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Multi-elemental analysis of some traditional medicinal plants from Marghalla Hill National Park, Pakistan

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Abstract

Multi-nutritional effect of plants is a gift from nature. Instead of having a great source of food, large variety of plants are also used as medicine due to presence of large amount of nutritive and non-nutritive compounds. The present study was conducted on the traditional medicinal plants from Marghalla Hills National Park in 2020-2021 in the Department of Botany, PMAS Arid Agriculture Lab for their multi-elemental analysis. In the qualitative phytochemical analysis most of plants species were found rich in term of nutritive and non-nutritive compounds. While in the quantitative analysis, *C. arvensis, S. mocroofitiana and P. plebeium* showed high total phenolic content (120.86 ± 0.44 µg GAE/mg, 113 ± 0.26 µg GAE/mg, 103.94 ± 0.19 µg GAE/mg) and flavonoid content (83.47 ± 0.32µg QE/mg, 46.32 ± 0.29 µg QE/mg and 38.52 ± 0.30 µg QE/mg). High scavenging ability and total antioxidant ability was observed in the extracts of *S. heteromalla* (98.37 µg/ml; 99.3637 µg/ml) and *C. arvensis* (147.096 µg/ml; 102.34637 µg/ml). While high reducing ability was observed in *P. plebeium.* The high nutritive value was observed in *C. arvensis* and *L. aphaca* with the values of 407.28 ± 0.49 kcal/100 g and 407.78 ± 0.59 kcal/100g. Several micro and macro minerals were observed in different concentration in. The results of the present study revealed that the traditional medicinal plants are high in their nutritive and non-nutritive components which provide the strong relationship of their medicinal effect. This study will provide a base for the researcher to explore these plants for their biological activities.

Keywords: Antioxidants, Marghalla Hills, Medicinal plants, Multi-elemental analysis

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Introduction

Multi-nutritional value of plants is a valuable gift from nature. Due to this multi-nutritional nature plants have played important role in the lives of mankind from time immemorial for their use as a source of food as well as medicine (Saboon et al., 2019). The medicinal effect in the plants are due to the presence of large variety of nutritive and non-nutritive substances commonly known as ‘‘Phytochemicals’’ (Li et al., 2016). The nutritive compound of plants includes substance like Chlorophyll, proteins, nutrients, vitamins, minerals, fatty acids, fiber and sugars an essential part of diet. While non-nutritive includes Saponins, Phytates, Oxalates, Tannins, Glycosides, Flavonoids and Polyphenols (Radha et al., 2021). These non- nutritive compounds from plants are also very valuable for the treatment of different disorder. These compounds are made up of unique carbon skeleton which imparts them different properties due to which they used as a medicine. But some time the high doses of these non- nutritive compounds are toxic for human body due to great complexity of their structures, and it is a sad reality that the indications and contraindications knowledge for using an herbal medicine is insufficient (Bode & Dong, 2015). Therefore it is necessary to have a well-informed knowledge about the application of medicinal plants in human body.

Asia is one of the great reservoirs for the medicinal herbs and more than its half population still relays on herbal medicine. This continent is rich in vegetation due to its extraordinary variation in climate and geography which provides a vast spectrum of environmental conditions for the growth of versatile plant species. Approximately 38,660 species of medicinal plants are found in Asia, from which almost 78 species are grown for commercial purposes (Astutik et al., 2019). This commercialization of the medicinal plants are from several Asian countries include, Pakistan, China, India, Indonesia, Nepal and Bangladesh (Phumthum et al., 2017). Pakistan is one the wealthiest country in term of vegetation, having a large diversity of medicinal plants due to the very diverse climate zones. There are approximately 6000 species of higher plants, out of which 3000 reported from the north area of Pakistan, approximately 5000 flowering plants are native to Pakistan, out of theses flowering plants about 650–750 are used as medicine and about 124 of these are from the north area of Pakistan including Marghalla Hills (Alamgeer et al., 2018). But unfortunately, only 10% of the total plant species in Pakistan have explored for their medicinal values (Shaheen et al., 2014).

In our recent research work we mainly focus on the Margalla Hills National Parks, Pakistan. Margalla Hills National Park is rich in term of vegetation. This places have very large diversity of medicinal plants showed by the work of (Shinwari and khan, 2000; Ahmad et al., 2009). The present study aimed to explore the safety level and nutritional value of the plants and their antioxidant effect as most of the herbal formulation and remedies used by the local people are not explored for their potential toxicity people just consumed them on the bases of traditional knowledge which sometime may cause severe toxicity. The objectives of our study include phytochemical analysis, proximate analysis and antioxidant activity of plant extracts.

Materials and Methods

Study area

Margalla Hills National Park, Pakistan (North east of Islamabad) was selected for the study. This area is rich in term of vegetation and have a vast diversity of flora. The Margalla range has an area of 12,605 hectares, with the elevation of 5,262ft and the Latitude: 33° 44' 23.99" N and Longitude: 73° 02' 18.00" E**.**

Selection and identification of plants

In our recent work we selected different plants *Lactuca serriola* L., *Saussurea heteromalla* (D. Don) Hand.-Mazz., *Salvia moorcroftiana* Wall. ex Benth., *Lathyrus aphaca* L., *Sida cordata* (Burm. f.) Boiss., *Malva neglecta* Wallr., *Polygonum plebeium* R. Br., *Silene conoidea* L., *Ipomoea purpurea* and *Calendula arvensis* (Vaill.) L.) on the basis of ethnobotanical survey showed in Table 1.

