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PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL EVALUATION OF *IFLOGA SPICATA* (FORSSK.) SCH. BIP. IN LEISHMANIASIS, LUNGS CANCER AND OXIDATIVE STRESS

SYED MAJID SHAH[1,2], FARHAT ULLAH[2], MUHAMMAD AYAZ[2*], ABDUL WAHAB[1] AND ZABTA KHAN SHINWARI[3,4,5]

[1]*Department of Pharmacy, Kohat University of Science & Technology, Kohat, 26000, Pakistan*
[2]*Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa, 18800 Pakistan*
[3]*Pakistan Academy of Sciences, Islamabad 44000, Pakistan*
[4]*Department of Biotechnology, Quaid-i-Azam University, Islamabad 44000, Pakistan*
[5]*Department of Eastern Medicine and Surgery, Qarshi University, Lahore 54000, Pakistan*
[*]*Corresponding author's email: Ayazuop@gmail.com,*

Abstract

Ifloga spicata (*I. spicata*), is an ethno-medicinally important plant. Its crude extract (Is.Cr) and subsequent fractions: *n*-hexane (Is.Hex), chloroform (Is. Chf), ethyl acetate (Is.EtAc) and aqueous (Is.Aq) were evaluated for potential applications in leishmaniasis, lungs cancer and oxidative stress. Total phenolic content (TPC), Total flavonoid contents (TFC), Total Antioxidant Capacity (TAC) were determined colorimetrically. Samples were screened for antileishmanial effect using *Leishmania tropica* promastigotes, for cytotoxicity using NCI-H460 cell lines, and for antioxidant potentials via DPPH, ABTS assays. In the phytochemical analysis Is.EtAc exhibited highest amount of TPCs (141.01±0.12µg GAE/mg DW) and TFC of (52.75±1.22µg QE/mg DW). Is.Cr, Is.Hex, Is.Chf, Is.EtAc and Is.Aq demonstrated antileishmanial effect with IC_{50} values of 184, 573, 212, 52, 483 µg/ml respectively. Is.Chf showed prominent cytotoxic effect (IC_{50} 79± 2.9µg/mL) against lung cancer cell line. In DPPH and ABTS assays, Is.EtAc was most potent with IC_{50} of 30.44 and 31.79 µg/ml respectively. These findings indicate that *I. spicata* crude extract and its derived fractions are safe sources of natural antioxidant, cytotoxic and antileishmanial compounds and thus need further studies.

Key words: *I. spicata*, Phytochemical analysis, Leishmaniasis, Oxidative stress, NCI-H460 cell line.

Introduction

Medicinal plants play an important role in controlling numerous diseases (Majid *et al*., 2015; Zohra *et al*., 2018). It is assessed that approximately 25% of medicines are resulting from medicinal plants (Colares *et al*., 2013). Phytomedicines have been used since long time in various forms including the crude extracts, powdered sample, decoction, essential oil and isolated compounds (Ahmad *et al*., 2016; Ayaz *et al*., 2017a; Ovais *et al*., 2018a). Phytochemicals are mainly divided into two groups namely primary and secondary metabolites (Ovais *et al*., 2018b). Plants have their medicinal and therapeutic values due to the presence of particular secondary metabolites (Ayaz *et al*., 2014 and Rashid *et al*., 2013). Pakistan is particularly rich in medicinal flora (Khalil *et al*., 2014 and Ayaz *et al*., 2017b). Majority of the global population are dependent on the medicinal plant based therapies (Ovais *et al*., 2018c; Shinwari, *et al*., 2013). Such types of evidences are usually collected through interviewing native tribes and conducting ethnomedicinal surveys (Butt *et al*., 2015).

Leishmaniasis is well-known tropical disease of the underdeveloped countries around the world with prevalence of 0.5 million and 1.5–2.0 million cases of the visceral and cutaneous forms of the diseases causing 70,000 deaths per year respectively. Cutaneous leishamniasis (CL), caused by *Leishmania tropica* is common in Pakistan, affecting mainly the exposed parts of the body (Shah *et al*., 2019a, 2019b) It is generally self-healing within 3–18 months, but the lesions leave disfiguring marks which leads to public isolation, mental stress and lesser chances of economic success (Hailu *et al*, 2016; Shah *et al*., 2014a; Ullah *et al*., 2016). Antimonials are used as a gold standard treatment for Leishamniasis (Khalil *et al*., 2017), but it is reported that the parasite is gaining resistance, necessitating alternative treatment strategies (Hassan *et al*., 2018). Antileishmanial activity has been reported for twenty species of Asteraceae family (García *et al*., 2011). Here, we report for the first time the antileishmanial activity of different extracts of *I. spicata* of Asteraceae family with the aim to validate the antileishmanial potential of this plant.

