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Research Article

Phytochemical Analysis and GC-MS Determination of *Lagenaria breviflora* R. Fruit

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ABSTRACT
The fruit of *Lagenaria breviflora* R. was obtained and subjected to methanol extraction. The chemical compound in the extract was analyzed using a Gas chromatography coupled with mass spectrophotomter (GC-MS). The screening of the fruit extract revealed important phytochemicals such as Phenols, Alkaloids Carotenoids and Flavonoids. The analysis of the GC-MS revealed that hydrocarbons as the most abundant compound in the extract and a total of 30 compounds were identified. The major components are octadecane (19.25%), hexacosane (10.9%), docosane (9.15%0, 2 methyl-E,E-3,13-octadecadienol (8.33%), heptadecane (8.18%), tricosane (4.36%), tridecane (3.71%), 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (3.63%), tetracontane, 3,5,24-trimethyl- (3.38) and 9,12-Octadecadienoic acid (Z,Z)- (2.64%). Some of the compounds obtained have been reported to have potentials for prophylactic and therapeutic treatment of diseases in both man and animals.

Keywords: *Lagenaria breviflora R.*, chemical compounds, phytochemicals.

INTRODUCTION

Plants are one of the most significant sources of medicines and most of the modern drugs used today are derived plant products[1]. Plant and plant products are commonly used ingredients in the practice of ethno medicine this is hinged on the fact that plants and their products are easily accessible, cheap and effective[2]. Numerous plants have been used to treat and prevent diseases in both man and animal especially in the rural areas, one of such numerous plants used in ethno medicine in West Africa is *Lagenaria breviflora* Robert. It is traditionally known as "Tagiri" in the South West of Nigeria, it belongs to the family Cucurbitaceae[3] and is one of those numerous plants with characteristic antibacterial and antiviral herbal remedies in local communities[4]. The family consists of diverse plants which grow in the temperate regions but it also thrives in the hot arid region of the world[5]. The family consists of 110 genera and 640 species[6]. There are about 21 genera existing in Nigeria of which many of them are of great economic importance[5]. *Lagenaria breviflora* plant is a perennial climber ascending to the forest canopy by means of axillary tendrils[5,7]. The leaves are simple, alternate and palmately veined scabrid and sandpapery[5,8]. The stem has an unpleasant scent when crushed, and a decoction prepared from it is used in Western Nigeria for headache and as a vermifuge[9]. The fruit colour is green with cream-coloured narrow blotches. The pulp is said to be bitter and the seeds can number up to 400 in an average-size fruit which contain fixed oil[5].

The fruit is use in treating and preventing diverse diseases, it is used to the treat cold[10] and schistosomiaisis in man[9]. According to[11], *Lagenaria breviflora* has a broad spectrum antibacterial activity. The use of *Lagenaria breviflora* in the treatment of digestive disorders, measles in man as well as wound antiseptics, and its use in the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry has been well documented[11,12,13]. Furthermore, the anti-fertility and erythropoiesis stimulating effects in experimental rats, likewise the miracidial and cercacidial activities of the fruit have also been reported[9,14,15].

Medicinal plants contains secondary metabolites also known as phytochemicals, which are responsible for their wide range of biological activities. These plant metabolites work through diverse mechanisms and may inhibit microorganism like bacteria and also provide clinical values for the treatment of infection as a result of resistant microbes[16]. Therefore, this study is undertaken to evaluate the proximate composition, phytochemicals and the active ingredients present in the fruit of *Lagenaria breviflora.*

MATERIALS AND METHODS

Plant specimen and collection

The whole fruit of *Lagenaria breviflora* Robert was harvested in February from Abeokuta environ. The fruit was identified and authenticated at Herbarium Unit of Botany Department, Federal University of Agriculture, Abeokuta, Ogun state.

Phytochemical screening of Lagenaria breviflora

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Table 1: Phytochemical composition of *Lagenaria breviflora* fruit.

