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Nutraceuticals in Leaves of *Eucalyptus Citriodora* and *Eucalyptus Camandulensis*

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ABSTRACT

Two essential oils bearing plants (*Eucalyptus camadulensis* and *Eucalyptus citriodora* leaves) were investigated for their nutraceutical properties. Results showed the percentage proximate composition of *E.citriodora* and *E. camadulensis* as ; moisture (4.32 and 4.11), ash (2.26 and 4.15), crude fibre (16.74 and 16.55), crude fat (3.87 and 3.96), crude protein (5.26 and 7.13), and carbohydrate (84.29 and 80.64), respectively while the level of the mineral elements are; potassium (1.42 % and 1.53 %), calcium (2.72 % and 2.5 %), manganese (0.24 ppm and 0.26 ppm), iron (0.81 ppm and 0.42 ppm) and copper (0.03 ppm and 0.034 ppm). Nickel and Zinc were not detected in *E.citriodora* but present in *E.camadulensis* (0.003 ppm, 0.05 ppm) respectively. The samples fixed oils were analyzed for fatty acid concentration by gas chromatography. The percentage saturation, monounsaturation and polyunsaturation of *E. citriodora* are; 40.85, 37.79, 21.36 while that of *E.camadulensis* are; 41.83, 36.35, 21.20 respectively. The fatty acid composition of the two species contains a healthy mixture of all the types of fatty acids.

Introduction

The use of medicinal plants has always been part of human culture and is wide spread in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than expensive imported drugs. Also in Nigeria, a large percentage of the populace depend on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach of common man (Lawal *et al*., 2012).

Medicinal plants play an important and vital role in traditional medicine and are widely consumed as home remedies, a number of minerals essential to human nutrition are accumulated in different parts of plants and most wild plant species provide minerals, fibre, vitamins, essential fatty acids and enhance taste and colour in diets (Bianco *et al*., 1998).

Amongst the medicinal plants commonly use in Nigeria for management and treatment of various types of ailments are the Eucalyptus species. The *Eucalyptus*, a native genus from Australia, belongs to the *Myrtaceae* family and comprises about 800 species (Ogunwande *et al*., 2004). The different species had been studied for their content of essential oil. In fact, for many years there had been intense interest in the species essential oils as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infections and as natural food preservatives (Shuenzel and Harisson, 2002; Tepe *et al*., 2004). A number of studies have demonstrated the antimicrobial properties of *Eucalyptus* species essential oils against a wide range of microorganisms (Cimanga *et al*., 2002; Tyagi and Malik, 2012).

Even though the leaf extracts of *Eucalyptus* have been approved as food additives (Takahashi *et al*., 2004), the various species have been undervalued and underexploited as sources of dietary fibre, mineral elements and essential fatty acids. Most times when the essential oils are distilled off, the residual leaves are left to waste thus contributing to environmental pollution. Thus the present study investigates the proximate composition, level of mineral element of leaves and fatty acids profile of oil of two essential oil bearing plants; *Eucalyptus citriodora* and *Eucalyptus camadulensis* with a view to searching for nutraceuticals.

Materials and methods

Sample collection

Samples of clean mature young leaves of *E.citriodora* were collected from the Eucalyptus tree in front of the old Information and Communication Technology (ICT) building in Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Oyo state and *E.camadulensis* was collected from Ogbomoso High School compound, along Oyo road, Ogbomoso, Nigeria.

Sample preparation

The samples were dried under laboratory shade for 3 weeks. They were ground to powder using a KW 10 food blender, stored in an air tight container prior to analyses.

Proximate analysis

The proximate composition of the sample was determined using the method of AOAC (1990). Drying loss content (moisture and essential oil) was obtained by heating the samples to a constant weight in a thermostatically controlled oven at 105 ^{0}C. The ash content by igniting a 0.5 g test sample in a muffle furnace at 550 ^{o}C until light grey ash results, protein was determined using the Kjedhal method (N X 6.25) The dried pulverized sample was extracted with petroleum ether (boiling point 40-60 ^{0}C) for 6 hours using a soxhlet apparatus to obtain the crude lipid content while crude fibre content was estimated

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by consecutive acid and alkali digestion of sample followed by washing, drying, ashing at 600 ^{0}C and calculating the weight of ash free fibre and carbohydrate was calculated by difference.

Determination of nutritive value of samples

Nutritive value of the dry powder of the leaves was calculated based on the energy value available per kg of the macronutrient. Proteins, carbohydrates and fats yield 4.0, 4.0 and 9.0 Kcal of energy per g respectively. The nutritive value (NV) is calculated as [(4x % protein) + (4 x % carbohydrate) + (9 x % fat)] (Indrayan *et al*., 2005; Chinnasammi *et al*., 2011).