Table 1 The ethnobotanical documentation of study plants

Plants name	Ethnobotanical uses	References
Calendula arvensis	Fortify eye sight, heart diseases, liver disorders, gastrointestinal, Gynecological diseases, wounds and burn healing, as a sedative, disinfectant, antispasmodic, diuretic, anti-inflammatory, antitumor, antipyretic agent, sudorific, emmenagogue, diaphoretic, Toothache. As a herbal tea and the plant is also used for ornament purposes	Rehman et al., 2015, Ranfa and Bodesmo, 2017; Passalacqua et al. 2007, Abbasi et al., 2010, Dall'Acqua et al., 2008, Tiwari 2008, Khan et al., 2018, Nacakcı, and Dutkuner, 2018
Slavia moorcroftiana	Healing wounds, for insect bites, as analgesic and antipyretic, antiseptic, ant diabetic, Wound healing, Antidiarrheal, antitussives, digestive problem and stomach pains. As emetic, anthelmintic Guinea worms, itches, , Use for body cracks, anticancer, antibiotic, topical application on skin to release puss, aphrodisiac, araunesaa, for treatment of piles, Fresh leaves are used as a food.	Rehman et al., 2015, Abbasi et al., 2010, Bibi et al., 2014, Ahmad et al., 2009, Ahmad et al., 2015, Hassan et al., 2017, Khan et al., 2015, Khan et al., 2018, Khan et al., 2016, Rahman et al., 2019
Polygonum plebeium	Used against Pneumonia, bowl disorders, hypertension, asthma, cough, Eczema, cholera, plant used as a liver-tonic, heart burn, galactagogue, for thickness of semen, and for dementia. whole plant is also used to make tea and as a food	Bibi et al., 2014, Amjad et al., 2017, Ali et al., 2018, Umair et al., 2019, Sandey and Sharma 2019, Shah et al., 2016, Fatima et al., 2019, Rahman et al., 2019
Lactuca serriola	Applied to burns, used for rheumatism & gout, gonorrhea & urinogenital irritation, wound healing, Liver diseases, Kidney ailments, Digestive disorders, as Expectorant. Whole plant is used as a salad or in cooked form. From the root excretion of this plant Chewing gum is obtained.	Ahmad et al., 2009, Çakılcıoğlu et al., 2010; Şığva and Seçmen 2009; Tetik et al. 2013; Nacakcı, and Dutkuner, 2018, Arı et al., 2015
Saussurea heteromalla	Use as a tonic for liver, kidney, Nerve, as aphrodisiacs, for removing phlegm, Root is tonic and effective in skin diseases and wound healing. Used as anti-venom, anti-inflammation and anti-arthritis, anthelmintic, antitussives, used against reproductive disorder of women, for rheumatism, paralysis and against slipped disc	Rehman et al., 2015, Amjad et al., 2017, Ali et al., 2018, Shah et al., 2016, Ahmad et al., 2018
Malva neglecta	Effective against constipation as a laxative and emollient, tonsils, asthma, swelling of the feet, boils, digestive, stomach and kidney disorders, gynecological disorders, abscess disorders, aphrodisiac, Demulcent broken bones, and as anti-spasmodic, anti-inflammatory, anti-diabetic, antitussives. Used for wound healing, body pain, joints pain, fatigue, toothache and for rheumatism. Used in veterinary medicine. Plant leaves are cooked as vegetable.	Rehman et al., 2015, Bibi et al., 2014, Ahmad et al., 2009, Khan et al., 2015, Khan et al., 2016, Ali et al., 2018, Arı et al., 2015, Korkmaz and Karakurt, 2015
Sida cordata	Topically applied on cuts and bruises, used against diarrhea, dysentery, leucorrhoea, bleeding piles, gonorrhea, and rheumatism. Having properties of anti-pyretic, diuretic, demulcent, and astringent. Seeds are aphrodisiac and	Amjad et al., 2017, Quamar et al., 2014, Shukla et al., 2010, Kadirvelmurugan et al., 2014

	Laxative, leaf juice as body coolant, as cystitis, strangury, hematuria.	
Silene conoidea	used in curing pimples and backache, used against lungs disorders, as a coolant, relive cough, against wound and skin infection and stop bleeding. Shoots of the plant used as vegetable. Fruit of the plant cause slight fever and drowsiness.	Rehman et al., 2015, Khan et al., 2016, Liu et al., 2008, Abbasi et al., 2013
Ipomea purpurea	Used against bronchitis and bowl problem, used as antimicrobial.	Amjad, 2015, Korkmaz and Karakurt, 2015, Ibrar et al., 2007
Lathyrus aphaca	Used against skin infection and pain. Ripen seeds were consider to have narcotic and flowers were used as resolvent, Plant shoots are used as a food.	Bibi et al., 2014; Fatima et al. 2019; Abbasi et al., 2013

Collection of plant material

All the Plants were collected from the Margallah Hills National Park, Pakistan, in march-April 2020 dried at room temperature and stored at dark place. All the collected samples were identified with the help of literature and herbarium of Quaid e Azam University Islamabad (Ali & Qaiser, 2013).

Drying and extraction procedure for collected medicinal plant samples

All the Plant material was carefully cleaned with tap water, rinsed in distilled water to avoid any contamination and dried under shade at room temperature. The dried material was pulverized to fine powder. Extraction of powder plant material was done using cold maceration technique (Ewansiha et al., 2012). Twenty-gram powder of each plant material was soaked in 100 mL methanol (Sigma-Aldrich, USA) separately and placed on a mechanical shaker for continuous stirring, for 72 h, filtration was done. The filtrate solvent was than evaporated by the rotary evaporator (Heidolph, Germany) at 40^{o}C under vacuum to get extract. The residue was reprocessed in the same way and filtered; the process was repeated three times to gain the maximum yield. The percentage extraction yield was calculated by using the following formula (Saboon et al., 2019):

% Yield = weight of extract / weight of sample × 100

The extract was stored at 4 ^{o}C for the use.

Organoleptic evaluation of selected plants

All the collected plants were organoleptically evaluated by studying their vegetative characters, floral characters, their taste and odour. To study the organoleptic characters, fresh specimen was collected and their morphological features were evaluated by the use of Light microscope and by human perception (Khan et al., 2016).

Qualitative analysis of phytochemicals

Qualitative phytochemical analysis of (Tannins, Flavonoids, Alkaloids, PhytoSterol, Cardiac glycoside, Phenolics, Coumarins, Anthraquinones, Terpenoids, Phlobatannins, Saponins, carbohydrates and amino acids) the crude methanolic extract (CME) was done by following the protocol of (Yadav et al., 2014).

Quantitative analysis of phytochemicals

Total phenolic contents (TPC) extraction

The crude methanolic extracts (CME) of all 10 plants were subjected for Determination of total phenolic contents by Folin-Ciocalteu calorimetric method (Iqbal et al., 2015) with some modifications. 1mg plant extract was dissolved in 1mL methanol. Gallic acid was used as a standard with the range of dilutions (15.62 – 500 µg). From each extract and control 300 µL was mixed with 2.5 mL Folin-Ciocalteu phenol reagent, after 5 min 2.5 mL, Sodium Carbonate (6%) was added. The mixture was than incubated for 90 min at room temperature. After which the absorbance was measured at 725 nm. TPC of extracts was calculated form the standard calibration curve of Gallic acid (mg GAE/g).

Total flavonoid contents (TFC) extraction

Total flavonoid contents of all CME of all plants were done by aluminium chloride calorimetric method (Stankovic, 2011) with some modifications. 1mg plant extract was dissolved in 1mL methanol. Quercetin was used as a Standard different dilution (15.62 – 500 µg) of quercetin was prepared for developing of standard calibration curve. 500 µL of each CME of plant and control was taken with1.5 mL of methanol, 100 µL of 10% aluminium chloride solution, 100 µL of 1M Potassium acetate solution and 2.8 ml of distilled water to make the total volume up to 5 ml. The mixture was than incubated at room temperature for 30 min. Absorbance was taken at 415 nm. From Standard calibration curve of Quercetin results of all plant extracts were measured as mg Quercetin equivalent per gram (mgQE/g).

Proximate and nutritive analysis

Proximate analysis was carried out of edible medicinal plants of our research. The analysis was carried out on powdered plant material by using the official method of AOAC (2000) Dry matter, moisture contents, ash value, lipid content, crude proteins and fiber were carried out. 30 g of plant powder was

weighed and placed in a hot oven at 105 ^{o}C up to constant weight. Difference in weight was calculated as moisture content, remaining was the dry matter. 5 g plant powder was ignited in an ashing furnace (weiber) at 600 ^{o}C, upon formation of white ash, ash content was calculated. Total carbohydrates and available carbohydrates were calculated from the formulas (Shukla et al., 2014).

