

COMPARATIVE STUDY OF AGNIMANTHA SPECIES FOR ANTI - INFLAMMATORY POTENTIAL

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ABSTRACT

Premna integrifolia Linn and *Clerodandrum phlomidis* Linn are known under the common name Agnimantha-dvaya [two types]. Roots of both plants are potent inflammatory [Shothahara] and are considered to be useful in the treatment of variety of ailments. According to classical texts, both possess the same properties, so the present study was aimed at evaluating the two roots for their Anti-inflammatory potential by using decoction of drugs. Pharmacognostical study containing organoleptic, Microscopic and physicochemical analysis have been performed. In Experimental study Anti-inflammatory activity was performed on rat paw edema model by using decoction of drugs. This activity defines that both drugs, *Premna integrifolia* Linn and *Clerodandrum phlomidis* Linn show Anti-inflammatory activity by inhibiting the paw volume increase induced by the chemical carrageen. By the experimental study it is clear that both the drugs show anti-inflammatory activity but *Premna integrifolia* Linn is more potent drug than *Clerodandrum phlomidis* Linn.

Keywords: *Premna integrifolia* Linn, *Clerodandrum phlomidis* Linn, Anti-inflammatory activity

INTRODUCTION

Several Anti-inflammatory [Shothahara] Drugs are mentioned in the literature of Ayurveda. Among these *Dashmula* [ten roots] is one of the important group of plants. This is used in Ayurveda as Vataghna [pacify vata] which results in reduction of pain and inflammation. Out of these, *Agnimantha* is one of the potent drug which is used single as well as in various formulations mentioned in classics [1] [2]. In Ayurvedic literature, two types of *Agnimantha* are mentioned as Bruhat (*Premna integrifolia* Linn.) and Laghu (*Clerodandrum*

phlomidis Linn.) variety. Both type of *Agnimantha* show same properties as mentioned by classical texts [3]. It has properties like Appetizer [Deepana], Digestive [Pachana], Diabetes [Pramehaghna], Analgesics [Vedanasthapana, Shula prashamana], Antipyretic [Jwraghna] and Anti-inflammatory [shothahara] etc [4]. Various source plants from the genus *Clerodendrum* and *Premna* are used in different regions of the country. Harshitha Kumari et al. had carried out work on two species of *Premna* for anti-inflammatory activity against carrageen in-

duced rat hind paw edema [5]. Present study covers the pharmacognostic, physicochemical and anti-inflammatory experimental of both type of drug.

MATERIAL AND METHODOLOGY

Collection of plant material: The fresh roots of the drug *Premna integrifolia* Linn was collected from Vile, Bhagad, Raigad and *Clerodandrum phlomidis* Linn is collected from Jeur, Pune, and Maharashtra. Roots were identified with the help of different floras [6] and authenticated at Agharkar Research Institute and at Department of Botany Pune. Matured roots were cleaned properly, cut in to pieces, shade dried and coarsely powdered (10 mesh). The sample was analyzed by using different organoleptic, qualitative and quantitative parameters.

Organoleptic evaluation: In the organoleptic evaluation various sensory parameters such as color, odor, taste and texture were investigated.

Physico-chemical studies: The physico-chemical parameters such as loss on drying, total ash content, pH, and extractive values, (water-soluble and alcohol soluble) were determined. These parameters were analyzed in accordance with the Ayurvedic Pharmacopeia of India. [7]

Chromatographic study: Thin layer chromatography [TLC] of Alcohol extract was carried out for the normal phase separation of components. [8]

EXPERIMENTAL STUDY

Animals

After permission and approval from the animal ethics committee (reference no.

BVDUMC / 184 / 2014-2015), experimental study was carried out at IRSHA, BharatiVidyapeeth, Deemed University, Pune. 48 Albinos Wister rats were taken and all arranged in 8 groups containing 6 rats in each group. Animals were maintained at room temperature at 25 °C, with 12 hours day and dark cycles. Standard laboratory diet was given with an unlimited water supply of drinking water. The animals are randomly selected, marked to permit individual identification and kept in their cages for 3 day prior to dosing to allow for acclimation to the lab condition.

Dose fixation and schedule-

The human dose of decoction prepared from both the plants is 96-100 ml/day. Considering adult human dose of both the plants, the dose for experimental study was calculated by converting the human dose to animal dose based on the body surface area ratio using the table of Paget and Barnes. [9]

The study was carried out using three drug dose levels as [X/2, X, 2X] (4.5ml/kg, 9ml/kg, and 18ml/kg). Control group was given plain distilled water. As a Positive control, standard anti-inflammatory drug diclofenac was taken.

Route of drug administration-

The test drugs were administered by the oral route with the help of No. 6 gastric catheter sleeved onto a syringe.

