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ORIGINAL ARTICLE

Evaluation of *In Vitro* Cytotoxic and Antioxidant Activity of *Datura metel* Linn. and *Cynodon dactylon* Linn. Extracts

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ABSTRACT

Aim: To evaluate *in vitro* cytotoxicity and antioxidant activity of *Datura metel* L. and *Cynodon dactylon* L. extracts. **Materials and Methods:** The extraction of plants parts (*datura* seed and fruit pulp) and areal parts of *durva* was carried out using soxhlet and cold extraction method using solvents namely methanol and distilled water. The total phenolic content (TPC) and total flavonoid content (TFC) was determined by established methods. The *in vitro* cytotoxicity assay was performed *in vero* cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. *In vitro* antioxidant activity of the extract was performed by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method. **Results:** We found that the highest amount of TPC and TFC in methanolic extracts of seed (268.6 µg of gallic acid equivalence/mg of dry plant material) and fruit pulp (8.84 µg of quercetin equivalence/mg dry plant material) of *D. metel,* respectively prepared by Soxhlet method. The methanolic extract of *C. dactylon* prepared using soxhlation has shown potent free radical scavenging activity with 50% inhibitory concentration (IC_{50}) value of 100 µg/ml. The IC_{50} of a methanolic cold extract of *datura* fruit was found to be 3 mg/ml against vero cell line. **Conclusion:** We observed that plant parts of *C. dactylon* and *D. metel* have a high antioxidant activity. Further research is needed to explore the therapeutic potential of these plant extracts.

Key words: 2, 2-diphenyl-1-picrylhydrazyl radical, Antioxidant, Cytotoxicity, Total phenolic content, Total flavonoid content

SUMMARY

- In the present study we observed a positive correlation was between the phenolic and flavanoid content of the *Datura metel* and *cynodon doctylon* (durva) extracts with the free radical scavenging activities. Both were found to have a high antioxidant activity.

Abbreviations used: BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, CC50: 50% cell cytotoxic concentration, CNS: Central nervous system, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, IC50: 50% inhibitory concentration, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), TFC: Total flavonoid content, TPC: Total phenolic content.

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INTRODUCTION

Biological combustion involved in various processes produces free radicals which lead to oxidative damage to the body. Many human diseases are caused by oxidative stress that results from an imbalance between the formation and neutralization of pro-oxidants.[1] Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, and cardiovascular diseases.[2,3] Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids, and lipid peroxidation,[4] thus, inactivating antioxidant defense. Plant and its products are rich sources of phytochemicals and have been found to possess a variety of biological activities including antioxidant potential. Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, as well as a food additive.[5]

Cynodon dactylon traditionally known as *durva*, is a medicinal plant used as a folk remedy for anasarca, alaculus, cancer, convulsions, epilepsy, hypertension, bronchitis, cough, and diarrhea. According to Ayurvedic system of medicine, it acts as an appetizer, antihelminthic, antipyretic, alexiteric agent and has a wound healing activity. In homeopathic system of medicine, it is used to treat all types of bleeding and skin diseases. It, also, has a central nervous system depressant activity.[6,7] Another less studied Indian medicinal plant is *datura,* botanical name: *Datura metel*, although known for its toxicity, also contains medicinal properties. *Datura* intoxication typically produces delirium (as contrasted to hallucination), hyperthermia, tachycardia, bizarre behavior, and severe mydriasis with resultant painful photophobia that can last several days. However, *datura* also has medicinal properties. *Datura* has long been used as an extremely effective treatment for asthma symptoms. The active anti-asthmatic agent is atropine, which causes paralysis of the pulmonary branches of the lungs, eliminating the spasms that cause the asthma attacks. The leaves are generally smoked either in a cigarette or a pipe. This practice of smoking *datura* to relieve asthma has its origins in traditional Ayurvedic medicine in India.[8,9]

Several synthetic antioxidant agents including butylated hydroxyanisole and butylated hydroxytoluene are commercially available, however, are reported to be toxic to animals including human beings.[10] Furthermore, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, flavonols, and proanthocyanidins.[11]

D. metel has long been known for its sedative action and known to cure hydrophobia in Ayurveda and *C. dactylon* is known to possess

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anti-inflammatory and immunomodulatory activities. In the present work, the polyphenolic content of these two medicinal plants were quantified, and their antioxidant and cytotoxic potential was evaluated.

