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RELATIVE EFFICACY AND TOXICITY STUDIES ON THREE WILD MEDICINAL PLANTS OF FABACEAE: A PHARMACEUTICAL PERSPECTIVE

UROOJ KHALIL[1], IRAM FATIMA[1,2], SOBIA KANWAL[3*] AND TARIQ MAHMOOD[1*]

[1]*Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad-45320, Pakistan*
[2]*Department of Biotechnology, Fatima Jinnah Women University, Rawalpindi, Pakistan*
[3]*Department of Biology, Allama Iqbal Open University, Islamabad, 44000, Pakistan*
[*]*Corresponding author's email: tmahmood@qau.edu.pk; sobiakanwal16@gmail.com*

Abstract

Naturally derived compounds with biological activities have positive effect on human health. In present study, ethanol, methanol and chloroform extracts of different parts of three plants viz. *Dumasia villosa* DC., *Trifolium repens* Linn. and *Medicago laciniata* var. laciniata belonging to family Fabaceae were prepared and various phytochemical tests were performed. Total phenolic and flavonoid contents were determined, and antioxidant activity was evaluated by DPPH scavenging assay, total reducing power assay and total antioxidant capacity. To determine toxicity potential of these extracts, radish seeds (phytotoxicity) and brine shrimp (cytotoxicity) assays were carried out. Methanol extract of *D. villosa* fruit revealed highest antioxidant and phytotoxic potential due to the presence of total phenolic and flavonoid contents while chloroform extracts of *M. laciniata* leaves and *D. villosa* fruit displayed highest cytotoxic potential. Hence, *D. villosa* fruit could be utilized in pharmaceutical industries against different ailments.

Key words: Fabaceae, Phytochemicals, Antioxidant, Radish seeds, Brine shrimps.

Introduction

Medicinal plants are effectively used against asthma, bronchial diseases, cough, cold, malaria, chronic fever, dysentery, diabetes, diarrhea, skin diseases, arthritis, insects bite and in treatment of hepatic, gastric, cardiovascular, and immunological disorders for thousands of years (Najafi & Deokule, 2010; Ahmad *et al*., 2016; Zahoor *et al*., 2021). Numerous herbs, fruits and vegetables are cultivated and utilized as food, fibre, and medicinal drugs by the human beings (Kose *et al*., 2017; Khan *et al*., 2017; Najeebullah *et al*., 2021). Medicinal species consist of natural compounds and are preferred over synthetic drugs as they are cheap, easily affordable and have less side-effects. According to an estimate, 25 % of all the drugs recommended for various ailments are derived from the plants (Cornwell *et al*., 2004; Khan *et al*., 2018, Shinwari *et al*., 2020). Moreover, they are also used as antioxidants to increase the shelf life of food in food industry (Granato *et al*., 2017; Nikmaram *et al*., 2018; Ayatollahi *et al*., 2019).

Pakistan possesses unique floral diversity comprising of about 6000 species of flowering plants (Shinwari *et al*., 2006). Family Fabaceae or Leguminosae, also known as bean or legume family, can be easily distinguished by fruits (legume/ pod). It is the third largest family of angiosperms after Asteraceae and Orchidaceae consisting of 751 genera and above 19,000 species (Christenhusz & Byng, 2016). The members of this family possess significant nutritional value due to the presence of proteins in their leaves and seeds (Graham & Vance, 2003). Agriculturally important plants of family Fabaceae includes *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Arachis hypogaea* (peanut), *Glycine max* (soybean), *Phaseolus mungo* and *Cajanus cajau*. Some species of family Fabaceae are also used in medicines to cure wide range of human diseases (Dzoyem *et al*., 2014).

