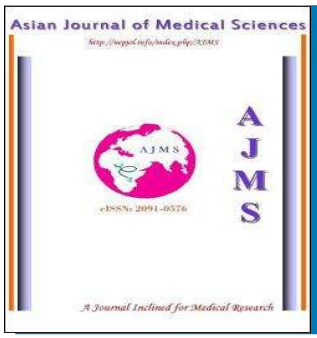

PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF THE AQUEOUS ROOT EXTRACT OF *TRICLISIA DICTYOPHYLLA*

ORIGINAL ARTICLE, Vol-4 No.1

Asian Journal of Medical Science, Volume-4(2013) http://nepjol.info/index.php/AJMS

[1]Anslem O. Ajugwo, [2]Anthony C. Ezimah, [3]Francis M. Awah, [4]Philippe E. Mounbegna, [4]Celestine C. Azikiwe and [1]Andre M.Abouo. [1]Department of Haematology and Blood Transfusion, Madonna University Elele. [2]Department of Physiology/Anatomy, Federal University Ndufu Abakaliki Ebonyi State. [3]Department of Biochemistry, Madonna University Elele. [4]Department of Pharmacology, Anambra State University Uli.

CORRESPONDENCE:

Anslem o. Ajugwo
Department of haematology and blood transfusion
Madonna University Elele
Nigeria
Email: slemjugwo@yahoo.com
Phone: +234 8033343128

"Triclisia dictyophylla has potential to be formulated into antibiotics"

ABSTRACT

Triclisia dictyophylla have been used traditionally for the treatment of different ailments. The root was therefore subjected to phytochemical analysis and antimicrobial/antifungal activity against some hospital-strain disease causing microorganisms. Standard methods were used for the phytochemical screening. The extract was subjected to antimicrobial/antifungal activity using *Staphylococcus aureus, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa* and *Candida albicans*. The sensitivity pattern was compared to that of ciprofloxacin, cotrimoxazole and ofloxacin. Phytochemical analysis revealed mostly alkaloids and protein while tannins, glycoside and saponin were revealed in trace (+) amounts. The extract showed a good antimicrobial activity on *Staphylococcus aureus* isolated from sputum and on *E. coli* from both urine and sputum with no antifungal activity. The extract contains active components which could be harnessed for formulation of antibiotics.

Keywords: *Triclisia dictyophylla*, phytochemical, antimicrobial, antifungal, antibiotics

INTRODUCTION

Triclisia dictyophylla, a member of the family Menispermaceae (Moon seed) is a medicinal plant that is indigenous to Africa and has been shown to possess anticoagulation activity[1]. In Igbo speaking parts of Delta state, the name is derived from its uses, thus: *okwuite*, as the leaves were used as lid to provide for tight covering during the early days of cooking with clay pot and *akwukwo oji* as the leaves are used in the preservation of fresh kolanuts [1].

Earlier works on *Triclisia dictyophylla* led to isolation of morphinan alkaloids[2]. It is indigenous to West Africa and has been used natively as medicinal plant in the treatment of several ailments like oedema, anaemia and spasm [3].

Recent surveys carried out indicate that substantial numbers of people are using complementary health treatments including herbs and herbal products [4,5].

Several well-known drugs, such as quinine and artemesinin used as antiprotozoan agents; have their origin in plants [6,7,8].

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties [9,10]. Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases [11]. Given the alarming incidence of antibiotic resistance in bacteria of medical importance[12], there is a constant need for new and effective therapeutic agents[13].

Phytochemical and antimicrobial screening of extracts from *Peterocapus osun* stem, showed significant antimicrobial activities due to different classes of newly-isolated constituents[14]. While [15] reported the antimicrobial activity of the leaves of *Bryophyllum pinnatum* at a concentration of 25mg/ml.

Phytochemical study and other research have shown that garlic and, or onion oils (genus Allium) have potentials as wide antimicrobial agents, antitumors, hypolipidaemic, antimicrobial and hypoglycaemic agents [16].

This present work, was therefore carried out to determine the phytochemical and antimicrobial properties of *Triclisia dictyophylla*.

