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Identification of antibacterial component from extract of *Garcinia indica* fruit rinds using LC/MS/MS

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1. Introduction

Medicinal plants have played an important role in drug discovery with many pharmaceutical products originating from plant. Isolation and characterization of antibacterial compounds is still relevant because of continuous development of resistance of bacteria to antibiotics.

1-1. Garcinia indica

Garcinia indica, a plant from the mangosteen family (*Clusiaceae*), commonly known as 'Kokum' is used for culinary purposes in India. *Garcinia indica* is indigenous to the Western Ghats region of India located along the western coast of the country. Fruits of *Garcinia indica* have been suggested in the Indian system of medicine for a number of diseases as a remedial drug[1].

Fig. 1 Garcinia indica

Classification of *Garcinia indica* [1]

Kingdom	: Plantae
Division	: Mangoliophyta
Class	: Magnoliopsida
Order	: Malpighiales
Family	: Clusiaceae
Subfamily	: Clusioideae
Tribe	: Garcinieae
Genus	: Garcinia
Species	: indica

1-2. Garcinol

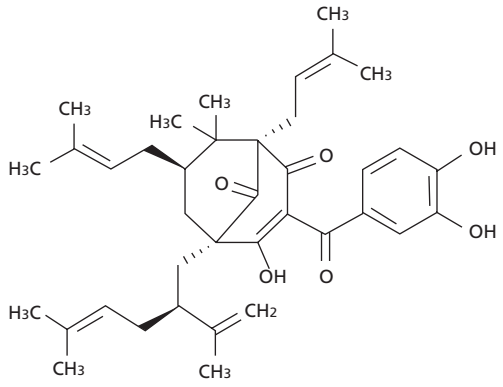

Fig. 2 Garcinol

Garcinol, a polyisoprenylated benzophenone, is extracted from the rind of the fruit of *Garcinia indica*, a plant found extensively in tropical regions (shown in Fig. 2). There is ample data to suggest potent antioxidant properties of this compound which have been used to explain most of its observed biological activities. However, emerging evidence suggests that garcinol could be useful as an anti-cancer agent, and it is increasingly being realized that garcinol is a pleiotropic agent capable of modulating key regulatory cell signaling. Garcinol is an example of a prenylated chalcone, which has many beneficial effects in human health and disease[2][3].

In present work, we have studied antibacterial activity of extract from rinds of *Garcinia indica* by using Bioautography technique and identified this antibacterial compound by LCMS-8040 triple quadrupole mass spectrometer. The feature of 'optimisation of method' present in LCMS-8040 provides qualitative information along with Multiple Reaction Monitoring (MRM) optimisation which can be used for the probable structural assignment.

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2. Method of Analysis

2-1. Preparation of plant extracts

Garcinia indica fruits were collected from Ratnagiri area in the state of Maharashtra in India. The fruits rinds were separated from the fruit. Shed dried fruit rinds were powdered using mixer grinder, sieved through the mesh size 40 and stored in the air-tight PET bottles at room temperature.

2-2. Bioautography

Target plant pathogen : *Xanthomonas axonopodis pv.punicae*

Information about target organism : *X. axonopodis* pv.*punicae* is a bacterial pathogen that causes serious blight disease of pomegranate. The disease was first reported in India where it is a major threat to pomegranate cultivation due to lack of effective control measures. The blight can cause 50 to 100 % production loss depending on severity of disease. In oily spot of pomegranate (also called bacterial blight, black spot) the main damage is observed on fruits, which develop black spots resulting in enormous yield losses. The disease has become an increasingly serious threat for pomegranate growers of Andhra Pradesh, Maharashtra, and Karnataka. For example in 2006-2007 in Maharashtra, oily spot was found to be the main disease with 100 percent infection in some orchards.

Plant used : Matured *Garcinia indica* fruit rinds but not fully ripe.

Extraction : Soxhlet extraction was done for 18 to 24 hrs using Ethyl acetate. Solvent was evaporated after extraction. The dried plant extract was reconstituted in Methanol.

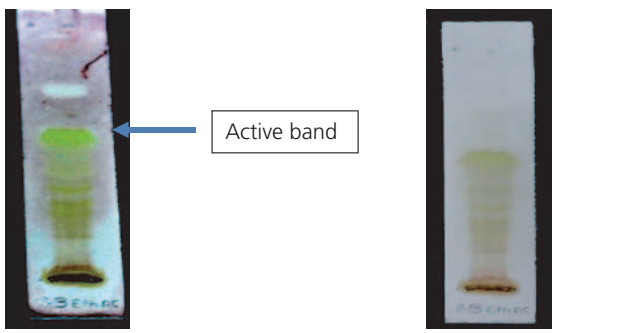
TLC : The extract was reconstituted in methanol and used for TLC.

Mobile phase used : Toluene : Ethyl acetate : Formic acid (4 : 1 : 0.5 v/v/v) Chamber saturation : 30 mins.

