WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 1, 1755-1765. Research Article ISSN 2277– 7105

INVITRO ANTI INFLAMMATORY ACTIVITY OF *GYMNOSPORIA EMERGINATA*

***B. Nirmala Devi, K. Naga Lakshmi and D. Swarnalatha**

Department of Pharmacognosy, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Y.S.R. Dist. - 516126, A.P., India.

Article Received on
20 November 2022,
Revised on 10 Dec. 2022,
Accepted on 31 Dec. 2022
DOI: 10.20959/wjpr20231-26866

***Corresponding Author**
B. Nirmala Devi
Department of Pharmacognosy, Annamacharya College of Pharmacy, New Boyanapalli, Rajampet, Y.S.R. Dist. - 516126, A.P., India.

1. INTRODUCTION

Medicinal plants have always been considered a healthy source of life for all people. Therapeutically properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted.[1] A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. A number of plants have been used in traditional medicine for many years due to their antimicrobial properties.[2] Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body.[3]

The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds. These phytochemicals are toxic to microbial cells. Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs

with fewer side effects is necessary from medicinal plants origin. Herbal products are often perceived as safe because they are "natural".[4] In India, in recent years, there is increased research on traditional ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied. Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources Inflammation is defined as a localised reaction that produces redness, swelling, to the tissue and as a result of infection, irritation damage the cells. There are several reasons for inflammation such as Etiologic agents - viruses, bacteria, fungi, parasites, Hypersensitivity – body reacts against itself, Physical and chemical agents - trauma, sunburn, acid, Necrosis - anoxia, trauma and Cardinal features: Rubor (Redness), Tumor (Swelling), Calor (Heat), Dolor (Pain), Hypersensitivity Reactions, Physical Agents and Irritant And Corrosive Chemicals.[5,6]

There are different types of inflammation such as acute inflammation and chronic inflammation. In Acute inflammation the stimulation results in increased movement of plasma an white blood cells into injured tissues. It is the initial response of the body to the harmful stimuli. The inflammatory response is continuous with the process of repair. Acute inflammation is short, lasting only a few hours to days. Longer inflammatory responses are referred to as chronic inflammation.[7-9]

Chronic inflammation, like its acute cousin, is a host response to an inciting stimulus. There are, however, some distinct differences. First and foremost is the time factor. Chronic inflammation is considered to be inflammation of prolonged duration - weeks to months. Second, rather than being just exudative, chronic inflammation usually is productive or proliferative. Chronic inflammation is rarely gooey.[10,11] Cells in the chronic inflammatory process tend to produce substances that add new tissue, such as collagen and new blood vessels. Many of these changes also represent the repair process and there is a blurry continuum between chronic inflammation and the whole repair process. In general, chronic inflammation is characterized by inflammation, tissue destruction, and attempts at repair all happening at once.

The effects of inflammation can be both local and systemic. The systemic effects of acute inflammation include fever, leukocytosis and vascular changes. These will be discussed in more detail later in this unit. The local effects are usually clearly beneficial, for example the destruction of invading microorganism, but at other times they appear to serve no obvious

function, or may even be harmful Enzymes such as collagenases, elastases and other proteases may degrade normal tissues, resulting in their destruction. For example in type III hypersensitivity reactions and in some types of glomerulonephritis small vessels are damaged. There are several anti-inflamamtory medications are available in the market.

Gymnosperia emerginata (Wild) grow in moderately fertile, moist but well drained soil in full sun with midday shade. World wide interest in natural products as preventive and therapeutic agents as led to a greater appreciation of the rich heritage of traditional system of medicine. The selection of scientific and systemic approach for the biological evaluation of plant world on the basis of their use in the traditional system of medicine forms a basis for art ideal approach in the development of the new drugs from plants.[12,13]

The present aim to evaluate the anti inflammatory of *Gymnosporia Emerginata* leaves extract. The objective of the present study includes: Preparation of ethanolic extract of Gymnosporia Emerginata using soxhalation process, To perform phytochemical investigation of ethanolic extract of *Gymnosporia emerginata*, To assess the in vitro anti inflammatory activity of *Gymnosporia Emerginata* by employing the method such as protein denaturation.