MATERIALS AND METHODS

Reagents and chemicals

Gallic acid and quercetin were purchased from Sigma Chemical Co. (USA). HPLC grade ethanol, methanol, Folin Ciocalteu's Phenol reagent, sodium carbonate, and aluminum chloride were purchased from Merck (Germany). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (Himedia, India).

Collection of plant material

Datura fruit and *durva* plant was collected from the phool market, Dadar, Mumbai. Plant specimen was authenticated and deposited in Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai.

Preparation of plant extracts

The extraction of plants parts (*datura* seed and fruit pulp) and areal parts of *durva* was carried out using soxhlation and cold extraction method. The solvents used for extraction method were methanol (MeOH) and distilled water (D/W). In brief, plant material was thoroughly washed with water, shade dried on a clean filter paper and grinded to a fine powder. A total of 25 g of powder of fruit and seed was wrapped in a thimble made of Whatman filter paper No. 1 and placed into the soxhlet apparatus containing 300 ml solvent. The temperature of the heating mantle was adjusted to the boiling point of the solvent. The extraction process is stopped once the solvent in the side arm flask becomes colorless. For cold extraction, a total of 25 g of powder of *datura* fruit and seed is soaked in 300 ml solvent (methanol and water) for overnight. The extracts were then filtered and concentrated to dryness using a rotary evaporator to remove the solvent. The dried plant extracts obtained was weighed and stored in the refrigerator until *in vitro* assays.[12]

Determination of total phenolic content

The total phenolic content (TPC) of Soxhlet and cold extracts of *datura* (fruit and seed) and *durva* were determined by using the Folin–Ciocalteu method.[13,14] Standard solutions of gallic acid of concentration 1.56–100 μg/ml were prepared in water. 50 μl of extracts or standard solution were added to 50 μl of distilled water. 50 μl of 10% Follin–Cicocalteu's phenol reagent and 50 μl of 1 M sodium carbonate solution were added to the mixture in a 96-well plate. Distilled water was used as blank. The reaction mixture was incubated for 60 min at room temperature and protected from light. The absorbance was measured at 750 nm with a Microplate Reader (Biotek, USA.). TPCs were expressed as μg gallic acid equivalents (GAE)/mg of dry plant material.

Determination of total flavonoid content

Total flavonoid content (TFC) and cold extracts of *datura* (fruit and seed) and *durva* were determined by the aluminum chloride colorimetric assay.[14,15] Standard solutions of quercetin of concentration 1.56–100 μg/ml were prepared in 80% ethanol. 50 μl of extracts (1 mg/ml) or standard solution was added to 10 μl of 10% the aluminum chloride solution and followed by 150 μl of 95% ethanol. 10 μl of 1 M sodium acetate was added to the mixture in a 96-well plate. 80% ethanol was used as the reagent blank. All reagents were mixed and incubated for 40 min at room temperature protected from light. The absorbance was measured at 415 nm with a Microplate Reader (Biotek, USA.). TFCs were expressed as μg quercetin equivalents (QE)/mg dry of plant material.

In vitro cytotoxicity assay

The cytotoxic activity of *datura* fruit and seed extracts was carried out using MTT assay. Vero cells (NCCS, Pune) were trypsinized and cultured onto a 96-well plate at the density of 0.2×10^6 cells/ml. Once the monolayer is formed after 24 h, different concentrations (0.1–4 mg/ml) of *datura* fruit and seed extracts were serially diluted and added to each culture wells in triplicate and incubated at 37°C with 5% CO_2 for 24 h. After incubation, the medium with extracts was removed and 10% of 5 mg/ml MTT (100 μl) was added to each well and incubated for 4 h at 37°C. After incubation, MTT (100 μl) was removed and the crystal formed (formazan) was solubilized by adding DMSO to each well. The absorbance was read at 550 nm with a Microplate Reader (Biotek, USA). The cytotoxic effect (50% inhibitory concentration [IC_{50}]) was measured using following equation.[16]

% Cytotoxic effect = [(Absorbance of control − Absorbance of sample)/(Absorbance of control)] ×100.