Type of test	Observation	Phytochemical present
Ferric chloride test	bluish- green colour	Phenols
Dragendoff's test	reddish-brown precipitate	Alkaloids
sulphuric acid test	blue colour	Carotenoid
Shinoda'a test	pink or red colour	Flavonoids
H_2SO_4 and $KMNO_4$ test	faint pink color	Oxalate
Acetic Anhydride and H2SO4 test	blue, green rings	Terpenoids
Frothing test	honey comb froth	Saponin
HCL and ammonium thiocynate test	slightly brownish yellow colour	Phytate
Ferric chloride test	blue or greenish-black colour	Tannin

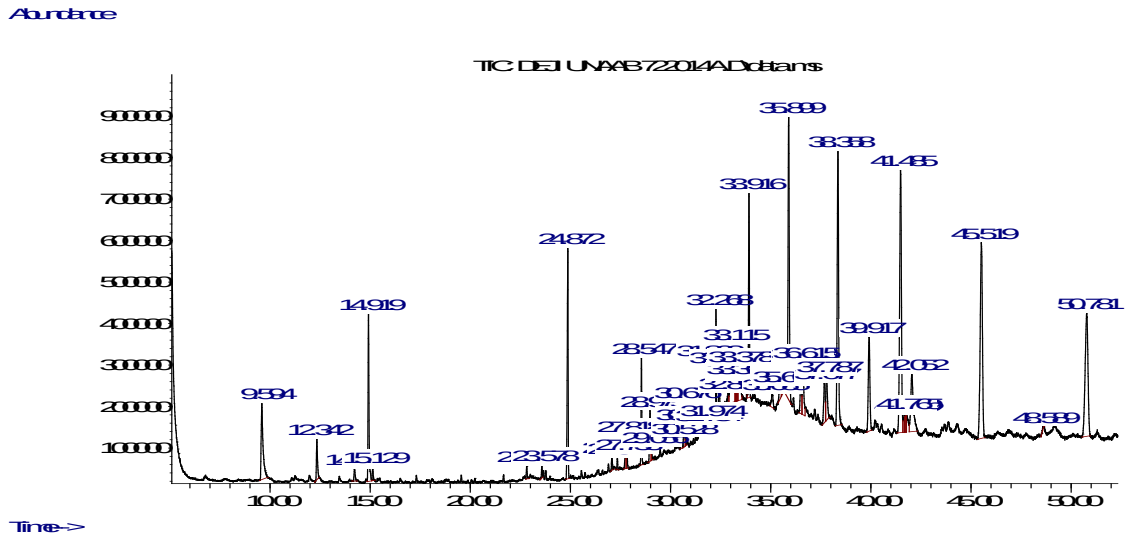

Figure 1: GC-MS chromatogram of methanol *Lagenaria breviflora* extract.

Phytochemical screening of the whole fruit was carried out using standard procedures to determine the presence of saponin, oxalate, tannin, terpenoid, alkaloid, phenol, carotenoid, phytate and oxalate content.

Dragendoff's test for alkaloid

To the extract, 1ml of dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate was taken as an evidence of the presence of alkaloids

Ferric chloride test for tannin

Three drops of diluted solution of FeCl3 was added to the extract, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins

Frothing test for saponin

The powdered leaves (0.5g) was placed in a test tube and 10ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins

Shinoda's test for flavonoid

Few magnesium chips were added to 3ml of the extract solution and 2 drops of dilute hydrochloric acid was added and warmed. A pink or red colour indicates the presence of flavonoids

Ferric Chloride test for phenols

The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and Potassium Ferro cyanide. Formation of bluish- green colour is taken as positive phenols.

Test for terpenoids

The extract was added to 2 ml of Acetic Anhydride and Concentrated H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids).

Test for carotenoids

The filtered solution of extract was treated with 85 % sulphuric acid. A blue colour at the interface showed the presence of carotenoids.

Test for oxalate

Sample was treated with 3N H_2SO_4 and then filtered. The filtered solution of extract was pipette into a flask and heated to near boiling. It was then titrated against 0.05M $Kmno_4$ until when a faint pink color appeared that persisted for at least 30second

Test for phytate

Sample was treated with HCL and allowed to soak for 3hrs, it was filtered and 10ml of 0.3% ammonium thiocynate solution was added. A slightly brownish yellow colour indicate the presence of phytate.

