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"ISOLATION AND CHARACTRIZATION OF CHEMICAL CONSTITUENTS FROM GARCINIA MANGOSTANA LINN FRUITS"

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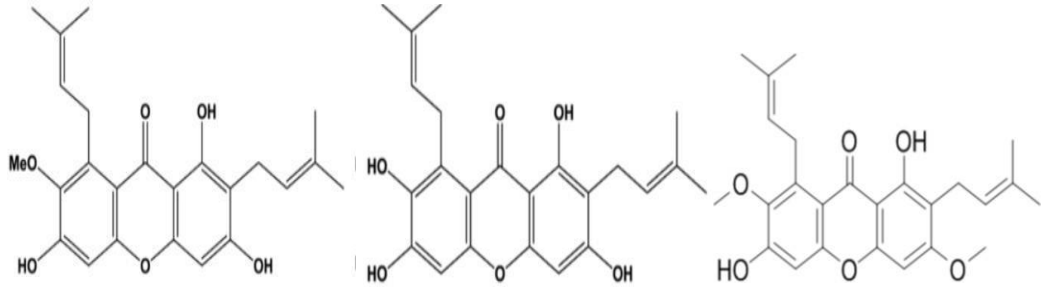
ABSTRACT

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, C^{13} 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 compound shows the purity above 90%.

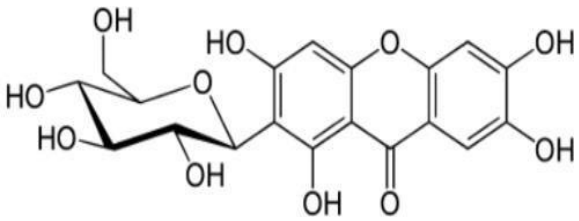
Keywords: *Garcinia mangostana*, HPLC, Mass Spectroscopy, H-NMR, C^{13} NMR.

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.



α-Mangosteen **γ-Mangosteen** **β Mangosteen**



Xanthonoids

Figure 2.1 Chemical constituents from *Garcinia mangostana* Linn.

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 C_{18} (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).

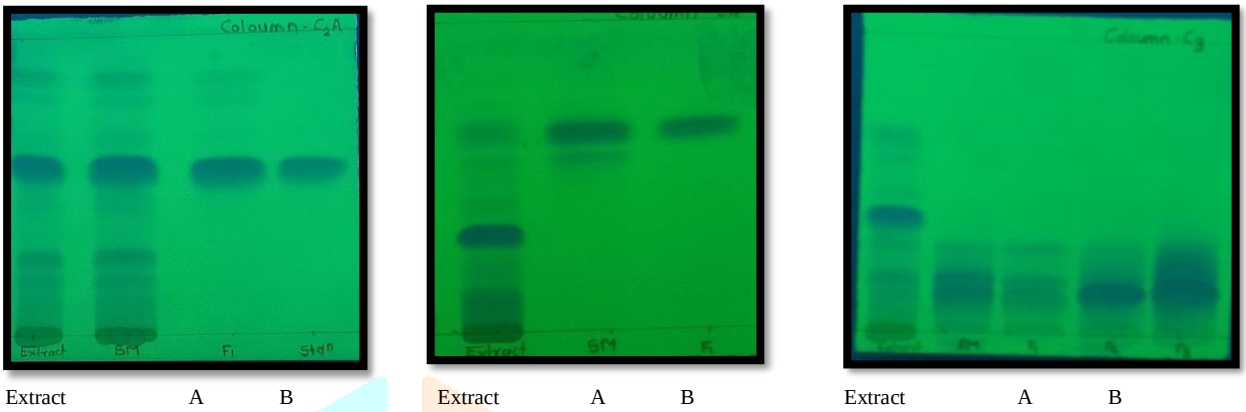


Figure 2: TLC Profile of compound

Table No. 1: TLC details of the Compound

Sr. No	Compound	RF value
1	A	5
2	B	4.5
3	C	6.4

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

Time (Min)	% A	% B
0.01	25	75
10.0	10	90
15.0	0	100
25.0	0	100
25.0	25	75
35.0	25	75