Free radicals are produced in cell during normal metabolic process as a result of respiration and metabolism and damage macromolecules (Ayaz *et al*., 2017c; Sadiq *et al*., 2018). Accumulation of these free radicals in cell also causes various diseases. Living cells have best endogenous defense system in the form of various detoxifying enzymes which counter the damaging effect of these free radicals by scavenge them (Majid *et al*., 2015; Ali *et al*., 2017). The exogenous natural and synthetic antioxidants are available but synthetic antioxidants are less used because of their safety concern (Ayaz *et al*., 2015; Mir *et al*., 2019). Medicinal plants have natural antioxidant constituents which reduce the harmful effects of free radicals by scavenging them and thus these phytochemicals can be used in controlling many ailments (Shah *et al*., 2014b; Kamal *et al*., 2015; Jafri *et al*., 2017).

Lung cancer is the principal reason of cancer-associated death across the globe. More than fifty percent of people with lung cancer die within a year. About eighty percent of all lung cancer is identified with non-small cell lung cancer. Whole invasive resection is one of the key treatment options however, the recurrence occurs in 30%–75% cases. Thus, an effective plan for reducing the reappearance of novel therapeutic approaches is required to control lung cancer. (Ma *et al*., 2015; Lee *et al*., 2019). Natural products from plants have been known for diverse therapeutic applications like cancer (Cragg & Newman, 2005; Habiba *et al*., 2016) and currently sixty percent

anti-cancer agents are obtained from natural sources (Newman *et al*., 2003; Cragg *et al*., 2011).

I. spicata is an annual herb with short tap root. The stem is herbaceous from the base. Leaves linear with tapering ends. Fruit is achne which containing pappus. It is distributed in Canary Island, Afghanistan, Pakistan and India. *I. spicata* belongs to family Asteraceae, used as whole plant for skin diseases like dermatosis, allergies and cardiac diseases in decoction form (Ali & Nasir, 1990; Hammiche & Maiza, 2006; Abouri *et al*., 2012; Osman *et al*., 2014). In view of the medicinal importance of *I. spicata*, the present work was carried out to assess the phytochemical and biological potentials of methanolic extract and its derived fractions to explore potent phytomedinces for leishmaniasis, oxidative stress and lung cancer.

Material and Methods

Reagents and chemicals: ABTS, DPPH and reference compounds including quercetin, gallic acid were obtained from Sigma–Aldrich USA. Solvents including methanol, chloroform, ethyl acetate, *n*-hexane, were obtained from Daejung Korea. The *L. tropica* strains were grown in particular Medium199 purchased from Gibco, Invitrogen, USA.

Plant collection and processing: Plant was collected in the start of April 2016 from District Karak, KP, Pakistan, and identified by taxonomist, Waheed Murad, Department of Plant Sciences, Kohat University of Science and Technology, Kohat and deposited in Department Herbarium having voucher number (KUH 1002). The plant (18 kg) was dried under shade and grinded to form coarse powder. Plant material maceration was carried out in methanol for 25 days and 600 gm methanolic extract was obtained. The methanolic extract (Is.Cr) 500 gm was dispersed in pure water (500 ml) and consecutively partitioned with organic solvents with increasing polarity to get *n*-hexane (Is.Hex), chloroform (Is.Chf), ethylacetate (Is.EtAc) and aqueous (Is.Aq) fractions respectively (Shah *et al*., 2014c).

Qualitative phytochemical analysis: Phytoconstituents in Is.Cr were detected by means of usual procedures with slight modifications (Evans, 2009; Ayaz *et al*., 2014; Khan *et al*., 2016). The formation of constant froth was a sign of existence of saponins in the Is.Cr after mixing it with 5 ml of distilled water in test tube and shaken strongly. Is.cr was combined with 2 ml of 2% solution of NaOH. Deep yellow color was observed indicating the presence of flavonoids. A brown ring formation show the occurrence of cardiac glycosides after mixing crude extract with 2 ml of glacial acetic acid and concentrated H2SO4. Terpenoids were confirmed by getting grayish color after mixing Is.Cr, 2 ml of chloroform and 2 ml concentrated H_2SO_4. Alkaloids were detected by the formation of precipitate after adding 2 ml of 1% HCl followed by the addition of Mayer's & Wagner's reagents to the Is.Cr. The existence of phenolic compounds was reflected by dark green color formation by dispersing Is.Cr in 5 ml of distilled water followed by the addition of few drops of 5% ferric chloride solution.

Determination of total phenolic content (TPC): The TPC was estimated according to standard procedure with slight adjustment. The extract solutions were prepared in strength of 1 mg/ml in solvent dimethyl sulfoxide. The mixture of 20 µl test solution and 90 µl of Folin–Ciocalteu reagents were added to 96 well plate and kept at room temperature for 5 min followed by the addition of 90 µl of 6% sodium carbonate. After 90 min, absorbance of each mixture was checked at 630 nm using microplate reader. A calibration curve (y = 0.0136x + 0.0845, R2 = 0.9861) was achieved in parallel using similar working settings, keeping gallic acid (6.25–50 µg/ml) as standard drug. The TPC was appraised three times and the results were showed as µg gallic acid equivalent in one miligram of plant extract weight (µg GAE/mg extract weight) (Fatima *et al*., 2015).

