

## ANTI-INFLAMMATORY, ANALGESIC AND ANTIOXIDANT POTENTIAL OF THE STEM BARK OF *SPONDIAS MANGIFERA* WILLD.

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**Abstract** - The anti-inflammatory and analgesic activities of the ethyl acetate (EAFSM) and n-butanol (NBFSM) fractions of the alcoholic extract of *S. mangifera* bark were evaluated using carrageenan-induced rat paw edema and by the tail-flick method in rats. The radical scavenging activity of the ethanolic extract, aqueous extract and fractions was determined with the DPPH radical scavenging capacity assay. Two fractions of the alcoholic extract, EAFSM and NBFSM, at doses of 75, 150, 300 mg/kg b.w. administered orally, showed a significant reduction in paw volume when compared with the respective control group challenged by carrageenan. Different doses of extract fractions also showed a significant prolongation of the tail-flick latency of the rat ( $P < 0.01$ ). Different concentrations of alcoholic, aqueous extracts and fractions of alcoholic extract showed significant free radical scavenging capacity against DPPH generated free radicals.

**Key words:** Amora, inflammation, pain, herbal remedies, carrageenan challenge

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### INTRODUCTION

Inflammation is the initial response of the body to tissue damage caused by mechanical, chemical, or microbial stimuli. Cytokines are the physiological messengers of the inflammatory response and some of the principal molecules involved are tumor necrosis factor-alpha (TNF $\alpha$ ), interleukins (IL-1 and IL-6), interferons, and colony stimulating factors (CSFs) (Davis and Hagen 1996). The main cells involved in the inflammatory response are monocytes/macrophages, polymorphonuclear leukocytes (PMNs) and endothelial cells. When these cells become activated, they aggregate and infiltrate tissue where they undergo a respiratory burst, increasing their oxygen use and the production of cytokines, reactive oxygen species (ROS), and other mediators

of inflammation. ROS can initiate and also perpetuate inflammatory cascades and cause subsequent tissue damage. It is becoming more apparent that ROS do this through the upregulation of a number of different genes involved in the inflammatory response (Conner and Grisham, 1996). The scope of ROS-mediated diseases is believed to be broad, and herbs that scavenge reactive oxidant chemicals before they damage tissue may prevent or slow down these processes (Nelson and Perrone, 2000).

*Spondias mangifera* Willd. (F. Anacardiaceae) is a fast growing tree allied to *Mangifera*, commonly known as Hog-plum (*Amora*) or Bile-tree and *Amrata* in Ayurveda, and is widely distributed in the tropics and abundantly in the eastern and in north-east regions of India. All parts of the plant

produce a fetid turpentine-like odor when broken or brushed (Anonymous 1992). It is a tree with a rich tradition in the ancient health system of Ayurveda and north-eastern people for the management of rheumatism (Kangilal and Das, 1984). The bark is aromatic, astringent, refrigerant and is used to give tone and treatment of rheumatic articular and muscular pain (Kritikar and Basu, 1975). It is given to prevent vomiting and in the treatment of dysentery and diarrhea (Nandkarni, 1976). The root powder is recommended for the regulation of menstruation (Sharma, 2002). Earlier isolated constituents from the aerial parts of the plant *S. mangifera* are ellagitannins, galloyl-geranin, lignoceric acid,  $\beta$ -carotene, vitamin-A, thiamine, riboflavin, ascorbic acid, minerals, etc. (Rastogi and Mehrotra, 1979). Based on these ethnobotanical clues, work was undertaken for the first time to investigate the anti-inflammatory, analgesic and radical scavenging activity of the different fractions of ethanolic extract of *S. mangifera* stem bark.

#### MATERIALS AND METHODS:

##### *Collection and authentication of plant materials*

The stem bark of *S. mangifera* was collected from the Jokoai forest of Dibrugarh, Assam in January 2006. The plant specimen was authenticated by Prof. Muhibul Islam, Dept. of Life Sciences, Dibrugarh University, Assam, with Voucher Specimen No L-32/06.

##### *Chemicals and drugs*

Carrageenan was purchased from the Sigma Chemical Co., St. Louis, MO., ibuprofen and pentazocine from the Ranbaxy labs, New Delhi, 1'-Diphenyl-2-picryl-hydrazyl (DPPH) was procured from Sigma-Aldrich Co MO, USA.

