Vol 15 | Issue 1| 2024 | 1-7.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

IN VITRO STUDY OF ANTI-INFLAMMATORY ACTIVITY FROM ETHANOLIC LEAF EXTRACT OF ORMOCARPUM COCHIN CHINENSE

Hariyadharshini A[1]*, Hemalatha R[1], Hepzihbah H[1], Jayakumar M[1], Dr. Karthi J[2]

[1]Bachelor of Pharmacy, Pallavan Pharmacy College, Kanchipuram , Tamil Nadu, India.
[2]Principal, Department of Pharmacognosy, Pallavan Pharmacy College, Kanchipuram Pallavan Pharmacy College, Kanchipuram, Tamil Nadu, India.

ABSTRACT
Ormacarpum cochichinase is a shrub Which is extremely efficacious in mending bone fractures,but at present it's use is known only to handful of villagers in the tropical dry evergreen forest areas of Tamil Nadu for healing bone fractures . Other than bone mending this plant have some more pharmacological potentials like anti-inflammatory, anti-arthritic, muscitocidal, anti-microbial and so on. This study aims on phytochemical analysis and the study of anti-inflammatory potential. The phytochemical analysis was done by various chemical tests and the anti-inflammatory potential was studied by using egg albumin denaturation assay. Further research works should be carried out for the better understanding of this plant and it`s pharmacological potential.

Keywords: Ormocarpum Cochinchinese, Anti-Inflammatory Activity, Leaf Extract, Denaturation.

INTRODUCTION

The Ormocarpum genus has numerous species of medicinal herbs that are important to human health from a biological standpoint. One species of the Ormocarpum genus that has utilized to treat bone fractures is Ormocarpum Cochinchinense [OC]. About 17 of the approximately 25 species in the genus Ormocarpum plant. FAMILY: Fabaceae Papilionoideae are found in Africa's tropical regions. A number of therapeutic plants such as Ormocarpum kirkii S. Moore in this genus, Ormocarpum trichocarpum [Taub.], Ormocarpum Cochinchinense, Ormocarpum Keninse Gilllet, Ormocarpum Sennoides and Ormocarpum [Lour.] Merr. Subspecies of Sennosides Zanzibaricum have been extensively utilized to heal wounds, oedema, headaches, fever, stomach discomfort, hernia, diarrhoea, gastro intestinal issues, rheumatism and STD.

This is explained by the abundance of phytochemicals found in plants in this genus secondary metabolites that are present in the Ormocarpum including coumarins, phytosterols, flavonoids, iso flavonoids, bioflavonoids and triterpenoids. There have also been reports of pharmaceuticals characteristics in Ormocarpum species, including anti-inflammatory, anti-Plasmodial, cytotoxic, antioxidant, antimalarial and antibacterial actions. The herb belonging to the genus Ormocarpum is famous for its high natural supply of phytoconstituents. The leaves, bark, stem and root of the plant are the portions that are most commonly utilized medicinally. Traditional bone setters employ a miraculous herb called Ormocarpum Cochinchinense [OC] to treat bone fractures.Ormocarpum Cochinchinense[OC] is one of the species of herb which has lots of medicinal properties. OC is called Elum Botti [Bone-to join] in Tamil [10]. Plant extracts were successful in the treatment of bone defects. There was the release of anti-inflammatory mediators which accelerated bone healing.

TOXONOMY:
KINGDOM : Plantae
PHYLUM : Tracheophyta
CLASS : Magnoliopsida
ORDER : Fabales
FAMILY : Fabaceae
GENUS : Ormocarpum
SPECIES : Cochinchinense [Lour.] Merr.

MORPHOLOGY:

- Erect subshrubs. Leaves pinnately 10-13-foliolate; leaflets alternate, obovate-oblong, obtuse; petiole slender; stipule ovate. Flowers in slender axillary, 6-10 long, racemes.

Corresponding Author: **Hariyadharshini A**

- Plant growth form : Tree (Small (6m-15m)), Shrub
- Mode of nutrition : Autotropic
- Plant shape : Irregular
- Height range : 7.5

INFLAMMATION:

Inflammation is a normal defensive response tissue injury, wide array of process, including enzyme activation, mediators release, fluid extra vasation, cell migration tissue break down repair are involved in inflammation.