2. MATERIALS

In the present study, the leaves of *Gymnosporia Emerginata* were collected from local areas of Tirupathi and authenticated by Prof. Madhav chetty, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. The authenticated leaves of *Gymnosporia Emerginata* were air dried and subjected to size reduction to get coarse powder and was subjected to standardization with different parameters. All the solvents and reagents used in the present work are of analytical grade required chemicals were procured from Qualigeris chemicals pvt. Ltd.

3. METHODS

3.1. Plant Extraction

The leaves of *Gymnosporia Emerginata* belonging to family celastraceae were reduced to coarse powder (40 size mesh) and 150 gm of powder was subjected to successive hot continuous extraction (soxhlet) with ethanol. After the effective extraction, extracts were concentrated using rotary flash evaporator. The extracts obtained were weighed and percentage yield was calculated.

3.2. Preparation of ethanolic extract

The plant material was washed and then dried in hot air oven at temperature not more than 500C and reduced to coarse powder the powdered material 150gms was subjected to solvent extraction in soxhlet apparatus with ethanol the extraction procedure was continued until the colourless solution was obtained and the solution was concentrated with rotary evaporator under reduced pressure and the dried extract was weighed the percentage yield were presented in experimental results.

Figure 1: Soxlhlet extraction and distillation of *Gymnosporia emerginata.*

3.3. Qualitative phyto chemical analysis[14]

Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. In phytochemical evaluation the powdered leaves were subjected to phytochemical screening for the detection of various plant constituents, characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis.

3.4. Qualitative chemical investigation

Qualitative chemical tests were conducted for all the extracts of leaves of *Gymnosporia Emerginata* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded.

1) Tests for Carbohydrates

Preparation of test solution: The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 2N HCl and subjected to following chemical tests.

a) Molisch's test: To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H2SO4 from sides of the test tube was observed for violet ring at the junction of two liquids.

b) Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions were mixed and boiled for one minute. Equal volume of test solution was added. Heated in boiling water bath for 5-10 min and was observed for yellow, then brick red precipitate.

c) Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

d) Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

f) Tests for Non-Reducing Sugars: Test solution does not give response to Fehling's and Benedict's test.

g) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

2) Tests for Proteins

Preparation of Test Solution: The test solution was prepared by dissolving the extract in water.

a) Biuret test (General test): To 3 ml T.S added 4% NaOH and few drops of 1% CuSO4 solution observed for violet or pink colour.

b) Millon's test (for proteins): Mixed 3 ml T.S. with 5 ml Millon's reagent, white precipitate obtained. Precipitate warmed turns brick red or precipitate dissolves giving red color was observed.

c) Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H2SO4 observed for white precipitate.

d) Precipitation test:

The test solution gave white colloidal precipitate with following reagents: Absolute alcohol, 5% HgCl2 solution, 5% CuSO4 solution, 5% lead acetate, 5% ammonium sulphate.

3) Tests for Steroids

Preparation of test extract solution: The extracts were re fluxed separately with alcoholic solution of potassium hydroxide till complete saponification. Saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the Chloroform.

a) Salkowski reaction: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H2SO4 was added. Shake well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

b) Libermann-Burchard test: Mixed 2ml of extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentrated H2SO4 from the side of test tube observed for first red, then blue and finally green color.

c) Libermann's test: Mixed 3 ml of extract with 3 ml of acetic anhydride. Heated and cooled. Added few drops of concentrated H2SO4 and observed for blue color.

4) Tests for Amino Acids

a) Ninhydrin test (General test): 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. Observed for purple or bluish color.

b) Test for Tyrosine: 3 ml T.S. and 3 drops Millon's reagent. Solution observed for dark red color.

c) Test for tryptophan: To 3 ml T.S. added few drops glycoxalic acid and concentrated H2SO4 observed for reddish violet ring at junction of the two layers.

