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Research Article

PHARMACOGNOSTICAL, PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT ACTIVITY OF HYDROMETHANOL EXTRACT OF *GORDONIA DIPTEROSPERMA* KURZ.

PHAIBIANG LAPASAM*, ANDREW LALTHASANGA, FREDDY TEILANG NONGKHLAW, ZOTHAN PUIA

Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Mizoram University, Zemabawk, Aizawl, India.
Email: phaibianglapasam14@gmail.com

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ABSTRACT

Objective: This research is to evaluate the pharmacognostic parameter, phytochemical analysis, and *in vitro* antioxidant properties of hydromethanol extract.

Method: This study carries out the collection and authentication of the plant, extraction, pharmacognostic study, preliminary phytochemical screening, and antioxidant property of leaves hydromethanol extract were evaluated by scavenging the following free radicals – DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide, nitric oxide, and reducing power. One-way analysis of variance (ANOVA) followed by Dunnett's test was performed. The minimum value of *p<0.05 considered as significant, **p<0.01 and ***p<0.001.

Results: Powder microscopy of the leaves showed the presence of stomata, calcium oxalate crystals, trichome, fibers, and oil glands. The total ash was considered to be 3.75%, water-soluble ash 1.25%, and acid-soluble ash 3%. Hydromethanol (3:7) extract yielded 38.8%, moisture content 15.6%. Preliminary phytochemical screening of the extract showed the presence of carbohydrates, glycoside, saponin, phenol, tannin, flavonoid, and steroid. The total flavonoid content was considered to be 32.25 mg/g of quercetin and the total phenolic content of the extract was found 610 mg/g of gallic acid. The IC_{50} radical scavenging effect of extract and gallic acid was considered to be 3.62 and 3.46 for 2,2-diphenyl-1-picrylhydrazyl, 10.4 and 24.73 for hydrogen peroxide, and 48.76 and 58.83 for nitric oxide.

Conclusion: The phytochemical constituents of the extract were well-known pharmacologically active chemicals and significant antioxidant potential was shown by the extract. This study finds out the rationality of the use of this plant as a medicinal plant. Further studies would be needed to explore their potential as a treatment for fever, diarrhea, and dysentery.

Keywords: *Gordonia*, Meghalaya, Antioxidants, Free radicals, Pharmacognostical parameters, Extraction.

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INTRODUCTION

Nature has got a complete storehouse of remedies to cure all ailment of humankind. Since prehistoric, humans have roamed the earth in search of food and they have learned through their experienced which can be edible and which are not [1]. Traditional medicine is popularly practiced in a different part of the world, especially people residing in remote areas, they depend on traditional medicine as their first line of service for health care. Various natural sources are utilized, such as plants, animals, microorganisms, and marine organisms, in medicine to alleviate and treat diseases [2]. According to the WHO, traditional medicine includes diverse health practice, approach knowledge and beliefs, incorporating plants, animals, and/or mineral-based medicines, spiritual therapies, manual techniques, and exercise which can be used to maintain well-being as well as to treat, diagnose, or prevent illness [3].

Gordonia is a genus of flowering plants, family – Theaceae, related to *Franklinia, Camellia*, and *Stewartia* of roughly 40 species. *G. dipterosperma* Kurz. is an evergreen tree growing to 10–20 m tall. They are discovered in the East Himalayas, Nepal to Northeast India, China, Virginia, Florida, and Louisiana [4-6]. The species is adapted to acidic soils. They required high rainfall. The flowering time is in the late winter to early spring [7,8].

Free radicals are substances which are highly reactive and unstable that are produced in the body as a by-product of metabolism or exposure to toxins in the environment such as tobacco, smoke, and ultraviolet light since they are unstable, they tend to donate or accept an electron from another molecule, therefore, they act as oxidants or reluctant which lead to chains reaction which damage cell of an organism. An antioxidant is a molecule that terminates the chain reaction to balance the oxidative stress the plants and animals produce their antioxidant molecules such as glutathione, enzymes, or the dietary antioxidant; Vitamin C and Vitamin E [9].

