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IDENTIFICATION OF PHYTOCHEMICALS IN *CONVOLVULUS PLURICAULIS*, *MICHELIA CHAMPACA* AND *CHROMOLAENA ODORATA* BY ORBITRAP HIGH RESOLUTION LIQUID CHROMATOGRAPHY MASS SPECTROSCOPY (O-HRLCMS) AND ITS PHARMACOLOGICAL SIGNIFICANCE

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ABSTRACT

Background: For drug discovery and development of novel therapeutic agents against diseases, understanding phytochemistry in plants is very crucial. **Objective:** The aim of this research was to identify the phytochemical profile of ethanolic extract of *Convolvulus pluricaulis* (CP), *Michelia champaca* (MC), and *Chromolaena odorata* (CO) by using LC-MS technique. **Materials and Methods:** The extract of selected plants by soxhlet extraction technique was prepared by using ethanol as a solvent. The extract was subjected to LC-MS analysis and the identified constituents were further reviewed for their pharmacological potentials. **Results:** LC-MS findings has provided significant contribution in identification of phytoconstituents in herbs belonging to a) phenolic compounds - flavonoids, phenylpropanoids; b) alkaloids - amide, proline and quinoline and c) pentacyclic triterpenes. Phenolic compounds - Rutin, Quercetin-3-β-D-glucoside, Genistein, Biochanin A, Puerarin,

Orientin, Luteolin, Cynaroside, Tangeretin, Nobiletin, Sakuranetin, Hesperetin, 7-hydroxycoumarin, 6-Gingerol and 2-Methoxyresorcinol were identified in plants. Alkaloids – Piperine, DL-Stachydrine and 8-hydroxyquinoline were identified. Pentacyclic triterpenes betulin was identified. **Conclusion:** In this research study, different phytoconstituents of pharmacological significance was identified in all the three plants by LC-MS technique. Hence, these plants are indicative of potential for medicinal use and the further researchers could work with these plants individually or by combination (polyherbal formulation) using related *in-vitro* and *in-vivo* models.

Keywords: Phytoconstituents, LC-MS, *Convolvulus pluricaulis*, *Michelia champaca*, *Chromolaena odorata,* Pharmacology

INTRODUCTION

Phytochemicals from plant extracts would help in the investigation of various groups of compounds and have potential in the identification of new bioactive compounds. Phytochemicals are plant-derived secondary metabolites, which may exert many pharmacological activities including anticancer properties **[1-3]**. Phytochemicals can be found in various parts of plant like stems, leaves, roots, seeds, fruits, and flowers. However, many phytochemicals, notably color compounds, are found in high concentration in the outer layers of plant tissues. Phytochemicals are classified as primary and secondary metabolites, based on their function in plant metabolism. Primary metabolites are necessary for plant life and include carbohydrates, amino acids, proteins, lipids, purines, and pyrimidines of nucleic acids. Secondary metabolites are the remaining plant chemicals produced by the cells through metabolic pathways derived from the primary metabolic pathways which have been described as an antiviral, antifungal, and antibiotic, which are responsible for protecting plants from pathogens. Ethnobotanical and ethnopharmacological surveys has confirmed the medicinal effects of many plants and the biological activity come from these secondary metabolite molecules. Various tissues and organs of medicinal plants could have peculiar medicinal properties at specific development phases and these days, they are associated with valuable industries such as pharmaceutics, cosmetics, and fine chemicals **[4]**.

Based on the biosynthetic pathway, secondary metabolites in plants are classified into three main categories ; (a) nitrogen-containing compounds such as alkaloids, glucosinolates, and cyanogenic glycosides, (b) phenolic compounds such as phenylpropanoids and flavonoids, and

(c) terpenes. Alkaloids are a class of nitrogen-containing compounds produced in plants in response to biotic or abiotic environment which endows alkaloids to possess remarkable biological activities and structure diversity. Cyanogenic glycosides are amino acid-derived plant components found in more than 2500 plant species and are widely distributed among 100 families of flowering plants. The toxicity of cyanogenic glycoside derivatives is based on the release of hydrogen cyanide. Glucosinolates contain sulfur and nitrogen produced in some plants and are chemically stable under normal conditions. The non-protein amino acids are structurally similar to protein amino acids and particularly participate in plant defense against stress and act as essential mediators in response to abiotic factors. Amines as low molecular weight are nitrogenous compounds which are naturally present in plants and are responsible for many biological effects such as acting as important precursors of hormones. Phenolics components are derived from shikimate, pentose phosphate, and phenylpropanoid pathways in plants and have an aromatic ring with one or more hydroxyl groups. Flavonoids are the largest group of polyphenolic compounds that are present in high concentrations in medicinal plants. These compounds majorly contribute to the proclaimed pharmacological properties of the plants and have also been widely reported to possess therapeutic effects as individual compounds. Flavonoids are important class of natural products and are further subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. These subgroups are: flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones. Flavonoids carry out a number of protective functions in the human body and they are evolved as bioactive compounds that interfere with nucleic acid or proteins and show pharmacological properties. Glycosides are usually organic molecules isolated from plant sources and consist of one or more sugars incorporated with phenol, alcohol, or a complex molecule such as a steroid nucleus. Terpenoids are the most abundant group of plant secondary metabolites typically produced in flowers, vegetative tissues, and roots. They show a broad range of biological activities which result in lower cholesterol, triglycerides, or LDL-cholesterol, as well as blood pressure **[4, 5]**.

70-80% of the global population depend on herbal medicines and it has been expanding in numerous countries over the

world. Also, the phytochemical compounds obtained from plants have always been a source for the discovery of new medicinal drugs and because of advances in modern technology, it is now possible to assess the pharmacology and mechanisms related to function of many medicinal herbs.

The selected plants - *Convolvulus pluricaulis*, *Michelia champaca* and *Chromolaena odorata* are well known ethnobotanical herbs which are used in traditional medicine. The phytochemical analysis in these selected plants would provide researchers with the grater validity to the findings. Therefore, the aim of this study was to investigate the phytochemical profile, especially the phenolic compounds found in the whole plant extracts of CP, MC and CO by using Liquid Chromatography Mass Spectrometry (LC-MS). LC-MS is a powerful analytical technique with very high sensitivity and specificity. LC-MS is combination of Liquid Chromatography (LC) and Mass Spectrometry (MS). With the Liquid Chromatography (LC) the separation of components can be done and then the sample eluents from LC are transferred into Mass Spectrometry (MS) where the detection, identification and determination of masses of components can be done in presence of other components **[5]**. The researchers have interpreted the results based on mass elucidation with structural identification of different classes of compounds and the studies provide researchers with a view that the selected plant extracts elucidated good number of flavonoids along with some alkaloids and terpenes.

