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Research Article

Invitro anti-inflammatory activity of Ethanol Extract of *Glochidion Ellipticum.*

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Abstract
The study was aimed at evaluating the in vitro Anti inflammatory activity of ethanol extract of leaves of *Glochidion ellipticum* (family: Euphorbiaceae). The drug was evaluated using different Anti inflammatory. The various Anti inflammatory activity activities were compared to synthetic drugs such as ascorbic acid. When compared with ethanolic extract methanolic extract showed higher Anti inflammatory effect.
Keywords: *Glochidion ellipticum*, Anti inflammatory activity.

1. Introduction
In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future. Majority of crude herbs come from wild sources and it is collected to assess quality parameters by which presence of various phytochemicals can be confirmed. Standardization of natural products is complex task due to their heterogenous composition in form of whole plant. authentication, pharmacognostic evaluation, phytochemical analyses are few basic protocols for standardization of herbals. [1,2]

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Since the dawn of the human civilization, the importance of medicinal plants in the treatment of variety of human ailments has been immense. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites. Plants are the best source of active secondary metabolites which are beneficial to mankind in treating many diseases (Sandhya. S, 2011).Genus Glochidion have been used for a varied of biological activities in traditional medicine and also have been using by many ethnic groups. It is a vast genus in which many plants explored chemically, but most of the species in this genus were not standardized pharmacognostically. [2,-5]

Detailed literature review states that the plant has broad spectrum of the activities which were claimed traditionally and some are

proven scientifically. Most of species in this genus were explored on the basis of the chemical constituent but not on pharmacognostical and pharmacological basis. Medicinal plants are rich in Secondary metabolites, less in quantity with more value compounds and are potential sources of drugs and essential oils. Many of these compounds are having tremendous values in treatment of various ailments. Traditional herbal practices are now-a-days becoming familiar due to the Natural drugs having no side effects when compared to that of chemical drugs.

Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Natural products, which come out from medicinal plants are also important for pharmaceutical research and for drug development as a sources of therapeutic agents. India is perhaps the largest producer of medicinal herbs and rightly called the botanical garden of the world, which are used for thousands of years in the indigenous system of medicine like Ayurveda, Siddha and Unani. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Their role is twofold in the development of new drugs either for development of a medicine or used for the treatment of diseases. Since ancient times many plants have been utilized by man for extracting and utilizing secondary metabolite products.

The Euphorbiaceae are mostly monoecious herbs, shrubs, and trees, sometimes succulent and cactus-like, comprising one of the largest families of plants with about 300 genera and 7,500 species that are further characterized by the frequent occurrence of milky sap. The leaves are mostly alternate but may be opposite or whorled and they are simple, or compound, or sometimes highly reduced. Stipules are generally present but may be reduced to hairs, glands or spines. The flowers are unisexual and usually actinomorphic. They may be highly reduced by suppression of parts, in the extreme form consisting of a naked stamen as a male flower and a naked pistil as a female flower. A specialized type of miniature inflorescence called a cyathium occurs in about 1,500 species comprising the genera Euphorbia and Chamaesyce. The cyathium consists of a single naked pistillate flower surrounded by cymes of naked staminate flowers, each consisting of a single stamen. These flowers are all enclosed in a cup-like involucre that typically is provided with peripheral nectaries and petaloid appendages such that the whole aggregation closely resembles a single flower. In other members of the family the flowers and inflorescences are more ordinary in appearance, with male and female flowers typically bearing a 5-merous calyx and corolla of distinct segments, although the corolla is sometimes absent. In these forms the androecium most commonly consists of 5, 10 or sometimes numerous distinct or monadelphous stamens. The gynoecium of female flowers consists of a single compound pistil of typically 3 carpels, an equal number of styles or primary style branches, and a superior ovary with typically 3 locules, each bearing 1 or 2 collateral, axile-apical pendulous ovules. The fruit is usually a capsular schizocarp. [6-7]

Materials and Methods

All the chemicals and reagent used were of laboratory grade and were procured from manufactures ofResearch lab fine chemicals, Mumbai., Loba Chemie, Mumbai, Sigma-Aldrich, Mumbai., Hi Media Lab Mumbai, Finar reagents, Ahmadabad, Merck, Mumbai, Genuine Chem., Mumbai, Labin, Mumbai, Moly Chem, Mumbai)

Collection of plant:

The plant was collected from the forest regions of Koyna dam of karaddist, satara. It was authenticated by Dr. Sanjay S. Sathe, Asso. Professor, Dept. of Botany, PDVP, Mahavidyalaya Tasgaon, Dist- Sangli. A herbarium was prepared and deposited in the Dept. of Pharmacognosy for further reference. The plant was identified as *Gochidion elipticum*. (Euphorbiaceae) and was certified under Voucher No: RCP-SNG/ ph'cog/ 2009-10/003.