Reactive oxygen species (ROS) mostly damages the cells and tissues, process known as oxidative stress. These radicals are inactivated by the defense mechanisms of antioxidants (Umamaheswari & Chatterjee, 2008). Different methods are used to determine the antioxidant capacity of plants (Fatima *et al*., 2019; Panthi *et al*., 2020). Flavonoids and phenols present in plants primarily act as free radical scavengers (Cai *et al*., 2004). Phytotoxic potential of plant extracts is most commonly analyzed by observing the growth stimulatory or inhibitory effects on the radish seeds while cytotoxic potential of these species is usually assessed by using brine shrimps as a model organism as it exhibits significant correlation with cytotoxic activity in human tumor cells (Turker & Camper, 2002; Gilani *et al*., 2010). Hence, present studies were designed to determine the antioxidant and toxicity potential of different parts of three species of family Fabaceae.

Materials and Methods

Plant collection and preparation of extracts: *Dumasia villosa* (stem, leaves and fruit), *Trifolium repens* (stem, leaves and flowers) and *Medicago laciniata* (stem and leaves) were collected from Rawalpindi and District Bagh (Azad Kashmir) during March to April 2017. All plant parts were thoroughly rinsed with distilled water and then shade-dried at room temperature for 8-10 days. Plant parts were powdered and then extracted with three different solvents viz., ethanol, methanol and chloroform (30 g/ 300 ml each). After 48 hours, extracts were filtered, and the remaining plant residue was again soaked in 300 ml of respective solvents. This process was repeated three times and then the filtrates were concentrated in rotary evaporator. The selected plant extracts and their abbreviations are given in (Table 1).

Table 1. List of names of different parts of selected plants and their abbreviations.

Plant name	Parts used	Solvent	Abbreviation
Dumasia villosa	Stem	Ethanol	DVStE
	Leaves	Ethanol	DVLvE
	Fruit	Ethanol	DVFrE
	Stem	Methanol	DVStM
	Leaves	Methanol	DVLvM
	Fruit	Methanol	DVFrM
	Stem	Chloroform	DVStC
	Leaves	Chloroform	DVLvC
	Fruit	Chloroform	DVFrC
Trifolium repens	Leaves	Ethanol	TRLvE
	Stem	Ethanol	TRStE
	Flower	Ethanol	TRFlE
	Leaves	Methanol	TRLvM
	Stem	Methanol	TRStM
	Flower	Methanol	TRFlM
	Leaves	Chloroform	TRLvC
	Stem	Chloroform	TRStC
	Flower	Chloroform	TRFlC
Medicago laciniata	Leaves	Ethanol	MLLvE
	Stem	Ethanol	MLStE
	Leaves	Methanol	MLLvM
	Stem	Methanol	MLStM
	Leaves	Chloroform	MLLvC
	Stem	Chloroform	MLStC

Qualitative phytochemical analysis: Plants extracts were analyzed using standard protocols to determine various phytochemical compounds i.e. flavonoids, phenols, saponins, tannins, steroids, terpenoids, glycosides, anthocyanins and coumarins (Sofowora, 1993; Parekh & Chanda, 2007).

Determination of total phenolic and flavonoid contents: Phenolic content in each plant extract was analyzed by Folin-Ciocalteu reagent (Clarke *et al.*, 2013). Gallic acid (400 µg/ml) was used as positive control and phenolic content was expressed as equivalent of gallic acid. Absorbance was measured at 630 nm. Flavonoid content in each plant sample was analyzed by aluminum colorimetric method (Chang *et al.*, 2002). Quercetin was used as a standard in this process and the absorbance was taken at 415 nm. Total flavonoid contents were expressed as mg QE/g.

Antioxidant assays

DPPH free radical scavenging assay: Stock solution (2 mg/ 1 ml DMSO) of each sample was prepared and ascorbic acid was used as positive control. About 180 µl of DPPH solution was added in 20 µl of each sample followed by the incubation at room temperature for 40 minutes (Clarke *et al*., 2013). Absorbance was recorded at 517 nm and IC_{50} values were determined using graphpad prism.