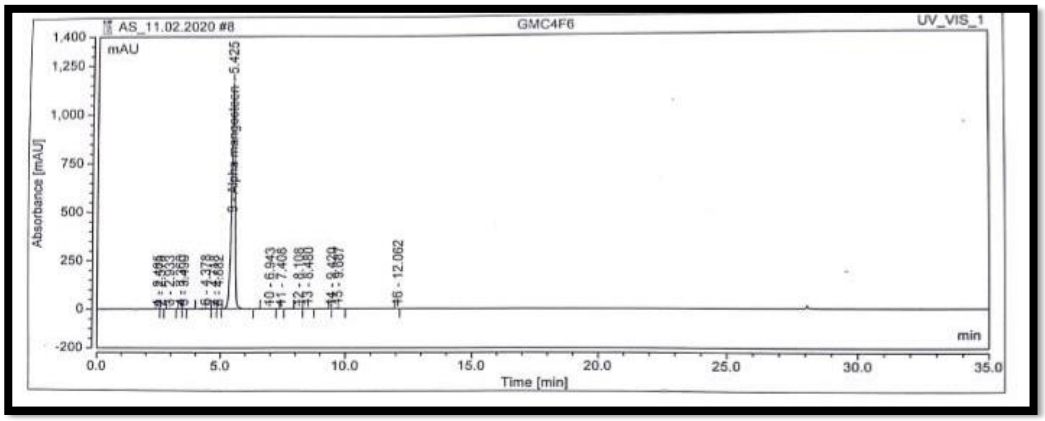


Fig 3. HPLC chromatogram of Compound A

Fig 4. HPLC chromatogram of Compound B

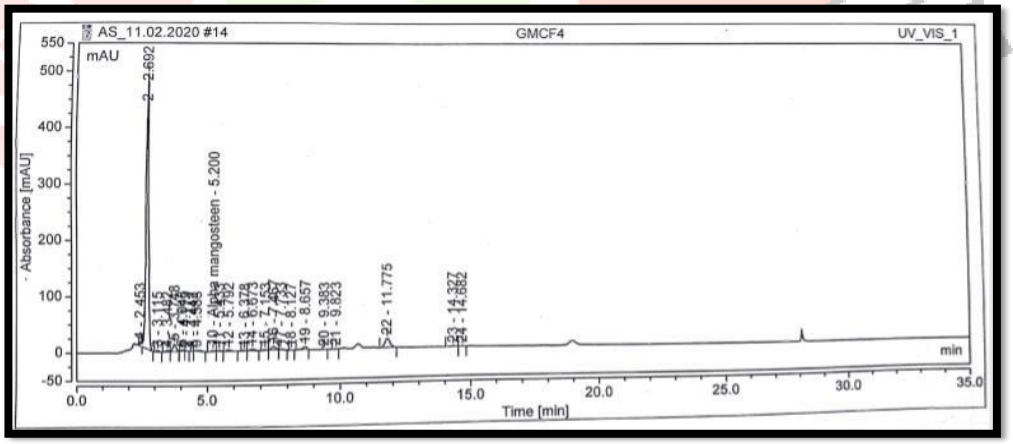
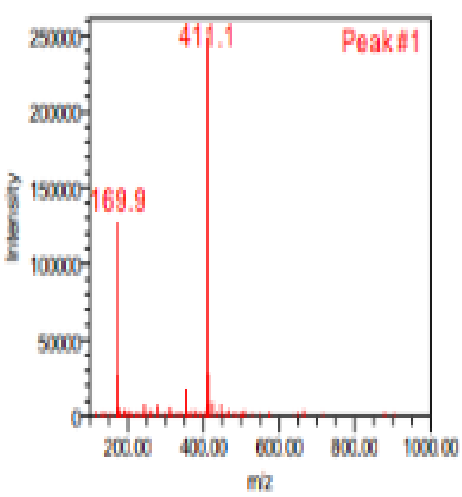
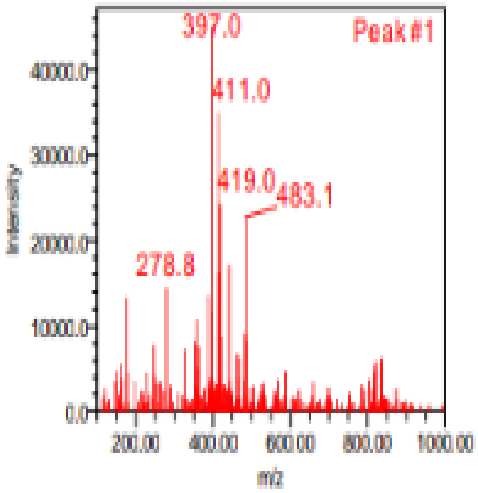


