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Chemical Composition, Mineral Profile and Antimicrobial Activity of *Stachys parviflora* and *Calotropis procera*

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In the present study, two medicinally important plants, namely *Stachys parviflora* and *Calotropis procera* were explored for their chemical composition, mineral profile and antimicrobial activity. For this purpose, the selected plans were collected from their natural habitat and their various parts (roots, stems, leaves, flowers, fruits and seeds) were analyzed for moisture, ash, fibre, fat, protein and carbohydrate contents. The results showed that both the plants were nutritionally rich with good computed energy. Regarding the mineral analysis, sufficient amount of essentials macro- (Na, K, Ca, Mg and P) and micro- (Fe, Zn, and Cu) mineral were found in all parts of the plants. Both the plants accumulate variable quantities of these elements which can provide a part of the recommended dietary allowance (RDA). The antimicrobial activity of the aqueous, ethanol, ethyl acetate and n-hexane extracts of both the plants exhibited quantifiable inhibitory effect against the pathogenic six bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus atrophaeus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* and a fungal strain (*Candida albicans*). Based on the results obtained, the studied plants were found to be a potential source of food diet with natural minerals and good antimicrobial activity.

Key words: Antimicrobial activity, Chemical composition, Mineral profile, *Stachys parviflora*, *Calotropis procera*.

Stachys parviflora is commonly known as Baggibuti. It is a perennial herb, distributed in the temperate and tropical regions of Pakistan. It belongs to family *Lamiaceae* and genus *Stachys*[1,2]. Phytochemical investigation of genus *Stachys* has demonstrated a large number of chemical constituents, which include polyphenols, tannin, alkaloids, flavonoids, diterpenoids, saponins, phenylethanoid glycosides, phenylethly alcohol glycosides, iridoids, terpenoids, diterpenes, fatty acids, essential oils and some others[3-6]. Ahmad *et al.* have isolated two new phenethyl alcohol glycosides namely parviflorosides A and B[1] and two new triterpenoidal saponins, stachyssaponin A and B[2] from the butanolic extract of whole plant of *S. parviflora*. Similarly, the essential oil composition of the aerial parts of *S. parviflora* was analyzed by GC-FID and GC-MS and the results showed twenty-three compounds, representing 99.9% of the oil. Muurolol (48.4%) and oxygenated sesquiterpenes (71.4%) were the major components of oil and essential oil, respectively[7]. The reported biological activities of *Stachys* species include antimicrobial, antifungal, antioxidant, anxiolytic, anti-inflammatory, antinociceptive, antiproliferative, anti-allergic,

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cytotoxic, hypotensive, hyaluronidase, neuroprotective and antinephritic properties. In the traditional medicine, *Stachys* species have been used as disinfectant, antispasmodic, cough relieve, asthma, earaches, in wounds healing and to inhibit the development of genital tumor and cancerous ulcer[3-6].

Calotropis procera is commonly known as giant milk, belongs to family Asclepiadaceae and genus *Calotropis*. It is a xerophytic, erect shrub and can grow about 6 m in height. It is widely distributed in arid and semi-arid regions of the world including Pakistan. Various phytochemical constituents reported from *C. procera* include alkaloids, flavonoids, flavonoids quercetin, flavones, steroids, cardiac glycosides, tannins, sterols, saponins, saponin glycosides, natural hydrocarbons, anthocyanins, triterpenes, triterpenoids, norditerpenic esters, organic carbonates, cysteine protease procerain, resins, numerous cardenolides and a large number of other chemicals[8-12]. The chemical analysis of *C. procera* showed the presence of various metals (Al, As, Cu, Ca, Cr, Cd, Fe, K, Mn, Na, Pb, and Zn), total protein (27-32%)[7], saturated and unsaturated fatty acids[13]. The biological potentials of *C. procera* are very well known. The common reported activities are; antibacterial, antifungal, ascaricidal, larvicidal, insecticidal, nematocidal, molluscicidal, cytotoxic, anticancerous, schizonticidal, anthelmintic, anti-inflammatory, antidiarrhoeal, antinociceptive, spasmolytic, inflammatory, antipyretic, antioxidant, wound healing protective, analgesic, antiulcer, anti-fertility and anticoccidial[8-10,14,15]. In the folk medicine, the plant is used for the treatment of ulcers, tumors, leprosy, elephantiasis, fever, menorrhagia, malaria and snake bite, ringworm, syphilitic sores, purulent wound infections, purgative, antihelmintic, digestive problems, asthma, boils, dysentery, eczema, piles, diseases of liver and spleen disorders[10]. Beside its phytomedicinal potentials, the plant can also be used as a potential biosorbent and bioindicator[16-18]. As part of our continuous research program on the exploration of medicinal plants[19-29], the present work was undertaken. In the current study, another two medicinally important plants namely; *S. parviflora* and *C. procera* were explored for their chemical composition, mineral profile and antimicrobial potentials.