Extraction methods

- Preparation of various extract of medicinal plants

- Aqueous extraction

Aqueous extracts *Gochidione lipticum* were carried out by cold maceration. In this process, solid ingredients were subjected to cold maceration with chloroform: Water I.P (2:98) (Indian Pharmacopoeia (I.P.); 1996). Powder was placed in 2 liters round bottom flask for about 7 days at room temperature in a warm place. The flask was securely plugged with absorbent cotton and was shaken periodically with frequent agitation until soluble matter is dissolved. The mixture was filtered and after most of the liquid has drained, the filtrate was concentrated to residue at constant temperature bath at temperature 50^0C.

Note: Chloroform water I.P.

2.5 ml of chloroform was shaken with 900 ml of water until dissolved and diluted to 1000 ml with water.

Successive solvent extraction

The dried leaves of the plant of *Gochidion elipticum* were reduced to coarse powder (40 size mesh) and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet apparatus) with petroleum ether (60-80^0C), chloroform, ethyl acetate and ethanol to about 10 cycles per batch for 1 batches. The extraction was continued until the solvent in the thimble became clear. Each time before extracting with next solvent the powdered material was dried at room temperature.

After the effective extraction, solvent was distilled off using rotary vacuum evaporator and the extracts were concentrated at low temperatures. The dried concentrated extracts were used for phytochemical investigation, isolation, pharmacological activity. [8,9]

- **Alcoholic extraction**

About 500 gms of fresh air-dried Leaves and stem bark of *Gochidion elipticum* were extracted with ethanol by using soxhlet extractor. The extract was filtered and concentrated with the help of rotary vacuum evaporator.

In-vitro anti-inflammatory activity

Materials: Bovine serum Albumin, tris buffer, Ibuprofen and diclofenac sodium, distilled water was prepared in analytical; department of A.B.C.P.Sangli.

Sample preparation:

Stock solution of crude extract was prepared 10mg/kg in distilled water and from the stock solution the samples was prepared into different concentration.

1. **Antidenaturation activity by using Bovine serum albumin:**

A solution of 0.2% w/v of BSA was prepared in a Tris Buffer Saline and pH was adjusted to 6.8 using glacial acetic acid From these stock solutions (Stock solutions of 10 mg/ml.) The control consists of 5ml of 0.2% w/v BSA solution with 50µl ethanol. The standard consists of 100µg/ml of Ibuprofen in ethanol with 5ml 0.2% w/v BSA solution. different concentrations of 100, 200 and 500µg/ml were prepared by using ethanol as a solvent. 50µl (0.05ml) of each extract was transferred to tubes with 5ml of 0.2% w/v BSA was added to tubes. The test tubes were heated at 72ºC for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using a UV-VIS Double beam spectrophotometer (ELICO SL 244) at a wavelength of 660nm. Each experiment was carried out in triplicate and the mean absorbance was recorded.

2. **Protein denaturation activity by using fresh egg albumin:**

The control and standard consists of 0.4ml egg albumin and 5.6ml phosphate buffer saline with pH 6.4 with 4 ml saline and diclofenac sodium respectively. Different concentrations of 100, 200 and 500µg/ml in 4 ml/kg reaction mixture incubated at 37^0C for 15 min then heated at 70^0C for 5 minutes and then cooled under tap water. The absorbance of these solutions was determined by using a spectrophotometer at a wavelength of 660nm. Each experiment was carried out in triplicate and the mean absorbance was recorded.

Result and Discussion

In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by BSA

Table no. 1- Concentration effective in inhibiting heat induced albumin denaturation as dose dependant manner.

Sr. no	Conc.(µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of leaves
1	10	46.36 ±2.14	41.26 ±2.14
2	20	64.59 ±2.13	62.23 ±1.16
3	30	74.59 ±2.11	72.26 ±2.18
4	40	78.96 ±2.10	75.59 ±2.12
5	50	82.26 ±2.09	79.25 ±2.11

Values were expressed as mean ± SD Table no. 1 indicates at concentration 10-50 µg/ml was effective in inhibiting heat induced albumin denaturation as dose dependant manner. The maximum inhibition 82.26 % at the conc. of 50 µg/ml was observed from ethanolic extract of *Glochidion elipticum*.

Fig. 1- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by BSA.

