Research Article

Evaluation of anti-inflammatory activity of alcoholic, hydroalcoholic and aqueous extract of *Amoora rohituka* and *Soymida febrifuga* in albino rats

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Abstract

Objective: The objective of present paper to evaluate anti-inflammatory activity of *Amoora rohituka* and *Soymida febrifuga* belongs to family Meliaceae. The Meliaceae plants are known to be rich sources of limonoids. A number of limonoids have been isolated from several genera of Meliaceae and some of this exhibit anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many various activities. **Materials and Methods:** Anti-inflammatory effect of the alcoholic, hydro-alcoholic (40:60) and aqueous extracts of the *Amoora rohituka* and *Soymida febrifuga* were studied in albino rat using the carrageenan – induce rat paw oedema methods. The hydro-alcoholic extracts of both belongs to inhibit the carrageenan – induce rat paw oedema at dose of 100 mg/kg p.o. where the standard drug was Indomethacin at dose 10 m g/kg p.o. **Results and Conclusion:** The results indicated that the hydro-alcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* produced significant ($p<0.05$) anti-inflammatory effect with Indomethacin as standard at dose 10mg/kg p.o. The alcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* resulted 33.4% and 55.2% inhibition at the dose of 100mg/kg p.o. The aqueous extracts of *Amoora rohituka* and *Soymida febrifuga* resulted 45.2% and 56.6% inhibition at the dose of 100mg/kg p.o. The hydroalcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* resulted 53.3% and 66.2% inhibition at the dose of 100mg/kg p.o.

Keywords: Anti-inflammatory, *Amoora rohituka, Soymida febrifuga,* Indomethacin

Introduction

Meliaceae family has 40 genera and 600 species. The Meliaceae plants are known to be rich sources of limonoids. A number of limonoids have been isolated from several genera of Meliaceae and some of these exhibits anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many various activities (Conolly et al., 1976).

Amoora rohituka (Roxb.) wight & arnet family Meliaceae is a large handsome evergreen tree (Figure 1a), with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8m in girth distributed in the sub-himalayan tract from Gonda eastword to Bengal, Sikkim and Assam. In Western Ghats, chhota Nagpur, Andaman and adjoining hills from Poona to South word to Tinnevelly. Petroleum extract of the air dried bark gave a new tetra-nortriterpenold, Aphanamixinin ($C_{27}H_{34}O_7$). The bark appears to be an effective immunosuppressive drug similar to prednisolone. The bark is strongly astringent and is used in disease of the liver and the spleen and for tumors and abdominal complaints. Bark is astringent and used for treatment of enlarged glands, and disease of the liver and spleen. (Choudhury et al., 2003*)*

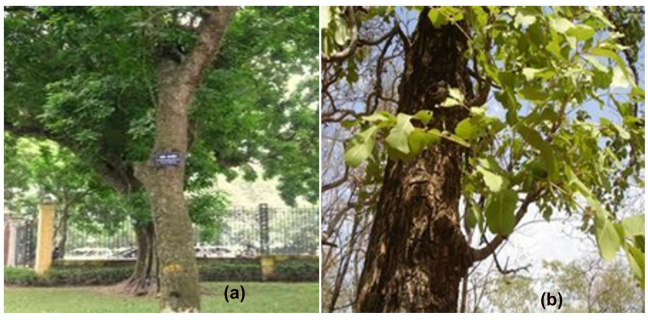

Figure 1. Plant of (a) *Amoora rohituka* and (b) *Soymida febrifuga*

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Soymida febrifuga A. Juss. Family Meliaceae, (common name is Indian redwood) is a lofty dicedous trees with 22-25m height with rough bark exfoliating in large plates or scales (Figure 1b). It is distributed in Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh.

The stem bark contains mainly Alkaloids, flavanoids, saponinand cyanogenic glycoside the stem bark also gives positive result of tannins. The chemical constituents of *Soymida febrifuga* are Deoxyandirobin, epoxyfebrinin B and its 14,15-dihydro derivative, febrinolide, methylangolensate, Beta-sitosterol, lupeol, febrifugin, myricetin, ampelopsin, naringenin and quercetin phytochemicals. Bark used in the treatment of diarrhoea, dysentery and fever and also as a general tonic; decoction used in gargles, vaginal infections, rheumatism swellings and as enemata (Ananata et al., 2013).

