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Original Research Article

# Preliminary Phytochemical Analysis and Oral Acute Toxicity Study of the Leaves of *Baccaurea ramiflora and Microcos paniculata*

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**Abstract:** The current study was planned to evaluate the phytochemical analysis and oral acute toxicity study of the leaves of *Baccaurea ramiflora* and *Microcos paniculata*. The soxhlated extraction and standard methods were used for extractions and phytochemical analysis. The drug was administration orally, animal's observed from cage side observations and mean body weight was taken for 14 days. There phytochemical analysis of ethanolic extract of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* revealed the presence of alkaloids, carbohydrates, glycosides, tannins, phytosterols, saponins and flavonoids. The oral acute toxicity study showed no noticeable clinical signs of toxicity and mortality during 14 days of the study period. No statistically significant alterations in body weights, compared to control group. We conclude that the outcome of the present study may add scientific information of ethanolic, chloroform extracts and acute toxicity study of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* to the system of medical specialization. Ethanolic extract of leaves of *Baccaurea ramiflora* and *Microcos paniculata* was found to be nontoxic up to 5000 mg/kg body weight, so the LD50 is greater than 5000 mg/kg body weight. **Keywords:** Phytochemicals, Toxicity study, *Baccaurea ramiflora* and *Microcos paniculata* leaves

# INTRODUCTION

For centuries, medicinal plants are the basis for the treatment of various diseases. Nearly 80% of people living in developing countries still depend on plantbased traditional medicine for their primary health care and almost three fourths of the herbal drugs used worldwide are derived from medicinal plants. However, the quality control of herbal medicine remains a challenge owing to the fact that there is a high variability in the active constituents involved. Hence, World Health Organization (WHO) has approved fingerprint technique or standardized extract for quality assurance of herbal medicines. In recent years, phytochemicals are increasingly purported to exert potent beneficial actions to support health and may play a role in reducing synthetic drug use for the treatment of metabolic complications. To this effect, research has focused on the identification and isolation of compounds from natural products. Many plants synthesize compounds that are useful for the maintenance of health in humans and animals, like aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many of these compounds extracted from natural products could be useful lead compound in the production of drugs [1].

Herbal medicines are popular and extensively used in the developing world. In Nigeria, they offer a more wide available and affordable alternative to pharmaceutical drugs and natural food supplements. There are over a hundred chemical substances that have been derived from plants for drugs and medicines [2]. However, many plants have also been reported to be toxic to both human and animals. It should therefore, be emphasized that the traditional use of any plant for medicinal purposes, by no means, warrants the safety of such plant. Plants in folk medicine should therefore, be evaluated for safety or toxicity and necessary recommendations made on their use. The data of the acute and sub-chronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in its safety to humans, particularly for use in the development of pharmaceuticals [3].

*Baccaurea ramiflora* (family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Penninsular Malaysia, Tibet and Andaman islands. It is an evergreen tree reaching a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diameter with leathery pericarp, three seeded arillus embedded in pinkish white pulp. The leaf is simple, alternately arranged, with petiole [4]. It is ovate to ovate lanceolate in shape and  $10-20 \times 4-9$ cm in size. The petiole is 1-8 cm long with lanceolated and fimbriated stipules. It grows in evergreen forests on a wide range of soils. The common names include Latkan or Bhubi (Bengali), Letuk (Assamese), Leteku (Hindi), Mafai (Thai) and Burmese grape (English). It is used medicinally to treat skin diseases. The whole plant of B.ramiflora is utilized as an antiphlogistic and anodyne against rheumatoid arthritis, cellulitis, and abscesses and to treat injuries. Young leaves of B. ramiflora are used as vegetable, flavoring agent with curries, fresh bark is chewed or juice is used orally for constipation [5].

