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Full Length Research Paper

Rationalizing some medicinal plants used in treatment of skin diseases

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The hydroethanolic extracts obtained from ten plant species being used as components of skin disease remedy in Southwest Nigeria were subjected to phytochemical analysis and screened for antimicrobial activity. The antimicrobial activity was determined against *Escherichia coli* NCIB 86, *Staphylococcus aureus* NCIB 8588, *Klebsiella pneumoniae* NCIB 418, *Pseudomonas aeruginosa* NCIB 950, *Proteus vulgaris* NCIB 67, *Bacillus subtilis* NCIB 3610, *Candida albicans* and *Aspergillus flavus* by agar diffusion method. Terpenes, flavonoids, tannins and saponin were detected in the active plants through phytochemical screening and confirmed by thin layer chromatography (TLC). The plant extracts demonstrated antimicrobial effect against bacteria and the fungal cultures used in this study at different levels. The plant species *Funtumia elastica, Raphyostylis beninensis, Butyrospermum paradoxum, Serataria caudula, Parkia biglobosa and Curculigo pilosa* showed significant antimicrobial activities against the test organisms. Curculigo *pilosa, F. elastic and P. biglobosa* gave the highest zone of inhibition of 30 mm at 80 mg/ml against *Aspergillus flavus, Bacilus subtilis* and *Proteus vulgaris* while *Curculigo pilosa* also gave the least zone of inhibition of 2 mm at 80 mg/ml against *Klebsiella pneumoniae*. This relative high active index explains the antimicrobial effects of the remedy for skin diseases.

Key words: Phytochemical analysis, antimicrobial activity, TLC, skin disease, *Funtumia elastica.*

INTRODUCTION

The skin is man's largest organ, and essential to the man's health are the plants showing dermatological properties and the ability to stop bleeding to heal wounds and burns (Lewis and Elvin-Lewis, 1977).

Skin diseases occur in various forms, basically classified as non-contagious and contagious diseases, the primary of which are bacterial, fungi, viral and parasitic diseases. These diseases occur the world over, but are prevalent in the rural and tropical regions (Davis et al., 1980). Despite the plethora of antibiotics afforded by lower plants such as fungi, microbial diseases are still on the rise in developing countries due to relative unavailability of medicines and the emergence of wide spread drug resistance (Okeke et al., 2005). More startling was the report in 1996 that infectious diseases are actually on the increase in developed countries, (Pinner et al., 1996). Thus, the search for antimicrobial com-pounds in higher plants is vigorously pursued by many phytochemical laboratories (Hamburger and Hostettmann, 1991). Yet, the search for compounds yielding a spectrum of activity complementary to existing drugs and novel lead structures remains a task to be overcome (Mitscher and Rao, 1984).

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Parts of the ten plant extracts used in this research work are being used by traditional medical practitioners in Western Nigeria though they are available for sales in other countries. In Cote D'ivoire for example, *Khaya senegalensis* (Desr.) is available for sale in medicinal plants. The plants, bark are used against various fungal infections. *Ceiba pentandra* (L.) Gaertn, belongs to the family Bombacaeae and its Cork (bark) is used for treating Sores, ulcers, cancerous sores while the Bark of *Parkia biglobosa* (Jacq.) R. Br. Ex. G. Don which belongs to family Meliceae is also used to treat Ringworm, wound, localized skin eruption, measles, chicken pox, athlete's foot and other fungal infections. The other seven plants are *Rhaphiostylis beninensis, Parquetina nigrescens, Colocynthis citrullus, Funtumia elastica, Butyrospetmum paradoxum, Curculigo pilosa* and *Setaria caudula.* They belong to the families Icacinaceae, Periplocaceae, Curcubitaceae, Apocynaceae, Sapotaeceae, Hypoxidaceae and paniceae. They are used for the local treatment of bacterial infection (Canon, 1999), septic sore, heat rashes, wound or snake bite, hemorrhoids (Nwosu, 2002) or diarrhea in Nigeria (Agunu et al., 2005; Kubmarawa, 2007) and rashes respectively. Ofeimun and Onwukaeme (2006) reported that alkaloids, steroidal saponin, glycosides and tannins were detected in the powdered crude extracts of *R. beninensis*. The methanol extracts of this *R. beninensis* reportedly showed dose- related analgesic and anti inflammatory activities in the respective tests (Ofeimun and Onwukaeme, 2006). Though *Ceiba pentandra* reportedly had antimicrobial activity but it had been reported to be used as abortifacients in the Sangmelima region of Southern Cameroon (Noumi and Tchakonang, 2001). Adebayo-tayo and Adegoke (2008) reported that *K. senegalensis* and *P. biglobosa* among some plant extracts variously exhibited antimicrobial activity against some extended spectrum beta lactamase producing bacterial pathogens. Consequently, the aim of this research was to confirm the phytochemistry and antimicrobial activity of the crude extracts of the notable ten medicinal plants being used for treatment of skin diseases.

