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Full Length Research Paper

Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma

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Essential oil was obtained from the dried leaves of *Euphorbia hirta,* commonly called asthma plant. Analysis of the oil was carried out on a combined gas chromatograph-mass spectrometer fitted with an HP-5 MS (5% phenylmethyl siloxane) column at a temperature programme of 120°C (5 min) increased at 5°C/min to 320°C and held for 5 min. The major components identified include 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 6,10,14-trimethyl-2-pentadecanone, hexadecanal, phytol and n-hexadecanoic acid adding up to 61.01%. Minor constituents include 2-butoxyethanol, tetradecane, phthalic acid, butyl tetradecyl ester, oleic acid, 13-heptadecyn-1-ol, 2-methyl-1-hexadecanol and 1,2-benzene dicarboxylic acid diisooctylester. The possible roles of the components in the treatment of asthma and other diseases are discussed. The oil may function as a repellent against *Anopheles* species and thus useful for malaria control.

Key words: *Euphorbia hirta*, asthma, arthritis, essential oil, medicinal properties, insect repellent, GC-MS.

INTRODUCTION

Herbal medicine is currently in the lime light and is given more popularity than ever before as sales figures in some countries, for example the USA, have risen beyond the expectations of some producers. The reasons for this change are complex but clearly are connected with what could be described as the 'greening of medicine' (Ernst, 2000). Plants and their extracts continue to provide effective treatment for diseases of all kinds including asthma. Asthma is a chronic inflammatory disorder of the airways. Airway inflammation causes various symptoms of asthma which are often associated with widespread airflow obstruction and also cause an associated increase in airway responsiveness to a variety of stimuli (Sugita et al., 2003). Asthma causes an attack accompanied by wheezing, shortness of breath, chest tightness and coughing. Obstruction is often reversible either spontaneously or after treatment with a variety of drugs. Asthma medications are mostly taken with an inhaler which allows the medicine to reach the lungs effectively. The cornerstone of modern asthma therapy is the regular use of inhaled corticosteroids (ICS) (Angus, 2002). Many plants have been alleged to have curative properties for asthma. Among these plants is *Euphorbia hirta* (Sonibare and Gbile, 2008). It is well known and used by the major tribes in Nigeria. In Nigeria the name in Yoruba is Emi-ile. The plant *E. hirta* is commonly called asthma plant because of its alleged efficacy in East and West Africa in the treatment of asthma and various respiratory ailments (Odugbemi, 2008; Brown, 1995; Oliver, 1959). The plant is used as a diuretic, febrifuge, galactogogue, purgative and vermifuge. It is reported as medication for intestinal amoebic dysentery (Ogbulie et al., 2007; Stuart, 1979). Traditionally, the plant is squeezed in water and the extract taken orally as a remedy for asthma. The phytochemicals in *E. hirta* include volatile oil, alkaloids, tannins, saponins and steroids (Hashemi et al., 2008). The analgesic, antipyretic and anti-inflammatory properties of the plant have been reported (Lanhers et al., 1991). The ethanolic extracts of the plant have been found to demonstrate antibacterial activity and toxicological potential (Ogbulie et al., 2007). *In vitro* antifungal and

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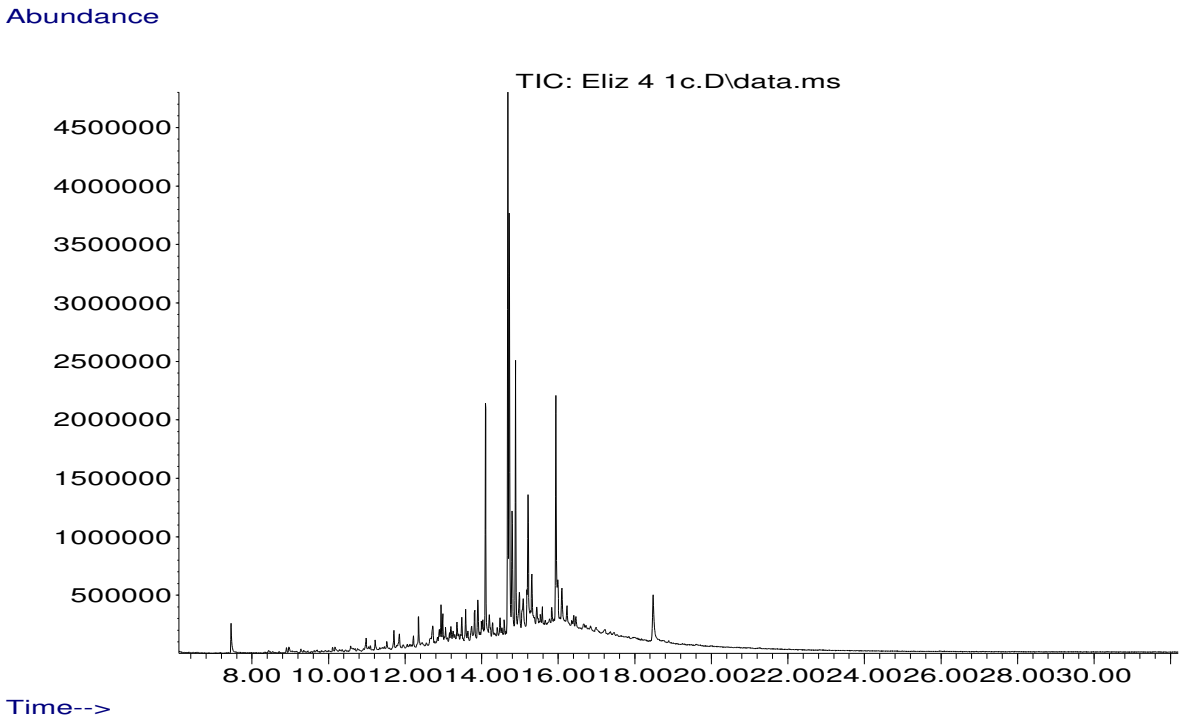


