



Research Journal of
Phytochemistry

ISSN 1819-3471



Academic
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Analysis of Essential Oil Constituents in Hydro-Distillates of *Calotropis procera* (Ait.) R.Br

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Abstract: The essential oil from the dried leaves of *Calotropis procera* was analyzed by GC-MS. The three major components in the oil are phytol and its isomers 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol (37.59%) and 6,10,14-trimethyl-2-pentadecanone (15.31%). The essential oil was collected in two modes: one mode is a continuous distillation for 4 h and another mode involves hourly collection of fractions over a period of 4 h, thus providing fractionated samples. This novel procedure makes it possible to identify other components which might not have been detected in the unfractionated sample. Such other components include tetradecanal, isophytol and 1-docosanol. The usefulness of phytol in the management of inflammatory diseases suggests that the plant may be useful in the management of arthritis. 6, 10, 14-trimethyl-2-pentadecanone, a mosquito repellent may be useful for malaria control.

Key words: *Calotropis procera*, asthma, essential oil, arthritis, malaria control
GC-MS

INTRODUCTION

Asthma is a medical terminology used as a blanket term to cover conditions characterized by episodes of breathlessness caused by intermittent narrowing of the bronchial tubes, or airways within the lungs. Asthma is accompanied by features in which the airways within the lungs are inflamed and become very sensitive to specific factors such as triggers which cause the airways to become narrow, reducing airflow through them and making the patient breathless and sometimes wheezy (Ayres, 2008). The factors that contribute to the development of asthma include genetic factors such as inheritance as well as mother smoking during pregnancy (Bracken *et al.*, 2002). Passive smoking in childhood (Miyuki and Kenichi, 2005), allergens such as house dust mite, infections and occupational exposures such as chemicals can also be causative agents for asthma (Becher *et al.*, 1996). The obstruction of the airways caused by asthma is often reversible either spontaneously or with treatment with a variety of drugs.

Asthma medications are often administered with an inhaler which allows the drug to penetrate right into the lungs. The medicines are usually of two types, namely, the long-term control drugs and the quick-relieve drugs. The mainstay of persistent asthma management is the regular use of inhaled corticosteroids (ICS) (Chipps, 2009). The ICS have both local and systemic side effects which are normally accompanied with undesirable consequences. The

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ICS are normally the preferred treatment for young children but may reduce the growth rate of children in all ages (Daley-Yates and Richards, 2004). In elderly adults, long-term use of high-dose ICS therapy has the potential to cause decreased bone mineral density, skin thinning and bruising as well as cataracts (Dahl, 2006). Some of the other side effects of these drugs include restlessness, difficulty in concentrating, irritability, nausea, poor appetite, stomach ache and even seizures.

Many plants including, *Calotropis procera*, known as giant milk weed or Sodom apple, have been alleged to be useful in the management of asthma. *Calotropis procera* belongs to the family of Asclepiadaceae and in Nigeria it is used by several tribes and the names in the various languages are well documented (Odugbemi, 2008). The roots, leaves, bark and latex are used as medications for the treatment of diarrhoea, dysentery, elephantiasis, leprosy, chronic eczema, ringworm, inflammation, cough, asthma and convulsion. The plant parts are also used as emetic, diaphoretic, antipyretic and abortifacient agents. It is one of the plants listed as being used for treating eye infections in Nigeria. The leaves, when crushed and rubbed on the breasts of nursing mothers, increases milk production (CSIR, 1992; Odugbemi, 2008; Ogunlesi *et al.*, 2008; Zailani and Ahmed, 2008; Aliero *et al.*, 2001). The latex is processed to give a medication acclaimed to be effective in treating vertigo, baldness, toothache, intermittent fevers, rheumatism as well as paralysis. The latex has been reported to produce potent anti-inflammatory, analgesic and weak antipyretic effects in various animal models (Kumar and Basu, 1994; Sangraula *et al.*, 2002; Dewan *et al.*, 2000a, b). However, the neat latex can cause blindness (Zailani and Ahmed, 2008). Various tribes of Central India use the root, bark and leaves as a curative agent for jaundice (Samvatsar and Diwanji, 2000). Different parts of the plant have been reported to exhibit antimicrobial, antioxidant and cytostatic properties (Kumar and Arya, 2006). Methanol extracts of the leaves are reported to exhibit strong antioxidant activity (Yesmin *et al.*, 2008).

