Research Article

Preliminary Phytochemical Screening and Toxicity Assessment of *Dodonaea viscosa*

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Abstract | Preliminary screening of phytochemicals is an essential process for identifying bioactive compounds in medicinal plants, which can potentially pave way for drug discovery and development. In this study, different methods were utilized for phytochemical screening of *Dodonaea viscosa*. The leaves of *D. viscosa* were collected and ground into a fine powder after drying. Extracts of varying concentrations were prepared using the powdered leaves. Cytotoxicity analysis of leaves extract of *D. viscosa* was done using brine shrimp (*Artemia salina*). Plant extracts were tested to figure out the various phytochemicals present. The presence of these phytochemicals (alkaloids, coumarins, flavonoids, phenols, saponins and tannins) was determined through various methods. Different surveys were performed in different areas to check the ethno medicinal uses of *D. viscosa*. The presence of alkaloids, coumarins, flavonoids, phenols, tannins ad saponins was confirmed in *D. viscosa*'s leaves extract. Proximate analysis was performed to evaluate and determine that the leaves of *D. viscosa* contain 9.6 % moisture, 90.4 % dry matter, 11.38 % crude protein, 2 % Crude fat, 11.5 % crude fiber and 6 % total ash. Rate of seed germination was found to be decreasing with increasing extract concentration while the rate of root inhibtion was increasing with increasing extract concentration applied. Root length of radish plant decreased up to 81.25 %. During surveys, information regarding ethno botanical aspects of *D. viscosa* was collected. Percentage of plant parts used as medicines were leaves 42.38%, fruits 14.76%, seeds 16.19%, whole plant 7.61%, roots 6.96%, all aerial parts 2.85%, legumes 1.90%, flowers 1.90%, shoots 0.95% and latex 5.45%. The current work concluded that the methanol extract of *D. viscosa* leaves exhibited a toxic effect on radish seeds. The result was confirmed as the number of nauplii death was found to be increasing by increasing extract concentration. Results indicated that alkaloid, coumarins, saponins, flavonoids, phenols, and tannins are present in *D. viscosa*.

Received | June 24, 2024; **Accepted** | November 6, 2025; **Published** | March 24, 2026
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Citation | Adnan, M., I. Munir, S.K. Chaudhari, S. Ashraf, H. Fatima, R. Ramash, A. Fatima and M. Nasir. 2026. Preliminary phytochemical screening and toxicity assessment of *Dodonaea viscosa*. *Sarhad Journal of Agriculture,* 42(1): 511-518.
DOI | https://dx.doi.org/10.17582/journal.sja/2026/42.1.511.518
Keywords | Phytochemicals, *Dodonaea*, Ethnobotany, Phytotoxic, Proximate analysis

Introduction

The Sapindaceae family, belonging to the order Sapindales, comprises approximately 1500 species of dicotyledonous flowering plants. These plants are predominantly found in tropical and subtropical regions (Xu and Deng, 2017). Among the 135 genera in the Sapindaceae family, *Dodonaea* has 68 species, most of which are small trees and shrubs. See Figure 1.

Dodonaea viscosa is an evergreen shrub popularly known as hop bush or Sanatha. While the word viscosa is taken from the Latin word viscosus (sticky) (Lawal and Yunusa, 2013). Despite being an Australian native, it can also be found in South Asia, South Africa and North America's subtropical, tropical regions and climates (Muhammad *et al*., 2016).

