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Research Paper

ANTIBACTERIAL AND ANTIDIARRHOEAL ACTIVITIES OF DICHROSTACHYS CINEREA AGAINST SOME ENTERIC PATHOGENS

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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 ap- preciable antibacterial and antidiarrhoeal activities.

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is *Dichros- tachys cinerea* .*cinerea* and the Latin word:*konis* word: 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, ex- tract is said to be an effective *Dichrostachys cinerea* 2004). antidiarrhoeal plant commonly used by Mwaghavul tribe in mangu, hence the need to scientifically validate the use of this plant as a medicinal plant.

SDHOTMATERIALS AND ME

ollection and Authentication of PlantC

were collected from Sherry Hills area of Jos *D. cinerea* The leaves of 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.

traction ProceduresxE

D. Extraction of the active ingredient from the plant sample of were done using different principal solvents for the *cinerea* 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 100C.For the aqueous extract, a 50g of was put in a conical flask and 500mls *D. Cinerea* powdered leaves of 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 ex- tract 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 preweighed sterile sample container.

مقدمة

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 generatio verbally or in writing (Sofowora, 1993). Diarrhoea is one of the clinical findings in gastrointestinal 2004). According to NNDIC (2003), *et al.,* infections (Jawetz, 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 *Shigella dysenteriae* ,*Escherichia coli* Typhi,*Salmonella* includes: , among others (Garner, *Vibro cholerae* ,*Campylobacter jejuni*, 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 2005). Disease and *et al.,* leading cause of infant mortality (Bryce death caused by diarrhoea is a global problem, but it is especially 2003). Furthermore, *et al.,* prevalent in developing countries (Kosek adults are also affected and need special attention in treatment and management, especially in acute and long –term care residents, because of their multiple comorbidities, immunosenescence, frailty, and poor nutritional status (Trinh and Prabhakar, 2007).Several in the treatment *Dichrostacys cinera* studies have reported the uses of *hrostachys cDi*-of many diseases and ailments. The bark of has been used by the local tribes of Mayurbhanji district of *cinerea* Odisha for the treatment of diabetes mellitus. In addition, the plant is *et al.,* used in veterinary medicine in India (Handa are traditionally used as antimicrobial, *ichrostachys cinerea D*2003). 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 2013).The *et al.,* conditions, arthritis, pile and eczema (Swetha generic name Dichrostachys means coloured spike and cinerea refers to the greyish hair of the typical sub-species obtained from the Greek

, was given Loperamide (10mg/kg body weight orally **roup IVG** 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.

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In the result below, table 1 shows the result of the phytochemical Ethanolic and petroleum *D. cinerea.* screening of the leave extract of 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 *Shigella* Typhi and*Samonella* petroleum ether extracts for both whose MIC's were at 25 mg/ml.In addition, figures 1, 2 *dysenteriae* 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 at all, but was *Escherichia coli* and*Shigella dysenteriae* against Typhi except at 12.5 mg/ml and 6.25 *Samonella* effective against Typhi was not susceptible to all the *Samonella* mg/ml. However, 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 standard antidiarrhoeal drug while 200 mg/ml ($P > 0.05$) *D. cinerea* water is not a treatment.Water vs means that there is no significant difference between the two 200 mg/ml is not more effective than *D. cinerea* treatments i.e. 400 mg/ml ($P < 0.05$) means there was a *D. cinerea* water.Water vs 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 200 mg/ml *D. Cinerea* with increasing concentration.Loperamide vs 400 mg/ml (all P-values less than 0.05)*D. Cinerea* and Loperamide vs means that loperamide was more active than both doses of the extract.

400 mg/ml ($P > 05$) *D. Cinerea* 200mg/ml vs*inerea c. D* .1 means there is no significant difference between the two doses.

NOISSISCUD

Dichrostachys The results of this study indicates that the extracts of has an appreciable antibacterial activity as was earlier *cinerea* indicated. All the test organisms used showed a marked degree of at 100 mg/ml *Dichrostachys cinerea* susceptibility to the extracts of and 50 mg/ml. This shows that its activity is dose dependent, thus (2003) report which *et al.* showing concordance with Okpekon elucidated the relationship between the antimicrobial activities of medicinal plants (e.g. plants with chemotherapeutic actions) with *Dichrostachys* concentration.Extracts from the leaves of Typhi, *Escherichia coli, Salmonella* inhibited the growth of*cinerea* at most of the concentrations used for the *Shigella dysenteriae* and Typhi *Salmonella* susceptibility test though at different degrees. were the most susceptible to the aqueous extract *Escherichia coli* and

Preparation of Plant Extracts Concentrations

One gram of each aqueous, ethanol and petroleum ther extracts preprepared (each separately) were taken and the aqueous ex- tract 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.

ources of Test OrganismsS

The test organisms were gotten from the bacteriology laboratory of National Veterinary Research Institute, Vom, as purified stock *Escherchia coli,* cultures. The organisms are as follows: using a standard operating , *Shigella dysenteriae,* Typhi*Salmonella* 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 (106 CFU/ml) was obtained (Cheesbrough, 2006).

ntibacterial AssayA

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 37oC, under aerobic conditions for 24 hours.

(MIC)f Minimum Inhibitory Concentrationsoermination tDe

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).

Dichrostach- ys* Phytochemical Screening of the Leaf Extract of *cinerea

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

cute toxicity studyA

The acute oral toxicity study was carried out as per the 423 guideline set by Organization for Economic Cooperation and Development (EOCD). The extracts were administered at the dose level of 2000 mg/kg. One tenth of the median lethal dose (LD50) 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.

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

were given 2ml/100g distilled water – Negative control.**roup III G**

while the three (3) test organisms were all susceptible to the ethanolic Typhi was the only organism susceptible to *Salmonella* extract. But 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 extract at 400 mg/ml as compared to the *Dichros- tachys cinerea* of same extract at a concentration of 200 mg/ml. This indicates that the antidiarrhoeal activity of the extract increases in a dose-dependent manner

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استنتاج

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 prepa- ration 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.

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