Moisture content = weight of the sample before drying- weight of sample after drying

Total carbohydrate = 100 - (% Moisture + % Ash + % Protein + % Crude lipid)

Available carbohydrates = % Total carbohydrates - % Crude fiber

Total energy value of plant was calculated by formula and express in Kilocalories/100 gram.

Nutritive value = (4 × % Protein) + (9 × % Crude fat) + (4 × % Total carbohydrates)

Mineral analysis

Analysis of different macro and micro-minerals (Na, K, Ca, Mg, Mn, Zn, Ni, Fe, Cu, S, Si, Cr, Cd, Pd, P and Al) was carried out through wet digestion method (Uddin *et al*., 2016). Stock solutions of different salts were prepared by dissolving them in distilled water having various ppm for the determination of Micro and macronutrients. 0.25g of Plant powder was mixed in nitric acid, sulphuric acid and perchloric acid with the ratio of 5:1:0.5 to make the volume upto 6.5 ml for each plant, the mixture is left for overnight for initial digestion, the mixture was than heated with sulphuric acid for 1hr. in fume hood on hot plate (150 ^{0}C), until the dense white fumes was seen, digestion was continued for 30 minutes after white fumes. After cooling the volume of mixture was raised to 50ml by adding distilled water, filtered and stored. Further analysis was done by Atomic Absorption Spectrophotometer, to analyses Concentration of micro-minerals in the samples and Flame Photo photometry was used to know the concentration of macro-minerals.

Free radical scavenging activity

The DPPH (Sigma- aldrich, USA) assay was be carried out by following the protocol of (Pyrzynska and Pękal, 2013). 2.4 mg of DPPH was dissolved in 100 mL of methanol to prepare the stock solution. For working, DPPH solution was than diluted with methanol to attain an absorbance of 0.980 ± 0.02 using the spectrophotometer (CE 7400 Double Beam UV, Buck Scientific, USA) at 517 nm. 2ml of this working solution was add to 200µl of plant extract at varying concentration. After 15 min incubation the absorbance was taken at 517 nm. The scavenging percentage activity and milligram ascorbic acid equivalents per 100 gram (AA mg/100g) was calculated by the following formulas:

Scavenging effect (%) = [(control absorbance-sample absorbance)/ (control absorbance)] × 100

Ascorbic acid equivalents activity = IC50 of ascorbic acid/ IC50 of sample × 105

Reducing power ability assay

The reducing ability of the plant sample was evaluated according to the procedure of (Sherikar and Mahanthesh, 2015). 200µl of plant extract was mixed with, 2 ml of phosphate buffer (0.2M, pH 6.6) and 2 mL of 1% potassium ferricyanide, the mixture was than incubated at 50 °C for 20 min. After that 2 mL of 10% Trichloroacetic acid was added to the mixture. The tubes were then centrifuged, and supernatant up to 2mL was than collected and dissolved with 2mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride was added, the mixture was again incubated for 10-min and after that the absorbance was measured at 700 nm. The result are calculated by standard calibration curve of Ferrous sulfate heptahydrate and expressed as mmol $Fe^{2+/}$100 g.

Total antioxidant activity

The total antioxidant activity of plant sample was determined by the phosphomolybdenum assay according to the protocol of (Maswada, 2013). 200µl of different dilutions (62.5 – 500 µl) of plant extract and control was dissolve with 2mL of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate). The mixture was than incubate in a water bath (Hh6, OEM, Hunan, China) at 95 ^{0}C for 90 min. after that the absorbance of the mixture was measured at 765 nm against a blank. Inhibition percentage was measured by following formula. While milligram ascorbic acid equivalent activity per 100g was measured by the standard calibration curve of ascorbic acid.

Inhibition (%) = (1- absorbance of sample/ absorbance of control) × 100

Statistical analysis

The experiment was carried out in triplicates so the mean, standard deviation, standard error mean, and 50% inhibition concentration (IC_{50}) was measured with the help of graph pad prism software 5.0, San Diego, USA

Results and Discussion

Organoleptic evaluation

Organoleptic evaluation was done with fresh plant material by using human sense of taste, smell and sight presented in Table 2. Different plants showed different characteristics in term of

odour, taste and colour. Even the different parts of same plants were different in those characteristics.

Yield of plant extracts

Extraction done by cold maceration technique in methanol showed different percentage yield for different plants. The percent yield of different plants is represented in the form of graph in Fig. 1. The higher % yield was observed in *L. serriola* (5.73% in 20g/100ml), followed by *M. neglecta* (5.67%), *S. heteromalla* (5.4%), *P. plebeium* (5.32%), *L. aphaca* (4.6%), *C. arvensis* (4.32%), *I. purpurea* (4.15%), *S. conoidea* (4.03%), while the lowest was obtained from *S. mocroofitiana* (3.98%) and *S. cordata* (3.89%).

Qualitative analysis of nutritive and non-nutritive compounds

The crude methanolic extract (CME) of different plants were found rich in terms of nutritive and Non-nutritive compounds. The date is given in Table 3. All the plants showed the presence of nutritive primary metabolites (carbohydrates and amino acids) while the studied plants were found different in terms of secondary non-nutritive metabolites. The richest plants in our study in term of secondary non-nutritive metabolites were *L. serriola, C. arvensis, S. heteromalla, S. mocroofitiana* and *S. conoidea*. These plants showed the presence of most nutritive and Non-nutritive metabolites such as Tannins, Flavonoids, Alkaloids, PhytoSterol, Cardiac glycoside, Phenolics, Coumarins, Anthraquinones, Terpenoids, Phlobatannins, Saponins, carbohydrates and amino acids. It was also observed that some plants showed the absence of Coumarins. The CME of *S. conoidea* showed the presence of all primary and secondary metabolites except Anthraquinones. While, CME of *I. purpurea* was found negative for alkaloids and Cardiac glycoside in our study.

In almost all of the plants carbohydrates and amino acids are observed this is because these compounds are primary nutritive metabolites and every plant synthesizes it during their metabolism for their normal functioning. While the non-nutritive metabolites were different in different plants because it varies according to the challenges faced by the plant. Such a study is also carried out by (Ali et al., 2020) on different ethno pharmacologically important plant including *L. serriola* from their study it is confirmed that different plants are different in term of their chemical compounds. Our studies are in also in agreement with that of (Gulnaz and Savitha 2013) worked on *S. cordata*, Nayeem et al., 2019 carried out preliminary analysis on *M. neglecta* and (Gani et al. 2019) worked on *S. mocroofitiana* showed that these plants contain wide variety of these non-nutritive compounds. Most of these secondary non- nutritive metabolites are well known for their therapeutic potential, such as phenolic compounds which was reported by many researchers for their vast therapeutic potential like, anti-inflammatory, anti-oxidant, anti-microbial and anti-tumor activities.

