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An efficient extraction and isolation of Mangiferin from mango leaves by using cold maceration: Include the study of effect of solvents and comparison with Soxhlet extraction

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ABSTRACT

Analysis of extraction method using various solvents for efficient yield of biologically active compounds from natural source is very essential because there is large importance for the natural compounds in food, pharmacy and other allied industries. Mangiferin which is a xanthone compound present in high quantity in mango leaves and is endow to have anti diabetic activity along with other pharmacological activities. In the present investigation two different extraction methods like Soxhlet extraction and cold maceration method were potentially used for the extraction and isolation of Mangiferin from mango leaves and compared for the first time. The Mangiferin obtained from both methods was shown same properties and the yield was similar. The solvent used for Soxhlet extraction is methanol and the for cold maceration is water and methanol and water in 1:1 ratio. The compound was identified by using FTIR, Melting point LCMS and UV and confirmed as Mangiferin.

I.INTRODUCTION

Mangiferin indica commonly known as mango is a popular and well known plant in the south Asian countries. The stem bark and leaves of mango tree contain high quantity of neutraceutical, polyphenolic, xanthone compound Mangiferin(1). Due to its complexity in structure it is very difficult to synthesize it chemically, so extraction and isolation from natural sources is best way(2). Various reports are available on its medical uses including Anti diabetic activity, Analgesic activity(3). Extraction is considered as first basic approach in medicinal plant research because the preparation of extracts from raw materials is the basic point for the separation and purification of chemical constituents(4).

Much evidence is found in literature for Pharmacological effects Mangiferin is a tremendous Bio-active molecule against numerous Disorders (5, 6). It has excellent Pharmacological activities like Cardio protective (7), Anti HIV (8), Antitumor (9, 10), Antibacterial (11), Antiviral (12), Immunomodulatory(13, 14), Anti diabetic(15,16) ,analgesic effects (17),neuroprotective(18) . The present research summarises the complete information regarding Mangiferin include its chemistry and pharmacological activities. However there were limited studies on quantification of mangiferin from mango leaves.

Many methods reported in the literature for the extraction and isolation of Mangiferin but the present developed technique is easy and cost effective. In this work M.Indica leaves was used as a starting material for Mangiferin extraction. The traditional techniques for extracting biologically active ingredients from plant source are based on the augmentation of solvent effect integrated with the aid of physical force. The extraction techniques operated at room temperature without any stress or pressure to prevent denaturation of compound. The required compound was extracted from plant source using single solvent and combined solvents frequently under constant agitation. The leaves were collected, washed and dried and powdered. The powder was passed through sieve no 20 to remove ununiform particles. Part of powder was done with Soxhlet extraction using methanol as solvent. The extraction was performed for 24 hours and the product was isolated by using ethyl acetate. In cold maceration the powder was mixed with water and kept aside for maceration (19).

II.MATERIALS AND METHODS

3.1 Materials: Mango leaves collected from the medicinal garden of srikrupa institute of pharmaceutical sciences, Velikatta.Leaves were washed with tap water and shade dried (at a room temperature for 4-5 days) and grinded to get powder. The powder of the leaves was stored at the room temperature. All organic solvents Methanol and Ethyl acetate were AR grade purchased from Tarnath Chemicals, India.

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3.2 Extraction of Mangiferin:

***Soxhlet extraction*:** 25.5 gm dried leaves powder was taken in Soxhlet apparatus with 300 ml solvent such as methanol per batch, and extraction was carried out for 48 hours. After completion of extraction, Methanolic extract (free from leaves powder) collected at the end. The extract was distilled off and dried extract was obtained and analyzed by reverse phase HPLC to determine concentrations of the extracted Mangiferin. The process of Soxhlet extraction for Methanolic extraction is described as follows: Mangiferin Indica dried leaves is subjected to exhaustive Soxhlet extraction with methanol as a solvent to isolate Mangiferin. The point of completion of extraction is determined by reaction with iodine vapours. A small spot is applied on a TLC plate by using capillary tube and placed it in iodine chamber, if colourless spot is observed that indicates completion of extraction.

3.3 Cold maceration: 25.5 grams of dried leaves powder was taken in conical flask and solvent (water), solvent mixture was added (water and methanol in 50:50 ratio). The solution was macerated for 24 hours. After 24 hours filtered and the filtrate was concentrated.

3.4 Fractionation and isolation of Mangiferin from extract

The concentrated extract obtained from Soxhlet extraction and cold maceration was fractionated with different solvents like Petroleum ether, Diethyl ether, methanol and Ethyl acetate. Fractionization was done by different methods based on solvent. Fractionization with Ethyl acetate on magnetic stirrer given a good result.

3.5 IDENTIFICATION OF MANGIFERIN:

Melting point: The melting point if isolated Mangiferin was determined by using Melting point apparatus. The drug was filled in capillary tube and inserted in apparatus. Temperature observed at which the drug was melted and exist as liquid.