MATERIALS AND METHODS

After proper identification at Igbodo in Delta State in the old Mid Western part of Nigeria. The plant was uprooted and the roots were air dried for 4 days to reduce the water content which can cause dilution effect on the active components. The root was chopped into bits and pounded in a wooden mortar. The entire content was weighed and immersed in 2 liters of distilled water and left to stand for 48 hours. Undissolved particles were sieved out to remove root wood and fibres. Filtration was done using Whatman's No 1 filter paper to obtain the brownish filtrate. The filtrate was poured into the conical flask of rotary evaporator. The pH and boiling points were taken. The filtrate was evaporated into a dry semisolid black-brown residue which was poured into an evaporating dish and put in an incubator at 60^0C for 3 days. Solid extract was obtained, weighed and refrigerated.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Tests for flavonoids, tannins, carbohydrates/ glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard methods [17,18].

Test for tannins

Pulverised sample (0.5 g) of each plant was boiled in 20 ml of distilled water in a test tube and then filtered with Whatman No. 1 filter paper. Then 0.1 % $FeCl_3$ was added to the filtrate and observed for brownish green or a blue black

colouration, which shows the presence of tannins.

Test for saponins

A quantity (2 g) of pulverised samples of each plant was boiled together with 20 ml of distilled water in a water bath and filtered. Then 10 ml of the filtered sample was mixed with 5 ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Test for resins

Pulverised plant material (0.5 g) was extracted with 15 ml of 96 % ethanol. The ethanol extract was then poured into 20 ml of distilled water in a beaker. A formation of resinous precipitate indicates the presence of resins. Furthermore, 0.12 g of the extract was extracted with chloroform and the extract concentrated to dryness. The residue was re-dissolved in 3 ml acetone and 3 ml concentration HCl added. This mixture was heated in a water bath for 30 min. A pink colour that changes to magnets red confirmed the presence of resins.

Test for alkaloids

To 0.5 g of pulverised plant material, 5 ml of 1% HCl were added and boiled for 5 min. in a steam bath. This was filtered and 1 ml of the filtrate treated with a few drops of Draggendorf's reagent, a second 1 ml portion treated similarly with Wagner's reagent and a third portion with Mayer's reagent. The formation of red, reddish-brown and creamy white precipitates respectively was an indication of the presence of alkaloids.

Test for glycosides

To 0.5 g of pulverised plant samples, 10 ml of distilled water was added and boiled for 5 min. This was filtered and 2 ml of the filtrate hydrolyzed with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2 ml of Benedict's qualitative reagent and boiled. A reddish–brown precipitate showed the presence of glycosides.

ANTIMICROBIAL TESTING

Pure cultures of bacterial and fungal isolates were procured from Medical Microbiology Laboratory of Madonna University Teaching Hospital (MUTH) Elele. The pure cultures were subcultured on nutrient agar and Sabroaud agar for bacterial and fungal isolates respectively. Subsequently, incubated at 37^0C for 24 hours and room temperature for 48 hours for bacterial and fungal isolates respectively. Different concentrations of the aqueous extract of *Triclisia dictyophylla* were prepared at concentrations of 10µg, 100 µg, 1000 µg and 10,000 µg. These different concentrations were impregnated unto sterile sensitivity disc. 1ml of $1x10^7$ cells/ml suspension of each bacterium/fungus in saline was prepared and poured unto preset nutrient agar, swirled for even distribution and allowed to stand for 5 mins. The various discs were placed on the preset nutrient agar at 3 – 5 cm away from one another. Then incubated at 37^0C for 24 hours and at room temperature for 48 hours for bacteria and fungi respectively. It was repeated for every test organism and sensitivity was determined by measuring the degree of zone of inhibition of the extract and other antibiotics (ciprofloxacin, cotrimoxazole and ofloxacin).

RESULTS

Aqueous Extraction:

After the grinding of the root, the weight that was obtained was 202g. This was then immersed in 2 liters of distilled water and left for 48 hours. Fibers and undissolved particles were sieved out. Whatman number 1 filter paper was used to obtain a brownish filtrate. The pH of the filtrate was

measured, and gave a pH of 5.0 while the boiling point was recorded at 94.5^{0}C. Evaporating dish was used to evaporate the filtrate, and evaporation point of 98^{0}C was also recorded. After evaporation, the filtrate yielded a dry semisolid brown-black residue which was incubated at 60^{0}C for 3 days. After evaporation, the filtrate yielded a dry semisolid brown-black residue which was incubated at 60^{0}C for 3 days. The solid extract was then weighed and it gave 16.8g.

