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Antioxidant And Anti-Inflammatory Potential Of Blepharis Edulis

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Abstract: The present work emphasizes the comprehensive ethano-medicinal uses of *Blepharis edulis*, to enlighten its phytochemical constituents and pharmacological uses which may useful in various types of diseases. Theo bjective of the present study was to evaluate the antioxidant and anti-inflammatory activity of extract of *Blepharis edulis* leaves and seeds extract for scientific validation of the folklore claim of the plant.

Introduction: Natural products have been the main sources of new drugs. The different strategies have been developed to find the new drugs based on natural products. The traditional and ethic medicines have provided information on the therapeutic effects and resulted in some notable drug discovery of natural products. More than 80 of 371 pharmaceutical substances included in Ninth Edition of International Pharmacopoeia are natural products or natural products derivatives [1, 2]. In ancient time of facing the challenge of unknown disease, the natural products were discovered by errors and trials. The ancient written documents originated from North Africa, India and China showed the earliest record of clinical practices [3]. As the progression of isolation of active compound from natural products, structural identification becomes new direction of drug discovery from natural products. In 1940s, Robert Burns Woodward first introduced physical means to identify the structure of natural products, which greatly improved the level of research on the structure of compounds [4]. The history of drug discovery based on therapeutic effects from natural products could retrace to 4,000 years before. During the long period of development, the strategies of passive discovery from therapeutic effects of the natural products have evolved into active searches for new medicines. Natural products, as precursors for semi-synthesis of drugs and templates for chemical synthesis, broad ideas for structural modification in drug design of natural products and have become effective strategies of new drug development. Antioxidants are substance having potential to quench free radicals and significantly delay or inhibit oxidation of the substrate, thus protect biological systems against potential harmful effects of free radicals; in

low concentrations [5]. Plants produce a large number of antioxidants to control the oxidative stress caused by sunbeams and oxygen, thus plants contains potent compounds with antioxidant activity [6]. These compounds are able to scavenge reactive oxygen species due to their electron donating properties [7]. In search of novel anti-oxidants with low toxicity over past few years, medicinal plants have been studied extensively for their radical scavenging activity. The several categories of compounds showed predominant antioxidant potential. The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. The present work emphasizes the comprehensive ethano-medicinal uses of *Blepharis edulis*, to enlighten its phytochemical constituents and pharmacological uses which may useful in various types of diseases. Blepharis is a genus of plant in family Acanthaceae and it contains around 126 species found in seasonally dry to arid habitats with a tropical and subtropical distribution. Traditionally whole plant powder have pharmacological efficacy establish plant for treatment of anti-inflammatory activity. The proposed work aimed to investigate the different extract of *Blepharis edulis* seeds and leaves for antioxidant and anti- inflammatory activity.

Material and methods:

Collection and identification of plant material: Blepharis edulis herbs along with inflorescence were collected from tribal region of Amravati (Maharashtra) in the month of October. Blepharis edulis seed purchased from local market Bhopal. Plant material were identified and authenticated in the Department of Botany, Government College Khimlasa, Sagar (M.P.). The plant materials were dried in shade, powdered moderately and pass through sieve No. 10.

Preparation of the extract: The powdered plant material (250 gm) were successively extracted in a soxhlet apparatus with petroleum ether (60-80°C), Chloroform, Ethyl acetate, methanol and finally with water (by maceration process). After each extraction test was performed to see whether the drug had been completely exhausted or not. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent. The extract obtained with each solvent was weighed to a constant weight and percentage w/w yield was calculated

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Anti-oxidant Activity: For the assessment of free radical scavenging activity, the extracts of selected plants were dissolved in 5% DMSO. DPPH, Nitric oxide, hydroxyl radical, superoxide radical methods were carried in the present study.

CBEL: Chloroform extract of *blepharis edulis* leaves

MBEL: Methanol extract of *blepharis edulis* leaves

ABEL: Aqueous extract of *blepharis edulis* leaves

CBES: Chloroform extract of *blepharis edulis* seeds

MBES: Methanol extract of *blepharis edulis* seeds

ABES: Aqueous extract of *blepharis edulis* seeds

Determination of DPPH radical scavenging activity of extracts: The free radical scavenging activity of the extracts were evaluated using 1,1 diphenyl-2- picryl hydrazyl (DPPH) [8]. In its radical form, DPPH absorbs at 517nm, but upon reduction by an antioxidant or a radical species, the absorption decreases.1ml of 0.25mM solution of DPPH in DMSO was added to the different concentrations of selected plant extracts which were dissolved in DMSO (100- 500µg/ml). After 30 min, the absorbance was measured at 517nm by UV-Visible spectrophotometer (shimadzu UV-Vis 1800). All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control. Ascorbic acid was used as a standard. The extracts were added with three ml of DPPH (0.5 mM/ L) in methanol. The absorbance was recorded at 517 nm after 30 minutes of incubation at 37˚C. The percentage of scavenging activity was calculated using following formula,

