



PHARMACOGNOSTICAL & PHYTOCHEMICAL EVALUATION OF
OLDENLANDIA CORYMBOSE

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The current study has been done on oldenlandia corymbosa, reflect that their botanical name oldenlandia corymbosa linn, common name khet papra, white diamond flower, synonyms hedyotis corymbosa linn., oldenlandia scabrida dc. It is an annual slender herbaceous weed upto 50 cm tall. its show various pharmacological activity hepatoprotective activity, anti-malarial activity, antioxidant activity, anti-bacterial activity, anti-malarial activity, anthelmintic activity, abortifacient activity, anti-diabetic activity, hepatoprotective activity, anticancer activity, antipyretic activity, antioxidant activity, antifungal activity, the object of the study is to choose plants from conventional medical systems and look into their pharmacological profiles related to Type 2 Diabetes & Alzheimer's Disease. After collection and authentication we done proximate analysis of the plant drug, which included total ash value, loss of drying, foaming index, total tannin content. Extraction is done using soxhlet apparatus using ethanol as a solvent in last we performed pharmacological activity like Cholinesterase enzyme inhibitory activity, Glucosidase enzyme inhibitory activity, Scavengers of nitric oxide, Reducing Power Assay of O. corymbosa, The UV-Vis spectra of the 70% ethanol extract of leaf of OC had absorption maxima at 241.6 nm, within the range of characteristic absorption for flavonoids.

Key Words: Oldenlandia corymbosa, Abortifacient, Cholinesterase, Extraction, Antipyreti

1. INTRODUCTION

Diabetes is a complex metabolic disease characterised by hyperglycemia brought on by insufficient or ineffective insulin. 425 million people worldwide were estimated to have diabetes mellitus in 2017. By 2045, there are expected to be 629 million diabetics globally. (2018) Cho et al. In those with type 1 diabetes, the pancreatic beta cells are attacked by the immune system, which results in hyperglycemia (Forbes and Cooper, 2013).

There is no denying that Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM) are two of the most prevalent disorders affecting people in their senior years. The most prevalent major neurocognitive illness, AD accounts for 60% to 80% of all causes of dementia in the aged population (de Matos et al., 2018). A progressive memory loss and a steady deterioration in cognitive ability are two clinical markers of AD. The patient dies before their time a few years following the diagnosis.

2. OVERVIEW OF OLDENLANDIA CORYMBOSE

2.1 Taxonomical classification (Sivarajan and Balachandran, 1994)

<u>Domain</u>	“Eukaryota”
<u>Kingdom</u>	“Plantae”
<u>Phylum</u>	“Tracheophyta”
<u>Class</u>	“Magnoliopsida”
<u>Order</u>	“Rubiales”
<u>Family</u>	“Rubiaceae”
<u>Genus</u>	“Oldenlandia”
<u>Species</u>	“Oldenlandia Corymbosa”

Table no 1. Taxonomical classification

2.2 Botanical Name- Common Name: White Diamond Flower (Khet Papra) Synonyms include *Oldenlandia scabrida* DC and *Hedyotis corymbosa* Linn. Localised Name *O. corymbosa* is known by a variety of names in different parts of India. Parpata, Parpataka in Sanskrit Name in English: Diamond Flower Name in Hindi: Pitpapra, Daman pappar Parpatakapullu, Parpatakam are its Malayalam names.

2.3 Other Botanical Characters

Habitat

At elevations ranging from sea level to 2000 meters, there are various types of cultivated and disturbed ground, including grassland, crop fields, mountain scrub, shallow soil on rocks, lawns, gardens, waste places, and roadsides (Figure 1) (Das et al., 2019).



Figure no 1. Natural habitat of *Oldenlandia corymbosa*



Figure no 2. Natural habitat of *Oldenlandia corymbosa*

Growth: It is an annual slender herbaceous weed up to 50 cm tall in (Figure no 2.)

Leaves: Oldenlandia corymbosa has simple, sub-sessile, or extremely short petioles on its leaves (Figure 3). Linear, narrowly or delicately lanceolate, oblong, obovate, or elliptical are some of the leaf shapes. The leaves have noticeable midribs and measure 1-4 cm long by 1-6 mm wide. The stipules are fused to the bases of the petioles, the base and apex of the leaf are acute, and the margin is complete.