D. viscosa has numerous therapeutic properties, and indigenous peoples have utilized it for various purposes. It is a form of conventional medicine that is applied to the skin or taken orally to cure a variety of illnesses all over the world (Rani *et al*., 2009). *D. viscosa* was traditionally utilized in treatment of infections of skin, rheumatism, diarrhea, pain in stomach, splenic pains, and uterine colic. It is also used as antipruritic in rashes, used for treating dermatitis, hemorrhoids, and throat problems. An infusion of leaves was utilized as treatment of rheumatism, snake bites, gout hemorrhoids, and fractures (Rojas *et al*., 1996; Meenu *et al*., 2011). The initial phytochemical screening showed that *D. viscosa* contains alkaloids, carbohydrates, gums, glycerides, flavonoids, fixed oil and fat, mucilage, phenolics, saponins, reducing sugar, steroid and tannins (Lai-Bin *et al*., 2012; Kumar *et al*., 2013; Shobana *et al*., 2014; Shafek *et al*., 2015; Al-Snafi, 2017). *D. viscosa* is known for its antidiabetic (Akhtar *et al*., 2011; Rani *et al*., 2012), antimicrobial (Naidoo *et al*., 2012), insecticidal (Kannaian *et al*., 2012), antioxidant (Shafek *et al*., 2015), cytotoxic (Shafek *et al*., 2015), antifertility (Kumar *et al*., 2013), anti-inflammatory and analgesic, antiulcer (Arun *et al*., 2008), anti-diarrheal (Rajamanickam *et al*., 2010) and detoxification (Sivanesan *et al*., 2009) properties.

Materials and Methods

Sample preparation and experimental detail

The plant's leaves were collected from Bhakkar, Pakistan, cleaned and dried at room temperature (in lab), then ground, and made a fine powder of these leaves. All of the chemicals and compounds used in this experiment, as well as those listed below, were obtained from The University of Lahore. The methanol extraction process comprised of adding 250 g of plant powder in 750 ml of methanol. This mixture was subjected to a Soxhlet rotary apparatus for 36 hours, ensuring that the temperature stayed below the boiling point of methanol. After extraction, the filtrate was filtered using Whatman No. 1 filter paper. The extract was then transferred to beakers and was allowed to sit at room temperature for one week to allow solvent evaporation. Subsequently, the extracts were dried and concentrated by means of a laboratory vacuum rotary evaporator at 40°C. After concentration, the extracts were weighed, labeled, and stored in sterilized bottles at 4°C for further analysis.

Phytotoxic effect

The phytotoxic activity of *D. viscosa* was determined using two different assays. (Turker and Camper, 2002; Islam *et al*., 2016). Five different amounts of methanol extract (0, 10, 100, 500, and 1000 ppm) were used in the root length inhibition and radish seed germination assay. The radish seed germination assay and root inhibition were conducted following Turker and Usta protocol.

Determination of root length inhibition

All seeds underwent sterilization using sodium hypochlorite for ten minutes, followed by rinsing with distilled water. Each 90 mm diameter petri dish was lined with two sheets of Whatman No. 1 filter paper. In each petri plate, 5 ml of methanol extract at

Figure 1: *inflorescence of Dodonaea viscosa*

five different concentrations (10, 100, 500, and 1000 ppm) was dispensed using a pipette. After allowing the solvent to evaporate, 5 ml of distilled water was added to each petri plate. Subsequently, ten seeds were placed in each petri plate, which was tightly sealed and then incubated at 23°C. The length of the roots of all seeds was measured after 1, 3, and 5 days of incubation. The percentage of growth inhibition was calculated using the formula provided in the study.

Growth inhibition percentage = (100[PC – PT])/PC

PT represents the length of roots of seeds treated with the extracts, while PC represents the length of roots of seeds not treated with the extracts (control group).

Determination of germination rate

This experiment aimed to evaluate the potential toxicity of methanolic extracts from *D. viscosa*. Petri dishes lined with filter papers were prepared similarly to the root inhibition experiment. However, this experiment utilized a larger number of radish seeds (hundred seeds) and three different extract concentrations (10, 100, and 1000 ppm). The germination process was monitored daily, and the germination rate was recorded over a period of five days. A germination index was calculated using a specific formula. A control group with no extract treatment was included as a standard reference. The petri dishes were maintained at room temperature, and the daily germination rate was observed and recorded for five days.