Figure 1. Gas chromatogram of the essential oil from *Euphorbia hirta*.

antibacterial properties as well as ulcer-protective effect and hepatoprotective activity of the plant extract in rats have also been demonstrated (Somchit et al., 2001; Rao et al., 2003; Edwin et al., 2007).

The purpose of this study is to analyze the essential oil of *E. hirta* by GC-MS and identify any of the components that may be useful for the management of asthma.

MATERIALS AND METHODS

Collection of samples

Several batches of fresh leaves of *E. hirta* were purchased from Mushin Market in Lagos in July 2007. Identification was carried out by Mr. T.K. Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a sample with voucher number FHI 107760 was deposited in the Herbarium of the Department of Botany.

Hydro-distillation of samples

In a typical experiment, 2.4 kg of the fresh leaves were cut into small pieces and air-dried in a dust-free environment giving 620 g which was blended to obtain the powder. Batches of 100 g of the powder were each added to 3 l water and the essential oil obtained by hydro-distillation was collected into hexane. The hexane solution of the essential oil was concentrated by evaporation at room temperature.

GC-MS analysis of oil

Analysis of the oil was carried out on a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (Agilent Technologies Ltd.) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m x 250 µm x 0.25 µm. Helium was used as carrier gas. The column temperature was initially 120°C (5 min) which was thereafter increased at 5°C/min to 320°C and held for 5 min. For MS, electron impact ionization was achieved with ionization energy of 70 eV. The essential oil was diluted with hexane and 2 µl of the diluted sample was injected automatically in splitless mode. Identification of the constituent compounds was by the Chem-Office software along with the MS library.

RESULTS AND DISCUSSION

The gas chromatogram of the essential oil from the leaves of *E. hirta* is shown in Figure 1 while the thirteen constituent compounds with abundance of 1% of total and above and their corresponding mass spectra are reported in Table 1. The retention times (R_T) are reported in minutes. The five major compounds identified in the essential oil are 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer (R_T 14.690, 14.800 and 14.881, 26.46%) which are methylated unsaturated long chain fatty acid alcohols, 6,10,14-trimethyl-2-pentadecanone, a methylated long chain fatty acid ketone, (R_T 14.727, 12.37%), phytol which is an isomer of 3,7,11,15-tetramethyl-2-hexadecene-1-ol (R_T 15.942, 8.29%), hexadecanal a long chain fatty acid aldehyde, (R_T 14.104, 7.63%) and n-hexadecanoic acid, a fatty acid, (R_T 15.217, 6.26%). These major compounds add up to 61.01% of total constituents. The remaining eight compounds are two aromatic dicarboxylic acid esters namely phthalic acid

Table 1. Results of the analysis by GC-MS.