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed by reported method.[17] 20 μl of extracts (1 mg/ml) in respective solvents was added to 180 μl of DPPH (2 mg/100 ml) reagent prepared in methanol in the 96-well plate. Absolute methanol was used as the reagent blank. The reaction mixture was incubated for 30 min at room temperature, protected from light. The absorbance was measured at 517 nm with a Microplate Reader (Biotek, USA). The percentages of the DPPH free radical scavenging activity were calculated as follows.[15,18]

% Scavenging effect = [(Absorbance of control − Absorbance of sample)/(Absorbance of control)] ×100.

Statistical analysis

Data are expressed as mean ± standard deviation from three separate experiments. The cytotoxicity assay and antioxidant assay was calculated using GraphPad Prism version 5.0 software (California corporation, USA).

RESULTS

Determination of total phenolic acid content

The amount of phenolic acid content was determined by Folin–Ciocalteu method and was found to be higher in methanolic extract of *D. metel* seed (268.6 μg GAE/mg of dried sample), extracted with soxhlation. In the *C. dactylon* (244.6 μg GAE/mg of dried sample), high amount of phenolic acid content was observed in cold methanolic extract [Figure 1].

Determination of total flavonoid content

The TFC was determined by using aluminum chloride method. The higher amount of flavonoid content was found in methanolic extract of *D. metel* fruit (8.84 μg QE/mg of dried sample), extracted by soxhlation method [Figure 2].

In vitro cytotoxicity assay

Different concentrations (0.1–10 mg/ml) of soxhlet and cold extracts of *datura* seed, fruit and *durva* were added onto vero cell line (0.2×10^6 cells/ml) in the 96-well plate and were analyzed by MTT based cytotoxicity assay. The IC_{50} of Soxhlet extracts of *datura* seed (water and methanol) was found to be 7.5 mg/ml and 5 mg/ml, respectively. Whereas, IC_{50} of cold extracts of *datura* seed (water and methanol) was found to be 3.5 mg/ml and 5.5 mg/ml, respectively. The IC_{50} of soxhlet extract of *datura* fruit (water and methanol) was found to be 5 mg/ml and 4 mg/ml, respectively whereas of cold extracts of *datura* fruit (water and

methanol) was found to be 7 mg/ml and 3mg/ml, respectively [Figure 3]. The *durva* (water and methanol) soxhlet extracts was not found to be cytotoxic whereas the IC_{50} of *durva* (water and methanol) cold extract was found to be 8.17 mg/ml and 9.20 mg/ml, respectively. The IC_{50} was calculated and the percent cytotoxicity was represented by using GraphPad Prism version 5.0 software [Figure 4].

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity

Datura fruit and seed (Soxhlet and cold) extracts of different concentration (50–800 μg/ml) were used for antioxidant assay by DPPH radical scavenging method. The ascorbic acid was used as a standard for DPPH scavenging assay and the absorbance was read at 517 nm [Figure 5]. The IC_{50} of *datura* fruit (water and methanol) soxhlet extracts were found to be 650 ± 22.21 μg/ml and 600 ± 23.71 μg/ml, respectively and of *datura* fruit (water and methanol) cold extracts were found to be 800 μg/ml [Figure 6], whereas the IC_{50} of *datura* seed (water and methanol) Soxhlet extracts were found to be 600 μg/ml, whereas of *datura* seed (water and methanol) cold extracts were found to be 700 ± 19.08 μg/ml and 600 ± 18.71 μg/ml, respectively. The IC_{50} was calculated, and the percent inhibition was represented by using GraphPad Prism version 5.0 software [Figure 7].

Durva (Soxhlet and cold) extracts of different concentration (50–800 μg/ml) was also used for antioxidant assay by DPPH radical scavenging method. The absorbance is read at 517 nm. The IC_{50} of *durva* (water and methanol) Soxhlet extracts was found to be 150 ± 18.42 μg/ml and 100 ± 18.18 μg/ml, respectively; whereas, of *durva* (water and methanol) cold extracts were found to be 200 ± 15.53 μg/ml. The IC_{50} was calculated and the percent inhibition was represented by using GraphPad Prism version 5.0 software [Figure 8]. We found higher free radical scavenging potential in methanolic extract of *C. dactylon*, extracted with soxhlation method.