The significant antifungal activities of the extracts of *C. procera* confirmed its pharmacological and therapeutic potentials (Hassan *et al.*, 2006). The n-butanol extract of the flowers has been reported to have antibacterial activity (Larhsini *et al.*, 2001). Phytochemical analysis conducted on the plant parts revealed the presence of saponins, glycosides and simple sugars in the leaves while the root bark was found to contain tannins in addition to those listed (Tahir and Chi, 2002). The purpose of this study is to identify the constituents in the essential oil of *C. procera* and find out if any of them is relevant to the alleged efficacy of the plant in treating asthma.

MATERIALS AND METHODS

Collection of Samples

Several batches of fresh leaves of *C. procera* were purchased from Mushin market, Lagos, between July and August 2007. The plants were identified by Mr. T. K. Odewo of the Department of Botany, Federal Research Institute of Nigeria (FRIN), Ibadan. A voucher assigned number FHI 107882 was deposited in the Herbarium in the Department of Botany at FRIN.

Hydro-Distillation of Samples and GC-MS Analysis of Essential Oil

The plants were cut into small pieces and air-dried in a dust-free environment for 21 days. In a typical experiment, 2.02 kg of fresh leaves gave 505 g dry weight (24.6% yield). The dried samples were powdered and the essential oil obtained by hydro-distillation of batches of 100 g of powdered leaves in 3 L of water. The vapour of the essential oil was

condensed and passed into hexane. In one mode, fractions of essential oil were collected hourly over a 4 h period. In another mode, a single collection was made over the 4 h period. The hexane in the extracts was evaporated at room temperature to concentrate the samples; however dilution with hexane was carried out prior to gas chromatographic analysis and 2 μ L of each extract was injected automatically into the gas chromatograph. The analysis was carried out on GC-MS model HP 6890 (Agilent Technologies Ltd.) fitted with HP-5 MS (5% phenylmethylsiloxane) capillary column 30.0 m \times 250 μ m \times 0.25 μ m, using helium as carrier gas. The column temperature was initially 120°C (6 min) and increased at 5°C min⁻¹ to 320°C (6 min). Mass spectra were recorded using ionization energy of 70 eV. The detector was 5973 inert MSD. Identification of the fragment ions was made possible by the Chem-Office software and the library of the MS.

RESULTS

The chromatograms of the fractions collected in the 1st, 2nd, 3rd and 4th h are shown in Fig. 1-4, respectively while that of the essential oil collected in continuous 4 h stretch is shown in Fig. 5.