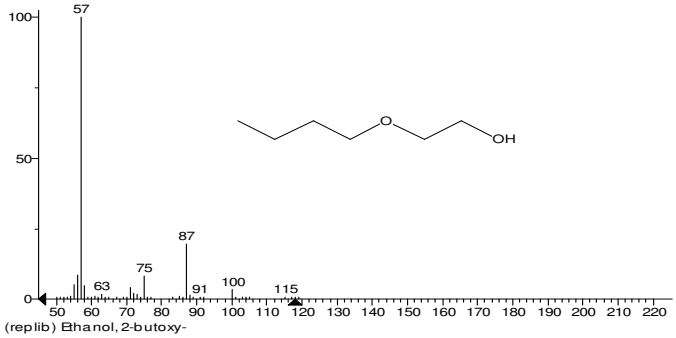
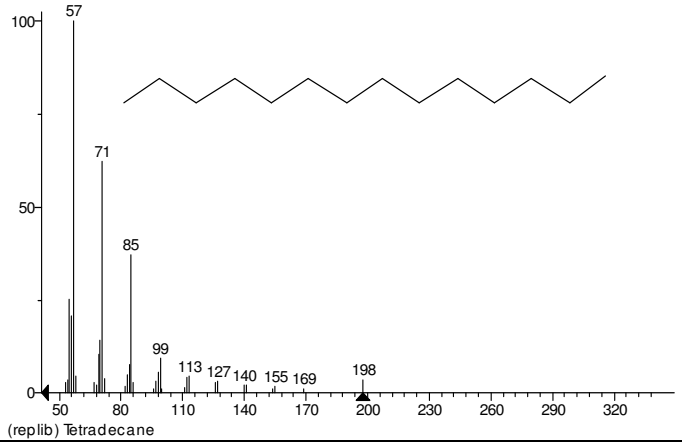
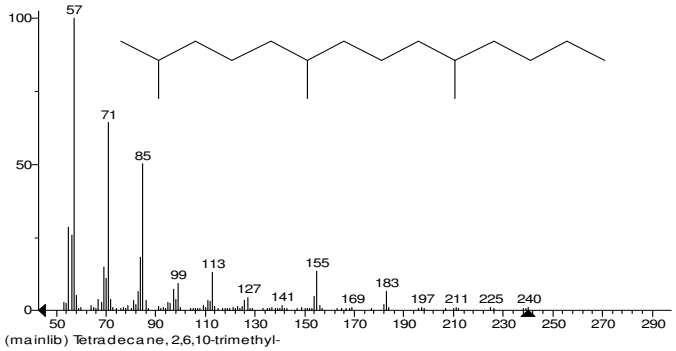
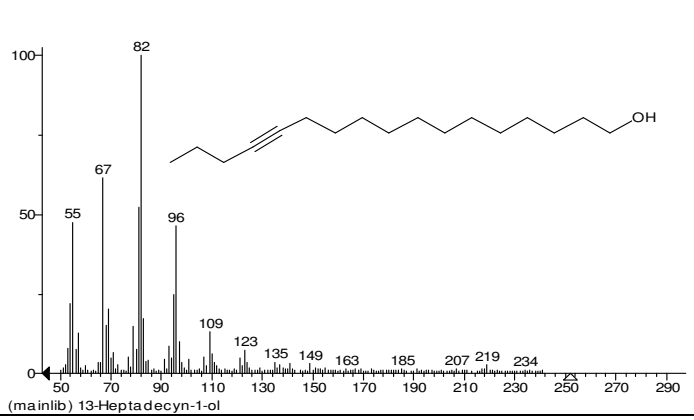
S/N	Retention time (min)	Percent of total	Compound	Structure
1.	7.456	1.13	2- butoxy ethanol	
2.	12.355	1.01	Tetradecane	
3.	12.940	1.24	2,6, 10 – Trimethyl tetradecane,	
4.	13.585	1.18	13-Heptadecyn-1-ol	

Table 1. contd.

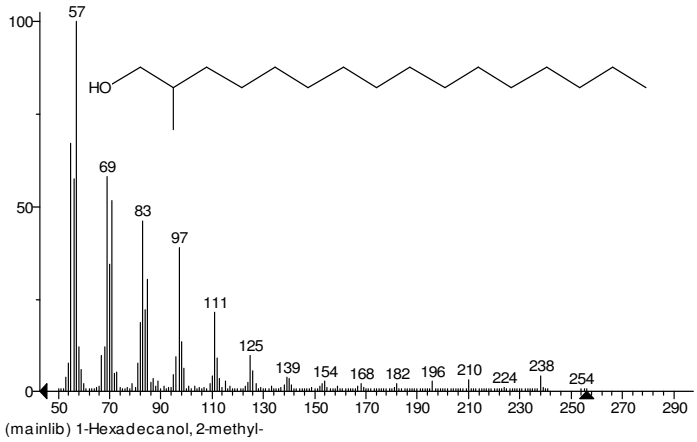
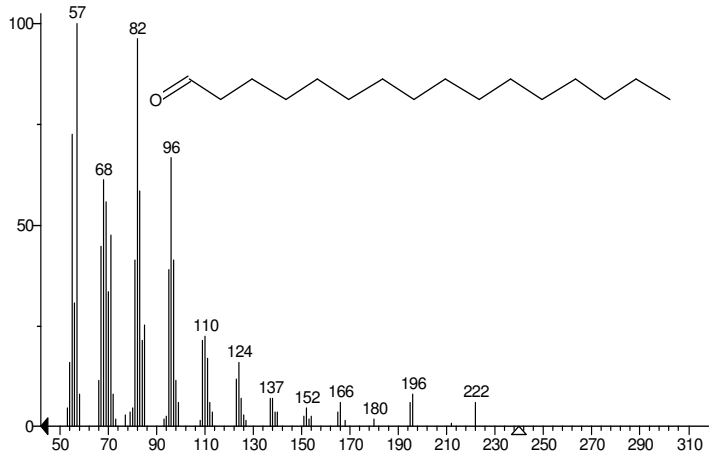
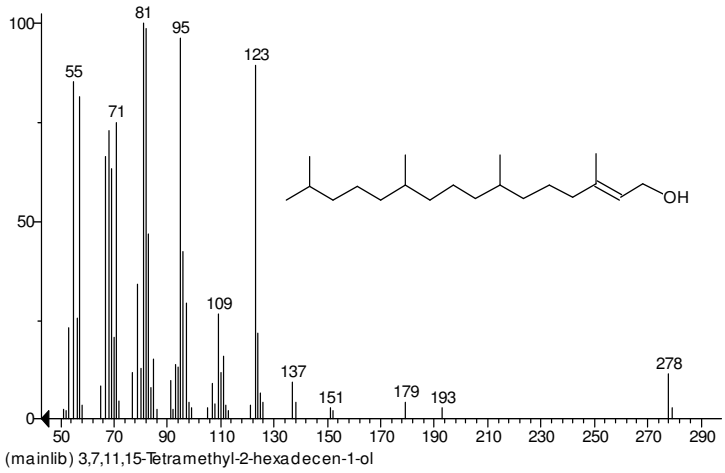
5.	13.907	1.40	2-Methyl hexadecanol,	
6.	14.104	7.63	Hexadecanal	
7.	14.690	13.31	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	
8.	14.800	4.74	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Same as 7
9.	14.881	8.41	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Same as 7

