

Original article

Estimate the Types and Contents of Phenolic Acid in *C. Parviflorus* Lam and *C. Salviifolius* L Plants Growing at Al-Gabal Al-Khder region

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In this study, the leaves, stems, and roots of *C. parviflorus* Lam and *C. salviifolius* plants that are growing in the Al-Gabal Al-Akhder region of Libya were selected to estimate the contents and types of phenolic acids using by GCMS instrument. The study included information on chemical structure definitions and the effects of solvents. The types and contents of phenolic acid compounds and their derivatives detected in the studied samples showed small variations in their contents; some phenolic acids showed relative increases in alcoholic extracts when compared to aqueous extracts. The types and values of the phenolic compounds were dependent on the polarity of the solvents that were used in this study. In general, the types of phenolic compounds that were detected using the solvents that were used in this study are: chlorogenic acid, caffeic acid, 3,4-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid, cinnamic acid, gallic acid, geraniol, tannic acid, phloridzin, and quercetin. The phenolic acid concentration of the alcoholic and aqueous extracts varied little, according to the results.

Keywords. Determination, Phenolic Acid Types, Concentration, GC Mass Analysis.

Introduction

The identification of novel medicinal agents is made possible by natural products like plant extracts [1]. Since they are a vast source of beneficial phytochemicals that will aid in the creation of new medications, medicinal plants are currently regarded as being quite important. Most phytochemicals derived from plants, including flavonoids and phenols, have been shown to improve health and prevent cancer [2]. Since ancient times, people have used medicinal plants to treat ailments [3]. The therapeutic qualities of the plants are ascertained by their chemical constituents [4]. The usage of medicinal plants by indigenous people from around the world has been documented by the World Health Organization (WHO). In the latter part of the 1990s, the use of plant derivatives as therapeutic agents became increasingly common. About 80% of the world's population relies on herbal therapy, and most developing nations employ traditional medicine and medicinal plants as a standard base for sustaining good health. Many secondary metabolites, including phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols, are typically produced by plants and are significant sources of biocides and numerous other pharmaceutical medications [5].

Drug development and pharmacological research both benefit from the use of medicinal plants [6]. Plants are the source of around 7,000 of the therapeutic substances listed in the Western Pharmacopoeia [7]. Because they generate a wide variety of bioactive chemicals, the majority of which most likely developed as chemical defenses against infection or predation and antioxidant substances, plants have been a rich source of medicinal products [8]. Numerous plants have antibacterial properties, and various actions are employed to treat various illnesses. In rural Libya, medicinal plants have been utilized in traditional medicine; regions have comparatively lower costs than contemporary medicine, and they have been extensively utilized as topical anti-inflammatory drugs, diuretics, and hemostatic [9]. The *Cistus* plant that is flourishing at Al-Gabal is the subject of this investigation. Al-Khder (Libya) was chosen, and the following is a summary of this plant's description: *Cistus* plant: There are 180 species in the family Cistaceae, which includes 8 genera. Species, five Mediterranean-native genera (*Halimium*, *Cistus*, *Tuberaria*, *Fumaria*, and *Helianthemum*). There are 21 species in the genus *Cistus* [3]. *Cistus* (derived from *kistos*, a Greek term). The name of this family is Rock. Rose plants [10]. These plants are lovely bushes that are classified into several types based on the color of their pink or white blooms [11]. Spread throughout the regions of western Africa, Asia, Europe, and the Mediterranean [12]. It is well known that the Mediterranean region is the typical home of the genus *Cistus* [13]. These plants can thrive in challenging soil, rocky, and climate conditions. [14]. The heterosexual In Mediterranean traditional medicine, many of these have been utilized as herbal plants to cure colds and intestinal issues, as well as extracts for therapy for illnesses. The illnesses and ailments that are being targeted include anxiety, bronchitis, arthrosis, asthma, and many malignancies, duodenosis, cardiopathies, catarrh, bacterial and fungal diseases, headache, dyspnea, diarrhea, and dysentery [10].

Tea made from *C. salviifolius* leaves is used to treat cancer. In Libya, *Cistus* species are utilized in traditional medicine. For a variety of reasons. For instance, a root decoction is used to treat infertility among women in Artvin. Various kinds of *castus* are utilized in Jordanian traditional medicine to treat numerous illnesses, including gout, ulcers, intestinal disorders, and anti-inflammatory diseases, as well as reducing blood sugar [15]. Current scientific studies have concentrated on the identification and isolation of substances found in

resins and extracts made from several *Cistus* species. Additionally, research has examined its pharmacological and biological properties. This has therapeutic qualities for a variety of illnesses [16].

