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RESEARCH ARTICLE

Antioxidant and Analgesic Activity of Leaf Extracts of *Artocarpus heterophyllus*

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ABSTRACT:
Background: Traditional Indian medicine has always found its way for the treatment of multiple diseases and conditions. *Artocarpus heterophyllus* is a common plant and can easily be exploited for its rich medical heritage.
Methods: Two leaf extracts were made. Both the aqueous and ethanolic extracts were subjected to phytochemical screening. Analgesic property was established with the help of Eddys hot plate ,acetic acid induced writhing ,Tail Flick methods. One way ANOVA was used to correlate the significance $p<0.005$. Antioxidant properties were evaluated with DPPH and Nitric oxide scavenging activity.
Results: The ethanolic extract shows a statistically significant ($p<0.005$) correlation towards the analgesic activity in both the methods namely hot plate method and writhing method. The ethanolic leaf extracts showed the following phytochemicals—Flavonoids, phenols, terpenoids, saponins, steroids while the aqueous extracts showed the presence of flavonoids, phenols, saponins and steroids. Both the extracts showed significant antioxidant activity.
Conclusion: This study shows the Phytochemical evaluation and analgesic activities of both the extracts. Hence further studies should be made to isolate the necessary constituent for both the activities.

KEYWORDS: *Artocarpus heterophyllus*, Analgesia, Jack Fruit leaves.

INTRODUCTION:

Artocarpus heterophyllus traditionally known as the Jack fruit tree and a member of the family Moraceae (Shrinath Baliga et.al 2011)[1]. It is a fairly wild growing, medium-size evergreen tree typically reaching 8–25 m (26–82 ft) in height, consisting of glossy, alternate ,leathery leaves to 22.5cm in length. (Torane Rasika Charudatta et. al 2015)[2] indigenous to the ever green forests at an height of 500-1, 300m and cultivated throughout the hotter parts of India. It is well suited to tropical lowlands and has been used as traditional folk medicine. (Om Prakash et.al 2013)[3].

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The tree can provide many environmental services. In home gardens, the dense jackfruit canopy can provide a visual screen and is very ornamental. It is highly wind tolerant and therefore makes a good component in a windbreak or border planting. Growing in pastures, it can provide fallen fruit for livestock, shade, and longterm timber. (Craig R. Elevitch et.al 2006)[4]. The fruit is the edible part and has an average weight of 10 kg. The yellowish bulbs containing the perianth portion of the fruit are fleshy, fibrous, and rich in sugars as well as carotenoids. It contains a rich source of carbohydrates, minerals, carboxylic acids, dietary fibres, and key vitamins such as ascorbic acid (vitamin C) and thiamine (vitamin B1) (Haidy S. Oma et.al 2011)[5]. Leaves are dark green, alternate, entire, simple, glossy, leathery, stiff, large i. e up to 16 cm length (6 inches), and elliptic to oval in form. Leaves are often deeply lobed within when small and on young shoot (Craig R. Elevitch et.al 2006)[4]. Hot water extract of mature jack leaves is recommended by ayurvedic and medical practitioners as a treatment of diabetes mellitus (Raja

sekhar K.K et.al 2010)[6]. In herbal homes, leaves of this plant are used for the treatment of hepatic disorders, hypertension, and diabetes (Oluwatosin Adekunle et al 2014)[7]. *heterophyllus* exhibit numerous medicinal properties such as antibacterial, antioxidant, antidiabetic, anti-inflammatory, anti-diuretic, and have been useful in the treatment of fever, skin diseases, convulsions, constipation, ophthalmic disorders and snake bite (Shirajummunira et.al 2014)[8].

Taxonomical classification: (Prakash O et.al 2009) [9]
Kingdom: Plantae
Subkingdom: Tracheobionta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Hamamelidae
Order: Urticales
Family: Moraceae
Genus: *Artocarpus*
Species : *Artocarpusheterophyllus* Lam.

