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ANTIMICROBIAL EFFICACY OF *GUIERA SENEGALENSIS* AND *PROSOPIS AFRICANA* LEAVE EXTRACT ON SOME BACTERIAL PATHOGENS

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ABSTRACT: *The bioactive components of the leaves of Guiera senegalensis and Prosopis africana were extracted using ethanol, aqueous and crude extraction methods. Qualitative phytochemical analysis showed that extracts contain alkaloids, tannins, saponins, flavonoids, glycosides and steroids, while glycosides and alkaloids were absent in P. africana and G. senegalensis respectively. Quantitative phytochemical analysis of G. senegalensis showed 1.352mg/100g of flavonoids and 14.59mg/100g of phenols. Prosopis africana quantitatively showed 3.041mg/100g flavonoids and 10.22mg/100g phenol content. The various extracts were investigated for their antibacterial activity using agar diffusion methods of susceptibility testing against the test organisms. The ethanolic extract of Prosopis africana demonstrated the highest activity against Staphylococcus aureus, Escherichia coli, and Salmonella typhi (4.7mm, 4mm and 4mm zones of inhibition respectively) while the least activity was demonstrated by aqueous extract against Escherichia coli (1mm inhibition zone). The ethanolic extract of G. senegalensis also inhibited Salmonella typhi, Staphylococcus aureus and Escherichia coli with (3.5mm, 3mm, and 2.8mm of zones of inhibitions respectively). The crude and the aqueous extracts of both plants showed lower zones of inhibition against all the three organisms. This study shows that the use of G. senegalensis and Prosopis africana leaves as traditional medicine has a lot of potential in treatment of antimicrobial infections with further standardization.*

KEYWORDS: Antimicrobial efficacy, leave extract, phytochemical, bacteria, inhibition.

INTRODUCTION

Higher plants are employed as medicine by different people of both rural and urban areas all over the world who have been using them as sources of food and medicine since the dawn of civilization (Sofowora, 1993). Infectious disease is the world's major threat to humans and account for almost 50,000 deaths everyday (Ahmad and Beg, 2001). The situation has further been complicated with the rapid development of multidrug resistance by microorganisms to the antimicrobial agent available.

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Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (*Buenz et. al*., 2004). The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Kubmarawa *et al*., 2008).

Guiera senegalensis commonly known as Sabara in Hausa is a shrub of the savannah region of west and central Africa. It is widely being used in traditional medicine for the remedy of many ailments/diseases. Its leaves extract is being used against dysentery, diarrhoea,gastrointestinal pain and disorder, rheumatism and fever (Sule and Mohammed, 2006). The astringent property of this plant was reported to be due to its tannin content (Koumare *et al*., 2002).

Prospopis africana (known as kiryaa in Hausa) is found in sub tropical and tropical regions of America, Africa, West Asia and South Asia. It is known to contain a myriad of complex chemical compounds which is health wise beneficial to humans and animals (Edeoga *et al.*, 2005). It is a plant used as chewing stick and is carefully selected for such properties as foaminess, hardness or bitterness. Almost all parts of the tree are used in medicine; the leaves in particular for the treatment of headache and toothache; the back is used for eyewashes; the roots are used for treatment of gonorrhea, tooth and stomach ache (Tagboto and Townson, 2001).

Medicinal properties of plants are hinged on the presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and essential oils amongst others. This necessitates the need for continued screening of medicinal plants, not only to determine the scientific basis for their usage, but also to discover new active principles (Karou *et al*., 2006). The galls of *Guiera senegalensis* demonstrated pronounced antioxidant potential, showed high polyphenols, totals tannins and totals flavonoids contents (Sombié *et al.*, 2011a, b). The flavonoids are not only present in plants as constitutive agents but have also accumulated in plant tissues in response to microbial attack (Galeotti *et al.*, 2008).

The primary benefits of using plant derived medicines are that they are relatively cheaper than synthesized alternatives offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine.
The rapid increase in the antibiotic resistance of microorganism to the available synthetic drugs and the discomforting side effect of synthetic antibiotic necessitate the search for alternative source of antibiotics. This is because natural extracts have significant effect and alternative therapy of some bioactive ingredient of natural source from plants that will enhance the quality of the treatment of diseases.

