraction of essential oils

Essential oil was extracted from the synthesized spice as described with some modifications [9, 10, 11]. 100 g of the powder was placed in a 1 L conical flask and connected to the distillation apparatus. 500 mL of distilled water was added to the flask and heated to the boiling point. The steam in combination with the essential oils were distilled into a graduated cylinder for 5 hours and then separated from the aqueous layer. The oil was dried with anhydrous sodium sulphate and then stored in an airtight container. The oil was kept refrigerated below 4 °C until required for further analysis.

2.2.1. Characterisation of essential oils

The GC-MS was used to characterize the extracted essentials oils, using a Perkin-Elmer Clarus 680/600 chromatogram with built in auto sampler using a fused DB-5 capillary column (length 30 m × 0.25 mm internal diameter (ID), film thickness 0.25 µm), equipped with a Elite-5 MS capillary column and FID detector. The oven temperature was programmed at 50 °C-260 °C, at a rate of 5 °C/min. The temperature of the injector was set at 250 °C, at a volume of 1.0 µL. The run time was 46 min. Helium gas was used as the carrier gas, with a flow rate of 1.1 mL/min [12].

2.3. Determination of phytochemicals

The tannin content of the sample was determined as described by Harbone [13], using 0.1 g of the powdered sample in distilled water with boiling and filteration, 5 ml filterate was added to 10 ml of freshly prepared 17 % sodium carbonate and 2.5 ml of Folin Denis reagent, allowed to stand for 20 min for colour development. Absorbance was read 520 nm with standard tannic acid curve and blank.

Alkaloids were quantitatively determined as described [13] using 5 g sample with 200 ml 10 % acetic acid in ethanol, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Conc. NH_4OH was added dropwise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

For the determination of Oxalates, the methods described by Pearson [14] and Harbone [13] was used, with the ashing of 10.0 g sample in a muffle furnace at 500 °C, washing the ash with 40 ml concentrated hydrochloric acid (HCL), 60 ml of distilled water and 3 drops of concentrated nitric acid ((NHO_3), followed by boiling the mixture and cooling. 0.5 ml filterate was taken into a beaker. 1 ml of 30 % citric acid solution and 5 ml ammonium chloride solution were added. The volume was made up to 100 ml with distilled water and was boiled. 10 drops of Bromocresol green solution (0.04 %) and 30 ml of warm and saturated ammonium oxalate solution was added. The solution was neutralized very slowly with ammonia solution, stirred vigorously until the pH changes slightly. The mixture was then heated, cooled, filtered, 50 ml warm sulphuric acid solution was added in a volumetric flask and then made up to 100 ml with distilled water. The filtrate was heated to 70-80 °C and filtered with 0.2 M potassium permanganate solution (3.16 g/l) until pink colour persisted.

The residue in the beaker was filtered again with filter paper. The filtrates were combined and shaken with benzene either mixture and the alcoholic extracts were contracted under reduced pressure, crystal deposit was removed by filtration, followed by the addition of 25 ml off N-Butanol (to dissolve the crystal) the filtrate was washed with the distilled water and dissolved on phosphate buffer solution (311.2 g of NaH_2 PO_42H_20) per liter which was added to 28.39 g of NaH_2PO_4) to give pH 7.0, the filtrate was re-crystallized with 95 % ethanol to give brownish colour crystals. The crystals were then weighed.

The flavonoid content of the spice was determined by the gravimetric method as was described by Harborne [13]. 5 g of the powdered sample was placed into a conical flask and 50 ml of water and 2 ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered and the residue was then placed in an oven to dry at 60 ^oC, it was cooled in a desiccator and weighed.

Phytates was determined using colorimetric method [15]. Briefly, 5 g of sample was weighed into a 250 ml conical flask, 100 ml of 0.1 M HCL was added and extracted for 1 hour at room temperature and centrifuged. Supernatant was decanted. 1 ml of 2.4 % extract supernatant was diluted to 25 ml with distilled water. 10 ml of diluted sample was passed through the AG1-X8 chloride anion exchange column (0.5 g). Phytate was eluted with 0.7 M NaCl. 3 ml of 0.7 M eluent fraction was pipetted into 15 ml conical test tubes, and mixed on a vortex mixer for 5 seconds, and centrifuged for 10 minutes. Absorbance of supernatant was read at 500 nm using water to zero the spectrophotometer.

