Antibacterial Activity and Phytochemical Analysis of *Amaranthus Pinosus* and *Ipomea Sarifolia*

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SUMMARY

Aim: To investigate antibacterial activity in *Amaranthus pinosus* and *Ipomea sarifolia* using the disc diffusion method. The phytochemical constituents of the two plants were also analyzed.

Methods: Twenty (20) grammes each of *Amaranthus pinosus* and *Ipomea sarifolia* were extracted using each of methanol, hexane, chloroform and water. Aqueous solutions of the four extracts each of *Amaranthus pinosus* (0.9% concentration each) and *Ipomea sarifolia* (1% concentration each) were prepared. Isolates of *Pasteurella multocida, Salmonella typhi, Salmonella typhimurium, Klebsiella pneumoniae, Escherichia coli, Streptococcus pyogenes* and *Streptococcus pneumoniae* were subcultured overnight at 37°c on nutrient agar plates. In a dry petri dish 10 per microorganism of Mueller-Hinton agar were flooded with the appropriate isolates suspended in sodium chloride solution (0.9%). Sterile 6mm diameter absorbent paper was placed on each plate. The plates were incubated at 37°c for 24 hours after which diameters of zones of inhibition were measured in millimeter. Phytochemical analysis was carried out using the method of Mamoud et al[16].

Results: The results of antimicrobial activities showed that *Amaranthus pinosus* and *Ipomea sarifolia* have antimicrobial activities against *Escherichia coli* and *Pasteurella multocida,* but lack activity against *Salmonella typhi, Salmonella typhimurium, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Streptococcus pyogenes*. Phytochemical analysis revealed that the two plants contain flavonoid, saponin and tannin but lack steroid. However, *Amaranthus pinosus* showed the presence of alkaloid which was not present in *Ipomea sarifolia;* and *Ipomea sarifolia* contained glycoside that was not present in *Amaranthus pinosus*.

Conclusion: The test plants may be used to treat infectious diseases caused by *Escherichia coli* and *Pasteurella multocida*. Presence of flavonoid and alkaloid in hexane, chloroform, methanol and water extracts of *Amaranthus pinosus* and *Ipomea sarifolia* may be responsible for their antimicrobial activities. But *Ipomea sarifolia* may be more toxic because it contained glycosides in addition to other chemical substances. It is recommended that the plants should be tested in vivo.

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INTRODUCTION

Amaranthus pinosus L. (spiny amaranth) belongs to the family Amaranthaceae, an annual herb: erect, branched, about 60cm high with oval leaf which is 7cm by 5cm being broadest near the base; obtuse, acuminate petiole which is long and reddish[1]. Axillary spines occur at the leaf base; inflorescence is terminal: a spike with a small glomerulus of greenish flowers densely packed along the axis that may be branched giving rise to lateral spikes[1]. Fruits are capsules which dehisce circularly. It is mainly of tropical distribution[1].

Traditionally, *Amaranthus pinosus* is used to treat gastrointestinal disorders, haemorrhoids, arthritis[2], eczema, snakebite, ulcers[1] and gout[3]. Phytochemically, the plant contains tannins, saponin and hydrocyanic acid[4].

Ipomea sarifolia belongs to the family convolvulaceae, a herbal perennial plant with thick creeping stem branched and hollow with simple leaves that are alternate and glabrous[1]. The lamina is oval and cordiform, about 10cm long and wide; basally cordate but rounded at apex; inflorescence is borne on axillary peduncle, 8-18cm long[1]. Flowers are mauve to white and 7-9cm broad; calyx is 5-3cm long, while the fruit, usually a capsule, is oval with a diameter of 10mm with 4 rounded cells containing brown seeds[1]. The plant is widespread in Africa South of Sahara and has medicinal uses in the traditional treatment of cough, epilepsy, rheumatism and guinea worm infestation; it is an abortifacent[1]. However *Ipomea sarifolia* contains lysergic-acid-like substance which elicits nervous signs in animals; it is trysinergic in characters[5]. It is also poisonous to lambs[6].

Multi-drug resistant gram-negative microbes are emerging worldwide and carbapenems and fluoroquinolones are currently the drugs of last resort used against such microbes[7, 8]; aminoglycosides, including amikacin and tobramycin are, however, still potent agents for use against resistant bacili. The proliferation and dissemination of clinical isolates that produce metallo-β-lactamases and acquire mutations in gyrA and ParC genes have become a global threat[9, 10]. A most common resistance mechanism against aminoglycosides is the production of enzymes such as acetyl transferases, phosphorylases and adenyltransferases that modify aminoglycosides[11]. Therefore, the changing pattern of bacterial aetiology of infections and the altered sensitivities to antimicrobial agents employed in their treatment call for regular searching and exploration of our indigenous plants for antimicrobial activity[12]. In view of these, this study was designed to screen *Amaranthus pinosus* and *Ipomea sarifolia* for antimicrobial activity and phytochemical components.

