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Chemical Composition, Cytotoxicity and Antioxidant Activity of Essential Oils of *Acalypha hispida* Flowers

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Abstract: *Acalypha hispida* (Burm.) F. flowers (Euphorbiaceae) were subjected to distillation using a hydro-distiller (all-glass Clavenger apparatus) to extract the essential oil present in the plant samples. GC and GC/MS analysis were carried out on the essential oil and was found to contain 44 compounds constituting 99.98% of the total oil composition. The compounds were identified by spectral comparison to be mainly alcohols, esters, ketones, terpenes and hydrocarbons. The principal constituents are 15, 16-Epoxylabda-13 (16), 14-dien-8α-ol (12.75%), 8, 14-Cedranoxide (12.19%), Curcumene (10.14%), 1-Hexandecene (8.37%) and Ethyl vanillin (6.87%) while others were present in trace amounts. Brine shrimp lethality assay was carried out using brine shrimps at 10, 100 and 1000 ppm to determine the toxicity of the oils to living organisms (shrimps). LC_{50} value (μg mL^{-1}) of 4.3715 obtained showed that the essential oil of *A. hispida* flower is toxic. The antioxidant properties of essential oils of *A. hispida* flowers were investigated using the UV/Visible spectrophotometer. The oil exhibited weak activity as a radical scavenger in the experiment using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), indicating that *A. hispida* oil has very weak ability to donate hydrogen when compared with the standard Butylatedhydroxylanisole (BHA). The absorption is stoichiometric with respect to the number of electron taken up. At 20 μg mL^{-1}, the oil activity was 0.9360±0.037 (26.5% inhibition) which was less than that of BHA (70.2%).

Key words: *Acalypha hispida*, cytotoxicity, hydrodistillation, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), gas chromatography, mass spectroscopy

INTRODUCTION

Antioxidant are agents that neutralizes harmful compounds called free radicals which damage living cells, spoil food and degrade materials such as rubber, gasoline and lubricating oils. Antioxidants can take the form of drugs e.g., enzymes in the body, vitamin supplements or industrial additives. They are routinely added to metals, oils, foodstuffs and other materials to prevent free radical damage. Free radicals also play very important roles in human health and are beneficial in combating several diseases. However, excess formation is harmful to the body organs. Any molecule can become a free radical by either losing or gaining an electron. Once initiated these free radicals get involved in chain reaction with stable types. The compounds thus formed have longer stability in the body and increase the potential for cellular damage. Free radicals damage the cell at the site of their operation causing serious health disorders. Antioxidants therefore, work to control the level of free radicals before they do oxidative damage to the body (Alan and Miller, 1996; Gill, 1992; Halliwell, 1999; Newman *et al*., 2000).

Essential oils are employed as antioxidants due to their small molecular size and their ability to easily penetrate the skin tissue. They are lipid soluble and are capable of penetrating the membranes easily even in conditions where oxygen deficiency leads to hardening of membranes. Studies reveal that essential oils serve as powerful antioxidants that produce adverse environment for damaging free radicals thus, preventing mutations and oxidants in cells. They therefore function as scavengers for free radicals. Essential oils may be extracted from plants, fruits, flowers, barks, roots and seeds with each possessing unique characteristics (Potterat, 1997; Bray, 1999).

The plant *Acalypha* belongs to the sole genus subtribe Acalyphinae of the family Euphorbiaceae which comprises about 570 species, a large portion of which are weeds while others are ornamental plants. They are found in the tropics of Africa, America and Asia. Some of the species are well known in folklore medicine and a few have actually appeared in homeopathic pharmacopoeia. *Acalypha hispida* also known as chenille plant or Philippine medusa appears as a small shrub growing to a