5) Tests for Glycosides

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

Tests for Cardiac Glycosides:

a) Baljet's test: A test solution observed for yellow to orange color with sodium picrate.

b) Bromine water test: Test solution dissolved in bromine water giving yellow precipitate

c) Legal's test (For cardenoloids): To aqueous or alcoholic test solution, added 1ml pyridine and 1ml sodium nitrprusside observed for pink to red colour.

Test for anthraquinone glycosides, cyanogenetic glycosides and Tests for Alkaloids also performed. In addition Tests for Flavonoids, Test for Vitamins, Saponins, Tannins and Phenolic compounds were also performed.

3.5. In vitro anti inflammatory activity

Method

Protein denaturation method by Egg albumin

Principle

The mechanism of denaturation probably involves alteration electrostatic hydrogen, hydrophobic and disulphide bonding. The increase in absorbance of plant extract and reference drug with respect to control indicated the stabilization of albumin protein.

Procedure

In-vitro anti-inflammatory activity of *Gymnosporia emerginata* was studied by using protein denaturation technique. Which was studied according to (Mizushima et al and sakat et al). followed with minor modifications. The following procedure was followed for evaluating the percentage of inhibition of protein denaturation :-Control solution of 5ml made by 2ml of egg albumin(from fresh hens egg), 2.8ml of phosphate buffer(pH 6.4) and 2ml distilled water. Standard drug solution 5ml made by 2ml egg albumin, 2.8ml of phosphate buffer and various concentrations of standard drugs (Diclofenac sodium) concentration of 200, 400, 600, 800 and 1000 microgram per ml. Test solution of 5ml made by 2ml of egg albumin, 2.8ml of phospahte buffer and various concentrations of plant extract concentration of 200, 400, 600, 800 and 1000 microgram per ml. All of the above reaction mixture were incubated at 370C for 15 minutes and heated at 700C for 5min. After cooling the absorbance of the above solutions was measured using UV visible spectrophotometer at 660nm. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation can be calculated as using the formula.

% INHIBITION = Absorbance of Control – Absorption X 100

Absorbance of Control- The Percentage protection from denaturation is calculated by using the formulae tabulated

Preparation of diclofenac sodium solution

10 mg of standard diclofenac sodium powder was dissolved in 5ml of water in a 10 ml volumetric flask then adjust the volume up to 10ml water then 1ml of this solution was diluted to 50ml of diluted water the concentration of solution of this solution was 20µg.

3.6. Statistical analysis

Invitro data were expressed as mean percentage inhibition ± SD(N=3). All analysis were

carried out in graph pad prism (version 5.0) software.

4. RESULTS AND DISCUSSION

Gymnosporia emerginata extraction has been studied for various parameters and the results have been presented in Table 1 to 4.

Table No 1: Macroscopic characteristics of leaves of *gymnosporia emerginata.*

S. No.	Parameters (Physical Tests)	Observation of flowers
1	Texture	Smooth
2	Colour	Greenish black
3	Odour	Characteristic
4	Taste	Characteristic

Table 2: Analysis of extracts of Leaves of *Gymnosporia Emerginata*.

S. No.	Extract	Colour Of Extract
1	Ethanol	Brownish Red

Table 3: Qualitative Phytochemical analysis of ethanolic extract of *Gymnosporia Emerginata*.

S.NO	CHEMICAL CONSTITUENTS	ETHANOLIC EXTRACT
1	Alkaloids	+
2	Carbohydrates	+
3	Anthraquinone Glycosides	+
4	Cardiac Glycosides	+
5	Saponin Glycosides	+
6	Flavanoids	+
7	Steroids And Tri Terpinoids	+
8	Tannins	+
9	Proteins	+
10	Gums And Mucilage	+

Table 4: Effect of ethanolic extract of *gymnosporia emerginata* on anti inflammatory activity by egg albumin denaturation.

