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Research Article

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Extraction, Characterization and Antibacterial Screening of Oil from the Heartwood of *Pterocarpus Erinaceus* (African Rosewood Powder)

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ABSTRACT

Oil was extracted from Pterocarpus erinaceus (African rosewood) powder with n-hexane using a soxhlet apparatus. The percentage yield of the oil was 5.1%. The oil had saponification value of 144 mg KOH/g; iodine value of 3.03 g I2/100 g; acid value of 3.78 mg KOH/g; peroxide value of 3.4 m Eq/Kg; refractive index of 1.473 and specific gravity of 0.7424. Thin-layer chromatography of the oil with hexane/chloroform (3:1) revealed three different components with retention factors of 0.4328, 0.2388 and 0.1493. The components of the oil were determined using Gas Chromatography-Mass Spectrometry. The fatty acids detected include palmitic acid, linoleic acid and stearic acid. Some saturated hydrocarbons were also identified. The antibacterial activity of the oil was tested on Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella dysenteriea, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella spp and Salmonella typhae using Agar-diffusion method. The results of the physicochemical parameters and antimicrobial activity study show that the oil obtained from Pterocarpus erinaceus powder is non-drying oil containing saturated hydrocarbons and fatty acids. It can find application in the soap and cosmetic industries. It can also be used as hydraulic brake fluid and as lubricant, but it cannot be used as antibacterial agent.

Keywords: Soxhlet apparatus; Physicochemical parameters; Mass spectrometry; Antibacterial activity; Fatty acids; Iodine value; Saponification value

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INTRODUCTION

Fats and oils usually contain mixtures of triglycerides in different proportions. These triglycerides are known to vary according to the species of the plant and the part of the plant where they are found [1]. The characteristics of the triglycerides present in oil usually determine the properties of the oil [2]. Oils can be put to different use varying from industrial application as fluids and lubricants to edible purposes such as food. The diverse applications for which oils can be used depend on the yield obtained, the composition of the oil and their physical and chemical properties [3]. Physicochemical measurements such as iodine value, saponification value, peroxide value, acid value, free fatty acids, can give information about the degree of saturation of fats and oils; their degree of rancidity, their edibility and their suitability for industrial applications. The combined separation and analytical technique, Gas Chromatography-Mass Spectrometry (GC/MS) can be used to characterize fatty acids in fats and oils (triacylglycerols) from plant and animal sources as their fatty acid methyl ester derivatives [4]. Gas chromatography (GC) is employed to separate the fatty acid methyl esters while mass spectrometry (MS) is used to identify the individual components separated.

Pterocarpus erinaceus is known as African rosewood or African teak. Other common English names include African kino, barwood, black camwood, Gambia gum, molompi wood tree, Senegal rosewood, West African kino and West African rosewood [5]. *Pterocarpus erinaceus* is native to Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan and Togo [5]. It has a wide range of applications. Its leaves and seeds are edible when cooked properly. Its foliage and immature pods serve as feed for sheep, goats, cattle and horses. Leaves are used in abortifacient mixtures and as a febrifuge. In Northern Nigeria, the bark is occasionally an ingredient in abortifacient prescriptions. It is used in injection for urethral discharges. A decoction of the bark and resin is used as an astringent for severe diarrhoea and dysentery. The pulverized bark is mixed with kola nut and taken in regular doses as a restorative. The grated root can serve as cough remedy [5]. Considering the vast usefulness of *Pterocarpus erinaceus* especially for therapeutic purposes, the research work is aimed at extracting oil from the heartwood of *Pterocarpus erinaceus*, characterizing the oil and subjecting the same to antimicrobial screening.

