SCIENTIFIC JOURNAL OF THE MODERN EDUCATION & RESEARCH INSTITUTE

• The Kingdom of Belgium

COMPARATIVE ANALYSIS OF THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SOME SPECIES OF CLIMACOPTERA

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Abstract

In this study, complete comparative phytochemical analyses of the component composition of some plants of the genus of *Climacoptera* were conducted for the first time. The data for quantitative determination of biologically active compounds and the study of amino, fatty and mineral composition of some species of *Climacoptera* family *Chenopodiaceae* were presented. 20 amino acids, 8 known fatty acids and 11 macro– and microelements have been identified in the studied objects; their quantitative composition has been established and compared. Abroad range of biological activities such as immunomodulatory, antifungal, antioxidant, antibacterial, antidiabetic and anticancer activities have been ascribed to different species of *Climacoptera*. In addition, Kazakh species of *Climacoptera* plants are also valued as a rich source of saponins and flavonoids. This review focuses on the biological activities and detailed profile of high-value bioactive phytochemicals.

Key Words: *Climacoptera*, halophytes, amino acids, fatty acids, macro– and microelements.

INTRODUCTION

The largest number of halophytes is included in Chenopodiaceae and it consists of about 550 halophyte species, while other families that include halophytes are Poaceae, Fabeaceae and Asteraceae however less than 5% of the species in these families are halophytes. Arid soils cover 34% of Kazakhstan. A multitude of halophytes grow on them. *Climacoptera* Botsch. belongs to the family Chenopodiaceae, a family comprising of probably about 100 genera and 1400 species, which is represented in Kazakhstan by 47 genera and 218 species. It mostly comprises perennial herbs or shrubs mostly xerophytic or halophytic. There are 23 species of the plant genus *Climacoptera*; 14 of these are indigenous to Kazakhstan [1–4].

Kazakh species (C. obtusifolia, C. ferganica, C. lanata, C. brachiata, C. crassa, C. affinis, C. subcrassa, C. korshinskyi, C. ambylostegio, C. aralensis) are belonging to the obligate halophytes plants have not been subjected to in depth studies before for their chemical constituents and biological activities. Therefore, the study of the chemical composition, the development of methods for isolation of biologically active substances from these plants and the study of their biological activity in order to create new drugs is an urgent task.

Phytochemical studies of this plant mainly showed the presence of triterpenoid glycosides (Annaev et al., 1983 [5-7]; Annaev and Abubakirov, 1984 [8]; Eskalieva et al., 2004 [9]; Yeskaliyeva et al.,

2006 [10]; Kipchakbayeva et al., 2016 [11]) and flavonoids and their glycosides (Baeva and Zapesochnaya, 1980 [12]; Yeskaliyeva et al., 2006 [10]; Kipchakbayeva et al., 2012, 2016 [13, 14]; Seitimova et al., 2014 [15]).

Plants of the genus *Climacoptera* are known for antifungal, immunomodulatory activities [4, 10]. It is known that *Climacoptera* has long been used for the artisanal mining of soda and also as the autumn and winter forage for camels. Chemical analyzes of most plants of the family Chenopodiaceae point to their high nutritional value [2].

As part of our current interest in the medicinal plants of Kazakhstan, we investigated the chemical constituents of the aerial parts of *Climacoptera* obtusifolia (Schrenk.) Botsch. The authors found that *Climacoptera* obtusifolia contains pharmacologically active substances, which showed strong antidiabetic and antitumor activities [16, 17].

The purpose of this study: investigation of the chemical composition and biological activity of some species of *Climacoptera*, which growing on the salt soils of the territory of Kazakhstan.

MATERIALS AND METHODS

Fatty and amino acids analysis

The composition of the saturated and unsaturated carboxylic acids (fatty acids) in plants is determined by gas-liquid chromatography apparatus «CARLO-ERBA-4200» using helium as a carrier gas, flame ionization detector, carrier gas velocity 30 ml/min, detector temperature 188˚C, oven temperature 230˚C, adsorbent Cellite 545 on Chromosorb WAW. The chloroform extract of plant species is added to 10 ml of methanol and 2-3 drops of acetyl chloride and then carried out methylation at 60-70˚C in a special system for 30 minutes. Methanol was removed using a rotary evaporator, and the samples are extracted with 5 mL of n-hexane and analyzed by gas chromatography for 1 hour.

