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GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN ETHANOLIC EXTRACT OF *PUNICA GRANATUM* PEEL AND *VITIS VINIFERA* SEEDS

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ABSTRACT

Plants have been an important source of medicine with high antioxidant activity for thousands of years. *Vitis vinifera* and *Punica granatum* are medicinally important plants belonging to the family *Vitaceae* and *Lythraceae* respectively. The peels of *Punica granatum* is a powerful astringent and cure for diarrhea, and also for stomatitis it can be drunk, used as a mouthwash, douche or enema. The *Vitis vinifera* seeds possess antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardio protective, hepatoprotective, and neuroprotective effects. In the present study the ethanolic extract of *Punica granatum* peel, *V.vinifera* seeds were subjected to GC-MS analysis. Twenty six and sixteen compounds were identified in *Punica granatum peel* and *Vitis vinifera* seeds. The major chemical constituents in *Punica granatum peel* were Glycerin, Hydroxymethylfurfurole, Guanosine and Pyrogallol. Constituents mainly present in *Vitis vinifera* seed were Linoleic acid, Palmitic acid, gamma-Sitosterol and Glycerin.

KEYWORDS

Punica granatum, Vitis vinifera, Pyrogallol, GC-MS analysis.

INTRODUCTION

Punica granatum L. commonly called as Pomegranate is one of the important and oldest edible fruit of tropical and subtropical regions, which originated in the Middle East and India and has been used for centuries in ancient cultures for its medicinal purposes. It is widely reported that pomegranate exhibits antivirus, antioxidant, anticancer, antiproliferative activities [1-3]. The pomegranate is a symbol of life, longevity, health, femininity, fecundity, knowledge, morality, immortality and spirituality; if not divinity [4]. The plant is cultivated in Iran, California, Turkey, Egypt, Italy, India, Chile and Spain. The world pomegranate production amounts to approximately 1,500,000 tons [5], where the peels amounts to approximately 60% of the pomegranate fruit weight [6]. For centuries, the barks, leaves, flowers, fruits, and seeds of this plant have been used to ameliorate diseases [7].The fruit has been widely used by traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases [8-10]. In Cuban traditional medicine the fruits of *P. granatum* have been used to treat acidosis, dysentery, microbial infections, diarrhea, helminthiasis, haemorrhage, and respiratory pathologies [11]. Pomegranate peels are exploited in traditional medicine because of their strong astringency, making them a popular remedy throughout the world. In the form of an aqueous decoction (i.e., boiling the hulls in water for 10-40 minutes), it was used for dysentery and diarrhea, and also for stomatitis. It can be drunk and used as a mouthwash, douche or enema [12]. The phytochemistry of pomegranate has also been widely studied by some researchers and this fruit is found to be a rich source of polyphenolic compounds [13]. Both flavonoids and tannins are more abundant in the peels[14]. Peels of *Punica granatum* L. contains a wide variety of phytochemical compounds like gallotannins, ellagic acid, gallagic acid, punicalins, punicalagins, as it was previously stated by many researchers [15,16].

Vitis vinifera is a member of the Vitaceae family, native to the Mediterranean region, central Europe and southwest asia and cultivated today in all temperature regions of the world [17]. *Vitis vinifera* is used in conditions like hemorrhages, anemia, leprosy, skin diseases, syphilis, asthma, jaundice, bronchitis, anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties[18-20]. *V.vinifera* seed contains lipid, protein, carbohydrates and 5-8% polyphenols [21]. The grape seed extract (GSE) has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects. The seeds of the grape are used in herbal medicine and as a dietary supplement[22]. GSE is considered as a powerful antioxidant nutritive supplement that prevents premature ageing and diseases[23]. Oil produced from grape seeds is considered a rich source of polyphenolics with strong antioxidant activity [24]. Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals, and that they have antimutagenic and anticarcinogenic. It has a protective effect on oxidant-induced production and deposition of extracellular matrix components [25]. Hence the objective

of the present study is to identify the phytochemical constituents of ethanolic extract of Punica granatum peel and *Vitis vinifera* seeds with the aid of GCMS technique.

EXPERIMENTAL

Collection of plant material

The fresh fruits of *Punica granatum* and *Vitis vinifera* were collected from Koyambedu market, Chennai. The selected fruits were identified and authenticated PRAC/2009/360 and PRAC/2009/361 by Prof.P.Jayaraman, Plant Anatomy Research Center, West Thambaram, Chennai, Tamilnadu, India.

Preparation of powder and extract

The *P.granatum* peels and *V. vinifera* seeds were shade-dried and pulverized to fine powder in a mechanical grinder. The powder (100g) was extracted with 1 litre of ethanol as solvent. The extracts were concentrated under reduced pressure in a rotary evaporator. The ethanolic extracts were used for GC-MS analysis.

