



PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF EXTRACTS OF SELECTED MEDICINAL PLANTS FROM NEPAL

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ABSTRACT

Aqueous and metholic extracts of twelve different species of antidiabetic plants belonging to different plant groups (liverworts, ferns and flowering plants) were screened for the presence of seven different groups of secondary metabolites. The same extracts were also subjected to quantification of total phenolic and flavonoid content. Antioxidant potential of those extracts was also compared using DPPH radical scavenging activity. The qualitative phytochemical assessment showed presence of more phytochemicals in methanol extracts than aqueous extracts. Furthermore, the extracts of none of the species possessed all the phytochemicals tested. The methanolic extracts also contained quantitatively more metabolites like total phenolics and total flavonoids than aqueous extracts. The highest value of total phenolic content (TPC) and total flavonoid content (TFC) were found respectively in methanol extracts of *Rhus succedanea* (162.71 ± 0.23 mg GAE/g) and *Asterella wallichiana* (70.5 ± 0.58 mg QE/g). The lowest value of TPC (17.86 ± 0.3 mg GAE/g) and TFC (2.63 ± 0.34 mg QE/g) was found in aqueous extracts of *Hedychium coronarium*. DPPH radical scavenging activity revealed high antioxidant potential in species with relatively high TPC values like *O. wallichii*, *Rhus chinensis*, *R. succedanea*, *Rubus niveus* and *Smilax ferox*.

Keywords: medicinal plants, phytochemical screening, TPC, TFC, RSA

INTRODUCTION

Nepal, a country in the central Himalaya is regarded as repository of medicinal plants. It among the countries with low government expenditure (ca. 5% of GDP) in health sector (MOHP, 2022). Furthermore, the health facilities are mostly confined to urban areas and costly. The people in the remote rural areas do not have access to these facilities. Since, one third of Nepalese population is poor (Sachs *et al.*, 2022) and cannot afford modern health facilities, traditional medicine is the only primary health care facility available to them.

Himalayan region as a whole is regarded as abode of gods and is rich in medicinal plant diversity. Nepal Himalaya alone houses 1950 species of medicinal plants (Ghimire, 2008). The occurrence of well over 100 different ethnic groups and the existence of different traditional medicinal practices has contributed to such high diversity of medicinal plants which amounts to ca. one third of total vascular plants reported from the country. Therefore, there is an urgent need for phytochemical screening and bioactivity testing of such diverse medicinal plants from Nepal.

Available literature shows that almost all of the plants studied for phytochemical screening and activity testing consist of vascular plant, especially the angiosperms while there are very few plants from the lower groups like bryophytes, hornworts and mosses included in such studies. This disparity caused by preferential selection of flowering plants over other groups for phytochemical analysis can be justified by the fact that angiosperms alone account for 90% of total plant species on earth, while bryophytes and pteridophytes *sensu lato* account for 3.34 and 2.3 percent of total land plant diversity, respectively (Corlett, 2016).

The present work aimed to compare the secondary metabolites present in extracts of medicinal plants belonging to bryophytes (*Asterella wallichiana* and *Marchantia polymorpha*), pteridophytes (*Adiantum incisum*, *Oleandra wallichii* and *Tectaria coadunata*) species) and flowering plants (*Hedychium coronarium*, *Rhus chinensis*, *R. succedanea*, *Rubus niveus*, *Smilax aspera*, *S. ferox* and *Sonchus arvensis*) and to quantify TPC and TFC in those extracts. Furthermore, the extracts were also assessed for their antioxidant potential.

