World Journal of Science and Research. 1(1): 24-27 (2015)

ISSN: 2455 2208

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World Journal of Science and Research

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Research Article **Botany**

PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY ACTIVITY OF *Aegle marmelos* LEAF

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ABSTRACT

To evaluate the pytochemical and anti-inflammatory activity of *Aegle marmelos* leaf in rats. The phytochemical screening of *Aegle marmelos* showed that the presence of flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids and triterpenoids, polyohenol, steroids, phlobatannins and anthroquinones.Vitamin C and D present in the *Aegle marmelos* extract.The results of the present investigation confirmed that *Aegle marmelos* possess significant anti-inflammatory activity is directly proportional to the concentration and time. The maximum activity of *Aegle marmelos* leaves were observed at a dose of 500mg/kg. The anti-inflammatory activity increases gradually at 2 hrs and then decreases were observed.The anti-inflammatory activity of *Aegle marmelos* which might have been due to the phytochemicals present in it.

***Citation*:** K. Sathya and G. Viji Stella Bai. (2015) Phytochemical screening and anti-inflammatory activity of *Aegle marmelos* leaf. World Journal of Science and Research. 1(1): 24-27.

Article Info:

Received on 1 April 2015

Accepted on 03 May 2015

Keywords:

Aegle marmelos, anti-inflammatory activity, Phytochemicals

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INTRODUCTION

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. Many of these diseases are debilitating and are becoming increasingly common in our aging society. Rheumatoid arthritis and osteoarthritis are the major inflammatory diseases affecting people worldwide. Rheumatoid arthritis is an inflammatory condition that usually affects multiple joints. It affects 0.3–1.0% of the general population and is more prevalent among women in developed countries. Persistent inflammation leads to joint destruction, but the disease can be controlled with drugs. Osteoarthritis, which is characterized by loss of joint cartilage that leads to pain and loss of function primarily in the knees and hips, affects 9.6% of men and 18% of women aged more than 60 years. Increases in life expectancy and aging populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020 (Woolf and Peger, 2003; Smit, 2005)

A number of natural products are used in the traditional medical systems in many countries. Alternative medicine for treatment of various diseases is getting more popular. Making medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory

chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity; they have various and severe adverse effects. Therefore, agents of natural origin with very little side effects are required as substitute chemical therapeutics. In order to evaluate the anti-inflammatory activity of *Aegle marmelos* leaf in rats. The following aspects were analyzed to the anti- inflammatory activity.

MATERIALS AND METHODS

Collection of Plant material and Preparation of extract

The leaves of *Aegle marmelos* (L) Corr. (Fig 1) were collected from Senthamarikan, Thiruvarur district, Tamil Nadu. The powder material of *Aegle marmelos* leaves were macerated with 50% methanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

Chemicals:

Carrageenan, Mercury, Ethanol and Trichloro acetic acid (TCAs were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Animals:

Male albino rats of Wistar strain approximately 3-4 months weighing approximately 140-160g were used in this study. They were healthy animals procured from IAS, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºc and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatization to the environment for 1 week prior to experimental use.

Phytochemical analysis

Chemical tests were carried out on the alcholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Qualitative analysis of Vitamins (Pearson, 2004; Patel, 2005).

Experimental design:

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema according to the technique of Winter *et al*. (1962). After 12hrs fast rats were divided into five groups of six each. Each animal was marked for identification and monitoring. Group I served as control group received carrageenan only. Group II further sub divided into three groups of each. Group II a, IIb, and IIc animals received ethanolic extract of *Ocimum basilicum* at a dose of 100, 250 and 500 mg/kg respectively. Group IId, IIe, and IIf animals received ethanolic extract of *Lucas aspera* at a dose of 100, 250 and 500 mg/kg respectively. Group III was orally administered 2mg/kg (ip) Dexamethasone as a standard drug. The animals were pretreated with the extract half an hour before the administration of carrageenan. Acute inflammation was produced by the subplantar administration of 0.1 ml of 1% carrageenan in normal saline in the right paw of the control and experimental rats. The paw was marked with in at the level of lateral malleous and immersed in mercury up to the mark and measured by mercury volume displacement methods. The paw volume was measured ½, 1, 1½, 2 and 2½hours after injection of carrageenan to each group. The difference between the readings was taken as the volume of oedema and the percentage of anti-inflammatory activity was calculated.

$$\% \text{ of inhibition rate} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c is the oedema value of the control group and V_t is the oedema value of treated groups.

Statistical analysis:

The results were expressed as mean ± S.D. Statistical significance was determined by analysis of variance and subsequently followed by stutends tests. P values less than 0.05 were considered as indicative of significance.

RESULTS AND DISCUSSION

The present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Aegle marmelos (L.) Corr*. investigated a summarized in Table 1.

Table 1. Qualitative Phytochemical screening of *Aegle marmelos* (L) Corr

Test	Result
Tannin	+
Phlobatannins	+
Saponin	+
Flavonoids	+
Steroids	++
Terpenoids	+
Triterpenoids	++
Alkaloids	+
Carbohydrate	+
Amino acid	++
Anthroquinone	+
Polyphenol	++
Glycoside	++

(+) Presence (-) Absence

Analysis of vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the

molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2003).