% of inhibition = (A1 – A2)/A1 × 100

Where, A1 = Absorbance of DPPH

A2 = Absorbance of the reaction mixture with extract (DPPH with Sample)

Determination of Nitric Oxide (NO) radical scavenging activity of extracts: Nitric oxide radical scavenging activity the method used was based on the standard methods with some modification [9]. Nitric oxide (NO) was generated from sodium nitro prusside (SNP) and was measured by the griess reagent (1% w/v sulfanilamide, 2%w/v H3PO3 and 0.1% w/v N-(1-Naphthyl) ethylene diamine dihydrochloride). SNP in aqueous solution at physiological PH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be

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estimated by the use of griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. SNP (1ml of mM) was mixed with 1ml of selected plants extracts in different concentrations in (Di methyl sulphoxide) DMSO. The mixture was incubated at 25oc for 180 minutes. To 1ml of the incubated solution, 1ml of griess reagent was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-naphtyl) ethylene diamine dihydrochloride was read at 546nm by UV-Visible spectrophotometer (Shimadzu UV-Vis1800). All the test analysis were run in triplicate and averaged. Lower absorbance of reaction mixture indicates higher free radical scavenging activity. Ascorbic was used as a positive control. The percentage inhibition was calculated by

Percentage of Nitric oxide scavenged = [(A0–A1)/A0] X 100,

Where A0 = Absorbance of the control, and

A1 =Absorbance of the extract/ standard.

Anti-Inflammatory Activity by Carrageenan induced hind paw edema: The anti-inflammatory activity of suspension of methanol and aqueous extract of *blepharis edulis* leaves and seeds were evaluated by the carrageenan-induced rat hind paw edema method [10]. Wistar albino rats of either sex were used for experimental study. The animals were housed in cages at 25 ± 2ºC, and relative humidity (50 ± 5%) with 12 h light, and 12 h dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water ad libitum. All procedures were performed according to the Institutional Animal Ethics Committee's approval.

Group I: Disease control

Group II: Standard (Diclofenac 10mg/kg p.o)

Group III MBEL Methanol extract of blepharis edulis leaves (200 mg/kg)

Group IV MBEL Methanol extract of blepharis edulis leaves (400 mg/kg)

Group V ABEL Aqueous extract of blepharis edulis leaves (200 mg/kg)

Group VI ABEL Aqueous extract of blepharis edulis leaves (400 mg/kg)

Group VII MBES Methanol extract of blepharis edulis seeds (200 mg/kg)

Group VIII MBES Methanol extract of blepharis edulis seeds (400 mg/kg)

Group IX ABES Aqueous extract of blepharis edulis seeds (200 mg/kg)

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Group X ABES Aqueous extract of blepharis edulis seeds (400 mg/kg)

The initial paw volume was measured using vernier callipers at each individual group of animals. The specific dose of drug diclofenac 10mg/kg or test trial having concentration 200 mg/kg via orally administered to animals. Now, 0.1 ml of carrageenan was injected in the right hind leg after 2 h addition of drug. The edema formed in the paw was measured by digital vernier calipers after 3 hours. The degree of swelling provoke was assess by the proportion of the degree of hind paw previous to to after carrageenan treatment. The percentage inhibition was resolute by allowing for edema induced by carrageenan alone was as 100% induction. The statistical as mean ± SEM was performed by one way analyses of variance (ANOVA) with GraphPad Istant3. The $P<0.05$ was considered as statistically significant. Edema was expressed as the increment in paw thickness due to carrageenan administration. The paw volume was measured using a mercury plethysmometer at the time intervals of 30, 60, 90, 120, 180, 240, 300, 360 minutes after administration of carrageenan. Percent inhibition of edema volume between treated and control group was calculated as follows:

$$\text{Percent inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc and Vt represented mean increase in paw volume in control and treated groups respectively.

