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PHYTOCHEMICAL CONSTITUENTS AND TERMICIDAL ACTIVITY OF ESSENTIAL OILS FROM SYZYGIUM AROMATICUM (CLOVE BUD)

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ABSTRACT

The present study was carried out to determine the phytochemicals constituents and termicidal potential of essential oils from Syzygium aromaticum as anti-termites activity in Dutse. The essential oil (Syzygium aromaticum) was extracted by macerated method with petroleum ether. The chemical composition of the oil was analyzed by GC-MS. After the extraction with petroleum ether, maceration with water was subsequently carried out. The water extract was thereafter, phytochemically screened. The aqueous extract contains flavonoids, cardiac glycosides, tannins, and terpernoids respectively. After this the GC-MS showed that extract contained cyclopentane, cyclopropane, caryhylene, humulene, phenol, D-limonene, α-terpenes and carene. The result of the contact and repellence test showed that the activities of the syzygium aromaticum against captotermesformasanus increase with increase in dose and time. The result revealed that the contact activity of the oil of syzygium aromaticum showed toxicity against coptotermesformasanus as a result of effective usage of the essential oil which differs significantly; while the repellence activity showed 100% mortality of coptotermesformosanus with a dose of 0.5ml/L of the essential oil after 10 minutes of exposure. Hence the oils of the syzygium aromaticum proved to be effective against coptotermesformasanus.

