# CHAPTER 4 (a)

**Proximate, elemental composition, qualitative phytochemical and antimicrobial analysis of six selected ethnomedicinal plants found in Churachandpur district, Manipur, India**

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## 4a.1. Introduction

According to the World Health Organization, 80 percent of the world's population in developing nations rely on herbal medications and traditional therapies, which are not only inexpensive but also often the only source of care available due to their accessibility (Mukherjee, 2002). Many indigenous cultures rely on plants for survival. Considering today's high health-care expenses, the affordability of traditional medicines for most of the population makes them more appealing than other conventional treatments. India is rich in medicinal plants and is renowned as the world's greatest grower and botanical garden. Three biodiversity hotspots (Himalay, Western Ghats, and Indo-Burma) are found in India, all of which are severely endangered ecoregions. India's North-Eastern states are home to some of the world's most diverse collections of medicinal and fragrant plants. This region is also noted for its unique human culture and for being home to a significant variety of Indian ethnic groups. Manipur is one of the Indian subcontinent's eight northeastern states and part of the Indo-Myanmar hotspot zone. This region is ranked 8th among the world's 34 biodiversity hotspots (Sajemet et al., 2008). In Manipur, traditional healthcare techniques are still widely practiced. In certain rural parts of Manipur, these are the main or only medical facilities available (Lee, 2005). The majority of research investigations concentrate on the phytochemical elements found in medicinal plants, which are primarily employed for healing and curing various human disorders. The mineral and proximate content of plants can reveal a lot about their medicinal and nutritional value. When compared to other plants, medicinal plants have a higher concentration of trace elements (Chitturi et al., 2015). The trace elements found in medicinal plants must be measured because they are necessary for the human body's optimal functioning and metabolic activity. In the fight against disease, trace elements are both curative and preventative. The potential for utilizing the preventative medical properties of certain trace elements is enormous. Mineral and trace element concentrations in plants are so low that their value

has been overlooked for a long time. In most of the elemental studies of medicinal plants the concentrations are below the toxic levels. The doses consumed by the population are far from toxic doses, and therefore, both medicinal plants and herbal medicines can be supplementary sources of several essential elements useful for the human organism.

Herbal medicines have been used by humans for millennia. Traditional medicine practitioner have highlighted the therapeutic usefulness of several indigenous plants for a variet y of ailments. The advent of diseases and the growth of scientific understanding about he rbal medicines as major alternatives or complementary treatments for diseases justify the therapeutic substances derived from plants. A large variety of medicinal plants have been identified as important sources of natural antibacterial chemicals that may be beneficial in the treatment of these troublesome bacterial infections (Iwu et al., 1999). Many plants have been employed for their antibacterial properties, which are attributed to phytochemicals produced in the plant's secondary metabolism (Dahanukar et al., 2000; Cowan, 1999). Secondary metabolites found in plants include tannins, alkaloids, phenolic compounds, and flavonoids, which have been shown to have antibacterial activities in vitro (Duraipandiyanet et al., 2006; Djeussi, 2013). The advent of diseases and the growth of scientific understanding about herbal medicines as major alternatives or complementary treatments for diseases justifies the use of therapeutic substances derived from plants. Many pharmacological medicines start with these bioactive molecules as a starting point. As a result of these concerns, the need for alternative preservatives that are possibly effective, healthier, safer, and natural has grown (Mostafa et al., 2017; Nasar-Abbas et al., 2004). Antibiotic resistance has thus been recognized as a major worldwide health problem in recent years (Rossolini et al., 2014). As a result, efforts have been made to combat the threat of antibiotic resistance while also looking into other antimicrobial agents, such as medicinal plants (Anand et al., 2019). Antibiotics that have a limited efficiency in the treatment of human and animal diseases to tackle the burden of these pathogens, antibiotics with reduced efficacy in treating human and animal diseases due to antibiotic resistance must be substituted with novel medications. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (WHO, 2002). As a result, medicinal plants are expected to be the most reliable source of a wide range of medications. Because medicinal plants' contents have a wide range of structural and biological variety, they may be an excellent renewable resource for the identification of possible new medications. Higher plant natural products could provide a new

supply of antibacterial compounds with potentially novel modes of action (Runyoro et al., 2006; Shahidi et al., 2004). A great number of researchers from all around the world have investigated the impact of plant extracts on bacteria (Reddy et al., 2001). In India, ethnomedicinal plants have gotten a lot of attention (Maheshwari et al., 1986). To this end, the hunt for a novel antibiotic derived from natural products is an important part of modern medicine's effort to combat the socio-economic and health consequences of multidrug-resistant microorganisms.

As a result, in the current study, an attempt has been made to focus on the nutritional and elemental composition, which is equally responsible for the living beings' diverse biological and physiological activities, which are required for the maintenance of good health in both plants and animals. Also, it's critical to investigate more plants that are widely available and inexpensive locally as potential sources of innovative remedies (especially for chronic illnesses and food-borne illnesses) to help with disease management. The goal of this study was to analyse the efficacy of six plant extracts utilising three different approaches that have been used as traditional remedies by Manipur residents to help achieve this goal. Therefore, the current study's findings can be used as a database for treating diseases and other pharmaceutical applications.

## 4a.2. Materials and methods

### 4a.2.1. Collection and Identification of the plants

The medicinal plants were obtained from various locations in Manipur's Churachandpur district. Plant parts were collected based on the information provided based on a survey conducted among the informants. Each specimen/plant material was labeled, numbered, noted with the date of collection and their medicinal uses were recorded. Plant taxonomists of the Botanical Survey of India (BSI) Eastern Regional Centre, Shillong, and the Ethnomedicinal Plants Research Centre, Kangpokpi, Manipur identified the plants. Six different medicinal plants were taken for analysis belonging to different families. Table 4a.1. shows the botanical name, family, sample code, parts used, and ethnomedicinal use of plants.

**Table 4a.1:** Details of ethnomedicinal plants and the diseases treated

| Species name | Family | Sample code | Parts used | Ethnomedicinal uses |
|---|---|---|---|---|
| *Passiflora edulis* Sims | Passifloraceae | BSI/ERC/Tech/2019/613/1 | Leaves | Hypertension |
| *Plantago major* L | Plantaginaceae | BSI/ERC/Tech/2019/419/12 | Leaves | Boil sepsis, Hypertension, Intestinal wall thickness, Kidney stones |
| *Clerodendrum glandulosum* Lindl. | Lamiaceae | BSI/ERC/Tech/2019/613/4 | Leaves | Hypertension |
| *Solanum indicum* L | Solanaceae | BSI/ERC/Tech/2019/613/18 | Seeds | Hypertension, Mouth ulcers, Stomache |
| *Centella asiatica* (L) Urb. | Apiaceae | BSI/ERC/Tech/2019/613/16 | Leaves | Asthma, Gastrointestinal problem, Hypertension |
| *Phlogocanthus thyrsiformis* (Roxb.ex.Hardw.) Mabb. | Acanthaceae | FEEDS/EMRC/Plant Tax/PlantIden./2021/01/04 | Leaves | Cough and cold, Fever Hypertension, Skin itching |

### 4a.2.2. Preparation of Plant Extract

The plant portions were properly washed under running tap water and rinsed in distilled water before being air dried at room temperature in the shade (23-27°C) and cut to size. Each plant species' dried plant material was pulverized into a fine powder that passed through a 100 mm sieve. The samples were extracted using three different techniques viz. solvent extraction, ultrasound extraction, and supercritical fluid extraction under various conditions as indicated in Table 4a.2.

**Table 4a. 2:** The various extraction condition

| Solvent Extraction | Ultrasound Assisted Extraction – Xu et. al (2017) | Supercritical fluid Extraction - Ouédraogo et. al (2018) |
|---|---|---|
| Extraction time - 24hrs<br>Rotation - 200 rpm<br>Temperature - 30°C | Extraction time - 30 mins<br>Power - 200 W<br>Temperature - 37 to 40°C | Extraction time – 45 mins<br>Pressure – 250 bars<br>Temperature - 55°C |

Because ethanol has few or no negative effects on the human body and the environment, it was utilised as a solvent throughout the extraction process. The solvent was removed by evaporation using a Rotary evaporator, and the aqueous residue was placed in a lyophilizer to remove non-polar solvents and dry the extracts. By utilizing a lyophilizer to freeze-dry the extract, it was further concentrated to dryness. After drying, the dry extract was collected and placed into airtight bottles, where the resulting dried mass was packed into a glass vial and stored in a desiccator over silica gel and kept in a refrigerator at -80°C until use (Sasidharan et al., 2011; Kenneth-Obosi, 2017).

### 4a.2.3. Proximate Analysis

The proximate composition was assessed in triplicates on a dry weight basis using standard analytical techniques from the Association of Official Analytical Chemist methods (AOAC, 2000) with minor modifications (Anonymous, 2000). Moisture content was determined by the air-oven drying method (130°C for 1 h). Crude protein content by the micro-Kjeldahl method (% protein= %N × 6.25). Fat content was determined using the Soxhlet apparatus. The ash content was determined by combusting the plant materials in silica crucibles in a muffle furnace at 620°C for 3h. Total carbohydrates were calculated using Equation 4a.1.

Total carbohydrates (%) = 100 - % (moisture + crude protein + crude fat + ash) Eq$^{n}$ – 4a.1

### 4a.2.4. Scanning Electron Microscope and Energy Dispersive X-ray Spectroscopy (SEM-EDX)

The investigation was carried out on six distinct medicinal plants using SEM-EDX (JEOL JSM-6390 LV, SEM, USA). SEM-EDX is a highly competent analytical technique for the

identification and quantification of distinct elements in diverse biological and environmental samples, among the several analytical techniques used for elemental analysis. C $CaCO_3$, O $SiO_2$, Mg MgO, Al $Al_2O_3$, Si $SiO_2$, P GaP, S $FeS_2$, Cl KCl, K MAD-10, Ca Wollastonite, Fe Fe were used as a standard. The approach is non-destructive and more favorable in multi-elementary analysis than other existing methods such as ICP-AES, ICP-MS, AAS, and INAA, in addition to being a strong instrument for such analysis (Ramamurthy and Kannan, 2009)

### 4a.2.5. Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds. Lyophilized dried and powdered samples were screened for the presence of saponins [Foam test], phenols [Libermann's test], tannins [Ferric Chloride test], alkaloids [Wagner's test], flavonoids [Ammonium test] and glycosides [Borntrager's test] as described in literatures (Sofowara, 1993; Harborne, 1973; Ogbuewu, 2008).

### 4a.2.6. Antimicrobial screening

#### 4a.2.6.1. Determination of zone of inhibition

In vitro antibacterial activities were examined for different extracted samples. The antimicrobial activity of the plant extracts was determined by agar well diffusion method using the method of (Chollet et al., 2008; Oke et al., 2009) with some modifications. Tetracycline was used as a reference medication in control trials, which were conducted under similar conditions. After 18 to 24 hours of incubation at 37°C for bacteria, the zones of growth inhibition around the discs were assessed. Plates were examined after incubation for the formation of a clear zone around the well, which correlates to the antibacterial activity of the substances studied. The diameters of inhibitory zones (including the diameter of the disc) on the agar surface around the disc were measured to evaluate the microorganism species' sensitivity to plant extracts. The zone of inhibition (ZOI) was measured in millimetres, and values less than 8 millimetres were considered inactive against microorganisms.

#### 4a.2.6.2. Test microorganisms

The reference bacterial species; ***E.Coli*** **(ATCT11774)**, ***S.Aureus*** **(ATCC12600)**, ***L.Monocytogenes*** **(ATCC13932)**, ***B.Cereus*** **(ATCC10876)** and ***B.Subtilis*** **(ATCC9199)**

were collected from Microbiology Laboratory, Department of Food Engineering and Technology, Tezpur University, Assam.

### 4.2.7. Statistical analysis

All results were expressed as mean ± SEM. Statistical significance between the groups was analyzed by one-way analysis of variance (SPSS 18.0, SPSS Inc., Chicago, USA) followed by Tukey's post hoc multiple comparison test for comparison between groups. For the tests, $p < 0.05$ was considered statistically significant.

## 4a.3. Results and Discussion

### 4a.3.1. Proximate composition

Essential nutritional ingredients including carbohydrates, protein, fat and fibre are present in medicinal plants. These substances are crucial for the needs of the human body and are used in a variety of physiological, metabolic, and morphological processes (Radha et al., 2021; Cheeke, 2009). The ingredients in medicines, dietary supplements, and other healthcare items are obtained from natural plants. Plants and their phytochemical contents, such as antioxidants, hypoglycemic and hypolipidemic components, are essential in the discovery of new beneficial pharmaceutical components. Many medicines are derived either directly or indirectly from plant resources, which are frequently great suppliers of medicines (Petrovska, 2012; Dagli et al., 2015). The selected plants namely, *Passiflora edulis* Sims*., Plantago major* L*., Clerodendrum glandulosum* Lindl*., Solanum indicum* L*., Centella asiatica* (L.) Urb*. and Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. respectively, grow indigenously in Manipur, northeastern region of India and are taken for proximate, elemental and phytochemical analysis. The proximate compositions of the six different plants are presented in Table 4a.2.

The ash percentage of the six medicinal plant species ranged from 4.24±0.37 to 13.7±1.35 %. Leaves of *P.major* had the highest ash content (13.7±1.35%), and the seeds of *S.kurzii* had the least amount of ash (4.24±0.37%). The analyzed samples could be good ash sources. The consistency of a food substance is determined by its ash content, which also reveals the sample's organic, inorganic, and impurity content and identifies the material as carbon-free. The total ash content predicts the soluble and insoluble minerals in the sample (Ilodibia et al., 2016). The high ash content of the plants indicates that they are good mineral sources for human nutrition. This demonstrates the presence of both physiological

and non-physiological ash, the latter of which is produced from plant tissue. This is comparable to a study done in India, where it was determined that the total ash was 17.83±0.20% (Builders et al., 2015).

Moisture levels ranged from 11.06±1.43 to 17.35±0.55%. The leaves of *C.asiatica* had the highest total moisture content (17.35±0.55%), followed by *P.edulis* (13.75±0.99%), *P.thyrsiflorus* (11.06±1.43%), *P.major* (11.81±0.93%), and *C.glandulosum* (11.55±1.05%) respectively (Table 4a.3). The leaves of *P.thyrsiformis* had the lowest moisture content (11.06±1.43%). The pharmacopoeial monographs limit the water content material in vegetable sources at 8% and 14%. High moisture content frequently equates to an excessive amount of free water, which could change a variety of physical and chemical characteristics of goods that are meant to be used or stored dry. It also promotes the development of harmful bacteria, yeasts, and moulds (Saohin et al., 2007). Since the influence of water content on the stability and safety of crude drugs is significant, the European Agency for the Evaluation of Medicinal Products recommended that the moisture content be included in the list of comprehensive specifications for herbal materials and finished natural products that can be solids (Anjoo, 2012). The increased level of food spoiling is mostly explained by the leaves' (75% higher) higher moisture content.

The crude protein content of all six plant species ranged from 3.86±0.41 to 7.36±0.52%. The leaves of *C.glandulosum* had the highest protein content (7.36±0.52%), and the leaves of *C.asiatica* had the lowest crude protein content (3.86±0.41%) of all the plants studied Table 4a.3. In a study conducted by Bapan et al., 2018, *C. asiatica* plant contains 4.98% v/v protein which is a little higher compared to the current study. In the last decade, plant-based nutrition has gotten a lot of attention. In affluent countries, the large amount of animal protein consumption raises both health and environmental issues. As a result, these plants could serve as good sources of plant-based protein diet.

While analyzing the fat contents in the selected six medicinal plant varieties, the crude fat content ranges from 1.3±0.34 to 4.42±0.69 %. The leaves of *C.asiatica* had the highest crude fat content (4.42±0.69%), which is extensively higher than the other plants studied. The leaves of *C.glandulosum* had the lowest crude fat content (1.3±0.34%). The studied samples had a low-fat content, which will aid in the prevention of numerous cardiovascular disorders. In a study conducted in the Cold Desert of Western Himalaya on some selected medicinal plants (Radha et. al 2021) the crude fat ranges from 1.42 to 2.53% which agrees with the current study

The crude fiber content of the six plant species ranged from 10.76±0.62 to 23.9±2.12 %. Seeds of *S.kurzii* had the highest crude fibre content (23.9±2.12%), and the leaves of *P.major* had the lowest overall crude fibre content (10.76±0.62%) shown in Table 4a.3. Because all six plants include a significant quantity of fiber, they can help maintain the digestive tract healthy by eliminating harmful toxins from the body and preventing the absorption of excess cholesterol. It also adds weight to the diet and keeps the intake of starchy meals to a minimum. Whelton and colleagues found that fibre intake resulted in a slight drop in diastolic blood pressure (1.65 mmHg, 95% CI, 2.70 to 0.61) but had no association with systolic blood pressure in a thorough meta-analysis of 25 randomised controlled trials including 1,477 people (Whelton et al., 2005).

Carbohydrate content varies between 67.28 and 77.24 percent in all six plant species. The seeds of *S.kurzii* had the highest carbohydrate content (77.24%), and *P.major* had the lowest carbohydrate content of all the species.

Table 4a.3: Proximate composition of six selected medicinal plants

| Plant name | Plant parts | Ash (%) | Moisture (%) | Protein (%) | Fat (%) | Fiber (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *Passiflora edulis* Sims | leaves | 6.4±0.6$^{a}$ | 13.75±0.99$^{a}$ | 4.8±0.23$^{a}$ | 1.3±0.34$^{a}$ | 12.13±1.64$^{a}$ | 73.75±1.47$^{c}$ |
| *Plantago major* L | leaves | 13.7±1.35$^{d}$ | 11.81±0.93$^{a}$ | 6.25±0.77$^{b}$ | 1.84±0.26$^{a}$ | 11.61±1.70$^{a}$ | 66.41±1.49$^{a}$ |
| *Clerodendrum glandulosum* Lindl. | leaves | 8.71±0.34$^{b}$ | 11.55±1.05$^{a}$ | 7.36±0.52$^{b}$ | 1.41±0.16$^{a}$ | 12.01±2.14$^{a}$ | 69.11±5.13$^{b}$ |
| *Solanum indicum* L | seeds | 4.24±0.37$^{a}$ | 11.13±1.27$^{a}$ | 4.53±0.46$^{a}$ | 4.42±0.69$^{b}$ | 23.9±2.12$^{b}$ | 75.67±2.73$^{c}$ |
| *Centella asiatica* (L) Urb. | leaves | 5.24±0.62$^{a}$ | 17.35±0.55$^{b}$ | 3.86±0.41$^{a}$ | 4.02±0.59$^{b}$ | 13.38±1.24$^{a}$ | 69.53±0.47$^{b}$ |
| *Plogocanthus thyrsiflormis* (Roxb. Ex. Hardw.) Mabb. | leaves | 10.43±0.52$^{c}$ | 11.06±1.43$^{a}$ | 5.79±0.87$^{ab}$ | 3.71±0.93$^{b}$ | 10.76±0.62$^{a}$ | 69.02±1.83$^{b}$ |

Values are represented as means±standard deviation, and values with different superscripts are different at $p<0.05$

### 4a.3.2. Elemental composition

Elemental compositions were performed using the Electron Dispersion X-ray and indicated the presence of thirteen different types of elements (Table 4a.3) and the SEM-EDX images and micrographs are shown in Fig. 4a.3 & 4a.4. The elements included are C, O, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Br, and Mo. C, O, Si, Cl, K, and Ca are the only elements found in all six plant species. As can be seen in Table 4a.2, all of the plant species had the highest percentages of C and O. The proportion of C was found in the range from 53.59 to 62.42 wt. %. The highest C content was identified in *S.kurzii* (62.42 wt.%), while the lowest was reported in *C.asiatica* (53.59 wt.%). The second highest element was Oxygen with *C.asiatica* having the highest content at 41.37 wt. % and *P.edulis* having the lowest content at 34.97 wt. %. Oxygen is contained in every major organic component in the body, including proteins, carbohydrates, lipids, and nucleic acids, and plays a key role in metabolism and respiration. Carbon serves primarily as a structural element, producing the "backbone" of many organic compounds.

