Pak. J. Bot., 46(5): 1677-1684, 2014.

STUDY ON *CARALLUMA TUBERCULATA* NUTRITIONAL COMPOSITION AND ITS IMPORTANCE AS MEDICINAL PLANT

BASHIR AHMAD[1*], SAYED JAFFAR ABBAS[1], FARRUKH HUSSAIN[2], SHUMAILA BASHIR[3] AND DAWOOD AHMAD[4]

[1]*Centre of Biotechnology & Microbiology,* [3]*Department of Pharmacy,University of Peshawar, Pakistan.*
[2]*Department of Botany, Bacha Khan University Charsadda,*
[4]*Institute of Biotechnology and Genetic Engineering, Agricultural University Peshawar, Pakistan*
[*]*Corresponding author e-mail: sayedjaffarabbas@hotmail.com*

Abstract

This study was conducted on *Caralluma tuberculata,* a famous traditional medicinal plant in the northern territory of Pakistan to assess its importance. Analysis were performed on its nutritional, proximate and microchemical composition for evaluation as food plant, while experiments of cytotoxicity, phytotoxicity and as phytobiocide were conducted to study its importance as medicinal plant. A method for vegetative propagation of *C. tuberculata* was optimized using cut stems without roots give better result and also more economical, from single stem multiple plant can be produced. It contains good amount of nutrients and proximate contents. Due to high inhibitory effect results of *C. tuberculata* against *Alternaria alternata* it can be recommended as selective fungicide for controlling *Alternaria* sp., born diseases. From the results it becomes clear that *C. tuberculata* had some anti-bacterial compounds which had very minimum inhibitory effect on the growth of bacterial species. This plant also showed significant activity against *Artemia salina, Lemna minor* and can be used as cytotoxic and phytotoxic agents in concentrated states.

Keywords: *Caralluma tuberculata,* Pamankay, Phytotoxicity, Cytotoxicity, Nutritional analysis.

Introduction

Plants are vital for fundamental needs of food, clothing and shelter for human beings. According to the hypothesis of early herbalists known as the "Doctrine of Signature" plant parts resembling parts of the human body were considered useful to treat ailments of those body parts (Shinwari *et al*., 2013). This hypothesis is still widely followed by all tribes living in the Federally Administrated Areas (FATA) of Pakistan with the slight modification "plant fruits resembling any part of the human body are considered useful for that part of the body". Medicinal and aromatic plants have been utilized as therapeutic agents since ancient times. (Girach *et al.,* 2003). In Arabic and IndoPak traditional medicines, various species of *Caralluma* are used for the treatment of diabetes, cancer, snake and scorpion bites, inflammation and skin rashes (Abdel-Sattar *et al.,* 2007; Adnan *et al*., 2014). More than 200 species have been reported in the genus *Caralluma* (Al-Massarani *et al.,* 2012) widely distributed in the dry regions of Africa, tropical Asia and the southern Mediterranean. The word *Caralluma* is derived from the Arabic phrase "Qarh Al-Luhum" which translates as "wound in the flesh".

Caralluma tuberculata grows abundantly on the mountains in the FATA and its surrounding districts in Pakistan. *C. tuberculata* in a local Pashto language is called Pamankay, which is combination of Paman and kay (paman translates as pimple, kay translates as thing). Due to its appearance people use this plant to regulate blood pressure and also to treat pimples. It is also used to reduce sugar levels in diabetes patients. Peoples eat their raw fleshy leaves either as salad or cook with minced meat which removes its bitter taste. The present study focuses on the nutritional, proximate, cytotoxicity, phytotoxicty and pytobiocidal values of *C. tuberculata* in order to assess its beneficial value as a medicinal plant.

Material and Methods

Samples collection: *C. tuberculata* plant samples were collected during winter from the Lachi and Shakardara mountains of Kohat district, Pakistan.

Sample preparation: Mature plants were selected, cut into small pieces and shed dried. For the vegetative propagation experiment green plant material was used. After complete drying the plant material was blended into powder form using a blender. The powdered samples were then analyzed.

