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Thin Layer Chromatography based Chemical Profiling and Antioxidant Activity of selected Nepalese Medicinal Plants

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ABSTRACT

Introduction: Numerous locally accessible plants in Nepal are left unseen. So, exploring their antioxidant activity for medicinal purposes can be beneficial in treating various diseases. Antioxidants have great importance in terms of reducing oxidative stress that causes damage to biological molecules. The qualitative analysis of chemical constituents using a chromatographic technique like TLC plays a pivotal role in this aspect. The present study aimed at performing chemical profiling using thin-layer chromatographic technique and evaluation of the antioxidant activity of selected medicinal plants.

Methods: Firstly, TLC profiling of 70% methanolic extracts of eighteen medicinal plants was done using preparative TLC plate in two different ratio of chloroform, methanol and water. Then, antioxidant activity was tested by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay by using a 96 well plate method at wavelength 510 nm in which Trolox was taken as standard.

Results: Flavonoids, tannins, saccharides, and phenols were identified from TLC profiling. The yield value of Sapium insigne leaves extracts i.e. 20.52% was highest and Monochoria vaginalis leaves possess the least i.e. 3.93%. Solena heterophylla leaves extract with IC_{50} amount i.e. 21 µg/ml was found most potent among all the plant extracts.

Conclusion: The results imply that the extract of ethnomedicinal plants is rich with a variety of phytochemicals, which can be used as natural antioxidants. However, further studies are warranted to isolate and identify the chemical and biological properties of obtained extracts for the provision of scientific evidence for traditional uses.

Keywords: *Antioxidant, DPPH, TLC profile, IC_{50}, 96 well plate.*

INTRODUCTION

Free radicals are responsible for the degenerative and pathological conditions like cancer, aging, inflammation, coronary heart disease, diabetes.[1] Among the Phyto compounds present in natural plants, great attention has been sought by antioxidants among researchers since they can reduce or neutralize the free radicals and protect the human body.[2] Some of the main methods to express constituents are high-performance liquid chromatography (HPLC), gas chromatography (GC), and Thin-layer chromatography (TLC), etc. TLC is a classical, simple, quick, inexpensive, and commonly used procedure by which several components in the mixture can be identified. TLC is commonly used for the analysis of natural medicines included in various pharmacopeias and also for the qualitative determination of small amounts of impurities.[3]

Several pieces of research have been done on separation and chemical characterization of plant constitutes and their different parts. However, there is a lack of research on the various ethnomedicinal plant for their antioxidant activity and phytochemical constituents. In the case of Nepal, many locally available plants are left unnoticed and this provides the rationale behind the study. This study may also give preliminary data on phytochemical compounds and is the basis for the isolation of chemical compounds with biological activity from the extract with potent antioxidant properties.

In this connection, we have selected locally available medicinal plants and evaluated the antioxidant property, mainly focusing on their ethnomedicinal uses. Besides this, we also investigate the TLC fingerprint profile of the crude extracts.

MATERIALS

Plant material:

Eighteen medicinal plants were collected from different places of Nepal from October 15, 2015, to November 20, 2015. The detailed information like scientific and common name, family, parts of the plant used, ethnomedicinal uses[4-7] are given in Table 1.

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The authentic samples of plants and herbarium were preserved in the Laboratory of Pharmacognosy, School of Health and Allied Sciences, Pokhara University.