Vitamins work with other substances in the body like enzymes and minerals. Together they perform such functions as strengthening bones, healing wounds, keeping the skin healthy, building cells, and helping to resist infections. Vitamins are separated into two groups, fat soluble and water soluble. The fat soluble vitamins are A, D, E, and K, and can dissolve in dietary fats and are stored in the liver and body fat. The body stores them for a longer amount of time, so they are not needed every day. Too much of these vitamins can become toxic and cause health problems. The water soluble vitamins are made up of 8 B vitamins and vitamin C. Water soluble vitamins dissolve in water, and are not stored in the body. Rather they travel through the bloodstream and need to be replenished every day. These vitamins are easily destroyed during food preparation and storage. In the present study, the vitamine D and C found to be in *Aegle marmelos leaves* (Table 2).

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys (Clark, 2008).

Vitamin C, or ascorbic acid, is one vitamin humans cannot make; they have to get it from food. Vitamin C helps hold the cells together, heal wounds, and build bones and teeth. The best sources for vitamin C are citrus fruits, strawberries, melons, and leafy green vegetables. Vitamin C also helps to absorb and use Iron. It is important to protect the vitamins in fruits and vegetables from being destroyed; simple ways of doing this include refrigeration, washing them before cutting them, storing them in airtight containers, and avoiding high temperatures and long cooking times (Okwu, 2003).

Table 2 Qualitative phytochemical analysis of vitamins

Vitamins	Result
A	--
C	+
D	+
E	--

(+) Presence (-) Absence

Anti-inflammatory activity of *Aegle marmelos (L.) Corr.*

The present study establishes the anti-inflammatory activity of the ethanol extract of *Aegle marmelos (L.) Corr*. in carrageenan induced anti-inflammatory models tested.

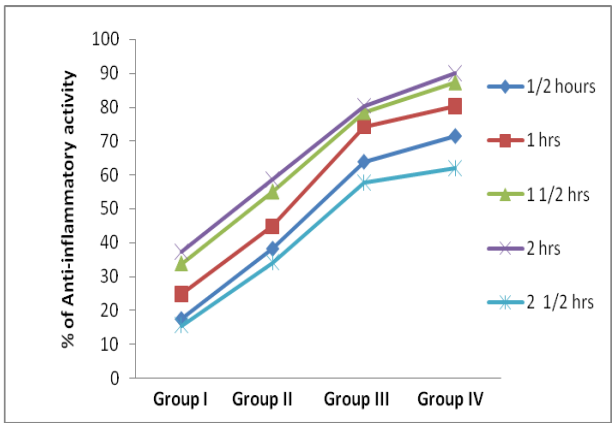
The effect of *Aegle marmelos* on carrageenan induced paw oedema is shown in the table 3. The rats foot pad become oedemateous after injection of carrageenan. Administration of *Aegle marmelos* reduces the paw oedema to inflammatory rats at a dose of 100, 200 and 300mg (kg body weight). The dose dependent a significant decrease of paw oedema and the reference drug dexamethasone (2mg/kg body weight) exhibited significant decrease. Among the various doses, the 500mg/kg (body weight) of *Aegle marmelos* extract possess potential anti-inflammatory activity as compared to other doses. The anti-inflammatory activity increases gradually at 2 hrs and then decreases were observed.

Table 3 Effect of *Aegle marmelos* leaf on carrageenan induced paw oedema

Treatment Groups	Doses (mg/Kg)	½ hr	1 hr	1 ½ hr	2 hr	2 ½ hr
Group I (Control)		--	--	--	--	--
Group IIa	100	17.5 ± 0.52	24.79 ± 1.05	33.75 ± 1.61	37.36 ± 1.42	15.48 ± 0.36
Group IIb	200	38.18 ± 1.14	44.77 ± 1.34	55 ± 1.85	58.68 ± 1.66	34.11 ± 1.32
Group IIc	300	63.75 ± 1.91	74.28 ± 2.69	78.5 ± 2.85	80.31 ± 1.50	57.64 ± 1.12
Group III (Dexameth-asone)	2	76.45 ± 2.29	94.46 ± 2.83	95.13 ± 2.82	95.17 ± 2.13	62.22 ± 1.86

Values were expressed as %

Figure 3 Effect of *Aegle marmelos* on carrageenan induced paw oedema



Carrageenan induced inflammation is a biphasic phenomenon and is a useful model to detect oral actions of anti-inflammatory agents (Meier *et al*., 1950). The development of oedema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances (Winter *et al*., 1962). Due to the increasing frequency of intake of Non-steroidal anti-inflammatory drug's (NSAID'S) and their reported common side effect, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. In Indian system of medicine, certain herbs are claimed to provide relief of pain and inflammation (Mujumdar *et al*., 2001). So there is continuous search for indigenous drugs, which can provide relief on inflammation.

Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic response. The early phase (0-1 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents (Brito and Antonio 1998). The *Aegle marmelos* produced dose dependent and inhibition of carrageenan-induced paw oedema over a period of 2 h.

The effect of *Aegle marmelos* against inflammations produced by individual mediators was studied. The *Aegle marmelos* also suppressed the inflammation produced by the putative inflammatory mediators viz. histamine and serotonin (highest inhibition in case of serotonin, 80.31% and 88.86 % at 500 mg/kg body wt.). It suggests that the *Aegle marmelos* inhibited the inflammatory mediators involved in the inflammatory process.

The phytochemical screening of *Aegle marmelos* showed that the presence of flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids and triterpenoids, polyohenol, steroids, phlobatannins and anthroquinones.Vitamin C and D present in the *Aegle marmelos* extract.The results of the present investigation confirmed that *Aegle marmelos* possess significant anti-inflammatory activity is directly proportional to the concentration and time. The maximum activity of *Aegle marmelos* leaves were observed at a dose of 500mg/kg. The anti-inflammatory activity increases gradually at 2 hrs and then decreases were observed.The anti-inflammatory activity of *Aegle marmelos* which might have been due to the phytochemicals present in it.

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