The Composition and Antifungal Properties of the *Erythrophleum Suaveolens* Guill and Perr. (Leguminosae) Seeds and Oil

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Abstract: This study investigated the composition of the *Erythrophleum suaveolens* seed and oil, as well as the antifimgal activity of the oil extracted on some dermatophytic fungi were analyzed. The moisture content of the healthy *E. suaveolens* seeds was 7.50 + 0.61%; while the oil yield (quantity of oil) was 36.00 + 0.34%; 17.50 + 1.28% protein and 27.37 + 0.86% crude fiber. The oil extracted from the *E. suaveolens* seed (quality of oil) was edible and non-rancid with free fatty acid value of 2.17 + 0.34%; peroxide value of 2.57 + 0.89 meq/kg; iodine value 3.14 + 0.68%; unsaponsifiable matter of 16.54 + 0.40 g/kg and refractive index of the oil at 40 °C was 1.52 + 0.32. The oil showed significant antifungal activity against the dematophytes tested *Aspergillus flavus*, *A. niger*, *A./vanity*, *Candida albicans* and (*Microsporum gyseum*), above the 10 mm recommended standard for a good inhibition. The antifungal activity of the orthodox antibiotic, Nystatin, was significantly higher than that of oil on the fungi tested. [Life Science Journal. 2006;3(4):61 - 64] (ISSN: 1097 - 8135).

Keywords: antifungal activity; *Erythrophleum suaveolens*; seeds; oil quality; heavy metals

Abbreviations: AAS: atom absorption spectrometer; FFA: free fatty acid; SDA: saboraud dextrose agar

1 Introduction

The tropical rainforest in Africa is endowed with yet fully exploited economic trees. One of such trees is *Erythrophleum suaveolens* Guill and Perr. in the family lequninosae, commonly called sasswood or red water wood. It is called "Ingi" or "orachi" in Igbo and "Emin" or "Obo" in Yoruba[1]. In Nigeria, *E. suavelons* is cultivated in the southeastern states and eaten mainly by Igbos. The seeds of *E. suaveolens* are used as a soup thickener for various dishes. The seed of *E. suaveolens* are relatively cheap to purchase thus used by natives to replace melon "egusi" seeds in preparing the staple vegetable soup in the southeastern Nigeria. In Uganda, the fruit is a favorite food of elephants and they are reported to be responsible for dispersing the seeds[2]. The bark of *E. suaveolens* tree is used as an arrow poison. In large does, the bark extracts occasions a progressive loss or mental reflexes, when applied to animal, that later leads to muscular relaxation, paralysis of the heart and eventual death. Analysis of the bark extracts yields an alkaloid called erytrophleine[3]. *E. suaveolens* bark extracts was claimed to be used in the treatment of heart disease traditionally in Nigeria, though not adopted in modern medicine, but it is said to be of use in the treatment of spasmodic asthma[2].

Seeds are usually one of the sources of the propagation of plants as well as source of food[4]. During harvesting and storage, fungi, bacteria, insects etc attack seeds. Some workers have studied the nutritive value of seeds, which include groundnuts[5], maize[6], palm kernels[7], cocoa beans[8], and melon seeds[9]. These workers have helped evaluate the nutritive value of the seed and seed oil quality. To confirm the identity of most oils and fats, it is normally considered sufficient to determine the iodine value, saponification value, unsaponifiable matter, free fatty acid (FFA) value and peroxide value coupled with qualitative tests for appropriate adulterants[10]. The rancidity of the oil will also indicate the quality of the oil and affect its uses for soap, cream production and edibility.

The FFA and peroxide value can be used to measure rancidity of the oil[11,12]. The composition of the seed, and oil extracted from the seed of *E. suaveolens*, as well as the antifungal activity of the extracted oil have not been reported in literature.

As a continuation of studies in this laboratory on the composition of indigenous African seed plants food, the composition of the *E. suaveolens* seed and its oil is presented here. Also reported are some of the biochemical properties (Saponification value, unsaponifiable matter, peroxide, FFA value and Iodine value), and antifungal properties of the oil extracted from the *E. suavelons* seed. This is

to help access and document the quality of some African foods with respect to *E. suaveolens* seeds.