Various parts of the plant used:
Apart from whole plant, seeds, fruits, bark, root, leaves and latex are also used.

Materials and Methods: Collection of materials:
The leaves of *Artocarpus heterophyllus* were collected from a local herbarium in Chennai, Tamilnadu and the authentication was carried out in Dept. of Botany, Vels University. The leaves are shade dried.

Animals:
Swiss albino mice of 20-25g weight of both sex were used. The animals were fed with pellet diet and maintained in standard conditions. The usage of animals has been approved by the Institutional Animal Ethics Committee XVI/ VELS/ PCOL/ 04/ 2000/ CPCSEA/ IAEC/ 25.11.14.

Preparation of Plant Extracts:
Preparation of Extracts:
The leaves were washed with tap water and then with distilled water and dried in shade. The leaves were made to powder and extracted with ethanol and water. The yield of aqueous extract was found to be 37.45%w/v while ethanolic extract was 29.34% w/v.

Phytochemical screening:
The plant extracts were subjected to Phytochemical screening for the presence of alkaloids (Dragon draff's method and Wagner reagent), glycosides, proteins, aminoacids, saponins, terpenoids etc.

Analgesic Activity:
Eddy's Hot Plate Method :
The hot plate test is used to calculate analgesic activity by the method explained by Eddy and Leimbach with minor modifications. In this method, mice were divided in five groups of six animals in each group. Mice were placed on a hot plate having a stable temperature of 55±1 ^{0}C. Each mice was individually placed on the hot plate in order to find the animal's reaction to electrical heat-induced pain such as licking of the forepaws and eventually jumping. The time taken for either paw licking or jumping was recorded. The latency until mice showed first signs of discomfort like hind paw lifting, hind paw licking, or jumping was recorded, before (baseline). The response of the mice was determined at 30, 60 and 120 min after the administration of normal saline, *Artocarpus heterophyllus* (50, 100, and 200 mg/kg), and aspirin (10 mg/kg). The cut off time was considered as 15 seconds.(Masoume Rezaee-Asl et.al 2014)[10].

Acetic acid-induced writhing method:
The acetic acid induced writhing method is adopted for analgesic behavioral observation assessment. It demonstrates a noxious stimulation in mice. The pain reaction is characterized as a writhing response which includes stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). (Swati Paul et.al 2012)[11] .In this method, mice were divided in five groups of six each. The animals were previously treated with drugs 45 minutes before induction of writhing. The animals received the standard drug aspirin (10 mg/kg, i.p.) which served as reference standard. Analgesic activity of *Artocarpusheterophyllus* (50,100, and 200 mg/kg, i.p.) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml/kg i.p.). The number of writhes observed per animal was counted for the next 20 minutes. Percentage protection against abdominal constriction was taken as an index of analgesia (R.S. Bachhav et.al 2009)[12]. It was calculated as: (Number of writhing in control group – Number of writhing in treated group ÷ Number of writhing in control group)X 100

Tail Flick Method:
The tail flick examination was used to calculate analgesic activity by the method defined by D'amour and Smith 1941, with minor alterations in the procedure. The tail flick method is used to study the antinociceptive activity in mice. A radiant heat automatic tail flick analgesiometer is utilized to measure reaction latencies. Basal reaction time of animals to radiant heat was recorded by locating the tip (last 1-2 cm) of the tail on radiant heat source. The tail removal from the radiant warmth was taken as end point. The cut off time of was considered as15 seconds in order to avoid tail injury by heat. Mice were divided into five groups (n=6). Mice were treated with aspirin (10 mg/kg), normal saline, and *Artocarpus heterophyllus* (50, 100, and 200 mg/kg). The latent period of the tail-flick response was determined at 15, 30, 45 and 60minutes after the administration of drugs (Masoume Rezaee-Asl et.al 2014)[13].