Active ingredient screening of Lagenaria breviflora Robert (GC-MS procedure)

The whole fruit of *Lagenaria breviflora* Robert was cut into pieces, crushed and 100g of the *Lagenaria breviflora* was soaked in methanol in a 50ml cleaned bottle for a week. The mixture was shaken vigorously and allowed to stand 30 minutes in order to form a suspension. The organic solvent with extract was collected by filtering into a quartz beaker; the process was repeatedly carried out for two consecutive times. The aliquot collected were

Table 2: Active ingredients in *Lagenaria breviflora.*

Compound	RT	Area %	Formula
Tetracontane, 3,5,24-trimethyl-	9.596	3.38	$C_{43}H_{88}$
Octane, 4-ethyl	12.342	1.01	$C_{10}H_{22}$
2,6-Octadienal, 3,7-dimethyl-	14.219	0.32	$C_{10}H_{16}O$
Tridecane	14.917	3.71	$C_{13}H_{28}$
2-Hexene, 2-methyl-	22.819	0.26	C_7H_{14}
1,7-Dimethylene-2,3-dimethylindole	23.58	0.24	$C_{10}H_{11}N$
3-Eicosene, (E)-	27.017	0.29	$C_{20}H_{40}$
Oxalic acid, allyl decyl ester	27.345	0.18	$C_{15}H_{26}O_4$
Benzothiazole, 2-methyl-	27.735	0.39	C_8H_7NS
Pentadecanoic acid, 14-methyl-, methyl ester	27.82	0.6	$C_{17}H_{34}O_2$
1,2-Benzenedicarboxylic acid, butyl octyl ester	28.547	1.95	$C_{20}H_{30}O_4$
Octacosyl trifluoroacetate	29.056	0.24	$C_{30}H_{57}F_3O_2$
3-Eicosene, (E)-	30.527	0.26	$C_{20}H_{40}$
9,12-Octadecadienoic acid (Z,Z)-,methyl ester	30.676	1.16	$C_{19}H_{34}O_2$
Cyclooctene, 3-ethenyl-	30.767	0.45	$C_{10}H_{16}$
9,12-Octadecadienoic acid (Z,Z)-	31.734	0.09	$C_{18}H_{32}O_2$
5-Isopropenyl-2-hydroxy-2,4,6-cycl	31.803	1.6	$C_{10}H_{12}$
9,12-Octadecadienoic acid (Z,Z)-	31.974	0.07	$C_{18}H_{32}O_2$
Heptacosane, 1-chloro-	32.266	1.77	$C_{27}H_{55}Cl$
3-Eicosene, (E)-	32.427	0.72	$C_{20}H_{40}$
2-Methyl-Z,Z-3,13-octadecadienol	32.896	0.33	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.113	3.5	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.228	0.16	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.302	0.21	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.302	0.38	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.353	0.65	$C_{19}H_{36}O$
2-Methyl-Z,Z-3,13-octadecadienol	33.376	1.7	$C_{19}H_{36}O$
Tricosane	33.914	4.36	$C_{23}H_{48}$
12-Methyl-E,E-2,13-octadecadien-1-ol	35.087	0.53	$C_{19}H_{36}O$
9,12-Octadecadienoic acid (Z,Z)-	35.499	0.68	$C_{18}H_{32}O_2$
9,12-Octadecadienoic acid (Z,Z)-	35.556	0.24	$C_{18}H_{32}O_3$
9,12-Octadecadienoic acid (Z,Z)-	35.608	0.11	$C_{18}H_{32}O_4$
Octadecane	35.9	9.03	$C_{18}H_{38}$
9,12-Octadecadienoic acid (Z,Z)-	36.512	1.45	$C_{18}H_{32}O_2$
9,17-Octadecadienal, (Z)-	36.615	1.74	$C_{18}H_{32}O$
2-Methyl-Z,Z-3,13-octadecadienol	37.679	1.4	$C_{19}H_{36}O$
9-Oxabicyclo[6.1.0]nonane, cis-	37.788	1.52	$C_8H_{14}O$
Docosane	38.36	9.15	$C_{22}H_{46}$
1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	39.917	3.62	$C_{16}H_{22}O_4$
Hexacosane	41.484	10.9	$C_{26}H_{54}$
9,12-Octadecadienoyl chloride (Z,Z)	41.679	0.51	$C_{18}H_{31}ClO$
9-Oxabicyclo[6.1.0]nonane, cis-	41.719	0.45	$C_8H_{14}O$
5-Undecyne	41.765	0.18	$C_{11}H_20$
Octadecane	45.518	10.22	$C_{18}H_{38}$
1-octadecene	48.591	0.05	$C_{18}H_{36}$
Heptadecane	50.783	8.18	$C_{17}H_{36}$