Among the selected species, some like *Rhus chinensis*, *R. succedanea* and *Rubus niveus* are used for treatment of various ailments in Sowa Rigpa system of traditional medicine (Ghimire *et al.*, 2021). *Oleandra*

wallichii is used the treatment of headache, and dislocation and fracture of bones (Malla, 2018) *Tectaria coadunata* is used in the treatment of diarrhea and dysentery (Adhikari *et al.*, 2019). *Sonchus arvensis* is used in fever, indigestion, typhoid and dysuria (Manandhar, 2002). *Hedychium coronarium* is used in relieving the allergic effects of *Rhus succedanea* and in treatment of fever in children by people of Magar community in Rolpa (Budha-Magar *et al.*, 2020). These medicinal plants have also been demonstrated to show various activities both in vivo and vitro. The extracts of all of the species showed antidiabetic activities in vitro (Pant *et al.*, 2021). Similarly, extract of *Adiantum incisum* has also been reported to show antidiabetic activities in vivo as well (Bilal *et al.*, 2022). Silver nanoparticles from root extracts of *Smilax aspera* have shown antibacterial, anti-fungal and anti-inflammatory activities (Negi *et al.*, 2022). Flavonoids from bryophytes like *Marchantia polymorpha* have shown strong hepatoprotective activity (Zhang *et al.*, 2022) while the endophytes from the same plant have shown anticancer potential (Stelmasiewicz *et al.*, 2021).

MATERIALS AND METHODS

Sample collection and extract preparation:

Plant samples of 12 species of medicinal plants namely *Adiantum incisum* Forssk, *Asterella wallichiana* (Lehm.) Grolle, *Hedychium coronarium* J. Koenig, *Marchantia polymorpha* L., *Oleandra wallichii* (Hook.) C. Presl, *Rhus chinensis* Mill., *R. succedanea* L., *Rubus niveus* Thunb., *Smilax aspera* L., *S. ferox* Wall. ex Kunth, *Sonchus arvensis* L. and *Tectaria coadunata* (Wall. ex Hook. and Grev.) C. Chr. belonging to nine genera representing different evolutionary lines of vascular plants were selected for the study. These species were primarily selected based on their reported use as antidiabetic in various ethnomedicinal practices. Plant samples were collected from different parts of central Nepal. The collection involved collection of samples and voucher specimens. The samples were air dried and subjected to extract preparation. The collection sites, specimen photographs and details of extract preparation have already been published in Pant *et al.* (2021).

Qualitative phytochemical analysis

Crude extracts of selected plant material were used to screen the presence of bioactive compounds using various protocols described in various literatures (Table 1).

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Table 1

Summary of qualitative phytochemical tests

S.N.	Phytochemicals	Test	Results	Reference
1.	Saponin	0.5 mg extract + 2 mL Water, vigorous shaking	Formation of stable foam	Tiwari <i>et al.</i> (2011)
2.	Alkaloids	Plant Extract + 1-2 drops of Dragendorff's Reagent-	Reddish Brown precipitate	Singh and Kumar (2017)
3.	Flavonoids	1 mL Plant Extract + 2 mL 2% NaOH + few drops of dilute HCl	An intense yellow color which disappears on addition of HCl	Gul <i>et al.</i> (2017)
4.	Steroids	5 mL aq extract + 2 mL Chloroform + 2 mL conc. H ₂ SO ₄	Pink ring/red colour on lower layer	Singh and Kumar (2017)
5.	Diterpenes	Aq extract + (CH ₃ COO) ₂ Cu solution (few drops)	Green color formation	Tiwari <i>et al.</i> (2011)
6.	Phenol/Tannin	1 mL plant extract few drops of 2% FeCl ₃ solution (aq)	Dark Green or bluish black color	Yadav and Aggrawala (2011)
7.	Glycosides	Plant extract + 2 mL chloroform + 2 mL acetic acid (in ice bath) + few drops of conc. H ₂ SO ₄	Color change violet to blue to green	Yadav and Aggrawala (2011)

Quantitative Phytochemical Analysis:

TPC estimation:

TPC was determined following the protocol of Ainsworth and Gillespie (2007) TPC. One hundred microlitres of respective plant extract (2.5mg/mL) was separately mixed with 1 mL working solution (10X dilution in distilled water) of Folin phenol reagent (Fisher Scientific, India). Then 800 μ L of 1M solution (aq.) of washing soda (Na₂CO₃) (Merck India Ltd.) was added. After 15 minutes of incubation at room temperature, the mixture was subjected to spectrometric measurement of absorbance at 765nm. TPC in the plant extract were then quantified by using standard calibration curve ($y=0.006x+0.0656$; $R^2 = 0.9947$) of Gallic acid solution (25-250 μ g/mL) in methanol and water (50:50 v/v) and expressed in mg GAE/g.