Table 1: List of selected medicinal plants

S. N.	Scientific name	Common name (Nepali)	Family	Parts used	Ethno-medicinal uses
1	Ageratum conyzoides	Gandhe	Compositae	Whole plant	Wound, leprosy, to set dislocate bone
2	Calotropis gigantea	Aank	Asclepiadaceae	Roots, barks, leaves, latex, Flowers	Febrifuge,anthelmintic, expectorant
3	Cheilanthes albomarginata	Ranisinka	Pteridaceae	Rhizome	Peptic ulcer, cuts, and wounds
4	Chromolaena odorata	Banmara	Asteraceae	Young leaves	Skin wound, rashes, diabetes, and as an insect repellent
5	Cissampelos pareira	Batulpate	Menispermaceae	Roots, leaves	Fever, indigestion, snakebites, swelling of gums
6	Citrus aurantifolia	Jyameer	Rutaceae	Fruits, barks	Digestive, rheumatism
7	Costus speciosus	Betlauree	Zingiberaceae	Rhizome	Astringent,purgative, depurative, stimulant, anthelmintic,
8	Desmostachys bipinnata	Kush	Graminae	Whole plant	Cooling, aphrodisiac, diuretic, asthma, jaundice
9	Ficus hispida	Khasreto	Moraceae	Fruits, seeds, barks	Purgative, emetic and astringent
10	Ficus palmata	Bedu	Moraceae	Young shoots, Leaves, latex	Curdling milk, to remove spine lodged deeply in the flesh
11	Mallotus philippensis	Sinduri	Euphorbiaceae	Root, barks, Leaves	Body pain, typhoid, bronchitis
12	Melastoma malabathricum	Angeri	Melastomaceae	Leaves, roots, shoots	Used in cuts or wounds, high blood pressure, diabetes
13	Mesua ferra	Nareshwore	Guttiferae	Barks, Leaves,Flowers, oils	Astringent, stomachic, and used in cough, dysentery.
14	Monochoria vaginalis	Neelo Jalakumbhi	Pontederiaceae	Roots, leaves	Diuretic, tonic
15	Premna barbata	Gineri	Labiatae	Woods, barks	Chilblain, wounds, fever
16	Sapium insigne	Khirro	Euphorbiaceae	Roots, barks, latex	Diarrhea, stomach disorder
17	Solena heterophylla	Golkaankree	Cucurbitaceae	Whole plant	Indigestion, throat infection, abdominal ulcer, peptic ulcer
18	Zanthoxylum acanthopodium	Timur	Rutaceae	Seeds and barks	Tonic in fever

Materials used

The chemicals used in this study were, Chloroform (Fischer Scientific Pvt. Ltd., India), Dimethyl Sulfoxide, Ethanol and Methanol (Fisher Scientific, India), DPPH and Trolox (Wako Pure Chemical Co. Ltd., Osaka, Japan), Ferric chloride (S.D. Fine Chemicals Limited, India), Hydrochloric acid (Fischer Chemical Pvt. Ltd., India), Preparative TLC plate (Merck) and Sulphuric acid (Qualigens Fine chemicals, India). The instruments used were; Rotatory Evaporator (Model Rotavapor R-215) of Buchi, Switzerland, Double beam UV Spectrophotometer (Model UV-1800) of Schimazdu, and Falcon 96 well plate.

Extraction, TLC Profiling, Solvent systems preparation, and Data analysis.

Extraction of plant material

Extraction was performed by maceration. Five grams of each dried plant were macerated using 100 ml of 70% methanol in 250 ml conical flask in the water bath at 60°C for 5 hours at room temperature for 24 hours. Then the extract obtained was filtered using a cotton plug and concentrated in the rotatory evaporator and dried in a vacuum desiccator. Finally, the yield value of each extract was calculated.

TLC Profiling:

The preparative TLC plates of analytical grade were used for TLC profiling of the exact.

Solvent systems and reagents preparation:

The different solvent system, $CHCl_3$: MeOH: H_2O (7:3:0.5), $CHCl_3$: MeOH: H_2O (6:4:1), 10% (v/v) H_2SO_4 spray/heat, 10% (w/v) Aq. $FeCl_3$, DPPH (2, 2-diphenyl-1-picrylhydrazyl, stock solution, tests solution), DPPH solution, phosphate buffer, Trolox solution, 50% DMSO solution, EtOH in H_2O were prepared according to the standard procedure.[3,8,9]

Procedure for TLC:

Concentrated dry extract of crude drug sample was diluted with 70% methanol. Samples were adjusted up to 1 ml volume, which was further spotted in preparative TLC plates of size 7cm×5cm which was run in two solvent systems of Chloroform, Methanol, and distilled water in 7:3:0.5 and 6:4:1 ratio and dried. The observation was performed in short UV with a wavelength of 254 nm and long UV with a wavelength of 365 nm. Finally, the plates were sprayed with different spraying agents as 10% (v/v) H_2SO_4/heat, 10% (w/v) aqueous Ferric chloride, dipping in DPPH solution and observation was done.