Proximate composition: Standardized methods of Anon., (2000) were employed for determining crude protein, crude fat, moisture, and ash content as shown in Table 1. Nitrogen free extract was calculated by recording the difference, this difference in value was obtained by subtracting the sum of the percentages of moisture, ash, crude fiber, crude protein and crude fat from 100. Energy value was computed by multiplying the protein and carbohydrate percentages by 4.1 and that of crude fat by 9.1 constants values (Khalil & Saleemullah, 2004).

Minerals composition: Powdered sample of 1g was weighted and placed in a digestion tube. 10 ml concentration of nitric acid was added into it, incubated over night and 5ml perchloric acid ($HCLO_4$) was added to it on the next day. It was transferred to heater and temperature was raised to 200^oC slowly and kept at this temperature till the white dense fumes of perchloric acid ($HCLO_4$) disappeared. The flask was cooled after digestion and the content was filtered through watt-man filter paper NO.42. In a100 ml volumetric flask it was diluted with deionized water upto the mark.

Table 1. Proximate analysis of *Caralluma tuberculata*.

Replications	Moisture content %	Ash %	Fiber %	Protein %	Fat %	NFE %
R1	58.33	14.07	19.38	5.26	1.44	1.52
R2	58.45	14.19	19.19	5.13	1.31	1.4
R3	58.21	14	19.69	5.39	1.57	1.64
Mean ± SE	**58.33 ± 0.08**	**14.08 ± 0.06**	**19.42 ± 0.17**	**5.26 ± 0.09**	**1.44 ± 0.09**	**1.52 ± 0.08**

	Energy (Kcal)	% Total sugar	pH	% Total acidity	Vitamin C (mg/g)
R1	40.902	4	4.44	0.832	1.218
R2	38.694	4.1	4.44	0.505	1.554
R3	43.11	4	4.44	0.512	1.428
Mean ± SE	**40.90 ± 1.56**	**4.03 ± 0.04**	**4.4**	**0.61 ± 0.13**	**1.4 ± 0.12**

The digest of *C. tuberculata* was used for the analysis of different elements with a double beam atomic absorption spectrophotometer (AAS), Perkin Elmer Model 700, which uses both air acetylene and nitrous oxide flames with laminar flow burner and hollow cathode lamps. A air acetylene flame was used for calcium, manganese, cobalt, sodium, nickel, lead, chromium, cadmium, zinc, copper, magnesium, iron and potassium elements, while a nitrous oxide flame was used for strontium, yttrium, silver, molybdenum, silicon, antimony, and barium element analysis.

The instrument was calibrated using standards and then digested samples were introduced into the device. The atomic absorption spectrophotometer device was programmed for the analysis of specific elements by setting a specific light source for each element. The absorbance reading that appeared on the screen was used to calculate the concentration of each element using the following formula:

$$\mu g/g = \frac{\text{Instrumental reading x Dilution factor}}{\text{Weight of sample}}$$

Biochemical composition: The detection of biochemicals in *C. tuberculata* samples was conducted following the methods of Evans, 2002.

Phytotoxicity and cytotoxicity measurement: Phytotoxic activity of the *C. tuberculata* 75% methanolic crude extracts was assessed against *Lemna minor* following the method of McLaughlin *et al*., (1991), and the cytotoxic activity of the 75% methanolic crude extracts was assessed against *Artemia salina* (Shrimps) following the method of Meyer *et al*., (1982).

Phytobiocides activity measurement: *C. tuberculata* plant dried material was saoked for 15 days in 75% methanol. Crude extract of *C. tuberculata* was obtained by filtering the soaked material and methanol was separated from the plant extract by using a rotavapour machine. The plant extract was then diluted in water to different concentrations and used as a suspension for *In vitro* studies.

Evaluation of phytobiocides against fungus in *In vitro*: The test isolates of *Alternaria alternata, Asperigulas Flavus,* and *Penicilium expansum* were taken from lab cultures in the Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan. Each isolate was plated on Potato Dextrose Agar (PDA) medium. After autoclaving, *C. tuberculata* extract was added at a ratio of 5ml extract per 500ml medium after cooling. In Laminar Air flow hood, 0.5cm plug of the test isolate was placed in the center of each petri plate. Petri plates without extract served as control. The plates were incubated at 25˚C for one week. Taking the colony diameter along two perpendicular lines and then their mean determined radial growth of the fungus. The numbers of spores were counted by using a haemocytometre. The micrometry of *Alternaria alternata* spores was also determined using a micrometer. The experiment was laid out as a completely randomized design with four replications.

