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PHYTOCHEMICAL INVESTIGATION AND ANTIDIARRHOEAL ACTIVITY OF VARIOUS LEAF EXTRACTS OF *DALBERGIA SISSOO* (ROXB.) IN EXPERIMENTALLY INDUCED DIARRHOEA IN MICE

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ABSTRACT

Diarrhoea has long been recognized as one of the most important health problems in the developing countries. About 80% of people in developing countries depend on traditional systems of medicine for primary health care. *Dalbergia sissoo* leaf has been used traditionally for the treatment of eye ailments, sore throats, heart problems, dysentery, syphilis and gonorrhea Therefore, the present study was undertaken to evaluate the antidiarrhoeal potential of *D.sissoo* leave extracts in castor oil induced diarrhea model. For this Swiss albino mice of either sex were used. all the crude extracts of *D.sissoo* leaf such as ethanol, ethyl acetate, n-butanol and petroleum ether were tested for antidiarrhoeal activity at 200 and 400 mg/kg body weight. Where as loperamide (3 mg/kg) were used as standard drugs and given orally. The pet. ether extract of *D.sissoo* leave was found to be effective in a dose dependent manner against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body weight.

KEYWORDS: *Dalbergia sissoo,* antidiarrhoeal, n-butanol.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries. According to world health organization, it is the one of the most common cause of morbidity and mortality in many developing countries effecting mainly the infants and children's.[1] WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.[2] it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. The tribal areas of

Baipariguda, Koraput (District) of Eastern Orissa. due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *Dalbergia sissoo* (family: fabaceae.) also known as sisu (Oriya) is frecuntly distributed. And extensively used traditionally by the tribal people. The plant species are found generally in many tropical areas of the globe, particularly Africa, Asia, central and southern America where they are used to manage a number of ailments.[3,4,5] Some *Dalbergia* species have been investigated and found to possess antimicrobial, antioxidant, anti-inflammatory and ani-diarrhoela ctivities.[6,7,8,9] Traditionally Different parts such as roots, bark, wood, leaves and seeds are being used as remedy in many diseases including skin diseases, blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, aphrodisiac, expectorant. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis and gonorrhea. In India and Nepal rural people use *Dalbergia sissoo* leaves to treat animals suffering from non-specific diarrhea.[10] Chemically leves contains sissotrin and an isoflavon-O-glycoside all so reported.[11]

MATERIAL AND METHODS

Drugs and chemicals

Loperamide was procured as gift sample from Provizer Pharma, Surat, Gujarat, India The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C, petroleum ether AR 40-60°C, Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

Collection of Plant Material

The leaves of *Dalbergia sissoo* were collected from the tribal belts of the local area of Patrapur of Koraput district. (India) in the month of October 2015. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-207/15, dated 10.5.2015). After authentification leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1000 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.[12,13,14]

Phytochemical investigation

The crude ethanol, ethyl acetat, n-butanol and petroleum ether extracts of the leave of *Dalbergia sissoo* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis.[13,14]

Evaluation of Antidiarrhoea Activity

Animals

Healthy albino mice of Swiss strain of either sex were used. They were housed in standard conditions of temperature (25±2°C), 12 hours light per day cycle, relative humidity of 45-55% in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition.

Drugs

Loperamide (3 mg/kg) and a dose of 200 & 400 mg/kg of different *D. sissoo* leave extracts used for activity study and the route of administration for both standard and test drug was orally.

Experimental protocol

Animals were selected, weighed (25-30 g) and devided in to ten groups (n=3), namely control group, standard group and eight groups belonging to four different leave extracts of *D. sissoo.* All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar, Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies

The acute toxicity was performed according to OECD 423, 2001. The selected female albino rats were used to determine the dose. The animals were divided into twelve groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the different leave extracts of *Dalbergia sissoo* and administered orally as following doses of 100, 300,600,1000 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively. Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *Dalbergia sissoo* leaves extract would be non-toxic in living body.[15,16]