ANTI-INFLAMMATORY ACTIVITY:

Inflammation is a clinical condition characterized by four cardinal indicators: redness, swelling, heat, and pain. Autoantigens build up in the synovial joints of people with rheumatoid arthritis (RA), an inflammatory illness that causes joint inflammation. Both steroidal and non-steroidal anti-inflammatory drugs are available to treat inflammatory illnesses. Sadly, these drugs have major side effects that include stomach lesions, renal failure, heart failure, and GI tract damage.

Ormocarpum cochinense was extracted using ethanol as a solvent, the plant showed better anti-inflammatory action. Overall, the several solvent extracts of *O.cochinchinense* leaves demonstrated the presence of different phytochemicals. Alkaloids were present in ethanol, water, methanol, and ethyl acetate. For the anti-inflammatory effect, it is necessary

Although herbal treatments have been used for a long time to treat a wide range of disorders, scientific research on these herbs is increasingly crucial due to their low side effects and affordability. Numerous herbs are used to treat rheumatism and inflammation.

Aim and Objective:

In many regions of the world, the traditional strategy has been using plant extract to treat various disease. It has been discovered that the plant extracts work well in their mode of action and have no negative effects on the patient, the experiment shows that the *ormocarpum* cochinchinese has a wide range of phytochemical physiological and pharmacological, pharmacologically and drug support the discovery of new therapeutic uses. Additional research is required to investigate several other significant, essential and have no negative effects on the patient. The experiment shows that the *ormocarpum cochinchinense* has a wide range of physiological and pharmacological effects and is a source of pharmacokinetically, pharmacologically and medicinally bio active substances. As a result, this drug supports the discovery of new therapeutic uses. Additional research is required to investigate several other significant, essential and unidentified benefits.

Research and Methodology:

Soxhlet apparatus extraction process: -

About 20g of powdered leaf was poured into the muslin cloth and packed. The packed leaf powder was kept into the Soxhlet apparatus. 99.9% ethanol was used as a solvent for extraction. The extraction process was done for about 8 hours at the temperature of 35-40^{o}C. The resultant extract was collected on a China dish and concentrated and the solvent were evaporated by applying heat. The extract of about 3g was obtained.

CHROMATOGRAPHY:

Thin Layer Chromatography:

The sample was tested by using TLC method. It is a technique used to separate wide range of compounds of biochemical interest. The aqueous extract was subjected to thin layer. chromatography about 0.1-0.2 ml of conc. The extract was loaded on the plate by using capillary tube. During spotted plates were carefully dried and used for elution purpose. Later different combinations of solvents were tested depending on polarity basis. The different solvent systems were used as mobile phase for TLC, which consisted of chloroform: methanol (9:1, 8:2), pure chloroform, chloroform: ethyl acetate (1:1) and methanol: hydrochloric acid (9:1) solvent combinations. The spots were observed under visible light. The spotting plate was carefully dried and used for elution purpose. Different solvent systems ranging from lower polarities to higher polarities were tested for the separation of bioactive components. The TLC plates were observed and the separated spots were marked. The eluted spotted plates were dried at room temperature and they were placed in iodine chambers for the development of chromatogram. The Rf values of spot were calculated.

$$RF = \frac{\text{Distance moved by the substance(cm)}}{\text{Distance moved by the solvent(cm)}}$$

HPLC

Procedure:

The investigation was carried out on a Varian HPLC system (USA) consisting of a Pro Star 430 autosampler and Pro Star 210 pumps. The system also included a Photodiode Array (PDA) detector
and a computer running Varian workstation version 6.42 software for data acquisition and processing. Preparation of sample solutions

For the extract, approximately 0.01 g of the dried sample was resuspended in 10 mL of water and ethanol :water (1:1 v:v) solution, respectively. The samples were stored at 4°C until analysis. Ranges of the calibration curve Aliquots of the standard stock solution were transferred to volumetric flasks and diluted to get concentration in the linear range. HPLC instrumentation and chromatographic conditions