Flower Inflorescence has 2 to 5 axillary, cymose blooms. The pedicels range in length from 3 to 11 mm. The calyx is hairless. A subglobose or ellipsoid part of the hypanthium measures 0.4–0.8 mm. Base depends on the limb lobes. The lobes are somewhat triangular, whole to ciliate, and measure between 0.6 and 1.2 mm. Corollas are funnel-shaped, rotating tubes that range in colour from white to pinkish-purple and have an interior that is pubescent or glabrous. The flower's anthers and stigma measure 0.2–0.6 mm and 0.2–0.5 mm, respectively.



Figure no 3 Inflorescence & Flowers

Stem The plant is 4-angled to flat, glabrous, and the angles are stout to the wing.



Fruits & Seeds (Fig. 5) is capsular, subglobose, ovoid, and ranges in size from 1-2 mm. It dehisces through a flat to broadly rounded apex, and, if present, has a 0.5 mm beak. As the fruit matures, the peduncles and pedicels typically lengthen quickly. Smooth and dark brown seeds (Fig. 6) are present (Chopra, 1956; Wagner et al., 1999).



Figure no 5 Fruits



Figure no 6 Seed

2.4 Folk use

When cooked alongside other vegetables like Amaranthus and Cucurbita, the young, fresh leaves and stems serve as a tenderizer for other prepared vegetables. These are a significant ascorbic acid source. Burning the leaves and stems, combining the ash with water, filtering it, and using the resulting liquid as a tenderizer for cooking other vegetable components are all options (Das et al., 2019).

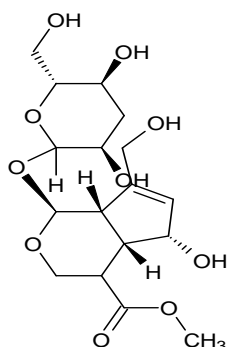
2.5 Ethnomedicinal Uses:

To treat gastrointestinal issues, the leaves are pounded, steeped in warm water, and the resulting beverage is consumed. To relieve irritated eyes, they are applied. An anthelmintic, anti-rheumatic, depurative, digestive, diuretic, pectoral, and stomachic are all uses for the entire plant. The herb is frequently found in Indian concoctions that are taken internally to treat recurrent fevers, soothe gastrointestinal discomfort, and serve as tonics. Jaundice can also be treated with it. It is utilised during birthing in African nations. When a patient has a high fever, the hands and feet are treated with the plant's decoction to reduce the temperature. According to reports, the roots have vermifuge qualities. They are tinctured for usage (Das et al., 2019; Nguyễn, 1993).

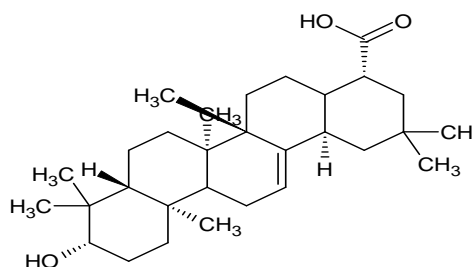
2.6 Chemical Composition:

The following list includes the chemical components that have been found in various Oldenlandia corymbosa plant sections. (1) "Flavanoids, proteins, carbohydrates, phenols, terpenoids, tannins, saponins, steroids, coumarin, and glycosides have all been identified by

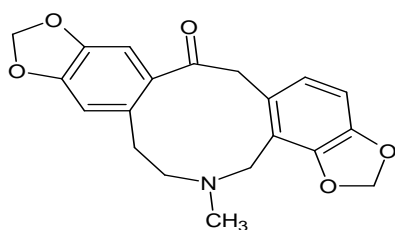
phytochemical research. Asperulosidic acid, asperuloside, deacteylasperuloside, 10-o-p-hydroxy benzoylscandoside methyl ester, rutin, and (+)-lyoniresinol-3-alpha -o-beta glucopyranoside” are some of the compounds from Oldenlandia corymbosa plants that have been found in studies.