This study is to access the antimicrobial efficacy of *Guiera senegalensis* and *Prosopis africana* leave extracts against some bacterial pathogens.

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MATERIALS AND METHODS

Sample collection
G. senegalensis and *P. africana* leaves were collected from healthy plants in Gwallameji, within Bauchi metropolis and transported to the Biology/Microbiology laboratory of Science Laboratory Technology Department, Federal Polytechnic Bauchi in sterile polythene bags.

Sample preparation
The leaves collected were washed and dried in the laboratory away from sun light for seven days to prevent the loss of active components. Some of the leaves were ground and then sieved to obtain powder. Some fresh leaves were also preserved in the refrigerator while others were ground for immediate use.

Crude extraction methods
The extraction method described by Omoregbe (1997) was used for crude extraction. The fresh leaves of G*uiera senegalesis* and *Prosopis africana* were washed to remove any debris; they were then ground using mortar and pestle and squeezed out into a clean sterilized beaker. The extracts were stored in screw cap bottles and labelled.

Aqueous Extraction
The procedure used for aqueous extraction was that described by Predrag *et al*, (2005). This was obtained by weighing 250g of the plant powder. 500ml of distilled water was added to it and was heated for 20mintues in a water bath. It was then filtered, the mixture and the filtrate were concentrated over the water bath and the extract was stored in a sterile bottle and labeled.

Ethanol Extraction
40g of the powdered plant sample was weighed and mixed with 300ml of ethanol. The mixture was kept for two days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. This mixture was filtered through muslin cloth. The extracted liquid was subjected to water bath evaporation to remove the solvent. The water bath temperature was adjusted to 40^{o}C. The same procedure was used for the aqueous extract. The semi-solid extract produced was kept under a ceiling fan to dry. The extract was weighed and portion of it used for phytochemical screening while the rest was used for the susceptibility test.

Phytochemical screening
Phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, glycosides, anthraquide, steroids and terpense was carried out according to standard procedures as reported by Sofowora, (1993).

Test for glycoside ($FeCl_3$ test)
To about 0.5 of the extract, 5ml of concentrated tetra-oxosulphate (VI) acid (H_2SO_4) was added and boiled for 15 minutes. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Three drops of ferric chloride solution was added to one of the portions. And a green to black precipitate indicated the presence of glycoside.

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Test for saponins (frothing test)
5ml extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponin.

Test for steroids
Five drops of concentrated H_2SO_4 was added to 1ml of extract in a test tube. Red colouration was observed, which indicated the presence of steroids.

Test for Triterpenes
5 drops of acetic anhydride and a drop of concentrated H_2SO_4 were added to 1ml of the extract. The mixture was then steamed for 1h and neutralized with NaOH, followed by the addition of chloroform. Absence of blue-green colour indicated the absence of triterpenes.

Test for Tannins To 1 ml of the extract, 1ml of freshly prepared 10% Potassium hydroxide (KOH) was added. A dirty white precipitate showed the presence of tannins.

Test for Glycoside
3ml of the extract a little amount of magnesium powder (2g) and a few drops of hydrochloric acid were added to the extract. A red coloration indicates the presence of reddish brown steroid ring indicates the presence of cardiac glycosides.

Test for alkaloids
To 3 ml of the extract, 1ml of 1% hydrochloric acid (HCl) was added in a test tube. The mixture was heated for 20minutes, cooled and filtered. Two drops of Mayer's reagent was added to 1ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

Quantitative determination of the chemical constituency
Preparation of Fat Free Sample
The sample was de-fatted with 100ml of di-ethyl ether using a soxhlet apparatus for 2hours. This was done according to the method of Edioga and Gomina, (2000).

Determination of Quantitative Phenols and Flavonoids by Spectrophotometric Method
The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15minutes. 5ml of the extract was pipetted into a 50ml flask, and then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl-alcohol were also added. The samples were made up to mark and left to react for 30minutes for colour development. This was measured at 505nm (Edioga and Gomina, 2000).
For determination of flavonoid content, the samples were extracted repeatedly with 100ml of 80% of aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42. The filtrate was later transferred into crucible and evaporated into dryness over a water bath and weighed to a constant weight. (Bohm and Kocipai-Abyazan, 1994).

