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Isolation of phenolic esters from the seed kernel of *Mangifera Indica* and their biological studies

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ABSTRACT

*From the air dried seed kernels of Mangifera indica Linn., three known secondary metabolites: Methyl gallate (**1**), Gallic acid (**2**) and 1,2,3,4,6- Penta-O-galloyl-β-D-glucose (**3**) were isolated from active fraction of folklore medicinal plant. Their structures were elucidated on the basis of extensive spectroscopic analysis and comparison with known compounds. The methanolic extract, its fractions and isolated compounds were examined for in-vitro elastase inhibition activity and found that methanolic extract and ethyl acetate fraction showed an excellent activity and also two of the compounds showed good synergistic activity.*

Keywords: *Mangifera indica*, phenolic compounds, elastase inhibition

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INTRODUCTION

The tropical plant *Mangifera indica* Linn., which belongs to Anacardaceae family. The plant is widely distributed in Asia, in the Americas and in tropical Africa. From ancient times mango tree and its fruit – both green and ripe - are intricately linked to Indian culture. The leaves and fruits are part of Indian festivals and religious ceremonies. Besides other parts of the plant, including the twigs, bark, and resinous gum from the bark, dried flowers, seed kernel, seed fat and sap is described in the botanical and traditional literature of various regions of this plant [1].

Generally, Mango skin is discarded by humans while eating the fruit and sometimes provided to animals as feed. It is also reported that compounds like quercetin, mangiferin and norathyriol present in mango skin help fight some metabolic diseases such as diabetes and some forms of cancer in view of their modulation of protein proteosome alpha receptors [2]. The seed kernel is generally put to veterinary usage. Also, a fat, high in stearic acid content is known to be isolated from the seed kernel, and a butter from the kernel isolate is commercially available to be used to soothe and nourish the skin. The kernel is astringent, anthelmintic, stimulant, anti-inflammatory, antibacterial, antifungal, antispasmodic, antiscorbutic and is given in diarrhoea, asthma, diabetes, ulcers and nasal bleeding [1]. The ash of leaves is used for its wound healing properties in burns, scalds and diabetes. The flowers are acrid, astringent, refrigerant, cooling, haemostatic, vulnerary, constipating and are useful in haemorrhages, wounds, ulcers, leucorrhoea and anaemia. Seed kernel is sweet refrigerant, anthelmeintic etc [3]. The mango kernel is used as feed for cattle and poultry, as anthelmintic and also as astringent in bleeding piles [1]. Fumes from the burning leaves are inhaled for relief from hiccups and affections from throat. The bark of the tree is used for treat uterine haemorrhage and seed are used in asthma treatment [4].

The mango peel was reported to be good source of dietary fiber containing large amounts of total extractable polyphenolics, which showed high antioxidant property by in-vitro methods [5]. The composition obtained from stem bark of *Mangifera indica* contains polyphenols, terpenoids, steroids, fatty acids and microelements, which have anti-oxidation, anti-inflammatory, analgesic and anti-spasmodic properties thereby conferring to high value dietary supplements to improve the quality of life of patients suffering from degenerative diseases, anti-aging treatments [6]. The mangiferin was obtained from the leaf extract, which had shown cardiotonic and diuretic activity [7]. The compound, mangiferin has also been indicated as possessing anti-ultraviolet, anti-collagenase and anti-elastase activity in addition to its anti-free radical and anti-tyrosinase activity [8]. An alcoholic extract of the seed kernel of *M. indica* exhibited significant anti-inflammatory activity in acute, sub acute not chronic cases of inflammation [9].

Based on our continuous interest on the isolation of bioactive secondary metabolites from plants for personal care applications [10-16], we under took a chemical examination of the seed kernels of *Mangifera indica*. We report here the isolation and structure elucidation of three known compounds **1**-**3**. Structure of the compounds was deduced by NMR spectral data and comparison with those reported in the literature. The present paper also describes the elastase inhibition activity of the crude extract, fractions, isolated compounds and their synergy studies.

MATERIALS AND METHODS

General procedures:
Melting points were reported are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded CD_3OD on Bruker spectrometer, operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C NMR. ESIMS was recorded on Jeol SX 102/DA 600 mass spectrometer. IR spectra were recorded on a Shimadzu IR Prestige 21. UV spectra were recorded on Shimadzu UV spectrophotometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh). Purity of the samples was checked by TLC on pre-coated aluminum sheets, silica gel 60 F_{254} (20 X 20 cm, 0.2mm thickness, Merck) and compounds were detected under UV light (254 & 366 nm) and spraying with 5% sulphuric acid in methanol followed by heating the plates at 110^{o}C for 5 min. The chemical shift values are reported in ppm (□) units and the coupling constants (*J*) are in Hz.