Table 4a.4. Elemental concentrations (weight %) of six different ethnomedicinal plants determined by SEM-EDX analysis

| | C | | O | | Mg | | Si | | Cl | | K | | Ca | | P | | S | | Mo | | Al | | Fe | | Br | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) | Wt. (%) | At. (%) |
| *P.edulis* | 60.9 | 68.9 | 34.9 | 29.7 | 0.38 | 0.21 | 0.42 | 0.2 | 0.48 | 0.18 | 0.86 | 0.3 | 1.05 | 0.36 | nd | nd | nd | nd | 1.09 | 0.15 | nd | nd | nd | nd | nd | nd |
| *P.major* | 55.5 | 64.9 | 37.4 | 32.4 | 0.29 | 0.17 | 1.53 | 0.76 | 0.77 | 0.3 | 1.28 | 0.46 | 1.5 | 0.52 | 0.27 | 0.12 | 0.55 | 1.24 | 0.21 | 0.17 | 0.56 | 0.29 | 0.57 | 0.14 | 0.67 | 0.12 |
| *C.glandulosum* | 59.0 | 66.8 | 37.5 | 31.9 | 0.38 | 0.21 | 0.24 | 0.12 | 0.39 | 0.15 | 1 | 0.35 | 0.79 | 0.27 | 0.28 | 0.12 | 0.34 | 0.14 | 0.73 | 0.1 | nd | nd | nd | nd | nd | nd |
| *S.indicum* | 62.4 | 69.9 | 35.4 | 29.6 | nd | nd | 0.22 | 0.11 | 0.31 | 0.11 | 1.2 | 0.41 | 0.33 | 0.11 | 0.37 | 0.16 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| *C.asiatica* | 53.5 | 61.8 | 41.3 | 35.8 | 0.56 | 0.32 | 2.06 | 1 | 0.26 | 0.1 | 1.19 | 0.42 | 0.64 | 0.22 | nd | nd | nd | nd | nd | nd | 0.5 | 0.25 | nd | nd | nd | nd |
| *P.thyrsiformis* | 59.0 | 66.9 | 36.8 | 31.3 | 0.62 | 0.35 | 0.38 | 0.18 | 0.71 | 0.27 | 1 | 0.34 | 1.22 | 0.41 | 0.31 | 0.14 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

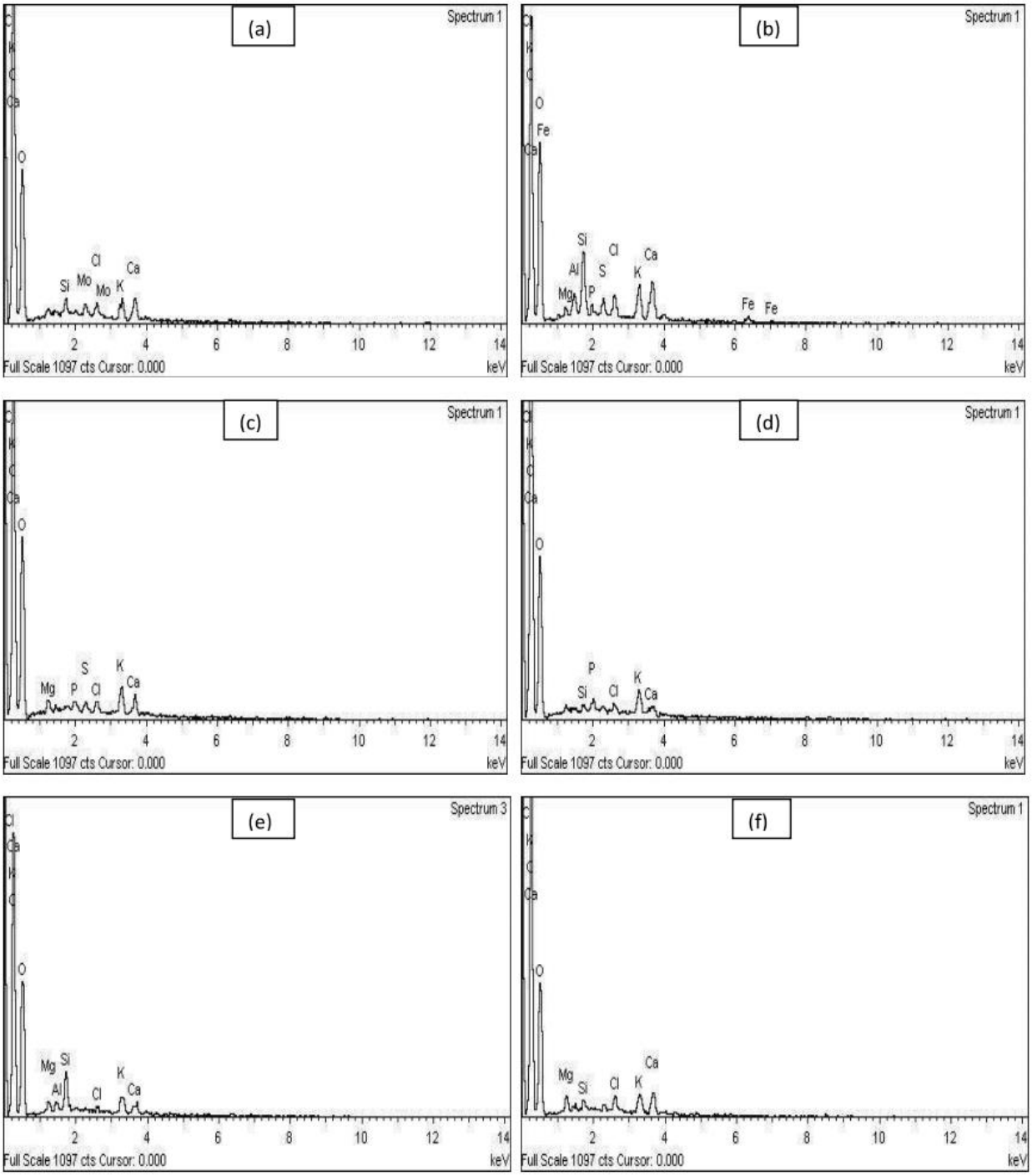



**Fig. 4a.1:** SEM-EDX spectra for elemental analysis of six ethnomedicinal plants of Manipur, (a) *Passiflora edulis* Sims; (b) *Plantago major* L*;* (c) *Clerodendrum glandulosum* Lindl; (d) *Solanum indicum* L*;* (e) *Centella asiatica* (L) Urb*;* (f) *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb.

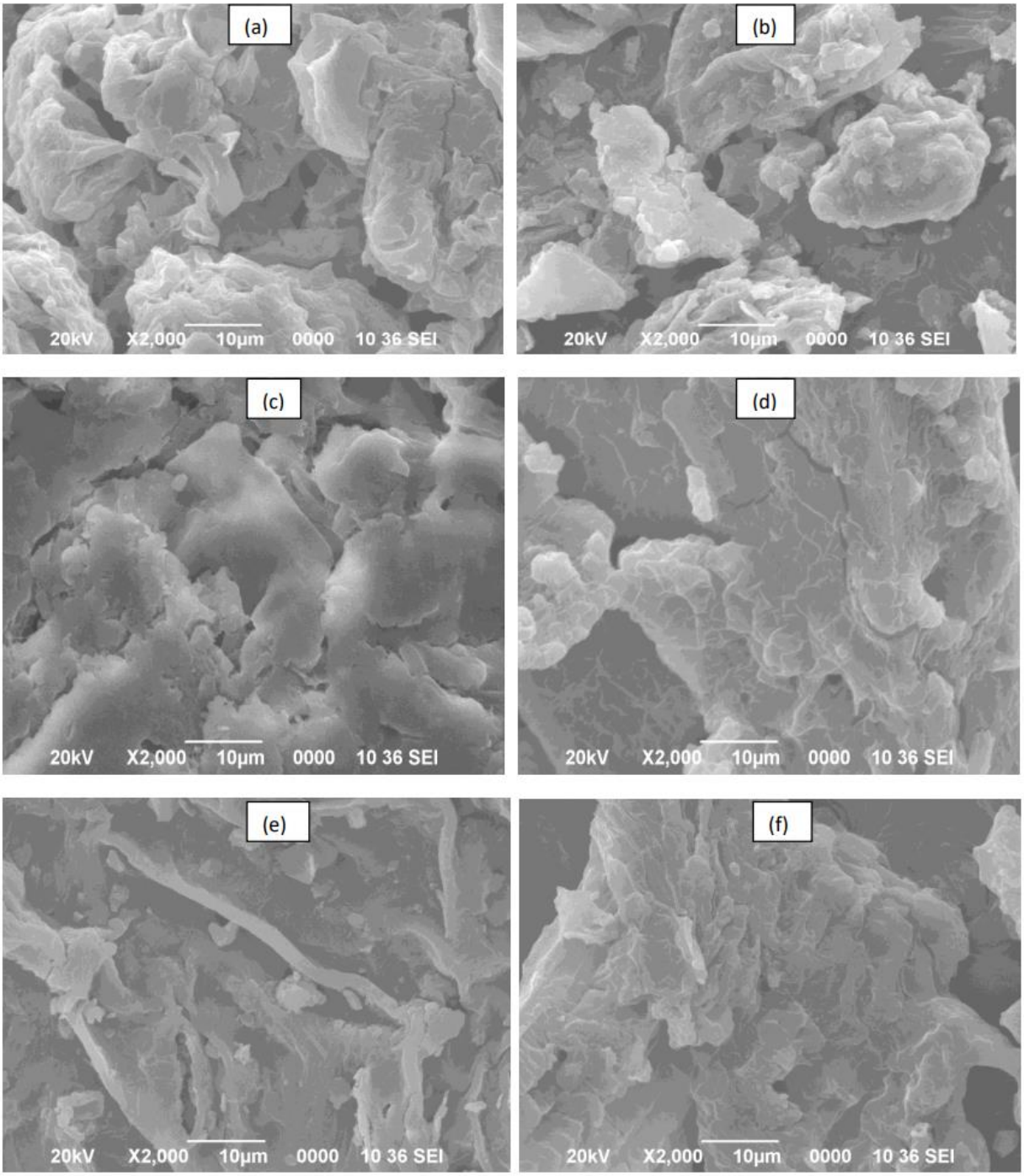



**Fig. 4a.2:** SEM-EDX images of six ethnomedicinal plants of Manipur, (a) *Passiflora edulis* Sims; (b) *Plantago major* L; (c) *Clerodendrum glandulosum* Lindl; (d) *Solanum indicum* L; (e) *Centella asiatica* (L) Urb: (f) *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb.

The Mg content of the various medicinal plant species ranged from 0.29 to 0.62 weight %, however it was not detected in the seeds of *S.kurzii*. This could be due to the greater

concentration of Mg in plant leaves compared to the rest of the plant. Except for *S.kurzii*, which was a seed/berry plant, all of the other plants studied were leaves. *P.major* has the lowest Mg concentration (0.29 wt. %) and *P.thyrsiformis* with the highest (0.62 wt. %). Mg is important for bone and muscle growth and function, as well as the prevention of high blood pressure and depression (Smith & Hammarsten, 1958). It's also involved in the activation of several enzymes, as well as enzyme co-factor kinesis. It also has a significant impact on cholesterol metabolism and heart disease (Yagi et al., 2013).

The Si content ranged from 0.22-2.06 weight percentages. The lowest concentration (0.22 wt. %) was identified in *S.kurzii* seeds, while the maximum concentration (2.06 wt. %) was observed in *C.asiatica* leaves. When compared to the other elements discovered in the various plant species, the amount of Si in *C.asiatica* was found to be extremely high. Si plays an important function in bone development and preservation, according to a growing body of scientific evidence. It also improves the quality of the bone matrix and accelerates bone mineralization (Price et al., 2013). Silicon's role in a range of tissues, including bones, has been the subject of increased investigation since 2002 (Chumlea, 2007).

Phosphorus content was highest (0.37 wt.%) in *S.kurzii* seeds and lowest (0.27 wt.%) in *P.edulis*. This element, however, was not found in two plant species, *P.edulis* and *C.asiatica*, respectively. In mammals, phosphorus is a significant intracellular anion that serves a variety of roles. It keeps blood sugar levels stable and allows for proper cardiac contractions and is required for bone growth and renal function, as well as normal cell growth and repair (Linder, 1991).

Among the six plant species analyzed, sulfur was found in two samples. All four other plant samples were not detected for it. Sulphur *P.major* had 0.55 wt.% and *C.glandulosum* had 0.34 wt.%, respectively. Sulfur is included in a range of metabolites that are vital for cell structure and biological functions. The AAs cystine, cysteine, and methionine include sulphur, which is found in extracellular compartments. Disulfide bridges are formed by the covalent bonding of sulfhydryl groups between molecules, and they are responsible for the tertiary structure of proteins, which is required for the activity of enzymes, insulin, and other proteins. Heparin, chondroitin in bones and cartilage, thiamin, biotin, pantothenic acid, and S-adenosyl methionine are all made up of it (SAM-e). It's also found in glutathione, a powerful antioxidant (Szulc, 1993).

Among the plants studied, *P.major* had the highest chlorine concentration (0.77 wt.%). Cl concentrations range from 0.26-0.77 weight %. With 0.26 wt. percent, the leaves of *C.asiatica* had the lowest quantity of Cl. Cl, along with sodium, is the most critical extracellular anion for maintaining electroneutrality and osmolality. In the extracellular fluid compartment, it is the most common anion linked with sodium (Walker et al., 1990).

After carbon and oxygen, potassium was the most abundant element, with concentrations ranging from 0.86 to 1.28 wt%. The leaves of *P.major* (1.28 wt. %) had the highest concentration of K, whereas the leaves of *P.edulis* had the lowest (0.8 wt. %). The most essential physiological function of K is to regulate the excitability of muscles and neurons. The cardiovascular system is the most vulnerable during periods of potassium deficiency. Cardiac muscle cells rely on their ability to adjust their electrical potentials, as well as the accompanying K flux, when they are subjected to the right stimuli, to cause muscle contraction and nerve conduction. K is the most abundant intracellular cation and aids in maintaining osmotic pressure and pH balance. It may also have a function in intracellular volume regulation, protein and carbohydrate metabolism (Muhammad et. al 2010). Hypertension and sclerosis are greatly reduced as a result of these interrelationships in eating and foods which are excellent sources of potassium helps in the protection of hypertension and helps to improve bone health (Weaver, 2013).

Calcium is another element that was discovered in all of the analyzed samples. The leaves of *P.edulis* had the highest Ca content (1.5 wt. %), whereas the seeds of *S.kurzii* had the lowest (0.33 wt. %). One of the most significant minerals and a major component of the human skeleton is calcium. In humans, it is essential for membrane permeability, muscle contraction, proper nerve impulse transmission, and neuromuscular excitability. Ca is necessary for the formation and maintenance of strong bones and teeth, as well as human blood and extracellular fluids. It is also important for the heart's function and blood coagulation. It assists rennin in the stomach's coagulation of milk. It is also required for the coagulation of blood (Heaney, 1993).

The only elements found in the leaves of *P.major* were iron and bromine. It was not found in any of the other five plant species. The Fe concentration was 0.57 wt. % and the Br content was 0.67 wt. %. Fe is required for the survival of all living species. Almost all cellular and physiological activities rely on Fe-containing proteins and metabolic pathways. Hemoglobin, an erythrocyte protein that transports oxygen from the lungs to the

tissues, contains the majority of Fe in the body. It is also a necessary component of myoglobin, a protein that transports oxygen throughout the body's muscles. It is required for regular cellular functioning, growth, development, and the manufacture of certain hormones and connective tissue (Erdman et al., 2013).

Molybdenum was found in three of the six plant samples viz.*P.major* (1.21 wt. %), *P.edulis* (1.09 wt. %), and *C.glandulosum* (0.73 wt. %). Mo has been shown to be a fascinating trace mineral that is required for life to exist. Sulfite oxidase, xanthine oxidase, aldehyde oxidase, and mitochondrial amidoxime reducing component all require Molybdenum as a cofactor (Novotny, 2011). In addition to various enzymatic reactions, it is linked to electron transport (Chan et al., 1998).

**4a.3.3. Phytochemical analysis**

Preliminary phytochemical screening of ethnolic extracts of the leaves of *Passiflora edulis* Sims*, Plantago major* L*, Clerodendrum glandulosum* Lindl*., Solanum indicum* L, *Centella asiatica* (L) Urb*.,* and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. and are presented in Table 4a.5 and Fig. 4.3. revealed that saponins, tannins, alkaloids, flavonoids, phenolics, and glycosides, were the most abundant compounds (Sunitha and Devaki, 2009; Adom et al., 2017; Adom et al., 2015; Sharma et al., 2017; Saranya et al., 2017; Kumar et al., 2017). Alkaloids were not found in extracts of *Clerodendrum glandulosum* Lindl. and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. Furthermore, glycosides were not found in any of the *Plantago major* L and *Centella asiatica* (L)Urb extract samples. The findings demonstrated that ethnolic extracts from a variety of plants reduce the growth of food pathogens and spoilage bacteria with varying degrees of efficacy. The current study's preliminary findings on most ethnomedicinal plants demonstrate that they all contain phytochemicals. The presence of these bioactive chemicals presents in plants inhibited the development of reference bacterial strains. Many plants have been employed for their antibacterial properties, which are attributed to phytochemicals produced in the plant's secondary metabolism (Medina et al., 2005; Romero et al., 2005). Secondary metabolites found in plants include tannins, alkaloids, phenolic compounds, and flavonoids, which have been shown to exhibit antibacterial activities in vitro (Duraipandiyan et al., 2006; Djeussi et al., 2013). Medicinal plants' antibacterial capabilities are rapidly being reported from all over the world. The rise and spread of multidrug-resistant organisms have put conventional antibiotic therapy in jeopardy. This has forced a search for new antimicrobial

substances, such as plants, which produce a wide range of bioactive chemicals with recognised medicinal capabilities.

**Table 4a.5:** Phytochemical screening of six medicinal plants extracts

| Scientific name | Extraction techniques | Saponins | Tannins | Alkaloids | Flavonoids | Phenolics | Glycosides |
|---|---|---|---|---|---|---|---|
| *Passiflora edulis* Sims | SES | ++ | ++ | + | + | ++ | ++ |
| | UAE | ++ | ++ | ++ | ++ | ++ | ++ |
| | SFE | ++ | ++ | ++ | ++ | ++ | ++ |
| *Plantago major* L | SES | + | ++ | + | ++ | ++ | _ |
| | UAE | ++ | ++ | ++ | ++ | ++ | _ |
| | SFE | ++ | ++ | ++ | ++ | ++ | _ |
| *Clerodendrum glandulosum* Lindl. | SES | ++ | ++ | _ | ++ | ++ | ++ |
| | UAE | ++ | ++ | _ | ++ | ++ | ++ |
| | SFE | ++ | ++ | _ | ++ | ++ | ++ |
| *Solanum indicum* L | SES | ++ | + | ++ | + | + | + |
| | UAE | ++ | + | ++ | ++ | ++ | + |
| | SFE | ++ | + | ++ | ++ | ++ | + |
| *Centella asiatica* (L) Urb. | SES | ++ | + | ++ | ++ | ++ | _ |
| | UAE | ++ | ++ | ++ | ++ | ++ | _ |
| | SFE | ++ | ++ | ++ | ++ | ++ | _ |
| *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. | SES | ++ | ++ | _ | + | ++ | ++ |
| | UAE | ++ | ++ | _ | ++ | ++ | ++ |
| | SFE | ++ | ++ | _ | ++ | ++ | ++ |

+ indicates low detection of phytochemicals agents; ++ indicates abundant detection of phytochemicals agents; – indicate non-detection or absence of phytochemical agents.

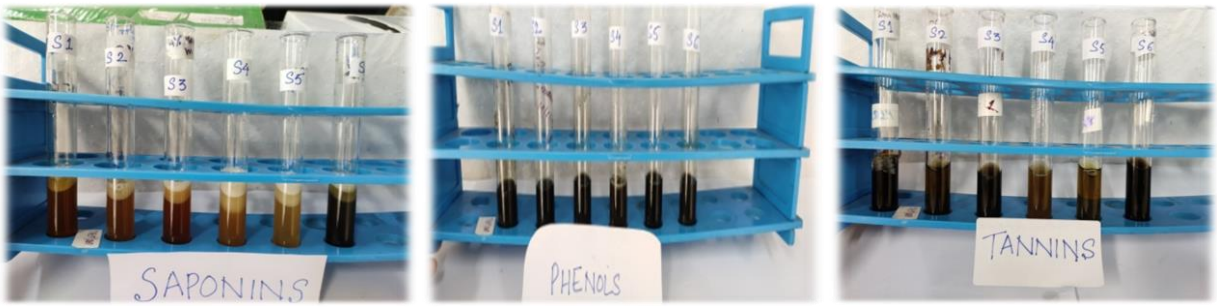



**Fig 4a.3:** Qualitative phytochemicals screening of different extracted samples

### 4a.3.4. Antimicrobial analysis

From the six different medicinal plants (*Passiflora edulis* Sims*, Plantago major* L*, Clerodendrum glandulosum* Lindl.*, Solanum indicum* L*, Centella asiatica* (L) Urb. and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. These bioactive compounds were found to have potential antibacterial activity against the studied human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes***,** *Bacillus cereus*, and *Bacillus subtilis* in most extracts. In terms of ethnolic extracts' antibacterial effectiveness against bacterium reference strains, all plant extracts were found to have some antimicrobial activity against the reference strains. Table 4a.6. presents results on mean zones of inhibition recorded for the ethnolic extracts against the clinical bacteria isolates.