Chemical analyses carried out on various *Cistus* species showed that terpenes, flavonoids, and phenolic compounds make up their constituents. acids, bornyl esters, and resacetophenone glucoside. lipids (from the seeds), gum, resin, and essential oils. The basic oil composition of *Cistus* species is examined. The existence of aromatics, sesquiterpenes, and oxygenated monoterpenes Sesqui terpenes with oxygen and trace amounts of carbonyl compounds. *Cistus* species' leaves have conerved structures that release Terpenoids make up most of the resin and basic oil [17]. This study aims to estimate the types and contents of phenolic acids in plants collected from the Al Gabal Al Khder region, Libya.

Methods

Sampling

Two plants (*C. parviflorus* and *C. salviifolus*) were chosen IN this study because of their significant medical uses. There are numerous plants used in the AL-Gabal AL-Khder region of Libya. The samples were gathered in the spring of 2023 from the Al-Gabel Al-Kadar region. Taxonomy of Plants: The Seliphium Herbarium, Botany Department, Faculty of Science, Omar Al-Mukhtar University, identified the samples that were gathered. The taxonomy of plants was shown in (Figures 1 & 2), as well as (Table 1).



Figure 1. *C. Parviflorus* Lam



Figure 2. *C. Salviifolius* L

Table 1: The taxonomy of the studied plants.

Kingdom	Plant	
Clade	Tracheophytes and Angiosperms	Tracheophytes and Angiosperms
Family	<i>Cistaceae</i>	<i>Cistaceae</i>
Genus	<i>Cistus</i>	<i>Cistus</i>
Species	<i>C. Parviflorus</i> Lam	<i>C. Saiviifolius</i> L
Vernacular name	Torrashe Ahmar, Birabash Ahmar	Torrashe Abiad, Birabash Abiad

Sample preparation

The plants under study had their leaves, stems, and roots removed and repeatedly washed with distilled water. After that, the samples were dried in a dry, dark environment. Following a mortar grind, the samples were kept in plastic bottles pending analysis [18-30].

Extraction

Two different solvents were used in this study: Water (aqueous) and Ethanol (Alcoholic), 10 grams of each sample (leaves, stems, or roots) were transferred to beakers containing 100 ml of each solvent, and the samples were mixed. Then it is input in the evaporator system until dryness [31-48].

Chemical studies

The Determination of phenolic acids GC mass: At Alexandria University's Central Lab in Egypt, the phenolic acids were estimated using a GC-MS equipment [49-51].

Results

There are several types and amounts of phenolic acids recorded in the studied samples; the results indicated

that there are small variations in the detected phenolic acids of the samples by the GC-MS analysis. The following phenolic acids were found in the extracts of the chosen plants used in this investigation: Chlorogenic acid; caffeic acid; 3,4-dihydroxybenzoic acid; 3,5-dihydroxybenzoic acid; 4,5-dihydroxybenzoic acid; cinnamic acid; gallic acid; geraniol; tannic acid; phloridzin; quercetin; and chlorogenic acid. The results of this study were given in (Tables 2 & 3): where the aqueous phenolic acid concentrations ranged as follows: 0.012–0.042, 0.014–0.080, 0.0019–0.0081, 0.010–0.062, 0.038–0.077, 0.0015–0.0037, 0.0047–0.048, 0.0029–0.0064, 0.0043–0.070, 0.032–0.076, 0.0051–0.038, and 0.019–0.029 µg/g for the phenolic acid compounds mentioned above, in that order. Conversely, the concentrations of phenolic acids in alcoholic extracts varied between 0.016 and 0.053, 0.015 and 0.073, 0.0017 and 0.0064, 0.016 and 0.071, 0.041 and 0.079, 0.0021 and 0.0051, 0.0052 and 0.046, 0.0034 and 0.0076, 0.0054 and 0.084, 0.043 and 0.092, 0.0043 and 0.033, and 0.016 and 0.054 µg/g. for the phenolic acid compounds mentioned above. Alcoholic extracts have a comparatively higher phenolic acid concentration than water extracts. The types and amounts of phenolic acids in the aqueous extracts of the plants under study are listed in (Tables 2 & 3).

