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# Antioxidant, Phytochemical and TLC Analysis of *Desmodium gangeticum* Linn. DC. Vulnerable Medicinal Plants of Narayanpur, Bastar, Chhattisgarh

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**ABSTRACT**
*Desmodium gangeticum* Linn. DC. belongs to the family Fabaceae, subfamily Papillionaceae popularly known as 'Shalaparni' is a woody perennial herb. *D. gangeticum* (L.) DC. has been used in folk medicine in the treatment of various ailments which are widely used drugs in Ayurveda due to its wide uses in formulations. Research on the potential of diversity, ethnobotany and ethnopharmacology and bioactivity of plants used by local tribes in Chhattisgarh state is still relatively inadequate. The present study is aimed to analyze the presence of phytoconstituents, TLC analysis and antioxidant properties of crude leaves extracts of *D. gangeticum* (L.) DC.. In the present investigation, considering the highly significant therapeutic role of antioxidant phytochemicals, effort was made to find out the antioxidant capacity of various polarity extracts (acetone, chloroform, petroleum ether and aqueous) were used for determination of DPPH radical scavenging assay, reducing power assay, metal chelating activity and total phenol assay were performed due to their high speed and sensitivity. Results of qualitative phytochemical screening revealed the presence of alkaloids, phenols, reducing sugar, saponins, tannins, terpenoids, flavonoids, fixed oil and fats, anthraquinones as the major bioactive compounds. While cardiac glycoside was found to be absent. Further characterization of bioactive compounds was performed using TLC analysis. TLC results confirmed the presence of phenols, saponins and flavonoids with different Rf values showing maximum separation bands for *D. gangeticum* (L.) DC. 7 bands in acetone, phenols with $R_f$ value (0.9, 0.966) showed 2 bands, flavonoids with $R_f$ value (0.253, 0.893, 0.993, 1.00) showed 4 bands and saponins with $R_f$ value (0.966) showed only 1 band. While in chloroform and petroleum ether extract with 6 bands. Antioxidant activity results showed that the percentage inhibition of DPPH radicals of *D. gangeticum* (L.) DC. leaf extracts were maximum in acetone extract 73.34% inhibition absorbance at 517 nm. The DPPH free radical scavenging activity of positive control/standard antioxidant [ascorbic acid (10 mg/ml)] was found to be 75.27%. The reducing power in chloroform extracts of *D. gangeticum* (L.) DC. leaf extracts were the highest 0.056 in terms of absorbance ($\lambda$max at 700 nm). The reducing power assay of positive control/standard antioxidant [ascorbic acid (10mg/ml)] measured at A700 nm was found to be 0.014. The metal chelating capacity in *D. gangeticum* (L.) DC. leaf extracts were found to be highest in acetone extract with an absorbance of 80.34%. The metal chelating activity of positive control/standard antioxidant [ascorbic acid (10mg/ml)] was found to be 87.56%. The total phenol content of *D. gangeticum* (L.) DC. leaf extracts were highest in acetone extract 15.22 mg/kg of gallic acid equivalents. The total phenol content of positive control/ standard antioxidant [ascorbic acid (10mg/ml)] was found to be 18.56 mg/kg. From this study it is clear that *D. gangeticum* (L.) DC. possesses a potential antioxidant capacity. So, our result can serve as a preliminary source of knowledge for sustainable use, further isolation, characterization and identification of the compounds that can serve as a source of raw material for pharmaceutical and pharma industries as a natural source with low cost and very less side effects using clinical trials. However, additional work is encouraged to study other beneficial properties, especially as antimicrobial, antioxidant and anti-cancer drugs. Also to elucidate the possible mechanism of action of these extracts. Plant extract from these plants provided a great help in a new discovery in the area of chemical diversity because of the unknown availability either as standardized extract or as pure compound. Therefore, extracts from these plants could be seen as a good source of useful drugs and facilitate novel drug discovery.

**Keywords:** Medicinal and aromatic plants, secondary metabolites, phytocompound, TLC analysis, antioxidant potential, DPPH free radical scavenging assay, reducing power assay, metal chelating capacity, total phenolic content.

  

## I. INTRODUCTION

Medicinal plants are an important source of antioxidants and play an important role as a health-protected factor due to the presence of many active compounds [46]. Many plants possess compounds having large amounts of antioxidants with free radical scavenging activities. A wide range and diversity of naturally occurring antioxidants are found in medicinal plants which are different in their composition, physical and chemical properties and site of actions [60]. They are found in all parts of plants such as roots, stems, barks, leaves, flowers, seeds and fruits [37]. An enormous number of aromatics, medicinal, herbs, spice and other plants containing chemical compounds exhibiting antioxidant properties are commonly employed in industries, food preservations, flavors, perfumes, cosmetics and bio regulatory properties [3].

Antioxidant compounds contain phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl that lead to inhibit the oxidative mechanisms and cause degenerative diseases. Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules *viz.,* nucleic acids, proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation with subsequent tissue injury. Trapping of free radicals is the dominant characteristic of an antioxidant. Free radicals like hydroxyl and reactive oxygen radicals are produced in our body during the normal metabolism are extremely reactive in nature and damage almost every molecule found in living cells, affect human health and lead to several degenerative diseases including atherosclerosis, hypertension, heart attack, diabetes, arthritis, immunosuppression, neurodegenerative diseases, parkinson and alzheimer diseases, cancer, emphysema, cirrhosis well as premature body aging ([18], [38], [19], [20], [12], [43]). Natural antioxidant agents have attracted much significance because of their capability to scavenge free radicals [48]. The occurrence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases i.e., ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders and for prevention and treatment of diseases ([17], [53], [47]).