Keywords: Extraction, Essential oil, *Syzygium aromaticum*, Termicidal activity

INTRODUCTION

Phytochemicals have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical and chemical characteristics (Meagher *et al*., 1999).Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. These complex materials are referred to as secondary metabolites. Plants secondary metabolites have recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidant effect, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Halliwell and Gutteridge, 1992; Farombi *et al*., 1998). Antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the a etiology of many diseases and in food deterioration and spoilage (Halliwell and Gutteridge, 1992; Kasaikina, 1997; Farombi, 2000; Koleva *et al*., 2000).Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. In wide-range dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, roots, stems, leaves, flowers, fruits or herbs and spices (Mathai, 2000). Some are in cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources ((Mathai,2000). Termites are large and diverse group of insects consisting of over 2600 species worldwide. With over 660 species, Africa is by far the richest continent in termite diversity (Eggleton, 2000). The species known to damage crops, trees and rangeland belong to the family Termitidae. This family consists of four subfamilies: Macrotermites, Nasutitermitinae, Termitinae and Apictotermitinae. It is estimated that less than 20% of members of the termite causes damage in agriculture, forestry and urban settings is distributed to members of the Macrotermitinae (Pomeroy *et al.*, 1991; Mitchell, 2002), which build the large mounds (hereafter called termitaria) that form the spectacular features of the African landscape (Glover, 1997, Malaisse, 1998).Cloves (*Syzygium aromaticum* L.) are the aromatic dried flower buds of a tree in the family of *Myrtaceaae*. The clove tree is an evergreen which grows to a height ranging from 10-20m, having large oval leaves and crimson flowers in numerous groups of terminal clusters. The flower buds are at first of a pale color and gradually become green, after which they develop into a bright red, long calyx, terminating in four spreading sepals, and four unopened petals which form a small ball in the center (Kim *et al*., 1998). Clove is native to Indonesia and used as a spice in virtually all of the world's cuisine (Kim *et al*., 1998). The name derives from French *clou*, a nail, as the buds vaguely resemble small irregular nails in shape(Kim *et al*., 1998).Cloves are harvested primarily in Indonesia and Madagascar; It is also grown in India, Sri Lanka, and the "Spices Islands" Moluccas, Indonesia known as the Bandas Islands (Kim *et al*., 1998). Cloves can be used in cooking, either whole or in a grind form, but as they are extremely strong, they are used sparingly (Kim *et al*., 1998). The spice is used throughout Europe and Asia and is smoked in cigarettes (also known as kreteks) in Indonesia and in occasional coffee bars in the West, mixed with marijuana to create marijuana spliffs (Kim *et al*., 1998). Cloves are also an important incense material in Chinese and Japanese (Kim *et al*., 1998). Clove essential oil is used in aromatherapy, and oil of cloves is widely used to treat toothache in dental emergencies (Kim *et al*., 1998). It was reported that *Syzygium aromaticum* has been successfully used for asthma and various allergic disorders by oral administration (Kim *et al.*, 1998).Clove essential oil is used in aromatherapy, and oil of cloves is widely used to treat toothache in dental emergencies (Kim *et al*., 1998). The essential oil of cloves has anesthetic and antimicrobial qualities and is sometimes used to eliminate bad breath or ameliorate the pain of a bad tooth (Adorjan and Buchbauer, 2010). Sesquiterpenes found in clove were investigated as potential anti-carcinogenic agents (Zheng *et al*., 1992).The whole essential oil or its main component (eugenol , $C_{10}H_{12}O$; 4-ally-2-methoxy phenol) is used by dentists to calm the nerve inside a tooth after the removal of deep decay and is the characteristic odor of a dentist' office. When mixed with zinc oxide, eugenol forms a cement used by dentists (Lee and Shibamto, 2001). Clove oil is used in the traditional blend of products (clove oil in mineral oil) and is applied to Japanese sword blades to prevent tarnishing of the polished surface (Cai and Wu 1996; Baytop 1999; Lepage *et al.*, 1993). Also, eugenol derivatives or methoxylphenol derivatives in wider classification are used in perfumery and flavoring. They are used in formulating insect attractants and UV absorbers, analgesics, biocides and antiseptic. They are also used in manufacturing stabilizers and antioxidants for plastics and rubbers (Lee and Shibamoto, 2001).Three essential oils are available from clove species: clove bud oil, clove stem oil, and clove leaf oil. Each has different chemical and flavor. Clove bud oil is the most expensive and the best quality product. It contains eugenol (80-90%), eugenol acetate (15%) and beta caryophyllene (5-12%). It is well known that the amounts of secondary compounds like essential oils are affected by genetic factors, climate, soil and cultivation techniques (Burt, 2004: 1995; Verzar-Petri *et al*., 1995; Arslan *et al*., 2004). Over the past decades, there has been an increase in effort to reduce the reliance on synthetic insecticides in the prevention of diseases due to its harmful effects in the environment and human. These synthetic chemicals have been found to cause numerous health problems such as cancer endocrine disruption and lower immunes system. It is then important to find an alternative to the synthetic insecticides. Essential oils, which are naturally found in plant are easily biodegradable and can be an alternative to the synthetic insecticides used. The aim of this research is to study the phytochemical constituents and the termicidal activity of *Syzygium aromaticum* (clove bud).This can be achieved by extracting essential oil from seeds of *Syzygium aromaticum*, components analysis of essential oils using GS-MS, the repellant activity and contact activity of essential oils.

MATERIALS AND METHODS

Plant Materials: Clove bud (*Syzgium aromaticum)* was purchased from the local market (Ultra-modern market) in Dutse. The seed was collected from Dutse, Jigawa State, Nigeria in 20thJuly, 2016 and transported to the Herbarium Unit, Department of Biological Sciences, Faculty of Science, Federal University Dutse, Jigawa State for identification. The seeds were air-dried and ground to a coarse powder using a mortar and pestle.

Termites Collection

Secondary nests of (*Coptotermesformosamus)* were collected from termite mould at Federal University Dutse and placed in black garbage bags. The nests were immediately transported to the laboratory and placed inside 100 liter plastic containers with lids and kept in a room at 25^{o}C for three days. Distilled water was sprayed on the sides of the container to keep the relative humidity above 80%.

Extraction of the Seed

About 150g of the coarse powder of the Clove bud (*Syzygium aromaticum)* seeds was macerated with petroleum ether in a sealed container for 5 days at room temperature with occasional shaking and decanting until exhaustion of the extraction oil. The oil was concentrated by a rotary evaporator. Thereafter the extraction of the essential oils were stored in airtight small bottles in a refrigerator at 4^{o}.

