Phytochemical Screening and Proximate exploration of *Diapensia himalacia* leaf extract

[1]Arnab Bagchi

[1]Assistant Professor, Himalayan Pharmacy Institute

Majhitar, Rangpo, East Sikkim. Pin- 737136, India.

[2]Bapi Ray Sarkar*

[2]Associate Professor, Department of Pharmaceutical Technology

University of North Bengal(NBU), Raja Rammohunpur, Darjeeling, West Bengal . Pin-734013, India.

[3]Prosanta Pal

[3]Assistant Professor, Department of Pharmaceutical Technology

University of North Bengal(NBU), Raja Rammohunpur, Darjeeling, West Bengal . Pin-734013, India.

***Corresponding Author**

Dr. Bapi Ray Sarkar

Associate Professor, Department of Pharmaceutical Technology,

University of North Bengal (NBU),

Darjeeling, West Bengal . Pin-734013, India.

E-mail: **brspublication@gmail.com**

ABSTRACT:
The goal of the current study was to examine the phytochemical and proximate profile of Diapensia himalacia leaf extracts. A key source of treatment for a variety of human maladies has been medicinal plants. An important criteria to spot drug adulteration or inappropriate treatment was the pharmacognostic evaluation of the plant material. Ash values of Diapensia himalacia leaves were examined in the current study. The extractive yields of leaves from Diapensia himalacia were determined using five different solvent extracts (petroleum ether, chloroform, ethyl acetated, methanol, and water). Both the leaf powder and the solvent extracts underwent a fluorescence analysis. In both leaf samples, total ash levels were found to be higher than acid insoluble and water soluble ash. When extracted in a methanol solvent, the leaf extracts under study demonstrated substantial extractive yields. The highest amounts of extractive yield, total ash, water soluble ash, and acid insoluble ash were found in Diapensia himalacia. Diapensia himalacia leaf extracts and leaf powder underwent fluorescence analysis, which exhibited unique colouring with various chemicals.

KEYWORDS: Diabetes mellitus, Diapensia himalaica, Phytochemicals, Ash value, Extractive yield.

I. INTRODUCTION:

To combat oxidative stress, plants have evolved a variety of defence mechanisms (antioxidant systems). There are several distinct antioxidants in these systems, each with a unique composition, mechanism, and location of action, including ascorbic acid, gluthione, uric acid, tocopherol, carotenoids, and (poly) phenols[1]. Diabetes mellitus (DM) is a severe, long-lasting, and complicated metabolic condition with numerous aetiologies and dangerous acute and long-term effects. Diabetes, or DM as it is also called, affects people in both industrialised and developing nations, creating a significant socioeconomic challenge. This disease is thought to afflict 25% of the world's population. The development of diabetes is greatly influenced by both genetic and environmental factors. Because insulin, a peptide hormone that controls blood glucose, does not operate adequately on target tissues throughout the development of diabetes, the body's cells are unable to efficiently metabolise sugar[2]. The most recent survey in 219 nations found that 382 million people aged 20 to 79 had diabetes mellitus, and it is predicted that this number would rise to 592 million by 2035[3]. The medicinal plants that treat diabetes could be useful in the search for safer hypoglycemic drugs. 1,200 uncommon plants are thought to have antidiabetic potential worldwide, according to ethnobotanical statistics[4]. Because they contain a wide range of chemical substances that can have a clear physiological effect on humans, medicinal plants have been utilised to cure human diseases for thousands of years. Alkaloids, tannins, flavonoids, terpenoids, saponins, and phenolic compounds are the most significant of these substances[5].

II. MATERIALS AND METHODS

Materials:

Chemicals, Reagents: HPTLC grade petroleum ether, chloroform, methanol, toluene, ethyl acetate, water, and formic acid were purchased from Merck (India). Concentrated sulphuric acid (conc. H_2SO_4), concentrated hydrochloric acid, glacial acetic acid, Fehling's solution A & B, dragendroff's reagent, ammonia, aluminum chloride ($AlCl_3$), magnesium ribbon, iodine, potassium iodide, potassium acetate, L-ascorbic acid, trichloroacetic acid, ferric chloride, potassium ferricyanide, gelatin, Folin-ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate and methanol were purchased from Sisco Research Laboratories (SRL) Pvt Ltd. All other chemicals were used analytical grade.

