Phytochemical analysis and laxative activity of the stem bark of baccaurea ramiflora lour.

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Abstract

As per the Indian traditional system of medicine, Baccaurea ramiflora stem bark is used for the treatment of constipation. However, no studies have explored the laxative effects of aqueous extract (juice) of baccaurea ramiflora stem bark which may be due to the lack of availability of this indigenous wild edible plant. The present study was undertaken to investigate the phytochemical constituents and the laxative activity of the stem bark of baccaurea ramiflora. Fresh stem barks of baccaurea ramiflora were collected, dried, extracted with water and subjected to phytochemical screening to investigate the primary and secondary metabolites viz. carbohydrates, alkaloids, glycosides, tannins, phenols, terpenoids, flavonoids, proteins, fixed oils, gums and mucilage. The aqueous extract of the stem bark of baccaurea ramiflora at doses (200 and 400 mg/kg, p.o) were screened for the laxative activity. The laxative activity was determined by the weight of faeces matter at 8th and 16th h. The results were compared with standard Senna extract. Phytochemical investigation revealed the presence of glycoside, phytosterols, terpenoids, carbohydrate, gums and mucilage in the stem bark. Aqueous extract of the stem bark of baccaurea ramiflora showed significant laxative activity. The laxative property can be due to the phytochemicals present in the aqueous extract. Phytochemicals reported gave information regarding the various active constituents present in the drug. The laxative property can be due to the phytochemicals present in the aqueous extract of the stem bark. These findings showed that oral administration of the aqueous extract of the stem bark of baccaurea ramiflora produce a significant increase in fecal output in wistar rats and stimulation of gastrointestinal motility. Moreover, isolation of the active constituent is required to identify the compound responsible for laxative activity.

Keywords: Baccaurea ramiflora, Bark, Euphorbiaceae, Laxative activity, Phytochemical analysis.

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Introduction

One of the most common gastrointestinal complaints globally is constipation. It is an ailment of bowels when defection of faeces is difficult due to slow passage of digesting food through the intestine. It affects around 20 % of the general population [1]. Defecation is an important part of everyday life in which individuals excrete waste from ingested food. Adults, elderly, woman during pregnancy, after giving birth, after surgical treatment, after taking medications usually suffers from constipation. To treat patients with constipation, herbal laxative drugs are given which are safer on the body as compared to synthetic drugs. These drugs are taken by oral route or in the form of suppository. Senna, Aloe vera, Isapghul are few well known herbal drugs used to treat constipation.

Traditional medicine or Indigenous medicine consists of information that developed before the era of modern medicine and is passed on from one generation to another generation. Southeast countries like Asia and Africa, 80 % of total population depends on indigenous medicine for their wellbeing. Meghalaya is the rainiest state of India [2]. The maximum temperature in this region barely goes beyond 28°C, whereas winters temperatures are below zero degrees. Meghalaya has unique vegetation which is due to the abundant rainfall, diverse topography, different climatic and edaphic conditions across the state. Due to absence of modern medicinal facilities, many of the medicinal plants in this region is botanically unexplored or under explored to the world. One of such plant is Baccaurea ramiflora Lour.

Baccaurea ramiflora Lour., belongs to the family Euphorbiaceae. It is an evergreen, dioecious tree which is native to countries of Southeast Asia. In India, it grows at an altitude of 900 m as cultivated or wild plants in sub-Himalayan region. It is known by several vernacular names such as Burmese grape (English), Kataphal (Hindi), Leteku (Assam), Bhubi and Latka (Bengali). It blossoms during April to May [3]. The fruits ripen during the rainy seasons. Cultivation of trees is carried out for its edible fruits whose shape varies from round to oval. The fruit turns yellowish brown after ripening. Fresh fruit yields more pectin than the stored ones and are used in preparation of jellies and wine. The pulp, peel and seeds of Baccaurea ramiflora fruit is used as an antioxidant. Leaves yields green dye which is used for coloring. The juice of the bark is used in wide range of ailments, including constipation

as per the Indian Traditional medicine. The bark contains friedelin, epi-friedelanol, β-sitosterol, methyl-betulinate and an unknown ester. Studies showed that the stem bark has antimicrobial, cytotoxic and antioxidant activities [4]. Traditionally, Baccaurea ramiflora plant is used for the treatment of pain in rheumatoid arthritis and injuries. The leaves contain phenolic compound and has antioxidant properties, hypolipidemic and hypoglycemic activities, which is possibly due to the presence of tannins, flavonoids, terpenes and steroids. The whole plant of Baccaurea ramiflora is used to cure stomach ulcer, stomach ache and colic. Seeds contain 4.8 to 6 % of annatto dye (orange-red) which is used for coloring of cotton and silk fabric. Seed oil contains saturated fatty acids (palmitic acid 33.67 %, stearic acid 19.38 %, arachidic acid 9.69 %, oleic acid 24.48 and total fatty acids 60 %), unsaturated fatty acids 12.75 % and trans-11-eicosenoic acid. Seeds are crushed to cure diarrhoea. Fruits of Baccaurea ramiflora was reported to possess antioxidant and antiviral activity and diuretic activity was observed in the stem bark [5].

