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Research Paper

Comparative Study of the Phytochemical Screening and Antibacterial Potential of the Methanol and Chloroform Extracts of the Root of *Securidaca longipenduculata*

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Abstract: *The phytochemical screening and antibacterial potential of the chloroform and methanol extracts of the root of securidaca longipenduculata were compared. The crude chloroform extract indicated the presence of tannin, Steroidal glycoside, alkaloid, terpenoid and phenol but absence of saponin and flavonoid while the crude methanol extract contained saponin, tannin, flavonoid, Steroidal glycoside, phenol but terpenoid was not present. These extracts were tested against four human pathogens. The crude methanol at a concentration of 100mg/mL was bactericidal against Staphylococcus aureus and Escherichia coli with inhibition zone diameter (IZD) of 30mm and 27mm respectively but bacteriastatic against Streptococcus faecalis and Pseudomonas areuginosa with IZD of 20 mm respectively while the crude chloroform extract at the same concentration was bactericidal against Staphylococcus aureus, Streptococcus faecalis and Escherichia coli with IZD of 30mm, 28 mm and 25mm respectively but bacteriastatic against Pseudomonas areuginosa with IZD of 20mm. Ampiclox that was used as the control or standard drug showed IZD of 31mm for Staphylococcus aureus, 33mm for Escherichia coli, 30mm for Streptococcus faecalis and 25mm for Pseudomonas areuginosa at a concentration of 500mg/mL.*

Keywords: Bactericidal, Control, Extract, Phytochemical, Root

Introduction

Since the dawn of humanity, war against diseases has been part of everyday life and the use of plant materials in the treatment of sicknesses is as old as man [1-2]. Some of the crude drugs in the past are still valued in recent phytotherapeutics[3]. For instance cinchonine from chinchona plant used to treat fever and malaria is still often used in its natural and unrefined form [4] although, some other herbal drugs have been refined by isolating the pure active principle. These pure active principles from plants have helped the advancement of scientific medicine.

The use of plant extracts and phytochemicals, both with known antimicrobial properties could be of great significance in therapeutic treatment. Many plants had been used because of their their antimicrobial traits which are due to compounds synthesized during the secondary metabolism in the plant [5].

Securidaca longipendunculata known as 'ipeta' in Yoruba language of Western Nigeria, 'eze ogwu' in Ibo language, 'ofodo' in Hausa, 'alali' in Arabic and 'Violet tree' in English language is a semi-deciduous shrub that grows to 12m, spiny with straggly looking crown. It belongs to the *Plantae* kingdom and the family of *Polygalaceae* [6]. It had been used for different ailments in Africa [3]. It has been greatly used in herbal medicine to effectively treat such simple ailments as headache and severe cases of arthritis. It had been used in the treatment of schizophrenia, boils, inflammation, diarrhoea, gonorrhoea, and cough and in the management of HIV patients [7-8]. The stem of *Securidaca longipendunculata* is used in herbal medicine to treat sexually transmitted diseases [9]. Phytochemicals such as tannin, alkaloid, saponin, flavonoid and glycoside had been reported to be present in the plant [10].

The emergence of pathogenic bacteria that were resistant to multiple antibiotics represented a growing threat to human health and has given additional driving force for the search for novel antibiotics drugs. New antibiotics had been produced in the last decades but resistance to these drugs by microorganisms has increased. This was because bacteria had the genetic ability to transmit and acquire resistance to drugs which were utilized as therapeutic agents [11].

In the present study, extraction of the compounds that might be present in the root of this plant species using two different solvents chloroform and methanol was done. The extracts were screened for phytochemicals and their antibacterial potency was tested on four human pathogens. Comparison was made between the two solvents as to which of them would be the best solvent to be employed in the extraction of active principles present in the root, this was based on the extract that gave the best result against the test organisms.

Material and Methods

Plant Sample

Fresh root sample of *Securidaca longipendunculata* was purchased from an open Market in Kwara State of Nigeria in the month of January 2014.The plant sample was authenticated by comparison with voucher specimens at Applied Biology Department Ebonyi State University Abakaliki, Nigeria.

Test Organisms

Four clinical bacterial isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas areuginosa* and *Streptococcus faecalis* were obtained from the Applied Microbiology Department of the University. These were isolated and purified using standard methods and characterized based on biochemical methods [12].

Extraction

The sample was chopped when fresh, dried at room temperature and ground to 3 mm particle size and stored at 4^oC prior to extraction. In a typical run, the ground material (approximately 1000g) was suspended in chloroform ($CHCl_3$) 2500mL with occasional stirring for 96h and filtered, and then the filtrate was dried under vacuum. The dried residue (50g) was recovered. The same method was used for a fresh sample of 1000g suspended in 2500mL methanol (MeOH) for 96h, 64g dried residue was recovered.

Phytochemical Screening [13]

Sample Preparation

The sample used for the phytochemical tests was prepared by dissolving 2g of the plant sample in 20mL of distilled water.

