IJBPAS, September, Special Issue, 2021, 10(9): 387-395
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

COMPARATIVE LCMS ANALYSIS OF THE METABOLITE PROFILE OF *CORIANDRUM SATIVUM*, *MENTHA PIPERITA* AND *EUCALYPTUS CITRIODORA*

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Received 25th June 2021; Revised 28th July 2021; Accepted 29th Aug. 2021; Available online 25th Sept. 2021

https://doi.org/10.31032/IJBPAS/2021/10.9.1029

ABSTRACT

The metabolites from the coumarin and quercitin class, present in tissues of plants belonging mainly to the *Myrtaceae*, *Lamiaceae* and *Apiaceae* families, included compounds with high chemical diversity. These health-promoting components are recognized for their valuable biological activities in herbal preparations but also for their phototoxic effects. A comparative LCMS study to analyse the metabolite profile of *Coriandrum sativum*, *Mentha piperita* and *Eucalyptus citriodora* was done. This analytical approach was applied to investigate the metabolite compositions of leaves extracts obtained from *Coriandrum sativum*, *Mentha piperita* and *Eucalyptus citriodora*. The individual compounds were tentatively annotated using database correlations, retention time (Rt), accurate *m/z* data obtained by electrospray ionisation (ESI) (+)-HR-MS, proposed molecular form, theoretical mass, mean concentration in ppm and LCMS fragmentation patterns. Moreover, the identification was based on transforming the exact mass ratio (*m/z*) for the protonated molecular ions $[M + H]^+$ of the observed metabolites, which made it possible to graphically present the ion peaks. Caffeic acid-hexose, Coumaric acid-hexose, Quercetin, Kaempferol, Naringenin, Dicaffeoylquinate

and Tricaffeoylquinicate were reported in *Coriandrum sativum belonging to Apiaceae* family. Salicylicate, Protocatechuic, Ferulicate, Sinapicate, Myricetin, and Astragalin were reported in *Mentha piperita* belonging to *Lamiaceae* family. Vanillic acid Kaempferol, Rutinand Malvidin-3-(caffeoyl)- rutinoside-5-O-glucoside were reported in *Eucalyptus citriodora* belonging to *Myrtaceae* family.

Keywords: *Coriandrum sativum*, *Mentha piperita* and *Eucalyptus citriodora,* retention time, LCMS, metabolites

INTRODUCTION

Several metabolites are ordinarily found in both edible and non-edible plants, and they have been reported to have many biological effects, including antioxidant activity. Crude leave extracts rich in essential metabolites are increasingly of interest in diverse industries because they decelerate oxidative degradation of compounds and therefore improve the quality and commercial value of final products. The importance of the plant metabolites in the maintenance of health and preservation from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers as the tendency of the future is moving toward functional food with specific health effects. Some plant metabolites have been suggested to play a protective role in the improvement of cancer and heart disease **[1]**.

Coriander (*Coriandrum sativum*) is an annual herb plant belonging to the family *Apiaceae* **[2]**. The plant of *C. sativum* is well-known throughout the world as being edible and its fresh leaves and dried seeds (as a spice) being most traditionally used in cooking **[3]**. The female inflorescences rich in polyphenolic components and acylphloroglucides are widely used to in cooking and to give it a characteristic aroma and flavour **[4-6]**. Peppermint (*Mentha piperita*) is a hybrid mint, a cross between water mint and spearmint. The plant of *M. piperita* is well-known throughout the world as sources of menthol and menthone and is among the oldest herbs used for both culinary and medicinal products **[7]**. Mentha have long been used for medicinal targets. In particular, mentha preparations were mainly suggested for the treatment of sleeping disorders, as a mild sedative, and for the activation of gastric function as bitter stomachic. In order with a growing interest in the health benefits of plants used in traditional medicine, *M.piperita* has received considerable interest by the researchers and, as a result, an important number of articles have been published **[8]**. Eucalyptus *(E. Citriodora)* is also called as Lemon-Scented Gum, Lemon Scented

Eucalyptus **[9]**. It belongs to family *Myrtaceae.* The plant of *E. citriodora*is well-known throughout the world as sources of Citronellal, an essential oil found in most Eucalyptus species is reported to be mutagenic when used in isolation **[10]**.