Table 2: The types and contents of phenolic acids of aqueous extracts of the studied plants

Phenolic acids µg/g	Samples					
	Parvifloru leafs	Parvifloru Stems	Parvifloru Roots	Salviifolius leafs	Salviifolius Stems	Salviifolius Roots
Chlorogenic acid	0.012	0.016	0.042	0.019	0.022	0.031
Caffeic acid	0.020	0.014	0.039	0.025	0.080	0.032
3,4-Dicaffeoyl guinic acid	0.009	0.0081	0.0040	0.0053	0.0076	0.0019
3,5-Dicaffeoyl guinic acid	0.047	0.036	0.062	0.041	0.010	0.029
4,5-Dicaffeoyl guinic acid	0.077	0.054	0.038	0.065	0.070	0.064
2,5-dihydroxy Benzoic acid	0.0020	0.0031	0.0015	0.0037	0.0028	0.0043
Cinnamic acid	0.031	0.048	0.030	0.0047	0.0080	0.0065
Gallic acid	0.0060	0.0029	0.0063	0.0064	0.0029	0.0057
Geraniol	0.027	0.045	0.070	0.052	0.0090	0.0043
Tannic acid	0.076	0.032	0.058	0.078	0.039	0.060
Phloridzin	0.0085	0.020	0.0051	0.038	0.0070	0.0090
Quercetin	0.023	0.019	0.034	0.029	0.024	0.028
Average	0.028	0.024	0.032	0.030	0.023	0.022
Total	0.338	0.291	0.389	0.367	0.382	0.275

The types and amounts of phenolic acids in the alcoholic extracts of the plants under study are shown in (Table 3).

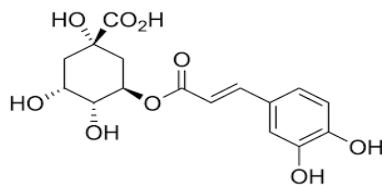
Table 3: The types and contents of phenolic acids of alcoholic extracts of the studied plants

Phenolic acids µg/g	Samples					
	Parvifloru leafs	Parvifloru Stems	Parvifloru Roots	Salviifolius leafs	Salviifolius Stems	Salviifolius Roots
Chlorogenic acid	0.016	0.018	0.053	0.031	0.041	0.028
Caffeic acid	0.018	0.015	0.042	0.019	0.073	0.026
3,4-Dicaffeoyl guinic acid	0.007	0.0064	0.0035	0.0062	0.0081	0.0017
3,5-Dicaffeoyl guinic acid	0.052	0.038	0.071	0.046	0.016	0.033
4,5-Dicaffeoyl guinic acid	0.065	0.080	0.041	0.072	0.079	0.071
2,5-dihydroxy Benzoic acid	0.0043	0.0040	0.0021	0.0046	0.003	0.0051
Cinnamic acid	0.046	0.039	0.034	0.0052	0.0090	0.0083
Gallic acid	0.0055	0.0034	0.0070	0.0076	0.0038	0.0061
Geraniol	0.032	0.068	0.084	0.049	0.0075	0.0054
Tannic acid	0.057	0.044	0.063	0.092	0.043	0.074
Phloridzin	0.0090	0.015	0.0046	0.033	0.0065	0.0089
Quercetin	0.043	0.016	0.030	0.045	0.032	0.054
Average	0.029	0.028	0.036	0.034	0.026	0.0267
Total	0.354	0.346	0.435	0.410	0.321	0.321

Discussion

Chlorogenic acid

As an intermediary in the production of lignin, chlorogenic acid (CGA) is the ester of caffeic acid and (–) quinic acid. A related class of esters known as "chlorogenic acids" includes quinic acid and hydroxycinnamic acids (caffeic acid, ferulic acid, and p-coumaric acid) [52].



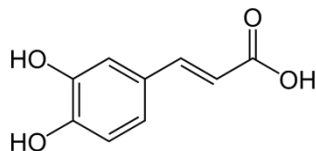
Among its many significant and medicinal functions are antioxidant activity, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, antimicrobial, anti-hypertensive, free radical scavenger, and central nervous system stimulator. Chlorogenic is a biologically active dietary polyphenol. A potential chemical sensitizer linked to respiratory allergies to specific plant materials has been investigated: chlorogenic acid. Chlorogenic acid lowers blood pressure a little, whether taken as a dietary supplement or in coffee [53].