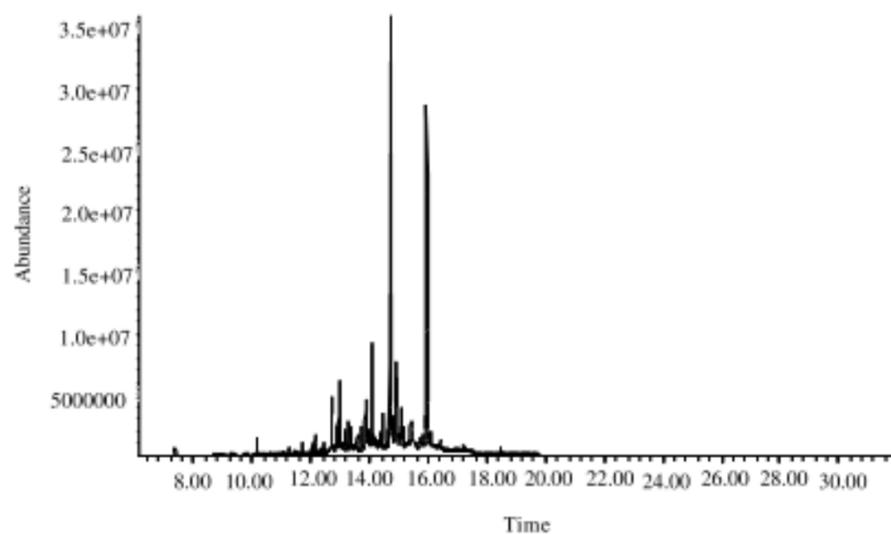


Fig. 1: Chromatogram of the oil collected during the 1st h

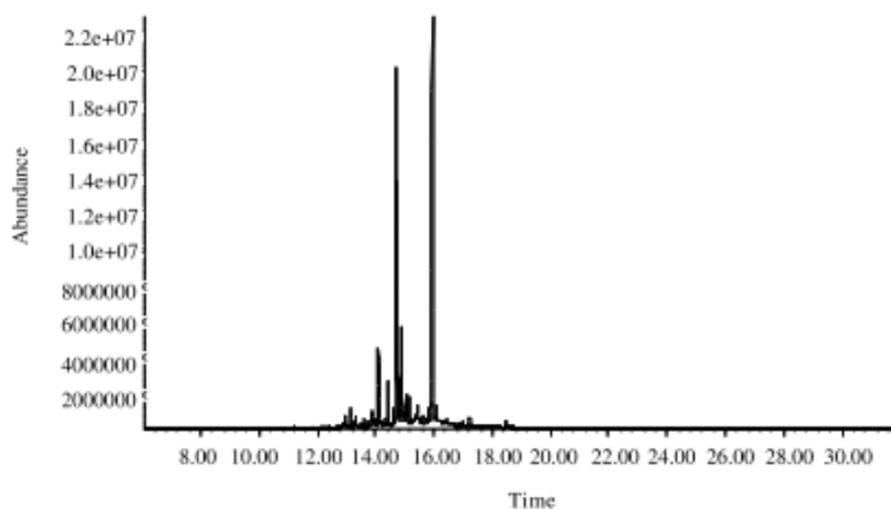


Fig. 2: Chromatogram of the oil collected during the 2nd h

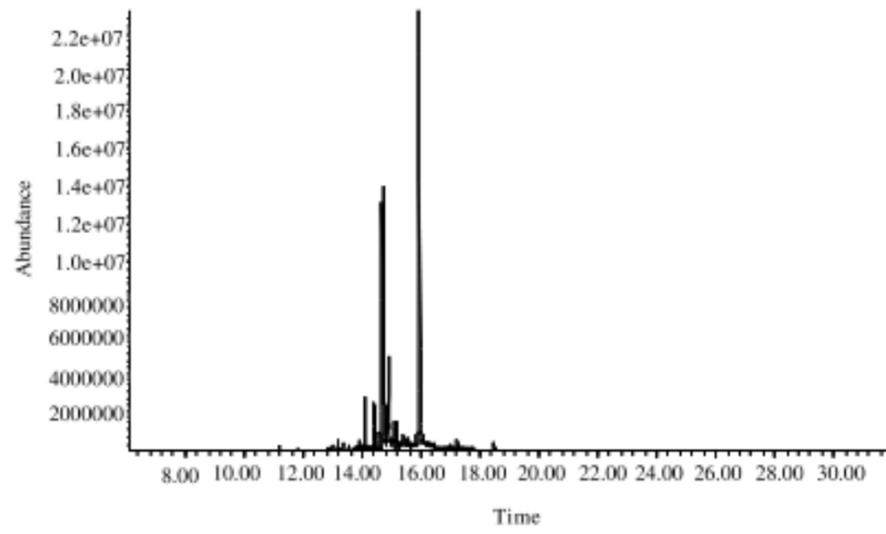


Fig. 3: Chromatogram of the oil collected during the 3rd h

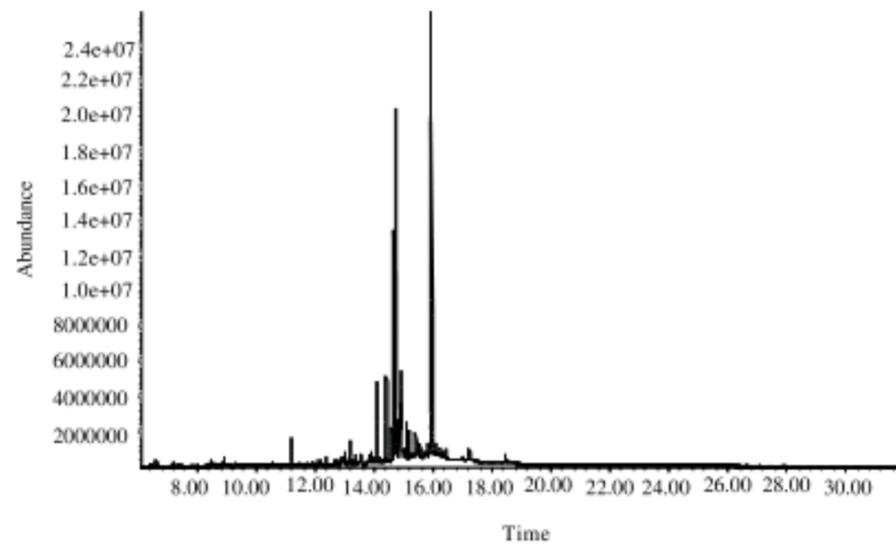


Fig. 4: Chromatogram of the oil collected during the 4th h

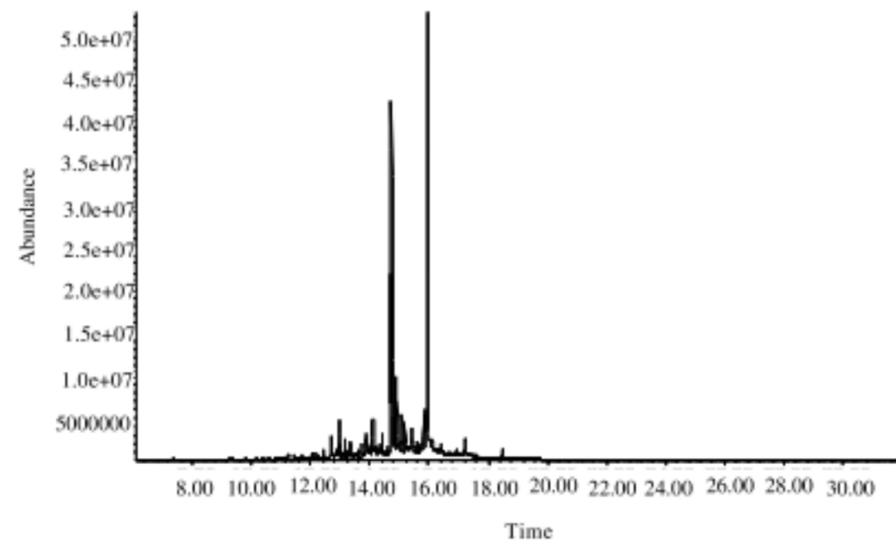


Fig. 5: Chromatogram of the essential oil-single collection over 4 h

The constituents of the essential oil fraction collected in the first hour include phytol (14.44%, 15.968 min), 6, 10, 14-trimethyl-2-pentadecanone (14.44%, 14.734 min), 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer (4.74%, 14.690 min and 4.62%, 14.800 min, respectively), 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (3.18%, 12.706 min), 3-buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (2.43%, 12.984 min), 9, 17-octadecadienal (2.84%, 13.885 min), hexadecanal (4.14%, 14.104 min), Z-5-nonadecene (3.25%, 14.917 min) and 2-methyl-1-hexadecanal (2.08%, 15.408 min).

The essential oil fraction collected in the second hour contains the constituents identified in the fraction collected in the first hour except 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl and 9-nonadecene instead of Z-5-nonadecene. However, in addition it contains 5, 9, 13-pentadecatriene-2-one, 6, 10, 14-trimethyl (E, E), isophytol and docosanol.

The constituents of the third hourly fraction are essentially those contained in the second hourly fraction with the exception of 9, 17-octadecadienal and 1-docosanol. However, in addition, tetradecanal and 4, 8, 12, 16-tetramethylheptadecan-4-olide were present.

The constituents in the fourth hourly fraction are almost the same as those in the third hourly fraction except 4, 8, 12, 16-tetramethylheptadecan-4-olide but with the addition of 1-docosanol.