Isolation and identification of test Organism
Pure culture of test organisms were collected at El-salem Medical Laboratory, Bauchi and transported to the microbiology/biology laboratory of Science Laboratory Technology department, Federal polytechnic Bauchi for the study. The morphological, physiological and

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biochemical test were carried out to confirm these organisms; *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli*, according to methods described by Cheesbrough, (2000). The following biochemical tests were carried out to confirm and authenticate the organisms: catalase, coagulase, indole, hydrogen sulphide and sugar fermentation test were carried out.

Antimicrobial activity of extracts

Staphylococcus aureus, *Salmonella typhi* and *Escherichia coli*, were re-isolated and the pure culture sub cultured on nutrient agar slants. They were stored at 4^0C until required. An in-vitro test using the agar diffusion method was carried out. All the test bacteria used were incubated and introduced into nutrient agar broth respectively. About 20ml of sterile molten nutrient agar in a petri-dish was seeded with 1.0ml of standardized broth cultures of the bacteria ($5.9x10^7$cfu/ml) and swirled gently to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After then, 6mm diameter wells were bored in the agar with sterile cork borer and filled with 0.5ml of various dilutions of the two extracts, thus, 60%, 70%, 80%, 90% and 100% with distilled water. The petri-dishes were allowed to stand for 1hr and then incubated for 37^oc for 24hr. At the end of incubation, zone of inhibition that developed were measured with a transparent ruler and calculated. Distilled water was used as control (Cheesbrough, 2000).

RESULT

Table 1 shows the phytochemical component of *Guiera senegalensis* leaves extract based on crude, aqueous and ethalolic extraction methods. Tannin, flavonoid, saponin, steroid, triterpenes and glycoside were present while alkaloid was absent in all extraction methods.
In *Prosopis africana*, tannin, flavonoid, saponin, steroid, triterpenes and alkaloid were present while glycoside was absent in both aqueous and ethanol extraction (Table 2).
Table 3 shows the quantitative phytochemical determination of both plants. The total phenol and flavonoid contents of *Guiera senegalensis* are 14.52mg/100g and 1.352mg/100g respectively. And the total phenol and flavonoid contents of *Prosopis africana* are 10.22mg/100g and 3.041mg/100g respectively.

Tables 4 and 5 show the antimicrobial pattern of crude, aqueous and ethanol extractions of *Guiera senegalensis* and *Prosopis africana* against some bacteria, in which the ethanolic extracts show the highest zones of inhibition for all the concentrations been prepared. *P. africana* ethanolic extract at 100% concentration had the highest zones of inhibition 4.7mm, 4.0mm and 4.0mm against *S. aureus*, *E. coli* and *S. typhi* respectively. *Guiera senegalensis* ethanolic extract at 100% concentration also had its highest zones of inhibition of 3.0mm, 2.8mm and 3.5mm against *S. aureus*, *E. coli* and *S. typhi* respectively. At 60% and 70% concentrations, the crude and aqueous extracts of *Guiera senegalensis* and *Prosopis africana* didn't inhibit the growth of any of the organisms. The ethanol extract of *Prosopis africana* at 60% concentration showed 1mm zone of inhibition against both *S. aureus* and *S. typhi* and no inhibition against *E. coli*, while ethanol extract of *Guiera senegalensis* at 60% concentration showed 1mm zone of inhibition against *S. aureus* and no inhibition against *E. coli* and *S. typhi*.

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Table 1: Phytochemical component of *Guiera senegalensis* leaf extract

	Crude	Aqueous	Ethanol
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Triterpense	+	+	+
Alkaloids	-	-	-
Glycoside	+	+	+

Table 2: Phytochemical component of *Prosopis africana* leaf extract

	Crude	Aqueous	Ethanol
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Triterpense	+	+	+
Alkaloids	+	+	+
Glycoside	+	-	-

Table 3: Determination of quantitative phenolic and flavonoid content of *Guiera senegalensis* and *Prosopis africana*

Plant samples	Phenol (mg/100g)	Flavonoid (mg/100g)
Guiera senegalensis	14.52	1.352
Prosopis africana	10.22	3.041

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Table 4: Antimicrobial pattern of *Prosopis africana* leave extracts on some bacteria