The percentage yield was calculated thus:

$$\frac{\text{Weight of final yield}}{\text{Weight of initial yield}} \times 100$$

$$\frac{16.8\text{g} \times 100}{202\text{g}} = 8.3\%$$

Table 1: Qualitative phytochemical constituents

EXTRACT	Flavonoids	Tannins	Alkaloids	Terpenoid	Glycoside	Saponins	Resin	Protein
ROOT	+	+	+++	-	+	+	-	++

\+ present in trace amount
++ present in high amount
+++ present in very high amount
\- absent

Table 2: Antimicrobial sensitivity of *Triclisia dictyophylla* and some other antibiotics

Organism	Sensitivity of Triclisia dictyophylla				Others		
	10 µg	100 µg	1,000 µg	10,000 µg	cipro	Cotri	Oflox
Staphylococus aureus(sputum)	R	10mm	12mm	20mm	10mm	10mm	20mm
Staphylococus aureus (urine)	R	R	R	R	8mm	6mm	10mm
Escherichia coli (sputum)	R	4mm	10mm	18mm	10mm	10mm	20mm
Escherichia coli (urine)	R	4mm	10mm	10mm	10mm	R	6mm
Psuedomonas aeruginosa (sputum)	R	4mm	10mm	20mm	10mm	R	20mm
Klebsiella spp (sputum)	R	6mm	10mm	18mm	10mm	R	10mm
Klebsiella spp (urine)	R	R	R	R	R	R	10mm
Candida albicans	R	R	R	R	R	R	R

Cipro - Ciprofloxacin
Cotri - Cotrimoxazole
Oflox - Ofloxacin
R - Resistant
mm - millimeter (Zone of inhibition)

DISCUSSION

Triclisia dictyophylla which is of the family Menispermacae has a unique characteristic which is location. Such plants are seen only in some selected regions of any particular country [19]. The search for this plant took us to old Mid Western states where the plant was eventually discovered in Igbodo Delta State having previously been identified [1].

Phytochemical analysis on the root extract yielded the presence of alkaloids(+++) and protein (++). Also tannins, glycoside and saponin were revealed in trace (+) amounts. Morphinan alkaloids have been demonstrated in *Triclisia dictyophylla* [3]. Plant extracts have been widely used and studied as antimicrobial agents [20]. Many plants are used in traditional medicine for treatment of diseases, fever and cough [21]. The antimicrobial activity of the extract was also studied. This was done by preparing 10, 100, 1,000 and 10,000µg of the extract of *T. dictyophylla* and compared with other antibiotics (ciprofloxacin, cotrimoxazole and ofloxacin). The antimicrobial activity of *T. dictyophylla* was concentration-dependent. The extract showed a good antimicrobial activity on *Staphylococcus aureus* isolated from sputum while the same *Staphylococcus aureus* from urine was resistant. It also showed a good antimicrobial activity on *E. coli* from both urine and sputum. This antimicrobial activity could be as a result of the presence of alkaloids, flavonoids and tannins. These active ingredients have also been described as been responsible for antimicrobial activity of methanolic extract of *Bombax buonopozense* [22]. Flavonoids have also been shown to possess antimicrobial activity[23]. The extract showed a poor antifungal activity. Some plants that have antimicrobial activity also recorded poor antifungal activity[24]. This is an indication that the extract contain active components which may be used to source for antibiotic substances.

