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Full Length Research Paper

Phytochemical screening and antibacterial activity of *Gmelina arborea* fruit extracts

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Extracts of *Gmelina arborea* fruits have been locally used in Benue State, Nigeria by traditional practitioners for the treatment of wounds, sores, burns, vaginal discharges, etc. The antibacterial activity of *Gmelina arborea* fruit extracts (neat methanol and hexane) was investigated using the disk diffusion method. The test organisms were hospital/diagnostic laboratory isolates: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus morganis*, *Salmonella typhi* and *Pseudomonas aeruginosa* obtained from wound swab, ear swab, stool and urine. Neat methanol extracts had the lowest minimum inhibitory concentration (MIC) of 0.001 µg each implying greatest activity while hexane extract had the highest MIC (100.0 µg). The neat extract inhibited growth of *Staphylococcus aureus*, *Streptococcus pyogenes, Escherichia coli* and *Proteus morganis*. The methanol extract inhibited *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Proteus morganis,* and *Pseudomonas aeruginosa*. Hexane extract inhibited only *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The width of inhibition zone is highly concentration dependent. Preliminary pytochemical analysis of aqueous and methanol extracts revealed the presence of saponins, tannins, reducing sugar, steroids, flavonoids and glycosides respectively. The findings of this study support the tradi-medical use of the plant fruit on wounds and other bacterial infections in Nigeria.

Key words: *Gmelina arborea*, growth, inhibition, extract photochemical screening.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence shows immense potential of medicinal plants used in various traditional systems.

A wide range of antimicrobial agents that has the ability to kill or inhibit the growth of pathogenic or non-pathogenic microorganisms exists. These effects may be physical or chemical in nature. However, parts of some plants such as leaves, roots, bark or fruits are known to contain components usually chemical that can inhibit or prevent the growth of micro-organisms.

Extracts of *Gmelina arborea* fruits have been locally used in Benue State, Nigeria by traditional practitioners for the treatment of wounds, sores, burns, vaginal discharges, etc. The local use of the fruit of this plant motivated this investigation. However, the study was conducted to investigate the antibacterial activity of *Gmelina arborea* fruit using the disk diffusion method and to screen the fruit extracts for phytochemical properties. Information obtained from this study may be useful in improving treatment of wound infections.

MATERIALS AND METHODS

Media preparation

The media used were nutrient agar, blood agar and nutrient broth.

Extraction of *Gmelina arborea* fruit juice

Fresh ripe fruits picked from Makurdi metropolis, Benue

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State and identified by botanists in the department of Biological Sciences, University of Agriculture Makurdi, were properly washed with sterile water and allowed to drain. One hundred grams of the fruits were mashed with glass metal and filtered with a sterile filter paper. The filtrate (extract) was used for antibacterial activity assays, as described by Stein et al. (1981).

Aqueous extraction

Twenty grams of *Gmelina arborea* fruit were sliced with knife and boiled in 400 ml of distilled water over a Bunsen burner for thirty minutes. The resultant liquid was allowed to cool for one hour and then filtered. The filtrate was then used immediately for photochemical tests.

Hexane and methanol extraction

Forty grams of the dry and pulverized fruit was transferred to the thimble which was stopped with wool. Three hundred milliliters of hexane was poured into the round bottom flask of the soxhlet apparatus. The apparatus was mounted on the heating mantle and heated for eight hours to ensure complete extraction of the soluble constituents.

After heating, the solvent was recovered from the extract using the distillation apparatus. The crude extract was transferred into a 100 ml beaker and placed on a water bath to evaporate any hexane still left in the extract; the extract was then concentrated to a constant weight and placed in a fume cupboard in a beaker covered with paraffin wax.

Same procedures as described in hexane were used for methanol extraction. It should be noted that the same sample in the thimble was used for both extractions because the components of these plants can only be extracted by hexane while others by methanol.

Test organisms

The test organisms used were clinical isolates of *Staphylococcus aureus* from human adult (male) wound swab, *Streptococcus pyogenes* from human adult (female) urine, *Escherichia coli* from human adult (male) stool, *Pseudomonas aeruginosa* from adult (male) ear swab. Pure culture of each isolate was used for identification.

Determination of minimum inhibitory concentration (MIC) of *Gmelina arborea* fruit extract on test organisms

The minimum inhibitory concentration (MIC) tests were carried out using the methods of Mukherjee et al. (1995). Different concentrations of the *Gmelina arborea* fruit extract were obtained by ten-fold serial dilutions of the extract in appropriate blanks, using sterile water.