Total reducing power assay: About 200 µl of 0.2 M of phosphate buffer and 250 µl of potassium ferricyanide solution were added to 100 µl of each plant sample. Reaction mixture was incubated (20 minutes) at 50ºC, then acidified with trichloroacetic acid (200 µl) and centrifuged for 10 minutes at 3000 rpm. Then 150 µl of supernatant was mixed with 50 µl of 0.1% ferric chloride solution and then absorbance was taken at 630 nm. Ascorbic acid was used as a control (Aliyu *et al*., 2009).

Total antioxidant capacity (TAC): TAC of each plant sample was determined using the procedure described by Farokhzad *et al.*, (2006). 100 µl of stock solution of each plant extract (2 mg/ml extract in DMSO) was stirred with 900 µl of reagent solution (0.6 M sulfuric acid, 4 mM phosphomolybdate and 28 mM sodium phosphate). The reactions mixtures were then incubated (90 minutes) at 95°C and then absorbance was measured.

Toxicological studies

Radish seed bioassay: Radish seed bioassay was performed to check the allelopathic potential of medicinal plant samples as described by Turker & Camper (2002) with few modifications. Water was used as positive control. Twenty radish seeds sterilized with $HgCl_2$ (0.1%) solution were placed in all petri dishes along with 10,000 µg/ml of each plant extract and then incubated at 25°C. After five (5) days, the number of seeds germinated as well as root length of radish seeds was measured carefully. The obtained data was analyzed by ANOVA.

Brine shrimp lethality assay: Different plant concentrations (50, 100 and 150 µg/ml) were poured in vials and the volume was made up to 5 ml with the help of saline water. Ten brine shrimps were added in each vial and then incubated for 24 hours at 32°C. After 24 hours, number of alive shrimps were counted and then percentage mortality and LC_{50} values were calculated (Sirajuddin *et al.*, 2012).

Results

Phytochemical's analysis: Qualitative analysis revealed the presence of different secondary metabolites in varying concentrations in all plant extracts. Most of the compounds were strongly present in the fruits and flowers extracts compared to the leaves and stem extracts. Among different extracts, compounds were detected in decreasing order of methanol > ethanol > chloroform. However, among plants, *D. villosa* revealed the presence of most of the secondary compounds compared to the *T. repens* and *M. laciniata* (Table 2). Total phenolic contents ranged from 16.94 ± 0.99 mg GAE/g to 91.58 ± 2.74 mg GAE/g while total flavonoids contents ranged from 4.06 ± 1.76 mg QE/g to 32.67 ± 1.89 mg QE/g in selected species. Highest phenolic and flavonoid contents were observed in the methanol (91.58 ± 2.74 mg QE/g and 32.67 ± 1.89 mg GAE/g) and ethanol (83.37 ± 1.01 mg QE/g and 30.45 ± 1.61 mg GAE/g) extracts of *D. villosa* fruit (Table 3).

Antioxidant assays: Highest DPPH scavenging activity was detected in ethanol (IC_{50} = 51.16 ± 3.12 µg/ml), methanol (IC_{50} = 32.88 ± 2.87 µg/ml) and chloroform (IC_{50} = 140.4 ± 2.45 µg/ml) fruit extracts of *D. villosa.* However, lowest activity was observed in the stem extract of *M. laciniata* in ethanol (IC_{50} = 373.3 ± 4.05 µg/ml), methanol (IC_{50} = 295.0 ± 3.75 µg/ml) as well as chloroform (IC_{50} = 759.5 ± 3.27 µg/ml) extracts. Ascorbic acid revealed IC_{50} value of 16.91 ± 2.57 µg/ml while among plants, *D. villosa* extracts showed remarkable scavenging activity (Table 3).

Table 2. Qualitative phytochemical analysis of selected plant extracts.