Analysis of amino acids was carried out chromatographically using helium as carrier gas, flame ionization detector 300˚C and condenser temperature 250˚C on Chromosorb WAW. Aqueous extract of the plant was hydrolyzed in HCl for 24 hours. The resulting hydrolyzate was evaporated to dryness in a rotary evaporator at 40˚C, after centrifugation at 2.5 thousand revolutions per minute the resulting precipitate was dissolved in sulfosalicylic acid and amino acids are eluted through an ion exchange column Dausk-50. On freshly obtained elutes 2, 2-dimethoxypropane and propanol saturated with HCl were added. The resulting mixture is heated at 110˚C for 20 minutes, then addition of a freshly prepared acylating reagent (1 volume of acetic anhydride and 2 volumes of triethylamine and 5 volumes of acetone), evaporation of the sample to dryness, addition of ethyl acetate and saturated aqueous solution of NaCl. Finally, the ethyl acetate layer is analyzed on the amino acid analyzer (Carlo-Erba) [18-20].

Mineral analysis: ashing approximately 2.0 g dried and ground sample in a muffle furnace at 550°C. The ash was analyzed for macro and microelements by atomic absorption spectrophotometer Shimadzu 6200.

Collection of plant materials

The aerial parts of ten medicinal plants, namely *Climacoptera* obtusifolia, C. ferganica, C. lanata, C. brachiata, C. crassa, C. affinis, C. subcrassa, C. korshinskyi, C. ambylostegio, C. aralensis, were collected during flowering and fruiting period from different regions of Kazakhstan. The plant material was taxonomically identified, authenticated by professors of botany at Institute of Botany and Phytointroduction and Department of Biodiversity and Bioresources, al-Farabi Kazakh National University, Almaty. The aerial parts of the plant were air dried, powdered to particle size in the range 6.0-8.0 mm, according to regulatory documents, sieved, weighed and transferred into airtight containers with proper labeling for future use.

RESULTS AND DISCUSSION

The moisture content, total ash, extractive materials, qualitative and quantitative contents of biologically active constituents of *Climacoptera* were determined according to methods reported in the State Pharmacopoeia of the Republic of Kazakhstan I edition techniques [21].

Moisture content is an important factor because appearance and stability of dried plants depends on the amount of water they contain and the propensity of microorganisms to grow depends on their water content.

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, environment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant and is useful in determining authenticity and purity of sample and these values are important qualitative standards. The ash content is a measure of the total amount of minerals present within a plant, whereas the mineral contents are a measure of the amount of specific inorganic components present within it.

Extractive materials of medicinal plants conventionally called complex organic and inorganic substances extracted from plant material with an appropriate solvent and quantified as a dry residue.

The data quantitative determination of ten medicinal plants is shown in Table 1. The results revealed the presence of biologically active compounds in the ten plants studied.

Table 1. **Qualitative and quantitative screening of the powdered aerial parts of some species of Climacoptera**

Species	Contents, %										
	Moisture content	Ash	Extractives materials 70% – aqueous alcohol	Saponins	Flavonoids	Phenols	Alkaloids	Amino acids	Carbohydrates	Organic acids	Coumarins
C. obtusifolia	8.33	28.27	53.53	2.60	1.75	2.20	1.06	4.75	1.39	1.62	0.10
C. ferganica	5.70	42.90	65.40	2.30	1.50	2.10	0.71	6.19	1.60	1.20	0.70
C. lanata	4.54	13.67	28.82	3.42	1.29	2.52	0.80	0.22	1.20	1.05	0.09
C. crassa	4.27	42.40	68.54	1.32	1.20	0.75	0.39	0.28	2.17	0.63	0.17
C. brachiata	4.75	40.70	62.37	2.27	1.33	0.90	0.31	0.20	1.18	0.51	0.20
C. affinis	8.27	26.20	41.85	2.94	1.78	0.80	0.96	3.35	2.28	0.58	0.10
C. aralensis	2.37	15.53	28.23	2.95	1.64	2.34	0.75	0.27	1.7	0.54	0.13
C. subcrassa	6.02	12.87	28.82	2.42	1.2	1.5	0.03	1.87	1.7	1.09	0.80
C. korshinskyi	7.37	19.53	36.23	3.90	1.1	0.9	0.08	1.40	1.1	1.54	0.13
C. ambylostegio	7.36	21.12	35.65	1.21	0.80	0.70	0.03	1.23	1.9	1.07	1.03