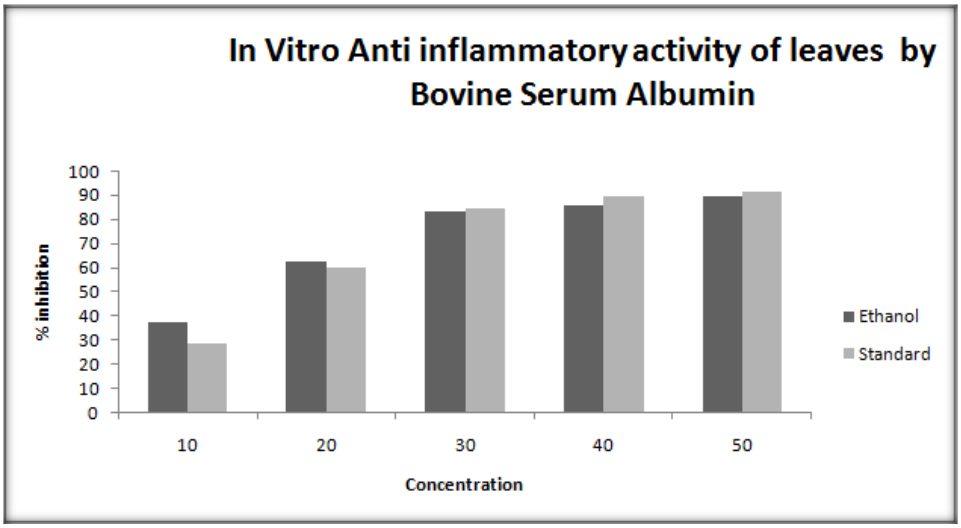


Table No. 2- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by BSA

Sr. no	Conc .(µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of leaves
1	10	46.36 ±2.14	43.21 ±2.10
2	20	64.59 ±2.13	58.26 ±2.11
3	30	74.59 ±2.11	68.59 ±2.13
4	40	78.96 ±2.10	70.25 ±3.10
5	50	82.26 ±2.09	72.23 ±3.14

Values were expressed as mean ± SD Table no. 2 indicates that aqueous extract at concentration 10-50 µg/ml was less effective in inhibiting heat induced albumin denaturation. The maximum inhibition 82.26 % at the conc. of 50 µg/ml was observed from ethanolic extract of *Glochidion elipticum*. [11-15]

Fig. 2- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by BSA.

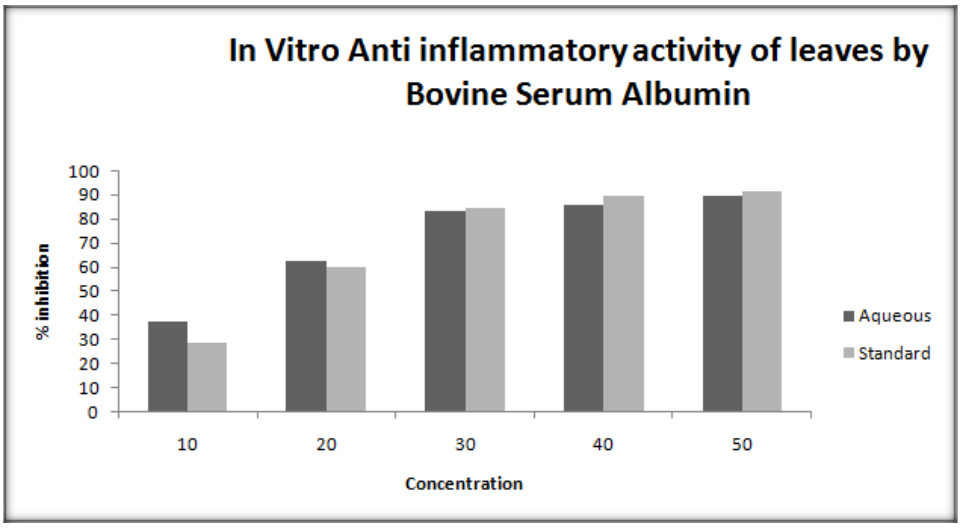


Table No. 3- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by BSA.

Sr. no	Conc .(µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of stem bark
1	10	46.36 ±2.14	35.26 ±2.10
2	20	64.59 ±2.13	43.58 ±2.11
3	30	74.59 ±2.11	62.28 ±2.12
4	40	78.96 ±2.10	68.89 ±2.13
5	50	82.26 ±2.09	71.25 ±2.17

Values were expressed as mean ± SD From this Table no.3, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* stem bark was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 71.25 % inhibition than standard 82.26 %.

Fig. 3- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by BSA.