Determination of fatty acids profil

Fatty acid profile was determined using Gas Chromatography with column dimensions 30 m x 0.25 mm x 0.25 µm, column type HP INNOWAX, initial temperature 60 ^{0}C using Flame Ionisation Detector (FID) and detector temperature 320 ^{0}C and nitrogen as a carrier gas at a flow rate of 22 psi.. A 50 mg of the extracted oil from the sample was esterified for five (5) minutes at 95 ^{0}C with 3.4 ml of aqueous 0.5 M KOH. The mixture was neutralized by using 0.7 M HCl and 3 ml of 14 % boron triflouride in methanol. The mixture was heated for 5 minutes at the temperature of 90 ^{0}C to achieve complete methylation process. The fatty acid methyl esters were extracted thrice from the mixture with redistilled n-hexane. The content was concentrated to 1 ml for gas chromatography analysis and 1 µl was injected into the injection port of GC. The fatty acids were identified by comparing their retention times with those of standards. The content of fatty acids was expressed as percentage of total fatty acids.

Quantification of mineral elements

The mineral elements of the samples were analyzed using X-Ray fluorescence (XRF) transmission emission technique at the Center for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife, Nigeria, with model: PX2CR Power Supply and Amplifier for the XR-100CR Si Detector. The samples were pulverized, pelletized and then irradiated with X-Ray for 1000 s, to obtain the characteristics spectral, each spectral was made up of peaks which was characteristics of certain elements contained in the sample. The spectrum was checked on the computer system and then interpreted for quantitative determination of elements by direct comparison of count rates.

Statistical analysis

All data are reported as mean ± standard deviation of three different determinations.

Results and discussion

The proximate constituents of both leaves are reported in Fig. 1. The level of moisture and volatile content (drying loss) is low, this is an advantage as low drying loss indicates that microorganisms will not have favourable condition to thrive, thus the samples will have long shelf life when properly kept against external conditions. The protein in *E. camadulensis* is higher than that in *E. citriodora*, although the level is lower than 12% protein recommended for a food to be considered as a protein source (FNB, 2002) the protein content is higher when compared to that of some commonly consumed vegetables such as lettuce, spinach, parsley and cabbage which contain protein of 1-3 % range (Mccollum, 1992; Chinnasammi *et al*., 2011). The ash content in *E.camandulensis* is higher than that of *E.citriodora*, ash is an indication of level of inorganic materials present in a sample, both samples contain higher ash content compared to herbal leaves of *Mucuna utilis* with ash content of 0.11% (Ujowundu *et al*., 2010). Both samples contain low quantities of fat, the leaves could be recommended as beverage for people requiring low fat diet. A diet providing 1-2 % of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging (Antia *et al*., 2006).

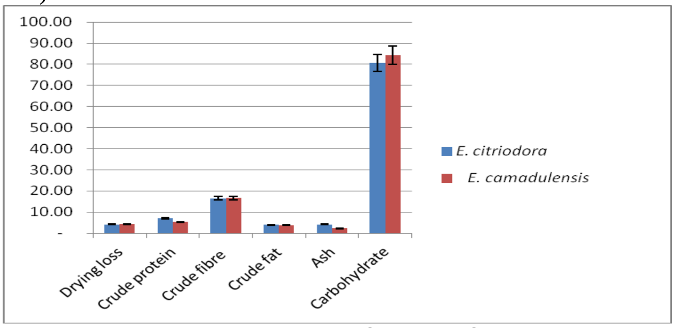

Fig. 1: Proximate constituents of leaves of *E. citriodora* and *E. camadulensis* (g/100g)

Dietary fibre is a nutraceutical in the prevention of various colon and rectum diseases. Non-starchy vegetables are richest sources of dietary fibre and are employed in the treatment of diseases such as obesity, diabetes, cancer and gastrointestinal disorders (Agostoni *et al*., 1995). The crude fibre (CF) in both Eucalyptus species compared favouraly with 19.25 % CF reported for *Moringa oleifera* leaves (Sharma *et al*., 2012). The CF was also high when compared to those of some *Acalypha* species used in the treatment of various ailments as antimicrobial agents; *Acalypha racemosa* (7.20 % CF), *Acalypha hispada* (10.25 % CF) and *Acalypha marginata* (11.5 % CF) (Iniaghe *et al*., 2009). Infact, the higher crude fibre could also complement the CF of some commonly consumed vegetables which are lower in CF; *Talinum triangulare* (6.20 %), *Piper guineeses* (6.40 %), *Cochorus olitorius* (7.0 %) and bitter leaves, *Vernonia amygdalina* (6.5 %) (Akindahunsi and Salawu, 2005).

The observed carbohydrate (80.64; 84.29 g/100g) and nutritive calorific value (386.72; 393.03 K cal/g) in *E. citriodora* and *E. camadulensis* respectively may be an indication that the samples could provide energy to power the cells and tissues of the body on consumption.