combined and concentrated on a steam berth to about 5ml. This was purified by passing through a Pasteur pipette packed with anhydrous sodium sulphate on a membrane and air dried to about 2ml for gas chromatographic (GC) analysis.

The extract of the sample was subjected to GC/MS analysis. The gas chromatographic Model: 7890A (GC) analysis was performed interfaced to a Mass Selective Detector model: 5975C (MSD). The electron ionization was at a 70v with an ion source temperature at 250^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 80^{o}C and held for 5 minutes and increased to 250^{o}C. The holding time was 16 minutes at the rate of 4 degrees/minute, 1µl was auto injected for a final run time of 50 minutes. Each element /compound present in the fruit were identified since each compound had different retention time; these elements tried to spurt from the sample once its retention time had been attained in the gas chromatographic system.

RESULTS AND DISCUSSION

The phytochemicals present in the whole fruit of *Lagenaria breviflora* Robert is presented in Table 1. The presence of antioxidants (phenols and flavonoids) suggests that the fruit of *Lagenaria breviflora* can positively contribute to the immune system of an organism, thereby improving their performance. This is in line with the report of [17] that flavonoid has wide range of biological activities like antimicrobial, ant-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant

Table 3: Summary of the active ingredients and their biological activities.

Chemical group	Compound	Total area %	Biological activities
Alkane	Tetracontane, 3,5,24-trimethyl-	3.38	NF
Alkane	Octane, 4-ethyl	1.01	NF
Terpenoids	2,6-Octadienal, 3,7-dimethyl-	0.32	antimicrobial, food component (flavour), synthesis of vitamin A
Alkane	Tridecane	3.71	NF
Alkene	2-Hexene, 2-methyl-	0.26	NF
Indole derivative	1,7-Dimethylene-2,3-dimethylindole	0.24	NF
Alkene	3-Eicosene, (E)-	1.27	NF
Akyl ester	Oxalic acid, allyl decyl ester	0.18	NF
Benzothiazoles	Benzothiazole, 2-methyl-	0.39	antimicrobial,anti cancer,anthelmintic and anti diabetic
Palmitic acid methyl ester	Pentadecanoic acid, 14-methyl-, methyl ester	0.6	antioxidant
Dicarboxylic acid ester	1,2-Benzenedicarboxylic acid, butyl octyl ester	1.95	antimicrobial, antifouling (Khalil et al., 2014)
Esters	Octacosyl trifluoroacetate	0.24	NF
linoleic acid methyl ester	9,12-Octadecadienoic acid (Z,Z)-,methyl ester	1.16	Antimicrobial, Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic
Alkene	Cyclooctene, 3-ethenyl-	0.45	NF
linoleic acid	9,12-Octadecadienoic acid (Z,Z)-	2.64	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
Tropones	5-Isopropenyl-2-hydroxy-2,4,6-cycl	1.6	anti-fungal,anti-bacteria
Chlorinated alkane	Heptacosane, 1-chloro-	1.77	NF
Terpenoid	2-Methyl-Z,Z-3,13-octadecadienol	8.33	Pesticide, herbicide, insecticide, pheromone
Alkane	Tricosane	4.36	NF
Fatty alcohol	12-Methyl-E,E-2,13-octadecadien-1-ol	0.53	antibacteria phenolic agent
Alkane	Octadecane	19.25	Anitmicrobial, antioxidant, anticancer
Aldehyde compound	9,17-Octadecadienal, (Z)-	1.74	Antimicrobial, Antiflammatory
Oxabicyclic compound	9-Oxabicyclo[6.1.0]nonane, cis-	2.42	NF
Alkane	Docosane	9.15	Antibacterial activity
Benzoic/carboxyilic acid	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	3.62	Antifungal, anti retroviral, anti tumor,anti diabetic anti cancer,antioxtioxidant, scabies anti inflammatory, potent antimicrobial agent
Alkane	Hexacosane	10.9	Antifungal
linoleic acid chloride	9,12-Octadecadienoyl chloride (Z,Z)	0.51	Anit-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic,