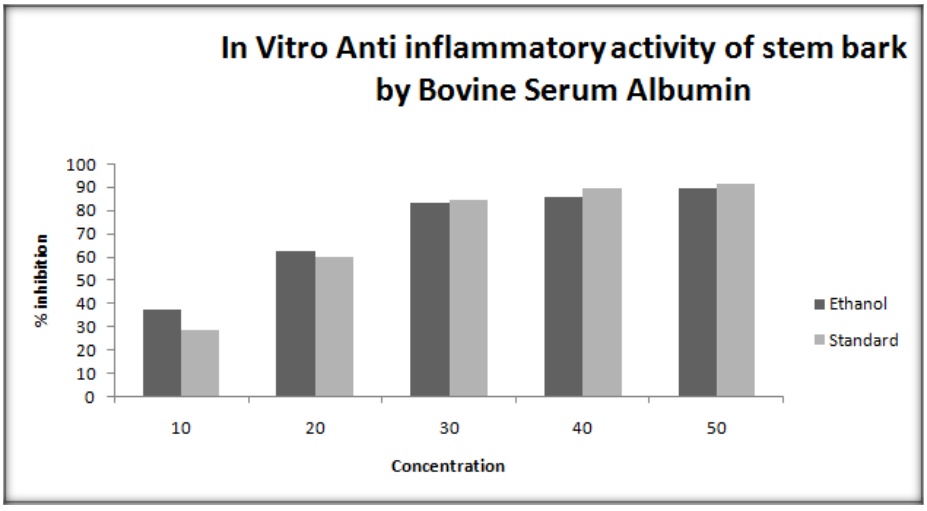


Fig. 4- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by BSA.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of stem bark
1	10	46.36 ±2.14	30.26 ±2.10
2	20	64.59 ±2.13	42.58 ±2.01
3	30	74.59 ±2.11	62.25 ±2.11
4	40	78.96 ±2.10	68.59 ±2.16
5	50	82.26 ±2.09	70.26 ±2.18

Values were expressed as mean ± SD From this Table no.4, it is indicate that anti-inflammatory activity aqueous extract of *Glochidion elipticum* stem bark was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 70.26 % inhibition than standard 82.26 %.

Fig. 4- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by BSA

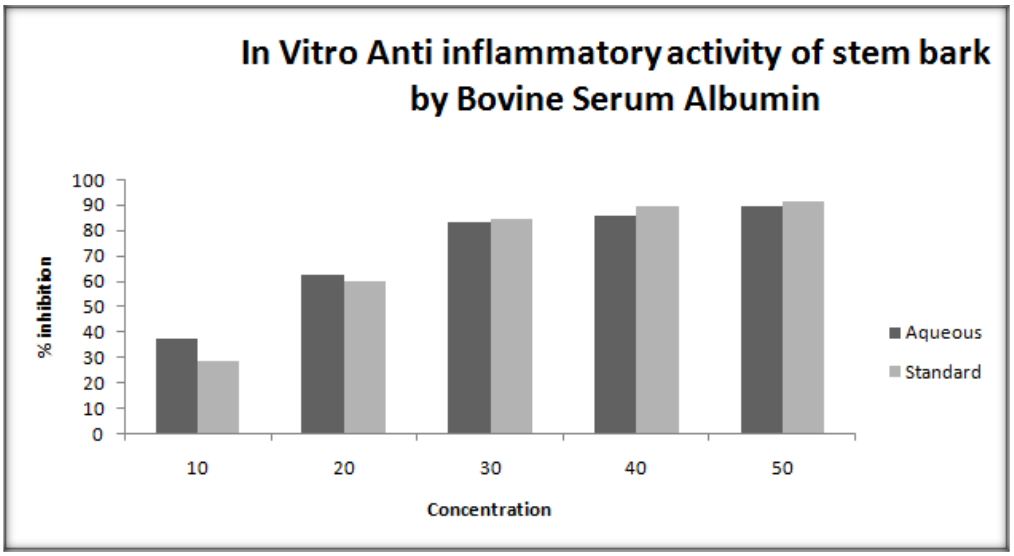


Table No. 5- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by BSA.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of root
1	10	46.36 ±2.14	43.25 ±2.13
2	20	64.59 ±2.13	61.29 ±2.18
3	30	74.59 ±2.11	65.58 ±2.15
4	40	78.96 ±2.10	68.69 ±2.12
5	50	82.26 ±2.09	71.26 ±2.11

Values were expressed as mean ± SD From this Table no. 5, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* root was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 71.26 % inhibition than standard 82.26 %.

Fig. 5- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by BSA.

