

Evaluation of Combined Anti-Inflammatory Activity of *Colocasia esculenta* Leaves and *Madhuca longifolia* Seeds

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ABSTRACT:

The objective of this current study is to evaluate the anti-inflammatory activity of herbal plants, we have selected leaves of *Colocasia esculenta* and seeds of *Madhuca longifolia* extract that can be used as Substitute to artificial medicament in prospective. Natural plant is rich sources for discovery of new drug because of their chemical diversity. There are hundreds of phytoconstituent reported to have many pharmacological activities. The effect of NSAIDs is so much useful for treating of various type of pain like dysmenorrheal, pyrexia, gout, migraine, and certain types of trauma cases, ankle sprain, and soft tissue injuries. Anti-inflammatory activity is evaluated by both in vivo and in vitro evaluation. Anti-inflammatory agents are use in the remedy of inflammation. *Madhuca longifolia* and *Colocasia esculenta* is emerging plant of interest of multipurpose use. This plant has shown various pharmacological activities such as analgesic, anti-inflammatory, antihelminthic, immunosuppressive, wound healing, hepatoprotective, antidiabetic, nephroprotective, antioxidant, antimicrobial, anticonvulsant, and antipyretic activity. Preliminary phytochemical screening revealed the presence of saponin, glycoside, flavonoid, alkaloid and tannins and phenol compound in the ethanol extract of the plant under study. The photochemical constituent present in this ethanol extract may be responsible for the anti-inflammatory activities of the plant. Hence, in this article we have to evaluate the anti-inflammatory activities of mentioned plants. I have studied about two methods for evaluation that are in vitro and in vivo methods. From these two methods we have done invitro method for

further investigation. It includes so many methods like membrane stabilization, Protein denaturation activity, Albumin denaturation activity, Antilipoxygenase activity. From above all methods we have go through the RBC membrane stabilization method for check the activities in different concentration of *Colocasia esculenta* leaves and *Madhuca longifolia* seeds and aspirin was taken as reference drug and we have concluded that different concentration of drug has been possessed inhibition of inflammation.

KEYWORDS: Anti-inflammatory, phytoconstituent, *Colocasia esculenta*, *Madhuca longifolia*, NSAIDs, Secondary constituent and human diseases.

I. INTRODUCTION:

1.1. Inflammation

Inflammation is a sectarian or bounded defensive replication educe by damage or annihilation of part of an organism consisting of an aggregate of cells, which serves to destroy dilute or wall off injured tissues. Inflammation is currently treated by NSAIDs. The use of herbal medicines becoming faddish due to harmful and complexity of allopathic medicines. Remedial plants play an extensive role in the evaluation of significant curative agent.^[1]

Some examples of traditional medicinal plants are *Zingier officinal*, *Zea Mays*, *asparagus racemosus*, *Allium sativa*, *Embolic officinal* is (Euphorbiaceous), and *Bacopa monnieri* Linn. (Scrophulariaceae), *Bryophyllum pinnatum*, *Portulaca oleracea*, *Centella Asiatic*, *Aloe vera*, *Cannabis sativa*.^[2]



Fig.1.1: Inflammation

There are mainly two types of inflammation which are as follows^[3,4]

- A) Acute inflammation - It is short time process and redness heat swelling is the sign of acute inflammation.
- B) Chronic Inflammation-It is long time process and body pain skin rashes mucous production is the sign of chronic inflammation.

Anti-inflammatory

Anti-inflammatory is a non-steroidal drug which is used for the treatment of inflammation and reduces pain swelling and redness. It blocks certain substances in the body a chemical name Arachidonic acid that cause inflammation. Many patients which are suffering from orthopedic condition including arthritis tendonitis and bursitis are prescribed non-steroidal anti-inflammatory drug.^[5]

Table 1.2: Many of the Herbal plant which shows Anti-inflammatory Activities are as follows^[6]

S.No.	Plants	Botanical source	Chemical Constituents	Family
1.	Ginger	Zingier officinal is	Gingerol	Zingiberaceae
2.	Haldi(turmeric)	Curcuma longa linn	Cucruminoids	Zingiberaceae
3.	Padma	Nelumbo officinalis	Betuliniaceae	Nymphaeaceae
4.	Madura(Kathy)	Cyperustegetumrox	Cyprusrotundas	Cyperaceous