Microcos paniculata (family: Euphorbiaceae) is a shrub that is abundant in secondary forests and also grown as hedges. Microcos is tall semi-deciduous tree, sometimes shrubby. Leaves 10-15 cm long, ellipticoblong, acuminate, entire or slightly and irregularly toothed [6, 7]. Flowers small, yellow, in terminal panicles. Fruits globose or slightly obovoid, about 10 mm across. The plant is used in indigestion, eczema, itch, small-pox, typhoid fever, dysentery and syphilitic ulceration of the mouth. Tripuras in Chittagong Hill Tracts use leaves of this plant along with turmeric and shell of snail for the treatment of jaundice. A decoction of the roots is used to treat coughs. A drink prepared from the roasted and boiled leaves is given to children as a vermifuge [8]. It has been used traditionally to prepare herbal medicines and traditional teas, while a limited number of reports concerning the chemical constituents and biological activities of M. paniculata have appeared in the literature. Previous phytochemical investigations revealed in M. paniculata the presence of flavones and alkaloids and it have been reported that the water extract of M. paniculata not only has significant analgesic effects, but that it also exerts beneficial pharmaceutical and preventative effects for coronary heart disease and angina pectoris [9-13]. The chloroform and ethanol-extract of M. paniculata could also act as a pesticide. Based on previous research, it is evident that M. paniculata which contains a variety of active components has diverse pharmacological and dietary values [14, 15].

## MATERIALS AND METHODS Collection of plant materials

The leaves of *Baccaurea ramiflora* and *Microcos paniculata* belonging to family Euphorbiaceae were collected from local market of Belonia, Tripura, India during May – July and authenticated (ID No. is BOT/HEB/AC23072011 and BOT/HEB/AC23072512) by Dr. B. K. Datta, Professor

of Botany, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura, India.

### **Preparation of Extracts**

After collection of the plants, the leaves of both the plants were rinsed thoroughly in tap water and dried in shade for about 20 days under controlled temperature  $(25 \pm 2 \,^{\circ}\text{C})$ . Then the crude material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage. Coarsely powdered and dried leaves were successively soxhlated using petroleum ether, chloroform, ethanol and water for 72h. The extracts were filtered and the solvents were evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C to 45 °C. The leaves extract was subjected to phytochemical evaluation.

# Phytochemical analysis

The phytochemical analysis of alkaloids, carbohydrates, glycosides, flavonoids, phytosterols, tannins, saponins, proteins and mucilages were performed according to standard methods [16, 17].

# 1. Test for glycosides

A portion of the extract was hydrolysed with HCl and the hydrolysate was subjected to Legal's and Borntrager's test to detect the prescence of different glycosides.

## a) Legal's test

To the extract, 1ml of pyridine and few drops of sodium nitroprusside were added and it was made alkaline with NaOH. Appearance of pink to red colour shows the presence of glycosides.

## b) Borntrager's test

Extract was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink colour showing the presence of glycosides.

## 2. Test for saponins

#### a) Froth test

Place 2ml of extract in water in a test tube. Shake well, stable froth (Foam) is formed. Hence saponins are present.

## 3. Test for alkaloids

a) Draggendorf reagent

Drug extract when treated with potassium bismuth iodide solution gives reddish brown ppt. Hence alkaloids are present.

#### b) Mayer's reagent

Drug extract when treated with potassium mercuric iodide solution gives cream colour ppt. Hence alkaloids are Present.

# 4. Test for tannins

To the extract few ml of chromic acid was added. No ppt was found. Hence tannins are present.

# 5. Test for flavonoids

#### a) Shinoda test

To the extract add few magnesium turnings and conc. HCl drop wise. Pink scarlet, crimson red or occasionally green to blue colour appears after few minutes. Hence flavonoids are present.

## b) Zinc hydrochloride test

To the extract add a mixture of Zn dust and conc. HCl. gives red colour after few minutes. Hence flavonoids are present.

# 6. Test for mucilage

To the extract ruthenium red solution is added, pink colour is obtained. Hence mucilage is present.

# 7. Test for carbohydrates

#### a) Molisch test

To the extract add few drops of alcoholic  $\alpha$ -naphthol, and then add few drops of con. H2SO4 through sides of the test tube, violet colour ring is appeared at the junction. Hence Carbohydrates are present.

# 8. Test for proteins

a) Xanthoprotein test

To 5ml of extract add 1ml of conc. nitric acid and boil. Yellow ppt was obtained. After cooling add 40% NaOH solution. Orange colour was obtained. Hence proteins are present.

# 9. Test for phytosterols

## a) Salkowski test

To the extract add few drops of conc. H2SO4, red colour at the lower layer indicate the presence of sterol and yellow colour indicates the presence of phytosterols.

## Animals

Fifteen adult female Wistar rats of age around six months and weighing close to 200-210 g were used for the experimental study. The rats were acquired Inhouse from Sainath Agencies, Hyderabad, and Telangana, India. The rats were acclimatized to the laboratory conditions for a week before the start of the experiments; they were maintained as per the Institutional ethical committee (IAEC) norms. The rats were maintained with 12 hour dark and light cycle with food and water at ad libitum. The study procedures involving the treatment and handling of animals were approved by the Institutional ethical committee Regd. No. 1662/PO/Re/S/12/CPCSEA.