DISCUSSION

Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure, and degenerative diseases as a result of deficient natural antioxidant defense mechanism. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research.

In vitro antioxidant activity of *D. metel* (seed and fruit) and *Cynodon doctylon* (*durva*) were estimated by DPPH radical scavenging method and the absorbance is read at 517 nm. The IC_{50} of *datura* fruit (water and methanol) soxhlet extracts were found to be 650 ± 22.21 μg/ml and 600 ± 23.71 μg/ml, respectively and of *datura* fruit (water and methanol) cold extracts were found to be 800 μg/ml. Whereas the of *datura* seed (water and methanol) Soxhlet extracts (IC_{50}) were found to be 600 μg/ml and of *datura* seed (water and methanol) cold extracts were found to be 700 ± 19.08 μg/ml and 600 ± 18.71 μg/ml, respectively. The IC_{50} of *durva* (water and methanol) Soxhlet extracts were found to be 150 ± 18.42 μg/ml and 100 ± 18.18 μg/ml, respectively; whereas, of *durva* (water and methanol) cold extracts were found to be 200 ± 15.53 μg/ml. The IC_{50} was calculated and the percent inhibition was represented by using GraphPad Prism version 5.0 software [Figure 8]. We found higher free radical scavenging potential in methanolic extract of *C. dactylon*, extracted with soxhlation method [Table 1].

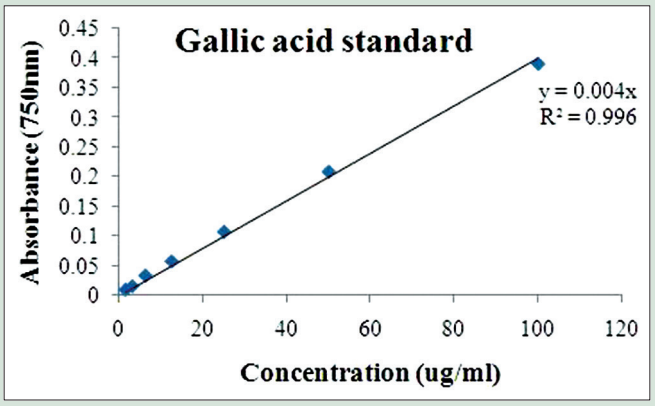

Figure 1: Total phenolic content of both Soxhlet and cold extracts of *datura* (fruit and seed) and *durva* were calculated by using a standard curve of gallic acid (y = 0.004×, R^2 = 0.996) and the absorbance is read at 750 nm

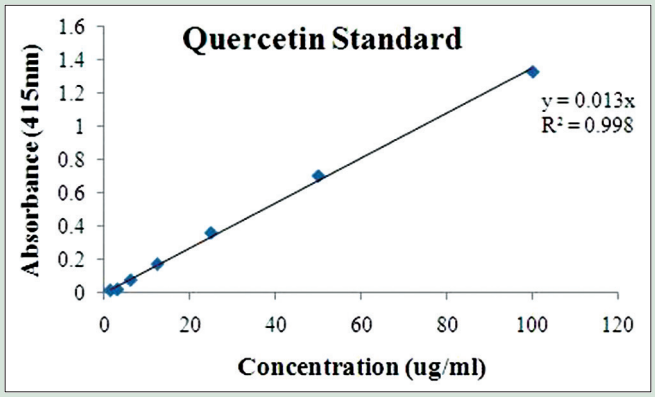

Figure 2: Total flavonoid content of both Soxhlet and cold extracts of *datura* (fruit and seed) and *durva* were calculated by using a standard curve of quercetin (y = 0.013×, R^2 = 0.998)

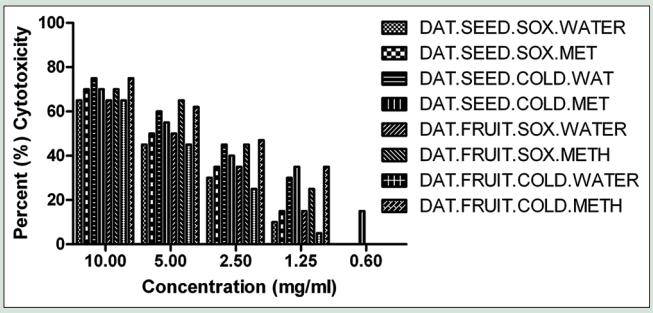

Figure 3: *In vitro* cytotoxicity assay of *datura* (fruit and seed) extracts of different concentration (0.6–10 mg/ml) was performed *in vero* cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