G. dipterosperma Kurz. is used as a medicinal plant by Jaintia tribal people of Meghalaya, India, both bark and leaves are prepared by simple decoction and filtered, the filtrate is orally consumed for the treatment of fever, diarrhea, and dysentery. Since the plant is not properly documented, pharmacognostic studies are performed for standardization and quality control of the leaves. Phytochemical screening is important to find out the presence of active constituents present in the leave which gives the therapeutic response.

The present study has been made to evaluate the pharmacognostic parameter, preliminary phytochemical screening, total phenolic content, total flavonoid content, and *in vitro* antioxidant activity of hydromethanolic extract of *G. dipterosperma* Kurz. leaves.

MATERIALS

Collection of plants

The leaves of *G. dipterosperma* Kurz. were collected in July 2019 from Khlookynrin village, Meghalaya, India. The leaves were subsequently processed cleaned, washed to remove dust, and unwanted matter then shade dried and pulverized into coarse powder for extraction.

Authentication
The plant was gathered during the flowering seasons for preparing the herbarium sheet and identified by Dr. Chaya Deori (scientist in charge) Botanical Survey of India, Shillong, bearing a voucher specimen no. (BSI/ERC/Tech/2019-20/200).

Macroscopical evaluation
Macroscopical study is the morphological description of the leaf which can be seen by naked eyes and it was performed by following the standard methods to determine the shape, size, color, and odor of the leaves of *G. dipterosperma* Kurz. [10].

Microscopical evaluation
Microscopical evaluations are the histological character of the organized drug and it was made on a qualitative basis [10].

Transverse section of leaves
A fine section of the leave was cut with a razor blade and mounted in a glass slide with a drop of glycerin, covered with a coverslip, place the slide on the stage of Digital Microscope (Truechrome-2) Zeiss, and observed using 10X and 45X lens, each distinguishes character was noted down.

Powder microscopy
For powder analysis, the shade-dried leaves were pulverized and passed through sieve no. 40. This was observed under a Digital Microscope (Truechrome-2) Zeiss for the presence of plant's components such as stomata, trichome, fibers, starch, and calcium oxalate crystals.

Physicochemical parameters

Determination of ash value

A 4 g of powder drug was taken in a tarred silica crucible and incinerated at 450°C in muffle furnace till the ash was free from carbon or it becomes white or pale white. The crucible was cooled in a desiccator and weighed. The percentage of total ash was calculated regarding air-dried drugs [11,12].

$$\%\text{Total ash} = \frac{X - Y}{N} \times 100$$

Where,
X=The weight of crucible dish + ash obtained
Y=The weight of the empty dish
N=Weight of crude drug taken

Acid-insoluble ash
The total ash was boiled with 25 ml of 2 N HCl for 5 min, filtered with ashless filter paper, washed the insoluble matter with hot water, and incinerated at 450°C in a muffle furnace. Then, it was cooled in a desiccator. The weight of the remaining ash was taken. The percentage of acid-insoluble ash was calculated regarding the air-dried drug.

$$\%\text{Acid - insoluble ash} = \frac{X - Y}{N} \times 100$$

Where,
X=The weight of crucible dish + ash obtained
Y=The weight of the empty dish
N=Weight of crude drug taken

Water-soluble ash
The total ash was boiled with 25 ml of water for 5 min, filtered with ashless filter paper, washed the insoluble matter with hot water, and then ignited in muffle furnace not exceeding 450°C. The weight of insoluble matter was subtracted from total ash; the percentage of water-soluble ash was calculated regarding the air-dried drug.

$$\%\text{Water - soluble ash} = \frac{X - Y}{N} \times 100$$

Where,
X = The weight of crucible dish + ash obtained
Y = The weight of the empty dish
N = Weight of crude drug taken

Determination of moisture content
A 5 g of the powder crude drug was taken in a tarred porcelain dish and dried in the oven for 5 h at 100–105°C, cooled in a desiccator, and weighed. The percentage of loss on drying was calculated regarding the air-dried drug [13].

$$\%\text{Moisture content} = \frac{X - Y}{N} \times 100$$

Where,
X=The weight of porcelain dish + crude drug obtained
Y=The weight of the empty porcelain dish
N=Weight of crude drug taken

Fluorescence analysis
A small quantity of powder drug was treated with various reagents and observed in visible light, under UV with short wavelength (254 nm) and long wavelength (365 nm). The color observed by application various reagents in different radiation was recorded [14,15].

