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A COMPARATIVE PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *Boswellia dalzielii, Bridelia ferruginea* AND *Prosopis africana*

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ABSTRACT
This study was on the comparative phytochemical analysis and antimicrobial activity of Boswellia dalzielii, Brideliaferruginea and Prosopis africana against the clinical isolates of Staphylococcus aureous and Escherichia coli. The plants stem bark extracts revealed the presence of Alkaloids, Flavonoids, Tannins, Saponins and Cardiac glycosides. While Steroids and Phenols were absent. The concentrations of Alkaloids and Flavonoids were higher in Prosopis africana.Tannins and Saponins were higher in Boswellia dalzielii and that of Cardiac glycosides was higher in Brideliaferruginea .There were significance differences at P< 0.05 among the values of Alkaloids, Flavonoids, Tannins, and Cardiac glycosides in the three plant species. However, there was no significance difference between the values of Saponins in Boswellia dalzielii andProsopis africana.but there was significance difference between the values of the two plants and that of Brideliaferruginea. The results of the antimicrobial screening revealed that Boswellia dalzielii and Brideliaferruginea were effective on Staphylococcus aureousand Escherichia coli, while Prosopis africana was effective on Staphylococcus aureous only. However the extracts were not as effective as Ciprofloxacin used as standard. The antimicrobial activity of the three plants species might be due to the high concentrations of Alkaloids and Flavonoids. The plants may serve as potential source of useful drugs.

INTRODUCTION

Medicinal plants represent a growing alternative to clinical -medicine in the management of health challenges and diseases. Plant exhibits wide biological and pharmacological activities such as anti-inflammatory, diuretic, laxative, anti- plasmodic, anti-hypertensive and anti-microbial functions due to the presence of pharmacological bioactive phyto constituents available in these plants (Onobrudu and Harcourt, 2017). Demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries Abere*et al.,* (2010). The World Health Organization (WHO) estimated that about 80 % of developing world's population meets their primary healthcare needs through traditional medicine Abere *et al*., (2010). Despite an apparent lack of scientific evidence for their quality, safety and efficacy Jadeja *et al.,* (2011). Most plants consumed locally in Nigeria have not been thoroughly evaluated for their phytochemical profile and antimicrobial properties Musa *et al*., (2005).

Boswellia dalzielii is a tree that belongs to the family of *Burseraceae*, from the genus of *Boswellia* and species of *B. dalzielii*. It is about 13m high of the wooden savanna with a pale papery bark peeling and ragged characteristic... Both the root and the bark are used as an antidote for arrow poison (Choi, 2003). *Bridelia ferruginea* belongs to the family *Euphorbiaceae* which is commonly found in Savannah regions. Its common names are Kizni (Hausa), Marehi (Fulani). The tree is 6 - 15 m high (Ekanem *et al*., 2008). *B. ferruginea* has diverse uses, a decoction of the leaves has been used to treat diabetes, the bark extract has been used for the coagulation of milk (Burkil, 1994). *Prosopis africana* also known as African mesquite belongs to the family "Mimosoideae". The common vernacular names of the plant include "kirya" (Hausa) Ayanwuyi *et al*., (2010). The plant is a quick-growing tree about 17 m tall (Burkhill, 1995). The bark decoction is used locally in Senegal for toothache. The crushed bark is placed over skin infections (Burkhill, 1995).

Phytochemicals are chemical compounds produced by plants, generally to help them thrive competitors, predators, or pathogens. Some phytochemicals have been used as poisons and others as traditional medicine (Igwenyi *et al*., (2011).

It is well-known that plants produce these chemicals to protect themselves but recent research demonstrated that they can also protect humans against diseases (Igwenyi *et al.*,(2011).The aim of this research work is to compare qualitatively and quantitatively the phytochemicals in the stem bark extracts of *Boswellia dalzielii*, *Prosopis africana* and *Bridelia ferruginea* and also test their antimicrobial activities.

MATERIALS AND METHODS

Collection and Preparation of Plants Samples

The Plants Stem bark collected were from their natural habitat, about 5km away from Gusau to Magami road, at Tungar Danrani Village, Bungudu Local government, Zamfara State, each was scratched using hoe, aid field guide and hand lens, the plants were identified and authenticated in the Department of Botany, Kebbi State University of Science and Technology Aliero. Four hundred Gram (400g) each of the powdered plant was added to 1.5 liter of distilled water inside conical flask and plugged with cotton wool. After 74 hours the mixture was filtered using white cloth and then through Whatman Paper No.11. The filtrate was concentrated by evaporating in water bath at temperature of 50^0 C and stored in refrigerator at temperature of 5^oC. (Iweala and Okeke, 2005).

