Elemental, nutritional, phytochemical and biological evaluation of *Hypericum perforatum* Linn.

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Abstract: This study was carried out to study elemental, nutritional, phytochemical and biological evaluation of *Hypericum perforatum* collected from Swat in 2010. The elemental analysis showed that Ca was highest (5600 µg/g) in leaves and lowest (2500µg/g) in flowers. The potassium was highest (840µg/g) in fruit and lowest (80µg/g) in leaves. Magnesium was highest (260µg/g) in stem and lowest (200µg/g) in flowers. Sodium was highest (4900µg/g) in stem and lowest (4700µg/g) in leaves and flowers. Copper was highest (26µg/g) in stem and lowest (10µg/g) in leaves. Iron was highest (5000µg/g) in flowers lowest (1200 µg/g) in stem. Zinc was highest (80µg/g) in flowers and lowest (46 µg/g) in stem. Nickle, cadmium and Cobalt were <5µg/g for all plant parts. The nutritional analysis showed that the dry matter was in the range of (97.61%) in stem and (96.38%) in leaf, ash (5.43%) in flowers and (1.90%) in stem, crude protein (12.63%) in leaf and (6.15%) in stem, crude fibre (64.74%) in flowers and (13.0%) in leaf, ether extract (10.98%) in fruit and (1.88%) in stem and nitrogen free extract was (65.80%) in leaf and (10.98%) in flower, respectively. *Hypericum perforatum* did not show cytotoxic, insecticidal and antibacterial activity *in vitro* at different doses. The % activity was zero% in cytotoxic and insecticidal activities. However, *H. perforatum* plant parts revealed phytotoxic activity. The phytotoxic activity of leaf and fruit remained same (44.0%) at highest dose (500µg/ml). The phytochemical screening showed the presence of mucilage, tannins, anthraquinones, saponins, fats and oils and proteins in all parts of the plant. Calcium oxalate was found in all parts except the fruit. Lignin and catechin was found in all parts except the leaf. Cutin was found only in stem and flower while chlorophyll was found only in stem and leaf. In various localities (Shartangaar, Panj Pali and Sharanko) of Swat fresh leaves were used while in Barani and Jaba fresh as well as dried leaves were used as stimulant, in fever, cough, diphtheria and as an anthelmintic only in Shartangaar.

Keywords: *Hypericum perforatum,* elemental, nutritional, antibacterial, phytochemical screening.

INTRODUCTION

The genus *Hypericum* occurs widely in temperate regions. It contained xanthones, phloroglucinols, flavonoids, naphtodianthrones and essential oils (Tala *et al*., 2013). The consumption of products of *H. perforatum* has increased in recent years and are available as phytopharmaceuticals, nutraceuticals, teas and tinctures. Plant is used in eczema, burns, and diseases of the alimentary tract. Plant had phenolic compounds, including hypericin, hyperforin and their derivatives, rutin, hyperoside, quercetin, chlorogenic acid, flavonols and flavones. Hypericin had antibacterial, antiviral and anti-inflammatory activities while hyperforin is antidepressive (Mašković *et al*., 2011). The inflorescence of the plant is used as sedative, tonic while an oil infusion is effective to treat sciatica, neuralgia and to speed wound healing (Crockett *et al*., 2011).

Elements play a vital role in the medicinal value of a plant and therapy and in health and disease. Similarly, nutrients and biochemical's like carbohydrates, fats and proteins also play an important role in satisfying human needs for energy and life processes (Adnan *et al*., 2010). Many workers investigated medicinal plants for minerals and proximate analysis e.g., (*Polygonatum verticillatum* Khan *et al.,* 2012; *Anethum graveolens*, *Sisymbrium irio*, *Vernonia anthelmintica* Fatima *et al.,* 2012; *Myrtus communis,* Aydin and Ozcan, 2007; *Zingiber officinale*, *Allium sativum* and *Parkia biglobosa,* Odebunmi *et al*., 2010; *Amaranthus hybridus,* Akubugwo *et al.,* 2007; *Aerva javanica*, *Calotropis procera*, *Datura alba* and *Nepeta suavis,* Hussain *et al.,* 2011).