MATERIALS AND METHODS

Soxhlet apparatus was used for the extraction. The solvent used is n-hexane. Reagents used for the determination of the physicochemical properties include tetrachloromethane, Wij's solution, potassium iodide, sodium thiosulphate, starch indicator, distilled water, alcoholic potassium hydroxide, phenolphthalein, hydrochloric acid, diethyl ether, ethanol, sodium hydroxide, glacial acetic acid and chloroform. *Pterocarpus erinaceus* powder (locally called *osun*) was obtained from herb sellers at *Oja Oba* market in Akure. It is reddish and usually gotten from the heartwood of the tree. The dried, ground and weighed sample was packed into a defatted white handkerchief and loaded into the thimble of a soxhlet. Hexane was used as the extracting solvent and the extraction lasted six hours, after which hexane was distilled off. The extract was poured in the beaker and the remaining solvent was left to evaporate. The oil obtained was yellow and partly solidified after a few hours. The percentage yield was calculated. The oil was stored in a glass bottle and kept in a refrigerator.

The solutions used to determine the physicochemical properties of *Pterocarpus erinaceus* oil were prepared and standardized according to methods described by Pearson [6] and AOAC [7]. The physicochemical parameters determined are iodine value, saponification value, acid value, peroxide value, refractive index and specific gravity.

Iodine Value

Iodine value was determined by Wij's method according to Pearson [6]. 10 ml of carbon tetrachloride was added to the oil samples. 20 ml of Wij's solution was also added. The blank was prepared concurrently by adding carbon tetrachoride and Wij's solution without the oil. 15 ml of 10% potassium iodide solution was added to the mixtures after they were allowed to stand in the dark for 30 mins. 100 ml distilled water was also added. The mixtures were then titrated with 0.1 M sodium thiosulphate using starch as indicator before the end point. The titre value was taken when the yellow solution turned milky. The Iodine value was then calculated thus:

$$Iodine\ value = \frac{(b-a)\times 1.269}{weight\ of\ sample(g)}$$

b represents the titre value for the blank while a stands for the sample.

Saponification Value

Alcoholic potassium hydroxide solution was first prepared according to the method described by Pearson [6]. 0.33 g of the oil was weighed into a round bottom flask and alcoholic KOH solution was added. A reflux condenser was attached to the flask and the flask was heated in boiling water for one hour. 1 ml of phenolphthalein (1%) solution was added and the mixture was titrated with 0.5 M HCl. The titre value was recorded as *a* ml. A blank was carried out at the same time with titre value of *b* ml. Saponification value was then calculated as:

$$Saponification\ value = \frac{(b-a)\times 28.05}{weight\ of\ sample(g)}$$

Acid Value

The acid value was evaluated according to Pearson [6]. 25 ml diethyl ether was mixed with 25 ml ethanol and 1 ml of 1% phenolphthalein solution. 1g of the oil was dissolved in the mixture and titrated with aqueous 0.1 M NaOH. The mixture was shaken constantly until a persistent pink colour was obtained. Acid value was calculated as:

$$Acid\ value = \frac{Titre\ Value \times 5.61}{Weight\ of\ Sample(g)}$$

Peroxide Value

The peroxide value was determined according to AOAC [8]. About 1 g of the oil was weighed into a dry boiling tube. 1 g powdered KI and 20 ml of solvent mixture (glacial acetic acid and chloroform) were added to the oil in tube. The tube was placed in boiling water for not more than 30 seconds. The contents of the tube were quickly poured into a flask containing 20 ml of 5% of KI solution. The boiling tube was washed out twice with 25 ml water and titrated with 0.002 N $Na_2S_2O_3$ solution using starch as indicator. A blank determination of the reagent was also performed. Peroxide value was obtained using the formula:

$$Peroxide\ value = \frac{2(v_1 - v_2)\ mEq\ /\ kg}{weight\ of\ sample(g)}$$

V_2 represents the blank titre value while V_1 is the sample titre value.