Qualitative Phytochemical Analysis

The Tests for Alkaloids and Phenol were carried out using the method described by Ibukun *et a*l. (2013) and Harathi *et al*. (2017). Flavonoids and Steroids were tested using the method reported by (Amah and Eze, 2010) and Adebayo *et a*l., (2012). Tannins was tested using the method reported by Ogbada *et al*., (2017) (Ameh and Eze, (2010). The presence of Saponins was tested using the method reported by Ekweme *et al*., (2015), Ibukun *et al.,* (2013), and Cardiac Glycosides was with the method reported by Ibukun *et a*l., (2013) and Sahu *et al*. (2014)

Quantitative determination of Phytochemicals

Determination of Alkaloids content:

Alkaloids were determined using the method reported by Ibukun *et al*., (2013) and Adebiyi *et a*l., (2012). 100 cm^3 of 10% acetic acid in ethanol was added to 5 g each of powdered plant sample in a 250 cm^3 beaker and was allowed to stand for 4 hours. The extract was concentrated on the water bath to one-quarter of their original volume followed by addition of 0.1M ammonium hydroxide drop wise until the precipitation was completed. The solution was allowed to settle and the precipitate was collected and washed with 20 cm^3 of 0.1 M ammonium hydroxide and then filtered using filter paper (no.11 $cm^{3)}$, the residue was allowed to dry in oven and weighed. Percentage of alkaloids was calculated mathematically using equation 1.

$$\% \text{ Alkaloids} = \frac{\text{weight of the residue}}{\text{weight of the sample}} \times 100 \text{ ----------1}$$

Amount in mg per 1 g was Converted using the following expression

x g = 100 g,x ×1000 mg = 100 g,q mg = 1g, q = x1000 / 100, Where x is the amount in gram obtained, q is the amount in milligram obtained

Determination of Flavonoids Content

Flavonoids were determined according to the method followed by Ibukun *et al*.,(2013). The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. About 100µl of the sample extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of sample extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions.

Determination of Saponins

Saponins were determined using the method adopted by Adebiyi *et al*., (2012) and Hamed *et a*l., (2013). 5 g each of the samples was taken in to a conical flask. 50 ml of 20% aqueous ethanol was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residues were re-extracted with another 50 ml 20% ethanol. The combined extract was reduced to 10 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml reparatory funnel and 5 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated twice.60 ml of n-butanol was added, The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, it was dried in the oven. The Saponins content was calculated as a percentage using equation 3.3 bellow.

$$\% \text{ saponins} = \frac{\text{weight of the residue}}{\text{weight of the sample}} \times 100 \text{ ----------2}$$

Amount in mg per 1 g was Converted using the method described in method one

Determination of Tannin Content

Tannins was determined using the method adopted by Ibukun *et al* ., (2013) and Ameh *et al.*, (2010) . 5 gram of the plant samples was weighed in to the 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. Then 5 ml of the filtrate was measured into a test tube and mixed with 2 ml of, 0.1M $FeCl_3$, 2ml 0f 0.1M HCl and 2ml of 0.008 M potassium ferro-cyanate. The absorbance was measured at 395 nm after 10 minutes. A blank was prepared without the sample and the absorbance was taken at the same wavelength. Standard curve was also prepared using tannic acid (1.00 mg/ml), varying concentrations (0.02–1.0 mg/ml) of the standard tannic acid solution was measured into five different test tubes to which all the reagents above were present. The absorbance was read at the same wavelength to obtain a calibration curve.

Tannins concentration was calculated using equation 3 bellow

$Y = mx + C$ ----------3

Y = Absorbance of the sample, M = Slope, X= Equivalent of tannic acid, C = Intercep

Determination of Cardiac Glycosides

Cardiac glycosides were determined using the method adopted by Adebiyi *et al*., (2012).1g of the fine powder of plant sample was soaked in 10ml of 70% alcohol for 2hrs. And then filtered. 8ml from the filtrate obtained was mixed with 8 ml of 12 % lead acetate (to precipitate resin, tannin and pigments), this mixture in 100 ml volumetric flask was filled to the mark with distilled water and filtered.50 ml of the filtrate was measured in to another 100 ml volumetric flask, 8 ml of 4.7 % disodium hydrogen phosphate (Na_2HPO_4) solution was added (to precipitate excess lead). The mixture was made up to the mark with distilled water. The mixture was filtrated twice with filter paper.10 ml of Buljet's reagent (containing 95ml aqueous picric acid and5ml of 10% aqueous NaOH was added to 10 ml of the filtrates. The blank sample was prepared by addition of 10 ml buljet's reagent in to 10 ml distilled water. The two mixtures were allowed to stand for one hour (time maximum for color development) . The intensity of color was read at 495 nm spectrophotometer against the blank The percentage of total glycosides was calculated using the equation 3.6 below.