Determination of total flavonoid content (TFC): TFC was checked following colorimetric aluminum chloride as we reported previously (Rashid *et al*., 2013; Ayaz *et al*., 2014). The mixture of plant samples 20 µl (concentration of 1 mg/ml prepared in DMSO), 10 µl aluminum chloride, 1.0 M potassium acetate, and 160 µl of distilled water were transported subsequently to each well of 96 well plate kept at room temperature for half hour and subsequently absorbance values of the resulting mixture were recorded via microplate reader at wavelength of 415 nm. The assay was repeated three times and the resultant TFC was calculated in µg equivalents of Quercetin in one mg of plant extract weight (µg QE/mg extract weight). The comparison got for standard drug quercetin calibration curve was y = 0.0268x + 0.00753 (R2 = 0.9986) (Jafri *et al*., 2017).

Anti-leishmanial assay: Stock solutions for samples were prepared in concentration of 10 mg/ml and diluted successively using DMSO in the wells of a 96-well plate. *L. tropica* promastigotes were cultured in Medium 199 containing penicillin, streptomycin and 10% fetal bovine serum. About 180 µl of M199, 100 µl of *L. tropica* log phase culture and 20 µl of sample were added to wells followed by incubation for 72 hrs at 24°C. After three days, 15 µl from each well was taken on a Neubauer chamber and was counted with the help of microscope. Glucantime was used as standard drug. The IC_{50} values were calculated using Graphpad Prism 6 (Shah *et al*., 2014a).

DPPH anti-radicals assay: The purpose of this assay is to scavenge free radical, 1, 1-diphenyl, 2-picrylhydrazyl, by the plant sample. DPPH solution was prepared by dissolving 24 mg of it in 100 mL of methanol. Stock solutions of test samples (1 mg/mL) were made in methanol followed by serial dilution in concentrations ranging from 62.5-1000 µg/mL. One micro liter from DPPH and extract solutions were combined and kept at 23°C for half hour. UV Spectrophotometer was used to check absorbance at 517 nm taking Ascorbic acid as positive control. Antioxidant effect was calculated as follows,

$$\text{Antioxidant effect (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Median inhibitory concentrations were calculated using GraphPad prism program and assay was repeated three times (Kamal *et al*., 2015).

ABTS radical scavenging assay: The aim of this experiment is to measure the scavenging ability of the plant samples of free radical, 2,2-azinobis [3-ethylbenzthiazoline 1,1-]-6-sulfonic acid (Kumaran & Karunakaran, 2007; Okokon *et al*., 2014). Absorbance value was adjusted to 0.7 at 745 nm by the addition of 50% methanol to ABTS solution. Free radicals were formed by mixing solutions of ABTS (7 mM) and potassium persulphate (2.45 mM) without exposure to light for 12-16 hours. The mixture of 300 µl sample and 3.0 ml of ABTS solution was put in cuvette and reading was taken on spectrophotometer using AA as positive control and percent scavenging effect was calculated as follows:

$$\% \text{ Scavenging effect} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Total antioxidant capacity: TAC was found by phosphomolybdenum method by mixing 0.1 ml of each fraction with 1 ml of Ammonium molybdate reagent solution. The mixture was heated at 95 oC for 90 min and then cooled. Absorbance of the mixture was noted at 695 nm by using DMSO and ascorbic acid (AA) as negative and positive controls respectively. The antioxidant capacity of samples were presented AA equivalents per mg of extracts i.e., µgAAE/mg DW and experiment was performed three times (Jafri *et al*., 2017).

Anti-cancer sulphorhodamine-B assay (SRB): Is.Cr and its resultant fractions were investigated against cancer cell line NCI-H460 (Okokon *et al*., 2014). The cancer cells (10000 cells/100 µL) were protected for one day at 37ºC in a CO2 incubator and afterwards 100 µL of various dilutions of both sample and standard solutions were added in each well of 96-well plate and incubated for two days. Afterwards 50 µL of trichloroacetic acid was provided slowly in the concentration of fifty percent and kept it for half hour, washed with pure water and allowed to dry. Hundred micro liter of SRB solution (0.4% w/v in 1% acetic acid) was supplemented to wells and then acetic acid and tris base were added to remove stains. Microplate reader was used to measure absorbance at 515 nm. The growth inhibition, of the methanolic extract and its resultant fractions were calculated and represented as GI_{50}.