Globalization interfere with infectious disease control at the national level while microbes moves freely around the world, unhindered by borders, human response to infectious diseases are conditioned by jurisdictional boundaries (Stepanovic *et al*., 2003). Bioactive compounds are often toxic to *Artemia salina* shrimp larvae (Carballo *et al.,* 2002). Lethality assay has been used successfully to biomonitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent compounds from plants (Krishnaraju *et al*., 2005). Due to limited studies a thorough screening is needed to find out the cytotoxic activity of plants that can be possibly useful forerunners for anti-tumour compounds. In *Lemna* assay, it is observed that natural antitumour compounds can inhibit *Lemna* growth. It was also discovered that some substances stimulate frond proliferation making it useful to detect new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant

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growth stimulants may someday be filled with natural products detected by the simple and convenient *Lemna* bioassay (Atta-ur-Rahman *et al*. 1991). *Euphorbia wallichii* (Ali *et al*., 2009) and *Polygonatum verticillatum* (Saeed *et al*., 2010) have shown phytotoxicity.

Keeping in view the significance of minerals, nutritional value and biological activities of medicinal plants in general as reported by various scientists it was thought to investigate the *Hypericum perforatum* which was used locally in different parts of Swat for the treatment of various diseases.

MATERIALS AND METHODS

Elemental analysis

Plant was collected from Shartangar (Swat) where it grew abundantly in summer, and flowered in April-June, fruiting on the onset of July but in Shartangar it had fruiting in June. The plant was oven dried at 65ºC for 72 h. The ground samples were stored in plastic bags for elemental and proximate analysis. Plant was identified with the help of flora of Pakistan and was deposited in Peshawar University Herbarium, Department of Botany, University of Peshawar.

A small amount i.e., 0.5g of the powdered plant material was taken in a 50ml conical flask and 10ml nitric acid (HNO_3) was added in it and allowed to stand overnight. Next day, 4ml perchloric acid was added in it and it was boiled on a hot plate in a fume hood. After few minutes the yellowish color of plant material changed into white fumes and it indicated that digestion was completed. The flasks were removed, cooled down and 100ml distilled water was added. It was filtered with filter paper and filtrates were collected in labeled plastic bottles. These solutions were analyzed for the elements through atomic absorption spectrometer (Zafar *et al*., 2010).

Proximate analysis

The proximate analysis was carried out following the standard procedures of (AOAC, 1990). Dry matter (DM) was obtained by oven drying the plant sample at 65°C for 72 hours method. Ash was calculated following (AOAC, 1990). Proteins were determined by micro Kjeldahl method. It included sample digestion, distillation followed by titration in order to calculate the nitrogen value, the precursor for proteins. The nitrogen value was converted to protein by multiplying a factor of 6.25. Fiber contents were obtained from the loss in weight of the crucible on ignition. For crude fat Soxhelt's apparatus was used. Nitrogen free extract was calculated after Galyean (1985).

Biological activities

Insecticidal activity

Preparation of test compound

One concentration of test sample 1572.69µl/cm^2 was prepared from 20% stock solution.

Rearing techniques

The stored grains pests were reared in the laboratory under control temperature and humidity, so that the insects of uniform age and size were used. The pairs of insects were reared in 9.0cm diameter and 11cm high plastic bottles containing 250gm of breeding media. Bottles were covered with muslin cloth tied by means of rubber bands, or small jars or wide mouthed bottles sealed with filter paper (Whatman No 29, black) and paraffin wax (to prevent contamination) were suitable. The media should be sterilized at 60ºC for one hour. ***Procedure*** The insects were exposed to extracts by contact method using filter paper. 1ml of the concentration of the extract was applied by micropipette to 90 mm diameter filter papers and then placed in the petri dishes. After that 10 adult insects of the same size and age in each batch were transferred to petri dishes. A check batch was treated with solvent for determination of solvent effect. A control batch was kept for the determination of environmental effects. Another batch supplemented with reference insecticides e.g. coopex and decis (synthetic pyrethroids) were used. All they were kept without food throughout 24 hours exposure period. Mortality counts were done after 24 hours exposure period (Naqvi and Perveen 1991).