The preliminary and Real test

The real test was performed from the preliminary test by the selection of the test concentration 0.1 mg/ml, which shows equal absorbance with Trolox.

DPPH Free Radical Scavenging Activity Analysis

The antioxidant assay is based on DPPH free radical scavenging assay using the method proposed by Hazra et al., with slight modification[9] using Falcon 96 well plate method for the antioxidant activity analysis of a large number of samples of plant extracts in short duration of time. A standard antioxidant Trolox was used as a positive control. Samples stored in the dark for 30 minutes to measure the absorbance at 510 nm using UV-Visible Spectrophotometer. The scavenging activity calculated by using the following formula:

$$\text{Scavenging activity}(\%) = \frac{A - B}{A} \times 100\%$$

Where A is the absorbance of the DPPH solution without the sample, B is the absorbance of the DPPH solution in the presence of the sample (crude extract/ Trolox). The scavenging activity or % inhibition was then graphed against concentration. IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis with Microsoft Office Excel 2007.

RESULTS

Yield obtained:

The extraction of plant material with the modified maceration results in various extraction values have been depicted in Table 2. The result of extraction yield value revealed the highest value in Sapium insigne leaves extract, i.e., 20.522% and Monochoria vaginalis leaves possess the least yield value, i.e., 3.93%.

Table 2: Extraction yield value of 70% methanolic extract

Plants	Parts used (Symbols)	% Yield value
Cheilanthes albomarginata	Leaves (1)	8.87
Cissampelo spareira	Leaves (2)	7.15
Zanthoxylum acanthopodium	Leaves (3)	15.18
Solena heterophylla	Leaves (4)	7.19
Citrus aurantifolia	Leaves (5)	13.12
Mallotus philippensis	Leaves (6)	17.80
Chromolaena odorata	Leaves (7L)	4.05
	Stems (7S)	5.99
	Flowers (7F)	10.40
Mesua ferra	Leaves (8L)	12.15
	Small Branches (8S)	9.19
Desmostachys bipinnata	Leaves (9)	4.48
Ageratum conyzoides	Whole plant (10)	11.23
Sapium insigne	Leaves (11L)	20.52
	Stems (11S)	6.08
Ficus hispida	Stems (13S)	11.26
	Leaves (13L)	12.53
	Fruits (13Fl)	18.10
Ficus palmate	Leaves (12L)	9.76
Monochoria vaginalis	Leaves (15)	3.93
Melastoma malabathricum	Leaves (16a)	9.91
Premna barbata	Leaves (16)	10.41
Calotropis gigantean	Leaves (17)	14.08
Costus speciosus	Rhizome (18)	4.86

TLC Profiling:

The TLC profiling of different extracts in the solvent system chloroform: methanol: water (7:3:0.5) and of chloroform: methanol: water (6:4:1) are given in Figure 1 and Figure 2 respectively.

$CHCl_3$: MeOH: H_2O (7:3:0.5)

From the figure of the TLC profile, spots are seen in the lower level of the TLC plate due to the less polarity of solvents after observing in short UV and long UV.

CHCl3: MeOH: H2O (6:4:1)

From the figure of the TLC profile, spots are seen in the upper layer of the TLC plate due to more polarity of solvents after observing in short UV and long UV.

10% (v/v) H2SO4 / heat:

In both solvent systems, spraying of 10% H2SO4 and after heating, most of the plant extract showed the presence of flavonoids with yellow color spots and saccharides through the black color spots.

10% (w/v) aqueous Ferric chloride:

The presence of phenol through brownish color spot and tannin through the blue color spot in both solvent systems was observed through this spray in our experiment.

DPPH solution:

After dipping in DPPH solution in the dark, observation of pale yellow or no color in both solvent systems suggested the presence of potent antioxidants in most of the plants in our research.

