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FORMULATION OF OIL CONTAINING *PLUCHEA LANCEOLATA* EXTRACT OBTAINED THROUGH DIFFERENT ORGANIC SOLVENTS AND EVALUATION OF ITS ANTI-INFLAMMATORY ACTIVITY BY TOPICAL APPLICATION

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ABSTRACT

Pluchea lanceolata has been used in massage oil as well as in traditional ayurveda as a potent pain reliever. The traditional Method of obtaining oil is simply based on taking water as the medium for extraction. The water extract obtained is then boiled with oil, till the water is completely evaporated. Here, our aim is to replace water by different organic solvents and obtain oil which has much better efficacy than the traditionally extracted oil. The idea here is to obtain more efficacious oil than the marketed sample which is majorly extracted using water as solvent. Here, the different organic solvents used are methanol, ethanol, petroleum ether and chloroform. The oil obtained was checked for its anti-inflammatory activity with the carrageenan induced rat paw edema. The prepared oil was compared with the marketed sample of mahanarayan oil. The ethanolic extract has showed to be having high extract yield in literature.

INTRODUCTION: Anti-inflammatory and analgesic are the most widely used class of drugs. Synthetically prepared drugs are their severe side effect of GIT distress and bronchoconstriction. The oil prepared from the herbal drugs will have no or less side effects as it will be applied topically and it will not undergo first pass metabolism.

Pluchea lanceolata has been used in massage oil as well as in traditional Ayurveda as a potent pain reliever. It is one of the most potent herbal drug for relieving pain. *Pluchea lanceolata* (Family:-Asteraceae) also popularly known as rasna has been used in Ayurveda since early ages. The plant is used for the inflammations and bronchitis, psoriasis, cough and piles. It is also used as antipyretic, analgesic, laxative and nervine tonic.

The decoction of plant is used to prevent the swellings of joint in arthritis, rheumatism and neurological diseases. The roots are antipyretic, bitter, laxative and thermogenic and are used for allaying the pain caused by the sting of scorpions. Plant extract is used as a cooling agent in summer. The leaves are aperient and used as a laxative, analgesic and antipyretic [1, 2, 3]. The Plant also contains many essential chemical constituents that give the effect of relieving the pain.

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The constituents like Pluchine, stigmasterol are the main constituents. It also contains many other chemical constituents of different classes like glycosides, alkaloids, etc. They act by inhibiting the inflammatory mediators in the body.

MATERIALS AND METHODS:

Materials: The formulation contains the known potent drugs in ayurveda. This herb has been established as potent anti-inflammatory as well as analgesic action in the literature. The drug to be used in preparation of the formulation is *Pluchea lanceolata.* Different organic solvents like methanol, ethanol, chloroform and petroleum ether were used for extracting. The formulation contained a suitable base which was sesame oil [4, 5].

Methods:

1. **Preparation of formulation:** The plant parts were obtained from the botanical garden from the campus. The plants were collected and washed thoroughly and cut into small parts. The respective plant parts were allowed to shade dried and then boiled in about 5 times of volume of water/solvent until 2 times of water was left. The heating was continued for over two hours until 2 times of water was left. The left over water/solvent extract was filtered and boiled with the suitable base (Sesame Oil) until all the water/solvent is evaporated. The oil obtained was filtered for any residual particles if seen and was stored in suitable container as amber color container. Three different oil were prepared, one having more amount of *Pluchea lanceolata* (25% more than the actual concentration in other combination) and *Pluchea lanceolata* (25% more than the actual concentration in other combination) respectively [6].

2. **Pharmacological activity:** The anti-inflammatory activity of the prepared poly herbal formulation was checked with the help of carrageenan induced rat paw edema model. The carrageenan was digested in saline for 24 hrs before injecting it into the rat. The oil was applied on the hind rat paw before thirty minutes of the injecting the carrageenan solution. The observations for Inflammation were taken at regular intervals of one hour, two hour and twenty four hour. Comparisons of data were made to evaluate the efficacy of the oil with the marketed preparation of anti-inflammatory oil [7, 8].

Study Plan:

TABLE 1: THE STUDY PLAN FOR THE CARRAGEENAN INDUCED RAT PAW INFLAMMATION MODEL [9, 10, 11]

Group	I	II	III	IV	V	VI	VII
Treatment	**Normal Control (carrageenan)**	**Standard treatment (mahanarayan oil)**	**Treated with Combination I + Carrageenan#**	**Treated with Combination II + Carrageenan$**	**Treated with Combination III + Carrageenan***	**Treated with Combination IV + Carrageenan+**	**Treated with Combination V + Carrageenan+**
Dose (mg/kg/day)	0.5 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Number of animals	3	3	3	3	3	3	3
Duration of treatment	Once	Once	Once	Once	Once	Once	Once
Route of administration	Sub-cutaneous	Topical Application + S.C.	Topical Application + S.C.	Topical Application + S.C.	Topical Application + S.C.	Topical Application + S.C.	Topical Application + S.C.
Parameters to be evaluated	Paw Volume will be evaluated at different time interval (1, 2, 3 hr) up to 24 hour.						
Statistical Analysis	The data will be statistically evaluated by one wav analysis of variance followed by the comparison test to determine the level of significant difference between different combinations and optimizing the efficacy of the oil.						