Plant extracts	Flavonoids	Phenolics	Tannins	Saponins	Terpenoids	Glycosides	Steroids	Anthocyanins	Coumarins
DVStE	+	+	+	-	-	+++	++	++	-
DVLvE	+	++	++	+	-	+	+++	+	-
DVFrE	++	++	-	++	+	++	+++	-	+
DVStM	+	+	+	-	+	++	++	+	+
DVLvM	+	++	++	++	+	+++	++	+	-
DVFrM	++	+++	+	++	+	+++	+++	+	+
DVStC	+	+	-	-	-	+	+	-	-
DVLvC	+	+	+++	-	+	-	-	++	+
DVFrC	++	+	-	-	+	+	-	-	+
TRLvE	++	++	++	+	+	-	+	+	-
TRStE	+	+	++	++	++	++	+	-	-
TRFlE	+	+	++	+	-	++	++	-	++
TRLvM	++	+	+	+	-	+	+	++	-
TRStM	+++	+	+	+	+	++	++	+	++
TRFlM	++	+++	+++	++	+++	+	++	+	-
TRLvC	+	+	-	-	++	-	-	++	+
TRStC	++	+	-	-	-	-	-	+	+
TRFlC	++	+	-	+	++	+	++	+	+
MLLvE	+	+	++	-	+	+	+	+	+
MLStE	++	+	+	-	+	-	+	-	-
MLLvM	+	+	+++	++	+	++	++	+	-
MLStM	++	++	+	++	+	+++	++	+	++
MLLvC	+	+	+	+	-	-	-	+	+
MLStC	++	+	-	-	++	-	-	+	+

+++ Strongly present; ++ Moderately present; + Weakly present; - Absent

Table 3. Total phenolic and flavonoid contents and antioxidant activity of selected medicinal plant extracts.

Plant extracts	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)	Antioxidant assays		
			DPPH assay (IC_{50} value)	Total antioxidant capacity (mg/g)	Total reducing power assay (mg/g)
DVStE	49.22 ± 2.20^{f}	18.88 ± 1.36^{gh}	97.34 ± 0.75^{o}	24.44 ± 2.09^{def}	16.46 ± 0.96^{def}
DVLvE	67.19 ± 1.79^{d}	28.34 ± 0.97^{bc}	86.05 ± 1.53^{p}	26.79 ± 1.76^{cde}	20.12 ± 1.25^{bc}
DVFrE	83.37 ± 1.01^{b}	30.45 ± 1.61^{ab}	51.16 ± 2.71^{s}	42.15 ± 3.23^{a}	36.20 ± 1.49^{a}
DVStM	51.13 ± 2.96^{f}	20.85 ± 1.66^{f}	75.21 ± 0.39^{q}	25.62 ± 0.87^{cde}	17.95 ± 1.91^{cd}
DVLvM	72.75 ± 3.99^{c}	29.87 ± 0.21^{b}	61.49 ± 2.04^{r}	28.68 ± 2.67^{bcd}	22.49 ± 1.71^{b}
DVFrM	91.58 ± 2.74^{a}	32.67 ± 1.89^{a}	32.88 ± 2.87^{t}	44.08 ± 0.98^{a}	37.41 ± 1.58^{a}