1.3. PLANT PROFILE

1.3.1. Mahua (Madhuca longifolia)

It is emerging plant of interest of multipurpose use. Boiled leaf extracts are useful in the management

of skindisease and nervedisorder cough burning sensation. Its raw flower increases lactation and flower fried with ghee used to cure piles.^[8]

Table1.3.1: Taxonomical Classification of Mahua Plant^[7]

Botanical name	Madhucalongi folia
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Subclass	Ebenales
Family	Sapotacea
Genus	Madhuca
Species	Madhuca longifolia



Fig.1.3.1: Seeds of Mahua Plant

Arabi (Colocasia esculenta)

Colocasia esculenta belongs to family Araceae it is also known as arum Esculenta. It is wild plant and cultivated throughout the hotter part of India and Ceylon. Its juice is used in the treatment of arrest atrial hemorrhage, juice of the corm is used in cases of alopecia.^[9,10]

Table1.3.2: Taxonomical Classification of Arabi Plant^[9]

Botaniacal name	Colocasia esculenta
Kingdom	Plantae
Family	Araceae
Superdivision	Spermatophyta
Division	Magnoliophyta

Class	Monocotyledons
Subclass	Asecidae



Fig.1.3.2: Dried leaves of Arabi Plant

II. MATERIALS AND METHODS:

The materials and method used for this study is specified as follows:

2.1. Collection of plant:

Arabi (*Colocasia esculenta*)

The leaves of *Colocasia esculenta* were collected in the month of June–July from the

village dharamjaigarh district Raigarh and were air dried in the sun. Cleaned and dried under temperature the dried leaves of *Colocasia esculenta* were methodized to fine particles. The leaves powder stored in paper bags and stored in air tight container.^[11]



Fig.2.1: Dried Leaves of Arabi Plant

Mahua (*Madhuca longifolia*)

The seed of *Madhuca longifolia* was collected in the month of April from the village

ofkachkoba district Raigarh and was air dried in the sun cleaned and dried under room temperature. The dried seed were homogenized.^[13]



Fig.2.1: Dried Seed of Mahua Plant

2.2. Extraction of plant:

20gms of the powdered leaves of Colocasia esculent and seed of Madhuca longifolia sample were extracted by using ethanol in Soxhlet apparatus for 9 hours at 70°C through a

regulated heating mantle. The obtained extracts were concentrated under vacuum at 70°C using a rotary vacuum evaporator till solvent fully evaporated. The obtained extracts were stored in refrigerator for further use.^[11,12]



Fig.2.2: Extraction of Arabi leaves & Mahua Seeds

Table 2.3: Specification of chemicals reagent and instruments used:

S.No.	Chemicals	Reagent	Instrumentation
1	Petroleum ether	Ninhydrin reagent	Sox let apparatus
2	N hexane	Wagner reagent	Beaker
3	Chloroform	Benedict reagent	Funnel
4	H2SO4	Meyers reagent	Pipette
5	Ethanol:		Refrigerator
6	Acetic anhydride		Test tube
7	Lead acetate		Round bottom flask
8	Ammonia		Centrifugation tube
9	Iso amyl alcohol		Uv spectrophotometer

2.4. Methods for evaluating anti-inflammatory Agents (in vitro activity):^[14]

There are so many invitro activities which are used for treatment of inflammation like

- A) RBC membrane stabilization
- B) Protein denaturation activity
- C) Albumin denaturation activity
- D) Antilipoxygenase activity

So, in these so many activities I have done the activity of RBC membrane stabilization.

A) RBC membrane stabilization method

1. Blood have been collected from the healthy volunteer human body and mixed with Elsevier solution in a centrifuged tube. (Elsevier solution is made by mixing dextrose + sodium citrate+ citric acid+ sodium chloride)

2. Blood was centrifuged at 300 rpm and solution was washed with isosaline solution i.e.: NaCl solution.

3. The four extract was taken separately in different centrifuged tube and in each tube.

First tube	Colocasia esculenta extract+1 ml phosphate buffer+2 ml of hypo saline solution +0.5ml of Rbc suspension at different concentration
Second tube	Madhuca longifolia extract+1 ml of phosphate buffer+2 ml of hypo saline solution+0.5 ml of Rbc suspension at different concentration
Third tube	Combination of Madhuca longifolia and Colocasia esculenta extract+2 ml of hypo saline extract+0.5 ml of Rbc suspension taken at different concentration
Fourth tube	Reference drug i.e., aspirin was taken at different concentration

4. Aspirin was used as reference drug and distill water was used in control group.
5. The assay mixture was incubated at 37⁰c for 30 minute and centrifuged were done again.
6. The haemolysis content of the RBC suspension was estimated using uv spectrophotometer at 340 nm.