Combination I - Methanol as organic solvent for *Pluchea lanceolata* + Sesame oil as base

Combination II - Ethanol as organic solvent for *Pluchea lanceolata* + Sesame oil as base

Combination III - Water as organic solvent for *Pluchea lanceolata* + Sesame oil as base

Combination IV - Petroleum Ether as organic solvent for *Pluchea lanceolata* + Sesame oil as base

Combination V - Chloroform as organic solvent for *Pluchea lanceolata* + Sesame oil as base

Experimental Animals: Albino Wistar rats weighing 160-240gm were procured from the college animal house with the prior permission of CPCSEA. Each group consisted of six rats each.

CPCSEA Number: IICP/UG/08-2011/03

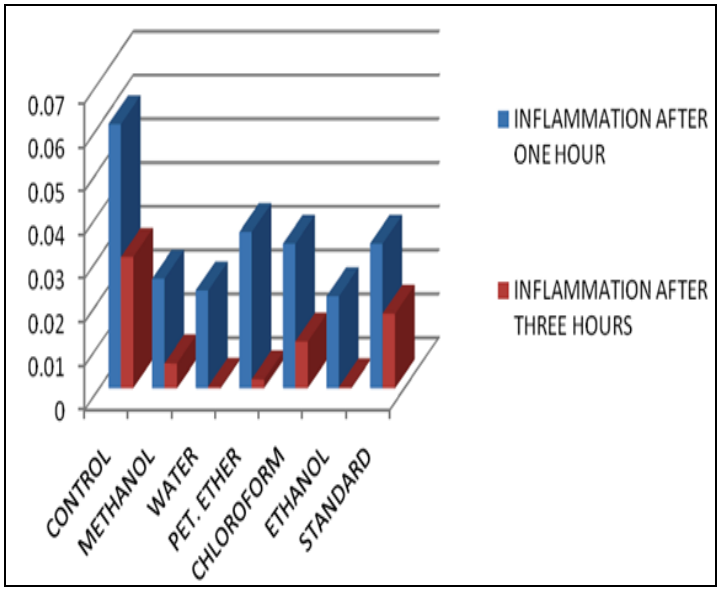
RESULT: The oil obtained was checked for its anti-inflammatory activity with the carrageenan induced rat paw edema. The prepared oil was compared with the marketed sample of mahanarayan oil. The ethanolic extract has showed to be having high extract yield in literature. The result of the application of oil extracted by using ethanol showed highest activity from all of the oils prepared using different organic solvents (**tables 2 and 3, graph 1 and 2**).

TABLE 2: THE MEASUREMENTS OBTAINED BY DIGITAL VERNIER CALIPERS AFTER APPLICATION OF OIL

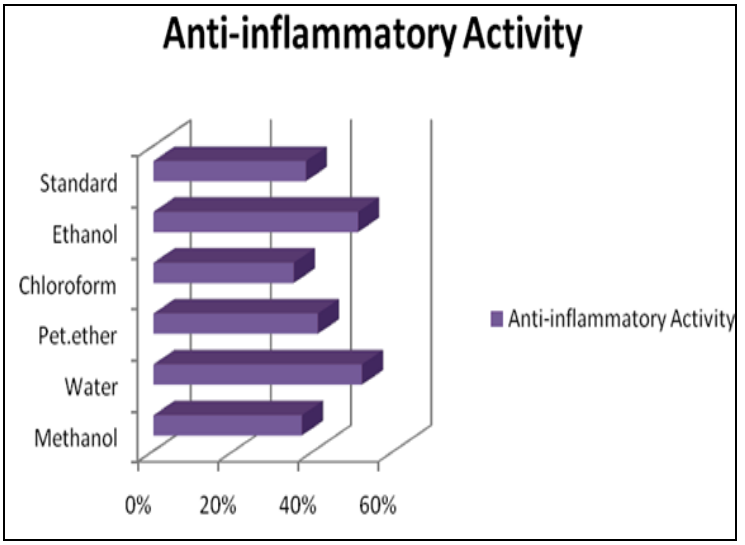
	Initial Readings			Readings after one hour			Inflammation			Mean
	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3	
Combination III	0.186	0.2	0.198	0.228	0.211	0.227	0.042	0.011	0.029	0.027333
Combination II	0.176	0.19	0.17	0.205	0.211	0.195	0.029	0.021	0.025	0.025
Combination I	0.171	0.18	0.185	0.186	0.248	0.201	0.015	0.068	0.016	0.033
Combination IV	0.18	0.15	0.192	0.2	0.198	0.221	0.02	0.048	0.029	0.032333
Combination V	0.19	0.16	0.2	0.21	.208	0.221	0.02	0.038	0.021	0.039
Control	0.199	0.174	0.166	0.257	0.231	0.224	0.058	0.057	0.058	0.057667
Standard	0.165	0.155	0.157	0.207	0.195	0.192	0.042	0.04	0.035	0.039

TABLE 3: THE RESULT OF THE DIFFERENT EXTRACTS USED IN CONVERSION

Extract (used for extracting active constituents)	Activity
1. Methanol	Good
2. Water	Excellent
3. Petroluem ether	Very Good
4. Chloroform	Good
5. Ethanol	Excellent
6. Standard	Good



GRAPH 1: INFLAMMATION AFTER DIFFERENT TIME DURATIONS



GRAPH 2: THE GRAPH SHOWS THE PERCENT INFLAMMATION INHIBITED BY THE VARIOUS SOLVENTS

CONCLUSION: The result of the application of oil extracted by using ethanol showed highest activity from all of the oils prepared using different organic solvents. Water as an extracting medium also showed good activity in alleviating the inflammation in the rat paw.

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