The levels of macro and micro mineral elements in the leaves samples are reported in Figs. 2 and 3. Minerals are important in the diet because they serve as cofactors for many physiologic and metabolic functions. The biological effects of the trace elements in living system strongly depend upon their concentration and thus should be carefully controlled especially when herbs and drugs are used in human (Jacob, 1994).

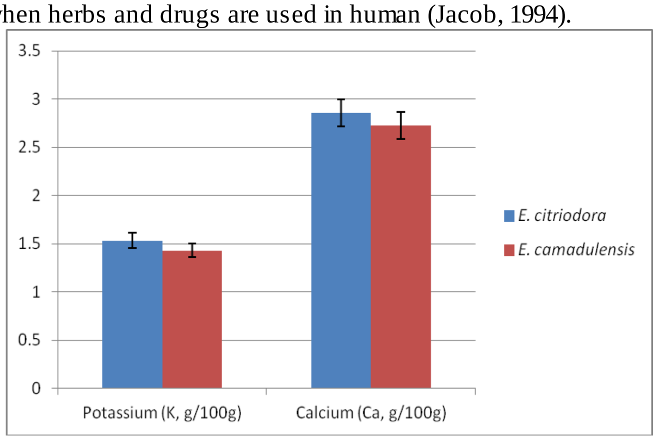

Fig. 2: Level of potassium and calcium in leaves of *E. citriodora* and *E. camadulensis*

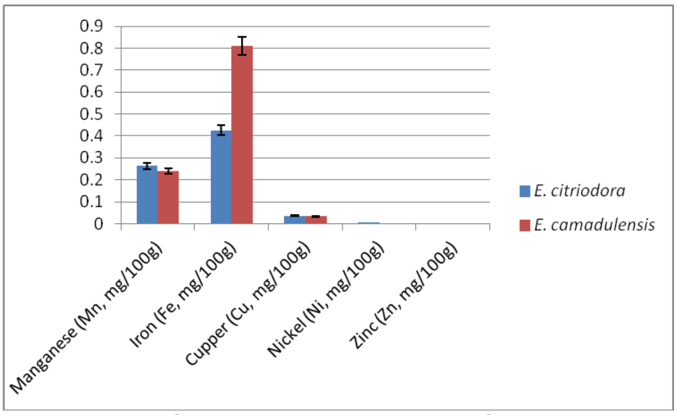

Fig. 3: Level of micro elements in leaves of *E. citriodora* and *E. camadulensis*

The elements quantified by XRF showed the following decreasing order Ca> K> Fe> Mn> Zn> Cu> Ni in *E. camadulensis* and also Ca> K> Fe> Mn> Cu. Ni, Zn were below the detection limit in E. *citriodora*. The level of calcium is highest in both species. Most calcium in the body is contained in the bones, but about 1 % is used for nerve impulses and muscle contractions including heart, kidney and other organs that sustain life and provide movement. Calcium participates in the protein structuring of RNA and DNA, so it affects the genetic structure and genetic mutations in the body's constant cellular replacement programme. Recommended daily allowance (RDA) of calcium is reported to be 1200 mg (Kocatepe and Turan, 2012). Less than 50 g of both species of Eucalyptus could provide the RDA of calcium. The higher level of potassium in the leaves could also enhance the normal functioning of the body system, Potassium has been reported to serve as an electrolyte that interacts with sodium to conduct nerve impulses and many other functions in the cells (Kocatepe and Turan, 2012) Mn is a component of several enzymes including manganese-specific glycosyltransferase and phosphoenol pyruvate carboxykinase and essential for normal bone structure. Mn deficiency can manifest as transient dermatitis and hypocholesterolemia.

Cu is universally important cofactor for many hundreds of enzymes. It functions as a cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. Cu deficiency can result in decrease in the tinsel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment (Tilson, 1982).

Zinc is a part of every cell in the body and forms a part of over 200 enzymes that have functions ranging from proper action of body hormones to cell growth. Because the body readily uses zinc for many different functions, it constantly needs to be replaced. Sufficient levels of zinc are very important for the body's immunity and strength (Kocatepe and Turan, 2012). *Eucalyptus camadulensis* and *citriodora* contained all these nutraceutical elements in varying proportion; zinc however was not detected in *E.citriodora*.