Filter paper disc impregnated with each concentration of the extract were placed in sterile Bijou bottles and stored in a refrigerator. Overnight culture of each isolate in nutrient broth was uniformly streaked onto a NB/BA agar plate. The filter paper discs impregnated with different concentrations of the extract were placed on the agar plates. Each plate was incubated at 37°C for 24 h after which zones of inhibition if any were measured in millimeters. This was done three times for each organism to ensure accuracy.

The lowest concentration that inhibited the growth of the organism was recorded as the minimum inhibitory concentration of the extract. Ten micrograms (10µg) of Peflacine (a standard antibiotic was used as a positive control and sterile water was used as a negative control).

Phytochemical screening of *Gmelina arborea* fruit

The phytochemical components of the extracts were determined according to the method of Culei (1982). This was necessary because it permits the identification of the bioactive agents from medicinal plants and allows for pharmacological research leading to synthesis of new drugs which are more effective (Ebana et al., 1991).

Statistical analysis

Means were compared using the Fishers Least Significant Difference (FLSD) and significant level was considered at $P < 0.05$.

RESULTS

Table 1 shows the effect of different concentrations of neat extract of *Gmelina arborea* fruits on the bacteria isolates. The extract showed highest activity on *Staphylococcus aureus* and *Streptococcus pyogenes* followed by *E. coli* and *P. morganis.* No activity was observed on *Salmonella typhi* and *Pseudomonas aeruginosa*.

Table 2 shows the different concentrations of methanol extract of *Gmelina arborea* fruit on bacterial isolates. The extract showed highest activity on *Escherichia coli* followed by *S. pyogenes*, *S. aureus* and *P. morganis*. The least was observed on *P. aeruginosa.* No activity was recorded on *S. typhi*.

Table 3 shows the effect of different concentrations of hexane extract of *Gmelina arborea* fruit on bacterial isolates. The extract showed the highest activity on *Staphylococcus aureus* followed by *E. coli*. The least was on *P. aeruginosa*. However there was no activity of the hexane extract on *S. pyogenes*, *P. morganis* and *S. typhi.*

Table 4 shows the minimum inhibitory concentration (MIC) of neat extract of *Gmelina aborea* fruits on test organisms. The MIC of the extract was observed to be lowest on *S. aureus* and *S. pyogenes* with 0.001 µg each. It was highest on *E. coli* and *P. morganis* with 1.0 µg

Table 2. Effect of graded concentrations of methanol extract of *Gmelina arborea* fruits on bacterial isolates.

Concentration (v/v.µl)	Zones of inhibition (mm)					
	S. aureus	***S. pyogenes***	***E. coli***	***P. morganis***	***S. typhi***	***P. aeruginosa***
1000.0	17.33	17.33	21.33	8.33	0.00	7.67
100.0	13.67	14.33	16.33	7.33	0.00	6.67
10.0	12.33	11.00	12.67	6.33	0.00	4.00
1.0	10.33	9.33	10.67	0.00	0.00	0.00
0.1	7.67	8.00	8.67	0.00	0.00	0.00
0.01	6.33	7.00	7.67	0.00	0.00	0.00
0.001	0.00	6.00	6.33	0.00	0.00	0.00
0.0001	0.00	0.00	0.00	0.00	0.00	0.00
S	0.00	0.00	0.00	0.00	0.00	0.00
P	28.67	17.00	13.33	13.67	19.00	21.33
FLSD (α = 0.05)	1.65	1.20	1.84	1.81	0.82	2.09

Keys: S = sterile water (negative control); P = Peflacine (positive control); FLSD = Fishers Lest Significant Difference.

Table 3. Effects of graded concentrations of hexane extract *Gmelina arborea* fruits on the bacteria isolates.

Concentration (v/v.µl)	Zones of inhibition (mm)					
	S. aureus	***S. pyogenes***	***E. coli***	***P. morganis***	***S. typhi***	***P. aeruginosa***
1000.0	12.33	0.00	7.33	0.00	0.00	4.33
100.0	7.33	0.00	0.00	0.00	0.00	0.00
10.0	0.00	0.00	0.00	0.00	0.00	0.00
1.0	0.00	0.00	0.00	0.00	0.00	0.00
0.1	0.00	0.00	0.00	0.00	0.00	0.00
0.01	0.00	0.00	0.00	0.00	0.00	0.00
0.001	0.00	0.00	0.00	0.00	0.00	0.00
0.0001	0.00	0.00	0.00	0.00	0.00	0.00
S	0.00	0.00	0.00	0.00	0.00	0.00
P	23.00	19.67	26.67	25.33	20.33	20.00
FLSD (α = 0.05)	1.49	0.82	1.16	0.62	0.31	2.11

Table 4. Minimum inhibitory concentration (MIC) of neat extract of *Gmelina arborea* fruit on test organisms.

