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Antibacterial and phytochemical analysis of *Gmelina arborea* fruits sap

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Abstract

In clinical utilization of anti-infective agents, resistance development by bacteria is increasingly compromising treatment outcome and novel antibacterial compounds are desirable. This study was carried out to investigate the antibacterial properties and phytochemical constituents of *Gmelina arborea* fruits sap against some clinically important pathogens. The ripe, mature green and immature fruits sap of *G. arborea* were each investigated against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella dysentariae*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus pneumoniae* and relevant typed cultures of *Shigella dysenteriae* ATCC 69559, *Salmonella typhi* ATCC 13311, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumoniae* ATCC 49619. The ripe fruits sap inhibited all the test bacteria species but most inhibited *S. aureus* and *S. aureus* ATCC 25923 with zone of 28 mm and least inhibited *S. dysenteriae* with a zone of 22 mm. Also the mature green fruits sap inhibited all test bacteria species with highest zone of 26 mm each on *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 32218 and least inhibition of 15 mm on *P. aeruginosa*. The immature green fruits sap could not inhibit *P. aeruginosa* but created highest inhibition of 25 mm on *Staphylococcus aureus* ATCC 25923 and least inhibited *Shigella dysentariae* with a zone of 15 mm. The antimicrobial activity of standard antibiotic Ampicillin was studied in comparison with the fruits' saps inhibition. The MIC of ripe fruits sap ranged from 12.5 – 50 mg/ml and MBC from 25 – 150 mg/ml. The mature fruits sap MIC ranged from 25 – 50 mg/ml and MBC from 50 - 150 mg/ml while from the immature fruits sap MIC was from the ranged of 50 – 200 and MBC from 150 – 250 mg/ml. Valuable phytochemical constituents known for bacteria inhibition were identified from the fruits sap. Steroids, glycosides and saponin were present in all fruits sap.

Keywords: Antibacterial, phytochemical, comparison, fruits sap, plant

1. Introduction

While herbs and spices have been well studied and documented for antimicrobial activity, recently fruits and vegetable are being given more attention. Thus, many fruits and vegetables are nowadays well known to have antimicrobial effect against different pathogenic and spoilage microbes. Fruits and vegetables generally contain phenolics and organic acids that are well known to possess antimicrobial activity. For example, the antimicrobial activity in Capsicum was reported to be due to the phenolic compound and 3-hydroxycinnamic acid (coumaric acid) [1]. Flavonoids from bergamot peel, a byproduct of citrus fruit processing, were found to be active against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas putida*, *Salmonella enterica*) and the antimicrobial potency of flavonoids was increased after enzymatic deglycosylation [2].

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The multidrug resistance by pathogens to commercial antibiotics and their side effects has lead to the pursuit of natural drugs present in plants to cure and prevent diseases of microbial origins. Infection with various microorganisms is one of the leading causes for a number of diseases [3]. Infectious diseases are usually characterized by clear symptoms, so it is likely that traditional healers have been able to recognize such diseases and have developed effective therapies. In the recent past, there has been tremendous increase in the use of plant based products in developing as well as developed countries resulting in an exponential growth of herbal products globally [4]. A variety of phytochemicals are accumulated in plants accounting for their constitutive antimicrobial activities. World Health Organisation (WHO) noted that the majority of the world's population depends on traditional medicine for primary health care [5]. *Gmelina arborea* is an unarmed, moderately sized to large deciduous tree with a straight trunk. It is wide spreading with numerous branches forming a large shady crown, attains a height of 30 m or more and a diameter of up to 4.5 m. Bark smooth, pale ashy-grey or grey to yellow with black patches and conspicuous corky circular lenticels. Inside surface of bark rapidly turns brown on exposure and exfoliates into thick woody plates or scurfy flakes. Blaze pale orange and mottled with a darker orange colour. The fruit is a drupe with 1.8-2.5 cm long, obovoid, seated on the enlarged calyx, glossy and yellow when ripe; exocarp succulent and aromatic; endocarp bony and usually 2-celled. Seeds are 1-3, lenticular, exalbuminous. In Nigeria *G. arborea* is majorly used as ornamental plant and wind breaker. However, this plant is used as domestic medicine for the treatment of some diseases.