The major constituents of the essential oil collected over the entire period of 4 h are the same as those in the hourly extraction and are phytol (25.19%, 15.952 min), 6, 10, 14-trimethyl-2-pentadecanone (15.31%, 14.734 min), 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer (6.80%, 14.690 min and 5.60%, 14.880 min, respectively). The minor constituents identified are 2-butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (1.57%, 12.706 min), 3-buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (1.48%, 12.984 min), 9, 12-octadecadienoyl chloride (1.53%, 13.885 min), hexadecanal (1.71%, 14.104 min), Z-5-nonadecene (2.27%, 14.917 min), 5, 9, 13-pentadecatriene-2-one-6, 10, 14-trimethyl (E, E) (1.88%, 15.09 min), 2-methyl-1-hexadecanol (1.95%, 15.408 min) and 4, 8, 12, 16-tetramethylheptadecan-4-olide (1.34%, 17.233 min). These results are presented in Table 1 for the major constituents and Table 2 for the minor constituents.

The major constituents identified in all the five samples including their retention times in minutes are presented in Table 1. Other components with a percentage of minimum of 1% total are presented in Table 2.

The major constituents are phytol, (2E, 7R, 11R)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, its isomer, 3,7,11,15-tetramethyl-2-hexadecen-1-ol and 6,10,14-trimethyl-2-pentadecanone. The data in Table 1 shows that the percentage of phytol in the extract increased from 14.44% in the 1st h to 22.41% in the 2nd h, to 30.97% in the 3rd h and decreased to 21.80% in the 4th h. The essential oil obtained in a single collection over a 4 h period contained

Table 1: The percentage composition of the major components in the essential oil from the dried leaves of *Calotropis procera*

Compound	1st	2nd	3rd	4th	Continuous collection over 4 h
I Phytol	14.44%	22.41%	30.97%	21.80%	25.19%
	R _T 15.968 min	15.942 min	15.942 min	15.942 min	15.952 min
II 6,10,14-trimethyl-2-pentadecanone	14.44%	16.63%	14.99%	13.83%	15.31%
	R _T 14.734 min	14.727 min	14.727 min	14.727 min	14.734 min
II 3,7,11,15-tetramethyl-2-hexadecene-1-ol	4.74%	10.28%	12.62%	8.46%	6.80%
	R _T 14.690 min	14.690 min	14.690 min	14.690 min	14.690 min
IV 3,7,11,15-tetramethyl-2-hexadecene-1-ol	4.62%	7.96%	10.59%	6.38%	5.60%
	R _T 14.800 min	14.800 min	14.800 min	14.800 min	14.880 min
Total percentages phytol and isomers of (i), (iii) and (iv)	23.80%	40.65%	54.18%	36.64%	37.59%

Table 2: The percentage composition of the minor constituents in the essential oil from the leaves of *Calotropis procera*

Retention time (min)	Collection				
	1st hourly	2nd hourly	3rd hourly	4th hourly	Single 4 h
12.706	2-Butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (3.18%)				2-Butanone-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (1.57%)
12.984	3-Buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (2.43%)	3-Buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (0.74%)	3-Buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (0.43%)	3-Buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (0.61%)	3-Buten-2-one-4, 2, 6, 6-trimethyl-1-cyclohexen-1-yl (1.48%)
13.885	9,17-Octadecadienal, (Z) (2.84%)	9,17-Octadecadienal, (Z) (1.49%)			9,12-Octadecadienoyl chloride (1.53%)
14.104	Hexadecanal (4.14%)		Tetradecanal (3.10%)	Tetradecanal (3.38%)	Hexadecanal (1.71%)
14.419		Hexadecanal (1.01%)	Hexadecanal (2.83%)	Hexadecanal (3.60%)	
14.595					
14.917	Z-5-Nonadecene (3.25%)	9-Nonadecene (2.77%)		1-Nonadecene (3.13%)	Z-5-Nonadecene (2.27%)
15.093	-	5,9,13-pentadecatriene-2-one, 6,10,14-trimethyl (E,E) (1.97%)	5,9,13-pentadecatriene-2-one, 6,10,14-trimethyl (E,E) (2.14%)	5,9,13-pentadecatriene-2-one, 6,10,14-trimethyl (E,E) (2.70%)	5,9,13-pentadecatriene-2-one, 6,10,14-trimethyl (E,E) (1.88%)
15.181		Isophytol (1.70%)	Isophytol (1.81%)	Isophytol (1.62%)	
15.408	1-Hexadecanol-2-methyl- (2.08%)	1-Hexadecanol-2-methyl- (1.54%)	1-Hexadecanol-2-methyl- (1.91%)	1-Docosanol (2.28%)	1-Hexadecanol-2-methyl- (1.95%)
15.891		1-Docosanol (1.29%)			
17.233			4,8,12,16-tetramethylheptadecan-4-olide (1.06%)		4,8,12,16-tetramethylheptadecan-4-olide (1.34%)