Table 1. contd.

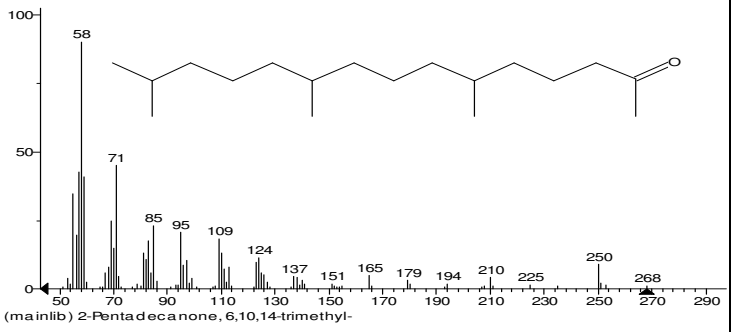
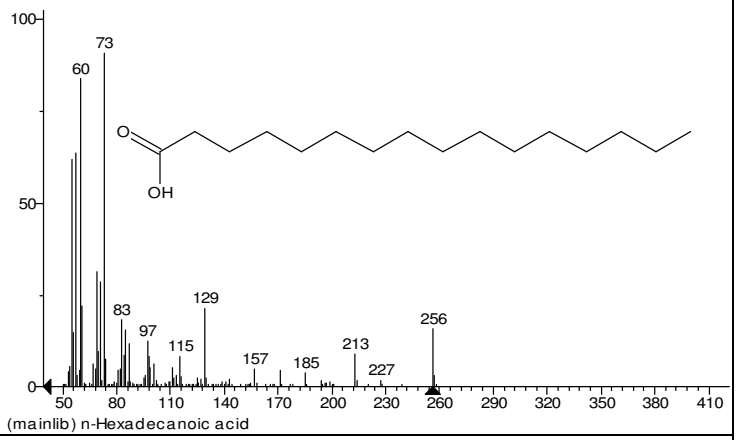
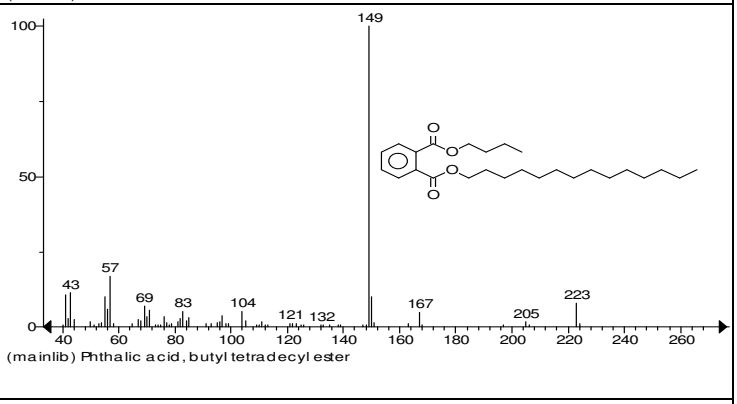
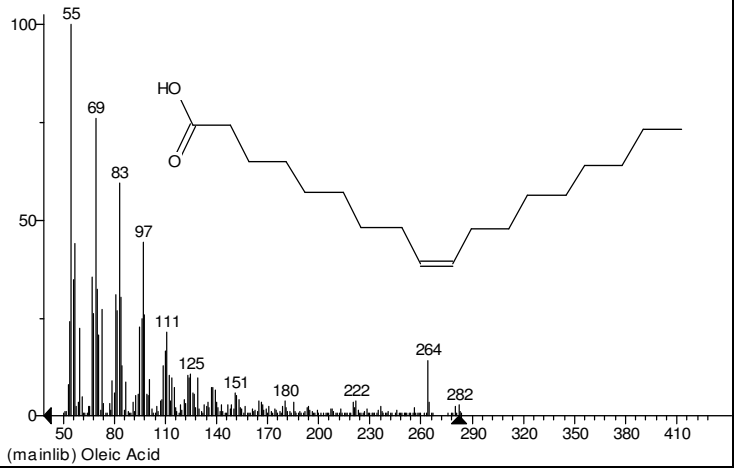
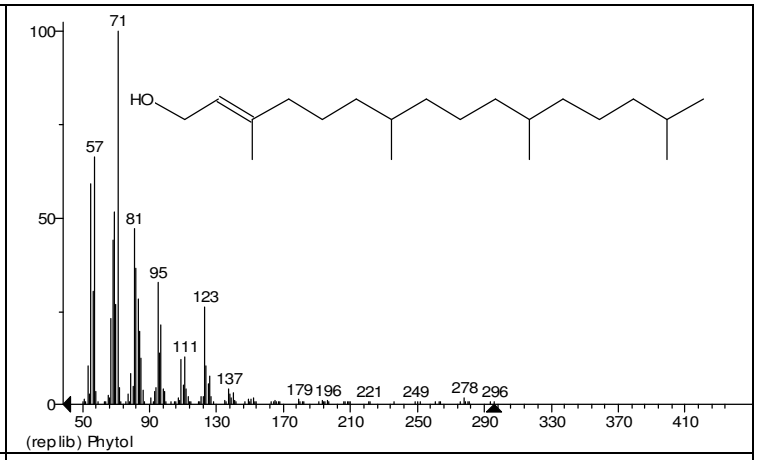
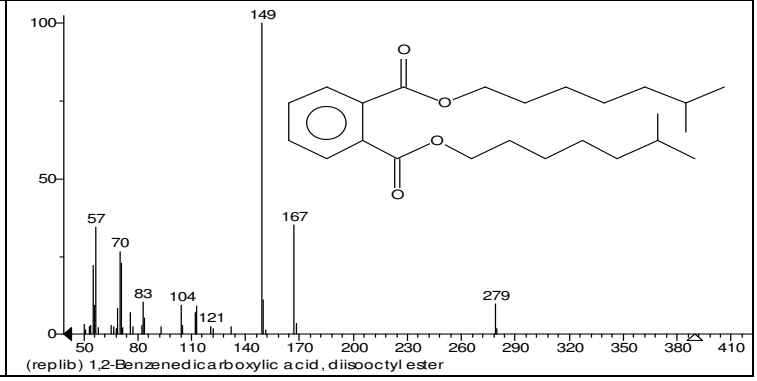
10.	14.727	12.37	6, 10, 14-Trimethyl 2-pentadecanone	
11.	15.217	6.26	Hexadecanoic acid	
12.	15.313	3.88	Phthalic acid, butyl tetradecyl ester	
13.	15.444	1.42	Oleic acid	

