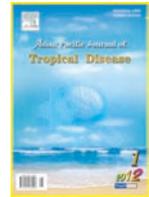
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Pharmacognostic study and anti – inflammatory activity of *Phyllanthus reticulatus* Poir. fruit

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ABSTRACT

Objective: To study pharmacognostic evaluation and anti – inflammatory activity of *Phyllanthus reticulatus* Poir. (*P.reticulatus*) fruit. **Methods:** The fruit sample was studied by macroscopical, microscopical and other WHO recommended methods for standardisation. The hydroalcoholic extract of ripe fruit was also screened for anti-inflammatory activity by carrageenan induced left hind paw oedema in rat at doses of 200 mg/kg and 400 mg/kg, orally. **Results:** Transverse section of fruit and powder study showed the presence of epicarp, mesocarp, endocarp, fibres and sclereids. Hydroalcoholic extract of fruit showed significant ($P<0.01$) reduction of paw volume when compared with diclofenac sodium. The extract showed anti – inflammatory activity at 200 mg/kg and 400 mg/kg and was comparable with diclofenac sodium. **Conclusions:** Pharmacognostic profile of *Phyllanthus reticulatus* provides the simplest and quickest means to establish the identity and purity and thereby assure the quality of sample. The hydroalcoholic extract shows significant anti – inflammatory activity at both doses when compared with standard drug diclofenac sodium (100 mg/kg).

1. Introduction

Phyllanthus reticulatus (*P.reticulatus*) (Family: Euphorbiaceae) is a large, often scandent, shrub. The plant grows throughout tropical areas of India, Bangladesh, China and the Malay Islands [1]. The biological work performed so far on this plant showed anti-diabetic [2], anti-plasmodial [3], hypocholesterolemic [4], antimicrobial and cytotoxic [5], hepatoprotective [6], antibacterial [1], Antinociceptive and antihyperglycemic [7], analgesic and anti-inflammatory [8] activities. The leaves of the plant are diuretic and also used for diarrhoea in infants [9]. The leaf juice is a remedy for spongy and bleeding gums [10]. Chemical studies demonstrated the presence of tannic acid, octacosanol, teraxerol acetate, friedeline, teraxerone, betulin, sitosterol, scopoletin [5], lupeol acetate, stigmasterol and lupeol [11]. The present work was carried out to study the pharmacognostic profile and anti-

inflammatory activity of *P. reticulatus*.

2. Materials and methods

2.1. Chemicals

Carrageenan was purchased from sd-fine chem. Limited, Mumbai, India. Phloroglucinol, glycerine, hydrochloric acid, potassium hydroxide and all other chemicals were of analytical grade used in the present study.

2.2. Plant material

Fruit of *Phyllanthus reticulatus* were collected from the campus of Kurukshetra University, Kurukshetra, India in the month of July – August and was authenticated by Dr. Singh HB, Scientist F & Head, Raw material Herbarium & Museum, NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium (NISCAIR/RHMD/consult/-2010-11/1472/70).

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2.3. Extract preparation

The dried ripe fruit of the plant was coarsely powdered using dry grinder and extracted with pet-ether (60–80 °C) in Soxhlet apparatus. The defatted plant material was then extracted with Hydroalcohol. The extract was concentrated at 45 °C under reduced pressure in rotary evaporator to yield a crude semi-solid mass. It was then dried and used.

2.4. Animals

Wistar rats of either sex, weighing about 150–250 gm were used in the study. Animals were maintained under standard environmental conditions (temp– 22 ±2) °C, relative humidity– 45–55% and fed with standard pellet diet and water ad libitum.

2.5. Pharmacognostic study

Fresh unripe fruit were taken for microscopic study. Coarse powder was used to study the powder characteristics. The detailed pharmacognostic study of the plant was carried out according to well known methods and procedures [12–16].

2.6. Anti – inflammatory activity

The carrageenan–induced rat paw oedema was performed according to the method described by Winter et al [17]. For anti – inflammatory activity the animals were divided into four groups ($n=6$). Group I served as control received the vehicle only (Normal saline). Group II served as standard, received diclofenac sodium at dose of (100 mg/kg/i.p.) Group III and Group IV served as test group, received hydroalcoholic extract of ripe fruit at dose of 200 mg/kg and 400 mg/kg p.o. respectively. 30min after administration of extract, 0.1ml of 1% w/v of Carrageenan suspension was injected to all animals in the left hind paw (plantar region). The paw volume, up to tibiotarsal articulation was measured by Plethysmometer (model 7140.Ugo basile, Italy). The measures were determined at 1, 2 and 3hr after drug treatment.

2.7. Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnett's test. P values less than 0.05 were considered as significant.