MATERIALS AND METHODS

Reagent and chemicals

All reagents and chemicals used in this study were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless stated otherwise. Distilled water was used throughout this work.

Collection and pre-treatment plant materials

The selected medicinal plants (*Stachys parviflora* and *Calotropis procera*) were collected from Timergara region of Khyber Pakhtunkhwa, Pakistan in their blooming season using standard botanical field collection methodology[27,28]. The plants were identified and authenticated by Dr. Samin Jan, Department of Botany, Islamia College University, Peshawar, Pakistan. The voucher specimens No. SJ-2010 and SJ-2010, respectively, for *S. parviflora* and *C. procera* were deposited in the Herbarium of the above-mentioned department, for future reference. The collected plant materials were transferred to laboratory and washed with distilled water for the removal of possible foreign particles.

Chemical composition

For the determination of chemical composition, the plant materials were divided into roots, stems, leaves, flowers, fruits and seeds. For the determination of moisture content, the plant materials were dried at 80 °C to a constant weight while the ash content was obtained by ignition of the samples at 550 °C for 6 h using muffle furnace. The crude fibers and fats were determined using the literature method[28,30]. Similarly, the nitrogen content (*N*) of the samples was estimated by Kjeldahl method and crude proteins were calculated as $N \times 6.25$[28,30]. The amount of total carbohydrates was obtained by the difference between weight of the sample taken and sum of its moisture, ash, total fiber, lipid and protein contents. The computed energy values were obtained by multiplying the values of carbohydrate, fat and protein with the factors 4, 9, and 4, respectively; then the products were summed and expressed in kcal/100 g[28].

Mineral analysis

Various mineral were also determined in the plant materials (roots, stems, leaves, flowers, fruits and seeds) using the reported methods. For the determination of metals (Na, K, Ca, Mg, Fe, Zn and Cu), an atomic absorption spectrophotometer

(Analyst 700, Perkin Elmer, USA) was used while for P analysis, the reported spectrophotometric method was adopted[24,27,28].

Preparation of plant extracts

The cleaned plant materials (whole plant of *S. parviflora* and leaves of *C. procera*) were shade dried at room temperature and coarsely grounded with ordinary grinder. The plant materials were soaked with ethanol for a week at room temperature using separatory funnel with occasional shaking. After a week, the extracts were filtered and evaporated under reduced pressure at 40 °C using a rotary evaporator[19,20,26]. The waxy masses were obtained after complete drying yield 11 and 13% for *S. parviflora* and *C. procera*, respectively. The residues were re-extracted with four different solvents namely; distilled water (aqueous), ethanol, ethyl acetate and n-hexane. The extracts were left at room temperature for 48 h and the solvents were again evaporated till dryness. The resulting crude extracts were stored at -4 °C till further use [19,20,26].

Antimicrobial activity

The antimicrobial activity of the crude extracts were tested against the common human pathogens including six bacterial strains out of which three were gram positive (*Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus atrophaeus*) and three were gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and a fungal strain (*Candida albicans*). The antibacterial activity was performed against the selected bacterial strains by the agar well diffusion protocol[19,20,26] while the antifungal activity of the extracts was evaluated by employing the agar tube dilution method[19,20,26]. The antimicrobial activity was determined by measuring the zone of inhibition in millimeter (mm). The standard drugs (azithromycin, ciprofloxacin and clotrimazole) and DMSO were used as positive and negative controls, respectively.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of the triplicate analysis. The data were statistically analyzed using the statistical program (Origin Version 5.1). The significant differences between means were calculated by a one-way analysis of variance (ANOVA) using Duncan's multiple-range test at $P<0.05$[28].