MATERIALS AND METHODS

The whole plants of CP, MC and CO were collected, dried, authenticated, and extracted by soxhlet extraction using 70:30 ratio of ethanol and water. And the extracts were subjected for LC-MS analysis at IIT Bombay.

The instrument used in the experimentation was as follows:

LC-MS instrument: Thermo Scientific fisher VANQUISH, USA; Q Exactive Plus – Orbitrap MS. Data Acquisition Software: Thermo Scientific Xcalibur, Version 4.2.28.14, Data Processing software: Compound Discoverer 3.2 SP1 Column: SB-C 18 RRHD 100×2.1 mm, 1.8 microns (Agilent Technologies).

Mobile phase: Solvent A: 0.1% formic acid in Milli-Q water, Solvent D:Acetonitrile.

The identification of compounds was primarily based on the matching of MS data against the mzCloud database.

RESULTS

LC-MS studies revealed the presence of different components from the CP, MC and CO extracts as mentioned **[Table 1**

to 3]. The LC-MS chromatogram of CP, MC, and CO extracts is shown in **Figure 1, 2 and 3**.

The phenolic compounds – Flavonoids [Table 1]: The flavonols Rutin and Quercetin-3β-D-glucoside were found in the CP extract having a characteristic peak at m/z 99.8 and m/z 98.2 **(Figure 4)**. The isoflavone genistein was found at m/z 96.0 in CP extract; and the Isoflavone biochanin A was found at m/z 99.99 in CO extract **(Figure 5)**. The Isoflavonoid Puerarin at m/z 70.9 and 88.4 was found in MC and CO extract **(Figure 5)**. The flavones orientin, luteolin, and cynaroside was found at m/z 90.4, 97.0 and 76.7 in CP extract **(Figure 6)**. The flavone tangeretin at m/z 94.5 and 96.8 was found in MC and CO extract. The flavone Nobiletin m/z 90.7, 94.9, 94.5 was found in all the three extracts **(Figure 6)**. The flavanones Sakuranetin, and hesperetin was found in CO extract at m/z 95.2 and 94.6 **(Figure 7)**. **The phenolic compounds – Phenylpropanoids [Table 1]:** The 7-hydroxycoumarin a class of phenolic phenylpropanoid compound was found at m/z 94.1 in CP extract. The phenol phenylpropanoid 6-gingerol at m/z 66.1 and m/z 74.2 was found in both CP and CO extracts. 2-Methoxyrecorcinol a member of phenol and methoxybenzenes was found at m/z 43.1 and 41.0 in both CP and CO extracts **(Figure 8)**. **Alkaloids [Table 2]:** The amide alkaloid piperine was found in the CP extract at m/z 96.5. The Proline alkaloid DL-Stachydrine [m/z 91.7, 94.3, 97.4] were found in all the three extracts. A quinoline alkaloid 8-hydroxyquinoline was found at m/z 99.6 and 99.4 in CP and CO extract **(Figure 9)**. **Terpenes [Table 3]:** The pentacyclic triterpene betulin was found at m/z 86.3 in CP extract and at m/z 59.8 in CO extract **(Figure 10)**.

Table 1 : Phenolic compounds by LC-MS technique found in CP, MC and CO extract

Sr. No.	Phytochemicals	Extract	Formula	Delta Mass (ppm)	Molecular weight	RT (min)	m/z	+Ve or -Ve mode	Figure number
	Phenolic compounds - Flavonoids								
1.	Rutin (flavonol)	CP	$C_{27}H_{30}O_{16}$	-0.83	610.1528	9.646	99.8	+Ve	4
2.	Quercetin-3β-D-glucoside (flavonol)	CP	$C_{21}H_{20}O_{12}$	-2.11	464.0945	9.819	98.2	-Ve	4
3.	Genistein (isoflavone)	CP	$C_{15}H_{10}O_5$	-2.43	270.0521	12.515	96.0	-Ve	5
4.	Biochanin A (isoflavone)	CO	$C_{16}H_{12}O_5$	-1.74	284.0679	14.634	99.9	+Ve	5
5.	Puerarin (isoflavone)	MC	$C_{21}H_{20}O_9$	-1.02	416.1103	15.468	70.9	+Ve	5
6.	Puerarin (isoflavone)	CO	$C_{21}H_{20}O_9$	-1.39	416.1101	15.47	88.4	+Ve	5
7.	Orientin (flavone)	CP	$C_{21}H_{20}O_{11}$	-0.79	448.1002	9.403	90.4	+Ve	6
8.	Luteolin (flavone)	CP	$C_{15}H_{10}O_6$	-2.65	286.0469	11.719	97.0	-Ve	6
9.	Cynaroside (flavone)	CP	$C_{21}H_{20}O_{11}$	-2.3	448.0995	10.33	76.7	-Ve	6
10.	Tangeretin (flavone)	MC	$C_{20}H_{20}O_7$	-0.75	372.1206	14.072	94.5	+Ve	6
11.	Tangeretin (flavone)	CO	$C_{20}H_{20}O_7$	-1.25	372.1204	14.078	96.8	+Ve	6
12.	Nobiletin (flavone)	CP	$C_{21}H_{22}O_8$	-1.24	402.1309	14.682	90.7	+Ve	6
13.	Nobiletin (flavone)	MC	$C_{21}H_{22}O_8$	-0.94	402.1310	14.711	94.9	+Ve	6
14.	Nobiletin (flavone)	CO	$C_{21}H_{22}O_8$	-1.85	402.1307	14.698	94.5	+Ve	6
15.	Sakuranetin (flavanone)	CO	$C_{16}H_{14}O_5$	-1.81	286.0836	14.528	95.2	+Ve	7
16.	Hesperetin (flavanone)	CO	$C_{16}H_{14}O_6$	-0.84	302.0787	12.673	94.6	+Ve	7
	Phenolic compounds - Phenylpropanoids								
17.	7-Hydroxycoumarin	CP	$C_9H_6O_3$	-0.19	162.0316	9.032	94.1	+Ve	8
18.	6-Gingerol	CP	$C_{17}H_{26}O_4$	-0.98	294.1828	14.602	66.1	+Ve	8
19.	6-Gingerol	CO	$C_{17}H_{26}O_4$	-0.37	294.183	17.262	74.2	+Ve	8
20.	2-Methoxyresorcinol	CP	$C_7H_8O_3$	-1.16	140.0471	4.326	43.1	+Ve	8
21.	2-Methoxyresorcinol	CO	$C_7H_8O_3$	-0.39	140.0472	4.326	41.0	+Ve	8