##### *Extraction of plant material*

The air-dried bark of *S. mangifera* was made into a coarse powder. 200 g of the powdered bark was ex-

tracted with 500 ml of 70% w/v ethanol by cold maceration process for 24 h and 72 h, respectively. Then the extract was filtered through muslin cloth and the filtrate was evaporated under reduced pressure and vacuum dried. The yield of ethanolic extract was 27% w/w. The extract was further successively fractionated with petroleum ether, chloroform, ethyl acetate and n-butanol. The percentage yields of petroleum ether, chloroform, ethyl acetate and n-butanol fraction were 1.60%, 1.76 %, 3.23 % and 3.85% w/w respectively.

##### *Preliminary phytochemical investigation*

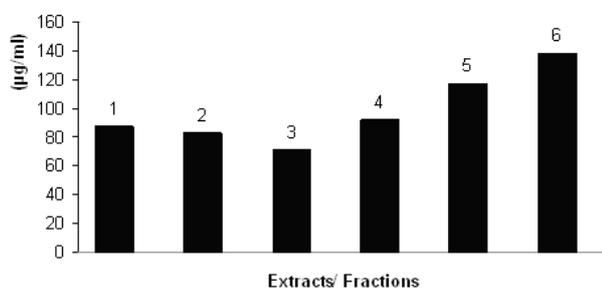
The ethanolic extract of the powdered bark was fractionated with various solvents such as petroleum ether, chloroform, ethyl acetate, and the ethyl acetate insoluble fraction was fractionated with n-butanol. The ethanolic extract, as well as different fractions, were subjected to preliminary phytochemical investigation for the presence of various phytoconstituents (Khandelwal, 2004).

##### *Animals*

Wistar albino rats (150–200 g) of either sex were used in this investigation. The animals were maintained under standard environmental conditions and had free access to feed and water ad libitum. For examining the anti-inflammatory and analgesic activities, the rats were divided into eight different groups (n=6 per group). The study protocol was approved by the Institutional Ethical Committee, GSVM Govt. Medical College Kanpur.

##### *Safety profile study*

An acute toxicity study of the n-butanol and ethyl acetate fractions of the ethanolic extract of *S. mangifera* was carried out for the determination of LD<sub>50</sub> by adopting the fixed dose method (Annexure 2d) of CPCSEA, OECD guideline no.420 (Veeraraghavan, 2000). Wistar albino rats (150-200 g) were used for the study. The number of dead or surviving rats after 24 h was recorded.



**Extracts:-** 1= Aqueous, 2= Ethanol,  
**Fractions of ethanolic extracts:-** 3= Petroleum ether, 4= Chloroform, 5= Ethyl acetate, 6= n-Butanol.

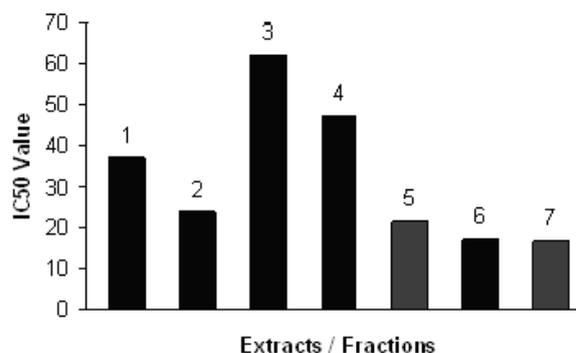
**Fig 1.** Total phenolic content of different extracts/fractions of stem bark of *Spondias mangifera*.

#### Determination of total phenolic contents

The total phenolic content was determined according to the method described by Taga et al. (Taga, Miller and Pratt, 1984). Suitable aliquots of the different extracts/fractions were taken in a test tube and made up to the volume of 1 ml with distilled water. Then, 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Then the tubes were vortexed, placed in the dark for 40 min and the absorbance was recorded at 725 nm. The amount of total phenolics was calculated as gallic acid equivalents/mg from the extract (Fig. 1).

#### DPPH radical scavenging capacity

The effect of extracts on DPPH radical was estimated by Yen and Chen (1995) with minor modification (Yen and Chen, 1995). In brief, 2 ml of DPPH in methanol ( $3.6 \times 10^{-5}$  M) were added to 50 µl of various concentrations of extracts, and fractions (0.025 mM – 1 mM). The mixture was vortexed for 15 s and left to stand at 37°C for 30 min. The decrease in the absorbance at 515 nm was continuously recorded in a spectrophotometer for 15 min at room temperature (Duan et al., 2006). All determination was performed in triplicate. The DPPH scavenging activity (decrease of absorbance at 515 nm) of extracts and compounds were plotted against time and the percentage of the



**Extracts:-** 1= Aqueous, 2= Ethanol, (7= Vitamin - E)  
**Fractions of ethanolic extracts:-** 3= Petroleum ether, 4= Chloroform, 5= Ethyl acetate, 6= n-Butanol.

