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Assessment of *in vitro* antiarthritic activity of *Manilkara hexandra* (Roxb.) Dubard leaf extract

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Abstract

The rationale of the current study was to assess the *in vitro* antiarthritic activity of methanolic (MEMH) and hydroalcoholic (HAMH) leaf extracts of *Manilkara hexandra* (Roxb.) Dubard. The *in vitro* models adopted for the study includes protein denaturation method and proteinase inhibition method.The culmination revealed the dose dependent increase in the antiarthritic activity of both the extracts. At concentrations 50,100, 200, 250 and 500 µg/ml, methanolic and hydro alcoholic leaf extracts of *M. hexandra* exhibited significant inhibition of protein denaturation and proteinase inhibition which were comparable to the standard diclofenac sodium. Hydroalcoholic extract exhibited significant protein denaturation and proteinase inhibition with IC_{50} values of 305 and 312 µg/ml which are comparable with standard IC_{50} values of 235 and 230 µg/ml, respectively.

Key Words: *Manilkara hexandra* (Roxb.) Dubard., *in vitro*, antiarthritic activity

1. Introduction

Arthritis is a systemic inflammatory disease, affecting mainly joints. There are two major types of arthritis, namely; rheumatoid arthritis and osteoarthritis. Gout is also a type of inflammatory disease, caused by the pathogenic deposition of uric acid crystals in joints and tissues. Conventional modern medicine is devoid of a satisfactory treatment to severe cases of arthritis. To a large extent, these diseases are treated symptomatically and the drugs used in the treatment have varying levels of toxic side effects. In traditional medicines, several herbal drugs are used to treat these diseases. However, their efficacy and safety are not clear. Herbal drugs are promising for the development of effective and safe drugs against arthritis. In the recent past, a few reports have appeared on this topic (Singh *et al.*, 2011; Arya *et al.*, 2011; Kaur *et al.*, 2012; Srikanth *et al.*, 2012; Subramoniam *et al.*, 2013).

Rheumatoid arthritis (RA) is a systemic autoimmune multisystem disease characterized by pain, synovial membrane inflammation, peripheral joint inflammation, morning stiffness, destruction of articular tissues and restricted joint movement (Paval *et al.*, 2009; Banji *et al.*, 2011; Patil *et al.*, 2012; Subramoniam, 2016). Arthritis can cause severe disability and ultimately affects a person's ability to carry out everyday tasks, restricts the quality of life (Murugananthan *et al.*, 2013). The various proinflammatory molecules including reactive oxygen species, prostaglandins, leukotrienes and cytokines released by macrophages are involved in the cause of this disorder (Snedegard, 1985; Henderson *et al.*, 1987; Subramoniam, 2016). Even though various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, they offer only temporary relief and produce severe side effects including gastrointestinal bleeding, renal morbidity and cardiovascular toxicity which limit their utility in the treatment of the disease (Pandey, 2010). Consequently, there is a need to develop antiarthritic agents with minimum side effects.

It is believed that RA is caused by the adverse response of the body to an infectious agent in genetically susceptible individual. Microvascular injury and an increase in the number of synovial lining cells are the initial changes, observed in synovitis in RA. Then, a perivascular infiltration with mononuclear cells occurs. As the disease-process continues, the synovium becomes edematous and protrudes into the joint cavity. The changes include hyperplasia, and hypertrophy of the synovial lining cells, micro-vascular injury, thrombosis, and neo-vascularization. When the disease progresses, periarticular soft tissue edema also appears (Lipsky, 2008).

Herbal medicines, excogitated from medicinal plants are used by about 80% of the world's population and are preferred due to lesser side effects and low cost (Padmini *et al.*, 2016). These drugs integrate plurality among all the officially recognized systems of health in India, *viz.*, Ayurveda, Yoga, Siddha, Homeopathy and Naturopathy (Iqbal, 2013; Pushpangadan, 2013, Subramoniam, 2014; Udupa, 2016).

Manilkara hexandra (Roxb.) Dubard (Family: Sapotaceae) is a small to medium sized evergreen tree. It is native to South Asia and Tropical countries and widely distributed in South, North and Central India, mainly in Rajasthan, Gujrat, Madhya Pradesh and Maharashtra (Malik *et al.*, 2012). Leaves, fruits, roots and barks of *M. hexandra* are renowned for their medicinal values (Parekh and Chanda, 2008).