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height of 1-3 m. In ethno-medical practices, the root and flower decoction is used for kidney ailments and as a diuretic. Leaf poultice is used as a cure for leprosy, the decoction of leaves and flowers are taken internally as laxative and for treatment of gonorrhea. Bark is used as expectorant and for asthma (Iwu *et al*., 1999; Kafaru, 2000; Sofowora, 2008). Previous work done on the leaves of *A. hispida* revealed the presence of phenolics, flavonoids, glycosides, steroids, saponins, phlobatannins, and hydroxyanthraquinones (Iniagbe *et al*., 2009; Okorondu *et al*., 2009). The antifungal, antibacterial, anti-ulcer and anti-tumor properties of extracts of leaves of *A. hispida* have been established (Ejechi and Soucey, 1999; Adesina *et al*., 2000; Gutierrez-Lugo *et al*., 2002). In this study we report the cytotoxicity and antioxidant activities of essential oils of *A. hispida* flowers. The goal was achieved by subjecting the essential oil of *A. hispida* to Brine shrimp lethality assay for determination of the toxic level. *In vitro* antioxidant assay determined by the effect on DPPH radical (2, 2-diphenyl-1-picrylhydrazyl) was carried out. DPPH radical gives strong absorption at 517 nm (deep violet colour) in visible spectroscopy. The absorption vanishes or is decolorized as the electron becomes paired off in the presence of a free radical scavenger.

MATERIALS AND METHODS

Plant materials: Fresh samples of the *A. hispida* flowers were collected in September, 2009 at the Botanical Gardens, University of Ibadan. Specimens were identified at the Botany and Microbiology Department, University of Ibadan, Oyo State, Nigeria. The volatile oil was immediately collected from the fresh plant material by hydrodistillation.

Reagents: Hexane and methanol (BDH chemicals), Butylated Hydroxyanisole (BHA) and 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) were obtained from were obtained from Sigma Chemical Co. (Germany).

Major equipment used: UV-Visible Spectrophotometer (Unicol 200 and Perkin Elmer lambda 25 models), GC-Mass spectrophotometer (Agilent Technologies), Hydro distiller-Clavenger apparatus.

Method

Isolation of essential oils: The oil was obtained by hydro distillation on a Clavenger type apparatus for 4 h in accordance with the British pharmacopoeia specifications (1980). The essential oil was collected and stored at 4°C until analysis. The oil yield was calculated relative to the dry matter.

Analysis of the essential oils

Gas chromatography: GC-MS analyses of the essential oil was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091)-413:325°C HP-5 column (30 m×320 μm×0.25 μm) was used with helium as carrier gas at a flow rate of 3.3245 mL min^{-1}. The GC oven temperature was initially programmed at 50°C (hold for 1min) and finally at 300°C (hold for 5 min) at a rate of 80°C min^{-1} while the trial temperature was 37.25°C. The column heater was set at 250°C and was a split less mode while the pressure was 10.153 psi with an average velocity of 66.45 cm sec^{-1} and a hold-up time of 0.75245 min was recorded. Mass spectrometry was run in the Electron Impact mode (EI) at 70 eV. The percentage compositions were obtained from electronic integration measurements using Flame Ionization Detector (FID), set at 250°C. The peak numbers and relative percentages of the characterized components are given in Table 1.

Gas chromatography-mass spectrometry: The essential oils were analysed by GC-MS using an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091)-413:325°C HP-5 column (30 m × 320 μm × 0.25 μm) was used with helium as carrier gas at a flow rate of 3.3245 mL min^{-1}. GC oven temperature and conditions were as described above. The injector temperature was at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z 30 to 500.

Identification of components: The individual constituents of the oil were identified on the basis of their retention indices determined with a reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns (NIST 08.L database/chemstation data system) with data previously reported in literature by Adams (2001), Joulain and Konig, (1998), Mclafferty and Stauffer (1989).

