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PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF SUDANESE DICHROSTACHYS CINEREA (L.) LEAVES

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ABSTRACT

The aim of this study is to investigate the chemical constituents of the methanolic extract of Dichrostachys cinerea (L.) (Mimosaceae) leaves and to evaluate its potential antimicrobial and antioxidant activity. Phytochemical analysis of the extract revealed the presence of (flavonoids and saponins) in high concentration, (alkaloids, tannins, triterpenes, steroids and glycosides) in moderate concentration and the absence of (coumarins and anthraquinones). The evaluation of antimicrobial potential against Escherichia coli, Pseudomonasa aruginosa, Staphylococcus aureus, Bacillus subtilis bacteria and the fungus Candida albicans showed (high, moderate, low) activity. An Antioxidant activity was evaluated by measuring the capacity of the extract against the stable DPPH radical. The study shows that this plant has therapeutic potential due to its high radical scavenging activity.

Keywords: Mimosaceae, leaves, chemical constituents, antibacterial, antifungal and antioxidant activity

INTRODUCTION

Traditional medicinal practices have been known for centuries in many parts of the world for the treatment of various human ailments. The use of synthetic medicines has revolutionized the treatment of urinary stones. However, their indiscriminate use has led to adverse effects, necessitating the development of new herbal medicines. In recent years, there has been a resurgence of interest in the study of natural resources for the development of such agents. 80% of the world's population relies on medicinal plants and in Sudan, the use of plants as therapeutic agents remains an important part of the traditional system of medicine. Dichrostachys cinerea (L.) Wight & Arn is a spiny, acacia-like sapling of the legume family

(known as Kadad) that is common in Africa from the sub-Saharan to the south under tropical and subtropical conditions [1]. The plant is a shrub that usually reaches 5-10 m in height. The leaves are compound and pinnate. The inflorescence consists of a pendulous spike. The flowers are bicolor - pinkish-white at the base and yellow at the end [2]. Earlier, the roots were reported to be bitter, astringent, acrid, anti-inflammatory, analgesic, diuretic and useful for kapha and vata [3]. It is also reported that the roots are used for urinary stones and kidney problems, diseases of the vagina and uterus, and joint pain [4]. The tender shoots of the plant are crushed and applied to the eyes to relieve eye ailments [5]. The leaves, believed to have a local anaesthetic effect, are used as a remedy for sore eyes and toothache. D.cinerea is also used to treat depressed fontanelles, influenza, cough, scabies, leprosy, and edema. The plant extracts of D.cinerea have been studied and found to possess considerable antibiotic activity[6,7]. Phytochemical studies performed on D.cinerea extracts have shown the presence of tannins, sterols and triterpenes, reductive compounds, polyphenols, flavonoids, as well as cardiotonic heterosides[8], saponins and steroids 10 . Ethanolic extracts of roots, fruits, leaves, and seeds of D. cinerea have been reported to have antibacterial activity[9,10]. Phytoconstituents of D.cinerea have antioxidant and anti inflammatory properties[11,12]. The aim of this study is to investigate the chemical constituents of the methanolic extract of Dichrostachys cinerea (L.) (Mimosaceae) leaves and to evaluate its potential antimicrobial and antioxidant activity.

EXPERIMENTAL

Plant material

Fresh leaves of Dichrostachys cinerea were collected from Alodiya town, West Kordofan State, Sudan in May 2021. The plant was taxonomically identified and authenticated by Dr. Ahmed Suliman – Gum and Forest Products Research Centre - West Kordofan University - Alnohud - Sudan.

Preparation of the plant extract

Powdered air-dried leaves of Dichrostachys cinerea were defatted with petroleum ether and macerated with 70% methanol for all tests. Plant material; dried in the shadow in air draft; was comminuted to powder and extracted for 48 hrs; the solvent is removed under reduced pressure then re-extracted using diethyl ether. The methanolic extract was obtained and stored at 4°C until experiments.

Phytochemical screening

Preliminary phytochemical screening for secondary metabolites was performed according to the classical procedures [13, 14] .

Test for Tannins

200 mg of crude plant extracts was mixed with 2 ml of 2% solution of FeCl 3 . Blue-green color was observed which indicates the presence of tannins.

Test for Flavonoids (Alkaline Reagent Test)

200 mg of extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid indicated the presence of flavonoids.

Test for Saponins

200 mg of extract was mixed with 5 ml of distilled water in a test tube and was shaken vigorously.

The formation of Stable foam (for 10 minutes) and add 1 ml HCl 2M, the foam persistent stable) was observed which shows an indication of the presence of saponins.

Test for Anthraquinones
0.5 g of the sample was boiled in 3ml of 1% HCl and filtered. The filtrate was shaken with 5 ml benzene and the benzene layer was removed, 10% NH 4 OH was added and pink/violet color in the alkaline phase was observed which indicated the presence of anthraquinones.

Test for Glycosides (Salkowski's Test)
200 mg of extract was mixed with 2 ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color was observed which indicated the presence of steroidal ring, that is, glycone portion of the glycoside.