DVStC	$25.91 \pm 1.02l^{m}$	4.060 ± 1.76^{p}	197.4 ± 1.17^{i}	11.34 ± 1.01^{jk}	8.666 ± 1.45^{ijk}
DVLvC	48.83 ± 2.47^{f}	19.44 ± 1.47^{fg}	175.3 ± 2.39^{j}	13.22 ± 0.78^{ij}	13.70 ± 1.76^{fgh}
DVFrC	60.99 ± 1.18^{e}	26.90 ± 1.71^{cd}	140.4 ± 2.45^{m}	23.25 ± 1.10^{efg}	22.08 ± 2.11^{b}
TRLvE	31.80 ± 1.71^{jk}	14.98 ± 1.08^{i}	139.8 ± 4.07^{m}	23.43 ± 1.76^{efg}	14.59 ± 4.23^{defg}
TRStE	29.81 ± 1.71^{kl}	$16.34 \pm 1.23f^{g}$	166.2 ± 3.11^{k}	19.77 ± 1.54^{gh}	10.46 ± 1.64^{hij}
TRFlE	39.91 ± 3.80^{gh}	23.36 ± 1.40^{e}	114.3 ± 2.00^{n}	29.13 ± 1.15^{bc}	20.40 ± 2.05^{bc}
TRLvM	35.96 ± 2.23^{hi}	12.38 ± 2.62^{jk}	117.6 ± 2.65^{n}	25.33 ± 0.98^{cde}	15.94 ± 1.23^{def}
TRStM	31.38 ± 2.59^{k}	17.99 ± 1.24^{hi}	152.8 ± 3.88^{l}	20.44 ± 1.47^{fgh}	11.69 ± 2.43^{ghi}
TRFlM	43.32 ± 2.75^{g}	25.27 ± 0.78^{de}	88.52 ± 2.33^{p}	31.89 ± 1.34^{b}	21.67 ± 2.91^{b}
TRLvC	19.69 ± 2.80^{no}	6.560 ± 2.52^{no}	388.4 ± 2.25^{d}	13.26 ± 1.51^{ij}	6.477 ± 1.30^{k}
TRStC	16.94 ± 0.99^{o}	10.04 ± 0.47^{klm}	442.0 ± 3.04^{c}	14.32 ± 2.98^{ij}	14.28 ± 3.30^{efg}
TRFlC	22.04 ± 2.13^{mn}	14.11 ± 0.62^{ij}	284.8 ± 3.98^{g}	17.62 ± 0.56^{hi}	11.86 ± 1.08^{ghi}
MLLvE	30.72 ± 1.17^{k}	8.220 ± 2.09^{lm}	345.2 ± 4.48^{f}	11.20 ± 0.76^{jk}	13.74 ± 2.91^{efgh}
MLStE	26.13 ± 3.24^{l}	10.95 ± 0.41^{kl}	373.3 ± 4.05^{e}	10.84 ± 0.98^{jk}	9.631 ± 1.23^{ijk}
MLLvM	35.45 ± 1.60^{ij}	8.610 ± 1.60^{lmn}	251.0 ± 3.65^{p}	14.55 ± 1.23^{ij}	17.10 ± 3.54^{cde}
MLStM	28.29 ± 3.57^{kl}	11.45 ± 1.09^{k}	295.0 ± 3.75^{h}	13.67 ± 1.78^{ij}	13.88 ± 3.76^{efg}
MLLvC	20.77 ± 3.53^{no}	4.430 ± 1.39^{op}	682.3 ± 2.36^{b}	10.68 ± 0.94^{jk}	7.901 ± 1.56^{jk}
MLStC	17.12 ± 1.65^{o}	7.970 ± 0.97^{mn}	759.5 ± 3.27^{a}	7.703 ± 1.20^{k}	6.691 ± 1.13^{k}