TFC estimation

TFC estimation was done by following the protocol of Roy *et al.*, (2011). The plant extracts dissolved in respective solvents at a concentration of 10mg/mL. Then, 250 μ L of each sample solution was separately mixed

with methanol (750 μL), 10% aqueous solution of AlCl_3 (50 μL), 1M aqueous solution of CH_3COOK (50 μL) and distilled water (1400 μL). After vigorous shaking and subsequent incubation at room temperature for half an hour, absorbance was measured at 415nm using a spectrophotometer. TFC was quantified based on calibration curve of Quercetin ($y = 0.0068x + 0.025$; $R^2=0.9962$) solution in methanol (10-100 $\mu\text{g}/\text{mL}$) and expressed in terms of mg QE/g, i.e. milligram of quercetin per gram of dry weight of the extract.

Antioxidant activity

DPPH radical scavenging activity of extracts was used to determine the antioxidant activity following the method of Blois (1958). Solution of stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical (0.2 mM) was prepared in methanol. Different concentration of plant extract and Ascorbic acid (10-100 $\mu\text{g}/\text{mL}$) were prepared in respective solvents. Equal volumes of the sample and DPPH solution was taken in a 2 mL polypropylene tubes, shaken well and kept in dark for 30 minutes. After incubation period, absorbance was measured at 517 nm.

The antioxidant activity was determined in terms of percentage free radical scavenging activity (RSA) of the plant samples as:

$$\text{RSA} = \frac{\text{A517 control} - \text{A517 sample}}{\text{A517 control}} \times 100$$

RSA values of extracts of different species were compared by plotting graph of the percentage scavenging activity against the concentration for both extracts.

Data analysis

All data were taken thrice and mean values were reported. All the data analysis was done using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The extracts of most of the species showed higher number of phytochemicals in methanolic extracts compared to aqueous extracts. However, none of the extracts of none of the species showed the presence of all the phytochemicals. The methanolic extracts of 4 species, namely *Rhus chinensis*, *R. Sucedanea*, *Rubus niveus* and *Smilax ferox* showed the presence of all the phytochemicals tested. Similarly, extracts of 3 species

(*Oleandra wallichii* and *Smilax aspera* and *Tectaria coadunata*) showed the presence of six phytochemicals. The extracts of *Adiantum incisum*, *H. coronarium* and *M. polymorpha* showed the presence of five different phytochemicals while that of *Asterella wallichiana* and *Sonchus arvensis* showed the presence of 4 and 3 different phytochemicals, respectively. The distribution of phytochemicals in aqueous extracts of different species was such that there were 6 different phytochemicals in three species (*O. wallichii*, *Rhus chinensis* and *Rubus niveus*), five in two species (*Rhus succedanea* and *Tectaria coadunata*), 4 in two species (*Smilax aspera* and *S. ferox*), 3 in 2 species (*Adiantum incisum* and *H. coronarium*), 2 in *M. polymorpha*, and 1 in *Asterella wallichiana*. Furthermore, the aqueous extract of *Sonchus arvensis* did not show the presence of any phytochemicals tested (Table 2).

Table 2

Summary of qualitative phytochemical screening of aqueous extracts

Species	Alkaloids		Phenols		Flavonoids		Glycosides		Saponins		Steroids		Terpenoids	
	Aq	Me	Aq	Me	Aq	Me	Aq	Me	Aq	Me	Aq	Me	Aq	Me
AD	+	+	-	+	-	-	-	+	-	-	+	+	+	+
AS	-	-	-	+	-	-	-	+	-	-	-	+	+	+
HC	-	-	-	+	-	-	-	+	+	+	+	+	+	+
MP	-	-	-	+	+	+	-	+	-	-	-	+	+	+
OL	+	+	+	+	+	+	+	+	-	-	+	+	+	+
RC	+	+	+	+	+	+	-	+	+	+	+	+	+	+
RN	+	+	+	+	+	+	+	+	+	+	-	+	+	+
RS	+	+	+	+	+	+	-	+	-	+	+	+	+	+
SA	+	+	-	+	+	+	-	+	+	+	-	-	+	+
SC	-	-	-	+	-	-	-	+	-	-	-	-	+	+
SF	+	+	+	+	+	+	-	+	+	+	-	+	+	+
TC	+	+	-	+	+	+	+	+	+	+	-	-	+	+

