

Radical Scavenging Activity of Essential Oils from Some Nigerian Medicinal Plants and Spices

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ABSTRACT

Essential oils are plants' secondary metabolites widely used through ages for bactericidal, fungicidal, anti-parasitic and insecticidal applications. In recent years, there has been an increased interest in essential oils because of their potentials as pharmaceuticals. However, there is a dearth of information on the antioxidant activity of the essential oils from *Aframomum melegueta*, *Crassocephalum crepidioides*, *Monodora myristica* and *Ocimum gratissimum* from Nigeria. This study was designed to screen the essential oils from these plants for possible radical scavenging (antioxidant) activity. Essential oils were extracted from the leaves, stem-bark, roots and seeds of the plants through hydro-distillation using the Clevenger type apparatus. The oils were analysed by gas chromatographic and gas chromatographic/mass spectrophotometric techniques. The essential oils were composed of monoterpenoids and sesquiterpenoids. They were tested for antioxidant activity using 1-diphenyl-2-picrylhydrazyl (DPPH) method. The antioxidant activity ranged from 65.5 to 96.4%. Essential oils of *O. gratissimum* leaves exhibited the highest antioxidant activity (96.4%). The results obtained suggest possible applications of the essential oils from these plants as sources of pharmaceuticals.

Keywords: Medicinal plants, Essential oils, Antioxidant activity, Nigeria.

INTRODUCTION

Essential oils are plants' odorous secondary metabolites which have been known and utilized by mankind through ages. Thus they are one of the best studied groups of plants secondary metabolites (Pichersky, 2006). The various applications of essential oils are the reasons for the interests in their study. In recent years, the concern for nature and the preferences for things which are natural have been spearheading a new interest group. In simple term, it is now an era of 'green revolution' in which everything natural and nature-based consumer products are in large demand all over the world. The back-to-nature and ecological trends, as well as the growing demand for good odours, not only in perfumery and cosmetic products but also in everyday life, have created a growing demand for natural raw materials. This is enhanced by the fact that there is no way to make 100% replicate of a natural fragrance with synthetic materials (Betts, 2001).

Furthermore, environmental limitations in industrialized countries and the cost of waste treatment have increased the total cost of production of many aroma chemicals to such a level that products obtained

from plants, being usually of better quality than those from chemicals, are now more preferred. Additionally,

beyond their great value as natural fragrance, many of them have curative properties and are used as such in pure therapy (such as Aromatherapy), or their beneficial properties are used in cosmetic products and in Para pharmaceuticals (Wladyslaw, 1995). As the world resources of coal and petroleum continue to dwindle and the philosophy of going 'green' keeps gaining acceptance internationally, gradually these will limit the dependence of industries on petrochemicals and return the natural oils which are eco-friendly and renewable resource as the preferred alternatives.

The studies of plant extracts cum essential oils have often served as phytochemical leads for pharmaceutical developments. Of the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value (Joy *et al.*, 1998). Recent studies have shown that traditional plant medicines from various parts of the world can provide a rich source of antiviral, antiradical and antibiotic activities (Yip *et al.*, 1991, Hudson,

1995, Taylor *et al.*, 1996, Ananiel *et al.*, 2000). In Africa, bioactive extracts can be ethical phytomedicine, if appropriate phytochemical standardization and toxicology investigation are undertaken (Gbeassor *et al.*, 1996). Oxygen is essential for aerobic forms of life however, oxygen metabolites also known as free radicals: O₂*, OH*, RO*, ROO* and H₂O₂ are very toxic. Free radicals have been implicated in the pathology and physiology of numerous afflictions such as atherosclerosis, heart failure, liver injury, ageing, chronic inflammation, neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and ischemic and hemorrhagic stroke), cancer, diabetes mellitus, and a plethora of other diseases (Mon *et al.*, 2001; Khadri *et al.*, 2008).

The antioxidant ability of a substance can be attributed to its radical scavenging ability. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene are known to be toxic in humans (Velioglu *et al.*, 1998) hence; a large number of studies have examined the benefits of plant secondary metabolites as possible antioxidants to reduce neuronal death occurring in the pathophysiology of these disorders. Preventing or minimizing these oxidation-related diseases may involve the use of antioxidant substances that scavenge and eradicate free radicals (Silvia *et al.*, 2012). Thus in this work, we investigate the radical scavenging activity of the essential oils from *Aframomum melegueta* K. Schum, *Crassocephalum crepidioides* Benth S. More, *Monodora myristica* Gaertn, and *Ocimum gratissimum* Linn which are used as spices and in traditional medicines in Nigeria.

MATERIALS AND METHODS

General Experimental Procedures

All solvents and reagents used in this study were of 'analar' grade, purchased from Sigma-Aldrich representatives in Nigeria. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Steinheim, Germany), Ultraviolet-visible absorbance were recorded using the cuvette cell compartment of UV-Visible 752 (Techmel and Techmel, USA) model spectrophotometer. The GC-MS spectra were obtained on Agilent 6890N GC Coupled with MS-5973-634071 Series running on Agilent-Chemstation retention time locking software (Agilent Technologists, USA).