Table 2: Alkaloids found in CP, MC and CO extracts

Sr. No.	Phytochemicals	Extract	Formula	Delta Mass (ppm)	Molecular weight	RT (min)	m/z	+Ve or -Ve mode	Figure number
	Amide alkaloid								
1	Piperine	CP	$C_{17}H_{19}NO_3$	-1.29	285.13613	15.28	96.5	+Ve	9
	Proline alkaloid								
2	DL-Stachydrine	CP	$C_7H_{13}NO_2$	-0.26	143.09459	0.94	91.7	+Ve	9
3	DL-Stachydrine	MC	$C_7H_{13}NO_2$	-0.48	143.09456	1.151	94.3	+Ve	9
4	DL-Stachydrine	CO	$C_7H_{13}NO_2$	0.16	143.09465	1.231	97.4	+Ve	9
	Quinoline alkaloid								
5	8-hydroxyquinoline	CP	C_9H_7NO	-1.24	145.05258	4.604	99.6	+Ve	9
6	8-hydroxyquinoline	CO	C_9H_7NO	-0.3	145.05272	4.62	99.4	+Ve	9

Table 3: Terpene found in CP and CO extracts

Sr. No.	Phytochemicals	Extract	Formula	Delta Mass (ppm)	Molecular weight	RT (min)	m/z	+Ve or -Ve mode	Figure number
	Pentacyclic triterpenes								
1	Betulin	CP	$C_{30}H_{50}O_2$	-0.91	442.38068	22.128	86.3	+Ve	10
2	Betulin	CO	$C_{30}H_{50}O_2$	-1.29	442.38051	23.556	59.8	+Ve	10

