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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical and Pharmacological investigation of extract of *Manilkara zapota* Linn. Seeds

Rupali P. Lade[1*], Manure Md. Javeed Md. Yakub[1], Harshad P. Khade[2]
[1]Appasaheb Birnale College of Pharmacy, Sangli. Maharashtra – 416416, India
2Annasaheb Dange College of B Pharmacy, Ashta. Maharashtra – 416 301, India
Corresponding author's Email: dr.laderupali@gmail.com

ABSTRACT
Manilkara zapota is commonly named Chiku. Manilkara zapota belongs to the family Sapotaceae. It cultivated for its delicious fruits. It is an evergreen plant, every part of the tree used for various ailments in traditional medicine. The scenario of the present study is investigation of the phytochemical and pharmacological action of Manilkara zapota seed extract. Ethanolic extract of seed evaluated for phytoconstituents and pharmacological activity. Ethanolic extracts shows presence of alkaloid, flavonoid, glycoside, saponin, tannins and phenol. A plant shows excellent immunomodulatory activity by Neutrophil adhesion test. Ethanolic extracts Manilkara zapota seeds doses showed a significant increase in the neutrophils adhesion to nylon fibers. This might be due to the up-regulation of the β2 integrins, present on the surface of the neutrophils through which they adhere firmly to the nylon fibers. Hence, it was inferred that causes stimulation of neutrophils towards the site of inflammation. It shows significant immunostimulant activity. From the ethanol extract active constituent quercetin is isolated and characterized by FT-IR, NMR, UV and MASS spectroscopy.
Keywords: *Manilkara zapota, Seeds, Neutrophil adhesion test, Immunomodulatory activity*

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INTRODUCTION

Sapotaceae-family is a varied & eco-logically significant group of eight hundred varieties & nearly 65 genera [1]. These include shrubs and trees that extensively scattered in tropical-regions being American, Asian & African types [2]. The members of this family can be effortlessly acknowledged with characteristic creamy-latex & alternating leather leaf having comparable derived veins. The genus-*Manilkara* comprises about 30-32 species, most of them have economic importance & commercial advantages for fruits, wood & latex. Some of the related varieties are *Calocarpum-mamosum*, *Calocarpum-viride* & *Chrysophyllum-cainito* [3].

Manilkara zapota Linn seed (*Sapotaceae)* is vital fruit based medicinal Indian crop. Antioxidant nature of fruit is highly effective to counteract oxidative-stress [4,5]. The poly-phenolic, steroidal, Saponin, terpene, vitamin & carotinoid rich nutraceutical properties of fruits make the usefulness of drug. Phyto-chemical constituents similar to phenols, ascorbic-acid & carotenoid create straight impact on radical-scavenger perspective. Antioxidant can overcome or reduce degeneration speed in disorders like malignancy, rheumatoid-arthritis, heart-diseases, and neuronal disorders [6]. The seed of *Manilkara zapota* Linn are aperients, diuretic tonic, febrifuge and arthritic activity [7].

MATERIAL AND METHODS

Collection:

The new seeds of *Manilkara zapota* were collected from Kavate Ekand, near to Sangli in July and August 2020.

Authentication

From the part of plant like fruits flower and leaves it was studied and authenticated by Mr. M. D. Wadmare, H.O.D. of Botany Department, Smt. K.W. C. Sangli, Maharashtra, India.

Drying of plant material [8]

The 1 kilogram of seeds were cleaned and collected. It was separated and wipes carefully to eradicate muck and fragments. Seeds were spread and shade dried for 20-25 days. After natural drying of seed weighed, it was found 780 g. Dried seeds were coarsely powdered.

30 g of coarsely powder placed in soxhlet apparatus using 250ml of alcohol in flask. After six hours the extract is collected and concentrated by fading by water bath and stored at 4°C.The extract is tested for different chemical constituents.

The extract were evaluated for immunomodulatory activity by using *In-vivo* method are following -

a) Neutrophil Adhesion Test

Phytochemical screening [9, 10]

The ethanolic extract evaluated for phytochemical investigation. It was tested for alkaloids, flavonoids, glycosides, saponins, tannins and phenolic content.

Pharmacological Screening

Neutrophil adhesion test [11-14]

The rats were orally treated with vehicle and plant ethanolic extracts for fourteen days. On fourteenth day, blood were drained and collected from the retro-orbital plexus keen on heparinized vials and evaluated for differential leukocyte count (DLC). The preliminary counts, blood were incubated with 80 mg/ml of nylon fibers for 10 min at 37 °C. The incubated blood were evaluated for DLC. The % of neutrophils in the treated and untreated blood was calculated, and the variation was in use as an index of neutrophil adhesion.