Extraction
The leaves of *G. dipterosperma* Kurz. were extracted by maceration using hydromethanol (3:7) as a solvent. A 250 g of coarse powder was taken and place in a stoppered container, the solvent was added till the powder drug submerged in the solvent and kept for 3 days with frequent agitation, filtered with filter paper to separate the powder particle from the filtrate. The extract was concentrated using a rotary evaporator (RV-8. IKA) and the obtained extract was dried in a water bath till it becomes viscous and further dried at room temperature [16].

Preliminary phytochemical screening
The hydromethanol leaf extract was analyzed with various tests to find out the phytoconstituent such as alkaloids, carbohydrate, phenolic, flavonoid, glycosides, saponin, protein, and amino acid [17].

Total phenolic content
The total phenolic content of the extract was performed spectrophotometrically according to the Folin–Ciocalteu method. A 1 ml (50 µg/ml) of extract treated with 5 ml of Folin's reagent (10-fold dilution), after 4 min, 4 ml of 20% sodium carbonate was added, kept in dark at room temperature for 1 hour, and the absorbances were measured at 750 nm. Gallic acid was used as a reference standard. The total phenolic content was quantified by a calibration curve obtained by measuring the absorbance of the known concentration of gallic acid [18,19].

Total flavonoid content
The total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetry assay. To 1 ml of extract, 2 ml of methanol, 3 ml of 5% sodium nitrite, and 0.3 ml of 10% aluminum chloride were added and kept for 6 minutes. A 2 ml of sodium hydroxide (1 M) was added and the volume was made up to 10 ml with methanol and kept for 1 hour at room temperature. The absorbance was measured at 510 nm, the total flavonoid content was expressed as mg quercetin equivalent per gram of leaves of *G. dipterosperma* Kurz. [18,19].

***In vitro* antioxidant activity**

DPPH radical scavenging activity

DPPH radical scavenging activity was carried out as described by Shimada *et al.* (1992). A 1 ml of different concentrations of the extract was mixed with 5 ml freshly prepared 0.1 mM of DPPH. The reaction mixture was kept in the dark for 50 min at room temperature. Ascorbic acid was used as a reference standard. The absorbance

was measured at 517 nm. Radical scavenging activity of DPPH was calculated [20]

$$\text{Scavenging effect } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Nitric oxide scavenging activity

The extract was prepared at a concentration of 20–100 µg/ml and stored at 4°C. Griess reagent was prepared by mixing an equal amount of 1% sulfanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid immediately before used. A 0.5 ml of 10 mM sodium nitroprusside which was previously dissolved in phosphate buffer saline was taken and to this, 1 ml of different concentrations of the hydromethanol extract was added, incubated at 25°C for 180 min after incubating the extract was mixed with an equal volume of freshly prepared Griess reagent. Control was prepared to add all the reagents instead of extract, methanol was added. The absorbance was measured at 546 nm [21].

$$\text{Scavenging effect } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (40 mM/L) was prepared in phosphate buffer (pH – 7.4). A 0.6 ml of prepared hydrogen peroxide solution was taken and to this, 1 ml of different concentrations of the extract was added, keep for 10 min at room temperature, the absorbance was observed at 230 nm [22].

$$\text{Scavenging effect } (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Reducing power assay

The hydromethanolic extract was diluted at different concentrations (20, 40, 60, 80, and 100 µg/ml). A 1 ml of each dilution was mixed with 2.5 ml of phosphate buffer ($_{p}$H – 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min. After cooling, 2.5 ml of 10% TCA was added and centrifuge for 10 min at 3000 rpm (rotation/minute). A 2.5 ml of supernatant was diluted with 2.5 ml of distilled water, to this add 0.5 ml of freshly prepared 0.1% ferric chloride solution and mixed. The absorbance of the mixture was measured at 700 nm and recorded. Ascorbic acid was used as a reference standard. The higher absorbance indicates an increase in reducing power [22].