Phytochemical Analysis

Chemical tests were carried out on petroleum ether extracts for the qualitative determination of phytochemical constituents as described by Harborne,1973; Trease and Evans,1989;Trease and Evans, 2012 ; Sofowora, 1993).

Contact Effect

The contact effect of the essential oil against termite was evaluated on filter paper disc by treating a what man No. 1 filter paper with the clove bud essential oil diluted in 100% acetone. A micro-pipette was used to suck out 1µL, 2µL, 3µL, 4µL and 5µL of the essential oils and was diluted with 2ml of acetone to form concentrations of 0.5ml/L, 1ml/L, 1.5ml/L, 2ml/L and

2.5ml/L respectively. They were each poured and allowed to flow regularly on a disc of filter paper placed in a petri dish. The solvent was allowed to dry after which 10 termites (*Coptotermesformosanus)* were introduced into the petri dish and then closed. Percentage mortality of termites was observed every 10 minutes. Each experiment was conducted in triplicate. Control experiment was done using only acetone (Ayoola *et al*., 2008).

Repellant Effect

The repellant effects of the clove bud (*Syzygium aromaticum*) seed of the essential oil against termites (*Coptotermesformosanus)* were evaluated using the area preference method. Tested areas consisting of Whatman No. 1 filter paper cut in half. 1µL, 2µL, 3µL, 4µL and 5µL of the essential oils and was diluted with 2ml of acetone to form concentrations of 0.5ml/L, 1ml/L, 1.5ml/L, 2ml/L and 2.5ml/L respectively. Full discs were subsequently remade by attaching treated halves to untreated halves with clear adhesive tape. 10 termites (*Coptotermesformosanus)* of each species were released separately at the center of the filter paper disc and the petri dishes were subsequently covered and observations were made. Each experiment was conducted in triplicate. Percentage repellency (PR) values were calculated as follows; multiplying the concentration by a factor of 100 (Ayoola *et al*., 2008).

Gas Chromatography-Mass Spectrometer (GC-MS) Analysis: Gas chromatography and mass spectrometry analysis was performed on Agilent 6890N instrument equipped with a flame ionization detectors and HP 5MS (30m x 0.25mm x 0.25µm) capillary column, while the essential oil components were identified on an Agilent Technology 5973N mass spectrometer. The GC setting was as follows: the initial oven temperature was held at 60ºC for 1 min and ramped at 10ºC min^{-1} to 180ºC for1 min, and then ramped at 20ºC min^{-1} to 280ºC for 15min. The injector temperature was maintained at 270ºC. The samples (1µL) were injected neat, with a split ratio of 1:10. The carrier gas was helium at flow rate of 1.0mL min^{-1}. Spectra were scanned from 20 to 550m/z at 2 scans s^{-1}. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compound available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkenes (C_8 – C_{24}) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature. Component relative percentage was calculated based on GC peak areas without using correction factors.

Statistical Analysis

Data were analyzed by analyses of variance (ANOVA) using statistical software (SPSS) at 5% significance level.

RESULTS AND DISCUSSION

Results

The results for the phytochemical constituents, GC-MS compounds , the contact and repellent activity of essential oils of *Syzygium aromaticum* against *Coptotermesformosamus* are shown in the table 1.0, 2.0 3.0, 4.0, 5.0and 6.0 respectively. The phytochemical screening revealed the presence of flavonoids, cardiac glycosides, tannins and terpenes (in large amount). While the GC-MS result contains the following essential oils: Cyclopentane, Cyclopentane, Cyclopropane, Humulene, D-limonene, α-terpene, phenol and Carene respectively. From table 3.0 the result of contact activity of essential oil of *Syzygium aromaticum* on *Coptotermes formosanus* revealed that 0% mortality was recorded during the first 30min of exposure to the formulation with the exception of 2.5ml/L which recorded 100% mortality. Table 4.0 showed that the results of *Coptotermes formosanus* for mortality with the essential oil of *Syzygium aromaticum* after 6hrs, 12hrs and 24hrs of formulation all graded concentration recorded 100% mortality. The mortality of the *Coptotermes formosanus* increased with increase in dose, which showed that it is time and dose dependent. The result of the repellency tests on the *Coptotermesformosanus* by the essential oil of *Syzygium aromaticum* is shown in table 5.0 all dose recorded 100% repellence after 10minutes to exposure. After 6hrs, 12hrs and 24hrs of formulation of the essential oil, all graded concentration recorded 100% repellence (Table 6.0).