Castor oil induced diarrhea

The method described by Shoba and Thomas was followed for this study with slight modification. The animals were all screened initially by giving 0.5 ml of castor oil one week before the actual experiment. Only those showing diarrhoea were selected for the final experiment. thirty mice fasted for 24 h were randomly allocated to five groups of five animals each. Group I (received 1% tween 80 at a dose of 10 ml/kg) served as control group, Group II received the standard drug loperamide 3 mg/kg, p.o. Group III to X received the different root extracts of *D.sissoo* at the doses of 200 and 400 mg/kg p.o., respectively. One hour after administration, all animals received 0.5 ml of castor oil and then they were individually place in cages the floor of which was lined with transparent paper. During an observation period of 4 h, the time of onset of diarrhoea, the total number of faecal output (frequency of defecation) and weight of faeces excreted by the animals were recorded.[17]

Statistical analysis

The data are represented as mean ± SEM and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where $P < 0.05$ was considered statistically significant.[18]

Table no. 1: Phytochemical screening for the different solvent extracts of *Dalbergia sissoo leave.*

Extracts	Phytochemicals						
	Cadiac glycoside	Flavonoids	Steroids	Terpinoids	Tannins	Saponins	Phenols
Ethanol	+++	--	++	++	+++	_	++
Ethyl-acetate	+	--	-	+	+	_	+
n-butanol	++	--	++	+	++	_	++
Petroleum ether	++	--	++	+	+	--	+

+++, strong; ++, moderately; +, poor presence, --, absence.

Table no. 2: Effect of *Dalbergia sissoo leave* extracts on castor oil induced diarrhea in mice.

Group	Treatment Dose(mg/Kg)	Time of onset of diarrhoea(min.)	Total number of faeces in 4h(frequency of defecation in 4 h)	% Inhibition of defecation	Weight of stool(g)
Control	----------	91 ± 12.2	8.2 ± 1.3	---------	0.72 ± 0.06
Loperamide (standard)	3	223.7 ± 6.0	1±0.2	81.73	0.04 ± 0.02
Ethanol extract	200	132.4±8	4±0.7	42.30	0.28±0.023
	400	187.3±14.2	6±0.4	27.21	0.16±0.012
Ethyl acetate extract	200	127.3±17.6	4.7±0.6	42.37	0.21±0.014
	400	152.6±12.5	8±0.6	53.37	0.31±0.018
n-butanol extract	200	137.2±12	3.9±0.4	31.42	0.22±0.031
	400	181.4 ±18	5.7±0.3	33.31	0.23±0.021
Pet. ether extract	200	172 ± 14	3.4 ± 0.8	57.67	0.21 ± 0.02
	400	213 ± 11.3	1.2 ± 0.3	73.88	0.02 ± 0.06

The data are represented as mean ± SEM, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey post test where P < 0.05.

RESULT AND DISCUSSION

The preliminary phytochemical screening showed that the different solvent extracts of *D.sissoo* leave contain the glycosides, steroids, terpenoids, phenols and tannins were present in all the solvent extract, These constituents may be responsible for the *in vivo* anti-diarrhoea activity of *D.sissoo* leave which showed in [Table1]. Anti-diarrhoea activity was found in plants possessing tannins, alkaloids, saponins, flavonoids, steroids and terpenoids.[19,20] The pet. ether extract of leave of *D.sissoo* was found to be effective in a dose dependent manner against castor oil induced diarrhoea on experimental mice at the dose of 400 mg/kg body

weight, At the same dose, the extract showed significant antidiarrhoeal activity showing 73.88% (pet. ether) extract reduction in diarrhoea comparable to that of the standard drug loperamide that showed 81.73% reduction in diarrhea. Which showed in (Table 2). Tannins present in anti-diarrhoea plants denature proteins in the intestinal mucosa by forming protein tannates which may reduce secretion. Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation).[21] The activity showed by this extract is of considerable importance and justified its use in the diarrhea treatment.

CONCLUSION

Among all the extracts pet. ether extract showed dose dependant & significant anti-diarrhoea activity as compared to reference drug loperamide. The folklore claim of leave of *D.sissoo* used as an anti-diarrhoeal have been confirmed. Further studies to isolate and reveal the active compound present in the crude extracts of *D.sissoo* leave and to establish the MOA of anti-diarrhoeal activity.

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