**Pharmacologically** in *D. gangeticum* (L.) DC., it has been reported that the plant is found to possess various biological activities such as antimicrobial, antioxidant, anti-cancer, anti-ulcer, anti-diabetic, anti-inflammatory, analgesic, anthelmintic, antipyretic, anti-amnestic, anti-secretary, anti-leishmanial, anti-implantation, cardioprotective, hepatoprotective, renal protective, psychopharmacological, immunomodulatory activities, cytoprotective, hypocholesterolemic along with it is effective as anti-writhing and central nervous system (CNS) depressant activity, improving memory and in healing different types of wounds ([41], [29], [44], [36]).

**Chemical Constituents,** leaves contain alkaloids like N-methyltetrahydrofuran, 5-methoxy N, N-dimethyltryptamine, 5-methoxy dimethyltryptamine $N_b$-oxide, $N_b$ - methyl- $H_4$ - Harman, N, N dimethyl tryptamine their N-oxides, β-carbolineum cation, indole-3-alkyl-amines, hypaphorine, hordenine, candicine, N-methyl tyramine, β-Phenylethylamine, α-amyrone, lupeol and its acetate, stigmasterol have been isolated from aerial parts. The aerial portion gave indol-3-alkylamines and their derivatives. Also, Dat et al. (2015) reported the presence of three phenolic glucosides like methyl salicylate β-D-glucopyranoside, leonuri side A and syringaresinol-4'-O-β-D-glucopyranoside in *Desmodium gangeticum* water soluble leaf extracts [9]. Flavones like rutin, 4,5,7-trihydroxy-8-prenyl-flavone 4-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, 8-C-prenyl-5,7,5-trimethoxy-3,4-methylenedioxy flavone, Quercetin-7-O-β-D-glucopyranoside, Kaempferol-7-O-β-D-glucopyranoside, 5-O-methyl-genistein-7-O-β-D-glucopyranoside. Sterols like β-sitosterol, β-sitosterol-3-O-β-D-glucopyranoside, 24-ethylcholestrol-5,22-dien-3β-ol,24-ethylcholest-5-en-3β-ol and 24-methylcholest-5-en-3β-ol ([1], [62], [4], [13], [9], [57]). Roots contains pterocarpans such as gangetin, gangetinin, desmodin, desmocarpin and gangetial ([30], [25], [31], [32], [56]). Seeds contains amino glucosylglycerol lipid, trans-5hexadecenoic acid, salicylic acid, 5-O-methyl genistein-7-O-β-d-glucopyranoside, 3,4-dihydroxybenzoic acid, kaempferol-7-O-β-d-glucopyranoside and uridine triacetate [41].

## II. MATERIALS AND METHOD

### A. Sample Collection

Mature and disease-free leaves of *D. gangeticum* (L.) DC. was collected from Herbal Garden,

  

Bakhrupara Kasthagar and Raoghat Tropical dry deciduous forest area of Narayanpur district during their flowering seasons (October-December). Leaves were washed 2-3 times with running water and once with distilled water and then shade dried at room temperature for 1-2 weeks till the leaves became brittle enough to break easily. After complete drying, plant parts were crushed using an electrical grinder to fine homogenous powder and stored into air tight poly bags with proper labelling at room temperature.

**B. Sequential Soxhlet Extraction of Plant Material**

Extraction was carried out by Soxhlet extraction procedure as described by [23]. Coarse powders of *D. gangeticum* (L.) DC. plants taken for successive Soxhlet extraction in a hot continuous process [22]. About 25 g of powdered plant material was packed in manually prepared cones from Whatman filter paper Grade 1 and placed in the thimble chamber of Soxhlet. In the round bottom flask of Soxhlet was taken 250 ml of different solvent *viz.,* aqueous, acetone, chloroform and petroleum ether respectively. The process of cycling continues until the liquid droplet from the Siphon arm does not leave any residues and become colorless. After that the extracts were filtered through Whatman filter paper grade 1. After filtration, the extracts were concentrated under low pressure until dryness. Crude extracts were stored in Eppendorf tubes or airtight sterile brown bottles and then kept in the refrigerator for further analysis [64].

**C. Qualitative Phytochemical Screening**

Preliminary phytochemical screening was performed using standard procedures described by ([21], [54], [51], [52], [11], [28]).

**D. Purification of Bioactive Compounds Using Thin Layer Chromatography Analysis**

After preliminary tests, TLC analysis confirmed the presence of various bioactive phytocompounds in plant samples. Traditionally analytical TLC has found application in the detection and monitoring of compounds through a separation process Silica gel G was used as adsorbent for TLC plates as stationary phase. Steps wise preparation of TLC plate and development of different bands using the following four steps: 1) Preparation of TLC plates; 2) Spotting and development; 3) TLC Spraying Reagent; 4) Visualizing.

**Table I. List of spraying reagents used for identification of bands**

| Sr. No. | Active Compound | Spraying Reagent | Preparation method |
|---|---|---|---|
| 1. | Phenol | $FeCl_3$ | 10 gm $FeCl_3$ dissolved in 100 ml distilled water |
| 2. | Flavonoids | Ammonia solution | Ammonia solution |
| 3. | Saponins | Conc. HCl | Conc. HCl: Spraying Reagent |

**E. Antioxidant Assays**

To determine the antioxidant effects of plant extracts, different antioxidant assays for the plant extracts were performed according to standard procedures with minor modifications.