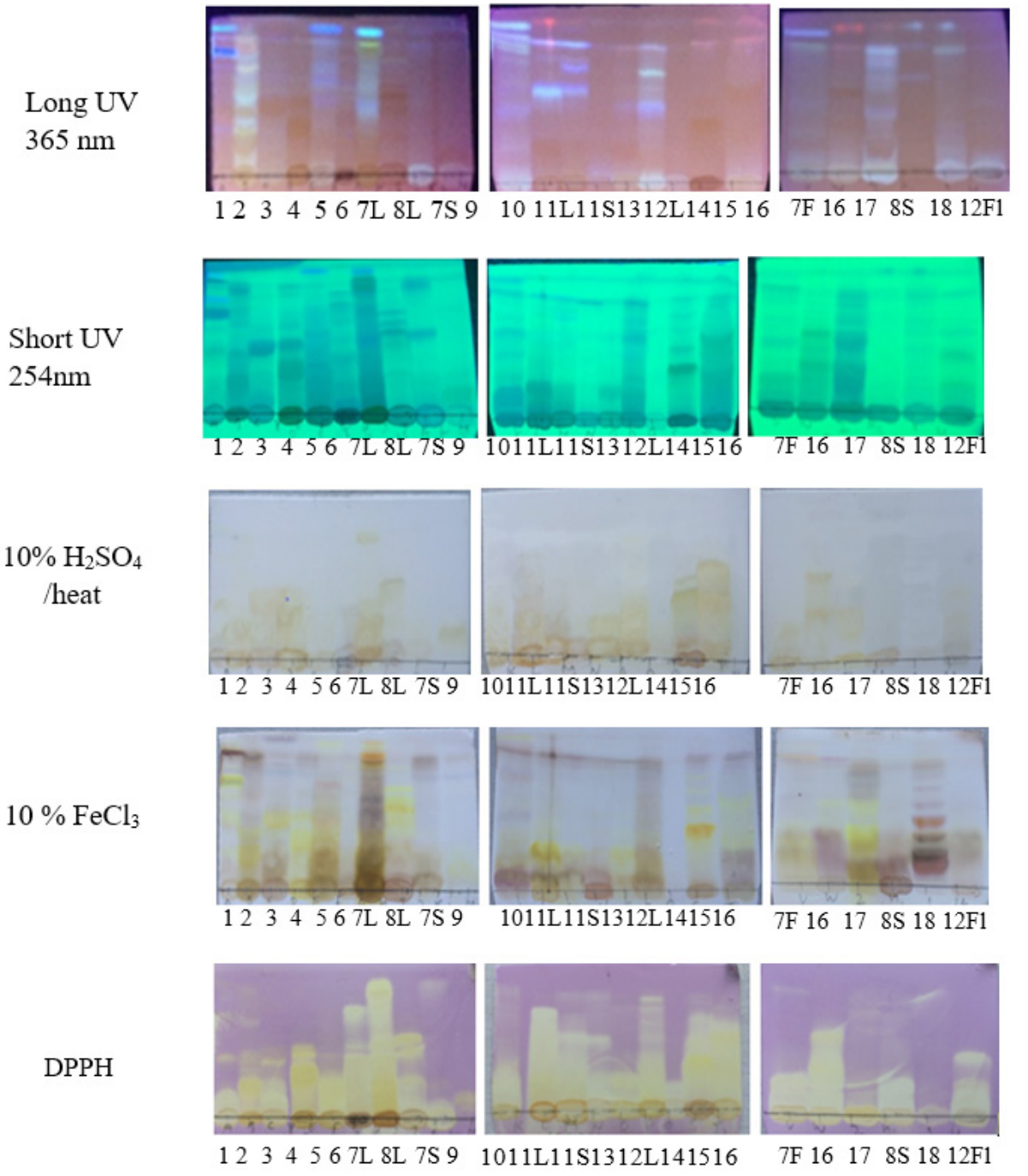

Figure 1: TLC profiling of chloroform: methanol: water (7:3:0.5) solvent system

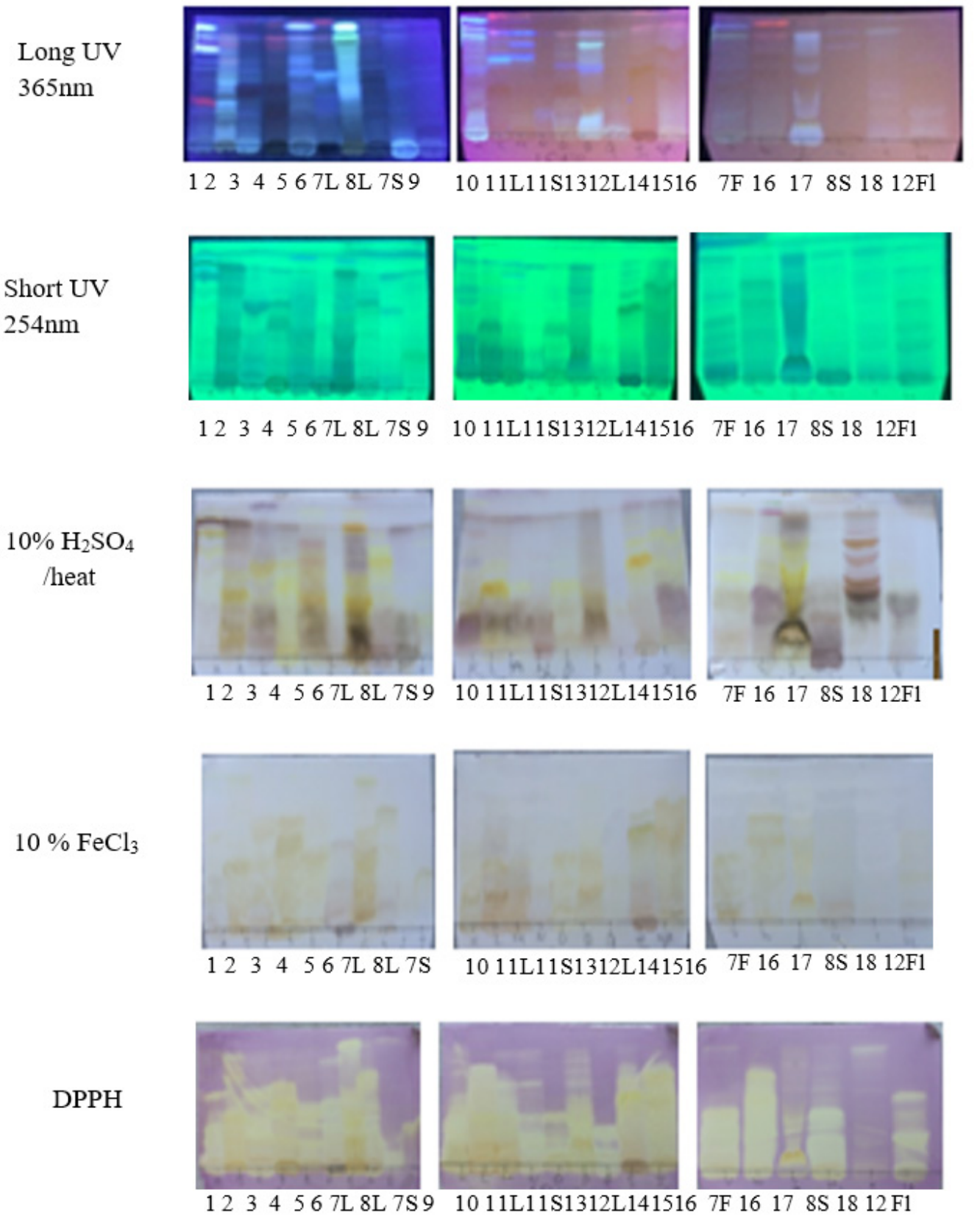

Figure 2: TLC profiling of chloroform: methanol: water (6:4:1) solvent system

DPPH Free Radical scavenging activity analysis:

From the preliminary test experiment, we found 1X=0.1 mg/ml and through real test % inhibition of DPPH free radical of 70% methanolic extract of 18 medicinal plants based on observation of the TLC profile in DPPH solution and through preliminary test experiment. DPPH free radical scavenging activities were evaluated to assess antioxidant activity. The present study revealed a dose-dependent increase in activity from 100 to 25 µg/ml for all the examined selected plants. We used different concentration of four replicates: 100 µg/ml, 75 µg/ml, 50 µg/ml and 25 µg/ml. IC_{50} of plant samples were determined, as shown in Table 3. Graphical representation of IC_{50} values of plant extracts and Trolox are given in Figure 4 and Figure 5 respectively.