Plant Material: In the month of June 2021, leaf samples of Diapensia himalaica were obtained in the North Sikkim region of India. The species was verified by Dr. Manoranjan Chowdhury of the Department of Botany at the University of Northbengal in West Bengal, India. For future use, the accession number for the plant is 11778.

Methods:

Preparation of the different solvent extracts from the leaves of Diapensia himalaica using soxhlet apparatus :

250 g of coarsely powdered leaf samples that were dried in the shade Using the hot continuous percolation method (Soxhlet), Diapensia himalaica was extracted with solvents of increasing polarity such as petroleum ether, chloroform, ethyl acetated, methanol, and water. The various extracts were then concentrated using a rotary vacuum evaporator (Buchi) at 50 °C, dried in a vacuum dessicator, and stored at - 20 °C until further use[6].

A. **Phytochemical Screening:** Phytochemicals (Greek: *phyton* = plant) are chemical compounds naturally present in the plants attributing to positive or negative health effects. The richest bioreservoirs of different phytochemicals are found in medicinal plants that are used to treat various illnesses and disorders. The phytochemical components of plants determine their therapeutic qualities. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other essential phytochemicals are found in diverse plant sections[7].

a) **Test for triterpenoids and steroids:**

1) **Libermann-Buchard test**:First, a test sample of 10 mg was placed in a test tube with 1 ml of chloroform and 1 ml of acetic anhydride. 2 ml of strong sulfuric acid were then added to the mixture. The existence of triterpenoids and steroids was established by the formation of a reddish-violet ring at the intersection of two layers. [8].
2) **Noller test:** In a test tube, 2ml of pure thionyl chloride containing 0.01% anhydrous stannic chloride was added. Five milligrammes of the test sample were added to the solution, turning it purple. Triterpenoids are present when the purple colour changes further to deep crimson [9].
3) **Salkowski Test:** Following the dissolution of 10 mg of the test material in 1 ml of chloroform, 1 ml of concentrated sulfuric acid was added. The presence of steroids is shown by the chloroform layer's reddish blue hue and the acid layer's green fluorescence [10].

b) **Detection of flavanoids:**

1) **Magnesium and hydrochloric acid reduction:** A small piece of magnesium ribbon, 5 ml of alcohol, and a few drops of concentrated HCl acid are added once the extract (50 mg) has been dissolved. If any pink to scarlet colour appears, flavanol glycosides are likely present[11].

c) **Detection of tannins and phenolic compounds:**

1) Ferric chloride test: It takes 50 milligrammes of the extract to dissolve in 5 ml of distilled water. A few drops of 5% natural ferric chloride solution are then added to this. Phenol compounds are indicated by a dark green colour [12].

2) Gelatin test: 5 ml of distilled water are used to dissolve the extract (50 mg), and 2 ml of 10% sodium chloride are then added. Phenol compounds are indicated by a white precipitate [13].

3) Lead acetate test: Three millilitres of a 10% lead acetate solution are added after the extract (50 mg) has been diluted in distilled water. Phenol compounds are indicated by a large white precipitate[14].

d) **Test for Saponins:** A tiny amount of the test sample was dissolved in some distilled water, added to a graduated cylinder, and agitated for 15 minutes. The presence of saponins was verified by the stable foam generation.A little amount of the test material was dissolved in methanol before being mixed with a 1% lead acetate solution. The appearance of white precipitate indicated saponins were present[15].

e) **Detection of alkaloid:** Solvent free extract, 50 mg is stirred with few ml of dilute HCL and filtered. The filtrate is tested carefully with various alkaloid reagents as follows:

1) **Mayer's test:** A drop or two of Mayer's reagent are added by the side of the

test tube to a few ml of filtrate. A white or creamy precipitate denotes a positive test result [16].

