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Phytochemical Screening and *In Vivo* Anti-inflammatory Activity of Hydroalcoholic Extract of *Embelia Ribes* Burm. F

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ABSTRACT

Embelia ribes Burm. f. (*E. ribes,* Myrsinaceae), first described by Nicolaas Laurens Burman in 1768, is a woody climber distributed in the primary lowland and mountain forests of Bangladesh, Burma, Cambodia, India, Laos, peninsular Malaysia, Thailand and Vietnam. Ayurvedic medicine prescribes the dried fruits or *vidanga* and used as antibacterial, antifertility activities, antiprotozoal, abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease, obesity, analgesic, anti-inflammatory and antioxidant. Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. So number of herbal medicines is recommended for the treatment of inflammation that has no side effects. The present study is aimed to evaluate the anti inflammatory activity of *E. ribes* on formalin induced paw edema in rats as for controlling inflammatory disorders. Acute toxicity of the extract (2000 mg/kg) was examined in wistar rats for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids ect. The total phenolics content of *E. ribes* extract was (1.155mg/100mg), followed by flavonoids (0.811mg/100mg) respectively. Hydroalcoholic extract up to 2000 mg/kg did not produce any toxic effects. The hydroalcoholic extract of *E. ribes* (100 and 200 mg/kg) inhibited the inflammation induced by formalin in rats in a dose dependent manner. The results of present study demonstrate that hydroalcoholic extract of the seeds of *E. ribes* possess significant ($P < 0.05$) anti-inflammatory potential.

Keywords: *Embelia ribes* Burm. f, Acute toxicity, Anti-inflammatory effect, Phytochemical screening, Flavonoid, paw edema

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INTRODUCTION

Herbal therapy, although still an unwritten science, is well established in some countries and traditions and has become a way of life in almost 80% of population in rural areas. Inflammation is one of the most important physiological reactions of a body to stimuli such as irritation, trauma, tissue injury and infection, but excessive or persistent inflammation results in a variety of pathological conditions or organ damage[1]. Usually, inflammation develops through infiltration of leukocytes to the injury sites and production of specific cytokines such as IL-1b and TNF-a. Reactive oxygen species (ROS) also are released during the inflammation process to exert a protective effect against invading pathogens[2, 3].Chronic anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration[4], the most common being gastrointestinal bleeding and peptic ulcers[5]. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine. Dried fruits of *E. ribes* belong to family Myrsinaceae is one of the most significant plants used from the prehistoric time in the form of the drug Baibidanga or Vidanga[6]. It has been used as an ingredient in most of the Ayurvedic formulation for the treatment of various ailments. Various formulations of *E. ribes* are used in ayurvedic system of medicine like asava, aristha, lauha and taila[6]. Commonly it is known as false black pepper. It is listed in red book as threatened species. In various literatures, it is found that the fruits of that plant used as an anthelmintic, diuretic, carminative, contraceptive, anti-bacterial, anti-inflammatory

astringent, antioxidant, anticancer agents and seed possessed antibiotic and antitubercular properties[7,8]. Seeds are used as antibiotic, anthelmintic, antituber-culosis, alterative and stimulative[9]. Leaves are astringent, demulcent, depurative and useful in pruritus, sore throat, ulcers of mouth, indolecent, skin diseases and leprosy[10]. Active constituent of pants contained Quinones derivatives; Embelin, Embelinol, Embeliaribyl ester and Embeliol and Vilangin, Quercitol, christembine, Sitosterol and daucosterol, Tannin, fatty substances, resiniod and volatile oil, phenolic acids like vanillic acid, cinnamic acid, caffeic acid, chrorogenic acid, and o-cumaric acid[9,11,12]. Therefore, the present study was designed to investigate anti-inflammatory activities of hydroalcoholic extract of twigs of *E. ribes* by using formalin -induced rat paw edema model.

MATERIALS AND METHODS

Plant material

Fresh seeds of *Embelia Ribes* were collected from area adjoining forests of Bhopal in the month of October, 2018.