Quantitative analysis of nutritive and Non-nutritive compounds

Estimation of total phenol and total flavonoids

Different concentrations of Gallic acid (15.62 – 500 µg) were used for preparation of Standard calibration curve, following linear equation ($y = 0.0043x - 0.0594$, $R^2 = 0.9997$) was used for determination of TPC of all ten plants. While the TFC Calculated by Different concentration of Quercetin standard calibration curve to obtained linear equation ($Y = 0.0047x + 0.5487$, $R^2 = 0.9992$) results are presented in Table 4. During this study it was observed that the CME of *C. arvensis, S. mocroofitiana and P. plebeium* are rich in term of total phenol with mean value of 120.86 ± 0.44 µg GAE/mg, 113 ± 0.26 µg GAE/mg, 103.94 ± 0.19 µg GAE/mg and flavonoids with the mean value of 83.47 ± 0.32µg QE/mg, 46.32 ± 0.29µg QE/mg, 38.52 ± 0.30 µg QE/mg followed by, *L. serriola, S. heteromalla, M. neglecta* and *S. conoidea*, while the lower concentration was observed in, *I. purpurea* and *Lathyrus aphaca* with total phenol (52.06 ± 0.31 µg GAE/mg, 49.91 ± 0.24 µg GAE/mg) and total flavonoids (18.53 ± 0.03 µg QE/mg and 16.89 ± 0.31 µg QE/mg), respectively. The high TPC and TFC confirm their traditional use against different aliments. Our results are confirmed by the work of (Shah et al., 2017; Gani et al., 2019) analyzed *S. cordata, P. plebeium* and *S. mocroofitiana* for quantitative compound analysis and showed the presence of considerable amount of phenolic and flavonoids. As the research described the strong relationship of these non-nutritive compounds with the therapeutic activities, the phenols an abundant group of non-nutritive phytochemicals are well describe for their antioxidant potential, some of these polyphenols are also using at the commercial level to protect the body from the free radical damage.

Proximate and nutritive analysis

Edible plant from our research study was selected for proximate analysis. Total eight edible plants (*L. serriola, C. arvensis, L. aphaca, S. cordata, M. neglecta, P. plebeium, S. mocroofitiana* and *S. conoidea*) were subjected for proximate analysis. In proximate analysis different parameters of plant were tested i.e., dry matter, moisture, Ash, Total Lipid, Protein, Fiber, Total Carbohydrate, available Carbohydrate and Total energy value (kcal/100 g)). The data from nutritional and mineral values of medicinal plant are very helpful to translate medicinal samples intakes, as intakes of food components. Because the quantity of different parameters (moisture, total protein, carbohydrates, fats, fiber, ash value, micro and macronutrients) have strong effect on human health, like the high ash value of plant sample shows the accumulation of high content of heavy metal. The result of this study is presented in Table 5 with mean standard error. The proximate analysis of *C. arvensis* and *L. aphaca* showed high nutritive values of 407.28 ± 0.49 kcal/100 g and 407.78 ± 0.59 kcal/100 g *C. arvensis* and *L. aphaca* showed high nutritive values of 407.28 ± 0.49 kcal/100 g and 407.78 ± 0.59 kcal/100 g with the dry weight of

82.76 ± 0.33 and 76.6 ± 0.25, moisture content 10.39 ± 0.12 and 9.26 ± 0.15, ash value 4.52 ± 0.03 and 7.54 ± 0.05, total lipids 4.62 ± 0.02 and 2.74 ± 0.01, total proteins 3.83 ± 0.01 and 11.42 ± 0.23, total fiber 9.49 ± 0.01 and 9.48 ± 0.03, total carbohydrate 76.63 ± 0.11 and 69.25 ± 0.30, and available carbohydrates 67.14 ± 0.11 and 59.75 ± 0.29. Followed by *P. plebeium*, *S. mocroofitiana* and *S. cordata* with the total energy value of 403.92 ± 0.92 kcal/100 g; 401.67 ± 0.75 kcal/100 g and 403.78 ± 0.55 kcal/100 g. However, the lowest nutritional value was observed in *M. neglecta* with nutritional value of 374.96 ± 0.40 kcal/100 g. Our study is in agreement with that of (Gani et al. 2019) worked on different species including *M. neglecta*, *Prunus avium*, *Cydonia oblonga* and *Taraxacum officinale* showed that different vegetable contain different amount of protein, fiber, carbohydrate and fats. During the study *S. conoidea* was observed of having high total carbohydrate and available carbohydrates values of 77.77 ± 0.21 and 71.26 ± 0.22, while high protein content was observed in *L. aphaca* 11.42 ± 0.23, lipids were found most abundantly in *C. arvensis* 4.62 ± 0.02, the high content of fiber was found in *P. plebeium* with the total value of 9.53 ± 0.01, respectively.

Table 2 Organoleptic evaluation of plants

Organoleptic evaluation	Plant Parts	*Saussurea heteromalla*	*Lathyrus aphaca*	*Lactuca serriola*	*Calendula arvensis*	*Silene conoidea*	*Sida cordata*	*Ipomea purpurea*	*Polygonum plebeium*	*Malva neglecta*	*Slavia mocroofitiana*
Colour	Flower	Pinkish-purple	Yellow	Pale Yellow	Yellow	Pink	Yellow	Purple	Pink	Purplish White	Lilac Blue
	Fruit	Dirty White	Green	Green	Light Green	Light Green	Dark Green	Brown	Black	Green	Dark Green
	Seed	Black	Green	Grayish Brown	Brown	Black	Brown	Black	Brown	Brownish Black	black
	Leaves	Green	Whitish Green	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green	Greyish Green
	Stem	Brownish Green	Whitish Green	Whitish Green	Dark Green	Drk Green	Ligh Green	Ligh Green	Redish Brown	Dark Green	Greyish Green
	Root	Brownish Green	Light Brown	Dark Brown	Light Brown	Brown	Light Brown	Light Brown	Brown	Ligh Brown	Dark Brown
Odour	Flower	Pungent	Odourless	Fetid Odor	Sweet Smell	Slight Musky Smell	Odourless	Slight Fragrance	Odourless	Slight Sweet Aroma	Minty Intense Aroma
	Fruit	Pungent	Odourless	Fetid Odor	Sweet Smell	Slight Musky Smell	Odourless	Odourless	Odourless	Slight Sweet Aroma	Minty Intense Aroma
	Seed	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Minty Intense Aroma
	Leaves	Unplesent Odour	Odourless	Fetid Odor	Sweet Smell	Slight Musky Smell	Odourless	Odourless	Odourless	Intense Aroma	Minty Intense Arom
	Stem	Unplesent Odour	Odourless	Fetid Odor	Sweet Smell	Slight Musky Smell	Odourless	Odourless	Odourless	Intense Aroma	Minty Intense Arom
	Root	Pungent	Odourless	Odourless	Odourless	Strong Smell	Odourless	Odourless	Odourless	Odourless	Minty Intense Arom
Taste	Flower	Slight Bitter	Bitter	Bitter	Sweet Camphour Like Taste	Bitter	Sour	Light Acrid	Acidulous	Acidulous	Acrid
	Fruit	Slight Bitter	Sweet	Bitter	Sweet Camphour Like Taste	Bitter	Sour	Acrid	Acidulous	Bitter	Acrid
	Seed	Slight Bitter	Sweet	Bitter	Sweet Aroma	Bitter	Bitter	Light Earthy Taste	Bitter	Acidulous	Slight Bitter
	Leaves	Slight	Sweet	Sweet	Sweet	Bitter	Sour	Acrid	Acidulous	Acidulous	Acrid

