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PHYTOCHEMICALS AND ANTIMICROBIAL SCREENING OF AN INDIGENOUS *Caesalpinia Coriaria* (Divi-Divi)

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ABSTRACT
Caesalpinia coriaria has been screened for their Phytochemicals and antimicrobial activity against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp. and Salmonella spp. The tannins extracted using water, acetone and methanol exhibited antimicrobial activity against Staphylococcus aureus, Bacillus subtillis, Escherichia Coli, Proteus vulgaris, Shigella dysenteriae and Candida albicans. The results showed that methanol extracts have more inhibitory effect than the other extracts. However, in this study the focus is on water extract which is the solvent used traditionally. The highest zone of inhibition of the water extract was found to be 22 mm of Bacillus subtillis compared with a standard Sparfloxacin 10µg/disc which gave 23 mm of Bacillus subtillis. Phytochemicals screening including alkaloids, tannins, saponin, steroid, terpenoids, flavonoid, carbohydrates, and glycosides were present. The proximate analysis of the extracts - moisture content, total soluble, pH, and total solid and total insoluble gave 4.00%, 50.88%, 5.59, 96.00% and 45.12% respectively. . It may be concluded that Caesalpinia coriaria pods contain bioactive compounds of potentially therapeutic and prophylactic significance and thus could be a promissory candidate for drug development.

Keywords: *phytochemicals, antimicrobial and Caesalpinia coriaria.*

INTRODUCTION

Infectious diseases are the number one cause of death worldwide, and in tropical countries, it accounts for approximately 50% of deaths [1]. This may be due to poverty and increasing incidence of multiple drug resistance. Bacterial resistance to almost all antibacterial agents has been reported [2]; this resistance is largely due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases. Apart from resistance, some antibiotics have serious undesirable side-effects which limit their applications, so there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side-effects and higher plants represent a potential source of novel antibiotic prototypes [2,3]. Rapid development of resistance to most conventional antimicrobial agents in use and high cost of the modern antimicrobial agents nowadays, necessitate the establishment of a plant extract which may serve as a lead for the synthesis of an effective, less toxic and cheaper antimicrobial agents

EXPERIMENTAL

Antimicrobial Screening

Antimicrobial activities of extracts from *Divi-divi* were determined using some pathogenic microbes; the microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria. The extract (1 g) was weighed and dissolved in 10 cm^3 of Dimethylsulphoxide (DMSO) to obtain a concentration of 100 mg/l. This was the initial concentration of the extracts used to check the antimicrobial activities of the extracts.

Mueller Hinton broth was the medium used as the growth medium for the microbes. The sterilization was carried out in an equitron autoclave at 121^{0}C for 15 min, the sterilized medium was then poured into sterile Petri dishes, and the plates were covered and allowed to cool and solidify. Sterilization of glass was carried out by diffusion method, which was used for screening the extracts. The sterilized medium was then seeded with 0.1 cm^3 of the standard inocualum; the inocualum was spread evenly over the surface of the medium with a sterile swab.

By the use of a standard cork borer of 6 mm in diameter, a well was then cut at the centre of each inoculated medium. 0.1 cm^3 of the solution of the extract of concentration 100 mg/ml was then introduced into each well on the medium. The inoculated medium was then incubated at 37 ^{o}C for 24 h, after which each plate was observed for zones of inhibition. The zones were measured with a transparent ruler recorded in millimetres.

Determination of MIC

The minimum inhibition concentration (MIC) of the extract was carried out using broth dilution method.

Preparation of Mueller Hinton broth: This was prepared by literature method [4,5] and dispensed into test tubes, sterilized at 12^{o}C for 15 min, and the broth was then allowed to cool. Mc- farland turbidity standard scale number 0.5 was prepared to give turbid solution based. Normal saline was prepared in 10 cm^3 and dispensed into sterile test tubes, the test microbes were inoculated and incubation was made at 37 ^{o}C for 61 min.

Dilution of the test microbe in the normal saline was done until the turbidity marched that of the Mac-farlands scale by visual comparison; at this point the test microbe has a concentration of about 1.5 x$10^{8.}$ Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 100, 50, 25, 12.5

and 6.25 mg/ml. The initial concentration was obtained by dissolving the 1g of extract in 10 cm^3 of the sterile broth. Having obtained the different concentrations of the extracts in the broth, 0.1 cm^3 of the test microbe in the normal saline was inoculated into the different concentrations of the extract in the broth; incubation was made at 37 ºC for 24 h. After which each test tube of the broth was observed for turbidity (growth), the lowest concentration of the extract in the broth which showed no turbidity was recorded in the minimum inhibition concentration, and assessment of the antimicrobial activity carried using the ratio. The antimicrobial activities were passed through the ratio mbc/mfc, to check whether the test microbes were killed or only their growth was inhibited.

Preparation of Mueller Hinton agar

Mueller Hinton broth was prepared and sterilized at 12 ºC for 15 min, poured into sterile Petri dishes and allowed to cool and solidify. The contents of the MIC in the serial dilution were then sub- cultured onto the prepared medium, incubation was made at 37 ºC for 24 h after which each plate was observed for colony growth as determined in equation.

