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Phytochemical Screening, Proximate Analysis and Antimicrobial Activities of Dichrostachys Cinerea (L)

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Abstract

Dischrastachys cinerea was collected from southern kordofan state (2020). And authentic by Dr. Yahiya Mohamed (Medicinal and Aromatic Plants and Traditional Medicinal Research Institute (MAPTMRI) 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.

Keywords: Dichrostachys cinerea; Pharmacognacy; Anti-atherosclerotic; Anti-inflammatory

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 biology and Pharmacognacy 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 botanist 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 aliments. This can be achieved by encouraging interested botanists and medical doctors 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 defences 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 end owed 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]. Phytochemistery 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 tradition ally used to treat dysentery, headache and elephantiasis. It is root infusions are used to treat epilepsy, gonorrhoea 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 [8-11].

Material and Methods

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 (27C-30C) for 14 days.

Plant extraction

The extraction was carry out according to protocol of WHO 1998. And successive extraction method.

Phytochemical screening

Phytochemical was carry out and show the present of tannin by addition of ferric chloride reagent to the filtrate was given blue colour indicate the presence of tannin Alkaloids by addition of few drop of Dragendorffs 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 colour was inductive the present 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 faltered H_2SO_4 was added to filtered to form lower layer reddish brown colour steroidal ring was appear, carbohydrate was determined by Mulish test, reduced sugar was determined by Fehling reagent.

Proximate analysis

Proximate analysis was carry out to show presence of fat the sample was hydrolyzed by hydrochloric acid at 70-8^{0}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 e method was based on drying sample under control temperature until constant weigh is obtained, Ash method was involve oxidation of all organic matter by incineration in a furnace at specific temp less than (55^{0}C) Ashing above 65^{0}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 tittered 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 H_2SO_4 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 H_2SO_4, 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 cute 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^{o}C for 24 h. the zones of inhibition were then recorded.

Results and Discussion

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 radical's .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 proximate analysis was carry out for quantitative determination and show the present of moisture 2.270 %, Ash 9.823%, protein 20.738%, fat 0.806% and fibe (Tables 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	+	-	+	+	+	-	+	+	+	-

Result of Antimicrobial Activity of D.Cinerea

The stem-bark and leafs 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.

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 (Tables 4,5).

Table 4: *Result of proximate analysis.*

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

Table 5: *Result of antimicrobial activity of D.cinerea.*

Mean diameter of inhibition zone mm				
Microorganism	**H_2O (STM)**	**H_2O (LV)**	**MET(STM)**	**MET(LV)**
S.aureus	20.44	21.03	22.88	23.00
P. aerugionsa	21.28	22.28	21.74	22.72
B.sublitis	18.20	18.44	19.82	18.33
E.coli	23.50	25.00	23.61	25.23
C.albicans	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. subltits was the least sensitive microorganism to the extracts.

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