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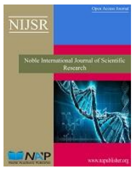

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Antibacterial, Compositional and Physicochemical Properties of *Guizotia Scabra* (Vis.) Chiov. Seed Oil Found on Jos-Plateau-Nigeria

Article History

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Abstract: The oil extract of G. scabra seeds was analyzed for its antibacterial, chemical and physical properties. Physical and chemical properties analyzed included moisture content (0.91%), acid value (13.65%), iodine value (132.27), peroxide value (18.58), saponification value (191.03), free fatty acids (6.86%) and density (0.87g/cm3). The oil was found to inhibit the growth of Staphylococcus aureus (SA), Escherichia coli (EC) and Salmonella typhi (ST) at an MIC of 50%. However, there was no effect on Shigella sonnei (SS). The fatty acid composition of the extracted oil was revealed using the Gas chromatography Mass spectrometry (GC-MS) method. A total of 18 components of the oil were identified. Oleic acid (27.07%), 10- undecenoic acid (19.99%), palmitic acid (10.56%) and stearic acid (6.22%) were detected as the dominant fatty acids in the G. scabra seed oil.

Keywords: Scabra, Seeds, Bacteria, Fatty Acid.

1. Introduction

Oils of plant origin have been largely used for food-based applications. They not only stand as non-polluting renewable resource but also provide wide diversity in fatty acids composition with varied applications. Vegetable oils constitute an important component of human diet. Prominent edible vegetable oils include those from soybean, canola, sunflower and groundnut (peanut). They serve as a source of edible fatty acids (saturated, monounsaturated and polyunsaturated), which play important roles in cellular metabolism as a way to store energy and making it available when required.

Vegetable oils are natural products with vegetable origin containing mixtures of esters derived from glycerol that have chains of fatty acid with 14 to 20 carbon atoms that have different degrees of unsaturation [1]. They have an important functional and sensory role in food products, because of their fatty acids composition and the fat-soluble vitamins (A, D, E, and K). They are also sources of energy and essential fatty acids like linoleic and linolenic which are responsible for growth and the health of organisms [2].

Guizotia comprises of 6-7 species distributed in the mountains of East Africa. Exception is the *Guizotia scabra*, which is found in the highlands of some east African countries, Nigeria and Cameroon [3]. In Nigeria, it grows wild and sometimes cultivated on the Jos Plateau where the leaves are eaten in soup or boiled and salted like cabbage. The seeds are pounded and used to garnish local bean meal called "bebal" or eaten raw. It is commonly called 'Kutul' by a number of ethnic groups in Central Plateau Senatorial District of Plateau state. Elsewhere in Africa, a leaf decoction is taken orally for stomach ache, gonorrhea, malaria, constipation, salmonella infection, ulcers, dyspepsia, gastritis, enteritis and syphilis. Leaf extract of *G. scabra* was found to be active against *Erwinia carotovora* L., bacteria responsible for Irish potato soft rot [4]. In Ethiopia, leaf of *G. scabra* is squeezed and its drop is applied to wound to aid healing [5].

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh plant of *G. scabra* was collected in November from Dong village- Jos North, Plateau State. It was identified at the Federal College of forestry, Jos Nigeria by Mr. Azila.

Healthy dried seeds sample of *G. scabra* were pulverized and stored in air tight polythene bags for subsequent use.

2.2. Extraction

The powdered seed of G. scabra was extracted with n-hexane using the Soxhlet Extractor at the boiling temperature of hexane.

2.3. Physical and Chemical Characteristics

Specific gravity of the oil was determined at 25°C with the help of a pycnometer. Moisture content was determined by AOAC [6] method. Chemical characteristics i.e. iodine value (Wijs), while the saponification value, acid value, percentage of free fatty acid (FFA), and peroxide value were determined according to the methods described elsewhere [7].

2.4. Invitro Antimicrobial Test Using the Agar Diffusion Method

Four microorganisms: *Staphylococcus aureus, Shigella sonnei, Escherichia coli, Salmonella typhi*. To inoculate the plate, one drop of the adjusted sub-cultured nutrient broth was applied to the surface of the nutrient agar and evened to cover the surface of the agars with microbes. One microbe was inoculated to one plate making a total of four plates for four microorganisms. After 30mins, three wells were punched on the plate using a sterile corn borer of 5mm diameter, one for the oil, 1 for negative control and 1 for positive control. A 0.1ml of the oil was dropped into each appropriately labeled well. Into the remaining two wells, distilled water and gentamycin of 10mg/ml concentration were introduced to serve as negative and positive controls respectively for the bacteria. The inoculated plates were left on the table for 1hour to allow for proper diffusion. Plates were incubated aerobically at 37^{o}C. Zones of inhibition produced after incubation was measured by linear measurement of diameter.