Statistical analysis

All measurements were taken in triplicates for each sample which was shown as Mean±SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's test. $p<0.05$ was considered statistically significant *$p<0.05$ and **$P<0.01$ when compared with control. All analyses were done with the statistical software GraphPad InStat.

RESULTS

Macroscopical characters

The morphological characteristic of the leaves shown in Fig. 2, it shows that the leaves have green color on both sides, the petiole is 0.5–1 cm in length, the midrib is prominent, the venation of the leaves is not prominent, the leaves are 6–15 cm in length and 3.5–5.5 cm in wide, it has a smooth surface with an elliptical shape and acuminate apex, the margin of the leaves is entire/denticulate, the leaves are odorless.

Microscopical evaluation

A fine transverse section of the fresh leaves shows the anatomy of the leaves such as lower epidermis, sclerenchyma, phloem, xylem, collenchyma, palisade, and the upper epidermis, as shown in Fig. 3.

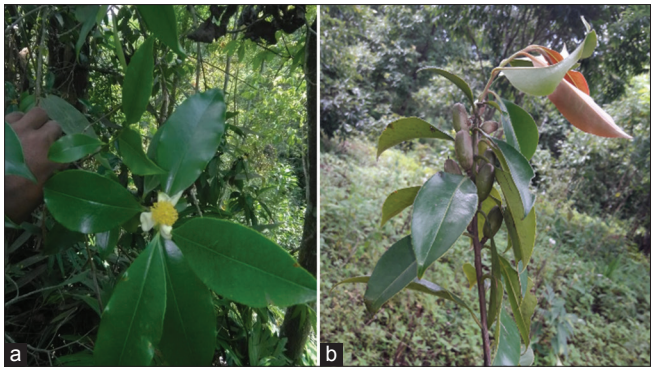

Fig. 1: (a) Flower and leaves, (b) fruit and leaves of *Gordonia dipterosperma* Kurz. (Theaceae)

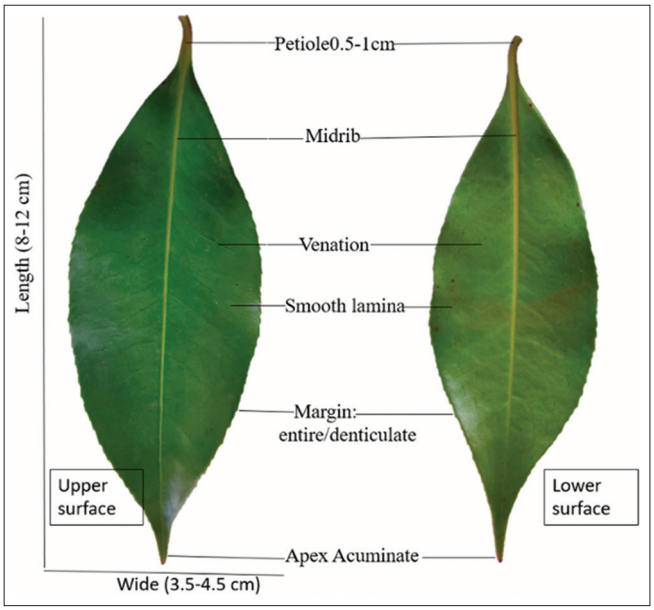

Fig. 2: Organoleptic and morphological characteristics of the leaf of *Gordonia dipterosperma* Kurz