A reversed-phase C18 HPLC column was used to separate phenolic compounds chosen among phenolic classes to draw a composition profile of the studied matrices. HPLC analysis was performed using a liquid chromatographer equipped with a binary pump, an online vacuum degasser, a diode array detector (DAD), an autosampler, a thermostatted column compartment, and features a direct access rack system. System control and data analyses were performed using

Lab solution software (Shimadzu). Before choosing the chromatographic conditions, several trials were carried out using different types of solvents and mobile phase concentrations to check the retention time (RT), peak shape, and tailing factor (peak symmetry) of the analyte. Separation was carried out in a Shim-pack VP-ODS C18 column (5 µm, 150 x 4.6 mm). The mobile phase consists of a gradient elution using the proportions of solvent A (1% formic acid in water) to solvent B (acetonitrile) as follows: initial 5% B; 0 - 7 min, 5% B; 7 - 18 min, 5 – 30% B; 18 - 35 min, 30 - 60% B; 35 - 40 min, 60 – 95% B with a flow-rate of 1 mL min-1 and the injection volume of 0.02 mL of samples and standards. The total running and post-running times were 40 and 5 min, respectively. The column temperature was maintained at 40°C throughout the analysis and the spectra were acquired in the 210 - 800 nm range. The quantification was made by external standardization using analytical curves and limits of detection (LOD) and quantification (LOQ).

IN-VITRO ANTI-INFLAMMATORY ACTIVITY

Aim:-

To study the anti-inflammatory potential of the *ormocarpum cochinchinese* ethanolic leaf extract by using egg albumin denaturation assay.

Principle involved in egg albumin denaturation assay:-

The main goal of the egg albumin denaturation assay is to ascertain whether certain substances or agents can prevent or impede the denatured state of egg albumin in specific situations. The process by which a protein loses its biological activity and undergoes structural alterations is known as denaturation. In the experiment, egg albumin is used as a model protein, and it is denaturized by subjecting it to high temperatures, acidic environments, or other denaturing agents. During denaturation, egg albumin's initial conformation is broken, altering its physical properties and rendering it non-functional. The egg albumin denaturation assay assesses a substance's ability to prevent or reduce egg albumin denaturation in order to determine whether or not it has anti-inflammatory properties. The denaturation assay for egg albumin is founded on the theory behind the egg albumin denaturation assay is that anti-inflammatory medicines would be able to maintain protein structures and avoid denaturation, which is commonly associated with tissue damage and inflammation. Therefore, substances or treatments that in this test considerably reduce the denaturation of egg albumin may have anti-inflammatory effects. Protein denaturation is thought to be one of the causes of inflammation. In addition to inhibiting the COX enzyme, NSAIDs stop protein denaturation. Egg albumin solution can be added to test samples at varying concentrations under carefully monitored experimental settings to allow reactions to occur. The absorbance can then be measured to determine the percentage of inhibition.

Materials required:-

Chemicals:-

- Egg albumin solution.
- Phosphate buffer solution (Ph 6.4).
- Distilled water.

Apparatus:-

- Pipettes and puppet tips.
- Test tubes.
- Incubator.
- Spectrophotometer.
- Water bath.

Preparation of 1% egg albumin solution: -

The 1% egg albumin solution was prepared by cracking the egg shell carefully and the albumin was collected on a beaker 1ml of albumin was taken and diluted using 100ml of distilled water and the resultant solution was used for the experiment.

Procedure: -

- Control solution: - 0.2ml of egg albumin from fresh hen`s egg was collected and 2.8ml of phosphate buffer solution (Ph 6.4) and 2ml of distilled water was added.
- Standard solution:-0.2ml of egg albumin solution was added with 2.8ml of phosphate buffer and 2ml of various concentration of standard drug aspirin (50 0µg/ml)
- Test solution: -0.2ml of egg albumin solution was added with 2.8ml of phosphate buffer and 2ml of various concentrations of test as (100,200,300,400,500 µg/ml). All of the above solutions were adjusted to pH 6.4 using a small amount of 1 N HCl.
- The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes.
- After cooling, the absorbance of the above solutions was measured using ultraviolet visible spectrophotometer at 660 nm.
- The percentage inhibition of protein denaturation was calculated using the following formula