**Fig 2.** DPPH radical scavenging activity of different extracts/fractions of stem bark of *Spondias mangifera*.

DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 15 min duration as follows

$$\text{Percent inhibition DPPH} = (\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Test}}) / \text{Abs}_{\text{Control}} \times 100$$

#### Carrageenan-induced rat hind paw edema

In all groups acute inflammation was induced by subplantar injection of 0.1 ml of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw of the rats (Mukherjee et al., 1997). The paw volume was measured using a plethysmometer (UGO Basil, Italy) before and 1 h, 2 h and 3 h after carrageenan challenge in each group. The animals were premedicated with vehicle (0.3% CMC p.o.), ethyl acetate and n-butanol fractions of *S. mangifera* (75, 150 and 300 mg/kg p.o.) and standard drug ibuprofen (50 mg/kg p.o.) 30 min before carrageenan injection. Percentage inhibition of the paw volume was calculated at 1 h intervals for 3 h by using this formula (Ajith and Janardhanan, 2001).

$$\% \text{ of Inhibition} = (1 - D / C) \times 100$$

D = Mean change of paw volume in treated group

C = Mean change of paw volume in untreated group

**Table 1.** Chemical test of different extracts/fractions of stem bark of *Spondias mangifera*.

Test	Aqueous	Ethanollic	Pet. ether	Chloroform	EAFSM	NBFSM
Alkaloids	–	–	–	–	–	–
Free sugars	+	+	–	+	+	+
Flavonoids	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Terpenoids	–	+	–	–	+	+
Tannins	+	+	–	–	+	+
Xanthoproteins	+	+	–	–	+	+

(+) = Present and (–) = Absent.

**Table 2.** Effect of ethyl acetate (EFSM) and n-butanol (NBFSM) fraction of stem bark *Spondias mangifera* on carrageenan induced rat paw edema.

Group (n=6)	Dose (mg/kg p.o)	Mean increase in paw volume (ml) (mean ± SEM)			
		0 hr.	1 hr.	2 hr.	3 hr.
Control	0.3% CMC	0.76 ± 0.024	1.28 ± 0.165	1.84 ± 0.192	1.93 ± 0.118
	75	0.71 ± 0.021	1.11 ± 0.019 <sup>ns</sup> (13.28%)	1.45 ± 0.036 <sup>a</sup> (21.19%)	1.40 ± 0.104 <sup>b</sup> (27.46%)
EAFSM	150	0.79 ± 0.031	1.00 ± 0.022 <sup>a</sup> (21.87%)	1.38 ± 0.033 <sup>a</sup> (22.82%)	1.35 ± 0.094 <sup>b</sup> (30.10%)
	300	0.74 ± 0.028	0.98 ± 0.028 <sup>a</sup> (23.44%)	1.37 ± 0.027 <sup>b</sup> (25.54%)	1.30 ± 0.056 <sup>b</sup> (32.64%)
	75	0.75 ± 0.030	1.05 ± 0.032 <sup>ns</sup> (17.97%)	1.44 ± 0.048 <sup>a</sup> (21.74%)	1.36 ± 0.080 <sup>b</sup> (29.53%)
NBFSM	150	0.82 ± 0.038	0.98 ± 0.025 <sup>a</sup> (23.44%)	1.39 ± 0.031 <sup>a</sup> (24.46%)	1.27 ± 0.063 <sup>b</sup> (34.20%)
	300	0.76 ± 0.042	0.96 ± 0.035 <sup>b</sup> (25.00%)	1.34 ± 0.022 <sup>b</sup> (27.18%)	1.21 ± 0.044 <sup>b</sup> (37.31%)
Ibuprofen	50	0.80 ± 0.034	0.95 ± 0.023 <sup>b</sup> (25.78%)	1.22 ± 0.027 <sup>b</sup> (33.7%)	1.11 ± 0.086 <sup>b</sup> (42.48%)

Each values are expressed in Mean ± S.E.M. one way ANOVA followed by Dunnett's test.

ns = non significant P: <sup>a</sup>p < 0.05 & <sup>b</sup>p < 0.01 compare to respective control group.