Gas Chromatography - Mass Spectrometry (GC – MS) Analysis

The GC- MS analysis of selected samples was performed with Shimadzu GCMS - QP2010. The inert gas helium (99.9995%) was used as carrier gas, at flow rate of 1.5 ml /min, Split ratio 10:1; sample size, 1µL injected using the splitless injection technique; fused capillary silica column HP-5 (30m × 0.25mm × 0.25µm). Temperatures: injector: 260˚C, detector: 300˚C, column: 70˚C, 10˚C min−1, 260˚C (10min). The total GC running time is at 35 min. The MS was taken at 70 eV. The MS scan parameters included a mass range of m/z 40-1000, a scan interval of 0.5 s, a scan speed of 2000 amu s−1, and a detector voltage of 1.0 kV. Identification of compounds was conducted using the database of NIST08, WILEY8 and FAME Libraries. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC-MS chromatogram of the ethanolic extract of *P.granatum peel* (Figure 1) showed 26 peaks indicating the presence of twenty six phytochemical constituents. On comparison of the mass spectra of the constituents with the NIST08, WILEY8 and FAME libraries the twenty six phytoconstituents were characterized and identified (Table 1). The major phytochemical constituent's mass spectra are Glycerin, Hydroxymethylfurfurole, Guanosine and Pyrogallol. The *V. vinifera* seed extract showed sixteen peaks in (Figure 2) GC-MS chromatogram indicating the presence of sixteen phytochemical constituents. On comparison with mass spectra sixteen phytoconstituents were characterized and identified (Table 2). The major phytochemical constituent's mass spectra are Linoleic acid, Palmitic acid, gamma-Sitosterol and Glycerin

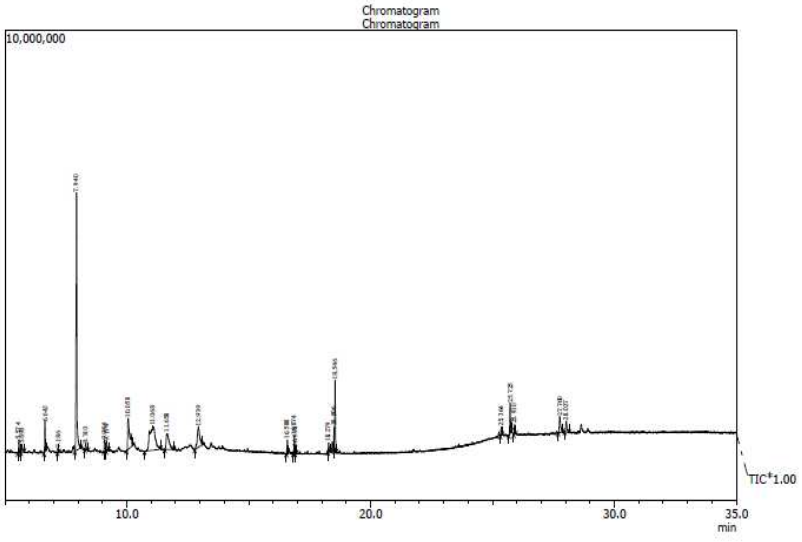

Figure 1
GC-MS Chromatogram of ethanolic extract of the Punica granatum peel

Table 1
Phytochemicals identified in the ethanolic extracts of the Punica granatum peel by GC-MS

Peak	R.Time	Name of the compound	Molecular Formula	Molecular Weight	Peak Area%
1	3.315	2-Hydroxycyclopent-2-en-1-one	$C_5H_6O_2$	98	0.64
2	4.333	Glycerin	$C_3H_8O_3$	92	27.03
3	4.725	Cymene	$C_{10}H_{14}$	134	0.32
4	5.574	2-Hydroxy-3-methyl-4-pyrone	$C_6H_6O_3$	126	0.77
5	5.693	2-Hydroxyacetylfuran	$C_6H_6O_3$	126	0.74
6	6.643	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144	1.76
7	7.186	(+-)-p-Menth-1-en-4-ol	$C_{10}H_{18}O$	154	0.23
8	7.940	Hydroxymethylfurfurole	$C_6H_6O_3$	126	21.18
9	8.310	n-Nitrosoazacyclononane	$C_8H_{16}N_2O$	156	0.53
10	9.084	1-Methylhexyl acetate	$C_9H_{18}O_2$	158	1.32
11	9.174	Cis-Dimethyl morpholine	$C_6H_{13}NO$	115	0.84

