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ANTIMICROBIAL EFFECT OF *STROPHANTUS HIPIDIS* AND *SECAMONE AFZELI* ON SOME PATHOGENIC BACTERIA AND THEIR DRUG RESISTANT STRAINS

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ABSTRACT

Two local medicinal plants *Strophantus hipidis* and *Secamone afzeli* were studied to verify their medicinal claims. The extracts of both the roots and leaves of these plants were shown *in vitro* to inhibit not only some pathogenic bacteria but also their multiple drug resistant strains. The organisms inhibited included, *Neisseria gonorrhoeae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Proteus mirabilis*. Laboratory induced antibiotic resistance of the various pathogens were also sensitive to the aqueous and ethanolic extracts of the plants. The plants were found to contain important phytochemical bases such as alkaloids, saponins, cardiac glycosides and polyphenols which may be the bioactive bases responsible for the antimicrobial property.

INTRODUCTION

Strophantus hipidis (Apocyanaceae) and *Secamone afzeli* (Asclepiadaceae) are two medicinal plants used by many people in South Eastern Nigeria. The roots and leaves of the plants are used by local herbalists for treatment of various diseases including rheumatism, stomach aches, malaria and venereal diseases. Limited knowledge about the practices of local heralists and lack of scientific studies of the plants they use have led to the neglect of potentially valuable drug containing plants (Sofowora, 1982). The two plants were studied to establish the scientific basis for using them as medicinal plants.

Medicinal properties of plants are normally dependent on the presence of certain phytochemical bases such as alkaloids, anthraquinones, cardiac glycosides, tannins and polyphenols (Harnborne, 1973, Trease and Evans, 1978; Gundiza, 1985)

This paper has screened the two plants for bioactive bases as well as tested the extracts of the plants on both pathogenic bacteria and their drug resistant strains.

MATERIALS AND METHODS

Test Organisms:

Local strains of the test organisms were collected from the University of Calabar Teaching Hospital. They included *Neisseria gonorrhoeae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes* and *Staphylococcus aureus.*

Isolation of Drug-Resistant Organisms

The organisms were mutated to show multiple drug resistance using the method described by Hopwood (1970). The antibiotics used in this study and their codes include gentamicin (g) tetracycline (t) ampicillin (a) penicillin (p) and genticin (g).

Test Plants:

The test plants *Strophantus hipidis* and *Secamone afzeli* (Schultes) K. Schum were collected with the help of native herbalists and identified by Dr. Madunagu in the Department of Biological Sciences, University of Calabar, Nigeria.

Phytochemical Screening:

This was carried out as described by Culier (1982); Sofowora (1984) and Gundidza (1985). The plants were screened for the presence of alkaloids, saponins, phlabatannins, anthraquinones, cardiac glycosides, polyphenols and tannins.

Alkaloids

A residue obtained by evaporating 10 ml of ether extract was dissolved in 2% HCl. A precipitate with Mayer's reagent indicated the presence of alkaloids.

Tannins:

The alcohol extract was diluted with water and then with $FeCl_3$. The presence of green blackish colour indicated catechol tannins while blackish blue indicated gallic tannins.

Saponins:

The residue obtained by evaporating the ether extract (4ml) was dissolved in methanol alcoholic solution of N. KOH and alcoholic solution of dinitrobenzoic acid was added. By heating, a disappearing violet colour was obtained.

Anthraquinones:

5g of plant extract was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. The presence of a pink, red or violet colour in the lower phase indicated the presence of free anthraquinones.

Table 1: *Phytochemical screening of the aqueous and ethanolic extracts of the plants*

Phytochemical Bases	S. hipidis				Plant Extract *S. afzeli* (Schultes) *K. schum*			
	SHLA	SHLE	SHRA	SHRE	SALA	SALE	SARA	SARE
Alkaloid	+	+	+	+	+	+	+	+
Saponins	+	—	+	—	+	—	+	—
Phlabat annins	+	—	+	—	—	+	—	—
Anthraquinones	—	+	+	+	—	+	—	+
Cardiac glycosides	+	+	+	+	—	+	—	+
Polyphenois	+	+	+	+	—	+	+	+
Tannins	+	—	+	—	+	—	+	—

Cardiac glycosides:

This was tested by thin layer chromatography and detected by spraying with a mixture of 1% chloramine solution and 25% trichloroacetic acid. The plates were heated for 10 minutes, examined under ultraviolet light for fluorescence of different colours.