Statistical analysis

One-way analysis of variance was used for obtained data. Duncan's multiple range test (MRT) was applied using SPSS 17 program and $p<0.05$ level was taken as significant. All the procedures were repeated three times and vales were expressed as means ± Standard error means (SEM).

Results

Phytochemical screening: Is.Cr was found to contain all tested phytoconsituents (Table 1).

Estimation of total phenolic content: The TPC of all extracts are presented in Fig. 1, which ranged from 141.01 ± 0.12µg GAE/g DW for the Is.EtAc to 30.91 ± 0.72 µg GAE/g DW for Is.Hex. This indicates that distribution of phenols depends upon the polarity of solvents. Phenolic compounds are important secondary metabolites having antioxidant, and antimutagenic potential (Kumaran & Karunakaran, 2007; Kumar *et al*., 2013).

Estimation of total flavonoid content: TFC was counted in all fractions. Is.EtAc was found to contain highest amount of TFC, approximately 52.75 ± 1.22 µg QE/mg DW followed by Is.Cr > Is.Chf > IS.Aq > Is.Hex fractions as shown in Fig. 2. The range of total phenolic and flavonoid contents in various fractions changes on the basis of nature of solvent and have been observed for antioxidant and medicinal properties of plants (Zohra *et al*., 2018).

Antileishmanial studies: The antileishmanial activity of the Is.Cr of I. spicata and resultant fractions were investigated using glucantamine as a reference (IC_{50} = 5 µg/ml). At various concentrations ranging from 10,000 to 62 µg/ml the extracts Is.Cr, Is.Hex, Is.Chf, Is.EtAc and Is.Aq demonstrated antileishmanial effect with IC_{50} values of 184, 573, 212, 52, 483 µg/ml respectively (Table 2).

DPPH assay: The antioxidants constituents in plant fractions scavenged the DPPH radical, indicated by color change due to formation of reduced DPPH (Ul-Haq *et al*., 2012). The results are summarized in Table 3. It is obvious that Is.EtAc showed highest activity (IC_{50} = 30.44 µg/ml) while Is.Hex showed lowest activity (IC_{50} = 435.35 µg/ml).

ABTS assay: The present study indicates that among different fractions, Is.EtAc was most potent (IC_{50} 31.79 µg/mL), followed by Is.Cr and Is.Aq fractions with IC_{50} of 66.72 and 184.37 µg/mL respectively as shown in Table 3. The antioxidant effect was compared with the standard ascorbic acid (IC_{50} 22.02 µg/mL).

Total antioxidant capacity: TAC was concentration dependent and was found to decrease in the order, such that Is.EtAc > Is.Cr f > IS.Aq > Is.Chf > Is.Hex Fig. 3.

Cytotoxicity against (NCI-H460) cell line: In Fig. 4 shows results of cytotoxicity against NCI-H460 cells in which Is.Chf was more bioactive fraction. It displayed growth inhibition (~26-64%) at 10-100 µg/mL against NCI-H460 cells and the GI_{50} value of 79 ± 2.9 µg/mL was observed. Is.Hex fraction at 250 µg/mL concentration exhibited cytotoxic effect reaching maximum of ~139%. It also demonstrated concentration dependent growth inhibition (~16-92%) between 10-250 µg/mL against (NCI-H460) cell line. The other extracts failed to display significant growth inhibitory effect at the tested concentration.The IC_{50} of doxorubicin (standard) was 0.5 ± 0.09 µg/mL.

Table 1. Phytochemical constituents in Is.Cr of *I. spicata*.

S.No.	Phytochemical class	Test performed	Observations	Results
1.	Saponins	Froth Test	Stable froth formation	+
2.	Tannins	Ferric chloride test	Green coloration	+
3.	Flavonoids	Ferric chloride test	Formation of yellow color which changed to colorless on acid addition	+
4.	Glycosides	Keller Killiani test	Brown ring at the interphase	+
5.	Terpenoids	Liebermann Burchard test	Appearance of grayish color	+
6.	Alkaloids	Mayer's And Wagner's test	Turbidity/precipitation	+
7.	Phenolic compounds	Ferric chloride test	Dark green color	+

Table 2. Results of antileishmanial activity of *I. spicata* methanolic extract and its derived fractions.