2.5. GC-MS Analysis

The GC-FID/MS analysis of *G. scabra* oil was performed on a Shimadzu GC-2010 Plus and on a Shimadzu GCMS-QP2010 Ultra, equipped with a Shimadzu auto injector AOC-20i.

3. Results

Table 1. Some Physicochemical properties of *G. scabra* oil

S/N	Parameters	Observed values
1	color	Light yellow
2	oil yield (%)	24.10%
3	Density (g/cm^3)	0.87
4	Acid value (mg KOH/g)	13.65
5	Iodine value (g I_2/100g)	132.27
6	Saponification value	191.03
7	Peroxide value (mEq/Kg) 18.58	18.58
8	Moisture (%)	0.91
9	Free Fatty Acids (%)	6.86

Source: Biochemistry Laboratory, NVRI, Vom-Plateau State-Nigeria

Table 2. Antibacterial Activity of *G. scabra* Oil Extract

	Concentration of extracts (%)/Diameter of zones of inhibition (mm)			
Bacterial isolates	100%	50%	25%	+C
SA	27	20	0	33
SS	0	0	0	27
EC	28	16	0	25
ST	34	30	0	30

Source: Bacteriology Laboratory, NVRI, Vom-Plateau State-Nigeria

Key: SA *Staphylococcus aureus*, SS *Shigella sonnei*, EC *Escherichia coli*, ST *Salmonella typhi*, +C positive control (Gentamycin 10mg/ml)

Table 3. Identified compounds of *G. scabra* seed oil

Peak #	Retention Time	Peak Area	Name of compound
1	15.464	0.24	Methyl-14-methylpentadecanoate
2	16.057	10.56	Hexadecanoic acid (palmitic acid)
3	16.994	2.19	13-Hexyloxacyclotridec-10-en-2-one
4	17.128	0.78	Methyl linoleate
5	17.175	0.57	Methyl-11-octadecenoate
6	17.401	0.39	Methyl stearate
7	17.778	27.07	Oleic acid
8	17.933	6.22	Stearic acid
9	18.532	4.71	9-octadecenal
10	18.947	3.52	2-monopalmitin
11	19.591	19.99	Undecyclenic acid
12	20.214	4.82	7.11-hexadecadienal
13	20.452	5.34	10-undecenal
14	20.842	3.08	2, 3-dihydroxypalmitate
15	21.570	1.53	1-(2, 2- dimethylcyclopentyl)ethanone
16	22.148	3.74	10-undecylchloride
17	22.429	4.96	(Z)-7-Tetradecenal
18	23.651	0.29	Squalene

Source: National Research Institute for Chemical Technology (NARICT), Zaria-Nigeria

4. Discussions

The WHO/FAO guideline sets the maximum allowable limit for edible oils quality parameters including moisture (0.2%), acid value (0.6 mg potassium hydroxide/g oil) and peroxide value of 10 mill-equivalents oxygen/kg oil [8]. The studied physicochemical properties of the crude oil from *G. scabra* seeds are presented in Table 1. The color of the oil was light yellow. It was an odorless liquid at room temperature. The percentage oil yield of the oil was 24.10%. This indicates that *G. scabra* seeds could be suitable for industrial production of vegetable oil. The relatively high saponification value showed that the oil has the potential for use in the soap industry [9]. The acid and Free Fatty Acid (FFA) values are used to show the level of rancidity of oils (edibility) as well as its suitability and applicability in paint production. With acid value of 13.65%, *G. scabra* seed oil might have undergone deterioration occasioned by the degradation of triglycerides. Although Consumption of rancid edible oil is unlikely to cause immediate health impact, it can significantly reduce the nutritional value of foods by degrading the essential fatty-acids and nutrients [10, 11]. The percent FFA value of 6.86% for the oil is higher than the allowable limit for FFA for edible oils 1.0- 3.0% [12]. The lower the FFA level, the better the quality of the oil for human consumption. The high level of FFA obtained for the *G. scabra* seed oil may be due to increased growth of microorganisms on the seeds which caused hydrolysis to take place in the presence of moisture [13]. The Iodine Value (IV) is a measure of the relative degree of unsaturation in oils. The greater the iodine value, the more the unsaturation and the higher the susceptibility to oxidation. Our results show that *G. scabra* seed oil had the value for IV (132.27) hence highly unsaturated. Hence, the observed higher iodine value in the oil indicated that it is likely to be healthy for consumption. Studies have recommended the switch from saturated to unsaturated fats because of the risk of cardiovascular disease associated with high consumption of saturated fatty-acids [10, 14]. Another important parameter used to assess the quality of vegetable oil is the peroxide value (PV), which is an indicator of the level of lipid oxidation. For *G. scabra* oil, Peroxide value of 18.58 mEq/Kg is only slightly higher than the Codex Alimentarius Commission [15] limit of 15 mEq/Kg. This can be attributed to the time gap between production and analysis, which was approximately 60 days. The exposure to light and elevated temperatures could have potentiated the formation of lipid peroxide molecules [16]. This is an indication that the oil is safe for human consumption if care is taken during handling processes.