Refractive Index and Specific Gravity

Abbe Refractometer was used to measure the refractive index of the oil at 20°C [7]. The prism was removed with the aid of the screw head and cleaned with a tissue paper moistened with ethanol. A drop of the oil was then introduced to the prism and it was placed back in the refractometer. The instrument was allowed to stand for a few minutes before taking the reading to ensure the regularization of the temperature of the oil and that of the instrument. The model of the Abbe Refractometer used is No. A81015, Bellingham Stanley Limited, England. The specific gravity bottle was weighed. The bottle was filled with 1 ml distilled water at room temperature and weighed again. The difference in weight gave the weight of water. The water was poured away and the specific gravity bottle was cleaned and dried. It was weighed again before 1ml of the oil was then poured into it and weighed at room temperature. The difference in weight taken gave the weight of oil. The various weights taken were recorded. Specific gravity was calculated as:

$$specific\ gravity = \frac{weight\ of\ oil(g)}{weight\ of\ water(g)}$$

GC/MS

The GC/MS analysis of the oil was done at National Research Institute for Chemical Technology (NARICT) in Zaria, Nigeria, with GCMS-QP2010 PLUS, SCHIMAZU JAPAN. The components of the oil were identified using their retention time indices and mass spectra in reference to those contained in the National Institute of Standards and Technology (NIST) library.

Antimicrobial Study

The agar-diffusion method was employed for the antibacterial test [9,10]. The activity of oil was tested on *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella dysenteriea, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella spp and Salmonella typhae*. A solution of nutrient agar was prepared in a conical flask by dissolving 2.8 g of agar in 100 ml of water. The conical flask was covered with cotton wool and foil paper and the solution was shaken. The nutrient agar solution was then put in autoclave for 30 minutes. The agar solution was then removed and allowed to cool. The plates were seeded with microorganisms and labeled accordingly. The cooled nutrient agar was poured in the plates, which were swirled gently to mix the micoorganism with the agar. After 15 mins of setting at room temperature, a hole was punctured in the set nutrient agar in the plates with the aid of a syringe tube. The punctured portions were removed and the extracts were applied to the holes created. The culture was incubated for 24 hours at 4°C for the growth of the indicator strain, after which they were observed for microbial inhibition.

RESULTS AND DISCUSSION

Extraction and Percentage Yield

The soxhlet extraction of *Pterocarpus erinaceus* powder with hexane gave a yield of 5.1%. This yield is low as expected because the oil was extracted from the dye of the plant and not the seed which usually contains a higher percentage of oil in most plants [11]. The results of the determination of the physicochemical properties of the oil are summarized in Table 1.

Table 1: Physicochemical characteristics of the oil extracted from the heartwood of *Pterocarpus erinaceus*

Physicochemical Parameter	Value
Percentage yield	5.10%
Iodine Value	3.03 gI_2/100 g
Saponification Value	144.5 mgKOH/g
Peroxide Value	3.4 mEq/Kg
Acid Value	3.78 mgKOH/g
Refractive Index	1.473
Specific Gravity	0.7424

Iodine Value

The Iodine Value obtained (3.03 gI_2/100 g) shows that the oil is rich in saturated compounds and will be quite stable against oxidation and rancidification [12]. However, the iodine value is low when compared to other saturated vegetable oils such as coconut oil (6.3-10.6 gI_2/100 g), babassu oil (10-18 gI_2/100 g) and palm oil (50-55 gI_2/100 g) according to Codex Alimentarius Commission [13]. Some refined oils have been reported to have low iodine values comparable to this. An example is hydroxylated African oil bean whose iodine value is 3.06 gI_2/100 g [14].

Pterocarpus erinaceus oil can be classified as non-drying oil as it is less than 100 g I_2/100g and non-drying oils have been reported useful in the manufacture of soaps [15]. It can also find extensive use as lubricants and hydraulic brake fluids, but it is not suitable in the paint and coating industry [16]. Since high degree of saturation renders the oil very stable against oxidative rancidity and confers on the oil a long shelf life, the oil can be safely stored at room temperature [17,18]. Coconut oil and palm kernel oil have been known for their use in high heat cooking. *Pterocarpus erinaceus* oil stands the chance to be stable for use at high temperatures because of its low iodine value.