Result and discussion:

Anti-oxidant Activity: Analysis of the free radical scavenging activities of the blepharis edulis leaves and seed extracts revealed a concentration dependent free radical scavenging activity resulting from reduction of DPPH, nitric oxide radical to non-radical form. The scavenging activity of Ascorbic acid, a known antioxidant used as positive control, was however higher.

DPPH radical scavenging activity of blepharis edulis leaves and seed extracts: DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was intiated by the lipid autoxidation. Percentage Inhibition of in vitro anti-oxidant result of various blepharis edulis leaves and seed extracts by DPPH Method and ascorbic acid as standard at various concentrations. In case of blepharis edulis leaves extracts the methanol extract were found to have greater reduction potential than other extracts.

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Nitric Oxide (NO) radical scavenging activity of blepharis edulis leaves and seeds extract:

Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study the fruit extracts of selected of blepharis edulis leaves and seeds checked for its inhibitory effect on Nitric oxide production. Results revealed that all the tested extracts showed the percentage of inhibition in a dose dependent manner.

The inhibitory effect of blepharis edulis seed extracts on Nitric oxide radical scavenging model were revealed that methanol extract of blepharis edulis seed at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The methanol extract of blepharis edulis leaves at varied concentrations showed remarkable inhibitory effect of nitric oxide radical scavenging activity compared to other extract. The methanol extract of blepharis edulis seed showed more activity than methanol extract of blepharis edulis leaves.

Anti-inflammatory activity by Carageenan Induced Rat Paw Edema Method: Carrageenan-induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory agents. Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical use for the release of inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF-α, etc.).

The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component Carrageenan-induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis. The course of acute inflammation is biphasic. First phase starts with the release of histamine, serotonin, and kinins after the injection of phlogistic agent in the first few hours . While the second phase is related to the release of prostaglandins like substances in 2-3 hours. Second phase is sensitive to both the clinically useful steroidal and nonsteroidal anti-inflammatory agent. There is a significant ($P < 0.05$) percentage inhibition of paw edema, 64.28% and 71.28 at doses of 200 and 400mg/kg of blepharis edulis seed methanol extract respectively, at 4th hour. Blepharis edulis seed methanol extract were found more potent than blepharis edulis seed methanol extract. The methanol extract of was found to be slightly potent than the aqueous extract.

Therefore, it can be inferred that the inhibitory effect of methanol extract on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis. The effect of blepharis edulis seed and leaves extract and standard drug as compared to carrageenan control at different hours in carrageenan-induced paw edema model using vernier caliper. The present results suggest that methanol extract of blepharis edulis seed and leaves suppresses the carrageenan-induced paw edema. The present study showed that methanol extract of blepharis edulis seed and leaves have anti-inflammatory properties.

Summary and conclusion: *Bblepharis edulis* is a small, perennial plant that include in ethnomedicinal literature at India. *B. edulis* is also used as food to increase sperm count and as aphrodisiac plant have been found to have following therapeutic activities in the literature. The whole plant is of good medicinal value and has been used as folk remedy for treating ailments like wounds, bone fracture, skin disease, urinary infections, cancer, diarrhea and leaves are useful in syphilis and dysentery. Traditionally whole plant powder with milk and or wheat or black gram floor is commonly used in healing bone fracture in various parts of India. Tribal people in southern India uses leaf juice heated in gingelly oil topically for wound healing. Very little information for both phytochemistry and pharmacological efficacy is available on this plant. The objective of the present study is to establish efficacy of this plant as antioxidant and anti- inflammatory potential. Analysis of the free radical scavenging activities of the blepharis edulis leaves and seed extracts revealed a concentration dependent free radical scavenging activity. Methanol extract of *Bblepharis edulis* seed possess significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions.

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Table 1: Results of blepharis edulis leaves extracts on DPPH radical scavenging model

Concentration	**200 µg/ml**	**400 µg/ml**
Ascorbic acid	76.14	92.14
CBEL	17.47	46.54
MBEL	32.87	59.87
ABEL	71.87	82.65

Table 2: *In vitro* 50% inhibition concentration (IC_{50}) of blepharis edulis leaves extracts on DPPH radical scavenging model

Extract /compound	**50% inhibition concentration (IC_{50}) of DPPH model (µg/ml)**
Ascorbic acid	64.5

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CBEL	502
MBEL	85.4
ABEL	394

Table 3: Results of blepharis edulis seeds extracts on DPPH radical scavenging model