The present study aims to find the antibacterial activity and major biologically active phytochemical constituents present in the fruits of *Gmelina arborea.*

2.Materials and Methods

Preparation fruits sap

Ripe, mature green and immature green fruits of *G. arborea* were directly plucked from tree located at Afe Babalola University campus, Ado Ekiti, Nigeria. The fruits were washed with 3% sodium hypochlorite and rinsed severally in distilled water. To aid sap withdrawal, the fruits were pressed with fingers. With the use of sterile needle and syringe, saps were drawn out from the fruits and emptied into sterile bottles until 150 ml quantity was obtained. This was filtered through No 1 Whatmans filter paper and finally through a Millipore filter.

Antibacterial activity

This was performed by the agar well diffusion method[6]. One milliliter each of overnight broth culture of the test bacteria species prepared to match McFarlands standard (10^6), were pour plated with molten Mueller Hinton agar in triplicates. The seeded plates were left for 2 h and then bored with a sterile cork borer to create wells. The wells were filled with 0.5 ml of whole fruits sap and 20 mg/ml of Ampicilin using a sterile syringe and needle. The plates were aerobically incubated for 24 – 48 h at 37 ^oC. The zones of inhibition exerted on the test bacteria species were measured with a transparent ruler in centimeter and then converted to millimeter to express degree of sensitivity.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms. Concentrations of between 6.25 – 300 mg/ml were prepared for each of the fruits sap. Under aseptic condition, 1 ml of overnight bacterial broth culture of McFarland's standard and one milliliter of each fruits' concentrations were added into 8 ml of sterile peptone water in test tubes. The tubes were incubated at 37 ^oC for 24 h. The tubes with least concentration where clear vision to the naked eyes were considered as the MIC point for each test bacteria species.

Determination of Minimum Bactericidal Concentration (MBC)

From every tube where clarity to the naked eyes were observed for each fruits sap concentration, 1 ml for culturing was obtained after shaking the tubes and pour plated with freshly prepared plate count agar. The plates were incubated for 24 – 48 hours at 37 ^oC. The fruits sap concentrations where bacterial colonies were observed on respective plates were considered as bacteriostatic effect, while the concentrations without bacterial growth on culture plates were reported as bactericidal effect.

Qualitative Phytochemical Analysis

Chemical tests were carried out to identify the presence of phytochemicals such as alkaloid, tannin, saponin, phlobatannins and flavonoids with the criteria of Sofowara, Trease and Evans and Harbone [6].

Saponins

To 5 ml of fruits sap, 5ml of distilled water was added in test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Reducing sugar

Fruits sap was mixed with distilled water, shaken and filtered. The filtrate was boiled with Fehling's solution A and B for 10 min. Red precipitate indicates the presence of reducing-sugar.

Tannins

Five milliliters of fruits sap was mixed with 2 ml of 2% solution of $FeCl_3$. Blue-green or black coloration indicated tannins.

Flavonoids

Five milliliters of fruits sap was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colorless on addition of few drops of diluted HCl. This reaction confirms the presence of flavonoids.

Steroid

Two milliliters of fruits sap was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids

Glycosides ***(****Salkowski's test)*

Two milliliters of fruits sap was mixed with 2 ml of chloroform. Thereafter, 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown appearance is an indication of the presence of steroidal ring, i.e., glycone portion of the glycoside.

Phlobatannins

To 3 milliliters of fruits sap, 2 ml of 1% HCl was added to and boiled. Deposition of a red precipitate was taken as an evidence for the presence of Phylobatannins

Alkaloids

Fruits sap was mixed with 2 ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

3. Results

The taste of the ripe fruits sap was of sweet and bitter combination while the mature and immature green fruits sap were mainly bitter. The odour of the fruits sap irrespective of stage was pungent and consistent was sticky. Though a lighter brown colour was observed from the ripe fruits sap than the mature and immature green fruits sap, all turned dark after few hours of extraction and were unidentified from each other (Table 1).