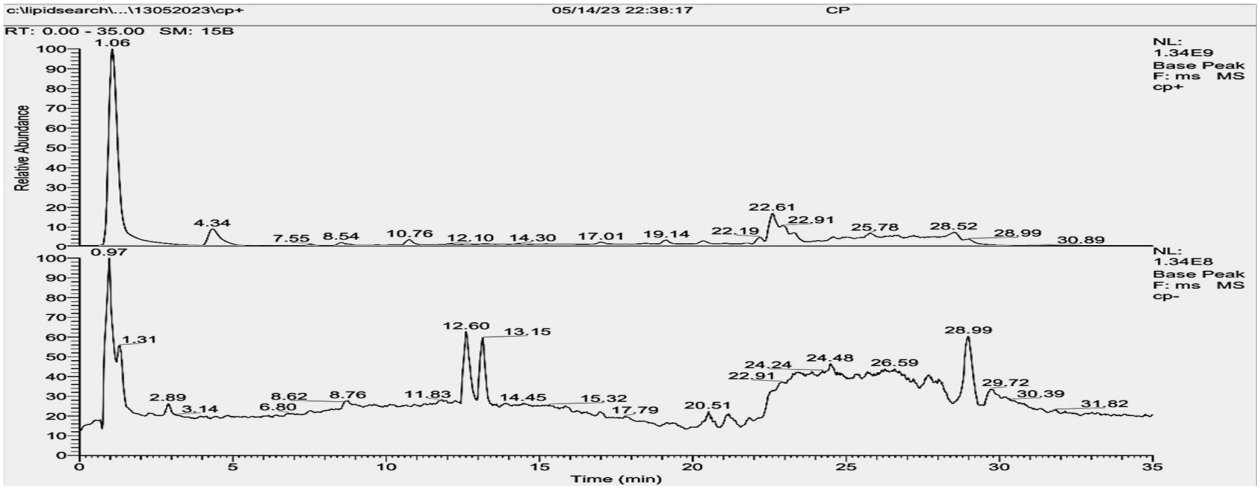

Figure 1 : LC-MS Chromatogram of ethanolic extract of *Convolvulus pluricaulis*

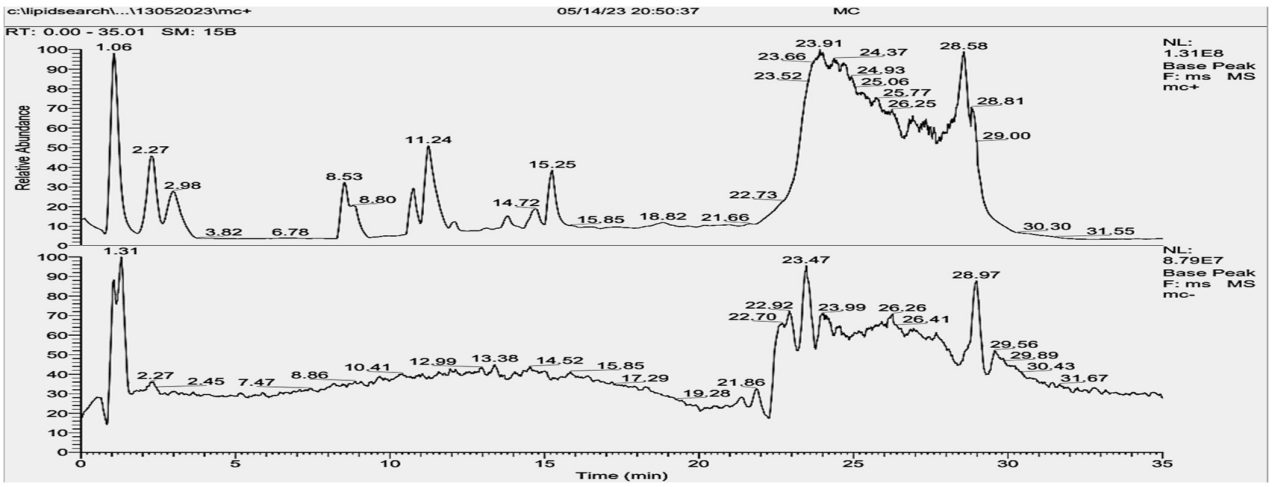

Figure 2 : LC-MS Chromatogram of ethanolic extract of *Michelia champaca*

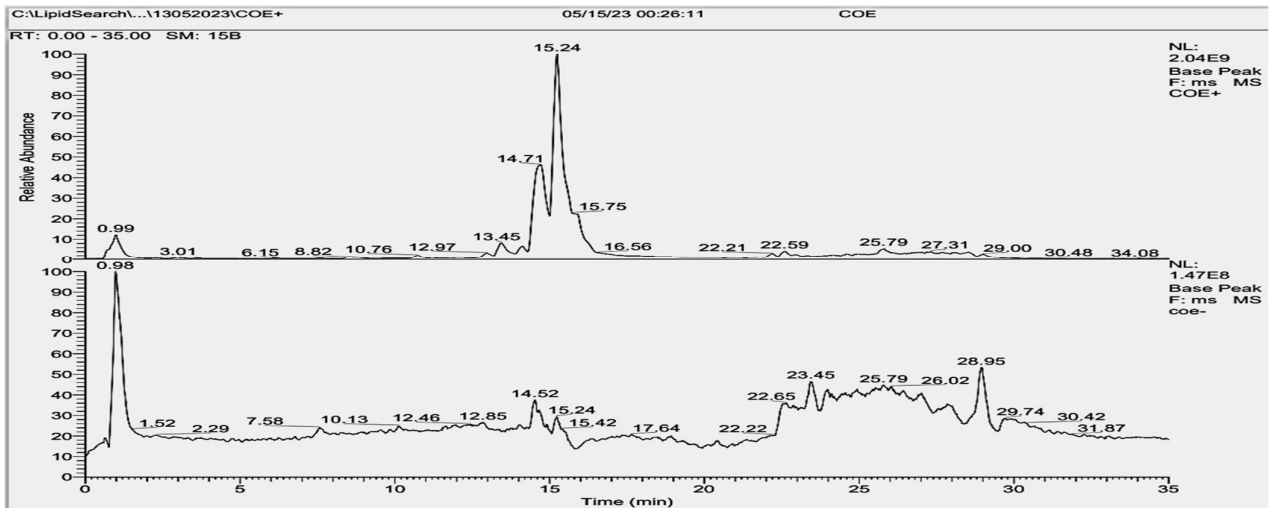

Figure 3 : LC-MS Chromatogram of ethanolic extract of *Chromolaena odorata*

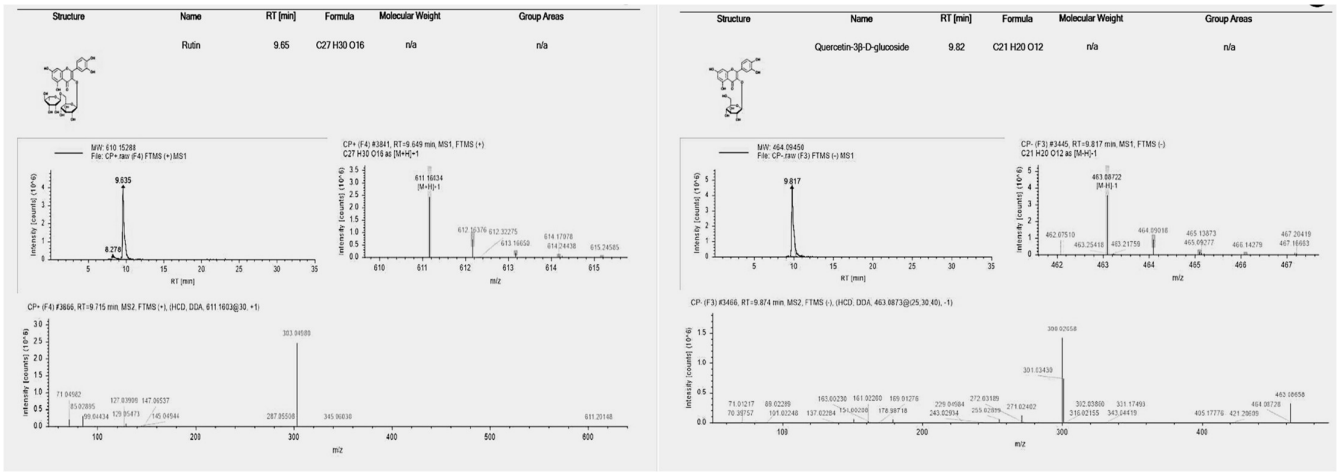

Figure 4 : Mass spectrum of Flavonols - Rutin and Quercetin-3β-D glucoside in CP extract