RESULTS

The chemical composition of the selected medicinal plants showed variable quantities of moisture, ash, crude fibers, fats, proteins and carbohydrates in their different parts (Table 1). The results showed that in all the studied parts of *S. parviflora*, the moisture and crude fiber contents were higher as compared to *C. procera*. However, *C. procera* showed larger quantities of crude fats, proteins and computed energy values in comparison with *S. parviflora* (in all the studied parts). Large amount of ash contents were recorded in all the studied parts of *C. procera* except roots and fruits. In case of total carbohydrates, greater quantities were found in the roots, flowers and seeds of *S. parviflora* while the stems, leaves and fruits of *C. procera* showed sufficient amount of total carbohydrates. The average percentage composition of the studied parameters in all the parts of the plants was in the order of; *S. parviflora*; moisture (78.51) > proteins (7.26) > fats (4.71) > carbohydrates (4.51) > crude fibers (2.90) > ash (1.90) and computed energy of 89.14 kcal/100 g and *C. procera* moisture (76.60) > proteins (9.43) > fats (5.15) > carbohydrates (4.32) > crude fibers (2.54) > ash (1.96) and energy (101.41 kcal/100 g) (Table 1).

In the elemental analysis, various mineral elements (Na, K, Ca, Mg, P, Fe, Zn and Cu) were detected in all the studied parts of the selected medicinal plants (Table 2). In all the studied elements, Mg was found in large quantity in *C. procera* as compared to *S. parviflora* while the amount of Na was high in *S. parviflora*. Similarly, Fe and Zn was recoded in sufficient quantity in the roots, stems, leaves, seeds and fruits of *S. parviflora* while concentration of K, P, Ca and Cu was found to be higher in most of the studied parts of *C. procera* (Table 2).

The antimicrobial activity of the aqueous, ethanol, ethyl acetate and n-hexane extracts of *S. parviflora* (whole plant) and *C. procera* (leaves) was determined using six bacterial and one fungal (*C. albicans*) strains. The results of the antimicrobial activity were measured in term of zone of inhibition in mm (Table 3). All the crude extracts showed low to significant activity. For the aqueous extracts, the zone of inhibition was from 9.0±0.16 to 17.0±0.82 mm and 11.5±0.32 to 15.0±0.51 mm for

Table 1. Chemical composition and energy value of *Stachys parviflora* and *Calotropis procera*

Plant Parts	Selected Medicinal Plants	Chemical Composition (%)[a] Moisture[b]	Ash	Fibers	Fats	Proteins	Carbohydrates	Energy (kcal/100 g)
Roots	*Stachys parviflora*	79.02±3.12	2.56±0.11	2.59±0.07	4.26±0.31	7.35±0.32	4.22±0.12	82.62±3.01
	Calotropis procera	76.63±2.37	2.26±0.06	2.46±0.12	5.14±0.22	10.32±0.61	3.19±0.09	100.30±4.43
Stems	*Stachys parviflora*	78.13±2.91	1.87±0.04	2.87±0.11	5.04±0.25	8.74±0.45	3.35±0.10	93.72±4.11
	Calotropis procera	74.56±2.64	2.63±0.12	2.85±0.06	5.48±0.33	9.68±0.55	4.80±0.21	107.31±5.42
Leaves	*Stachys parviflora*	80.24±3.01	1.44±0.01	3.63±0.23	4.31±0.19	6.81±0.47	3.57±0.13	80.31±2.98
	Calotropis procera	79.28±2.82	1.49±0.03	3.01±0.21	4.76±0.14	7.86±0.34	3.60±0.08	88.86±3.44
Flowers	*Stachys parviflora*	79.65±2.53	1.19±0.01	2.92±0.20	4.52±0.21	5.26±0.21	6.46±0.35	87.64±4.16
	Calotropis procera	77.54±3.21	2.01±0.10	2.47±0.15	4.95±0.18	8.45±0.43	4.58±0.11	96.67±4.55
Fruits	*Stachys parviflora*	78.33±2.55	2.60±0.05	2.65±0.09	5.17±0.32	6.78±0.35	4.47±0.22	91.53±5.10
	Calotropis procera	76.21±2.37	1.53±0.06	1.85±0.07	5.23±0.26	10.01±0.62	5.17±0.31	107.79±4.95
Seeds	*Stachys parviflora*	76.98±2.63	1.72±0.09	2.74±0.14	4.96±0.23	8.62±0.43	4.98±0.11	99.04±3.88
	Calotropis procera	75.34±3.61	1.86±0.07	2.59±0.09	5.34±0.41	10.27±0.58	4.60±0.07	107.54±4.63

a: Dry weight basis; b: Fresh weight basis.