Cytotoxic activity

Hypericum perforatum (leaf, flower and fruit) was ground to powders using an electric grinder. 10g of each sample was soaked in 50ml methanol for 72 hours. Each plant extract was passed through Whatman filter paper No. 1823. This process was repeated three times. Evaporating in a rotatory evaporator at 40°C, the concentrated extracts were obtained. These extracts were stored at 4°C prior to use. The methanol plant extracts i.e., test sample (20 mg) was dissolved in 2ml of dimethylsulphoxide (DMSO) and from this stock solution transferred three concentrations i.e., 5, 50 and 500µl corresponding to 10,100 and 1000µg/ml, respectively. The solvent was allowed to evaporate overnight and 5 ml seawater water solution (38 g/L) was added to each vial. Hatching tray of 22x32cm was half filled with filtered brine solution and 50mg of shrimp eggs were sprinkled into it. The tray was incubated for 24 h at 37°C. After 2-days of hatching and maturation as nauplii placed 10 larvae/vials, using a Pasteur pipette. It was incubated at 25-27°C for 24 hours under illumination. Supplement other vials with solvent, serving as negative controls. After incubation, the eggs were hatched and the test samples were applied to find its cytotoxicity. Bioactive compounds are often toxic to shrimp larvae. Hence *in vivo* lethality to shrimp larvae can be used as a rapid and simple preliminary monitor for bioactive compounds during the isolation of natural products. When placed in artificial sea-water, the eggs hatch within 48 hours, providing large number of larvae. These tiny shrimp larvae have been extensively used as a tool to monitor the cytotoxicity of samples under study. The eggs were stored at low temperatures (4°C) that

remained viable for years. The cytotoxic activity was done for methanolic extracts (Khuda *et al.,* 2012; Ahmad *et al.,* 2011).

Phytotoxic activity

Lemna aequinoctialis was used as a test organism. The experimental conditions included light intensity (9000 Lux), photoperiod (12 hours), incubation condition (28º C, 56 + 1% rh). Ten plants per flask were used. Number of plants per dose =Single with a rosette of three fronds. Phytotoxic activity of the extracts was carried out against the *L. aequinoctialis* following McLaughlin *et al*. (1991).

E-medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted between 5.5-6.0 by adding KOH pellets. Constituents in distilled water include: 1. Potassium dihydrogen phosphate (0.68gm/L). 2. Potassium nitrate (1.515gm/L). 3. Calcium nitrare (1.180gm/L). 4.Magnesium sulphate (0.492gm/L). 5. Boric acid (0.00286gm/L). 6. Magenous chloride (0.00362gm/L). 7. Ferric chloride (0.00540gm/L). 8.Zinc sulphate (0.00022gm/L). 9. Copper sulphate (0.00022gm/L). 10. Sodium molybdate. 11. Ethylene diamino tetracetic acid. The medium was autoclaved at 121ºC for 15 min. Fifteen (mg) of extract was dissolved in 1.5 ml of solvent (methanol) serving as stock solution. The solvent was allowed to evaporate overnight under sterilized conditions. Three sterilized flasks (for each concentration) were incubated with 5, 50 and 500µl of solution pipetted from the stock solution which was equivalent to 10, 100 and 1000µg/ml, respectively and added to the flasks containing *Lemna* plants. Twenty ml of medium was added to each flask. Other flasks were supplemented with E-medium only as control. Plants were examined daily during incubation. Flasks were placed in growth cabinet for 7 days. The number of fronds per flask were counted and recorded on seventh day. Results were analyzed as growth regulation in terms of percentage as described by (Nisar *et al*., 2011).