	Bitter			Camphour Like Taste						
Stem	Slight Bitter	Sweet	Sweet	Sweet Camphour Like Taste	Bitter	Sour	Acrid	Acidulous	Acidulous	Acrid
Root	Slight Bitter	Bitter	Bitter	Bitter	Bitter	Sour	Acrid	Bitter	Acidulous	Acrid

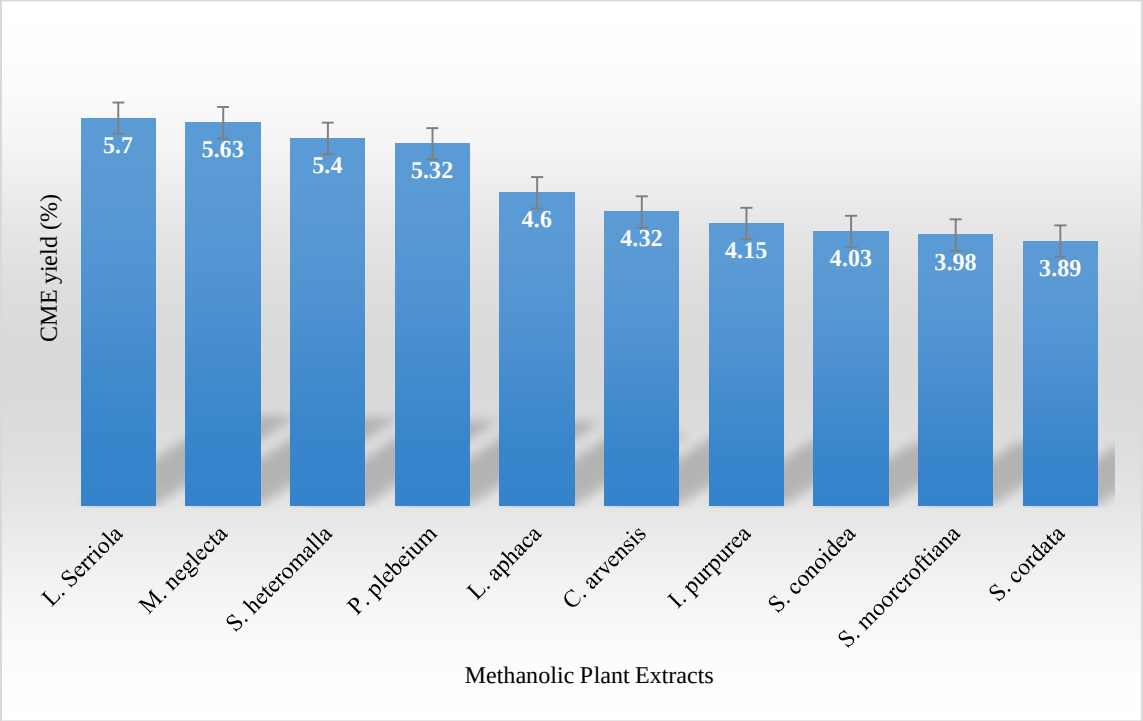

Fig. 1 Crude methanolic extracts yield percentage of selected plants

Table 3 Qualitative analysis of nutritive and non-nutritive compounds of studied plants

Metabolites	Presence/ Absence									
	Saussurea heteromalla	*Lathyrus aphaca*	*Lactuca serriola*	*Calendula arvensis*	*Silene conoidea*	*Sida cordata*	*Ipomea purpurea*	*Polygonum plebeium*	*Malva neglecta*	*Salvia moorcroftiana*
Tannins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Flavonoids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Alkaloids	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Sterol	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Cardiac glycoside	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
Phenolics	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Coumarins	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve
Anthraquinones	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve

Terpenoids	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Phlobatannins	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Saponins	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
Carbohydrates	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Amino acids	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

(present = +ve; absent = -ve)

Table 4 Total phenolic and flavonoid content

CME of Plants	Total phenolic (µg GAE/mg)	Total flavonoids (µg QE/mg)
Calendula arvensis	120.86 ± 0.44	83.47 ± 0.32
Slavia moorcroftiana	113 ± 0.26	46.32 ± 0.29
Polygonum plebeium	103.94 ± 0.19	38.52 ± 0.30
Lactuca serriola	94.4 ± 0.26	37.37 ± 0.37
Saussurea heteromalla	86.33 ± 0.31	32.95 ± 0.16
Malva neglecta	73.28 ± 0.21	29.31 ± 0.19
Sida cordata	72.81 ± 0.46	23.04 ± 0.28
Silene conoidea	66.34 ± 0.30	17.45 ± 0.33
Ipomea purpurea	52.06 ± 0.31	18.53 ± 0.03
Lathyrus aphaca	49.91 ± 0.24	16.89 ± 0.31

Mean ± SEM, n = 3

Table 5 Proximate and nutritive analysis

Parameters	*Malva neglecta*	*Polygonum plebeium*	*Sida cordata*	*Slavia mocroofitiana*	*Lathyrus aphaca*	*Silene conoidea*	*Lactuca serriola*	*Calendula arvensis*
Dry Matter	81.64±0.40	93.3±0.21	79±0.32	86.8±0.40	76.6±0.25	84.23±0.37	87.16±0.22	82.76±0.33
Moisture	12.36±0.19	13.42±0.22	9.38±0.19	10.42±0.06	9.26±0.15	8.76±0.11	9.44±0.22	10.39±0.12
Ash	5.75±0.06	5.19±0.10	6.47±0.04	6.31±0.06	7.54±0.05	5.11±0.09	8.13±0.13	4.52±0.03
Total Lipid	2.25±0.08	2.01±0.01	2.39±0.05	3.09±0.04	2.74±0.01	1.68±0.04	2.51±0.03	4.62±0.02
Protein	4.53±0.03	7.31±0.01	6.87±0.01	6.87±0.01	11.42±0.23	6.67±0.05	7.31±0.02	3.83±0.01
Fiber	6.21±0.01	9.53±0.01	8.47±0.02	8.98±0.02	9.48±0.03	6.52±0.02	8.35±0.04	9.49±0.01
Total carbohydrate	75.27±0.10	72.22±0.21	74.99± 0.11	73.33±0.16	69.25±0.30	77.77±0.21	72.73±0.35	76.63±0.11
Available Carbohydrate	69.06±0.10	62.69±0.20	66.52±0.09	64.35±0.14	59.75±0.29	71.26±0.22	64.38±0.32	67.14±0.11
Energy value (Kcal/100 G)	374.96±0.40	403.92±0.92	403.78±0.55	401.67±0.75	407.78±0.59	396.30±0.62	395.18±1.63	407.28±0.49

