Asian Journal of Pharmaceutical Education and Research
Vol -10, Issue-3, July- September 2021
ISSN:2278 7496

RESEARCH ARTICLE Impact Factor: 7.014

PHYTOCHEMICAL AND *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *AEGLE MARMELOS* ROOT

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Received 17 May 2021; Revised 22 May 2020; Accepted 29 May 2020, Available online 10 July 2020.

Cite this article as: Mehta J, Joshi PM, Verma S, Kumar KL, Acharya R and Sharma B. Phytochemical and *In-Vitro* Anti-Inflammatory Activity of Hydroalcoholic Extract of *Aegle Marmelos* Root. Asian Journal of Pharmaceutical Education and Research. 2021; 10(3) : 13-20.

https://dx.doi.org/10.38164/AJPER/10.3.2021.13-20

ABSTRACT

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. So, number of herbal medicines is recommended for the treatment of inflammation that has no side effects. This has given the impetus to the search for alternative medicines with no or less side effects.Bael (*Aeglemarmelos*) has been known to be one of the most important medicinal plants of India since Charak (1500 B.C). More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. However, their anti-inflammatory properties are yet to be explored. This study sought to evaluate the anti-inflammatory potentials of hydroalcoholic extract of root of *Aeglemarmelos*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total alkaloid was determined by the well-known test protocol available in the literature. This study, therefore, justifies the use of the plant in the treatment of inflammation.

Keywords: *Aeglemarmelos*, Phytochemical profile, Alkaloids, *In Vitro* anti-inflammatory Activity.

INTRODUCTION

Inflammation is considered as a primary physiologic defence mechanism that helps body to protect itself against infections, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as etiological factor for many chronic illnesses [1]. Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used in the treatment of pain and inflammation. Currently available NSAIDS are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDS usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa [2]. Hence there is a need for anti-inflammatory drugs

with fewer side effects. Plants have been an important source of medicine for 1000's of years. Herbal medicine is still the mainstay of therapy for about 75-80% of the whole population in developing countries for primary health care [3]. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries [4]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine [5]. World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases[6]. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain [7]. Bael (*Aeglemarmelos*) has been known to be one of the most important medicinal plants of India since Charak (1500 B.C) [8]. More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders [9-12]. Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial, antispermatogenic effects have also been reported on various animal models by the crude extracts of this plant [13-18]. Therefore, the present study was aimed to investigate in *vitro*anti-inflammatory activity of hydroalcoholic extracts of *Aeglemarmelos*.

MATERIALS AND METHODS

Collection of plant material

Fresh root plant material of *Aeglemarmelos* was collected from local area of Bhopal (M.P.). The plant materials were washed thoroughly with normal tap water followed by sterile distill water. Then plant material was dried under shaded condition at room temperature. Fresh plant materials of *Aeglemarmelos* were crushed to powder using grinding machine. Powder was stored at 4^{o}C in tight air container bottle.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). Atropine was kindly provided by Scan Research Laboratories, Bhopal (India). All other chemicals and solvents used were of HPLC and analytical grade.

Extraction Procedure

The maceration method was followed for the extraction. 50 gm powder of dried plant material was added into 100 ml of 70 % methanol in an Erlenmeyer flask (250 ml capacity) and resulting mixture was vortexed well. The maceration process was carried out in shaker incubator at with 50 rpm for 48-72 hrs.After this process, the extracts were filtereddried extract was stored in refrigerator for their future use in phytochemical analysis.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract[19].

