World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 5.111
Year - 2025
Volume: 4; Issue: 5
Page: 375-381

"EXTRACTION, IDENTIFICATION AND INVITRO ANTIOXIDANT ACTIVITY OF SOME IMPORTANT BIOFLVONOIDS FROM MANGO PEELS" (*MANGIFERA INDICA*)

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Article Received: 24 August 2025 | | Article Revised: 14 September 2025 | | Article Accepted: 5 October 2025

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DOI: https://doi.org/10.5281/zenodo.17368458

How to cite this Article: Syed Azhar Nizami, Neha Unnisa, Divya Shree J., Aishwarya, Afreen Zaina, Mishra Avartika Ratnesh (2025) "EXTRACTION, IDENTIFICATION AND INVITRO ANTIOXIDANT ACTIVITY OF SOME IMPORTANT BIOFLVONOIDS FROM MANGO PEELS" (MANGIFERA INDICA). World Journal of Pharmaceutical Science and Research, 4(5), 375-381. https://doi.org/10.5281/zenodo.17368458

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ABSTRACT

Flavonoids are a class of polyphenolic secondary metabolites found with rich contents in colours of fruits flowers and leaves of plants. Mangiferin is bioflavonoid, a phenolic compound, Xanthone glycoside found primarily in mangoes (*Mangifera indica).* The Fruits pulp peels and, seeds, leaves, and bark of mango tree, has demonstrated potent anti-oxidant, anti- inflammatory, and anti- diabetic properties. In this study we have reported a simple method of extraction, isolation and antioxidant potential of Mangiferin from mango peels. For the extraction of mangiferin we used water and acetone mixture (water / Acetone 30/70: v/v) as a solvents, the % yield was 68.03%. TLC was done to identify Mangiferin in the extract using Ethyl acetate: Methanol: Water: 7:1:2 as mobile phase. The Rf value of Mangiferin was found to be 0.72. Further, Nitric oxide (NO) reducing power method was employed for invitro Antioxidant activity using DMF as a solvent at different concentrations, viz. 20, 40, 60, and 80 µg/ml and ascorbic acid was used as standard drug. The results of the study suggested that Mangiferine revealed the peak at 546.0 nm in DMF, The investigation of invitro antioxidant activity data revealed that Mangiferin by nitric oxide (NO) method showed moderate antioxidant activity compared with standard ascorbic acid.

KEYWORDS: Flavonoids, Mangiferine, Acetone extract, Antioxidant activity, TLC.

INTRODUCTION

In human health flavonoids modulate numerous cellular signally pathways and enzymatic activities, making them important dietary compound for the prevention and management of chronic diseases. Mangiferin is bioflavonoid, a

phenolic compound, Xanthone glycoside found primarily in mangoes (*Mangifera indica).* The Fruits pulp peels and, seeds, leaves, and bark of mango tree. In recent years mango has gained attention in scientific and nutraceutical research for its rich nutritional research for its rich nutritional profile and diverse phytochemical composition.[1-5] Mangiferin's consists of a 1,3,6,7-tetrahydroxyxanthone core with a β-D-glucosyl residue attached to the 6-position via a carbon-carbon (C-glycosyl) bond (Fig no 1). This C-glucoside structure, featuring two aromatic rings and several hydroxyl groups, grants it a potent antioxidant and diverse pharmacological properties, including anti-inflammatory, antidiabetic, and antitumor activities.[6-8] Hence in this study, we have reported the extraction, identification and invitro antioxidant activity of Mangiferin from mango peels.