Chemical reagents

Diclofenac sodium (Themis Pharmaceuticals, Mumbai), formalin (Sigma Chemical Co, St Louis, MO, USA) were used in present study. All other chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Dried powdered 150 gm seeds of *E. ribes* were subjected to maceration extraction with (500 ml) 80% methanol for 24 hours. The extraction procedure was ensured by pouring a few drops of extract from thimble left no residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[13].

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures[14,15]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenolic contents

The total phenolic content was determined using the method of Olufunmiso et al[16]. A volume of 1 ml of *A. catechu* bark extracts or standard was mixed with 1ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoid contents

The total flavonoid content was determined using the method of Olufunmiso et al[16]. 1 ml of 2% AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Animals

In the present investigation the Wistar rats (150-200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic extraction of seeds of *E. ribes* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD). Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect[17].

Formalin induced hind paw oedema

Experimental designs

Group –1: Formalin control

Group –2: Diclofenac sodium (10 mg/kg, bw, Standard)

Group –3: Hydroalcoholic extraction of seeds of *Embelia Ribes* (100mg/kg, p.o.)

Group –4: Hydroalcoholic extraction of seeds of *Embelia Ribes* (200mg/kg, p.o.)

Anti-inflammatory activity was measured using formalin induced rat paw oedema assay. The rats were divided into 4 groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels). Group 1 was treated as formalin (0.2 ml of 2% v/v freshly prepared formalin solution prepared in distilled water) was used as edematogenic agent; Group 2 was administered Diclofenac sodium (10 mg/kg, bw) and considered as standard. Group 3 were treated with Hydroalcoholic extraction of seeds of *E. ribes* (100mg/kg, p.o.). Group 4 were treated with Hydroalcoholic extraction of seeds of *E. ribes* (200mg/kg, p.o.). The thickness was measured before injecting the formalin and after injecting the formalin everyday at a fixed time. The volumes of oedema of the injected were measured after the induction of inflammation using a Vernier caliper to calculate the percentage of paw oedema inhibition[18].

$$\text{Percentage Inhibition} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group, Vt- Edema volume of test group

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extract. The yield of extracts was found to be 5.88% w/w. The results of qualitative phytochemical analysis of the crude powder of seeds of *E. ribes* are shown in Table 1. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extracts of seeds of *E. ribes* showed the content values of 1.155. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extracts of seeds of *E. ribes* showed the content values of 0.811 Table 2 & Fig. 1 and 2. No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *E. ribes*. This indicates that 2000 mg/kg is maximum safe dose. So 1/20th and 1/10th *i.e.* 100 and 200 mg/kg of body weight of the maximum safe dose were selected for studying *in vivo* anti-inflammatory effects. Table and Fig. 3 shows the effect of hydroalcoholic extract of seeds of *E. ribes* and standard drug as compared to formalin control group in formalin-induced paw edema model using vernier caliper. Hydroalcoholic extract of seeds of *E. ribes* administered at a dose of 100 and 200 mg/kg showed 69.17 % and 72.27 % inhibition respectively, while Diclofenac sodium at a dose of 10 mg/kg prevented formalin induced paw edema with a percentage inhibition of 75.36%. Formalin-induced paw edema is one of the most suitable test procedures to evaluate chronic anti-inflammation, as it closely resembles human arthritis[19]. As shown in Table 3 and Fig. 3, administration of hydroalcoholic extract of seeds of *E. ribes* prevented formalin-induced paw edema in a dose-dependent manner showing significant anti-inflammatory effect, percentage inhibition shown was found to be 69.17% and 72.27% at dose of 100 and 200 mg/kg, respectively. Hence, it is suggested that hydroalcoholic extraction of seeds of *E. ribes* may provide benefits in the management of arthritis.