Antibacterial susceptibility assay: The antibacterial activity was determined against *Escherichia coli, Xanthonomonas campestris* and *Citrobactor sp.* The bacterial cultures were refreshed in nutrient agar plates at 37°C. Four concentrations of *C. tuberculata* extract (5g/L, 50 g/L, 75 g/L and 100 g/L) were used to determine antibacterial activity using a Disc Diffusion Assay (DDA). Sterile Nutrient Yeast Agar (NYA) plates containing 20 ml of media were inoculated with approximately 100 µl of seed culture, approximately 10^8 cfu/ml (test bacteria). 100 µl extract discs were loaded into the plates (for each concentration) and incubated at 37°C overnight, streptomycin and distilled water being the positive and negative control. The experiment was performed in triplicate. The antibacterial activity of each extract was recorded based on the zone of inhibition of bacterial growth by the extract

Statistical analysis: The data means and standard error were analyzed statistically in Microsoft software excels.

Results and Discussion

This is the first complete study of *C. tuberculata* to investigate morphological growth and development, proximate analysis and nutrient composition, biochemical composition, antimicrobial activity, phytotoxic and cytotoxic inhibitory effects of the plant extract.

Vegetative propagation: Propagation of *C. tuberculata* from seed failed for unknown reasons. Vegetative propagation of *C. tuberculata* from whole single stems with attached roots, whole single stems without roots, single cut stems with roots and single cut stems without roots were successful (Figs. 1-6). Mean and standard error comparisons of each measured trait for the different vegetative propagation methods are presented in Table 2. Results showed that mean plant stem height was (13.8-18.3cm), (15-21.7cm), (13-19.5cm), and (12-18cm) for whole single stem with attached roots, whole single stem without roots, single cut stem with roots and single cut stem without roots respectively. Mean stem diameter was (3.4-5.2cm), (4.5-5.3cm), (4-4.6cm) and (4.5-5cm), mean number of stems plant^{-1} was (46-147), (36-65), (24-39) and (37-223), mean number of spine-like protrusions stem^{-1} was (74-96), (100-104), (66-94) and (66-130), mean internode distance between spine-like protrusions was (0.8-1.3cm), (0.8-1.3cm), (0.8-1.3cm) and (0.8-1cm), mean number of roots plant^{-1} was (49-95), (29-71), (17-51) and (45-176), mean root size plant^{-1} was (9.5-15cm), (10.8-16cm), (10.3-13.5cm) and (11.5-15.5cm), mean weight plant^{-1} was (55-500g), (125-250g), (45-150g) and (50-475g). The highest average stem length (18.1cm), stem diameter (5cm) and number of spine-like protrusions stem^{-1} (101.5) were recorded for the whole main stems without roots, whereas the highest number of stems (102.8), maximum number of roots (92.5), maximum root size (13.9cm) and maximum weight (236.3g) were recorded for cut stems without roots. In Kohat local market each kilo gram of *C. tuberculata* is 2 to 3USD in winter and 5-6USD in summer season. Based on the yield and number of stems produced, vegetative propagation of *C. tuberculata* from cut stems without roots performs better than the other trialled methods of propagation, it is also more economical as from a single stem multiple plants can be grown. The number of stems is also an important character in *C. tuberculata* because stems are used as salad with a main meal. The young stems look very beautiful and prominent in salad dressing.