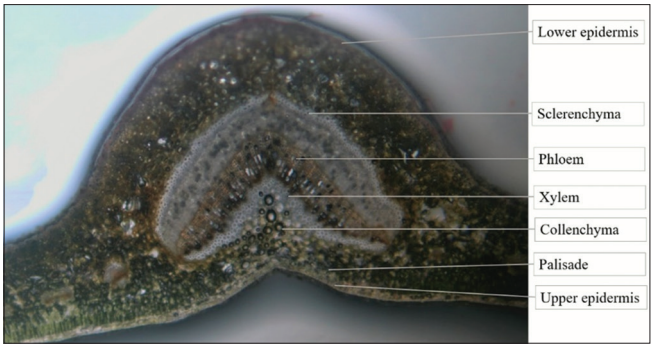

Fig. 3: T. S of leave of *Gordonia dipterosperma* Kurz. shows the anatomy of the leaves

Powder microscopy

Powder microscopy of the leaves identified the different components of the leaves, such as stomata, trichome, fibers, calcium oxalate crystal, and an oil gland, as shown in Fig. 4.

Physicochemical evaluation

Ash value of leaves powder

Moisture content
The moisture content of the leaf powder was found to be 15.6% w/w.

Fluorescence analysis
Fluorescence analysis of power leaves shown in Table 2.

Extraction
The percentage yield of hydromethanol (3:7) extracts was found to be 38.8% w/w.

Phytochemical screening
Phytochemical screening of hydromethanol extract showed positive for carbohydrate, phenols, tannins, flavonoids, glycoside, and saponin, as shown in Table 3.

Symbol (+++) indicates present in high concentration. (++) indicates present in moderate concentration.

Table 1: Percentage of total ash, water-soluble ash, and acid-insoluble ash

S. No.	Ash value	%w/w
1	Total ash	3.75
2	Water-soluble ash	1.25
3	Acid-insoluble ash	3

Total phenolic content
The total phenolic content of the extract was considered to be 610 mg per gram of gallic acid.

Total flavonoid content
The total flavonoid content of the extract was found to be 32.25 mg per gram of quercetin.

***In vitro* antioxidant activity**

DPPH radical scavenging activity

DPPH (2-2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay it is based on an electron transfer that produces a violet solution in methanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The percentage inhibition on DPPH radicals of the extracts was compared with the standard ascorbic acid. The study was performed in triplicate. The hydromethanol extract scavenging activities show dose dependent. The IC_{50} of hydromethanol extract and ascorbic acid is 3.62 and 3.46 mcg, respectively, presented in Table 4.

Nitric oxide scavenging activity
Leaves hydromethanol extracts exhibited antioxidant activity through competing with oxygen to scavenge for the nitrite radical which was generated from sodium nitroprusside at physiological pH in an aqueous environment. The maximum free radical scavenging activity and

Table 2: Fluorescence analysis of powder leaves

S. No.	Treatment of powder	Visible light	Short wavelength UV rays (254 nm)	Long wavelength UV rays (365 nm)
1.	Powder as such	Green	Dark brown	Dark green
2.	Powder + 50% H_2SO_4	Dark green	Greenish-brown	Dark green
3.	Powder + 50% HNO_3	Yellow	Greenish-brown	Dark green
4.	Powder + 5% KOH	Yellowish-brown	Brownish-green	Dark green
5.	Powder + Methanol	Light green	Brownish-green	Dark green
6.	Powder + 1N HCl	Pale green	Dark brown	Dark brown
7.	Powder + Cold H_2O	Green	Dark brown	Dark brown
8.	Powder + Picric acid	Yellowish-green	Greenish-yellow	Green
9.	Powder + Hot H_2O	Light green	Dark brown	Black
10.	Powder + 1N NaOH	Reddish-brown	Greenish-brown	Dark green
11.	Powder + Formic acid	Dark green	Greenish-brown	Dark green
12.	Powder + Calcium chloride	Green	Dark brown	Brown
13.	Powder + Ammonia (25%)	Red	Reddish-brown	Dark brown
14.	Powder + diethyl ether	Green	Brown	Brownish-green
15.	Powder +Acetic acid	Pale yellow	Dark green	Dark green
16.	Powder + $FeCl_3$	Dark brown	Greenish-brown	Dark green

