SUPPLEMENTARY MATERIAL

LC/MS characterization of phenolic antioxidants of Brindle berry (*Garcinia gummi- gutta* (L.) Robson)

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Abstract

Characterization of antioxidant fraction was done in the fruit of *Garcinia gummi- gutta* using Liquid Chromatography Mass Spectroscopic analyses. Total poly phenolics and radical scavenging activity of various extracts such as acetone, methanol and hydroalcohol were estimated spectrophotometrically. The active extract was analysed by LC/MS in order to identify the molecular mass and tentative structures of major compounds.

Key words: Phenolics, *Garcinia gummi- gutta*, DPPH, LC/ MS

Experimental

Plant materials

The plant materials (fruits) were collected from Herb garden of Arya Vaidya sala, Kottakkal, Kerala and all the materials were authenticated by Plant Systematics and Genetic Resources Division, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala Kottakkal, Kerala, India. The voucher specimen was deposited in CMPR herbarium (04871, CMPR).

Chemicals and reagents

Folin-Ciocalteu reagent was procured from Sisco Research Laboratory (SRL), Mumbai, India. DPPH, catechin, gallic acid and quercetin were procured from Sigma Chemicals Co. (USA). LCMS solvents were also procured from Sigma Chemicals Co. (USA). All other chemicals employed were of standard analytical grade from Merck India.

Extraction

Dried fruit powder (10 g) was extracted with acetone, methanol and hydro alcohol (Ethanol: water 50:50) for 48 hrs in a soxhlet apparatus. After filtration, the filtrate was concentrated to dryness by rotary evaporator at 48⁰C, then weighed and diluted to 25 ml with respective solvents.

Estimation of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent (Singleton et al., 1965). TPC was expressed as gallic acid equivalents (GAE) in mg / g of sample.

DPPH assay

DPPH radical scavenging activity was measured spectrophotometrically (Tepe et al. 2005). Catechin was used as standard.

HPLC analysis

The most active extract against DPPH radical was further analysed by HP-LC/MS. The hydro alcoholic extract was subjected to HPLC analysis using Agilent 1200 preparative high pressure liquid chromatographic system equipped with prep pump, a Rheodyne injector, and diode array detector in combination with Chem32 and Chemstation software. Gradient elution was performed with water/0.05% formic acid (solvent A) and acetonitrile (solvent B) in a ratio of

40(A): 60 (B) at a constant flow rate of 1 ml/min with a total run time of 15 minutes. The DAD signal was recorded at 265 nm.

LC-ESI-MS/MS analysis

LC-ESI/MS analysis was conducted on Agilent 6520 accurate mass Q-TOF LC/MS coupled with Agilent LC 1200 equipped with Extend-C18 column of 1.8 μm , 2.1 x 50 mm. Gradient elution was performed with water/0.05% formic acid (solvent A) and acetonitrile (solvent B) at a constant flow rate of 0.2 ml/min. The MS analysis was performed using ESI in the negative mode. The conditions for mass spectrometry were: drying gas (nitrogen) flow 5 L/min; nebulizer pressure 40 psig; drying gas temperature 325°C; capillary voltage - 3000 V; fragmentor volt 125V; Oct RF Vpp 750 V. The mass fragmentation was performed with varying collision energy 4 V/ 100 DA with an offset of 8V.

Statistical analysis

Results for TPC and DPPH EC_{50} were given as mean value of three readings, calculated by employing the statistical software (COSTAT, Monterey, CA 93940, U.S.A).

Figures

Fig.S1. HPLC Chromatogram of hydro alcoholic extract of *G. gummi-gutta*

Fig S2 LC/MS Base peak Chromatogram of hydro alcoholic extract of *G. gummi- gutta*

Tables

Table S1 TPC and DPPH EC₅₀ of various extracts of *G. gummi- gutta*

Extract	TPC (mg GAE)	EC ₅₀ µg/ml(DPPH)
Acetone	12.80± 0.16	8.64 ± 0.62
Methanol	9.25 ± 0.12	13.86 ± 0.16
Hydro alcohol	18.36± 0.36	6.38± 0.24
Catechin		3.40 ± 0.12

Table S2 LC MS/MS analysis of *G. gummi- gutta* hydro alcoholic extract

R _t	m/z [M-H]	MS/MS	Molecular formula	Tentative identification
1.61	461.07	285.04	C ₂₂ H ₂₁ O ₁₁	Luteolin 7-O- glucuronide
1.96	489.10	285.04	C ₂₃ H ₂₁ O ₁₂	Kaempferol 3-O-(6-O-acetyl) glycoside
2.13	515.12	359.01 179.0 135.12	C ₂₅ H ₂₃ O ₁₂	Dicaffeoylquinic acid

2.25	563.13	503.10	$C_{26}H_{27}O_{14}$	Apigenein-6-C-pentosyl-8-C-hexoside
		473.10		
2.80	337.08	173.02	$C_{16}H_{17}O_8$	p-Coumarylquinic acid

References

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