Table 2. Mineral composition of *Stachys parviflora* and *Calotropis procera*

Plant Parts	Selected Medicinal Plants	Mineral Composition (mg/100 g)[a] Na	K	Ca	Mg	P	Fe	Zn	Cu
Roots	*Stachys parviflora*	1.60±0.06	3.02±0.08	23.0±1.10	5.20±0.05	13.21±0.75	0.71±0.01	0.86±0.01	0.11±0.00
	Calotropis procera	1.10±0.05	2.40±0.04	25.04±1.12	17.21±0.87	14.44±0.81	0.22±0.00	0.17±0.00	0.16±0.00
Stems	*Stachys parviflora*	1.20±0.03	1.00±0.02	20.10±0.91	1.30±0.01	10.28±0.28	1.02±0.01	0.20±0.00	0.10±0.00
	Calotropis procera	0.70±0.01	1.05±0.01	21.12±0.82	24.6±2.82	12.45±0.53	0.76±0.01	0.11±0.00	0.09±0.00
Leaves	*Stachys parviflora*	2.15±0.04	7.02±0.11	14.50±0.10	10.03±0.09	12.01±0.32	1.70±0.012	0.81±0.01	0.14±0.00
	Calotropis procera	2.20±0.05	7.89±0.12	21.62±1.02	18.24±0.43	10.80±0.21	1.30±0.02	0.72±0.01	0.17±0.00
Flowers	*Stachys parviflora*	1.95±0.02	2.03±0.03	12.56±0.09	3.72±0.02	10.45±0.18	0.94±0.01	0.23±0.00	0.08±0.00
	Calotropis procera	1.56±0.02	2.47±0.02	9.98±0.04	15.33±0.22	8.75±0.09	1.52±0.02	0.43±0.01	0.11±0.00
Fruits	*Stachys parviflora*	0.70±0.01	2.35±0.04	10.24±0.05	1.21±0.01	9.03±0.08	0.41±0.01	0.15±0.00	0.06±0.00
	Calotropis procera	0.60±0.01	0.70±0.02	9.65±0.04	3.69±0.02	9.41±0.07	0.43±0.01	0.11±0.00	0.09±0.00
Seeds	*Stachys parviflora*	0.30±0.01	0.32±0.01	11.45±0.06	0.97±0.00	5.54±0.02	0.30±0.01	0.13±0.00	0.07±0.00
	Calotropis procera	0.12±0.00	0.60±0.01	7.96±0.06	6.33±0.05	7.75±0.04	0.19±0.00	0.16±0.00	0.09±0.00
	RDA (mg/day)	1300–1500	2000–2300	1000–1200	330–350	580–700	6-8	8–11	0.7–0.9
	TUL (mg/day)	2300	4700	2500	350	4000	45	40	10

a: Dry weight basis; RDA: Recommended dietary allowance; TUL: Tolerable upper limit.

Table 3. Antimicrobial activity of the crude extracts of *Stachys parviflora* (whole plant) and *Calotropis procera* (leaves)

Microbial Strains		Selected Medicinal Plants	Zone of Inhibition (mm) Crude Extracts				Standard Drugs
			Aqueous	Ethanol	Ethyl acetate	n-Hexane	
Bacterial Strains	Gram Positive	*Staphylococcus aureus*	12.0±0.91	9.0±0.56	14.5±0.92	9.5±0.36	23±1.06[a]
		Calotropis procera	12.5±0.86	11.5±0.73	11.0±0.65	10.5±0.56	
		Bacillus subtilis	*Stachys parviflora*	11.5±0.46	13.5±0.51	18.0±0.98	11.5±0.41	25±1.12[a]
			Calotropis procera	15.5±0.67	9.0±0.32	10.0±0.41	11.0±0.38	
		Bacillus atrophaeus	*Stachys parviflora*	17.0±0.82	12.5±0.43	18.5±0.87	12.5±0.47	22±1.35[a]
			Calotropis procera	15.0±0.51	10.5±0.32	12.0±0.66	8.5±0.29	
	Gram Negative	*Escherichia coli*	*Stachys parviflora*	11.5±0.32	14.0±0.46	13.0±0.53	12.5±0.31	41±2.27[b]
			Calotropis procera	11.5±0.35	9.5±0.27	10.0±0.28	8.0±0.22	
		Pseudomonas aeruginosa	*Stachys parviflora*	12.0±0.41	14.2±0.58	10.5±0.29	13.5±0.38	34±1.68[b]
			Calotropis procera	13.0±0.50	11.0±0.42	14.0±0.61	9.0±0.21	
		Salmonella typhi	*Stachys parviflora*	9.5±0.26	9.5±0.27	8.5±0.22	12.5±0.76	42±2.25[b]
			Calotropis procera	10.5±0.31	10.0±0.26	9.0±0.23	8.5±0.21	
Fungal Strain	*Candida albicans*	*Stachys parviflora*	9.0±0.16	9.0±0.22	10.0±0.26	9.5±0.27	32±1.43[c]
		Calotropis procera	11.5±0.32	11.0±0.36	7.5±0.14	9.5±0.25	