Antibacterial activity

Fifty grams of plant sample was soaked in 250ml 70% methanol for 72 hours. Plant extract was filtered through Whatman filter paper No. 1823. This process was repeated 3 times. Each extract was concentrated using rotatory evaporator. These extracts were stored at 4°C prior to use.

Requirements

Test organisms were available at HEJ bioassay laboratory, Karachi, Pakistan), test samples, nutrient broth, nutrient agar, petri dishes, micropipettes, sterile cotton swab, sterile cork borer, test tubes, incubator, laminar flow hood. Bacterial cultures used were *Bacillus subtilis, Corynebacterium diphtheriae, Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus* and *Streptococcus pyogenes*. The antibacterial activity of the *Hypericum perforatum* was carried out by agar well diffusion method followed Carron *et al*. (1987). Bacterial strains were first cultured on nutrient broth and incubated for 24 hours prior to experiments. Nutrient agar was melted, cooled to 40ºC and poured into sterilized Petri dishes. The wells were bored in media using 6 mm diameter with the help of sterile metal cork borer and keeping a distance of 24mm between two adjacent wells. The 4-8 hour old bacterial culture was spread on the surface of nutrient agar with the help of sterilized cotton swab. These processes were done 3 times turning the plate 60º between each streaking. About l00µl of 2mg/ ml of respective extract, dissolved in DMSO was then added to the wells. Other wells were supplemented with DMSO and 10µg (Tetracycline) served as positive and negative controls. The plates were then incubated for 24 hours at 37ºC. The plates were then observed for zones of inhibition. The phytochemical screening was done following (Edeoga *et al*., 2005).

RESULTS

Elemental analysis

Calcium was highest (5600µg/g) in leaves and lowest (2500µg/g) in flowers. Potassium was highest (840µg/g) in fruit and lowest (80µg/g) in leaves. Magnesium was highest (260µg/g) in stem and lowest (200µg/g) in flowers. Sodium was highest (4900µg/g) in stem and lowest (4700µg/g) in leaves and flowers. Lead was highest (2200µg/g) in stem and lowest (10µg/g) in leaves. Copper was highest (26µg/g) in stem and lowest (10µg/g) in leaves. Iron was highest (5000µg/g) in flowers lowest (1200µg/g) in stem. Manganese was highest (85µg/g) in leaves and lowest (60µg/g) in stem. Zinc was highest (80 µg/g) in flowers and lowest (46µg/g) in stem. Nickle, cadmium and Cobalt were <5µg/g for all plant parts (table 1).

Nutritional value

Dry matter was highest (97.61%) in stem and lowest (96.38%) in leaf of *H. perforatum.* The ash was highest (5.43%) in flowers and lowest (1.90%) in stem. Crude protein was highest (12.63%) in leaf and lowest (6.15%) in stem. Crude fiber was highest (64.74%) in flowers and lowest (13.0%) in leaf. Ether extract was highest (10.98 %) in fruit and lowest (1.88%) in stem. Nitrogen free extract was highest (65.80%) in leaf and lowest (10.98%) in flower (table 2).

Biological activities

In antibacterial activity the highest zone of inhibition (35mm) was shown by *Corynebacterium diphtheriae, Escherichia coli, Salmonella typhi* followed by *Staphylococcus aureus* (30mm) and the lowest zone of inhibition (14mm) was shown by *Pseudomonas aeruginosa* (table 4). The number of organisms were not sufficient to prove the antibacterial activity. In phytotoxic activity the leaf extract showed the highest inhibition

Table 1: Elemental analysis (µg/g) of various parts of *Hypericum perforatum* L.

Plant part	Pb	Zn	Cu	Ni	Mn	Cd	Co	Na	K	Mg	Ca	Fe
Stem	22	46	26	<5	60	<5	<5	4900	820	260	3000	1200
Leaf	10	63	10	<5	85	<5	<5	4700	80	240	5600	2800
Flower	20	80	12	<5	65	<5	<5	4700	90	200	2500	5000
Fruit	20	74	16	<5	80	<5	<5	4800	840	240	4700	4800

Table 2: Proximate analysis of various parts of *Hypericum perforatum* L.