Tests for alkaloid

Approximately 1.3g mercuric chloride ($HgCl_2$) and 5g potassium iodide (KI) were dissolved in distilled water and made up to 100mL with water to give Meyer's reagent. Two drops of this were added to 1mL of each extract. The formation of a creamy precipitate showed that alkaloid was present in the $CHCl_3$ but not in the MeOH extract.

1.3g of iodine with 2g of KI were dissolved in distilled water and made up to 100ML, this was Wagner's reagent. 0.5mL of this reagent was added to 1mL of each extract. Only the $CHCl_3$ extract gave slight reddish precipitate a positive test for alkaloid.

Tests for saponin

i**. Frothing (foaming) test**

About 5 mL of distilled water was added to 1 mL of each extract and shaken vigorously for about 2 min. frothing was present in the MeOH extract but not in $CHCl_3$ extract.

ii. Emulsion test

Approximately 5 drops of olive oil were added to 2mL of each extract and shaken vigorously. A stable emulsion formed in the MeOH extract but not in the $CHCl_3$ extract.

Tests for tannin

i. Potassium hydroxide (KOH) test

The addition of 2 drops freshly prepared 10% KOH to 1 mL of each extract yielded dirty white precipitate in the two extracts.

ii. Ferric chloride ($FeCl_3$) test

Test i was confirmed by adding 2 drops of 5% $FeCl_3$ to 1 mL of each extract, greenish precipitate was observed indicating the presence of tannin.

Tests for steroid

i. Salkowski's test

Five drops of conc. H_2SO_4 was added to 1 mL of each extract. A red colouration developed in both $CHCl_3$ and MeOH extracts.

ii. Lieberman Burchard's test

Acetic anhydride 1mL was mixed with 2 mL conc. H_2SO_4 and the solution added to 1 mL of each extract. Blue-green colouration appeared in both extracts.

Tests for flavonoid

The addition of 1mL 10% NaOH into 3 mL of each extract and a second test of the addition of two drops of $AlCl_3$ followed by few drops of conc. H_2SO_4 into each of the extract produced yellow colouration in MeOH extract but not in $CHCl_3$ extract.

Tests for glycoside

About 5mL of 50% H_2SO_4 was added to 5mL of each extract. The mixture was warmed on a steam bath for 15 min and allowed to cool. To this was added 5mL of

Fehling's solution and boiled. A brick red precipitate was observed in MeOH extract.

Tests for phenol

Each extract was mixed with distilled water in the ratio of 1:1, to each sample was introduced 2 drops of 5% NaOH. Orange coloured precipitate developed in the two extracts. In a separate test, a mixture of 0.5mL of each extract and 4 mL of distilled water was heated and few drops of 1 M $FeCl_3$ solution added. Dark-brown precipitate developed in the two samples.

Antibacterial Activity

The agar-difussion method was used in this study [14]. The microorganisms were maintained on agar slants and subcultures were freshly prepared before use. The inocula were made in 5mL Nutrient broth (Merck), and grown for 24h at 37.5^{o}C. The final inocula was prepared with Nutrient agar medium (5mL, 48^{o}C), seeded with the test organism (0.5%). Plates were prepared by pouring 20mL of freshly prepared Nutrient agar into 20mm x100mm Petri dishes and adjusted to 45^{o}C. About 5 mL inoculum was poured over the surface of prepared plates and allowed to solidify for 5 min, stainless steel cylinders were applied to the surface of the inoculated plates with sterile forceps. 100mg/mL of each sample were inoculated through each cylinder and the plates incubated for 24h at 37^{o}C. After the incubation period, inhibition zones were recorded as the diameter of the growth free zones. Ampiclox 500mg/mL was used as the positive control.

Results and Discussion

The result of the phytochemical screening of the plant sample is shown in Table 1. The antibacterial activity is shown in Table 2 and the antibacterial activity of the control drug is shown in Table 3.

The chloroform extract showed strong indication of tannin, alkaloid and phenol while steroidal glycoside was indicated though terpenoid as single component was fairly indicated, flavonoid was absent. The methanol extract showed strong indication of steroidal glycoside and saponin, phenol and flavonoid were indicated, and tannin was present but alkaloid and terpenoid were absent.The role of saponin and alkaloid had been discovered to be very potent against clinical pathogens such as *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*[15]. Saponins especially steroidal saponins had been found to have anticancer, antimicrobial and antifungal activities. Phenols and flavonoids have antioxidant properties, tannins like alkaloids are known for their bitter tastes and antimicrobial activity [16]. The presence of tannin, alkaloid and phenol were responsible for the antibacterial activity observed in the $CHCl_3$ extract which showed a broad spectrum action compared to the MeOH extract. As can be seen in Table 2, this extract showed high IZD values against three of the pathogens, *staphylococcus aureus*, *Streptococcus faecalis* and *Escherichia coli.* The methanol extract was only active against two of the pathogens, *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of the methanol extract might be attributed to the strong indication of the presence of saponin. The control drug ampiclox at a high concentration of 500mg/mL compared to the plant extracts gave almost identical IZD values.