Table 1: Phytochemical Analysis of aqueous extract of S*yzygium aromaticum*

Phytochemicals	Aqueous extract of S*yzygium aromaticum*
Flavonoids	+
Cardiac glycoside	+
Saponins	-
Tannins	+
Alkaloids	-
Terpenes	+

Key: += present, - = absent

Table 2: GC-MS Result of Various Compounds of aqueous extract *Syzygium aromaticum*

Retention time (RT) Mins	Compounds of *Syzygium aromaticum*
2.130	Cyclopentane
2.395	Cyclopropane
8.300	Caryphylene
7.543	Humulene
7.055	Phenol
5.347	D-Limonene
5.205	α Terpene
4.450	Carene

Table 3: Contact mortality of essential oil of *Syzygium aromaticum* against *Coptotermesformosanus* after an hour of exposure

Exposure	Concentration (ml/L)					
Time (min)	Control	0.5	1	1.5	2	2.5
10	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
20	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	50.00±5.80[b]
30	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	100.00±0.00[c]
40	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	50.00±0.00[b]	100.00±0.00[c]
50	0.00±0.00[a]	0.00±0.00[a]	50.00±0.00[b]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
60	0.00±0.00[a]	50.00±0.00[b]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

Table 4: Contact mortality of essential oil of *Syzygium aromaticum* against *Coptotermesformosanus* after several hours of exposure

Exposure	Concentration (ml/L)					
Time (hrs)	Control	0.5	1	1.5	2	2.5
6	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
12	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
24	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

Table 5: Percentage repellence activity of essential of *Syzygium aromaticum* against *Coptotermesformosanus* after an hour exposure

Exposure	Concentration (ml/L)					
Time (min)	Control	0.5	1	1.5	2	2.5
10	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
20	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
30	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
40	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
50	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
60	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

Table 6: Percentage repellence activity of essential oil of *Syzygium aromaticum* against *Coptotermesformosanus* after several hours of exposure

Exposure	Concentration (ml/L)					
Time (hrs.)	Control	0.5	1	1.5	2	2.5
6	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
12	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]
24	0.00±0.00[a]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]	100.00±0.00[c]

The result shows the mean ± SD of three replicates. Data within a row followed by the same letter are not significantly different at P<0.05.

DISCUSSION

From the GC-MS analysis; result obtained revealed the various compounds in the plant include; cyclopentane, cyclopropane, caryhylene, humulene, phenol, D-limonene, α-terpene and carene. The results of the GC-MS gave a supportive evidence for this reason the contact activity and repellent activity occur as a result of the various compounds or component present in the essential oil from *Syzygium aromaticum.*

The results of the contact activity revealed that the essential oil of *Syzygium aromaticum* had significant insecticidal effects on the adults of *Coptotermesformosamus* as compared to the control. They were found to be very effective in causing mortality of termites. A highly significant differences ($P<0.05$) were found among all the treatments. Mean mortality of the adult's *Coptotermesformosamus* exposure to five concentration levels of the essential oil as presented in table 3.0, 4.0, 5.0 and 6.0 a direct mortality rate and repellent rate was observed at different exposure time. It means that the toxicities of this essential oils increased with increase in dosage as well as increase in the period of exposure to the essential oils, this is same as interpreted by (Ayoola *et al*.,2 008). And the results indicated that the entire essential oil significantly reduced the number of termites. In general, toxicity for the essential oil was observed at the lowest concentration of the essential oils (0.5ml/L) the essential oil of clove bud (*syzygium aromaticum*) were found to be more toxic in the contact treatment and repellent treatment. This toxicity could be attributed to the volatiles substances from the test oil. The termites showed 100% mortality within 60 minutes of exposure essential oils (table 3.0). Mortality (100%) of termites *(coptotermsformosamus)* was observed after 60 minutes of exposure time. Termite (adult's insect) are susceptible to the toxicity of the essential oil at different exposure time significantly.

