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Research Paper

Antibacterial and Antidiarrhoeal Activities of Dichrostachys Cinerea Against Some Enteric Pathogens

Microbiology

KEYWORDS : Dichrostacys cinera, Antibacterial, Antidiarrheal, Enteric, Castor oil.

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ABSTRACT

The in vitro antibacterial activity of the aqueous, ethanol and petroleum ether extracts of Dichrostachys cinerea were tested on some bacteria associated with diarrhoea namely; Escherichia coli, Salmonella Typhi and Shigella dysenteriae using agar well diffusion band broth dilution method. The extract inhibited the growth of almost all the test organisms though with different zones of inhibition. Although the petroleum ether extract was not effective against Shigella dysenteriae and Escherichia coli at all, but was effective against Salmonella Typhi except at 12.5 mg/ml and 6.25 mg/ml. Generally, ethanolic and aqueous extracts were the most effective solvents against all the test organisms. The Minimum Inhibitory Concentration (MIC) of the ethanol, aqueous and petroleum ether extract for E. coli were 12.5 mg/ml in all cases. For Salmonella Typhi, the MIC for the aqueous and petroleum ether extracts were 12.5 mg/ml respectively while it was 25 mg/ml for the ethanolic extract. In addition, the MIC for Shigella dysenteriae was 12.5 mg/ml for both the aqueous and ethanolic extracts while it was 25 mg/ml for the petroleum ether extract. The phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, carbohydrates, steroids and terpenes, cardiac glycosides and saponins. However, the plant does not contain anthraquinones. Antidiarrhoeal activity of the ethanolic plant extract demonstrated better activity of at 400 mg/ml than 200 mg/ml which is indicative of the fact that the activity of the plant is dose dependent. Therefore, it can be concluded that the Dichrostachys cinerea has appreciable antibacterial and antidiarrhoeal activities.

INTRODUCTION

Traditional medicine can be described as the total combination of knowledge and practices whether explicable or not, use in diagnosing, preventing, or eliminating a physical mental or social disease and which rely exclusively on past experience and observations handed from generations to generation verbally or in writing (Sofowora, 1993). Diarrhoea is one of the clinical findings in gastrointestinal infections (Jawetz, *et al.,* 2004). According to NNDIC (2003), diarrhoea is a loose, watery stool occurring more than three times in a day. It may be caused by a temporary problem such as an infection, or chronic problem such as intestinal disease. It causes include viral, bacterial intestinal disease and or functional bowel disorder. However, the commonly identified causes of bacterial diarrhoea includes: *Salmonella* Typhi, *Escherichia coli*, *Shigella dysenteriae*, *Campylobacter jejuni*, *Vibro cholerae*, among others (Garner, 2002).

Diarrhoea disease is still the leading cause of mortality in developing countries. An expert committee of the World Health Organization recently estimated that diarrhea causes 18% of the 11 million death among children under the age of 5 years and is the leading cause of infant mortality (Bryce *et al.,* 2005). Disease and death caused by diarrhoea is a global problem, but it is especially prevalent in developing countries (Kosek *et al.,* 2003). Furthermore, adults are also affected and need special attention in treatment and management, especially in acute and long –term care residents, because of their multiple co-morbidities, immunosenescence, frailty, and poor nutritional status (Trinh and Prabhakar, 2007).

Several studies have reported the uses of *Dichrostacys cinera* in the treatment of many diseases and ailments. The bark of *Dichrostachys cinerea* has been used by the local tribes of Mayurbhanji district of Odisha for the treatment of diabetes mellitus. In addition, the plant is used in veterinary medicine in India (Handa *et al.,* 2003). *Dichrostachys cinerea* are traditionally used as antimicrobial, anticonvulsant, astringents (the root of the plant), antihelminthics, purgatives, laxatives and diuretics (the bark). In medicine, the bark is used to alleviate headache, toothache, dysentery, elephantiasis among others. Also, the root infusions are consumed to treat leprosy, syphilis and cough. Furthermore, its powder can be used in the massage of fractures. The leaves are used to treat inflammatory conditions, arthritis, pile and eczema (Swetha *et al.,* 2013).

The generic name Dichrostachys means coloured spike and cinerea refers to the greyish hair of the typical sub-species obtained from the Greek word: *konis* and the Latin word: *cinerea*. *Dichrostachys cinerea* is one of the very useful wild medicinal plants which originates from Africa and spreads to many tropical areas in Asia, Australia, America and the Caribbean. It is also one of many very useful wild medicinal plant of semi-arid areas in Kenya, Eritrea and Somalia (Rukangira, 2004). *Dichrostachys cinerea* extract is said to be an effective antidiarrhoeal plant commonly used by Mwaghavul tribe in mangu, hence the need to scientifically validate the use of this plant as a medicinal plant.