$$\%\ \text{Glycoside} = \frac{\text{Absorbance of the sample}}{17} \times 100$$ ----------4

17 is a constant value of standard digitoxins ($C_{41}H_{64}O_{13}$

Amount of Glycoside in mg per 1g was Converted as expression methodone

Antimicrobial Screening

Test Bacteria

The test bacterial strains used for the study were *E. coli*, and *Staphylococcus aureus*, Stock cultures were obtained from Department of Microbiology, Kebbi State University of Science and Technology Aliero .

Preparation of the Media

All media used for this research was prepared according to the manufacturer instruction as contained in the laboratory. The media used in this research work were Nutrient agar, Mueller Hinton agar and nutrient broth.

Antibacterial Activity Assay

The agar well diffusion method was used to determine the antibacterial activity of the plant s extracts as described by Zaharadeen *et al.*, (2016) and (Nawsher M. and Ali M,2017). 0.1 ml of standardized organisms (0.5 Mac Farland = 10^6 Cfu ml^{-1}) was inoculated on the surface of sterile Mueller Hinton Agar plates and allowed to set and then solidified. A sterile cork borer 6mm was used to punch holes (5 wells) in the inoculated agar. Five wells that were formed in each Petri dishes were filled with different concentrations of the extracts which were labeled accordingly, 200,400,600,800 and 1000 mg/ml. while the 5th well contained the solution used for the research to serve as control, Ciprofloxacin 250 mg/ml, was used as control. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters.

Determination of Minimum Inhibitory Concentration (MIC) of the crude extract

The MIC was determined according to the method proposed by (Nas and Ali, 2017). The standard drug used was Ciprofloxacin, the stock Concentration of was 250 mg/ml. stock solution of each of the extracts (stem bark of *B. dalzielii ,P. africana* and *B. ferruginea*) was 1000 mg. Sterile test tubes were used for each extracts, 2ml of sterile nutrient broth was dispensed from test tube 1-12. 1000 mg of each crude extract was dissolved into 4ml of distilled water. 2ml of the stock solution was dispensed aseptically in to tube 1 and 2ml, from the contents of test tube 2 a doubling dilution was performed using 2ml transfer to tube 10, leaving tube 11 and 12. 2ml was taken out of tube 10 and discarded.

The concentration in each tube from tube 1 to 10 was 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.9, 1.95 and 0,98 mg/2ml respectively. 1:100 (10^{-2}), broth culture of the test bacteria (*Staphylococcus aureus*, and *E. coli)* were prepared separately and the dilution of the broth culture were compared with 0.5 McFarland turbidity standards. 2ml of the culture was dispensed into each test tubes with exception of test tube 11 and 2ml of sterile nutrient broth was added to the test tube 11, and were then incubated at 37^{o}C for 24 hours. After 24 hours, the test tubes were examined for turbidity in order to determine the MIC. Test tube 11 served as control for the sterility of broth , while test tube 12 served as control for the viability of the test bacteria . The MIC was the concentration in the tube that fails to show evidence of growth (turbidity), just immediately after the last one that shows growth.

STATISTICAL ANALYSIS

Data for the quantitative analysis are presented as mean ± standard deviation and were analyzed using One way Analysis of variance (ANOVA) and statistical program SPSS version 20.1.The values were considered significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

The results for the phytochemical screening of the extracts from the three plant samples are presented in table 1. The results revealed the presents of Alkaloid, Flavonoids, Tannins, and Saponins and Cardiac glycosides. Alkaloids were present in lower concentration in *Boswellia dalzielii* and *Bridellia ferrugine.* Where as *Prosopis Africana* had the highest amount*.* Flavonoids were present in higher amount in *Prosopis africana,* while the lowest amount observed in Boswellia *dalzielii*. Tannins had the highest amount in *Boswellia dalzielii* where as the lowest amount was in *Prosopis africana.* The highest concentration for Saponins was observed in *Boswellia dalzielii*a and *Prosopis Africana* while the lowest value was in *Bridelia ferruginea* . Similarly, Cardiac glycoside was the highest in *Boswellia dalzielii* where as the lowest was obsearved in *Prosopis africana* and *Bridelia ferruginea* . Phenols and Steroids were absent in all the three plants species.