Proximate composition analysis

Proximate analysis was utilized to determine the percentages of moisture content, dry matter, protein, fat, and crude fiber. The moisture content was determined using Nancy Trumann's approach.The crude protein percent was estimated using the well-known Kjeldahl method also followed by Onwuka, 2005, and fat was extracted using Soxhlet apparatus.

Moisture content percentage

Nancy Trumann's and Tom Richard's approach was used to determine the moisture content of *D. viscosa*. After weighing a little container, 1g of plant material powder was placed in it and oven dried at 105-110°C for 24 hours of time. Finally, the sample was reweighed, the container's weight was removed, and the moisture content was estimated using the formula below.

$$Mn = \frac{(Ww - Wd)}{Ww \times 100}$$

Mn stands for the moisture content (%) of the substance n, Ww for the sample's wet weight, and Wd for the sample's dry weight.

Dry matter percentage

Dry matter was calculated using the formula.

Dry matter percentage = 100 – Moisture content

Protein percentage

To calculate samples, total nitrogen content, micro Kjeldahl was used. In a digestion flask, 1 gram of leaf powder of *D. viscosa* was combined with 3 grams of mercury sulphate digestion mixture ($HgSO_4$) and potassium sulphate (K_2SO_4) in a 1:9 ratio and 20 ml concentrated H_2SO_4. In a digesting machine, the samples were boiled for the duration of two hours until clear contents were obtained. A 250 mL amount of the digested product was diluted. In the micro Kjeldahl distillation apparatus, ten ml were added, where they were distilled with 50 mg of Zn dust and 10 ml of NaOH (40 percent). A receiver flask containing 5 mL of boric acid (2%) and methyl red as a color indicator was used to capture the distillate. The receiver's contents were titrated against sulfuric acid until they achieved the end point of a pale pink color. The volume of acid was used to compute the nitrogen percentage, and the %age of protein was obtained by using the following formula:

$$Nitrogen\ Percentage = \frac{(\text{volume of 0.1 N H2SO4} \times 0.0014 \times 250 \times 100)}{(\text{sample weight} \times 10)}$$

Protein Percentage N × 6.25

Fat percentage

To quantify the lipid contents and eliminate the ether soluble component, petroleum ether, a dried sample was extracted (400–600C) in a Soxhlet system. To obtain lipid content, following formula was used:

Fat Percentage = (Weight of either extract × 100) / (Weight of sample)

Crude fiber extract

The plant material was boiled in 1.25 percent NaOH and 1.25 percent H_2SO_4 to dissolve acid soluble and alkali components. To get a consistent weight, crude fiber remnants were dried. The crude fiber was calculated using the weight loss on igniting in a muffle furnace at 500°C.

Ash percentage

On an oxidizing flame, a dried 1 g sample was carbonized until no fumes emerged. It was then warmed in a muffle furnace at 600 degrees Celsius to burn any organic stuff.

Ash percentage = Weight of Ash × 100

Qualitative phytochemical analysis of plant extracts

Qualitative phytochemical tests for determining presence or absence of alkaloids, flavonoids, phenol, coumarins, saponins and tannins were conducted following standard protocols of Ismail *et al.* (2017).

Questionnaire

- **Name of participant** .....
- **Date**.....
- **Location**.....
- **Gender**.....
- (a) Male(b) Female
- **Age**
- (a)24-34(b)35-44(c) 45-55(d) 55-64 (e)65- 75 (f) 75 and above
- **Occupation**
- Residence of Participant .....
- (a) Rural(b) Urban
- **Parts of plants which are used**
- (a)Leaf (b) stem (c) Bark (d) Flower (e) Fruit (f) root (g) seed (h) whole plant
- **Method of utilization**
- (a)Decoction (b) powder (c) infusion (d) Raw (f) cooked (g) paste (h) juice (i) jam
- **Disease Category**
- (a) antidote(b) Dermatological problems(c) Gastrointestinal diseases (d) Glandular Disorders (I) Gynecological diseases (f) Infectious diseases (g) Musculoskeletal Disorders (h) Respiratory diseases(i) Respiratory diseases (j) Urogenitalproblems (k) Others.