Table 1. contd.

14.	15.942	8.29	Phytol	
15.	18.483	2.90	1,2-Benzene dicarboxylic acid diiso octyl ester	

butyltetradecyl ester (R_T 15.313, 3.88%), 1,2-benzene dicarboxylic acid diisooctyl ester (R_T 18.483; 2.90%), oleic acid, an unsaturated fatty acid (R_T 15.444, 1.42%), 2-methyl hexadecanol, (R_T 13.907, 1.40%), 2,6,10-trimethyl tetradecane, a methylated long chain alkane, (R_T 12.940, 1.24%), 13-heptadecyn-1-ol a long chain fatty acid alcohol, (R_T 13.585,1.18%), 2-butoxy ethanol (R_T 7.456, 1.13%) and tetradecane, a long chain alkane, (R_T 12.355, 1.01%) all adding up to 14.16% of total constituents.

It is observed that 3,7,11,15-tetramethyl-1-hexadecene-1-ol was eluted at retention times 14.690, 14.800 and 14.881 min suggesting isomeric forms and large abundance. These compounds are synonymous with a mixture of cis and trans phytol which are terpenoids. Thus phytol and its isomeric forms add up to 34.8%. Phytol is a reactive oxygen species-promoting substance. In an extensive study, experiments showed that treatment of rats with phytol increased oxidative burst *in vivo* and thereby corrected the effect of the genetic polymorphism in arthritis-prone $Ncf1^{DA}$ rats (Hulqvist et al., 2006).

In the same study phytol treatment was also found to decrease the auto immune response and to ameliorate both the acute and chronic phases of arthritis. The results showed that a single injection of phytol mediated a surprisingly long suppression of arthritis. An increase in oxidative burst capacity was evident the day after phytol injection and persisted for several weeks. It was found that subcutaneous administration of phytol resulted in best preventive effect compared to intraperitoneally or intranasally (Hulqvist et al., 2006).

However, the users of *E. hirta* as a phtomedication for the management of asthma adopt oral administration of the aqueous extract.

In the study by Hulqvist et al. (2006), the increase in oxidative burst after phytol injection was found not to be restricted to one anatomical compartment and was observed in the blood, bone marrow, spleen and lymph node. It was further stated that thymus could also be affected by the treatment. Other effects of phytol documented in the study are as follows: the increase in oxidative burst alters cell distribution, it suppresses collagen-induced arthritis and collagen type II autoimmunity, it inhibits arthritis induced without adjuvant oil such as pristine, it decreases arthritis severity in established disease, the phytol-induced suppression of arthritis is reversible (for example with histamine dihydrochloride) and its efficiency is comparable to standard treatments (Hulqvist et al., 2006). For example, the prevention mediated by phytol was found to be more pronounced than that of etanercept and while no significant reduction of disease severity was seen after a single etanercept administration at a certain stage of the

disease, phytol significantly lowered the disease scoring. In comparison with methotrexale, phytol was found to be valid as a potential therapeutic agent. Thus phytol showed comparable or better therapeutic effects in comparison with two of the major drugs currently used for rheumatoid arthritis (Hulqvist et al., 2006). Phytol was also found to be effective at different stages of the arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species-promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases.