**1. DPPH radical scavenging activity**

*In vitro* antioxidant activity of the extract was measured with the DPPH method [50] with slight modifications. A solution of DPPH was freshly prepared by dissolving 6mg DPPH in 50mL methanol (about 0.3mM). The extract (2.5mL) with varying concentrations (60-220 µg/mL) and DPPH solution (2.5mL) were mixed together in a test tube. The test tube was then incubated in the dark for 20 minutes at room temperature. The decrease in absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Absorbance of the DPPH radical without antioxidants i.e., blank, was also measured. The percentage inhibition of radicals was calculated using the following formula:

% scavenging of DPPH = $[(A_0 - A_1) / A_0]$ x 100

Where $A_0$ was the absorbance of a DPPH solution without extract and $A_1$ was the absorbance in the presence of a plant extract.

**2.5.2. Reducing power assay**

The method described by [8] was applied in this work to determine the reducing power of plant extract. This reducing power was investigated by observing the transformation of $Fe^{3+}$ to $Fe^{2+}$. The extract was diluted with distilled water. The diluted extract (0.5mL) was mixed with phosphate buffer (2.5mL, pH 6.6) and potassium ferricyanide (2.5mL, 1% w/w) in a test tube, followed by incubating in a water bath at 50°C for 30 minutes. After cooling, put trichloroacetic acid (2.5mL, 10% w/v) into the tube and centrifuged at 3000 rpm for

  

10 minutes whenever necessary. The supernatant (2.5 mL) was mixed with distilled water (2.5mL) and a freshly prepared ferric chloride (0.5mL, 0.1% w/w). The mixture was mixed thoroughly and its absorbance was measured at 700 nm using a UV-VIS spectrophotometer. Control was prepared in a similar manner excluding samples. Ascorbic acid was used as a standard. Increased absorbance of the reaction mixture shows an increase in reducing power.

**2.5.3. Determination of metal chelating capacity**

Metal chelating activity was measured by the technique described [7]. 0.1 mM $FeSO_4$ (0.2mL) and 0.25 mM of ferrozine (0.4mL), forming a $Fe^{2+}$-ferrozine complex, were subsequently added into 0.2mL of plant extract. After incubation at room temperature for 10 minutes, absorbance of the mixture was recorded at 522 nm. Chelating activity was calculated using the following formula:

Metal ion chelating activity = $[(A_0 - A_1) / A_0] \times 100$

Where, $A_0$ was the absorbance control/ blank (without plant extract), and $A_1$ was the absorbance in the presence of a plant extract.

**2.5.4. Determination of total phenolic content**

Total phenolic contents (TPC) of the aqueous, acetone, chloroform and petroleum ether extract of selected plants were estimated using the Folin-Ciocalteu reagent as described by [39] with Gallic acid as the standard with minor modifications. The dried extract was dissolved in distilled water to a concentration of 50 µg/mL. The calibration curve was established using gallic acid (0-60 µg/mL). Diluted extract or gallic acid (1.6mL) was added to 0.2mL Folin-Ciocalteu reagent and mixed thoroughly for 3 minutes. Sodium carbonate (0.2mL, 10% w/v) was added to the mixture and the mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 750 nm using a UV-VIS spectrophotometer. TPC was expressed as a milligram of gallic acid equivalent per gram (mg GAE/g sample).

## III. RESULTS AND DISCUSSION

**3.1. Phytochemical screening of *D. gangeticum* (L.) DC.**

**Table II** below shows the results for phytochemical screening tests obtained during the experiment on *D. gangeticum* (L.) DC.. Maximum no. of phytochemical compounds was extracted in acetone and aqueous plant extract, as compared to chloroform and petroleum ether. Where '+' sign indicates the presence and '-' sign indicates the absence of phytocompound.

**3.2. Results of Thin Layer Chromatography (TLC)**

Preliminary investigations used TLC to separate the extracts, with a mixture of petroleum ether: benzene: methanol (16:3:2) as the developing solvent. Thin layer chromatography spots were observed with different color and $R_f$ values determined by measuring the distance traveled by solute/plant extract and distance traveled by the solvent. $R_f$ value is used to characterize the different phytochemical compounds that are present in the extract. Results of TLC profiling are summarized in **Table III**. *D. gangeticum* (L.) DC. aqueous leaves extracts (phenols, flavonoids and saponins) showed no color change after adding spraying coloring reagents **(Photoplate No. 1(a))**. In acetone extract phenols with $R_f$ value (0.9, 0.966) showed 2 bands, flavonoids with $R_f$ value (0.253, 0.893, 0.993, 1.00) showed 4 bands and saponins with $R_f$ value (0.966) showed only 1 band **(Photoplate No. 1(b))**. In chloroform extract phenols with $R_f$ value (0.8) showed only 1 band, flavonoids with $R_f$ value (0.22, 0.3, 0.626, 1.00) showed 4 bands and saponins with $R_f$ value (0.8) showed only 1 band **(Photoplate No. 1(c))**. In petroleum ether extracts phenols with $R_f$ value (0.592, 0.728) showed 2 bands, flavonoids with $R_f$ value (0.264, 0.428, 0.942) showed 3 bands and saponins with $R_f$ value (0.728) showed only 1 band **(Photoplate No. 1(d))**.

