“ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS FROM GARCINIA MANGOSTANA LINN FRUITS”

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ABSTRACT

The chemical studies were carried out on the fruits of *Garcinia mangostana*. A chemical constituent was isolated from the Methanolic extract of *Garcinia mangostana*. The structure of isolated constituents was analysed by HPLC, Mass Spectroscopy, H-NMR, C13 NMR. The Extract of *Garcinia mangostana* or Chemical Constituents of garcinia mangostana Show the biological activity. The pure isolated compounds show the anti- bacterial, Larvicidal activity. The xanthone compound are isolated Methanolic extract of *garcinia mangostana* which shows the potential activity. The yield of the extract of Methanolic extract was found and isolated compoud shows the purity above 90%.

**Keywords:** *Garcinia mangostana*, HPLC, Mass Spectroscopy, H-NMR, C13 NMR.

INTRODUCTION

*Garcinia mangostana* is the tropical green tree to have originated in the island and Indonesia. *Garcinia mangostana* grow in mainly southern Asia and tropical southern American countries. *Garcinia mangostana* is the belonging clusiaceace family. The mangosteen fruit is highly in juicy and its texture. The plant of mangostana show the activity against cancer treatment according cultivation in Java, Sumatra, Indochina and Philippines. Nature source of Xanthones is the mangostana which shows the anti-oxidant and anti-inflammatory and anti-fungal activity. The chemical xanthones from garcinia mangostana is α- Mangostana which shows against cytotoxic activity and inhibition of human leukaemia cell. The chemical constituent reported in garcinia mangostana Xanthones was 1. α - Mangosteen, 2. B- mangosteen, 3. γ - mangosteen, 4. 3-isomangosteen, 4. Stigmastone.
MATERIALS AND METHODS

General Experimental Procedure:

Plant material

_Garcinia mangostana_ Linn. fruits were collected from Sava Herbal Health Care Pvt. Ltd. Pune. The Fruits were air dried for 2 days and later crushed into smaller sizes and kept in tightly closed container in dark places until subjected to the extraction process.

General material

Thin layer chromatography (TLC) was performed over pre-coated silica plates 60 F254 (Merck). Borosilicate Glass column (55×4.5cm ID) used for column chromatography. Millipore filters (Pall corporation, 0.22µm) were used for sample filtration. HPLC column Phenomenex C18 (250 × 4.6 mm, 5 µm) used for HPLC analysis.

Solvents and Reagents:

Laboratory grade Ethanol (Fisher Scientific) was used for extraction. Laboratory grade methanol (Merck), n-hexane (Merck), ethyl acetate (EMPLURA) were used for liquid-liquid extraction. Analytical grade methanol (Rankem) was used for HPLC analysis.

Extraction:

Dried Fruits of _Garcinia mangostana_ was (500 gm) and extracted with methanol (2.5 L) for 3 hours at 50-60 ºC. The extract was then filter and again extract with fresh solvent. Then extract was filtered using Whatmann filter paper and concentrated by using a rotary evaporator. The yield of the extract was 12%. The crude extract was collected and stored at cool place for further process.
Sequential liquid-liquid extraction

The 100gm of ethanol extract was extracted with ethyl acetate. Ethyl acetate soluble fraction subjected to the column chromatography. And insoluble fraction will be discarded.

Thin layer chromatography:

Development of TLC profile for separation of Phytoconstituents present in ethyl acetate enriched extract. The TLC was performed on pre-coated silica plates 20x20 (0.20 mm) silica gel 60 F254. The mobile phase was selected Ethyl acetate: n-hexane (7: 3) as mobile phase.

Isolation of Chemical Constituents:

The 25 gm ethyl acetate extract was subjected to column chromatography on silica gel (100- 200 mesh). The slurry of silica gel was made with n-hexane and transfer into column with vigorous shaking so that silica gel settles down in the column. Then mixture of ethyl acetate extract was adsorbing on silica gel in order to form solid mass. These solid mass then loaded on silica which was settled in column. The column was then eluted with 100 % n-hexane and collected total 4 fractions. Then 8 %v/v ethyl acetate in n-hexane for that 6 fractions were collected. Then column was eluted with 15% v/v ethyl acetate in n- hexane, total 5 fractions was collected at the flow rate of 6-8 ml/min. After column was eluted with 22% Ethyl acetate (5 fractions) and then 40% methanol (3 fractions).

Preparation of Solutions:

Preparation of Sample Solution

10 g of compound was weighed in 10 ml volumetric flask to make 1000 μg/ml concentration of sample prepared in methanol then sonicate for 20 min and filtered through Millipore filters and Then was directly used for analysis.