Legend: AD- Adiantum incisum, AS- Asterella wallichiana, HC- Hedychium coronarium, MP- Marchantia polymorpha, OL- Oleandra wallichii, RC- Rhus chinensis, RN- Rubus niveus, RS- Rhus succedanea, SA- Smilax aspera, SC- Sonchus arvensis, SF- Smilax ferox, TC- Tectaria coadunata; Aq-Aqueous, Me-Methanolic

Extraction involves separation of different phytochemicals present in test material by using solvents of varying polarity and different extraction procedures. Though both water and alcohols are considered polar solvents, water has higher polarity (1.0) than alcoholic solvents like methanol (0.762). Alcoholic solvents are universal solvents for phytochemical extraction (Zhang *et al.*, 2018) and are suitable for extraction of a wide range of polar as well as non-polar phytochemicals, while water is suitable

for extraction of mostly polar compounds. The presence of most of the tested phytochemicals in the methanolic extracts of different species and poor presence of the same in aqueous extracts of respective species may be attributed to the predominance of non-polar phytochemicals in those species. In addition to the nature of solvent used, the phytochemicals present within the plant vary also with species and parts of the plant material used (Handa *et al.* 2008). The differences in the nature of phytochemicals present in aqueous and methanolic extracts of different species may be due to the same reason.

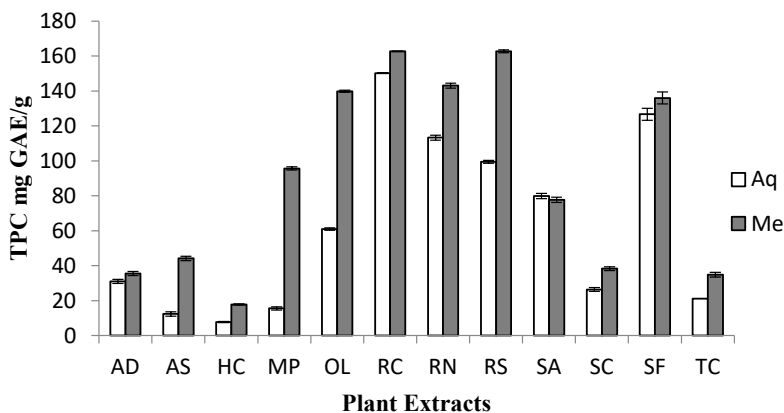
Total Phenolic Content (TPC)

The values of TPC in plant extracts based on the standard curve of Gallic acid is shown in Figure 1. The methanolic extracts possessed relatively higher TPC than aqueous extracts in most of the species. In the case of *Smilax aspera* however, the aqueous extracts possessed high TPC than methanolic extracts.

Among the aqueous extracts the TPC was found highest (150.20 ± 0.13 mgGAE/gm) in *Rhus chinensis* and the lowest (7.84 ± 0.34 mg GAE/gm) in *H. coronarium*. The differences in TPC in aqueous extracts of different species were statistically significant ($P < 0.5$).

Figure 1

TPC values in different plant extracts **Legend:** AD- *Adiantum incisum*, AS- *Asterella wallichiana*, HC- *Hedychium coronarium*, MP- *Marchantia polymorpha*, OL- *Oleandra wallichii*, RC- *Rhus chinensis*, RN- *Rubus niveus*, RS- *Rhus succedanea*, SA- *Smilax aspera*, SC- *Sonchus arvensis*, SF- *Smilax ferox*, TC- *Tectaria coadunata*; Aq- aqueous extract; Me- methanolic extract.