MATERIALS AND METHODS

Collection and Authentication of Plant

The leaves of *D. cinerea* were collected from Sherry Hills area of Jos Plateau State and was authenticated in the Department of Plant Science University of Jos. The leaves were dried in an open air under room temperature to prevent the ultra violet rays in activating the chemical constituents there-in. The dried leaves

were pulverized in a mortar with pestle.

Extraction Procedures
Extraction of the active ingredient from the plant sample of *D. cinerea* were done using different principal solvents for the extraction process; ethanol, petroleum ether and water (aqueous extraction). 100g of the pulverized samples were weighed and wrapped in a filter paper and inserted into a Soxhlet apparatus, and 1000ml of absolute ethanol as the solvent was poured into a quick fit round bottom flask.

The Soxhlet apparatus was connected to fit the bottom flask and a condenser was connected to the solvent for 72 hours, after which the Soxhlet was dismantled and the wrapped sample was removed. The same procedure was repeated for petroleum ether as solvent. The Soxhlet was reconnected to recover the solvent from the flask leaving the extract behind. The extract was concentrated using a rotary evaporator. The dried extract was stored in the refrigerator at 10^0C.

For the aqueous extract, a 50g of powdered leaves of *D. Cinerea* was put in a conical flask and 500mls of cold water was added. The mixture was stirred to homogeneity using a stirrer, covered and left at room temperature for 24 hours. After 24 hours, the extraction was filtered and the aqueous liquid containing the extract was put in a stainless plate and allowed to evaporate in an oven at 45°C. After drying, the sample was scrapped out and put in a pre-weighed sterile sample container.

Preparation of Plant Extracts Concentrations
One gram of each aqueous, ethanol and petroleum ther extracts pre-prepared (each separately) were taken and the aqueous extract was dissolved in 10ml sterile distilled water, while the ethanol and petroleum ether extracts were dissolved in 10ml of DiMethyl Sulphoxide (DMSO). Thus 100 mg / ml of stock was obtained as a standard concentration of aqueous, ethanol and Pet. ether extracts respectively. Different concentrations of extracts were prepared using water and DMSO as solvents. Different working concentrations (100mg/l, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were prepared using doubling dilution of the prepared stock solution of 100mg/ml concentration.

Sources of Test Organisms
The test organisms were gotten from the bacteriology laboratory of National Veterinary Research Institute, Vom, as purified stock cultures. The organisms are as follows: *Escherchia coli, Salmonella* Typhi*, Shigella dysenteriae,* using a standard operating procedure/technique. A 24h culture of the bacterial culture isolates were diluted with physiological saline solution and the turbidity corrected by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (10^6 CFU/ml) was obtained (Cheesbrough, 2006).

Antibacterial Assay
A cork borer of diameter 5mm was sterilized by dipping into alcohol flipped and flamed to red hot. The sterilization was done at intervals after boring seven holes in a plate that was inoculated with only one strain of organism. About 0.3ml of the diluted extract concentration (both ethanol, petroleum ether and aqueous extracts) were introduced into the holes in clockwise fashion starting with the highest concentration to the lowest. The sixth hole contained distilled water while the seventh olehole contained 0.2ml of the standard drug, ciprofloxacin. This was done aseptically. The plates were allowed to stand for 1hour for pre-diffusion before they were incubated at 37°C, under aerobic conditions for 24 hours.

Determination of Minimum Inhibitory Concentrations (MIC)
This was determined using broth dilution method as described by Junaid (2006). The dilutions that showed no turbidity were taken and recorded as the Minimum inhibitory concentration (MIC).

Phytochemical Screening of the Leaf Extract of *Dichrostachys cinerea*
The ethanolic, aqueous and petroleum ether extract of *Dichrostachys cinerea* was subjected to a standard phytochemical screening for the presence of resins, saponins, alkaloids, tannins, glycosides, flavonoids and steroids to the methods of Trease and Evans (1983).

Acute toxicity study
The acute oral toxicity study was carried out as per the 423 guideline set by Organization for Economic Co-operation and Development (EOCD). The extracts were administered at the dose level of 2000mg/kg. One tenth of the median lethal dose (LD_{50}) was taken as an effective.

Castor Oil Induced Diarrhoea Assay
Twenty rats was used for this study, the rats were starved for 12hours before the commencement of the experiment, having access to only water. The rats were separated into four groups of five rats each.

Groups I and II were given graded doses of the extract. (200mg/kg, 400mg/kg, respectively).

Group III were given 2ml/100g distilled water – Negative control.

Group IV, was given Loperamide (10mg/kg body weight orally positive control).

The rats were housed singly in cages lined with white blotting paper. One hour after the above treatment, all the rats in the groups will be given castor oil orally. The rats were observed for 6 hours for watery (wet) or unformed faeces. The watery faeces from each rat will be counted hourly for up to 6 hours. At the end of the experiment the group mean is obtained and the percentage of protection was calculated.