The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolizedby cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation. The present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory activity in rats (Balasubramanian et al., 2005).

Material and methods

Plant material

The stem bark of *Amoora rohituka* (Roxb.) wight & arnet and *Soymida febrifuga* A. Juss. family Meliaceae was collected from Jawaharlal Nehru Krishivishwavidyalaya, Jabalpur Madhya Pradesh, India. The authentication was done by Dr. A.B. Tiwari, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur Madhya Pradesh, India.

Experimental animals

Albino rats weighing between 150-200 gm bodyweight were selected for anti-inflammatory activity. The rats were divided into different (eight) groups, each group consisting of 6 animals.

Reagents and drugs

Carrageenan is a mixture of polysaccharides composed of sulphated galactose units and is derived from Irish Sea moss, *Chondru scripsus*. The oedema that develops in a rat paw after carrageenan injection is a biphasic event. The initial phase is attributed to the release of histamine, 5-HT and serotonin the oedema maintained between the first and the second phase to kinin like substances and the second phase to prostaglandin like compounds (Winter *et al*., 1962).

Indomethacin: The indomethacin is used as standard drug for study of anti-inflammatory activity, which is a non-steroidal anti-inflammatory drug (NSAID). Indomethacin works by reducing hormones that cause inflammation and pain in the body.

Standardization of powdered plant material

Standardization of *Amoora rohituka* and S*oymida febrifuga* stem barks were done accordingly WHO guidelines for herbal drugs, for following parameters:

Foreign organic matter

Foreign organic matter is the material consisting Part of organ or organs from which the drug is derived other than the part named in the definition and description or for which the limit is prescribed in individual monograph. Weighed 100 grams of drug or original sample and spread it out in a thin layer and Inspected the sample with an unaided eye or with use of 6X lens and separated the foreign organic matter manually as completely as possible. Finally Weighed and determined the percentage of Foreign organic matter from the weight of drug taken (WHO, 1998). Data has shown in table 1.

Total ash value

Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at as low as temperature as possible (about 450° C) to remove all the carbons. At higher temperature, the alkali chlorides may be volatile and may be lost by this process. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash – which is derived from the plant tissue itself and non-physiological ash- which is the residue of the adhering material to the plant, *e.g.,* sand and soil. Placed about 2-4 gm of the air dried material, accurately weighed, in a previously ignited and tared crucible (usually of platinum or silica), spread the material in an even layer and ignite it by gradually increasing the heat to 500-600C until it is white, indicating the absence of carbon. Cooled in a desiccators and weighed without delay. The content of total ash calculated in mg per gm of air dried material. Data of Ash Value has shown in table 2.

Acid insoluble ash value

To the crucible containing total ash, add 25ml of hydrochloric acid (~70g/l) TS, coverd with a watch glass and boiled gently for 5 minutes. Rinse the watch glass with 5ml of hot water and add this liquid to the crucible. The insoluble matter collected on ash less filter paper and wash with hot water until the filtrate is neutral. The filter papers

containing the insoluble matters were transferred in to the original crucible, dried on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccators for 30 minutes, then weighed without delay. The content of acid-insoluble ash was calculated in mg per g of air dried material (WHO, 1998).

Water soluble ash value

To the crucible containing the total ash 25ml of total water was added and boiled for 5 minutes. Insoluble matter collected in a sintered-glass crucible or on ashless filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450C. subtract the weight of this residue in mg from the weighed of total ash. The content of water soluble ash was calculated in mg per g of air dried material (WHO, 1998).

Determination of extractive values

This method determines the amount of active constituents in a given quantity of medicinal plant material when extracted with solvents. It is employed for that material for which no chemical or biological assay method exist. According to Indian Pharmacopoeia 1996 and British Pharmacopoeia 1980, the determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drugs which are not readily estimated by other means.

Cold extractive value

Macerated 5gm of air dried powdered drug with 100 ml of solvents (ethanol 99% v/v) of specific strength in a closed flask for 24 hrs. Shaking frequently during 6hr and allow to stands for 18 hrs. Filtered rapidly taking precaution against loss of solvents. Evaporated 25 ml of filtrate against dryness in a tarred flat bottomed shallow dish Dried at 105ºC and weighed. Calculate percentage of extractives with reference to air dried drug.