Mean ± SEM, n = 3

Mineral analysis

The essential minerals are required by the body for normal functioning. But some of these mineral, such as heavy metal are harmful for human health if present in large amount. The amount of minerals varies from plant to plant due to various environmental factors and soil conditions. Similarly plants also vary in their ability to extract minerals from soil and convert them into the useful substance such as protein, enzymes or vitamins. Sodium and potassium play an important part in cell signaling while calcium is an important part of bones, the essential mineral iron is the part of blood hemoglobin protein without which the body is unable to carry oxygen. The researcher also correlates the presence of minerals with the bioactivity of plants. They showed that the minerals have synergistic effect with the chemical in plants to enhance the bioactivity and production of phenolic compounds (Iqbal et al., 2015). The macro and micro-mineral analysis of plant showed that most the plants are richest in term of Na, K, Ca, Mg, Mn, Cu, Zn and Fe, while the heavy metals are also found in the permissible limits. The richest mineral in our studied plants were sodium (Na) range from 65 to 250 mg/100 g, followed by potassium (K) 100 to 220 mg/ 100 g, calcium (Ca) 125 to 400 mg/ 100 g, while magnesium (Mg) was found in the range of 20 mg/100g to 98 mg/100 g. The higher concentration of these minerals was observed in *M. neglecta, P. plebeium* and *C. arvensis.* The higher concentration of sodium was observed in *M. neglecta* 185.19 ± 0.96 mg/100 g, the higher concentration of calcium was observed in *M. neglecta* 381.27 ± 2.73 mg/100 g, while the higher concentration of potassium, magnesium and iron was observed in *C. arvensis* 218.19 ± 1.61, 97.8 ± 1.68

mg/100 g and 12.60 ± 0.15 mg/100 g. During this study it was observed that all heavy metal were found at the permissible rang in those plants. The lower level of heavy metal was observed in *S. conoidea* (copper 0.248 ± 0.03 mg/100 g, chromium 0.07 ± 0.01 mg/100g, nickel 0.04 ± 0.01 mg/100g, cadmium 0.04 ± 0.01 mg/100 g and lead 0.03 ± 0.01 mg/100 g). While the higher concentration of copper, zinc and cadmium was observed in *M. neglecta* 7.74 ± 0.06 mg/100 g, 5.136 ± 0.03 mg/100 g and 0.171 ± 0.01 mg/100 g. All the values were presented with standard error mean and were found significantly (P<0.05) presented in Table 6.

Table 6 Macro and micro-mineral analysis of plants

Minerals mg/100g	*Malva neglecta*	*Polygonum plebeium*	*Sida cordata*	*Slavia mocroofitiana*	*Lathyrus aphaca*	*Silene conoidea*	*Lactuca serriola*	*Calendula arvensis*
Na	185.19±0.96	154.59±0.56	114.87±0.60	83.339±0.18	116.84±0.54	67.70±0.27	75.70±0.44	142.04±2.04
K	148.6±0.56	194.79±0.59	190.62±0.41	137.31±0.76	102.68±1.65	188.01±0.44	177.82±0.55	218.19±1.61
Ca	381.27±2.73	244.77±0.61	237.43±1.18	178.52±0.31	166.94±0.27	127.41±0.28	134.22±0.19	186.31±0.64
Mg	50.57±0.59	20.45±0.29	23.19±0.20	26.53±0.58	42.51±0.90	30.07±0.46	55.08±1.05	97.8±1.68
Fe	9.44±0.04	4.73±0.02	1.67±0.05	1.038±0.02	7.743±0.10	3.443±0.04	3.159±0.06	12.60±0.15
Cu	7.74±0.06	4.78±0.08	0.15±0.01	3.65±0.04	0.612±0.02	0.248±0.03	2.218±0.02	0.66±0.01
Zn	5.136±0.03	3.53±0.03	0.07±0.01	0.545±0.02	0.28±0.01	1.737±0.03	1.12±0.01	2.14±0.01
Cr	0.236±0.02	0.177±0.01	0.013±0.01	0.38±0.01	0.08±0.01	0.07±0.01	0.09±0.01	0.05±0.01
Ni	0.36±0.01	0.10±0.01	0.07±0.01	0.12±0.01	0.25±0.01	0.04±0.01	0.98±0.01	0.78±0.01
Mn	22.66±0.19	23.51±0.29	14.72±0.11	8.86±0.05	18.47±0.24	7.62±0.03	13.00±0.09	17.42±0.26
Cd	0.171±0.01	ND	ND	0.131±0.01	0.093±0.01	0.04±0.01	0.05±0.01	0.05±0.01
Pb	0.25±0.02	ND	ND	1.51±0.01	0.15±0.01	0.03±0.01	0.06±0.01	0.04±0.01
P	0.51±0.01	9.60±0.09	0.37±0.02	2.04±0.03	14.42±0.22	2.39±0.04	1.39±0.03	0.90±0.04
Si	ND	1.46±0.05	0.01±0.01	ND	ND	ND	0.15±0.01	ND
Al	ND	1.13±0.01	ND	0.93±0.02	0.15±0.01	0.033±0.02	ND	0.061±0.01
S	ND	1.53±0.01	0.031±0.01	13.33± 0.185	1.53±0.03	ND	ND	ND

ND = Not detected, Mean ± SEM, n = 3

Free radical scavenging activity

The free radical DPPH was used to check the scavenging ability of plant extracts. 50% inhibitory concentration IC_{50} of control and plant were calculated by graph pad prism 5.0 software, San Diego, USA by fitting the data to a non-linear regression curve. The mean IC_{50} value of ascorbic acid was calculated as 3.52 $x10^{-3}$ by repeating concentrations. The IC_{50} value of each extract was also found by same using graph pad prism. The ascorbic acid equivalents antioxidant activity was than calculated with help of formula. While the IC_{50} of % scavenging ability of free radical DPPH is presented in the form of graphs in Fig. 2. The result of our study showed that *S. heteromalla* and *C. arvensis* exhibit high scavenging potential for stable free radical DPPH with the scavenging ability with the IC_{50} value of 98.36 µg/ml and 147.096 µg/ml at highest concentration as compared to other. There ascorbic acid equivalent values are also high as compared to other plants, with mean of 216.28±0.15 mg AA/100 g and 208.2 ± 0.35 mg AA/100 g followed by *S. moocrofitiana* 198.33 ± 0.23 mg AA/100 g, *L. serriola*194.29 ± 0.33 mg AA/100 g and *P. plebeium* 160.32 ± 0.28 mg AA/100 g, respectively. While the lower scavenging ability was observed in *L. aphaca* and *I. purpurea* with mean values of 83.17 ± 0.25 mg AA/100 g and 62.14 ± 0.24 mg AA/100 g. Similar study conducted on *L. serriola* by (Abd-ElGawad et al., 2019; Al-Laith et al., 2019) showed that the plants exhibited significant antioxidant activity with the IC50 value of 257.9 µL L^{-1}. The research also showed that the non-nutritive compounds in this plant have strong relationship with its scavenging ability, as many volatile phenolic compounds are identified in the leaves of this plant.