**3.3 Antioxidant activity**

Antioxidant properties of medicinal plants cannot be evaluated by a single method due to a variety of complex phytochemicals [34]. The antioxidant properties of *D. gangeticum* (L.) DC. have been evaluated by measuring their DPPH radical scavenging activity, reducing capacity, metal chelating activity and total phenolic activity provided clear insights into the curative properties of these plants.

**3.3.1. DPPH radical scavenging activity**

DPPH is a stable radical with a purple color. After receiving a proton from an antioxidant compound, which results in a visually noticeable discoloration from purple to yellow. Reduction in the color of DPPH can be measured spectrophotometrically at 517 nm. In the DPPH assay, the antioxidants are able to reduce the stable DPPH radical (purple) to the non-radical form DPPH-H (yellow) ([50], [24], [16]). In Radical scavenging activity of different solvent extracts of *D. gangeticum* (L.) DC. (DPPH). The percentage inhibition of DPPH radicals of *D.*

  

*gangeticum* (L.) DC. leaf extracts were maximum in acetone extract 73.34% inhibition absorbance at 517 nm. Whereas aqueous extracts with 68.56% inhibition, in chloroform extracts with 41.23% inhibition and lowest in petroleum ether extracts with 35.23% inhibition represented in **Fig 1**. The DPPH free radical scavenging activity of positive control/standard antioxidant [ascorbic acid (10 mg/ml)] was found to be 75.27% represented in **Table IV**.

**3.3.2. Reducing power assay**

The reducing power in chloroform extracts of *D. gangeticum* (L.) DC. leaf extracts were the highest 0.056 in terms of absorbance (λmax at 700 nm). Whereas petroleum ether extracts with 0.025, acetone extracts with 0.012, and aqueous extracts with 0.010 are represented in **Fig 2**. The reducing power assay of positive control/standard antioxidant [ascorbic acid (10mg/ml)] measured at A700 nm was found to be 0.014 represented in **Table IV**.

**3.3.3. Metal chelating scavenging activity**

The metal chelating capacity in *D. gangeticum* (L.) DC. leaf extracts were found to be highest in acetone extract with an absorbance of 80.34%. Whereas in aqueous extracts with absorbance of 80.12%, in chloroform extracts with absorbance of 45.23% and lowest in petroleum ether extracts with absorbance of 34.56% represented in **Fig 3**. The metal chelating activity of positive control/standard antioxidant [ascorbic acid (10mg/ml)] was found to be 87.56% represented in **Table IV**.

**3.3.4. Total phenolic content (TPC) (mg/Kg in terms of gallic acid equivalents)**

Phenols are another important plant constituent due to their free radical scavenging ability because of hydroxyl groups [40]. The antioxidant activity of medicinal plants is usually because of the presence of phenolic and flavonoids in them. The antioxidant properties are primarily because of redox properties possessed by phenolic compounds found in the medicinal plants.

The total phenol content of *D. gangeticum* (L.) DC. leaf extracts were highest in acetone extract 15.22 mg/kg of gallic acid equivalents. Whereas in aqueous extracts 12.34 mg/Kg, in chloroform extracts 10.56 mg/Kg and lowest in petroleum ether extracts 8.23 mg/Kg gallic acid equivalents are represented in **Fig 4**. The total phenol content of positive control/ standard antioxidant [ascorbic acid (10mg/ml)] was found to be 18.56 mg/kg represented in **Table IV**.

The DPPH scavenging ability and reducing power assays provides preliminary information on the reactivity of the test compound with a free radical and its hydrogen-donating tendency and the reduction capability of the DPPH radical [46]. Many researchers have reported positive correlation and observed that high reduction of DPPH is related to the high scavenging activity and larger amounts of antioxidants present in the sample [14]. The potential DPPH radical scavenging activity exhibited by various solvent extracts in reported works includes, [59] reported that the ethanolic extract of *D. gangeticum* possesses strong total phenolic content, total flavonoid content, DPPH radical scavenging, reducing power, chelating ability. [58] reported the free radical scavenging action of methanol extracts of plants in the order of *D. gangeticum* > *A. caudatus* > *S. nigrum* > *P. longum* > *E. alba* > *O. sanctum*. The highest total phenolic content was found to be in *O. sanctum*. The extracts, which showed the strongest DPPH radical scavenging activity were *D. gangeticum* and *A. caudatus* while the others showed moderate antioxidant properties. [55] reported strong antioxidant activities of the 10 *Desmodium* species were DPPH and ABTS scavenging activity in order *Desmodium sequax* (DSE) > *D. heterocarpon* (DH) > *D. microphyllus* (DM) > *D. uncinatum* (DU) > *D. intortum* (DI) > *D. gangeticum* (DG) > *D. scorpiurus* (DSC) > *D. triflorum* (DTR) > *D. tortuosum* (DTO) > *D. renifolium* (DR). Reducing power assay in order DSE > DH > DM > DU > DI > DG > DSC > DTR > DTO > DR. Total phenolic contains in order DSE > DH > DM > DU > DG > DI > DSC > DTR > DR > DTO respectively. *D. gangeticum* aerial part [15], *D. gangeticum* roots aqueous extract [33]. The potential Metal ion chelating activity exhibited by various solvent extracts in reported works includes, leaf extract of *D. gangeticum* [16], *D. indica* methanol extracts [49], *D. indica* ethanolic fruits extract [5]. Similarly, using others plants [10] reported the antioxidant and metal chelating activity in *M. pruriens*, *K. pinnata* in alcoholic leaf extracts [6], [61] among the six tropical medicinal plants tested, *C. nutans*, *C. formosana* and *H. diffusa* showed strong activity. *C. nutans* and *C. formosana* could potentially be used for the isolation of potent antioxidant and anti-diabetic compounds, *T. tetraptera* pulp extract exhibited stronger activity than whole fruits and seed extracts [2]. Reports on the potential total phenolic content exhibited include aerial parts of *D. gangeticum* (L.) DC. [58], roots and aerial parts of *D. gangeticum* (L.) DC. [42], leaves of *D. gangeticum* water-soluble extract [9]. [26] reported that higher absorbance was shown by ascorbic acid