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Traditionally, it is used in medicinal herbal drugs to cure various diseases such as jaundice, ulitis, odontopathy, fever, colic dyspepsia, helminthiasis, hyperdyspepsia and burning sensation (Joshi, 2000). It purifies the blood and beneficial in swelling, abdominal colic, gout, rheumatism and toxicosis (Rao *et al*., 1985). It contains a variety of components which possess various biological activities such as anti-inflammatory, diuretic, antiurolithiatic, analgesic, antipyretic and antimicrobial activity (Khare, 2007). The bark of this plant species is astringent, refrigerant, febrifuge, sweet, tonic and is used traditionally to treat a wide range of gastrointestinal disorders (Shah *et al*., 2004). Seed oil of *M. hexandra* is demulcent and emollient (Anjaria and Parabia, 1997). The leaves of *M. hexandra* are used in asthma (Anjaneyulu and Sudarsanam, 2013), infertility (Gunasekaran) and as antimicrobial agent (Parekh and Chanda, 2008).

The supporting evidence for taking up this research was, the notable antiarthritic activity perceived in the similar genus, *Manilkara zapota* (Madan Singh *et al*., 2011)

2. Materials and Methods

2.1 Collection of plant material

Fresh plant leaves of *Manilkara hexandra* (Roxb.) Dubard (Sapotaceae) were collected from Tirumala Hills, Tirupati, Chittoor District, Andhra Pradesh, India in January 2016. Dr K. Madhava Chetty, Taxonomist, Department of Botany, Sri Venkateshwara University, Tirupati, India, identified and authenticated the plant material. An herbarium (Voucher Specimen No. 1257) of the plant was deposited in the Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India.

2.2 Extraction

The dried leaves of *M. hexandra* are pulverized and the material was taken in round bottomed flask and refluxed with methanol. The extract was collected four times at every 1 h interval with the addition of fresh solvent. The resulting extract is methanolic extract of *M. hexandra* (MEMH). Similar extraction procedure was adopted with 60 % methanol and 40 % water (hydroalcohol) to obtain hydroalcoholic extract of *M. hexandra* (HAMH).

All chemicals used in this study are analytical reagent grade of Merck India Co. Ltd., and purified according to the standard procedures.

2.3 *In vitro* antiarthritic activity

2.3.1 *Protein denaturation method*

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of test extracts (100 and 250 mcg/ml of final volume). pH was adjusted at 6.3, using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control test. 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin (Deshpande *et al.*, 2009). The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percentage inhibition} = \frac{\text{Abs. of control} - \text{Abs. test sample}}{\text{Abs. of control}}$$

where Abs. = Absorbance

The control represents 100% protein denaturation.

2.4 Proteinase inhibition study

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml 25 mM tris-HCI buffer (pH 7.4) and 1.0 ml aqueous solution of sample extract. The mixtures were incubated at 37°C for 5 min. Then, 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 min. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated. Diclofenac sodium was used as standard (Oyedapo and Famurewa, 1995).

2.5 Statistical analysis

The simple statistical analysis was carried out for calculating the mean and the standard error of mean. IC_{50} values were calculated using standard graph.

3. Results

3.1 Protein denaturation method

The distinction of antiarthritic activity of both the extracts, MEMH and HAMH by protein denaturation method is depicted in Table 1 and Figure 1. The activity of both the extracts increased significantly with the upsurge in the concentrations. The result also accompany the fact that HAMH demonstrated efficient inhibition of protein denaturation with 57.98% at maximum concentration of 500 µg/ml which is comparable to the standard percentage inhibition 68.5% at similar concentration. The IC_{50} value of HAMH (305 µg/ml) appears to be proximate to the standard IC_{50} value of 235 µg/ml (Table 3).

3.2 Proteinase inhibition method

The proteinase inhibitory activity of MEMH and HAMH extracts, were shown in Table 2 Figure 2. Both the extracts (MEMH and HAMH) and the standard revealed a dose dependent proteinase inhibitory activity. The inhibitory activity of HAMH is found to be 74.6% at 500 µg/ml; which is comparable to standard inhibition of 84.11%. The IC_{50} value of HAMH is found to be 312 µg/ml. (Table 3).

Table 1: Effect of MEMH and HAMH extracts on protein denaturation

Concentration (µg/ml)	Inhibitory activity of MEMH (%)	Inhibitory activity of HAMH (%)	Inhibitory activity of Standard (%)
50	3.8±0.008	6.9±0.026	7.2±0.181
100	8.3±0.023	10.1±0.076	12.8±0.479
200	17.5±0.303	22.9±0.416	30±0.316
250	24.8±0.069	27.5±0.063	34.9±0.286
500	49.62±0.548	57.98±0.120	68.5±0.080