MATERIALS AND METHODS

Collection and authentication of plant materials

Barks and fruits of the ten listed plant species (Table 1) as components of a remedy used to treat skin diseases were bought from *Fresh Herb* Shop, Ibadan, South-Western Nigeria. The plant species were identified locally and scientifically by Dr. K. Ajibesin at Department of Pharmacognosy, University of Uyo, Nigeria

Preparation of extracts

About 450 g of each of the ten plant material was collected, air dried and reduced to powder. It was separately macerated with 50% ethanol, allowed to stand for 72 h and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at -20°C.

Microorganisms

Escherichia coli NCIB 86, *Staphylococcus aureus* NCIB 8588, *Klebsiella pneumoniae* NCIB 418, *Pseudomonas aeruginosa* NCIB 950, *Proteus vulgaris* NCIB 67, *Bacillus subtilis* NCIB 3610, *Candida albicans* and *Aspergillus flavus* were used as test microorganisms. They were obtained from Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Phytochemical screening

To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff's reagent were used to treat 1ml of the filterate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. This is called Dragendorff's test.

For saponin test, about 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, to test for presence of tannins, about 0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins. Borntrager's test was used in this research work for detecting the presence of anthraquinones. In this test, 0.5 g of the plant extract was shaken with benzene, benzene layer was separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone.

The presence of cardiac glycosides was confirmed by Liberman's test, Salkowski test and Keller-Killani test (Culer, 1982; Sofowora, 1993; Trease and Evans, 2002)

Thin layer chromatography (TLC)

This was carried out to confirm the phytochemical results. To prepare the TLC plates, one part of silica gel was shaken with two part of water to form slurry. The washed and dried 20 X 20 cm glass plates were mounted horizontally on a metal template. The plates were then carefully cleansed with acetone to remove grease. The slurry was poured into the spreader which ran over the plates, moving in one direction to provide uniform adsorbent coating. The plates were air-dried and later activated in an oven at 100°C for 1 h after which they were allowed to cool and stored in desiccators.

The solvent systems used for the TLC analysis were: ethyl acetate-methanol (4:1) and chloroform-methanol-ammonia (7:3:0.5). All the dissolved sample spots (placed at equidistance apart) of about 2 mm in diameter of the sample were applied 1cm from the bottom of the plate. The spots were then allowed to dry in air at ambient temperature for 30 min in order to be fixed on the adsorbent and evaporated the solvent from the spotted sample. The plates were inserted into the saturated tanks which were covered and allowed to stand until the solvent had travelled to within 1cm of the TLC plates. The plates were then removed and the solvent front marked. Spots on the TLC plates were detected using UV light type A4Q9 (λ max 366 nm). The R_f (Retention factor) for each spot was calculated using the formula and the ingredients confirmations done using various detecting reagents

Screening for antimicrobial activities

The antimicrobial screening was carried out by adopting agar diffusion method. A measure of 2 ml of a broth culture of each test

Table 1. Phytochemical screening of the ten plant component.