Caffeic acid

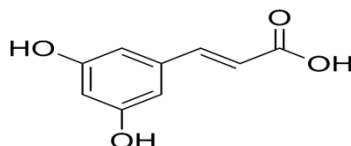
An organic substance categorized as a hydroxycinnamic acid is caffeine. Both phenolic and acrylic functional groups are present in this yellow solid. Since it is a crucial step in the manufacture of lignin, one of the main constituents of woody plant biomass and its byproducts, it is present in all plants. Ferulic acid is produced in plants by converting 4-hydroxycinnamic acid (left) to caffeic acid (center). In vitro research and animal models have shown that caffeine has a range of possible pharmacological effects. It has also recently been shown to have an oxidative mechanism that inhibits the proliferation of cancer cells in the human HT-1080 fibrosarcoma cell line [53]. Both in vitro and in vivo, caffeine has antioxidant properties. Additionally, caffeine has anti-inflammatory and immunomodulatory properties. With a more than 95% reduction in aflatoxin formation, caffeic acid fared better than the other antioxidants. These investigations are the first to demonstrate that caffeic acid can inhibit oxidative stress, which would otherwise cause or increase the synthesis of aflatoxin by *Aspergillus flavus*. Adding antioxidants to trees makes it possible to employ them as a natural fungicide. Research on caffeine's carcinogenicity has produced conflicting findings. It has been demonstrated to have carcinogenic effects in some experiments and to suppress carcinogenesis in others. Rats that received large oral dosages of caffeic acid developed stomach papillomas. Colon tumor growth was significantly inhibited by high dosages of combination antioxidants, including caffeic acid [52].

Caffeic acid Isomers

Umbellic acid (2,4-dihydroxycinnamic acid) and 2,3-dihydroxycinnamic acid are isomers belonging to the hydroxycinnamic acid family that share the same chemical formula.



3,4-Dicaffeoyl quinic acid

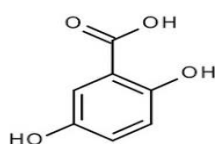


3, 5-Dicaffeoyl quinic acid

A carboxylic ester known as 3,5-di-O-caffeoyl quinic acid is produced when the carboxy group of trans-caffeic acid condenses with the hydroxy groups at positions 3 and 5 of (-) quinic acid. It has cytotoxic and hepatoprotective properties and was isolated from *Suaeda glauca* and Brazilian propolis. It functions as an antineoplastic, hepatoprotective, and metabolite. It is a carboxylic ester and cyclitol carboxylic acid. It comes from a trans-caffeic acid and a (-)-quinic acid [52].

2,5-Dicaffeoyl quinic acid

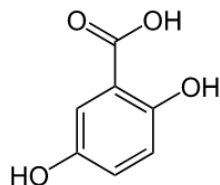
One type of dihydroxybenzoic acid is gentisic acid. The kidneys eliminate this small (1%) byproduct of the metabolic breakdown of aspirin, which is a derivative of benzoic acid. Except in certain pharmaceutical compositions, gentisic acid is used as an antioxidant and is easily oxidized as a hydroquinone [53].



Cinnamic acid

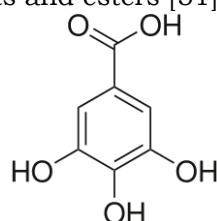
The organic chemical cinnamic acid has the formula $C_6H_5CH=CHCOOH$. It is a white, crystalline substance that dissolves readily in a wide range of organic solvents and just minimally in water. It is found in many

plants naturally and is categorized as an unsaturated carboxylic acid. Both a cis and a trans isomer exist; however, the latter is more prevalent. Flavorings, artificial indigo, and some medications all include cinnamic acid. Cinnamic acid is a precursor to the sweetener aspartame by enzyme-catalyzed amination to provide phenylalanine. It is also used extensively as a precursor to generate methyl, ethyl, and benzyl cinnamate for the perfume business. In non-polar solvents, cinnamic acid can dimerize, producing distinct linear free energy correlations [53].



Galic acid

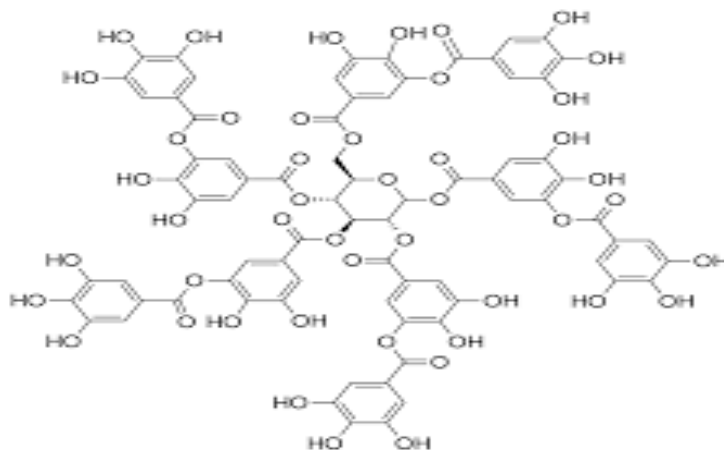
The tri-hydroxybenzoic acid, gallic acid (sometimes called 3,4,5-trihydroxybenzene acid, has the formula $C_6H_2(OH)_3COOH$. It falls within the category of phenolic acid. Gallnuts, tea leaves, witch hazel, sumac, oak bark, and other plants contain it. Although samples are usually dark due to partial oxidation, it is a white solid. The term "gallates" refers to gallic acid salts and esters [51].



