



PRELIMINARY PHYTOCHEMICAL SCREENING, ACUTE TOXICITY AND LAXATIVE ACTIVITY ON THE LEAVES OF *EUPHORBIA BALSAMIFERA* AIT (EUPHORBIACEAE)

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ABSTRACT

Euphorbia balsamifera [Ait] is a low shrub or a small tree belonging to the family Euphorbiaceae. It grows to a height of about 2-5 meters tall and is indigenous to the Canary Islands, North America, West Africa, Somalia and South of the Arabian Peninsula. The roots and leaves of the *E. balsamifera* are strongly laxative. The study was aimed at evaluating the phytochemical constituents, acute toxicity and laxative activity of the leaves of *E. balsamifera* in rats. The dried powdered leaves was macerated with 70% ethanol for 72 hours. The preliminary phytochemical screening using standard procedures, acute oral toxicity test based on OECD guidelines and laxative study were carried out. The preliminary phytochemical screening revealed the presence of steroids/triterpenes, tannins, anthraquinones and cardiac glycosides. The LD₅₀ of the extract was found to be greater than 5000 mg/kg when administered orally. There was increase in faecal output in both doses (400 and 800 mg/kg) and significant (at $p < 0.05$) only in the group administered 800 mg/kg of the extract. The leaves extract was found to be practically nontoxic and posses laxative potentials.

KEYWORDS: Extract; Nontoxic; Faecal.

INTRODUCTION

Euphorbia balsamifera Ait is known as Balsam Spurge (in English) and Aguwa (in Hausa) [1]. The plant varies greatly in height and it can be described either as a low shrub or as a small tree from 2-5 meters tall [2]. The parts of the plant used for medicinal purpose include leaves, roots and exudate [3]. The roots and leaves of the *E. balsamifera* are strongly laxative [4]. The leaves and exudate are used for its anthelmintic and vesicant (diuretic) properties [5]. Also, the leaves have been used to relieve tooth ache and gum troubles.

Previous phytochemical analysis of the ethanol extracts of the plant parts revealed the presence of tannins, saponins, steroids, terpenoids, flavonoids, cardiac glycosides and balsam [6]. Aqueous extract

of *E. balsamifera* leaves was reported to cause statistically significant decrease in the levels of Pack Cell Volume (PCV), hemoglobin concentration and Red Blood Cell (RBC) counts [7].

The use of laxatives is deeply rooted in medical and social traditions. Laxatives are useful in order to prevent undue straining at the stool which may result in hernia, rectal prolapse, fainting or even cerebrovascular incidents [8]. A typical example of plant with laxative activity is *Senna alata*, in which the laxative activity is attributed mainly to the anthraquinone glycosides, especially sennoside A and B [9].

MATERIALS AND METHOD

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Collection and Identification of the Plant

Fresh leaves of *E. balsamifera* were collected from Kwasai area in Sokoto South Local Government, Sokoto state, Nigeria in May, 2017. The plant was identified and given the Voucher number PCG/UDUS/Euph/0007 at the herbarium unit of the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences Sokoto. The leaves were air dried under shade, powdered and stored in an air tight container.

Extraction of Powdered Leaves of *E. balsamifera*

200g of powdered leaves of *E. balsamifera* was macerated in a glass jar with 1000ml of 70% ethanol for 3 days (72 hours) with frequent stirring. The content was decanted, filtered and concentrated at 50°C. The extract was kept in a closed air tight container.

Preliminary Phytochemical Screening

The extract was tested for the presence of steroids, flavonoids, tannins, alkaloids, anthraquinones and cardiac glycosides.

Acute Toxicity Screening of Extract

Rats of either sexes weighing between 120-150g maintained under standard laboratory condition were used for the acute toxicity test according to the Organization of Economic Cooperation and Development (OECD) guidelines 423 [10]. Three (3) rats received 2000mg/kg of the extract orally after 6 hours of fasting. Food was withdrawn for 4 hours after the extract was administered. The animals were observed 30 minutes after dosing and occasionally during 24 hours for behavioural changes and death. After 24 hours of without any death, another group of three (3) rats were administered 5000mg/kg of the extract orally and observed [11].

Laxative Activity Test

The study was carried out according to model described by Shankara and Sriram [12] with slight modification on rats of either sex. Wistar rats of either sex were fasted for 18 hours. The rats were divided into four groups of four animals each. Group I received 25 ml/kg of distilled water orally, Group II received castor oil (2 ml/kg orally), Group III and IV received *E. balsamifera* 400 and 800 mg/kg orally respectively.

The animals were separated in suitable cages for collection and weighing of the faecal output immediately after the dosing. Food and water were

given to all rats and faecal output was weighed after a period of 16 hours.

Laxative Activity on Loperamide Induced Constipation in Rats

This study was carried out, as described by Yadav *et al* [13]. Wistar rats were fasted for 18 hours and were placed individually in cages lined with clean filter paper. The rats were divided into four groups of five animals each. Group I received 5 ml/kg normal saline orally. Group II received castor oil (2 mg/kg orally). Group III and IV received *E. balsamifera* 400 and 800 mg/kg orally respectively. After one hour, all the animals received loperamide (5 mg/kg orally) by gavage. The faecal outputs in all four groups were monitored for 8 hours.