a: Azithromycin; b: Ciprofloxacin; c: Clotrimazole.

S. parviflora and *C. procera*, respectively. The activity of these extracts were high for gram positive bacterial strains, followed by gram negative bacterial strains and least for fungal strain. Similarly, the ethanolic of extract of *S. parviflora* (9.0±0.22-14.2±0.58 mm) showed better antimicrobial activity as compared to *C. procera* extract (9.0±0.32-12.5±0.43 mm). The overall activity of these extract were in the order of; gram negative bacteria > gram positive bacteria > fungal strain. In case of ethyl acetate extracts, *S. parviflora* exhibit low to significant (8.5±0.22-18.5±0.87 mm) antimicrobial activity in comparison with *C. procera* extract (7.5±0.14-14.0±0.61 mm). Again, the trend was more towards gram positive bacteria strains, followed by gram negative bacteria and fungal strain. The n-hexane extracts of both the plants displayed moderate to low antimicrobial activity against the tested microorganisms. In this case, the extracts were more active towards gram negative bacteria as compared to gram positive bacteria and fungal strain. The all the four extracts showed the following order of antimicrobial activity; ethyl acetate > aqueous > ethanol > n-hexane. The extracts displayed better antibacterial activity as compared to antifungal activity (Table 3).

DISCUSSION

The results showed that the studied plants could be a potential source of fibers, fats, proteins, carbohydrates and hence energy. Nutritional composition and elemental analyses of selected wild edible plants[31] medicinal plants of the family *Polygonaceae* and other have reported previously[32,33]. Similarly, the chemical composition, mineral profile and antioxidant potentials of the selected *Morus* species were reported by Imran *et al.*[28]. Plants that provide more than 12% of its calorific value from protein are considered good sources of protein. The total protein contents of the *S. parviflora* and *C. procera* were found to be the range of 5.26±0.21% (flowers) to 8.74±0.45% (stems) and 7.86±0.34% (leaves) to 10.32±0.61% (roots), respectively. The protein contents of *C. procera* were comparable with the reported literature[34-36].

The living organisms such as plants and animals as well as human beings required significant

amounts of various essential minerals for maintenance of life. The importance of dietary minerals for the prevention of various diseases is well-documented in the literature although, human body comprise only 4-6% of such minerals. In plants, the mineral content is influenced by the physico-chemical characteristics of the soil, climate, water supply, plant species and stages of development[24,27]. In the present study, the studied medicinal plants showed the presence of essential macro- (Na, K, Ca, Mg and P) and micro- (Fe, Zn and Cu) mineral elements. The concentration of mineral elements was found to be varied in the studied parts of the medicinal plants. According to our results, roots, stems and leaves accumulate higher amount of mineral elements as compared to flowers, fruits and seeds (Table 2). Accumulation of various mineral elements in different parts of the medicinal plants was also reported elsewhere[23,24,28,31].