Many terrestrial plants, including the aquatic plant *Myriophyllum spicatum*, the parasitic plant *Cynomorium coccineum*, and the blue-green alga *Microcystis aeruginosa*, contain gallic acid. Moreover, certain oak species, *Caesalpinia mimosoides*, and the stem bark of *Boswellia dalzielii* contain gallic acid. Gallic acid is present in many foods in varying concentrations, particularly fruits (bananas, strawberries, and grapes), teas, vinegars, and cloves.

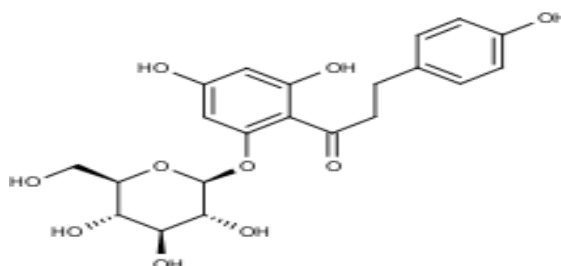
Tannic acid

Historically, it has been used to absorb poisons and act as an antidote. However, in everyday life, tannic acid is used to cure rashes, stop bleeding, and ease various soreness-related disorders [51].



Phloridzin

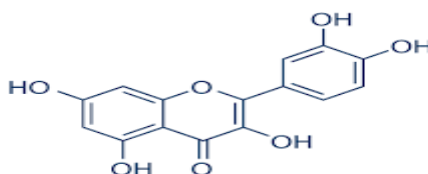
As a glucoside of phloretin, a dihydrochalcone belonging to the family of bicyclic flavonoids, phlorizin (also known as phloridzin or phloretin-2'- β -D-glucopyranoside) is a subgroup of the varied phenylpropanoid production pathway in plants.



Unripe *Malus* (apple) root bark is the main source of phenolizin, while minor levels have also been discovered in strawberries. It is more prevalent in seeds and vegetative tissues (like leaves and bark) in *Malus*. Phloridzin is absent from closely related species, including cherry, pear (*Pyrus communis*), and other Rosaceae fruit trees. One phytochemical that is a member of the polyphenol class is phenolizin. It can be found in natural sources alongside other polyphenols as rutin, procyanidins, epicatechin, quercetin, and catechin. Because it competes with D-glucose for binding to the carrier, phorizin is a competitive inhibitor of SGLT1 and SGLT2, which lowers renal glucose transport and blood glucose levels. More selective and promising synthetic analogs, like empagliflozin, canagliflozin, and dapagliflozin, have supplanted phorizin, which was investigated as a possible pharmaceutical treatment for type 2 diabetes. When used orally, phlorizin is almost completely broken down by hydrolytic enzymes in the small intestine, making it an ineffective medication [52].

Quercetin

It belongs to the flavonoid category of polyphenols and is a plant flavonol. Numerous fruits, vegetables, leaves, seeds, and grains contain quercetin; typical foods that contain significant levels of this compound include kale and red onions. Foods, drinks, and dietary supplements all contain quercetin, which has a bitter taste [53].



One flavonoid that is abundant in nature is quercetin. Originating from quercetum (oak forest), after the genus *Quercus*, the term has been in use since 1857. It is a polar auxin transport inhibitor that occurs naturally. The average daily intake of quercetin is between 25 and 50 milligrams, making it one of the most prevalent dietary flavonoids. Higher levels of quercetin are found in the outermost rings and the area nearest the root of red onions, with the latter having the highest concentration. According to one study, tomatoes cultivated organically had 79% more quercetin than those grown non-organically [54-55]. The GC-Mass instrument is one of the most methods used to separate and identify the chemical compounds as amino acids, hydrocarbons, flavonoids, fatty acids, many studies were carried out by used this methods [56-60] In this study The GC mass chromatograms of the studied compounds were given in the (Figures 3 - 14).

