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STANDARDIZATION AND ANTI-INFLAMMATORY POTENTIAL OF *AMOORA ROHITUKA* AND *MELIA AZEDARACH* BELONGS TO FAMILY MELIACEAE

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ABSTRACT

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 them exhibit anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many various activities. Anti-inflammatory effect of the alcoholic, hydroalcohalic (40:60) and aqueous extracts of the Amoora rohituka and Melia azedarach stem barks were studied in albino rat using the carageenan induced rat paw oedema method. The hydro alcoholic extracts of both the stem barks found to inhibit the carageenan induced rat paw oedema at dose of 100 mg/kg p.o., where the standard drug was Indomethacin at dose 10 mg/kg p.o. The results indicated that the hydro alcoholic extracts of Amoora rohituka and Melia azedarach stem barks produced significant ($p<0.05$) anti-inflammatory effect when compared with Indomethacin as standard at dose 10 mg/kg p.o.

Keywords: *Anti-inflammatory, Amoora rohituka, Melia azedarach, Indomethacin*

1. 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 exhibit anticancer, antimalarial, cytotoxic, antiprotozoal, antifeedant and many various activities. [1]

Amoora rohituka (Roxb.) wight & arnet Family *meliaceae* is a large handsome evergreen tree, with a dense spreading crown and a straight cylindrical bole up to 15 m in height and 1.5-1.8 m in girth. It is distributed in the sub-himalayan, Gonda eastword to Bengal, Sikkim and Assam. In Western Ghats, Chhota Nagpur, Andaman and adjoining hills from Poona to Southword to Tinnevelly. Petroleum ether extract of the air dried bark gave a new tetranortriterpenoid; 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 tumours and abdominal complaints. Bark is astringent and used for treatment of enlarged glands, and disease of the liver and spleen [2].

Melia azedarach Linn. Family *Meliaceae,* (common name is Chinaberry tree, Pride of India) is deciduous tree up to 9-12 m tall with a spreading crown and sparsely branched limbs. Bark smooth, greenish-brown when young, turning gray and fissured with age. It is distributed in Bangladesh, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, SriLanka, Thailand, and Vietnam.

Fig. no. 1: Plant of *Amoora rohituka*

Fig no. 2: Plant of *Melia azedarach*

Melia azedarach is often planted as an ornamental shade tree. Several compounds from Chinaberry have been isolated for medical purposes. Meliacine, a peptide isolated from leaves of *Melia azedarach*, exhibits potent activity against herpes simplex type 1 (HSV-1). *Melia azedarach* has also been used as an abortifacient, an antiseptic, a purgative, a diuretic, an insect repellent, etc [3].

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 metabolized by cycloxygenase (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 these indigenous herbs for anti-inflammatory activity in rats [4].

2. MATERIAL AND METHODS

2.1. Plant material

The stem bark of *Amoora rohituka* (Roxb.) wight & arnet and *Melia azedarach* Linn. family *Meliacea*e was collected from Jawahar lal Nehru Krishi Vishwavidyalaya ,Jabalpur M.P. The authentication was done by Dr. A.B. Tiwati, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, M.P. India.

2.2. 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.

2.3. Carrageenan (Sigma, USA)

Carrageenan is a mixture of polysaccharides composed of sulphated galactose units and is derived from Irish Sea moss, *Chondrus cripsus*. 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.

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 and manufactured by sigma-aldrich (merck) [5].

2.4. Standardization

Standardization of *Amoora rohituka* and Melia azedarach stem barks were done according to 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, quality control of crude drug, 1998) [6].

Table no. 1: Calculation of Foreign organic matter for *Amoora rohituka* and *Melia azedarach* stem barks

	A. rohituka	*M. azedarach*
Drug sample taken	100 gm	100 gm
Foreign organic matter	3.67	3.19
% Foreign organic matter	3.67	3.19

Total ash is designed to measure the total amount of material produced after complete incineration of the grounded drug at temperature (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 tarred crucible (usually of platinum or silica), spread the material in an even layer and ignited it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cooled in desiccators and weighed without delay. The content of total ash calculated in mg per gm of air dried material [7].