Plant material
The seed kernels of *Mangifera indica* (2.5kg) were collected from local market in Chennai, Tamil Nadu (India) in July 2008. A voucher specimen of this plant was deposited in Cavinkare Research Centre, Chennai, India

Extraction and Isolation of compounds
The seed kernel powder of *Mangifera indica* (400 g) were exhaustively extracted with methanol (3.0 L) by using soxhlet apparatus. The solvent was removed by rotary evaporator under reduced pressure at ~ 40^{o}C and 45 g crude extract was obtained. The crude methanolic extract (44g) was suspended in methanol : water (1:4) followed by fractionation with chloroform, ethyl acetate and saturated n-butanol to get corresponding fractions 2.2g, 23.0g, 3.0g respectively. The ethyl acetate fraction showed good elastase inhibition activity (IC_{50}=2.97µg /ml) than other fractions.

The resulting light brown residue of ethyl acetate fraction (5.0 g) was subjected to column chromatography on a silica gel (100-200 mesh, 200g) and the column was eluted with chloroform, chloroform: ethyl acetate (3:1, 1:1, 1:3) ethyl acetate and ethyl acetate : methanol (9:1). Combined homogeneous fractions based on the visualization of spots on TLC plate and divided into 4 major fractions (A to D). Fraction A (0.95 g) was subjected to silica gel column and eluted with hexane: chloroform (100:0 - 90:10) to give three fractions A1-A3. Fraction A1 (0.27g) was further purified over small silica gel column eluting with hexane: chloroform (95:5) to obtain methyl gallate (**1**, 160 mg) [17]. Fraction B (0.52 g) was subjected to silica gel chromatography eluted with chloroform: ethyl acetate (90:10-50:50) to obtain gallic acid (**2**, 90 mg) [18]. Fraction C found to be solid in nature, which contained lot of brown colored pigment along with compound. This fraction (2.4 g) was adsorbed on silica gel, eluted with chloroform: ethyl acetate (80:20 – 10:90) to get three sub-fractions (C1-C3). The sub-fraction C2 (1.30g) showed solid nature and was crystallized with ethyl acetate: methanol to get penta-O-galloyl-β-D-glucose (**3**,1.02g) [19]. Fraction D did not give any useful compound.

RESULTS AND DISCUSSION

Compound 1 (Methyl gallate) : Colorless crystals obtained chloroform: hexane, mp: 200-202^{o}C, UV ($CHCl_3$) $\square_{max}$: 214; IR (KBr) $\square_{max}$: 1622, 1595, 1114, 1070 and 833 cm^{-1}, ESIMS m/z: 183 $[M-H]^-$ (calc. for $C_8H_80_5$). ^{1}H NMR (CD_3OD, 400 MHz): $\square\square$3.80 (3H, s), 7.03 (2H, s). ^{13}C NMR (CD_3OD, 100 MHz): $\square$ 50.7 (C-8), 108.5 (C-2 & 6), 119.9 (C-1), 138.2 (C-4), 145.0 (C-3 & 5), 167.5 (C-7).

Compound 2 (Gallic acid): Amorphous powder, mp: 248-50^{o}C, UV (MeOH) $\square_{max}$: 272; IR (KBr)$\square_{max}$: 3400 (hydroxyl), 1720 (acid carbonyl), 1618 (aromatic), 10 cm^{-1}; ESIMS m/z 169 $[M-H]^-$; ^{1}H NMR (CD_3OD, 400 MHz): $\square\square$7.04 (2H,s, H-2 & 6). ^{13}C NMR (CD_3OD, 100 MHz): $\square$ 108.8(C-2 & 6), 120.5 (C-1), 138.1(C-4), 144.9(C-3 & 5), 168.9 (C-7).