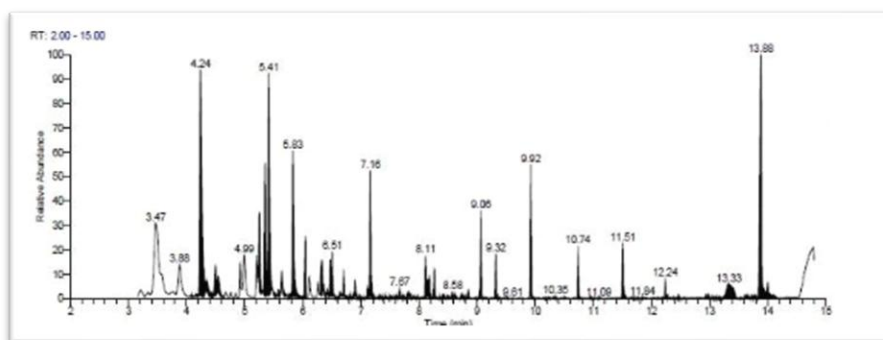


Figure 3: GC –Ms analysis of aqueous extract of leaves of *C. Parviflorus* Lam

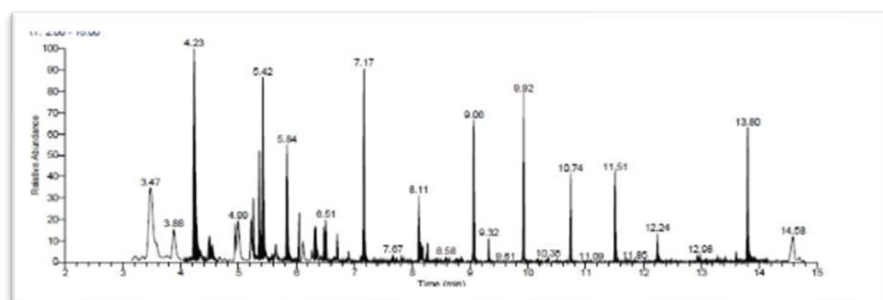


Figure 4: GC –Ms analysis of aqueous extract of stems of *C. Parviflorus* Lam.