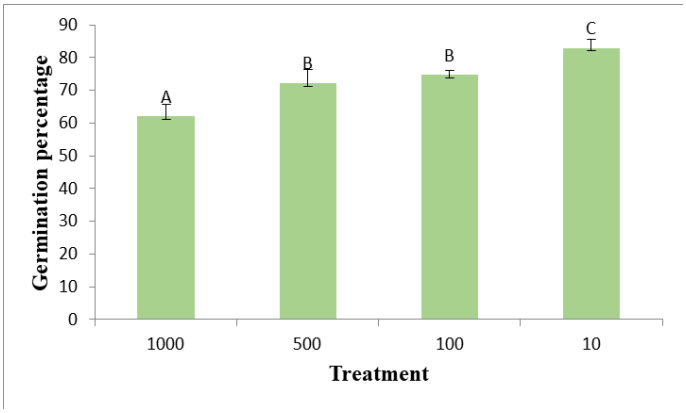

Figure 2: *Effect of Dodonaea viscosa extract on germination of radish seeds*

Results and Discussion

Phytotoxic potential of D. viscosa leaves extract

Radish Seed Germination Assay

Effect of D. viscosa on Radish seed Germination

Data in Figure 2 explain the effect of *D. viscosa* on germination of radish seeds. Seed germination rate decreased by the increase in concentration of extract applied. In 0 ppm, 10 ppm, 100 ppm, 500 ppm and 1000 ppm, means of seeds germination were 89.667, 83.00, 74.667, 72.33and 62.00.

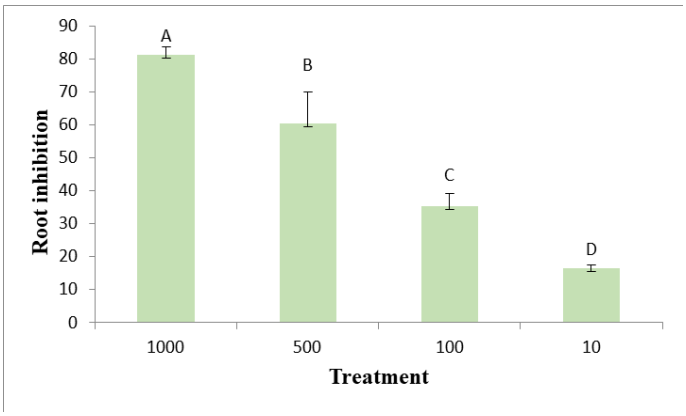

Figure 3: *Effect of Dodonaea viscosa on root length inhibition of radish seed*

Root length inhibition by D. viscosa

Data in Figure 3 explains the effect of *D. viscosa* extract on root inhibition of radish seeds. The rate of root inhibtion increased with increasing extract concentration applied. Root length of radish plant decreased up to 81.25 %. Means of root inhibition were 16.458%, 35.42%, 60.42%, 81.25% in 10ppm, 100ppm, 500ppm and 1000ppm respectively. *D. viscosa* is thought to have considerable allelopathic potential and could be studied for weedicidal and insecticidal properties. (Barkatulllah *et al.*, 2010).

Cytotoxicity assessment of D. viscosa leaves extract

The cytotoxic potential of *D. viscosa* was investigated using Brine shrimp (*Artemia salina)*. The result shown in Table 2 was confirmed as the number of nauplii death was found to be increasing by increasing extract concentration. Ten-ppm showed mortality rate 13.33

Table 1: *Cytotoxicity*

Oncentration	No. of nauplii that were taken	No. of nauplii that shown dead	Total survivors	Percentage of mortality	LC50 µg/ml
10	30	04	26	13.33	186
100	30	13	17	43.33	
1000	30	22	08	73.33	