Sample	Concentration (mg/ml)	% inhibition of *Leishmania tropica*	IC_{50} (mg/ml)
Is.Cr	10	100.0 ± 0.00	0.184
	5	98.90 ± 0.96	
	2.5	81.16 ± 0.86	
	1	70.98 ± 0.72	
	0.5	66.65 ± 0.98	
	0.25	51.93 ± 0.67	
	0.125	44.16 ± 0.86	
	0.062	37.65 ± 0.98	
Is.Hex	10	100.0 ± 0.00	0.573
	5	89.36 ± 0.57	
	2.5	77.08 ± 0.47	
	1	54.41 ± 0.55	
	0.5	45.43 ± 0.76	
	0.25	31.56 ± 0.69	
	0.125	18.58 ± 0.56	
	0.062	15.54 ± 0.45	
Is.Chf	10	100.0 ± 0.00	0.212
	5	92.87 ± 0.26	
	2.5	79.85 ± 0.97	
	1	76.17 ± 0.72	
	0.5	62.42 ± 0.57	
	0.25	51.74 ± 1.29	
	0.125	47.05 ± 0.75	
	0.062	27.74 ± 0.68	
Is.EtAc	10	100.0 ± 0.00	0.052
	5	100.0 ± 0.00	
	2.5	93.58 ± 1.12	
	1	86.03 ± 0.48	
	0.5	78.44 ± 0.58	
	0.25	73.90 ± 0.96	
	0.125	65.70 ± 1.60	
	0.062	57.42 ± 0.43	
Is.Aq	10	100.0 ± 0.00	0.483
	5	96.61 ± 0.43	
	2.5	64.93 ± 0.67	
	1	57.34 ± 0.98	
	0.5	48.05 ± 0.75	
	0.25	36.74 ± 0.68	
	0.125	27.42 ± 0.57	
	0.062	19.42 ± 0.57	

Standard drug; Glucantime IC_{50} = 5.40 ±0.44 µg/ml. Values expressed as Percent inhibition. (Mean ± SEM of n = 3) and IC_{50}

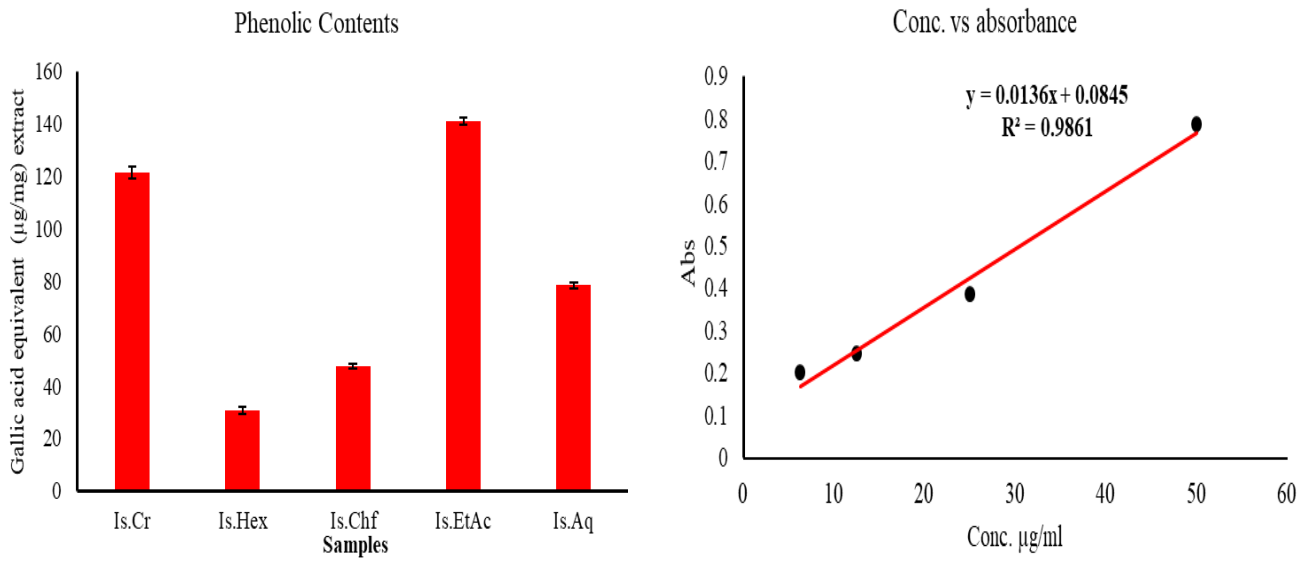

Fig. 1. Total Phenolic Contents (TPC) of various fractions isolated from *I. spicata*.

