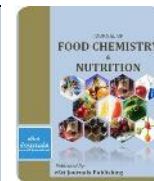




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ANALYSIS OF PHENOLIC COMPOUNDS, PHYTOSTEROLS, LIGNANS AND STILBENOIDS IN GARLIC AND GINGER OIL BY GAS CHROMATOGRAPHY

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ABSTRACT

The phenolic compounds, phytosterols, lignans, stilbenoids alongside the physicochemical, proximate and fatty acid composition of garlic and ginger obtained from a local farm in Nigeria were determined. We set out to confirm the presence of bioactive substances in these samples as found in our locality with the aim of dispelling or confirming previously stated bioactive substances obtained from same samples but from different regions of the world. Gas chromatography analysis revealed that the major phenolic compound in the samples was caffeic acid. The result of the analysis of phytosterol in the samples showed sistosterol as the major phytosterol in them. Analysis of the lignans showed that (9E, 12E, 15E)- 9,12,15- octadecatrien-1-ol was the major lignan, occurring up to 46% in the samples. Determination of the stilbenes showed that resveratrol was the major stilbenes present in them while unsaturated fatty acids were the major fatty acids present in the samples. These findings showed that the samples contained similar bioactives compounds alongside some additional bioactive compounds that were not detected in the previous studies. These findings further highlight high proportion of bioactive compounds in these samples especially lignans and stilbenes which may be of value in many biological processes and medical conditions such as in reducing the risk of cancer in humans.

Keywords: Lignan, stilbene, garlic, ginger.

INTRODUCTION

Characterization of oils and fats involve determination of the bulk physicochemical and bioactive compounds often present in them. Also, it has been found that the unsaponifiable matter in oils have important bioactive, nutritional and characteristic compositional properties that affect the quality of individual oils (Mitel *et al.* 2009). Among phytochemicals possessing these properties is phenolic compounds (Jahangir *et al.* 2009). Phenolic compounds commonly referred to as polyphenols are present in all plant but in different concentrations. It is a generic term that refers to a large number of compounds which are characterised by having at least one aromatic ring with one or more hydroxyl group(s) attached (Maria *et al.* 2011). It has been reported that intake of these phenolic compounds help to reduce the risk of coronary disease and cancer

(Maria *et al.* 2011). Sterols are a group of naturally occurring substances derived from hydroxylated polycyclic isopentenoids. They occur as a mixture of different compounds although their structures are closely related and varied depending on the extent of modifications of the ring system and side chain variations. Sterols are known to have a wide range of biological activities and physical properties such as: inhibition of cholesterol absorption (Abidi, 2001), lowering of plasma cholesterol, acting as useful emulsifiers for cosmetic manufacturers, supplying the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals (Abidi, 2001).

Stilbenes are phenolic-based compounds, of which the most widely recognized is resveratrol (3,4',5-trihydroxystilbene) (Aggarwal *et al.* 2004). Stilbenoids are chemical compounds, belonging to the family of phenylpropanoids including resveratrol, pterostilbene and piceatannol which can be found in grape skins and

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seeds, wine, nuts, peanuts (Norton, 2011). Resveratrol has attracted immense attention because of its biological properties including its anticancer effects (Aggarwal *et al.* 2004). Stilbenes especially resveratrol functions as a chemopreventive agent and has been shown to inhibit ribonucleotide reductase and certain other cellular events associated with initiation, promotion, and progression of carcinogenesis (Jang *et al.* 1997). Lignans are a group of chemical compounds found in plants. They are polyphenolic substance derived from phenylalanine via dimerization of substituted cinnamic alcohols (Milder *et al.* 2005). Lignans have multiple physiological functions in the body such as decreasing blood lipids (Hirata *et al.* 1996) and arachidonic acid levels (Ghafoorunissa, 2004). They also act as antioxidants thereby providing anti-inflammatory functions (Hsu *et al.* 2001).