Plant part	Dry matter (%)	Percent on dry matter basis				
		Ash (%)	Crude Protein (%)	Crude Fibre (%)	Ether extract (%)	Nitrogen Free Extract (%)
Stem	97.61	1.90	6.15	34.80	1.88	55.30
Leaf	96.38	4.66	12.63	13.00	3.96	65.80
Flower	96.43	5.43	11.24	64.74	7.70	10.98
Fruit	96.46	4.77	11.16	24.70	10.98	48.39

Table 3: Phytochemical screening of various parts of *Hypericum perforatum* L.

Plant part	Alk	Muci	Anth	CO	Cellulose	Sap	Tannin	Lignin	Cutin	Starch	Fats & oils	Protein	Chl	Catechin
Stem	-	+	+	+	-	+	+	+	+	-	+	+	+	+
Leaf	-	+	+	+	-	+	+	-	-	-	+	+	+	-
Flower	-	+	+	+	-	+	+	+	+	-	+	+	-	+
Fruit	-	+	+	-	-	+	+	+	-	-	+	+	-	+

Key: + = Present; - = Absent; Alk = Alkaloids; Anth = Anthraquinone; CO, Calcium oxalate, Chl = Chlorophyll; Muci = Mucilage, Sap = Saponin

Table 4: Antibacterial activity of *Hypericum perforatum* L.

S. No	Microorganisms	Inhibition Zone (mm)	Reference drugs	Inhibition Zone (mm)	Antibacterial activity (%)	Remarks
1	*Bacillus subtilis*	0	Tetracycline	22	0%	No activity
2	*Corynebacterium diphtheriae*	0	Tetracycline	35	0%	
3	*Escherichia coli*	0	Tetracycline	35	0%	
4	*Klebsiella pneumoniae*	0	Tetracycline	25	0%	
5	*Proteus vulgaris*	0	Tetracycline	22	0%	
6	*Pseudomonas aeruginosa*	0	Tetracycline	14	0%	
7	*Salmonella typhi*	0	Tetracycline	35	0%	
8	*Shigella dysenteriae*	0	Tetracycline	25	0%	
9	*Staphylococcus aureus*	0	Tetracycline	30	0%	
10	*Streptococcus pyogenes*	0	Tetracycline	25	0%	

(44.0%) at 500µg/ml followed by (42.85%) at 5 µg/ml. The flower extract showed the highest inhibition (36.0%) at 500µg/ml followed by (35.17%) at 5 µg/ml. The fruit extract showed the highest inhibition (44.0%) at 500 µg/ml followed by (32.14%) at 5µg/ml. The reference inhibitor (paraquat) indicated 100% inhibition (table 7). The leaf, flower, and fruit extract of *H. perforatum* did not show insecticidal activity against any grain pests and mortality mean % was zero for all the plant parts (table 5). The three concentrations of leaf, flower, and fruit extract of *H. perforatum* had no cytotoxic effect and these concentrations did not significantly cause the mortality of brine shrimps (table 6). The phytochemical screening showed the presence of mucilage, tannins, anthraquinones, saponins, fats and oils and proteins in all parts of the plant (table 3).

Ethnomedicinal uses

Hypericum perforatum was found at Shartangaar (1696m), Panj Pali (1727m), Baranai (1757m), Sharanko (1973m) and Jaba (2158m). It was also found in green meadows in Baranai and Jaba and under trees in Sharanko. It was abundant in Shartangaar as compared to other areas. The growth of the plant was good in Shartangaar, Panj Pali and Baranai as compared to Sharanko and Jaba. It was observed that at lower altitudes the growth of the plant was better. The fruiting time was the onset of July but early fruiting stages were seen somewhere in Shartangaar suggesting that lower altitude

Table 5: Insecticidal activity of various parts of *Hypericum perforatum* L.