Evaluation of *in vitro* anti-inflammatory activity

Anti-inflammatory activity of the *Aeglemarmelos* root extract was evaluated by protein denaturation method asdescribed by Padmanabhan and Jangle [20]. Diclofenac sodium, a powerful non steroidal anti-inflammatorydrug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of*Aeglemarmelos*extract (100-500 µg/mL) or standarddiclofenac sodium (100-500 µg mL-1) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation wasinduced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbancewas measured at 660 nm by using double distilled water as blank. The percentage inhibition of protein denaturation was calculated by using thefollowing formula:

$$\% \text{ Inhibition} = \frac{Control\ Absorbance - Test\ Absorbance}{Control\ Absorbance}\ x\ 100$$

The plant concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

The yield of *Aeglemarmelos*Hydroalcoholic root extracts was 4.56% w/w. Preliminary phytochemical screening of *Aeglemarmelos*Hydroalcoholic root extracts revealed the presence of various components such as Alkaloids, Carbohydrate, Saponinsand the results are summarized in Table 1. The total alkaloid content of the extracts was expressed as percentage of atropine equivalent per 100 mg dry weight of sample. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance. Total alkaloid content mg/100mg in Hydroalcoholic root extracts was found to be 1.059 mg/100mg AE Table 2. The *In-vitro* anti-inflammatory effect of *Aeglemarmelos*hydroalcoholic root extracts was determined using protein denaturation method, the percentage inhibition of Diclofenac sodium was found to be 250.95µg/ml and *Aeglemarmelos* root extract was found to be 293.50 µg/ml Table 4.

Table 1: Result of Phytochemical screening of root extract of *Aeglemarmelos*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Hager's Test:	+ve
2.	**Glycosides**	
	Legal's Test:	-Ve
3.	**Flavonoids**	
	Lead acetate Test:	-Ve
	Alkaline test:	-Ve
4.	**Diterpenes**	
	Copper acetate Test:	-Ve
5.	**Phenol**	
	Ferric Chloride Test:	-Ve
6.	**Proteins**	
	Xanthoproteic Test:	-Ve
7.	**Carbohydrate**	
	Fehling's Test:	+Ve
8.	**Saponins**	
	Froth Test:	+Ve
9.	**Tannins**	
	Gelatin test:	-Ve

Estimation of total alkaloid content (TAC)

Calibration curve of Atropine

Table 2: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	40	0.325
2	60	0.457
3	80	0.609
4	100	0.721
5	120	0.849