Table 1 Phytochemical screening of extract of *E. ribes*

S. No.	Test	Extracts
		Hydro alcoholic extract
1	**Alkaloids**	-ve
2	**Carbohydrates**	+ve
3	**Glycosides** Anthraquinones Saponins Flavonoids Cardiac	 -ve +ve +ve -ve
4	**Proteins and amino acids**	-ve
5	**Sterols**	-ve
6	**Tannins**	-ve
7	**Phenolic compounds**	+ve
8	**Acidic compounds**	-ve
9	**Resins**	-ve
10	**Fats and oils**	-ve

Table 2 Estimation of total phenolics and total flavonoids content

S. No	Extract	Total phenolic content(mg/100mg of dried powder)	Total flavonoids content(mg/ 100 mg of dried extract)
1	Hydroalcoholic	1.155	0.811

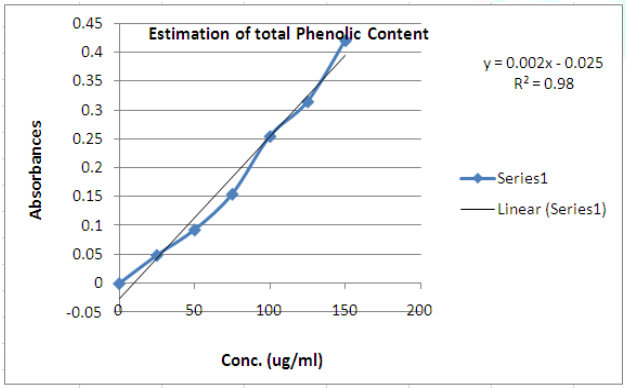

Fig 1 Graph of estimation of total phenolic content

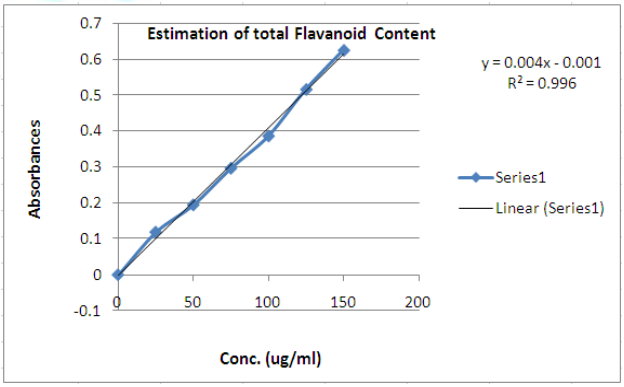

Fig 2 Graph of estimation of total flavonoids content

Table 3 Effect of hydroalcoholic extract of seeds of *E. ribes* on paw edema induced by formalin in rats

Group	Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	% of Inhibition
Group I	Control	0.2 ml of 2% v/v freshly prepared formalin solution	3.23 ±0.60	--
Group II	Diclofenac sodium	10	0.90±0.60 ***	75.36
Group III	Extract of *E. ribes*	100	1.10±0.50 **	69.17
Group IV	Extract of *E. ribes*	200	1.00±0.40 ***	72.27

Values are expressed as mean ± SD. *P < 0.05-significant compared to formalin treated group

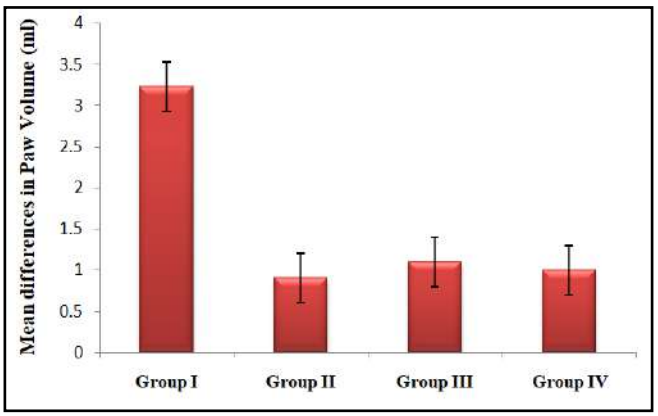

Fig 3 Effect of hydroalcoholic extract of *E. ribes* on paw edema induced by formalin in rats

CONCLUSION

Altogether, the present study results confirmed that hydroalcoholic extract of seeds of *E. ribes* possess significant anti-inflammatory activity, which may be devoted to major secondary active metabolite present in it. In conclusion we suggest that the future studies on *A. catechu* could be useful for the management of inflammatory diseases and oxidative stress.

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