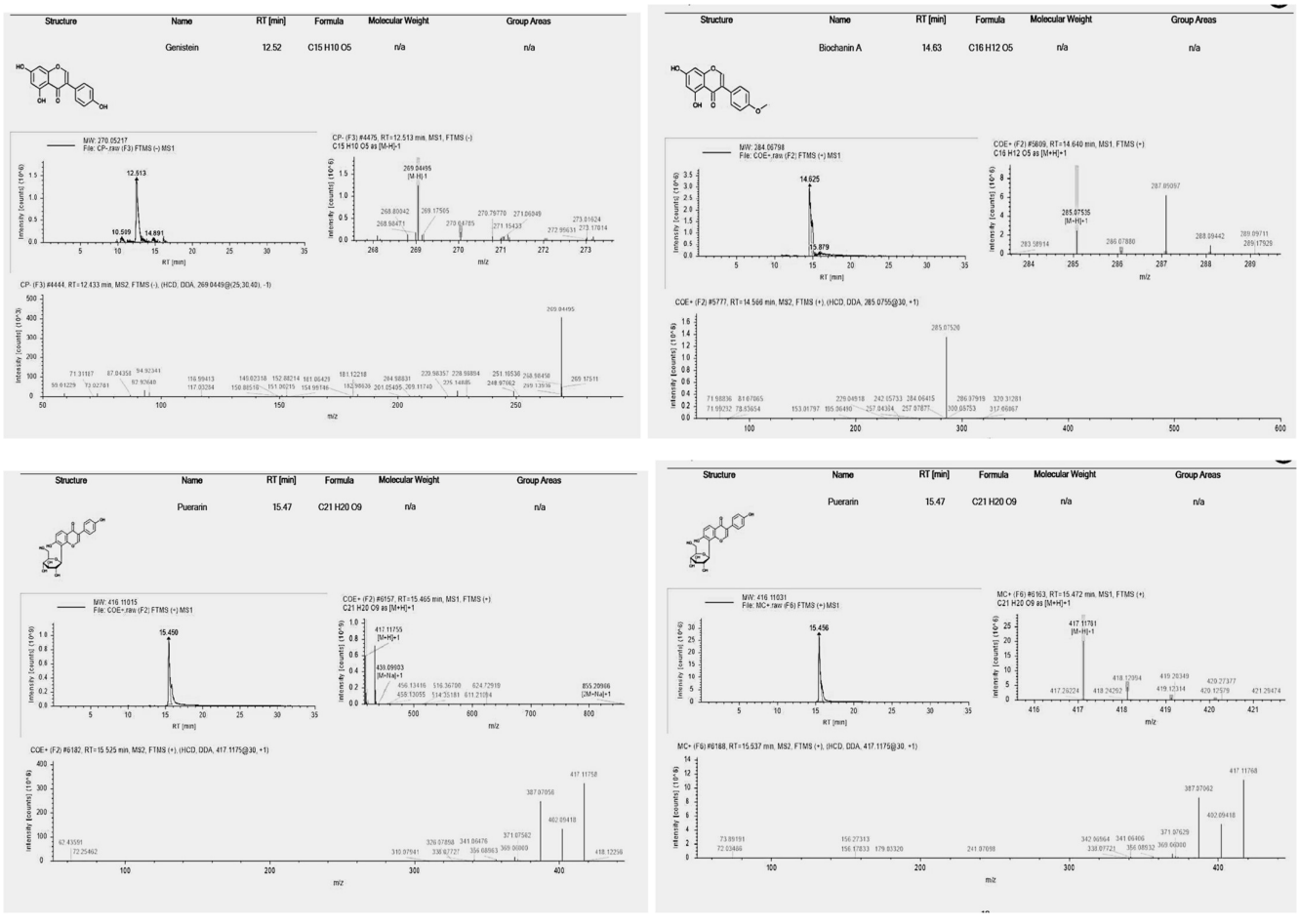

Figure 5 : Mass spectrum of Isoflavones - Genistein (CP), Biochanin A (CO), and Puerarin (MC &CO)

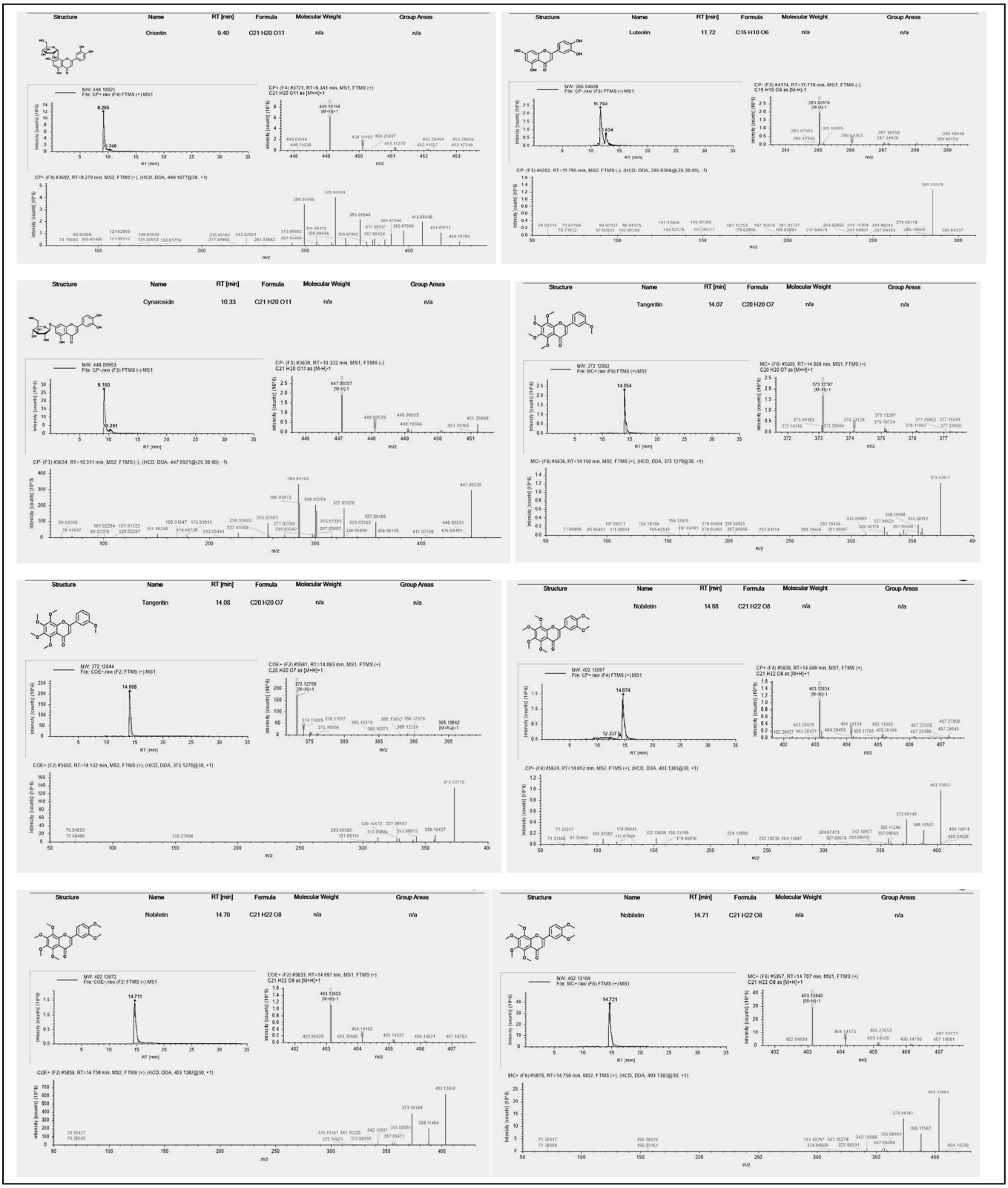

Figure 6 : Mass spectrum of flavones - Orientin (CP), Luteolin (CP), Cynaroside (CP), Tangeretin (MC&CO), Nobiletin (CP, MC & CO)

