

PHYTOCHEMICAL SCREENING, PROXIMATE ANALYSIS AND ANTIMICROBIAL
ACTIVITIES OF *DICHROSTACHYS CINEREA* (L)Fatehalrahman F. Magbool^{1*} and Mahmoud A. Ibrahim²¹Assistant Professor of Pharmaceutics, Red Sea University - Sudan.²College of Pharmacy, Department of Pharmaceutical Analysis, Ribat National University, Sudan.

*Corresponding Author: Fatehalrahman F. Magbool

Assistant Professor of Pharmaceutics, Red Sea University - Sudan.

Article Received on 24/06/2025

Article Revised on 14/07/2025

Article Accepted on 04/08/2025

ABSTRACT

Medicinal plants and their bioactive molecules are always in demand and are a central point of research. To date, herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. Preliminary phytochemical screening and antimicrobial activity of *Dischrastachys cinerea* was carried out to assess the chemical contents, biological and antimicrobial activity. The extraction was carry out according to protocol of WHO 1998 successive extraction method. The phytochemical screening was carry out to show the present of Tannin, Saponins, Alkaloid, Flavonoids, Steroids and sugar. proximate analysis was done to show the present of Moisture 2.270, Ash 9.823, Protein 20.738, Fat 0.806, Fiber 60.390, and carbohydrate 5.973. And antimicrobial was tested against four stander bacteria species: Gram positive bacteria *staphylococcus aureus* (ATCC 25923)20.44 and *Bacillus subtilis* (NCTC8236) 18.2 Gram Negative bacteria *Escherichia coli* (ATCC 25922) 25.50 and *pseudomonas aeruginosa* (ATCC 27853)21.28 and one stander fungal strain VIZ, *Candida albicans* (ATCC 7596) 19.94 using disc diffusion method. The study illustrate that, all the extracts of various *D. cinerea* plant exhibited antibacterial and anti-candidal activities. All the extract were effective against all microorganism that use .Of the two extraction solvent methanol extracts gave better inhibition zones as compared to water extract.

KEYWORDS: Medicinal plants, Bioactive molecules, Phytochemical screening, Antimicrobial activity, Extraction, Bioactive molecules.

INTRODUCTION

Medicinal plants are still invaluable source of safe, lower price available and reliable natural resource of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparation to treat themselves^[1] therefore, it is useful to investigate the potential of local plants against these disabling diseases.^[2] Sudan represents one of the largest African countries and characterized by rich flora described by many botanists. They observed that Sudan medicine represents a unique blend of indigenous cultures with Egyptian, Arabia, west and east African culture. In an attempt to collect information on Phytochemistry of Sudanese medicinal plants it is important to collect information about the plants used by herbalists in different part of Sudan. Form the observations of many pharmacists working in the field of medicinal plants, a lot of work need to be done to identify species that are used by traditional herbalists over years for curing specific ailments. This can be achieved by encouraging interested pharmacists and clinicians in Sudan to collect

information from their respective regions by working very close with the established herbalists.^[3] In developing countries medicinal plants continue to be the main source of medication. The medicinal plants contain many active constituents such as tannins, flavonoids, alkaloids and saponins. Tannin is responsible for the antimicrobial effect by different mechanism, include inhibition of the extracellular microbial enzymes, deprivation of the substrates required for the microbial growth or direct action on microbial metabolism through the inhibition of oxidative phosphorylation. A further mechanism involving iron deprivation is proposed. Many microorganism can overcome plant defenses based on tannins.^[4]

Dichrostachys cinerea is one of the very useful wild medicinal plants in many areas Despite substantial efforts by ethno botanical researchers to document majority of medicinal plants used in indigenous health systems few researchers have examined and documented their safe dosages and extinction threats posed to habitat-

specific species.^[5] Plants are endowed with free radical scavenging molecules. Such as vitamins flavonoids, phenolic acids, lignins, stilbenes, tannins, betalains, and other metabolites, which are rich in antioxidant activity studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities.^[6] Phytochemistry have been instrumental in rationalization of the use of various herbal medicines however unscreened herbal products still find their way to markets owing to their high demand. For instance, the bark of *D. cinerea* is used to prepare concoction traditionally used to treat dysentery, headache and elephantiasis. Its root infusions are used to treat epilepsy, gonorrhea coughs and sore eye and also serve as an anthelmintic, laxative and strong diuretic.^[7] Seeds of this plant are edible and the leaves are good fodder for domesticated animals. In India it's called the mother of healing.^[8]

MATERIALS AND METHODS

Materials

Plant material

Plant was collected from southern Kordofan state 2020 and sample was identified and authenticated by the taxonomists of the medicinal and aromatic plant and traditional medicine research institute (MAPTMR) Khartoum, Sudan. They were washed and then air dried under light exposure (27°C-30°C) for 14 days.