From the table 1, it could be seen that, amino acids, carbohydrates, phenols and coumarins, flavonoids and saponins were present in all the plants and it should be noted the predominance of saponins, flavonoids and phenolic compounds in *Climacoptera*.

Some of heavy metals (Fe^{+++}, Cu^{++} and Zn^{++}) are essential for plants. The availability of heavy metals in medium varies, and metals such as Cu^{++}, Zn^{++}, Fe^{+++}, Mn^{++} and Ni^{++} are essential micronutrients, whose uptake in excess to the plant requirements result in toxic effects. Halophytes tolerate salinity through the uptake or repulsion of ions, increasing of organic solutes, change of stomata, water content and other physiological changes [22].

The elemental analysis of the ten species showed the presence of eleven macro (calcium, magnesium, potassium, sodium) and microelements (iron, nickel, zinc, copper, manganese, cadmium, lead) with different levels and percent with the highest percent of K^+, Na^+, Mg^{++}, Ca^{++} and Fe^{+++}. Na^+ was found with the highest level in C. crassa (36000.0 µg/g) and C. brachiata (25500.0 µg/g) followed by K^+ (9425.0 and 6785.0 µg/g) and Mg^{++} (209.0 and 296.5 µg/g) respectively, while in C. subcrassa Ca^{++} represented the high level by 404.71 µg/g. The results showed that the concentrations of cadmium and lead in the analyzed plants did not exceed the maximum allowable concentration (MAC).

GC analysis of the amino acids constituents of the aerial parts of the *Climacoptera* (table 2) revealed the presence of twenty amino acids but differs in their percentages, the major amino acids in studied *Climacoptera* plants were glutamic acid (2.26-3.89%), alanine (0.42-1.89%), aspartic acid (0.13-1.25%), proline (0.46-0.96%), tyrosine (0.10-0.96%) and arginine (0.21-0.62%).

These plants can be used in winter and spring as wild feed for sheep and cattle owing to the high contents of glutamic and aspartic acids [23].

A comparison of the fatty-acid analyses for these plants detected eight fatty acids (table 3).

The dominant fatty acids in all plants with respect to quantity were oleic and linoleic. This fact and the rapidly renewable properties [24] together with high drought and freezing resistance and broad distributions on low and highly saline soils, i.e., those of little value for agriculture, supported our hypothesis about the feed value of these *Climacoptera* plants.