Statistical analysis

Data were expressed as the Mean \pm Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

RESULTS

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the extract showed the presence of steroid/triterpenes, alkaloids, tannins, anthraquinones and cardiac glycosides.

Acute Toxicity Study

The oral median lethal dose (LD₅₀) of the 70% ethanol extract of *E. balsamifera* administered to rats was found to greater than 5000mg/kg body weight.

Effect of 70% Ethanol Extract of *E. balsamifera* Leaves on Laxative Activity

Oral administration of 70% ethanol extract of *E. balsamifera* leaves extract at the dose of 400mg and 800mg/kg showed a dose dependent increase in faecal output with a significant increase at a dose of 800mg/kg compared to the control group as shown in Table 1.

Effect of 70% Ethanol Extract of *E. balsamifera* Leaves on Loperamide Induced Constipation in Rats

In the loperamide-induced constipation, the 70% Ethanol Extract of *E. balsamifera* leaves at the doses of 400 and 800 mg/kg orally, increased the total number of faeces in a dose dependent manner, and the results were statistically significant as shown on Table 2. There was less significant effect with the dose of 400 mg/kg of the extract compared with control. The reduction of the loperamide induced constipation at 800 mg/kg orally was found to be almost comparable with that of reference group.

DISCUSSION

Phytochemical analysis of the extract revealed the presence steroid/triterpenes, tannins, anthraquinone, and cardiac glycosides which in line with previous report [6]. Compounds demonstrating steroidal activity are of importance and interest in pharmacy due to their relationship with sex hormones [14]. It has been reported that several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens [15] hence their use as antimicrobials. The presence of anthraquinones in the extract can be attributed to its laxative properties [8].

Acute toxicity studies of the plant extract showed no sign of behavioral changes and no mortality was recorded in maximum dose of 5000 mg/kg and also 10000 mg/kg of the aqueous extract as shown [7]. Hence the LD₅₀ is higher than 5000mg/kg and practically non-toxic [16]

The laxative activity of 70% ethanol extract of *E. balsamifera* leaves was studied in rats. The results showed that an oral administration of the 70% ethanol extract of *E. balsamifera* leaves produced significant and dose dependant increase in faeces output of rats in regards to the accumulation of water in intestinal loop and the stimulation of gastrointestinal motility. These effects were similar with that of castor oil (standard drug) at high dose of 800 mg/ kg body weight. Castor oil is a fixed oil obtained from the seed of *Ricinus communis* (Euphorbiaceae) that affects electrolyte transport and smooth muscle contractility in the intestine. Its cathartic action is due to water accumulation in the intestine [17]. The observed activities therefore suggest that laxative activity of 70% ethanol extract of *E. balsamifera* leaves may be mediated through this mechanism.

Similarly, our results have indicated that the higher dose of 70% ethanol extract of *E. balsamifera* leaves exert opposite effects with loperamide on the gastrointestinal function. Loperamide abolishes

experimental osmotic diarrhea by acting on intestinal motility and consequently reducing the flow entering the colon [18]. These results suggest that 70% ethanol extract of *E. balsamifera* leaves had stimulated Na⁺, K⁺ and Cl⁻ secretion. Most of the naturally laxative exert their effects on the colonic epithelium by stimulating Cl⁻ secretion and/or inhibiting Na⁺ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility [19].

Phytoconstituents such as terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids [20] have been previously found to be responsible for laxative activities in plants. These results suggest that the laxative activity of 70% ethanol extract of the leaves of *E. balsamifera* may be because of presence of these phytoconstituents in the plant extract.

CONCLUSION

The present studies have established the presence of some phytochemicals such as steroids, tannins, anthraquinones, and cardiac glycosides in 70% ethanol extract of *E. balsamifera* leaves which is practically non-toxic. The results of the study also justify the use of *E. balsamifera* leaves as laxative.

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Table 1: Effect of 70% ethanol extract of *E. balsamifera* leaves on the faecal output of rats

Group	Treatment	Dose	Faecal output (mean \pm SEM)
Control	Distilled water	25ml/kg	2.002 \pm 0.475
Reference	Castor oil	2ml/kg	4.920 \pm 0.129*
Experimental	Extract	400mg/kg	2.053 \pm 0.264
Experimental	Extract	800mg/kg	3.421 \pm 0.257*

Values were analysed using, one-way analysis of variance (ANOVA) followed by Dunnett's test. Value is statistically significant at *P<0.05, compared with control group.

Table 2: Effect of 70% ethanol extract of *E. balsamifera* leaves on the faecal output of loperamide induced constipation in rats

Group	Treatment	Dose	Faecal output (mean \pm SEM)
Control	Normal Saline	25ml/kg	1.502 \pm 0.082
Reference	Castor oil	2ml/kg	3.163 \pm 0.126*
Experimental	Extract	400mg/kg	1.556 \pm 0.467
Experimental	Extract	800mg/kg	2.823 \pm 0.248*

Values are analysed using, one-way analysis of variance (ANOVA) followed by Dunnett's test. Value is statistically significant at *P<0.05, compared with control group.

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