Concentration	**200 µg/ml**	**400 µg/ml**
Ascorbic acid	76.14	92.14
CBES	19.25	48.54
MBES	34.52	81.65
ABES	73.87	88.15

Table 4: *In vitro* 50% inhibition concentration (IC_{50}) of blepharis edulis seeds extracts on DPPH radical scavenging model

Extract /compound	**50% inhibition concentration (IC_{50}) of DPPH model (µg/ml)**
Ascorbic acid	64.5
CBES	498.7
MBES	76.4
ABES	321.6

Table 5: Effect of blepharis edulis leaves and seeds extracts on percent Edema Inhibition in Carageenan Induced Rat Paw Edema Method

Groups	**Treatment**	**Edema Inhibition (%)**			
		1 h	**2 h**	**3 h**	**4 h**
Group I	Disease control	-	-	-	-
Group II	Standard	47.56	56.28	68.62	79.11
Group III	MBEL (200 mg/kg)	34.16	43.18	51.37	59.28
Group IV	MBEL (400 mg/kg)	40.16	47.18	58.37	66.28

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Group V	ABEL (200 mg/kg)	30.16	39.18	48.37	55.28
Group VI	ABEL (400 mg/kg)	36.16	42.18	54.37	62.28
Group VII	MBES (200 mg/kg)	38.16	47.18	55.37	64.28
Group VIII	MBES (400 mg/kg)	44.16	52.18	62.37	71.28
Group IX	ABES (200 mg/kg)	32.16	41.18	50.37	57.28
Group X	ABES (400 mg/kg)	39.16	45.18	57.37	65.28

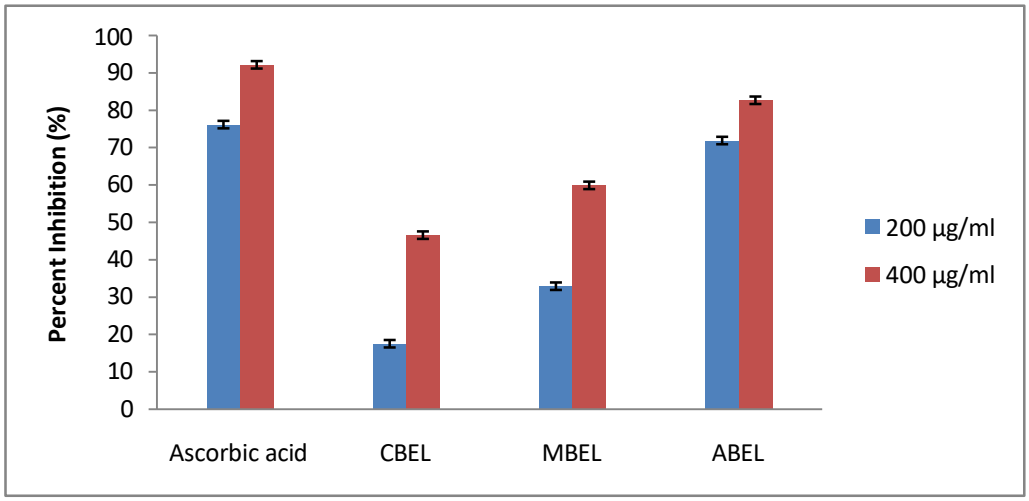

Figure 1: Results of blepharis edulis leaves extracts on DPPH radical scavenging model