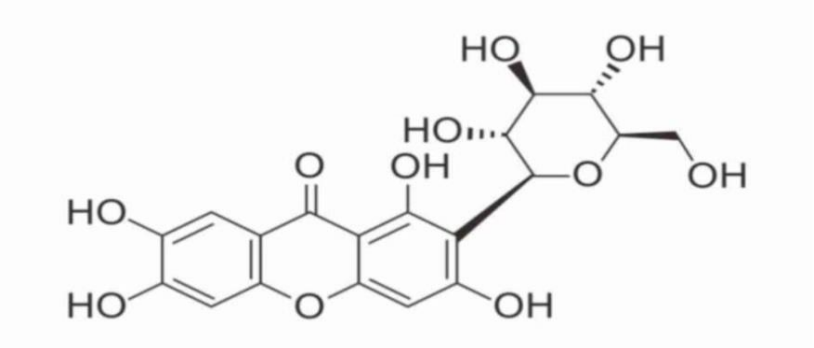

Fig. 1: Structure of Mangiferin

2. METERIALS AND METHODS

2.1. Chemicals

Solvents such as Acetone, DMF, nHexane, silica gel G and other solvents and reagents was used in the study were of analytical grade, purchased from SD fine chemicals pvt ltd Mumbai India and Merck India ltd Mumbai, India. TLC technique was followed using silica coated glass plates by using an appropriate solvent mixture. TLC spots of the compounds were visualised in Iodine chambers. For quantification of the extract double beam spectrophotometer (SHIMADZU-1700 series. Japan) was used.

2.2 METHODS

2.2.1. Method of Extraction

Locally available fresh mango fruits "*Mangifera indica*" were obtained from local market of Channapatna, Banglore south dist. few of them was washed and removed from the peels using a sharp knife. The peels were cut into small pieces placed them in a clean and dry glass jar added sufficient amount of water and acetone. The ratio of solvent to mango fruit was 30:70. The jar was tightly closed and shaken gently to mix the solvent and the mango peel. The jar was then stored in a cool place for 48 hrs to allow the solvent to extract the bioflavonoids such as Mangiferine and other compounds from the mango fruit peels. After the desired extraction time the mixture was filtered through a muslin cloth to remove any solid particles. The filtrate was collected in a clean glass container. (Fig no-2)

2.3. EVALUTION OF PHYTOCONSTITUENTS IN THE EXTRACT

The extract was subjected to preliminary phytochemicals studies the identification test for extract containing bioflavonoids was done on the basis of chemical test. TLC was performed for the identification of mangiferin using silica gel G as a stationary phase and n-butanol was used as mobile phase.

2. 3. 2. Detection method

To detect the Mangiferin contents in the extracted material, thin layer chromatography (TLC) method was performed. The extract was dissolved in Methanol and a spot was placed on the TLC plates with the help of a capillary tube. The sample was applied on the marked points. The plates were then kept in the beaker containing Ethyl acetate: Methanol: Water: 7:1:2 as the mobile phase and allowed to travel up to three-fourth of the length. It was then removed from the beaker and the solvent front was marked on the plates and dried.

Rf = (distance covered by the sample) / (distance covered by the solvent)

2.2.3. Invitro anti-oxidant activity of Mangiferin.[9,10]

For the determination of invitro antioxidant potential of Mangiferin in Mango peels extract Nitric Oxide (NO) Scavenging method was employed at different concentrations, and ascorbic acid was used as standard.

2.2.4. Nitric Oxide (NO) Scavenging Activity

The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent (1% sulfanilamide, 2% Phosphoric acid (H_3PO_4), and 0.1%N-(1-naphthyl) ethylene di amine di hydrochloride). Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. 1mL of sodium nitroprusside (10mmol) and 1.5mL of phosphate buffer saline (0.2M, pH7.4) were added to different concentrations (20, 40, 60 and 80μg/mL) of the test sample and incubated for 150 min at 25^0C and 1mL of the reaction mixture was treated with 1mL of Griess reagent. The absorbance value of the reaction mixture was recorded at 546nm. Nitric oxide scavenging activity was calculated using the formula,

$\%(NO_2)$ scavenging = control absorbance - simple absorbance / control absorbance$\times$100

3. RESULTS AND DISCUSSION

Mangiferin was isolated from mango fruit peels *Mangifera indica*. Conventional solvent extraction procedure was performed the yield was found to be 68% per 100 grms of mango peels. (**Fig no. 01**)

Figure 2: isolation of mangiferin from mango fruits peels: *Mangifera indica.*

Table no 1 Phytochemicals present in the mango fruit peels.

Parts of fruits used	Solvent for extraction	Phytochemicals present
peels	acetone	Bioflavonoids (Mangiferin)

Table 2: Identification Test for bioflavonoids.