Results are interpreted as mean ±SD (n=3), column superscripts (a-p) depict means with significant differences (p<0.05), as determined by LSD, all pair-wise comparison test (ANOVA)

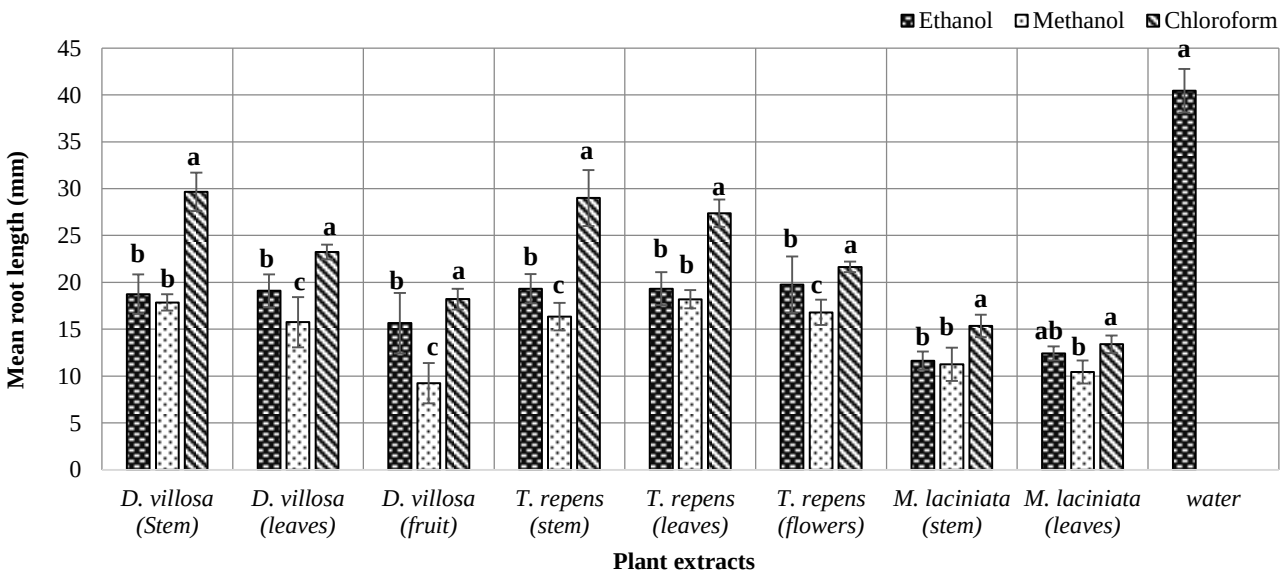

Fig. 1. Mean root length of radish seeds after 5 days of incubation showing phytotoxicity potential of selected plant extracts. Vertical bars indicate means ± standard error of three replicates and different letters (a–c) are not significantly different at $p<0.05$.

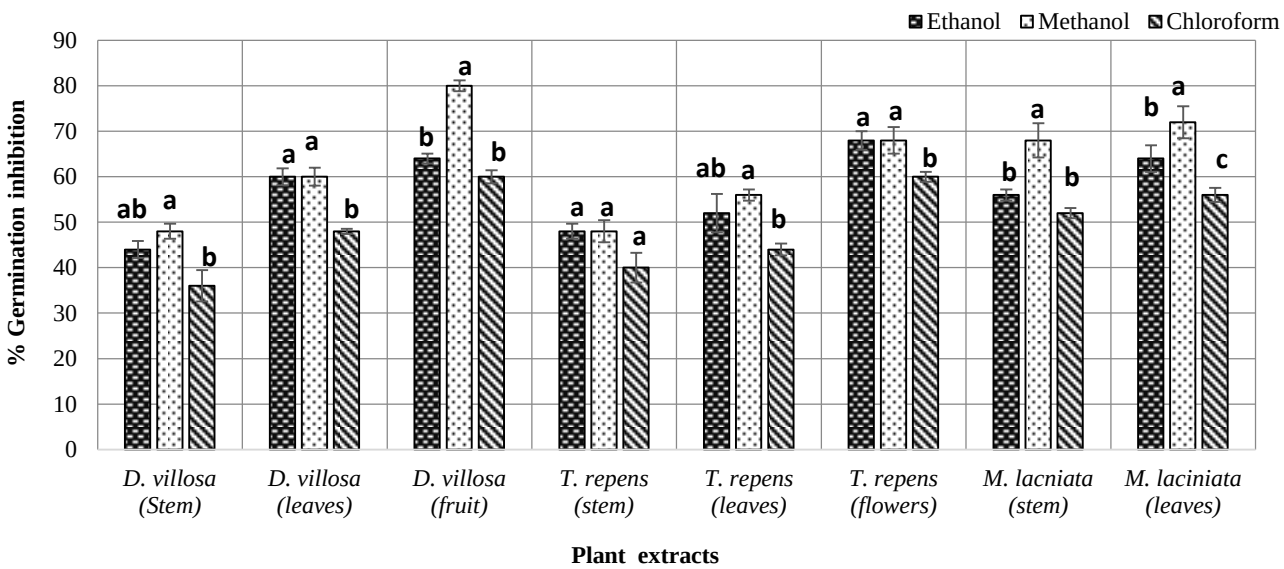

Fig. 2. Percentage germination inhibition of radish seeds after 5 days of incubation showing phytotoxicity potential of selected plant extracts. Vertical bars indicate means ± standard error of three replicates and different letters (a–c) are not significantly different at $p<0.05$.