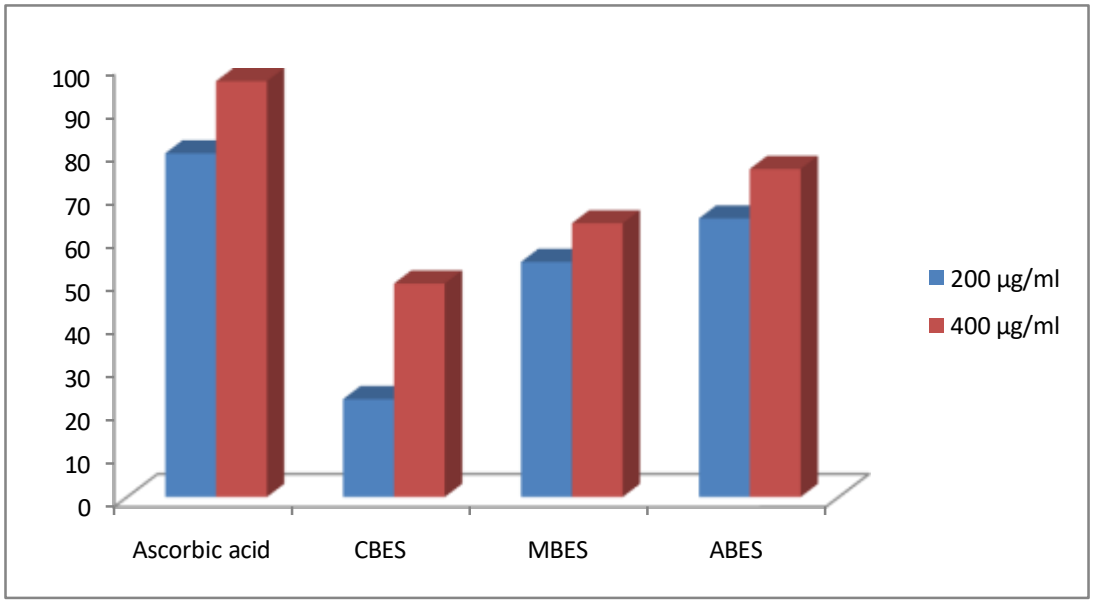

Figure 2: Results of blepharis edulis seeds extracts on DPPH radical scavenging model

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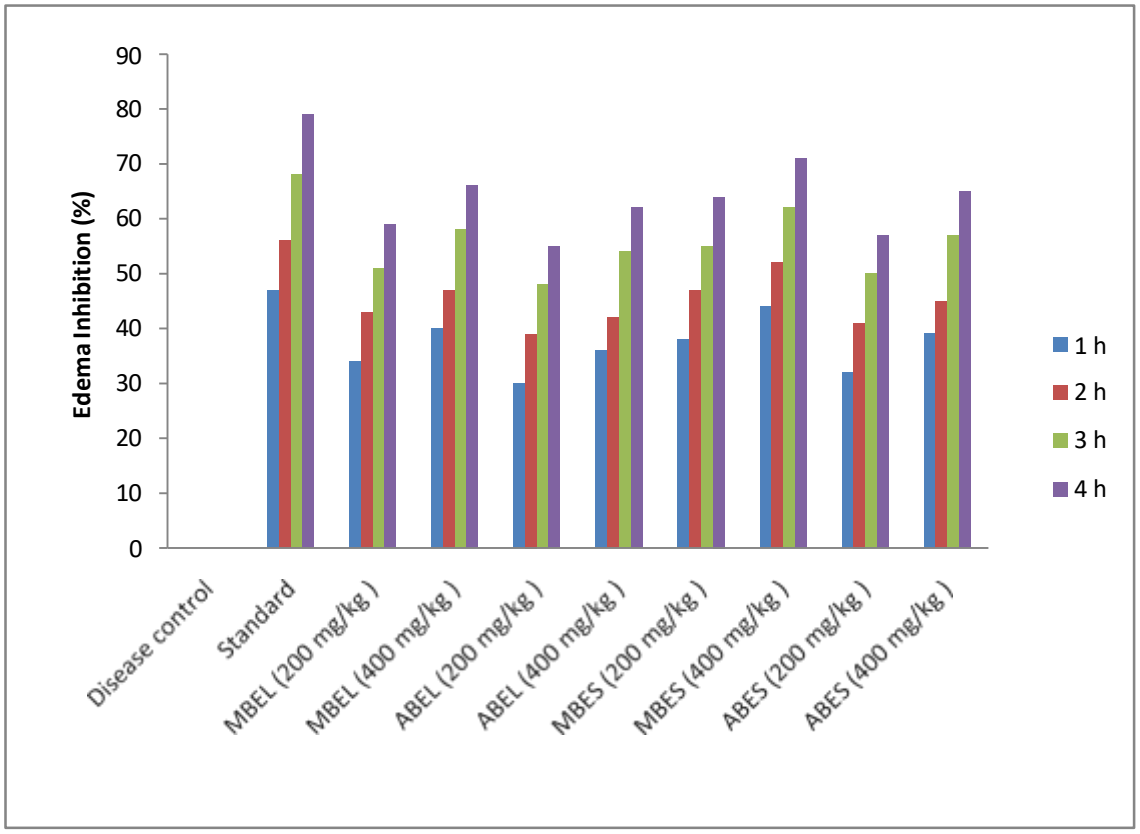

Figure 3: Table 6.19: Effect of blepharis edulis leaves and seeds extracts on percent Edema Inhibition in Carageenan Induced Rat Paw Edema Method