Nitric oxide scavenging activity:
Nitric oxide scavenging activity was measured spectrophotometrically. The extracts were prepared from a 10 mg/mL ethanol crude extract and was added to different test-tubes in varying concentrations such as 0.25, 0.5, 1, 1.5, 2, 2.5 mg/ml. Sodium nitroprusside (5mM) in phosphate buffer is added to each test tube in order to make the volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes.(S. Banerjee et.al 2011)[14]. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use (Fadzai Boora et.al 2014)[15]. 1.5ml of Griess reagent was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard. (S. Banerjee et.al 2011)[15].

DPPH method:
The free radical scavenging capacity of the compounds was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method with minor modifications. Test compounds were allowed to react with stable free radical, DPPH for 30mins at 37°C. The concentration of DPPH was 100 mM. The plants extracts were dissolved in DMSO (Dimethyl sulfoxide), while the DPPH solution was prepared in methanol n-Propyl gallate and 3-t-butyl-4-hydroxyanisole were used as standards. Five dilutions for each compound and standards were tested. Each dilution was tested in triplicate. After incubation, decrease in absorbance was measured at 517 nm using microplate reader. Percent radical scavenging activity (% RSA) of samples is determined in comparison with DMSO treated as control group by using the following formula: (Jamshed Iqbal et.al 2012)[16].

$$\% \text{ RSA} = \frac{(100 - \text{Absorbance of test compound})}{\text{Absorbance of control}} \times 100$$

Statistical analysis:
The statistical analysis was performed using one way ANOVA and the p value of $p<0.005$ is considered significant.

RESULTS:

The following results were obtained in this study:

Phytochemical screening:
The ethanolic leaf extracts showed the following phytochemicals—Flavanoids, phenols, terpenoids, saponins, steroids while the aqueous extracts showed the presence of flavonoids, phenols, saponins and steroids.

Analgesic activity:
The analgesic activity was reported in table no 1, 2 and 3.

Antioxidant activity:
The antioxidant activity was reported in table no 4 and 5.

Table 1: Eddy's hot plate

S.no	Groups	Paw licking time			
		Before	30 mins	60mins	120mins
I	Control(Normal saline)	0.36±0.22	5.42±0.16	5.30±0.32	5.06±0.46
II	Aqueous extact (250mg/kg)	5.32±0.47	14.26±0.21*	17.01±0.16**	13.30±0.32*
III	Aqueous extact (500mg/kg)	5.16±0.12	16.32±0.29*	19.32±1.00**	15.45±0.29**
IV	Ethanolic extact(250mg/kg)	6.00±0.30	19.21±0.17*	22.16±0.15*	18.28±0.32**
V	Ethanolic extact(500mg/kg)	4.57±0.52	23.10±0.19**	28.32±0.45**	25.32±0.42
VI	Aspirin(10 mg/kg)	5.12±0.32	26.22±0.32	31.55±0.32	27.32±0.45

All values are expressed in mean ± SEM(n=6) *p<0.005 ,** p<0.001

Table 2: Acetic acid induced writhing method:

Group	No. of writhes 30mins (mean ± SEM)	% Inhibition
Control (NS)	65.40±3.27	—
Aqueous extact (250mg/kg)	37.12 ±6.10*	43.24
Aqueous extact (500mg/kg)	31.10 ±5.42*	52.44
Ethanolic extact(250mg/kg)	34.24 ±4.10*	47.64
Ethanolic extact(500mg/kg)	24.16 ±52*	63.05
Aspirin(10 mg/kg)	21.45 ±2.72*	66.59

All values are mean ± SEM(n=6) one way ANOVA was performed*p<0.001

Table 3: Tail Flick method:

Group	Treatment	Dose	Basal reaction time	Reaction time (after)			
				15 min	30min	45min	60min
1	NS	10ml/kg	2.10±0.27	2.33±0.34	2.41±0.33	2.51±0.25	2.34±0.12
2	Aspirin	10mg/kg	2.16±0.32	4.21±0.24	5.17±0.16	6.81±0.15	8.41±0.51
3	Aqueous extract	250mg/kg	2.32±0.32	5.17±0.12	5.47±0.25	$6.97\pm0.12^{*}$	$8.53\pm0.41^{*}$
4	Aqueous extract	500mg/kg	2.16±0.16	4.21±0.46	$5.21\pm0.17^{*}$	$6.17\pm0.05^{*}$	$7.59\pm0.19^{*}$
5	Ethanolic extract	250mg/kg	2.00±0.11	$3.16\pm0.26^{*}$	$4.14\pm0.12^{*}$	$5.16\pm0.45^{**}$	$5.56\pm0.12^{**}$
6	Ethanolic extract	500mg/kg	2.05±0.52	$2.49\pm0.34^{**}$	$3.10\pm0.21^{**}$	$3.52\pm0.15^{**}$	$3.96\pm0.18^{**}$

All values are mean ±SEM, *p<0.01, **p<0.001.

Table 4: Nitric oxide radical scavenging property of aqueous extract and Ethanolic extract with Ascorbic acid

S. No	Concentration (µg/ml)	% Inhibition		
		Aqueous	Ethanolic	STD
1.	50	24.13 ± 0.41*	44.12 ± 0.49**	61.65 ± 0.71**
2.	100	26.03 ± 1.01*	56.45 ± 0.81**	72.85 ± 0.32**
3.	200	31.98 ± 0.91**	68.12 ± 1.08**	81.21 ± 0.29*
4.	400	38.76 ± 0.32**	80.15 ± 0.93*	86.06 ± 0.41**
5.	800	40.86 ± 1.13*	85.12 ± 1.13**	90.96 ± 0.87**
6.	1000	48.65 ± 1.28*	93.45 ± 0.96**	96.61 ± 1.09**
7.	IC_{50}	108 µg/ml	52 µg/ml	41.12 µg/ml

Values are mean ± SEM of 6 parallel measurement.
All the values are significant, **P< 0.01 when compared against control
All the values are significant *P< 0.01 when compared against standard.

Table 5: DPPH Free radical scavenging property of aqueous extract and Ethanolic extract with Ascorbic acid

S. No	Concentration (µg/ml)	% Inhibition		
		Aqueous	Ethanolic	STD
1.	50	24.10 ± 0.27*	51.15 ± 0.21*	57.12 ± 0.81**
2.	100	29.69 ± 0.63*	60.12 ± 1.04*	61.24 ± 0.63*
3.	200	32.73 ± 1.80**	65.06 ± 1.81*	67.25 ± 1.80*
4.	400	34.81 ± 1.62*	74.18 ± 1.12*	76.07 ± 1.62**
5.	800	40.25 ± 1.40*	81.97 ± 1.32**	82.19 ± 1.40*
6.	1000	43.12 ± 0.81*	88.79 ±1.28*	94.19 ± 0.81
7.	IC_{50}	129 µg/ml	45.8 µg/ml	42.24 µg/ml

Values are mean ± SEM of 6 parallel measurements.
All the values are significant, **P< 0.01 when compared against control

DISCUSSION:

The presence of phytochemicals such as flavonoids and steroids are also elicited in the study done by M. Binumol et al 2013[17]. This study also showed no toxic effects of both the extracts upto a dose of 2000mg/kg. The ethanolic extract shows a statistically significant correlation towards the analgesic activity in both the methods namely hot plate method and writhing method. This result is also similar to study on a different plant extract by Om Prakash et al [3].

The aqueous extract also shows a statistically significant correlation towards the analgesic activity in both the methods namely hot plate method and writhing method. This result is also similar to study on a different plant extract by Om Prakash et al[3]. The presence of antioxidant activity by both the methods was similar to the study conducted by Omar HS et al[18]. Further studies are recommended for the isolation of constituents responsible for the analgesic activity.

CONCLUSION:

This study shows the Phytochemical evaluation and analgesic activities of both the extracts. Compound isolation and spectra studies are recommended for further research on these properties of Arthocarpus species.

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