3. Results and discussion

3.1. Characterisation of essential oils from synthesized food spice

Table 1 presents the respective retention time and concentrations of compounds present in the extracted oil. Totally, over ten compounds were identified out of the traceable peaks in the essential oil examined in the gas chromatogram (Figure 1). The components were identified based on the elution pattern on DB-5 column and comparison with library data.

Among all the components, the predominant compounds are 2-Pyrrolidinone, 1 methyl (35.21 %), Cyclohexanecarboxylic acid (24.00 %), Diethyl Phthalate (15.46 %) and 2-Pyrimidinamine, 4,6-dimethyl (5.60 %) respectively. In literature, it has been reported that essential oils are very complex mixtures of compounds and many variations have been found in their chemical composition [11, 12, 16]. According to Singh *et al* [11] and Wang *et al* [17], some variations in the chemical composition of distilled oils is considerably not only due to the existence of different subspecies, but also can also be attributed to the varied agro-climatic condition (climatic, seasonal, geographic) of the regions, stage of maturity, adaptive metabolism of plants, distillation conditions, the plant part analyzed and some other

factors. Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70 %) compared to other components present in trace amounts. Generally, these major components determine the biological properties of the essential oils [18, 19]. Our present study is in agreement with previous studies, as the extracted oil contains these components in ratios conforming to earlier studies.

Table 1 Chemical composition of essential oils from synthesized food spice

Compound	R.T (Mins)	Concentration (%)
2-Pyrrolidinone, 1-methyl	4.068	35.21
tert-Butyldimethylsilyl nitrile	4.570	0.24
2-Pyrimidinamine, 4,6-dimethyl	4.862	5.60
Cyclohexanecarboxylic acid	5.166	24.00
Diethyl Phthalate	11.166	15.46
aR-Turmerone	11.975	2.76
Hexadecanoic acid, methyl ester	14.652	2.30
Oleic Acid	15.021	2.41
Cyclopentadecane	16.094	1.27
3-Eicosene	17.083	2.58
2-Propionyloxytetradecane	19.895	4.90

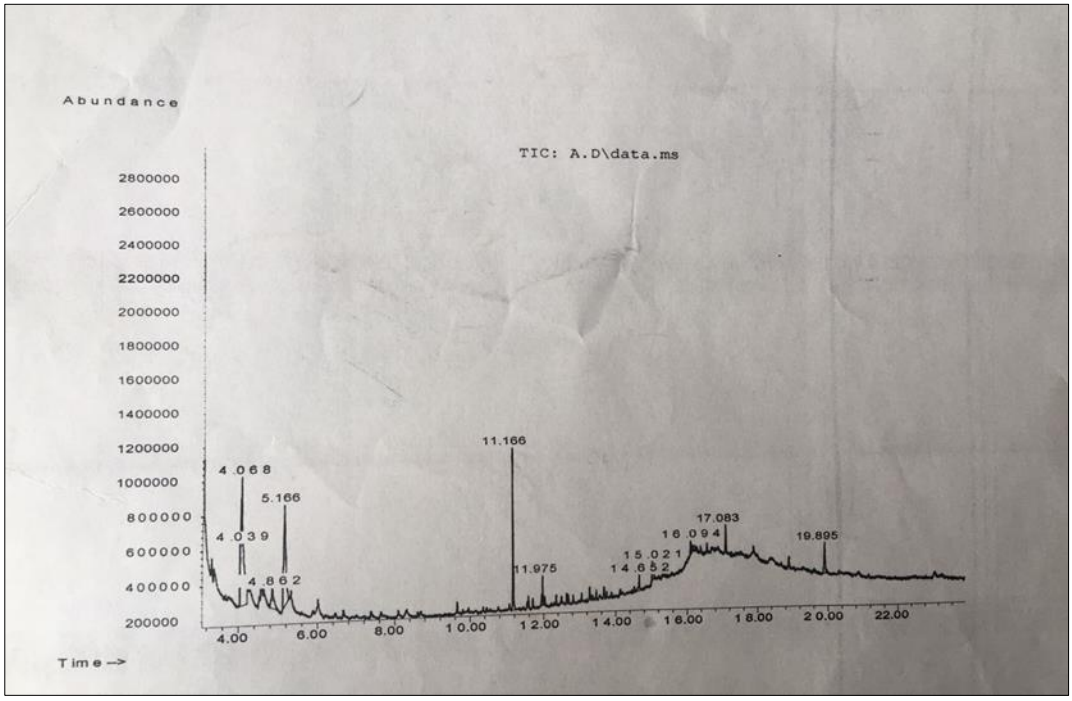

Figure 1 Chromatogram of the chemical composition of essential oils from synthesized spice