FTIR studies: One (1) mg of the isolated Mangiferin crystals were measured using potassium-bromide (KBr) pellet method in FTIR spectrometer (Bruker-Alpha).IR data of isolated compound was compared with the reference standard of Mangiferin.

Mass spectroscopy: Mass spectrometry was performed on a Maldi-TOF Synapt XS HD Mass Spectrometer IN Punjab university, Chandigarh.

Determination of lambda max: Lambda max of the compound was determined by using UV Visible spectroscopy. For this the drug was dissolved in methanol and scanned in 200-800 nm range. The wavelength at which more absorbance found is considered as lambda max.

3.6 Construction of Standard calibration curve:

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In methanol:

Preparation of solutions for Calibration curve:
Stock solution 1: Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of drug in 100 ml solution of methanol (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.
Stock solution 2: 10 ml of this (stock solution 1) is diluted to 100ml with methanol to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.
Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml,) in each 10ml volumetric flask and makeup the volume upto10 ml to produce (2, 4, 6, 8, 10, 12, µg/ml) respectively.

In pH 6.8 Phosphate buffer

Preparation of solutions for Calibration curve:
Stock solution 1*:* Stock solution of drug (1mg/ml) is prepared by dissolving 100 mg of drug in 100 ml solution of pH 6.8 Phosphate buffer (to get 1000 µg/ml drug solutions) with vigorous shaking and further sonicated for about 10 minutes.
*Stock solution 2***:** 10 ml of this (stock solution 1) is diluted to 100ml with pH 6.8 Phosphate buffer to get a stock solution containing 100 µg/ml of drug. The stock solution was filtered through Whatmann filter paper No.41.
Dilutions: Take the respective samples (0.2ml,0.4ml, 0.6ml, 0.8ml, 1ml, 1.2ml,) in each 10ml volumetric flask and makeup the volume upto10 ml with pH 6.8 Phosphate buffer to produce (2, 4, 6, 8, 10, 12, µg/ml) respectively.

Preparation of Calibration curve:

The standard solutions for the drug having concentration 2, 4, 6, 8, 10, 12µg/ml was prepared with phosphate buffer pH 6.8 from the stock solution. The absorbance of solutions of pure drug were measured at 242 λ max and a calibration curve was plotted between absorbance v/s concentration to get the linearity and regression equation which has shown in fig. 2.

Melting point: The melting point of isolated compound was found to be 275 ± 0.5°C and the standard melting point 275-280°C

Determination of Lambda max: Lambda max of isolated Mangiferin was found as 242 nm which is near to the standard compound so isolated compound confirmed as Mangiferin.

IV.RESULT AND DISCUSSION

Melting point: The melting point of isolated compound was found to be 275 ± 0.5°C and the standard melting point 275-280°C

Determination of Lambda max: Lambda max of isolated Mangiferin was found as 242 nm which is near to the standard compound so isolated compound confirmed as Mangiferin.

FTIR studies:

Absorbance peak	Functional group
2936,2891cm^{-1}	C-H Stretching
3367.4 cm^{-1}	O-H Stretching
1648,1621 cm^{-1}	C=O Stretching
1255.9,195.6 cm^{-1}	C-O Stretching

Table1:FTIR interpretation

Calibration curve of drug in phosphate buffer

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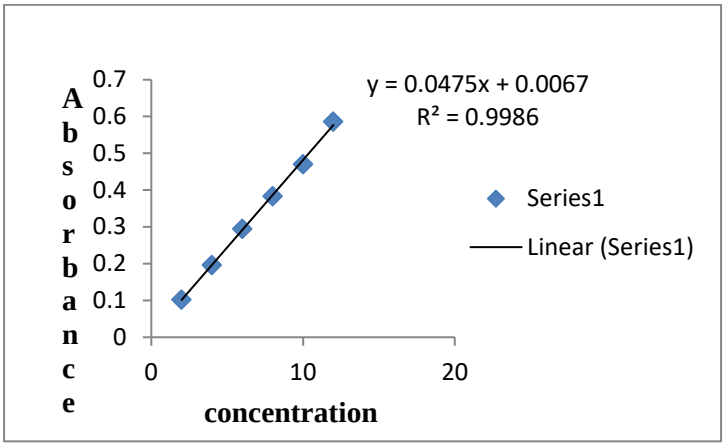