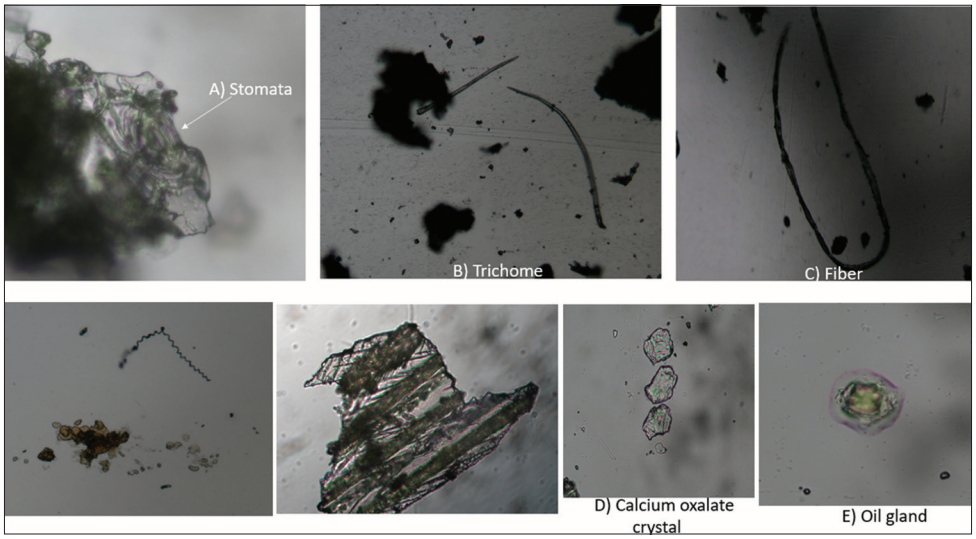

Fig. 4: Powder microscopy of the leaves observed under Digital Microscope (Truechrome-2). All the components are observed using ×10 and ×45

Table 3: Phytochemical screening

S. No.	Phytochemical test	Maceration (hydromethanol extract)
1.	Carbohydrates	++
2.	Phenols and tannins	++
3.	Flavonoid.	++
4.	Glycoside	++
5.	Saponin	+++

Table 4: DPPH radical scavenging activity of hydromethanol extract and standard ascorbic acid

Concentration	Ascorbic acid (Mean±SEM)	Extract (Mean±SEM)
20	46.33±0.08**	53.30±0.41**
40	81.86±0.30**	73.48±0.20**
60	90.28±0.15**	85.75±0.20**
80	95.03±0.08**	93.48±0.05**
100	96.27±0.02**	97.92±0.08**
IC_{50}	3.46 mcg	3.62 mcg

All values represented as Mean±SEM, n=3. One-way analysis of variance (ANOVA) followed by Dunnett's test (compare all vs. control) was performed. p<0.05 considered significant, **p<0.01, ***p<0.001.

Table 5: Nitric oxide scavenging activity of methanol extract and gallic acid

Concentration	Gallic acid (Mean±SEM)	Extract (Mean±SEM)
20	40.92±0.748**	41.71±0.239**
40	60.23±1.743**	70.33±0.692**
60	75.10±0.598**	71.90±0.403**
80	78.27±0.951**	72.74±0.369**
100	79.64±1.741**	72.43±1.363**
IC_{50}	24.73 mcg	10.41 mcg

All values represented as Mean±SEM, n=3. One-way analysis of variance (ANOVA) followed by Dunnett's test (compare all vs. control) was performed. p<0.05 considered significant, **p<0.01, ***p<0.001.