Table 3: IC_{50} value of 70% methanolic extract of selected medicinal plants and Trolox

Plants	Parts used (Symbols)	IC_{50} value (µg/ml)
Cheilanthes albomarginata	Leaves (1)	114
Cissampelo spareira	Leaves (2)	151
Zanthoxylum armatum	Leaves (3)	109
Solena heterophylla	Leaves (4)	21
Citrus aurantifolia	Leaves (5)	72
Mallotus philippensis	Leaves (6)	64
Chromolaena odorata	Leaves(7L)	170
	Stems(7S)	46
	Flowers(7F)	57
Messua ferra	Leaves(8L)	48
	Stems(8S)	110
Desmostachys bipinnata	Leaves (9)	45
Ageratum conyzoides	The whole plant (10)	34
Sapium insigne	Leaves(11L)	104
	Stems(11S)	108
Ficus hispida	Stems (13)	280
	Leaves (14)	140
	Fruits(12FL)	155
Ficus palmate	Leaves(12L)	100
Monochoria vaginalis	Leaves (15)	61
Melastoma malabathricum	Leaves(16a)	85
Premna barbata	Leaves (16)	29
Calotropis gigantean	Leaves (17)	109
Costus specious	The rhizome (18)	379
Trolox	Trolox	100

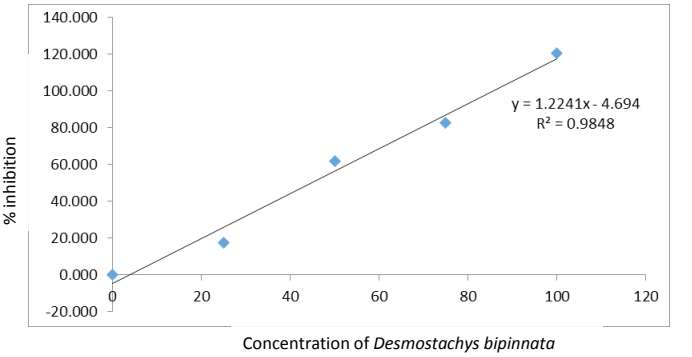

Figure 3: Linear equation curve between % inhibition absorbance versus concentration of Desmostachys bipinnata Leaves.

Figure 3 Illustrate the IC_{50} value of Desmostachysbipinnata.
From the linear equation, Y=mx+C
Here y= 50, m= 1.224, c= 4.694
So, $50= 1.224^{x} - 4.694$
Therefore, ^{x} = 45 µg/ml.
Hence the IC_{50} value of Desmostachys bipinnatais 45 µg/ml.

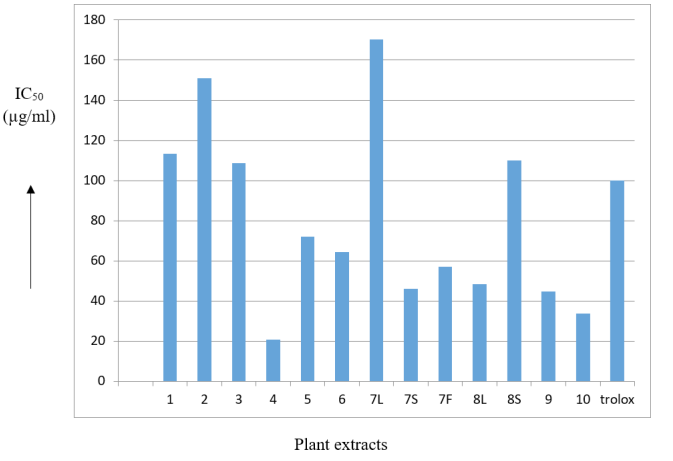

Figure 4: Graphical representation of IC_{50} values of plant extracts and Trolox

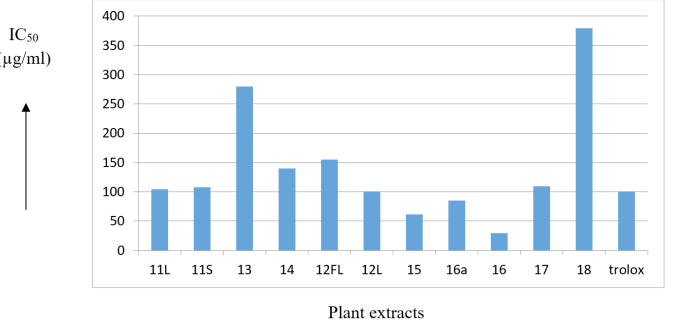

Figure 5: Graphical representation of IC_{50} values of plant extracts and Trolox

DISCUSSION

The traditional knowledge and ethnomedicinal practices that are reported can be used to explore the plants.[10,11] In this study, we have selected eighteen medicinal plants based on ethnomedicinal uses and extracted the extracts for TLC profiling and DPPH radical scavenging activity analysis.