Many microorganisms which cause damage to human health, exhibits drug resistance due to inadequate use of antibiotics. Certain species of bacteria such as *B. subtilis*, *S. aureus* and *B. atrophaeus* were found to be more opposed to ordinary antibiotics as compared to others. The antimicrobial properties of secondary metabolites from a variety of plants have been assessed time to time. To search for traditionally used medicinally plants with potent antimicrobial properties, the various solvent extracts of the selected medicinal plants were explored which showed moderate to significant activity against the tested common human pathogens (Table 3). So, the studied medicinal plants could be used to develop new natural drugs. Dulger and Aki[37] investigated the antimicrobial activities of the ethanolic extract of *Stachys pseudopinardii* (leaves) which exhibit moderate activity against the tested microorganism (*B. subtilis, B. cereus, S. aureus, E. coli, Proteus vulgaris, Salmonella typhimurium, P. aeruginosa, C. albicans, Debaryomyces hansenii, Kluyveromyces fragilis* and *Rhodotorula rubra*). In another study, the of antimicrobial activity of diethyl ether and ethyl acetate extracts obtained from the aerial parts of *Stachys* species (*S. germanica, S. iva, S. plumosa a*nd *S. scardica*) was investigated which showed significant activity against the tested microbes (*S. aureus, E. coli, Klebsiella pneumoniae, P. aeruginosa, S. enteritidis, Aspergillus niger* and *C. albicans*)[4]. The antimicrobial activity of volatile constituents of eight *Stachys* species was tested against the selected pathogens and it was found essential oils showed better activity against bacterial species than against fungi[38]. Previously, Yesmin *et al.*[39] has investigated the antibacterial activity of methanol and aqueous extracts of *C. procera* leaves against the selected gram positive and negative bacterial species. The results showed that the both the extracts exhibited no inhibition to moderate activity. In a previous study, the antimicrobial activity of *C. procera* leaves and flowers extracts were tested against *B. pumilis, E. coli, A. niger* and *Fusarium oxysporum*. The results revealed that maximum antimicrobial activities were exhibited by 80% ethanol extract of leaves whereas lowest by 80% acetone extract of flowers[15]. According to Kareem *et al.*[40] reported that the aqueous extract of *C. procera* leaves was not effective against *P. aeruginosa*, however, latex extract showed good activity against the same species. The ethanolic extract of *Paeonia emodi* (aerial parts) did not show the antimicrobial activity against the tested bacteria and fungi[19]. Similarly, the aqueous and chloroform extracts of selected medicinal pants display moderate to good activity against the gram positive bacteria, very low activity against gram negative bacteria and no antifungal activity[29]. The antibacterial activity of various crude extracts (n-hexane, chloroform, ethyl acetate and methanol) of different parts (roots, stem and leaves) of *Theraxicum officinale* were tested against common human pathogens and only the methanolic extract of roots showed better activity.[41]

CONCLUSIONS

In the present study, analytical investigations were carried out to ascertain the chemical composition, minerals profile and antimicrobial potentials of *S. parviflora* and *C. procera*. The results showed that both the studied plants were nutritionally rich with sufficient amount of natural fibers, proteins, carbohydrate and hence energy. The plants could also be good sources of essential macro- and micro-minerals with significant antimicrobial activity which further increases their nutritive as well as phytomedicinal potentials. The present study will also provide a

step towards the standardization of such plants as potential healthy source which may also be used in food and pharmaceutical industries. It would be of great interest if further research is carried out for the isolation of biologically active compounds present in the studied medicinal plants.