Table 1: Phytochemical screening

S.NO	TEST	PROCEDURE	OBSERVATION	INFERENCE
1.	Mayer's test	Few ml filtrate + 1-2 drops of mayor's reagent (along the sides of test tube)	A creamy white / yellow precipitate	Presence of Alkaloids
2.	Lead acetate test	1ml plant extract + Few drops of 10% of lead acetate solution	A yellow precipitate	Presence of Flavonoids

3.	Iodine test	1 ml extract + Few drops of dil. Iodine solution	A transient red colour	Presence of phenolic compounds
4.	Quinin's test	1ml extract + Concn. H2so4	Red colour	Absent of quinins
5.	Ferric chloride test (or) Braymer "s test	1ml extract + 3ml dis. Water + 3drops ferric chloride	Blue green screening of blue to green colour	Presence of tannins
6.	Foam test	5ml extract + 2ml dis.water + Shake for 15mins	2cm layer foam	Presence of saponins
7.	Test for steroids	1ml extract + Chloroform and concn. H2so4	Red colour	Absent of steroids
8.	Ninhydrin test	1ml extract + 1ml linhydrin's reagent	Purple colour	Presence of amino acid
9.	Million's test	1ml extract + Few drops of million's reagent	White precipitate turn red on gently heating	Presence of protein
10.	Fehling's test	2ml extract + Few drops of fehling's reagent A & B	Brick red colour	Presence of Glycosides

Table 2: RESULT

S.NO	CHEMICAL CONSTITUENTS	INFERENCE
1	ALKALOIDS	PRESENT
2	FLAVONOIDS	PRESENT
3	PHENOL	PRESENT
4	QUININES	ABSENT
5	TANNINS	PRESENT
6	SAPONINS	PRESENT
7	STEROIDS	ABSENT
8	AMINO ACID	PRESENT
9	PROTEIN	PRESENT
10	GLYCOSIDES	PRESENT

Ormocarpum cochinchinese whole plant	Ormocarpum cochinchinese flower
	
Ormocarpum cochinchinese	**Soxhlet apparatus**

Egg albumin denaturation Assay value :

FORMULA:

$$\text{Percentage inhibition} = 1 - \frac{Absorbance\ of\ standard}{Absorbance\ of\ control} \times 100$$

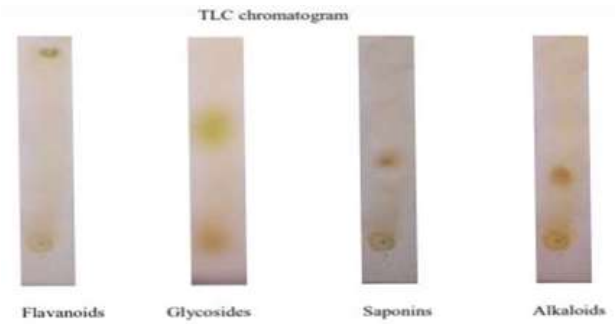

Fig1 : TLC chromatogram

S.No	Constituents	Rf Value
1	Alkaloids	0.28
2	Saponins	0.33
3	Glycosides	0.57
4	Flavonoids	0.92

The TLC indicated the presence of chemical constituents including flavonoids, alkaloids, saponins and glycosides.

HPLC system

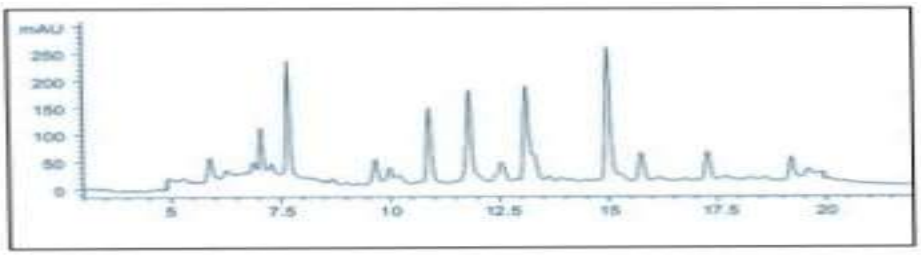

Fig 2: HPLC chromatogram

RESULT

The ethanolic leaf extract of ormocarpum cochinchinese was tested for anti-inflammatory potential by using egg albumin denaturation assay and the absorbance value obtained for the test sample was compared with the standard drug aspirin`s absorbance value. From the table the test sample shows 53% activity

