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Analytical Methods

Uni-dimensional double development HPTLC-densitometry method for simultaneous analysis of mangiferin and lupeol content in mango (*Mangifera indica*) pulp and peel during storage

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ABSTRACT

Mango (*Mangifera indica*) fruit is one of the important commercial fruit crops of India. Similar to other tropical fruits it is also highly perishable in nature. During storage/ripening, changes in its physico-chemical quality parameters viz. firmness, titrable acidity, total soluble solid content (TSSC), carotenoids content, and other biochemicals are inevitable. A uni-dimensional double-development high-performance thin-layer chromatography (UDDD-HPTLC) method was developed for the real-time monitoring of mangiferin and lupeol in mango pulp and peel during storage. The quantitative determination of both compounds of different classes was achieved by densitometric HPTLC method. Silica gel $60F_{254}$ HPTLC plates and two solvent systems viz. toluene/EtOAC/MeOH and EtOAC/MeOH, respectively were used for optimum separation and selective evaluation. Densitometric quantitation of mangiferin was performed at 390 nm, while lupeol at 610 nm after post chromatographic derivatization. Validated method was used to real-time monitoring of mangiferin and lupeol content during storage in four Indian cultivars, e.g. Bombay green (*Bgreen*), *Dashehari*, *Langra*, and *Chausa*. Significant correlations ($p < 0.05$) between of acidity and TSSC with mangiferin and lupeol in pulp and peel during storage were also observed.

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1. Introduction

In India, mango (*Mangifera indica*, Linn, Anacardiaceae) fruit is designated as "The King of Fruits" and is one of the most exported tropical fruit. It is considered as one of the most preferred fruits of the world due to its excellent nutritional properties, delicious taste, wide flavors and attractive and vibrant color (Anonymous, 2011). Both ripe and unripe mango are utilized for products like puree, nectar, leather, chutneys, pickles, juice, squash and canned slices, etc. worldwide. In India, it is cultivated in more than 2300 ha with a total produce of 15.03 million tons, contributing to 40.48% of the total world production of mango. Apart from seedling varieties, there are more than thousand horticultural races of mango have been grown in seven genetic diversity centers of India and most of them are vegetatively propagated (Praksh & Dinesh, 2007). However, on commercial-scale there are only about 30 varieties grown in different states of India (ICAR, 2012).

Mango fruits are mainly used for nutritional purposes in the form of different food and culinary preparations and also for therapeutic purposes in folklore and traditional medicines (API, 2007) (Table 1). Mango fruit is rich of source of anti-oxidant, nutritional and therapeutic compounds. In addition to a number of phenolics, flavonol-3-*O*-glycosides and xanthone-C-glycosides, mangiferin is predominantly found in stem bark, pulp, peel, and leaves of the mango (Augustyn, Combrinck, & Botha, 2011; Schieber, Berardini, & Carle, 2003; Selles et al., 2002). Mangiferin is a plant natural polyphenol of C-glycosylxanthone structure and has various pharmacological activities. It exhibits a number of pharmacological/biological activities such as, analgesic, antidiabetic, antisclerotic, antimicrobial and antiviral, cardio-, hepato-, and neuroprotective, antiinflammatory, antiallergic, monoamine oxidase (MAO) inhibiting and memory improving, as well as radioprotective against X-ray, gamma, and UV radiation (Adam, Piotr, Edyta, & Dorota, 2013). Another phytochemical, lupeol, a well-known triterpene, is also found in several medicinal plants and fruits, including mango (Saleem, 2009). It also acts as an anti-inflammatory,

Abbreviations: UDDD, uni-dimensional double development; HPTLC, high performance thin layer chromatography; TSSC, total soluble solid content; *Bgreen*, Bombay green; LOD, limit of detection; LOQ, limit of quantitation.

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Table 1
Phytochemical flux of different parts of Mango tree and their uses.