Organism	Concentration (g/ml)										
	1000.00	**100.0**	**10.0**	**1.0**	**0.1**	**0.01**	**0.001**	**0.0001**	**S**	**P**	**MIC(µl)**
S. aureus	+	+	+	+	+	+	+	-	-	+	0.001
S. pyogenes	+	+	+	+	+	+	+	-	-	+	0.001
E. coli	+	+	+	+	-	-	-	-	-	+	1.0
P. morganis	+	+	+	+	-	-	-	-	-	+	1.0
S. typhi	-	-	-	-	-	-	-	-	-	+	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	+	-

Keys: + = inhibition; - = no inhibition; S = sterile water (positive control); P = Peflacine (positive control).

each. There was no inhibition on *S. typhi* and *P. aeruginosa*. The lowest MIC indicates greater susceptibility to the bacterial is isolates.

Table 5 shows the minimum inhibitory concentration (MIC) of methanol extract of *Gmelina arborea* fruits on test organisms. The MIC of the methanol extract on *S. pyogenes* and *E. coli* was 0.001 µg, followed by 0.01 µg on *S. aureus*, while the highest inhibitory concentration (10.0 µg) was observed on *P. morganis* and *P. areuginosa*.

Table 5. Minimum inhibitory concentration (MIC) of methanol extract of *Gmelina arborea* fruits on bacterial isolates.

Organism	Concentration (g/ml)										
	1000.00	100.0	10.0	1.0	0.1	0.01	0.001	0.0001	S	P	MIC (µl)
S. aureus	+	+	+	+	+	+	-	-	-	+	0.01
S. pyogenes	+	+	+	+	+	+	+	-	-	+	0.001
E. coli	+	+	+	+	+	+	+	-	-	+	0.001
P. morganis	+	+	+	-	-	-	-	-	-	+	10.0
S. typhi	-	-	-	-	-	-	-	-	-	+	-
P. aeruginosa	+	+	+	-	-	-	-	-	-	+	10.0

Keys: + = inhibition; - = no inhibition; S = sterile water (positive control); P = Peflacine (positive control).

Table 6. Minimum inhibitory concentration (MIC) on hexane extract of *Gmelina arborea* fruits on bacteria isolates.

Organism	Concentration (g/ml)										
	1000.00	100.0	10.0	1.0	0.1	0.01	0.001	0.0001	S	P	MIC (µl)
S. aureus	+	+	-	-	-	-	-	-	-	+	100.0
S. pyogenes	-	-	-	-	-	-	-	-	-	+	-
E. coli	+	-	-	-	-	-	-	-	-	+	1000.0
P. morganis	-	-	-	-	-	-	-	-	-	+	-
S. typhi	-	-	-	-	-	-	-	-	-	+	-
P. aeruginosa	-	-	-	-	-	-	-	-	-	+	-

Table 7. Comparative minimum inhibitory concentration (MIC) of neat, methanol and hexane extract of *Gmelina arborea* fruits on bacterial isolates.

Organism	Neat extract (µl)	Methanol extract (µl)	Hexane extract (µl)
S. aureus	0.001	0.01	100.0
S. pyogenes	0.001	0.001	-
E. coli	0.01	0.001	1000.0
P. morganis	1.0	10.0	-
S. typhi	-	-	-
P. aeruginosa	-	10.0	-

Table 6 shows the MIC of hexane extract of *Gmelina arborea* fruits on bacterial isolates. The MIC of the hexane extract was 100.0 µg on *S. aureus*, while it was 1000.0 µg on *E. coli*. There was no inhibition for *S. pyogenes*, *P. morganis*, *S. typhi*, and *P. aeruginosa.*

Table 7 compares the MIC of neat, methanol and hexane extracts of *Gmelina arborea* fruits on test organisms. The results showed that neat extract had the lowest MIC on *S. aureus* and *S. pyogenes* at 0.001 µg. However, methanol extract had the lowest MIC of 0.001µg on *S. pyogenes* and *E. coli*. Hexane extract had the highest MIC on *E. coli* at 1000.00 µg.