Brine shrimp lethality test: The Brine Shrimp Lethality Test (BST) was used to predict the presence in the oils, of cytotoxic activity (Meyer *et al*., 1982). The shrimp's eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100, and 10 μg mL^{-1}) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h the vials were examined against a lighted

Table 1: Chemical composition of the essential oil from the flowers of *A. hispida* by GC and GC/ MS analysis*

Peak No. compounds	Composition (%)
Propyl butyrate	0.89
Anisole	0.49
α-Thujene	0.52
α-pinene	1.79
Trans-1-butyl-2-pentyl cyclopropane	2.52
Acetophenone	0.60
Furfuryl propanoate	0.46
Citronellal	0.51
Pulegol	0.47
2, β-pinene oxide	0.39
4-Hydroxy benzoic acid	0.94
Iso-dihydrocarveol	3.78
n-Iso-dihydrocarveol	0.47
Methyl citronellate	3.51
Sabinyl acetate	0.41
Cumin alcohol	1.42
Benzyl butyrate	2.10
Geranyl acetate	2.82
p-Anisyl acetate	3.70
Trans-Cinnamyl acetate	0.44
Ethyl Vanillin	6.87
Curcumene	10.14
15,16-Epoxylabda-13 (16), 14-dien-8a-ol	12.75
8,14-Cedran oxide	12.19
Furfuryl octanoate	3.36
Tridecanol	0.39
1-Hexadecene	8.37
β-Optopenone	0.43
5-Cedranone	3.33
τ-Mooroloil	1.01
Coniferyl alcohol	0.42
β-Bisabolol	0.53
Cadalene	0.80
Apiole	0.39
α-Bisabolol	0.39
Trans-Farnesol	0.64
β-*cis*-santalol	0.98
cis-Farnesol	0.41
3,5-bis-(1,1-dimethylethyl)-1,2-benzenediol	0.52
E-Coniferyl alcohol	3.48
Oplopanone	2.02
Silicic acid, diethyl bis (trimethyl silyl) ester	0.44

background and the average number of larvae that survived in each vial was determined. The concentration at fifty percent mortality of the larvae (LC_{50}) was determined using the Finney computer programme.

ANTIOXIDANT ACTIVITY

Scavenging effect on DPPH: A 0.5 mM of the radical source 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) solution in methanol was prepared, and 3 mL of this solution was mixed with 1 mL of the oil sample in methanol (Koleva *et al*., 2002; Oloyede and Farombi, 2010). The decrease in absorption at 517 nm of DPPH was measured after 10 min of incubation. The actual decrease in absorption was measured against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on butylatedhydroxylamisole (BHA) a known antioxidant. All test and analysis were run in triplicates and the result obtained was averaged. The activities were determined as a function of their %Inhibition which was calculated using the formula:

$$I(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

RESULTS AND DISCUSSION

The yield of the *A. hispida* flower oil was 0.40% (w/w) from 300 g of the fresh flowers used. The colourless essential oils with characteristic smell were analyzed both by GC and GC/MS systems using a polar column, resulting in the identification of only 44 constituents in the hydrodistilled sample, representing 99.98% of the total essential oil. The oil yield of *A. hispida* is low considering the fact that flowers are known to be rich in essential oils. Overall, alcohols, esters, terpenes and hydrocarbons were found in the sample as the dominating group of compounds (Table 1) for the hydro distilled samples. Many chemical compounds of medicinal importance are been reported from *A. hispida* (Iniagbe *et al*., 2009; Okorondu *et al*., 2009). The nor-labdane-type diterpene 15, 16-Epoxylabda-13 (16), 14-dien-8α-ol reported in this plant has also been isolated from another specie of *Acalypha* (Gina *et al*., 2006). The cytotoxicity result of the essential oil showed an average death of 10 (10000 ppm), 16 (1000 ppm) and 22 (100 ppm). An LC_{50} value of 4.3715 μg mL^{-1} with lower and upper confidence limits of 253.7717 and 426221.1000 μg mL^{-1}, respectively showed that the essential oil of *A. hispida* flower is toxic and therefore, its use at higher concentrations should be monitored. The LC_{50} (μg mL^{-1}) results is further corroborated by the presence in the oil of hydrocarbon molecules which accounts for the high toxicity of the oil. The free radical scavenging activity was evaluated by the decrease in absorption of the stable hydroxyl radical 2,2-diphenylpicryl hydrazyl radical (DPPH) at 517 nm. *A. hispida* essential oil decolorized DPPH due to its hydrogen donating ability. The activity of the essential oil of *A. hispida* flower on the stable radical DPPH decreased with decrease in concentration. The free radical scavenging activity was compared with the activities of a known antioxidant Butylated hydroxyanisole (BHA). At 20 μg mL^{-1}, the oil activity was 0.9360±0.037 (26.5% inhibition) which was less than BHA (70.2%), at 40 μg mL^{-1} it was 0.7781±0.023 (21.3%) which was also less than BHA (65%) and at 60 μg mL^{-1} the activity was 0.6421±0.010 (5.26%) which was also less than BHA (62.5%). Hence the oil of this plant has low antioxidant activity. The absorption is stoichiometric with respect to the number of electron taken up. The percentage inhibition is concentration dependent. DPPH is known to