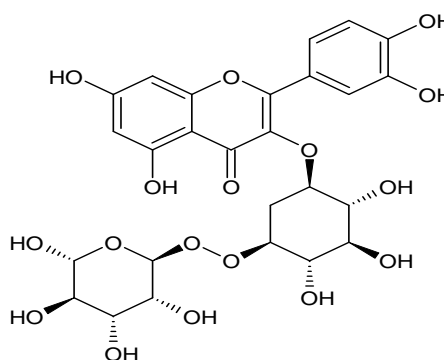
6- alpha hydroxygeniposide



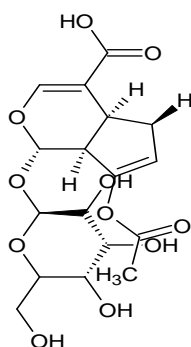
oleanolic acid



biflorine



Rutin



Asperulosidic acid

2.7 Ethnopharmacological Information

1. The plant has reportedly been utilised to treat various malignancies in China and has immunopotential potential (Khare, 2007).
2. It is used to treat recurrent fever with nervous depression and gastrointestinal irritation as well as fever brought on by disturbed bile and air flow.
3. In Konkan, the juice is used to soothe the burning sensation experienced in the hands' and feet's soles. To soothe the burning pit of the stomach, juice is administered internally together with a small amount of milk and sugar. The decoction is helpful for heat outbreaks, recurrent fever, and applied to the body's surface as well. The plant extract is used as an an

4. thelmintic and for jaundice. In Indo China, the plant is employed as a febrifuge. (Warrier et al., 1995).

2.8 Pharmacological Action

Acute Oral Toxicity Test, Cytotoxic Activity (Nikolajsen et al., 2011)., Hepatoprotective Activity (Patel and Jain, 2018).

3. METHODOLOGY

3.1 Selection of Plant

The selection of the right plant is essential because making the wrong choice will waste time and resources.

There are three methods for choosing medicinal plants, according to Elisabetsky and Moraes.

a) Randomized Method: The investigation follows an arbitrary path.

b) Chemotaxonomically/phylogenetic: Species are chosen based on the chemical type of substances found in a genus or family.

c) Ethnopharmacological: Plants are chosen based on their medicinal value to a specific ethnic group.

Another point to which everybody agrees is that the chances of research success are increased if plants are chosen based on their traditional use.

After considering the above factors, we chose the following plants based on ethnopharmacology, traditional use and availability.

Oldenlandia corymbosa. belongs to family: Rubiaceae

3.2 Collection, Identification And Authentication:

Selected plant components will be gathered or purchased from the local market, in Meerut, India and then authenticated by Dr Anju Pal, Scientist at G.B.P.U.A.T., Pantnagar, Uttarakhand, India. individuals.

3.3 Assessment of the quality of the plant materials:

Selected plant material will be subjected to the determination of foreign material and analysis for proximate parameters according to WHO guidelines.

3.4 Preparation of extracts

Various extracts will be prepared by using solvents of different polarity from leaves of *Oldenlandia corymbosa* will be extracted.

3.5 Preliminary phytochemical evaluation of extracts:

All extracts obtained from leaves of *Oldenlandia corymbosa* will be investigated for the presence of different chemical constituents.

3.6 Phytochemical profiling

Phytochemical profiling will be performed by the means of total phenolic, flavonoids content etc.

3.7 Determination of foreign matter

The contamination of either plant was examined for any evidence of mold, insects, or other animal contamination.

3.8 Macroscopic evaluation

Oldenlandia corymbosa and Grangea maderaspatana fresh plant parts were examined for colour, flavour, and aroma as well as for form, size, surface properties, and other attributes.

3.9 Powder Microscopy

Oldenlandia corymbosa's leaves demonstrated the substantial quantity of rap hides present in its powder. The epidermal cell walls of Oldenlandia corymbosa's calyx are arranged in a net-like fashion (Patel and Jain, 2018).

3.10 Proximate Analysis

3.10.1 Total Ash value

The amount of material that is still present after ignition is represented by the total ash. This contains both "physiological ash," which comes from plant tissue, and "non-physiological ash," which is left over from foreign objects that adhere to the surface of the plant. In a silica crucible that had already been lit and tarred, approximately 4 g of the ground, air-dried powdered material were precisely measured. The substance was spread out evenly, heated up to 500–600 C over time, and ignited when it turned white, indicating a lack of carbon. It was weighed after cooling in a desiccator. Total ash content was estimated as mg of ash per gramme of air-dried material (Patel and Jain, 2018).

3.10.2 Acid insoluble ash

The amount of silica, particularly in the form of sand and siliceous earth, is measured by acid insoluble ash. 25 ml of 2N hydrochloric acid was added to a crucible containing the whole ash, and the crucible was covered with a watch glass and slowly heated for 5 minutes. After being rinsed with 5 ml of hot distilled water, the watchglass was placed in the crucible. Hot water was used to wash the insoluble material off of ashless filter paper until the filtrate was neutral. To ignite to a consistent weight, transfer the filter paper holding the insoluble material to the original crucible, dry on a hotplate, and ignite. After 30 minutes of cooling in a suitable desiccator, weigh the residue. Determine how much acid insoluble ash there is in each gramme of air-dried material.