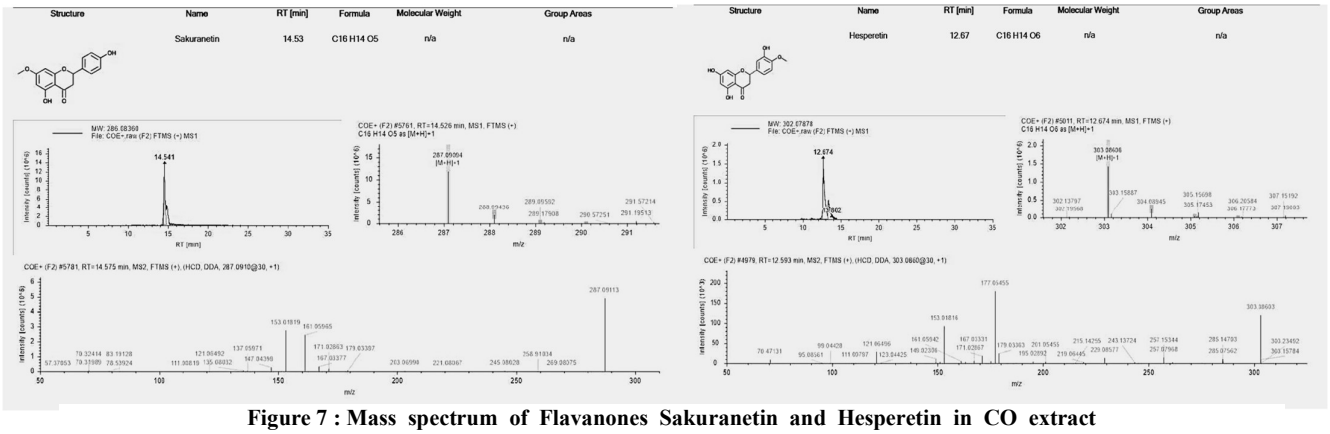

Figure 7 : Mass spectrum of Flavanones Sakuranetin and Hesperetin in CO extract

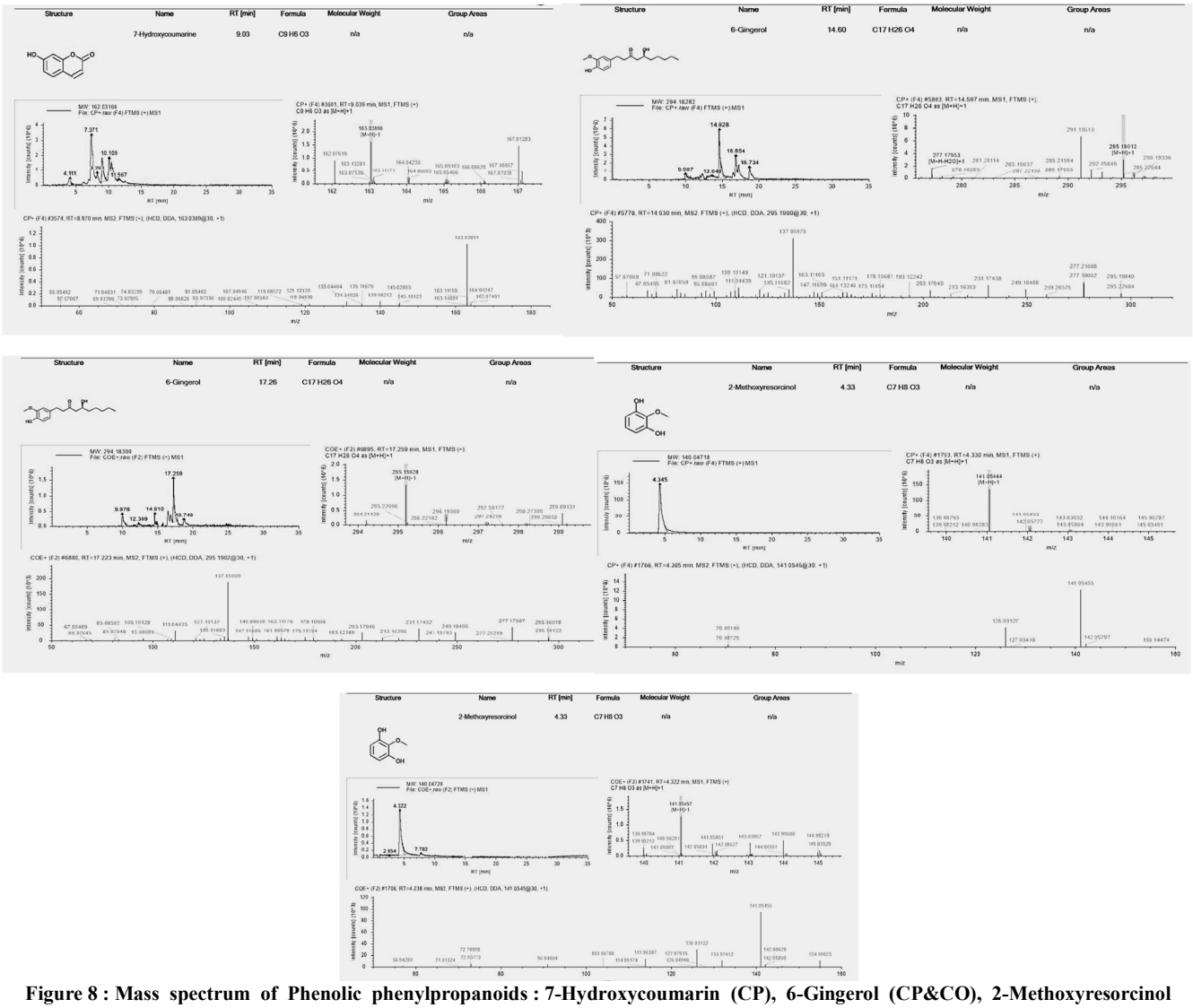

Figure 8 : Mass spectrum of Phenolic phenylpropanoids : 7-Hydroxycoumarin (CP), 6-Gingerol (CP&CO), 2-Methoxyresorcinol (CP&CO)