Total antioxidant capacity was determined by phosphomolybdate assay which revealed that methanol extract has significant ability to reduce Mo (VI) to Mo (V) than the ethanol and chloroform extracts. Total antioxidant capacity ranged from 7.703 ± 1.20 mg/g to 31.89 ± 1.34 mg/g in selected species. Fruits and flower extracts of the plants showed highest antioxidant capacity compared to the leaves and stem of selected species. In reducing power assay, methanol and ethanol fruit extracts of *D. villosa* (37.41 ± 1.58 mg/g and 36.20 ± 1.49 mg/g) showed highest reducing power ability while chloroform extracts of *T. repens* leaves (6.477 ± 1.30 mg/g) and *M. laciniata* stem (6.691 ± 1.13 mg/g) showed lowest reducing power compared to the other plants. Among plants, *D. villosa* extracts showed remarkable reducing power ability compared to *T. repens* and *M. laciniata* extracts (Table 3).

Toxicological studies: In case of phytotoxicity, ethanol, methanol and chloroform extracts revealed significant phytotoxic potential. Mean root length and percentage germination inhibition of the plants were measured after five days of germination. Water was used as a positive control which revealed maximum root length having no germination inhibition as expected (Fig. 1). Highest germination inhibition was detected in the methanol extracts of *D. villosa* fruits (80%) and *M. laciniata* leaves (72%) followed by the methanol and ethanol extracts of *T. repens* flowers (68%) while lowest germination inhibition was observed in the chloroform extracts of *D. villosa* stem (36%) and *T. repens* stem (40%). Overall, *D. villosa* and *M. laciniata* were as more phytotoxic than *T. repens*. Among different plant extracts, methanol extracts exhibited more phytotoxic potential compared to the ethanol and chloroform extracts (Fig. 2).

Cytotoxic potential of the selected plant extracts was notably increased with increase in concentration. Vincristine sulphate (positive control) showed LC_{50} value of 2.810 ppm. Among ethanol and methanol extracts, *M. laciniata* leaves (LC_{50} 33.594 ppm and 30.088 ppm) and *M. laciniata* stem (LC_{50} 34.675 and 35.087 ppm) showed highest cytotoxic potential. However, among chloroform extracts *M. laciniata* leaves (LC_{50} 17.896 ppm) and *D. villosa* fruit (LC_{50} 27.576 ppm) revealed highest cytotoxic potential compared to other extracts (Table 4).

Table 4. Cytotoxicity potential of selected plant extracts.

Plant extracts	LC_{50} (ppm)	95 % Confidence interval
DVStE	56.697	29.398 – 109.34
DVLvE	62.465	17.987 – 106.58
DVFrE	50.953	31.139 – 83.377
DVStM	50.747	29.225 – 88.119
DVLvM	59.198	18.890 – 185.51
DVFrM	47.945	25.799 – 89.100
DVStC	44.788	26.741 – 75.016
DVLvC	46.270	22.842 – 93.726
DVFrC	27.576	11.606 – 65.517
TRLvE	42.041	16.360 – 108.03
TRStE	38.621	14.670 – 101.67
TRFlE	41.452	20.923 – 82.125
TRLvM	37.662	17.341 – 81.797
TRStM	36.738	19.229 – 70.192
TRFlM	40.621	23.091 – 71.457
TRLvC	35.994	19.247 – 67.311
TRStC	38.761	19.958 – 75.277
TRFlC	40.621	23.091 – 71.457
MLLvE	33.594	18.018 – 62.635
MLStE	34.675	14.064 – 85.490
MLLvM	30.088	9.3310 – 97.021
MLStM	35.087	10.918 – 112.75
MLLvC	17.869	2.7570 – 115.81
MLStC	29.948	11.939 – 75.121
VS (positive control)	2.8100	1.9700 – 4.0100