Plant extract of *Prosopis africana*	Concentration (%)	*Staphylococcus aureus(mm)*	*Escherichia coli(mm)*	*Salmonella typhi(mm)*
Crude	100	2.0	3.0	2.0
	90	1.3	1.5	1.0
	80	1.0	1.3	1.5
	70	-	-	-
	60	-	-	-
Aqueous	100	2.7	2.0	1.5
	90	1.2	1.0	1.3
	80	1.0	-	1.0
	70	-	-	-
	60	-	-	-
Ethanol	100	4.7	4.0	4.0
	90	3.0	3.3	3.5
	80	2.0	3.0	3.0
	70	1.5	2.0	2.7
	60	1.0	-	1.0

Key: - = Negative, % = Percentage, mm= millimeter zone of inhibition

Table 5: Antimicrobial pattern of *Guiera senegalensis* leave extracts on some bacteria

Plant extract of *Guiera senegalensis*	Concentration %	*Staphylococcus aureus(mm)*	*Escherichia coli(mm)*	*Salmonella typhi(mm)*
Crude	100	2.6	1.5	2.0
	90	2.0	1.2	1.5
	80	1.0	-	-
	70	-	-	-
	60	-	-	-
Aqeous	100	2.7	1.8	1.3
	90	1.0	1.5	1.0
	80	-	1.0	-
	70	-	-	-
	60	-	-	-
Ethanol	100	3.0	2.8	3.5
	90	2.5	2.0	2.0
	80	2.7	1.5	2.5
	70	2.0	1.0	1.5
	60	1.0	-	-

Key: - = Negative, % = Percentage, mm= millimeter zone of inhibition

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DISCUSSION AND CONCLUSION

In this study, the result obtained indicates that leaves extracts of *G. senegalensis* and *P. africana* at different concentrations were quite effective against some of the test organisms. This shows that the extract of *G. senegalensis* contain substances that can inhibit the growth of some micro organisms. Williams *et al*., (2009) also reported that leave extract of *G. senegalensis* inhibited the growth of various microorganisms at different concentrations. Literature reports several recorded use for *G.sene*galensis in traditional medicine to treat various illnesses (Aniagus, *et al*., 2005). The observed antibacterial effect of the isolate is believe to be due to the presence of tannins, flavonoids and saponins which have been shown to possess anti-bacterial properties (Osadebe, 2004). This is in agreement with Luttrodt *et al*., (1999) who carried out similar work on plant extract of *Eucalyptus Camaldulensis* against *Salmonella typhi* and *Escherichia coli*, and attributed the action of the plant to this active ingredient.

Quantitative phytochemical screening was also carried out on the plants; flavonoid and phenolic contents present in *Guiera senegalensis* were 1.352 mg/100g and 14.52 mg/100g respectively; while that of *Prosopis africana* were 3.041mg/100g and 10.22 mg/100g respectively. This result conforms with the result of Osadebe (2004) whose result of flavonoid and phenolic contents while working with *Guiera senegalensis* were 1.461mg/100g and 14.63 mg/100g respectively. The presence of flavonoids also agrees with work of Singh and Bhat, (2003) that flavonoids were responsible for antimicrobial properties in some ethno medicinal plants. The presence of high amount of phenolic content in this study may also be responsible for it antimicrobial effectiveness.

The antimicrobial pattern of this study shows that ethanolic extract has the highest zones of inhibition in both plant extract followed by aqueous and crude. This is in line with the findings of (Srinivasan *et al.,* 2001) who stated that different solvent have different spectrum of solubility for the phytochemical constituent. The ethanolic extract was more effective and aqueous extract was also effective against the test organism compared to crude and cold extract because the active ingredient in the plant dissolved better in ethanolic solvent. This is in line with the findings of Aggrarwal,*et al.,* (2007) who carried out similar work on plant extract against *Staphylococcus aureus*.

Conclusions

The result of the studies shows that the various extracts of *Guiera senegalensis* and *Prosopis africana* show some antimicrobial activity against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus.* This indicates that the use of *Guiera senegalensis and Prosopis africana* leaves as traditional medicine has a lot of potential in treatment of antimicrobial infections with further standardization.

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