Table 2 presents diameter of zones of inhibition on bacterial growth with the crude fruits' sap. The ripe fruits sap had higher inhibition on most of the isolates than the mature and immature green fruits' saps. Highest inhibition of 28 mm each was expressed on *Staphylococcus aureus*, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 35218) with the ripe fruits sap and lowest inhibition of 20 mm on *Pseudomonas aeruginosa*. However, other test bacteria species were inhibited with zones that ranged from 22 – 27 mm. The mature fruits sap exhibited highest inhibition of 27 mm on *S. aureus* (ATCC 25923) and least inhibition of 15 mm on *P. aeruginosa.* Meanwhile, other test bacteria species were inhibited with zones of between 16 – 26 mm. The highest inhibition zone displayed by the immature fruits sap was 25 mm also on *S. aureus* (ATCC 25923). While *P. aeruginosa* was not inhibited with this fruits sap, other test bacteria species were inhibited with zones of between 15 – 24 mm (Table 2).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ripe fruits sap was between 1.25 mg/ml to 50 mg/ml and 50 mg/ml to 150 mg/ml respectively.

Klebsiella pneumoniae, *Streptococcus pneumoniae* (ATCC 49619), *S. aureus*, *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35218) were suppressed with MIC value of 1.25 mg/ml while MIC value of 25 mg/ml each was effective on *Shigella dysenteriea*, *Bacillus cereus*, and 50 mg/ml each on *P. aeruginosa* and *E. coli*. Value of 50 mg/ml was effective against *B. cereus* and *E. coli*. With MBC value of 100 mg/ml, *Salmonelle typhi* and *Shigella dysenteriea* were inhibited, while with 150 mg/ml, *P. aeruginosa* and *E. coli* were cleared. However, all test reference bacteria (*P. aeruginosa* ATCC 27853, *S. pneumoniae* (ATCC 49619), *E. coli* (ATCC 35218) and *S. aureus* ATCC 25923) were cleared with MBC value of 25 mg/ml each (Table 3). The MIC value against the test bacteria species with matured green fruits sap was at 50 mg/ml except on *S. dysenteriae* (25 mg/ml). MBC value of this fruits sap exhibited total inhibition on *S. typhi* and *B. cereus* while other test bacteria were potentially cleared with 150 mg/ml (Table 3). The immature fruits sap displayed least MIC values on test bacteria species where with 100 mg/ml each, *S aureus* and *E. coli* were suppressed. Other test bacteria species were suppressed with MIC values of 100 and 200 mg/ml. MBC value of 150 mg/ml exhibited total inhibition on *S. aureus* while others were cleared with 200 – 250 mg/ml respectively (Table 3).

Valuable phytochemical constituents known for bacteria inhibition were identified from the fruits sap. Steroids, glycosides and saponin were present in all fruits sap (ripe, matured green and immature green). Reducing sugar was present in the ripe and mature green fruits sap while it was absent from the immature green fruits sap. However, alkaloids, tannins and phlobatannins were absent from all fruits categories (Table 4).

Table 1. Physical characteristics of *G. arborea* fruits sap

Characteristics	Ripe	Matured green	Immature green
Colour	Light brown	Dark brown	Dark brown
Taste	Sweet/bitter	bitter	bitter
Consistency	Sticky	Sticky	Sticky
Odour	Slightly aromatic	Slightly aromatic	Characteristic

Table 2. Inhibition of bacterial isolates with *G. arborea* fruits sap

	Diameter of inhibition zone (mm)			
	Ripe	**Matured green**	**Immature green**	**Ampicilin**
P. aeruginosa	20	15	-	18
P. aeruginosa (ATCC 27853)	26	26	20	21
S. typhi	26	22	20	6
S. dysenteriae	22	16	15	19
S. pneumoniae	26	18	16	-
S. pneumoniae (ATCC 49619)	27	20	17	4
B. cereus	25	20	20	5
S. aureus	28	25	20	23
S. aureus (ATCC 25923)	28	27	25	25
E. coli	25	25	20	-
E. coli (ATCC 35218)	28	26	24	-

Table 3. Minimum inhibitory and minimum bactericidal concentrations (mic/mbc) of fruits sap.