%, 100ppm showed 43.33 % and 1000 ppm showed 73.33 %. LC50 value for *D. viscosa* was 186 µg/ml. Similar to our results, Prakash *et al.* (2012) also discussed that *D. viscosa* leaf extracts are toxic to shrimp larvae, with EEA extract showing the most toxicity with 70% mortality (LC50=95.46 mg/ml). Both non polar and polar extracts were deadly to Brine shrimp. Aziz *et al.* (2013) reported that defense secondary metabolites, such as flavonoids, phenols, saponins, or other compounds, may be responsible for the cytotoxicity of extracts.

Table 2: *Phytochemical analysis of Dodonaea viscosa*

Name of compound	Result (Presence/absence)
Alkaloid	Present
Coumarins	present
Flavonoids	Present
Phenols	Present
Saponins	Present
Tannins	Present

The cytotoxic effects of *D. viscosa* leaves extract has also been checked on animals. Ramasamy *et al.* (2022) investigated *D. viscosa's* cytotoxicity. The MCF Cell Line and the Trypan Blue Assay were used to test *viscosa* leaf extract for inhibition of cancer cell lines. The research confirmed the therapeutic *D. viscosa* potential against the MCF-7 cell line. Shafek *et al.* (2015) checked cytotoxic effects of leaves extract of *D. viscosa* on a breast cancer cell line (MCF7). Compared to the standard treatment (cisplatin), which had an IC50 of 5.48 g/ml, the 80 percent ethanolic extract of *D. viscosa* had a far higher cytotoxic activity, with an IC50 of 19.4 g/ml. According to a study, the standard growth delay of *D. viscosa* by ethanol extract fractions was 0.66, 0.08, 0.29, and 0.20, respectively, and the percentage increase in life span (ILS) was 33.33%, 38.09%, and 52.35% (Table 1).

Proximate analysis of D. viscosa

Proximate analysis was performed to evaluate and determine that the leaves of *D. viscosa* contain 9.6 % moisture, 90.4 % dry matter, 11.38 % crude protein, 2 % Crude fat (Figure 4).

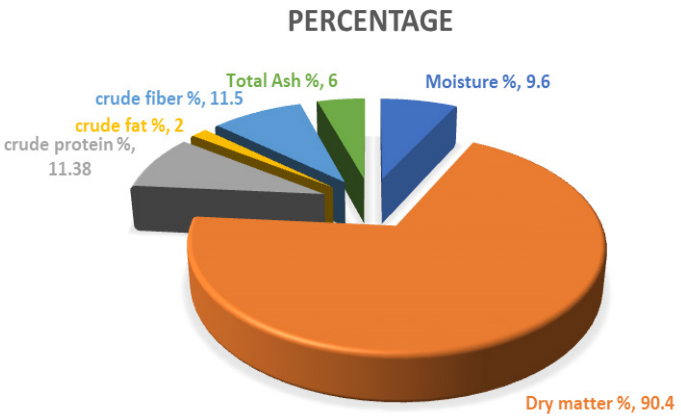

Figure 4: *Proximate analysis of Dodonaea viscosa*

Phytochemical analysis of D. viscosa

Chemical compounds known as active ingredients are in charge of therapeutic actions like pharmacological and anti-microbial capabilities. To determine the existence of these active ingredients, preliminary screening of plant crude extracts is commonly performed (Kar, 2007). This class of phytochemicals are also referred to as vital nutrients. *D. viscosa* had all of the necessary chemicals such as alkaloids, flavonoids, phenols, tannins, gum sticky, saponins, steroids and sugar and these findings are in agreement with the findings of Boncler *et al.* (2014). The current