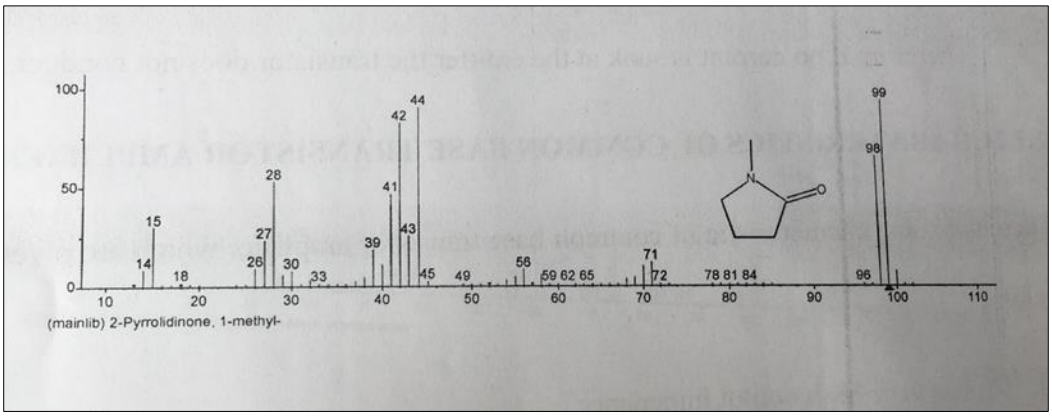

Figure 2 Chromatogram of 2-Pyrrolidinone, 1 methyl in synthesized spice oil

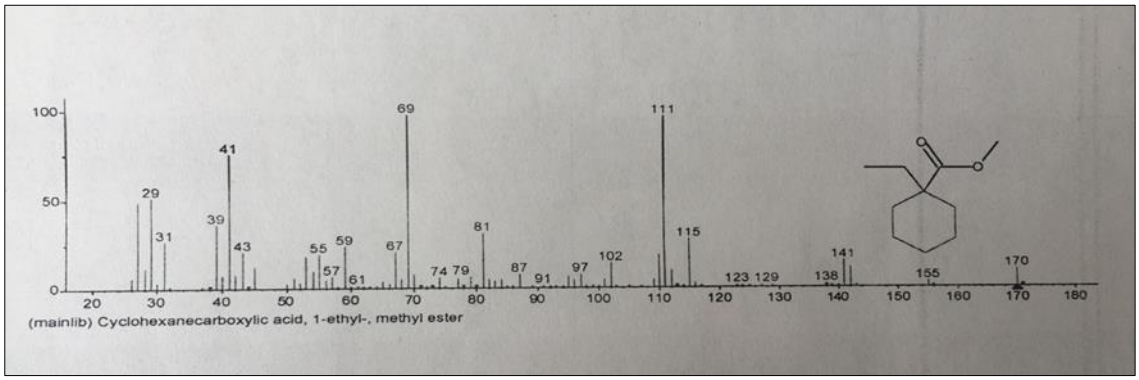

Figure 3 Chromatogram of Cyclohexanecarboxylic acid in synthesized spice oil

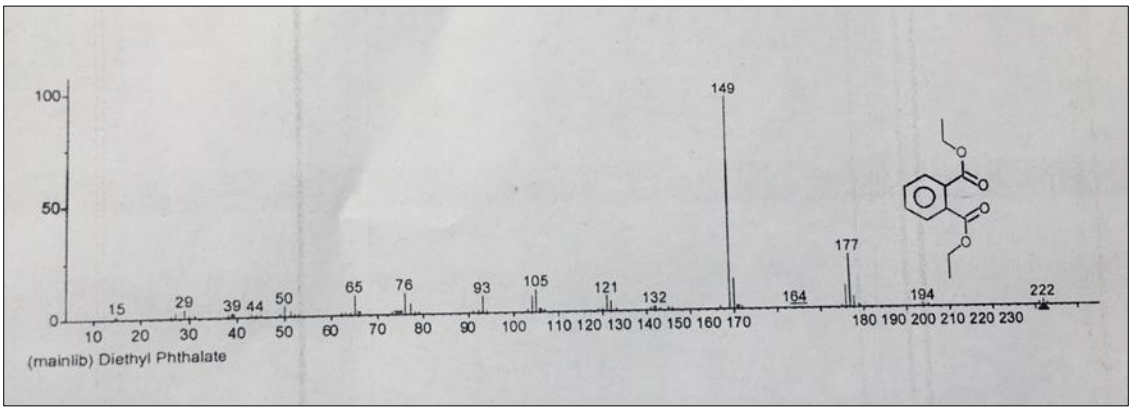

Figure 4 Chromatogram of Diethyl Phthalate in synthesized spice oil

3.1.1. Anti-nutritional factors in synthesized food spices

Table 2 presents the anti-nutritional factors of the food spices phytate, oxalate and tannin were found to be 0.71, 1.4 and 0.51 mg/100 g respectively. The presence of anti-nutritional factors in the samples is of significant importance since they are known to have some deleterious effects on both humans and other animals. Oxalate is a chelating agent, which binds calcium very effectively. Plants with high oxalate content may produce acute metabolic calcium deficiency (Hypocalcemia) when such plant products are used as a main food source [20]. One of the main health concerns about

oxalate is that it can bind to minerals in the gut and prevent the body from absorbing them. The concentration of oxalate (1.4 mg/100 g) in the present study seems to be on the low side when compared to reported values in some plants [21].

Tannin is known to evoke growth-depressing effects in rats. In this study, the tannin level for the food spice (0.51 mg/100 g) is relatively low in comparison with tannic acid in some literatures [21, 22, 23], the tannin content of the samples in this study is less than the tannin content of some dry plants (84.3 mg/100 g) as reported by Andualem and Gessesse [20]. The tannin content of this spice may not be as such harmful, as expected for consumption. High amount of tannins are well known to form complex with proteins and reduce the solubility of proteins, hereby making the protein less susceptible to proteolytic attack than the same proteins alone. However, relatively, some amount of tannin may have a potential role as protective factors against free radical mediated pathologies, such as cancer and atherosclerosis, in humans [24].