Fig1: Calibration curve of drug in phosphate buffer

Calibration curve of drug in methanol

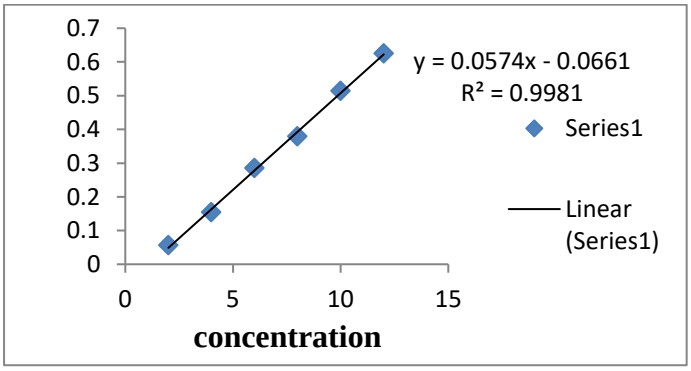

Fig 2: Calibration curve of drug in methanol

Impact of extraction technique and solvent on extraction:

s.no	Technique	Solvent	%yield
1.	Soxhlet extraction	Methanol 50%	10%
		Methanol 100%	20%
		Methanol Water	22%
2.	Cold maceration	Methanol 50%	10%
		Methanol 100%	20%
		Methanol Water	22%

Table 2: Impact of extraction technique and solvent on extraction

Impact of solvent on isolation:

S.no	Solvent	%Recovery
1	Petroleum ether	60
2	Diethyl ether	40
3	methanol	80
4	Ethyl acetate	90

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Table 3: Impact of solvent on isolation

LCMS results:

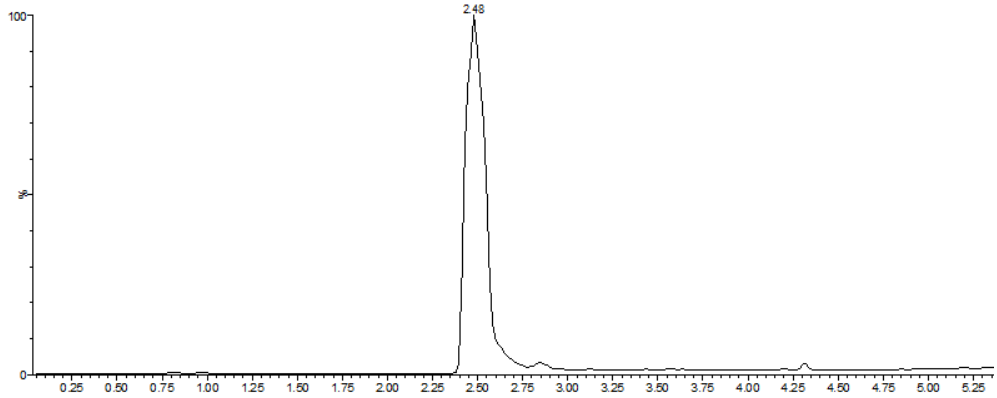

Fig 2:LCMS spectra of Mangiferin

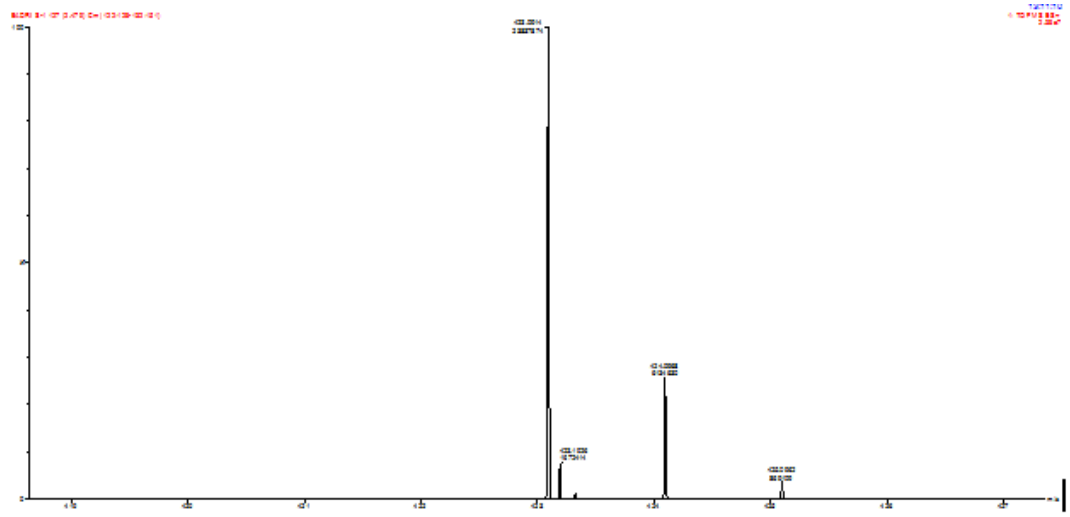

Fig 3:LCMS spectra of Mangiferin

V.CONCLUSION

The present study concluded that the isolated compound was Mangiferin and Soxhlet extraction and cold maceration methods given similar yield. so we can use cold maceration also for the extraction. Isolation with ethyl acetate is giving pure compound compared to other solvents. The FTIR spectrum of isolated compound is same as that of pure drug and the Mass spectrum of isolated compound showing molecular mass of 423 which is same as the standard compound, so the isolated compound was confirmed as Mangiferin.

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