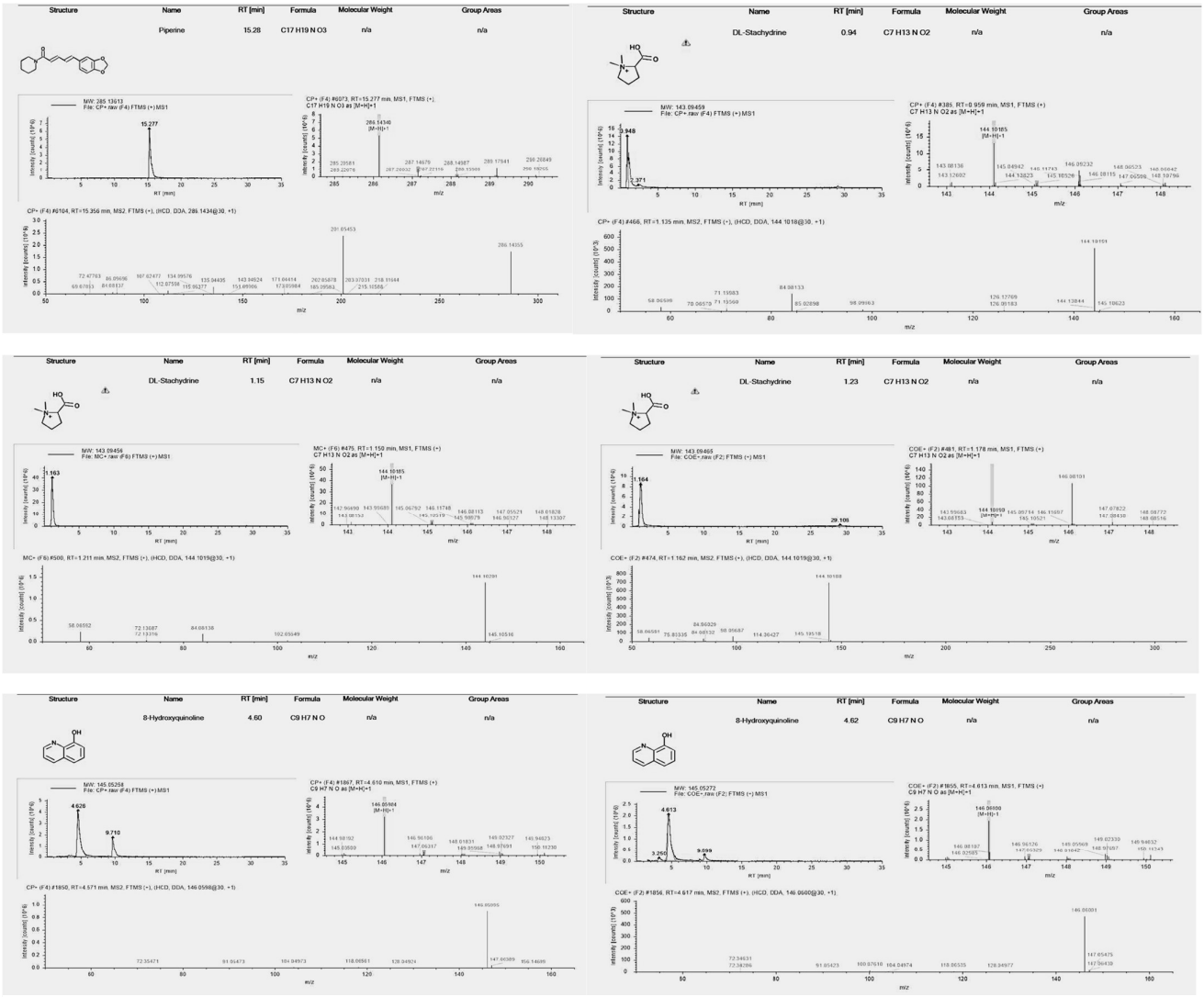

Figure 9 : Mass spectrum of alkaloids - Piperine (CP), DL-Stachydrine (CP, MC & CO) and 8-Hydroxyquinoline (CP&CO)

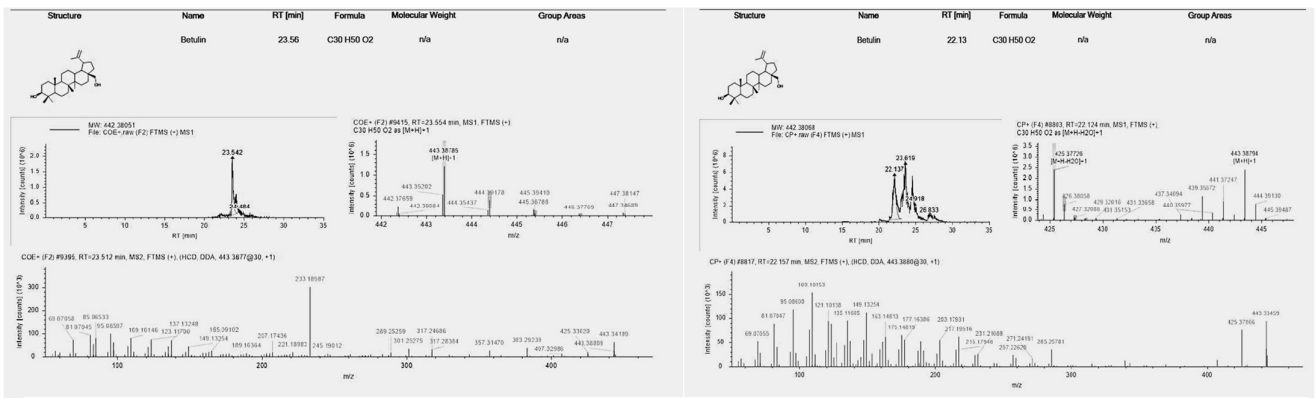

Figure 10 : Mass spectrum of Pentacyclic triterpene Betulin (CO & CP)

DISCUSSION/CONCLUSION

LCMS contributed in identifications of various phytoconstituents in CP, MC, and CO extracts. The alkaloids, phenolic compounds and triterpenes are reported from the extracts. Majority of flavonoids are identified in the extracts in this study. Flavonoids are commonly found as mixtures in various plant extracts, however, similarities in their structures and polarities have made identification of each compound difficult. Emerging powerful advanced techniques of liquid chromatography coupled with mass spectroscopy (LC-MS) has made the identification of flavonoids quiet easily **[6, 7]**. Flavonoids belonging to different subgroups were identified in the extracts such as flavonols, flavone, isoflavones, flavanones.

Flavonols act as scavengers of nitric oxide synthesis, inhibitors of xanthine oxidase, growth regulators, photosensitisers and chemotaxonomic markers.[8-10] They inhibit cyclooxygenase as well as lipoxygenase enzymes resulting in reduced platelet activation and aggregation, offers protection against cardiovascular diseases and inflammation, anticancer, anti-viral, anti-spasmodic, anti-hepatotoxic, anti-osteoporotic, anti-allergic, anti-spasmodic, and antiulcer agents **[11-18]**. The flavonols - rutin and Quercetin-3-β-D-glucoside were identified in CP extract and various studies have proven the immunomodulatory potential of rutin and quercetin. **Rutin** has many pharmacological effects, including antioxidant, anti-inflammatory, antibacterial and immune regulation. Scientific reports rationalize their protective effect on humoral and cell-mediated immunity. **Quercetin** and its derivatives are naturally occurring phytochemicals with promising bioactive effects. The anti-inflammatory, antioxidant, antimicrobial, antidiabetic, cardiovascular and wound-healing effects of quercetin have been extensively investigated. Also, anticancer activity against different cancer cell lines has been recently reported **[19-21]**.