Table 1: RF Values of Phenolic Components of Ethyl Acetate Extracts of Acid- Hydrolyzed Divi-divi

	A	**B**	**C**	**D**
Components	6%Acetic acid	n-ınol/acetic/H_20 (14:1:	$K_3Fe(CN)_6$- $FeCl_3$(1:1)	Potassium Iodate
Mono-o-galloyl-cyclohexane trans-1,2-diol	0.67	0.79	Blue	Brown
1-o-Gallolyaquinide	0.54	0.26	Blue	Brown
3-o-feruloylquinic acid	0.65	0.43	Blue	Pale brown

Table 2: Phytochemicals Screening

Constituents	Test	Water	Methanol	Acetone
Carbohydrates	Molisch	+	+	+
	Fehling's	+	+	+
Glycosides	Fehling's	+	+	+
	$FeCl_3$	+	+	+
Anthraquinone	Free	-	-	-
Bontrajer's test	Combine	-	-	-
Saponin	Frolling test	+	+	+
Steroids & triterpene	Steroid	-	-	-
(Lieberman buchard test)	Triterpene	+	+	+
Tannins	$FeCl_3$	+	+	+
	Lead sub-acetate	+	+	+
Flavonoid	NaOH	+	+	+
	Slunoda	+	+	+
Alkaloids	Mayer's	+	+	+
	Wayne's	+	+	+
	Drafferd of	+	+	+

Table 3: Proximate Analysis of Vegetable Tannin Extracts

Constituents	%
Moisture content	4.00
Total soluble	50.88
pH of liquid extract	5.59
Total solid	95
Total Insoluble	45.12

Table 4: Antimicrobial Activities of Divi-divi Plant Extracts

Test organism	Acetone extract	Methanol extract	Water
Staphilococcus aureus	S	S	S
Streptococcus pyogenes	R	R	R
Bacillus substilis	S	S	S
Corynebacterium ulcerans	R	R	R
Escherichia coli	S	S	S
Pseudomonas aeniginosa	R	R	R
Proteus vulgaris	S	S	S
Klebsiella pneumonia	R	R	R
Shigella dysenteriae	S	S	S
Candida albicans	S	S	S
Candida tropicalis	R	R	R

Key: S =>sensitive, R => resistance

Table 5: Zone of Inhibition of the Extracts against the Test Microorganism

Test organism	Acetone extract (mm)	Methanol extract (mm)	H_2O Extract (mm)
Staphilococcus aureus	24	26	20
Streptococcus pyogenes	0	0	0
Bacillus substilis	27	25	22
Corynebacterium ulcerans	0	0	0
Escherichia coli	24	27	20
Pseudomonas aeniginosa	0	0	0
Proteus vulgaris	25	25	21
Klebsiella pneumonia	0	0	0
Shigella dysenteriae	24	28	20
Candida albicans	20	24	21
Candida tropicalis	0	0	0

DISCUSSION

Paper chromatography separation and formation of discrete spots were achieved by first hydrolysing the tannins with HCl and extracting with ethyl acetate as shown in Table 1. One dimensional paper chromatography was employed in the separation of individual polyphenols. Paper chromatography of the Divi-divi extracts on Whatman number 1 chromatography paper using 6% acetic acid and n-butanol /acetic acid/H_2O as the mobile phase indicated three (3) spots each. These spots showed up as blue in $K_3Fe(CN)_6$-$FeCl_3$ (1:1) and pale brown-brown in saturated potassium iodate. The Rf values of 0.67, 0.54 and 0.65 in 6% acetic acid are attributed to Mono-0-galloylcyclohexane trans-1, 2-diol, 1-0-Galloylaquinide and 3-0-feruloylquinic acid respectively. Also the Rf values of 0.79, 0.26 and 0.44 in n-butanol/acetic/water are attributed to the respective components. The assertion is in accordance with literature [6].

The Phytochemicals detected using standard methods in the crude extracts of *Caesalpinia coriaria* pods using different solvents (water, acetone and methanol) are shown in Table 2. and confirm qualitatively the presence of carbohydrates, glycosides, Saponin, triterpene, tannins, flavonoid and alkaloids. The phytochemicals were analyzed primarily because it reveals compounds whose presence undoubtedly lends particular properties to the extract but which individually cannot be classified as tannins [7]. This attests to the fact that *Caesalpinia coriaria* pods contain bioactive compounds of potentially therapeutic and prophylactic significance and thus could be a promissory candidate for drug development and validates folkloric claim, as a cure for tuberculosis and other bacterial and fungal infections.