			anti-eczemic, anti-acne, anti-androgenic, antiarthritic, anti-coronary, insectifuge
Akyl group	5-Undecyne	0.18	NF
Alkene	1-octadecene	0.05	Antibacteria, antioxidant, anticancer
Alkane	Heptadecane	8.18	Anitmicrobial, antioxidant, anticancer

properties. The presence of saponin and tanin is also indicative of its antioxidant; antimicrobial and anticarcinogenic properties[18]. Elujoba et al.[19] reported the presence of triterpenoid saponins in the phytochemical screening of *Lagenaria breviflora* fruit, and it has been reported to be effective in hypercholesterolaemia, hyperglycaemia, anti-inflammatory and body loss[20]. According to[21], saponin is also an important expectorant i.e. it is useful in the treatment of respiratory infection. The presence of terpenoid found in the fruit of *Lagenaria breviflora* is suggestive of its antifungal and antibacterial activities and this can be attributed to their membrane disruption and inhibitory action on bacterial cell or fungus[22]. The extract of the fruit will serve as alternative to banned growth/antibacterial drugs usage in the poultry industry and likewise curb the incidence of bacteria resistance. The presence of carotenoids in this fruit is suggestive of its ability to reduce the risk of degeneration diseases such as cancer, cardiovascular disease and cataract formation[23] likewise its positive influence on laying birds by influencing their yolk colouration and the satisfy the crave for full organic products devoid of extraneous drugs or their residues[24].

The GC_MS chromatogram of *Lagenaria breviflora* extract is presented in Figure 1 below. The analysis of the active ingredients in the fruit of *Lagenaria breviflora* indicated the presence of chemical compounds that could contribute towards the medicinal properties of the plant (Table 2). Hydrocarbon group dominated the chemical constituent of the test material. Octadecane was the major active component in the tested fruit in this study; it is used in the cosmetic industries as emollients, perfume agent and skin conditioning solvents. The presence of octadecane and heptadecane compounds in algae and plants have been reported to show potent antioxidant, anticancer and antimicrobial activity[25,26]. These components are possibly responsible for its effectiveness against selected pathogenic organisms observed by[24]. These compounds are active against S*taphylococcus aureus* and *Salmonella typhimurium*[27] and can be used as an alternative option in the treatment or control of enteric bacterial infections in livestock animals.

Hexacosane also have anti-inflammatory property as it is reported to be one of the major active ingredient in some herbal plant e.g. Trichodesma amplexicaule[28]. Singh and Singh[29] also identified the compound hexacosane to be active against *E. coli*. The presence of tricosane was indicated in the analysis; this component is also present in several plants like *Azadirachta indica*, Staphylea *sp*. which are known for their antimicrobial activity[30, 31]. The analysis also shows the presence of 1,2-benzenedicarboxylic acid, mono[2-ethylhexyl] ester, its presence in the tested fruit is suggestive of its anti-oxidant and anti-inflammatory properties as this compound has been reported to act as an anti-oxidant and anti-inflammatory substance[32]

CONCLUSION

The presence of active ingredients and their biological activities in the fruit of *Lagenaria breviflora* makes it a prospective alternative to synthetic drugs in preventing and treating diseases in both man and animals. This study further gives credence to the folkloric use of *Lagenaria breviflora* plant in treating microbial infection in both man and animals.

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