

ANTI-ARTHRITIC ACTIVITY OF ETHANOLIC EXTRACT OF *MANILKARA ZAPOTA* L. BY BOVINE SERUM DENATURATION METHOD

K. Chandra lekha, P. Poornima, B. Dinesh kumar, P. Srinivasa Babu, R. Karthikeyan

Vignan Pharmacy College, Vadlamudi, Guntur (Dist.) -522 213, Andhra Pradesh, India

<p>*For Correspondence: Vignan Pharmacy College, Vadlamudi, Guntur (Dist.) -522 213, Andhra Pradesh, India</p>	<p>ABSTRACT Manilkara zapota L. (Sapotaceae) seeds have been reported to exhibit antibacterial activity. The present study was carried out to investigate phytochemical and antioxidant profile of seeds of Manilkara zapota L. The aim of this research is to explore the anti-anthritic potential of this selected plant material. Five successive dilution of crude extract of its seeds were subjected for in vitro anti-arthritis activity using inhibition of protein denaturation method. The successive methanol extract has shown potent anti-arthritis activity by inhibition of protein denaturation method with increase in concentration. All the concentrations prepared were paving dose dependent anti-arthritis activity.</p>
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INTRODUCTION

When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. The response to the stress of tissue damage is called as inflammation. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Whether loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion [1]. Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, 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 [2]. Both inflammation and free radical damage are inter-related aspects that influence each other. As said above proteins are susceptible to undergo denaturation by formation of free radicals and the mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species from activated neutrophil and macrophages. This over-production leads to tissue injury by damaging the macromolecule and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin-1, tumor necrosis factor- α , and interferon-

y, which stimulate recruitment of additional neutrophil and macrophages. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [3-5]. Thus, our study aims to find a natural remedy that will be useful to treat both inflammation and free radical damage. *Manilkara zapota* L. belongs to the family Sapotaceae. It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico and Central America. The seeds are aperients, diuretic tonic and febrifuge. Bark is antibiotic, astringent and febrifuge [6-7]. Chicle from bark is used in dental surgery. Fruits are edible, sweet with rich fine flavour. Bark is used as tonic and the decoction is given in diarrhoea and peludism [8]. The leaves are used to treat cough, cold, and diarrhoea [9-10] Bark is used to treat diarrhoea and dysentery. Antimicrobial and antioxidant activities are also reported from the leaves. The objective of the present study was to evaluate various phytochemicals and anti-inflammatory profile of seeds of *Manilkara zapota* L. seeds.



Figure 1. *Manilkara zapota* (L.) seeds.

2. Materials and methods

2.1. Collection and preparation of extracts

The plant material was collected from the plant *Manilkara zapota* L. which are collected during the month of December at Vadlamudi, Guntur (Dist.) of Andhra Pradesh. Then it was authenticated by Dr. P. Satyanarayana Raju, professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur. The seeds were extracted with soxhlet apparatus using methanol as solvent (yield 3.7%). The samples were prepared and used for anti-oxidant activity.

2.2 Chemicals and instruments

All chemicals used in the study were pure. Reference standard Diclofenac sodium obtained from Symed Pharma Pvt Ltd, Hyderabad. Bovine serum albumin fraction was used for the estimation of anti-arthritis activity. Systronics 220 (Double beam) spectrophotometer was used for the estimation of anti-inflammatory activity.

2.3. Preliminary phytochemical screening

Systematic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism [6]. The plant material is subjected to preliminary phytochemical screening for the detection of various plant constituents. Preliminary phytochemical screening was performed by using standard protocol. [5]

2.4 Anti-arthritic activity by inhibition of protein denaturation method

The method of Williams et al 8, was employed for antidenaturation assay. A solution of 0.2% W/V of BSA was prepared in Tris buffer saline and PH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 10,000µg/ml of all extracts were prepared by using methanol as a solvent. From these stock solutions 4 different concentrations of 1,2,4,6,8 and 10 µg/ml were prepared by using methanol as a solvent. 50µl of each extract was transferred to Eppendorf tubes using 1ml micro pipette. 5ml of 0.2% W/V BSA was added to all the above Eppendorf tubes. The control consists of 5ml 0.2% W/V BSA solution with 50 µl methanol. The standard consists of 100 µg/ml Diclofenac Sodium in methanol with 5ml 0.2% W/V BSA solution. The test tubes were heated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL-196) at a wavelength of 660nm. The % inhibition of precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula [11].