Test for Alkaloids
200mg of extract was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1ml of the filtrate was added 6 drops of Wagner reagent. Brownish-red precipitate was observed which indicated the presence of alkaloids.

Test for Terpenoids (Salkowski's Test)
5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form layer. A reddish brown coloration of the interface was formed which was an indication of positive results for the presence of terpenoids.

Test for coumarins
A small amount of moistened plant sample was placed in a test tube, and the tube was covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube is then placed in aboiling water bath for several minutes. The paper is removed and exposed to ultraviolet light. A yellow green or blue fluorescence appearing within a few minutes indicates the presence of coumarins.

Test for Steroids
To 2 ml of acetic anhydride was added 0.5 g of the sample followed by an addition of 2ml H_2SO_4. The color changed from violet to blue green indicating the presence of steroid.

ANTIMICROBIAL ASSAY

Preparation of bacterial suspensions
Aliquots of 1 ml of a 24-hour broth culture of the test organisms were aseptically spread on culture medium and incubated at 37°C for 24 hours. Bacterial growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of normal saline to produce a suspension containing approximately 108-109 colony forming units per ml. The suspension was stored in a refrigerator at 4°C until use. The average number of viable organisms per ml of the stock suspension was determined using the surface viable counting technique. Serial dilutions of the stock suspension were prepared in sterile saline in tubes, and a drop volume (0.02 ml) of the corresponding dilutions was transferred to the surface of dry nutrient agar plates using an adjustable volume micropipette. The plates were left at room temperature for two hours to allow the droplets to dry and then incubated at 37°Cfor 24 hour described in the Committee for Clinical Laboratory Standards guidelines [15].

Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar and incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline. The suspension was stored in a refrigerator until clotrimazole was used as a standard [16] .

Testing for antibacterial activity

The cup-plate agar diffusion method was used to evaluate antibacterial activity with some minor modifications. (2 ml) of the standardized bacterial strain suspension was mixed with 200 ml of sterile molten nutrient agar maintained at 45°C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed onto sterile Petri dishes. The agar was settled and two cups per half (10 mm diameter) were cut into each of these plates, which were divided into two halves, using a sterile cork borer (No. 4), with each of the halves designated for one of the test solutions. Separate Petri dishes were used for the standard antibacterial chemotherapeutics (ampicillin). The agar discs were removed, and the alternate dishes were filled with 0.1 ml samples of the test solution using an adjustable volume microliter pipette and allowed to diffuse for two hours at room temperature [17] .

Method of antioxidant assay

The test samples were allowed to react with the stable free radical 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH) for half an hour at 37°C, maintaining the DPPH concentration at (6x10-2mM). The test samples were dissolved in methanol, while DPPH was prepared in ethanol [18] . After incubation, the colour change of absorbance was measured at 515nm using a spectrophotometer. The percent radical scavenging activity of the samples was determined in comparison to a control group treated with ascorbic acid [19].

RESULTS AND DISCUSSION

Phytochemical Analysis

Phytochemical study of Dichrostachys cinerea (Table 1) shows that the methanolic extract has presence of polyphenolic compounds, flavonoids, tannins, steroids, alkaloids, saponins, glycosides and triterpenes in different intensity reactions, and absence of Coumarins and Anthraquinones.

Table1: Phytochemical screening of the leaves of Dichrostachys cinerea

No	Constituents	Dichrostachys cinerea extract
1	Tannins	+
2	Flavonoids	++
3	Saponins	++
4	Anthraquinones	-
5	Glycosides	+
6	Alkaloids	+
7	Terpenoids	+
8	Coumarins	-
9	Steroids	+

++ = Present in a high concentration; + = Present in a moderate concentration; - = Absence

Antimicrobial Activities of the plant

The antimicrobial response of the methanolic extract of Dichrostachys cinerea is shown in Table 2. The methanolic extract showed high significant antibacterial activity against Bacillus subtilis,

moderate activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa and low activity against Candida albicans fungus at the concentration 100mg/ml.

Table 2: Antimicrobial activity Dichrostachys cinerea methanolic extra

No	Microorganism	Methanolic extract (100mg/ml)
1	Bacilus subtilis	18.3
2	Staphylococcus aureus	13.5
3	Escherichia coli	14.0
4	Pseudomonas aeruginosa	11.0
5	Candida albicans	8.60

Antioxidants activity of extract

The methanolic extract of Dichrostachys cinerea showed a high value for absorption inhibition at a concentration of 63.8 µg/ml and a high antioxidant capacity (91.6) in the DPPH assay, which was comparable to the value of the positive reference (96.4) (Table 3), due to the presence of flavonoids and tannins [20] .

Table 3: Antioxidant activity of the methanolic extract of Dichrostachys cinerea

No	Sample	(RSA ± Sd) % (DPPH)
1	Crude extract	91.6 ± 0.09
2	Ascorbic acid (Standard)	96.4 ± 0.01

CONCLUSION

Medicinal plants appear to be rich in secondary metabolites, which are commonly used in traditional medicine to combat and cure various diseases. The results of this study have shown that the leaves of Dichrostachys cinerea have various significant antimicrobial and antioxidant activities, so the formulation with different proportions of these extracts may prove to be good. However, further studies are required to quantify the identification of the various phytochemical constituents.