Compound 3 (1,2,3,4,6- Penta-O-galloyl-β-D-glucose): Buff colored amorphous powder, mp: 248^{o}C, UV (CD_3OD)$\square_{max}$: 268 nm; IR (KBr) $\square_{max}$: 3380 (hydroxyl), 1687 (ester carbonyl), 1612 (aromatic), 1344, 1024 and 765 cm^{-1}, ESIMS m/z 939 $[M-H]^-$. ^{1}H NMR (CD_3OD, 400 MHz) : $\square\square$ 4.34-4.44 (2H, m, 6-H), 4.50 (1H, d, *J* = 10.8 Hz, 5-H), 5.58 (1H, dd, *J* = 8.4, 9.6 Hz, 2-H), 5.60 (1H, d, *J* = 8.7, 9.5 Hz, 4-H), 6.23 (1H, d, *J* = 8.4 Hz, 1-H), 5.89 (1H, t, J=8.3 Hz, 3-H), 6.23 (1H, d, J=8.3Hz, H-1), 6.89 (2H, s), 6.94 (2H, s), 6.98 (2H, s), 7.04 (2H, s), 7.10 (2H, s). ^{13}C NMR (CD_3OD, 100 MHz): $\square$ 63.1, 69.8, 72.2, 74.1, 74.4, 93.8, 110.4 X 8, 110.6 X 2, 119.7, 120.0 X 2, 120.1, 121.0, 140.1, 140.1, 140.2, 140.3, 140.4, 140.7, 146.2 X 2, 146.3 X 2, 146.4 X 2, 146.5 X 4, 166.2, 166.9, 167.0, 167.3, 167.9.

Elastase inhibition activity: The elastase inhibition activity of crude extract, different fractions, compounds and its combinations and ursolic acid (control) cell free system. The assay method is most reliable and reported in the literature [20]**.** Fresh solution of 300 µl (0.6 mg) of succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide (the enzyme substrate), 1200 µl of buffer and varying amounts of the elastase inhibitor under testing are incubated at 37^{o}C for 20 minutes. The hydrolysis is measured by the spectrophotometric measurement of the release of p-nitroaniline at a wavelength of 410 nm. In this method, the methanolic extract, EA fraction and isolated compounds were tested and the results documented in the table.1

Table1: Elastase inhibition data

Extract/Fraction/Compound	Inhibition (µg/ml)
Ursolic acid	IC_{50}= 13.1
Methanolic extract	IC_{50}=2.48
Ethyl acetate fraction	IC_{50}=2.97
Compound **1**	11.15% at 43.5
Compound **3**	56.6% at 70

Synergy studies

The individual compounds, **1** and **3** were not shown good activity when compared with crude extract or ethyl acetate fraction but combinations of isolated compounds **1** and 3 at different concentrations showed good synergy. The results were tabulated in Table 2.

Table 2: Elastase inhibition-synergy studies of 1 and 3 at different concentrations.

Sl. No.	Ratio of Compounds (1:3)	Inhibition (%)
1	0:100	49
2	20:80	64.5
3	40:60	58.8
4	60:40	43.9
5	80:20	31.7
6	100:0	3.05

CONCLUSION

The mango seed kernel showed an excellent elastase inhibition when compared to control, ursolic acid. The ethyl acetate fraction of methanolic extract also retained the activity. The silica gel chromatography of ethyl acetate active fraction yielded only three compounds, methyl gallate (**1**), gallic acid (**2**) and penta-O-galloyl-β-D-glucose (**3**). Two of the isolated compounds (**1** and **3**) have shown moderate activity. Further, the isolated compounds

showed good synergy with different combinations. So, the mango seed kernel extract or its isolated compounds can be used for skin care applications either singly or in combinations with other materials.

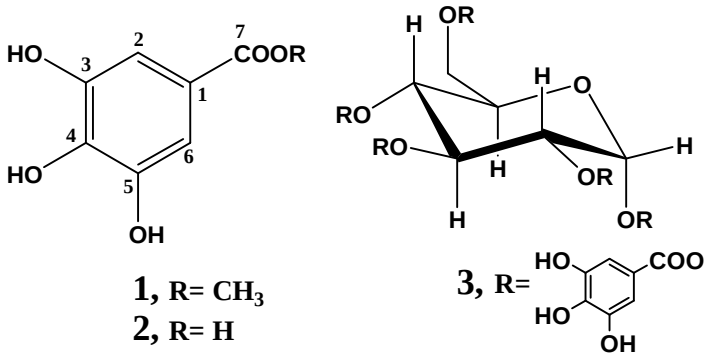

Fig: Compounds from *Mangifera indica* Linn.

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