3. Results

3.1. Pharmacognostic study

3.1.1. Macroscopic characteristics

Phyllanthus reticulatus is usually a dense deciduous shrub or small tree. Fruits are greenish and firm when young; however,

the ripe fruit is fleshy or become purple black and has characteristic odour and taste (Figure 1). Seeds are irregularly trigonous.

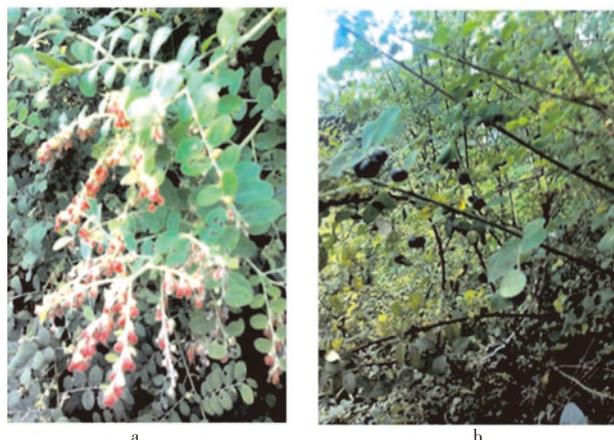


Figure 1. a: Flowering plant; b: Fruiting plant.

3.1.2. Microscopic characteristics

3.1.2.1. Fruit microscopy

The thin transverse section of fruit showed all the general microscopic characters of fruit i.e. presence of epicarp, mesocarp and endocarp which are well differentiated (Figure 2).

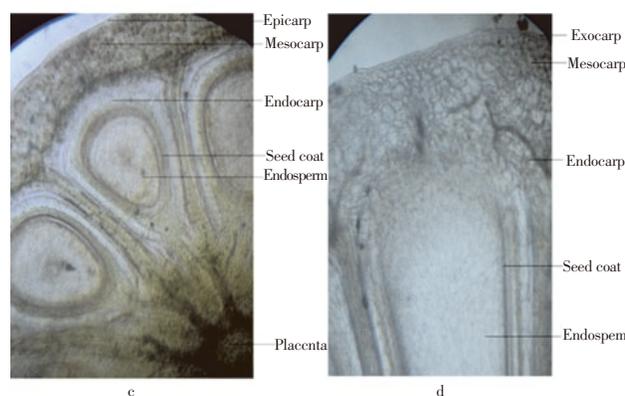


Figure 2. Transverse section of fruit.

Epicarp: It is the outermost layer of fruit made up of thin rectangular cells.

Mesocarp: Mesocarp is made up of many layers of thin compactly arranged parenchymatous cells.

Endocarp: It is made up of simple large parenchymatous cells which envelope the seeds.

3.1.2.2. Powder analysis

The fine powder of fruit was mounted with glycerine as well as stained with (Phloroglucinol+HCL). Observation under microscope showed the presence of cells of endocarp, mesocarp, epicarp, sclereids and unicellular fibres (Figure 3).