DISCLOSURE STATEMENT

Conflict of interest: the authors declare that they have no conflict of interest.
Authors contributions: All authors contributed equally in this work.
Availability of samples: samples of the preparations are available from the author.

REFERENCES

1. Palgrave K. C. Trees of Southern Africa, 4th ed.; C. Struik Publishers: Cape Town, (1984); p 254.
2. Mann A, Gbata M, Umar A. Medical and Economic plants.Jube Evans Books and publications, Bida, Nigeria, (2005); 161.Google Scholar
3. Vaidyaratnam PS.Varier. Indian Medicinal Plants. A compendium of 500 species. vol-I, Orient Longman Ltd, Chennai, India; (1998); 330-331.www.jpronline.info
4. Kirthikar KR and Basu B.D.Indian MedicinaPlants.Publications and Information Division,vol II,CSIR, New Delhi, India; (1975); 912-913.
5. Anonymous.The wealth of India. Publications and information irectorate.CSIR,(1952); 55-56.
6. Gelfand M, Mari S, Drummond RB, Ndemera B. The Traditioner Medical Practitional in Zimbabwe. 1st Ed. Mambo Press: Harare: (1985); 141-142. https://doi.org/10.4236/as.2014.560500
7. Eisa MM, Amaghoul Az, Omer MEA, Elegami AA (1). Fitoterapia.71(3): (2000); 324 – 347.

8. Tillement JP, Albengres E. L'approche pharmacologique de l'utilisation rationnelle des hétérosides cardiotoniques [Pharmacological approach to the rational use of cardiotonic heterosides]. Coeur Med Interne. 1977 Apr-Jun;16(2):239-48. French. PMID: 891123. https://pubmed.ncbi.nlm.nih.gov/891123/
9. Eisa, M.M., Almagboul, A.Z., Omer, M.E.A. and Elegami, A.A. Antibacterial activity of Dichrostachys cinerea. Fitoterapia71: (1999); 314-327. http://www.internationalscholarsjournals.org/login
10. Banso A, Adeyemo SO. Evaluation of antimicrobial properties of tannins isolated from Dichrostachys cinerea. Afr. J. Biotechnol., 6(15): (2007); 1785-1787. https://doi.org/10.5897/AJB2007.000-2262
11. Jung WK, Choi I, Oh S, Park SG, Seo SK, Lee SW, Heo SJ, Jeon YJ, Je JY, Ahn CB, Kim JS, Oh KS, Kim YM, Moon C, Choi IW: Anti-asthmatic effect of marine red alga (Laurencia undulate) polyphenolic extracts in a murine model of asthma.Food Chem Toxicol 47:(2009), 293-297. https://doi.org/10.1016/j.fct.2008.11.012
12. Kiessoun K, Souza A, Meda NTR, Yacouba Coulibaly A, Kidrebeogo M, Lamien-Meda A, Lamidi M, Millogo-Rasolodimby J, Nacoulma GO: Polyphenols content, antioxidant and anti- inflammatory activities of six Malavceae species traditionally used for treatment of hepatitis B in Burkina Faso. European Journal of Scientific Research 44:(2010), 570-580. https://doi.org/10.12691/ajmr-4-5-3
13. Harborne JB: Phytochemical Methods: A guide to Modern Techniques of Plant Analysis.Chapman and Hall, Londres;(1973):279. https://doi.org/10.12691/ajmr-4-5-3
14. Konan, A.B., Datté, J.Y. & Yapo, P.A. Nitric oxide pathway-mediated relaxant effect of aqueous sesame leaves extract (Sesamum radiatum Schum. & Thonn.) in the guinea-pig isolated aorta smooth muscle. BMC Complement Altern Med 8, 23 (2008). https://doi.org/10.1186/1472-6882-8-23.
15. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Wayne, Pensilvania docu-mentVol.19. (1999), M100-S9,
16. Rodriguez, Tudela,Estrella el al, Standardization of antifungal susceptibility variables for a semiautomated methodology, Journal of Clinical Microbiol, 39(7)(2001); 2513-2517. https://doi:10.1128/JCM.39.7.2513-2517.2001
17. Bauer AW, Kirby WMM, Sherris JC, Turck M ().Antibiotic susceptibility testing by a standardized single disk method. Am. J.ClinPathol.,43,(1966); 493–496.
18. Blois MS, "Antioxidant determination by the use of stable free radical", Nature,181, (1958); 1119-1120.
19. Shimada K, Fujikawa K, Nakamura T, Antioxidant properties of xanthan gum on antioxidation of soybean oil in cyclodextrin emulsion, Journal of Agricultural and Food Chemistery, 40(6)(1992),: 945-948. https://agris.fao.org/agris-search/search.do
20. williams, maclver, et al, The c-terminal domian of Drosophila beta Heavy-spectrin exhibits autonomous memberane association and modulates membrane area, Journal Cell scientific, 117(5) (2004),: 771-782. https://doi:10.1242/jcs.00922.