DISCUSSION:

- In natural products and its extracts presence of phenolic moiety comprises Alkaloids, Flavonoids which is usually associated with the Anti inflammatory against free radical pathology.
- Ormacarpum cochichinase is a shrub which is extremely efficacious in mending bone fractures, but at present its use is known only to handful of villagers in the tropical dry evergreen forest areas of Tamil nadu for healing fractures .
- The objective of the current investigation was to examine the antioxidant and anti-inflammatory properties of O. cochinchinense ethanolic extract in vitro. Antioxidants have been shown in scientific studies to be effective in treating a range of inflammatory conditions.
- The main source of naturally occurring antioxidants is plants. It should be mentioned, nevertheless, that the herb's anti-inflammatory and antioxidant properties change depending on where it is harvested. In addition, a host of other factors, including harvest time, soil properties, and weather circumstances, influence the activity of natural products. Moreover, there is a paucity of research describing the anti-inflammatory qualities of a specific herb, such as OC.

CONCLUSION:

The purpose of this study is to highlight Ormocarpum cochinchinense anti-inflammatory properties. The leaf extract demonstrated effective inhibition of protein denaturation and membrane stability.Both in traditional medicine and modern medicine, herbs are essential. The majority of people experience inflammation. This study demonstrated that Ormocarpum Cochinchinense, one of the plants, had anti-inflammatory properties. The bioactive components found in the ethanolic leaf extract of OC were responsible for the anti-inflammatory activity, supporting the use of OC in the treatment of inflammation.

REFERENCES

1. Wallis TE. Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs. Third Edition. Boston: Little, Brown and Company; 1965.
2. Junaid R Shaikh and Patil M K – Qualitative tests for preliminary phytochemical screening: An overview – International Journal of chemical studies, 8(2), 2020, 603-608
3. Setyowati, E., Ikawati, Z., Hertiani, T. and Pramantra, I.D.P, HMG-CoA reductase and lipase enzyme inhibition from combination of Gynura procumbens and Curcuma xanthorrhiza aqueous extract, *Food Research* 5(2), 2021, 386 - 392.
4. Karthikumar S, Vigneswari K, Jegatheesan, *et al*. Screening of antibacterial and Antioxidant activities of leaves of Eclipta prostrata (L) *Scientific Research and Essay*, 2(4), 2007, 101-104.
5. Supinya Tewtrakul, Sanan Subhadhirasakul and Sopa Kummee Songklanakarin, *et al*. Anti-HIV-1 integrase activity of medicinal plants used as self-medication by AIDS patients. *J. Sci. Technol*. 28(4), 2006, 785-790.
6. Abu Hanif, Md. Shahadat Hossan, Md. Manzurul Kadir Mia, Mohammad Jahirul I slam, Rownak Jahan, Mohammed Rahmatullah 2009. Ethnobotanical Survey of the Rakhain Tribe Inhabiting the Chittagong Hill Tracts Region of Bangladesh, *American-Europian Journal of Sustainable Agriculture*, 3(2), 2009, 172-180.
7. Bettina m ruppel, mem. Inst, oswaldo cruse, Rio de janerio, *et al*. Pharmacological screening of plants recomonded by folk medicine as anti-snake venoum analgesic anti-inflammatory activities, 86(II), 1991, 203-205.
8. V. Gopiesh Khanna and K. Kannabiran, *et al*. Full Length Research Paper Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre, and Eclipta prostrata against Culex qinquifaciatus mosquito larvae. *African Journal of Biotechnology* 6(3), 2007, 307-311.
9. G.Arunachalam, N.Subramanian. Anti-inflammatory activity of methanolic extracts of Eclipta prostrata L(Asteraceae), *African Journal of Pharmacy and Pharmacology* 3(3), 2007, 097- 100
10. A.Chakraborthy. Preliminary studies on anti-inflammatory and analgesic activities of Spilanthes acemella in experimental animal models, *Indian journal of pharmacology*, 36, 2004, 148-150.
11. C.S. Kumari, S. Govindasamy. Lipid lowering activity of Eclipta prostrate in experimental hyperlipidemia. Journal of Ethanopharmacology 105(3), 2006, 332-5.