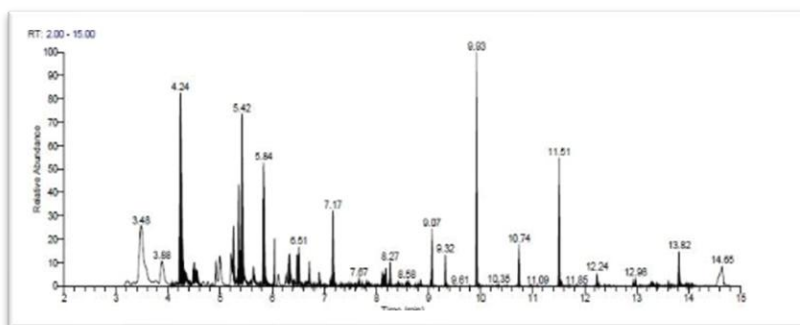


Figure 5: GC –Ms analysis of aqueous extract of roots of *C. Parviflorus* Lam

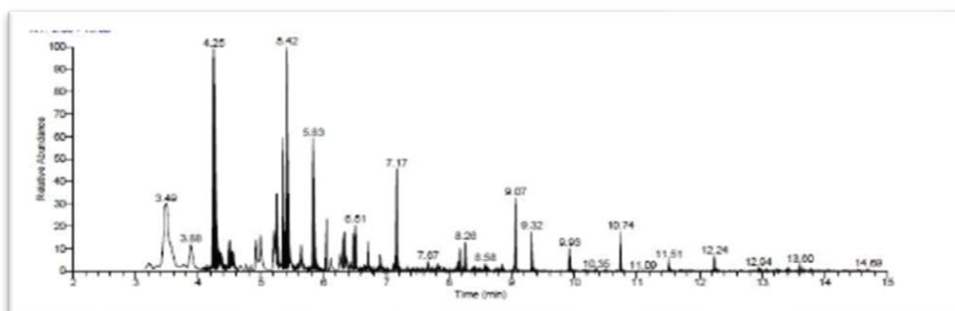


Figure 6: GC –Ms analysis of alcoholic extract of leaves of *C. Parviflorus* Lam

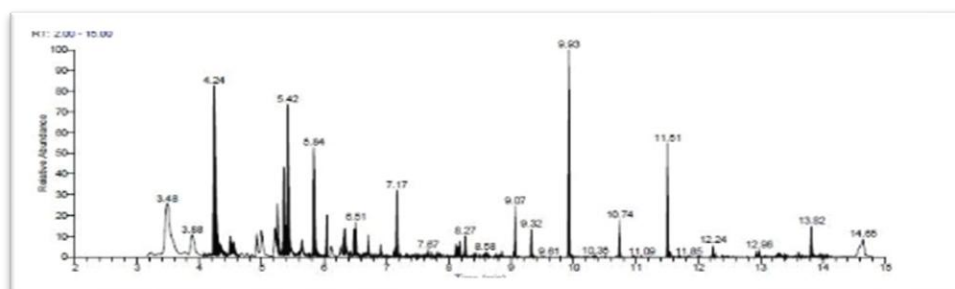


Figure 7: GC –Ms analysis of alcoholic extract of stems of *C. Parviflorus* Lam

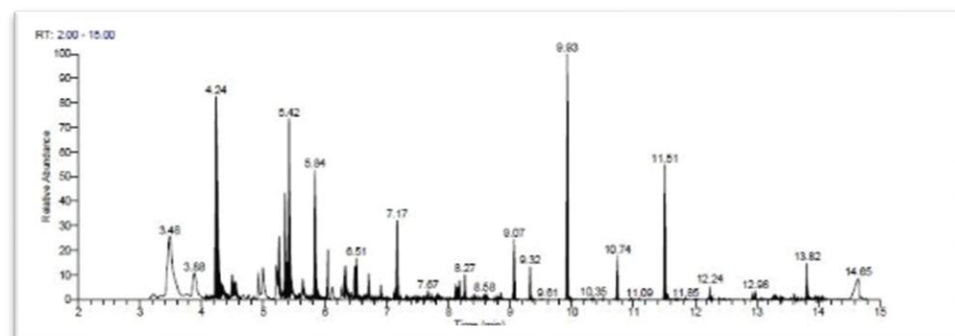


Figure 8: GC –Ms analysis of alcoholic extract of roots of *C. Parviflorus* Lam

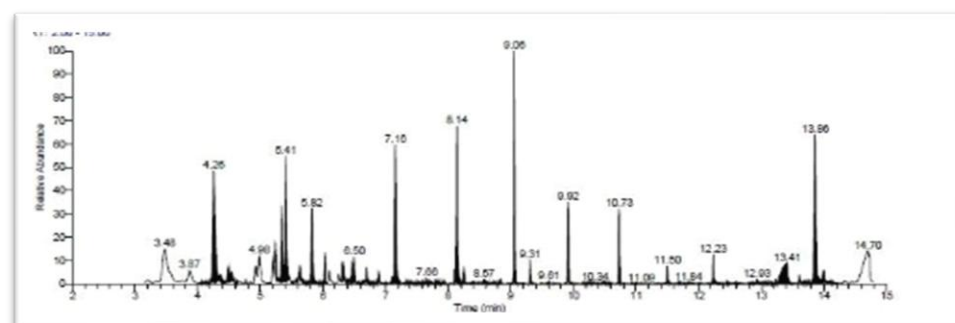


Figure 9: GC –Ms analysis of aqueous extract of leaves of *C. Savifolius* L

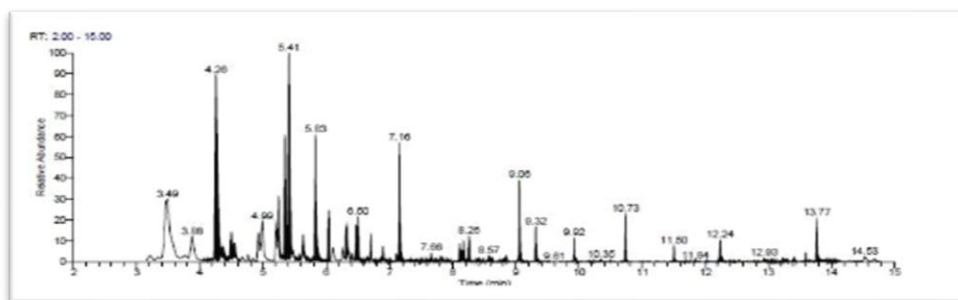


Figure 10: GC –Ms analysis of aqueous extract of stems of *C.Savifolius L*

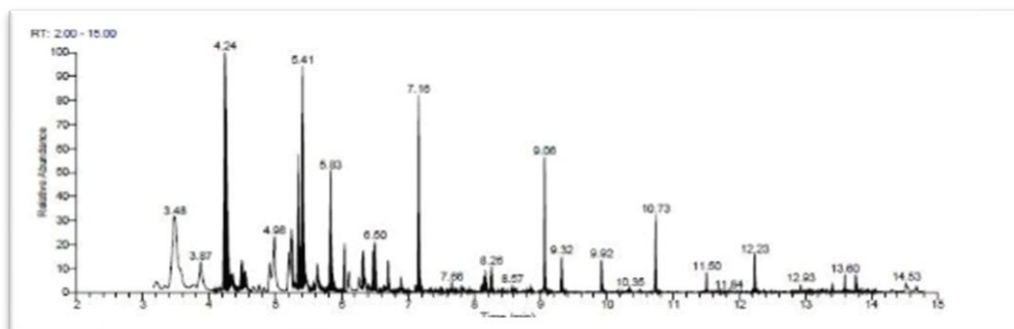