The essential oils from *syzygium aromaticum* exhibited potent repellent activity against adult's termites (C*optotermsformosanus)* table 5.0 and the repellency value increases with concentration and exposure period. The essential oils of S. *aromaticum* had the strongest repellency using same amount of concentration (Table 6.0). Repellency activity of the essential oils against termite *C. formosanus* adults gradually increased with increasing exposure time.

Contact Activity

The findings of this work showed that the contact activity of the essential oil of *S. aromaticum*. Table 3.1 and 3.2 shows that increase in exposure time and concentration could lead to increase in mortality rate. In Table 3.1 the 1ml/L (concentration) yielded 100% mortality rate after 60minutes exposure time, while 2.5ml/L yielded 100% mortality rate after 30minutes exposure time. After several hours of exposure, 0.5ml/L (concentration) yielded 100% mortality rate after 6hrs exposure time. While in Table 3.2 the 1.5ml/L (concentration) yielded 100% mortality rate after 60minutes exposure time and 2.5ml/L (concentration) yielded 100% mortality rate after 50minutes exposure time.

Repellent Activity

Table 5.0 and 6.0 showed the repellent activity of essential oil from *Syzygium aromaticum* against termite. These table show increase in repellent rate with increasing in concentration. Table 5.0 shows that in 0.5ml/L concentration which is the lowest concentration yield 100% repellent rate in 10minutes exposure time and 100% repellent rate was observed in 6hrs exposure time (Table 6.0).Concentration of 2.5ml/L which achieved 100% at 10minutes exposure time. Considering the lowest level it is observed that increase in concentration lead to increase in repellent. *Syzygium aromaticum* showed strong repellency and anti-termites activity. The finding of this study shows that, the essential oils contain termicidal principles and also *syzygium aromaticum* is more effective, efficient and contains more termicidal activity. It can be concluded that the essential oils studied are a potential termite killer (*Coptotermsformosanus*).

CONCLUSION

The presence of phytochemicals in the plant samples may not be unconnected with its termicidal activity. The study showed that exposure time and quantity of *Syzgium aromaticum* of essential oil increases as the rate of termite's knockdown also increased. It could therefore be concluded that the essential oils is a potential killer of termites. Regarding the harmful effects of synthetic pesticides, this study demonstrates that these essential oils can play an important role in protection of wood from insect invasion. This technology is cheap, safe, environmental friendly and easy to adopt.

RECOMMENDATION

Further studies are required to increase the lethality of these components due to the synergy achieved by the presence of other minor components of the oil. Since the technology is cheap, safe, environmentally friendly and easy to adopt by small-scale farmer and large scale farmer, strongly recommend the use of *Syzygium aromaticum* essential oil as an alternative means for wood protection and fumigation purposes. Environmental and health implications indicate that it would be safer and more environmental friendly to use the botanical fumigant in insect pest control than to use the poisonous and hazardous synthetic fumigants. Solid formulation should be made. Active ingredient should be synthesized.

REFERENCES

Adorjan B and Buchbauer G., (2010) Biological properties of essential oils. An updated review, *Flavour and Fragrance Journal, 5:407 – 426*.

Ayoola, G. A., Lawore, F. M., Adelowotan, T., Aibinu, I. E.Adenipekun, E., Coker, H. A. B. and Odugbemi, T. O (2008). Chemical analysis and antimicrobial activity of the essential oil of *S. aromaticum* (clove). *African Journal of Microbiology Research* 2: 162 166.

Arslan, N., Gurbuz, B. and Sanhan, E.O. (2004). Variation in essential oil content composition: In Turkish anise (*Pimpinellaanisum L.*) Populations, *Turk. J. Agric.* 28, 173-177.