Figures in parentheses indicate the % of anti-inflammatory activity.

**Table 3.** Analgesic activity of ethyl acetate and n-butanol fraction of *Spondias mangifera* stem bark on tail-flick response in rat.

Treatment mg/kg	Reaction intervals (seconds) at time (min)					
	30	60	90	120	180	
Control 0.3% CMC	2.98 ± 0.150	3.45 ± 0.178	3.54 ± 0.118	3.37 ± 0.092	3.78 ± 0.178	
EAFSM	75	3.54 ± 0.115 <sup>ns</sup>	4.58 ± 0.112 <sup>b</sup>	4.88 ± 0.089 <sup>b</sup>	4.12 ± 0.097 <sup>b</sup>	3.98 ± 0.175 <sup>ns</sup>
	150	3.63 ± 0.245 <sup>a</sup>	5.07 ± 0.124 <sup>b</sup>	5.21 ± 0.087 <sup>b</sup>	4.47 ± 0.178 <sup>b</sup>	4.20 ± 0.172 <sup>ns</sup>
	300	4.03 ± 0.136 <sup>b</sup>	5.18 ± 0.107 <sup>b</sup>	5.34 ± 0.100 <sup>b</sup>	5.19 ± 0.087 <sup>b</sup>	4.58 ± 0.172 <sup>b</sup>
NBFSM	75	3.61 ± 0.225 <sup>ns</sup>	4.73 ± 0.138 <sup>b</sup>	4.96 ± 0.115 <sup>b</sup>	4.63 ± 0.067 <sup>b</sup>	4.02 ± 0.078 <sup>ns</sup>
	150	3.71 ± 0.179 <sup>a</sup>	5.30 ± 0.119 <sup>b</sup>	5.39 ± 0.086 <sup>b</sup>	5.20 ± 0.093 <sup>b</sup>	4.23 ± 0.086 <sup>a</sup>
	300	4.28 ± 0.112 <sup>b</sup>	5.80 ± 0.151 <sup>b</sup>	5.83 ± 0.135 <sup>b</sup>	5.40 ± 0.169 <sup>b</sup>	4.72 ± 0.052 <sup>b</sup>
Pentazocin	10	4.39 ± 0.113 <sup>b</sup>	6.31 ± 0.171 <sup>b</sup>	6.53 ± 0.164 <sup>b</sup>	5.67 ± 0.142 <sup>b</sup>	5.39 ± 0.082 <sup>b</sup>

Each values are expressed in Mean ± S.E.M. one way ANOVA followed by Dunnett's test.

ns = non significant P: <sup>a</sup>p < 0.05 & <sup>b</sup>p < 0.01 compare to respective control group.

### *Analgesic activity*

The tail-flick latency test was performed by an analgesiometer (INCO, Ambala, INDIA) (Chakraborty et al., 2004). The tail-flicking response from heat was taken as the end point. Reaction time was noted at 30, 60, 120 and 180 min after the drug dosing. As the reaction time reaches 10 s, it was considered to be maximum analgesia and the tail was removed from the source of heat to avoid tissue damage. The strength of the current passing through the naked nichrome wire was kept constant at 6 amps. Animals were treated with vehicle (0.3% CMC p.o.), ethyl acetate and n-butanol fractions of the ethanolic extract of *S. mangifera* (75, 150 and 300 mg/kg p.o.) and reference drug pentazocine (10 mg/kg i.p.).

### *Statistical analysis*

The data were calculated as the means  $\pm$  S.E.M. Statistical significance was determined by one-way ANOVA followed by Dunnett's test. Values of P less than 0.05 were considered significant.

## RESULTS AND DISCUSSION:

Preliminary phytochemical screening of the different fractions of the ethanolic extract revealed the presence of carbohydrates, flavonoids, terpenoids and tannins as major phytoconstituents. A phytochemical test of the n-butanol and ethyl acetate fractions showed the presence of terpenoids, flavonoids, tannins and phenolic compounds (Table 1). Hence these two fractions were selected for the anti-inflammatory studies.

From acute toxicity studies it was observed that the administration of n-butanol and ethyl acetate fractions of *S. mangifera* to rats did not induce drug-related toxicity and mortality in the animals. The rats tolerated the fractions well and exhibited normal behavior up to 1.5gm/kg orally. All animals were alert with normal grooming, touch response, pain response and there was no sign of passivity, stereotypy, and vocalization.