Garlic (*Allium Sativum*) is cultivated worldwide and the potential medical properties of garlic have been recognised for thousands of years (Beato *et al.* 2011). Garlic compounds were reported to have tremendous antioxidant property which exerts actions by scavenging reactive oxygen species (ROS), enhancing cellular antioxidant enzymes and increasing glutathione in the cells (Borek (1981). Garlic health properties depend on the bioactive compounds of garlic especially organosulphur compounds (which are responsible for the pungent flavour of garlic), (Beato *et al.* 2011) and phenolic compounds which have interesting pharmacological properties (Lanzottili (2006). Garlic is claimed to help prevent heart diseases including: atherosclerosis, high cholesterol, high blood pressure and cancer (Block (2010). Animal studies and some early research studies in humans have suggested possible cardiovascular health benefits of garlic. A Czech study found garlic supplementation reduced accumulation of cholesterol on the vascular walls of animals (Durak *et al.* 2002).

Ginger is known botanically as *Zingiber Officinale*. It is used as a spice because it has a distinctive flavour and aroma, thus they are used to season foods. The characteristic odour and flavour of ginger is caused by a mixture of Zingerone, shogaols and gingerols (Abitogun & Badejo (2010). Phytochemical studies showed that the plant is rich in a large number of substances including zingiberene bisabolene (Jolad *et al.* 2005). Ginger is one of the most commonly used herbal supplements. Traditionally, ginger has been used to treat a wide range

of ailments such as stomach aches, abdominal spasm, nausea, vomiting as well as arthritis.

Although, previous studies (Beato *et al.* 2011; Gorinstein *et al.* 2008; Drozd *et al.* 2011) have identified various bioactive compounds present in garlic and ginger, majority of the studies were done outside the locality of the present study. The composition of seeds or fruits may vary with environmental and climatic factors. Hence, we set out to establish whether our local garlic and ginger contain the same or additional bioactive substances as shown by previous authors with the aim of further highlighting their economic, medicinal and nutritional values.

MATERIALS AND METHODS

Garlic and ginger roots were obtained from local farms around Ado- Ekiti, Ekiti State, Nigeria. All solvents and reagents used in this work were of analytical grade. The roots were washed several times with distilled water, cut into smaller pieces and air dried. The roots were milled using electric grinder.

Extraction of the oils: The powders were extracted with petroleum ether in a soxhlet apparatus for 6hrs. The extracted oil was concentrated using rotary evaporator.

Physicochemical analysis: The acid value, saponification value and iodine value were determined by standard methods as described by Association of Official Analytical Chemists (AOAC (1990). The refractive index was determined with calibrated Abbey refractometer and viscosity was also determined by a calibrated viscometer (Stress Tech Rheological). The metal compositions of the samples were determined using an Atomic Absorption spectrophotometer S series 711430VI.26 following the manufacturer's specifications.

Proximate Analysis: Standard methods of AOAC (1990) were used to determine the moisture, ash, crude fat, crude fibre, crude protein and carbohydrate contents of each sample.

Fatty Acid Methyl Ester Analysis: 50mg of the extracted fat content of the sample was saponified for five minutes at 95°C with 3.4ml of 0.5M KOH in dry methanol. The mixture was neutralised by using 0.7M HCl. 3ml of 14% boron trifluoride in methanol was added. The mixture was heated for 5 min at 90°C to achieve complete methylation process. The Fatty Acid Methyl Esters (FAME) was extracted thrice from the mixture with redistilled n- hexane. The content was

concentrated to 1ml for gas chromatography analysis and 1µl was injected into the injection port of GC.

Sterol Analysis: Aliquots of the extracted oil were added to the screw-capped test tubes. The sample was saponified at 95°C for 30 minutes, by using 3ml of 10% KOH in ethanol to which 0.2ml of benzene had been added to ensure miscibility. Deionised water (3 ml) was added and 2ml of hexane was used in extracting the non-saponifiable materials. Three extractions each with 2ml of hexane were carried out for 1 hour, 30 minutes and 30 minutes respectively to achieve complete extraction of the sterols. The hexane mixture was concentrated to 1ml in the vial for gas chromatography analysis and 1µl was injected into the injection port of GC. The GC used for both FAME and sterol analysis and the conditions of the instrument are: HP 6890 Powered with HP chemstation Rev. A 09.01 [1206] software. Injection temperature- split injection, Carrier gas – nitrogen, Split ratio - 20:1, inlet temperature 250°C, Column type - HP INNOWax, Oven program - initial temperature @ 60°C first ramping @ 10°C/min to 20 min, second ramping @ 15°C/min for 4 min.