Bioautography : Bioautography is a microbiological screening methods commonly used for the detection of antimicrobial activity. The screening can be defined as the first procedure, which is applied to sample, in order to establish the presence or absence of given analytes. It is a simple measurement providing a "yes/no" response. They are simple, cheap, time-saving and do not require sophisticated equipment. Bioautography screening methods are based on the biological activities, e.g. antibacterial, antifungal, antitumor, and antiprotozoae of the tested substances. This detection method can be successfully combined with Planar Chromatography techniques, such as Thin-Layer Chromatography (TLC), High-Performance Thin-Layer Chromatography (HPTLC), Overpressure Layer Chromatography (OPLC) and Planar Electrochromatography[4]. This work was performed by Direct Bioautography technique. Nutrient broth with selected pathogen in the actively growing phase was sprayed over the TLC plates and plates were incubated for stipulated time at 28°C.To visualize the microbial growth, aqueous solution of *p*-Iodonitrotetrazolium (INT) dye (2 mg/mL) was sprayed over the plates and they were further incubated at room temperature for 6 hrs[5].

Inference : Bacterial growth led to emergence of purple-red color on the TLC plates, formed due to reduction of INT into formazan. Clear zones (yellow) indicated the inhibition of bacteria due to the antibacterial compound present in that band with particular Rf ,(Retention factor) on the TLC chromatogram (shown in FIg. 3).

Isolation of active band and identification : The active band which is yellow in colour was scrapped out and taken in methanol and further subjected to LC/MS/MS for structural identification.



a. *Garcinia indica* extract against *Xanthomonas axonopodis pv.punicea*

b.*Garcinia indica* extract reference TLC plate.

Fig. 3 Results of Bioautography

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2-3. LC/MS/MS analysis

Column	: Connector
Flow rate	: 0.6 mL/min
Oven temperature	: 40°C
Detector	: UV detector at 238nm
Mobile phase	: A: 10 mM Ammonium acetate B: methanol
Gradient program (%B)	: 70
Injection volume	: 20 µL
MS interface	: Electro spray Ionization (ESI)
Nitrogen gas flow	: Nebulizing gas 1.5 L/min; Drying gas 10 L/min
MS temperature	: Desolvation line 250°C; Heat Block 400°C

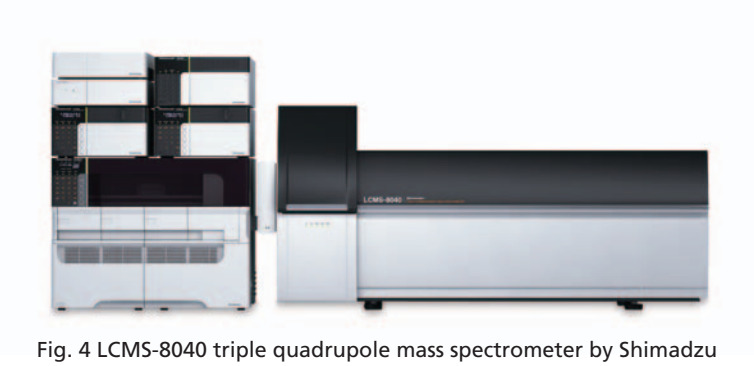


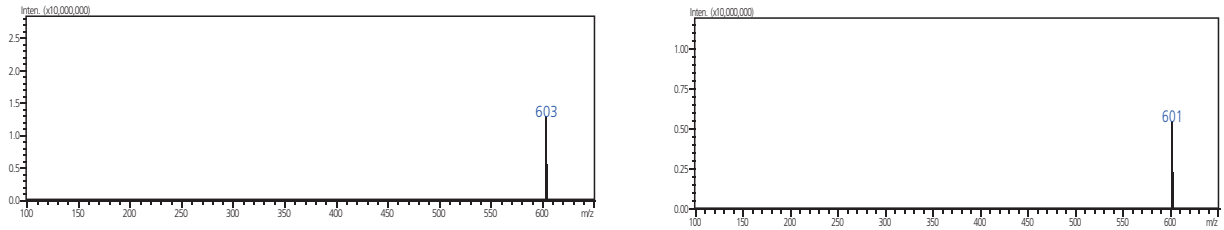
Fig. 4 LCMS-8040 triple quadrupole mass spectrometer by Shimadzu

3. Results

3-1. LC/MS/MS results

Q3 full scan was performed on ESI ionization mode using both polarities. The molecular ion of *m/z* 603 (positive ESI) and *m/z* 601 (negative ESI) were obtained (shown in Fig. 5).

It confirmed the molecular weight of antibacterial compound is 602.



a) ESI positive ionization mode

b) ESI negative ionization mode

Fig. 5 MS spectra of Garcinol

LCMS-8040 (shown in Fig. 4) has a feature of 'Optimisation of method' in which the mass spectrometer selects the best product ion(s) and optimises voltages and collision energies for the precursor to product transition.