Beginning of the second half of the 20th century, several phytochemical studies were accomplished to investigate the constitution of Citronellal and other parts of the plant, leading to the isolation and identification of pharmacologically relevant compounds. It is known that these naturally occurring molecules show antibacterial, antioxidant, antiinflammatory and anticancer activities. In recent years, researchers have been proving to define the bioactive ingredients in these three plants to illuminate the underlying molecular mechanisms by which they perform their activities was done **[11]**.

Therefore, a comparative LCMS was analysed to understand the metabolite profile of *Coriandrum sativum*, *Mentha piperita* and *Eucalyptus citriodora.*

METHODS

Plant materials: Leaves of *C. sativum*, *M.piperita* and *E. citriodora* were collected from Vadodara region of Gujarat, India. The plant (CS#01/2018, CS#02/2018 and CS#03/2018) was identified by Dr. Vinay Raole, Taxonomist, MS University, Vadodara, India. The dried plant material (100 g, each) were subjected to Soxhlet extraction.

Liquid chromatography-Mass Spectrometry (Applied BioSystem®):

The 75% methanol/water extract enabled separation by C18-reversed-phase LC and detection by both PDA and MS of semipolar metabolites. Software was metAlign. Running time was 50 minutes **[12]**. Flow rate was 1.0 ml/min and Volume was 5µl. Chemicals included Methanol (absolute)-HPLC preparatory grade, Formic acid (98%), Acetonitrile-HPLC preparatory grade, Ultrapure water (MilliQ), Liquid nitrogen or nitrogen gas generator for supplying gas to the mass spectrometer ionization source, Argon (99.999% pure) for supplying gas to the mass spectrometer collision cell, Leucine enkaphaline (95% pure) for online mass correction. Reagents and Solvents included Sample extraction solution-0.133% (v/v) formic acid (FA) in pure methanol, HPLC mobile phase: 0.1% FA (v/v) in ultrapure water (eluent A), and eluent B is 0.1% FA (v/v) in acetonitrile (eluent B), MS calibration solution: 1 mL of a 0.05% (v/v) phosphoric acid solution in 50% acetonitrile/ultrapure water. Load into the gas-tight glass syringe, Lock mass solution like leucine enkaphaline in 50% (v/v) acetonitrile/ ultrapure water to obtain a fi nal concentration of 0.1 mg/mL. The column temperature (6-24°C) was programmed at 3°C/min with final hold

time of 10 min. Identification of constituents were done on the basis of retention time, Retention Index (RI, determined with reference to homologous series of n-alkanes (C9-C24, Polyscience Corp., Niles IL) under identical experimental condition) in both polar and non-polar column, coinjection with standards (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data **[13-15]**.

RESULTS

The metabolite compositions of leaves of *C. sativum* grown in Vadodara region of Gujarat, India were analysed using LCMS. The analysis led to the identification of 14 constituents. The Metabolites identified by LC-MS in leaf extracts of *Coriandrum sativum* is represented in **Table 1, Figure 1**. The metabolites identified in leaves were given in **Table 1** in order of their elution on DB-5 (30 m x 0.32 mm) column. The odor and flavor of leaves of coriander are completely different. Aliphatic compounds are mainly comprised of C8-C16 aldehydes and alcohol predominate in the steam-volatile oil extracted from leaves of *C. sativum* and are responsible for its peculiar, fetid-like aroma. While the major metabolites in the leaves of coriander include Tricaffeoylquinic acid as major constituents. Three compounds were identified in leaf of *C. sativum*. The leaf from *C. sativum* was dominated by aliphatic compounds; while hexoses were the major class of compounds in leaves of *C. sativum*. Caffeic acid-hexose, Coumaric acid-hexose, Quercetin-hexose-deoxyhexose-pentose, Quercetin-Glc-rhamnose, Kaempferol-Glc-rhamnose, Naringenin chalcone-hexose presented as major constitutes. Aliphatic compounds like Naringenin, Naringenin chalcone, Dicaffeoylquinic acid, Quercetin-hexosedeoxyhexose-pentosep-coumaric acid were recorded too.