Separation and isolation:

The separation of active constituents done by various chromatography techniques like thin layer chromatography and column chromatography. R_f value is calculated and reported.

Thin Layer Chromatography [15]

In TLC mobile phase used is chyclohexen: ethyl acetate: formic acid (7:3:1) and R_f value is calculated by

$$Rf = \frac{\text{Distance travelled by solute front (cm)}}{\text{Distance travelled by solvent front (cm)}}$$

Column chromatography of ethanol extract of seed

Silica gel passed through 60-120 mesh size and triggered for one hour in hot oven at 110 ± 1°C. Prepare slurry with benzene and rand transferred into the glass column which is equipped with a glass wool plug. The partial quantity of solvent could stay on top of the column (about 2cm). The air bubbles in the column have been removed by moderate tapping to generate an adsorbent bed identical. The column chromatography details are as follows:

Table 1. Column chromatography of ethanol extract of seed

Adsorbent	Silica gel activated at 110°C ± 2 for 1 hour
Column length	45 cms
Adsorbent length	35 cms.
Diameter of the column	Outer 3 cms, inner 2.8 cms.
Rate of elution	1 ml /min.
Volume of the elute collected	20 ml each
Gradient elution	chyclohexen: ethyl acetate: formic acid (7:3:1)

Sample preparation

A quantity (10 g) of extract was used for part isolation. The extract was solved in a minimal amount of oil ether and adsorbed in silica gel for a dried powder, which poured over the chromatographic bed and was coated in cotton wool. For isolation, a solvent mixture of increasing polarity, beginning with a petroleum ether, was eluted to the column, and then chyclohexen: ethyl acetate: formic acid (7:3:1). set volume fractions (e.g. 20ml), cumulative 5 fractions from the column, and therefore more fractions. The chromatography TLC of the above compound can dry after column growth. Distance travelled between solvent and solvent was labeled and measured and sharp pointer was added to the Rf values of each isolated compound of the individual position. Every spot was collected in separate Rf-value test tubes and further study of its identification was carried out by means of analytical methods such as FTIR, GC-MS and NMR.

RESULT

Authentication:

Collected parts of plants were authenticated by Mr. M. D. Wadmare, H.O.D. of Botany Department, Smt. K.W. C. Sangli, Maharashtra, India. It was *Manilkara zapota* (L) van Royen (Sapotaceae).

Phytochemical investigation of extract

Qualitative analysis of extract:

Following phytochemicals are present in ethanol extract.

Table 2. Phytochemical investigation of extract

Sr. No.	Phytochemical constituent	Test	Ethanol extract of seed
1.	Alkaloids	Dragendroff's test	+
		Mayer's test	+
		Hager's test	+
		Wagner's test	+
2.	Flavonoids	Shinoda test	+
		Lead acetate solution	+
3	Glycosides	Baljet's Test	+
		Legal's Test	+
		Killer-Killiani test	+
		Borntrager's test	+
		Modified Borntrager's test	+
4	Saponins	Foam test	+
5	Tannin	5% $FeCl_3$ Solution	+
		Lead acetate solution	+
		Gelatin solution	+
		Bromine water	+
		Acetic acid solution	+
		Potassium dichromate	+
		Dilute iodine solution	+
		Dilute HNO_3	+

+ = Present, - = Absent

Pharmacological screening

Incubation of blood with nylon fibres produced an increase in the neutrophil counts due to adhesion of neutrophils to the fibres. Effect of ethanolic extracts of *Manilkara zapota* seeds s on the neutrophil adhesion is shown in table 10.

Activity of ethanol extract of seed tested by the neutrophil adhesion test is shown in following tables

Table 3. Neutrophil count of ethanol extracts *Manilkara zapota* seeds

Group	Before Incubation	Neutrophil Count (%)	After Incubation	Neutrophil Count (%)
Normal	32	32.00±1.0	25	24.66±0.57
	33		24	
	31		25	
Standard (Levisamazole)	33	32.66±1.52**	17	16.33±2.08**
	31		14	
	34		18	
Aq Test I	50	49.00±1.0***	31	28.00±3.00***
	48		28	
	49		25	

All values are expressed in ±SD ***p<0.001,**p<0.01,*p<0.05 significant when compared to normal.