Mean ± SEM

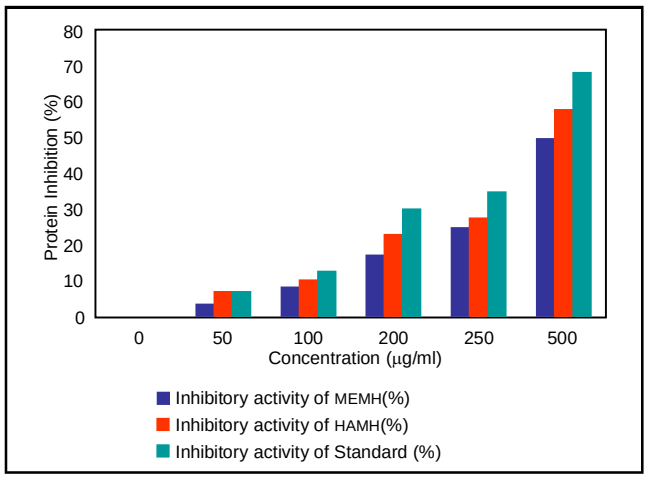

Figure 1: *In vitro* antiarthritic activity of *M. hexandra* in bovine serum albumin

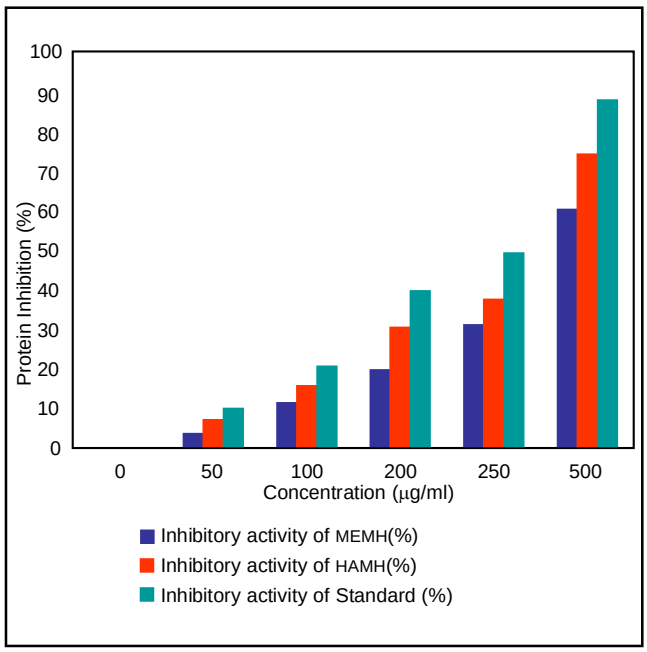

Figure 2: *In vitro* antiarthritic activity of *M. hexandra* by proteinase inhibition

Table 2: Effect of MEMH and HAMH extracts on proteinase inhibition

Concentration (µg/ml)	Inhibitory activity of MEMH (%)	Inhibitory activity of HAMH (%)	Inhibitory activity of Standard (%)
50	5.01±0.112	6.72±0.035	10.10±0.479
100	11.32±0.080	15.1±0.021	21.00±0.454
200	22.5±0.011	30.0±0.020	40.44±0.385
250	30.4±0.092	37.45±0.253	47.72±0.448
500	61.22±0.374	74.6±0.401	88.11±0.434

Mean ± SEM

Table 3: IC_{50} values of MEMH and HAMH extracts

Extracts	Protein denaturationic$_{50}$ (µg/ml)	Proteinase inhibition IC_{50} (µg/ml)
MEMH	350	380
HAMH	305	312
STANDARD	235	230

4. Discussion

The methanolic and hydroalcoholic leaf extracts of *M. hexandra* were found to possess antiarthritic activity. Protein denaturation is a process in which proteins lose their secondary structure and tertiary structure by application of external stress or compounds such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. For example, enzymes lose their activity, because the substrates can no longer bind to the active site (Denaturation, Wikipedia., 2010).

Denaturation of protein is one of the cause of rheumatoid arthritis was documented. Production of auto antigen leads to denaturation of protein in certain arthritic disease. Modulation of electrostatic, hydrogen, hydrophobic and disulphide bonding in denaturation of protein, which is the mechanism of protein denaturation. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation (Sangeetha *et al*., 2011; Chandra *et al*., 2012).

From the result of the present study, it can be stated that both the extracts of *M. hexandra* are capable of controlling the production of auto-antigen and, thereby it inhibit the denaturation bovine albumin and proteinase enzyme in dose dependent manner and its effect was compared with the standard drug diclofenac sodium. Hydroalcoholic extract of *M. hexandra* unveiled utmost inhibition of protein denaturation than methanolic extract owing to presence of phytocompounds with antiarthritic activity.

5. Conclusion

In conclusion, the current study disclosed the *in vitro* antiarthritic activity of methanolic and hydroalcoholic extracts of *M. hexandra*. The presence of flavanoids, sterols and the polyphenols which are reported to be present in the leaf of *M. hexandra* may be attributed for exhibiting antiarthritic activity. Our further aim is to find the phytochemical constituent responsible for antiarthritic activity and to carry out *in vivo* studies.

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Conflict of interest

We declare that we have no conflict of interest.

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