be a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). The toxic nature of essential oil from *A. hispida* flower shows that it has medicinal importance as it has been established by other workers that secondary metabolites from plants which are active medicinally are most times toxic to Brine shrimp larvae *Artermia silina* nauplii which is a living organism with no advance nervous system (Aiyelaagbe *et al*., 2009; Oloyede *et al*., 2010). The results obtained from free radical scavenging activities of the plant by using scavenging effect on 2,2-diphenylpicryl hydrazyl radical (DPPH) and hydrogen peroxide method has shown that *A hispida* is effective as an antioxidant; this result is also in agreement with results obtained by other workers in screening medicinal plants for antioxidant activities (Soares *et al*., 1997; Alma *et al*., 2003; Mutes *et al*., 2010).

CONCLUSION

The essential oil composition of *A. hispida* flower investigated revealed the presences of 44 compounds as determined by GC and GC/MS analysis constituting 99.98% of the total oil composition. The compounds were identified by spectral comparison to be mainly alcohols, esters, ketones, terpenes and hydrocarbons. The principal constituents are 15,16-Epoxylabda-13(16),14-dien-8α-ol (12.75%), 8,14-Cedranoxide (12.19%), Curcumene (10.14%), 1-Hexandecene (8.37%) and Ethyl vanillin (6.87%) Brine shrimp lethality assay showed that the essential oil of *A. hispida* flower is toxic (LC_{50} value (μg mL^{-1}) of 4.3715). The antioxidant properties of essential oils of *A. hispida* flowers revealed weak activity as a radical scavenger in the experiment using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) indicating that *A. hispida* oil has very weak ability to donate hydrogen when compared with a standard Butylatedhydroxylanisole (BHA). The absorption is stoichiometric with respect to the number of electron taken up. At 20 μg mL^{-1}, the oil activity was 26.5% which was less than that of BHA (70.2%). Thus the ability to scavenge free radicals is an important property in order to minimize oxidative damage to living cells. Synthetic free radicals available e.g., BHA and BHT have been found to be toxic, responsible for liver damage, promoters of carcinogenesis and general consumer rejection of synthetic food additives hence the need for their replacement with natural antioxidants (Gulcin *et al*., 2002) that will be supplied to human and animal organisms as food supplements or as specific pharmaceutics (Azuma *et al*., 1995). This plant may be found useful as antitumour, anticancer or antimicrobial agents due to its high toxicity but its use must be monitored at high dosage level. Also the nor-labdane-type diterpene 15, 16-Epoxylabda-13 (16), 14-dien-8α-ol is a potential source of anti tumour agent. This compound has also been isolated from another specie of *Acalypha* (Gina *et al*., 2006). Cedranoxide found in the plant is a good source of insect pheromone employed as sex attractant. The bisabolane sesquiterpenes curcumene is used as insecticides, repellents, and insect feeding deterrents.

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