TPC among the methanolic extracts was found to be highest (162.71 ± 0.23 mgGAE/gm) in *R. succedanea* and lowest (17.86 ± 0.53 mg GAE/gm) in *H. coronarium*. In case of other species TPC values ranged between these two extremes. The differences in TPC in different species were statistically significant ($P < 0.05$).

The TPC values in the range of 95.80 ± 3.60 to 169.35 ± 0.25 mg GAE/gm have been reported in methanolic extracts of different Nepalese indigenous medicinal plants (Sharma *et al.*, 2015). The TPC values in methanolic extracts of different species in present study are in the range reported by Sharma *et al.* (2015), while that in methanolic extracts of five species, namely, *Adiantum incisum*, *Asterella wallichiana*, *H. coronarium*, *Sonchus arvensis* and *T. coadunata*, however, were much lower. Similarly, Chai *et al.* (2015) reported TPC values in aqueous extract of different fern species in the range of 42.57 ± 0.55 to 143.79 ± 5.19 mg GAE/gm. The TPC values in aqueous extracts of fern species *Adiantum incisum*, *O. wallichii* and *T. coadunata* in present study were much lower.

Since the synthesis of phenolic compounds in plants is triggered by UV radiation (Tegelberg and Julkunen-Tiitto, 2001), they are believed to have protective role in plants, especially against radiation. In addition, they are also involved in defense against pathogens (Beckman, 2000). Among the plant species used in present study the species with highest TPC values were reported from tree and lowest TPC from shade loving plants also indicate towards the role of radiation in TPC content of plants. Besides, the genetic differences among the test plants representing different evolutionary lines may also have played role in variation in TPC. Furthermore, differences in extraction procedure may also have contributed to differences in TPC values.

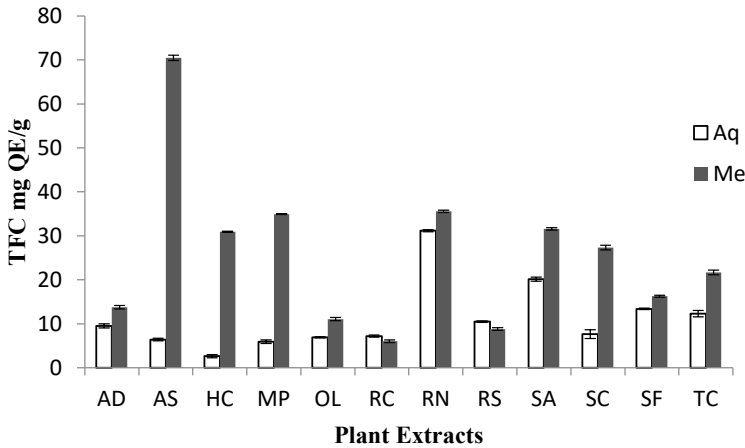
Total Flavonoid Content (TFC)

Total flavonoid content (TFC) in aqueous and methanolic extracts of selected species of medicinal plants based on the standard curve of quercetin is shown in Figure 2. For all the species (except *Rhus chinensis* and *R. succedanea*) methanolic extracts possessed higher TFC compared to aqueous extracts. Among the aqueous extracts the highest TFC (31.16 ± 0.21 mg QE/g extract) was found in *Rubus niveus* and the lowest (2.63 ± 0.34 mg QE/g extract) in *H. coronarium*. In other species TFC value ranged between

these two extremess. The differences in TFC among different species were statistically significant ($P \leq 0.5$). However, the TFC value was highest (70.5 ± 0.58 mg QE/g extract) in *Asterella wallichiana*, and lowest (6.04 ± 0.3 mg QE/g extract) in *Rhus chinensis* among the methanolic extracts. The differences TFC in methanolic extracts of different species were statistically significant ($P \leq 0.5$).

Figure 2

TFC values in different plant extracts. **Legend:** AD- *Adiantum incisum*, AS- *Asterella wallichiana*, HC- *Hedychium coronarium*, MP- *Marchantia polymorpha*, OL- *Oleandra wallichii*, RC- *Rhus chinensis*, RN- *Rubus niveus*, RS- *Rhus succedanea*, SA- *Smilax aspera*, SC- *Sonchus arvensis*, SF- *Smilax ferox*, TC- *Tectaria coadunata*; Aq- aqueous extract; Me- methanolic extract.