Proximate and nutritional composition: Mean value of the data showed that *C. tuberculata* contains 14.087% ash content (Table 1). Ash content shows a strong correlation with energy, fat and protein plus a moderate correlation with nitrogen free extract and a low correlation with moisture content. This plant species has a low amount of energy in (K Cal) and fat content. The fiber content of *C. tuberculata* was high at 19.42%. Nutritionally, it has sufficient fiber to aid in reduce absorption of cholesterol and absorption of trace elements in the gut (Abolaji *et al.,* 2007). As a cactus related species, it has high moisture content with capacity to tolerate water stress. The digestible carbohydrate or nitrogen free extract (1.52%) and protein content (5.26%) was recorded. Recommended daily dietary dose of Ca is between 500 and 1000 mg (Lokhande, 2010) while calcium content was found 3.66mg/g in *C. tuberculata,* while the. Manganese is an essential element required for various biochemical and enzymatic processes in the body (Weber and Konieczyński, 2003), eliminating fatigue and reduces nervous irritability (O'Dell & Sunde, 1997). Our selected plant high manganese content was observed, 25.667µg/g. Manganese plays an important role in Antimony element content was 28.533µg/g. (Tschan *et al.,* 2008) recorded a high amount of antimony (41mg/kg) in maize plant species. Antimony, a nonessential element, is toxic to humans when its uptake exceed 100 mg/d (Tschan *et al.,* 2008). The amount of Molybdenum element was recorded at 18µg/g, this element is involved in iron absorption, to promote normal growth and development. The adequate range of intake is 75 to 250µg/g. Table 3 shows that this plant contains high potassium (2706.7µg/g) and low sodium (205.53µg/g) concentrations. The regulation of potassium and sodium are largely interdependent (Lokhande, 2010). Through the action of the Na±, K±-ATPase (sodium pump), Potassium regulates ion exchange in human cells and is also an activator of some enzymes and co-enzymes required for normal growth and muscles development (Seiler *et al.,* 1994a). The elements cadmium (34.9µg/g) and lead (83.033µg/g) were found in higher amounts in *C. tuberculata* than average toxic levels of 3-30 ppm and 10 ppm respectively (Lokhande, 2010). Copper is an important constituent of many enzymes, which catalyze the oxidation of ferrous iron to ferric iron. Copper is required for absorption and transport of iron, which plays the main role in hemoglobin synthesis (Rajendran *et al.,* 2007). *C. tuberculata* contained 10.5µg/g copper.

It also contained 53.9 µg/g chromium element which comes under the reported range of 45-78 mg/kg recorded for the medicinal plants (Adnan *et al.,* 2010). (Macholz, 1987) reported that for many plant species Cr proved to be toxic at 10 mg/kg. Thus the studied plant has manifolds higher concentration of Cr as compared to that of recommended. The function of chromium is directly related to the function of insulin, which plays a vital role in diabetes (Lokhande, 2010). The iron is clearly associated with hemoglobin and the transfer of oxygen from lungs to each and every cell. Iron deficiency is the most prevalent nutritional deficiency in human's especially in females and is commonly caused by insufficient dietary intake, menstrual flow or multiple births which in most cases results in anemia. *C. tuberculata* (222.83µg/g) has high amount of iron. These results are inline with (Lokhande, 2010). *C. tuberculata* contains 39.333µg/g Zinc which plays an important role in diabetes as a cofactor for insulin but at optimum concentration. The Co content was observed 7.7µg/g which plays a vital role in thyroid metabolism in humans (Seiler *et al.,* 1994b), the recommended daily intake of vitamin B12 for adults is 3 mg which contains 0.13 mg of Co. Magnesium is the macro element which plays an important role in the carbohydrate and fat metabolism. 670.40µg/g of magnesium was recorded in *C. tuberculata* which also play a major role in the release of insulin. Elements silicon (0.2667µg/g), silver (0.6333µg/g) and strontium (50.8µg/g) were recorded respectively.

Fig. 1. Vegetative propagation of *Caralluma tuberculata*.

Fig. 4. Pod structure of *Caralluma tuberculata*.

Fig. 2. Vegetative growth in 1st year.

Fig. 5. *Caralluma tuberculata* pod with seeds.

Fig. 3. Flower development during 2nd year of growth.

Fig. 6. *Caralluma tuberculata* single seed.