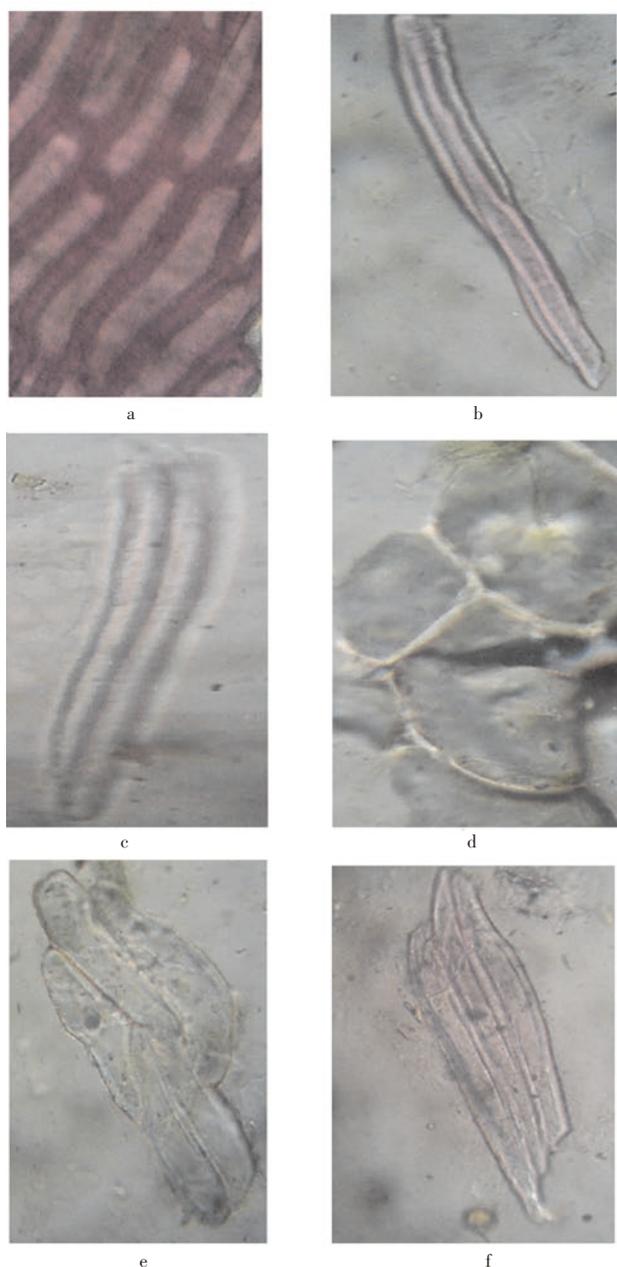


Figure 3. a: Fragment of endocarp; b: Sclereid; c: Unicellular fibre; d: Epicarp cells; e: Endocarp cells; f: Palisade like irregular shaped sclereid of testa.

3.1.3. Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of tannins and phenolic compounds, flavonoids, steroids and carbohydrates.

3.1.4. Physicochemical parameters

Ash value of a drug simply represents inorganic salts or impurities while extractive values are indicative of chemical constituents soluble in particular solvents. Total ash value, acid insoluble ash value, water soluble ash value, water soluble extractive value, hydroalcohol soluble extractive value, methanol soluble extractive value, chloroform soluble extractive value, pet ether soluble extractive value and loss on drying were found to be 8.24, 2.54, 2.12, 29.25, 67.75, 20.40, 6.65, 26.35 and 2.90 (% w/w) respectively.

3.2. Anti-inflammatory activity

Hydroalcoholic extract of *P. reticulatus* significantly inhibited carrageenan induced rat paw oedema. Anti – inflammatory activity was compared with diclofenac sodium (100 mg/kg). Both the doses (200 and 400 mg/kg) showed comparable effects with diclofenac sodium (Table 1).

4. Discussion

Evaluation of crude drugs provides the simplest and quickest means to establish the identity and purity. Microscopic evaluation is an indispensable tool for the identification of medicinal herbs [18]. Extractive values are useful to evaluate specific chemical constituents soluble in a particular solvent [19]. Ash values can be used for detecting adulteration [20]. All these parameters will be helpful in identification and authentication of the plant material. Preliminary phytochemical screening will be useful to find out the chemical nature of the drug [21]. Transverse section of fruit showed the presence of epicarp, mesocarp and endocarp which are the essential characteristic parts of fruit. Phytochemical analysis revealed the presence of tannins and phenolic compounds, flavonoids, steroids and carbohydrates. The hydroalcoholic extract was also screened for anti – inflammatory activity. Inflammation is the cardinal last defense response to injury [22]. Carrageenan induced rat paw oedema is a suitable experimental animal model for evaluating the anti–edematous effect of natural products [23]. This model is known as acute inflammatory model. Two phases are found in the development

Table 1

Anti-inflammatory activity of hydroalcoholic fruit extract (mean \pm SEM).

Group	Treatment	Paw Volume (ml)			
		0 hr	1 hr	2 hr	3 hr
Group I	Normal saline	0.770 \pm 0.042	1.986 \pm 0.070	2.418 \pm 0.082	2.04 \pm 0.071
Group II	Diclofenac sodium(100 mg/kg)	0.898 \pm 0.042	1.617 \pm 0.094**	1.26 \pm 0.032**	1.21 \pm 0.071**
Group III	Fruit extract (200 mg/kg)	0.8700 \pm 0.069	1.762 \pm 0.067**	1.40 \pm 0.076**	1.39 \pm 0.020**
Group IV	Fruit extract (400 mg/kg)	0.8917 \pm 0.032	1.78 \pm 0.059**	1.30 \pm 0.117**	1.27 \pm 0.021**

** P <0.01

of oedema. In first phase histamine and serotonin are released and in second phase bradykinin and prostaglandin are released [24]. *Phyllanthus reticulatus* hydroalcoholic fruit extract showed significant ($P < 0.01$) anti – inflammatory activity at doses of 200 and 400 mg/kg. The anti – inflammatory activity of both the doses was comparable with diclofenac sodium. The presence of tannins and phenolic compounds may be responsible for anti – inflammatory activity. However, the main active constituent should be isolated from the plant that is responsible for the activity. In conclusion, the pharmacognostic study of *P. reticulatus* Poir of fruit has been carried out for the first time. This could be useful in the identification and preparation of a monograph on the plant and hydroalcoholic extract of the plant has potent anti – inflammatory activity.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgement

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