Table 2 Phytochemical screening of synthesised spice

Phytochemicals	**Composition**
Tannins (mg/100 g)	0.51
Alkaloids (%)	1.15
Oxalates	1.4
Flavonoids (%)	0.33
Saponins (%)	1.0
Phytin (%)	0.71
Total Terpernoid	0.17

4. Conclusion

The presence of essential oils in medicinal plants makes these plants have bioactive activities, mostly defined by the nature, structural composition, and the functional groups present in these essential oils. In literature, essential oils are known to contain a variety of volatile compounds having bactericidal, virucidal, and fungicidal potentials. Essential oils are known to affect the cell membrane of implicated microorganism, finally disrupting the cell respiration and enzyme system.

From the results of this study, it can be concluded that the synthesized spice has a considerable amount of compounds which might be useful for diverse applications both for food processing, pharmaceuticals and other none food uses.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the Federal Polytechnic Ado Ekiti CRID-!BR for sponsoring this research work, and all the Staffs of the Research Laboratory, S.S.C.S, Federal Polytechnic Ado Ekiti for their invaluable help and support in the course of this study.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Yanishlieva NV, Marinova E, & Pokorný J. Natural antioxidants from herbs and spices. *European Journal of lipid science and Technology*. 2006. 108(9), 776-793.

[2] Sivasothy Y, Chong WK, Hamid A, Eldeen I M, Sulaiman S F, Awang, K. Essential oils of *Zingiber officinale* var. rubrum and their antibacterial activities. Food Chemistry. 2011. 124: 514–517.

[3] Kamatou GPP, Viljoen AM, Gono-Bwalya AB, van Zyl RL, Van Vuuren SF, Lourens ACU & Steenkamp P. The *in-vitro* pharmacological activities and a chemical investigation of three South African Salvia species. *Journal of ethno-pharmacology*. 2005. *102*(3), 382-390.