25.19% phytol. The isomers of phytol, 3,7,11,15-tetramethyl-2-hexadecan-1-ol showed a similar pattern. In one of them the percentages are 4.74, 10.28, 12.62, 8.46 and 6.80% in the same sequence as above. Adding the percentages of phytol and the isomers gives 23.80, 40.65, 54.18, 36.64 and 37.59%. The essential oil collected in the third hour contained the highest percentage of phytol. However, in the case of 6,10,14-trimethyl-2-pentadecanone, the essential oil collected in the 2nd h contained the maximum amount of 16.63% but this is marginally higher than the values in the 1st, 3rd and 4th h.

The minor components are presented in Table 2. An inspection of the Table shows that 2-butanone,4-2,6,6-trimethyl-1-cyclohexen-1-yl with retention time 12.706 min was extracted essentially within the first hour. 3-Buten-2-one-4-2,6,6-trimethyl-1-cyclohexen-1-yl was also extracted within the first hour, although lower quantities, (<1%) were extracted in the 2nd, 3rd and 4th h. These two constituents are the most volatile and hence were extracted essentially in the 1st h. 9,17-octadecadienal, (Z) was extracted in the 1st and 2nd h but was not observed in the single 4 h collection instead 9,12-octadecadienoyl chloride was observed at this retention time in the single 4 h collection. It is pertinent to note that this latter constituent was not observed in any of the hourly collection but was probably present in low concentrations and accumulated over 4 h and was observed at a low concentration in the single 4 h collection.

Hexadecanal is present in all the fractions but was eluted at different retention times. However, tetradecanal was observed present in the 3rd and 4th hourly fractions but not in the 1st and 2nd nor in the single 4 h collection. Tetradecanal being of lower molecular

weight than hexadecanal would be expected to be the more volatile constituent and should be present in the 1st hourly collection; the absence may be due to the fact that the hexadecanal eluted at R_f 14.104 masked the tetradecanal.

Isophytol, R_f 15.181, was found present in the 2nd, 3rd and 4th hourly fractions but not in the 1st hourly fraction or in the single 4 h collection. The mode of hourly collection has thus made it possible to detect isophytol as a constituent of the essential oil.

Docosanol, R_f 15.408, in the 3rd hourly fraction and R_f 15.891 in the 2nd hourly fraction was not detected in the single 4 h collection. This mode of collection has also thus made it possible to detect docosanol. In order to enhance the potentialities of this mode of collection, half-hourly fractions of the hydrodistillate may be collected. It may be necessary to increase the mass of the powdered plant material by 50-100% to obtain appreciable quantities of the fractions especially in plants where the essential oil obtainable is low in quantity.

DISCUSSION

Phytol, which is (2 E, 7R, 11R)-3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol and its isomer 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol, the major constituents of the essential oil (35%) are terpenoids. Phytol is a reactive oxygen species-promoting substance and has been shown to increase 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 extensive study, it was found that a single injection of phytol exhibited a surprisingly long suppression of arthritis, persisting for several weeks. Subcutaneous administration was found to be the preferred mode for effective drug delivery. The efficiency of phytol was found to be more pronounced in comparison to two of the major drugs used for rheumatoid arthritis at the time of the study. Phytol was also found to be effective at different stages of arthritis, with good preventive and therapeutic results against the disease. The study also showed that the phytol-induced suppression of arthritis could be reversed, for example with histamine dihydrochloride. It was also found that the increase in oxidative burst after phytol injection was observed in several anatomical compartments including the blood, spleen, bone marrow, thymus and lymph node. This suggests that phytol would be effective as an anti-inflammatory agent in such parts of the human anatomy. Thus apart from the prevention and therapeutic effects observed in arthritis, it may treat inflammatory conditions in some other parts of the body.