Table no.2: Different ash values for *Amoora rohituka* and *Melia azedarach* stem barks

Crude drug	Total ash value (mg/g)	Acid insoluble ash value (mg/g)	Water soluble ash value (mg/g)
Amoora rohituka stem bark	331	118	108
Melia azedarach stem bark	354	186	93

Acid insoluble ash value

To the crucible containing total ash, added 25 ml of hydrochloric acid (~70g/l) TS, covered with a watch glass and boiled gently for 5 minutes. Rinsed the watch glass with 5 ml of hot water and added this liquid to the crucible. The insoluble matter collected on ash less filter paper and washed with hot water until the filtrate was neutral. The filter papers' containing the insoluble matters was transferred in to the original crucible, dried on a hot plate and ignited to constant weight. Allowed the residue to cool in suitable desiccators for 30 minutes, and then weighed without delay. The content of acid-insoluble ash was calculated in mg per gm of air dried material. (WHO, quality control of crude drug, 1998) [8].

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 an ashless filter-paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtracted the weight of this residue in mg from the weighed of total ash. The content of water soluble ash was calculated in mg per gm of air dried material. (WHO, quality control of crude drug, 1998) [9].

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 the material for which no chemical or biological assay method exists. 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 about 5 gm of air dried powdered drug with 100 ml of solvents (ethanol 99% v/v) of specific strength in a closed flask for 24 hrs. Shaken frequently during 6 hrs and allowed to stand for 18 hrs. Filtered rapidly taking precaution against loss of solvents. Evaporated 25 ml of filtrate against dryness in a tarred flat bottomed dish dried at 105°C and weighed. Calculated percentage of extractives with reference to air dried drug.

Hot extractive value

The extractive value, which was 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 with solvent and set condenser. Started the assembly and run for 6 hrs or until side tube appeared colorless. Extract from round bottom flask was collected and made up the volume to 100 ml either by evaporation or by dilution. 25 ml of the extract was taken in flat bottomed dish and evaporated on water bath at 50-60°C, finally weighed the extract and calculated the extractive value in percentage with reference to air dried drug. (WHO, quality control of crude drug, 1998) [10].

Table no.3: Cold and hot extractive values for *Amoora rohituka* and *Melia azedarach* stem barks

Crude drug	Cold extractive value	Hot extractive value
Amoora rohituka stem bark	4.8 %	10.2%
Melia azedarach stem bark	10.53%	10.75

Loss on drying

This parameter is used to determine the amount of moisture present in a particular sample. The powdered drug (10 gm) sample was placed on a tarred evaporating dish. The tarred evaporating dish is dried at 105°C for 6 hours and weighed. The drying was continued until two successive reading matched each other or the difference between two successive weighing was not more than 0.25% of constant weight. (WHO, quality control of crude drug, 1998). The loss on drying for *Amoora rohituka* and *Melia azedarach* stem bark was found 7.41% and 7.46%.

Swelling index

Many medicinal plant material display specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicellulose. The swelling index is the volume in ml taken up by the swelling of 1gm of plant material under specific conditions. A glass stopper measuring cylinder was used. The internal diameter of cylinder should be 16 mm. The length of graduated portion was about 125 mm, marked in 0.2 ml division from 0 to 25 ml in an upward direction. 1gm of powered plant material was taken in cylinder. 25 ml 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 *Melia azedarach* stem bark was found 0.97 ml and 1.16 ml.

2.5. Preparation of extract

Dried and coarsely powdered stem barks (250 gm) were extracted with solvents Alcohol (99%), aqueous and hydro alcohol (40:60) using hot soxhlet extraction method, for 24 hrs. Filtered the extract then vacuum evaporated the filtrate and got the crude extracts [11].

2.6. 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.

Table no. 4: The preliminary phytochemical screening for *Amoora rohituka* and *Melia azedarach* stem barks

Phytochemical	Test performed	Alcoholic extract of *Amoora rohituka*	Alcoholic extract of *Melia azedarach*
Carbohydrate	Molish test	+	+
	Benidict test	+	+
	Fehlings test	+	+
	Borfoed test	+	+
	Pentose sugar test	+	+
	Tollens test	+	+
	Iodine test	–	–
	Tannic acid test	–	–
Amino acids	Ninhydrine test	+	+
	Tyrosine test	+	+
	Cystiene 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	–	–

Continued…

Phytochemical	Test performed	Alcoholic extract of *Amoora rohituka*	Alcoholic extract of *Melia azedarach*
Cardiac glycoside	Legal test	–	–
	Keller killiani test	–	–
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	+	+

2.7. Anti inflammatory activity:

1% solution of carrageenan was prepared. 0.1 ml of this solution was injected into the right hind paw of the each rats of eight groups. The standard drug/plant extract at varying doses based on the design of the experiment and control vehicle were given orally 30 min. prior to the injection of carrageenan. The paw volume was measured just before and 1, 2, 3, 4, 5th hrs after administration of carrageenan by the volume displacement methods using a plethismometer.