Test plant components	R.b	C.p	P.n	B.p	P.b	C.c	C.p	K.s	F.e	S.c
Alkaloids	-	+	-	-	-	+	+++	-	-	-
Saponins	+++	+++	+++	+++	+++	++	-	-	++	+++
Anthraquinones	++	-	++	-	-	+++	-	++	++	-
Cardiac glycosides										
i)Liberman's	++	++	++	++	++	++	++	-	++	+++
ii)Salkowski	++	+	++	+++	+++	++	++	+++	++	+++
iii)Keller Kiliani	++	++	++	++	+++	+++	++	++	++	+++
Flavonoids	++	++	++	++	++	++	++	++	++	++
Terpenes	++	++	++	++	+++	++	++	+	++	++
Tannins										
Ferric Chloride	++	++	++	++	++	+++	-	+++	+	-
Bromine water	++	+	++	+++	+++	++	-	+++	+	+

Key: +++= High concentration, ++=Moderate, +=Trace, R.b= *Rhaphiostylis beninensis,* C.p= *Curculigo pilosa*, P.n= *Parquetina nigrescens*, B.p= *Butyrospermum paradoxum*, P.b= Parkia biglobosa, C.c=*Colocynthis citrulus*, C.p= *Ceiba pentandra*, K.s= *Khaya senegalensis,* F.e= *Funtumia elastica*, S.c= *Setaria caudula,*

organism inoculated for 24 h (bacteria) and 7 days (fungi) was thoroughly mixed with 25 ml of molten agar and introduced aseptically into sterilized Petri-dishes near an open Bunsen flame and allowed to set. Equidistant wells bored on the surface of each medium contained 150 µl solution of different concentrations (40 and 80 ml) of each extract. One of the wells bore 2 µg/ml of either Chloramphenicol (bacterial plates) or Nystatin (fungal plates) as standard/comparative drug. The plates were allowed to stand for 1 h to allow complete diffusion of the extract. The bacterial plates were then incubated at 37°C for 24 h while the fungal plates were incubated for 7 days. Inhibition zones were calculated as the difference between diameter of the cork borer (6 mm) and the diameters of inhibition (Hewitt and Vincent, 1989). The mean inhibition zones were used to calculate the activity index. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug (Singh et al., 2002).

RESULTS

Phytochemical screening

The phytochemical results of the bark and fruits of 10 plant species revealed the presence of classes of compounds. Some of these compounds were present in moderate concentration while others in trace amount (Table 1). Compounds such as saponins and cardiac glycosides occurred in high concentration.

Thin layer chromatography

Various colours such as blue, yellow, brown, purple and red were observed under UV light (λ max 366nm) and their respective retention factors calculated (Table 2). Some of the extracts showed one tailing spot under the UV (λ max 366 nm), while plant species such as Parquetina *nigrescens, Colocynthis citrullus, Funtumia elastica* and *Curculigo pilosa* showed between two and three spots. Their light blue, purple and yellow connoted compounds like flavonoids, terpenes and saponins respectively.

Antimicrobial screening

The ten plant extracts were found to be effective against all tested microorganisms used in this study in different levels (Table 3). *P. biglobosa* exhibited the highest antibacterial effects against *E. coli* with the 18 mm as zone of inhibition and Activity Index (AI) with respect to the standard drug of 0.74 at the concentration of 80 mg/ml. However at lower concentration of 40 mg/ml, it shared the same zone of inhibition (that is, AI = 0.32) with *Seteria caudula.*

Highest antibacterial effect (Zone of Inhibition, Z.I. = 30) against *Proteus vulgaris* NCIB 67 was exhibited by *P. biglobosa* and this compared equally with the effect exhibited by the standard drug, Chloramphenicol, 10 µg, giving rise to A.I of 1. Other plants also showed relatively high antibacterial activities of 18 mm, 18 mm, 22 mm and 28 mm with corresponding A.I. of 0.6, 0.6, 0.73 and 0.93 for the crude extracts of *R. beninensis, Ceiba pentandra, B. paradoxum* and *S. caudula* respectively at concentration of 80 mg/ml. On the contrary at 40 mg/ml, *Colocynthis citrullus* and *P. biglobosa* exhibited higher bacterial activity (each with Z.I = 28 mm, A.I = 0.67) than the previous 4 plant extracts of which *S. caudula* had the highest activity (Z.I = 16 mm, AI = 0.53).

We also observed an inhibition zone of 20 mm (A.I = 0.8) by both extracts of *F. elastica* and *P. biglobosa*

Table 2. Thin Layer Chromatography Result of 10 Plant spp.