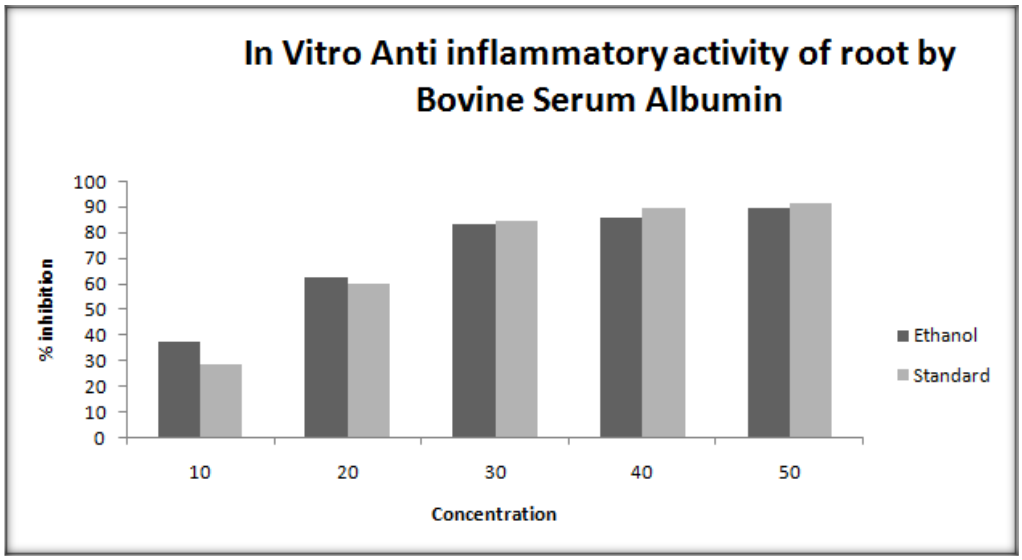


Table No. 6- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by BSA.

Sr. no	Conc .(µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of root
1	10	46.36 ±2.14	41.26 ±2.14
2	20	64.59 ±2.13	58.59 ±2.12
3	30	74.59 ±2.11	61.28 ±2.14
4	40	78.96 ±2.10	65.59 ±2.13
5	50	82.26 ±2.09	68.59 ±2.12

Values were expressed as mean ± SD From this Table no. 6, it is indicate that anti-inflammatory activity aqueous extract of *Glochidion elipticum* root was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 68.59 % inhibition than standard 82.26 %.

Fig. 6- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by BSA.

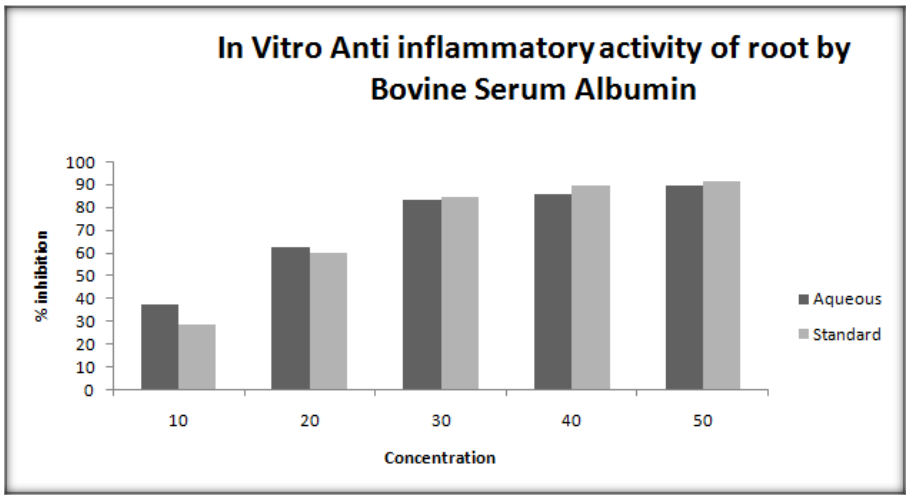


Table No.7- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of leaves
1	10	56.36 ±2.18	61.29 ±2.13
2	20	67.58 ±2.22	72.26 ±2.17
3	30	78.59 ±2.12	82.35 ±2.14
4	40	82.26 ±2.24	85.65 ±2.13
5	50	85.65 ±2.10	89.54 ±2.11

Values were expressed as mean ± SD From this Table no.7, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 89.54 % inhibition than standard 85.65 %.

Fig. 7- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by egg albumin denaturation.