Fig 4. HPLC chromatogram of Compound C

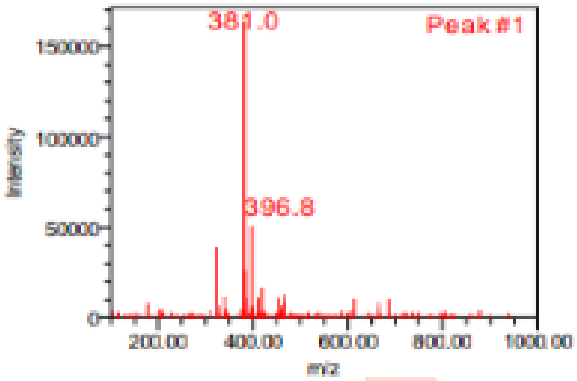
Mass Spectroscopy of Isolated Compound



(A)



(B)



(C)

Fig 5. Mass Spectroscopy of compounds

(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 164-166ºC. 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).**

REFERENCE:

1. Abdul Rohman, Mohamad Rafi, Chemical composition and oxidation studies of underutilized part of mangosteen (*Garcinia mangostana* L.) Fruits, Department of pharmaceutical chemistry, Faculty of pharmacy, Gadjah mada university, Indonesia, Journal of applied pharmaceutical science, Volume-9(08), pp047-052, Aug2019.
2. B. Martindale, The extra pharmacopeia, the pharmaceutical press, London, UK,28th edition, 1982.
3. Bruna Lais A., Geronimo P., Evaluation of Antimicrobial and antitumor activity of *Garcinia mangostana* Linn growth in southern brazil, Department of biological science, Technical procedure, original article model biological, Volume-29,2014.
4. C. Yang, advance in isolation and synthesis of xanthones derivatives, Chinese herbal medicines, Volume -4, Page no:102,2012.
5. Consolacion Y. R, Chemical constituents of *Garcinia mangostana* pulp and seeds, Department of chemistry, National Research institute of Chinese medicine, Taiwan, International Journal of pharmaceutical and clinicalresearch1166-1169,.2016.
6. Geetha R. V, Lakshmi T, Anitha Roy, In vitro evaluation of anti mycotic activity of ethanolic fruits extract of *Garcinia mangostana* Linn, International journal of current research and review, Volume-3, Page No:20-32.2012.
7. H. Hajimedhdipour, S. D. Sadat, three tetra oxygenated xanthones from swetia longifolia, pharmaceutical biology, Volume-41, Page no: 497-499,2003.
8. H. Liu, Separation method for pharmacologically active xanthones, journal of chromatography, Volume, Page no- 165-175.2004.
9. H. M. G. Hazimi, G. A. Miana, naturally occurring xanthones in higher plants and ferns, journal of the chemical society of Pakistan.
10. Carpenter, H. D. Locksley, and F. Scheinman, Xanthones in higher plants: biogenetic proposals and chemotaxonomic survey, Voliume-8. Page no: 2013-2025,1969.

11. Irene S., G. Cheng Lian Ee, Two new chemical constituents from the stem Bark of Garcinia mangostana, Department of chemistry, faculty of science, University putra Malaysia, molecule 19,2014.

12. J. S. Negi, P. Singh, fingerprinting, and RP- HPLC analysis and antidiabetic activity of swetia puniculata, natural product, Volume- 5, Page no: 910,2010.

13. Jebalsy L., Prince C., Biological activities of the *Garcinia mangostana* Linn, Asian journal of the pharmaceutical and clinical research, Research department of plant biology, Chennai, Tamilnadu, Volume-10,2017.

14. Jose P. Noemi C, Medicinal properties of Garcinia (Mangosteen),3 Article in Food and chemical toxicology, 46(2008), 3227-3239.2008.

15. K. Irshiguro, S. Nagata, BisXanthones from Hypericum japonium inhibit of PAF-induced hypotension, planta medica, Volume-68, Page Np:258-261.