S.NO	CONCENTRATION (µg/ml)	%INHIBITION	
		GYMNOSPORIA EMERGINATA	DICLOFENAC SODIUM
1	200	45.427±0.567	49.280±0.612
2	400	63.327±0.630	64.503±0.775
3	600	70.377±0.851	72.847±0.628
4	800	81.617±0.687	84.553±0.612
5	1000	90.590±0.609	92.597±0.744

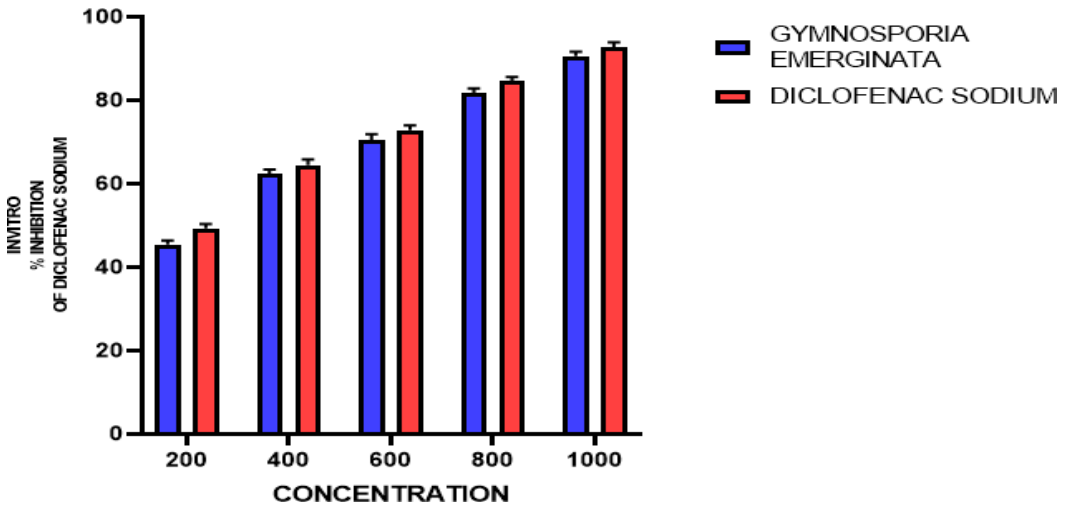

Figure 2: Graph Showing Effect of Ethanol Extract of *Gymnosporia Emerginata* on *Diclofenac.*

In the present study, shade dried leaves of *Gymnosporia Emerginata* belonging to family Celastraceae, having medicinally important bioactive constituents is reviewed, with special emphasis on the biological activities. The plant material was air dried and reduced to coarse powder. The powder material was subjected to solvent extraction in soxhlet apparatus with ethanol. The soxhelation was continuedand untill the coloursless solution was obtained and the solution was concentrated in rotary evaporator and reduced pressure and yield of extract is 14.0625 g. The colour of extract was greenish black in colour with characteristic taste.

Protein Denaturation is a process in which proteins lose their tertiary structure andsecondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation.

The ethanolic extract of *gymnosporia emerginata* should anti inflomatory activity by protein denaturation egg albumin method and maximum percentage of inhibition was found at1000µg/ml is 90.59%. Diclofenac sodium should anti inflomatory activity by protein denaturation egg albumin method and maximum percentage of inhibiton was found at 1000µg/ml is 92.59%.

CONCLUSION

At percent research an attempt has been made to find out the therapeutic activity like anti - inflammatory of the *Gymnosporia emerginata* plant. From the literature review the leaves of *Gymnosporia emerginata* (Celastraceae) was selected for the study and the following parameters were studied. Selection, identification and collection, Extraction and preliminary phytochemical analysis and Invitro anti- inflammatory activity. The ethanolic extract of *Gymnosporia emerginata* was identified for the presence of flavonoids steroids, triterpinoids, alkaloids, carbohydrates. The literature review reveals that *Gymnosporia Emerginata* has been in use since ancient times to treat wide range of diseases in traditional system of medicine. So the present target was find out Protein denaturation of the *Gymnosporia Emerginata* results showed dose dependent inhibition activity. *Gymnosporia Emerginata* maximum 90.59% inhibition of egg albumin 1000µg/ml compare to standard diclofenac sodium maximum 92.59%..it shows may be anti inflammatory activity. The presence of above mentioned phytoconstituents may be responsible for anti inflammatory activity.