Table 2. **Composition of the amino acids contents in Climacoptera, %**

Amino acids	Content, %									
	South Kazakhstan region		Mangystau region			Almaty region			Kyzylorda region	
	C. obtusifolia	C. affinis	C. crassa	C. ferganica	C. brachiata	C. subcrassa	C. korshinskyi	C. ambylostegio	C. lanata	C. aralensis
Ala	0.72	0.73	0.42	0.89	0.72	0.88	0.78	0.65	1.21	1.82
Gly	0.27	0.29	0.18	0.30	0.24	0.48	0.35	0.18	0.21	0.35
Val	0.30	0.36	0.13	0.41	0.20	0.26	0.15	0.11	0.75	0.49
Leu	0.59	0.60	0.53	0.62	0.93	0.32	0.22	0.17	0.53	0.76
Ile	0.52	0.50	0.21	0.43	0.31	0.29	0.19	0.09	0.44	0.53
Thr	0.32	0.33	0.14	0.44	0.16	0.24	0.19	0.05	0.51	0.94
Ser	0.47	0.47	0.30	0.05	0.38	0.52	0.32	0.31	0.47	0.53
Pro	0.59	0.61	0.46	0.92	0.48	0.96	0.75	0.56	0.86	0.82
Met	0.18	0.19	0.08	0.21	0.08	0.07	0.04	0.03	0.11	0.35
Asp	1.12	1.19	0.93	1.13	1.25	1.25	1.05	0.85	0.13	0.48
Cys	0.07	0.07	0.03	0.08	0.13	0.02	0.02	0.002	0.04	0.09
O-Pro	0.001	0.002	0.06	0.004	0.004	0.001	0.003	0.002	0.26	0.35
Phe	0.29	-*	0.40	0.43	0.40	0.32	0.19	0.10	0.13	0.12
Glu	2.58	2.61	2.26	2.68	2.62	3.89	2.65	2.59	2.93	2.58
Orn	0.001	0.002	0.01	0.003	0.003	0.005	0.003	0.002	0.04	0.07
Tyr	0.52	0.53	0.96	0.52	0.15	0.29	0.17	0.10	0.39	0.40
His	0.38	0.32	0.08	0.31	0.08	0.30	0.21	0.20	0.09	0.08
Arg	0.63	0.65	0.45	0.62	0.38	0.45	0.27	0.35	0.21	0.30
Lys	0.31	0.32	0.18	0.32	0.23	0.31	0.30	0.11	0.41	0.44
Trp	0.18	0.18	0.11	0.21	0.15	0.18	0.14	0.08	0.22	0.21