The objective of this research work was to investigate the laxative activity of Baccaurea ramiflora stem bark and to provide scientific proof for its traditional use. However, no studies have explored the laxative effects of aqueous extract (juice) of Baccaurea ramiflora stem bark (Figure 1(A,B)).

Figure 1. (*A) Outer and (B) inner surface of the stem bark of baccaurea ramiflora*.

Materials and Methods

Drugs and chemicals

All the chemicals and solvents used for extraction and phytochemical study were of analytical grade purchased from S. D fine chemicals Pvt. Ltd. Mumbai, India [6].

Collection of plant materials

The fresh stem barks of Baccaurea ramiflora Lour. (Euphorbiaceae) were collected in bulk from the Experimental Botanic Garden, Barapani under Botanical Survey of India, Shillong, in the month of July, and was authenticated by the Botanist -E, Botanical Survey of India, Eastern Regional Centre, Shillong, India. The voucher specimen was preserved for future reference [7,8]. The herbarium was deposited in S. K Patel College of Pharmaceutical Education and Research, Department of Pharmacognosy, Ganpat University, India. The stem barks were shade dried separately at room temperature (30 – 35°C) for 10 days, coarsely powdered and stored in air tight containers for further studies.

Extraction of the plant materials

The powdered crude drug was taken and subjected to successive solvent extraction using Soxhlet apparatus. The extraction were carried out at room temperature 30-35°C with the solvents in the increasing order of the polarity i.e. petroleum ether (60-80°C), chloroform, acetone, methanol and aqueous extracts [9]. All the extracts were collected separately. The extracts were concentrated using rotary evaporator under reduced pressure. The extracts were then weighed and the percentage yields (w/w) were calculated and stored in a refrigerator at 4°C, until it is used for phytochemical screening and biological testing.

Preliminary phytochemical screening

The powdered crude drug was subjected to phytochemical screening to investigate the chemical constituents present in different extracts of Baccaurea ramiflora leaves by using standard procedures.

Animals

Albino wistar rats of either sex, weighing 150-200 g were obtained from the Central Animal facility, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Gujarat, India. All the animals were housed at an ambient temperature 22 ± 1°C and relative humidity 55 ± 5 and 12:12 h light-dark cycle [10]. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. The present study was performed in accordance with the guideline provided by the CPCSEA (Ministry of fisheries, Animal husbandry and Dairying, Government of India) and the protocol was approved by Institutional Animal Ethics Committee (IAEC) of S. K. Patel College of Pharmaceutical Education and Research, Gujarat, India.

Acute toxicity

Acute Oral Toxicity (AOT) of aqueous extract of Baccaurea ramiflora stem bark was determined as per OECD (Organization for Economic Cooperation and Development) guideline 425. The LD50 of the test extract was calculated using 'AOT 425' software provided by Environmental Protection Agency, USA. Healthy albino wistar rats of either sex weighing 150-200 g were used for the study. The two doses of 2000 mg/kg and 5000 mg/kg of the test samples were given orally to two groups with five rats in each group. The mortality and general behavior of treated groups were monitored for 14 days. The aqueous extract of the stem bark was devoid of any toxicity in rats when given in doses up to 5000 mg/kg by an oral route [11]. Hence, for further studies 200 and 400 mg/kg doses of the extracts were used.

Evaluation of laxative activity

The test was performed on rats of either sex, fasted for 12 h before the experiment, but with water provided ad libitum. The animals were divided into four groups of six animals in each

group. The first group of animals served as normal control and received distilled water 25 ml/kg orally, the second group served as reference and received aqueous extract of Senna at 30 mg/kg orally, while the third and fourth group received the test extract at dose 200 mg/kg and 400 mg/kg orally, respectively. Immediately after administration of dose, animals were housed separately in polypropylene cages suitable for collection of faeces [12]. After 8 h and 16 h of drug administration, the faeces were collected and weighed. Subsequently, food and water were given to all the animals.