Animal grouping-

The selected 48 Wistar rats were divided in 8 groups each group containing 6 numbers of animals. They were starved overnight with water prior to the day of experiment.

Table 1: Grouping of Animals for experimental study

Sr. no.	Groups (n=6)	Treatment	Dose
1.	Group I- NC	Carrageenan only- Negative Control	Distilled water
2.	Group II- DICLO	Diclofenac- Positive Control	10 mg/kg B.W.
3.	Group III- PI L	<i>P. integrifolia</i> Linn. lower dose	4.5 ml/kg B.W.
4.	Group IV- PI M	<i>P. integrifolia</i> Linn. middle dose	9 ml/kg B.W.
5.	Group V -PI H	<i>P. integrifolia</i> Linn higher dose	18 ml/kg B.W.
6.	Group VI -CP L	<i>C. phlomidis</i> Linn. lower dose	4.5 ml/kg B.W.
7.	Group VII- CP M	<i>C. phlomidis</i> Linn middle dose	9 ml/kg B.W.
8.	Group VIII- CP H	<i>C. phlomidis</i> Linn. higher dose	18 ml/kg B.W.

Procedure:

- The control group received plain distilled water orally, while other group received standard drug and test drugs respectively.
- On the day of experiment, animals were administered with test drugs to respective groups.
- After 1 hr. of drug administration, rats were challenged by a sub-plantar injection of 0.1 ml of 1% freshly prepared carrageen suspension into a planter side of the right hind paw.

Criteria For assessment:

The paw volume measured before (0 h), and at intervals of 1, 2, 3, 4, 5 and 6 h after car-

rageen injection using plethysmometer (ref. Orchid Scientific, Model PLM 01 PLUS).

The percent Inhibition of paw oedema on the basis of paw volume calculated using the formula % oedema inhibition = [1- (Vt / Vc)] X 100, Where, Vt is paw volume in the drug treated group and Vc is paw volume in control group.

OBSERVATIONS:

PHARMACOGNOSTICAL STUDY:

Organoleptic examination of drug: The morphological characteristics of the *Premna* and *Clerodandrum* are shown in Table 2, 3.

Table 2: Organoleptic characters of Roots of both species

Organoleptic Characters	<i>Premna integrifolia</i> Linn.		<i>Clerodandrum phlomidis</i> Linn.	
	Root	Decoction	Root	Decoction
Consistency	Coarse	Liquid	Coarse	Liquid
Colour	Dark brown	Light brown	Dark brown	Dark brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Bitter, Astringent	Bitter, Astringent	Sweet, Astringent	Sweet, Astringent
Touch	Rough	Warm	Rough	Warm

Table 3: Macroscopic features of root parts:

Character	<i>Premna integrifolia</i> Linn.	<i>Clerodandrum phlomidis</i> Linn.
Shape	cylindrical	cylindrical
Size	5-7 cm long & 1-3 cm thick	5-7 cm long & 1-3 cm thick

Bark	Thin, easily peeled	Thin, easily peeled
Outer surface	Lenticellate, wrinkled	Rough, wrinkle & lenticels in all samples, slightly smooth
Colour of bark	Root light brown to yellow brown,agreeably scented	root yellowish-brownwood light dull yellow
Fracture	Hard with sound	Hard with sound
Other feature	Root is solid.	Light in weight

Physico chemical analysis: In physical evaluation, moisture content, water and alcohol extractive values, Microbial count was determined.[Table 4]

Table 04:Physico chemical parameters of both species

Sr no.	Physiochemical analysis	Obtained values	
		<i>Premna integrifolia</i> Linn.	<i>Clerodandrum phlomidis</i> Linn.
1	Total ash	4.4128%	4.0795%
2	Acid insoluble ash	0.769%	0.5716%
3	Alcohol soluble extractive value	6.778%	7.139%
4	Water soluble extractive value	10.54%	8.382%
5	ph Value	6.86	5.60
6	Microbial Count	4*10 ⁴ cfu/gm	2*10 ⁴ cfu/gm

Thin Layer Chromatography- Thin-layer chromatography was executed for the normal phase separation of components of Alcohol extracts of root of *Premnaintegrifolia* and *clodandrumphlomidis*. For TLC screening, solvent system was prepared by taking Chloroform and Methanol in a proportion of 85:15. Stationary phase for the TLC profile

was silica gel G60F254. The spots obtained from the extract were examined under ultra violet light of wavelength 254 and 365 nm. The resolution factor was calculated by using the formula $Rf = \text{distance travelled by solute} / \text{distance travelled by solvent}$. The result is depicted in table no. 05