RESULTS
In the result below, table 1 shows the result of the phytochemical screening of the leave extract of *D. cinerea.* Ethanolic and petroleum ether extracts had flavonoids "very present" while aqueous and ethanolic extracts had tannins "very present". Also, ethanolic extract had steroids and terpenes "extremely present" but anthraquinones was absent in all the extracts.

Furthermore, tables 2 shows the results of the minimum inhibitory concentration (MIC) of all the extracts in different concentrations of 100, 50, 25, 12.5, 6.25 mg/ml. Almost all the tubes had their MIC's at 12.5 mg/ml except for ethanolic and petroleum ether extracts for both *Samonella* Typhi and *Shigella dysenteriae* whose MIC's were at 25 mg/ml.

In addition, figures 1, 2 and 3 shows the susceptibility test result for aqueous, ethanolic and petroleum ether extracts. The plant leaf extract inhibited the growth of almost all the test organisms though with different zones of inhibition. The petroleum ether extract was not effective against *Shigella dysenteriae* and *Escherichia coli* at all, but was effective against *Samonella* Typhi except at 12.5 mg/ml and 6.25 mg/ml. However, *Samonella* Typhi was not susceptible to all the extracts at 6.25 mg/ml. In summary, ethanolic and aqueous extracts were the most effective against all the test organisms.

On the other hand, for the antidiarrhoeal test results, Water vs Loperamide ($P < 0.001$) means of the two groups were significantly different. This was expected since loperamide is a stand-

ard antidiarrhoeal drug while water is not a treatment.

Water vs *D. cinerea* 200 mg/ml (P > 0.05) means that there is no significant difference between the two treatments i.e. *D. cinerea* 200 mg/ml is not more effective than water.

Water vs *D. cinerea* 400 mg/ml (P < 0.05) means there was a significant difference between the two treatments i.e. at 400 mg/ml, the extract exhibited an antidiarrhoeal activity when compared to water. This may mean that the activity of the extract may increase with increasing concentration.

Loperamide vs *D. Cinerea* 200 mg/ml and Loperamide vs *D. Cinerea* 400 mg/ml (all P-values less than 0.05) means that loperamide was more active than both doses of the extract.

D. cinerea 200mg/ml vs *D. Cinerea* 400 mg/ml (P > 0.05) means there is no significant difference between the two doses.

DISCUSSION

The results of this study indicates that the extracts of *Dichrostachys cinerea* has an appreciable antibacterial activity as was earlier indicated. All the test organisms used showed a marked degree of susceptibility to the extracts of *Dichrostachys cinerea* at 100 mg/ml and 50 mg/ml. This shows that its activity is dose dependent, thus showing concordance with Okpekon *et al.* (2003) report which elucidated the relationship between the antimicrobial activities of medicinal plants (e.g. plants with chemotherapeutic actions) with concentration.

Extracts from the leaves of *Dichrostachys cinerea* inhibited the growth of *Escherichia coli, Salmonella* Typhi, and *Shigella dysenteriae* at most of the concentrations used for the susceptibility test though at different degrees. *Salmonella* Typhi and *Escherichia coli* were the most susceptible to the aqueous extract while the three (3) test organisms were all susceptible to the ethanolic extract. But *Salmonella* Typhi was the only organism susceptible to petroleum ether extract only.

The ethanolic extract compared to the aqueous extract showed slight difference in its antibacterial activity. This may be attributed to the high solubility of phytochemicals in ethanol than in water which is contrary to the report of Swetha (2013) who argued that methanol and Ethyl acetate extracts show better activity against Gram positive and Gram negative organisms.

The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, cardiac glycosides, steroids and terpenes and saponins. However, the plant extract did not contain anthraquinone. In addition, ethanol was the most effective extraction solvent compared to water as the former showed a higher concentration of all the constituents. This is similar to the study carried out by Mishira (2009) which suggested that all the major plant constituents are responsible for the antibacterial activity of medicinal plants.

The antidiarrhoeal result indicates a better activity of *Dichrostachys cinerea* extract at 400 mg/ml as compared to the same extract at a concentration of 200 mg/ml. This indicates that the antidiarrhoeal activity of the extract increases in a dose-dependent manner.

CONCLUSION

This study has presented a scientific prove of the potential use of the extracts of *Dichrostachys cinerea* as an antibacterial and antidiarrhoeal agent. Although traditionally, it is taken by preparation with water, this work has provided evidence that ethanol is a better solvent for the extraction of its active constituents and was effective against all the test organisms. Perhaps, other organic solvents might be effective as well. The antidiarrhoeal activity shows that at 400mg/ml or more the extract is effective. This means that the extract is dose dependent.