Method

Plant extraction

Extraction was carried out according to method described by^[9]: 500 g of the *Dichrostachys cinerea* was extracted by soaking in 2500 ml of different solvents for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus. In order to obtain a completely dry extract, the resultant extract were transferred to glass dishes.

Phytochemical screening

Phytochemical screening of *Dichrostachys cinerea* extracts for the active constituents was carried out using the methods described by^[10,11,12,13], with many few modifications. Phytochemical was carried out and show the presence of tannin by addition of ferric chloride reagent to the filtrate was given blue color indicate the presence of tannin. Alkaloids by addition of few drop of Dragendorff's reagent turbidity was taken as indicative of presence of alkaloids, flavonoids indicated by addition of magnesium metal followed by the addition of few drop of conc. HCL the red color was inductive the presence of Flavonoids, saponins content was determined by boiling 1 g powder in 10 ml distilled water for 15 min and after cooling the extract was shaken vigorously to record froth formation, steroid was determined by dissolved the extract by chloroform and filtered H₂SO₄ was added to filtered to form lower layer reddish brown color steroidal

ring was appear, carbohydrate was determined by Mulish test, reduced sugar was determined by Fehling reagent.

Proximate analysis

Proximate analysis was carried out to show presence of fat the sample was hydrolyzed by hydrochloric acid at 70-80°C. protein, if any, can be dissolved in acid, cured fat manually extracted by diethyl and petroleum ether the solvent was removed by evaporation and the oil residue dried and weighted, Moisture method was based on drying sample under control temperature until constant weight is obtained, Ash method was involve oxidation of all organic matter by incineration in a furnace at specific temp less than (550°C) Ashing above 650°C volatilities inorganic salt like alkali chloride and a portion of ash fused and enclosed some carbon, preventing them from benign ignited. The residue left after incineration is the Ash content of the sample, protein method was based on digestion of protein and organic food with sulfuric acid in catalyst to release nitrogen from protein ammonium gas was liberated upon the addition of excess alkali and was distilled in to a boric acid solution to form ammonium borate complex the ammonium liberated was titrated with standardized HCL the amount of nitrogen was determined from Mg equivalent to acid uses crude protein was determined by multiplied nitrogen content with conversion factor to food matrix and fiber method was extracted by 2g ether then precipitate was formed and transfer to digestive flask unit with addition to espstous, 200mg of H₂SO₄ was added digestion flask was connected to condenser then boiled, funnel was fixed with piece of cloth and then added regular a hot water to wash H₂SO₄, then NaOH was added to the cloth precipitate until the alkali is removed.

Method of antimicrobial activity

The antimicrobial test was performed using agar diffusion method. The test microorganism were incubated on nutrient agar plate and separate uniformly using sterile glass separator. Wells of 5mm in diameter were made on the nutrient agar using sterile cork borer. The agar disks were carefully removed by the use of forceps sterilized by flaming then the extract was added to plate. The plate is allowed to stand for one hour at room temperature for diffusion of the substance to proceed before the growth of microorganism commenced. The plate were incubated at 37°C for 24 h. the zones of inhibition were then recorded.

RESULTS AND DISCUSSION

Preliminary phytochemical screening was performed to establish the profile of *D. cinerea* extract for its chemical composition. An evaluation on the phytochemical screening of *D. cinerea* extract revealed the presence of medicinally active constituents. The phytochemical active compounds of *D. cinerea* were screened and the results are presented in (Table 1-3).

Table 1: Phytochemical Screening of Petroleum Ether Extract of Stem, Bark and Leaves of *D. cinerea*.

	Sterols	alkaloids	Saponins	tannins	anthracenes	Flavonoids	cardiac	carbohydrates	Reduce sugars	coumarins
Bark	-	+	+	+	-	+	-	+	+	-
Leave	-	-	+	+	-	+	-	+	+	-

Table 2: Phytochemical Screening of Methanol Extract of Stem, Bark and Leaves of *D. cinerea*

	Sterols	Alkaloids	Saponins	Tannins	Anthracene	Flavonoid	Cardic glycoside	Carbohydrates	Reduce sugars	Coumarins
Bark	+	-	+	+	-	+	-	+	+	-
Leave	-	+	-	+	-	+	-	+	+	-

Table 3: Phytochemical Screening of Water Extract of Stem, Bark and Leaves of *D. cinerea*.

	sterol	alkaloid	saponins	tannin	Anthracene	flavonoid	Cardiac glycoside	carbohydrate	Reduce sugar	coumarin
Bark	+	+	+	-	-	+	+	+	+	-
leave	+	-	+	+	+	-	+	+	+	-