+ represents presence of chemicals, - represents absence of chemicals. SHL - *S. hipidis* leaf; SHR = *S. hipidis* root: SAL = *Secamone afzeli* leaf; SAR = *Secamone afzeli* root. A = aqueous extract; E = ethanolic extract

Polyphenols:

2g of pulverized plant material was heated with 10ml water for 30 minutes. A mixture of 1% FeCl, and 1% potassium ferricyanide was added and observed for appearance of a green blue colour.

Preparation of Extracts:

Twenty grams (20g) of dried pulverised plants parts (leaf or root) was soaked in 200ml of distilled water for 48 hours. It was then filtered through Whatman No. 1 filter paper and excess water was removed by concentration in vacuo using a rotary evaporator to 50ml and stored in the refrigerator for use as aqueous extract.

For the preparation of ethanolic extract, two hundred grams (200g) of the dried pulverised plant parts (leaf or root was separately soaked in 500ml ethanol (98%) in an Erlenmeyar flask. The flask was covered with aluminum foil and allowed to stand for 14 days for extraction.

Antimicrobial Test:

The antimicrobial activity of both the aqueous and ethanolic extracts of the undiluted plants parts was determined using the disc diffusion method as described by Stoke and Ridgway (1980). The zones of inhibition produced i.e. the difference between the test and control zones were measured in millimetres. Tests were carried out in triplicates and their means recorded. A control was set up by soaking the filter paper in distilled water and in 98% ethanol. Negative results were regarded as those in which no zone of inhibition were observed.

Table 2: *Antimicrobial spectrum of aqueous and ethanolic extracts of* **S. hipidis** *and* **Secamone afzeli** *(Schultees) K. schum.*

Test Organism	Aqueous Extract				Ethanolic Extract			
	SHL	SHR	SAL	SAR	SHL	SHR	SAL	SAR
Niesseria gonorrhoeae	19 ± 0.00	16 ± 0.01	18 ± 0.07	19 ± 0.38	16 ± 0.01	23 ± 0.00	17 ± 0.12	16 ± 0.20
Klebsiella pneumoniae	25 ± 1.68	16 ± 0.1	30 ± 1.15	23 ± 0.05	32 ± 1.5	23 ± 1.00	32 ± 2.0	28 ± 2.0
Proteus mirabilis	17 ± 0.08	18 ± 0.57	15 ± 0.09	17 ± 0.00	24 ± 1.00	26 ± 1.00	31 ± 1.0	26 ± 1.0
Pseudomonas aeruginosa	22 ± 0.08	21 ± 0.07	35 ± 0.58	20 ± 0.01	25 ± 1.00	34 ± 2.00	33 ± 1.5	25 ± 1.0
Staphylococcus aureus	18 ± 0.58	16 ± 0.00	23 ± 0.01	18 ± 0.01	23 ± 1.00	22 ± 0.01	28 ± 1.0	25 ± 1.0
Streptococcus pyogenes	15 ± 0.57	17 ± 0.88	16 ± 0.08	19 ± 0.88	24 ± 1.0	25 ± 0.05	27 ± 1.0	23 ± 1.0
Esherichia coli	24 ± 0.07	22 ± 0.00	23 ± 0.00	21 ± 0.07	28 ± 1.5	25 ± 1.0	30 ± 1.5	20 ± 0.00

SHL = S. hipidis leaf, SHR = *S. hipidis* root, SAL = *S. afzeli* leaf, SAR = *S. afzeli* root.

RESULTS AND DISCUSSION

The multiple drug resistant bacteria encountered in this study and their codes include *N. gonorrhoese*$^{tpag-}$, *K. pneumoniae*^{tpa}, *P. mirabilis*$^{tspg-}$, *P. aeruginosa*$^{tpag-}$ *S. pyogeness*$^{pa-}$, *S. aureus*$^{tspag-}$, *E. coli.* The isolates showed resistance to at least three of the antibiotics used in our study. *S. aureus* showed multiple drug resistance to all the five antibiotics used in this study while *S. pyogenes, K. pneumoniae* showed resistance to only three of the antibiotics. The other organisms *(P. aeruginosa, P. mirabilis and E. coli)* were resistant to four of the antibiotics.

Phytochemical screening of the Plants:

The two plant extracts (aqueous and ethanolic) were screened for their phytochemical components. The results are as illustrated in Table 1. As shown, the aqueous extract of the leaf of

S. hipidis had all the components tested for except anthraquinone whereas the ethanolic extract did not show the presence of saponins, phlabatannins and tannins. The aqueous extract of the root had all the phytochemical components tested for. The ethanolic extract of the root also showed absence of saponins, phlabatannins and tannins. For *Secamone afzeli* the aqueous extract of the leaf had three components, namely, alkaloids, saponins and tannins whereas the aqueous extract of the root had four namely, alkaloids, saponin, polyphenol and tannins. The ethanolic extract of the leaf had all chemical components except saponins and tannins whereas that of the root did not show the presence of three components namely, tannins, phlabatannins and saponins.