Plant part	Phytochemical reported so far	Biological activities	Food/culinary and other use	Folklore/traditional medicinal use
Leaves	Humulene, elemene, ocimene, linalool, nerol, homomangifirin	Anti-diabetic, α-amylase inhibitory	–	• Tender leaves chewed to avoid bleeding in the gums • Infusion prepared from fresh leaves used as anti-diabetic action • Mango leaves reduce vomiting sensation
Flower	Alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propyl gallate, n-pentyl gallate, n-octyl gallate, 4-phenyl gallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid; humulene, elemene, ocimene, linalool, nerol	Astringents in cases of diarrhea, chronic dysentery and chronic urethritis	Flowers and tender buds boiled in water and used as mouth-wash	• Juice of fresh flowers taken with curds diarrhea treatment
Stem bark	Indicoside A and B, manghopanal, mangoleanone, friedelin, cycloartan-3β-30-diol and derivatives, mangsterol, manglupenone, mangocoumarin, n-tetacosane, n-heneicosane, n-triacontane and mangiferolic acid methyl ester, Mangostin, 29-hydroxy mangiferonic acid and mangiferin, protocatechic acid, catechin, mangiferin, alanine, glycine, γ-aminobutyric acid, kinic acid, shikimic acid and the tetracyclic triterpenoids cycloart-24-en-3β,26diol, 3-ketodammar-24 (*E*)-en-20S,26-diol, C-24 epimers of cycloart-25 en 3β,24,27-triol and cycloartan-3β,24,27-triol	Antibiotic activity anthelminthic, antiallergic, cytotoxic [breast cancer cell lines MCF 7, MDA-MB-435 and MDA-N; colon cancer cell line (SW-620) and a renal cancer cell line (786-0)]; anti-inflammatory, analgesic and hypoglycaemic	*Ayuvedic formulations:* Nyagrodhadi churna, Nyagrodhadi Kwath churna, Candanasava, Grahanimihir taila, Mutra sangrhaniya Kasaya churna	• Juice of the fresh mango bark is in the management of menorrhagia, leucorrhoea, mucus and pus discharges from the uterus and bleeding or haemorrhages from uterus
Root	Chromones, 3-hydroxy-2-(4′-methylbenzoyl)-chromone and 3-methoxy-2-(4′-methyl benzoyl)-chromone	Anti-oxidant, anti-inflammatory, analgesic anti-diabetic, Anthelminthic, antiallergic, cytotoxic, Antispasmodic and Immunomodulatory	–	
Fruit/seed kernel and fruit pulp/peel	Vitamins A and C, β-carotene and xanthophylls, unusual fatty acid, cis-9, cis-15-octadecadienoic acid, 5-Alkyl- and 5-alkenylresorcinols	Antibiotics activity, immunomodulatory,	• Kosambir • Pickles, chutney, gojju • Mango paank, mango lassi, clams gravy, pickle, sasam, amras, ghashi/rosu/udid methi, burfi, rasam • Mango lassi • Aamchur • Navras Pak • Triphala mashi • *Ayuvedic formulations:* Pusaynuga Churna¸ Brahat Gangadhara churana • Ashokarishta	• Raw sour mango with salt and honey, to overcome loose motion, constipation and indigestion • Raw mango pieces with black pepper and honey to avoid jaundice • Mango seeds mixed with buttermilk taken twice a day helps people with piles and such disorders • Dried and powdered seed with honey is used as medicine for diarrhea • Seed paste is used for leucorrhoea, vaginitis treatment

(Source: Anonymous, 2011; APEDA, 2012; Adam, Piotr, Edyta, & Dorota, 2013; APl, 2007; Augustyn, Combrinck, & Botha, 2011).

anti-microbial, anti-protozoal, anti-proliferative, anti-invasive, anti-angiogenic, anti-arthritis, anti-diabetic and cholesterol lowering agent (Siddique & Saleem, 2011).

The huge diversity in chemical nature of secondary metabolites pose a challenge to separate, detect and analyze their content rapidly with acceptable sensitivity and reproducibility (Glassbrook, Beecher, & Ryals, 2000). Multidimensional techniques provide a tool to separate very complex mixtures (e.g., plant extracts). It can easily be applied in a gas chromatography technique. But in liquid chromatography (LC), it is a difficult task to switch over from one mobile phase to another due to challenge of miscibility and high back pressure (Petruczynik et al., 2008).

Electrospray ionization mass spectrometry (ESI-MS) (Barreto et al., 2008; Selles et al., 2002) and high performance thin layer

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