% inhibition of denaturation

$$= 100 - \frac{\text{O. D of test control} - \text{O. D of product control}}{\text{O. D of test control}} * 100$$

3.Results and discussion

The method of Anti denaturation of BSA was chosen to evaluate anti-inflammatory property of *Manilkara zapota* L. In antidenaturation assay the denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo nephritis etc [12]. Heat denatured proteins are as effetcive as native proteins in provoking delayed hypersensitivity. Moreover, it was already proved that Conventional NSAID's like phenylbutazone and indomethazine does not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme ut also by prevention of denaturation of proteins. Thus, antidenaturation assay is the covenient method to check the anti-inflammatory activity. In our results both the extracts have shown considerable anti-inflammatory activity and methanolic extract was found to be more potent than hexane extract. The secondary metabolites like Phenolic compounds and tannins which were found in preliminary phytochemical screening table 1, might be responsible for this activity. Anti-arthritic activity results were presented in table 2 and figure 2.

Table 1. Phytochemical present in methanolic extract of *Manilkara zapota* L seeds.

S. No.	Phytochemicals	Methanolic extract if seeds of <i>Manilkara zapota</i> L
1.	Carbohydrates	+
2.	Alkaloids	-
3.	Flavonoids	-
4.	Steroids	+
5.	Terpenoids	-
6.	Phenolics and tannins	+
7.	Glycosides	+
8.	Saponins	-
9.	Proteins/ amino acids	-
10.	Coumarins	+
11.	Cardiac glycosides	+

Table 2. Representates the % Inhibition of denaturation bovine serum albumin by methanolic seed extracts of *Manilkara zapota* L.

Concentration($\mu\text{g/ml}$)	Absorbance	% Inhibition of denaturation
1	0.007	97.42
2	0.009	96.69
4	0.011	95.95
6	0.023	91.54
8	0.031	88.60
10	0.036	86.76
Diclofenac sodium- 10	0.042	91.01

Note: The value of bovine albumin without extract the absorbance is 0.000 at 660nm so all the values are become zero

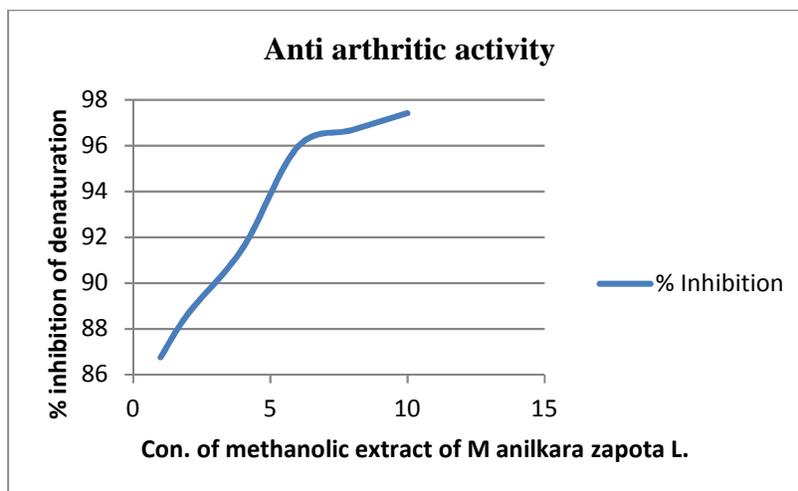


Figure 2. Representates the % Inhibition of denaturation bovine serum albumin by methanolic seed extracts of *Manilkara zapota* L.

CONCLUSION

Our investigation clearly demonstrates that methanolic extract of *Manilkara zapota* L seeds possess significant anti-inflammatory properties. Further studies are recommended to isolate the active principle responsible for these activities.

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