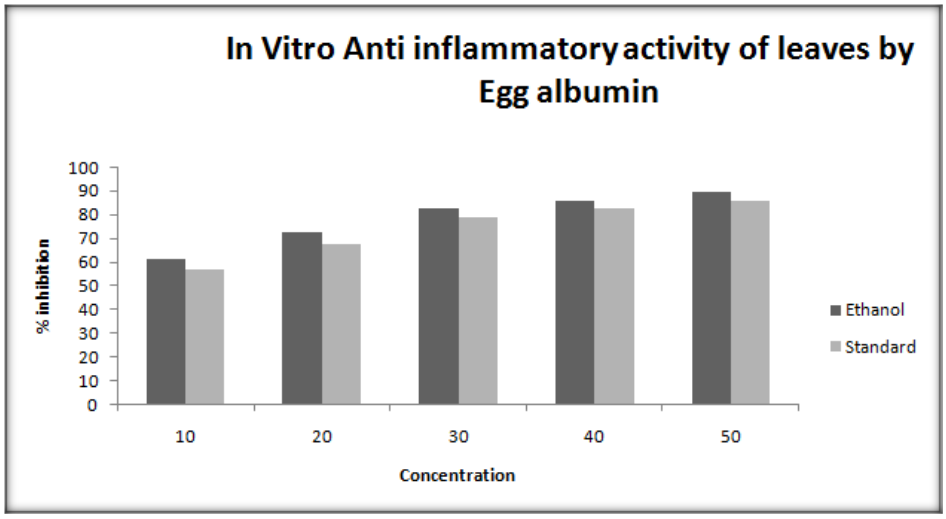


Table No. 8- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of stem bark
1	10	56.36 ±2.18	53.38 ±2.16
2	20	67.58 ±2.22	67.57 ±2.17
3	30	78.59 ±2.12	75.48 ±2.14
4	40	82.26 ±2.24	78.96 ±2.12
5	50	85.65 ±2.10	81.11 ±2.11

Values were expressed as mean ± SD From this Table no.8, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* leaves was shown good significant inhibition as

compared to standard drug aspirin. The extract concentration 50 µg/ml showed 81.11 % inhibition than standard 85.65 %.

Fig. 8- In Vitro Anti inflammatory activity of leaves *Glochidion ellipticum* by egg albumin denaturation.

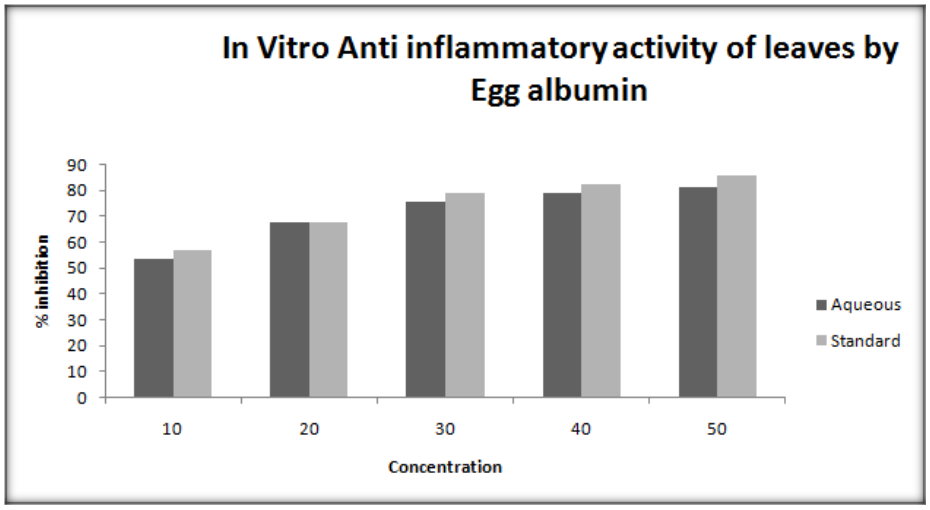


Table No.9- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of stem bark
1	10	56.36 ±2.18	56.59 ±2.19
2	20	67.58 ±2.22	71.25 ±2.14
3	30	78.59 ±2.12	81.59 ±2.14
4	40	82.26 ±2.24	82.26 ±2.15
5	50	85.65 ±2.10	84.46 ±2.16

Values were expressed as mean ± SD From this Table no. 9, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 81.11 % inhibition than standard 84.46 %.

Fig. 9- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum*by egg albumin denaturation.

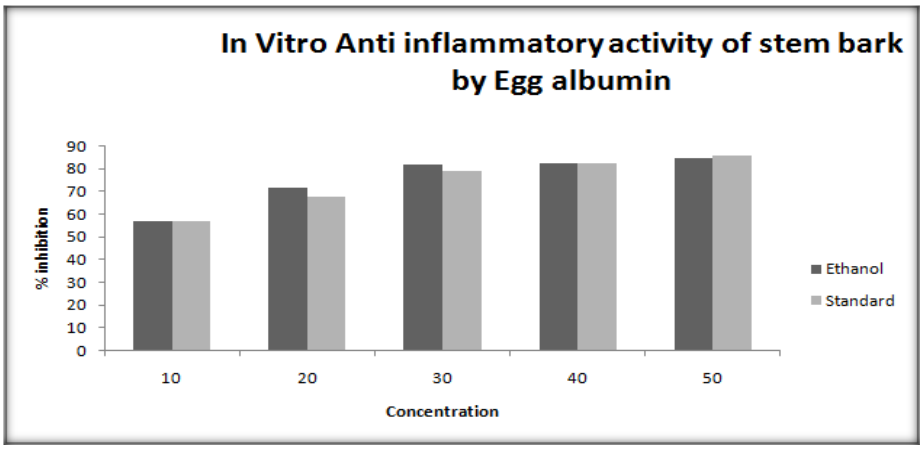


Table No. 10- In Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of stem bark
1	10	56.36 ±2.18	42.28 ±2.17
2	20	67.58 ±2.22	51.24 ±2.12
3	30	78.59 ±2.12	61.28 ±2.14
4	40	82.26 ±2.24	65.59 ±2.13
5	50	85.65 ±2.10	70.25 ±2.12

Values were expressed as mean ± SD From this Table no.10, it is indicate that anti-inflammatory activity aqueous extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 70.25 % inhibition than standard 85.65 %.