Saponification Value

The saponification value obtained (144.5 mg KOH/g) is lower than that of other saturated oils like coconut oil (248-265 mg KOH/g oil), babassu oil (245-256 mg KOH/g oil) and palm oil (190-209 mg KOH/g oil) according to Codex Alimentarius Commission [13]. However, the saponification value obtained is higher than that of beeswax (93 mg KOH/g) [19] and lower than that of *Demettia tripetala* fruit oil (pepper fruit) 159.33 mg KOH/g [20] which are commonly used in soap making. It can be safely inferred that *Pterocarpus erinaceus* oil will be suitable for soap production since its saponification value falls within the range of these oils and it is also close to that of African Pear oil 143.76 mg KOH/g which is good for soap making [21]. Besides, the saponification value obtained is also close to that of *Ricinodendron heudelotii* (Honey plum) which is 145 $\pm$ 5 mg KOH/g suggesting a preponderance of glycerides of low molecular weight [22]. This makes the oil useful in the manufacture of soaps, detergents and lather shave creams [23,24].

Peroxide Value

The peroxide value of *Pterocarpus erinaceus* oil obtained is 3.4 mEq/Kg of sample. This value is below 10 which characterizes the majority of conventional oils [18]. However, this low peroxide value can be attributed to the high degree of saturated bonds in the oil as indicated by the iodine value.

It is close to the Peroxide value of Jatropha oil (5.1 mEq/Kg) [25]. The range within which the onset of rancidity is generally observed is 20-40 mEq/Kg [6,25]. Peroxide values higher than 10 to 20 mEq O_2/Kg are commonly interpreted as rancidity. Oils with high peroxide values are unstable [26].

Acid Value

The acid value obtained (3.78 mg KOH/g) is higher than that of Palm Kernel seed oil (0.834 + 0.004 mg KOH/g) reported suitable for soap production [27] and lower than that of *Demettia tripetala* fruit oil (pepper fruit) 5.34 + 0.04 mg KOH/g also suitable for soap production [20].

The acid value therefore falls within the range suitable for soap production. The lower the acid value of oil, the fewer free acids it contains, which makes it less exposed to the phenomenon of rancidification [28]. The maximum acceptable acid value as recommended by the Codex Alimentarius Commission for groundnuts is 4 mg KOH/g, which is also the maximum acid value recommended for cold pressed and virgin oil [18].

Refractive Index

The value obtained is 1.473. This is slightly higher than the refractive indices of coconut oil (1.448-1.450 at 40°C), babassu oil (1.448-1.451 at 40°C) and palm oil (1.454 -1.456 at 50°C). The value is however closer to the refractive Index of breadnut oil 1.469, which agrees with values, obtained for some Nigerian fruits and seeds [17].

Specific Gravity

The value obtained (0.7424) is below the specific gravity of coconut oil (0.924 at 15°C), Diesel fuel oil 20 to 60 0.820-0.950 at 15°C, fuel oil (0.890 at 60 F) and kerosene (0.817 at 60 F). However, it is slightly above that of natural gasoline and the one used in vehicles which are 0.711 and 0.737 at 60 F respectively [29].

Thin-layer Chromatography

The mixture of hexane and chloroform (3:1) gave the best separation among the solvent systems employed. The chromatogram showed three spots, with retention factors (R_f values) of 0.4328, 0.2388 and 0.1493.