Determination of phenolic acids: 50mg of the sample was extracted with 5ml of 1M NaOH for 16 hours on a shaker at ambient temperature as described by Kelley *et al.* (1994) and Provan *et al.* (1994). After extraction, the sample was centrifuged, rinsed with water, centrifuged again and the supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2h to release the conjugated phenolic compounds. The heated extract was cooled, titrated with 4M HCl to pH < 2.0, diluted to 10ml, with deionised water, and then centrifuged to remove the precipitate. The supernatant was kept for subsequent purification and analyse using gas chromatography. The GC used for the analysis is HP 1 Column, column length – 30m, injection temperature – 250°C, Detector – FID, Carrier – nitrogen, initial temp – 60°C for 5 min, first rate – 15°C/min for 15 min, second rate - 10°C/min for 4 min.

Determination of stilbene: The samples were extracted with petroleum ether for 1hr in a soxhlet extractor at 40°C. The oils were concentrated with rotary evaporator. 1mg of the extracted oil was derivatized with 100µl of a mixture of 3.5:1:0.5 (v/v/v) bis-(trimethylsilyl) trifluoroacetamide dimethyl formamide- methanol in a 2ml GC vial according to

Rimando and Cody (2005). The vial was capped and heated at 70°C for 1 hr. After, the vial was cooled to room temperature. 2µl was injected and analysed by GC- MS. The GC used for the analysis is HP 6890 Powered with HP chemstation Rev. A 09.01 [1206] software. Injection temperature- split injection Carrier gas – hydrogen, Split ratio 20:1, inlet temperature 250°C, column type DB-5MS, oven program - initial temperature @ 40°C for 5 min, first ramping @ 10°C/min to 200°C, second ramping @ 8°C/min to 300°C.

Determination of lignan: The modified method of James *et al.*, 2006 was used for the analysis of lignan. The samples were extracted as described above. The total extracts were concentrated with a rotary evaporator. 1mg each of the oil was then purified in 5ml acetone for the GC- MS analysis. The oil was concentrated to 1ml in the gas chromatographic vial for the analysis. The GC used for the analysis is HP 6890 Powered with HP chemstation Rev. A 09.01 [1206] software. Injection temperature- split injection, Carrier gas – nitrogen, Split ratio 20:1, inlet temperature 250°C, column type HP 5, oven program- initial temperature @ 120°C ramping @ 10°C/min to 20 min, second ramping @ 8°C/min to 300°C.

RESULTS AND DISCUSSION

Table 1 presents the result of the proximate analysis of garlic and ginger flour. The result shows that both samples are rich in carbohydrate with garlic having relatively higher protein value than ginger and these are in agreement with work reported by other authors like: Okolo *et al.* (2012) and Otunola *et al.* (2012) but contrary to that by Nwinuka *et al.* (2005) where a high protein value for ginger (17.35%) and low value for garlic (8.58%) was reported. The discrepancy in these studies may be due to environmental conditions and methods of storage of samples. The result of the physicochemical characteristics (Table 2) showed that the saponification values of garlic and ginger oil is 162.12 and 169.70 mg/KOH/g respectively. The saponification values obtained in this work are lower than 192mgKOH/g reported by Gafar *et al.* (2012) for garlic oil and 213.18mgKOH/g reported for ginger by Abitogun and Badejo (2010). The differences in these results may be due to changes in climatic conditions. The observed high saponification values in this study showed that these oils could be used in soap making as earlier noted by other authors (Wara *et al.* 2011).

Table 1. Result of proximate analysis of ginger & garlic flour.

Parameters	Garlic	Ginger
Moisture content(%)	5.02	10.50
Crude ash (%)	4.37	3.50
Fat content(%)	0.52	4.82
Crude fiber(%)	2.22	3.56
Crude protein (%)	15.35	7.50
Carbohydrate (%)	72.52	70.12

The acid values obtained in this study, is in agreement with other previous report (Gafar *et al.* 2012) and the low acid value is an indication of the good storage ability of the oils. The elemental composition of the oils showed that both samples contain high percentage of phosphorous: 3.80 and 4.16% for garlic and ginger respectively. No trace of chromium was detected in them and this makes them safe for consumption.

Table 2. Physicochemical properties of garlic and ginger oil.