Bacterial isolates	Ripe	Matured green	Immature green
P. aeruginosa	50/150	50/150	-
P. aeruginosa (ATCC	1.25/25	50/50	50/150
S. typhi	25/100	50/100	150/250
S. dysenteriae	25/100	25/150	150/200
K. pneumoniae	1.25/50	50/150	200/250
K. pneumoniae (ATCC)	12.5/25	50/100	100/200
B. cereus	25/50	50/100	150/200
S. aureus	1.25/50	50/150	100/150
S. aureus (ATCC)	12.5/25	50/50	50/150
E. coli	50/150	50/150	100/200
E. coli (ATCC)	12.5/25	50/100	50/250

Table 4. Phytochemical components of *G. arborea* fruits sap

Phytochemicals	Ripe	Matured green	Immature green
Tannins	-	-	-
Steroids	+	+	+
Phlobatannins	-	-	-
Glycosides (Salkowski's test)	+	+	+
Reducing sugar	++	+	-
Alkaloids	-	-	-
Saponins	+	+	+
Flavonoids	+	+	+

4. Discussion

As naturally pure sap was directly collected from apparently healthy fruits under aseptic conditions, the sap are devoid of admixtures and physical impurities to interfere with results values. The fruits of *G. arborea* were found to be effective on both Gram negative, Gram positive and opportunistic pathogens of humans.

This antibacterial effectiveness was demonstrated with the agar well diffusion method as one of the methods of screening plants extracts for antibacterial activities.

The ripe fruits sap displayed higher antibacterial activity than the mature and immature fruits sap. Though same phytochemical constituents were determined from all fruits irrespective of development, reducing sugar was however more in the ripe fruits. This denotes that antimicrobial activity of natural compounds could be influenced by number of factors such as stage of development and method of extraction in addition to the composition, structure, and functional groups of the natural product. Sugars have been known to possess antimicrobial activity and this could be the more inhibitory effect exhibited with the ripe fruits sap.

As chemicals or any other treatment was not used for the extraction of the fruits sap, there is the tendency that there was no alteration in the active compounds to exhibit valuable antibacterial effects. [7, 8], have reported that direct extraction is a simple and safe procedure that prevents any possible alteration or destruction to the native structure of the active ingredients. For example, direct extract from guava and xoconostle pears were found to be effective against *Salmonella* spp. and *E. coli* O157:H7 respectively.

It was observed that *S. aureus* among the test isolates was the most susceptible to the fruit sap. Similar result was reported by [9]. From medical perspective, this suggests that this fruits sap could be adequate for treating staphylococcal infections and other Gram positive organisms such as *S. pyogenes* which also are responsible in wound infections. These organisms are invasive Gram-positive bacteria known as pyogenic (pus-producing) cocci which cause various supportive or pus-forming diseases in humans [10]. However, tannins as one of the identified phytochemicals from the fruits sap, have stringent properties and have been documented in healing of wound and inflamed mucous membranes [11].

Phytochemicals in plant and plant products help to exhibit inhibition characteristic model on microorganisms hence one of their reasons to be present in plants is microbial predation. The different phytochemicals identified from the fruits sap worked in combinative form to display the inhibition potency on the species of bacteria in the *in vitro* antibacterial efficacy.

All the phytochemical compounds identified from *G. arborea* fruits sap have been found to be beneficial to human in health care system. Phytochemicals with high medicinal activities also identified from *Gmelina arborea* fruits.sap include saponnins and flavonoids which are known to lower the risk of diseases in humans. Flavonoids from this fruit provide anti-inflammatory activity; this may be the reason for its use for the treatment of wounds, burns and ulcers [12].

The use of *G arborea fruits* to treat infections may be justified by the charisma of its possessed phytochemicals as effective antimicrobial. This study in respect to the results of antibacterial and phytochemical evaluated, confirmed the examined *G. arborea* fruits as large sources of bioactive chemicals as biological agents necessary in traditional and therapeutic utilization. The fruit alleviates pitta dosa and possesses heavy and oily attributes. Fruits are used for heart diseases, leprosy, vomiting and burning sensations.

Edible fruits are valuable resources for both primary health and complementary health care systems. Generally, fruits contain substances of medicinal value that have yet to be discovered, though large number of fruit juices are constantly being screened for their antimicrobial effect, these fruits may prove to be a rich source of compounds with possible antimicrobial activities [13]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. Steroids have been reported to have antibacterial properties [14].

5. Conclusion

Inhibition of the test bacteria species with the fruits sap was higher than the inhibitions exhibited with commercial antibiotic. The tested bacteria species were not resistant to the fruits sap except *P. aeruginosa* that was only resistant to the sap from immature fruits. The phytochemicals found in plants known for their antimicrobial activities were as well found in the fruits of *G. arborea*.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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