# Acute toxicity study

Acute toxicity study was carried out as per OECD guidelines- 425. At aggregate of twenty five rats were randomly divided into five groups, each containing five rats. The control group (C) was given normal standard diet. The four treated groups were given oral administration of a single dose of ethanolic extract of Baccaurea ramiflora 2000 mg/kg (EEBR2) and 5000 mg/kg body weight respectively (EEBR5) and also Microcos paniculata 2000mg/kg (EEMP2) and 5000 mg/kg body weight respectively (EEMP5) The oral administration was managed by utilizing a curved ball tipped intubation needle affixed to a 2 ml syringe. Immediately after administration the animal's behavior, toxic signs and mortality were continuously observed for the first thirty minutes and periodically at hourly intervals for during the first twenty four hours, special attention given during the first four hours, and daily thereafter for a total of 14 days.

# Cage side examination

The cage side examinations included changes in behaviour pattern, gait, and skin, condition of fur, tremors, convulsions, and eyes for dullness, eyeopacities, pupil diameter, ptosis, colour and consistency of faeces, diarrhoea, salivation and breathing abnormalities [18, 19].

# Body weight

The body weight of all control and treated rats was recorded on day zero (before 1st dose), day seven and day fourteen.

# RESULTS

## Preliminary phytochemicals

The preliminary phytochemical screening of petroleum ether and chloroform extracts of *Baccaurea ramiflora* leaves revealed the presence of alkaloids, glycosides, carbohydrates, tannins, phytosterols and flavonoids. Ethanol extract revealed the presence of proteins and saponins. Aqueous extract also revealed the presence of saponins and proteins (**Table 1**).

The preliminary phytochemical screening of petroleum ether and chloroform extracts of *Microcos paniculata* leaves revealed the presence of alkaloids, glycosides, carbohydrates, tannins, phytosterols and flavonoids. Ethanol extract revealed the presence of proteins and saponins. Aqueous extract revealed the presence of saponins (**Table 2**).

## Acute toxicity study

The acute toxic study results of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* showed no noticeable signs of acute toxicity and lack of death at all doses up to 5000 mg/kg body weight.

### Cage side observation

The study of behavior of treated rats carried out by general inspection of each animal on a day- today basis from the 0 days of study to the 14th day of the study at all dose levels showed no significant changes between control and treated groups (Table 3).

#### **Body weight**

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The body weight of all treated group rats was slightly diminished when compared with control, but the reduction was not statistically significant (Table 4).

Phytochemicals	Alk	Carb	Gly	Tan	Phytos	Flav	Sapo	Pro	Muci
Pet. ether	+	+	+	+	+	+	-	-	-
Chloroform	+	+	+	+	+	+	-	-	-
Ethanol	+	+	+	+	+	+	+	+	-
Aqueous	+	+	+	+	+	+	+	+	-

### Table 1: Phytochemical constituents of the leaves of Baccaurea ramiflora

Table 2: Phytochemical constituents of the leaves of Microcos paniculata										
emicals	Alk	Carb	Gly	Tan	Phytos	Flav	Sapo	Pro	Ι	
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Phytochemicals	Alk	Carb	Gly	Tan	Phytos	Flav	Sapo	Pro	Muci
Pet. ether	+	+	+	+	+	+	-	-	-
Chloroform	+	+	+	+	+	+	-	-	-
Ethanol	+	+	+	+	+	+	+	+	-
Aqueous	+	+	+	+	+	+	+	-	-
*									

The above table indicates the presence (+) or absence (-) of phytochemicals in petroleum ether, chloroform, ethanol and aqueous extract (Alk: Alkaloids, Carb: Carbohydrates, Gly: Glycosides, Tan: Tanins, Phytos: Phytosterols, Flav: Flavonoids, Sapo: Saponins, Pro: Proteins, Muci: Mucilages).