3.10.3 Water soluble ash

25cc of water was added to the crucible containing the whole ash, and it was gently heated for 5 minutes. On ashless filter paper, the insoluble material was collected, and hot water was used to wash it away until the filtrate was neutral. Transfer the filter paper holding the insoluble material to the original crucible, let it dry on a hot plate, then ignite it for 15 minutes at a temperature no higher than 450 °C to get a constant weight. After the residue has had 30 minutes to cool in a suitable desiccator, weigh it. Weight of the whole ash was deducted from weight of the residue. Determine the water-soluble ash concentration in mg per gramme of the air-dried material.

3.10.4 Loss on drying

I properly weighed 2 g of the air-dried plant material and put it in a flat weighing vial that had previously been tarred. heated the sample in an oven at 100 to 105 degrees for five hours

to dry it. Unless otherwise specified in the test method, dried until there was no more than a 5 mg difference between two consecutive weights. Then, determine the material's weight loss, expressed as a loss of mg/gm (Organization, 1994).

3.10.5 Extractive values

The extractive value determines how much active ingredients can be extracted with solvents from a given amount of plant material. Usually, it is determined by adding the extractive values for alcohol and water.

3.10.6 Alcohol soluble extractive value

In a conical flask with a glass stopper, 5.0 g of coarsely powdered, air-dried material was weighed. 100 cc of the alcohol should be macerated for 6 hours while stirring constantly, followed by 18 hours of standing time. The content was quickly filtered. In order to prevent solvent loss during filtration, 25 ml of the filtrate was transferred to a flat-bottomed dish covered in tar and dried out on a water bath. 105°C for 6 hours, 30 minutes of cooling in a desiccator, and immediate weighing of the extract. The amount of extractable material in mg per gm of air-dried material was calculated.

3.10.7 Water soluble extractive value

In a conical flask with a glass stopper, 5.0 g of coarsely powdered, air-dried material was weighed. 100 cc of the alcohol should be macerated for 6 hours while stirring constantly, followed by 18 hours of standing time. The content was quickly filtered. In order to prevent solvent loss during filtration, 25 ml of the filtrate was transferred to a flat-bottomed dish covered in tar and dried out on a water bath. 105°C for 6 hours, 30 minutes of cooling in a desiccator, and immediate weighing of the extract. The amount of extractable material in mg per gramme of air-dried material was calculated.

3.11 Determination of foaming index

Many therapeutic plant materials include saponins, which when mixed with an aqueous decoction can result in persistent froth. An aqueous decoction of plant materials and their extracts is evaluated according to its foaming capacity using a foaming index.

Procedure:

A conical flask with a volume of 500 ml was filled with 100 ml of boiling distilled water and precisely 1 g of the plant's powder. It was gently boiled for thirty minutes. The filtrate was filtered into a 100 ml volumetric flask, chilled, and then sufficiently diluted with distilled water was put through the filter. 10 test tubes with stoppers measuring 16 cm in height and 16 mm in diameter were filled with the decoction in amounts of 1 ml, 2 ml, 3 ml, etc. until 10 ml were reached. The liquid volume in each tube was then brought to 10 ml using water. 15 seconds were spent shaking the tubes longitudinally. two shakes per second. Allowed to stand for 15 minutes and the height of the foam was measured.

3.12. Foaming index: $1000 / a$

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

3.13 Determination of Total Tannin Content

Petroleum ether was used to extract 2gm of powdered material over the course of 20 hours. The leftovers were cooked in 300 cc of double-distilled water for two hours. The solution was filtered after cooling and diluting to 500ml. A conical flask was filled with 25 ml of the

filtrate from the infusion and then diluted with 750 ml of double-distilled water and 20 ml of indigo carmine solution. Then, 1ml at a time, of normal KMnO₄ solution was added to the solution to titrate it until the blue hue turned green. Then, a few drops at a time, were added until the solution's colour changed to golden yellow.

Similarly, the blank reading was taken by omitting the sample. Multiply the difference between two titrations by the factor to obtain value of total tannins.