Collection of Plants and Extraction of Essential Oils

The plant materials were sourced within Ondo State, South Western Nigeria. They were identified at the Herbarium of the Forest Research Institute of Nigeria, Ibadan where Voucher specimens were on file. The parts of the plants, leaves, stems, rhizomes and seeds were carefully separated, washed and then subjected to hydro-distillation separately for 2-3 h using an all glass Clevenger-type apparatus, according to *British Pharmacopoeia II* (1980) specification. The oil samples were stored in air-tight containers at 0°C before GC-MS analysis without any further treatment.

Gas Chromatography/Mass Spectrophotometric (GC/MS) Analysis

The essential oils were analyzed using Agilent (USA) 6890N GC Coupled with MS-5973-634071 Series. The capillary column type was DB-1 (fused-silica) [30.0m (length) X320.00µm (diameter) X1.00µm (film thickness)]. The carrier gas was Helium at constant flow rate of 1.0ml/min and average velocity of 37cm/s; the pressure was 0.78psi. The initial column temperature was set at 100°C (held for 5 minutes) to the final temperature of 250°C at the rate of 5°C/min. The injector was the split type and was set at 50:1, and volume injected was 1.0µL. The chromatograms were auto-integrated by Shem-Station and the constituents were identified by comparison of the GC-MS data with (NIST02) library spectra and data from literature (Adams, 1995).

Assay of Radical Scavenging (Antioxidant) Activity of Essential Oils

The free radical scavenging activity assay was carried out using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method (Brand-Williams, 1995). A methanolic stock solution (50.0 µL) of each sample of essential oil at different concentrations was placed in a cuvette, and 2.0 mL of 60.0 µM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Steinheim, Germany) was added. Absorbance measurements were made at 517 nm using a UV-Visible 752 spectrophotometer (Techmel and Techmel, USA) after 60 mins. of reaction at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution was used as negative control while butylated hydroxyanisole (BHA) (Sigma-Aldrich, Steinheim, Germany) was used as positive control. The percentage inhibition of the DPPH radical by the samples was calculated according to the following formula (eqn. 1):

$$\text{Scavenging effect \%} = \frac{(A_0 - A_1)}{A_0} \times 100 \dots (\text{eqn. 1})$$

Where A_0 was the absorbance of the control without extract (blank) and A_1 was the absorbance of the sample. Tests were carried out in triplicate and data were analysed using descriptive statistics.

RESULTS AND DISCUSSION

The major compositions of the Essential oils were determined and reported earlier (owokotomo *et al*; 2012a, b, c; 2014). The most important compounds of *A. melegueta* leaves volatile oil were identified as myrtenyl acetate (29.1%) and limonene (19.3%) while the stem-bark oil contained caryophyllene oxide (19.7%) and myrtenyl acetate (14.7%). The root oil comprised of myrtenyl acetate (22.7%) and pinocarvyl acetate (11.5%); while the volatile oil of the seeds consisted of α -caryophyllene (48.8%) and β -caryophyllene (32.5%). The main constituents of the leaves oil of *C. crepidioides* were α -caryophyllene (10.3%) and β -cubebene (13.8%) while the stem oil were mainly thymol (43.9%) and 4-cyclohexybutyramide (20.9%). The essential oil of the leaves of *O. gratissimum* afforded γ -terpinene (52.9%) while the seed volatile oil yielded γ -terpinene (48.2%) and caryophyllene (10.7%) as main constituents. The volatile oil of the seeds of *M. myristica* contained germacrene D-4-ol (25.5%) and linalool (15.1%) while the stem-bark oil yielded γ -cadinene (31.3%) and α -elemene (17.9%) as dominant constituents.

All the essential oils showed a proclivity to quench the DPPH radicals as indicated by their dose-dependent increase in percentage inhibition (Figures 1, 2, 3 and 4). The high percentages of inhibition corresponded to a rapid decrease in absorbance in the presence of the plant essential oils, indicating high antioxidant potency in terms of electron or hydrogen atom-donating capacity. The *O. gratissimum* leaf's essential oil had the highest DPPH radical scavenging activity (96.4%) while that of the roots of *A. melegueta* had the least DPPH radical scavenging activity (65.3%) at 20.0 mg/mL solution of essential oils. The antioxidant activities of the plants' essential oils compared very well with the butylated hydroxyl anisole (BHA) control.

Most of the antioxidant properties of medicinal plants have been attributed to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers (Hakkim *et al.*, 2007). However, because of large number of compounds with varying compositions in the essential oils coupled with the fact that radical reactions are difficult to predict, it is most likely that other mechanisms different from those above may be involved in the scavenging processes which might serve to explain the high antioxidant scavenging activity of the essential oils in this study.