Reducing power ability

The reducing power of plants was analyzed by their ability to reduce Fe^{3+} into Fe^{2+}. The more reduction of Fe^{3+} into Fe^{2+} give the higher absorbance value as shown in Fig. 3. Results were expressed as mmol Fe^{2+}/100 g by the standard calibration curve of $FeSO_4 \cdot 7H_2O$ ($y = 0.0031x + 1.4144$, $R^2 = 0.998$). The data is presented in Table 7 with mean standard error, all the results were found significantly (P<0.05). The result of our study showed that, *P. plebeium* have high ability to reduce Fe^{3+} into Fe^{2+} with the mean value of 1.70 ± 0.003 mmol Fe^{2+}/100 g, followed by *C. arvensis* 1.54 ± 0.002 mmol Fe^{2+}/100 g, *S. mocroofitiana* 1.37 ± 0.002 mmol Fe^{2+}/100 g, *S. heteromalla* 1.36 ± 0.006 mmol Fe^{2+}/100 g, *L. serriola* 1.32 ± 0.004 mmol Fe^{2+}/100 g, *and S. conoidea* 1.10 ± 0.003 mmol Fe^{2+}/100 g. While lower reducing ability was observed in *L. aphaca* and *M. neglecta* with the mean value of 0.36 ± 0.003 mmol Fe^{2+}/100 g and 0.46 ± 0.006 mmol Fe^{2+}/100 g, respectively. Most of the researcher (El-Esawi et al., 2017; Petkova et al., 2019) correlate the reducing power of plant extracts with presence of polyphenol which also confirms our results the extract with more polyphenol content showed good reducing ability.

Total antioxidants

Total antioxidant activity was measured by phosphomolybdate and showed in Table 7 with mean standard error. The data is measured in milligram equivalents ascorbic acid per 100 grams (AACA mg/100g), by the standard calibration curve of ascorbic acid (y = 0.0017x + 1.2334, R^2 = 0.9937). Result showed that *S. heteromalla and P. plebeium* have higher total antioxidant with the IC_{50} value of 99.36 µg/ml and 102.25 µg/ml and ascorbic acid values of 266.40 ± 0.33 mg AA/100 g, Followed by, *S. mocroofitiana* 240.20 ± 0.32 mg AA/100 g, *C. arvensis* 234.30 ± 0.28 mg AA/100 g *and L. serriola* 229.23 ± 0.40 mg AA/100 g (Fig. 4). While the lower total antioxidant values were observed in *L. aphaca* and *I. purpurea* with the mean value of 119.97 ± 0.31 mg AA/100 g and 116.98 ± 0.21 mg AA/100 g, respectively. All the results were found to be significant.

Table 7 Free radical scavenging, total antioxidant and reducing power ability of plant extracts

CME of plants	DPPH mg AA/100 g	Total Antioxidant mg AA/100 g	Reducing power mmol Fe^{2+}/100 g
Saussurea heteromalla	216.28±0.15	266.40±0.33	1.37±0.002
Calendula arvensis	208.2±0.35	234.30±0.28	1.54±0.002
Slavia moorcroftiana	198.33±0.23	240.20±0.32	1.36±0.006
Lactuca serriola	194.29±0.33	229.23±0.40	1.32±0.004
Polygonum plebeium	160.32±0.28	254.83±0.36	1.70±0.003
Malva neglecta	102.52±0.38	143.39±0.23	0.46±0.006
Sida cordata	93.32±0.34	136.96±0.30	0.53±0.003
Silene conoidea	91.90±0.34	151.27±0.48	1.10±0.003
Lathyrus aphaca	83.17±0.25	119.97±0.31	0.36±0.003
Ipomea purpurea	62.14±0.24	116.98±0.21	0.55±0.004