2) **Wagner's test**: By the side of the test tube, add a few drops of Wagner's reagent to a few ml of filtrate. A reddish-brown precipitate shows a positive test result [17].

f) **Dragendorff's test:** Few millilitres of filtrate Dragendroff's reagent is poured into the test tube in increments of 1 or 2 ml. A noticeable yellow precipitate indicates a positive test result [18].

1) **Hager's test:** By the side of the test tube, add 1 or 2 ml of Hager's reagent to a few ml of filtrate. A noticeable yellow precipitate indicates a positive test result [19].

g) **Detection of glycosides:** The following test is performed on 50 mg of extract after it has been hydrolyzed with strong hydrochloric acid for two hours on a water bath.

1) **Borntrager's test:** The presence of glycosides is detected by adding pink colour to 2 ml of filtered hydrolysated after adding 3 ml of chloroform and shaking. The chloroform layer is then separated, and 10% ammonia solution is added to it. [20].

2) **Legal test:** Pyridine is used to dissolve 50 mg of the drug extract, and then 10% sodium hydroxide is used to make the solution alkaline by adding a sodium nitroprusside solution. Pink hue indicates glycoside presence [21].

3) **Keller – killiani test:** A few drops of ferric chloride and concentrated sulfuric acid are added to a drug extract in glacial acetic acid. A reddish-brown hue develops [22].

h) **Detection of carbohydrate:** The extract (100gm) is dissolved in 5 ml of water andfiltered. The filtrate is subjected to the following test.

1) **Molish's test:** Alpha-naphthol alcoholic solution is added to 2 ml of filtrate in two drops, the mixture is thoroughly shaken, and 1 ml of conc. Slowly pouring sulfuric acid into the test tube's side, it is then left to stand. Carbohydrates are present as violet rings [23].

2) **Fehling's test**: A red ppt indicates the presence of sugar when 1 ml of filtrate is cooked on a water bath with 1 ml of Fehling solution A and 1 ml of Fehling solution B, respectively[24].

3) **Benedict's test:** 1ml of Benedict's reagent is added to 0.5 ml of filtrate. For two minutes,the mixture is boiled in a bath of boiling water. Precipitates with a distinctive colour indicate the presence of sugar [25].

4) **Barfoed's test**: Barfoed's reagent is added to 1 ml of filtrate and heated on a water bath for 2 minutes. Sugar is present when red precipitates are present [26].

B. Proximate analysis:

The crude medicine (leaves) underwent proximate analysis. Using the method outlined under each heading, the following conclusions were reached

1) **Moisture content**:

5g of the air-dried, raw medication were accurately weighed and placed in a tarred watch glass.The drug was dried in a hot air oven at 105°C for a while until it reached a constant weight. the different weight that was obtained. The weight difference determines the drug's moisture content [27].

2) Extractive value:

The coarsely powdered leaf samples of Diapensia himalaica was subjected to extraction. The extractive yield was expressed as[28].

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the Dry extract (g)}}{\text{Weight of the Sample used for extraction}} * 100$$

a) Water-soluble extractives:

Diapensia himalaica leaf powder weighing 5g should be macerated for 24 hours in a closed flask with 100ml of chloroform water. allowing for six hours of regular shaking, followed by 18 hours of standing. In order to prevent the solvent from leaking out, it was quickly filtered. Before being weighed at 105°C, a 25mL sample of the filtrate was dried to dryness in a shallow dish with a flat bottom and tarred bottom. Using the air-dried medication as a benchmark, the amount of the extractive value that is water soluble was estimated [29]

b) Alcohol soluble extractives:

5g of coarse, air-dried *Diapensia himalaica* leaf powder should be macerated in 100ml of 95% ethanol for 24 hours in a closed flask. allowing for six hours of regular shaking, followed by 18 hours of standing. In order to prevent the solvent from leaking out, it was quickly filtered. Before being weighed at 105°C, a 25 mL sample of the filtrate was dried to dryness in a shallow dish with a flat bottom and tarred bottom.using the air-dried medication as a benchmark to determine the proportion of extractive value that is alcohol-soluble [30].