Hot extractive value

The extractive value, which determined by hot extraction (by soxhlet) using ethanol as a solvent by taking 10 gm of coarsely powdered drug was taken in soxhlet apparatus. The round bottom flask was filled by solvent and set condenser. Start the assembly and run for 6 hrs or until side tube does not appear colourless. Extract from round bottom flask was collected and make up the volume to 100 ml either by evaporation or by dilution. 25 ml of this extract was taken in flat bottomed dish and evaporated it on water bath at50-60^{0}C, finally weighed the extract and calculate the extractive value in percentage wit reference to air dried drug (WHO, 1998). Data of percent hot extractive value shown in table 3.

Loss on drying

This parameter is used to determine the amount of moisture present in a particular sample. The powdered drug (10 g) sample was placed on a tarred evaporating dish. The tarred evaporating dish is dried at 105^{0}C for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25% of constant weight (WHO, 1998). The loss on drying for *Amoora rohituka* and *Soymida febrifuga* stem bark was found 7.41% and 7.71%.

Swelling index

Many medicinal plants have shown specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemi-cellulose. The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specific conditions. A glass stopper measuring cylinder was used. The internal diameter of cylinder should be 16mm. The length of graduated portion was about 125mm, marked in 0.2ml division from 0 to 25ml in an upward direction.1g of powered plant material was taken in cylinder 25ml of water was added and the mixture was shaken thoroughly the interval of 10 minutes for 1hr. The loss on drying for *Amoora rohituka* and *Soymida febrifuga* was found 0.97 ml and 1.02 ml.

Preparation of extract

Dried and coarsely powdered stem barks (250gm) were extracted with solvents Alcohol (99%), aqueous and hydro alcohol (40:60) using hot soxhlet extraction method, for 24 hours. Filter the extract then vacuum evaporated the filtrate and get the Crude extracts (Shuhag et al., 2000).

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out using the alcoholic extracts for different types of chemical constituents. The qualitative chemical tests give the general idea regarding the nature of chemical constituents of crude drugs. The extracts were subjected to preliminary phytochemical investigation for detection of Alkaloids, Glycosides, Flavonoids, Saponins, Sterols, Phenolic compounds, Carbohydrates, Proteins and amino-acids, Acidic compounds, Mucilage. The preliminary phytochemical screening for *Amoora rohituka* and S*oymida febrifuga* stem barks.

Anti-inflammatory activity

1% solution of carrageenan is prepared. 0.1 ml of this solution was injected into the right hind paw of each rats of eight groups. The test drug/plant extract at varying doses based on the design of the experiment and control vehicle are given orally 30 min. prior to the injection of carrageenan. The paw volume is measured just before and

1,2,3,4, 5th after administration of carrageenan by the volume displacement methods using a plethismometer.

Albino rats weighing between 150-200 gm body weights were selected for anti-inflammatory activity. The rats were divided into different groups each group consisting of 6 animals. Group-I was treated as negative control (received Normal saline 1ml/kg), Group-II served as positive control, (received Indomethacin 10 mg/kg p.o.) while the other groups received extracts (test compounds) from plants under study in the dose100 mg/kg p.o.by oral route.

The paw volume was measured using Plethysmometer immediately (measured within 30 sec. and referred as initial paw volume) i.e. 0 hr. and (final volume) 3rd hour and 5thhour after injection of carrageenan. The percent inhibition of oedema for the treated groups was calculated by following formula arc compared with the control group:

% Inhibition = 100 x [1 - Vt/Vc]

Where Vt and Vc are the mean changes of paw volume in the treated and control respectively (Al-Awadi et al., 2001).