Table 4. TLC count of ethanol extracts *Manilkara zapota* seeds at before incubation

Group	Reading	Total Leucocyte Count (/cumm) Before Incubation	Reading	Total Leucocyte Count (/cumm) After Incubation
Normal	10500	11100.66 ±1126.9	9900	9800.00 ±100.00
	12400		9800	
	10400		9700	
Standard	10900	11466.66 ±493.29*	7200	7100.00 ±100.00*
	11700		7000	
	11800		7100	
Aq Test I	10800	10666.66 ±152.75*	9800	9266.66 ±472.58*
	10500		8900	
	10700		9100	

All values are expressed in ±SD ***p<0.001,**p<0.01,*p<0.05 significant when compared to normal.

Chromatographic analysis

Plant constituent was separated by TLC; chyclohexen: ethyl acetate: formic acid (7:3:1) used as mobile phase. The UV spectrum of isolated compound from ethanol extract of *Manilkara zapota* seed showed characteristic band at ʎ= 370.10 nm, to ʎ= 333.90 nm. The band ʎ= 370.10 nm was compared with the standard it was found flavonoid quercetin. FT-IR spectra of isolated compound showed 3328.78 (OH streching), 1641.44 (C=O streching), 1462.80, 1433.63(Aromatic ring), 1392.14, 1349.70 (C-OH deformation) and 1183 (C-OH streching). Isolated compound showed ion peak at m/z 303.0 which is consistent with the molecular formula GCMS spectra. Literature survey of flavonoid suggests that the compound may be containing simple skeleton having good number of hydroxyl group. The compound is assigned to tentative structure of quercetin which has got molecular weight 302. NMR showed aromatic hydrogen peak at 8.1-8.2 and hydroxy peak at 7.3. From the interpretation of above data it was found quercetin.

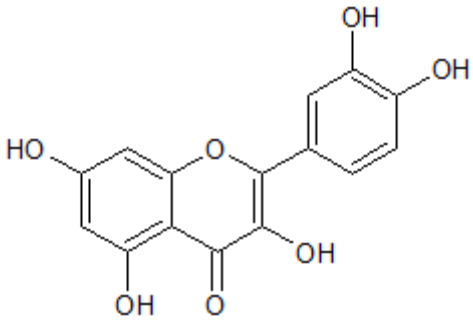

Figure 1. Isolated compound Quercetin

DISCUSSION

Manilkara zapota seed was extracted with ethanol. Ethanolic extract of seed evaluated for various phytochemical investigation. Ethanolic extract exhibit presence of alkaloids, flavonoids, glycosides, tannin, saponins and phenolic compound. Ethanolic extract also evaluated for pharmacological activity by neutrophil adhesion test (test for immunomodulatory action) .

Neutrophils are element of the cell-mediated immune responses liable for the innate immunity that donate to the authorization of foreign bodies by gratitude and immigration in the direction of the foreign body, phagocytosis and destroying the foreign agent. Cell adherence possessions of neutrophils are one of the initial responses of both immunological and physical injury. In the neutrophil adhesion test the cell adherence possessions of neutrophils was assessed in blood samples from diverse groups, by treating with nylon fibers to which the neutrophils adhere. Present study shows neutrophil adhesion ethanolic extracts of *Manilkara zapota* seeds. Ethanolic extracts Manilkara zapota seeds doses exhibits a noteworthy amplify in the neutrophils adhesion to nylon fibers. This capacity is due to the up-regulation of the β2 integrins, current on the surface of the neutrophils throughout which they adhere resolutely to the nylon fibers. Therefore, it was contingent that causes incentive of neutrophils towards the site of inflammation.

CONCLUSION

Manilkara zapota Linn plant have great importance and great food value throughout world. All parts of *Manilkara zapota* Linn have great source of active ingredients. The objective of this study is phytochemical and pharmacological evaluation of ethanolic extract of *Manilkara zapota* Linn seed. Phytochemical investigation of ethanol extract of seed shows presence of alkaloids, flavonoids, glycosides, tannin and phenol. For testing immunomodulatory activity of drug Neutrophil adhesion test was performed. Ethanolic extracts *Manilkara zapota* seeds doses showed a significant increase in the neutrophils adhesion to nylon fibers. This might be due to the up-regulation of the β2 integrins, present on the surface of the neutrophils through which they adhere firmly to the nylon fibers. Hence, it was inferred that causes stimulation of neutrophils towards the site of inflammation. It shows significant immunostimulant activity. From spectral analysis isolated compound was found quercetin.

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