  

at the lowest as well as highest absorbance among ethanolic extract of *Ocimum kilimandscharicum*, *Thymus serpyllum* and *Spilanthes acmella*. [27] tested the antioxidant activity of 21 medicinal plants in which 6 extracts of *B. monnieri*, *C. sinensis*, *C. arabica*, *C. longa*, *T. erecta* and *T. chebula* emerged as the most promising. These extracts exhibited elevated levels of phenolic 1378.19 mg gallic acid equivalents per gram.

## IV. CONCLUSION

The urge to discover novel plant compounds with antioxidant activity for healthcare and disease prevention is now an essential ingredient of contemporary pharmaceutical research. Leaves of *D. gangeticum* (L.) DC., exhibited noticeable antioxidant activities, making them potential candidates as phytomedicines. The total antioxidant potential of these medicinal plants was because of the high amount of polyphenol and other phytochemical compounds found in them. The free radical scavenging potential observed in these medicinal plant samples was because of the presence of some natural source such as phenol, flavonoids, or tannin contents. The strong antioxidant properties of these plants highly correlate with presence of phenolic compounds in appreciable amounts, which supports their use in traditional medicine particularly stress and immune related disorders. Thus, a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated with oxidative stress related degenerative diseases. These compounds also exhibit a wide spectrum of medicinal properties other than antioxidant properties, such as antimicrobial, anti-allergic, anti-inflammatory, anti-thrombotic, anti-carcinogenic, and anti-mutagenic activity, cardio-protective and vasodilatory effects. These observations may be used to substantiate the scientific reasoning that free radical scavenging is indeed the mode of operation of these plants in the treatment or prevention of the onset of deadly diseases. The survey of medicinal plants together with intense profiling research needs to be done in order to discover and progress these alternative choices of using phytochemical compounds. The targeted compounds should be employed in biomedical and pharmaceutical research ranging from *in vitro*, *in vivo*, and clinical trial steps to evaluate the safety, minimal or no toxicity, efficacy with fewer side effects, ease of incorporation in the health system due to their biological origins, ease to procurement and low manufacturing and trading costs. Furthermore, studies on the isolation and quantification of compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, and their effects through *in vivo* studies are needed to evaluate their natural biological function.

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**Table II. Qualitative phytochemical screening of *D. gangeticum* (L.) DC. leaf extracts**

| Chemical Constituents | Test Performed | Observation | Inference | | | |
|---|---|---|---|---|---|---|
| | | | AQ | AC | CF | PE |
| **Alkaloids** | Mayer's Test | Presence of turbidity | ++ | ++ | + | + |
| **Phenols** | Ferric Chloride Test | Bluish green color | + | ++ | + | + |
| **Reduced Sugar** | Fehling's Test | Brick red color | ++ | ++ | ++ | ++ |
| **Saponins** | Froth Test | Frothing persists 15 mins | + | + | + | + |
| **Tannins** | Ferric Chloride Test | Dark blue or greenish grey Cream ppt | + | ++ | - | - |
| **Terpenoids** | Salkowski's Test | Bluish-green color at interphase reddish color | +++ | - | - | - |
| **Flavonoids** | Ammonia Reduction Test | Yellow color | + | + | + | + |
| **Fixed oils and fats** | Filter Paper Test or Stain Test | Oil stain develops | +++ | +++ | +++ | +++ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Cardiac glycoside** | Keller- Killani Test | Violet ring below brown ring interphase | - | - | - | - |
| **Anthaquinones** | Borntrager's Test | Deep red or pink colour of aqueous layer | ++ | - | - | - |
| **AQ**- aqueous; **AC**- acetone; **CF**- chloroform; **PE**- petroleum ether. | | | | | | |
| '+++' relatively a strong presence; '++' relatively moderate presence; '+' relatively low presence, '-' indicates absence. | | | | | | |

**Table III. TLC of *D. gangeticum* (L.) DC. leaf extracts in mobile phase petroleum ether: benzene: methanol (16:3:2).**