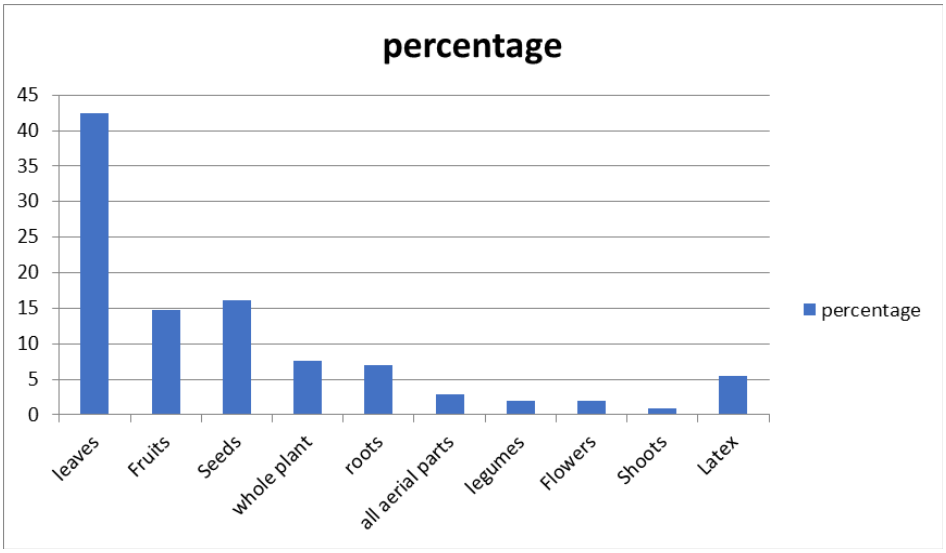

Figure 5: *Percentage of plant plants used as medicine*

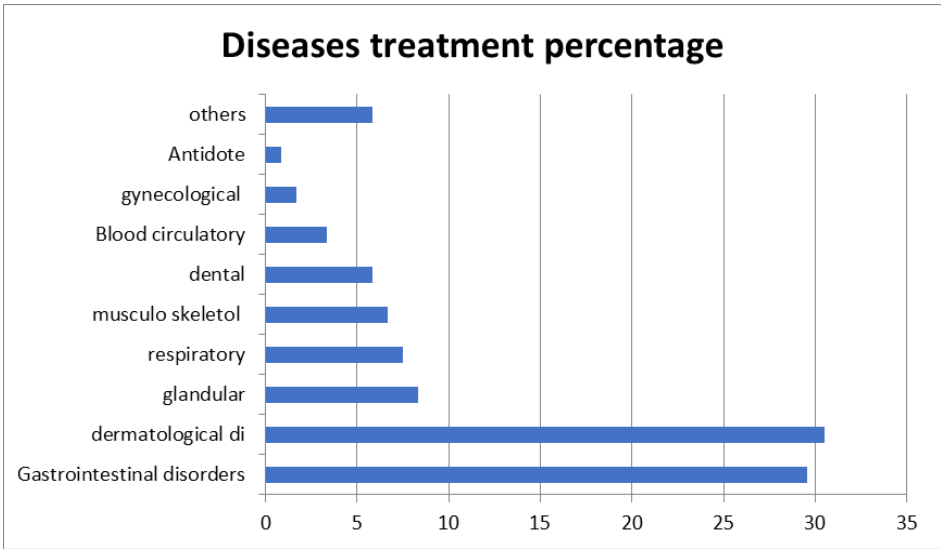

Figure 6: *Diseases treatment percentage of Dodonaea viscosa*

work used *D. viscos's* leaves methanolic extract to do phytochemical analysis, whereas ethyl acetate extract of *D. viscosa* also revealed that the flavonoids, alkaloids, terpenoid, tannins are present in the stem bark. These findings proposed various phytochemical components when extracted using different solvents. Kumar *et al.* (2013) discovered in their research that alkaloids, triterpenoids, amino acids, and cardiac glycosides were lacking, however flavonoids, saponins, tannins, reducing sugars, and steroids were abundant. The phytochemical analysis of *D. viscosa* revealed that it contains coumarins, flavonoids, saponins and tannins (Abdel mogib *et al.*, 2001; Veerapur *et al.*, 2010). Several scientists (Punitha and Manoharan, 2006) have identified coumarins, phenolic and flavonoids as bioactive anti-diabetic principles (Shani *et al.*, 1974). *D. viscosa's* (Sapindaceae) chemical study revealed the presence of alkaloids, carbohydrates, flavonoids, fixed oil and fat, glycosides, gums, mucilage, reducing sugars, saponins, tannins, steroid phenols and trace elements. *D. viscosa* shown different antioxidant, anti-diabetic, anti-microbial, anti-fertility, analgesic, anti-ulcer, anti-spasmodic cytotoxic, insecticidal and detoxifying properties in pharmacological tests. The findings are in agreement with those of Al-Snafi (2017). Phytochemical screening stated that *D. viscosa* include flavonoids, alkaloids, steroids, fixed fat and oils, phenols, saponins, gums, tannins, mucilage, reducing sugar, carbohydrates and glycerides according to the researchers (Kumar *et al.*, 2013; Jawahar *et al.*, 2004; Ramya *et al.*, 2011) (Table 2).

Ethno botanical aspects of D.viscosa

During surveys, information regarding ethno botanical aspects of *D. viscosa* were collected.Percentage of plant parts used as medicines was leaves 42.38%, fruits 14.76%, seeds 16.19%, whole plant 7.61%, roots 6.96%, all aerial parts 2.85%, legumes 1.90%, flowers 1.90%, shoots 0.95% and latex 5.45% (Figure 5).