Table 2. ***Caralluma tuberculata*** **vegetative growth parameters analysis.**

Replications	Stem length	Stem diameter	Number of stems	Number of spine-like protrusions/ Stem	Internode b/w spine-like protrusions	Number of roots	Root size	Weight/Plant
	Whole main stem with root							
R1	18.3	3.5	46.0	96.0	1.0	49.0	12.5	55.0
R2	17.0	3.4	80.0	88.0	1.3	53.0	15.0	100.0
R3	15.3	4.2	147.0	74.0	1.1	95.0	13.3	500.0
R4	13.8	5.2	71.0	74.0	0.8	58.0	9.5	150.0
Mean ± SE	**16.12 ± 1.13**	**4.05 ± 0.47**	**86 ± 24.9**	**83 ± 6.28**	**1.01 ± 0.12**	**63.75 ± 12.21**	**12.56 ± 1.32**	**201.25 ± 117.15**
	Whole main stem without root							
R1	17.7	4.5	65.0	100.0	1.3	71.0	13.5	125.0
R2	18.0	5.3	61.0	104.0	1.2	50.0	11.0	128.0
R3	21.7	5.0	36.0	102.0	1.1	37.0	10.8	225.0
R4	15.0	5.1	40.0	100.0	0.8	29.0	16.0	250.0
Mean ± SE	**18.08 ± 1.58**	**4.96 ± 0.19**	**50.5 ± 8.4**	**101.5 ± 1.1**	**1.06 ± 0.12**	**46.75 ± 10.58**	**12.81 ± 1.4**	**182 ± 37.47**
	Cut stem with root							
R1	14.5	4.6	26.0	90.0	1.2	30.0	13.5	50.0
R2	19.5	4.6	38.0	94.0	1.3	24.0	12.0	150.0
R3	13.0	4.5	39.0	86.0	0.9	51.0	10.3	100.0
R4	11.5	4.0	24.0	66.0	0.8	17.0	12.3	45.0
Mean ± SE	**14.62 ± 2**	**4.42 ± 0.16**	**31.75 ± 4.53**	**84 ± 7.18**	**1.01 ± 0.13**	**30.5 ± 8.46**	**12 ± 0.76**	**86.25 ± 28.41**
	Cut stem without root							
R1	18.0	5.0	52.0	130.0	0.9	97.0	14.5	200.0
R2	17.0	4.5	223.0	58.0	1.0	176.0	15.5	475.0
R3	13.0	5.0	99.0	62.0	0.7	45.0	14.0	220.0
R4	12.0	5.0	37.0	66.0	0.8	52.0	11.5	50.0
Mean ± SE	**15 ± 1.69**	**4.87 ± 0.14**	**102.75 ± 48.73**	**79 ± 19.72**	**0.8 ± 0.07**	**92.5 ± 34.78**	**13.87 ± 0.98**	**236.25 ± 101.79**

Table 3. Nutritional analysis of *Caralluma tuberculata*.

Replications	Nickle	Lead	Chromium	Cadmium	Zinc	Copper	Magnesium
R1	32.1	86.5	52.3	35.6	39.8	10.4	670.8
R2	35.9	82.1	55.1	34.1	38.9	10.6	669.9
R3	37.9	80.5	54.3	35.0	39.3	10.5	670.5
Mean ± SE	**35.3 ± 2.08**	**83.03 ± 2.19**	**53.9 ± 1.01**	**34.9 ± 0.53**	**39.33 ± 0.31**	**10.5 ± 0.07**	**670.4 ± 0.32**

Replications	Iron	Calcium	Manganese	Cobalt	Strontium	Yttrium	Silver
R1	225.5	3646.0	27.3	8.1	51.0	0.0	0.6
R2	220.0	3652.0	25.2	7.3	51.8	0.0	0.5
R3	223.0	3676.0	24.5	7.7	49.6	0.0	0.8
Mean ± SE	**222.83 ± 1.94**	**3658 ± 11.22**	**25.66 ± 1.03**	**7.7 ± 0.28**	**50.8 ± 0.78**	**0.0**	**0.63 ± 0.1**

Replications	Molybdenum	Silicon	Antimony	Barium	Sodium	Potassium
R1	17.4	0.4	0.0	1114.0	222.5	2707.0
R2	20.7	1.0	0.0	1024.0	205.7	2706.0
R3	16.9	2.5	85.6	967.2	188.4	2707.0
Mean ± SE	**18.33 ± 1.46**	**1.3 ± 0.76**	**28.5**	**1035.06 ± 52.34**	**205.53 ± 12.05**	**2706.66 ± 0.4**