LC_{50} = Lethal concentration fifty; VS: Vincristine sulphate

Discussion

In Pakistan, most of the people rely on medicinal plants for treatment of different diseases (Shinwari *et al.*, 2009; Ahmad *et al*., 2020). Natural flora exhibits vital compounds that play crucial role in different biological activities (Rodrigues *et al*., 2016; Espinosa-Leal *et al*., 2018). These species are traditionally used by local people to treat several diseases including stomach ailments, fever and hypertension (Saganuwan, 2010). Nowadays, medicinal plants are used for drug development. In present studies, relative efficacy and toxicity potential of various parts of three species i.e., *T. repens* (stem, leaves and flowers), *M. laciniata* (stem and leaves) and *D. villosa* (stem, leaves and fruit) were examined. In addition, some phytochemical tests were carried out to determine the presence of different compounds in plant extracts.

Qualitative tests depicted the presence of flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenoids, coumarins and anthocyanins in most of the plant extracts. Earlier findings of phytochemical tests carried out by Sudha *et al.*, (2011), Fatima *et al.*, (2018) and Palshetkar *et al.*, (2020) also correlate with the present studies revealing the presence of different secondary metabolites in medicinal plants. Saponins and tannins act as a self-defense against fungi, bacteria and other herbivore predators (Makkar *et al.*, 1995). Terpenoids play key role in inhibiting the formation of free radicals (Park & Pezzutto, 2002). Similarly, coumarins exhibit antimicrobial and antioxidant activities (Carpinella *et al*., 2005). However, phenolic and flavonoid compounds possess significant antioxidant and cytotoxic potential (Ragaee *et al*., 2006). Present studies correlate with the previous studies of Rodrigues *et al.*, (2013) in which 21.96 mg/g to 36.41 mg/g of phenolic contents have been reported in *Medicago* genus. However, so far no studies are conducted on the quantitative phytochemical estimation of *D. villosa*.

Free radicals or reactive oxygen species (ROS) results in oxidative stress at high concentration (Zheng *et al*., 2001). Antioxidants possess beneficial effects against free radicals by scavenging them (Chu *et al*., 2002, Hamza *et al*., 2020). In present studies, highest DPPH scavenging activity was observed in the ethanol extract of *D. villosa* fruit (32.88 ± 2.87 µg/ml) and lowest activity was detected in the chloroform extract of *M. laciniata* stem (759.5 ± 3.27 µg/ml). Similarly, highest reducing power and TAC was observed in the methanol extract of *D. villosa* fruit. Studies on the antioxidant potential of these species have not been carried out before. Previously, Sahreen *et al.*, (2011) confirmed that the phenols and flavonoids are the major contributors for the antioxidant activity.

The toxic effect of a plant on germination, growth and development of other plants due to release of toxic chemicals is called allelopathy or phytotoxicity. Natural herbicides can be made by determining the phytotoxicity of plant species using radish seed bioassay which is the simplest technique (Khan *et al*., 2011). Highest seed germination inhibition was detected in the methanol fruit extract of *D. villosa* (80%) and leaves extract of *M. laciniata* (72%). Moreover, brine shrimp lethality assay is most convenient assay used to evaluate the toxicity potential of medicinal plants (Saleh-e-In *et al*., 2016). Highest cytotoxic activity was observed in the chloroform extracts of *M. laciniata* leaves and *D. villosa* fruit. Previously, Avato and Tava, (2006) reported that the cytotoxic potential of genus *Medicago* is due to the presence of saponins. Moreover, present studies are also supported by the earlier findings of Sigaroodi *et al.*, (2012) according to which LC_{50} value of *T. repens* extracts ranged from 30 µg/ml to 50 µg/ml. However, no previous literature is available for the cytotoxic study of *D. villosa* and *M. laciniata* species.

In conclusion, extracts of *D. villosa* showed highest antioxidant, cytotoxic and phytotoxic potential as well as total phenolic and flavonoid contents and hence, can be used in the pharmaceutical industries. Further, *in vivo* studies and compound characterization needs to be carried out in future to confirm the relative efficacy and toxicity potential of these plant extracts.

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