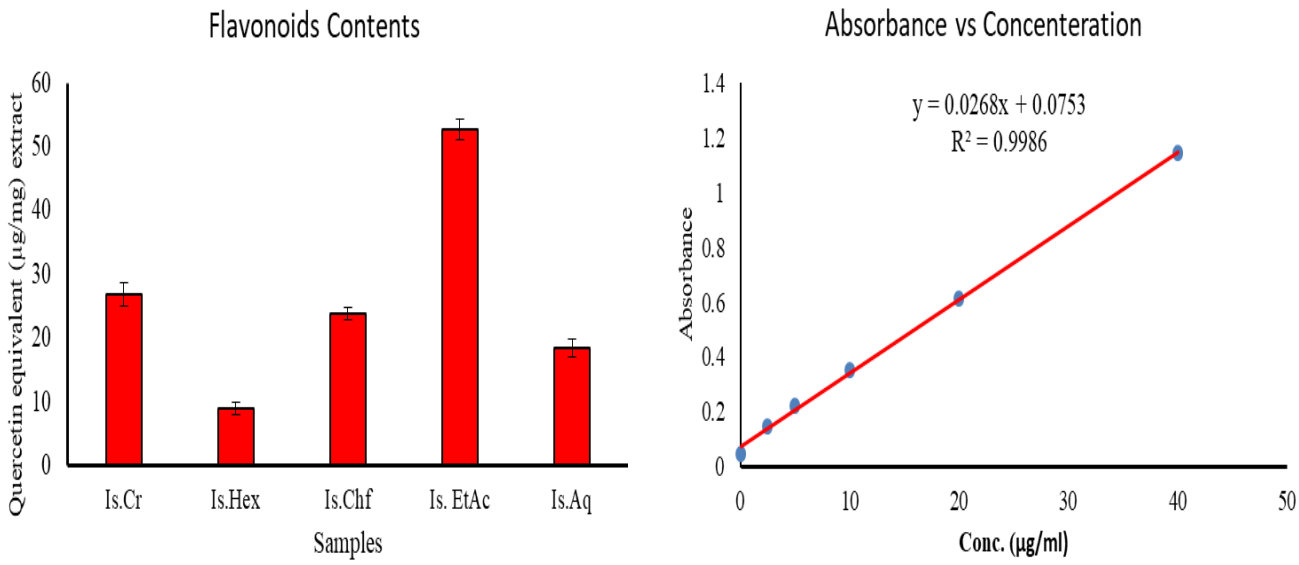

Fig. 2. Total Flavonoids Contents (TFC) of various fractions isolated from *I. spicata*.

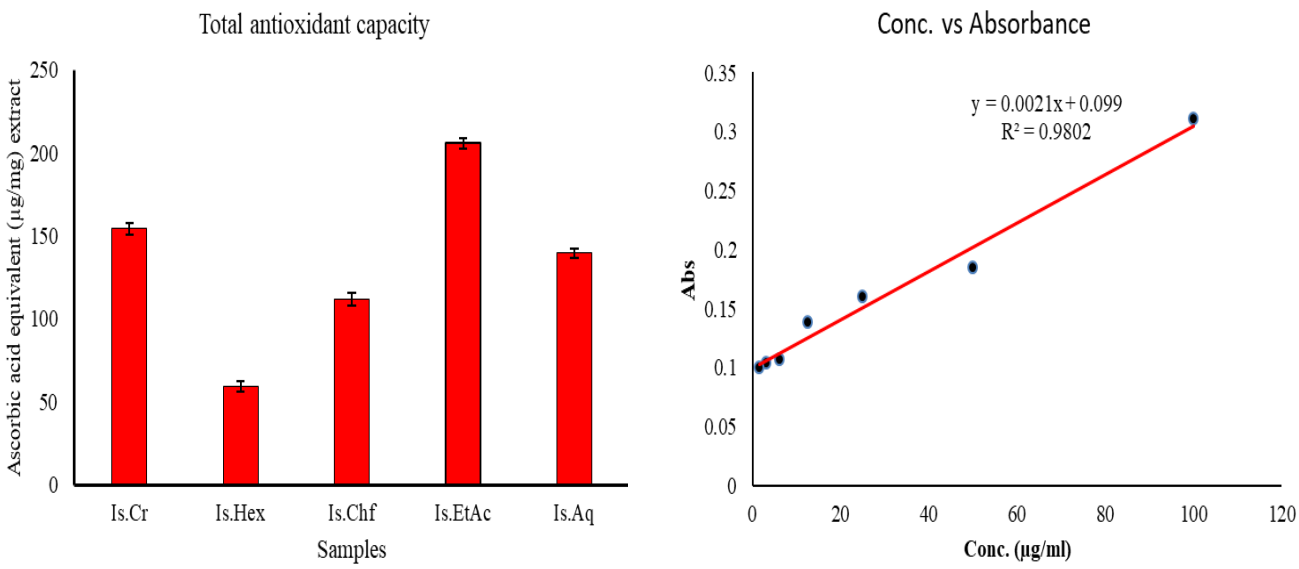

Fig. 3. Total Antioxidant Capicity (TAC) of various fractions isolated from *I. spicata*.