Table 1: Phytochemical Screening of the $CHCl_3$ and MeOH Extracts of the Root Sample

Phytochemical	$CHCl_3$ Extract	MeOH Extract
Tannin	+++	+
Steroidal glycoside	++	+++
Alkaloid	+++	-
Saponin	-	+++
Terpenoid	+	-
Flavonoid	-	++
phenol	+++	++

+++ strongly indicated, ++ indicated
+ mildly indicated, - = not indicated

Table 2: Antibacterial Activity of the $CHCl_3$ and MeOH Extracts on Test Organisms

Test organism	Inhibition zone diameter (mm) Concentration (100mg/mL)	
	$CHCl_3$ Extract	MeOH Extract
Staphylococcus aureus	30	30
Escherichia coli	25	27
Streptococcus faecalis	28	20
Pseudomonas areuginosa	20	20

25-30mm bactericidal, 20 bacteristatic.

Table 3: Antibacterial Activity of the Control Drug on on Test Organisms

Test organism	Inhibition zone diameter (mm) Concentration (500mg/mL) Ampiclox
Staphylococcus aureus	31
Escherichia coli	33
Streptococcus faecalis	30
Pseudomonas areuginosa	25

25-> = 30mm bactericidal.

The implication is that the plant samples when used at the same concentration of 500mg/mL might give higher IZD values and hence more potency

compared to ampiclox. Again, Chloroform would serve as a very good solvent compared to methanol in the extraction of phytochemicals present in the root of this plant. The chloroform and methanol extracts of the root of *Securidaca longipendunculata* are still under investigation to isolate the pure compounds, establish their structures through spectroscopic analyses and subject the pure isolates further to antibacterial tests in order to establish their potency either as pure active principles or as synergists.

Conclusion

The extracts of the root of *Securidaca longipendunculata* especially in this report proved to be potent antibacterial agents and confirmed its use in traditional herbal medicine. Thus it had shown that it might be one of the possible sources of new antibiotics of the coming decade.

References

1. Schimidt E. and McCleland W., Trees and Shrubs of Mpumalanga Kruger National Park, Jacana Publications Houghton Johannesburg, **46**, 21-44 (**2002**)

2. Akerele O., The Best of Both Worlds: Bringing Traditional Medicine up to Date, *Soc Sci.Med.,* **24**,177-181 (**1987**)

3. Ojewole J., Analgesic, Anti-inflammatory and Hypoglycaemia Effect of Securidaca Longipendunculata Root Bark Aqueous Extract, *Inflammopharmaco.*, **16 (4)**, 174-181(**2008**)

4. Evans W., Wichtland M. and Bradley P., Trease and Evans Pharmacology, 14th ed., Saunders, London 419 (**1985**)

5. Wondergem P., Senah K. And Glover E., Herbal Drugs In Primary Healthcare, Ghana: An Assessment of the Relevance of Herbal Drugs in PHC and Some Suggestion for Strengthening PHC. Amsterdam Royal Tropical Institute, 28-45 (**1989**)

6. Watt J., Breyer T. and Brankijk M., Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed., Livingstone Ltd. London (**1998**)

7. Iwu M., Handbook of African Medicinal Plant, Academic CRC Press Inc., Florida, 367 (**1993**)

8. Last M and Chavunduka G., The Professionalism of African Medicine. Manchester, Manchester University Press, 2nd Ed, 367-375 (**1986**)

9. Fabeku P., Traditional Medicine: The Art, Ways and Practice. In: Odugbemi,T., Outlines and Pictures of Medicinal Plants from Nigeria, University of Lagos Press, 13-24 (**2006**)

10. Oliver B., Medicinal Plant in Tropical West Africa, Cambridge University Press, Great Britain 374-375 (**1986**)

11. Cohen M., Epidemology of Drug Resistance: Implications for a Post antimicrobial Era, 1050-1055 (**1992**)

12. Wood G. and Washington J., Antibacterail Susceptibility Tests: Dilution and Disk Diffusion Methods: In Murray P, Baron E, Pfaller M, Tenover F. and Yolken R., 6TH ed., Manual of Clinical Microbiology Chapter 113, ASM Press, Washington D.C, 1327-1341 (**1999**)

13. Sofowora A., Man, Plants and Medicine in Africa. Inaugural Lecture, University of Ife, Ile-Ife, Nigeria: In Makinem 1988 African Philosophy, Culture and Traditional Medicine Ohio USA: 15-35 (**1981**)

14. George D. and Roger M., Encyclopaedia of Medicinal Plant, 5th ed. Spain(**2002**)

15. Ajibade V. and Falegan C., Antibacterial Effect of Acetone and Aqueous Extract of *Phyllanthus niruri* On *Escherichia Coli*. *J. Sci. Engr. Tech.,* **14(1)**, 7188-7197(**2007**)

16. Akinleye O., Adu O. and ayeni I., Studies of Some Biological and Chemical Characteristics of Mexican Sunflower (*Tithonia diversifolia*), *Consult. Res. J*., **1(1)**, 35-38(**1996**)