MATERIALS AND METHODS

Amaranthus pinosus and *Ipomea sarifolia* plants were collected from Katcha, Niger State and identified by a botanist in the herbarium of the Biological Department of Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Fresh leaves of *Amaranthus pinosus* and *Ipomea sarifolia* were collected, macerated and then dried in the sun during the dry season for 30 minutes to 1 hour and ground into powder using mortar and pestle. Into each of 4 conical flasks 20 grammes of the *Amaranthus pinosus* powder was weighed. Similarly into each of 4 other conical flasks 20 grams of the *Ipomea sarifolia* was also weighed. Next, into the respective conical flasks containing either *Amaranthus pinosus or Ipomea sarifolia powder* 100mls of either methanol, hexane, chloroform or water were added. Each mixture was thoroughly shaken and allowed to stand for 24 hours before being filtered through whatman No. 1 filter paper into measuring cylinders and evaporated to dryness using water bath and dessicator. The dried residue obtained was stored at 4°C until ready to use[13].

From the pilot study, it was found that the plants' extracts had no significant antimicrobial activity in concentrations of between 0.1% and 0.8%. This pilot study informed the use of solutions 0.9% of *Amaranthus pinosus* and 1.0% of *Ipomea sarifolia.*

From each of the dried hexane, methanol, chloroform and water extracts of

Amaranthus pinosus. 0.9g of was dissolved in 100ml of distilled water to prepare the required 0.9% solutions. In the same way, using 1.0g of each of the dried hexane, methanol, chloroform and water extracts of *Ipomea sarifolia*, 4 other 1% solutions were prepared. All the 8 solutions were sterilized by filtering through a 0.45 µm pore filter paper[4].

The isolates of *P. multocida, S. typhi, S. typhimurium, K. pneumoniae, E. coli, S. pyogenes* and *S. pneumoniae*, which were supplied by Veterinary Public Health Department of Usmanu Danfodiyo University, Sokoto, were sub-cultured overnight at 37°C on nutrient agar plates: ten plates per microorganism. The suspensions of each bacterial isolate were prepared as described by John et al[15], in isotonic sodium chloride solution. Dry Petri dish of Mueller-Hinton agar ten per each microorganism were flooded with the appropriate suspension of the bacterial isolates. Sterile 6mm diameter absorbent filter papers punched out from No.1 whatman paper were placed on the corresponding inoculated 140 plates: 70 plates per plant: ten each of *S. typhi, S. pneumoniae, S. pyogenes, K. pneumoniae, E. coli, P. multocida* and *S. typhimurium*. After the incubation at 37°C for 24 hours, all the plates were observed for zones of growth inhibition and the diameters of these zones measured in millimeter using measuring ruler.

Phytochemical analyses were carried out according to the method described by Mamoud et al[16].

RESULTS

Hexane and chloroform extracts of *Amaranthus pinosus* showed antimicrobial activities against *E. coli* and *P. multocida*. There was no activity shown by the methanol extract on any of the test microorganisms while the water extract showed activity against *E. coli* only (table 1).

Table 1

Antimicrobial Activity of *Amaranthus pinosus* leaf hexane, chloroform, methanol and water extracts

Microorganism	Antimicrobial activity of various extracts			
	Hexane	Chloroform	Methanol	Water
E. coli	+	+	-	++
K. pnemoniae	-	-	-	-
P. multocida	+	+	-	-
\S. typhimurium	-	-	-	-
S. pneumoniae	-	-	-	-
S. typhi	-	-	-	-
S. pyogenes	-	-	-	-

+ = Activity; - = No activity; ++ = Higher activity

Chloroform, methanol and water extracts of *Ipomea sarifolia* showed higher antimicrobial activities on *P. multocida* and *E. coli*. However there was no antimicrobial activity shown by any of the four extracts against other microorganisms (table 2).

Table 2

Antimicrobial Activity of *Ipomea sarifolia* Leaf hexane, chloroform, methanol and water extracts

Microorganism	Antimicrobial activity of various extracts			
	Hexane	Chloroform	Methanol	Water
E. coli	-	+	++	++
K. pneumoniae	-	-	-	-
P. multocida	++	++	-	-
S. typhimurium	-	-	-	-
S. pneumoniae	-	-	-	-
S. typhi	-	-	-	-
S. pyogenes	-	-	-	-

+ = Activity, - = No activity, ++ = Higher activity

Phytochemical analyses of the hexane, chloroform, methanol and water extracts of *Amaranthus pinosus* leaf are presented in table 3. All 4 extracts contain saponin while steroids and glycosides were absent in all 4 extracts. Tannin is present only in the methanol extract.

Table 3

Phytochemical components of *Amaranthus pinosus*

Extract	Alkaloid	Tannin	Glycoside	flavonoid	Steroid	Saponin
Hexane	-	-	-	+	-	+
Chloroform	+	-	-	+	-	+
Methanol	+	+	-	+	-	+
Water	+	-	-	-	-	+

+ = Presence, - = Absence

Phytochemical analyses of hexane, chloroform, methanol and water extracts of *Ipomea sarifolia* leaf revealed the presence of saponin and flavonoid while alkaloid and steroid were absent, table 4. Again, tannin is present only in the methanol extract.