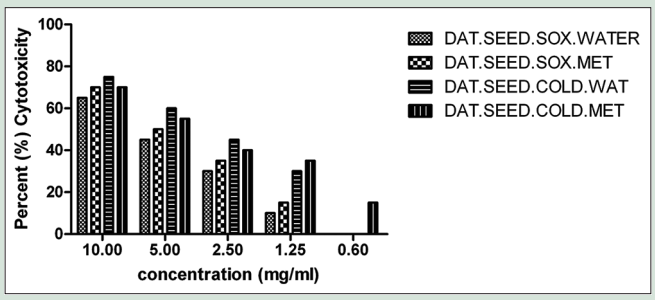

Figure 4: *In vitro* cytotoxicity assay of *durva* extracts of different concentration (0.6–10 mg/ml) was performed *in vero* cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

In the study it was observed that *durva* extracts showed a higher DPPH scavenging activity than *datura* extracts and overall *durva* extracts had a higher TPC and TFC than *datura* extracts and within the *datura* fruit and seed extracts it was found that *datura* seed extracts had a higher phenolic content (TPC) and flavonoid content (TFC) than *datura* fruit extracts. Thus in the present study, a correlation was obtained between antioxidant activity and phenolic content indicating that phenolic compounds contribute to the antioxidant activity.

It was reported that the antioxidant activity of the extracts might be due to the presence of phenolic and flavonoid compounds. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities.[19,20]

In a study by Sharma and Vig, the methanol and aqueous extracts of leaves of *Parkinsonia aculeata.* L. was evaluated by DPPH assay by and was found to inhibit 57.82% and 41.9%, respectively at the concentration of 1000 µg/ml.[13]

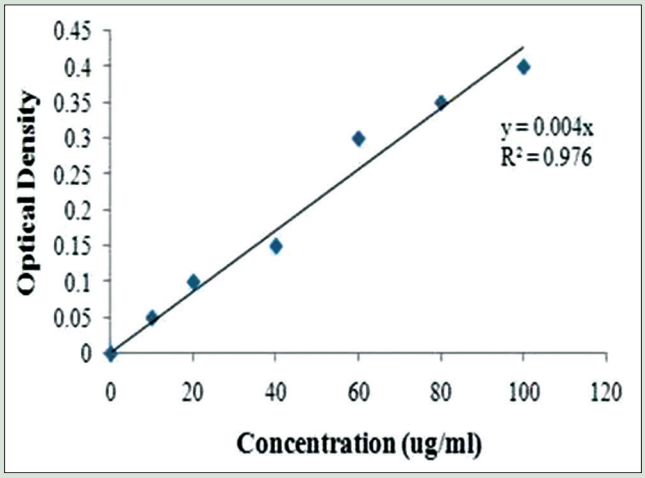

Figure 5: The ascorbic acid was used as a standard for 2, 2-diphenyl-1-picrylhydrazyl scavenging assay and read at absorbance of 517 nm (R^2 = 0.976)

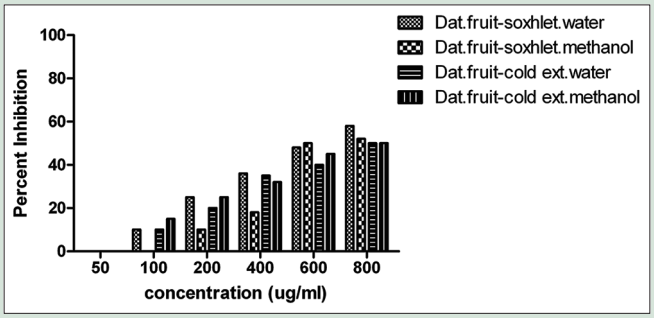

Figure 6: *In vitro* antioxidant activity of *datura* fruit (Soxhlet and cold) extracts was performed by 2, 2-diphenyl-1-picrylhydrazyl method