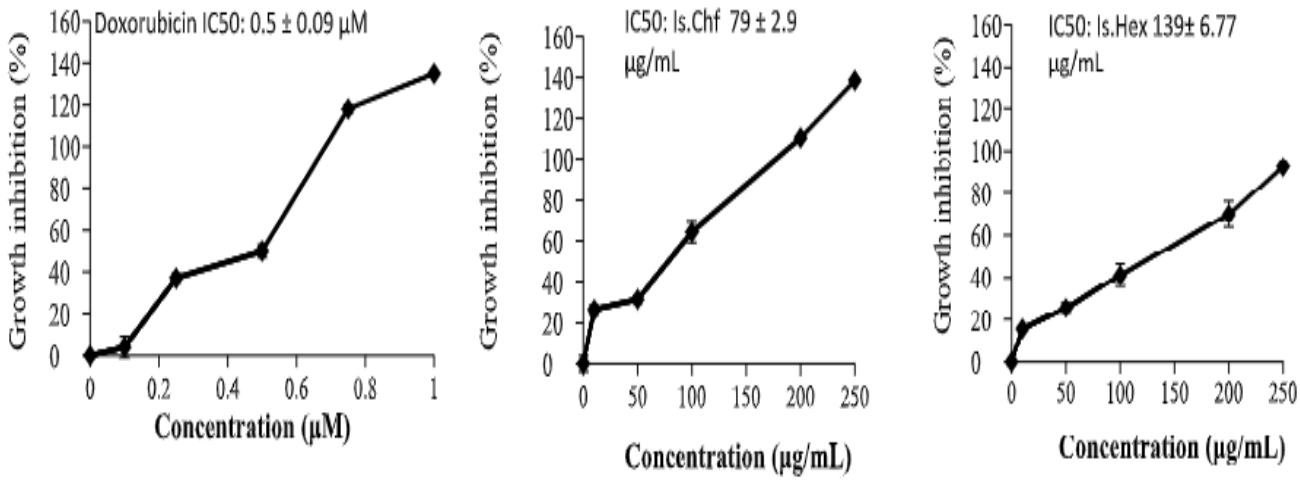

Fig. 4. Results of inhibitory effects of various fractions isolated from *I. spicata* against non-small lung cell line (NCI-H460) using Doxorubicin as positive control.

Table 3. Results of antioxidant potential of *I. spicata* against DPPH and ABTS free Radicals using ascorbic acid as standard.

Sample	Concentration (µg/ml)	% ABTS Scavenging	IC $_{50}$ (µg/ml)	% DPPH Scavenging	IC $_{50}$ (µg/ml)
Is.Cr	1000	80.79 ± 0.62	66.72	85.74 ± 0.61	37.68
	500	71.61 ±0.53		79.47 ± 0.56	
	250	63.58 ± 0.70		73.73 ± 0.64	
	125	61.75 ± 0.63		61.75 ± 0.58	
	62.5	47.58 ± 0.77		58.45 ± 0.49	
Is.Hex	1000	61.62 ± 0.74	526.54	57.55 ± 0.77	435.35
	500	56.66 ± 0.78		52.83 ± 0.56	
	250	28.37 ± 0.68		44.60 ± 0.80	
	125	18.58 ± 0.74		36.61 ± 0.77	
	62.5	11.65 ± 0.77		30.46 ± 0.63	
Is.Chf	1000	56.03 ± 0.86	418.11	76.66 ± 0.78	312.64
	500	56.25 ± 1.40		60.62 ± 0.74	
	250	53.61 ± 0.43		34.37 ± 0.68	
	125	26.51 ± 0.77		34.66 ± 0.78	
	62.5	22.52 ± 0.68		22.62 ± 0.74	
Is.EtAc	1000	98.54 ± 0.50	31.79	87.79 ± 0.63	30.44
	500	89.76 ± 0.58		86.63 ± 0.76	
	250	81.44 ± 0.51		85.68 ± 0.60	
	125	75.69 ± 0.77		79.74 ± 0.61	
	62.5	65.67 ± 0.61		51.81 ± 0.60	
Is.Aq	1000	77.53 ± 0.71	184.37	84.64 ± 0.75	143.22
	500	75.48 ± 0.64		81.85 ± 0.56	
	250	65.86 ± 0.60		57.48 ± 0.74	
	125	30.62 ± 0.74		44.62 ± 0.40	
	62.5	29.86 ± 0.60		34.60 ± 0.46	
Ascorbic acid	1000	90.68 ± 0.49	22.02	95.83 ± 0.56	16.55
	500	84.72 ± 0.66		88.55 ± 0.77	
	250	77.46 ± 0.63		82.65 ± 0.77	
	125	71.61 ± 0.77		76.58 ± 0.74	
	62.5	64.60 ± 0.80		71.37 ± 0.68	

Values significantly different as compared to positive control. Values expressed as Percent inhibition (Mean ± SEM of n = 3) and IC_{50}

Discussion

The traditional plant therapy is still experienced in various rural parts of developing countries including Pakistan and offers a base for the discovery of pharmacologically active drugs (Shinwari, *et al*., 2019). Phyto-investigations taking importance as diseases are more as compared to explored plants and are harmful to humans. Natural products are nontoxic, inexpensive and simply available, therefore preliminary phytochemical screenings, isolations, pharmacological studies are important in discoveries of novel phytomedicines for treatment of various ailments (Abbasi *et al*., 2010, Irum *et al*., 2019). Pakistan has very important medicinal plants because of particular soil composition and different climatic conditions. Safe and effective natural drugs can be found by proper exploration of these plants (Shah *et al*., 2015; Ayaz *et al*., 2019a). Pharmacological activities of medicinal plants are due to the bioactive phytocomponents. The present study reports that *I. spicata* contains all the tested secondary metabolites and explains its use in the traditional treatment (Duraipandiyan *et al*., 2006; Jones & Kinghorn, 2006) for skin and cardiac diseases (Abouri *et al*., 2012).