Plant part	Pests	Dose µl/cm^2	x̄ = x/n mortality mean %			Insecticidal activity	Remarks
			Control	Check	Test		
Leaf	Ts	1572.69	0	0	0	0%	No activity
Flower	Tg	1572.69	0	0	0	0%	
Fruit	Ca So Rd	1572.69	0	0	0	0%	

Key: Ts = *Tribolium castaneum*; Tg = *Trogoderma granarium*; Ca = *Callosobruchus analis*; So = *Sitophilus oryzae*; Rd = *Rhyzopertha dominica*

Table 6: Cytotoxic activity of various parts of *Hypericum perforatum* L.

Plate No.	Name of organism	Dose µg/ml	No of Shrimps	No of survivors	LD50 µg/ml	Reference drug	LD50 µg/ml	Cytotoxic activity (%)	Remarks
Leaf:									
1	*Artemia salina*	1000	30	10 10 10	-	-	-	0%	No activity
2		100	30	10 10 10	-	-	-		
3		10	30	10 10 10	-	-	-		
Flower:									
1	*Artemia salina*	1000	30	10 10 10	-	-	-	0%	No activity
2		100	30	10 10 10	-	-	-		
3		10	30	10 10 10	-	-	-		
Fruit:									
1	(*Artemia salina*	1000	30	10 10 10	-	-	-	0%	No activity
2		100	30	10 10 10	-	-	-		
3		10	30	10 10 10	-	-	-		

favored the plants growth. Fresh and dried leaves were used in the study areas. The plant was used as stimulant, in fever and cough in Shartangaar and Baranai. It was used in diphtheria and as an anthelmintic only in Shartangaar. For indigestion, the people of Sharanko used the plant while in gastric disorders it was used only in Jaba.

DISCUSSION

Elements such as Pb, Zn, Cu, Co, Ni, Mn, Cd, Na, K, Mg, Ca and Fe were detected in stem, leaf, flower and fruit of *Hypericum perforatum* (table 1). Calcium helps in hypertension, colon cancer and in bones strength (Sanjay *et al*., 2010; Chowdhary & Rasool, 2010). The necessary daily intake of Ca is between 350 and 1100mg/day (Stef,

Table 7: Phytotoxic activity of various parts of *Hypericum perforatum* L

Plate No.	Name of plant	Conc (µg/ml)	Number of fronds		Growth regulation (%)	FI50 µg/ml	Reference inhibitor	FI50 µg/ml	Phytotoxic Activity (%)	Remarks
			Sample	Control						
Leaf:										
1.	*Lemna aequinoctialis*	500	14	25	44.00	NI	Paraquat 100%	0.0000 0.125	44.0%	44% significant only at highest dose
2.		50	22	24	8.33	FP50 µg/ml	Reference promotor	FP50 µg/ml	8.33%	
3.		05	16	28	42.85	NI	NI	NI	42.8%	
Flower:										
1.	*Lemna aequinoctialis*	500	16	25	36.00	-	Paraquat 100%	0.0000 0.125	36.0%	36% significant only at highest dose
2.		50	20	24	16.66	FP50 µg/ml	Reference promotor	FP50 µg/ml	16.6%	
3.		05	18	28	35.17	NI	NI	NI	35.1%	
Fruit:										
1.	*Lemna aequinoctialis*	500	14	25	44.00	-	Paraquat 100%	0.0000 0.125	44.0%	44% significant only at highest dose
2.		50	21	24	12.50	FP50 µg/ml	Reference promotor	FP50 µg/ml	12.5%	
3.		05	19	28	32.14	NI	NI	NI	32.1%	

Key: FI50 = Concentration needed to inhibit 50% frond proliferation; FP50= Concentration necessary to promote 50% frond proliferation. NI= Not Investigated.