**Table 4a.6:** Antimicrobial analysis of six selected medicinal plants

(A) Zone of inhibition of Extracted sample of *Passiflora edulis* Sims

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control | 20.03±0.38$^{c}$ | 24.21±0.78$^{c}$ | 23.14±1.03$^{c}$ | 18.64±1.45$^{c}$ | 24.21±0.36$^{b}$ |
| Tetracycline 10 mg/disc | | | | | |
| SES sample | 12.97±0.15$^{a}$ | 11.5±0.3$^{a}$ | 11.17±0.66$^{a}$ | 11.5±1.56$^{a}$ | 15.2±0.62$^{a}$ |
| UAE sample | 14.67±1.14$^{ab}$ | 15.5±0.9$^{b}$ | 13.9±1.15$^{ab}$ | 15.63±1.55$^{b}$ | 16.1±0.56$^{a}$ |
| SFE sample | 15.6±0.6$^{b}$ | 16.63±0.75$^{b}$ | 14.93±0.51$^{b}$ | 14.7±0.7$^{b}$ | 17.67±1.40$^{a}$ |

(B) Zone of inhibition of Extracted sample of *Plantago major* L

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control Tetracycline 10 mg/disc | 20.03±0.38$^{b}$ | 24.21±0.78$^{c}$ | 23.14±1.03$^{c}$ | 18.64±1.45$^{b}$ | 24.21±0.36 |
| SES sample | 13.87±1.18$^{a}$ | 10.1±0.61$^{a}$ | 14.07±0.90$^{a}$ | 12.67±0.41$^{a}$ | 12.23±0.25$^{a}$ |
| UAE sample | 12.47±1.50$^{a}$ | 14.1±1.50$^{b}$ | 16.23±0.67$^{b}$ | 14±0.92$^{a}$ | 14.67±0.72$^{a}$ |
| SFE sample | 12.63±0.15$^{a}$ | 12.87±0.35$^{b}$ | 12.07±0.95$^{a}$ | 13.53±1.25$^{a}$ | 14.77±0.67$^{a}$ |

(C) Zone of inhibition of Extracted sample of *Clerodendrum glandulosum* Lindl.

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control Tetracycline 10 mg/disc | 20.03±0.38$^{b}$ | 24.21±0.78$^{b}$ | 23.14±1.03$^{b}$ | 18.64±1.45$^{b}$ | 24.21±0.36$^{b}$ |
| SES sample | 13.1±0.79$^{a}$ | 12.77±0.38$^{a}$ | 13.27±1.81$^{a}$ | 12.4±0.17$^{a}$ | 13.23±1.12$^{a}$ |
| UAE sample | 12.43±0.21$^{a}$ | 14.1±1.42$^{a}$ | 14.4±0.87$^{a}$ | 14.43±1.80$^{a}$ | 15.33±0.81$^{a}$ |
| SFE sample | 14.43±0.21$^{a}$ | 13.6±1.25$^{a}$ | 14±0.72$^{a}$ | 13.97±0.84$^{a}$ | 14.1±0.56$^{a}$ |

(D) Zone of inhibition of Extracted sample of *Solanum indicum* L

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control Tetracycline 10 mg/disc | 20.03±0.38$^{b}$ | 24.21±0.78 $^{b}$ | 23.14±1.03 $^{b}$ | 18.64±1.45$^{b}$ | 24.21±0.36$^{b}$ |
| SES sample | 14.1±0.95$^{a}$ | 12.93±0.31$^{a}$ | 12.97±0.89$^{a}$ | 12.6±1.15$^{a}$ | 13.7±1.3$^{a}$ |
| UAE sample | 13.3±1.82$^{a}$ | 14.53±1.42$^{a}$ | 14.17±0.9$^{a}$ | 13.4±0.69$^{a}$ | 15.03±1.31$^{a}$ |
| SFE sample | 14.17±1.15$^{a}$ | 14.5±1.82$^{a}$ | 15.37±0.90$^{a}$ | 14.6±0.6$^{a}$ | 14.8±0.53$^{a}$ |

(E) Zone of inhibition of Extracted sample of *Centella asiatica* (L) Urb.

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control Tetracycline 10 mg/disc | 20.03±0.38$^{b}$ | 24.21±0.78$^{c}$ | 23.14±1.03$^{c}$ | 18.64±1.45$^{c}$ | 24.21±0.36$^{c}$ |
| SES sample | 13.53±0.61$^{a}$ | 10.57±0.40$^{a}$ | 13.8±0.69$^{a}$ | 10.2±0.61$^{a}$ | 12.1±0.61$^{a}$ |
| UAE sample | 13.87±1.33$^{a}$ | 13.83±0.72$^{b}$ | 15.7±0.89$^{b}$ | 14.03±1.84$^{b}$ | 16.03±0.47$^{b}$ |
| SFE sample | 14±0.7$^{a}$ | 13.1±2.26$^{b}$ | 14.03±0.89$^{ab}$ | 14.7±0.82$^{b}$ | 16.77±2.38$^{b}$ |

(F) Zone of inhibition of Extracted sample of *Phlogocanthus thyrsiflormis* (Roxb. Ex. Hardw.) Mabb

| **Dry extract in solution** | ***E.Coli (ATCT11774)*** | ***S.Aureus (ATCC12600)*** | ***L.Monocytogenes (ATCC13932)*** | ***B.Cereus (ATCC10876)*** | ***B.Subtilis (ATCC9199)*** |
|---|---|---|---|---|---|
| Control Tetracycline 10 mg/disc | 20.03±0.38$^{b}$ | 24.21±0.78$^{c}$ | 23.14±1.03$^{c}$ | 18.64±1.45$^{b}$ | 24.21±0.36$^{b}$ |
| SES sample | 13.47±1.10$^{b}$ | 11.93±0.83$^{a}$ | 13.7±0.98$^{a}$ | 12.47±0.06$^{a}$ | 13.47±1.77$^{a}$ |
| UAE sample | 13.93±0.83$^{a}$ | 14.77±0.37$^{b}$ | 15.93±0.50$^{b}$ | 13.57±1.37$^{a}$ | 15.2±1.2$^{a}$ |
| SFE sample | 13.4±1.65$^{a}$ | 13.13±2.29$^{ab}$ | 14±1.41$^{ab}$ | 12.9±2.08$^{a}$ | 14.83±0.49$^{a}$ |

All the values are represented as means±standard deviation, and values with different superscripts are different at $p<0.05$

Tabulated values are given in diameter (mm) and it includes the agar well (6mm)

*SES – Solvent extracted sample

*UAE-Ultrasound extracted sample

*SFE-Supercritical fluid extracted sample

## 4a.4. Conclusions

The present study was an effort to know the biological properties of medicinal plants by proximate, elemental, phytochemicals and antimicrobial analysis. The extracts had potential phytochemicals and antibacterial properties against the bacterial strains tested, according to the findings. Considering the different nutrients and minerals compositions of the plants analyzed, it has been observed that these plants will help in the maintenance of various diseases. All the elements identified in this study were discovered in trace amounts or below the detection limit. It can be concluded from the present study that the plants will serve as good nutrition and most importantly essential sources of important elements necessary for carrying out many biological and physiological processes necessary for the maintenance of good health in our body. The concentration of elements is so meager that their importance was ignored for a long time. Moreover, SEM-EDX, a versatile tool has been employed for the analysis which is non-destructive and advantageous to most of the methods involved. This study will also serve as a basic and necessary step in the discovery of new drug development. The data obtained from the study can be used to evaluate the potentiality of these plants and explore more pharmacological actions of these plants. We also learned that the plants' antimicrobial characteristics (mostly antibacterial strains) were related to bioactive components found in many ethnomedicinal plants, implying that the medicinal plants tested are a rich source of phytochemicals. Ethanolic plant extracts exhibited good antimicrobial activity. Furthermore, more research can be done to see how successful they are at inhibiting the growth of parasites, viruses, and/or fungi.

# CHAPTER 4 (b)

## Quantitative phytochemicals analysis of the selected six herbal extracts and establishing their antihypertensive potentiality

### 4b.1. Introduction

Many phytochemicals, which are derived from plants, are used in commercial and pharmaceutical products as well as in the environmental and medical fields. Tiny chemical compounds known as secondary metabolites, which essentially have molecular weights of less than 3000 Da, are produced during the growth of plants from primary metabolites. Many plant species have different metabolite types and compositions. Although secondary metabolites make up the majority of the metabolites in natural plant products, there is no apparent distinction between primary and secondary metabolites, making their definition difficult to understand. Secondary metabolites are intriguing for a variety of different reasons, including their structural variety, their potential as therapeutic candidates, and/or their potency as antioxidants. Few examples of the chemical diversity of plant metabolites exist, making them complexes that the industry is unable to synthesise (Sanchez and Demain, 2000; Bourgaud, 2001).

New strategies are required to address problems in clinical therapy and drug development for a number of reasons. The rise of chronic diseases as leading causes of morbidity and mortality in industrialised countries, as well as increasingly in developing ones, may be the most significant factor. Most chronic illnesses don't exist as isolated entities. Instead, diverse illness symptoms are typically caused by several different biological pathways and networks, several etiological variables, and multiple mechanisms (Schadt, 2009; Barabasi et al., 2011). The use of many medications to address diverse targets and symptoms in order to prevent and treat these major chronic diseases has resulted in an increase in the frequency of negative interactions and side effects (Hopkins et al., 2006).

Plants contain polyphenols in abundance, and regular consumption of these compounds has been linked to a lower risk of developing a range of chronic illnesses, including cancer, cardiovascular disease (CVD), and neurological disorders. In recent decades, the chemical molecules called polyphenols, which are widely present in plants, have attracted increasing attention in the field of nutrition. A rising number of studies suggests that consuming

polyphenols may be essential for maintaining good health through controlling metabolism, weight, chronic disease, and cell proliferation. Although there are over 8,000 polyphenols, their short- and long-term health impacts have not yet been adequately described (Lecour and Lamont, 2011). According to epidemiological research, eating a diet high in polyphenols may reduce your chance of developing a number of illnesses, such as cardiovascular disease (CVD), certain types of cancer, and neurological diseases (Kuriyama et al., 2006).

The initial step in recovering essential bioactive compounds from herbal materials is called extraction, and it may be thought of as a mass transport phenomenon where components in a matrix are moved into the solvent (Lee et al., 2011). The consumer preference for natural additives and antioxidants over synthetic ones is related to the increased interest in bioactive substances from fruits and vegetables (Prasad et al., 2009a). Bioactive molecules, including phenolic chemicals, are often secondary metabolites of plants and are present at considerably lower concentrations than constitutive molecules (lipids, proteins, carbohydrates, and minerals). The main issue is that many bioactive substances, including flavonoids and anthocyanins, are found in insoluble structures (such as the vacuoles of plant cells or the bilayers of lipoproteins), making their extraction difficult (Corrales et al., 2008). Several of the organic chemicals found in herbal material are heat-sensitive, losing their integrity and biological activity when heated. The ideal extraction method is flexible, straightforward, secure, reasonably priced, quantitative, non-destructive, and time saving (Zhang et al., 2007; Lee et al., 2011).

In developed countries, cardiovascular disease (CVD), particularly coronary heart disease and stroke, is a leading cause of death. When it comes to the onset, progression, and development of CVD, a variety of hereditary and environmental factors are involved. For instance, smoking, consuming meals high in saturated fat, and not exercising are well-known environmental variables that raise the risk of CVD (Ambrose and Barua, 2004). The frequent consumption of foods high in polyphenols, such as fruits, vegetables, chocolate, tea, and wine, may have cardio-protective effects on people, according to a number of epidemiological and human intervention studies.

Many bacteria species found in the human gut play a crucial role in the metabolism of polyphenols and the health advantages they confer. Together with deglycosylation, the microbial population also exhibits dehydroxylation, demethylation, and degradation of

polyphenols. When these metabolites reach systemic circulation, they go through further metabolism. Polyphenols, which are also linked to human health, can be used to assess the make-up and activity of the gut microbiota. We must thus use in vitro and in vivo investigations to untangle the intricate biological functions of polyphenols and their metabolites in order to better comprehend their advantages for human health (Pathak et al., 2018).

Hydrolysable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthones, fatty acids, terpenoids, alkaloids, oligosaccharides, and peptide amino acids are just a few of the plant chemicals that have been found to have in vitro ACE inhibitory action (Park et al., 2003). Yet, there have only been a few reports of works on bioprospecting plant species targeting this molecular target (Somanadhan et al., 1999; Braga et al., 2000).

Since Manipur is in north-eastern India, which is said to have a rich flora, it may present an exceptional opportunity to discover new leads for the development of antihypertensive medications. This study was carried out to estimate the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities and in-vitro Angiotensin converting enzyme (ACE) of six plant samples from the Churachandpur district in Manipur, India and major phenolic acids present in these plants by RP-HPLC.

## 4b.2. Materials and methods

### 4b.2.1. Selected plants

*Passiflora edulis* Sims (Passifloraceae), *Plantago major* L (Plantaginaceae), *Clerodendrum glandulosum* Lindl. (Lamiaceae), *Solanum indicum* L. (Asteraceae), *Centella asiatica* (L.) Urb (Apiaceae) and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb (Acanthaceae). The plants were collected, identified by a taxonomist at BSI, Shillong and Ethno Medicinal Research Centre, Kangpokpi.

### 4b.2.2. Chemicals

Angiotensin converting enzyme (ACE) from rabbit lung, hippuryl-L-histidyl-L-leucine (HHL), hippuric acid (HA) were purchased from Sigma-Aldrich Co. (England). HCl, acetonitrile, boric acid, *Trifluoroacetic acid* (*TFA*), bmethanol (HPLC grade), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, $Na_2CO_3$, gallic acid,

quercetin, ascorbic acid, $FeSO_4$, EDTA, butylated hydroxyl toluene (BHT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), $FeCl_3$, NaCl, NaOH, Borax and dimethyl sulfoxide (DMSO) were also purchased from Sigma-Aldrich Co. (England). The solvent (solvent) was of analytic grade and procured from Merck, India. Thiobarbituric acid (TBA), linolenic acid, trichloroacetic acid (TCA) and all the high-performance liquid chromatography (HPLC) standards were purchased from Sigma, India.

### 4b.2.3. Extraction of the six selected plant samples

In the current study, three different extraction techniques were employed to extract maximum phytochemicals from the six selected plant samples. The tecnhiques – Solvent extraction, Ultrasound assisted extraction and Supercritical fluid extraction methods were used.

#### 4b.2.3.1. Solvent extraction method

For maximal extraction, the phyto-compounds from six powdered medicinal leaves were extracted in solvents (70%) using ethanol and double-distilled water. The extraction procedure took place over the course of 24 hours in a laboratory-scale shaking incubator (ORBITEK LETTD, India) at a rpm of 200 g at 30 ˚C. The extracts were centrifuged at 5000 g (5 ˚C) and the supernatant was collected, freeze-dried and stored at -20 ˚C for further analysis.

#### 4b.2.3.2. Ultrasound assisted extraction method

Ultrasound treatment for extraction of phenolics from powders of the six different plants were done using a laboratory scale ultrasound assisted system (Ultrasonic Homogenizer, Takashi), which was featured with a digital control panel system for controlling extraction time and ultrasonic power. One gram of the powder was used for solvent based ultrasound assisted extractions in aqueous solutions of ethanol in the same concentrations for all the six plant samples. The conditions of the ultrasound extraction parameters were power level (200W), extraction time (30 mins), solid-liquid ratio (1:30 g/mL), and ethanol concentration (75 %) at room temperature.

#### 4b.2.3.3. Supercritical fluid extraction method

Supercritical fluid extraction method for the plant extraction were conducted using Supercritical fluid extraction system (Waters, USA). The parameters taken for the extraction were 10g plant powder, pressure at 250 bar, temperature at 55°C, $CO_2$ flow rate at 2.25g/min for 30 mins.

### 4b.2.4. Determination of total phenolic content

Following a slight modification of the Folin-Ciocalteau assay (Slinkard and Singleton, 1977), the total phenolic content in the sample extracts was measured. For the analysis, separate test tubes containing 20 L of sample extract, a standard amount of gallic acid, and a blank were used. 1.58 ml of distilled water, 100 L of Folin-Ciocalteau reagent, and 300 L of sodium carbonate were then added to each test tube. The samples were immediately vortexed, and the tubes were incubated at 40°C for 30 minutes in the dark. A UV-Vis spectrophotometer was then used to detect the absorbance at 765 nm. The outcomes were given in mg GAE/100 g.

### 4b.2.5. Determination of total flavonoid content

The aluminium trichloride technique was used to determine the flavonoid concentration (Chang et al., 2002). In a nutshell, 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium trichloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of deionized water were combined with 0.5 ml of the sample extract. The reaction mixture's absorbance was measured at 415 nm against deionized water used as a blank in a UV-Vis spectrophotometer after being incubated at room temperature for 40 min (Cecil, Aquarius 7400). Findings were given as mg QE per 100 g of sample, or quercetin equivalent.

### 4b.2.6. Determination of free radical scavenging activity

#### 4b.2.6.1. DPPH radical scavenging activity

By calculating the rate at which the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical was inhibited, the sample extracts' ability to scavenge radicals was quantified (Brand-Williams et al. 1995). Exact amounts of 100 L of extracts and 1.4 ml of DPPH radical methanolic solution were used (10-4 M). Using a UV-Vis Spectrophotometer, the absorbance at 517

nm was measured after 30 minutes against a blank (100 l of methanol in 1.4 ml of DPPH radical solution). Radical scavenging activity was used to express the results. The radical scavenging efficiency (%) is calculated using Eq. (4b.1)

Radical scavenging activity (%) = [(Ao-As)/Ao] 100 (4b.1)

where, As is the sample extract's absorbance and Ao is the absorbance of the control blank.

The method described by Wolfenden and Willson (1982) was used to conduct the ABTS assay of the plant extracts. The Benzie and Strain (1996) method for ferric reducing antioxidant power measurement was used. The increased conversion of ferric to ferrous ions served as a gauge for the reducing power. Benzie and Strain (1999) used the ferric reducing ability of plasma FRAP assay as a gauge of antioxidant strength to calculate a sample's overall antioxidant capacity. The assay was based on a compound's reducing power (antioxidant). The ferric ion (Fe3+) will be converted to the ferrous ion (Fe2+) by a potential antioxidant. The latter produces a blue complex (Fe2+/TPTZ), which raises the absorbance at 593 nm. The acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl3 were combined at a ratio of 10:1:1 (v/v/v) to create the FRAP reagent. Each well received equal amounts of the reagent (3.4 L) and sample solutions (100 L), which were then well mixed. After 30 minutes, the absorbance was measured at 593 nm.

### 4b.2.7. Fourier transform infrared (FT-IR) spectra of different plant extracts

Spectroscopic analysis using FTIR maybe one of the most effective techniques for determining the kinds of chemical bonds (functional groups) present in compounds is the Fourier transform infrared spectrophotometer (FTIR). For the FTIR study, dried powders of various solvent extracts of each plant material were employed. To create a translucent sample disc, 2 mg of the dried extract powder was encapsulated in 50 mg of KBr pellet. Each plant specimen's powdered sample was placed into an FTIR Spectroscope (Nicolet Impact 410, UK) with an $8cm^{-1}$ resolution and a scan range of 4000 to 500 $cm^{-1}$.

### 4b.2.8. Determination of ACE inhibitory activity

The spectrophotometric approach described by Wu et al., (2002) was adapted for the purpose of measuring the ACE inhibitory activity. To achieve a concentration of

100mU/ml, commercial ACE (1 unit) was diluted in 50 mM Tris-HCl (pH: 7.5) containing 300 mM NaCl. Afterwards, 100-L aliquots were kept chilled at -20°C. Borate was used as the control, and 25 mL of plant extracts at various concentrations were added to a 100 mL solution of 5 mM Hippuryl-L-Histidyl-L-Leucine (HHL). In a pH 8.3 buffer comprising 300 mM NaCl and 100 mM Na-borate, plant extracts and HHL were produced. 10 L of ACE (100 mU/ml) was added after 10 min of 37 °C incubation, and samples were then incubated for 30 min at 37 °C with constant stirring. 100 mL of 1MHCl was added to halt the enzyme process. To separate the product and hippuric acid (HA) from HHL, the solution was filtered via a 0.45 m nylon syringe filter and then injected straight onto an asymmetric shield C18 column (4.6 mm 250 mm, 5 m, Waters). At a flow rate of 1 ml/min, 50% methanol in water (v/v) containing 0.1% TFA was used to elute the column. The absorbance was then measured at 228 nm. The comparison of the concentration of HA in the presence or absence of an inhibitor (control sample) served as the basis for the assessment of ACE inhibition. The HA peak areas obtained in the two cases were assessed following injection in HPLC of the control and the inhibitor-assisted test. ACE inhibition % can be calculated using the Eq. (4b.2)

$$\text{ACE inhibition \%} = (B\text{-}A/B\text{-}C) \times 100 \qquad 4b.2$$

where A is the relative area of the HA peak produced when an ACE inhibitor component is present, B is the relative area of the HA peak produced when ACE inhibitors are absent, and C is the relative area of the HA peak produced when ACE inhibitors are absent (corresponding to HHL autolysis during enzymatic assay).