% Total Tannins = (A-B) x Normality of KMnO₄ solution x 0.004157 x 1000

Weight of drug sample taken x 0.1

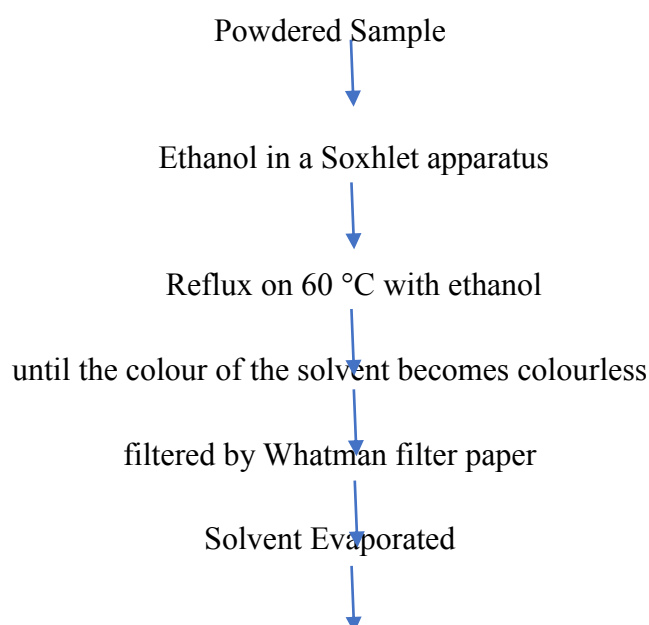
Plant Extraction & Fractionation

O. corymbosa's aerial components and roots were carefully cleansed with tap water before being rinsed with distilled water, butanol, and chloroform. The plant's leaves, stems, and roots were all dried separately at room temperature without exposure to sunshine. They were pounded to a powder when they had dried completely. The powder was then removed using the next technique.

Each of the dried powdered plant materials (100 g) was placed into a soxhlet apparatus (2 L) and subjected to a 6-hour, thorough extraction with 500 mL of diethyl ether at 75°C. Using a Cole-Parmer Desiccator (EW-06525-24, USA), the ether was evaporated, and the remaining ether was allowed to evaporate overnight at lab temperature. The test solution of each extract was prepared by dissolving 10 g of crude plant extract separately in 100 mL sterile distilled water in a 250 mL Erlenmeyer flask in a water bath (1083, Germany) at 80°C for 2 hours. Extracts were subsequently filtered through four folds of cheese cloth (Organization, 1994, 2000)

3.14 Soxhlet Extraction

For Soxhlet extraction, 300 ml of the 80% ethanol was added to 50 g of the powdered sample and heated to 60 °C in a Soxhlet apparatus until the solvent's colour turned colourless. To create a gummy crude extract, the organic layers were filtered through Whatman filter paper no. 1 and the solvent was evaporated using a vacuum rotary evaporator. In order to preserve the concentrated ethanolic extract for future research, it was placed in a beaker, covered with aluminium foil, and kept in a refrigerator at 4 °C



Gummy crude extract obtains
↓
preserved in a beaker

Fractionation of Crude Extract for Analysis

Water, butanol, chloroform, and hydroethanolic. For fractionation, crude extract from Soxhlet extraction was employed. 20 ml of butanol were used to dissolve 2 gramme of crude extracts, which were then magnetically swirled for 1 hour. To create a gummy crude extract, the solvent was evaporated under a vacuum rotary evaporator after the supernatant was recovered by decantation. The leftover precipitate was dissolved in 20 ml of ethyl acetate and agitated for an additional hour using a magnetic stirrer. By switching the solvent from ethanol to butanol, chloroform, and water, the same procedure was repeated. For in-vitro antioxidant analysis, all four extracts—chloroform, butanol, water, and ethanol—were gathered and kept in a refrigerator at 4 °C.

3.15 Phytochemical tests

Test for alkaloids

“Crude extract of plant when mixed with 2ml of 1% HCl and gently heated, followed by addition of Mayer’s and Wagner’s reagents to the mixture. Turbidity of the resulting precipitate will be taken as evidence for the presence of alkaloids”. (Yadav and Agarwala, 2011).

Test for carbohydrates:

Fehling’s Test:

“Equal parts of Fehling A and Fehling B should be combined to create crude extract, which should then be gently boiled. When a brick-red precipitate appeared at the test tube's bottom, reducing sugars were present”. (Yadav and Agarwala, 2011).

Benedict’s Test:

“A reddish-brown precipitate was produced when crude extract was combined with 2 ml of Benedict's reagent, indicating the presence of the carbohydrates”. (Yadav and Agarwala, 2011)

Molisch’s Test:

“Mixed crude extract with 2ml of Molisch’s reagent and mix it properly for 5 mins. After that, poured 1-2ml of concentrated H₂SO₄ carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate”. (Yadav and Agarwala, 2011)

Test for proteins:

Millon’s Test:

“The crude extract when mixed with 2ml of Millon’s reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein”. (Yadav and Agarwala, 2011).