According to information collected regarding *D. viscosa* uses, 29.53% plant parts were used for alimentary and digestive tract (stomach cooling, digestion, constipation, diarrhea, dysentery, smelly mouth, flatulence, nausea, pain in stomach, vermifuge and stimulant), 30.5% plant parts were used for dermatological and ear, nose and throat disorders (Burns, Eczema, cutaneous, antiseptic, secondary syphilis), 8.33% plant parts were used for glandular disorders (Diabetes, jaundice, spleen problems), 7.5% plants were used for respiratory diseases (Asthma, lungs diseases like cough, cold, bronchitis, whooping cough), 6.66% plants were used as muscular skeletal diseases (Backache, headache, joints pain as pain killer), 5.83% plants were used for dental disorders, 3.33% plants were used forblood circulatory disorders (blood purifiers, high blood pressure, heart inflammation), 1.66% plants were used for gynecological disorders, 0.83% plants were used for antidote (snake bite, insect bite),and 5.83% plants were used for other diseases (heat stroke, obesity, swelling of body, cancers, tumors, allergy, inflammation (Figure 6).

Conclusions and Recommendations

Our research concludes that *D. viscosa contains very essential phytochemicals which include* flavonoids, alkaloids, phenols, saponins, coumarins and tannins, and may have the medicinal value. We observed phytotoxic and cytotoxic effects of methanolic leaf extracts of *D. viscosa, which* exhibited its possible applications in pharmacological industry. The future research may be focused on isolation and characterization of the bio-compounds of *D. viscosa* to explore its specific role. Further, toxi-pharmacological

studies are recommended to be planned, so that therapeutic potential of *D. viscosa* compounds may be evaluated. Moreover, there is need for the sustainable cultivation of *D. viscosa to enhance its use in relevant industries (*herbal and pharmaceutical*).*

Novelty Statement

Concurrent phytochemical screening and in vivo acute toxicity assessment are what make this work novel. It provides fundamental evidence that connects the ethnobotanical use of the plant with pre-liminary biosafety parameters necessary for additional pharmacological research.

Authors' Contribution

Muhammad Adnan: : First draft writing
Iqra Munir: Statistical analysis
Sunbal Khalil Chaudhari: Supervision
Sana Ashraf: Conceptualization
Muhammad Nasir, Hira Fatima, Roha Ramash, Aimen Fatima: Final draft editing

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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