| Solvent extract | Solvent run | Peaks obtained (cm) | $R_f$ values | Color bands in visible light | Spraying Reagents | Appeared bands | Phytochemicalsdetected |
|---|---|---|---|---|---|---|---|
| **AQ** | 14.0 | 3.9 | 0.278 | Orange | $FeCl_3$ | - | Phenols |
| | 14.0 | 3.5 | 0.25 | Orange | Ammonia solution | - | Flavonoids |
| | 14.0 | 4.4 | 0.314 | Orange | Conc. HCl | - | Saponins |
| **AC** | 15.0 | 13.5 | 0.9 | Light green | $FeCl_3$ | Green | Phenols |
| | | 14.5 | 0.966 | Dark green | | Green | Phenols |
| | 15.0 | 3.8 | 0.253 | Orange | Ammonia solution | Orange | Flavonoids |
| | | 13.4 | 0.893 | Yellow | | Yellow | Flavonoids |
| | | 14.9 | 0.993 | Yellow | | Yellow | Flavonoids |
| | | 15.0 | 1.00 | Orange | | Orange | Flavonoids |
| | 15.0 | 14.5 | 0.966 | Dark green | Conc. HCl | Dark brown | Saponins |
| **CF** | 15.0 | 12.0 | 0.8 | Dark green | $FeCl_3$ | Green | Phenols |
| | 15.0 | 3.3 | 0.22 | Orange | Ammonia solution | Orange | Flavonoids |
| | | 4.5 | 0.3 | Yellow | | Yellow | Flavonoids |
| | | 9.4 | 0.626 | Yellow | | Yellow | Flavonoids |
| | | 15.0 | 1.00 | Yellow | | Yellow | Flavonoids |
| | 15.0 | 12.0 | 0.8 | Dark green | Conc. HCl | Dark brown | Saponins |
| **PE** | 14.0 | 8.3 | 0.592 | Light green | $FeCl_3$ | Green | Phenols |
| | | 10.2 | 0.728 | Dark green | | Green | Phenols |
| | 14.0 | 3.7 | 0.264 | Orange | Ammonia solution | Orange | Flavonoids |
| | | 6.0 | 0.428 | Yellow | | Yellow | Flavonoids |
| | | 13.2 | 0.942 | Yellow | | Yellow | Flavonoids |
| | 14.0 | 10.2 | 0.728 | Dark green | Conc. HCl | Dark brown | Saponins |

**Table IV. Antioxidant activity test in extracted solvent offers a *D. gangeticum* (L.) DC.**

| Sr.No. | Solvent Extracts | DPPH radical scavenging (% absorbance) | Reducing power assay activity | Metal chelating activity (% absorbance) | Total phenolic contents |
|---|---|---|---|---|---|
| **1.** | Aqueous | 68.56 | 0.010 | 80.12 | 12.34 |
| **2.** | Acetone | 73.34 | 0.012 | 80.34 | 15.22 |
| **3.** | Chloroform | 41.23 | 0.056 | 45.23 | 10.56 |
| **4.** | Petroleum ether | 35.23 | 0.025 | 34.56 | 8.23 |
| **5.** | Ascorbic acid (10mg/ml) | 75.27 | 0.014 | 87.56 | 18.56 |

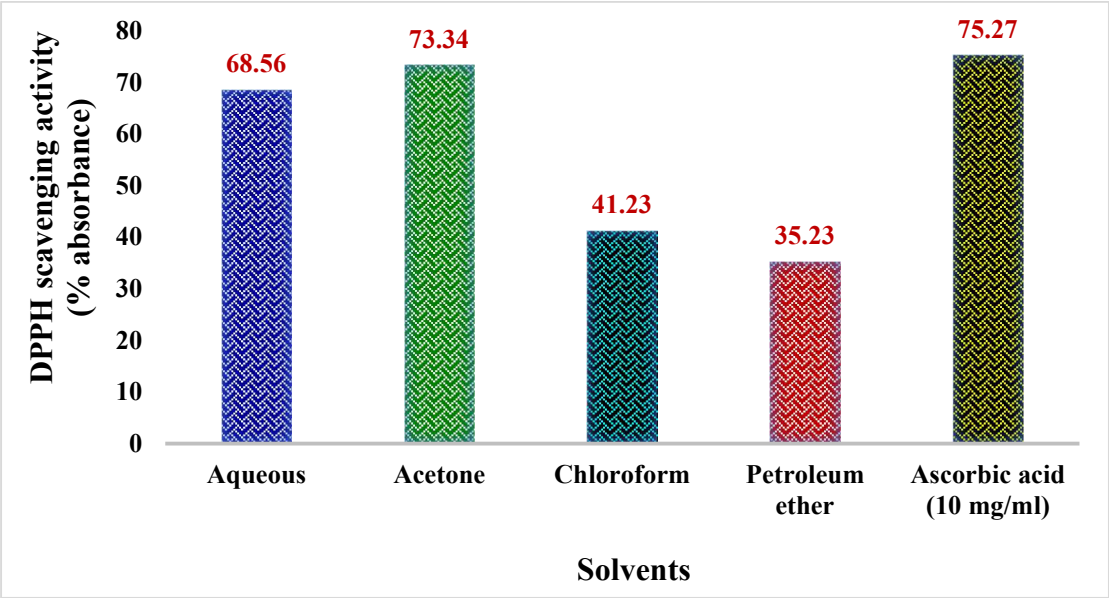



Fig. 1 DPPH radical scavenging activity of different solvent extracts of *D. gangeticum* (L.) DC. (leaf extracts).