Phytobiocidal activity

A. Fungal inhibition: Plant compounds from various wild and cultivated plants have been reported in the past to be effective against a wide range of micro-organisms (Hadizadeh *et al.,* 2009). This study showed that the plant extracts from *C. tuberculata* have potent anti *Alternaria alternata* activity in *In vitro* studies by inhibiting mycelial growth, while did not show any effect against the growth of *Asperigulas Flavus,* and *Penicilium expansum* . *C. tuberculata* inhibited 54.42% growth of *Alternaria alternata* (Figs. 7 and 8). The *C. tuberculata* extract induced more inhibitory results then previously reported by (Mishra, 2010) while less then that of (Ho *et al.,* 2007) who reported 100% inhibitory effect on *Alternaria* sp. by using different species of oriental medicinal plant extracts. *C. tuberculata* also greatly affect the *A. alternata* spores yield by decreasing their numbers (92.8%), length (23.3%) and width (67.6%). (Mishra, 2010)also reported that plant extracts affect the *Alternaria* sp. hyphal growth and spore formation. Due to high inhibitory effect results of *C. tuberculata* against *A. alternata* (Table 4) it can be recommended as selective fungicide for controlling the *Alternaria* sp. born diseases.

B. Bacterial inhibition: The minimum inhibitory concentration (MIC) exhibited by *C. tuberculata* extract (Table 5) against the tested organisms *Escherichia coli, Xanthonomonas campestris* and *Citrobactor sp.* ranged between 5 and 100 mg/ml of media but all the concentration give almost same result. *C. tuberculata* extract concentration ranges from 5-100gL^{-1} produced inhibition zone ranged from 1-1.3mm while (Mahesh & Satish, 2008) recorded higher ranges 13-18 mm of inhibition zones for E.Coli and *Xanthonomonas*. *C. tuberculata* extract had slightly more inhibitory effect on *Escherichia coli* and *Xanthonomonas campestris* as compared Citrobactor. (Pritima & Pandian, 2008) used Coleus aromaticus extract and obtained 14-30mm inhibitory zone against *Escherichia coli*. From the results it become clear that *C. tuberculata* had some anti-bacterial compounds which had very minimum inhibitory effect on the growth of bacterial species.

Fig. 7. Inhibitory effect of *Caralluma tuberculata* against *Alternaria alternate*.

Fig. 8. Inhibitory effect of *Caralluma tuberculata* extract (CC) against *Alternaria alternate*.

Table 4. Biocidal activity of *Caralluma tuberculata* against *Alternaria alternate*.

Treatment	Radial growth (cm)	spore length (µ)	spore width (µ)	Spore production
	Control			
R1	5.05	42	12	35000
R2	5.55	43	14	34000
R3	5.9	41	15	37000
R4	5.55	45	12	39000
Mean ± SE	**5.51 ± 0.2**	**42.75 ± 0.98**	**13.25 ± 0.86**	**36250 ± 1280.19**
	Caralluma tuberculata			
R1	2.45	32	4	2800
R2	2.65	35	3	2500
R3	2.6	31	5	2400
R4	2.35	33	5	2700
Mean ± SE	**2.51 ± 0.07**	**32.75 ± 0.98**	**4.25 ± 0.55**	**2600 ± 105.4**

Table 5. Biocidal activity of *Caralluma tuberculata* against bacterial species.

Treatment	Inhibition zone (mm)	Inhibition zone (mm)	Inhibition zone (mm)
	Xanthonomonas campestris	***Citrobactor* sp.**	***Escherichia coli***
5g/L			
R1	1.0	1.0	1.0
R2	1.0	1.0	1.0
R3	1.0	1.0	1.0
Mean ± SE	1.0	1.0	1.0
50g/L			
R1	1.0	1.0	1.0
R2	1.0	1.0	1.0
R3	1.0	1.0	2.0
Mean ± SE	1.0	1.0	1.33 ± 0.4
75g/L			
R1	1.0	1.0	1.0
R2	1.0	1.0	1.0
R3	1.0	1.0	1.0
Mean ± SE	1.0	1.0	1.0
100g/L			
R1	2.0	1.0	1.0
R2	1.0	1.0	1.0
R3	2.0	1.0	2.0
Mean ± SE	1.66 ± 0.4	1.0	1.33 ± 0.4