Results and discussion

Standardization of plant material

The *Amoora rohituka* and *Soymida febrifuga* belongs to family *Meliaceae,* found as standard crude drug by existing various physico-chemical characteristics as a standard crude drug, known as standardization of crude drug. The standardization of crude drugs mainly consists Ash values, extractive values, foreign organic matter, loss on drying swelling index as well as preliminary phytochemical screening. Preliminary phytochemical screening had shown that presence of terpenoids, which indicates to limonoids, saponine, flavones, and Anthraquionone, glycoside, carbohydrate, amino acids, organic acids and steroid in both stem barks. Total Ash Value of *Amoora rohituka* and *Soymida febrifuga* was found to be 331 and 357(mg/g) respectively. Foreign organic matter for *Amoora rohituka* and S*oymida febrifuga* stem barks was found to be 3.67 % and 2.90 % respectively. Cold extractive value (%) was found to be 4.8 and 8.12 along with hot extractive value (%) was found to be 10.2 and 9.62 for *Amoora rohituka* and S*oymida febrifuga* respectively. The loss on drying for *Amoora rohituka* and *Soymida febrifuga* was found 7.41% and 7.71% respectively

Table 1. Calculation of Foreign organic matter for *Amoora rohituka* and S*oymida febrifuga* stem barks

Parameters	*Amoora rohituka*	*Soymida febrifuga*
Drug sample taken	100 g	100 g
Foreign organic matter	3.67	2.90
Percentage Foreign organic matter	3.67 %	2.90 %

Table 2. Different ash values for *Amoora rohituka* and S*oymida febrifuga* stem barks

S. No.	Crude drugs	Total ash value (mg/g)	Acid insoluble ash value (mg/g)	Water soluble ash value (mg/g)
1.	*Amoora rohituka*	331	118	108
2.	*Soymida febrifuga*	357	210	105

Table 3. Cold and hot extractive values for *Amoora rohituka* and S*oymida febrifuga* stem barks

S. No.	Crude drug	Cold extractive value (%)	Hot extractive value (%)
1.	*Amoora rohituka*	4.8	10.2
2.	*Soymida febrifuga*	8.12	9.62

Table 4. The preliminary phytochemical screening for *Amoora rohituka* and S*oymida febrifuga* stem barks

Phytochemical	Test performed	Alcoholic extract of *Amoora rohituka*	Alcoholic extract of *Soymida febrifuga*
Carbohydrate	Molish test	+	+
	Benedict test	+	+
	Fehling's test	+	+
	Borfoed test	+	+
	Pentose sugar test	+	+
	Tollens test	+	+
	Iodine test	–	–
	Tannic acid test	–	–
Amino acids	Ninhydrine test	+	+
	Tyrosine test	+	+
	Cystine test	+	+
Phenols & tannins	5% ferric chloride test	+	+
	Lead acetate solution test	+	+
	Bromine water test	+	+
	Acetic acid solution test	+	+
	Pot. Dichromate solutions test	+	+
Proteins	Biuret test	–	–
	Millons test	–	–
	Xanthoprotein test	–	–
	5% lead acetate test	+	+
	5% copper sulphate test	+	+
	5% ammonium sulphate test	+	+
	5% mercuric chloride test	–	+
Alkaloids	Dragendroff test	–	–
	Hager test	–	–
	Mayer test	–	–
	Wagner test	–	–
Cardiac glycoside	Legal test	–	–
	Keller Killianitest	–	–
Anthraquinone glycoside	Borntrager test	+	+
	Modified Borntrager test	–	+
Cyanogenetic glycoside	Sodium picrate test	–	–
	Murcurous nitrate test	–	–
Coumarin glycoside	Alkali test	–	–
	Filter paper test	–	–
Saponine glycoside	Foam test	+	+
	Hemolytic test	+	+
Flavonoids	Shinoda test	–	–
	Lead acetate test	+	+
	Sod. Hydroxide test	+	+

+ means present and – means absent

Anti-inflammatory activity

In carrageenan induced acute model, Indomethacin with a dose of 10 mg/kg p.o. served as standard, resulted in 83% inhibition of Carrageenan Induced Rat Paw oedema. The alcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* stem bark, resulted 33.4% and 55.2% inhibition at the dose of 100mg/kg p.o. The aqueous extracts of *Amoora rohituka* and *Soymida febrifuga* stem bark, resulted 45.2% and 56.6% inhibition at the dose of 100mg/kg p.o. Data represented in figure 2.

The hydroalcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* stem bark, resulted 53.3% and 66.2% inhibition at the dose of 100mg/kg p.o. The alcoholic, aqueous and hydroalcoholic extracts of both stem barks given satisfactory result against the inflammation but, the alcoholic extract of *Amoora rohituka* showed good results against inflammation. The alcoholic extract of *Soymida febrifuga* showed better results against inflammation. The hydro-alcoholic extract of *Amoora rohituka* and *Soymida febrifuga*

Table 5. Effect of ethanolic extracts of *Amoora rohituka* and *Soymida febrifuga* stem bark on carrageenan induced rat paw oedema

Treatment	Dose (mg/kg, p.o.)	Change in paw volume after treatment (ml) ±SEM		% Inhibition in paw volume after treatment	
		3 Hrs	5 Hrs	3 Hrs	5 Hrs
(Normal saline) 1 ml	1	0.61±0.03	0.62±0.03	-	-
Indomethacin	10	0.15±0.02	0.10±0.03	77.1	83
Alcoholic extract of *Amoora rohituka* stem bark	100	0.42±0.02	0.40±0.02	30.2	33.4
Alcoholic extract of *Soymida febrifuga* stem bark	100	0.28±0.02*	0.25±0.04*	50.6	55.3

*p<0.05 when compared with control; Values are expressed as mean ± SEM (n=6).

Table 6. Effect of aqueous extracts of *Amoora rohituka,* and *Soymida febrifuga* in carrageenan induced rat paw oedema

Treatment	Dose (mg/kg, p.o.)	Change in paw volume after treatment (ml) ±SEM		% Inhibition in paw volume after treatment	
		3 Hrs	5 Hrs	3 Hrs	5 Hrs
(Normal saline) 1 ml	1	0.61±0.02	0.62±0.01	-	-
Indomethacin	10	0.15±0.03	0.10±0.03	77.1	83
Aqueous extract of *Amoora rohituka*	100	0.36±0.01	0.30±0.04	40.2	45.4
Aqueous extract *of Soymida febrifuga*	100	0.29±0.02*	0.26±0.03*	51.6	56.6

*p<0.05 when compared with control; Values are expressed as mean ± SEM (n=6).

Table 7. Effect of the hydro alcoholic extracts of *Amoora rohituka* and *Soymida febrifuga* on carrageenan induced rat paw oedema

Treatment	Dose (mg/kg, p.o.)	Change in paw volume after treatment (ml) ±SEM		% Inhibition in paw volume after treatment	
		3 Hrs	5 Hrs	3 Hrs	5 Hrs
(Normal saline) 1 ml	1	0.61±0.02	0.62±0.01	-	-
Indomethacin	10	0.15±0.03	0.10±0.03	77.1	83
Hydro alcoholic extract of *Amoora rohituka* stem bark	100	0.32±0.01*	0.30±0.04*	46.3	53.2
Hydro alcoholic extract of *Soymida febrifuga* stem bark	100	0.23±0.02*	0.20±0.03*	61.6	66.6

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple range test and differences below $p < 0.05$ are considered as significant.

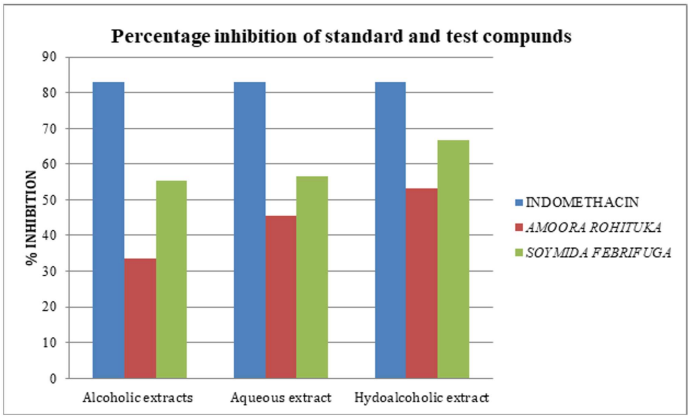

Figure 2. Anti-inflammatory activity of different extracts of *Amoora rohituka* and *Soymida febrifuga*

stem barks showed best results against inflammation. Results of paw volume changes are presented in table 5 and 6. Effect of ethanolic extracts was shown in table 5. Effect of Hydro Alcoholic Extracts was shown in table 7. Effect of aqueous extract is shown in table 6.

Conflicts of interest

The author declares no conflicts of interest.

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