Parameters	Garlic	Ginger
Acid value (mgKOH/g)	4.11	4.53
Iodine value (gI ₂ /100g sample)	134.0	96.0
Saponification value (mgKOH/g)	162.1	169.7
pH value	5.96	6.58
Viscosity	84.0	83.9
Refractive index	1.56	1.51

The result of the fatty acid composition is presented in Figure 1. The result showed that both samples contain high percentage of unsaturated fatty acid with their being present in relatively higher proportion in garlic oil than in ginger oil further highlighting their nutritional and health benefits respectively. This finding is in agreement with the report by Abitogun and Badejo (2010).

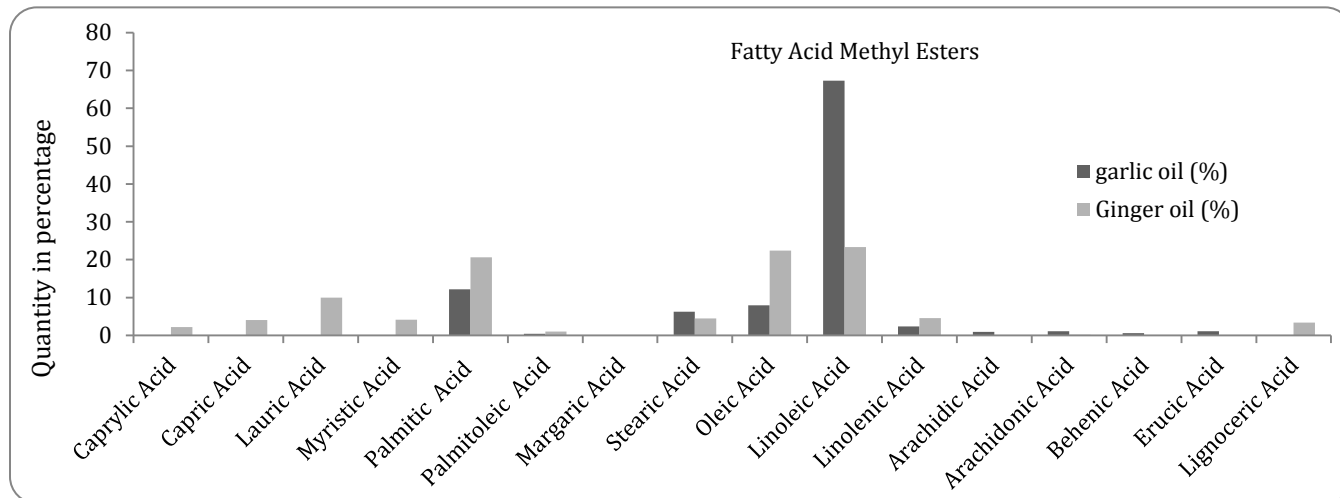


Figure 1. Percentage composition of fatty acid in garlic and ginger oil.

The high percentage of linoleic and oleic acid in the samples confirmed that the oil is liquid than solid. Therefore, the oil cannot easily congeal at ordinary temperature. It also implies that regular consumption of these sample oils is safe and can prevent the risk of heart problems because it offers better protection against increased blood pressure. (Abitogun and Badejo(2010).

The result of the phenolic compounds present in the samples showed that both garlic and ginger contained high percentage of caffeic acid; 46% and 45% for garlic and ginger respectively as shown in table 3 and figure 2. This is followed by 27% of ferulic acid in garlic. Percentages of protocatechuic acid, p – coumaric , p – hydroxybenzoic, piperic, chlorogenic, ellagic, rosmarinio were found to be approximately 3%, 3%, 5%, 5%, 4%, 3%, 1% in garlic oil.

Table 3. Phenolic compounds in garlic and ginger oil

Phenolic compounds	Garlic (%)	Ginger (%)
Protocatechuic acid	2.66	2.63
P – coumaric	3.46	3.80
Vanillic acid	0.15	7.25
O – coumaric	0.53	0.60
P – hydroxybenzoic acid	5.25	6.26
Gallic acid	0.36	4.32
Caffeic acid	46.13	45.77
Ferulic acid	27.08	3.39
Syringic acid	0.75	0.95
Piperic acid	4.48	5.41
Sinapinic acid	0.64	7.66
Chlorogenic acid	3.83	23.24
Ellagic acid	2.88	3.22
Rosmarinio acid	1.27	2.78

Ginger oil contain 23% chlorogenic acid, 7% vanillic acid, 6% p - hydroxybenzoic acid, 4 % gallic acid, 8% sinapinic acid, 3% ellagic acid, 3% protocatechuic acid, 4% p - coumaric acid, 3% ferulic acid, 5% piperic acid, 3% rosmarinio acid. This is in agreement with the result of Gorinstein *et al.* 2008 for phenolic acids in

garlic. Beato *et al.* and Drozd *et al.* did not detect any protocatechuic acid in the garlic samples they studied (Beato *et al.* 2011; Drozd *et al.* 2011). The discrepancy between these studies and the present study may be due to differences in environmental conditions and methods of analysis.