Table 05- TLC profile

Sr. no.	Detector	<i>Premnaintegrifolia</i> Linn.		<i>Clerodandrumphlomidis</i> Linn.	
		Rf value of	Colour of spots	Rf value of	Colour of spots
1	UV 365 nm	0.057	Blue	0.057	Blue
		0.19	Dark blue	0.86	Yellow
		0.28	Dark blue	0.90	Yellow

2	Iodine vapours	0.057	All Yellow	0.057	All yellow
		0.19		0.70	
		0.28		0.79	
		0.70		0.86	
		0.86		0.90	
		0.03			
3	5% methanol sulphuric acid	0.28	All Grey	0.57	All Grey
		0.70		0.70	
		0.86		0.79	
		0.93		0.86	
				0.90	

OBSERVATIONS OF EXPERIMENTAL STUDY

Table 06: Comparison percentage inhibition of paw oedema in 3rd and 6th hour of dosing

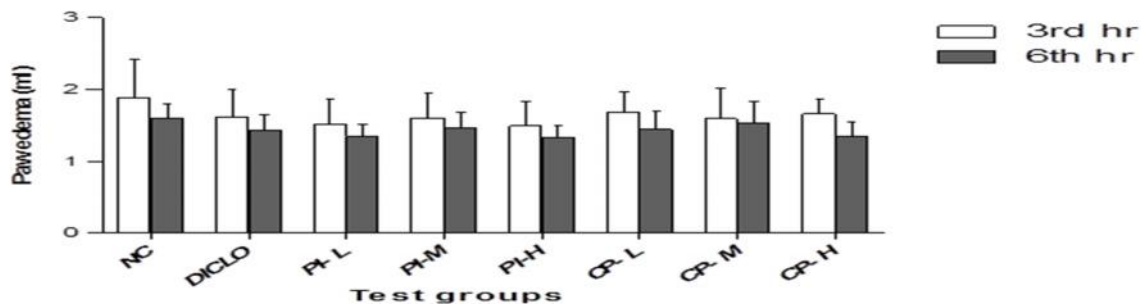
Group	inhibition of paw edema in 3 rd		inhibition of paw edema in 6 th hour	
	Avg	% inhibition	Avg	% inhibition
Group I- NC	1.89±0.534		1.60±0.192	
Group II- DICLO	1.61±0.374	14.37	1.44±0.211	10.10
Premna integrifolia- Lower, Medium, higher dose				
Group III- PI L	1.52±0.645	19.66	1.34±0.170	16.15
Group IV- PI M	1.60±0.342	15.26	1.47±0.221	8.44
Group V -PI H	1.49±0.346	21.08	1.33±0.176	16.98
Clerodandrum phlomidis -Lower, Medium, higher dose				
Group VI -CP L	1.68±0.287	11.02	1.44±0.249	9.90
Group VII- CP M	1.59±0.427	15.87	1.52±0.305	4.79
Group VIII- CP H	1.66±0.2143	12.26	1.35±0.207	15.94

The results showed that *Premna integrifolia* [4.5 ml, 9ml and 18 ml] and *Clerodandrum phlomidis* [4.5 ml, 9ml and 18 ml] showed 19.66 %, 15.26%, 21.08 % and 11.02, 15.87 %, 12.26 % of inhibition on carrageenan induced rat paw oedema at 3rd hour. Statistically significant inhibition of paw edema was observed at higher dose in 3rd hour and 6th hour in *P. integrifolia* Linn, while *Cler-*

odandrum phlomid higher dose showed 15.94 % inhibition at 6 hour.

Among both trial groups, the drug *Clerodandrum phlomidis* showed maximum % of inhibition of Odema [15.94 %] at sixth hour in higher dose, but it was less than *P. integrifolia* Linn higher dose [21.08% inhibition] at 3rd hour.

Graph anti-inflammatory (compatibility mode):



DISCUSSION

In *Ayurvedic* literature, several *Shothaghna Dravyas* are mentioned. *Agnimanthdwayam*, i.e. *P. integrifolia Linn* and *Clerodandrum phlomidis* both the drugs have been used in the treatment inflammation. The present experimental study was carried out to find out anti-inflammatory activity of varieties of *Agnimantha* i.e *Premna integrifolia* and *Clerodandrum phlomidis* by using carrageen induced rat paw oedema model.

Carrageenan induced rat paw oedema is commonly used in animal models for acute inflammatory agents and is of biphasic event. The initial phase is attributable to the release of histamine, 5-HT and various kinins in the first hour injection of carrageenan. Second phase is released of prostaglandin-like substance.[10]

Phytochemical analysis suggested that all the parameters are as per API guideline assure the quality of collected drug.

CONCLUSION

Both the plants can be used as the source plant of *Agnimantha* with respect to different aspects of drug. In experimental study it is clear that both the drugs show anti-inflammatory activity but *Premna integrifolia* is more potent drug then *Clerodandrum phlomidis*.

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