Table 4

Phytochemical components of *Ipomea sarifolia*

Extract	Alkaloid	Tannin	Glycoside	flavonoid	Steroid	Saponin
Hexane	-	-	+	+	-	+
Chloroform	-	-	-	+	-	+
Methanol	-	+	+	+	-	+
Water	-	-	-	+	-	+
+ = Presence, - = Absence						

DISCUSSIONS

Antibacterial activities of the hexane, chloroform and water extracts of the *Amaranthus pinosus* leaf against *E. coli* and *P. multocida* may explain the traditional use of the plant in the treatment of gastrointestinal disorders[2]. The results may also explain the rationale for the plant's use in the traditional treatment of snake bite and ulcers[1]. *E. coli* is one of the oral flora of snakes and gastrointestinal flora of man.

Amaranthus pinosus may be used in combating multidrug-resistant gram-negative mutant microbes that have been emerging worldwide. Although, carbapenems and fluoroquiolones are the last resort against infections caused by gram negative bacilli[7], aminoglycosides, including amikacin and tobramycin are still potent agents for use against resistant bacilli[11]. Despite the fact that *Amaranthus pinosus* has activity only on *E. coli* and *P. multocida* (narrow spectrum of activity), there is still a need for more research on its antimicrobial spectrum and to separate the plant constituents and test each of the component both in vitro and in vivo.

The antimicrobial activity shown by some of the tested leaf extracts of *Ipomea sarifolia* on *P. multocida* and *E. coli* may also explain the finding of Mann et al[1] that the plant has medicinal uses in cough because pasteurella multocida may cause respiratory infection that results in shallow breathing and a soft productive cough, which tends to increase with exercise[18]. *E. coli* is sometimes found as part of the normal flora of the upper respiratory and genital tracts and may cause incidental disease in human and animals[18]. Our findings of antibacterial activity in the extracts of the leaves of this naturally occurring plant support the report of Shidi Bonjar and Rashidi Farrokhi that natural resources, especially plants and microorganisms are potent candidates for new drugs[19].

A particular characteristic of plants is that different chemical substances are obtained in members of the same species in different geographical areas[20]. The narrow spectrum antibacterial plants may be used to treat infectious diseases which are the world's leading cause of premature deaths killing almost 50,000 everyday[21, 22]. The higher antibacterial activity exhibited by *Ipomea sarifolia* against *E. coli* and *P. mullicoda* as compared to *Amaranthus* pinosus may be due to differences in the concentrations of active principles of the two plants. Different methods of extractions may explain these different concentrations.

However, from the lack of antimicrobial activity shown by the plant *Ipomea sarifolia* against the remaining microorganisms we may conclude that their spectrum of activity is narrow.

The exhibited antimicrobial activity of the two plants against *E. coli* and *P. mullicoda* was not associated with steroid. However, the antimicrobial activities of *spiny amaranth* may be due to presence of flavonoid and alkaloids present in three out of the four extracts, whereas the antimicrobial activities of *Ipomea sarifolia* may be due to presence of flavonoid. This agrees with the reports of Jisaka el al that flavonoid has antioxidant and antimicrobial activities[23]. Our finding that the two test plants have pharmacological activity agrees with the earlier report[12] that the pharmacological activity of a

plant is dependent on its chemical constituents which may be exposed by different methods of extraction.

The presence of tannin in the methanol extract and saponin in all the four extracts of *Amaranthus pinosus* support the report of Gill that the plant contains tannins, saponin and hydrocyanic acid[4]. However our findings differed from that of Gill in that our study also revealed the presence of alkaloid in all the extracts of *Amaranthus pinosus* except the hexane extract and, flavonoid in all the extracts except the water extract, as well as absence of glycoside and steroid, which were not reported by Gill[4].

The absence of glycoside in *Amaranthus pinosus* may make the plant safer than *Ipomea sarifolia* because glycoside especially cardiac glycoside has excitatory effect on cardiac muscles. The presence of saponin, glycoside and flavonoid in all the four extracts and tannin in the chloroform extract of *Ipomea sarifolia* may make the plant poisonous. This support the previous reports[5], that *Ipomea sarifolia* contains lysergic-acid-like substance which elicit nervous signs in animals and that it is trysinergic in character. This in turn may explain the report of Ajagbonna et al[6] that *Ipomea sarifolia* is poisonous to lambs. It is also to be noted that saponin, tannin and alkaloid are antinutritional substances that can cause haemolysis, nutrient malabsorption and, abnormal haemopoeisis[24,25].

CONCLUSION

Hexane, chloroform, methanol and water extracts of *Amaranthus pinosus* and *Ipomea sarifolia* have antimicrobial activities against *E. coli* and *Pasteurella multocida*. However, all the extracts of the two plants lack antimicrobial activities against the remaining test microorganisms, including *K. pneumoniae, S. typhimurium, S. pneumoniae, S. typhi* and *S. pyogenes*. Presence of flavonoid and alkaloids in some test extracts of the two plants may be responsible for their antimicrobial activities. *Ipomea sarifolia* may be more toxic than *Amaranthus spinosus* because it contains glycoside.

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