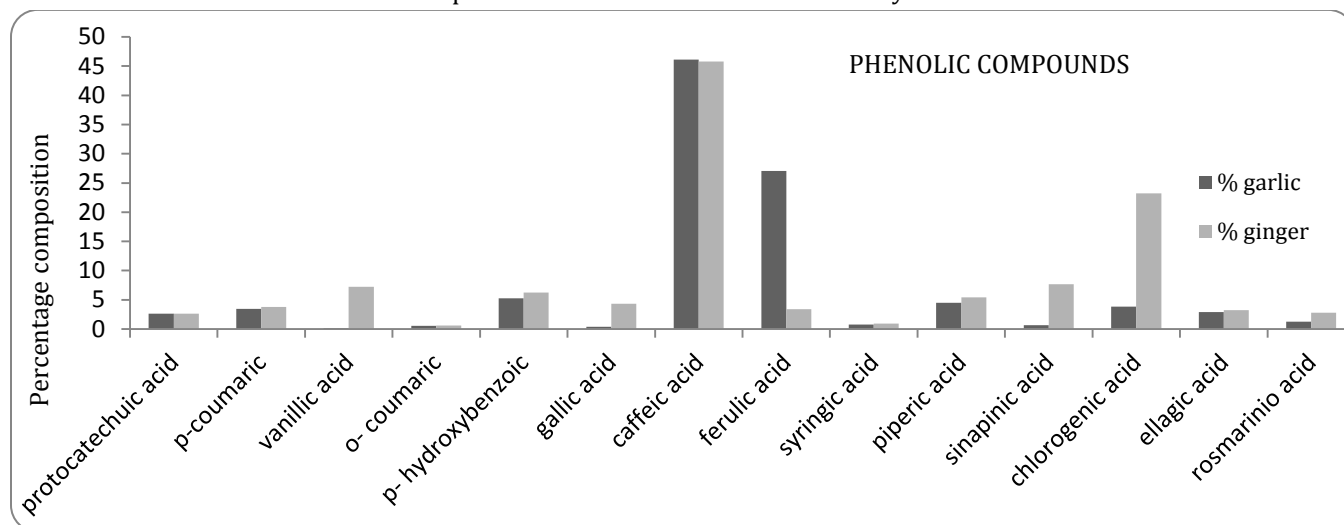


Figure 2. Percentage composition of phenolic compounds in garlic and ginger oil.

The result of the sterols (Figure 3) showed that both samples contain very high percentage of sitosterol; 86% and 87% for garlic and ginger respectively while campesterol, stigmasterol, and 5- avenasterol are present in approximately 7%, 2%, 5% and 6%, 3%, 4% for garlic and ginger oil respectively. Saturated oils like Cholesterol and cholestanol were found in very minute quantity in both samples. Sterols are known to inhibit

oxidative deterioration of oils; serving as potential antipolymerization agents for frying oils (Abidi (2011). The unsaturated analogues of phytosterols and their esters have been suggested as effective cholesterol - lowering agent by decreasing low density lipoprotein (LDL) cholesterol, mostly through interfering with the intestinal absorption of cholesterol thereby offering cardiologic health benefit (Law (2000).