For Garcinol, MRM was optimised for the precursor ion of *m/z* 603 using the above mentioned feature. During this optimisation, the precursor ion was subjected to a series of collision energies ranging from -10 V to -50 V with a step width of 10 V to obtain product ions ranging from higher *m/z* to lower *m/z* (shown in Table 1). The fragmentation pathway of Garcinol at different collision energies with probable structures are given in Fig. 6.

Table1 MS/MS data of Garcinol at different Collision Energies (CE)

CE	*m/z* of Product ions										
-10			137	177	233	287	343	411	467	535	585
-20			137	177	233	287	343	411	467		
-30		81	137	177	233	287	343	411			
-40		81	137	177	233	287	343				
-50	57	81	137	177	233						

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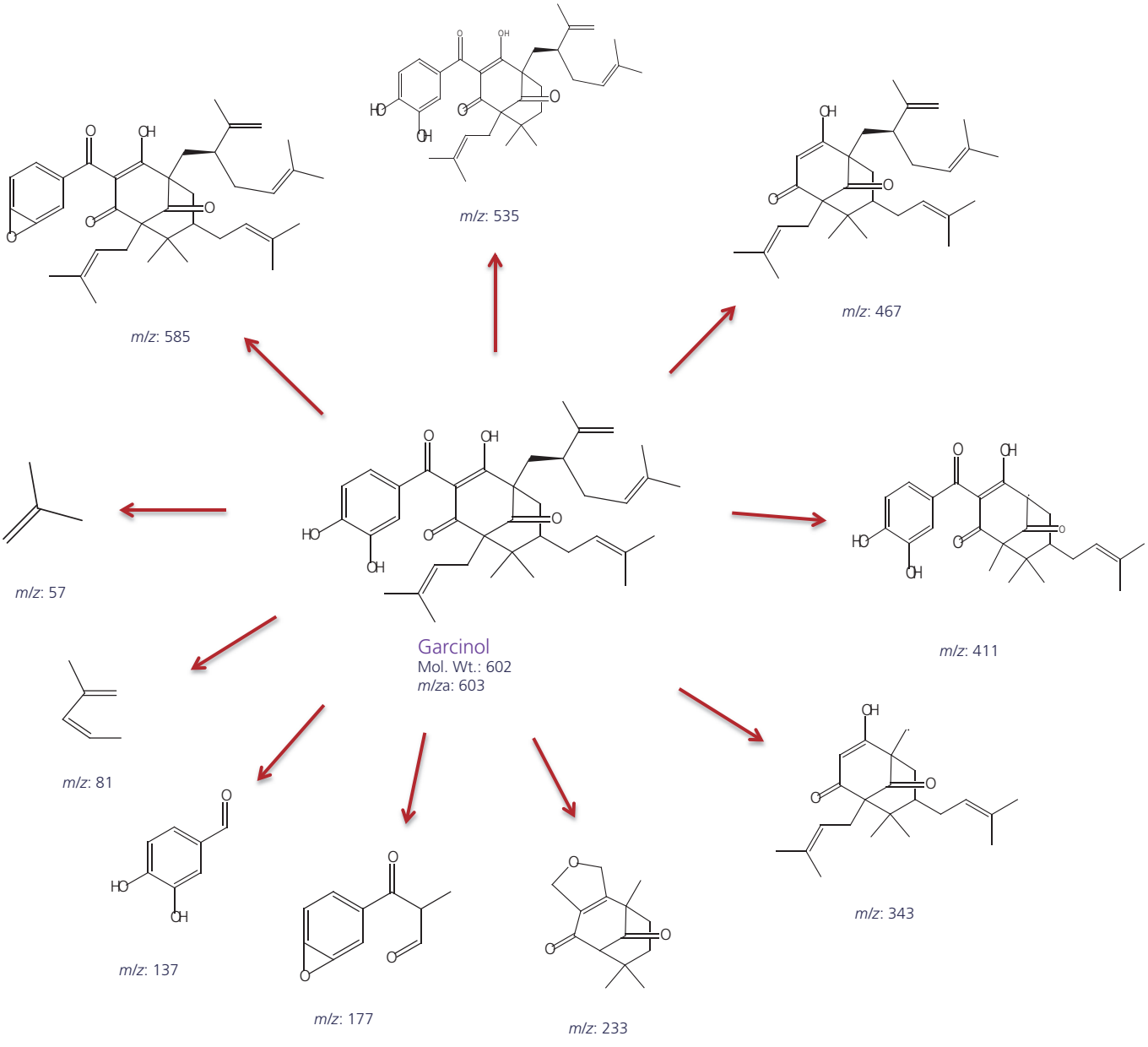

Fig. 6 Probable fragmentation pathway of Garcinol in ESI (+) tandem mass spectrometry

4. Conclusion

- The Bioautography technique was used to distinguish antibacterial component from the Garcinia indica fruit rinds extract.
- The antibacterial component scrapped out from TLC was subjected to LC/MS/MS analysis at different Collision Energies. Based on the product ions formed, the structure of antibacterial component was predicted and identified as 'Garcinol' .
- Further structural confirmation can be established by using HRMS and NMR techniques.

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5. References

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