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REFERENCES

1. Ahmad, V.U., Arshad, S., Bader S., Ahmed, A., Iqbal, S., Tareen, R.B. New phenethyl alcohol glycosides from *Stachys parviflora*. *J. Asian Nat. Prod. Res.*, 2006; **8**: 105-11.
2. Ahmad, V.U., Arshad, S., Bader, S., Iqbal, S., Khan, A., Khan, S.S., Hussain, J., Tareen, R.B., Ahmed, A. New terpenoids from *Stachys parviflora* Benth. Magn. *Reson. Chem.*, 2008; **46**: 986-89.
3. Vundac, V.B., Brantner, A.H., Plazibat, M. Content of polyphenolic constituents and antioxidant activity of some *Stachys taxa*. *Food Chem.*, 2007; **104**: 1277-81.
4. Lazarevic, S.J., Palic, R.M., Radulovic, N.S., Ristic, N.R., Stojanovic, G. Chemical composition and screening of the antimicrobial and antioxidative activity of *Stachys species*. *J. Serb. Chem. Soc.*, 2010; **75**: 1347-59.
5. Hassanzadeh, M.K., Emami, S.A., Asili, J., Tayarani, N.Z. Review of the essential oil composition of Iranian Lamiaceae. *J. Essent. Oil Res.*, 2011; **23**: 35-74.
6. Tundis, R., Peruzzi, L., Menichini, F. Phytochemical and biological studies of *Stachys* species in relation to chemotaxonomy: A review. *Phytochemistry,* 2014; **102**: 7-39.
7. Bashi, D.S., Emami, S.A., Iranshahy, M., Asili, J. Essential oil composition of aerial parts of *Stachys parviflora* L. from Iran. *J. Essen. Oil Bear. Plant.*, 2013; **16**: 261-64.
8. Silva, M.C.C., da Silva, A.B., Teixeira, F.M., de Sousa, P.C.P., Rondon, R.M.M., Junior, J.E.R.H., Sampaio, L.F.L., Oliveira, S.L., Holonda, A.N.M., de Vasconcelos, S.M.M. Therapeutic and biological activities of *Calotropis procera* (Ait.) R. Br. *Asian Pacif. J. Trop. Med.*, 2010; 332-36.
9. Shaker, K.H., Morsy, N., Zinecker, H., Imhoff, J.F., Schneider, B. Secondary metabolites from *Calotropis procera* (Aiton). *Phytochem. Lett.*, 2010; **3**: 212-16.
10. Sharma, R., Thakur, G.S., Sanodiya, B.S., Savita, A., Pandey, M., Sharma, A., Bisen, P.S. Therapeutic potential of *Calotropis procera*: A giant milkweed. *IOSR J. Pharm. Biol. Sci.*, 2012; **4**: 42-57.
11. Ibrahim, S.R.M., Mohamedb, G.A., Shaala, L.A., Banuls, L.M.Y., Goietsenoven, G.V., Kiss, R., Youssef, D.T.A. New ursane-type triterpenes from the root bark of *Calotropis procera*. *Phytochem. Lett.*, 2012; **5**: 490-95.
12. Nenaah, G. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World J. Microbiol. Biotechnol.*, 2013; **29**: 1255-62.
13. Khanzada, S.K., Shaikh, W., Kazi, T.G., Sofia, S., Kabir, A., Usmanghani, K., Kandhro, A.A. Analysis of fatty acid, elemental and total protein of *Calotropis procera* medicinal plant from Sindh, Pakistan. *Pak. J. Bot.*, 2008; **40**: 1913-21.
14. Jain, S.C., Sharma, R., Jain, R., Sharma, R.A. Antimicrobial activity of *Calotropis procera*. *Fitoterapia*, 1996; **67**: 275-77.
15. Ahmad, N., Anwar, F., Hameed, S., Boyce, M.C. Antioxidant and antimicrobial attributes of different solvent extracts from leaves and flowers of akk [*Calotropis procera* (Ait.) Ait. F.)]. *J. Med. Plants Res.*, 2011; **5**: 4879-87.
16. Ali, H., Muhammad, S.H. Biosorption of crystal violet from water on leaf biomass of *Calotropis procera*. *J. Environ. Sci. Technol.*, 2008; **1**:143-50.
17. Waleed, J.A. Response of *Calotropis Procera* for urban, suburban and sewage pollution. Umm Al-Qura Univ. *J. Sci. Med. Eng.*, 2006; **18**: 31-40.
18. Mubeen, H., Naeem, I., Taskeen, A. Phytoremediation of Cu(II) by *Calotropis Procera* Roots. *New York Sci. J.*, 2010; **3**: 1-5.
19. Khan, T., Ahmad, M., Khan, H., Khan, M.A. Biological activities of aerial parts of *Paeonia emodi* Wall. *Afr. J. Biotechnol.*, 2005; **4**: 1313-16.
20. Khan, T., Ahmad, M., Khan, R., Khan, H., Ejaz, A., Choudhary, M.I. Evaluation of phytomedicinal potentials of selected plants of Pakistan. *Am. Lab.*, 2006; **8**: 20-22.
21. Khan, T., Ahmad, M., Khan, R., Khan, H., Choudhary, M.I. Phytotoxic and insecticidal activities of medicinal plants of Pakistan: Studies on *Trichodesma indicum, Aconitum laeve* and

Sauromatum guttatum. *J. Chem. Soc. Pak.*, 2007; **29**: 260-64.