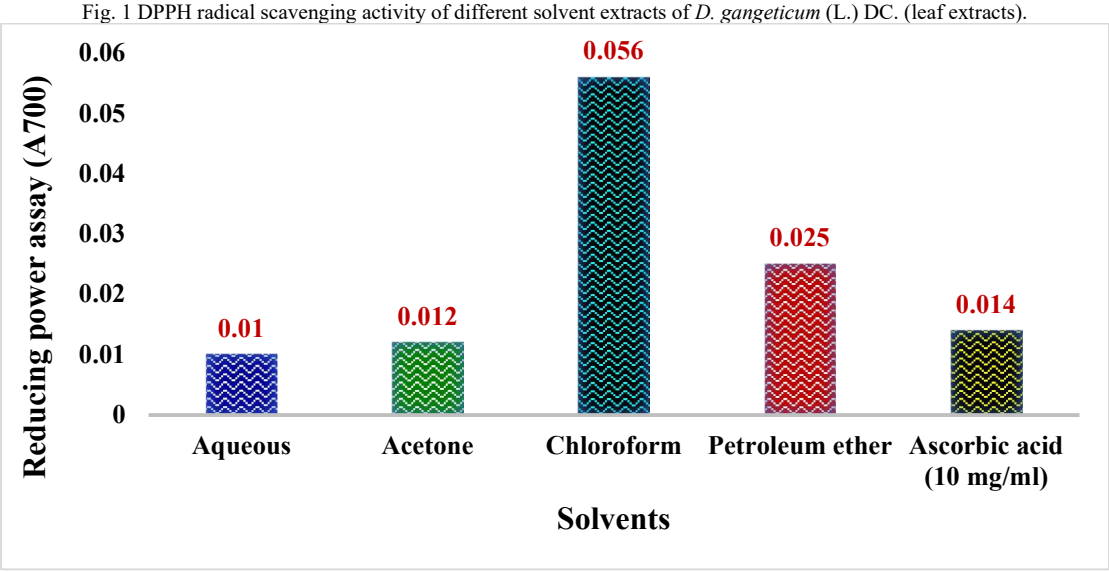



Fig. 2 Reducing power activity of different solvent extracts of *D. gangeticum* (L.) DC. (leaf extracts).

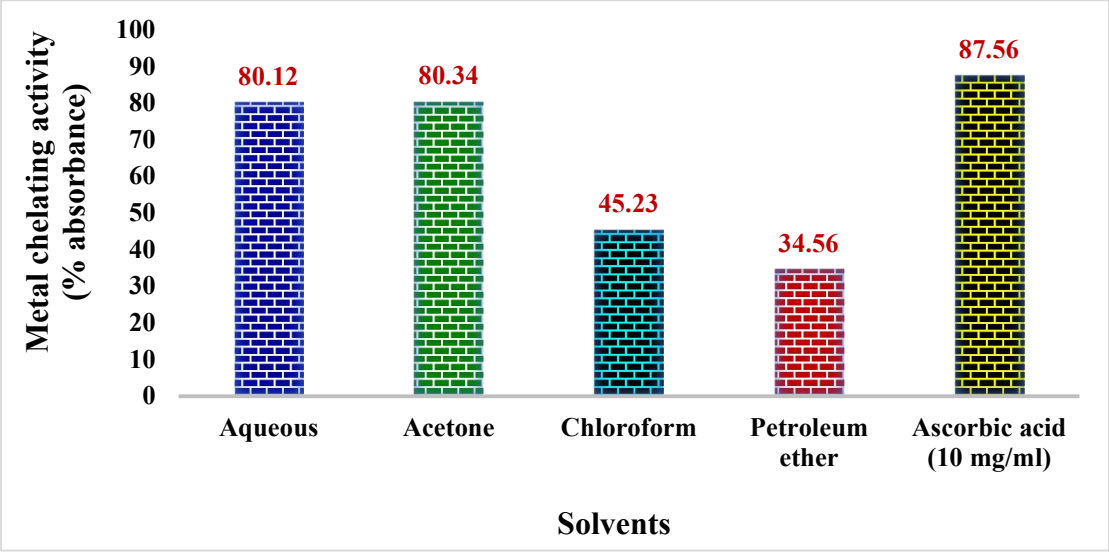



Fig. 3 Metal chelating activity of different solvent extracts of *D. gangeticum* (L.) DC. (leaf extracts).