Test	Observation
1. Alkaline Reagent Test (NaOH Test): a small amount extract was Dissolve in water, warmed and filter. Added10%aqueous sodium hydroxide (NaOH) to 2 ml of the solution.	Yellow colour appears which disappears after adding HCl
2. Shinoda's Test: a small amount of extract was dissolve in ethanol, warmed and filter. Added three pieces of magnesium chips to the filtrate, followed by a few drops of concentrated HCl.	Red colour appears
3. Lead acetate test: Add lead acetate solution to the extract.	Yellowish ppt.

Fig. 2: Identification Test for bioflavonoids.

3.1. Thin layer chromatography studies.

TLC was done to identify Mangiferin in the extract. The Rf value for Mangiferin was found to be 0.72

Fig. 3: Identification of Mangiferin by TLC.

3.2. INVITRO ANTI-OXIDANT ACTIVITY

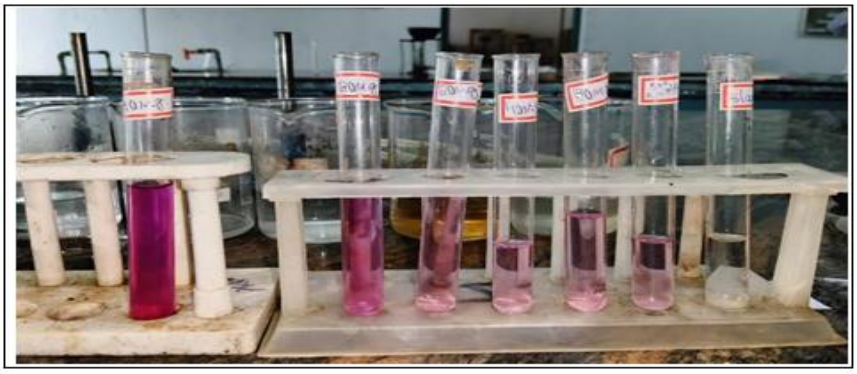

Fig 4: Colour Chromogen of Ascorbic acid and Mangiferin by nitric oxide (NO) method.

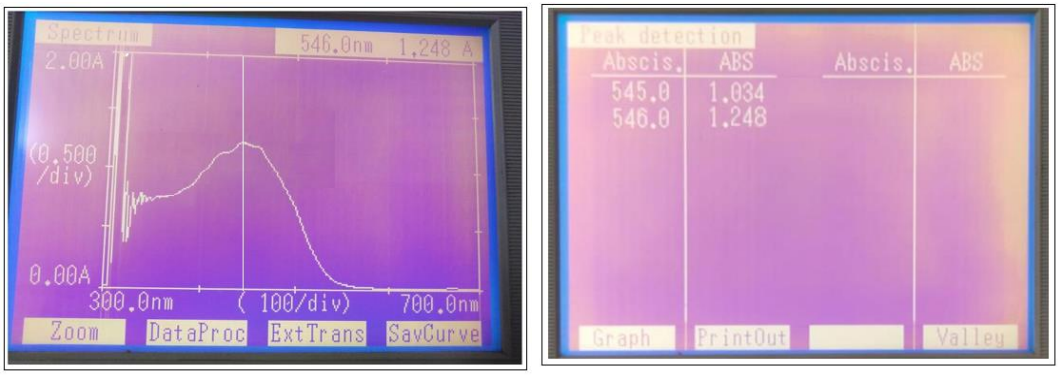

Fig 5: Absorbance maxima of Mangiferin at 546.0nm by nitric oxide (NO) method.