Table 6: Hydrogen peroxide scavenged activity of hydromethanol extract and standard ascorbic acid

Concentration	Ascorbic acid (Mean±SEM)	Extract (Mean±SEM)
20	53.77±0.016**	54.69±0.011**
40	63.65±0.016**	63.83±0.133**
60	78.95±0.092**	75.33±0.020**
80	86.50±0.021**	84.50±0.014**
100	91.59±0.021**	90.24±0.043**
IC_{50}	9.44 mcg	8.29mcg

All values represented as Mean±SEM, n=3. One-way analysis of variance (ANOVA) followed by Dunnett's test (compare all vs. control) was performed. p<0.05 considered significant, **p<0.01, ***p<0.001.

potency are presented in Table 5. The study was performed in triplicate. The hydromethanol extract scavenging activities show dose dependent. The scavenging activity was expressed in terms of percentage inhibition. IC_{50} values of gallic acid and extract were 24.73 μg and 10.41 μg, respectively.

Hydrogen peroxide scavenging activity

Hydrogen peroxide itself is not just very reactive but it can be toxic to cells as it provides radicals in the cell. Thus, removing H_2O_2 is of great important for the protection of food. The study was performed in triplicate. The hydromethanol extract scavenging activities show dose dependent. The IC_{50} of hydromethanol extract and ascorbic acid was found to be 8.29 and 9.44 mcg, respectively. H_2O_2 radical scavenging activity is given in Table 6.

Table 7: Rates of reducing power between concentration and absorbance

Concentration	Ascorbic acid (Mean±SEM)	Extract (Mean±SEM)
20	0.08±0.034	0.12±0.034
40	0.18±0.003	0.24±0.030
60	0.25±0.005	0.44±0.012
80	0.43±0.005	0.55±0.010
100	0.59±0.005	0.78±0.033

All values are represented as Mean±SEM, n=3.

Reducing power assay

In this assay, reducing ability was measured by the change of Fe^{3+} to Fe^{2+}, and the color changes from yellow to dark green when the reaction took place. The absorbance kept increasing with an increase in concentration which shows that the extract has a great potential and electron donor ability for stabilizing free radicals. The reading is given in Table 7.

DISCUSSION

Pharmacognostical parameters are the first step to evaluate the distinctive character and to establish the identity and quality of crude drugs. In this research work, the macro and microscopical of the leave are studied and the distinctive character of the leaves was identified. Ash value is the remaining residue after incineration of the crude drug which represents inorganic salt which naturally occurred in the crude drug. Ash value is used to determine the quality and purity of crude drugs. The moisture content of crude drugs should be minimal level to discourage the growth of bacteria yeast and fungi during storage, excessive moisture content of crude drugs can lead to decomposition either chemical change or microbial contamination. Fluorescence is a phenomenon exhibited by various chemical constituents present in the plant material. Organic molecules absorbed radiation at a specific range of wavelength and many of them reemit such radiation called luminescence. The phytochemical analysis shows the presence of various secondary metabolites such as phenol, flavonoid, glycoside, and saponin, which are potential for further studies of antimicrobial, anti-inflammatory, and anticancer activity. The total phenolic content and total flavonoid content of a hydromethanol extract are maximum and it shows significant antioxidant potential and it can be viewed as a source for natural antioxidant.

CONCLUSION

Traditional medicine is most trusted and reliable by many of the population as it has no side effect or less side effect compared to synthetic drugs and safe to take for the long term. The pharmacognostic study such as morphological, microscopical, ash value, and loss on drying is the parameter that helps in identifying the distinctive character of the plant to prevent adulteration and substitution and they are the basic tool for determining the percentage purity of crude drugs. Fluorescence analysis of powder crude drug shows varied fluorescence character which is essential for the standardization of plants. The phytochemical screening of hydromethanol extract finds out the secondary metabolite or bioactive compound present in the leaves of the plant. The hydromethanol extract possesses good antioxidant activity against free radical DPPH, nitric oxide, hydrogen peroxide, and ferric ions (Fe^{3+}). The presence of phenol, flavonoid, saponin, tannins, and glycosides supports the rationality of the use of this plant as a medicinal plant. Further studies would be needed to explore their potential as a treatment for fever, diarrhea, and dysentery.

CONFLICTS OF INTEREST

There are no conflicts of interest or financial support.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to this research work.

AUTHORS' FUNDING

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