Table 1. Phytochemical Constituents of *D. cinerea*

Constituent	Aqueous extract	Ethanolic extract	Petroleum ether extract
Alkaloid	+	+	+
Flavonoids	+	++	++
Saponins	-	+	+
Carbohydrate	+	++	+
Tannins	++	++	+
Cardiacglycoside	++	+	+
Steroids &Terpenes	+	+++	+
Anthraquinones	-	-	-

Key:
+ = Present
++ = Very present
+++ = Extremely present
- = Absent

Table 2: Minimum inhibitory concentrations (MIC) of the extracts *Dichrostacys cinera*

Test organisms	Extract	Concentrations (mg/ml)					MIC
S. Typhi	AE	-	-	-	*	+	12.5
	EE	-	-	*	+	+	25
	PE	-	-	-	*	+	12.5
Shigella dysentariae	AE	-	-	-	*	+	12.5
	EE	-	-	-	*	+	12.5
	PE	-	-	*	+	+	25

Key: AE = Aqueous extract, EE = Ethanol extract, PE = Petroleum Ether Extract
(-) = No growth
(+) = growth
(*) = MIC

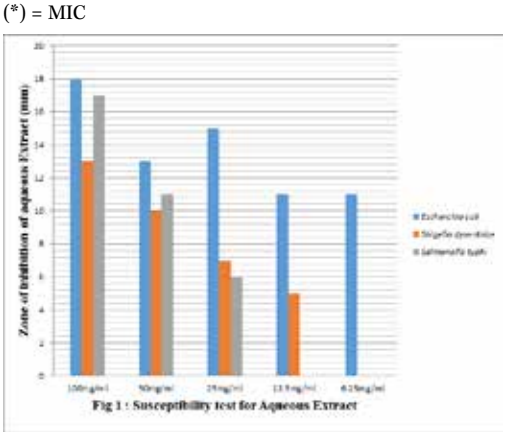

Fig 1 : Susceptibility test for Aqueous Extract

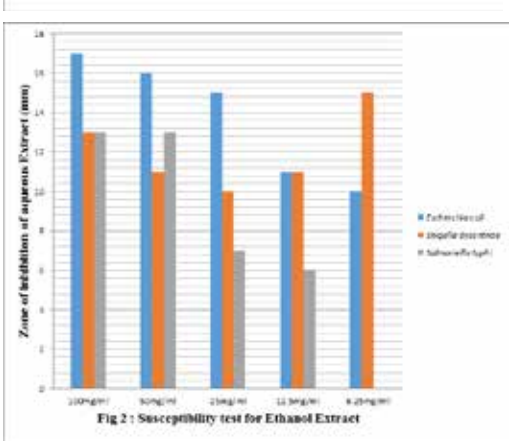

Fig 2 : Susceptibility test for Ethanol Extract

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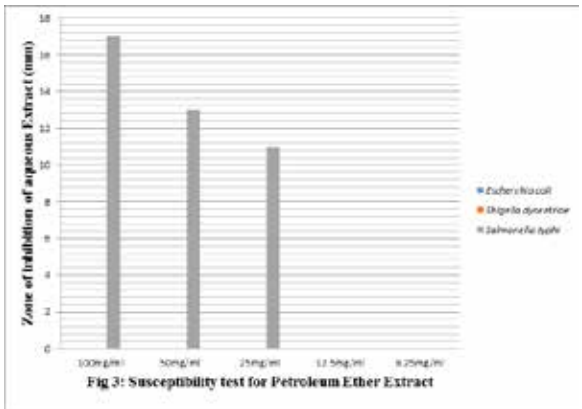
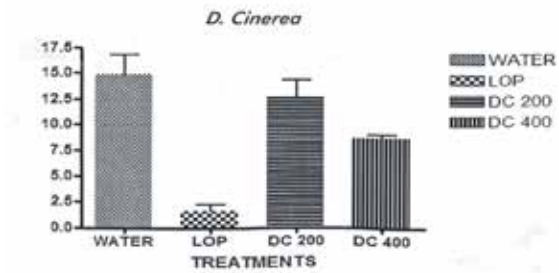


Fig 3: Susceptibility test for Petroleum Ether Extract

Table 3. Antidiarrhoeal Test Result.

	Tukey's Multiple Comparison Test	Mean Diff	q	P value	95% CI of diff
1	WATER vs LOP	13.20	9.564	P < 0.001	7.616 to 18.78
2	WATER vs DC 200	2.200	1.594	P > 0.05	-3.384 to 7.784
3	WATER vs DC 400	6.200	4.492	P < 0.05	0.6156 to 11.78
4	LOP vs DC 200	-11.00	7.970	P < 0.001	-16.58 to -5.416
5	LOP vs DC 400	-7.000	5.072	P < 0.05	-12.58 to -1.416
6	DC 200 vs DC 400	4.000	2.898	P > 0.05	-1.584 to 9.584



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