### 4b.2.9. RP-HPLC study of the polyphenols

The sample extract was prepared by extracting plant materials utilising solvent, ultrasonic, and supercritical fluid extraction methods in 70% ethanol. The extract was redissolved in 1 ml of HPLC-grade methanol, vacuum-evaporated, and filtered through a 0.22 m nylon filter (Himedia, India). The main phenolic acid composition of the examined samples was determined using the RP-HPLC (Waters system) gradient elution method. We employed a binary pump (Waters, 1525) and a UV-VIS detector (Waters, 2489) on a Symmetry 300TM C18 (5 m, 4.6 X 250 mm) column. Acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol were the mobile phases utilised (mobile phase B). Following the gradient approach of Saikia et al. (2015), the column was washed with 65% A (35-39 min), followed by 80% A (0-8 min), 65% A (9-12 min), 45% A (13-16 min), 30% A (17-20 min), 20% A (21-30 min), and 10% of A (31-

34 min) (40-45 min). 20 l of sample volume were used. The UV-Vis detector was employed at wavelengths of 254 nm and 325 nm, and the flow rate was kept constant at 0.8 ml/min. Gallic acid, narigenin, catechin, chlorogenic acid, caffeic acid, apigenin, syringic acid, ferulic acid, coumaric acid, rutin hydrate, kaempferol, and quercetin were the benchmarks utilised for comparison and identification.

### 4b.3. Results and discussion

#### 4b.3.1. Phytochemical properties of the selected plant samples

The leaves of *Clerodendrum glandulosum* Lindl. were extracted using ethanol as the extracting solvent in an ultrasound-assisted method, and the highest values of TPC and TFC were found to be 328±17.51 mg GAE/ 100 g and 169±39.37 mg QE/100 g, respectively. *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb extracts (UAE6) were closely behind (Table 1). In all of the samples, the levels were lower in samples extracted using solvent extraction techniques. Comparing samples extracted using solvent and supercritical procedures to those extracted using ultrasound aided methods, the highest values for TPC were seen in all of the extracted samples.

Table 4b.1 displays the ability of each extract to scavenge DPPH free radicals. At 80.05±5.31% at 100 g/mL, ultrasound-assisted extraction samples for *Clerodendrum glandulosum* Lindl. Mabb showed the highest activity. *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) sample showed comparable DPPH free radical scavenging activity, with the maximum activity detected at 100 g/mL (74.87±4.62%). For samples that were extracted using a solvent, all plants yielded lower results. Our findings indicated the extract's outstanding antioxidant capability by showing its ability to scavenge the DPPH radical.

According to estimates made using the FRAP test, the reduction activities of the ultrasound-assisted extracts were highest in *Clerodendrum glandulosum* Lindl. (UAE3) and lowest in SES2 *Plantago major* L. These values were found to be 1348.07±7.23 µM/100g and 419.14±10.73 µM/100g, respectively. An essential factor in determining the antioxidant activity of phenolics is the transformation of Fe (III) to Fe (II). The ABTS radical scavenging activity is shown in Table 4b.1. For *Clerodendrum glandulosum* Lindl. (UAE3 & SFE3), the samples extracted using the ultrasound assisted and supercritical fluid extractions approach had the maximum activity (91.56±3.53%). The figures were in line with the reference BHT standard (92.72±0.75 %). The samples extracted using the solvent extraction method gave the lowest values.

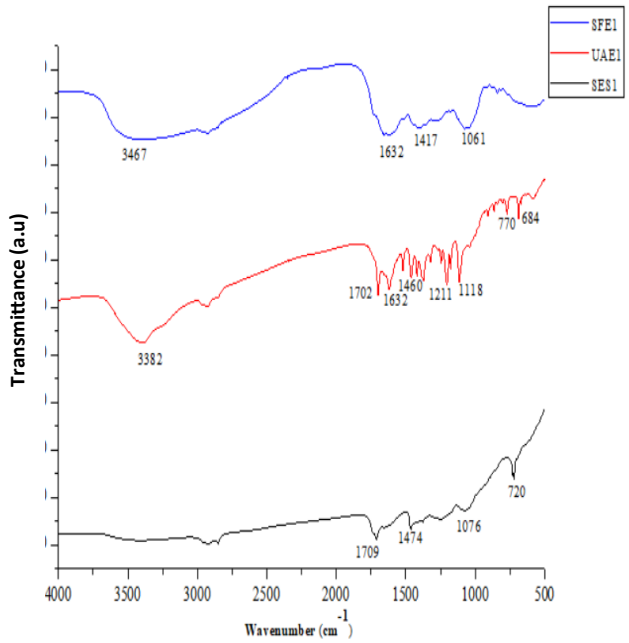
**Table 4b.1:** TPC, TFC, DPPH, ABTS and FRAP in dried extracts of eighteen different extracts of the six plants (Mean ± standard deviation)

| | TPC (mgGAE/100g) | TFC (mgQE/100g) | DPPH (%) | FRAP (µM/100g) | ABTS (%) |
|---|---|---|---|---|---|
| *Passiflora edulis* Sims | | | | | |
| SES1 | 130.69±8.02$^{cd}$ | 92.92±10.28$^{a}$ | 48.33±5.09$^{b}$ | 515.93±7.41$^{b}$ | 60.63±7.96$^{bc}$ |
| UAE1 | 227.43±12.53$^{fg}$ | 128.95±11.76$^{a}$ | 70.84±1.92$^{a}$ | 947.17±14.42$^{i}$ | 69.78±9.35$^{cd}$ |
| SFE1 | 223.22±22.3$^{f}$ | 119.66±16.32$^{a}$ | 72.22±7.48$^{hij}$ | 932.31±14.13$^{hi}$ | 68.47±2.69$^{cd}$ |
| *Plantago major* L | | | | | |
| SES2 | 86.27±5.47$^{a}$ | 74.03±23.08$^{a}$ | 38.25±6.06$^{a}$ | 419.14±10.74$^{a}$ | 49.75±6.31$^{a}$ |
| UAE2 | 141.76±8.35$^{d}$ | 113.28±9.21$^{a}$ | 61.96±3.03$^{def}$ | 914.03±7.91$^{gh}$ | 59.11±2.23$^{b}$ |
| SFE2 | 107.28±10.07$^{b}$ | 96.04±6.73$^{a}$ | 58.21±4.66$^{cde}$ | 779.79±10.89$^{e}$ | 58.36±4.61$^{ab}$ |
| *Clerodendrum glandulosum* Lindl. | | | | | |
| SES3 | 170.08±15.54$^{e}$ | 124.92±5.52$^{a}$ | 67.90±6.19$^{fgh}$ | 811.19±17.66$^{f}$ | 60.75±3.44$^{bc}$ |
| UAE3 | 328.03±17.51$^{j}$ | 169.47±9.37$^{a}$ | 80.05±5.31$^{ij}$ | 1348.07±7.23$^{n}$ | 91.56±3.53$^{f}$ |
| SFE3 | 252.07±7.75$^{h}$ | 131.52±7.75$^{a}$ | 77.24±3.58$^{j}$ | 1036.79±3.53$^{jk}$ | 91.56±3.53$^{f}$ |
| *Solanum indicum* L | | | | | |
| SES4 | 138.61±3.41$^{d}$ | 91.88±5.65$^{a}$ | 48.38±4.13$^{b}$ | 712.38±6.20$^{d}$ | 57.11±4.52$^{ab}$ |

| | | | | | |
|---|---|---|---|---|---|
| UAE4 | 244.43±8.58$^{h}$ | 144.15±11.02$^{a}$ | 68.21±2.86$^{fgh}$ | 1131.91±7.44$^{l}$ | 76.15±4.66$^{de}$ |
| SFE4 | 169.94±7.19$^{e}$ | 112.64±3.22$^{a}$ | 62.93±4.34$^{efg}$ | 888.68±12.81$^{g}$ | 68.99±2.63$^{cd}$ |
| *Centella asiatica* (L.) Urb | | | | | |
| SES5 | 121.79±3.71$^{bc}$ | 88.36±5.48$^{a}$ | 54.41±3.75$^{bcd}$ | 527.42±8.01$^{b}$ | 54.46±3.19$^{ab}$ |
| UAE5 | 281.13±3.79$^{i}$ | 149.92±7.66$^{a}$ | 60.77±5.09$^{cdef}$ | 1010.42±17.14$^{j}$ | 80.72±6.14$^{e}$ |
| SFE5 | 182.74±2.95$^{e}$ | 141.61±6.71$^{a}$ | 55.06±3.23$^{bcde}$ | 803.82±8.47$^{ef}$ | 67.96±3.79$^{cd}$ |
| *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb | | | | | |
| SES6 | 146.52±12.38$^{d}$ | 116.71±3.55$^{a}$ | 53.28±3.09$^{bc}$ | 655.85±6.93$^{c}$ | 56.49±2.71$^{ab}$ |
| UAE6 | 286.19±11.74$^{i}$ | 165.79±6.9$^{a}$ | 74.87±3.04$^{hij}$ | 1254.42±17.64$^{m}$ | 90.42±3.64$^{f}$ |
| SFE6 | 280.99±4.25$^{i}$ | 149.93±3.82$^{b}$ | 74.63±4.62$^{hij}$ | 1062.54±18.67$^{k}$ | 74.55±3.74$^{de}$ |

### 4b.3.2. FTIR spectral analysis of the six different plant extracts

The FTIR spectrum of the leaves of *Passiflora edulis* Sims, *Plantago major* L*, Clerodendrum glandulosum* Lindl*., Solanum indicum* L*., Centella asiatica* (L.) Urb., *and Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb*,* which were extracted using various extraction techniques, is shown in Fig. 4b.1. Based on the peak values in the region of IR radiation, the FT-IR spectrum is used to identify the functional groups of the active components present in the extract. As the concentrate is introduced to the FTIR, the practical groups of the components are separated, providing a clear glimpse of its peak percentage. We compared the vibration assignments for both stretching and bending to information from the literature (Szymanska-Chargot and Zdunek, 2013). The distinct eight locations in the MIR domain were identified with reference to the literature (Zavoi et al., 2011), and the fingerprint region was located between 900 and 1760 $cm^{-1}$. The ethanolic extracts of the plants contained N-H, O-H, C=C, C-H, C-O, and $CH_3$ functional groups, according to the results of FT-IR analysis. The objective of this investigation is to identify the functional groups present in the various extracts. Except for solvent extracted samples of *Passiflora edulis* Sims (SES1), *Plantago major* L (SES2), and *Solanum indicum* L, the strong instance peaks are identified at 3396-3467 $cm^{-1}$ and are ascribed to the H-bonded and O-H stretching vibration (SES4). Area A (1000 $cm^{-1}$) corresponds to out-of-plane C-H bending vibrations, area B (997-1140 $cm^{-1}$) to the absorptions of stretching C-O (mono-, oligo-, and polysaccharides) vibrations at 1047, 1054, 1069, 1076, and 1118 cm-1, and area C (1150-1270 $cm^{-1}$) to the stretching vibrations of carbonyl C-O.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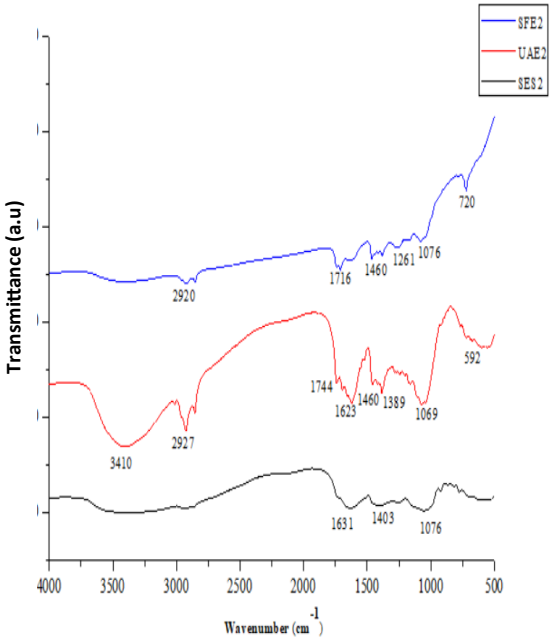
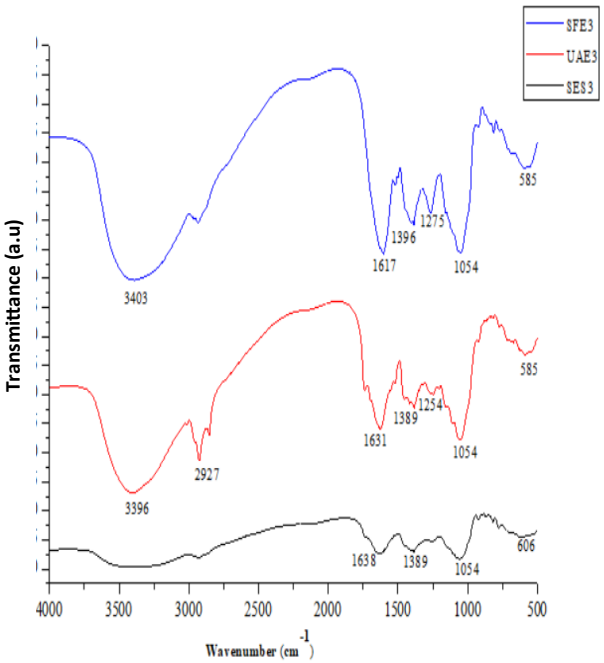
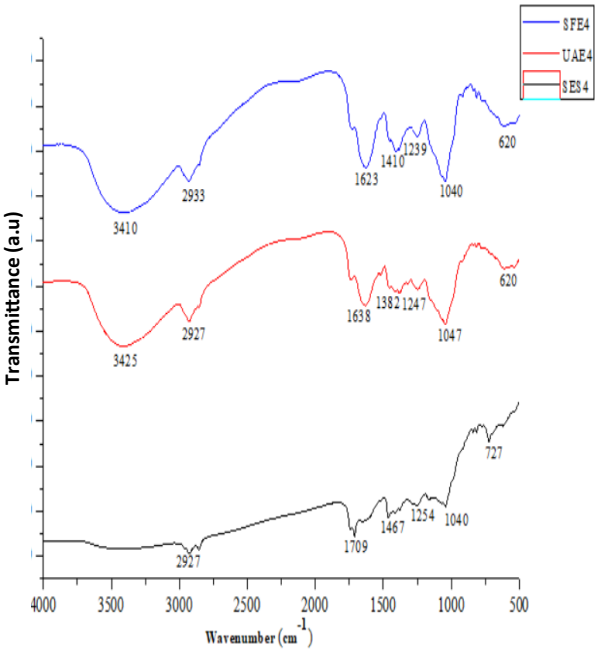
UAE1
SES1
3467
1632
1417
1061
3382
1702
1632
1460
1211
1118
770
684
1709
1474
1076
720
Transmittance (a.u)
Wavenumber (cm-1)


SFE2
UAE2
SES2
2920
1716
1460
1261
1076
720
3410
2927
1744
1623
1460
1389
1069
592
1631
1403
1076
Transmittance (a.u)
Wavenumber (cm-1)


SFE3
UAE3
SES3
3403
1617
1396
1275
1054
585
3396
2927
1631
1389
1254
1054
585
1638
1389
1054
606
Transmittance (a.u)
Wavenumber (cm-1)


SFE4
UAE4
SES4
3410
2933
1623
1410
1239
1040
620
3425
2927
1638
1382
1247
1047
620
1709
1467
1254
1040
727
2927
Transmittance (a.u)
Wavenumber (cm-1)

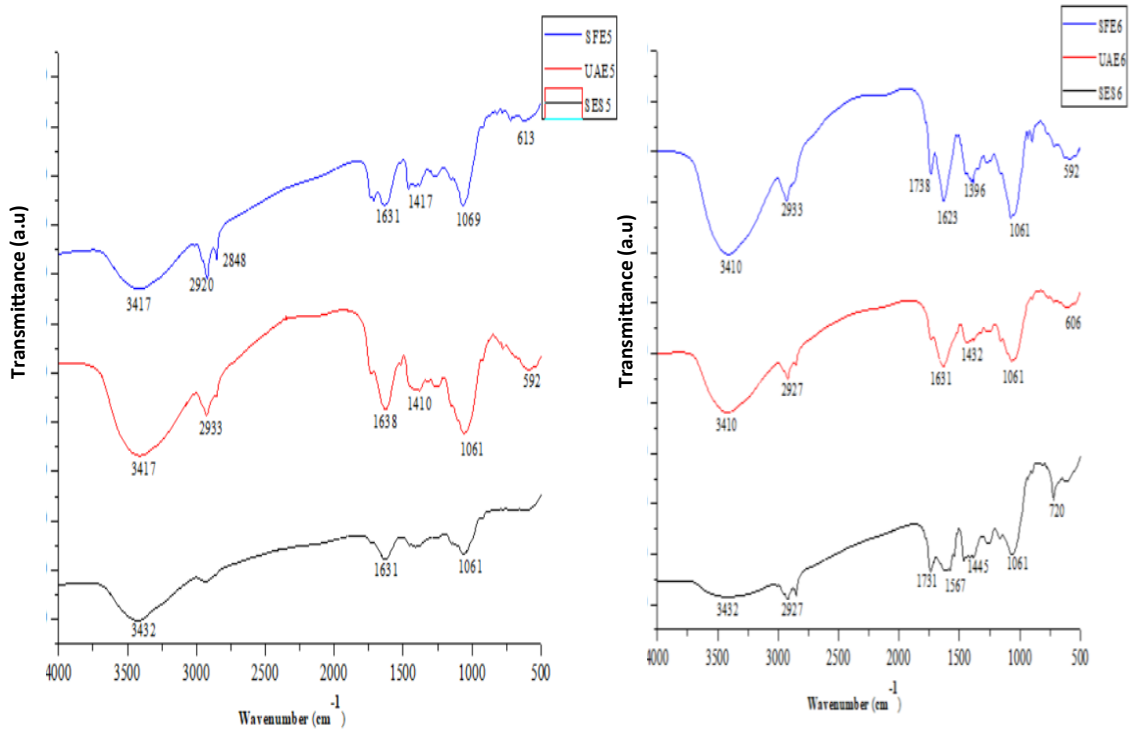



**Fig. 4b.1:** FTIR spectra of solvent, ultrasound assisted, and supercritical fluid extraction extracted samples

Region E (1500-1600 $cm^{-1}$) corresponds to aromatic domain and N-H bending vibrations, while area D (1300-1450 $cm^{-1}$) corresponds to stretching vibrations of CO (amide) and C-C from phenyl groups. Area E (1600-1760 $cm^{-1}$) is a complicated region that corresponds to bending vibrations of N-H (amino acids), C=O of related fatty acids (1710 $cm^{-1}$), and C=O of ester, such as glycerides (1740 $cm^{-1}$). Region F (2800-2900 $cm^{-1}$) corresponds to C-H stretching vibrations that are unique to methoxy derivatives of CH3 and CH2 from lipids. Stretching vibrations of OH groups correspond to Area G (3350-3600 $cm^{-1}$) in space (from water, alcohols, phenols, carbohydrates, peroxides). No absorbance between 2220 and 2260 $cm^{-1}$ suggests that there are no cyanide groups in any of the extracts. These findings demonstrate that there are no hazardous chemicals in any of the eighteen plant extracts.