C. Phytotoxicity and cytotoxicity: The results of the *C. tuberculata* cytotoxicity are given in Table 6. The LD_{50} was 93.66 against *A. salina* (shrimp). It has significant activity. Results of the phytochmical analysis in Table 7 revealed the presence of alkaloids, flavinoids, saponins, and calcium oxalate, while absence of mucilage, tannins and anthraquinone derivatives in the plant extract. The phytotoxic effect of the *C. tuberculata* crude extract is shown in Figure 9, which showed highest (85.45%) phytotoxic activity against *Lemna minor* at a concentration of the 1000 mg/ml, high (70.90%) at 100 mg/ml and moderate (54.54%) inhibition at a concentration of 10 mg/ml. The cytotoxicity study on shrimps and phytotoxicity study on *L. minor* by using *C. tuberculata* extracts were presented first time in this paper, while cytotoxicity study of *C. tuberculata* on mice was previously reported by (al-Bekairi *et al.,* 1992). *C. tuberculata* contians flavonoids, which are known to be involved in reducing RNA, increasing cytotoxicity and mutagenicity. (Qureshi *et al*., 1991). Beside cytotoxic biochemical compounds which breakdown nucleic acid on large scale, *C. tuberculata* is a rich source of saponins and other phyto-constituents which may be involved in protection of DNA and protein biosynthesis. Such compounds have been found to possess strong antimutagenic potential and it is also believed that these compounds are responsible for the efficacy of the herbal drugs (Shah *et al.,* 1989). The inhibition essay of *L. minor*, it is observed that natural anti-tumor compounds can inhibit Lemna growth (Rahman, 2011). These results showed that *C. tuberculata* can be used as cytotoxic and phytotoxic agents in concentrated states mentioned in the Table 6.

Table 6. Cytotoxic activity of *Caralluma tuberculata* against *A. salina.*

Plant name	Dose (ug/ml)	No. of shrimps	No of survivors	LD_{50} (ug/ml)
Caralluma tuberculata	10	30	17	93.66
	100	30	6	
	1000	30	4	
Standard drug		Etopoisde		7.46

Table 7. Microchemical screening tests of *Caralluma tuberculata*.

Alk	Muc	Sap	CaO	Fat	Pro	Sta	Cell	Tan	Ant
+++	-	++	+	+++	+++	++	+++	-	-

Key: Alk = Alkaloids, Muc = Mucilage, Sap = Saponins, CaO = Ca oxalate, Fat = Fats, Pro = Proteins, Sta = Starch, Cell = Cellulose, Tan = Tannins, Ant = Anthraquinone derivatives

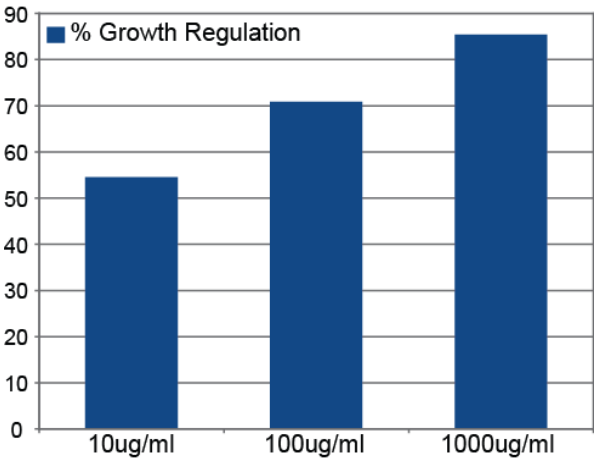

Fig. 9. Phytotoxic activity of different concentrations of *Caralluma tuberculata* against *L. minor*.

References

Abdel-Sattar, E., A.A. Ahmed, M.-E.F. Hegazy, M.A. Farag and M.A.A. Al-Yahya. 2007. Acylated pregnane glycosides from Caralluma russeliana. *Phytochemistry*, 68: 1459-1463.

Abolaji, O.A., A.H. Adebayo and O.S. Odesanmi. 2007. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica*, *Blighia sapida* and *Parinari polyandra*) commonly used by pregnant women in the Western part of Nigeria. *Pakistan Journal of Nutrition*, 6: 665-668.

Adnan, M., J. Hussain, M. Tahir Shah, Z.K. Shinwari, F. Ullah, A. Bahader, N. Khan, A. Latif Khan and T. Watanabe. 2010. Proximate and nutrient composition of medicinal plants of humid and sub-humid regions in North-west Pakistan.