Table 8 shows results of phytochemical screening of aqueous, methanol and hexane extracts of *Gmelina arborea* fruits. The results revealed the presence of saponnins, reducing sugar, steroids, flavonoids and glycosides for aqueous extract, saponnins, tannins, steroids, flavonoids and glycosides for methanol extract, and none for hexane extract.

DISCUSSION

Extract of *Gmelina arborea* fruits showed antibacterial activity. The results of this research demonstrate the effectiveness of the disc diffusion technique in screening plants for antibacterial activities. This varied according to extraction solvent and test organisms used. Neat extract showed higher activity on bacterial isolates than methanol and hexane extracts. Methanol extract showed some activity on *Pseudomonas aeruginosa*, this is because methanol was able to extract some active ingredients which could not be released in the neat and

Table 8. Photochemical components of aqueous, methanol, and hexane extracts of *Gmelina arborea* fruits.

Chemical component	Aqueous extract	Methanol extract	Hexane extract
Saponins	+	+	-
Tannins	-	+	-
Reducing sugar	+	-	-
Phlobatannins	-	-	-
Steroids	+	+	-
Flavonoids	+	+	-
Glycosides	+	+	-
Alkaloids	-	-	-

Keys: + = positive; - = negative.

hexane extracts. Hexane had the lowest inhibitory activity; consequently it is not a good solvent to be used for these fruits. The high potency of the neat extract on the bacterial isolates justifies the local use of the fruit on open wounds.

Given its antibacterial activity, the fruits of *Gmelina arborea* could be effective in treating Gram negative, Gram positive and opportunistic pathogens of humans. However, the fruit extracts did not have any activity against *S. typhi* and *P. aeruginosa*. The extracts may therefore not be of value in treating infections caused by these organisms. *S. aureus* was most susceptible to the fruit extract, and these findings support the use of these fruits in treating staphylococcal infections and other Gram positive organisms like *S. pyogenes* which are the commonest cause of wound infection. These organisms are invasive gram-positive bacteria known as pyogenic (pus-producing) cocci which cause various supportive or pus-forming diseases in humans (Joanne et al., 2008).

The lack of susceptibility of *P. aeruginosa* to the extracts could be attributed to the fact that this bacterium is naturally resistant to many antibiotics due to the permeability barrier of its outer membrane. However, its tendency to colonies in a biofilm form makes the cells impervious to therapeutic concentration of antibiotics since its natural habitat is the soil where it lives in association with bacilli, actinomycetes and moulds; it has developed resistance to a variety of their naturally occurring antibiotics.

The Fisher's Least Significant Difference (FLSD) value obtained implies that there is a significant difference between the different concentrations, thus a significant difference between the different concentrations in terms of zones of inhibition and that higher concentrations gave higher zones of inhibition. There was, however, no significant difference between the neat and methanol extracts and the standard antibiotic Peflacine justifying the use of this fruit in treating bacterial infections of man.

The phytochemical screening of *Gmelina arborea* fruits revealed that saponins, tannins, reducing sugar, steroids, flavonoids and glycosides were present. The saponin content of fruit extracts demonstrates the antihemorragic activity of the extracts and hence justifies the use of the fruit in treating wounds. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solution, hemolytic activity, cholesterol binding properties and bitterness (Okwu and Okwu, 2004).

These properties bestow high medicinal activities on the extracts from *Gmelina arborea* fruits. Apart from saponnins, other secondary constituents of *Gmelina arborea* fruits detected include flavonoids which in intestinal tract lower the risk of heart disease and act as antioxidants. Flavonoids from this fruit provide anti-inflammatory activity; this may be the reason for its use for the treatment of wounds, burns and ulcers.

Tannins have stringent properties and hasten healing of wound and inflamed mucous membranes; this perhaps explains why traditional practitioners use it in treating wounds and burns (Agoha, 1974). The presence of saponins and tannins is consistent with the findings of Akubue et al. (1983). These findings therefore support the medicinal use of the fruit locally as alternative to antibiotics.

Conclusion

Gmelina arborea fruits possess antibacterial activity. It has been found to be effective against some pathogenic bacteria involved in wounds and burns. This corroborates the rationale for the use of the plant in treating these ailments in traditional medicine. The fruits could provide cheaper substitutes for conventional drugs since it is easily obtainable. The neat extract can easily be made by simple process of squeezing and sieving. *Gmelina arborea* fruits extracts possess broad spectrum activity. This, therefore, suggests that constituents of the fruit extract could serve as a source of drugs useful in the chemotherapy of some microbial infections.

From the findings of this research, it is recommended that the fruits be used both domestically and industrially for treatment of wounds.

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