The tannins extracted using water, acetone and methanol exhibited antimicrobial activity against *Staphylococcus aureus, Bacillus subtillis, Escherichia Coli, Proteus vulgaris, Shigella dysenteriae* and *Candida albicans* (Tables 4,5,6 and 7). The results showed that methanol extracts have more inhibitory effect than the other extracts. The highest zone of inhibition of the water extract was found to be 22 mm of *Bacillus subtillis* (The antimicrobial properties of plants is due to the presence of tannins, alkaloids, flavonoids and terpenoids). In Table 2, the increase in antimicrobial effectiveness observed with increase in concentration of tannins is in agreement with the work reported elsewere [8,9,10].

Table 6: Minimum Inhibitory Concentration of the Extracts against the Test Organism

Test organism	Acetone Extract					Methanol Extract					Water Extract				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
Staphilococcus aureus	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
Streptococcus pyogenes															
Bacillus substilis	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
Corynebacterium ulcerans															
Escherichia coli	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
Pseudomonas aeniginosa															
Proteus vulgaris	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
klebsiella pneumonia															
Shigella dysenteriae	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
Candida albicans	-	-	ox	+	++	-	-	Ox	+	++	-	-	ox	+	++
Candida tropicalis															

Key: - No colony growth**,** **ox**-> MBC/MFC, **+** -> scanty colonies growth, **++** -> modorate colonies growth**,** **++++** -> heavy colonies growth.

CONCLUSION

There has been an increasing demand for new antimicrobial drugs due to the increased resistance of some of the microorganisms against antimicrobial drugs. It may be concluded that *Caesalpinia coriaria* pods contain bioactive compounds of potentially therapeutic and prophylactic significance and thus could be a promissory candidate for drug development.

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Table 7: Minimum Bactericidal/Fungicidal Concentration of the Extracts against the Test Microbes

Test organism	Acetone Extract					Methanol Extract					Water Extract				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**	**6.25mg/ml**	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
Staphilococcus aureus	OX	+	++	+++	++++	-	ox	+	++	+++	ox	+	++	+++	++++
Streptococcus pyogenes															
Bacillus subtilis	-	ox	+	++	+++	-	ox	+	++	+++	ox	+	++	+++	++++
Corynebacterium ulcerans															
Escherichia coli	-	-	ox	+	++	-	ox	+	++	+++	ox	+	++	+++	++++
Pseudomonas aeniginosa															
Proteus vulgaris	-	-	ox	+	++	-	ox	+	++	+++	ox	+	++	+++	++++
Klebsiella pneumoniae															
Shigella dysenteriae	-	-	ox	+	++	-	ox	+	++	+++	ox	+	++	+++	++++
Candida albicans	-	-	ox	+	++	Ox	+	++	+++	++++	ox	+	++	+++	++++
Candida tropicalis															

Key: - *No colony growth*, **ox**-*>MBC/MFC*, **+** *-> scanty colonies growth*, **++** *-> moderate colonies growth*, **++++** *-> heavy colonies growth*

REFERENCES

1. Iwu, M. (1993). Handbook of African medicinal plants. Edited by James, E. G., CRC press, Boca Raton. 4th edition. pp 55-72
2. Gvenwald, J. (1997). The herbal remedies market in the US market development, consumer, legislation and organization, phytopharm consulting communiqué. p 7.
3. Haslam, E. (1996). Natural Polyphenols (Vegetable tannins) as drugs: Possible modes of action, *Journal of Natural Product*; vol. 59 pp. 205.
4. Jigam, A.A., Akanya, H.O., Ogbadoyi, E.O., Dauda, B. and Evans, E.C. (2009). *In vitro* antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of lippie multiflora, mold. *Journal Medical Plants. Reg.* 3(3): 148-154.
5. Kurosoki, F. and Nishi, A. (1983). Isolation and antimicrobial activity of the phytoalexin-6-methoxymellein from cultured carrot cells. *Phytochemistry* 22(3): 669-672.
6. Ukoha, P.O., Ejila A., Maju C. and Asegbeloyin J.D. (2008) Tannic acid as a standard for the sepctrophotometric Determiantion of Tannins *Journal of chemical society of Nigeria*, Vol. 33(1):181-186
7. Singleton, V.L., Orthofer R., and Lamuela- Raventos, R.M. (1999). Analysis of total phenolics, By means of Follin- ciocallaeu reagent methods in Enzymology, oxidants and anti-oxidants. Part A. In: *Lester Packer* (Ed.) (1999). San Diego: Academic Press; 152- 178.
8. Erasto, P., Bbojase-moleta, G., and Majinda, R.R.T. (2004). Antimicrobial and antioxidant flavonoids from the roots wood of *Bolusathus speciosus*. *Phytochemicals* 65: 875-880.
9. Bassole, W.H., Quattara, A.S., Nebie, R., Ouottara, C.A.T., Kabore, Z.I., and Troore, A.S. (2003). Chemical composition and antimicrobial activities of the essential oil of *Lippia chevelieri* and *Lippia mutiflora* from Burkina Faso. *Phytochemicals.* 62:209-212.
10. Ajayi, I.A., Ajibade, O. and Oderinde, R.A. (2011). Preliminary Phytochemical Analysis of Some Plant Seeds. *Research Journal of Chemical Sciences* 1(3): 2011