Baytop, T. (1999). "*Therapy with medicinal plants in Turkey (Past and Present)*," No.3255, 2nd ed., Istanbul Publications of the Istanbul University, Istanbul.

Burt S. 2004. Essential oils: Their antibacterial properties and potential applications in foods – A Review. *International Journal of Food Microbiology* Vol. 94 (3):223-253. Science Direct:http://www.sciencedirect.com/science/article/pii/ S0168160504001680

Cai, L., and Wu, C.D. (1996). Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens, *Journal of Natural Products.* 59, 987-990.

Eggleton, P. (2000) *Global patterns of termite diversity*. Pages 25 – 51 in T. Abe, D.E. Bignell, and M. Higashi, editors. Termites: *Evolution, Sociality, Symbioses, Ecology*. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Farombi E, O (2000). Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol. Res* 42:75-80.

Farombi E.O, Nwamkwo J.O, Emerole G.O (1998). *Effect of methanolic extract of browned yam flour diet* on 7, 12-Dimethylbenzanthracene (DMBA) and 3-methylcholanthrene (3-MC) induced toxicity in the rat .*Proceedings Federal .African Biochemical. Molecular Biololgy*. 1: 5-10.

Glover, P.E. (1967) Further notes on some termites of northern Somalia. *East African Wildlife Journal* 5:121-132.

Halliwell B, Gutteridge JMC (1992). Free radicals, antioxidants and human diseases: where are we now? *Journal of Laboratory Clinical. Med*icine, 119:598-620.

Halliwell, B., and Gutteridge, J. M., (1999). Free Radical in Biology and Medicine, Claredon Press, Oxford. Pp. 617-783.

Harborne, J.B.(1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, 56-179.

Kim, H.M., Lee, E.H., Hong, S.H., Song, H.J., Shin, M. K., Kim, S.H., and Shin, T.Y. (1998). Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats, *Journal of Ethnopharmacology* 60; 125-131.

Koleva II, Niederlander H.A.G, Van Beek T.A (2000). An online HPLC Method for detection of radical scavenging compounds in complex mixtures. *Analytical Chem*. 72: 2323-2328.

Lee, K. G., and Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove buds (*Szygium aromaticum), Food Chemistry.* 74, 443-448.

Lepage, M., L. Abbadie, and A., Mariotti (1993). Food habits of sympatric termite species (Isoptera, Macrotermitinae) as determined by stable carbon isotope analysis in a Guinea savanna (Lamto, Cote d'Ivoire). *Journal of Tropical Ecology* 9:303 – 311.

Malaise, F. (1998) High termitaria. Pages 1279 – 1300 in M.J.A. Werger, editor. *Biogeography and Ecology* of Southern Africa. Dr. W. Junk The Hague, the Netherlands.

Mathai K (2000). Nutrition in the Adult Years. InKrause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.

Meagher E,and Thomson C (1999). *Vitamin and Mineral Therapy*. In Medical Nutrition and Disease, 2nd ed., G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc; pp33-58.

Mitchell, J.D. (2002) *Termites as pests of crops, forestry, rangeland and structures in Southern Africa and their control*. Sociobiology 40:47 – 67.

Sofowora, E. A. (1993). *Medicinal Plants and Traditional Medicinal in Africa.* Spectrum Books Limited, Ibadan, Nigeria. 238pp

Tokunda, G. Watanabe, H. (2007). Hidden celluloses in termites: revision of an old hypothesis *Biology letters.* 3(3): 336-339.

Trease G.E, and Evans W.C, (2002). Trease and Evans Pharmacognosy: *A Physician's Guide to Herbal Medicine*. 13th Edition, Bailliere Tindal London.

Verzar-Petri, G. Den. M and Meszaros, S. (1995). Formation of essential oil in clary sage under different conditions, In: *Proceedings of the 15th International Symposium on Essential oils.* Boston, 19-21.

Zheng, G. Q., Kenny, P. M., and Lam, K. T. (1992). Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarnogenic agents, *Journal of Natural products* 55; 999-1