Preparation of mobile phase:

0.1 % formic acid in water:

1 ml of Ortho-Phosphoric acid was dissolved in 1000 ml of double distilled water and, mixed it well and filtered through Millipore filter of 0.45 μm and then directly used for analysis.
RESULT AND DISCUSSION

Identify by TLC

Serval Mobile phase mixture were tried to separate the spot compound. TLC Chromatogram with goof resolution in (figure 1) was attained in the solvent system Ethyl acetate: n-hexane (7: 3).

![TLC Chromatogram](image)

Table No. 1: TLC details of the Compound

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Compound</th>
<th>RF value</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Identification by HPLC

The chromatographic separation of the drug was carried out on the shin pack C18 (250×4mm×5μm). The detection of the drug was carried out on the UV visible at a wavelength of 360 nm, 244 nm using the mixture of 0.1 % Ortho phosphoric acid in water: CAN. The flow rate 1ml/min

Table 2: Gradient method used for HPLC method

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>% A</th>
<th>% B</th>
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<tbody>
<tr>
<td>0.01</td>
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<td>75</td>
</tr>
<tr>
<td>10.0</td>
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<td>75</td>
</tr>
<tr>
<td>35.0</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>
Fig 3. HPLC chromatogram of Compound A

Fig 4. HPLC chromatogram of Compound B

Fig 4. HPLC chromatogram of Compound C
Mass Spectroscopy of Isolated Compound

(A) α-mangostin (1) (1 gm) was obtained as a yellow amorphous powder with melting point 181-184°C. The Mass spectrum showed the presence of a molecular ion peak at m/z 411.12 which, validated a molecular formula C24H26O6. $^1$H NMR, (600 MHz DMSO-D6) δ ppm 6.12(1H), 6.60(1H), 3.17(2H), 5.13(1H), 1.68(3H), 1.56(3H), 3.97(2H), 5.13(1H), 1.72(3H), 1.57(3H). $^{13}$C NMR, (600 MHz DMSO-D6) δ ppm 181.76, 165.45, 162.22, 160.20, 154.79, 154.79, 143.38, 137.09, 130.38, 130.27, 122.49, 110.05, 110.08, 101.36, 102.37, 91.73, 25.72, 24.60, 20.82, 16.93.

(B) γ-mangostin (2) (100 mg) was obtained as pale yellow crystals with a melting point of 203-206°C. The Mass spectrum showed the presence of a molecular ion peak at m/z 396.96. $^1$H NMR, (600 MHz DMSO-D6) δ ppm 1.580(3H), 1.580(3H), 1.780(3H), 1.780(3H), 3.260(2H), 4.780(2H), 5.168(1H), 6.168(1H), 6.450(1H) $^{13}$C NMR, (600 MHz DMSO-D6) δ ppm 184.81, 161.62, 157.62, 152.82, 152.65, 144.30, 131.27, 123.08, 119.53.

(C) 8-Desoxygartanin (2 mg) was isolated as yellowish crystal with a melting point of 164-166°C. The Mass spectrum showed a molecular ion peak at m/z 381.03 which corresponded to the molecular formula C23H24O6. $^1$H NMR, (600 MHz DMSO-D6) δ ppm 1.679(1H), 1.655(1H), 1.64(1H), 1.724(3H), 1.724(1H), 3.301(2H), 3.311(2H), 3.73(2H), 5.128(1H), 5.13(1H), 5.133(1H). $^{13}$C NMR, (600 MHz DMSO-D6) δ ppm 181.75, 162.28, 160.22, 157.81, 145.35, 143.35, 130.28, 130.28, 122.44, 12.44, 122.44, 118.66, 110.08, 102.38, 27.61.
22.16.

SUMMARY AND CONCLUSION

In the present study of fruits *Garcinia mangostana* were collected and dried. It was then reduced to small particle size and then subjected to extraction by using ethanol as solvent. The percentage yield of ethanol extract was found to be 12% and further ethanolic extract was subjected for sequential liquid-liquid extraction with solvent like hexane, chloroform and ethyl acetate. Column chromatography of ethyl acetate extract was done for isolation of active constituents. Fractions were collected and dried. The isolated compound was then purified and the quantity of those compounds were 1 gm, 300 mg, 200 mg respectively. The isolated compound was then characterized by using spectroscopic technique like HPLC, NMR and mass spectroscopy.

This study aimed to isolate flavonoids from the leaves of fruits of *Garcinia mangostana*. The isolated compounds were identified by HPLC, Mass and $^{13}$C NMR and $^1$H NMR spectra. The isolated compound was identified as α-mangosteen, γ-Mangosteen (Xanthones) and 8-desoxygartanin (Gartanin).

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