2 Materials and Methods

2.1 Source of plant materials

The seeds of *E. suaveolens* (1,000 g) were collected from Oyingbo market in Lagos State, Nigeria. The seeds were packed in polythene bags and stored in a refrigerator prior to use. The percentage moisture content of the seeds was determined at 103 °C for 17 hours as described by Agrawal[13].

2.2 Composition of *E. suaveolens* seed

The percentage carbohydrate content of the *E. suaveolens* seed was determined using the methods of Egan *et al*[14]. The methods of Lowry *et al*[15] was used to determine the percentage protein content of *E. suaveolens* seed, while the crude fiber content of the seed was determined using Diamond and Denman methods[16].

2.3 Extraction of oil

The method of extraction of oil is *E. suaveolens* seed was adopted from the oil extraction methods of Egan *et al*[14]. The seeds were ground using a ceramic pestle and mortar before blending in an electric blender. An amount of 20 g of the ground seed was packed into the extraction thimble before covering with a small ball of cotton wool. The thimble was inserted in a quick fit plain body soxhlet extractor. Petroleum either in the quantity of 200 ml (60 – 80 °C) was poured in a 250 ml round-bottom flask of known weight, which was connected to the extractor, and refluxed on an electric thermal heater for 5 hours. The ether was then collected in the plain body extractor and then separated from the flask that contained oil. The flask containing the oil was then heated in an oven at 103 °C for 30 minutes. It was cooled and weighed to get the final weight. The percentage oil content of the sample was calculated using the ratio of the amount of oil produced to the weight of sample used expressed as a percentage. The procedure was repeated until at least 250 ml of essential oil was extracted from the seeds.

2.4 Biochemical properties of the *E. suaveolens* seed oil

The quantity of oil extracted from the seed was determined as a percentage of the oil extracted, expressed over the weight of the seed used as described by Diamond and Denman[16]. The method of Anonymous[17] was used to determine the quality of oil extracted from the seeds, the saponification value, unsaponifiable matter, peroxide value and iodine value. The FFA value was determined according to the method of Egan *et al*[14].

2.5 Heavy metal determination

The method of Solomon[18] was used in the heavy metal determination. An amount of 4.4413 g of the ground seed was weighed into an already made carbon that was charging. This was then put into flurothen furnace at 580 °C for one hour at ash the seed. The ash was dissolved with 10 ml-distilled water in a 100 ml volumetric flask, and 1 ml of HCL was added and gently shaken for proper homogenization. The volume of the solution was made up to 100 ml mark of the flask using distilled water. The solution was then placed in an atom absorption spectrometer (AAS) to aspirate for the presence of elemental metal. Aspiration was done by putting hallow cathode lamp for each metal and the concentration of each heavy metal was measured against a standardized grade[19].

2.6 Antifungal activity of the oil extracted from *E. saveolens*, seeds

A modification of the paper disc diffusion method of Irobi and Daramola[20] was used. Spore or conidia suspension of $10^5 - 10^7$ cells were counted using haemocytometer. About 10 ml Saboraud dextrose agar (SDA) were poured into Petri dishes and allowed to solidify. A micro-pipette was used to introduce 0.1 ml of the spore or conidia suspensions onto the agar plate; spreading was done with a spreading rod under sterile conditions. The fungi, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus wentii Candida albicans* and *Microsporum gyseum* were obtained from infected skin of a patient at the College of Medicine, University of Lagos, Nigeria. Sterilized paper discs (6 mm, Whatman No, AA 201 7006) were soaked in *E. suaveolens* seed oil for 6 hours. Four of these soaked discs were spread on a fungal inoculum seeded plate with the help of sterile forceps. There were two controls, the first contained the SDA and Fungal inoculum but the discs were soaked in the antibiotics, Nystatin (l00 mg/ml). Three replicates were produced for each Fungus per treatment. All the plates containing the discs were then incubated at 28 – 30 °C. The zone of inhibition was measured after 48 – 72 hours of incubation. The experiment was repeated; the results were statistically analyzed to determine the standard errors[21].

3 Results

The moisture content of *E. suaveolens* seeds used in this study was 7.50 + 0.61%, while the mean oil content (quantity of oil) of the seeds was 36.00 + 0.34%. The FFA content of the *E.*

suaveolens seed oil was 2.17 + 0.14%; peroxide value was 2.57 + 0.8% meq/kg; iodine value was 3.14 + 0.68%; saponiflcation value was 904.81 + 19.18 mg/kg; unsaponiflable matter was 16.54 + 0.40 g/kg and refractive index of the oil at 40 °C was 1.52 + 0.23. Health *E. suaveolens* seeds contain 9.30 + 0.25% carbohydrate and 17.50 + 1 − 28% protein.