The phytochemical analysis conducted on *D. cinerea* extract revealed the presence of tannin, flavonoids, steroids, and saponins. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane bound enzymes such as the ATPase and phospholipase A2 and this property may explain the mechanisms of anti-oxidative action of *D. cinerea*. Flavonoids serve as health promoting compound as a results of its anion radicals. *D. cinerea* was also found to contain saponins known to produce inhibitory effect on inflammation. Tannins are known to be useful in the treatment of inflamed or ulcerated tissue and they have remarkable activity in cancer prevention and anticancer and possess antimicrobial activity. Thus *D. cinerea* containing this compound may serve as a potential source of bioactive compounds in the treatment of cancer. Alkaloid was also detected *D. cinerea* extracts. Alkaloids have been associated with uses for centuries and one of their common biological properties is their cytotoxicity success of *D. cinerea* extracts against both Gram positive and Gram negative bacteria are likely dependent on their content alkaloids to intercalate between DNA strands the presence of these phenolic compounds in *D. cinerea* extracts contribute to its ant oxidative properties and thus the usefulness of this plant in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. The various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants.^[14,15,16] They act as binders and for treatment of diarrhea and dysentery.^[17] Tannins also reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and is also used as diuretic.^[18] Plant tannin has been recognized for their pharmacological properties and is known to make trees

and shrubs a difficult meal for many caterpillars.^[19] Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants)^[20]; Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages.^[21] Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiostonic in nature and are reported to have anti-diabetic and anti-fungal properties.^[22,23] They are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. A large number of studies have been done in recent years on the antifungal and antibacterial activity of terpenoids of natural origin. The mechanism of action of triterpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic nature. Coumarins have been reported to stimulate macrophages which could have an indirect negative effect on infections. Plant steroids are known to be important for their cardiostonic, insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicines, cosmetics and they are routinely used in medicine because of their profound biological activities.^[24] Anthraquinones are structurally built from an anthracene ring (tricyclic aromatic) with a keto group each on carbon atom nine and ten. In plants, anthraquinones are found in a wide range of species. The effects of anthraquinones and anthrones are very diverse. Anthraquinones and anthrones are very reactive and have a broad pharmacological activities including, they are potent anticancer, antidiabetic, antimicrobial, antiinflammatory, and cathartic properties as well as its cardio-, hepato-, and neuroprotective qualities.^[25] Anthraquinones and xanthenes contain an aromatic core that serves as a scaffold for the attachment of diverse functional groups, resulting in a wide variety of molecules with distinct biological and biochemical characteristics.

Table 4: Proximate analysis of *D. cinerea*.

Moisture	Ash%	Protein%	Fat%	Fiber%	carbohydrates
2.270	9.823	20.738	0.806	60.390	56.7

The proximate analysis was carry out for quantitative determination (Table 4), and show the present of moisture 2.270 %, Ash 9.823%, protein 20.738%, fat 0.806% and fiber.

The stem-bark and leaves of *d-cinerea* family (*Mimosaceae*) was screened for antimicrobial activity against two gram positive bacteria (*B. subtilis* & *S. aureus*), two gram negative bacteria (*E. coli* & *P.*

areuginosa) as well as one fungi (*C. albicans*) using disc diffusion method (Table 5).

The extracts showed high activity (25.23mm & 22.72 mm) against gram negative (*E. coli* & *P. areginosa*) respectively and (23.00 mm & 19.82 mm) against gram positive bacteria (*S. aureus* & *B. subtilis*) respectively and also (22.14mm) against *C. albicans*.

Table 5: Antimicrobial activity of *D.cinerea* extracts.

Microorganism	Mean diameter of inhibition zone mm			
	H ₂ O (STM)	H ₂ O (LV)	MET (STM)	MET (LV)
<i>S.aureus</i>	20.44	21.03	22.88	23.00
<i>P. aeruginosa</i>	21.28	22.28	21.74	22.72
<i>B.sublitis</i>	18.20	18.44	19.82	18.33
<i>E.coli</i>	23.50	25.00	23.61	25.23
<i>C.albicans</i>	19.94	20.17	19.54	22.14

Key:H₂O (STM)stem-bark extract using water as solvent,H₂O (LV) Leave extract using water as solvent

MET (STM).....stem-bark extract using methanol as solvent

MET (LV).....leave extract using methanol as solvent

Interpretation of result MDIZ (mm):>15mm= sensitive, 12-15 =intermediate, <15 = resistant

All the extracts of various *D.cinerea* plant exhibited antibacterial and anticandidal activities. All the extract were effective against all microorganism that use. Of the two extraction solvent methanol extracts gave better inhibition zones as compared to water extract. Which might be attributed to the incomplete leaching of the antibacterial substance. *E. coli* was found to be the most sensitive microorganism while *B. sublitis* was the least sensitive microorganism to the extracts.