### 4b.3.3. RP-HPLC study of the polyphenols

The various polyphenols present in the *Passiflora edulis* Sims, *Plantago major* L, *Clerodendrum glandulosum* Lindl., *Solanum* kurzii, *Centella asiatica* (L.) Urb. and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. has been evaluated using RP-

HPLC (Reverse phase-high performance liquid chromatography). The retention time of the standard polyphenols has been recorded a

**Table 4b.2.** List of standards and their retention time

| Lists of HPLC standards | Retention time (mins) |
|---|---|
| Gallic acid | 6.83 |
| Catechin | 14.56 |
| Chlorogenic acid | 16.83 |
| Syringic acid | 17.54 |
| Ferulic acid | 19.44 |
| Rutin hydrate | 20.56 |
| Quercetin | 23.04 |
| Naringenin | 23.27 |
| Kaempferol | 25.45 |
| Apigenin | 25.28 |
| Coumaric acid | 27.89 |

254nm (Table 4b.2.), and the quantities of the polyphenols present in the six plant extracts are presented in Table 4b.3. The most widely used method for analyzing the polyphenols in various foods is RP-HPLC with C18 columns, despite the fact that procyanidin separation is subpar (Suarez et al., 1996). Since all phenolic chemicals exhibit strong UV absorption, a UV-vis multiwavelength detector was employed. The current technique is straightforward, user-friendly, and sufficient for identifying and quantifying the main phenolic chemicals in aromatic plants. Other writers have reported using a similar technique to examine the main flavonoid aglycons (Mattila et al., 2000; Justesen and Knuthsen, 2001). Figure 4b.2 shows a typical HPLC profile of a plant's phenolic components under analysis. Eleven phenolic acids were identified in the plant samples viz. apigenin (RT= 25.28 min), catechin (RT= 14.56 min), chlorogenic acid (RT= 16.83 min), coumaric acid (RT= 27.89 min), ferulic acid (RT= 19.44 min),gallic acid (RT= 6.83 min),kaempferol (RT= 25.45 min),naringenin (RT= 23.27 min), quercetin (RT= 23.04 min), rutin (RT= 20.56 min) and syringic acid (RT= 17.54 min). Only the 254 nm peak of the chromatographs was taken into consideration and reported (Table 4b.3 and Fig. 4b.3).

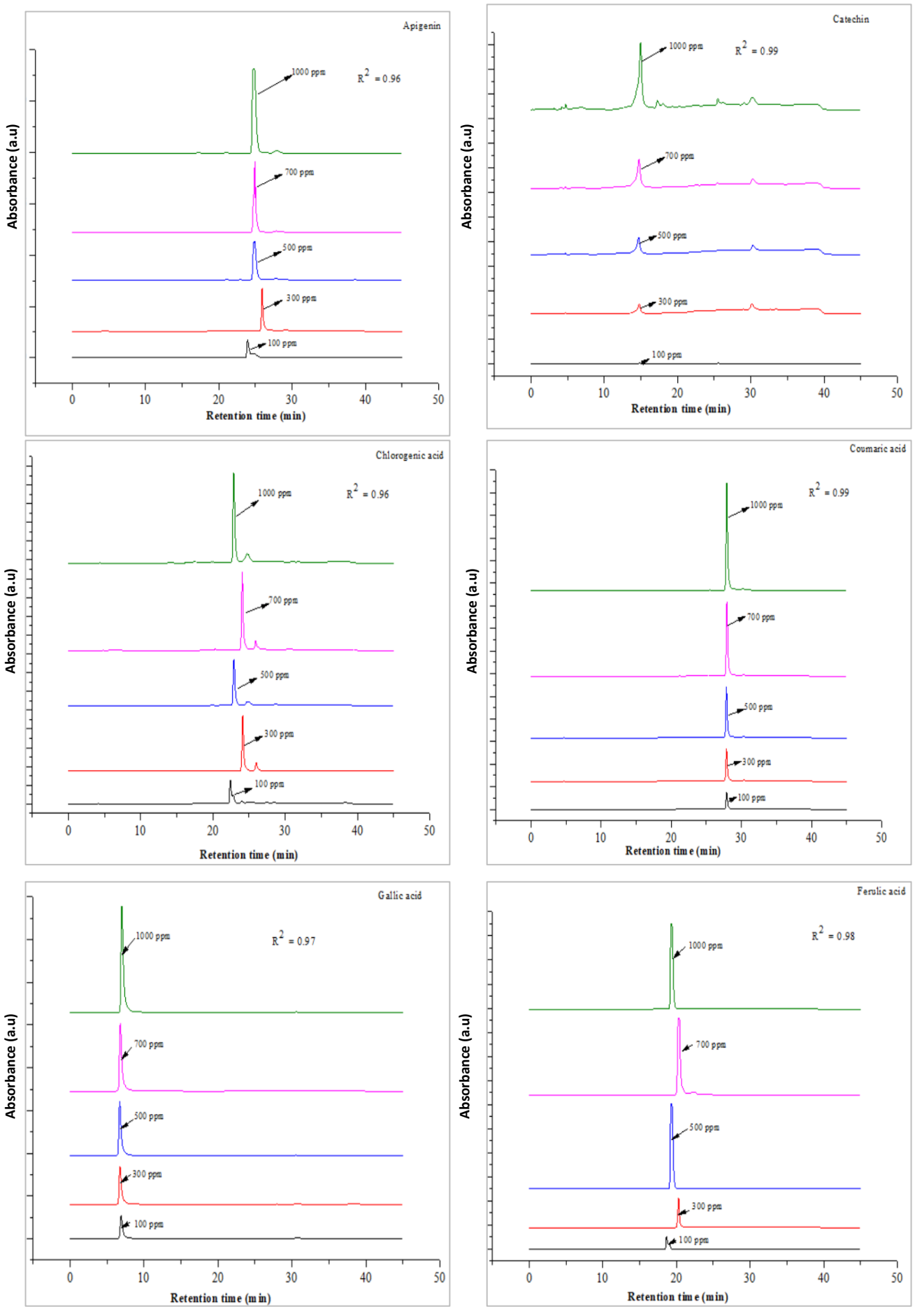
Table 4b.3. Content of phenolic acids in 18 plant extracts are determined by RP-HPLC expressed in mg/100g (fresh weight)

| Sample name | AP (a) | CT (b) | CGA (c) | CMA (d) | FA (e) | GA (f) | KF (g) | NG (h) | QC (i) | RH (j) | SA (k) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SES1 | 1.48±0.03 | ND | ND | 2.47±0.05 | 8.75±0.48 | 0.5±0.7 | ND | ND | ND | 4.17±1.78 | 1.86±0.38 |
| UAE1 | ND | ND | ND | 1.81±0.09 | 2.25±0.35 | 0.07±0.02 | 2.54±1.23 | ND | 2.47±2.61 | ND | 1.54±0.39 |
| SFE1 | ND | 1.06±0.06 | ND | 0.79±0.02 | 2.91±0.03 | 0.25±0.04 | 1.9±0.93 | ND | 0.77±0.62 | 2.45±2.22 | 1.63±1.25 |
| SES2 | ND | 3.12±0.08 | ND | 1.48±0.04 | ND | 0.04±0.02 | 1.3±1.67 | 2.96±1.51 | ND | ND | 4.72±0.38 |
| UAE2 | ND | 26.26±2.32 | ND | 1.18±0.15 | ND | 1.2±1.59 | ND | ND | ND | ND | 2.5±1.72 |
| SFE2 | 0.15±0.05 | 2.41±0.11 | ND | 1.20±0.03 | ND | 0.31±0.19 | ND | ND | 1.11±1.13 | 1.36±1.68 | 3.85±0.83 |
| SES3 | 0.78±0.02 | ND | ND | 4.78±1.74 | ND | 1.37±0.12 | 0.39±0.27 | ND | ND | 1.37±1.32 | 1.73±1.14 |
| UAE3 | 1.3±0.04 | ND | ND | 2.39±1.75 | ND | 0.91±0.16 | ND | ND | ND | 1.1±1.17 | 8.32±1.11 |
| SFE3 | 2.01±1.96 | ND | ND | ND | ND | 0.79±0.33 | ND | 2.66±1.91 | 1.86±1.33 | 1.64±1.57 | 10.05±2.44 |
| SES4 | ND | 4.11±1.13 | 0.22±0.12 | 1.68±0.17 | 1.6±0.09 | 0.56±0.2 | 1.01±0.32 | ND | ND | 1.49±1.97 | 1.82±0.84 |
| UAE4 | 0.76±0.05 | 2.5±0.04 | 0.15±0.01 | 0.2±0.04 | 0.72±0.05 | 0.41±0.16 | ND | ND | ND | 1.53±2.12 | 1.43±1.83 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SFE4 | 0.71±0.04 | 0.89±0.07 | ND | 0.69±0.02 | ND | 0.24±0.11 | ND | ND | 1.84±2.51 | ND | 0.88±0.23 |
| SES5 | ND | ND | 0.73±0.02 | 1.49±0.09 | ND | 0.11±0.08 | 5.66±2.19 | 3.49±2.87 | 1.67±1.9 | 1.68±1.39 | ND |
| UAE5 | 0.27±0.05 | 4.35±0.21 | ND | 0.29±0.07 | 5.81±0.42 | 0.14±0.05 | 1.29±1.64 | ND | 1.94±2.35 | ND | 0.75±0.94 |
| SFE5 | 0.38±0.1 | 3.67±0.02 | ND | 1.19±0.05 | 10.99±0.04 | 0.16±0.09 | ND | 5.24±1.08 | 2.11±2.12 | ND | 1.4±1.45 |
| SES6 | 0.71±0.07 | 2.89±1.25 | 0.9±0.09 | 1.15±0.21 | 60.39±0.94 | 0.14±0.13 | ND | 3.07±0.92 | ND | 2.7±2.32 | 4.36±2.64 |
| UAE6 | 0.24±0.02 | 11.44±0.27 | ND | 0.71±0.02 | 45.52±0.74 | 0.17±0.09 | 1.05±1.38 | ND | ND | 0.74±0.35 | ND |
| SFE6 | ND | 3.69±0.04 | ND | 1.01±0.08 | 11.5±0.54 | 0.13±0.05 | 2.15±1.76 | ND | ND | 1.6±1.25 | 0.66±0.82 |

AP-Apigenin; CT-Catechin; CGA-Chlorogenic acid; CGA-Coumaric acid; FA-Ferulic acid; GA-Gallic acid; KF-Kaempferol; NG-Naringenin; QC-Quercetin; RH-Rutin hydrate & SA-Syringic acid.

Apigenin could not be found in seven samples, however it was present in eleven extract samples. SFE3 had the highest concentration (2.01±1.96mg/100g), and SFE2 had the lowest concentration (0.15±0.05 mg/100g). Twelve samples tested positive for catechin, whereas just six samples did not. UAE2 had the highest concentration (26.26±2.32 mg/100g), and SFE4 had the lowest (0.89±0.07 mg/100g). Most plant extracts did not contain chlorogenic acid, and just four samples (SES4, UAE4, SES5, and SES6) were discovered to contain it. Except for SES3 with 4.78±1.74 mg/100g, nearly no plant extracts contained coumaric acid. Ten samples contained ferulic acid, while only eight samples did not. It was discovered to be exclusively high in plant extract sample SES6 (60.39±0.94 mg/100g) and lowest in UAE4 (0.72±0.05 mg/100g). Gallic acid, in contrast to other phenolics, was found in all plant extracts, ranging from (0.07±0.02) to 1.37±0.12 mg/100g. All of the plant samples included phenolics, but gallic acid was found to be the most prevalent. SES3 had the highest concentration (1.37±0.12 mg/100g), whereas UAE1 had the lowest (0.07±0.02 mg/100g). Nine samples had kaempferol present, while nine samples had it lacking. SES5 had the highest concentration (5.66±2.19 mg/100g), whereas SES3 had the lowest concentration (0.39±0.27 mg/100g). While Naringenin was found to be highest in SES5 (3.49±2.87 mg/100g). The concentration of quercetin was observed to range from 0.77±0.62 mg/100g to 2.47±2.61 mg/100g. Twelve samples were found to contain rutin hydrate, with SES1 having the highest concentration (4.17±1.78 mg/100g) and UAE6 having the lowest (0.74±0.35 mg/100g). Except for two samples, all the samples contained syringic acid. The concentration ranges from 0.66±0.82 to 10.05±2.44 mg/100g, with sample SFE3 having the highest level and SES4 having the lowest. The phenolic composition of plants clearly varies from those mentioned above. Due to their chemical complexity, phenolic compounds in plant material are challenging to isolate and quantify. Most of the plant extracts under investigation have very few papers available. The observed changes in phenolic levels are thought to be caused by harvesting timing and storage conditions. With a few exceptions, the plants examined in this study are generally good sources of phytochemicals and ought to be consumed because of their benefits to health.


Apigenin
1000 ppm
$R^2$ = 0.96
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)
Catechin
1000 ppm
$R^2$ = 0.99
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)
Chlorogenic acid
1000 ppm
$R^2$ = 0.96
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)
Coumaric acid
1000 ppm
$R^2$ = 0.99
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)
Gallic acid
1000 ppm
$R^2$ = 0.97
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)
Ferulic acid
1000 ppm
$R^2$ = 0.98
700 ppm
500 ppm
300 ppm
100 ppm
Absorbance (a.u)
Retention time (min)

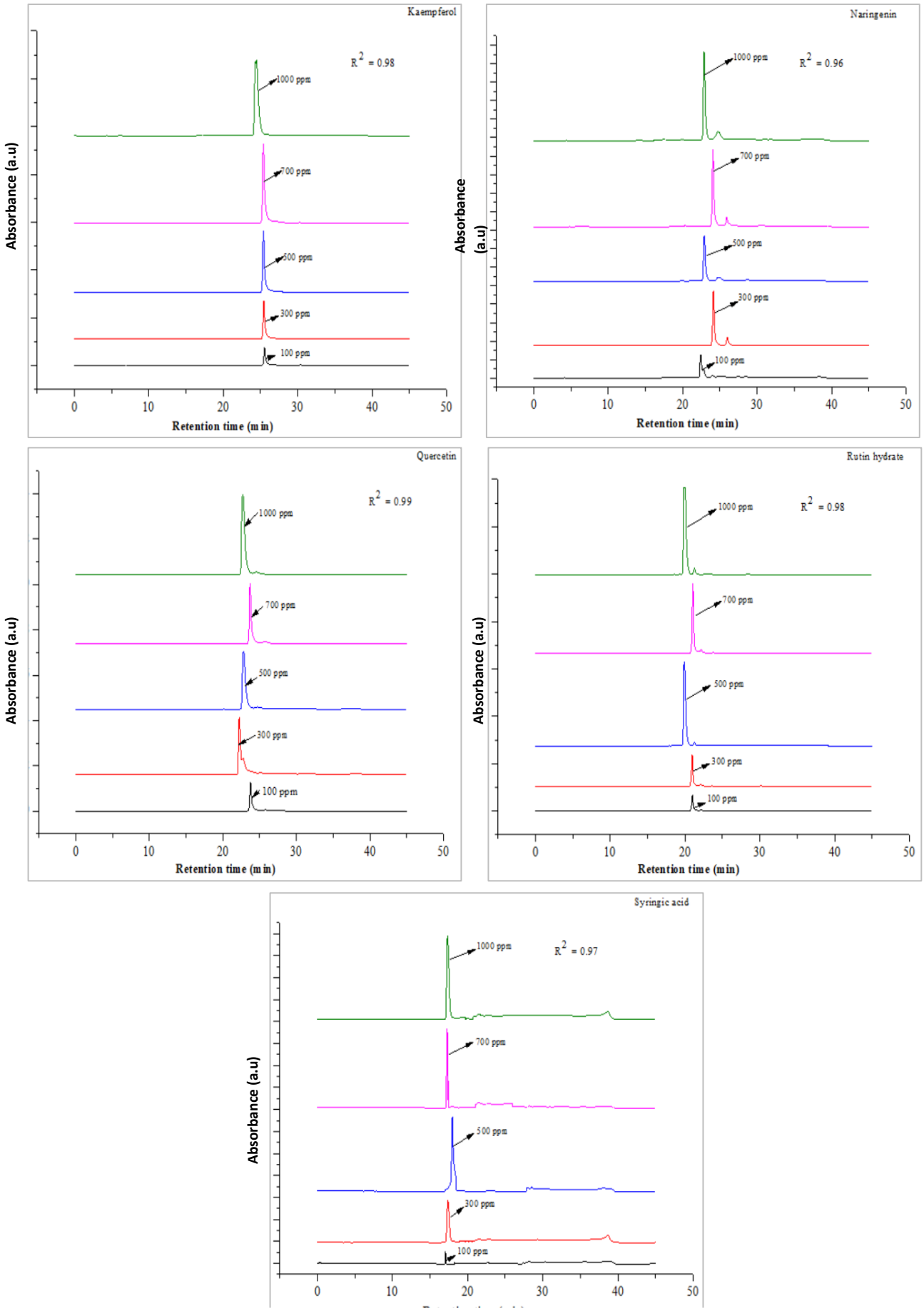
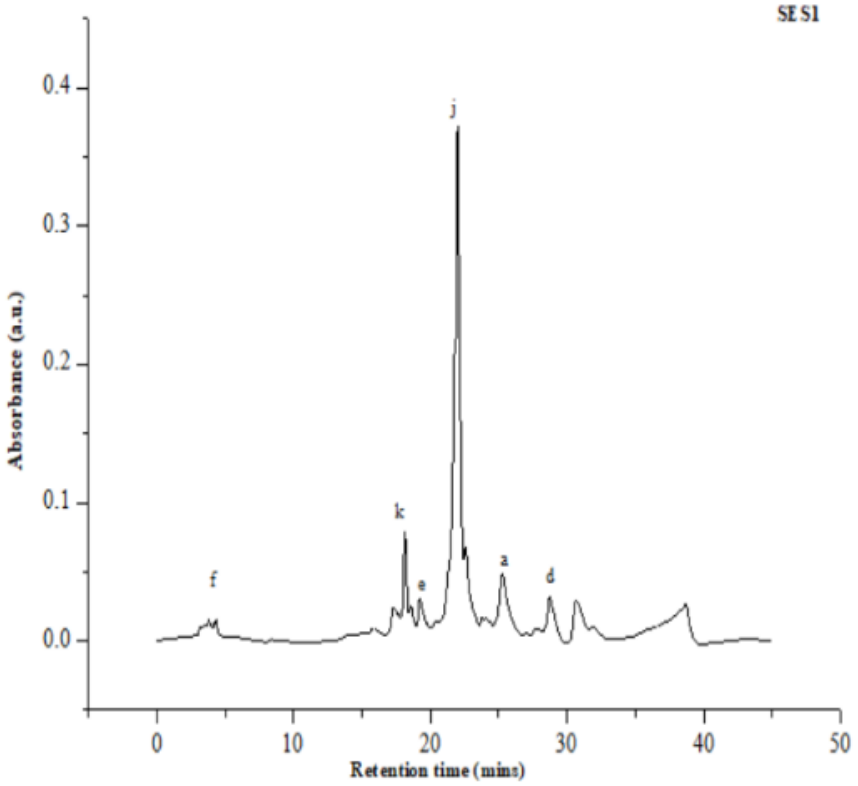