REFERENCES

1. Rios JL, Recio MC. Medicinal plants and Antimicrobial activity. Journal of Ethnopharmacology, 2005; 100: 80.
2. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, 1993.
3. Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A. In vitro antimicrobial and antioxi dant activities of the essential oils and various extracts of Thymus eigii M. Zohary et P.H. Davi s. J Agric Food Chem, 2004; 52: 11321137.
4. Marder VJ. Recombinant streptokinase- opportunity for an improved agent. Blood Coagel Fibrinolysis, 1993; 4: 1039- 1040.
5. celsus In ancient rome (30-38B.C.) and by Galen (A.D. 130-200B.C.) Division Of Science, University Of Luton, Luton, Uk.
6. Harshmohan "Text Book of Pathology", Jaypee brothers medical publishers, 2010, 6th edition, pp 134-137, 550, 591-593.
7. Harshmohan "Text Book of Pathology", Jaypee brothers medical publishers, 2010, 6th edition, pp 134-137, 550, 591-593. Feghali C. A., et al "Cytokines in acute and chronic.
8. Ofman JJ, Maclean Ch,Straus Wl,Et Al.Metaanalysis Of Severe Complications Of Nonsteriodal Anti Inflammatory Drugs. J Rheumatol, 2002; 29: 804-1.
9. Lijnen HR, Vanhoef B, DeCock F, Okada K, Ueshima S, Matsuo O. On the mechanism

of fib rin – specific plasminogen activation bystaphylokinase. J Biol Chem, 1991; 266: 1182611832.

10. K. Hemamalini, Uma Vasireddy, E .Rathna sundari Dept. Of Pharmacology, Meerpet, Hyderabad, AP, India. Dept. Of Pharmaceutics, Dhanavanthri College of pharmacy kothagudem Hyderabad, AP, India.
11. K MEDIGA KIRAN KUMAR, A.T.THIRUPATHI, K. NAGARAJU, B. DEEPIKA, KNV RAO K.RAJESWAR DUTT Dept of Pharmacology Nalandha college of Pharmacy, Nalgonda Correspondindavg author. Mediga Kiran Kumar, India. Email:- kumarswamy.gandla@gmail.com
12. Dharmassree BT Wijeratne, Vijaya kumar, M Uvais S Sultabawa journal of the ChemicalSociety, Pewrkin Transactions, 1981; 1: 2724–2726.
13. Yadav IK, Jaiswal D, singh H, Mishra A, Jain DA.Anti -HIV drugs from natural sources. pharmaceutical researches, 2009; 1: 93-100.
14. K Hemamalini et al. IJRP, 2013; 3(2): 154-169.
15. Harborne JB, Phytochemical methods of Analysis. London; Chapman and Hall Ltd, 1984; 1-7.
16. Sreeharsha, Nagaraja, Nimbagal Raghavendra Naveen, Posina Anitha, Prakash S. Goudanavar, Sundarapandian Ramkanth, Santosh Fattepur, Mallikarjun Telsang, Mohammed Habeebuddin, and Md Khalid Anwer. "Development of Nanocrystal Compressed Minitablets for Chronotherapeutic Drug Delivery." *Pharmaceuticals*, 2022; 15(3): 311.
17. Naveen, N. Raghavendra, Chakka Gopinath, and D. Subba Rao. "Isolation and assessment of natural mucoadhesive agent isolated from Abelmoschus esculents." *Journal of Pharmacy Research*, 2017; 11.5: 438-443.
18. Rizg, Waleed Y., et al. "QbD Supported Optimization of the Alginate-Chitosan Nanoparticles of Simvastatin in Enhancing the Anti-Proliferative Activity against Tongue Carcinoma." *Gels*, 2022; 8.2: 103.
19. Naveen, N. Raghavendra, et al. "Formulation Design and In Vitro Evaluation for Stomach Specific Drug Delivery System of Anti Retroviral drug–Acyclovir." *International Journal of Pharmacy and Life Sciences*, 2013; 4.3: 2506-2510.
20. Naveen, N. R. "Design and characterization of sustained release matrix tablets of glimepiride by using synthetic and natural polymers." *International journal of drug discovery and herbal research*, 2013; 3.1: 573-578.