Mean ± SEM, n = 3

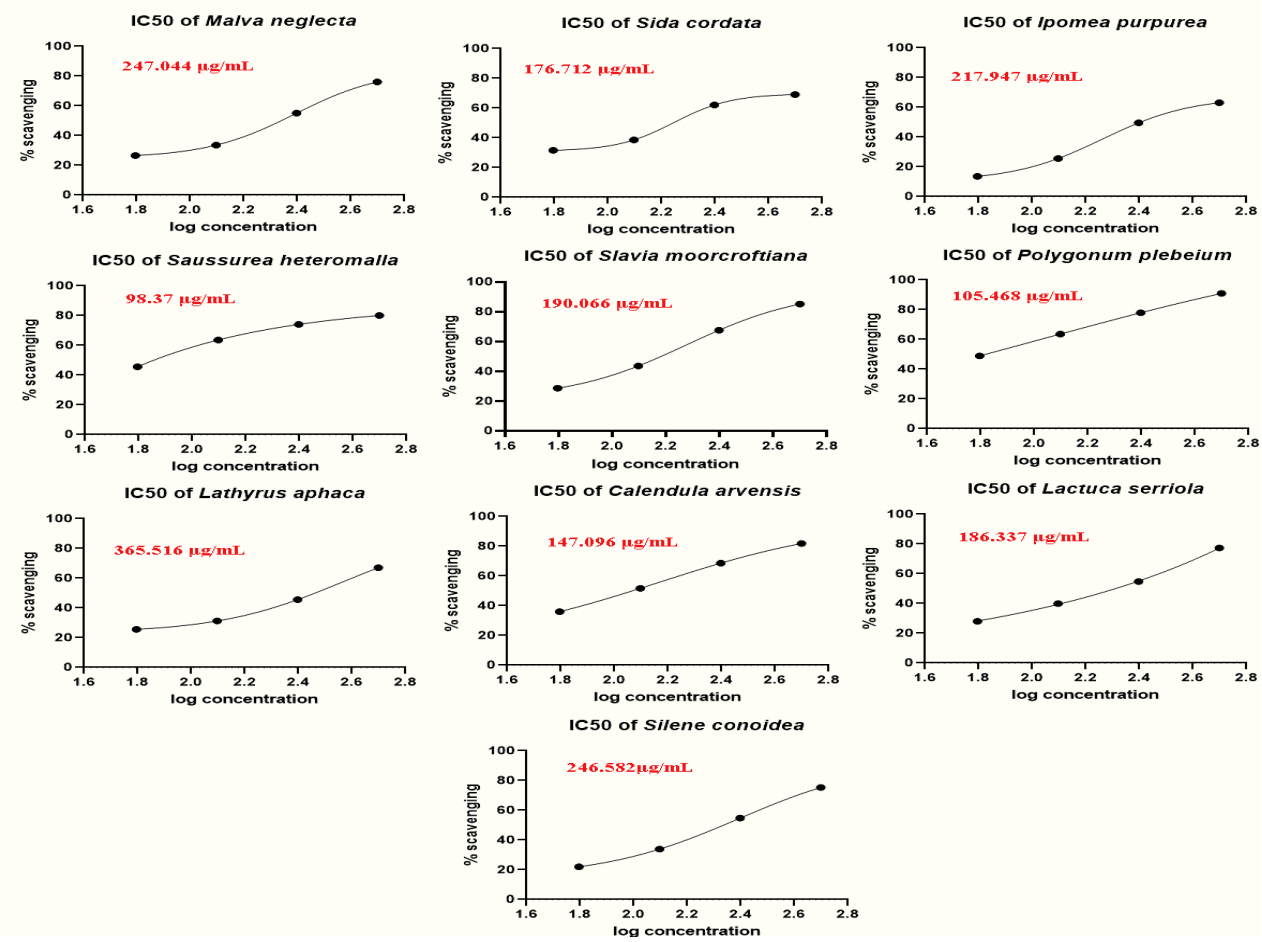

Fig. 2 Free radical scavenging (%) IC50 values of different plants

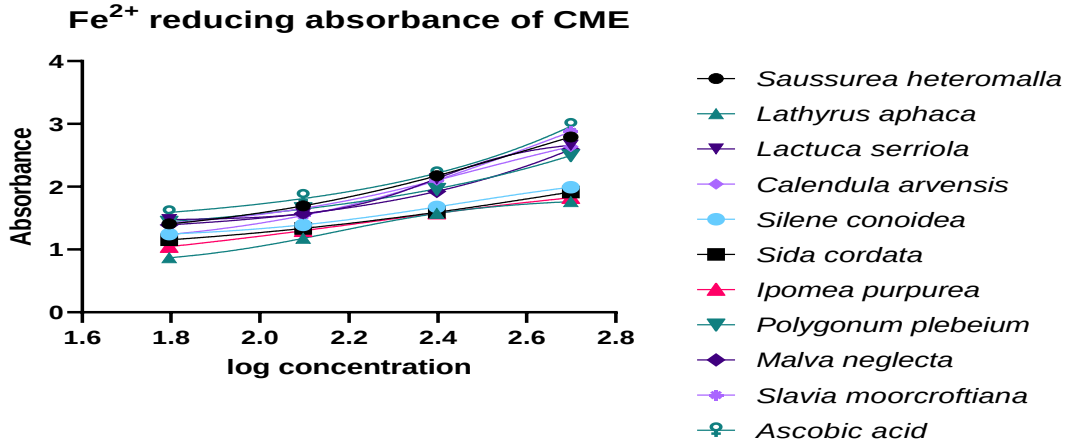

Fig. 3 Fe^{2+} absorbance of different plant extracts

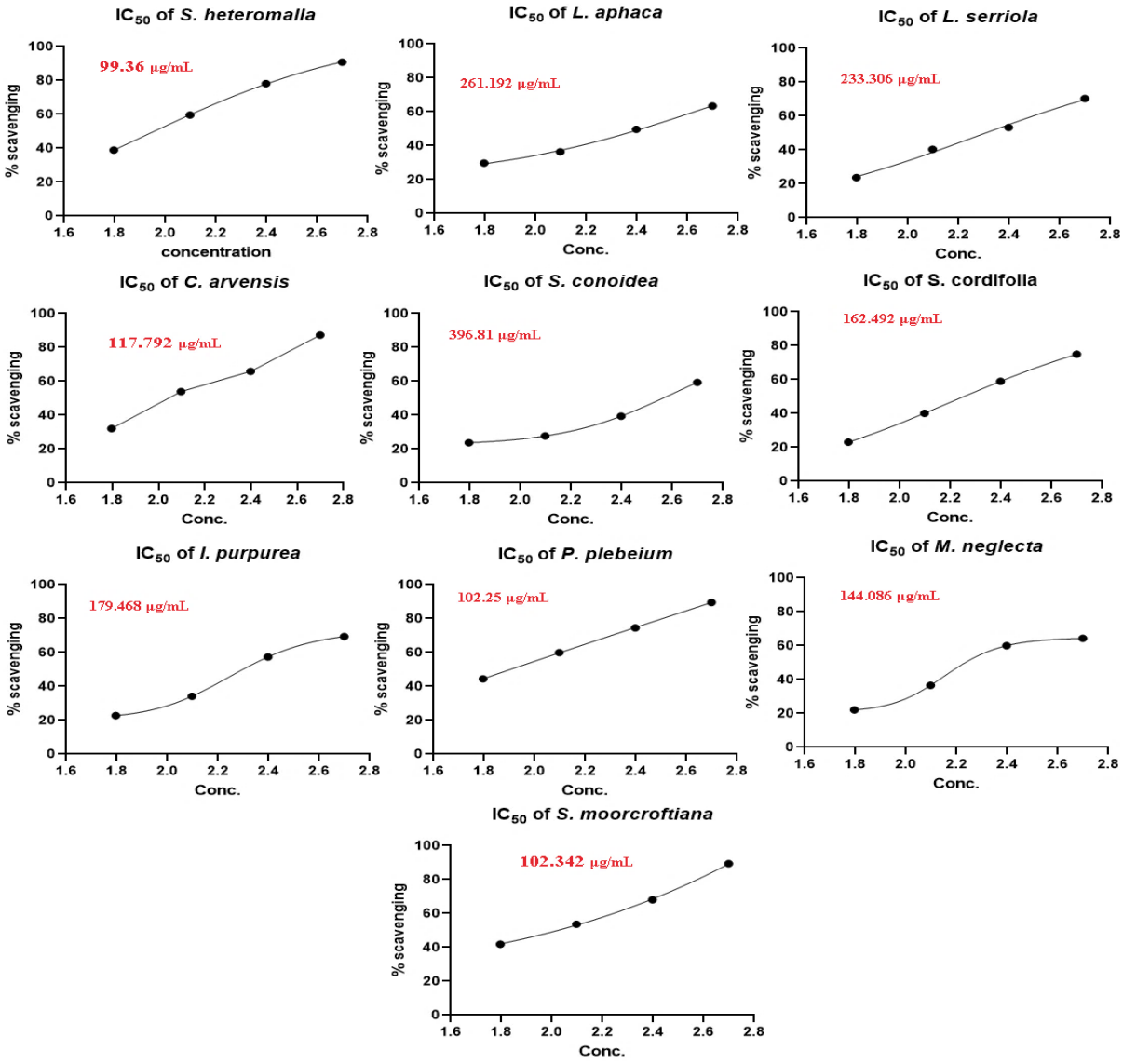

Fig. 4 Scavenging (%) and IC_{50} values of total antioxidant activity

Conclusion

Our observation revealed that the traditional medicinal plants are high in their nutritive and non-nutritive components which provide the strong relationship of their medicinal effect. In our study most of the plants are safe to use for the treatment of diseases with the specific dosages. The most nutrient rich plants were *C. arvensis* and *L. aphaca* with the total energy value of 407.28 ± 0.49 kcal/100 g and 407.78 ± 0.59. From our study we conclude that the nutritive plants are a good source of medicine.

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