Statistical analysis

The results were expressed as mean ± S.E.M for the number of animals in each group (n=6), where 'n' represents the number of rats. Statistical difference between two means determined by ANOVA for repeated measurements by using statistical computer software Graph pad Prism 5.0. Only the mean values showing statistical difference $P \leq 0.001$ were considered as statistically significant [13].

Results

Preliminary phytochemical screening

The preliminary phytochemical screening of petroleum ether, chloroform, acetone, methanol and aqueous extracts of the stem bark of Baccaurea ramiflora showed the presence of phytosterols, carbohydrate, gums and mucilage (Figure 2).

Acute toxicity studies

The acute toxicity studies showed the normal behavior of the treated rats. No toxic effects were observed at higher dose of 5000 mg/kg body weight. Hence there were no lethal effects, which indicated that it may have a reasonable safety margin with regards to acute toxicity [14] (Figure 3).

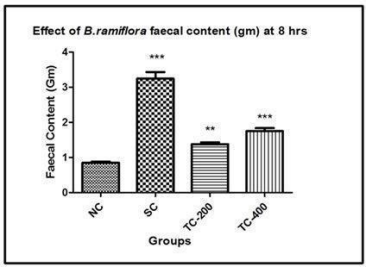
Laxative activity

Treatment with aqueous extract of the stem barks of Baccaurea ramiflora, orally given to rats showed a significant laxative effect when compared with standard Senna. The dose dependent response was observed at 200 and 400 mg/kg. The faecal output weight was increased as the dose increases at 8th h (Table 1).

Table 1. *Laxative activity of aqueous extracts of Baccaurearamiflora in rats.*

Treatment	Dose	Fecal output (mg)	
	mg/kg, p.o.	8 h	8-16 h
Control	-	0.853 ± 0.029	1.323 ± 0.106
Senna	30	3.250 ± 0.182***	4.217± 0.147***
Aqueous extract of Baccaurea ramiflora	200	1.383 ± 0.047**	2.183 ± 0.107**
Aqueous extract of Baccaurea ramiflora	400	1.750 ± 0.084***	2.767 ± 0.247***

Mean ± S.E.M. (n=6), **P ≤ 0.01, ***P ≤ 0.001 vs Control (Normal Saline)



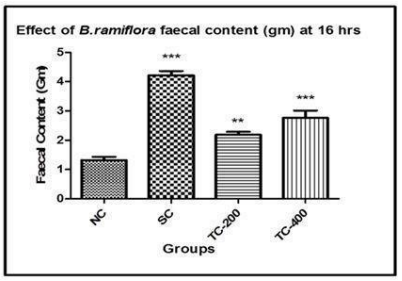
Mean ± S.E.M. (n=6), **P ≤ 0.01, ***P ≤ 0.001 vs Control (Normal Saline).

NC–Normal Control SC–

Standard Control TC–Test

Concentration

Figure 2. *Laxative activity of aqueous extracts of baccaurea ramiflora stem bark at 8 h.*



Mean ± S.E.M. (n=6), **P ≤ 0.01, ***P ≤ 0.001 vs Control (Normal Saline).

NC–Normal Control SC–

Standard Control TC–Test

Concentration

Figure 3. *Laxative activity of aqueous extracts of baccaurea ramiflora stem bark at 16 h.*

Discussion

Constipation is a condition of bowels when defection of faeces is difficult due to slow passage of digesting food through the intestine. It affects 5 to 20% of the general population. Adults at the age of 65 and above, pregnant woman, after childbirth or surgical treatment, after taking medicines usually suffers from constipation. To treat patients with constipation, herbal laxative or purgative drugs are given which are safer on the body as compared to synthetic drugs. The laxative or purgative drugs are taken by oral route or in the form of suppository. The present investigation has demonstrated that aqueous extract of the stem bark of Baccaurea ramiflora has laxative activity which was compared with Senna, a standard laxative drug [15]. The laxative property may be due to the compounds present in

the aqueous extract. This beneficial outcome of Baccaurea ramiflora stem bark showed that oral administration of the aqueous extract produce a significant increase in fecal output in wistar rats and stimulation of gastrointestinal motility.

Conclusion

The results obtained from the experiment confirmed that the aqueous extract of the stem bark of Baccaurea ramiflora possess laxative activity. Consumption of the aqueous extract (juice) of the stem bark could offer health benefits by increasing the fecal output and stimulation of gastrointestinal motility. However, further studies are needed for the isolation, characterization and biological evaluation of the active principle(s) of Baccaurea ramiflora extract.

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