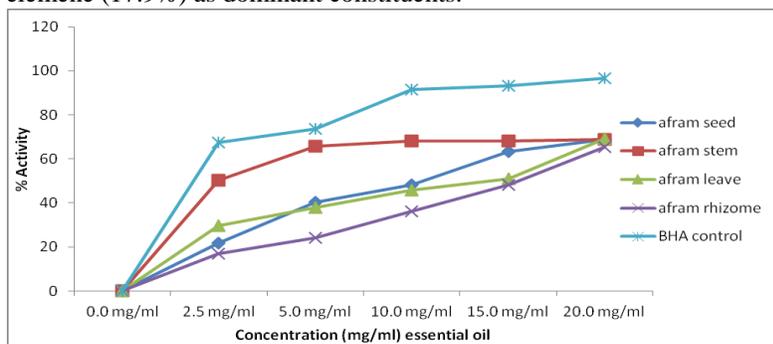


Figure 1: DPPH Scavenging activity of *Aframomum melegueta* and butylated hydroxyl anisole (BHA)

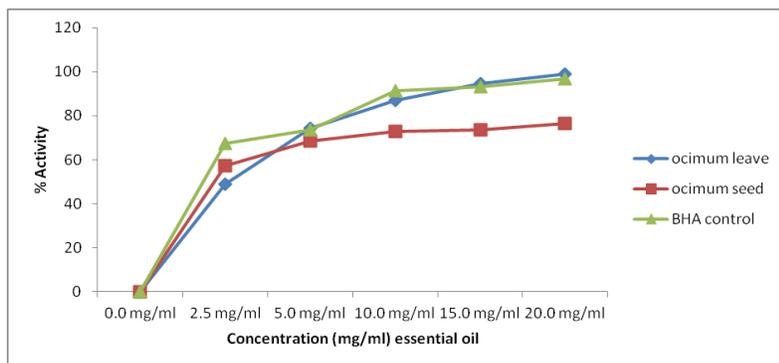


Figure 2: DPPH Scavenging activity of *Ocimum gratissimum* and butylated hydroxyl anisole (BHA)

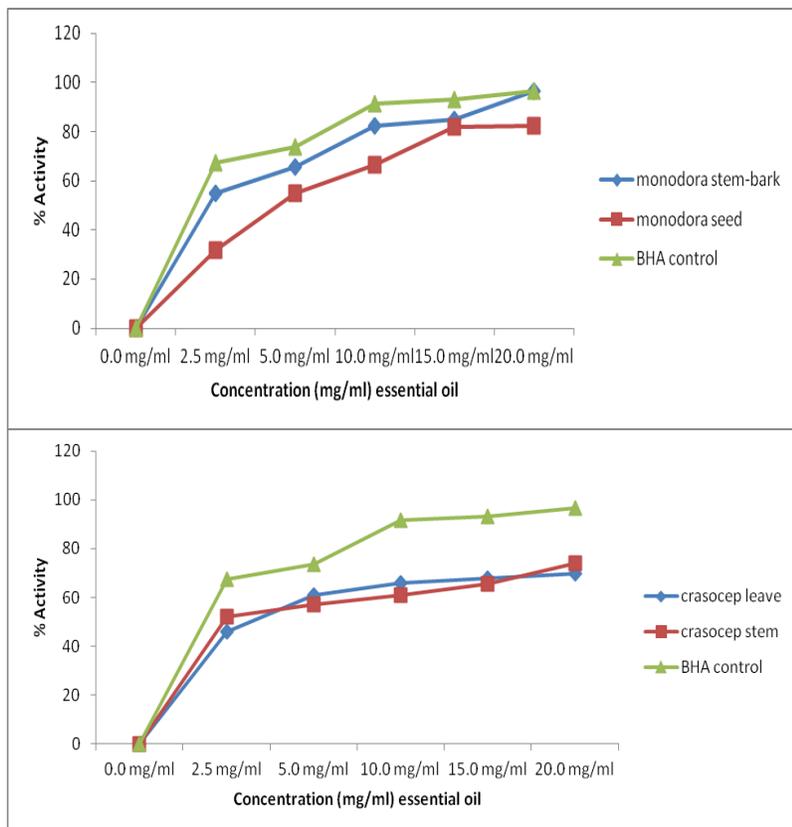


Figure 3: DPPH Scavenging activity of *Monodora myristica* and butylated hydroxyl anisole (BHA)

Figure 4: DPPH Scavenging activity of *Crassocephalum crepidioides* and butylated hydroxyl anisole (BHA)

CONCLUSION

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical widely used as the model system to investigate the scavenging activities of several natural compounds was used in this investigation. The results showed that the DPPH radical was scavenged by antioxidants present in the EOs through the donation of proton, forming the reduced DPPH and the colour changes from purple to light-yellow after reduction. All the essential oils showed strong activity (>50%) at 20.0 mg/mL test solution. *Ocimum gratissimum* demonstrated the highest activity, scavenging at $96.4 \pm 0.002\%$ thus comparable with the BHA standard activity of $96.7 \pm 0.002\%$ at the same dose level. Generally, the antioxidant activity of the EOs varied but all were dose-dependent in a linear order. From this study, it could be inferred that these plants' essential oils can be used as additives in foods to promote good health or as drugs of antioxidant activity. The results also corroborated the medicinal properties of these plants and strengthen their use in African ethno-medicinal practices, especially in Nigeria.

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