The results in our study show that phytol and its isomer 3,7,11,15-tetramethyl-2-hexadecen-1-ol present in *E. hirta* possibly contribute to the therapeutic effect of the plant in asthma patients.

In the study by Hulqvist et al, 2006, it was also reported that certain oils with alkane structure such as phytol and pristane (2,6,10,14-tetramethyl pentadecane) had an oxidative burst-inducing capacity *in vitro*. However, despite structural similarity, pristane induced arthritis whereas phytol protected against arthritis (Olofsson et al., 2003). In an investigation of the structure-function relationship using short saturated alkanes, those with 15 carbons or more such as pristane, induced arthritis where as shorter alkanes did not. C11 as well as C12 and C13 shown to increase oxidative burst activity *in vitro* were potent in reducing arthritis severity when administered 5 days before arthritis induction. However, C16 did not increase oxidative burst and did not protect against arthritis. However, the report is silent on C14 alkane. The 2, 6, 10-trimethyl tetradecane present in the essential oil of *E. hirta* may offer relief against asthma and other inflammatory diseases such as rheumatoid arthritis.

It has been reported that a combination of an antihistaminic compound with a terpenoid gave a pharmaceutical composition which when orally administered to human was found useful in the prevention and treatment of allergic rhinitis (hay fever), mild asthma and urticaria (Tschollar et al., 1998). It is pertinent to note that *E. hirta* is used for the management of hay fever in herbal medicine. Thus, if there is an antihistamine in the plant or human subject, then in the presence of phytol, the combination could relieve asthma. Phytol is produced from the hydrolysis of chlorophyll and it forms part of the molecules of vitamin E and vitamin K (Finar, 2001). Vitamin E administered orally has been found to suppress the increase in airway reactivity in guinea pigs sensitized to ovalbumin and was found to have membrane-stabilizing action (Deepika et al., 2005). In a study involving administration of vitamin K_2, minaquinone, to some patients, it was found to have clinical effects which resulted in significant relief in bronchial asthma (Kimur et al., 1975; Kimura et al., 1970). Thus phytol could produce some of the effects of vitamins E and K resulting in the relief of asthma.

A compound present in appreciable concentration is 6,10,14-trimethyl-2-pentadecanone, a C15 aliphatic methyl ketone. Some studies had established that long chain aliphatic methyl ketones showed repellence to arthropods, including blood-sucking insects (Ndungu et al., 1995; Blum et al., 1966; Torr et al., 1996; Barton, 2003; Roe, 2004; Gikonyo et al., 2002). In a study on the efficacy of aliphatic methyl ketones against *Anopheles gambiae*, a malaria vector, it was found that C7-C10 compounds had lower activity than those of C11-C15 indicating the significance of chain length on activity. It was also found that in the C11-C15 compounds, odd-carbon compounds including 2-pentadecanone were more effective than even-carbon compounds especially at high concentrations. The C15 compound compared favourably with N,N-diethyl-m-toluamide (DEET) a repellent to various insects including mosquitoes at 10% concentration (w/v) (Innocent, et al., 2008). Thus the essential oil from *E. hirta* could be effective repellent against *Anopheles* species because of the presence of 6,10,14 -trimethyl-2-pentadecanone and would therefore be effective in malaria control.

Many fatty acids are known to have antibacterial and antifungal properties (Russel, 1991). Hexadecanoic acid and oleic acids are known to have potential antibacterial and antifungal activity (McGraw et al, 2002; Seidel and Taylor, 2004; Dilika et al., 2000). Oleic acid in concentrations as low as 0.7% (v/v) has been found to be fungistatic against a wide spectrum of moulds and yeasts (Sheba et al., 1999). Free fatty acids including long chain C16-C20 unsaturated fatty acids were suggested to be responsible for the anti-inflammatory activity in the extract from *Tinospora smilacina* Berth (Li et al., 2004). Thus oleic acid and hexadecanoic acid can be of therapeutic value when infections are present in asthma patients. The anti-inflammatory activity of oleic acid is relevant in the management of asthma. The antibacterial, antifungal and anti-inflammatory activity identified is relevant in the use of the plant for use as anthelmintic and for the management of conjunctivitis and dysentery.

Unsaturated monoglyceride alcohols of chain length of 16 or 18 carbons were found to be extremely potent inactivators of some envelope viruses (Sands et al., 1979). Thus, the constituent 13-heptadecyn-1-ol, a long chain fatty acid alcohol in the essential oil, can be expected to exhibit viricidal activity.

1,2-Benzene dicarboxylic acid diisooctyl ester also known as diisooctyl phthalate (DIOP) constitutes 3% of the oil. In a report on Human Health Hazard Assessment on DIOP, the bulk of labeled phthalates ingested by humans were eliminated in urine within the first 24 hours and there was no significant tissue accumulation of DIOP (NICNAS, 2008). This constituent may therefore not be injurious to health.