**Fig. 4b.2:** HPLC chromatograms of standards


SE S1
Absorbance (a.u.)
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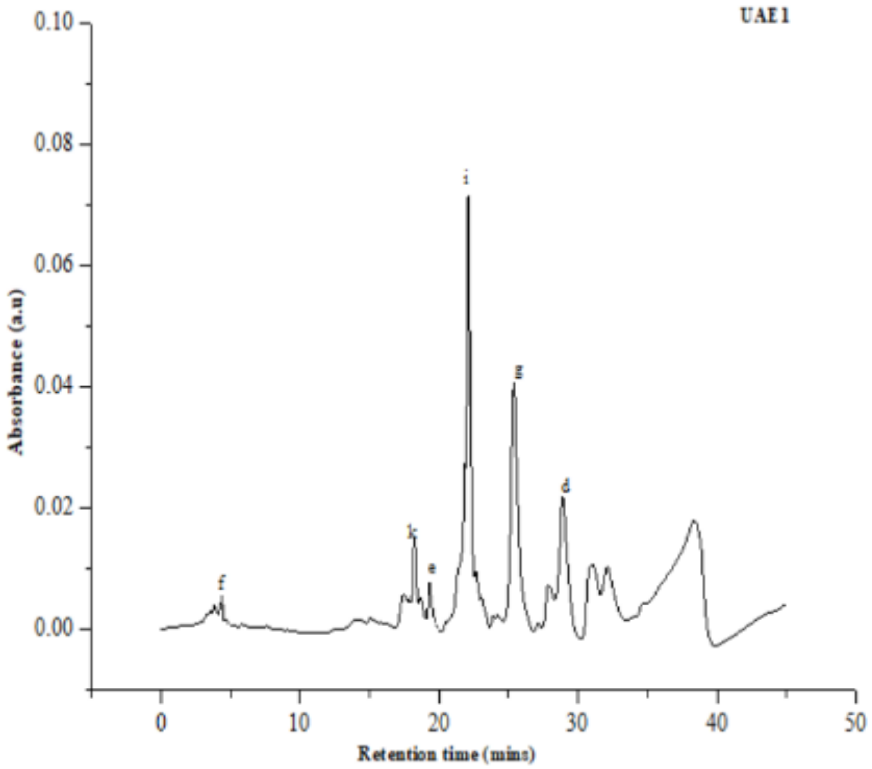
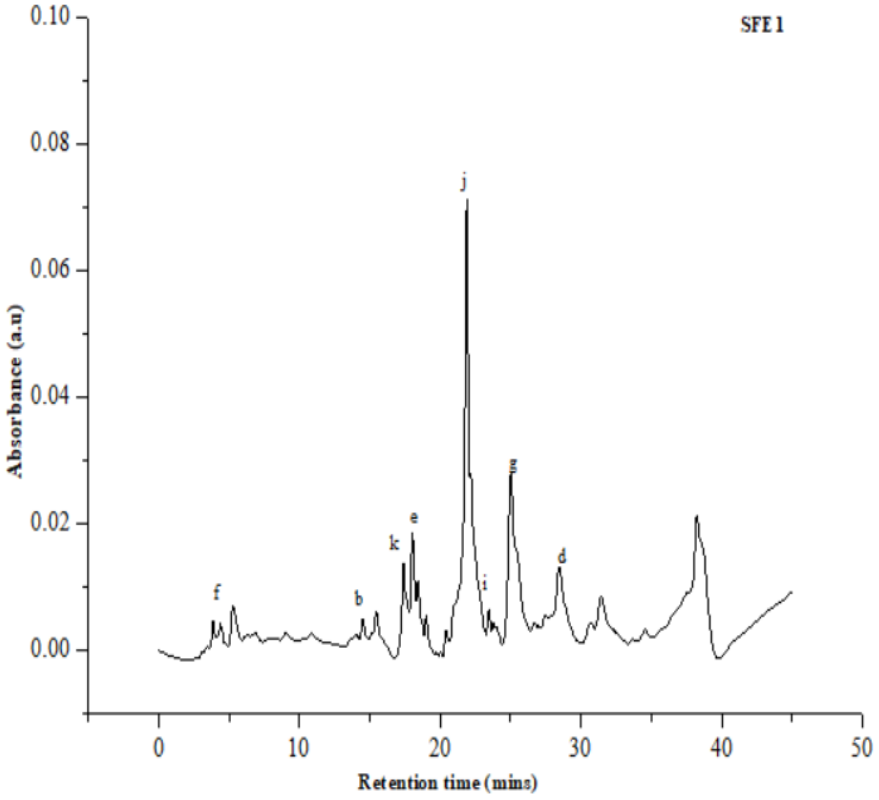
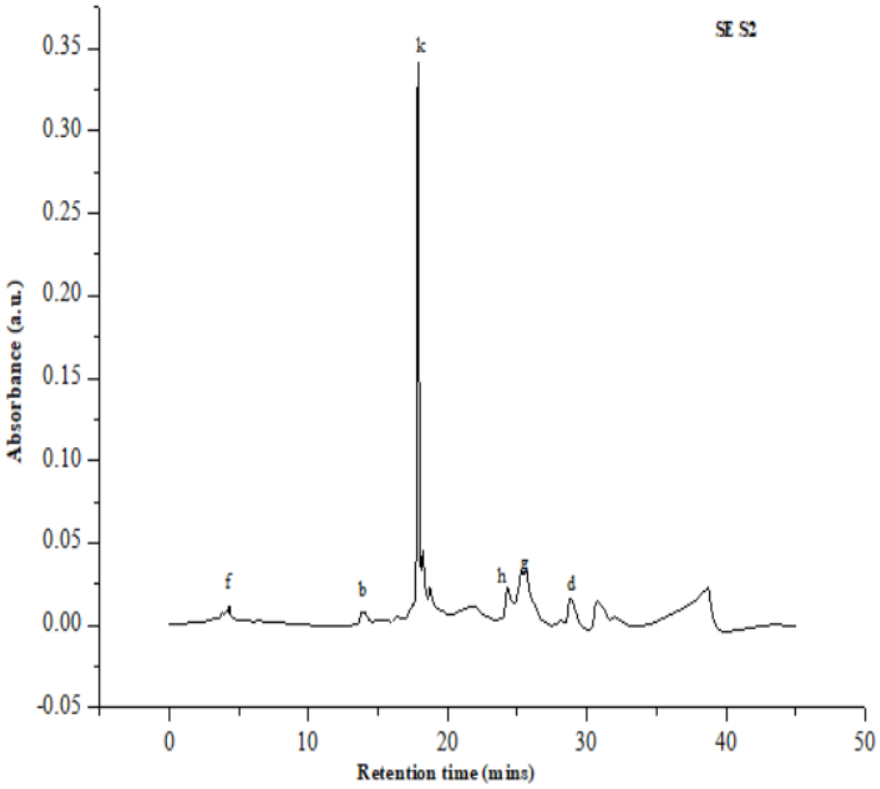
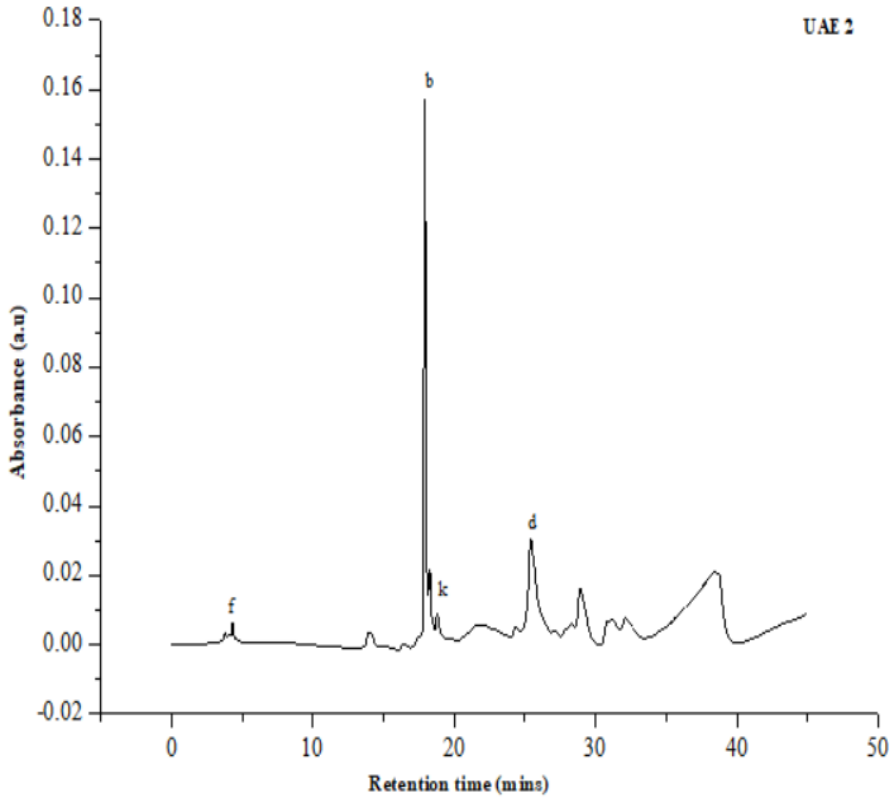
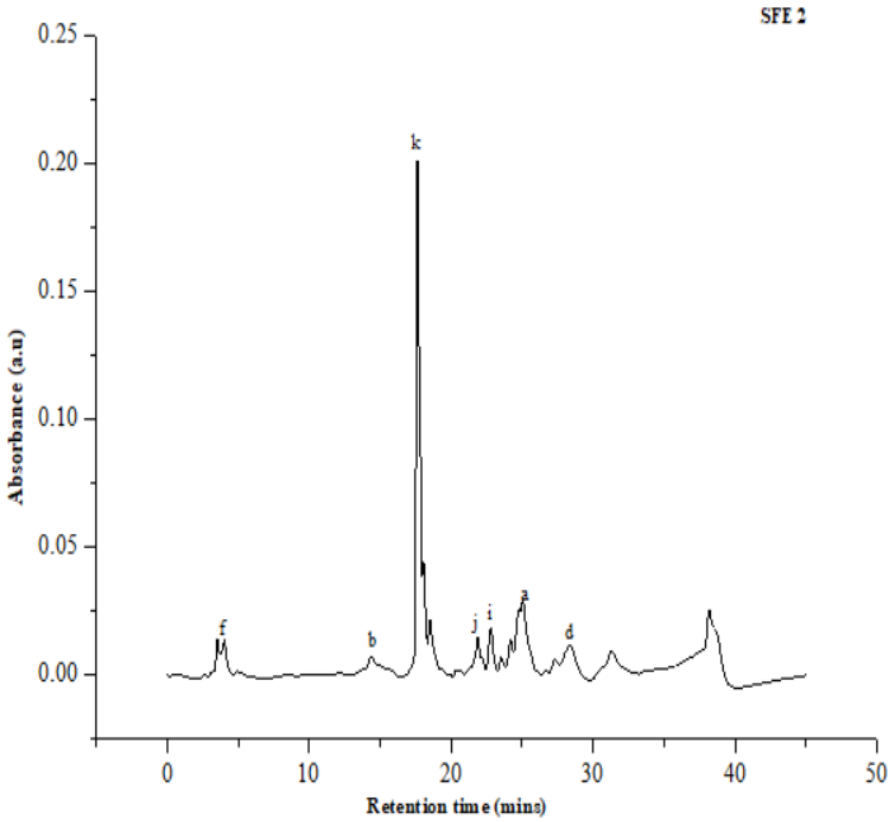
0.3
0.2
0.1
0.0
j
k
f
e
a
d
0
10
20
30
40
50
Retention time (mins)

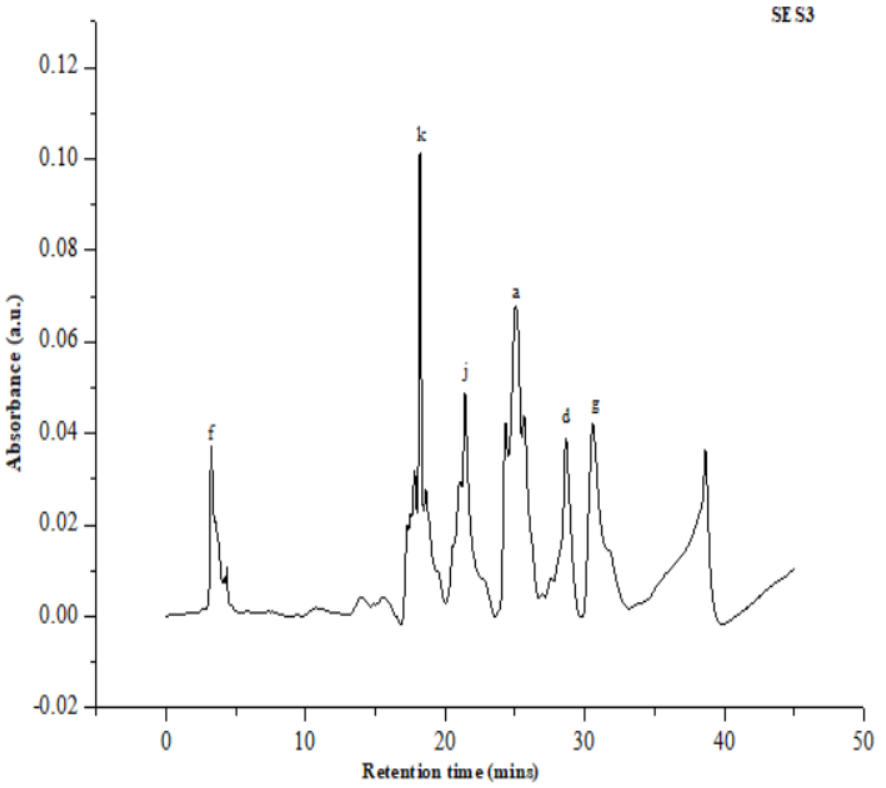

UAE 1
Absorbance (a.u)
0.10
0.08
0.06
0.04
0.02
0.00
i
g
d
k
e
f
0
10
20
30
40
50
Retention time (mins)

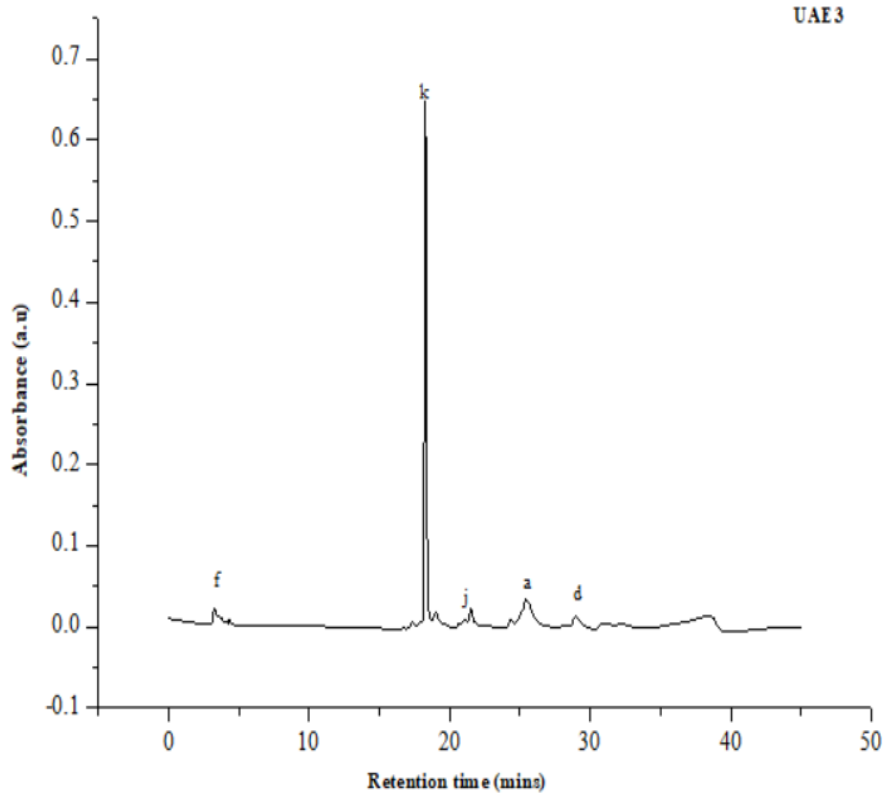

SFE 1
Absorbance (a.u)
Retention time (mins)
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
f
b
k
e
j
i
g
d

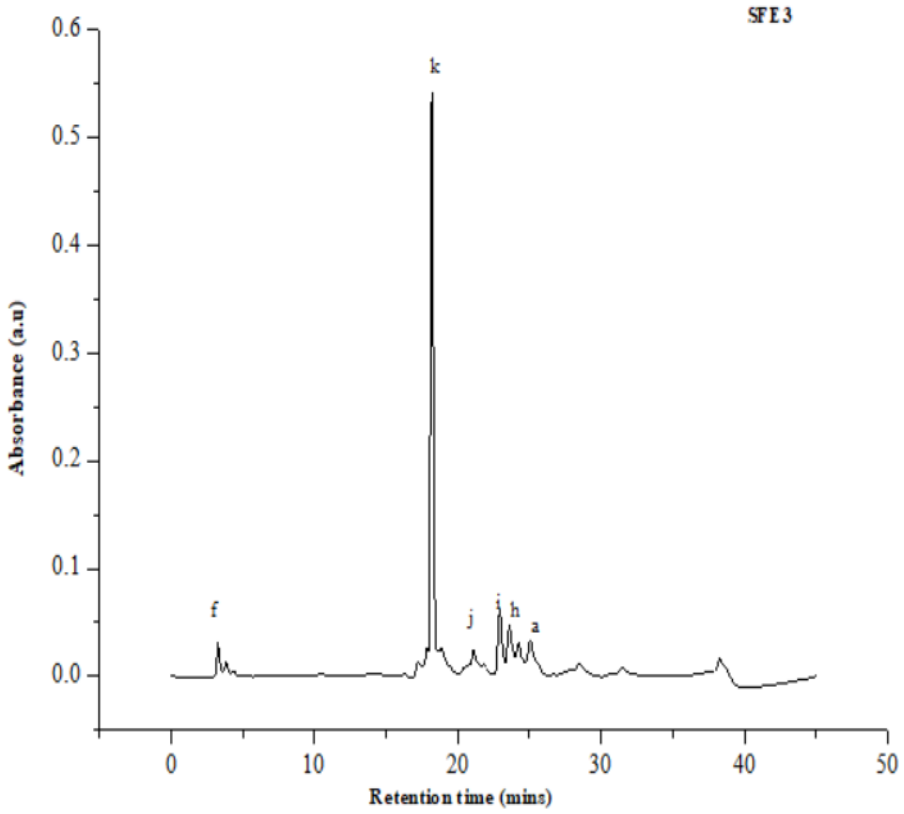

SE S2
Absorbance (a.u.)
Retention time (mins)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
10
20
30
40
50
f
b
k
h
g
d