### *Total phenolic content*

The extracts and fractions were found to have various phenolic levels (Fig. 1), ranging from 83 to 138  $\mu\text{g/ml}$ . The highest concentration of total phenolics was present in the ethyl acetate and n-butanol fractions. The content of total phenolic compounds in the ethyl acetate fraction was 117  $\mu\text{g/ml}$  and in the butanol fraction was 138  $\mu\text{g/ml}$ .

### *DPPH radical scavenging activity*

It was observed that the %-inhibition (at 15 min) with the  $\text{IC}_{50}$  values of the aqueous extract, ethanolic extract and petroleum ether, chloroform, ethyl acetate and n-Butanol fractions of the ethanolic extract of *S. mangifera* was found to be 37, 26.5 and 62, 47.5, 24, 17  $\mu\text{g/ml}$  compared to standard Vitamin E 16.8  $\mu\text{g/ml}$  (Fig. 2). Among the various fractions tested, only the ethyl acetate and n-butanol fractions showed good activity in scavenging DPPH radical with an  $\text{IC}_{50}$  value of 24 and 17  $\mu\text{g/ml}$ , respectively.

### *Carrageenan-induced rat hind paw edema*

The results obtained indicate that both n-butanol and ethyl acetate fractions, in all the doses administered, reduced the edema in the rats when compared to the control group. At 3 h of observation there was a statistically significant difference ( $P < 0.01$ ) between the control group and all doses of the fractions. It has also been observed that the inhibitory effect of the n-butanol fraction was higher than that of the ethyl acetate fraction (32.64% and 37.31%, respectively) (Table 2).

### *Analgesic activity*

The results obtained indicate that both the n-butanol and ethyl acetate fractions of the ethanolic extract showed a significant prolongation in the tail-flick latency of the rats ( $P < 0.01$ ). The peak analgesic effect of all doses of both the fractions was observed at 90 min of oral administration. The highest analgesic activity was shown by NBFMS (300 mg/kg b.w.) (5.90

sec at 90 min) followed by EAFSM (300mg/kg b.w.) (5.34 sec at 90 min) (Table 3).

#### CONCLUSION:

Carrageenan is a natural carbohydrate polymer used as a phlogistic agent. Carrageenan-induced edema is caused by the activation of platelet activating factors (PAF), prostaglandins and other inflammatory mediators. The first phase is attributed to the release of histamine, 5-HT and kinin, while the second phase (3 h) is related to the release of bradykinin and prostaglandin. Carrageenan also induces protein-rich exudates containing a large number of neutrophils (Lo, Almeida and Beveavan, 1982). All doses of EAFSM and NBFSM showed a reduction in paw volume from 1 h to 3 h, but the maximum reduction in paw volume was observed at 3 h of carrageenan challenge. From the data it is evident that NBFSM showed a greater percentage of decrease in paw volume than EAFSM.

The free radical scavenging capacity of the ethanolic extract and its various fractions were investigated by DPPH. The results from the DPPH assay revealed that the fractions, in particular the n-butanol separated from the crude alcoholic extract, showed an efficient quenching of DPPH and thus contain free radical quenching compounds, which act as primary radical scavengers that react with DPPH by providing a hydrogen atom or electron donating ability (Huang, Ou and Prior, 2005; Cotellet, 1996).

All the doses of both the fractions showed significant antinociceptive activity and NBFSM exhibited superior activity to EAFSM. Thirty minutes after drug administration the reaction time increased significantly for the groups treated with standard drug and test extracts when compared to the control group. Between the 60 and 120 min of observation a statistically significant difference ( $P < 0.01$ ) from the control group was found by all doses of both the fractions. It is possible that both the fractions EAFSM and NBFSM may modulate some neurotransmitters/neuromodulators involved in

the regulation of pain sensitivity. The bark extract of *S. mangifera* is reported to contain a phenolic group of compounds and tannins and flavonoids that have been reported to produce significant an opioid channel-mediated antinociceptive response similar to morphine (Yoganarasimhan, 1996). Thus it can be concluded that the antinociceptive activity of the plant may be due to the presence of a phenolic group of compounds, tannins and flavonoids. Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known. *S. mangifera* bark extract is suggestive of a high therapeutic potency against disease, especially in inflammatory disorders.

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