Parameter	Cage side observation				
	Control	Treated			
Behaviour pattern	Normal	Normal			
Gait	Normal	Normal			
Skin	Normal	Normal			
Condition of the fur	Normal	Normal			
Tremors	Nil	Nil			
Convulsions	Nil	Nil			
Eye-dullness	Nil	Nil			
Eye-opacities	Nil	Nil			
Pupil diameter	Normal	Normal			
Salivation	Normal	Normal			
Breathing abnormalities	Nil	Nil			

#### Table 3: Cage side observation control and treated animals

Table 4: Mean body weight control and treated animals in grams

Body Weight	Control	Treated					
	C (g)	EEBR2 (g)	EEBR5 (g)	EEMP2 (g)	EEMP5 (g)		
0 Day	$200.6 \pm 1.8$	$200.8 \pm 1.11$	$202.9\pm0.8$	$201.8 \pm 1.10$	$202.7 \pm 1.11$		
7 <sup>th</sup> Day	$212.4 \pm 1.7$	$209.3 \pm 1.5$	$210.3 \pm 1.2$	$211.4 \pm 1.6$	$212.5 \pm 1.4$		
14 <sup>th</sup> Day	$224.6\pm2.5$	$220.0\pm1.2$	$223.0\pm0.6$	$222.0\pm1.3$	$224.2\pm1.3$		

The data were expressed mean  $\pm$  SE, n=5. No statistical difference in body weight between control and treated. The P value is >0.05. C-control, EEBR2 - Ethanolic extract of the leaves of Baccaurea ramiflora 2000 mg/kg body weight, EEBR5 - Ethanolic extract of the leaves of Baccaurea ramiflora 5000 mg/kg body weight, EEMP2- Ethanolic extract of the leaves of Microcos paniculata 2000 mg/kg body weight and EEMP5- Ethanolic extract of the leaves of Microcos paniculata 5000 mg/kg body weight.

## DISCUSSION

Over a few decades medicinal value of plants has assumed an important dimension. The preliminary phytochemical screening investigations in plants, perhaps supportive in the recognition of the bioactive principles, may lead to the drug discovery and development. Further, these tests facilitate separation of pharmacologically active chemical compounds, their qualitative and quantitative estimation.

The preliminary phytochemical screening of ethanolic extract of the leaves of Baccaurea ramiflora and Microcos paniculata revealed the presence of alkaloids, carbohydrates, glycosides, tannins, saponins, phytosterols and flavonoids. Chloroform extract of leaves of Baccaurea ramiflora and Microcos paniculata revealed the presence of alkaloids, tannins, flavonoids and phytosterols. There were no previous reports on the phytochemicals of chloroform extract of leaves of Baccaurea ramiflora and Microcos paniculata. These outcomes suggest that the leaves of Baccaurea ramiflora and Microcos paniculata contains many secondary metabolites which are responsible for various medicinal properties and will be of great importance in phytomedicine like alkaloids which may have many pharmacological activities including analgesic, antihypertensive effects. antimalarial activity, antiarrhythmic effect and anticancer actions [20]. The tannins may have antioxidant activity, anticancer mechanisms, decrease in the risk of cardio- vascular diseases, anti-inflammatory properties and considered as anti-nutritional factors. The flavonoids may have antioxidant, antibacterial, hepatoprotective, analgesic, anti- Inflammatory, anti-ulcer, antiviral, anticancer activities, free radical scavenging capacity, coronary heart disease prevention, oestrogenic activity, antiallergic. The presence of sterols has pharmacological activity like lowering blood cholesterol and antiinflammatory.

The oral acute toxic study results indicated no significant alterations in final body weight with ethanolic extract of the leaves of *Baccaurea ramiflora* and *Microcos paniculata*. Further, during the study period of 14 days there were no noticeable signs of acute toxicity. Lack of death at all doses showed that the LD50 of ethanolic extract of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* is greater than 5000 mg/kg body weight. On that point are no late accounts in literature on acute toxicity studies of the leaves of *Baccaurea ramiflora* and *Microcos paniculata*.

# CONCLUSION

We resolve that the consequence of the present study may add to the scientific literature with respect to the phytochemical composition of the chloroform extract of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* as there is no data available in the literature. Further, the consequences of the acute toxicity study revealed that ethanolic extract of the leaves of *Baccaurea ramiflora* and *Microcos paniculata* was found to be nontoxic up to 5000 mg/kg body weight, so the LD50 of ethanolic extract of the leaves of *Baccaurea ramiflora* and Microcos panicula is greater than 5000 mg/kg body weight. This is likewise an important determination as the reports on the leaves extract are lacking. Therefore the present study offers a satisfactory preclinical proof of safety of leaves of *Baccaurea ramiflora* and Microcos panicula in maintenance of cellular architecture.

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