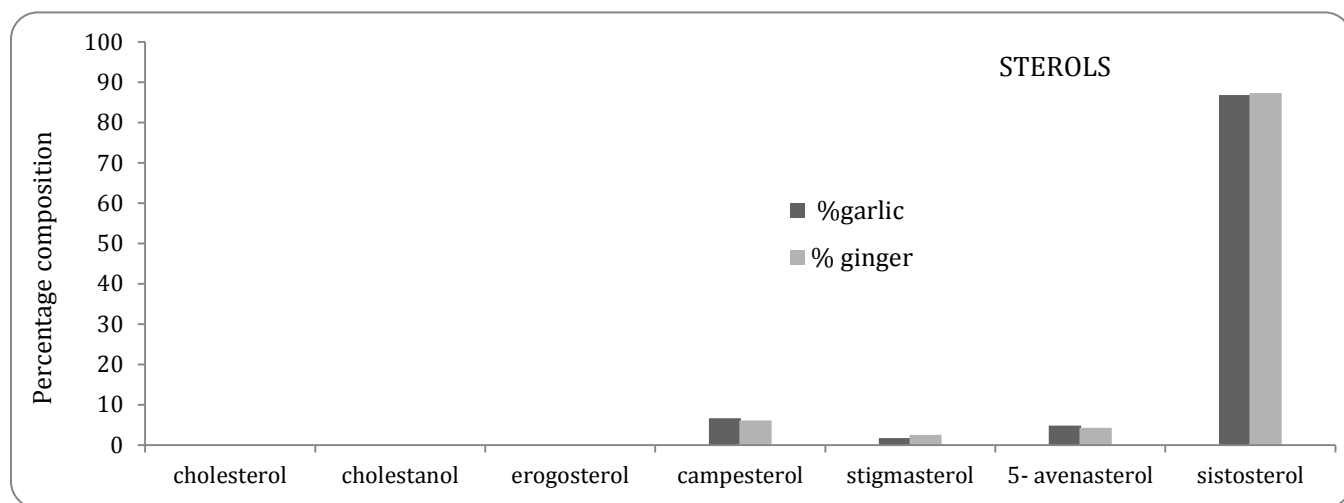


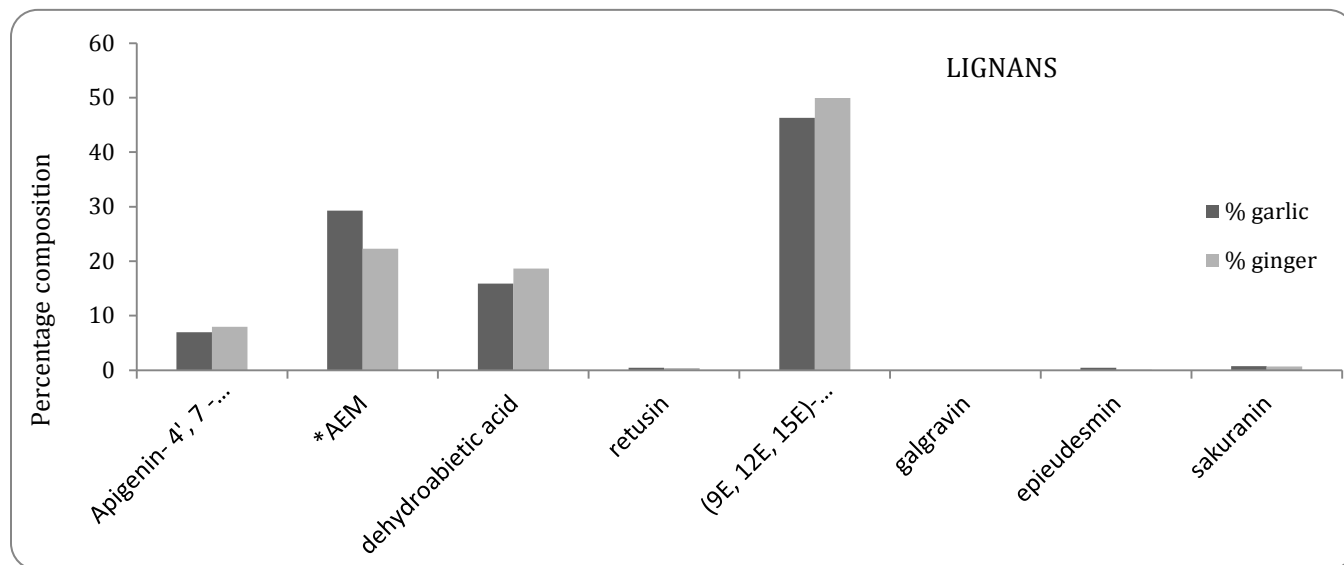
Figure 3. Percentage composition of sterols in garlic and ginger oil.