Figure 11: GC –Ms analysis of aqueous extract of roots of *C.Savifolius L*

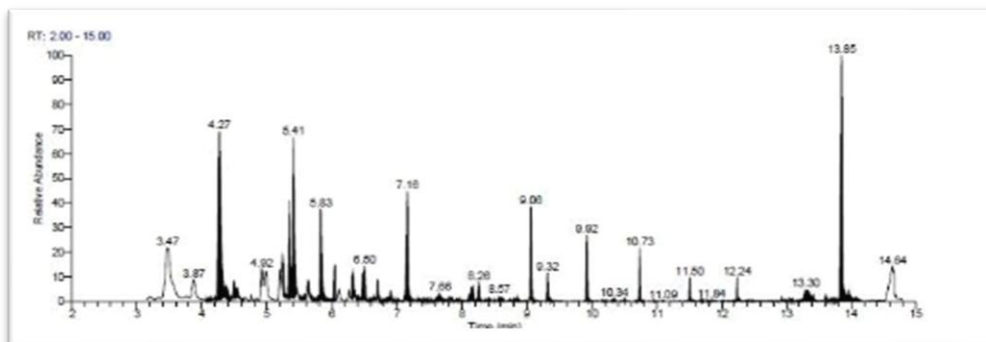


Figure 12: GC –Ms analysis of alcoholic extract of leaves of *C.Savifolius L*.

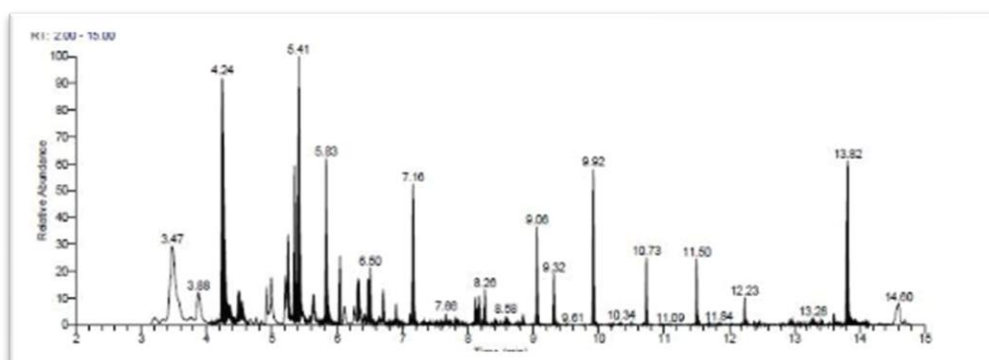


Figure 13: GC –Ms analysis of alcoholic extract of stems of *C.Savifolius L*.

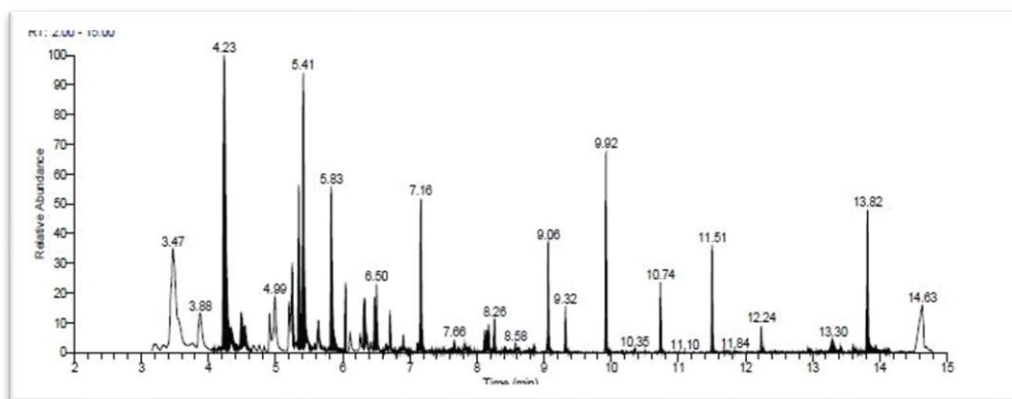


Figure 14: GC-MS analysis of the alcoholic extract of the roots of *C. Savifolius L*

Conclusion

The results of this study showed different types and contents of phenolic acids; there are small variations in their contents in the selected parts of the studied plants of leaves, stems, and roots.

Acknowledgement

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Conflict of interest. Nil

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