Fig. 10- Vitro Anti inflammatory activity of stem bark *Glochidion ellipticum* by egg albumin denaturation.

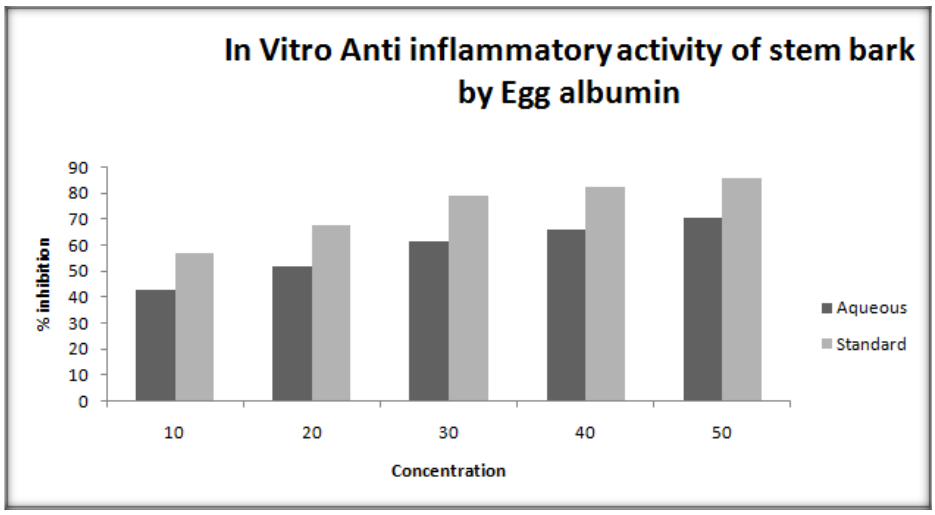


Table No.11- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Ethanol extract of root
1	10	56.36 ±2.18	53.29 ±2.10
2	20	67.58 ±2.22	59.57 ±2.11
3	30	78.59 ±2.12	68.24 ±2.11
4	40	82.26 ±2.24	71.29 ±2.16
5	50	85.65 ±2.10	72.29 ±2.14

Values were expressed as mean ± SD From this Table no. 11, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 72.29 % inhibition than standard 85.65 %.

Fig. 11- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by egg albumin denaturation.

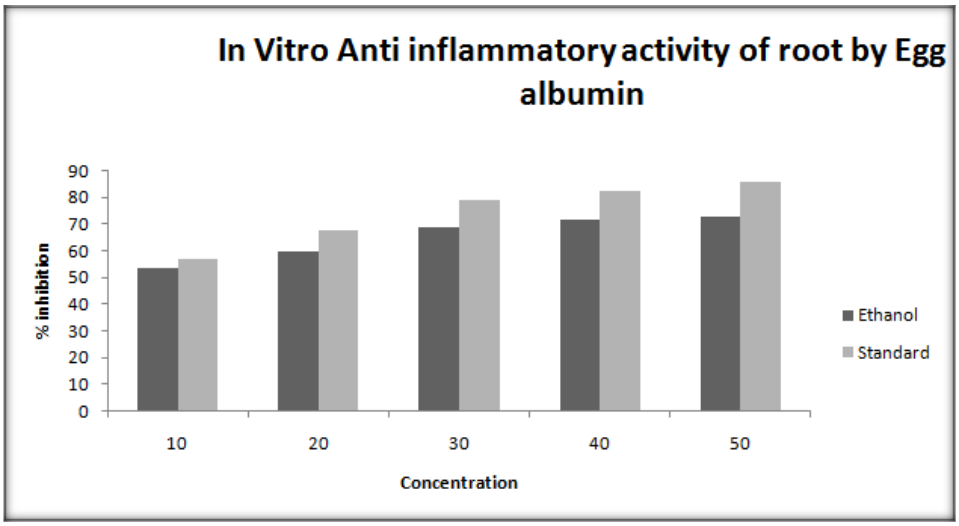
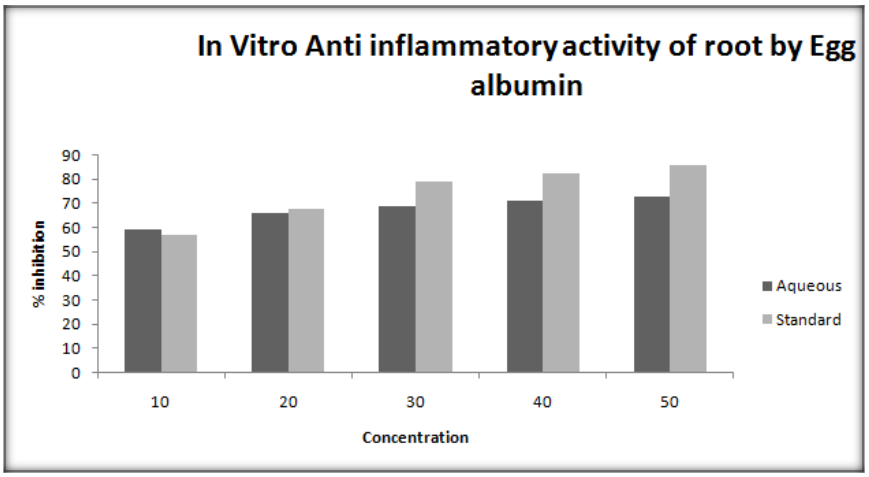


