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Research Article

ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF FLOWERS *MANILKARA ZAPOTA* L. BY HRBC MEMBRANE STABILIZATION METHOD

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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 methanolic crude extract of its seeds were subjected for *in-vitro* anti-oxidant activity by savenging of hydroxyl radical in p-NDA method. The successive methanol extract have shown potent anti-oxidant activity by p-NDA method with 25 µg/ml, 50 µg/ml and 100µg/ml respectively. The crude methanol extract has shown significant anti-oxidant activity by scavenging of hydroxyl radical in p-NDA method. All the concentrations prepared were paving dose dependent anti-oxidant activity.

Keywords: *Manilkara Zapota L, Methanolic Extract, p-NDA Method, Anti-Oxidant Activity*

INTRODUCTION
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 (Kaneria *et al.*, 2009; Nair and Chanda, 2008). 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 (Anjaria *et al.*, 2002). The leaves are used to treat cough, cold, and diarrhoea (Mohiddin *et al.*, 1992; Morton, 1982). 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.

MATERIALS AND METHODS
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 (District) of Andhra Pradesh. Then it was authentified 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.
Chemicals and Instruments
Sodium chloride, Sodium citrate, Dextrose, Citric acid and Buffer tablet were purchased from S.D fine chemicals, Bangalore. Ethanol was procured from national scientific with the brand of qualigen. Reference standard Diclofenac sodium obtained as a gift sample from Symed Pharm. Pvt. Ltd, Hyderabad. Systronics 220 (Double beam) spectrophotometer was used for the estimation of anti inflammatory activity.
Preliminary Phytochemical Screening
Preliminary phytochemical screening was performed by using standard protocol as followed in Khandelwal (2008).
Anti-inflammatory Activity by HRBC Membrane Stabilization Method
The anti-inflammatory activity of seed extract of *Manilkara zapota* L. was determined by HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium

chloride in water). The blood was centrifuged at 300 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) & 10% v/v suspension was made with isosaline. The assay mixture contained the drug. 1 ml phosphate buffer (0.15M, pH7.4), 2ml of hyposaline (0.36%) % 0.5 ml of HRBC suspension. Diclofenac was used as the reference drug. Instead of hyposaline, 2ml of distilled water was used as control. All the assay mixtures were collected at 370c for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using colorimeter at 560 nm. The percentage heamolysis was calculated by assuming the heamolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula.

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug sample}}{\text{Optical density of control}} * 100$$

RESULTS AND DISCUSSION

Phytochemical screening of the plant is preliminary and important aspect of the process of establishing herbal medicine quality. Preliminary phytochemical analysis is helpful in determining the chemical constituents of plant materials. They are also useful in locating the source of pharmacologically active chemical compounds.

Preliminary phytochemical study showed that the presence of steroid, cardiac glycosides, carbohydrates, tannins and phenolic compound are depicted in Table 1. Successively the crude methanol extracts have shown insignificant anti-inflammatory activity with 74.54 % and 87.28 % for 100 and 500µg/ml, respectively.

Table 2 showed % haemolysis and % protection of heamolysis produced by hyposaline *invitro*. The crude methanol extract showed significant and dose dependent protection of heamolysis from the HRBC membrane.

The preliminary phytochemical investigation revealed the presence of steroids compounds in the polar extracts of the plant.

Plant steroids are known to exhibit potent anti-inflammatory activity. Hence, the observed insignificant anti-inflammatory activity of methanolic extract of *Manilkara zapota* L. may be due to the presence of other constituents and its complexity in the extract.

The details of results of preliminary phytochemical analysis established in the present study will facilitate in identifying the genuine drug and will also be useful in preparation of monographs of this plant.

Table 1: Phytochemicals Present in Methanolic Extract of *Manilkara zapota* L Seeds

S. No.	Phytochemicals	Methanolic Extract if Seeds of *Manilkara zapota* 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 3: Evaluation of Anti-Inflammatory Activity of Ethanolic Extract of Seeds of *Manilkara Zapota* L. by HRBC Membrane Stabilization Method

S. No.	Name of the Drug	Concentration (µg/ml)	Absorbance (560 nm)	% Haemolysis	% Protection
1.	Control	-	2.526	100 %	0 %
2.	Ethanolic extract	250	0.718	74.54 %	25.46 %
		500	0.886	87.28 %	12.72 %
3.	Diclofenac	100	0.593	23.47 %	86.54 %
	sodium	500	0.362	14.33 %	95.67 %

Conclusion

In conclusion, this study provides evidences for the steroid and phenolic substance presence in the seed part of the drug. Study suggested to isolate the relevant phytochemical for the foresaid study. Hence, the complexity of phytochemicals in the plant extract would give two sort of activity such as either synergistic or antagonistic. In this study the activity was reduced because of the phtochemical complexity. However, further investigation is required to isolate the active constituents responsible for this activity and to elucidate the exact mechanism of action.

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Conflict of Interest

The authors are not showing any conflict of interest to publish this paper.

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