3) Fluorescence analysis:

Put 0.5g of plant powder into test tubes that have been cleaned and dried. Distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulphuric acid, nitric acid, hydrochloric acid, 5% $FeCl_3$, 5% I_2, picric acid, 1N NaOH, and 1N NaOH + methanol were all separately added to each tube in amounts of 5ml. The tubes were then shaken and let to stand for roughly 20 to 25 minutes. The resulting solutions were examined for their distinctive colour under visible daytime light, UV light of short wavelength (254 nm), and UV light of long wavelength (365 nm) [31].

4) Ash value Determination

Total ash, Acid Insoluble and Water soluble ash content were done as per standard procedure.

a) Determination of total ash

Diapensia himalaica leaf powder samples were correctly weighed in separate silicon crucibles that had been lit and weighed (350 °C for 1 hour). On the bottom of the crucible, dried leaf materials were distributed in the form of a thin layer. The crucible was burned in a muffle furnace (Nabertherm) at a temperature not to exceed 450 °C until it turned white, signifying they were carbon-free. The crucible was weighed after cooling. Calculating the percentage of total ash was done using the air-dried powder [32].

b) Determination of acid insoluble ash

The ash that was obtained in accordance with the instructions for calculating total ash was cooked in 25 ml of 2 N HCL for 5 minutes. On an ashless filter paper, the insoluble ash was gathered and then washed with hot water. Transferred into a pre-weighed silica crucible, the insoluble ash was burned for 15 minutes at a temperature no higher than 450 °C. When

calculating the proportion of acid-insoluble ash, the air-dried powder was used as a reference [33].

c) Determination of water soluble ash

The ash used to calculate total ash was cooked in 25 ml of water for 5 minutes. On an ash-free filter paper, the insoluble material was collected and then hot water was used to wash it away. Transferred into a silica crucible that had been previously weighed, the insoluble ash was fired for 15 minutes at a temperature no higher than 450 °C. To obtain the constant weight, the process was repeated. The weight of the total ash was calculated after deducting the weight of the insoluble material. The water soluble ash was assumed to be the difference in weight. When calculating the percentage of water soluble ash, the air-dried powder was taken into consideration [34].

III. RESULTS AND DISCUSSION

RESULTS:

Analyses of the phytochemistry of *Diapensia himalacia* leaf parts revealed the absence of glycosides and the presence of flavonoids, alkaloids, carbohydrates, tannin, saponins, and triterpenoids. The outcomes are presented in Table 1.

Table 1: Phytochemical Investigations of leaf extracts of *Diapensia himalacia*

Chemical Compound	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Water extract
Triterpenoids and Steroids	(+)	(+)	(+)	(+)	(+)
Flavonoids	(-)	(+)	(+)	(+)	(-)
Tannins	(-)	(-)	(+)	(+)	(-)
Saponins	(-)	(-)	(-)	(+)	(+)
Alkaloids	(-)	(+)	(+)	(+)	(-)
Glycosides	(-)	(-)	(-)	(-)	(-)
Carbohydrate	(+)	(+)	(+)	(+)	(+)

The moisture content of Diapensia himalacia leaf powder was assessed in the current study which was found 7.4% w/w. The results are shown in Table 2**.**

Table 2: Moisture Content Values

Fresh Weight (g)	Dry Weight (g)	Loss on Drying (g)	Moisture Content (% w/w)
5gm	**4.53**	**0.37**	**7.4**

The ash value of Diapensia himalacia leaf powder was assessed in the current experiment, it was observed that percentage of total ash, acid insoluble ash and water soluble ash were 10.86%, 0.27% and 0.10% reapectively. The results are shown in Table 3.