Table 1: Qualitative Phytochemical screening of aqueous extracts *Boswellia dalzielii, Bridelia ferruginea and Prosopis africana.*

Phytochemicals	*Boswellia dalzielii*	*Bridellia ferruginea*	*Prosopis africana*
Alkaloids	++	++	+++
Flavonoids	+	++	+++
Tannins	++	+	+
Saponins	+++	++	+++
Phenols	-	-	-
Cardiac glycosides	++	+	+
Steroids	-	-	-

Key : + = Presence in trace ,++ = Presence in moderate , +++ = Presence in large , - = not detected.

Table 2: Quantitative analysis of Aqueous extract of *Boswellia dalzielii, Bridellia ferrugenia and Prosopis africana in* mg /g

Phytochemicals	Amount(mg) *Boswellia dalzielii*	Amount(mg) *Bridellia ferruginea*	Amount (mg) *Prosopis africana*
Alkaloids	102.67 ± 3.06^{a}	122.00 ± 3.46^{b}	146.00 ± 5.29^{c}
Flavonoids	42.67 ± 2.52^{a}	56.00 ± 5.20^{b}	73.33 ± 5.77^{c}
Tannins	0.77 ± 0.02^{c}	0.65± 0.04^{b}	0.55 ±0.02^{a}
Saponins	122.00. ± 11.50^{b}	66.70 ± 1.50^{a}	113.30 ± 11.50^{b}
Cardiac glycosides	3.69±0.07^{b}	3.91 ±0.03^{c}	2.69 ± 0.03^{a}

Values reported as mean ± standard deviation. Mean followed different letters (a-c) in the same row are significantly different from each other at ($p < 0.05$), those with the same letter in the same row are not significantly different at ($P < 0.05$).

The quantitative Phytochemical composition of the three plants species as presented in Table 2 showed that the value of alkaloids present in *Boswellia dalzielii*s was higher compared with the values of 963.3mg/100g (9.633 mg/g) obtained in *Boswellia serrate* reported by Mohammed *et a*l.,(2010). The value of alkaloids obtained in *Bridellia ferruginea* was higher compared with the 11.70g/100g (117mg/g) for the same plant reported by Zaharadeen *et al.*,(2016).

The value obtained in *Prosopis Africana* was higher than 102.4 mg/g of *Prosopis africana* reported by Kolapo *et al*., (2009). However the values obtained in the three plants species were lower than 1.6g/g (160mg/g) for *Barbulaindica* reported by Adebiji *et al*., (2012). There was significant difference at ($p < 0.05$) between the values of alkaloids obtained in *Boswellia dalzielii, Bridellia ferruginea,* and *Prosopis africana.* The value of Flavonoids obtained in *Bridelia ferrugenia* was lower than the value of 2.07g/100g (20.7mg/g) reported by Adamu *et al., (2016).* Similarly, the values obtained in the three plants species were higher than 0.34g/100g (3.4mg/g) obtained in *Cleome nutridosperma* and 0.019g/100g (0.19mg/g) obtained in *Senecio biafrae* reported by Ibukun *et al.,* (2013). There was significant difference at ($p< 0.05$) between the values of Flavonoids obtained in *Boswellia dalzielii, Bridellia ferruginea* and *Prosopis africana*.The value for Tannins obtained in *Boswellia dalzielii* was lower than 240mg/g obtained in *Boswellia serrata* reported by Mohammed *et al*., (2010). Similarly, the value obtained in *Bridelia ferruginea* in this study was lower than the value of 8.33g/100g (83.3mg/g) obtained by Zaharadeen *et al.,* (2016), also higher than the value of 4.75 mg/g obtained in Leaf extract of *Senna mimosoides* reported by Ekwueme *et al., (2015)*. The value obtained in *Prosopis africana* was lower than the value of 85.9mg/g obtained from the same plants specie reported by Kolapo *et al*., (2009). There was significant difference at ($P< 0.05$) between the values for the three plants species.The value of Saponins obtained in *Bridelia ferruginea* was higher than 9.85g /100 g(98.5mg/g) obtained from the same plants specie reported by Zaharadeen *et al*., (2016). The value of Saponins obtained in *Prosopis africana* was higher than 110.0 mg/g obtained from the same plant specie reported by Okunade *et al.,* (2009). The values obtained for the three plants specie were higher than 0.56g/100g (5.6mg/g) obtained from *Sineciobiafrae* reported by Ibukun *et al.,* (2013) and higher than 2g/ 100g (20mg/g) from *Cleome nutridosperma* reported by Edeoga *et al*., (2005) , also higher than the value of 1.97 mg/g obtained in Leaf extract of *Senna mimosoides* reported by Ekwueme *et al., (2015)*. There was significant differences at $P < 0.05$ between the value obtained in *Boswellia dalzielii* and *Bridelliaferrugenia* and the value obtained in *Bridellia ferrugenia* with that obtained in *Prosopis africana*. Similarly, there was no significant difference between the value obtained in *Boswellia dalzielii* and the value obtained in *Prosopis africana*. The Presence and concentrations of Saponins indicated that the plants could be good source of Saponins. The value of Cardiac glycoside obtained in *Bridelia ferruginea* was lower than 5.48g/100 (54.8mg/g) obtained from the same plant as observed by Zaharadeen *et al.,* (2016). However The values obtained for the three plants species were higher than 0.06g/100g (0.6mg/g) obtained from *Sineciobiafrae* reported by Ibukun *et al* (2013)..There was significant different at ($P< 0.05$) between the mean values of Cardiac glycosides for the three plants species. **T**he presence of cardiac-glycosides in plants is an indication that the plants may have a strong and direct action on the heart, which helps in supporting its strength and rate contraction when it is failing.