A concluding remark in the study stated 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. It is therefore possible to infer that phytol and its isomers may be the therapeutic constituent in the essential oil useful for the management of asthma. Phytol and its isomers may thus be responsible for the reported anti-inflammatory activity of *C. procera* (Aliero *et al.*, 2001; Kumar and Basu, 1994; Sangraula *et al.*, 2002) and its use for the management of asthma in various communities (Odugbemi, 2008; Kumar and Basu, 1994).

A pharmaceutical preparation consisting of a terpenoid and an antihistaminic compound has been found useful in the prevention and treatment of mild asthma, hay fever and urticaria (Tschollar *et al.*, 1998). This may explain the therapeutic effect of the plant in asthma and hay fever (Odugbemi, 2008). Phytol is a product of the hydrolysis of chlorophyll and it forms part of the molecules of vitamins E and K (Finar, 2001). Vitamin E when administered orally was found to suppress the increase in airway reactivity in guinea pigs sensitized to ovalbumin

and it exhibited membrane-stabilizing action (Deepika *et al.*, 2005). Vitamin K₂, menaquinone, when administered on some patients exhibited clinical effects which resulted in significant relief in bronchial asthma (Kimura *et al.*, 1970, 1975). Thus phytol could produce the effects of vitamins E and K and thus relieve asthma. The presence of phytol in the essential oil of *C. procera* is thus scientific evidence in support of the use of the plant as a medicinal preparation for the relief of asthma.

A compound present in the essential oil in appreciable concentration (15%) is 6, 10, 14-trimethyl-2-pentadecanone, a C15 aliphatic methyl ketone. Long chain aliphatic methyl ketones have been reported to show repellence to arthropods including blood-sucking insects (Blum *et al.*, 1996; Ndungu *et al.*, 1995; Torr *et al.*, 1996; Gikonyo *et al.*, 2002; Roe, 2004). In a study on the efficacy of such compounds against *Anopheles gambiae*, a malaria vector, C11-C15 compounds were more effective than C7-C10 compounds and among the C11-C15 compounds, odd-carbon compounds were more effective than even-carbon compounds. The C15 compound was found to be of comparable activity as N, N-diethyl-m-toluamide (DEET) a repellent to mosquitoes and other insects at 10% concentration w/v (Innocent *et al.*, 2008). Thus, the essential oil from *C. procera* could be an effective repellent against *Anopheles* species due to the presence of 6,10,14-trimethyl-2-pentadecanone and the plant would therefore be effective in malaria control.

Docosanol, present in the 2nd and 4th hourly collections is a C22 fatty alcohol. Policosanol, a natural mixture of long chain unsaturated aliphatic alcohols containing C28, C26 and/or C30 alcohols was found to exhibit cholesterol-lowering effects with type II hypercholesterolemia and dyslipidemia due to type II diabetes mellitus with good safety and tolerability profiles and without evidence of drug-related adverse events were observed (Castano *et al.*, 2002). Octacosanol, another long chain fatty alcohol has been reported to suppress lipid accumulation in rats fed on a high-fat diet (Kato *et al.*, 1995) and also inhibit platelet aggregation (Arruzazabala *et al.*, 1994). Thus, apart from treating anti-inflammatory diseases such as rheumatoid arthritis and asthma, ingestion of the leaves may also suppress lipid accumulation and exhibit cholesterol-lowering effects. The essential oil from the plant is a potential material as repellent for insects including *Anopheles* species and could be a weapon in the effort of malaria control.

CONCLUSION

The essential oil from the leaves of *C. procera* has been found to be rich in phytol and its isomers and also contains appreciable concentration of 6,10,14-trimethyl-2-pentadecanone. The hourly mode of collection has been useful in detecting the presence of minor components such as tetradecanal, isophytol and docosanol in the essential oil.

ACKNOWLEDGMENT

The authors acknowledge the interest and assistance of Prof. Omowunmi Sadik of The State University of New York, (SUNY) Binghamton and appreciate her making the GC-MS facility available.

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