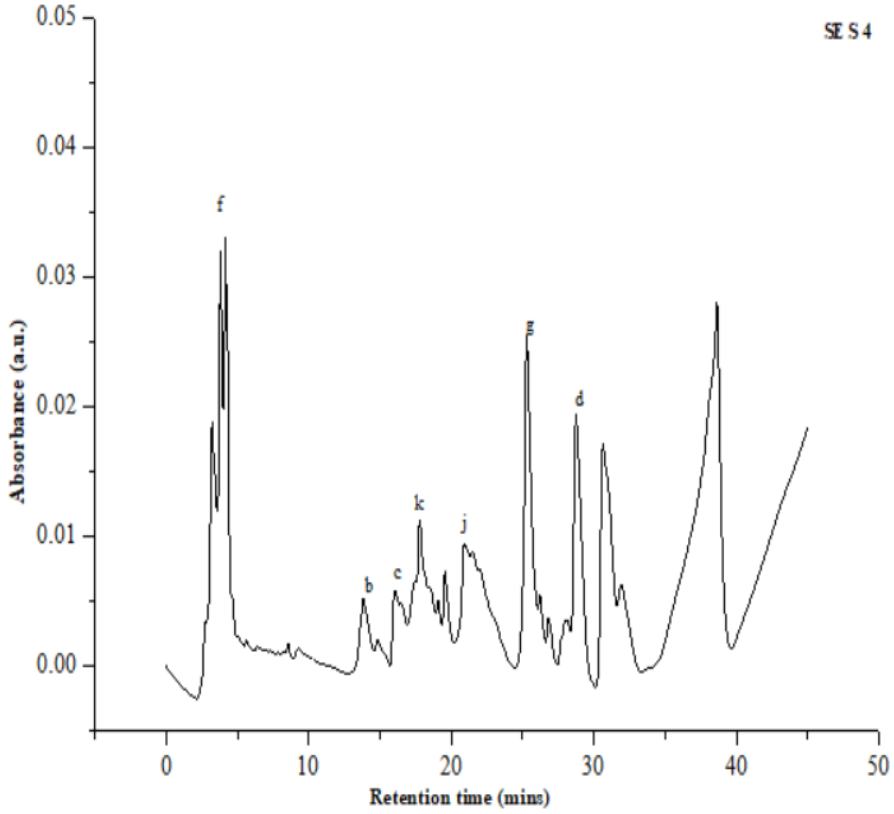

UAE 2
Absorbance (a.u)
Retention time (mins)
b
d
k
f

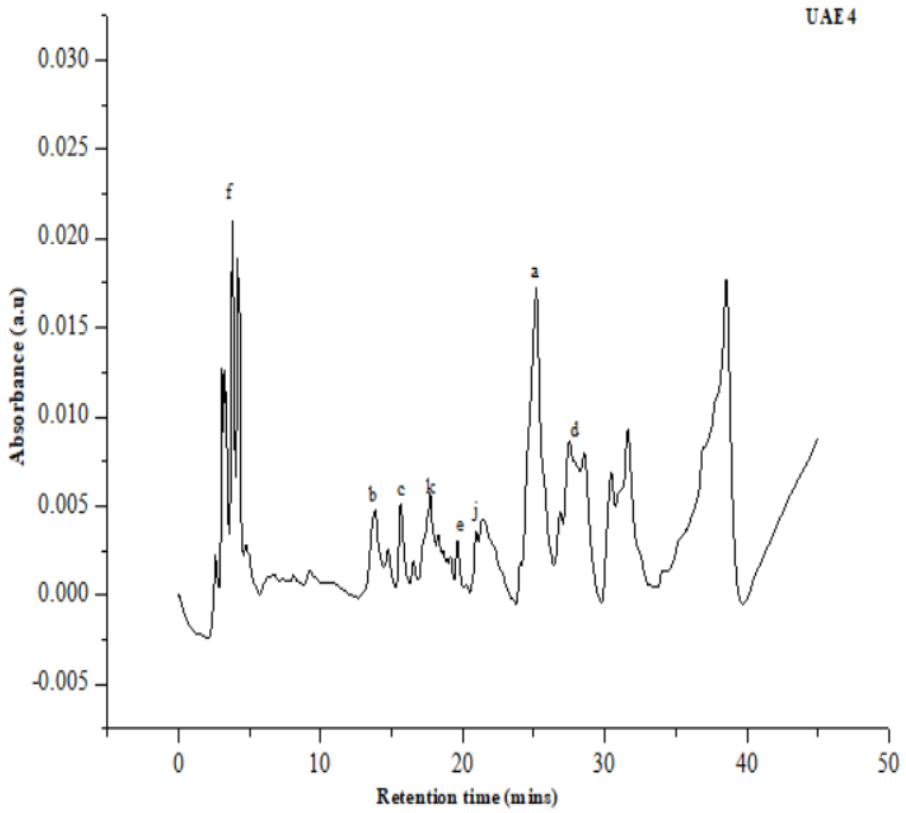

SFE 2
Absorbance (a.u)
Retention time (mins)
k
f
b
j
i
a
d

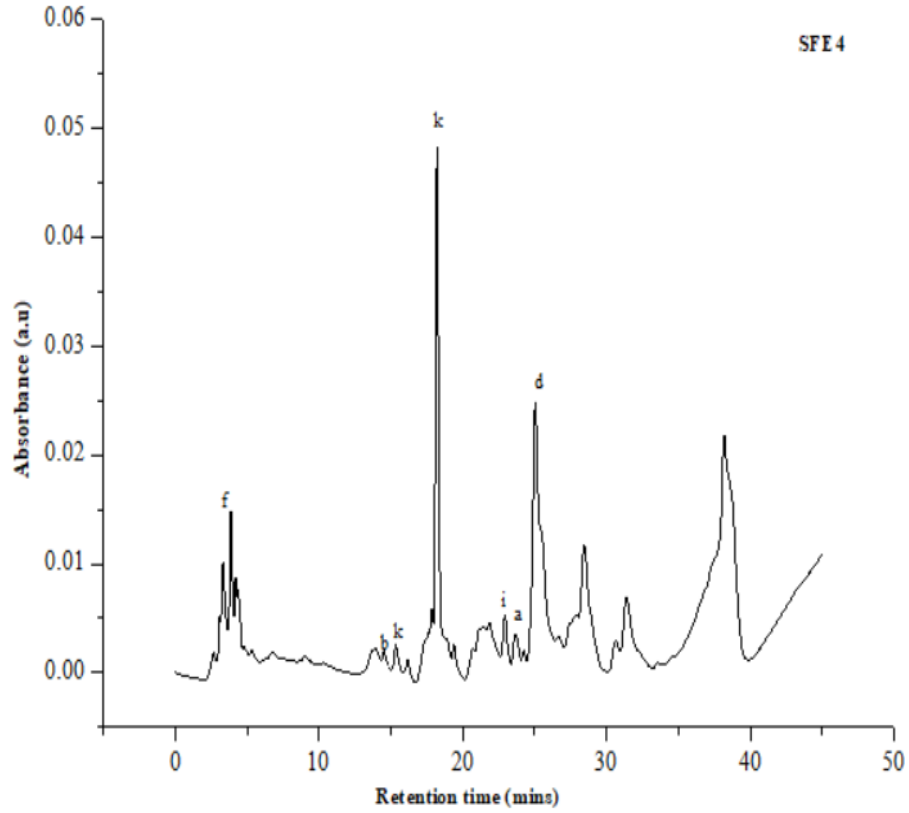

SE S3
Absorbance (a.u.)
Retention time (mins)
f
k
j
a
d
g

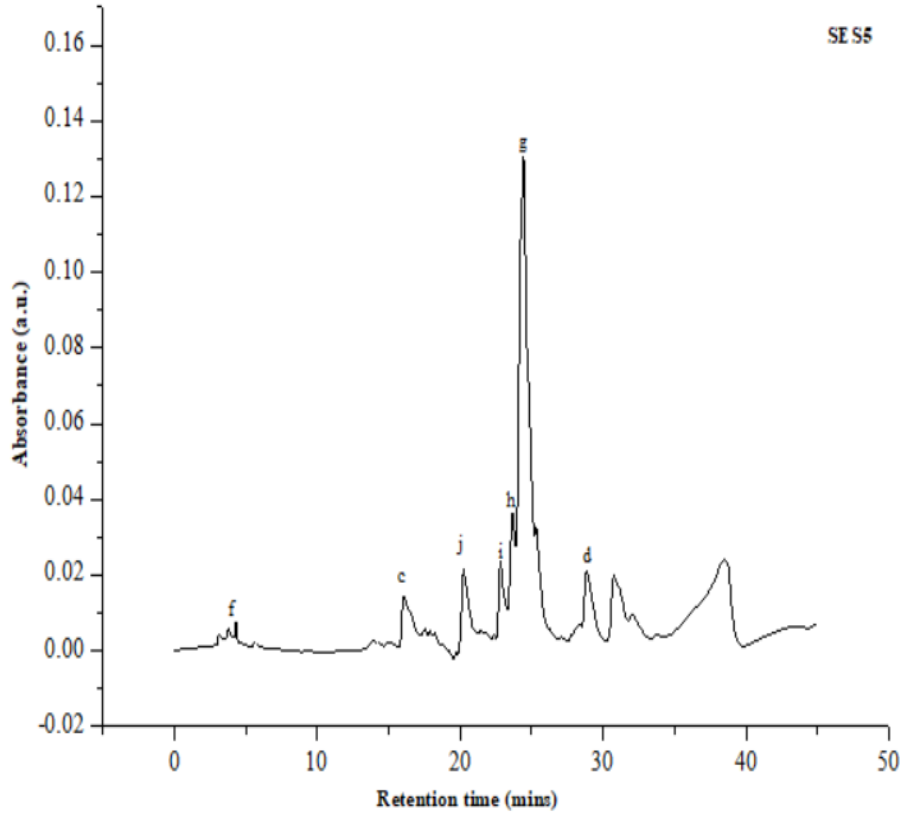

UAE 3
Absorbance (a.u)
Retention time (mins)
f
k
j
a
d

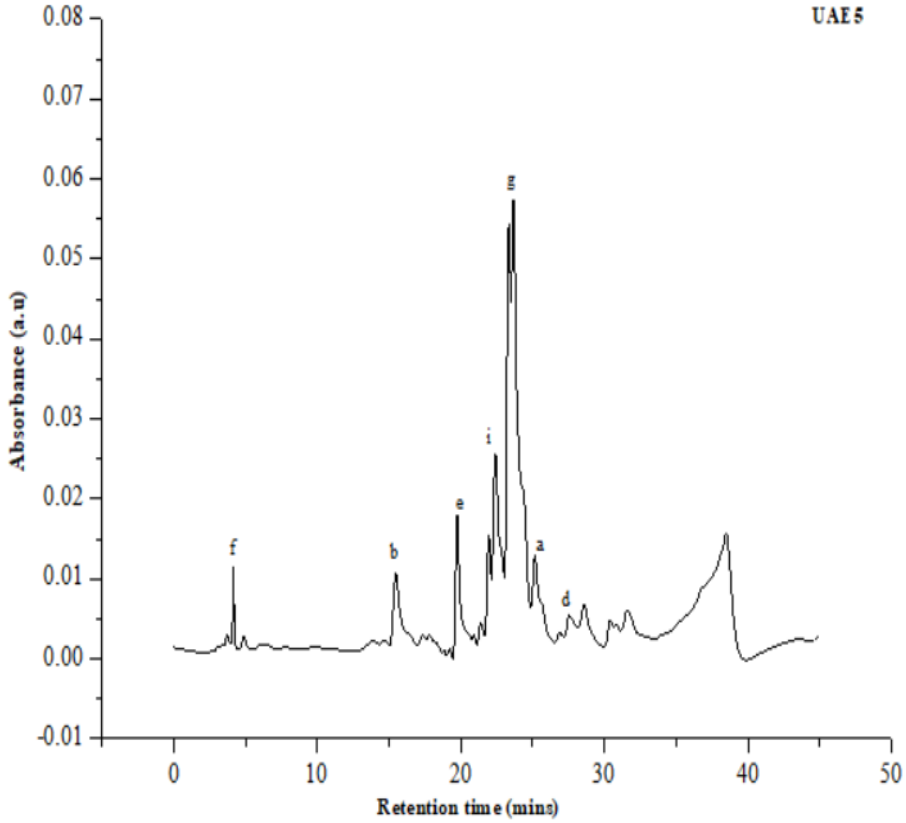

SFE3
Absorbance (a.u)
Retention time (mins)
k
f
j
i
h
a

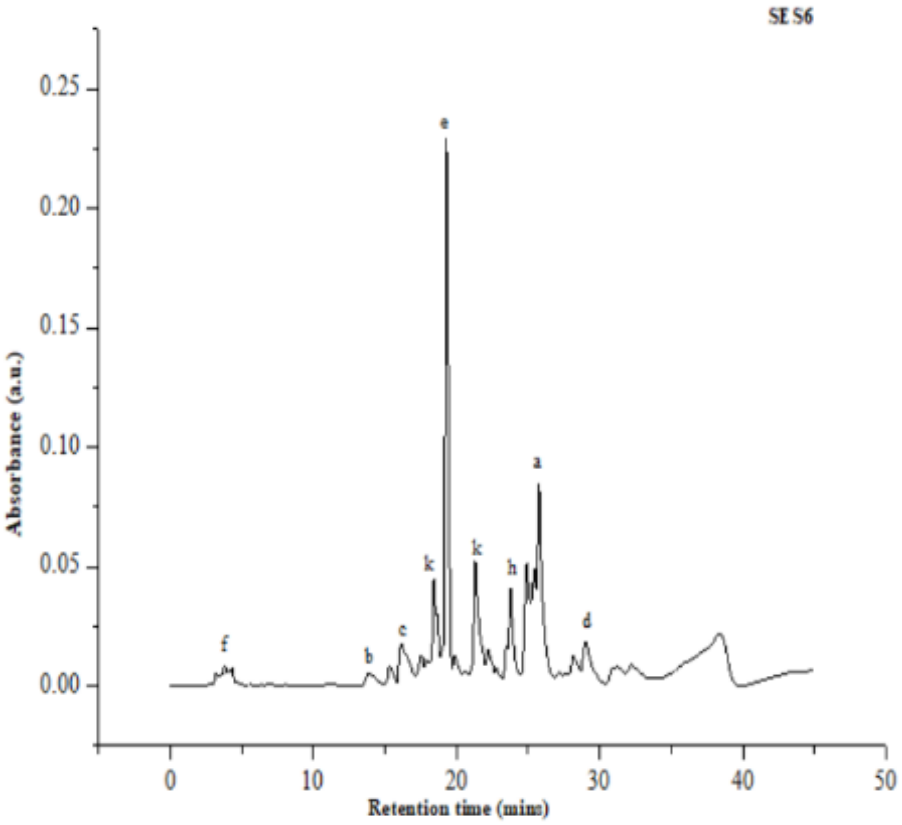

SES4
Absorbance (a.u.)
Retention time (mins)
f
b
c
k
j
g
d

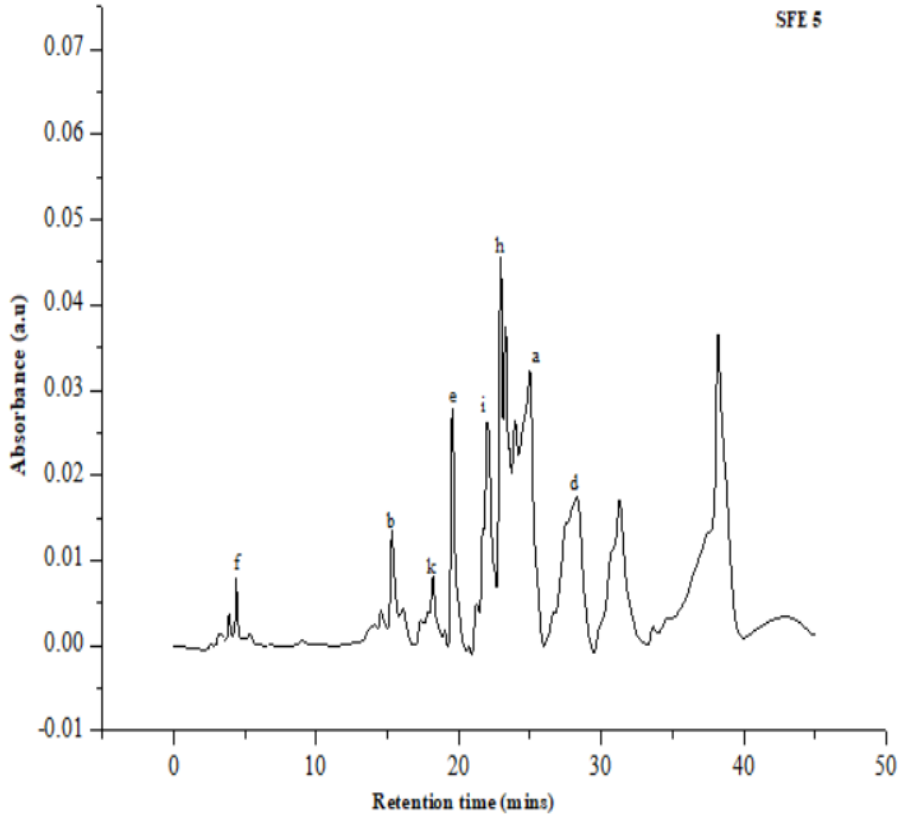

UAE 4
Absorbance (a.u)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
-0.005
f
b
c
k
e
j
a
d
0
10
20
30
40
50
Retention time (mins)


SFE 4
Absorbance (a.u)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
k
d
f
b
k
i
a
0
10
20
30
40
50
Retention time (mins)


SE S5
Absorbance (a.u.)
Retention time (mins)
f
c
j
i
h
g
d


UAE 5
Absorbance (a.u)
Retention time (mins)
f
b
e
i
g
a
d


SE S6
Absorbance (a.u.)
Retention time (mins)
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
e
a
k'
k
h
f
b
c
d


SFE 5
Absorbance (a.u)
Retention time (mins)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
0
10
20
30
40
50
h
a
e
i
d
b
f
k

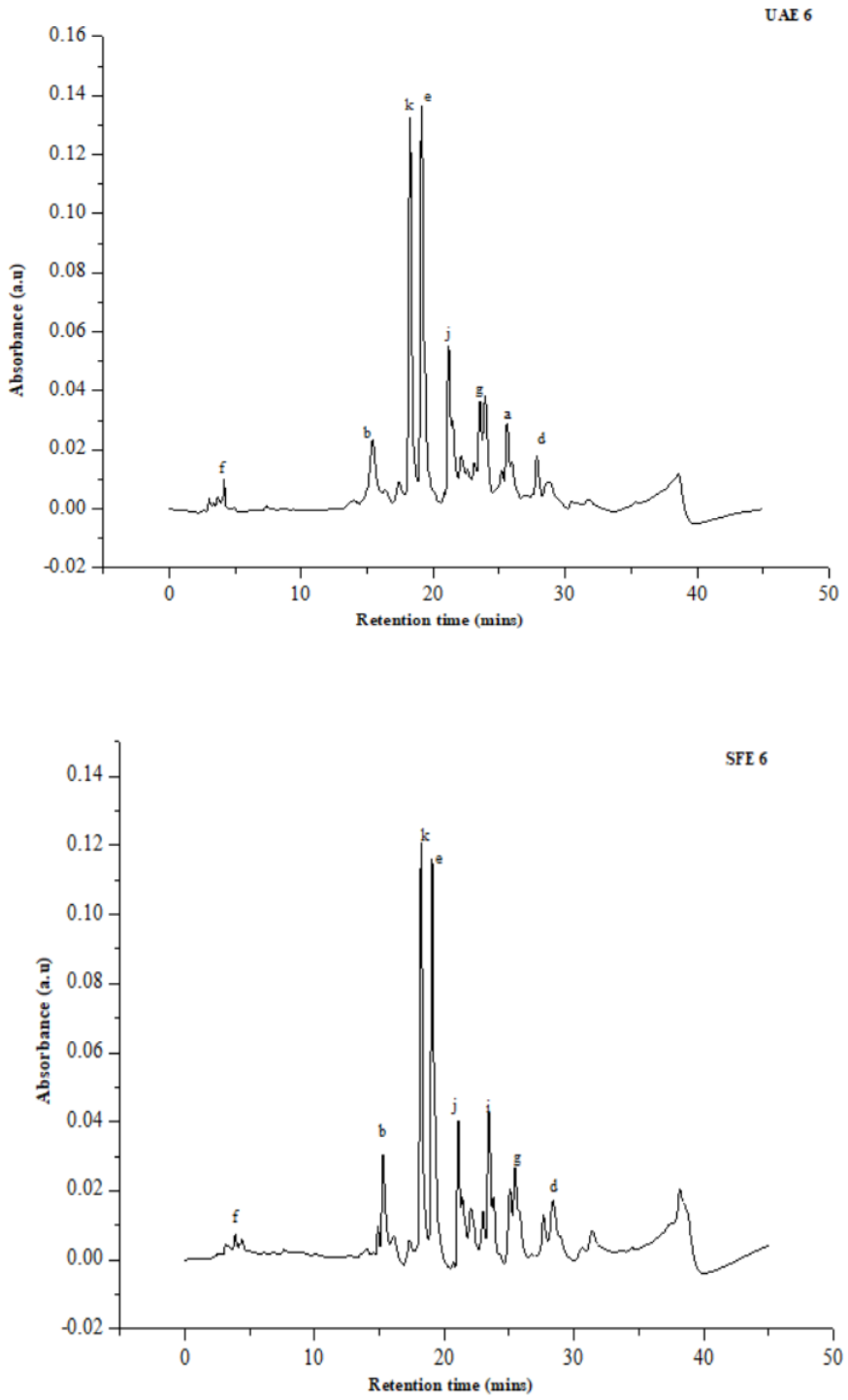



**Fig.4b.3.** RP-HPLC chromatograms of consisting of solvent extracted samples (SES1-SES6), ultrasound assisted extracted samples (UAE1-UAE6) and supercritical fluid extracted samples (SFE1-SFE6).

### 4b.3.4. In vitro anti-hypertensive study of six different plants in three different extraction techniques

The plant extracts were also examined for their ability to suppress ACE. Six herbal extracts out of a total of 18 showed more than 50% inhibitory action at a concentration of 1000 g/ml (Table 4b.4. & Fig.4b.4). These included *Solanum indicum* L in both ultrasound assisted extraction (59.85±0.24%) and supercritical fluid extracted sample (54.39±0.27%), *Clerodendrum glandulosum* Lindl. in ultrasound assisted extraction (64.52±0.61%), and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb in both ultrasound assisted extraction (52.67±0.57%) and supercritical fluid extracted sample (50.72± 0.52%). Another enzyme, ACE, which is stimulated to cause high blood pressure, influences metabolic syndrome.

Previous studies have shown that medicinal plant extracts can lower blood pressure by inhibiting ACE (Wu et al., 2011; Loizzo et al., 2008). Via two distinct reactions, the angiotensin I-converting enzyme (ACE) is a key player in the control of blood pressure and other cardiovascular processes. It inactivates the vasodilator bradykinin and catalyses the conversion of angiotensin I to angiotensin II, which lowers blood pressure (Shalaby et al., 2006; Xiong et al., 2013). One of the effective methods for treating hypertension is known as ACE inhibition (Chaudhary et al. 2013b). The intrinsic tissue renin-angiotensin-aldosterone system affects the tonic regulation of vascular resistance and local tissue function (such as kidney), which supports renal and cardiovascular homeostasis. ACE is the rate-limiting step in the renin-angiotensin-aldosterone system's production of angiotensin II. Angiotensin II is subsequently inhibited by ACE blockade (a potent vasoconstrictor and activator of aldosterone secretion). Since the dawn of time, phytomolecules originating from plants have been employed all over the world. Over the years, a variety of plant-based compounds have been screened for their potential antihypertensive effects in traditional medicines. These compounds include hydrolysable tannins, phenylpropanes (Jung et al., 2006), proanthocyanidins, flavonoids, xanthones, fatty acids, terpenoids alkaloids, oligosaccharides, and peptide amino acids. There have been reports of ACE inhibition capability in plants from the families Acanthaceae, Aloaceae, Amranthaceae, Apiaceae, Apocynaceae, Asclepiadaceae, Bignoniaceae, Combretaceae, Caesalpin1aceae, Fabaceae, Lamiaceae, Malvaceae, Moringaceae, Oleaceae, Piperacea (Braga et al. 2007). Based on the results, the ultrasound aided extracted sample of *Clerodendrum glandulosum* Lindl. showed the highest (64.52%) and

best ACE inhibitory activity compared to other extracted plant samples, making it a suitable candidate for further research.