12	10.058	Pyrogallol	$C_6H_6O_3$	126	6.45
13	11.068	Guanosine	$C_{10}H_{13}N_5O_5$	283	13.34
14	11.658	D-Allose	$C_6H_{12}O_6$	180	4.64
15	12.939	L-Glucose	$C_6H_{12}O_6$	180	5.17
16	16.588	Palmitic acid	$C_{16}H_{32}O_2$	256	0.89
17	16.874	Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.93
18	16.918	Oxandrolone	$C_{19}H_{30}O_3$	306	0.31
19	18.279	cis-Oleic Acid	$C_{18}H_{34}O_2$	282	0.59
20	18.506	Ethyl Oleate	$C_{20}H_{38}O_2$	310	1.11
21	18.546	(2E,6E)-9-(3,3-Dimethyl-2-oxiranyl)-3,7-dimethyl-2,6-nonadienyl phenyl sulfide	$C_{21}H_{30}OS$	330	4.23
22	25.364	Norolean-12-ene	$C_{29}H_{48}$	396	0.53
23	25.725	Methyl commate A	$C_{32}H_{52}O_4$	500	2.18
24	25.910	alpha.-Tocopherol- beta -D-mannoside	$C_{35}H_{60}O_7$	592	0.75
25	27.760	gamma.-Sitosterol	$C_{29}H_{50}O$	414	2.17
26	28.027	Cycloartenol acetate	$C_{32}H_{52}O_2$	468	1.34

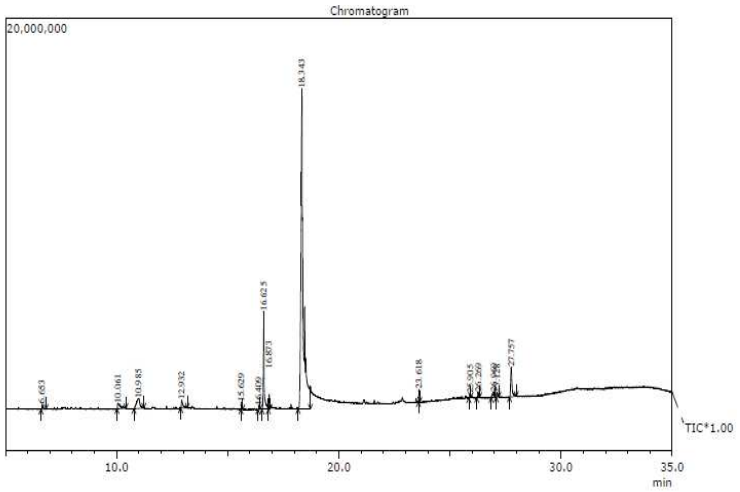

Figure 2
GC-MS Chromatogram of ethanolic extract of the Vitis vinefera seeds

Table 1
Phytochemicals identified in the ethanolic extracts of the Vitis vinefera seeds by GC-MS

Peak	R.Time	Name of the compound	Molecular Formula	Molecular Weight	Peak Area%
1	4.072	Glycerin	$C_3H_8O_3$	92	3.41
2	6.653	2,3- Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144	0.53
3	10.061	Pyrogallol	$C_6H_6O_3$	126	1.69
4	10.985	Guanosine	$C_{10}H_{13}N_5O_5$	283	3.38
5	12.932	4 -n-Propylresorcinol	$C_9H_{12}O_2$	152	1.17
6	15.629	Isobutyl phthalate	$C_{16}H_{22}O_4$	278	0.33
7	16.409	2-Hydroxycyclopentadecanone	$C_{15}H_{28}O_2$	240	0.19
8	16.625	Palmitic acid	$C_{16}H_{32}O_2$	256	9.05
9	16.873	Ethyl palmitate	$C_{18}H_{36}O_2$	284	1.04
10	18.343	Linoleic acid	$C_{18}H_{32}O_2$	280	72.70
12	25.905	alpha.-Tocopherol-.beta.-D-mannoside	$C_{35}H_{60}O_7$	592	0.29
13	26.269	delta.14-Serratene	$C_{30}H_{50}$	410	0.42
14	26.969	Lupenone	$C_{30}H_{48}O$	424	0.81
15	27.128	Stigmasterol	$C_{29}H_{48}O$	412	0.40
16	27.757	gamma-Sitosterol	$C_{29}H_{50}O$	414	3.97

CONCLUSION

In the present study twenty six and sixteen constituents have been identified from ethanolic extract of *Punica granatum* peel and *vitis vinifera* seeds by GC-MS analysis. Pyrogallol was present in both extract. The presence of various bioactive compounds justifies their use for various ailments by traditional practitioners Glycerin and Hydroxymethylfurfurole were present in high amount in *P. granatum peel* where as in *V. vinefera seeds* Linoleic acid and Palmitic acid were present in high amount. However, further studies are under taken to

study the combined effect of both extract on animal model to evaluate their bioactivity.

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