GC/MS

GC/MS analysis of the oil of identified two classes of compounds: alkanes and fatty acids in the form of their esters. The alkanes are of higher percentage. The three fatty acids detected include palmitic acid, linoleic acid and stearic acid at retention times of 15.3 min, 16.9 min and 17.2 min respectively (Table 2). Linoleic acid and palmitic acid are often used as indicators of oil deterioration because of their sensitivity to oxidation. The low values obtained for these two fatty acids confirm the resistance of *Pterocarpus erinaceus* oil to oxidation [30]. Palmitic acid is expected as it has been reported to be one of the most common saturated fatty acids found in plants and animals, which is useful in soap production [31]. Both palmitic and stearic acid find application in the production of detergents, soaps and cosmetics. Linoleic acid is known to impart nutritional and dietetic properties to oil. It belongs to one of the two families of essential fatty acids needed by humans and animals [32]. *Pterocarpus erinaceus* oil only has three out of the seven important fatty acids found in commonly used oils. Myristic acid, lauric acid, oleic acid and linolenic acid are absent. The high percentage of hydrocarbons shows the potential of the oil for use as fuel. The hydrocarbons detected include: decane, dodecane, 2,6,7-trimethyldecane, 7-methyltridecane, tridecane, 2,6,10-trimethyldodecane, tetradecane, n-hexadecane, pentadecane, cetane/n-hexadecane, 2,6,10-trimethyldodecane, n-hexadecane, n-pentadecane and n-heptadecane (Table 3).

Table 2: Retention time and index of the compounds in the oil extracted from the heartwood of *Pterocarpus erinaceus* as shown by GC/MS

Line	Compound	Retention Time (min)	Retention Index
1	Decane	5.6	1015
2	Dodecane	6.9	1214
3	2,6,7-trimethyldecane	7.1	1121
4	7-methyltridecane	7.9	1349
5	Tridecane	8.3	1313
6	2,6,10-trimethyldodecane	9.3	1320
7	Tetradecane	9.6	1413
8	n-hexadecane	10.4	1612
9	pentadecane	10.8	1512
10	cetane/n-hexadecane	12	1612
11	2,6,10-trimethyldodecane	13.2	1320
12	Palmitic acid, methyl ester	15.3	1878
13	n-hexadecane	16.2	1612
14	Linoleic acid, methyl ester	16.9	2093
15	Stearic acid, methyl ester	17.2	2077
16	n-pentadecane	18	1512
17	n-heptadecane	19.7	1711

Table 3: Mass spectrum of the oil extracted from the heartwood of *Pterocarpus erinaceus*

Line	Compound	Molecular weight	Base peak (Intensity)
1	Decane	142	43.00 (61052)
2	Dodecane	170	57.05 (172911)
3	2,6,7-trimethyldecane	184	57.05 (107843)
4	7-methyltridecane	198	57.05 (122807)
5	Tridecane	184	57.05 (296076)
6	2,6,10-trimethyldodecane	282	57.05 (98374)
7	Tetradecane	198	57.05 (322523)
8	n-hexadecane	226	57.05 (183901)
9	pentadecane	212	57.05 (259461)
10	cetane/n-hexadecane	226	57.05 (218747)
11	2,6,10-trimethyldodecane	212	57.05 (75110)
12	Palmitic acid, methyl ester	270	74.05 (140009)
13	n-hexadecane	226	57.05 (42557)
14	Linoleic acid, methyl ester	294	67.05 (63654)
15	Stearic acid, methyl ester	298	74.05 (60177)
16	n-pentadecane	212	57.05 (28282)
17	n-heptadecane	240	57.05 (24709)

Antimicrobial Activity

Pterocarpus erinaceus oil was tested against both Gram positive and Gram negative bacteria. The bacteria include *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Shigella dysenteriea*, *Pseudomonas aeruginosa*, *Klebsiella spp* and *Salmonella typhae*. *Pterocarpus erinaceus* oil was not effective against the bacteria as no zone of inhibition was observed. This result is similar to other antibacterial studies where the hexane extract had no inhibition at all, but more polar extracts might show some activity [33].

CONCLUSION

The oil of *Pterocarpus erinaceus* can be classified as non-drying oil and can find application in the soap and cosmetic industry. It can also be used as lubricants and hydraulic brake fluids but it is not suitable in the paint and coating industry. It is stable against oxidative rancidity and therefore has a long shelf life. The oil is a potentially edible oil because of the fatty acids detected and its low acid value. The oil is not effective against bacteria. GC-MS analysis shows that the oil has a large percentage of hydrocarbons, making it a potential source of fuel. However, the oil also contains palmitic, stearic and linoleic acids.

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