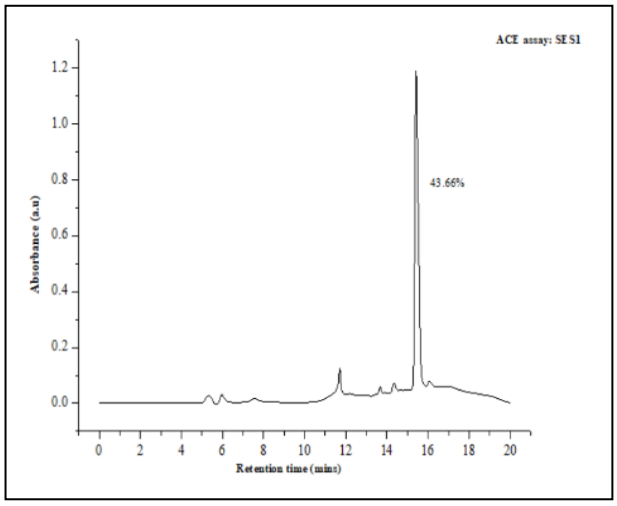
**Table 4b.4:** ACE inhibitory percentages of six different plants using three different extraction techniques

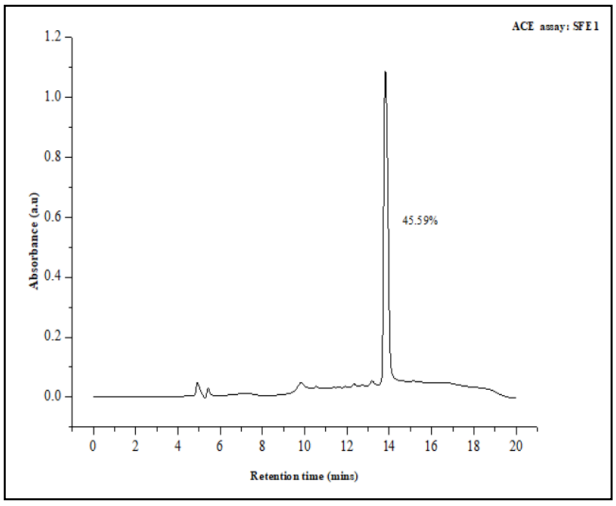
| Sample name | ACE value (%) | Sample name | ACE value (%) | Sample name | ACE value (%) |
|---|---|---|---|---|---|
| **SES1** | 43.66±0.58$^{d}$ | **UAE1** | 49.13±0.41$^{g}$ | **SFE1** | 45.59±0.71$^{e}$ |
| **SES2** | 37.59±0.58$^{a}$ | **UAE2** | 41.59±2.27$^{c}$ | **SFE2** | 39.80±0.35$^{b}$ |
| **SES3** | 48.03±0.21$^{f}$ | **UAE3** | 64.52±0.61$^{m}$ | **SFE3** | 62.05±0.14$^{l}$ |
| **SES4** | 44.97±0.35$^{e}$ | **UAE4** | 59.85±0.24$^{k}$ | **SFE4** | 54.39±0.27$^{j}$ |
| **SES5** | 45.79±0.40$^{e}$ | **UAE5** | 50.45±0.63$^{h}$ | **SFE5** | 49.16±0.46$^{g}$ |
| **SES6** | 43.57±0.56$^{d}$ | **UAE6** | 52.67±0.57$^{i}$ | **SFE6** | 50.72±0.32$^{h}$ |

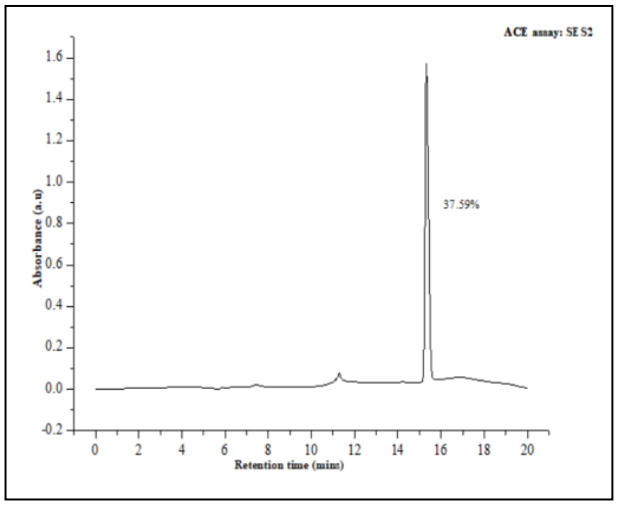
*SES- Solvent extracted sample

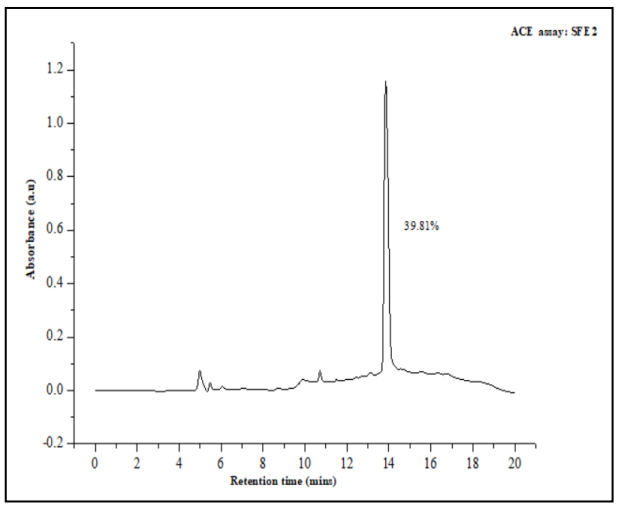
*UAE- Ultrasound assisted extracted sample

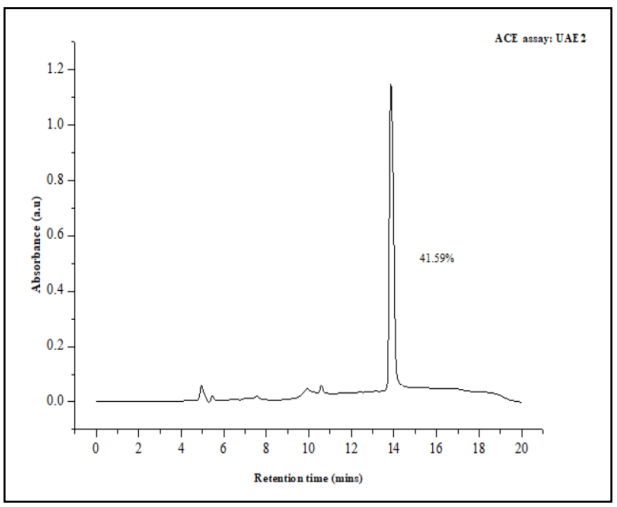
*SFE- Supercritical fluid extracted sample

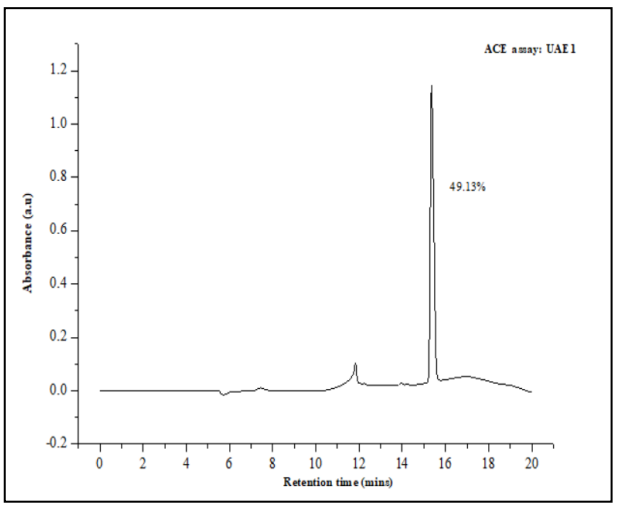

ACE assay: SES1
43.66%
Absorbance (a.u)
Retention time (mins)

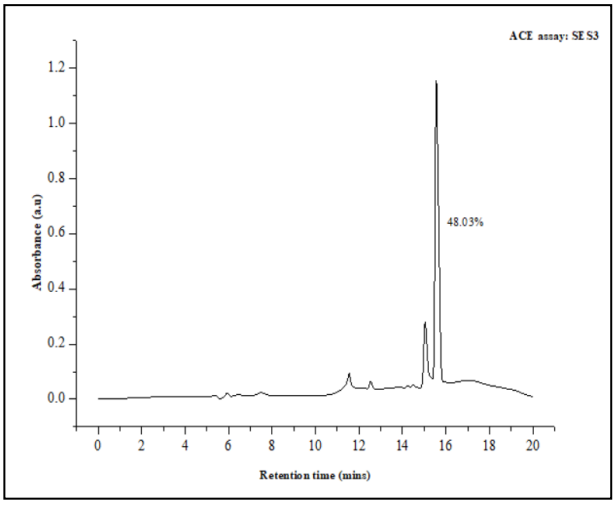

ACE assay: SFE1
45.59%
Absorbance (a.u)
Retention time (mins)

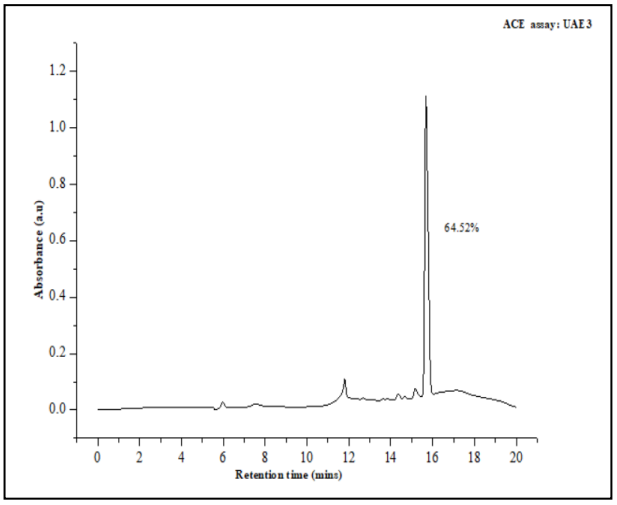

ACE assay: SES2
37.59%
Absorbance (a.u)
Retention time (mins)

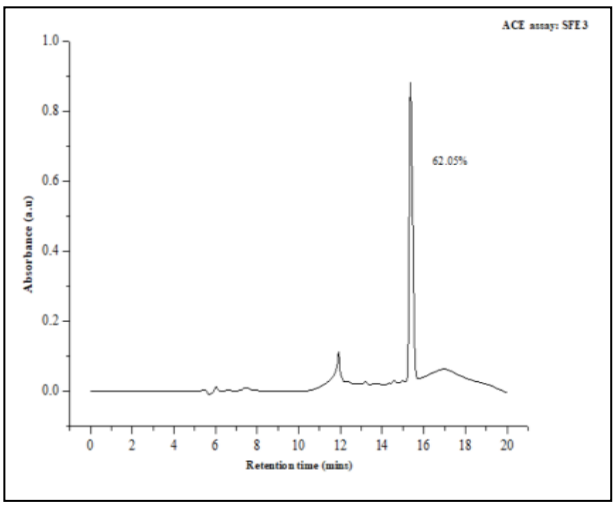

ACE assay: SFE2
39.81%
Absorbance (a.u)
Retention time (mins)

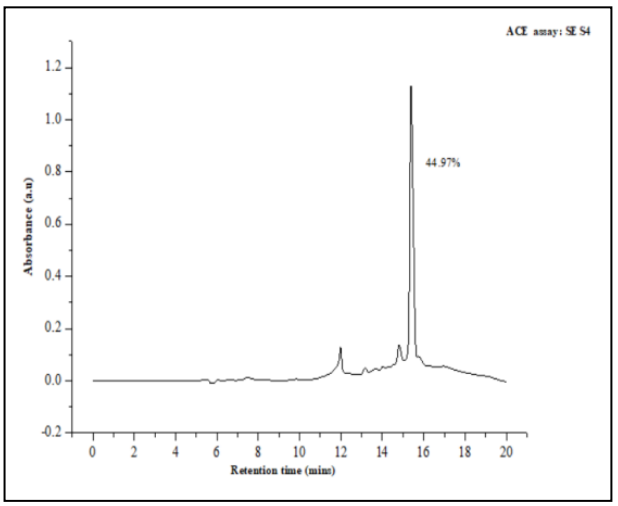

ACE assay: UAE2
41.59%
Absorbance (a.u)
Retention time (mins)

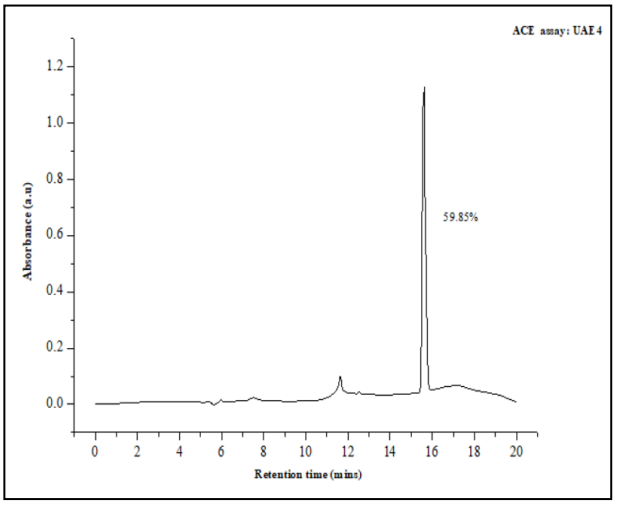

ACE assay: UAE1
49.13%
Absorbance (a.u)
Retention time (mins)

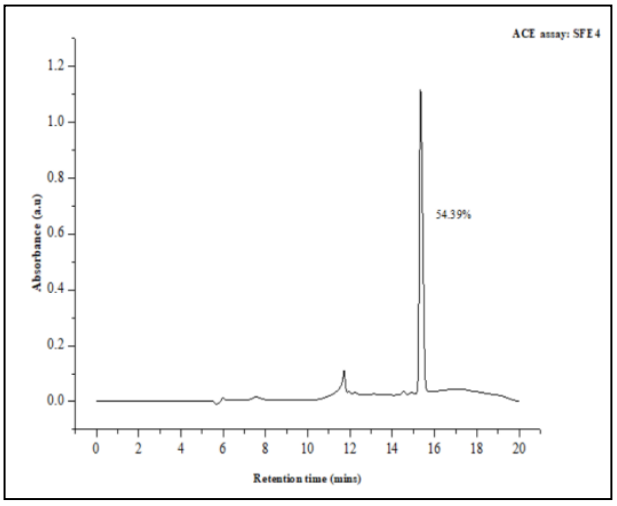

ACE assay: SE S3
Absorbance (a.u)
Retention time (mins)
48.03%


ACE assay: UAE 3
Absorbance (a.u)
Retention time (mins)
64.52%


ACE assay: SFE 3
Absorbance (a.u)
Retention time (mins)
62.05%


ACE assay: SE S4
Absorbance (a.u)
Retention time (mins)
44.97%


ACE assay: UAE 4
Absorbance (a.u)
Retention time (mins)
59.85%


ACE assay: SFE 4
Absorbance (a.u)
Retention time (mins)
54.39%

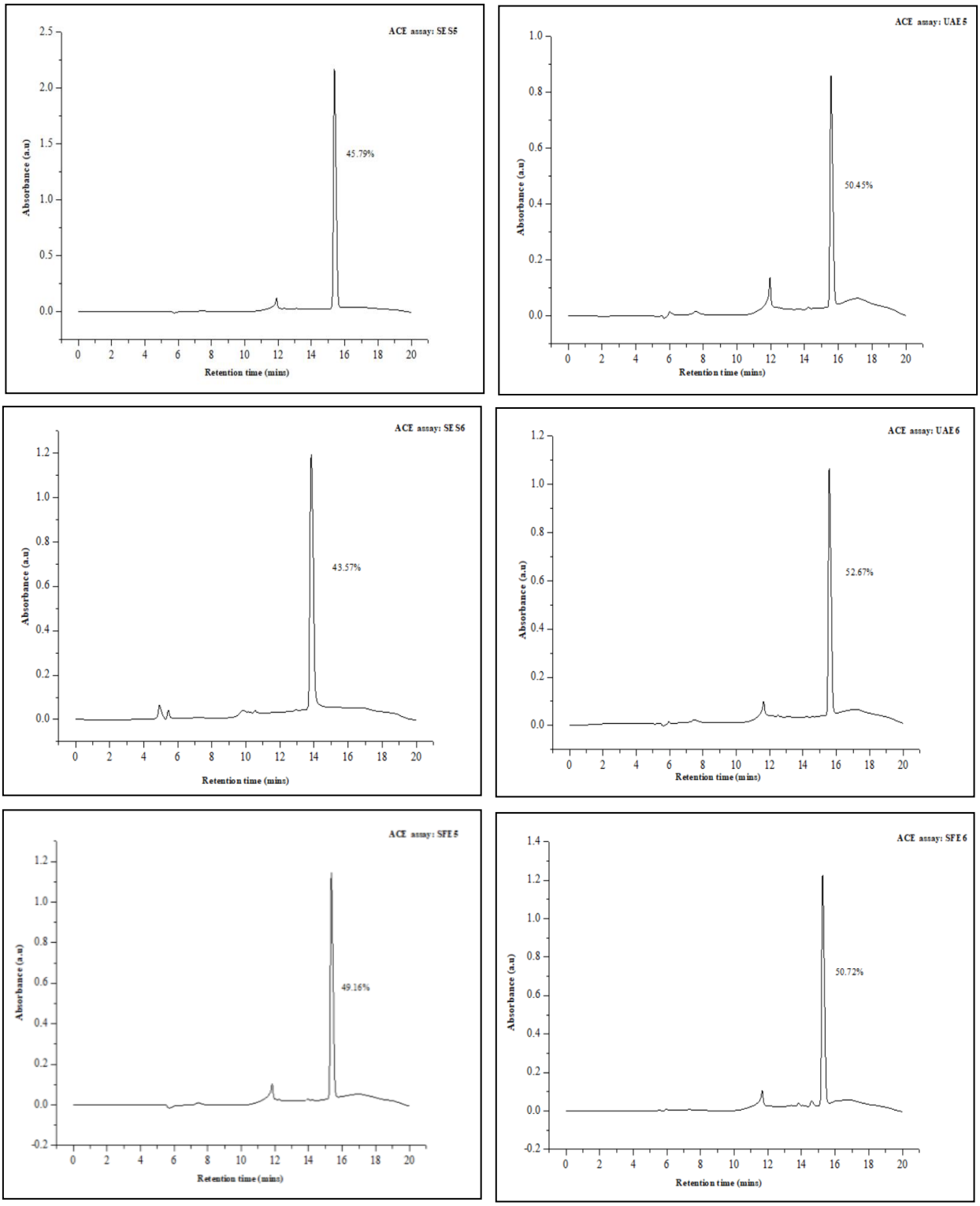



**Fig.4b.4:** HPLC chromatographs showing ACE inhibitory percentages of six different plants showing ACE inhibitory percentages using solvent, ultrasound assisted and supercritical fluid extraction techniques

## 4b.4. Conclusions

The present study demonstrated the extracts using three different techniques namely, Solvent extraction, Ultrasound assisted extraction and Supercritical fluid extraction techniques. The extracts of six different plants - *Passiflora edulis* Sims*, Plantago major* L*, Clerodendrum glandulosum* Lindl*., Solanum indicum* L*., Centella asiatica* (L.) Urb. *and Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb were analyzed. It was found that the leaves of *Clerodendrum glandulosum* Lindl. in an ultrasound-assisted method, and the highest values of TPC and TFC were found to be 308.69±4.80 mg GAE/ 100 g and 158.72±3.85 mg QE/100 g, respectively and followed by *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb. HPLC analysis in combination with antioxidant assays (DPPH, FRAP, ABTS), allowed the detection of ntioxidant compounds. While the extracts of all species showed antioxidant potentials, *Clerodendrum glandulosum* Lindl. and *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb were the most potent radical scavenger and reducing agent amongst all. This might be related to its higher phenolic content compared to other species. RP-HPLC analysis results showed that all of the plant samples included phenolics, but gallic acid was found to be the most prevalent. Besides, UAE extracted sample of *Clerodendrum glandulosum* Lindl. (64.52±0.61%) extracts were found to be the most effective inhibitor against ACE enzyme. Overall, the extracts of all the species, and particularly *Clerodendrum glandulosum* Lindl. & *Phlogocanthus thyrsiformis* (Roxb. Ex. Hardw.) Mabb could be regarded as viable sources of phyto-ingredients with pharmacological interest.