[4] Stoyanova A. Chemical diversity of *Lippia multiflora* essential oils from West Africa. *J. Essent. Oil Res.* 2006. 20, 49-54.

[5] Jirovetz L, Buchbauer G, Denkova Z, Stoyanova A, Murgov I, Gearon V & Geissler M. Comparative study on the antimicrobial activities of different sandalwood essential oils of various origin. *Flavour and fragrance journal*. 2006. *21*(3), 465-468.

[6] Jirovetz L, Buchbauer G, Stoilova I, Stoyanova A, Krastanov A & Schmidt E. Chemical composition and antioxidant properties of Clove leaf essential oil. *Journal of agricultural and food chemistry*. 2006. *54*(17), 6303-6307.

[7] Hamid AA, Diyelaagbe OO & Usman LA. Essential oils, its medicinal and Pharmacological uses. *Int. J. Current Research*. 2011. 3(2), 086-098.

[8] Buchbauer G. The detailed analysis of essential oils leads to the understanding of their properties. *Perfumer and flavourist*. 2000. 25: 64-67.

[9] Moyler D. Extraction and Analysis of Essential oils and Aroma. *J. Ethanopharmacol*. 1998. 28(7), 103.

[10] Masamba R, Kamanula JFM, Henry MT & Nyirenda GKC. Extraction and analysis of lemon grass oil. *J gricultural Sciences*. 2003. 2(1), 56-62.

[11] Singh G, Kapoor IPS, Singh P, Heluani GSD & Lampasona MPD. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Journal of Food and Chemical Toxicology*. 2008. 46: 3295 – 3302.

[12] Rakic T, Sinzar-Sekulic J, Filipovic B, Tadic V, Branka SB, Tan K. Ecophysiological and anatomical characteristics of the subtropical shrub Zanthoxylum acanthopodium (Rutaceae) in conditions of a temperate continental climate (Serbia). Arch. Biol. Sci., Belgrade. 2009. 61(2), 249-260.

[13] Harborne JB & Williams CA. Advances in Flavonoid research since 1992. Phytochemistry. 2000. 55, 481-504. http://dx.doi.org/10.1016/S0031-9422(00)00235-1

[14] Pearson D. The Chemical Analysis of Foods (7th Ed.). Longman Group Ltd, London. 1993.

[15] Eskin M & Latta M. Simple and rapid colorimetric method for phytate determination. J.of Agric. & Food Chem. 1980. 28, 1313-1315. https://doi.org/10.1021/jf60232a049

[16] El-Baroty GS, Abd El-Baky HH, Farag RS & Saleh MA. Characterisation of antioxidant and antimicrobial of Cinnamon and ginger essential oils. African Journal of Biochemistry Research. 2010. Vol. 4(6). 167-174.

[17] Wang R, Ruijiang W, Bao Y. Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. Innovative Food Sci. Emerging Technol. 2009. 10: 289–292.

[18] Bowles EJ. Chemistry of aroma therapeutic oils. Allen & Unwin, 2003.ISBN 174114051X.

[19] Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science. 2006. 311, 808–811

[20] Andualem B, Gessesse A. Proximate composition, mineral content and anti-nutritional factors of Brebra (Millettia *ferruginea*) seed flour as well as physicochemical characterization of its seed oil. Springer Plus. 2014. 3: 298.

[21] Umoren UE, Essien AI, Ukorebi BA, Essien EB. Chemical evaluation of the seeds of *Milletia obanensis*. *Food Chem*. 2005. 91: 195–201.

[22] Akinyede AI, Amoo IA & Eleyinmi AF. Chemical and functional properties of full fat and defatted *Dioclea reflexa* seed flours. *J. Food Agri. Envir.* 2005. 3(2): 112–115 Enujiugha VN. Chemical and functional characteristics of conophor. Pak. J. Nutr. 2003. (6): 335–338.

[23] Enujiugha VN. Chemical and functional characteristics of conophor. Pak. J. Nutr. 2003. (6):

335–338.

[24] Hangen L, Bennin MR. Consumption of black beans and navy beans (*Phaseolusvulgaris*) reduced azoxymethane induced colon cancer in rats. Nutr Canc. 2002. 44: 60–65.