CL is the most widespread form of leishmaniasis in all provinces of Pakistan caused by *lesihmania tropica* (dry lesions) and *lesihmania major* (wet lesions). About 90 % cases of CL present in all provinces of Pakistan and it affect 15000-20,000 people annually in the country (Hussain *et al*., 2017a, 2017b; Noor & Hussain, 2017). Scientists are in a constant search to develop safe, cost effective antileshmanial drugs having less parasitic resistance (Shah *et al.*, 2014a). Medicinal plants used to treat leishmaniasis in Pakistan are *Rhazya stricta*, *Aloe vera, Asparagus gracilis, Trachyspermum ammi*, *Citrullus colocynthis*, *Jurinea dolomiaea*, *Melia azedarach, Nepeta praetervisa*, *Onosma griffithii*, *Perotis hordeiformis*, *Physalis minima*, *Salvia bucharica* (Tariq *et al*., 2016). Antileishmanial effect of the plant extracts was checked against Leishmania tropica strain which causes cutaneous leishmaniasis. Different fractions of the plant used in this study indicated that Is.EtAc and Is.Cr fractions are most bioactive extracts, possibly because of high level of flavonoids and phenolic as previously reported for other medicinal plants, *Pluchea carolinensis*, *Consolida oliveriana*, *Kalanchoe pinnata*, and *Baccharis retusa* of same family Asteraceae (Muzitano *et al*., 2006; Marin *et al*., 2009; Grecco *et al*., 2010; García *et al*., 2011; Ramírez-Macías *et al*., 2012). Secondary metabolites like alkaloids and saponins also have been reported for antileishmanial activity (González-Coloma *et al*., 2012). Thus, it is dire need to investigate phytoconstuients of this plant both *In vitro* and *In vivo* to explore most effective and safe natural antileishmanial agents.

ROS (reactive oxygen species) cause potential harmful effects to humans, such as inducing cardiac diseases and inflammation etc. Currently, discovery of antioxidant agents is the most important area of modern research to stop ROS mediated diseases (Jones & Kinghorn, 2006; Reza *et al*., 2018, Ayaz *et al.*, 2019b). Different antioxidant mechanisms are reported and therefore several methods are developed to explore it (Shah *et al*., 2014b). Phenols and flavonoids are strong natural antioxidant candidates because of hydroxyl groups in their structure (Yıldırım *et al*., 2000; Jafri *et al*., 2017). Is.EtAc showed significant antioxidant activity because of high phenolic and flavonoid contents through different antioxidant mechanisms and hence it is the activity guided fraction for isolation of natural antioxidants.

Lung cancer causes approximately one million deaths each year (Sun *et al*., 2007). Cisplatin and vinblastine are the drugs of choice but still patients are not completely recovering. Natural products like etoposide, paclitaxel, vinorelbine and docetaxel obtained from different medicinal plants exert anticancer actions through different mechanisms (Choudhary *et al*., 2010; Wu *et al*., 2010; Ovais *et al* 2018d; Kamal *et al*., 2015). In previous studies natural products like withanolides from *Withania somnifera* leaves (Jayaprakasam *et al*., 2003a), cucurbitacins from *Cucurbita andreana* (Jayaprakasam *et al.,* 2003b), Chlorinated and diepoxy withanolides from *Withania somnifera* (Choudhary *et al*., 2010), sesquiterpene lactones from *Inula Britannica* (Fischedick *et al*., 2013) and Curcumin (Wu *et al*., 2010) were tested against NCI-H460 cell line with significant results. The current study indicates that phytconsituents in Is.Chf fraction can be safe source of cytotoxic agents against NCI-H460 cell line. A bio-guided fractionation will be performed on this fraction, in order to elucidate the active cytotoxic agents.

Conclusion

It is the first report on the phytochemical and pharmacological profile of extracts of *I. spicata.* Based on results Is.EtAc and Is.Cr extracts having the highest amount of TFC and TPC and thus having significant antioxidant and antileishmanial results and can be a potential source in the treatment of several types of free radicals and *Leishmania tropica* induced problems. The results of our investigations also showed that Is.Chf is activity guided fraction for the isolation of cytotoxic compounds against NCI-H460 cell line. All bioactive extracts are recommended for isolation of pure compounds responsible for the above mentioned experimental effects.

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