Table No.12- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by egg albumin denaturation.

Sr. no	Conc. (µg/ml)	% inhibition	
		Standard Aspirin	Aqueous extract of root
1	10	56.36 ±2.18	58.59 ±2.15
2	20	67.58 ±2.22	65.57 ±2.19
3	30	78.59 ±2.12	68.57 ±2.14
4	40	82.26 ±2.24	70.51 ±2.16
5	50	85.65 ±2.10	72.28 ±2.11

Values were expressed as mean ± SD From this Table no.12, it is indicate that anti-inflammatory activity ethanol extract of *Glochidion elipticum* leaves was shown good significant inhibition as compared to standard drug aspirin. The extract concentration 50 µg/ml showed 72.28 % inhibition than standard 85.65 %. [16]

Fig. 12- In Vitro Anti inflammatory activity of root *Glochidion ellipticum* by egg albumin denaturation.



Conclusion

In the present study, the study was aimed at evaluating the in vitro Anti inflammatory activity of ethanol extract of leaves of *Glochidion ellipticum* (family: Euphorbiaceae). The drug were evaluated using different Anti inflammatory. The various Anti inflammatory activity activities were compared to synthetic drugs such as ascorbic acid. When compared with ethanolic extract methanolic extract showed higher Anti inflammatory effect.

References

1. Schaeffer W. I., Friend K. Efficient detection of soft aga rgrown colonies using a tetrazolium salt. Cancer Letters1976; 1: 275-279.
2. Black MM and Speer ED, Effects of cancer chemotherapeutic agents on dehydrogenase activity of human cancer tissue in vitro, Am. ***J***. Clin. Phathol, 1953; 23:218-227.
3. Rao N. M., Joshi N. N., Shinde S. R., Avani S. H., Ghosh S. N. Breast size and breast cancer risk. European Journal of Cancer Prevention. 1996; 5(5): 343-350.
4. Marx J.L. Z-DNA: still searching for a function, Science. 1985; 230(4727):794–796.
5. Rice E. C. A., Diplock A. T. Current status of antioxidant therapy. Free radical and Biol. medicine. 1993; 15: 77-96.
6. Youdin, K.A. and J.A. Joseph: A possible emerging role of phytochemicals in improving age related neurological dysfunction a multiplicity of effects, Free Radic.Bio.Med,2001; 38:583-594.
7. Fridovich I. Oxidative Stress. Annual Review of Biochemistry. 1995; 64: 25.
8. Ray G., Hussain; S. A., Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol.. 2002; 40 (11): 1213-1232.
9. Pryor W. A. Oxy-Radicals and Related Species: Their Formation, Lifetimes and Reactions. Annual review of Physiology. 1986; 48: 657-659.
10. Cerutti P, A. Pro oxidant states and tumour promotion Science. 1985; 227(4685): 375-381.
11. Chessman K. H., Slater T. F. Free radicals in medicine, BritishmedicalBulletin. 1991; 49/3: 481-493.
12. Iwata E., Miyazakil I., Asanuma M., Gida A., Ogawa N. Protective effects of nicergoline against hydrogen peroxide toxicity in rat neuronal cell lineNeuroscience Letters. 1984; 251 (1): 49-52.
13. Gutteridge J.M.C.,. Lipid Peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem., 1995; 41(12), p. 1819-1828.
14. Percival, Mark., Antioxidants. Clinical nutrition insights, 1998; 1(96), p.1-4.
15. Leeuwen P.A.M. V., Nijveldt, R.J, Nood, E.V., Hoorn, D.E.C.V., Boelens, P.G., Norren, K.V., Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr, 2001; 74, p.418–25.
16. Sundararajan R., Haja NA., Venketesan K. Cytisusscoparius link--a natural antioxidant. BMC Complimentary and Alternative Medicine. 2006; 16:6-8.

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Conflict of Interest: None declared

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