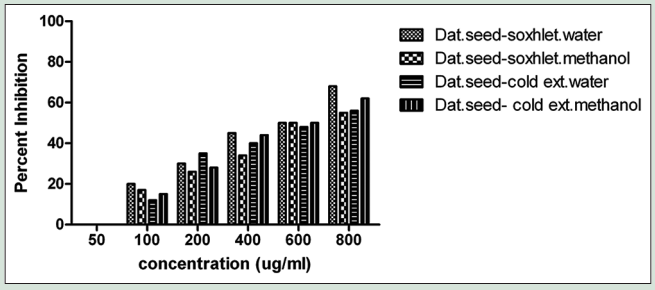

Figure 7: *In vitro* antioxidant activity of *datura* seed (Soxhlet and cold) extracts was performed by 2, 2-diphenyl-1-picrylhydrazyl method

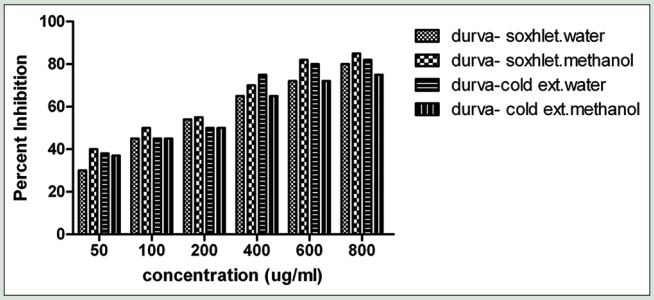

Figure 8: *In vitro* antioxidant activity of *durva* (Soxhlet and cold) extracts was performed by 2, 2-diphenyl-1-picrylhydrazyl method

Table 1: TPC, TFC, free radical activity, and cytotoxic activity of *Datura metel* L. and *Cynodon doctylon* extracts

Plant name	Plant parts	Extraction method	Solvents	TPCa (µg/mg)	TFCa (µg/mg)	DPPH activityb (µg/ml)	Cytotoxic activityc (mg/ml)
D. metel	Seed	Soxhlet	Methanol	268.6	5.4	600±20.62	5
		Cold	Distilled water	75.8	5.3	600±24.03	7.5
			Methanol	210.5	4.3	600±23.19	5.5
			Distilled water	185.1	ND[d]	700±21.58	3.5
	Fruit	Soxhlet	Methanol	249.3	8.84	600±23.72	4
			Distilled water	27.8	5.19	650±22.21	5
		Cold	Methanol	159.5	6.5	800±18.71	3
			Distilled water	106.2	3.38	800±19.08	7
C.dactylon	Areal	Soxhlet	Methanol	240.6	5	100±18.18	>10
			Distilled water	170.5	3	150±18.42	>10
	Areal	Cold	Methanol	244.6	4.5	200±15.53	9.20
			Distilled water	165.5	3.46	200±19.50	8.17

Values represent mean±SD of three different experiments. a-TPC and TFC are expressed as, µg of gallic acid equivalence/mg of dry plant material and µg of quercetin equivalence/mg dry plant material, respectively, b-IC50 of DPPH radical scavenging activity were expressed in µg/mland c-cytotoxicity were expressed in mg/ml. d-Not detected. SD: Standard deviation, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, IC50: 50% Inhibition concentration

In a study by Naima Saeed, antioxidant activity, total phenolic and TFCs of whole plant extracts *Torilis leptophylla* L. was evaluated and the IC_{50} value based on DPPH assay was found to be 41 μg/ml.[14]

In a study by Rahmat Ali Khan, the phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) showed DPPH radical scavenging activity at 4 μg/ml (IC_{50}).[17]

Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[21,22] The solvents and methods of extraction are an important factor to consider in drug discovery. In the present investigation, we found methanol and Soxhlet method as a suitable solvent and extraction method, respectively for the extraction of plant polyphenols.

CONCLUSION

The study has explored the potential *in vitro* antioxidant activity of *D. metel* and *Cynodon doctylon* extracts by means of DPPH radical scavenging activity. Further, *in vivo* toxicity and immunomodulatory studies are required to elucidate the biological property of these medicinal plants.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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