Solvent system	Plant extract	Number of spots	Colour under UV	Retention factor
ETOAC: MEOH 4:1	*Rhaphiostylis beninensis*	1	Light blue	0.01
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Parquetina nigrescens*	1	Purple	0.74
		2		0.11
		-	-	-
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Colocynthis citrullus*	1	Purple	0.39
		3		-
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Funtumia Elastic*	3	Light blue	0.01
		1		0.60
				0.70
				0.82
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Ceiba pentandra*	1	Light blue	0.60
		2		0.70
				0.82
				0.82
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Butyrospetmum paradoxum*	1	Light blue	0.01
				0.68
		2		0.82
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Parkia biglobosa*	1	Yellow	0.01
				-
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Curculigo pilosa*	3	Yellow	0.82
				0.50
				0.64
		1		0.78
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Seteria caudula*	1	Light blue	0.74
$CHCL_3$: MEOH: NH_3 (7:3:0.5)	*Khaya senegalensis*	1	Yellow	0.80

against *P. aeruginosa* NCIB 950 at concentration of 80 mg/ml. *F. elastica* still maintains a relative high activity, Z.I = 16 mm (A.I = 0.64) against the highly virulent, 'colonizer of surgical wound', *P. aeruginosa* at 40 mg/ml. For *S. aureus* NCIB 588, the Zone of Inhibition, Z.I. at concentration of 80 mg/ml was 20 mm and Activity Index, A.I. of 0.67 by the plant extract of *C. pentandra* just as each of *Rhaphiostylis beninensis* and *P. biglobosa* exhibited Z.I = 20 mm and A.I = 0.83 against *K. pneumonia* NCIB 418. Higher activity to this same atypical pathogen of pneumonia was exhibited by *F. elastic* than the standard drug; hence, an A.I. > 1 (that is, 1.08) at 80 mg/ml and Z.I = 18 mm, A.I = 0.75 at 40 mg/ml. The high potency of the extract of *F. elastic* reflected also against *B. subtilis* NCIB 3610 as it showed equal potency as the standard drug, Chloramphenicol 10 µg, hence an A.I = 1.0.

For *C. albicans*, the crude extracts varying activities *B. paradoxum* and *S. caudula* exhibited higher antifungal activity than all the other crude extracts with each having Z.I = 16 mm and A.I = 0.62 (with respect to Nystatin 100 µg).

DISCUSSION

Results obtained in this study showed that *R. beninensis, P. nigrescens, C. citrullus, F. elastica, C. pentandra, B. paradoxum, P. biglobosa, C. pilosa, S. caudula* and *K. senegalensis* have high antibacterial and antifungal activity against *E. coli (*the frequently implicated organism in gastroenteritis), *S. aureus* [that can bring about any of endocarditis, septic arthritis, toxic-shock syndrome, scalded-skin syndrome, food poisoning (Archer et al., 1994)], *K. pneumoniae* (aetiology of pneumonia), *P. aeruginosa* (pathogen of great threat in surgical wound manage-

Table 3. Effect of different extracts of the ten plant species on pathogenic microorganisms

Plant extracts	Test microorganism extract concentration zone of inhibition											
	Escherichia coli NCIB 86				*Proteus vulgaris* NCIB 67				*Pseudomonas aeruginosa* NCIB 950			
	80 mg/ml	A.I	40 mg/ml	A.I	80mg/ml	A.I	40 mg/ml	A.I	80 mg/ml	A.I	40 mg/ml	A.I
R.b	10	0.40	4	0.16	18	0.6	12	0.4	10	0.4	8	0.32
C.p	4	0.16	2	0.08	18	0.6	12	0.4	14	0.56	2	0.08
B.p	18	0.72	-	-	22	0.73	-	-	10	0.4	8	0.32
P.n	16	0.64	2	0.08	12	0.4	2	0.07	6	0.24	-	-
F.e	-	-	-		10	0.33	6	0.2	20	0.80	16	0.64
S.c	16	0.64	8	0.32	28	0.93	16	0.53	12	0.48	8	0.32
C.c	-		-		22	0.73	20	0.67	10	0.40	8	0.32
P.b	18	0.74	8	0.32	30	1.0	20	0.67	20	0.80	12	0.48
K.s	10	0.40	-		14	0.47	10	0.39	12	0.48	8	0.32
C.p	-	-	-		14	0.47	10	0.39	-	-	-	
CHL10	25		25		30		30		25		25	
NY100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 3 Contd.