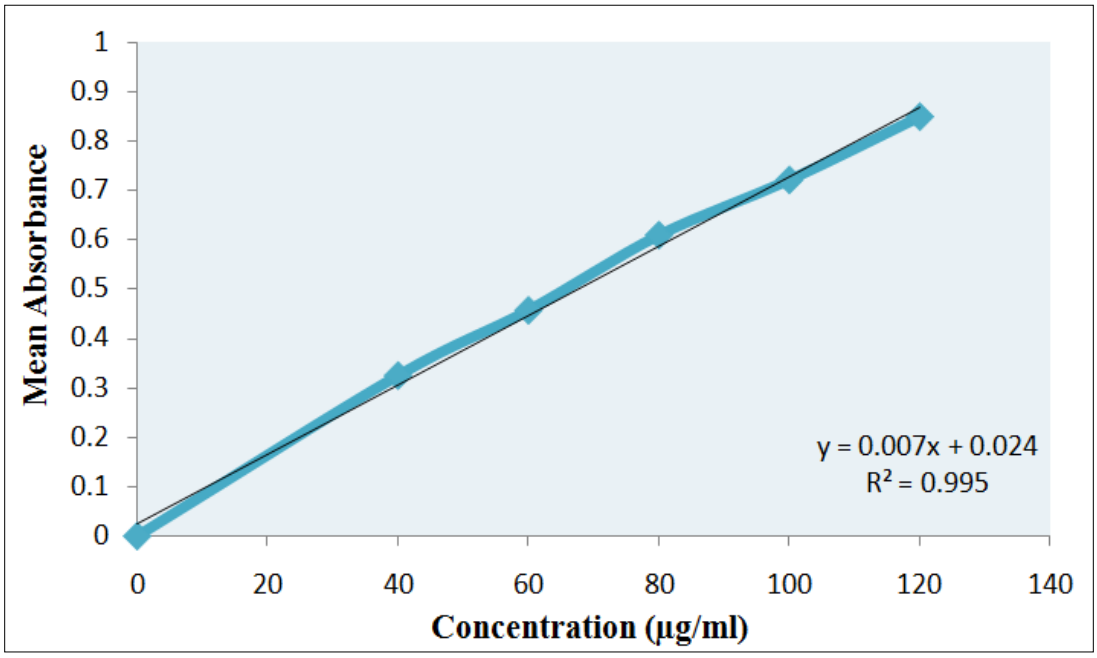

Figure 1: Graph of calibration curve of Atropine

Table 3: Result of total alkaloid content of *Aeglemarmelos* root extract

S. No.	Extract	(Total alkaloid content mg/100mg)
1.	Hydroalcoholic	1.059

Results of*in vitro* anti-inflammatory activity

Table 4:% Inhibition of Diclofenac sodiumand*Aeglemarmelos* root extract

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	*Aeglemarmelos* root extract
100	20.38	18.75
200	45.40	35.69
300	60.04	55.85
400	75.74	69.54
500	90.25	76.25
IC_{50}	**250.95**	**293.50**

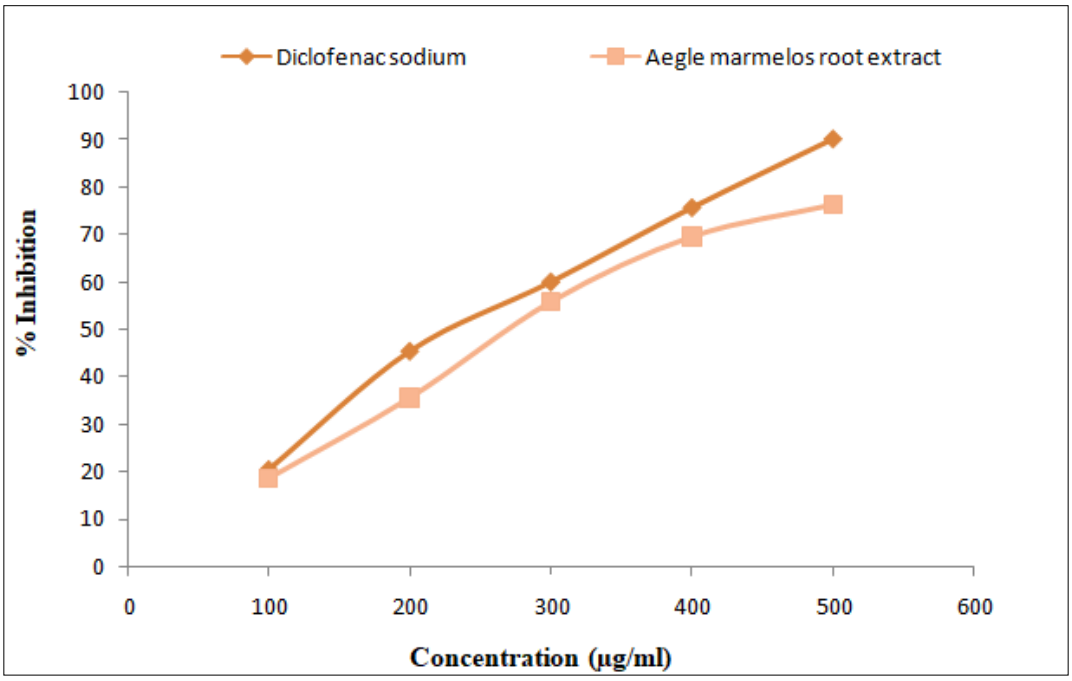

Figure 2: Graph of *in vitro* anti-inflammatory activity

Conclusion

Present Investigation evaluates the anti-inflammatory potentials of hydroalcoholic extract of root of *Aeglemarmelos*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total alkaloid was determined. The *In-vitro* anti-inflammatory effect of *Aeglemarmelos*hydroalcoholic root extracts was determined using protein denaturation method, the percentage inhibition of Diclofenac sodium was found to be 250.95µg/ml and *Aeglemarmelos* root extract was found to be 293.50 µg/ml. This study, therefore, justifies the use of the plant in the treatment of inflammation.

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