Butyl, tetradecyl phthalate ester is present in the oil. Phthalic acid esters (PAE) are plasticizers and di-n-butyl phthalates are used as insect repellents (Farm Chemicals,

1971). Thus the butyl tetradecyl phthalate ester in the essential oil may enhance its insect repellent activity in addition to the 6,10,14-trimethyl-2-pentade-canone present in the oil. The toxic effect of phthalate esters is of interest. The toxicity of di-n-butyl phthalate to fish has been found to be relatively low (Mayer and Sanders, 1973). However, PAE were found to accumulate in invertebrates to a similar degree as found with the same species of invertebrates exposed to organochlorine insecticides (Johnson et al., 1971). The phthalate was rapidly excreted. The percentage of butyl tetradecyl phthalate in the essential oil is low at about 4%. The PAE are not likely to have been synthesized in the plant but may be a contaminant from packaging materials.

2-Butoxy ethanol is synthetic glycol ether and is probably not synthesized by the plant but a contaminant. It is present in very low concentration, 1%, in the essential oil. Although it is readily absorbed following inhalation, oral and dermal exposure, it is metabolized by the body. The principal effect exerted by 2-butoxy ethanol and its metabolite is haematotoxicity and while rat is the most sensitive species, human red blood cells are not as sensitive (WHO, 1998).

The constituents, 1,2-benzene dicarboxylic acid diisooctyl ester, butyl tetradecyl phthalate esters and 2-butoxy ethanol are undesirable constituents of the essential oil and they should be removed from it to make it safe for use as a herbal medication.

Conclusion

Phytol and its isomer, 3,7,11,15-tetramethyl-2-hexadecene-1-ol as well as 2,6,10-trimethyl tetradecane present in the essential oil from the leaves of *E. hirta* have been shown by Hulqvist et al. (2006) to be relevant in the management of inflammatory conditions; hence this may be the compound that give relief to patients suffering from asthma which is a chronic inflammatory disorder of the airways. Oleic acid present in the oil also has anti-inflammatory activity (Li et al., 2004) which could also produce therapeutic effects in asthma patients. Hexadecanoic acid and oleic acid present in the essential oil have potential anti bacterial and anti fungal activity (McGraw et al., 2002; Seidel and Taylor, 2004; Dilika et al., 2000; Sheba et al., 1999). 13-Heptadecyn-1-ol, a long chain fatty acid alcohol in the essential oil is a potential antiviral agent (Sands et al., 1979). The oil has the potential to function as a repellent against Anopheles mosquito species because of the presence of 6,10,14-trimethyl-2-pentadecanone in appreciable quantity and thus it is useful for malaria control.

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REFERENCES

Angus RM (2002). Inhaled Corticosteroids (Budesonide): the Cornerstone of Asthma Therapy-What are the options? Pulmonary Pharmacol. Therapeutics, 15(6): 479-484.

Barton VM (2003). Trypsin modulating oostatic factor (TMOF) and non-peptidic analogs as novel insecticides and arthropod repellents. M.Sc. Thesis, North Carolina State University, Canada.

Blum MS, Warter SL, Traynham JG (1966). Chemical releaser of social behaviour-IV. The relation of structure of activity of ketones as releaser of alarm of *Iridomyrmex pruinosus* (Roger). J. Insect Physiol. 12: 419-427.

Brown D (1995). Encyclopaedia of herbs and their uses. Dorling Kindersley Limited, London.

Deepika J, Chhabra SK, Raj HG (2005). Effects of Vitamin E on airway responses and biochemical parameters in guinea pigs sensitized to ovalbumin. Respiratory Physiol. Neurobiol. 146(2-3): 231-238.

Dilika F, Bremner PD, Meyer JJ (2000). Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum:* a plant used during circumcision rites. Fitoterapia 71(4): 450-452.

Edwin JE, Dubey S, Gupta S, Gupta VB (2007). Hepatoprotective activity of *Euphorbia hirta*. Linn. Geobios. 34(1): 81-83.

Ernst E (2000). Herbal Medicine; A concise overview for professionals. Reed Professional Publishing Ltd, London Sciences, Preface 1.

Farm Chemicals (1971). Dictonary of pesticides. Meister Publishing Co., Willoughby, Ohio.

Finar IL (2001). Organic Chemistry, Vol. 2, 5th edition, Pearson Education, Singapore, p. 440.

Gikonyo NK, Hassanali A, Njagi PGN, Gitu PM, Midiwo JO (2002). Odour composition of preferred (Buffalo and Ox) and Non-preferred (Waterbuck) hosts of some Savanna tsetse flies. J. Chem. Ecol. 28: 961-973.

Hashemi SR, Zulkifli I, Hair Belo M, Farida A, Somchit M (2008). Acute Toxicity Study and Phytochemical Screening of selected Herbal Aqueous extracts in broiler Chickens. Int. J. Pharmacol. 4(5): 352-360.