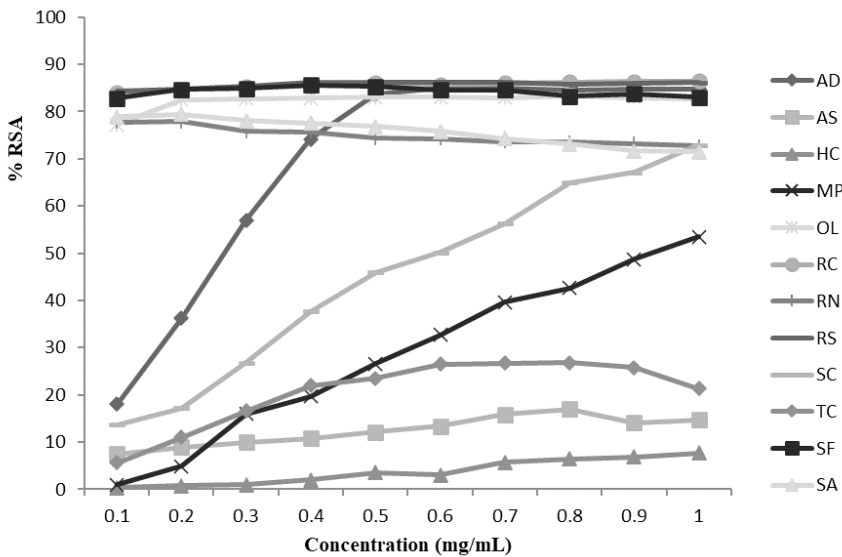
The TFC values in the range 10.70 ± 0.09 to 18.63 ± 0.30 mg QE/gm were reported in methanolic extracts of different Nepalese indigenous medicinal plants (Sharma et al., 2015). The values of TFC in methanolic extracts *Adiantum incisum*, *O. wallichii*, *Rhus chinensis*, *R. succedanea*, and *Smilax ferox* were comparable, while that in rest of the species were higher than reported by Sharma et al. (2015). Study carried out in aqueous extracts of different fern species have reported TFC values in the range 15.73 ± 1.55 to 367.88 ± 2.89 mg GAE/gm (Chai et al., 2015). The values of TFC in aqueous extracts of fern species in present study are comparable to the lower value reported by Chai et al (2015).

Antioxidant Activity

Figures 3 and 4 represent antioxidant activity of aqueous and methanolic extracts, respectively of different species of medicinal plants.

Figure 3

Percentage Radical Scavenging Activity of aqueous extracts. Legend: AD- Adiantum incisum, AS- Asterella wallichiana, HC- Hedychium coronarium, MP- Marchantia polymorpha, OL- Oleandra wallichii, RC- Rhus chinensis, RN- Rubus niveus, RS- Rhus succedanea, SA- Smilax aspera, SC- Sonchus arvensis, SF- Smilax ferox, TC- Tectaria coadunata.