2010). Magnesium helps in nerve and muscle function (Yamashita *et al.*, 2005). Some workers reported the variation of Pd and Cu in *H. perforatum* (Radanovic, 2002; Mężyk and Więckowski 1999; Radulescu *et al.*, 2013). Iron helps to maintain a healthy immune system and digestive action (Ahmed & Chaudhary, 2009). Iron was sufficiently found in flowers of *H. perforatum* (table 1). Several workers reported varying levels of Cd in *H. perforatum* as Marquard and Schneider (1995) reported 2.09mg/kg Cd; Chizzola and Franz (1996) found 0.179 ug/g Cd. Similarly, Djukić-Ćosić *et al.*, (2011) reported that Cd content varied significantly in *H. perforatum* samples. The lowest Cd level was from Rtanj localities (0.25mg Cd/kg), while the highest was from Ozren locality (1.24mg/kg).

The dry matter percentage was maximum only in stem while ash was high in flowers of *H. perforatum* (table 2). Crude protein was found high in leaf of *H. perforatum* (table 2). Epidemiological evidences showed that use of reasonable amount of dietary fiber (20-35g/day) had lower risk of coronary heart disease, type 2 diabetes mellitus and irritable bowel syndrome (Abidemi *et al.*, 2013). Crude fiber was highest in flowers of *H. perforatum* (table 2). The fruit had maximum ether extract as compare to other parts of *H. perforatum*. Nitrogen free extract was high in leaf of *H. perforatum* (table 2). Many workers such as Gul and Safdar (2009); Hassan and Umar (2006); Oladele and Oshodi (2007); Bhatt *et al*. (2008) reported variable amount of carbohydrates in different medicinal plants but these studies were not in line with the present study as they reported less carbohydrates.

The antibacterial activity showed that *Cornybacterium diptheriae, Escherichia coli, Salmonella typhi* were the most sensitive bacteria to extract of *H. perforatum* (table 4). Yousuf *et al*., (2012) reported that *H. perforatum* extract showed pronounced activity against *Salmonella typhi* and *E. coli*. This agrees with the present study. Stojanovic *et al*. (2013) stated that a number of studies of the biological activities of *Hypericum* species have shown that the most recognized species of this genus, *H. perforatum*, was not the most active. Several workers (Turi *et al*., 1997; Yesilada *et al*., 1999) reported the antibacterial activity of *H. perforatum* against various pathogens but the antibacterial activity was not strong. The results were a little different for phytotoxic activity as the plant revealed phytotoxic activity in all plant parts studied. The leaf and fruit had similar phytotoxic effect at the highest concentration and a significant dose dependant inhibition of fronds was observed for the three tested samples (table 7). Saponins have allelopathic potential (Chaieb, 2010; Lee *et al*., 2004). Saponins were detected in the present study and they might be responsible for the phytotoxic activity. Seabrook *et al*., (1999) reported the phytotoxic activity of extracts of *Hypericum* using cultures of human keratinocytes. The results confirmed the phytotoxic activity hence supporting the present study.

The phytochemical screening revealed the presence of mucilage, tannins, anthraquinones, saponins, fats and oils and proteins in all parts of the plant (table 3). Makovetskaya (1992) detected tannins in *H. perforatum* supporting the present results. Calcium oxalate was found in all parts except the fruit. Lignin and catechin was found in all parts except the leaf (table 3). Cutin was found only

in stem and flower while chlorophyll was found only in stem and leaf (table 3). Umek *et al*., (1999) screened six *Hypericum* species and the amount of most substances was found highest in flowers. Tala *et al*., (2013) reported bioactive components mainly xanthones, phloroglucinols, flavonoids, naphtodianthrones and essential oils in *H. perforatum*.

CONCLUSION

The present study showed the *Hypericum perforatum* is a rich source of minerals and different plant parts contained maximum proteins, fibers, and the leaf had the highest value of nitrogen free extract. Plant can be helpful for animals when it is grazed in various localities of Swat. Phytotoxic activity revealed the plant may have some herbicidal compounds that can be isolated. Ethno botanical uses suggested that this plant can be further studied for antipyretic, anthelmintic and gastroenteritis activities to prove the local people claims.

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