CONCLUSION

The preliminary phytochemical screening will be useful in finding the chemical nature of the drug. In this study, the preliminary phytochemical screening ascertained the presence tannin, flavonoids, steroids, and saponins, the various phytochemical compounds detected are known to have beneficial importance in industrial and medicinal sciences. Consequently, the therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. As this drug material is used for various diseases. Therefore, this study can be used as an useful information for identifying parameters to substantiate and authenticate the drug of this medicinally important plant.

REFERENCES

1. Amaral, FMM, Ribeiro MNS Barbosa-Fiho JM,, Reis AS, Nascimento FRF., Macedo RO., Plant and chemical constituents with giardicidal activity , Braz J pharmacogn, 2006; 16: 6969-920.
2. KoKo, SW., Antimalarial activity of *Xanthium brasiliicum* Vell*, In vitro, In vivo and toxicology approaches, Recent prog Med plants, 2005; 15: 1-10.
3. Huda, I. M. A., (2007), Biochemical evaluation and antimicrobial activity of seed oil of five species from family Combretaceae, A thesis of M.Sc department of Biochemistry, College of Applied and industrial Science, University of Juba, Sudan.
4. Ahmed M, Pin Lim C, AKyiremAKowuah G, Ismail NN., 2013; 5: 005.
5. Patel SS et al traditional medicine source of new drug pharmitime, 2000; 34(1): 17-18.
6. Farooqi Aa et al importance, present studies and future prospects of medicinal crop, 2003; 7: 69-72.
7. Horwitz W. (editor). Official method of analysis of AOAC international. 17th Edition. AOAC international, Maryland, USA, 2000; 920(39): P33.
8. Greenfield H. and Southgate DAT (1992). Food composition Data: Production, Management and Use. Elsevier Applied Science, UK.
9. Sukhdev. S. H; Suman. P. S. K; Gennaro. L and Dev. D. R. Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology, 2008; 116.
10. Martinez A, Valencia G: Marcha fitoquímica. (). In Manual de prácticas de Farmacognosia y Fitoquímica: 1999. 1st edition. Medellin: Universidad de Antioquia; Phytochemical screening methods, 2003; 59-65.
11. Sofowora, A. Medicinal Plants and Traditional Medicines in Africa. Chichester John, Willey & Sons New York., 1993; 256.
12. Harborne, J. B. Phytochemical methods. 2nd edition. Chapman and Hall, 1984.
13. Wall, M. E; Eddy, C. R; McClenna, M. L; & Klump, M. E. Detection and estimation of steroid and sapogenins in plant tissue. Analytical Chemistry, 1952; 24: 1337-1342.
14. Tyler VE, Brady LR, Roberts JE. Pharmacology. Lea and Ferbiger, Philadelphia. 1988; 85-90.
15. Awosika F. Local Medicinal plants and health of consumers. Clin. Pharm. Herbal Med., 1991; 9: 28-29.
16. Ogunleye DS, Ibitoye SF. Studies of antimicrobial activity and chemical constituents of *Ximenia Americana*. Trop. J Pharm Res., 2003; 2: 239-241.
17. Dharmananda S. Gallnuts and the uses of tannins in Chinese medicine. A paper Delivered at the Institute for Traditional Medicine, Portland, Oregon, 2003.
18. Heslem E. Plant Polyphenol: Vegetal Tannin Telisted- Chemistry and Pharmacology of Natural Products, 1st Edn., Cambridge University Press, Cambridge, Massachusetts, 1989; 169.
19. Aiyelaagbe O, Osamudiamen PM. Phytochemical Screening for Active Compounds in *Mangifera indica* Leaves from Ibadan, Oyo State, Plant Sciences Research, 2009; 1(2): 11-13.

20. Rauha JP, Remes S, Herinonen W, Hopia M, Kujala T, Pitinlaja K et al. Antimicrobial effects of finished plant extract containing flavanoids and other phenolic compounds. *Int. J Food Microbiol*, 2000; 56: 3-12.
21. Mark Percival. Antioxidants. *Clinical Nutrition Insights*, 1998; 31: 01-04.
22. Trease GE, Evans MD. A text book of Pharmacognosy, 13th Edn. Baillier, Tindal and Causel, London, 1989; 144-148.
23. Kamel JM. An extract of the mesocarps of fruits of *Balanite aegyptiaca* exhibited a prominent anti-diabetic properties in Mice. *Chem. Pharmacol. Bull.*, 1991; 39: 1229-1233.
24. Denwick PM. Natural Products A Biosynthetic Approach. 2nd Edn., John Wiley and Sons, Ltd., England, 2002; 241-243.
25. Izhaki I. Emodin - a secondary metabolite with multiple ecological functions in higher plants. *New Phytol*, 2002; 155(2): 205-217.