Table 3: Antibacterial sensitivity test of *Boswellia dalzielii, Bridellia ferruginea and Prosopis africana.*

Drug	Concentration in mg	Zone of inhibition (mm)	
		Staphylococcus aureus	*Escherichia coli*
Extract of *B. dalzielii*	125	8.00	7.00
	250	10.00	8
	500	11.00	10.00
	Mean	*9.67*	8.30
Extract of *B. ferruginea*	62.25	7.00	7.00
	125	9.00	8.00
	250	10.00	9.00
	500	11.00	10.00
	Mean	9.30	8.50
Extract of *Prosopis africana*	62.25	8.00	6.00
	125	9.00	6.00
	250	10.00	6.00
	500	11.00	6.00
	Mean	9.50	6.00
Control Ciprofloxacin	250	12.00	11.00

The results of antimicrobial activity for *Boswellia dalzielii, Bridellia ferruginea* and *Prosopis africana* on the Gram-positive and Gram-negative bacteria (*Staphylococcus aureus, Escherichia coli*) are presented in Table 3 Ciprofloxacin was used as standard drug. The sensitivity test showed that, *Boswellia dalzielii* and *Bridellia ferruginea* extracts were effective on all the microbes wi th higher zone of inhibition of 9.67mm and 9.30mm against *Staphylococcus aureus and Escherichia coli*, while *Prosopis africana* was only effective on *Staphylococcus aureus* with zone of inhibition of.30mm. Similarly, there was dose dependent increase in the zone as shown in Table 3 against the test bacteria for Extracts of *B dalzielii* and *B. ferruginea,* but no dose dependent increase was observed against *Escherichia coli for P. africanas Extract. A*ntimicrobial activity of the three plants species might be due to the high concentration of Alkaloid and Flavonoids in the plants Species Prashant *et al.*, (2011). The results were in agreement to a certain degree with the traditional usage of the plants.

CONCLUSION

This research had shown that the stem bark extracts of *Boswellia dalzielii, Bridellia ferruginea* and *Prosopis africana*, contained the active phytochemicals compounds (Alkaloids, Flavonoids, Tannins, Saponins and Cardiac glycosides). The composition of Alkaloids, Flavonoids, Tannins and Saponins in the three plants species indicated the significant differences at ($P < 0.05$), however there was no significant difference in the mean values of Cardiac glycosides of three species (*Prosopis africana, Bridellia ferruginea* and *Boswellia dalzielii).*It also showed that the plants might be serving as potential source of useful drugs, and the various medicinal uses of the plants might me due to this phytochemicals Presence in the plants.

RECOMMENDATION

These plants studied could be seen as a potential source of useful drugs. Further studies should be conducted in other to isolate, identify, characterize and elucidate the structure of these bioactive compounds. Similarly, more research should be carry out on the active ingredients responsible for the plant's antimicrobial activity , it is al so important to find out their toxicity

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