The metabolite compositions of leaves of *Mentha piperita* grown in Vadodara region of Gujarat, India were analysed using LCMS. The analysis led to the identification of 9 constituents. The Metabolites identified by LC-MS in leaf extracts of *Mentha piperita*is represented in **Table 2, Figure 2**. The metabolites identified in leaves were given in **Table 2** in order of their elution on DB-5 (30 m x 0.32 mm) column. The odor and flavor of leaves of *Mentha piperita* are characteristically different. Aromatic compounds predominate in the steam-volatile oil extracted from leaves of *Mentha piperita* and are responsible for its characteristic aroma. While the major metabolites in the leaves of coriander include Salicyclic acid as major constituents. The leaf from *Mentha piperita* was dominated by aromatic compounds;

while Protocatechuic acid were the major class of compounds in leaves of *Mentha piperita*. Ferulic acid, Sinapic acid, Myricetin, Chlorogenic acid (3-Ocaffeoylquinic acid), Feruloylquinic acid, Astragalin and Quercetin-3-O-trisaccharide were recorded too.

The metabolite compositions of leaves of *Eucalyptus citridora* grown in Vadodara region of Gujarat, India were analysed using LCMS. The analysis led to the identification of 08 constituents. The Metabolites identified by LC-MS in leaf extracts of *Eucalyptus citridora* is represented in **Table 3. Figure 3**. The metabolites identified in leaves were given in **Table 3** in order of their elution on DB-5 (30 m x 0.32 mm) column. The odor and flavor of leaves of *Eucalyptus citridora* are completely different. Aliphatic compounds are mainly comprised of C8-C16 aldehydes and alcohol predominate in the steam-volatile oil extracted from leaves of *Eucalyptus citridora* and are responsible for its peculiar, fetid-like aroma. While the major metabolites in the leaves of *Eucalyptus citridora* include p-Hydroxybenzoic acid as major constituents. Three compounds of 5-O-glucoside were identified in leaf of *Eucalyptus citridora*. These were Delphinidin-3-O-(caffeoyl)-rutinoside-5-O-glucoside, Petunidin-3-(caffeoyl)- rutinoside-5-O-glucoside and Malvidin-3-(caffeoyl)- rutinoside-5-O-glucoside. Kaempferol, 4-O-Caffeoylquinic acid, Vanillic acid and Rutinwere recorded too.

Table 1: Metabolites identified by LC-MS in leaf extracts of *Coriandrum sativum*

Metabolites identified by LC-MS in leaf extracts of *Coriandrum sativum*					
Ret time (min)	*Av m/z*	*Mol form*	*Theo. mass*	*Mean Δ(ppm)*	*Metabolites*
12.72	341.0883	$C_{15}H_{18}O_9$	341.0878	1.49	Caffeic acid-hexose
13.63	325.0929	$C_{15}H_{18}O_8$	325.0929	0.05	Coumaric acid-hexose
18.06	741.1870	$C_{32}H_{38}O_{20}$	741.1884	2.75	Quercetin-hexosedeoxyhexose-pentose
19.88	609.1451	$C_{27}H_{30}O_{16}$	609.1461	2.64	Quercetin-Glc-rhamnose
21.36	593.1505	$C_{27}H_{30}O_{15}$	593.1512	-1.08	Kaempferol-Glc-rhamnose
23.36	433.1135	$C_{21}H_{22}O_{10}$	433.1140	-121	Naringenin chalcone-hexose
24.70	271.0617	$C_{15}H_{12}O_5$	271.0612	1.84	Naringenin
26.16	271.0615	$C_{15}H_{12}O_5$	271.0612	1.15	Naringenin chalcone
29.59	515.1193	$C_{25}H_{24}O_{12}$	515.1195	0.53	Dicaffeoylquiniq acid
32.59	887.2246	$C_{41}H_{44}O_{22}$	887.2251	0.57	Quercetin-hexosedeoxyhexose-pentosep-coumaric acid
33.75	433.1137	$C_{21}H_{22}O_{10}$	433.1140	-0.81	Naringenin chalcone rhamnose
38.28	677.1503	$C_{34}H_{30}O_{15}$	677.1512	-1.28	Tricaffeoylquinic acid I
41.80	677.1493	$C_{34}H_{30}O_{15}$	677.1512	-2.56	Tricaffeoylquinic acid II
43.26	677.1569	$C_{34}H_{30}O_{15}$	677.1512	-2.88	Tricaffeoylquinic acid III