The summary of the heavy metal composition of *E. suaveolens* seed is shown in Table 1. The crude fiber content of the seed is 27.37 + 0.86%. The oil extracted from *E. suaveolens* seed showed antifungal activity against the dermatophytes tested, and the zone of inhibition was above 10 mm. The antibiotic, Nystatin, had zone of inhibitions higher than the oil (Table 2).

Table 1. Percentage heavy metal content of *E. suaveolens* seeds

Parameter	Percentage heavy metal content of seeds(%)
Zinc (Zn)	0.003
Iron (Fe)	0.060
Lead (Pb)	N.D*
Copper (Cu)	N.D
Nickel (Ni)	N.D
Sodium (Na)	N.D
Potassium (K)	0.250

* N.D means not detected

Table 2. Antifungal activity of the oil extracted from *E. suaveolens* seeds

Sample	Zone of Inhibition (mean + JS.E mm) Fungi				
	Aspergillus flavus	*Aspergillus niger*	*Aspergillus ventii*	*Candida albicans*	*Microsporum gyseum*
Control	0.00 + 0.00a*	0.00 + 0.00a	0.00 + 0.00a	0.00 + 0.00a	0.00 + 0.00a
Nystatin	26.69 ± 0.246	26.89 ± 0.31b	26.38 + 0.72b	28.00 ± 0.55c	27.89 ± 0.346c
Oil extracted from *E. suaveolens*	11.19 ± 0.89d	10.06 ± 0.10d	14.00 + 0.37e − *	11.00 ± 0.18d	14.05 ± 0.08e

* Zone of inhibition with similar letters show no significant difference at $P = 0.01$
Zone of inhibitions with different letters show significant difference at $P = 0.01$

4 Discussion

This investigations showed that the oil extracted from *E. suaveolens* seed is edible, because the qualitative properties of the oil fits the description of edible oil by Kirk[11] and International Seed Testing Association[22]. *E. suaveolens* seed has a good oil yield of 36.00% and comparable to the oil yield of *Arachis* of 38.50% and *Glycine soja* of 36.40%[10]. The peroxide value of *E. suaveolens* is 2.57 meq/kg. Perl and Krestchemer[23] explained that peroxide value below 10 meq/kg showed that the oil involved is a non-rancid oil. The FFA content of the *E. suaveolens* seed oil is 2.17% and below the 5.00% FFA content recommended for non-rancid oil[24,25], implying that the oil of *E. suaveolens* seed is non-rancid. The high saponification value of the oil indicates that it could be used as a base for soap manufacture. Ekundayo and Idzi[26] explained that saponification value has an inverse relationship with the chain length of the fatty acid in the oil, that is the higher the seed oil saponification value the lower the chain length and *vice versa*.

The oil from *E. suaveolens* seed probably has a broad-spectrum antifungal activity. The zone of inhibition is above the 10 mm mark recommended by Zygadlo and Grosso[27]. Even though the antifungal activity of the *E. suaveolens* seed oil is lower than that of the check antibiotic, Nystatin, it is of significance that the oil has antifungal properties, which provide an indication that probably soup made from *E. suaveolens* seed could be medicinal. The fact that the seed oil was not purified might be responsible for its lower antifungal activity to the nystatin. If the seed's oil active ingredient is isolated and used, it might be more potent than the nystatin at the same concentration.

The heavy metal composition of the seed sample used, had very low heavy metal component below the 0.95% heavy metal value, which is regarded as safe food for human consumption[28]. In some cases a few elements were not detected during the analysis.

These results indicate that the oil *E. suaveolens* seed is edible and non-rancid. It also shows that the oil is non-toxic (does not contain heavy metal) for human consumption, which can also be used as a base for body or hair cream production. Results here suggest that the *E. suaveolens* seed oil is fungi toxic and probably have a broad-spec-

trum antifungal property. The study also provides some evidence that the *E. suaveolens* seed oil is of high nutritional value, a justification for its consumption by the African natives.

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