Hulqvist M, Olofsson P, Gelderman KA, Homberg J, Holmdahl R (2006). A new arthritis therapy with oxidative burst inducers. Plos Med. 3(9): 1-17.

Innocent E, Gikonyo NK, Nkunya MHH (2008). Repellency property of long chain aliphatic methyl ketones against *Anopheles gambiae* s.s. Tanzania J. Health Res. 10(1): 50-54.

Johnson BT, Saunders CR, Saunders HO, Campbell RS (1971). Biological magnification and degradation of DDT and aldrin by fresh water invertebrates. J. Fish Res. Board. Can. 28: 705-709.

Li RW, Leach DN, Myers P, Leach GJ, Lin GD, Brushett DJ, Waterman PG (2004). Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina* Benth. Phytother. Res. 18: 78-83.

Kimur I, Tanizaki Y, Sato S, Saito K, Takahashi K (1975). Menaquinone (vitamin K2) Therapy for bronchial asthma..II Clinical effect of menaquinone on bronchial asthma. Acta. Med. Okayama. 29(2): 127-135.

Kimura I, Moritani Y, Tanizaki Y (1970). Menaquinone (Vitamin K2) therapy in bronchial asthma 1. Anti-allergic action. Arerugi, 19(11): 805-809.

Lanhers MC, Fleurentin J, Dorfman P, Mortier F, Pelt JM (1991). Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta*. Planta Medica, 57(3): 225-231.

Mayer Jr FL, Sanders HO (1973). Toxicology of phthalic acid esters in aquatic organisms. Environ. Health Perspect. 3: 153-157.

McGraw LJ, Jager AK, Van Staden J (2002). Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia, 73: 431-433.

Ndungu M, Lwande W, Hassaali A, Moreka L, Chhabra SC (1995). *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus* and maize weevil (*Sitophylus zeamais*) repellant. Entomol. Exp. Application, 76: 217-222.

NICNAS (2008). Existing Chemical Hazard Assessment Report;

Diisooctyyl Phthalate, Department of Health and Ageing, Sydney NSW 2001, Australia. www.nicnas.gov.au
Odugbemi T (2008). A Textbook of Medicinal Plants from Nigeria. University of Lagos Press, Lagos, p. 571.
Ogbulie JN, Ogueke CC, Okoli IC, Anyanwu BN (2007). Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta*. Afr. J. Biotechnol. 6(13): 1544-1548.
Oliver B (1959). Medicinal Plants in Nigeria. Nigerian College of Arts, Science and Technology, Ibadan, p. 138.
Olofsson P, Holmberg J, Tordsson J, Lu S, Akerstrom B (2003). Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nat. Genet. 33: 25-32.
Rao CV, Rao GMM, Kartik R, Sudhakar M, Mehrotra S, Goel RK (2003). Ulcer protective effect of *Euphorbia hirta.* J. Trop. Med. Plants, 4(2): 199-205.
Roe RM (2004). Method of repelling insects. United States Patent Application 20040242703, Kind Code A1.
Russel AD (1991). Mechanisms of bacterial resistance of non-antibiotics: food additives and food pharmaceutical preservatives. J. Appl. Bacteriol. 71: 191-201.
Sands J, Auperin D, Snipes W (1979). Extreme sensitivity of enveloped viruses, including Herpes Simplex, to long-chain unsaturated monoglycerides and alcohols. Antimicrob. Agents Chemoter. 15: 67-73.
Sheba DW, Saxena RK, Gupta R (1999). The fungistatic action of oleic acid. Curr. Sci. 76(8): 1137-1139.
Seidel V, Taylor PW (2004). *In-vitro* activity of extracts and constituents of Pelagonium against rapidly growing mycobacteria. Int. J. Antimicrob. Agent. 23: 613-619.
Somchit MN, Mutalib AR, Hasmawie MR, Murni A (2001). *In vitro* antifungal and antibacterial properties of *Euphorbia hirta.* J. Trop. Med. Plants, 2(2): 179-182.
Sonibare MA, Gbile ZO (2008). Ethnobotanical survey of anti-asthmatic plants in South-Western Nigeria. Afr. J. Trad. Complement. Altern. Med. 5(4): 340-345.
Sugita M, Kuribayashi K, Nakagomi T, Miyata S, Matsuyama T, Kitada O (2003). Allergic Bronchial Asthma; Airway Inflammation and Hyperresponsiveness. Inter. Med. 42(8): 636-643.
Stuart M (1979). The Encyclopaedia of Herbs and Herbalism. Orbis Publishing, London.
Torr SJ, Mangwiro TNC, Hall DR (1996). Response of *Glossinia pallidipes* (Diptera: Glossinidae) to synthetic repellents in the Field. Bull. Entomol. Res. 86: 609-616.
Tschollar W, Schmid B, Jurgens UR (1998). Oral Pharmaceutical Combinations of Antihistaminic Compounds and Terpenoids. World Intellectual Property Organization. Pub. No. WO/1998/001134.
WHO (1998). 2-Butoxyethanol. Concise International Chemical International Chemical Assessment Document 10: QD 341.E7. World Health Organization, Geneva.