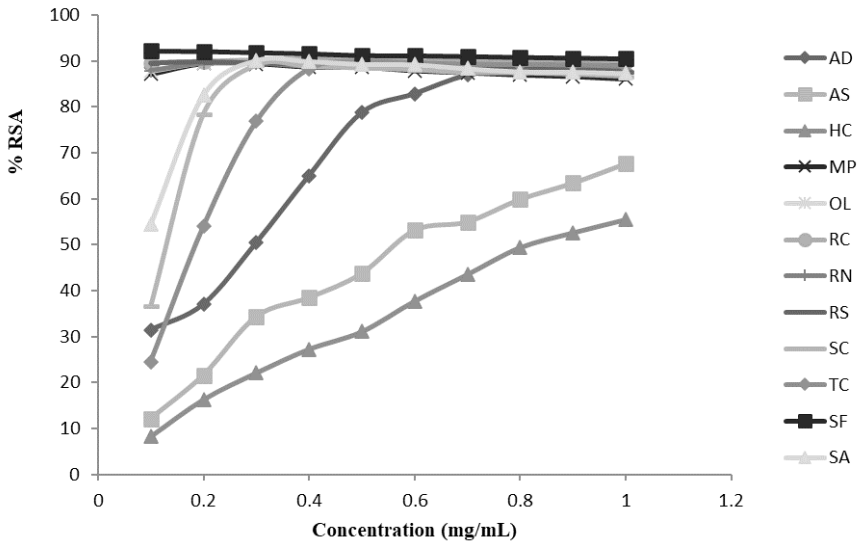
The percentage radical scavenging activity (RSA) was found to be higher for methanolic extracts than for aqueous extracts in all the species tested. *Asterella wallichiana* and *H. coronarium* extracts showed lowest RSA. In addition, the aqueous extracts of two more species *M. polymorpha* and *T. coadunata* also showed low RSA. *Sonchus arvensis* and *Adiantum incisum* showed moderate RSA in both the solvents. Similarly the RSA of extracts of *O. wallichii*, *Rhus chinensis*, *R. succedanea*, *Rubus niveus* and *Smilax ferox* were found to be high irrespective of the extraction solvent.

Antioxidants are basically reducing agents that protect the cells against oxidative damage (Evans, 2007) by scavenging free radicals such as peroxide, hydroperoxide etc. Various compounds occurring naturally in plants can serve as antioxidants. These include phenolics, nitrogen

containing compounds, carotenoids, ascorbic acid, etc. (Hall, 1997). The antioxidants are capable of preventing the onset of various diseases by inhibiting or neutralizing free radicals through different ways (Gupta and Sharma, 2014), this aspect of medicinal plants is among the most extensively studied aspects.

Figure 4

Percentage Radical Scavenging Activity of methanolic extracts. Legend: AD- Adiantum incisum, AS- Asterella wallichiana, HC- Hedychium coronarium, MP- Marchantia polymorpha, OL- Oleandra wallichii, RC- Rhus chinensis, RN- Rubus niveus, RS- Rhus succedanea, SA- Smilax aspera, SC- Sonchus arvensis, SF- Smilax ferox, TC- Tectaria coadunata



Sharma *et al.* (2015) reported the antioxidant activity in terms of free radical scavenging activity of different medicinal plants from Nepal and reported IC_{50} value of $6.48 \pm 0.08 \mu\text{g/mL}$ in *Bauhinia variegata* and $47.50 \pm 0.08 \mu\text{g/mL}$ in *Zizyphus mauritiana*. No direct comparison is possible between the results of present study and that of Sharma *et al.* (2015), however the values of RSA in present study are much lower.

Various phytochemicals, mostly the phenolics are associated with strong antioxidant properties (Velioglu *et al.*, 1998). The activities of these antioxidants help in prevention of systemic diseases like cancer, cardiovascular diseases, diabetes etc. caused by the action of free radicals

(Pourmorad *et al.*, 2006) either by scavenging the free radicals or by inhibiting their production through enhancement of gene expression leading to intracellular antioxidant production (Lu *et al.*, 2010). The high RSA in species like *O. wallichii*, *Rhus chinensis*, *R. succedanea*, *Rubus niveus* and *Smilax ferox* with high phenolic content in present study also supports these facts. Similarly, the low antioxidant activity in extracts of liverworts (*Asterella wallichiana* and *Marchantia polymorpha*) can also be attributed to low phenolic content in their extracts.

CONCLUSION

The comparison of phytochemical composition, TPC, TFC and antioxidant activity of medicinal plants belonging to different plant groups like bryophytes (liverworts), pteridophytes (ferns) and angiosperms did not reveal any specific pattern among different groups. From the study it appears that extracts of bryophytes were even superior to that of pteridophytes and flowering plants in some of the parameters studied. Therefore, it is likely that the exclusion of bryophytes from phytochemical studies of land plants is possibly due to the fact that they are less appealing to researchers due to their small size and low aesthetic value.

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