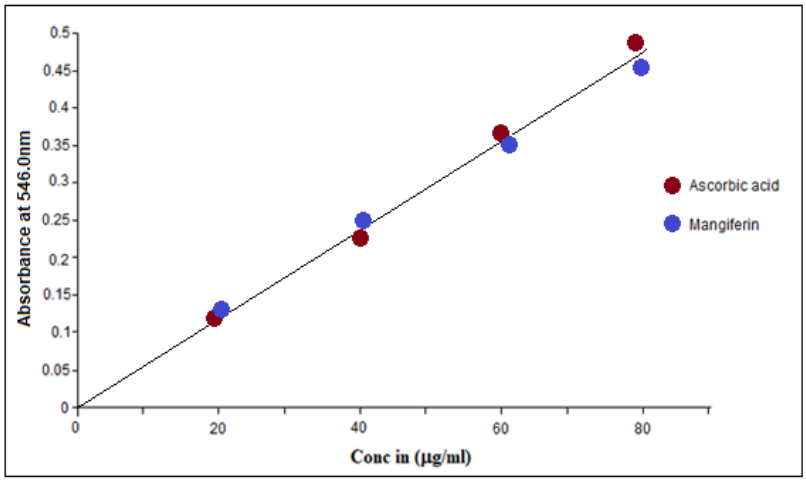

Fig 6: Standard Graph of Mangiferin and Ascorbic acid at 546.0nm data.

Table 3: Absorbance v/s concentration of Mangiferin and Ascorbic acid at 546.0nm data for standard curve.

Sr. No.	Concentration (µg/ml)	Absorbance at 546.0nm	
		Ascorbic acid	Mangiferin
1	20	0.142	0.133
2	40	0.264	0.272
3	60	0.413	0.421
4	80	0.512	0.508
5	DMF	0.00	0.00

Table 4: The in vitro antioxidant activity of Mangiferin in nitric oxide (NO) method.

Comp code	Concentration(μg/mL)				
	20	40	60	80	IC_{50}
Mangiferin	71.5 ± 0.85	74.3± 1.26	76.2± 0.44	76.2± 0.44	33.34± 0.23
Ascorbic acid	80.09± 0.22	81.18± 0.11	83.44± 0.22	85.55± 0.30	32.21± 0.42
Blank	----	----	----	----	----

(—) showed no scavenging activity. Values were the means of three replicates ± SD. IC50 value was calculated using the formula, **IC50 = [(ΣC/ ΣI) x 50]** where ΣC=sum of test concentration and ΣI= sum of percentage of inhibition concentration.

3.4. DISCUSSION

Mangiferin showed remarkable strong inhibition activity and is directly related to the concentration of Mangiferin (Figure 4, 5 & 6) which is summarized in Tables 2. The good radical scavenging activity is due to the presence of Phenolic hydroxyl groups are prone to donate a hydrogen atom to free radicals and extend conjugated aromatic system to delocalize an unpaired electron. Mangiferin was found to be powerful quenchers of nitric oxide radicals with significant IC_{50} value (33.34 µg/m). The IC_{50} value of the standard ascorbic acid by this method was found to be 32.21µg/mL.

4. SUMMARY AND CONCLUSION

Flavonoids or bioflavonoids are a class of polyphenolic secondary metabolites found in colours of fruits flowers and leaves of plants with rich contents of flavonoids. In human health flavonoids are acclaimed far their potent antioxidant properties and a wide range of biological activities including anti-inflammatory, anti-allergic, anti-microbial, anti-cancer, hepatic protective and cardio protective effects. They modulate numerous cellular signally pathways and enzymatic activities, making them important dietary compound for the prevention and management of chronic diseases. Mangiferin is bioflavonoid, a phenolic compound Xanthone glycoside found primarily in mango leaves, bark, and peel has demonstrated potent anti- oxidant, anti- inflammatory, and anti- diabetic properties. Mangiferin can be extracted easily from mango fruit, Hence, in this project we have reported a simple method of extraction identification and anti-oxidant activity of Mangiferin from mango peels by using water and acetone mixture (water / Acetone 30/70: v/v) as a solvents, the % yield was 68%. TLC was done to identify Mangiferin in the extract using Ethyl acetate: Methanol: Water: 7:1:2 as mobile phase. The Rf value of Mangiferin was found to be 0.72. The results of spectral analysis for Mangiferine revealed the peak at 546.0 nm in DMF, The investigation of invitro antioxidant activity data revealed that Mangiferin by nitric oxide (NO) showed moderate antioxidant activity compared with standard ascorbic acid, we conclude that mango peels, can be used in the regular diet, as it can provide flavonoid in significant amounts meeting the physiological requirements.

5. ACKNOWLEDGEMENT

The authors are thankful to Principal, Dr. HLT College of pharmacy for providing the necessary facilities and help in carrying out this project work.

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