** Not detected*

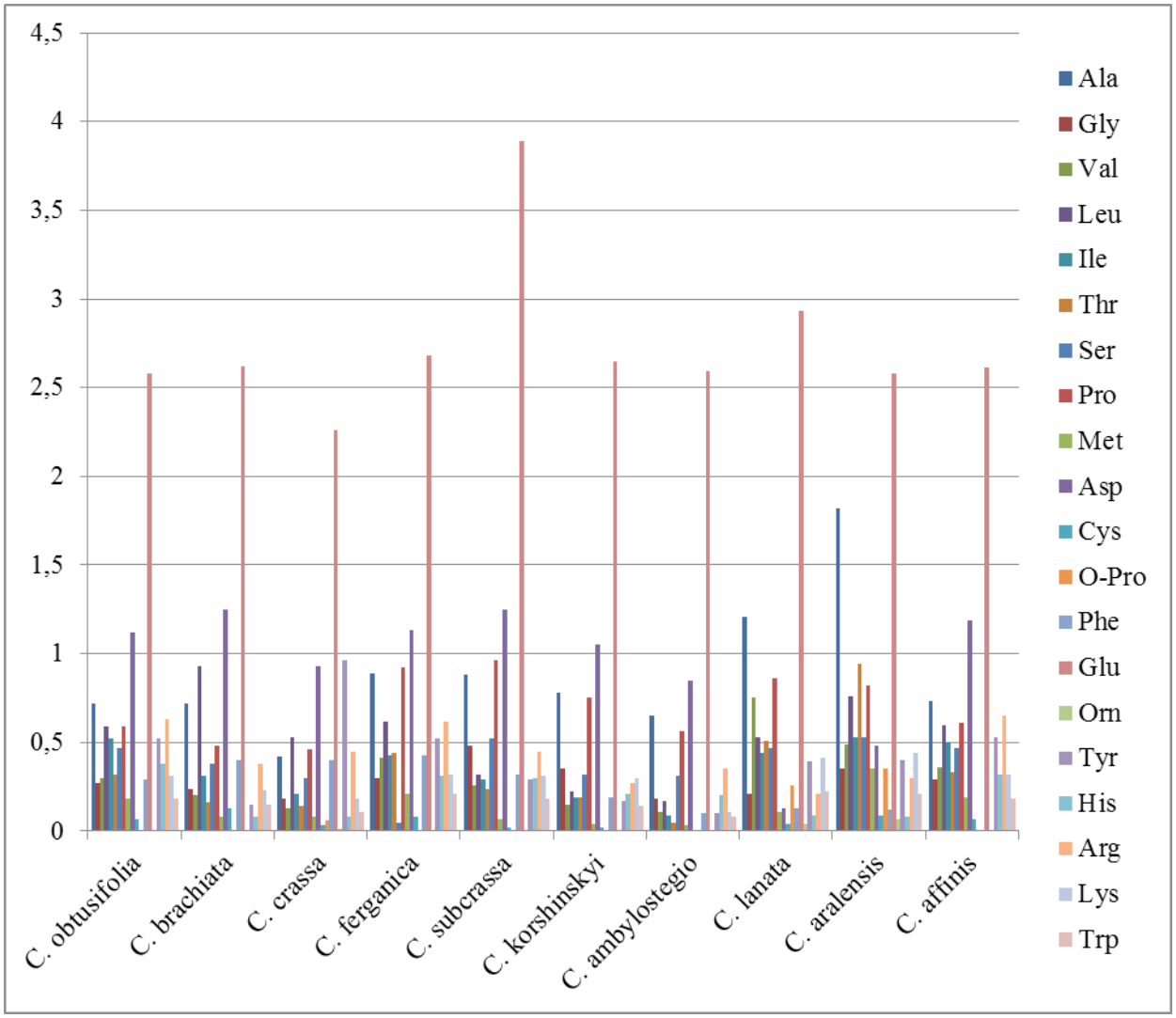

Figure 1. Composition of the amino acid contents in Climacoptera plants

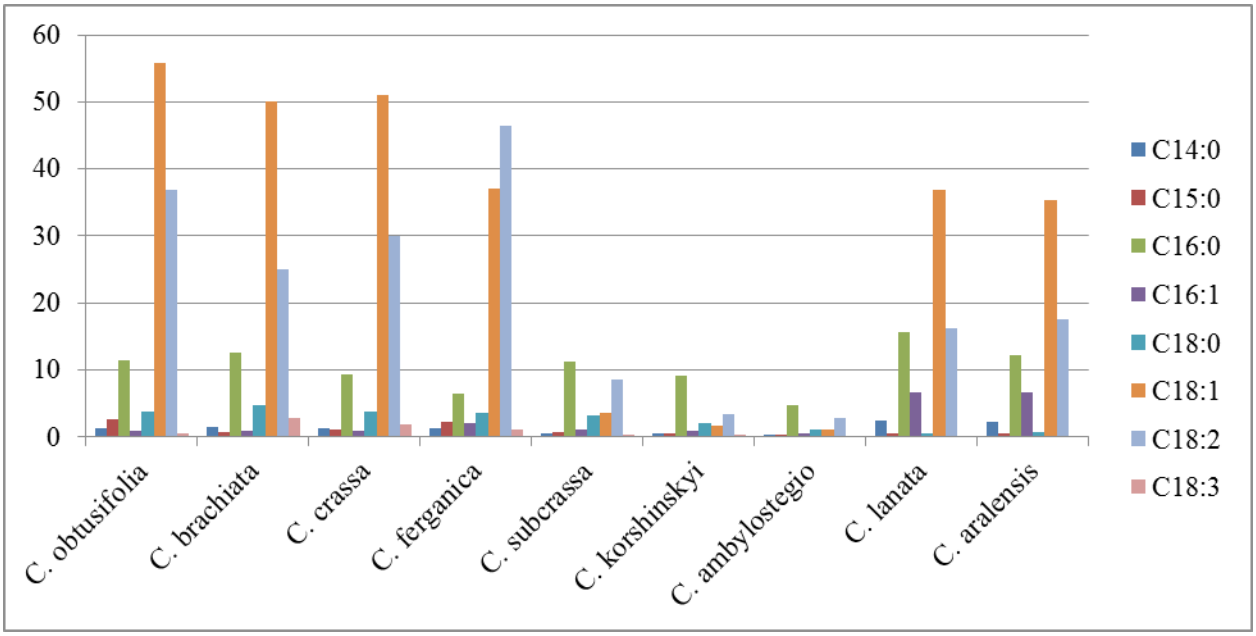

Figure 2. Composition of the fatty acid contents in Climacoptera plants

Table 3. **Composition of the saturated and unsaturated carboxylic acids (fatty acids) in Climacoptera plants, %**