TLC has many advantages including lower cost, short-time analysis, the possibility of multiple detections, and specific derivatization on the same plate.[12] TLC profiling of 70% methanolic extract is accurate for each plant species based on the pattern of separation, i.e., inferred with the termination based on the ratio of solvent systems consisting of chloroform: methanol: water (7:3:0.5 and 6:4:1) followed by detection using different detection systems. From the TLC profiling result, the maximum of the plant extracts showed the presence of flavonoids, saccharides, phenols, tannins, etc. after observation of the position and color of the spots in both solvent systems. With the use of spraying agents, 10% (v/v) H_2SO_4, and 10% (w/v) $FeCl_3$ observation of flavonoids, saccharides, tannins, and phenol were done. For most of the plant extracts, the presence of potent antioxidants was confirmed.

The result of our experiment with free radical scavenging assay ranges from 21 µg/ml to 400 µg/ml for most of the plant extract that had been tested using 70% methanolic extract for control Trolox with an IC_{50} of 100 µg/ml. Moreover, extract from Solena heterophylla with an IC_{50} value of 21 µg/ml, i.e., the lowest amount of extract needed to scavenge 50% of DPPH radicals. The plant is often used in gastrointestinal respiratory and vascular disorder and is used as a relaxant.[13] The plant is commonly used in Saradikhola of Kaski,[14] Nepal, for the treatment of the livestock disease, and its different species are used all over the world from Srilanka to India.[15]

Similarly, the excellent antioxidant properties from the extract of Leaves of Cheilanthes albomarginata revealed by the DPPH free radical assay with an IC_{50} value of 114 µg/ml. The potential antioxidant capacity of Cheilanthes sp. against DPPH free radical, ferric reducing power is parallel to that reported in the four species from Northern Western Ghats.[16] There might be some variation in IC_{50} value due to environmental effect or difference in the type of species or by the extract, i.e., made after the concentration followed by solubilizing using a different organic solvent, as in acetate fraction from Cheilanthes albomarginata with strongest DPPH free radical scavenging (82.54±0.48%).[17]

The IC_{50} value for the leaf extract from Citrus aurantifolia was found 72 µg/ml which is moreover comparable to that of anti-aflatoxigenic activity as reported from essential oil with a value of 50.1 µg/ml.[18]

The correlation of TLC profiling in DPPH solution and DPPH Radical Scavenging showed the presence of potent antioxidant compounds Solena heterophylla leaves, Desmostachys bipinnata leaves, Chromolaena odorata stems, Messua ferra leaves, Chromolaena odorata flowers, Monochoria vaginalis leaves, Mallotus philippensis leaves, Citrus aurantifolia leaves, Melastoma malabathricum leaves. It can be illustrated through observation of pale yellow or no color in DPPH solution and presence of higher aromatic phenol group and flavonoid derivatives groups in the plant extracts through the use of spraying agents 10% H_2SO_4 in heat and $FeCl_3$ with a change in color of spot and retention factor determination. Also, through the calculation of IC_{50} value, a quantitative analysis of antioxidants was done.

However, our study only provides preliminary data on phytochemical compounds, further studies are suggested for more precise quantitative separation of individual components and chemical compounds identification using Mass spectrometry or Nuclear Magnetic Resonance (NMR) technology.

CONCLUSION

The TLC study of extracts of different ethnomedicinal plants confirmed the presence of various secondary metabolites like flavonoids, tannins, phenols, and saccharides. The presence of a wide spectrum of phytochemical constituents proved the traditional medicinal usage of this species. Further studies are warranted to isolate and identify the chemical and biological properties of obtained extracts for the provision of scientific evidence for traditional uses.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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