Flavanones are another important class of flavonoids which are associated with a number of health benefits because of their free radical scavenging properties **[4]**. The flavanones sakuranetin and hesperetin were identified in CO extract. **Sakuranetin** belongs to the group of methoxylated flavones and has antiproliferative activity against human cell lines typical for B16BL6 melanoma, oesophageal squamous cell carcinoma and colon cancer. It also has antiviral activity towards human rhinovirus 3 and influenza B virus and was reported to have antioxidant, antimicrobial, anti-inflammatory, antiparasitic, antimutagenic

and antiallergic properties **[22, 23]**. **Hesperetin** a flavonoid suggests potential anti-inflammatory effect and may be potentially useful for modulating immune cell functions in physiological and pathological conditions. The reported anticancer effects of hesperidin have been found to be associated with its anti-oxidant and anti-inflammatory activities. Hesperidin interacts with numerous recognized cellular targets and inhibits cancer cell proliferation by inducing apoptosis and cell cycle arrest **[21, 24, 25]**. Flavones are among the important subgroups of flavonoids that are widely distributed in plant leaves, flowers, and fruits and are emerging as very important specialized metabolites involved in plant signalling and defense. Flavones have received increasing attention due to their anti-inflammatory, antimicrobial, and anticancer activities. One of the first beneficial effects ascribed to flavones were antioxidant activities, based on the ability of these compounds to scavenge reactive oxygen species (ROS) **[26]**. Preclinical studies have shown that flavone compounds possess a variety of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities as chemopreventive and chemotherapeutic agents because of their ability to inhibit angiogenesis, induce apoptosis, prevent carcinogenesis in animal models, reduce tumor growth *in-vivo*, and sensitize tumor cells to the cytotoxic effects of some anticancer drugs **[27, 28]**. The flavones orientin, luteolin and cynaroside were found in CP extract, tangeretin in MC and CO extract and nobiletin in all the three extracts. **Orientin** is a water-soluble flavonoid C-glycoside that contains medicinal properties of antioxidant, anti-inflammation, antiviral, vasodilation and cardioprotective, neuroprotective etc. **[29]**. **Luteolin** is commonly found in medicinal plants and has strong anti-inflammatory activity *in-vitro* and *in-vivo*. The biological effects of luteolin could be functionally related to each other, the anti-inflammatory activity may be linked to its anticancer property associated with the induction of apoptosis, and inhibition of cell proliferation, metastasis, and angiogenesis **[30, 31]**. The flavonoid **Cynaroside** exerts antibacterial, antifungal, antioxidant, hepatoprotective, antidiabetic, anti-inflammatory and anticancer effects. **Tangeretin** is a key member of flavonoids and has different biological activities such as antioxidant, anti-inflammatory, antitumor, hepatoprotective and neuroprotective effects. It also exerts anticancer activity by inhibiting the growth as well as the progression of cancer cells in both *in-vitro* and *in-vivo* studies **[32]**. The various

pharmacological activities of **Nobiletin** include neuroprotection, anti-inflammation, anticancer, cardiovascular protection, antimetabolic disorder and antioxidation **[33]**.

Isoflavonoids are flavonoid phenolic compounds which exhibit biologic effects through estrogen receptors, referred as phytoestrogens. In this study genistein was identified as in CP extract. **Genistein** is an isoflavone and belongs to the benzo-gamma-pyrones. Clinical studies have proven the significant role of genistein in reducing RA-induced inflammation by regulating the immune system in powerful ways **[34]**. Also, Genistein has been widely investigated for its anticancer properties, mainly against hormonally regulated breast and prostate cancers in animal models **[35]**. **Biochanin A** found in CO extract is an isoflavone possessing multiple pharmacological activities – antimicrobial, antioxidant, and anticancer, anti-inflammatory, neuroprotective and hepatoprotective properties **[36]**. **Puerarin** found in MC & CO extract is an isoflavone and exhibits immunomodulatory effect **[24]**. The literature suggest that Puerarin could be a promising immunomodulator to assist in the treatment of tumors **[37, 38]**.

The phenolic phenylpropanoid 7-hydroxycoumarin in CP extract and 6-gingerol in CP & CO extract was identified by LC-MS analysis. **7-hydroxycoumarin** is the simple coumarin hydroxylated, alkoxylated and alkylated derivatives of the parent compound, coumarin, along with their glycosides. The 7-hydroxycoumarin have antitumor activity against several tumor cell lines**.** The studies have investigated the anti-inflammatory and antipyretic effects of 7-hydroxycumairn in animal models and the results strongly suggested its inflammatory, analgesic and antipyretic effects related to COX-2 inhibition **[39, 40]**. **6-Gingerol** is the major pharmacologically-active component of ginger which exhibits variety of biological activities including anticancer, anti-inflammation, and anti-oxidation. 6-Gingerol has been found to possess anticancer activities via its effect on a variety of biological pathways involved in apoptosis, cell cycle regulation, cytotoxic activity, and inhibition of angiogenesis **[41]**. **2-Methoxyresorcinol** found in CP & CO extract has a wide range of biological activities, including anti-inflammatory, antioxidant, and anticancer properties.

Piperine is a type of amide alkaloid found in CP extract that displays numerous pharmacological effects such as antiproliferative, antitumor, antioxidant, anti-angiogenesis, antidiabetic,

antimicrobial, anti-inflammatory and immunomodulatory effects in various *in-vitro* and *in-vivo* experimental trials **[42, 43]**. **DL-Stachydrine** found in all the three extracts have been widely researched in various diseases including fibrosis, cardiovascular diseases, cancers, uterine diseases, brain injury, and inflammation **[44]**. The quinoline derivative **8-Hydroxyquinoline** found in CP & CO extract hold medicinal properties such as antineurodegenerative, anticancer, antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities **[45]**.

Betulin, a pentacyclic triterpene identified in CP & CO extract, show a wide spectrum of pharmacological properties such as anti-HIV, anti-inflammatory, anti-cancer **[46]**. Thus, this study of comprehensive composition and content of phenolic compounds showed that the selected plants can encourage the scientific community to address new studies to understand their potential on human health.

FUNDING

NIL

CONFLICT OF INTEREST

We declare no conflict of interest.

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