Fatty acids	Content, %,								
	C. obtusifolia	C. brachiata	C. crassa	C. ferganica	C. subcrassa	C. korshinskyi	C. ambylostegio	C. lanata	C. aralensis
$C_{14:0}$	1.3	1.5	1.3	1.3	0.6	0.5	0.3	2.5	2.2
$C_{15:0}$	2.7	0.8	1.1	2.2	0.8	0.6	0.3	0.5	0.6
$C_{16:0}$	11.5	12.5	9.3	6.4	11.2	9.2	4.7	15.7	12.3
$C_{16:1}$	1.0	0.9	0.9	2.0	1.1	1.01	0.6	6.7	6.7
$C_{18:0}$	3.8	4.7	3.8	3.6	3.2	2.1	1.2	0.6	0.8
$C_{18:1}$	55.8	50	51	37.0	3.6	1.6	1.06	36.8	35.4
$C_{18:2}$	36.9	25	30	46.4	8.5	3.5	2.8	16.2	17.5
$C_{18:3}$	0.5	2.9	1.8	1.1	0.4	0.3	0.1	-*	-*

** Not detected*

Different procedures have been devised for the isolation of bioactive components from *Climacoptera* plants.

In our recent studies, the most common extraction scheme has been opted for the extraction of flavonoids and saponins from the aerial parts of C. obtusifolia. Air-dried aerial parts were extracted with 80% methanol–H_2O and the extract was successively partitioned with hexane, $CHCl_3$, EtOAc, and n-BuOH. The butanol-soluble fraction was subjected to Diaion HP-20, ODS and polyamide column chromatography, followed by normal phase silica gel column chromatography and then identified by advanced chromatographic and spectroscopic techniques (Eskalieva et al., 2004, Yeskaliyeva et al., 2006). The given studies reported the isolation of new bidesmosidic saponins, known triterpenoid glycosides, several known flavonoids, for example, flavonols and their glucosides and some sterols.

In another study, powdered plant material of C. obtusifolia was soaked in 70% ethanol, and then ethanolic extract was concentrated. It was then divided into n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions. The chloroform extract of the aerial parts of C. obtusifolia has shown the presence of several secondary metabolites along with different flavonoids (Seitimova et al., 2014).

Moreover, for the isolation of total flavonoids and saponins from C.subcrassa, powdered plant material was extracted with 70% methanol–H_2O by using the method of supercritical fluid CO_2 extraction. For effective separation of butanol extract was used column chromatography over macroporous AB-8 resin. A quantitative analysis of flavonoid complex by HPLC was done and total saponins that were separated into pure compounds using Sephadex LH-20 gel (Kipchakbayeva et al., 2012, 2016).

Structures of the isolated substances established by modern spectral analysis methods (IR, UV, ^{13}C-NMR, ^{1}H-NMR, 2D NMR: COSY – 45°, HMBC, HMQC; HRESI-MS, FAB, ECD, EI and FD mass spectrometry).

A number of pharmacological and biological activities have been ascribed to different parts of *Climacoptera* plants. The scientific basis for such diverse biological functionalities of *Climacoptera* plants can be attributed to the presence of a wide array of biologically active and high-value components.

It was also reported the immunomodulatory activities of new saponins from C. obtusifolia [10]. The plant extracts were also revealed to contain saponins, flavonoids and their glycosydes, which are known as effective antioxidant and showed strong antidiabetic and anticancer activities [16, 17]. On the base of bioassay results these extracts has recommended for detail study as antidiabetic and anticancer agents.

As a result of bioscreening of biologically active complex from C.subcrassa showed antioxidant and antibacterial activities. Main components of an antioxidant activity are flavonoids, quercetin glycosides and

isorhamnetin. For antibacterial effect of biologically active complex are responsible arbutin, hypsogenin glycosides and adenine. Cytotoxic activity of the ethyl acetate extract was identified from plants of the genus *Climacoptera* for the first time.

CONCLUSIONS

A phytochemical comparative study of *Climacoptera* was carried out. The qualitative composition of amino, fatty acids, macro– and microelements of the plant genus *Climacoptera* has been studied by using method of paper chromatography (PC) and thin-layer chromatography (TLC), their quantitative composition of amino, fatty acids has been identified by gas chromatography.

The results revealed the presence of medicinally important constituents in the plants studied. The immunomodulatory, antifungal, antioxidant, antibacterial, antidiabetic and anticancer activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs.

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