Antimicrobial activity:

Tables 2 and 3 show inhibiting activity of the plant extract on test organisms including the drug resistant organisms. Table 2 shows the result of antimicrobial test of the aqueous extract. The leaf of *S. hipidis* showed its best inhibition on *K. pneumoniae, E. coli* and *Pseudomonas aeruginosa* whereas the root had its best inhibition on *E. coli* and *P. aeruginosa.* For *S. afzeli,* the table indicates that the aqueous extract of the leaf had very high inhibition on *K. pneumoniae* and *P. aeruginosa* and good inhibition on *S. aureus,* and *E. coli.*

This table also shows the result of the ethanolic extract of the two plants. These results indicate that the ethanolic extract showed higher diameter of zones of inhibition generally when compared to the aqueous extract.

Table 3: *Zones of inhibition (mm) produced by the plant extracts in susceptibility assays against the resistant organisms.*

Resistant micro-organisms	Aqueous Extract				Ethanol Extract			
	SHL	SHR	SAL	SAR	SHL	SHR	SAL	SAR
N. gonorrhoeae $^{tpag-}$	20 ± 0.00	17 ± 0.15	18 ± 0.57	21 ± 0.08	—	25 ± 0.00	—	—
K. pneumoniae $^{tpa-}$	26 ± 1.61	18 ± 0.01	31 ± 1.52	24 ± 0.05	34 ± 1.5	30 ± 1.5	35 ± 1.5	23 ± 1.00
P. mirabilis $^{tapg-}$	19 ± 0.12	19 ± 0.01	16 ± 0.00	17 ± 0.00	30 ± 1.0	25 ± 1.0	32 ± 2.0	28 ± 2.0
P. aeruginosa $^{tsag-}$	23 ± 0.01	21 ± 0.05	35 ± 0.08	21 ± 0.61	25 ± 1.0	35 ± 2.0	33 ± 1.5	25 ± 1.0
S. aureus $^{tspag-}$	20 ± 0.01	16 ± 0.00	24 ± 0.00	20 ± 0.07	25 ± 1.0	—	30 ± 1.0	25 ± 1.0
S. pyogenes $^{spa-}$	16 ± 0.15	20 ± 0.07	17 ± 0.08	20 ± 1.15	18 ± 0.1	16 ± 0.05	20 ± 1.0	19 ± 0.5
E. coli $^{spag-}$	24 ± 1.15	22 ± 0.00	24 ± 0.08	21 ± 0.08	28 ± 1.5	25 ± 1.0	30 ± 1.5	20 ± 0.0

— = absence of zone of inhibition; SHL = *S. hipidis* leaf; SHR = *S. hipidis* root;
SAL = *S. afzeli* leaf; SAR = *S. afzeli* root.

Table 3 gives the result of antibacterial activities of the aqueous and ethanolic extract on test organisms showing multiple drug - resistance. The aqueous extracts inhibited all the drug-resistant organisms encountered in this study. The results of the ethanolic extracts however, showed that the leaf of *S. hipidis* did not inhibit resistant *N. gonorrhoeae* and the ethanolic extract of the root also failed to inhibit the resistant *S. aureus* tested. Both the roots and leaves of a *Secamone afzeli* (schultes) K. schum inhibited all the drug - resistant organisms except *N.*

gonorrhoeae. These results are not peculiar to the drug - resistant organisms as the ethanolic extracts had similar effect on non-resistant organisms (Table 2). When the results of the activities of aqueous and ethanolic extracts were compared statistically using T-test, it was observed that in all treatments, (P 0.01) there was significant difference between the aqueous and ethanolic extracts in inhibiting the test micro-organisms, the latter showing higher inhibition than the former.

The use of alternative sources of antimicrobial substances to combat infections is justified, especially in attempt to control the drug resistant strains of bacteria encountered in many hospitals today.

This study has shown the presence of bioactive bases such as alkaloids, polyphenols, saponins, phlabatannins, anthraquinones, tannins and cardiac glycosides in the plants. The inhibition of pathogens like *E. coli, P. aeruginosa* and *K pneumoniae* by the leaf and root of *S. hipids* and *S. afzeli* points to the fact that the plant extracts contain potential biologically active substances that can be used in treating diseases caused by these organisms (Unaeze, 1987). The fact that the crude extracts of these local medicinal plants inhibited some medically important bacteria and their multiple drug resistant forms, proves that the plant might have some potential as an alternative source of antimicrobial substances.

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