Adnan, M., S. Jan, S. Mussarat, A. Tariq, S. Begum, A. Afroz and Z.K. Shinwari. 2014. A review on ethnobotany, phytochemistry and pharmacology of plant genus *Caralluma* R. *Br. J. Pharm. Pharmacol*., doi: 10.1111/jphp.12265.

Al-Bekairi, A.M., S. Qureshi, M.M. Ahmed, N.S. Qazi, Z.A. Khan and A.H. Shah. 1992. Effect of *Caralluma tuberculata* on the cytological and biochemical changes induced by cyclophosphamide in mice. *Food Chem. Toxicol*., 30: 719-722.

Al-Massarani, S.M., S. Bertrand, A. Nievergelt, A.M. El-Shafae, T.A. Al-Howiriny, N.M. Al-Musayeib, M. Cuendet and J.-L. Wolfender. 2012. Acylated pregnane glycosides from *Caralluma sinaica*. *Phytochemistry*, 79: 129-140.

Anonymous. 2000. Official Methods of Analysis Association of Official Analytical Chemist EUA

Girach, R.D., H. Khan and M. Ahmad. 2003. Botanical identification of Thuhar seldom used as Unani medicine. *Hamdard Medicus*. v. 46(1): 27-33.

Ho, W.C., T.Y. Wu, H.J. Su and W.H. Ko. 2007. Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from speedweed. *Plant Disease*, 91: 1621-1624.

Lokhande, R. 2010. Study of some Indian medicinal plants by application of INAA and AAS techniques. *Natural Science*, 02: 26-32.

Macholz, R. and D.C. Adriano. 1987. Trace elements in the terrestrial environment. Springer-Verlag. 31: 259-259.

Mahesh, B. and S. Satish. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World Journal of Agricultural Sciences*, 4: 839-843.

Mishra, A.S.A. 2010. Screening of some plant extracts against *Alternaria* sp., isolated from foot infections in Cancer patients. *International J of Pharm Tech Research*, 2: 1165-1170.

O'Dell, B.L. and R.A. Sunde. 1997. Handbook of Nutritionally Essential Mineral Elements. CRC Press. pp. 714.

Pritima, R.A. and R.S. Pandian. 2008. Antimicrobial activity of *Coleus aromaticus* (Benth) against microbes of reproductive tract infections among women. *African Journal of Infectious Diseases*, 1: 18-24.

Qureshi, S., A.H. Shah, M.A. Al-Yahya and A.M. Ageel. 1991. Toxicity of *Achillea fragrantissima* and *Thymus vulgaris* in mice. *Fitoterapia*., 62: 319-323.

Rahman, A. 2011. Studies in Natural Products Chemistry: Bioactive Natural Products. *Elsevier*,. pp. 1150.

Rajendran, A., V. Narayanan and I. Gnanavel. 2007. Study on the analysis of trace elements in *Aloe vera* and its biological importance. *J. Appl. Sci. Res*., 3: 1476-1478.

Seiler, H.G., A. Sigel and H. Sigel. 1994a. Hdbk on Metals in Clinical Analytical Chemistry. Dekker. pp. 780.

Seiler, H.G., A. Sigel and H. Sigel. 1994b. Handbook on metals in clinical and analytical chemistry. Marcel Dekker Incorporated. pp.780.

Shah, A.H., A.M. Al-Bekairi, S. Qureshi and A.M. Ageel. 1989. *Zizyphus sativa* fruits: Evaluation of some biological activities and toxicity. *Phytotherapy Research*, 3: 232-236.

Shinwari, Z.K., M. Saleema, R. Faisal, S. Huda and M. Asrar. 2013. Biological screening of indigenous knowledge based plants used in diarrheal treatment. *Pak. J. Bot*., 45(4): 1375-1382.

Tschan, M., B. Robinson and R. Schulin. 2008. Antimony uptake by *Zea mays* (L.) and *Helianthus annuus* (L.) from nutrient solution. *Environ. Geochem Health*., 30: 187-191.

Weber, G. and P. Konieczyński. 2003. Speciation of Mg, Mn and Zn in extracts of medicinal plants. *Anal Bioanal Chem*., 375: 1067-1073.

(Received for publication 16 April 2013)