22. Khan, A.L., Hamayun, M., Hussain, J., Khan, H., Gilani, S.A., Kikuchi, A., Watanabe, K.N., Jung, E.H., Lee, I.-J. Assessment of allelopathic potential of selected medicinal plants of Pakistan. *Afr. J. Biotechnol.*, 2009; **8**: 1024-29.
23. Khan, A.L., Hussain, J., Hamayun, M., Shinwari, Z.K., Khan, H., Kang, Y.-H., Kang, S.M., Lee I.-J. Inorganic profile and allelopathic effect of endemic *Inula koelzii* from Himalaya Pakistan. *Pak. J. Bot.*, 2009; **41**: 2517-27.
24. Hussain, I., Khan, L., Mehmood, T., Khan, I., Ullah, W., Khan, H. Effect of heavy metals on the growth and development of *Silybum marianum*, in various polluted areas of Peshawar, *Pakistan. J. Chem. Soc. Pak.*, 2005; **27**: 367-73.
25. Hussain, I., Khan, H., Saleem, M., Marwat, G.A., Iqbal, Y. Investigation of organic constituents of commercial tea brands and fresh tea leaves by high-performance liquid chromatography. *J. Chem. Soc. Pak.*, 2008; **30**: 571-76.
26. Hussain, I., Khan, F.A., Khan, H., Rehman, S.U., Badrullah. Antimicriobial, phytochemical and fluorescence studies on *Tribulus teresstris*. *J. Pharm. Res.*, 2011; **4**: 1556-58.
27. Hussain, I., Khan, H. Investigation of heavy metals content in medicinal plant, *Eclipta alba* L. *J. Chem. Soc. Pak.*, 2010; **32**: 28-33.
28. Imran, M., Khan, H., Shah, M., Khan, R., Khan, F. Chemical composition and antioxidant activity of certain *Morus* species. J. Zhejiang Univ.-Sci. B., 2010; 11: 973-80.
29. Al-Othman, A.M., Hussain, I., Khan, H., Rehman, M.U., Abdeltawab, A.A., Ullah, R., Rohullah, Noor, S., Talha, M. Phytochemical analysis and biological activities of selected medicinal plants. *J. Med. Plants Res.*, 2012; **6**: 4005-10.
30. AOAC (Association of Official Analytical Chemists), Official Methods of Analysis International, 17th edn., AOAC, Washington, DC, 2000.
31. Imran, M., Talpur, F.N., Jan, M.S., Khan, A., Khan, I. Analysis of nutritional components of some wild edible plants. *J. Chem. Soc. Pak.*, 2007; **29**: 500-08.
32. Hameed, I., Dastagir, G., Hussain, F. Nutritional and elemental analyses of some selected medicinal plants of the family *Polygonaceae*. *Pak. J. Bot.*, 2008; **40**: 2493-502.
33. Hameed, I., Dastagir, G. Nutritional analyses of *Rumex hastatus* D. Don, *Rumex dentatus* Linn and *Rumex nepalensis* Spreng. *Afr. J. Biotechnol.*, 2009; **8**: 4131-33.
34. Kalita, D. Hydrocarbon plant-New source of energy for future. Renew. *Sust. Energ. Rev.*, 2008; **12**: 455-71.
35. Kalita, D., Saikia, C.N. *Calotropis procera* and *Nerium indicum*-Two potential plants sources of energy and hydrocarbon. *Ind. J. Chem. Technol.*, 2001; **8**: 20-24.
36. Kalita, D., Saikia, C.N. Chemical constituents and energy content of some latex bearing plants. *Bioresour. Technol.*, 2008; **92**: 219-27.
37. Dulger, G., Aki C. Antimicrobial activity of the leaves of endemic *Stachys pseudopinardii* in Turkey. *Trop. J. Pharm. Res.*, 2009; **8**: 371-75.
38. Skaltsa, H.D., Demetzos, C., Lazari, D., Sokovic, M. Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece. *Phytochemistry*, 2003; **64**: 743-52.
39. Yesmin, M.N., Uddin, S.N., Mubassara, S., Akond, M.A. Antioxidant and antibacterial activities of *Calotropis procera* Linn. American-Eurasian *J. Agric. Environ. Sci.*, 2008; **4**: 550-53.
40. Kareem, S.O., Akpan, I., Ojo, O.P. Antimicrobial activities of *Calotropis procera* on selected pathogenic microorganisms. *Afr. J. Biomed. Res.*, 2008; **11**: 5-11.
41. Hussain, I., Ullah, R., AbdElsalam, N. M., Hayat, S., Uddin, K., Khan, J., Akhtar, N., Ullah Z. Antibacterial and preliminary phytochemicals screening of *Theraxicum officinale*. *J. Pure Appl. Microbiol.*, 2014; **8**: 535-38.