Plant extracts	Test microorganism extract concentration zone of inhibition															
	Staphylococcus aureus NCIB 588				*Klebsiella pneumonia* NCIB 418				*Bacillus subtilis* NCIB 3610				*Candida albicans*			
	80 mg/ml	A.I	40 mg/ml	A.I	80 mg/ml	A.I	40 mg/ml	A.I	80 mg/ml	A.I	40 mg/ml	A.I	80 mg/ml	A.I	40 mg/ml	A.I
R.b	18	0.6	10	0.34	20	0.83	10	0.42	20	0.63	18	0.56	10	0.31	-	-
C.p	20	0.67	2	0.07	-	-	-	-	12	0.38	4	0.13	-	-	-	-
B.p	18	0.6	8	0.27	12	0.50	10	0.42	18	0.56	18	0.56	16	0.52	16	0.50
P.n	8	0.27	-	-	2	0.07	2	0.07	4	0.13	4	0.13	8	0.26	8	0.25
F.e	12	0.40	12	0.40	26	1.08	18	0.75	30	1.00	28	0.88	-	-	-	-
S.c	16	0.53	3	0.10	16	067	10	0.42	16	0.50	12	0.39	16	0.5	16	0.50
C.c	4	0.14	2	0.07	-	-	-		-	-	-	-	6	0.19	6	0.19
P.b	10	0.34	6	0.21	20	0.83	16	0.67	16	0.50	16	0.52	20	0.62	16	0.50
K.s	12	0.40	-	-	12	0.50	12	0.5	12	0.38	10	0.31	6	0.19	-	-
C.p	6	0.2	-	-	-		-		12	0.38	10	0.31	20	0.62	6	0.19
CHL10	30		30		24		24		32		32		NA		NA	
NY100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	32		32	

R.b = *Rhaphiostylis beninensis,* C.p =*Curculigo pilosa*, B.p = *Butyrospermum paradoxum,* P.n = *Parquetina nigrescens*, F.e = *Funtumia elastic,* S.c = *Seteria caudula,* C.c = *Colocynthis citrullus,* P.b =*Parkia biglobosa*, K.s = *Khaya senegalensis,* C.p = *Cieba pentandra*, CHL10 = Chloramphenicol (10 µg), NY100 = Nystatin (100 µg).
A.I = Activity Index

Ment), *P. vulgaris* (pathogen in cross clinical implications), *B. subtilis* and *C. albicans* (Candidiasis).

The activity index above unitary value with respect to standard drugs and generally rich antimicrobial potentials might be as a result of their rich phytochemical constituents.

(Sofowora, 1993) The plants contained bioactive compounds known to contribute to the antimicrobial potential of plants (alkaloids, terpenes, saponins, tannins, flavonoids and cardiac glycosides) as their presence was confirmed by Thin Layer Chromatography, TLC. These bioactive compounds have been reported to have antimicrobial potency (Fadeyi and Akpan, 1990; Sofowora, 1993).

The two plant extracts, *P. biglobosa* and *F. elastic* which showed great potency and dynamism may likely do better and even showed double their present Activity Indices if they are both put together in combination therapy within the tissue tolerant concentration. The phytoche-mical and antimicrobial results of the *P. biglobosa* and *K. senegalensis* were in agreements with what we observed and reported in earlier related research (Adebayo-tayo and Adegoke, 2008). Also, early report by Ofeimun and Onwukaeme (2006) on the met-

thanol extracts of *R. beninensis* showed dose-related analgesic and anti inflammatory activities in the respective tests. This makes the plant a potential source of diverse drugs for diverse skin 'troubles' including epidermal effect of small pox (by *K. senegalensis)* and justifies its present use by traditional healers. In view of the aforementioned potency, this extracts and the others can be put into use in the management of HIV/AIDS whose clinical features might include rashes, inflammation and other skin troubles (Adegoke and Adebayo-tayo, 2008).

Conclusion

In conclusion, the detection of antibacterial and antifungal activity in extracts of *R. beninensis, P. nigrescens, C. citrullus. F. elastic, C. pentandra, B. paradoxum, P. biglobosa, C. pilosa, S. caudula and K. senegalensis* supports their traditional uses for the treatment of bacterial and fungal infections.

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