The iodine value equals the number of grams of iodine required to saturate the fatty acids present in 100 grams of the oil or fat. Technically it is the value of the amount of iodine, measured in grams, absorbed by 100 grams of a given oil sample. Iodine values are often used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds are present in the fat [17]. *G. scabra* seed oil has Iodine value of 132.27g I_2/100g. Although the iodine value is used primarily in industry, it is of value to us because it gives an indication of the oil's stability and health properties and the higher the iodine value, the greater amount of unsaturation [18].

The inhibitory effect of *G. scabra* seed oil was tested on four bacterial strains namely: *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Shigella sonnei* (SS) and *Salmonella typhi* (ST). The best activity was achieved with the oil on *Staphylococcus aureus, Escherichia coli and Salmonella typhi* at 100% concentration and an MIC of 50% (Table 2). *Shigella sonnei*, however, showed no susceptibility even at 100% concentration.

GC-MS analysis of the seed oil revealed 18 compounds (Table 3), among which are four fatty acids. The most abundant fatty acids were the oleic acid (27.07%) followed by undecyclenic acid (19.99%), palmitic acid (10.56%) and lastly stearic acid (6.22%). Both oleic and undecyclenic acids are the two major unsaturated fatty acids which together account for a large chunk of total fatty acids in *G. scabra* seed oil. Oleic acid is a monounsaturated omega-9 fatty acid found in many healthy high-fat foods, including vegetable and animal sources which include olive oil, avocado oil, and macadamia nuts. Omega-9 fatty acids are a family of unsaturated fats that have a carbon-carbon double bond at the omega−9 position. Unlike omega-6 and omega-3 fatty acids, omega−9 fats are not “essential fatty acids” because they can be synthesized from unsaturated fatty acids. Oleic acid was demonstrated to induce beneficial anti-inflammatory effects on auto immune diseases [19, 20] protective effect on breast cancer and improvement of immune system function [21]. Therefore, consumption of *G. scabra* seed oil has great propensity to avail such nutritional and medicinal benefits. Undecylenic acid is a naturally occurring unsaturated fatty acid found in the castor bean and also as a product of human sweat glands. It is mainly used for the production of Nylon-11 and in the treatment of fungal infections of the skin, but it is also a precursor in the manufacture of many pharmaceuticals, personal hygiene products, cosmetics, and perfumes [22]. Undecylenic acid can effectively control skin fungal infection, but the mechanism of its fungal inhibition is unclear. undecylenic acid inhibited biofilm formation of *C. albicans* effectively with optimal concentration above 3 mM [23]. Its presence in *G. scabra* seed oil in large concentration means the oil can be used in the same manner.

5. Conclusion

G. scabra seed oil portrays good quantitative estimates of a number of physical and chemical properties of oil. This is an indication that it could be used effectively for industrial purposes. The anti-microbial screening of the oil reveals the fact that the growth of pathogenic microorganisms such as *S. aureus, E. coli* and *S. typhi* were remarkably inhibited even at an MIC of 50%. It also has the potential to be effective when used topically on fungal infections owing to its high content of undecyclenic acid.

Nutritionally speaking, *G. scabra* seed oil can be a notable source of fatty acids. Our results indicated that the oil extracted from *G. scabra* seeds grown in Jos-North Central Nigeria is a rich source of fatty acids such as oleic, palmitic and stearic, and is of high potential for being used for nutritional purposes.

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