Table 3: Ash values

Ash Value	*Diapensia himalacia*
Total Ash	10.86%
Acid Insoluble Ash	0.27%
Water soluble Ash	0.10%

To determine the percentage yield of each solvent extracts, five different solvent extracts of Diapensia himalacia leaf samples were examined the percentage extractive value of Alcohol Soluble Extract and Water Soluble Extract were 38.66% (%w/w) and 13.33% (%w/w) respectively. The results are given in Table 4.

Table 4: Extractive values of leaf extracts of *Diapensia himalacia*

Sl. No.	Extracts	Extractive Value (%w/w)
1.	Alcohol Soluble Extract	38.66%
2.	Water Soluble Extract	13.33%

The powdered samples of Diapensia himalacia leaves were subjected to various chemical treatments for fluorescence analysis, and both visible light and UV light (356 nm) observations were made. Table 5 presents the results that were achieved.

Table 5: Fluorescence analysis of leaf extracts of *Diapensia himalacia*

Leaf Powder	Treatment With Chemical Reagent	Short Weave (254nm)	Long Weave (365 nm)	Visible Light
Leaf Powder	Alcoholic NaOH	Dark Green	Light Green	Radish Brown
Leaf Powder	Aqueous NaOH	Dark Green	Dark Green	Yellowish Brown
Leaf Powder	Con. H_2SO_4	Dark Brown	Dark Green	Dark Brown
Leaf Powder	Con. HNO_3	Dark Green	Light Green	Yellow
Leaf Powder	1N HCL	Dark Green	Green	Yellowish Brown
Leaf Powder	Acetic Acid	Dark Green	Dark Green	Dark Brown
Leaf Powder	$Fecl_3$	Dark Green	Light Green	Green
Leaf Powder	Iodine	Dark Green	Greenish Yellow	Mustard Yellow
Leaf Powder	Ammonia	Dark Green	Light Green	Mustard Yellow

The leaves of Diapensia himalacia include compounds from the phenolic and flavonoid classes, according to this phytochemical analysis. These compounds trap free radicals, act as an antioxidant naturally, and have anti-diabetic benefits on Diapensia himalacia leaves.The moisture content values demonstrated that it will not slow down microbial development and will extend the shelf life of stored samples. Ash value is a reliable indicator of the quality and purity of unprocessed medications [35]. The assessment of extractive values aids in the

analysis of the chemical elements present in the crude medicine. This number assists in determining which elements are specifically soluble in a particular solvent[36]. The formation of the bioactive components of medicinal plants is affected by a variety of intrinsic and external variables. A high alcohol soluble and water soluble extractive value indicates the presence of polar compounds such phenols, tannins, and glycosides [37]. Every chemical has a distinct, vivid colour. Plant materials take on a variety of colours when they are subjected to different substances. Some plant parts fluoresced in a particular visual range when seen in the sunlight [38]. When exposed to ultra violet light, many natural substances that do not clearly shine in the daylight illuminate. When specific reagents are added to a substance that isn't fluorescent on its own, it transforms into a fluorescent derivative or a breakdown product. Fluorescence analysis was a significant role in the pharmacognostic evaluation of crude pharmaceuticals, which is a prevalent sort of qualitative evaluation of crude pharmaceuticals [39].

IV. CONCLUSION

Pharmacochemical analysis of medicinal plants are a parameter of quality control. Thus, before using a plant in a research setting or for pharmaceutical formulation, it is vital to evaluate its pharmacognostic characteristics. It is possible to draw the conclusion from this study that the analysis demonstrated the purity of Diapensia himalacia leaf powders. Additionally, ethyl acetated extracts followed by methanol produced positive outcomes. In order to further research this plant material as a possible source for medicinal formulations, these extracts can be utilised.

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ABBREVIATIONS:

DM= Diabetes mellitus
$AlCl_3$ = Aluminum chloride
H_2SO_4= sulphuric acid
Na_2CO_3= sodium carbonate

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