The result of the analysis of lignan is presented in figure 4. The result showed that garlic oil contains (9E, 12E, 15E)-9,12,15 - octadecatrien-1- ol, 2- allyl-5- ethoxy -4-

mehtoxyphenol, dehydroabiatic acid, and apigenin - 4',7 - dimethyl ether in percentages 46, 29, 15, 7 respectively while ginger oil contains 22% (2- allyl-5- ethoxy- 4-

methoxyphenol), 49% (9E,12E,15E)9,12,15 - octadecatrien - 1- ol), 8% (apigenin - 4',7- dimethyl ether) and 18% (dehydroabietic acid). The major lignan found in both samples is (9E, 12E, 15E) 9,12,15 - octadecatrien - 1- ol. Intake of lignans has been associated with reduction in the risk of breast cancer, lung cancer and prostate cancer (Kanu *et al.* 2010). Lignans also help to reduce hair loss (Stephen (2006). The result of the

stilbenoids (Figure 5) showed that 98% of the total stilbenes in both samples is resveratrol while piceatannol and pterostilbene accounted for the remaining 2%. Resveratrol has been found to be a promising agent in promoting cardioprotection against coronary heart disease. It has angiogenic, antihypercholesterolemic and antidiabetic effects (Bacciottin *et al.* 2007) thus highlighting the health benefits of these plants.



*AEM - 2-allyl-5- ethoxy- 4- methoxyphenol

Figure 4. Percentage composition of lignans in garlic and ginger oil.

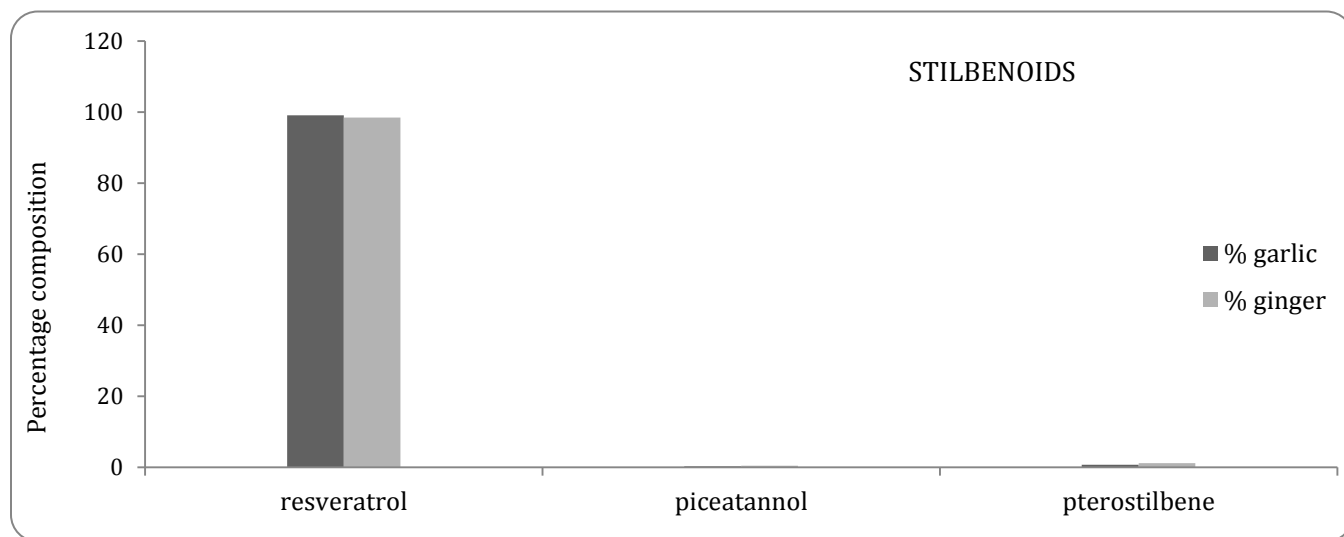


Figure 5. Percentage composition of stilbenoids in garlic and ginger oil.

CONCLUSION

This study highlights the presence of some previously identified composition of garlic and ginger alongside new compounds in them which were not detected hitherto by some previous authors and this shows that,

environmental conditions may have effect on the chemical composition of seeds and fruits. Also, the additional bioactive compounds found in garlic and ginger used in this study showed that their oil will offer better protection against many human health problems such as increased

blood pressure, cancer, heart diseases, hyperlipidemia and those diseases linked with lipid peroxidation and body tissue destruction by free radicals. There is need for more local studies on these samples to further unmask their hidden potentials with the aim of highlighting their economic, nutritional and medicinal values.

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