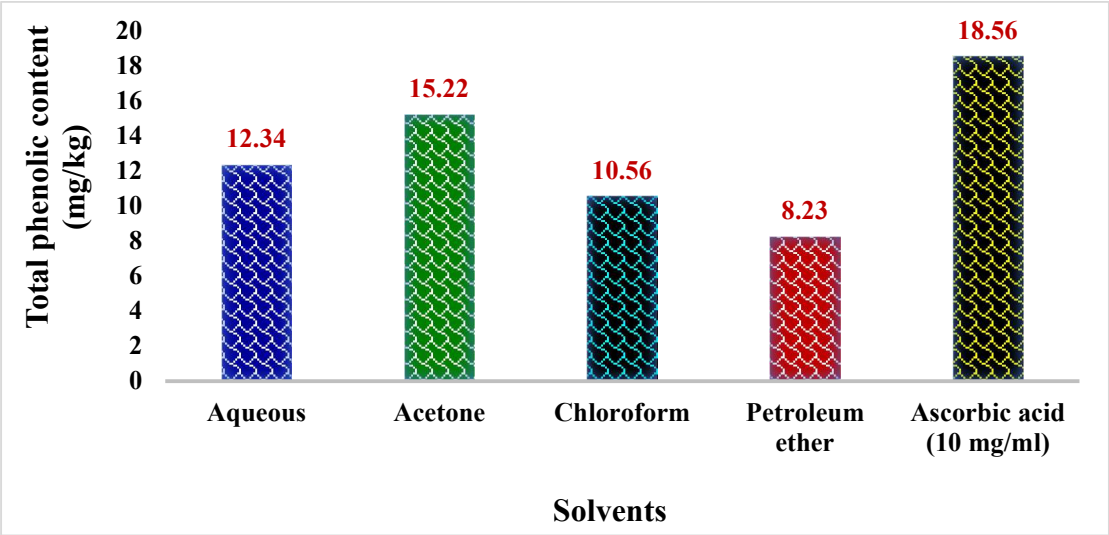
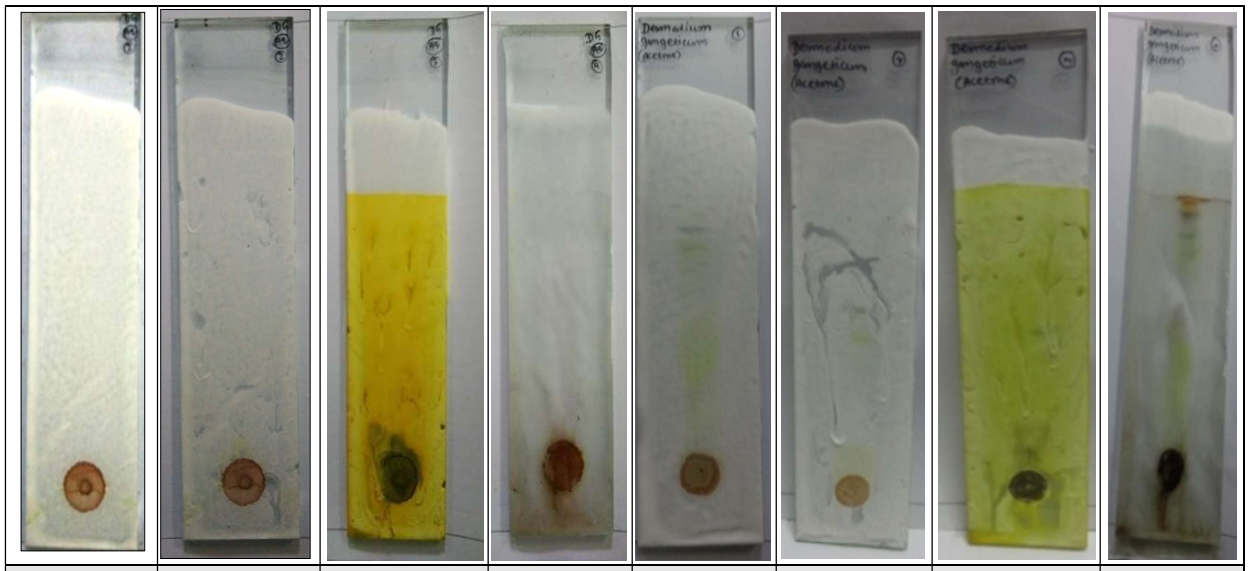



Fig. 4 Total phenolic content of different solvent extracts of *D. gangeticum* (L.) DC. (leaf extracts).

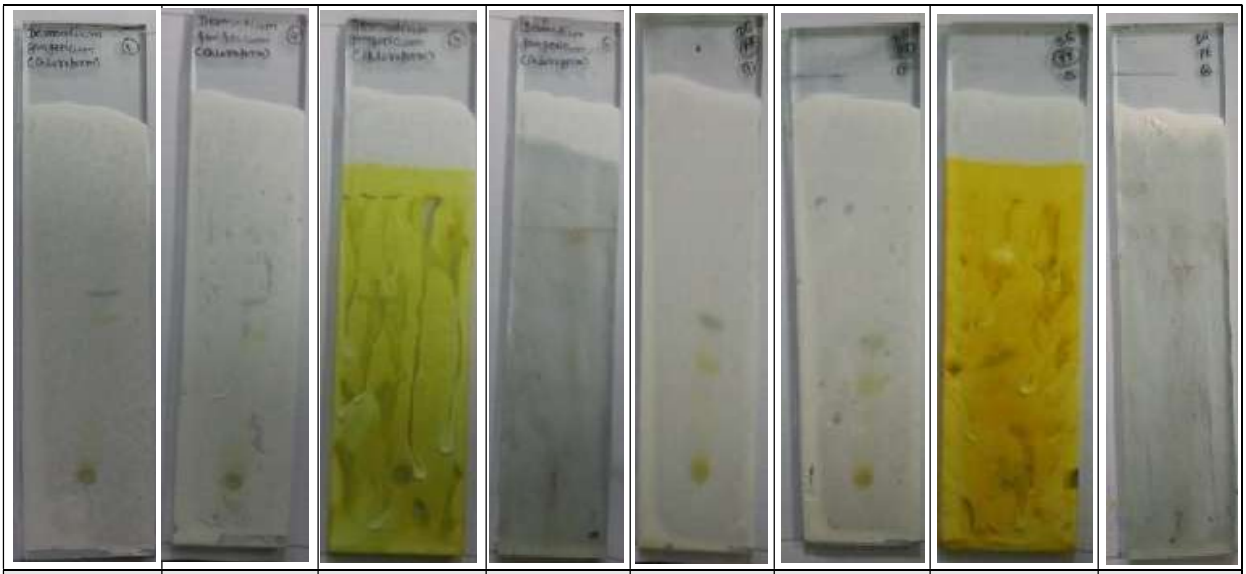
**Photoplate No. 1. TLC Chromatogram of *Desmodium gangeticum* (L.) DC. using different solvents leaf extracts**




**Photoplate No. 1(a)** TLC Plates of aqueous extracts of *D. gangeticum* (L.) DC.

**Photoplate No. 1(b)** TLC Plates of acetone extracts of *D. gangeticum* (L.) DC.



| | Phenols | Flavonoids | Saponins | | Phenols | Flavonoids | Saponins |
|---|---|---|---|---|---|---|---|

**Photoplate No. 1(c)** TLC Plates of chloroform extracts of *D. gangeticum* (L.) DC.

**Photoplate No. 1(d)** TLC Plates of petroleum ether extracts of *D. gangeticum* (L.) DC.