REFERENCES

1. Azikiwe, C.C., Amazu, L.U., Unekwe, P.C. and Nwosu, P.J.C. (2007), "Anticoagulation and antithrombotic effects of Triclisia dictyophylla (moon seed)", Nigerian Journal of Natural Plants Medicine. 11: 29 – 31.
2. Choi, H.S., and Sa, Y.S. (2001), "Fibrinolytic and antithrombotic protease from Spirodela polyrhiza", Biosci – biotech – biochem, 65 (4): 781-786.
3. Spiff, A.L., Zabel, V., Watsin, W.H., Zemaitis, W.A. and Ateya, A.M. (1981), "Constituents of West African Medical Plant xxxx, Tridictyophylline, a new morphinan alkaloid from Triclisia dictyophlla, Int. J Nat prod, 44 (2): 160 – 165.
4. Eisenberg, D. M., Kessler, R. C., Foster, C., Norlock, F. E., Calkins,D. R. and Delbanco, T. L. (1993), Unconventional medicine in the United States. Prevalence, costs and patterns of Use, N. Engl. J. Med. 328: 246 – 252.
5. Maclennan, A., Wilson, D. H. and Taylor, A. W. (1997), Prevalence and cost of alternative medicine in Australia. Mintel International Group, London.
6. Kirby, G. C. (1996), Medicinal plants and the control of parasites: medicinal plants and control of protozoal disease with particular reference to malaria, Trans. R. Soc. Trop. Med. Hyg. 90: 605 – 609.
7. Camacho, M. D., Croft, S. L. and Phillipson, J. D. (2000), Natural products as source of antiprotozoal drugs. Curr. Opin. Anti infect. Investig. Drugs 2: 47 – 62.
8. Tagboto, S. and Townson, S. (2001), Antiparasitic properties of medicinal plants and other naturally occurring products. Adv. Parasitol. 50: 1992 – 1995.
9. Cowan, M.M. (1999), Plant products as antimicrobial agents, Clinical Microbiology Reviews. 12: 564 – 582.
10. Palombo, E. A. and Semple, S. J. (2001), Antibacterial activity of traditional Australian medicinal plants, Journal of Ethnopharmacology. 77: 151 – 157.
11. Chariandy, C. M., Seaforth, C. E., Phelps, R. H., Pollard, G.V. and Khambay, B. P. (1999), Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of Ethnopharmacology. 64: 265 – 270.
12. Monroe, S. and Polk, R. (2000), Antimicrobial use and bacterial resistance, Current Opinion in Microbiology. 3: 496 – 501.
13. Bhavnani, S.M. and Ballow, C.H. (2000), New agents for gram-positive bacteria, Current Opinion in Microbiology. 3:528–534.
14. Ebi, G. C. and Ofoefule, S. I. (2000), Antimicrobial activity of Pterocarpus osun stems, Fitoterapia. 71 (4): 433 – 435.
15. Akinpelu, D. A. and Olorumola, F. O. (2000), Antimicrobial activity Of Bridella ferruginea fruit, Fitoterapia. 71 (1): 75 – 76.
16. Nostro, A., Canutelli, M. A., Morelli, I., Cioni, P. L., Bader, A., Marino, A. and Alonzo, V. (2002), Preservative properties of Calamintha officinalis essential oil with or without EDTA, Appl. Microbiol. 35 (5): 385 – 389.

17. Harborne, J. B. (1973), Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London. P 271.
18. Trease, G. E. and Evans, W. C. (1989), A Textbook of Pharmacognosy. Academic Press, London. P 22 – 40.
19. He, S. Z. (2000), A revision of species and distribution of medicinal plants in Guizhous province. Zhongguo – Zhong – Yao - Za – shi. 25 (9) : 521 – 524.
20. Hamoud, R., Sporer, F., Reichling, J. and Wink, M. (2012), Antimicrobial activity of a traditionally used complex essential oil distillate (Olbas, Tropfen) in comparism to its individual essential oil ingredients. Phytomedicine. (Epub ahead of print).
21. Mulaudzi, R. B., Ndhlala, A. R., Kulkarni, M. G. and Van Staden, J. (2012), Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. J. Ethnopharmacol. (Epub ahead of print).
22. Akuodor, G.C., Essien, A.D., Ibrahim, J. A., Bassey, A., Akpan,J.L., Ikoro, N.C. and Onyewenjo, S.C. (2011), Phytochemical and antimicrobial properties of the methanolic extracts of Bombax buonopozense leaf and root. Asian Journal of Medical Sciences. 2: 190-194.
23. Singh, G. and Kumar, P. (2011), Evaluation of antimicrobial efficacy of flavonoids of Withania somnifera. Indian J. Pharm. Sci. 73 (4): 473 – 478.
24. Mufti, F., Ullah, H., Bangash, A., Kahn, N., Hussain, S., Ullah, F., Jamil, M. and Jabeen, M. (2012), Antimicrobial activities of Aerva javanica and Paeonia emodi plants. Pak. J. Pharm. Sci. 25 (3) : 565 – 569.