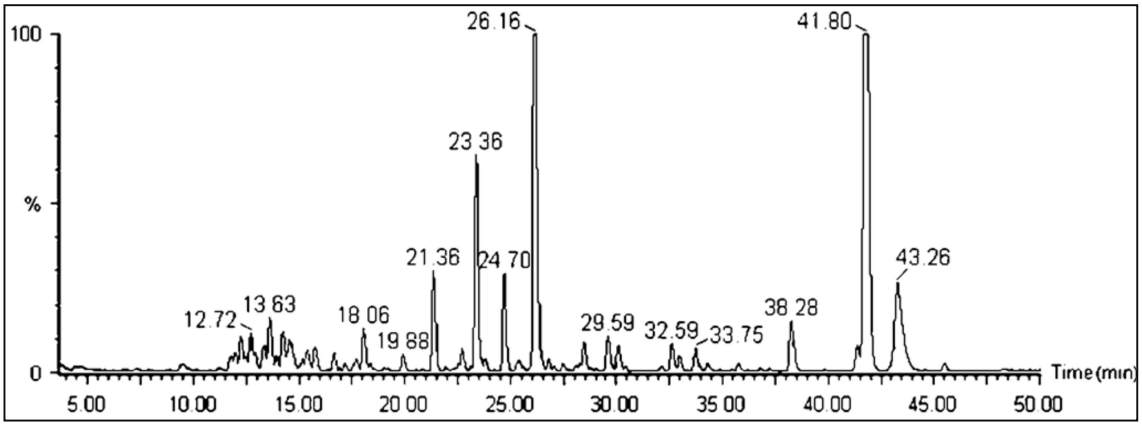

Figure 1: Graphical view of runtime of *Coriandrum sativum* metabolites identified through LCMS system

Table 2: Metabolites identified by LC-MS in leaf extracts of *Mentha piperita*

Metabolites identified by LC-MS in leaf extracts of *Mentha piperita*					
Ret time (min)	*Av m/z*	*Mol form*	*Theo. mass*	*Mean Δ(ppm)*	*Metabolites*
13.68	249.6723	$C_7H_6O_3$	138.0317	1.12	Salicylic acid
15.64	256.8214	$C_7H_6O_4$	154.0266	1.78	Protocatechuic acid
21.40	451.6928	$C_{10}H_{10}O_4$	194.0579	3.67	Ferulic acid
23.41	516.8725	$C_{11}H_{12}O_5$	224.0685	0.18	Sinapic acid
25.48	428.1097	$C_{15}H_{10}O_8$	318.0376	-2.56	Myricetin
33.75	872.4491	$C_{16}H_{18}O_9$	354.0951	1.48	Chlorogenic acid (3-Ocaffeoylquinic acid)
41.45	881.9210	$C_{17}H_{20}O_9$	368.1107	1.51	Feruloylquinic acid
41.85	896.4429	$C_{21}H_{20}O_{11}$	448.1006	0.89	Astragalin
43.27	913.1722	$C_{32}H_{38}O_{20}$	742.1956	0.97	Quercetin-3-O-trisaccharide

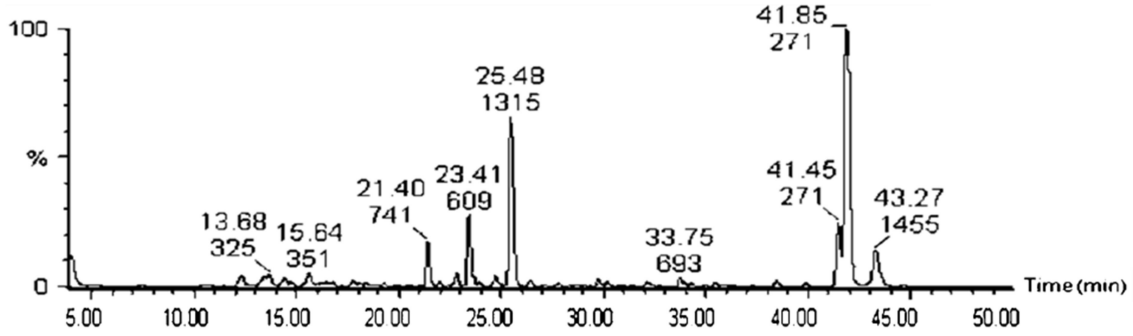

Figure 2: Graphical view of runtime of metabolites identified through LCMS in *Mentha piperita*