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 dose 100 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 5th hour 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 F.M *et.al.* 2001)

Results of paw volume changes are presented in observation tables-

Table no. 5- Effect of Ethanolic Extracts of *Amoora rohituka* and *Melia azedarach* 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	
		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 *Melia azedarach* stem bark	100	0.39±0.03*	0.36±0.02*	38.1	40.2

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

Table 6. Effect of Aqueous Extracts of *Amoora rohituka*, and *Melia azedarach* stem bark on Carrageenan Induced Rat Paw oedema.

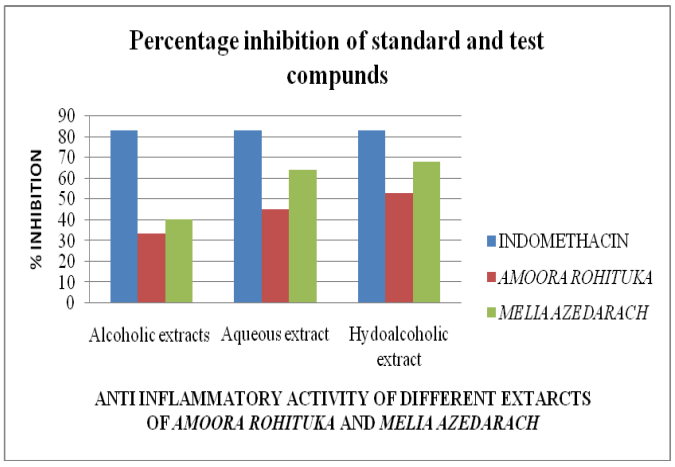
Treatment	Dose (mg/kg, p.o.)	Change in paw volume after treatment (ml) ±SEM		% Inhibition in paw volume	
		3 Hrs	5 Hrs	3 Hrs	5 Hrs
(Normal saline) 1 ml	1	0.61±0.02	0.62±0.01	-	-
Indomethacin	10	0.15±0.03	0.10±0.03	77.1	83
Aqueous extract of *Amoora rohituka* stem bark	100	0.36±0.01	0.30±0.04	40.2	45.4
Aqueous extract *Melia azedarich* stem bark	100	0.24±0.04*	0.20±0.03*	60.1	64.2

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

Table 7. Effect of Hydro alcoholic Extracts of *Amoora rohituka, Melia azedarach* 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	
		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 *Melia azedarach* stem bark	100	0.24±0.04*	0.19±0.03*	59.1	68.2

Fig.1: Anti inflammatory activity of different extarcts of *amoora rohituka* and *melia azedarach*



2.8. Statistical Analysis

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

3. RESULTS AND DISCUSSION

The *Amoora rohituka* and *Melia azedarach* 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. All the standardization parameters of crude drugs results positive and standard. Preliminary phytochemical screening results showed presence of saponine, flavones, and anthraquionone, glycoside, carbohydrate, amino acids, organic acids and steroid in both stem barks.

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 *Melia azedarach* stem bark, resulted **33.4% and 40.2%** inhibition at the dose of 100 mg/kg p.o.

The aqueous extracts of *Amoora rohituka* and *Melia azedarach* stem bark, resulted **45.2% and 64.2%** inhibition at the dose of 100 mg/kg p.o.

The hydroalcoholic extracts of *Amoora rohituka* and *Melia azedarach* stem bark, resulted 53.3**% and 68.2%** inhibition at the dose of 100 mg/kg p.o.

The alcoholic extract of *Melia azedarach* stem barks showed better results against inflammation. The hydroalcoholic extract of *Amoora rohituka* and *Melia azedarach* stem barks showed best results against inflammation.

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