The fatty acids profiles were reported in Figs 4 to 6. The main components of all fats are the fatty acids, which may be saturated, monounsaturated or polyunsaturated. Saturated and monounsaturated fats are not necessary in the diet as they can be synthesized by the human body. Two polyunsaturated fatty acids that cannot be synthesized by the body are linoleic acid and linolenic acid. These must be provided by diet and are known as essential fatty acids. The fatty acid composition of *E. camadulensis* was 41.83 % saturated (SFA), 36.35 % monounsaturated (MUFA) and 21.20 % polyunsaturated (PUFA) while that of *E. citriodora* was 40.84 % (SFA), 37.79 % (MUFA) and 21.36 % (PUFA). In both samples, palmitic acid accounted for more than 70 % of the saturated fatty acids, however, the quantity of palmitic acid in both leaves are lower than 41. 78 % palmitic acid in commonly consumed palm oil. The two species have lower level of SFA than 46.34 % in palm oil (Chowdhury *et al*.,2007) The proportion of unsaturated fatty acid is higher than saturation in both species. The leaves oils are very rich in oleic acid. Oleic acid has been reported as an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells (Carrillo *et al*., 2012) therefore, *E. camadulensis* and *citriodora* could have beneficial effects in inflammatory related diseases. The oils are also rich in bioactive linoleic acid, deficiency of the n-6 fatty acid linoleic acid leads to poor growth, fatty liver, skin lesions and reproductive failure (Connor *et al*., 1992) the Eucalyptus species could be a dietary source of this fatty acid in the prevention of the health related problems.

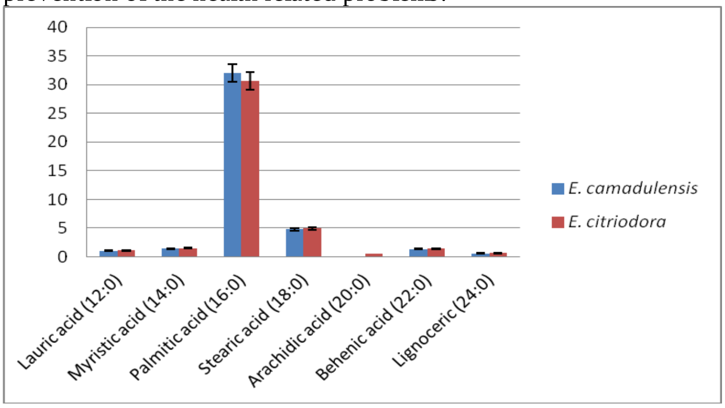

Fig. 4: Levels of saturated fatty acids (%) in leaves of *E. camadulensis* and *E.citriodora*

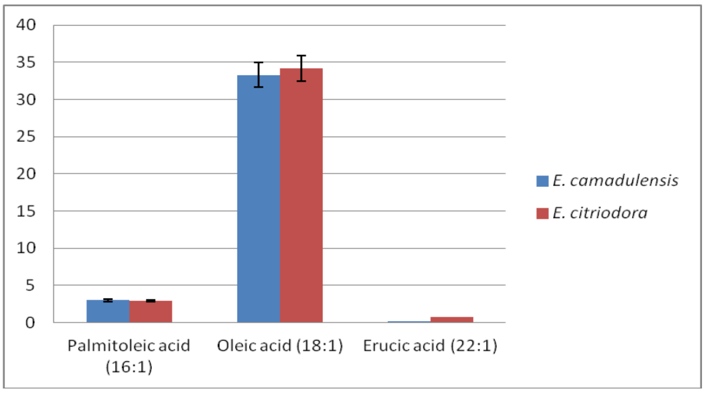

Fig. 5: Levels of monounsaturated fatty acids (%) in leaves of *E. camadulensis* and *E.citriodora*

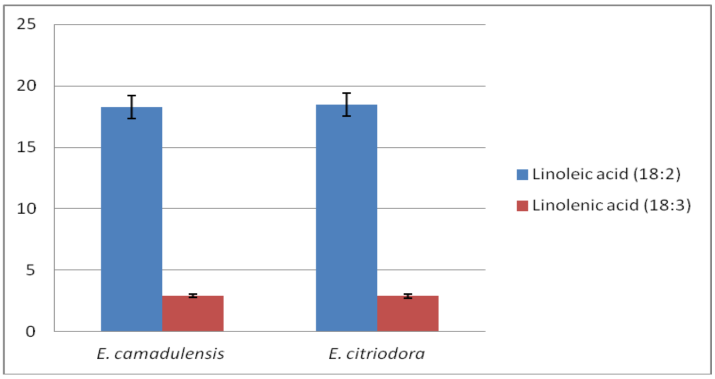

Fig. 6: Levels of polyunsaturated fatty acids (%) in leaves of *E. camadulensis* and *E.citriodora*

Conclusion

Eucalyptus citriodora and *Eucalyptus camadulensis* leaves will not yield only essential oils benefits but also nutritional benefits to its users. The leaves may serve as a constituent of human diet supplying the body with minerals and dietary fibre. The composition of their fixed oil are rich in bioactive nutraceutical such as oleic and linoleic acid. The two species could therefore be exploited as food supplement for their nutrient potentials.

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