Table 3: Metabolites identified by LC-MS in leaf extracts of *Eucalyptus citridora*

Metabolites identified by LC-MS in leaf extracts of *Eucalyptus citriodora*					
Ret time (min)	*Av m/z*	*Mol form*	*Theo. mass*	*Mean Δ(ppm)*	*Metabolites*
9.67	239.8734	$C_7H_6O_3$	138.0317	1.39	p-Hydroxybenzoic acid
13.48	259.9921	$C_8H_8O_4$	168.0423	1.82	Vanillic acid
21.22	397.0372	$C_{15}H_{10}O_6$	286.0477	3.76	Kaempferol
23.23	435.5182	$C_{16}H_{18}O_9$	354.0951	-21.88	4-O-Caffeoylquinic acid
24.55	669.2091	$C_{27}H_{30}O_{16}$	610.1534	0.59	Rutin
29.98	792.8162	$C_{42}H_{47}O_{24}$	935.2452	-0.14	Delphinidin-3-O-(caffeoyl)- rutinoside-5-O-glucoside
38.15	832.0481	$C_{43}H_{49}O_{24}$	949.2608	30.68	Petunidin-3-(caffeoyl)- rutinoside-5-O-glucoside
41.65	871.8285	$C_{44}H_{51}O_{24}$	963.2765	23.81	Malvidin-3-(caffeoyl)- rutinoside-5-O-glucoside

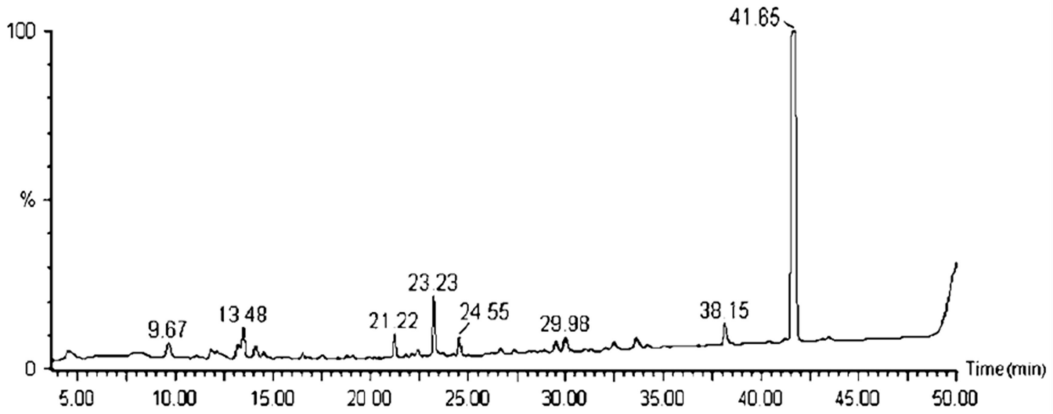

Figure 3: Graphical view of runtime metabolites identified through LCMS in *Eucalyptus citridora*

CONCLUSION

Caffeic acid-hexose, Coumaric acid-hexose, Quercetin, Kaempferol, Naringenin, Dicaffeoylquinate and Tricaffeoylquinicate were reported in *Coriandrum sativum belonging to Apiaceae* family. Salicylicate, Protocatechuic, Ferulicate, Sinapicate, Myricetin, and Astragalin were reported in *Mentha piperita* belonging *to Lamiaceae* family. Vanillic acid Kaempferol, Rutin and Malvidin-3-(caffeoyl)- rutinoside-5-O-glucoside were reported in *Eucalyptus citriodora* belonging to *Myrtaceae* family. This LC–MS analysis unambiguously demonstrated the presence of metabolites in leaves extract of *Coriandrum sativum*, *Mentha piperita* and *Eucalyptus citriodora*. This approach of LC–MS data interpretation can be used to understand complex mass spectra such as those of plant extracts.

Acknowledgments: The authors are thankful to the management of Parul

University for facilities and encouragement.

Conflict of interest: The authors declare that there is no conflict of interest.

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