Table 2.5: Qualitative analysis of photochemical constituents ^[15]

S.No	Phytoconstituent	Chemical used in test	Inference	Ethanol extract of Madhuca longifolia seed	Ethanol extract of Colocasia Esculenta leaves
1	Carbohydrate				
	Molish test	Test soln + few drops of alcoholic alpha naphthol and few drops of sulphuric acid through side of test tube	purple colour	+	+
	Barfoed test	1ml of barfoed reagent and 1 ml of test solution	Red cupric oxide	+	-
2.	Cardiac Glycosides				
	Legal test	2 ml of pyridine solution + few ml of alkaline sodium nitroprusside + few ml of test solution	Blood red colour	+	-
	Bal jet test	Few ml of picric acid and few ml of test solution	Orange colour	+	+
3.	Alkaloid test				
	Hager's reagent	2 ml of picric acid + few drops of extract sample	Yellow precipitate	+	+

	Wagner's reagent	2 ml of iodine potassium iodide solution + few drops of extract sample	Reddish brown precipitate	+	+
	Dragondroff reagent	2 ml of potassium bismuth iodide solution and few drops of extract sample	Reddish brown precipitate	+	+
	Mayer's reagent	Potassium mercuric iodide solution and few ml of extract sample	Cream color precipitate	+	+
4.	Tannin				
	Ferric chloride test	Few ml of ferric chloride + few ml of test solution	Green colour	+	+
5.	Reducing sugar				
	Benedict reagent	Few drops of Benedict reagent + few drops of test soln	Yellow color	-	-
6.	Protein test				
	Burette test	2 ml burette reagent +2 ml test solution	Violet color	-	-
7.	Amino acid				
	Million test	2ml of million reagents + few drops of test solution	White precipitate	-	+
	Ninhydrin test	2 ml of ninhydrin solution + few drops of test solution	Violet color	-	+

III. RESULTS AND DISCUSSION:

As per the result showed that all chemical used in this activity was evaluated by Invitro activity there are so many methods for the Invitro activity but the most common method which I have used is RBC membrane stabilization which is very useful and effective method for the evaluation of In vitro activity. Leaves of *Colocasia esculenta* and seed of *Madhuca longifolia* showed RBC membrane stabilizing activity and it shows

inhibition of *Colocasia esculenta* of 4.741%, *Madhuca longifolia* shows inhibition of 7.46%, combination of *Colocasia esculenta* and *Madhuca longifolia* shows inhibition of 2.193% and reference drug shows inhibition of 5.088% at different concentration. The result obtained from this study suggests that plant is a good natural source or Ant inflammatory therapy. Most of the anti-inflammatory drugs stabilize liposomal membrane and inhibit the inflammatory process by

restricting the release The herbal formulation has significantly decrease the development of Rbc membrane induced by aspirin as compared to the control and standard drug group. During the experiment which I have got results under the observation table and the percentage inhibition of

Colocasia esculenta is 230.63% inhibition Of Madhuca longifolia is 242.385% and inhibition of both Colocasia esculent and Madhuca longifolia is 4.3173275. The test sample is very good anti-inflammatory plant as compared to standard drug.

Observation table

Drug used during experiment	Percent inhibition of drug					
	0.1%	0.2%	0.3%	0.4%	0.5%	Inhibition of drug
Colocasia esculentaLeaves extract	1.721	0.586	0.837	0.553	1.044	4.741
Madhuca longifolia Seeds extract	0.260	1.997	1.763	1.768	1.772	7.56
Combination of Colocasia esculent and Madhuca longifolia	0.261	0.898	0.041	0.296	0.697	2.193
Reference drug (Aspirin)	0.759	1.24	1.684	1.151	1.273	1.2214

Calculated by % Inhibition formula = $\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$

IV. CONCLUSION:

Large number of polyherbal species has been used traditionally or as folk medicine against inflammatory disorder. Hence it is need of time that all herbal plant is considered for decisiveness of their physiochemical pharmacological action by insulation of single existence important for anti-inflammatory activity. In comparison to NSAIDs drug Indian medicinal plant are so much helpful and beneficial for the future prospect. From this review article we have come to the conclusion that by combining both the anti-inflammatory plant the anti-inflammatory activity has been inhibited and we found that the result is positive. The present review revealed that Colocasia esculenta and Madhuca longifolia is utilized for some common disease.

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