Ninhydrin Test:

“The crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins”.(Yadav and Agarwala, 2011).

Biuret Test:

“Crude extract when treated with an equal volume of 1% strong base (Sodium or Potassium Hydroxide) followed by few drops of aqueous copper sulphate. If solution turns purple, it indicates that the presence of proteins”. (Yadav and Agarwala, 2011).

Test for glycosides:

Liebermann’s Test:

“Crude extract when mixed with each of 2ml of chloroform and 2ml of acetic acid and carefully concentrated H₂SO₄ when added, colour change will from violet to blue to green indicated that the presence of steroidal nucleus”, i.e., glycine portion of glycoside(Yadav and Agarwala, 2011).

Salkowski’s Test:

“The crude extract when mixed with 2ml of chloroform and carefully concentrated H₂SO₄ when added a reddish brown colour indicated that the presence of steroidal ring, i.e., glycone portion of the glycoside”. (Yadav and Agarwala, 2011).

4. RESULTS

4.1 Foreign Matter: No Foreign matter was present in the drug.

4.2 Macroscopic evaluation

Table no 2 Macroscopic evaluation of Oldenlandia corymbosa

Parameters	Observations
Type	Simple
Phyllotaxy	Opposite
Size	3.5 cm long, 5 mm wide
Shape	Linear – lanceolate or Elliptic
Margin	Entire
Apex	Acute
Base	Acute
Venation	Reticulate
Surface	Glabrous
Color	Dark green
Odour	Characteristic
Taste	Characteristic

4.3 Proximate analysis

The results obtained from various determinations are compiled in Table. The ash values of a drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of exhausted or adulterated drug.

Table no 3. Physicochemical parameters of powder of O. corymbosa

Sr. No.	Parameters	Values (% w/w)
1	Loss on drying	8.00%

2	Ash value	
	Total ash	12.25%
	Acid insoluble ash	2.00%
	Water soluble ash	5.50%
3	Extractive value	
	Water soluble extractive	10.00%
	Alcohol soluble extractive	4.5%
4	Foaming Index	
		< 100

The values given here are expressed as percentage of air-dried material. Each value is average of three determinations. The morphological, microscopical and physicochemical parameters of *Oldenlandia corymbosa* can possibly help to differentiate the drug from its other species and the pharmacognostic profile of the plant presented here will assist in standardization viz., quality, purity and sample identification.

4.4 Extraction Yields (%)

The shade-dried, powdered plant material was extracted with 90% ethanol in water. The obtained crude methanolic extract was suspended into water and fractionated by successive solvent-solvent extraction with chloroform and n-butanol. Dried yield percent of 90% ethanol extract of OC from solvent extraction by soaking was found to be 20.58, 15.69 and 16.97, respectively. The fractionation of ethanolic extract produced the chloroform, n-butanol and residual aqueous fractions. Initially, ethanol showed the highest extractive capacity. As indicated by data, different extractive capacities of solvents used for fractionation were in descending order of residual aqueous > n-butanol > chloroform fraction of each solvent.

4.5 Determination of total tannin content

The powder of *Oldenlandia corymbosa* plant contains 1.09% of total tannins.

4.6 Extraction yield

Oldenlandia corymbosa plant powder was subjected to successive solvent extraction (except water extract which was prepared by decoction). The different extracts obtained with their % yield, color, consistency are recorded in Table.

Table no 4. Extraction yield of *O. corymbosa*

Sr. no.	Solvent	Color	Consistency	%Yield w/w
1	Petroleum Ether	Yellowish green	Slight Sticky	2.36
2	Chloroform	Dark green	Slight Sticky	2.02
3	Ethyl acetate	Green	Slight Sticky	1.78
4	Ethanol	Green	Slight Sticky	3.80
5	Water	Brown	Slight Sticky	9.50

4.7 Phytochemical chemical tests

The extracts obtained from successive solvent extraction process were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like steroids, carbohydrates, alkaloids, glycosides, phenolics and tannins etc.

Table no 5. Phytochemical screening of extracts of O. corymbosa

Chemical constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Water extract
Carbohydrate	-	-	-	+	+
Protein	-	-	-	-	-
Phenolics & Tannins	-	-	-	+	+
Saponins	-	-	-	+	++
Flavanoids	-	-	-	+	+
Terpenes	++	++	+	+	-
Steroids	++	++	-	-	-
Alkaloids	-	-	-	-	-

(+ - Positive test, - - Negative test)

4.8 The results obtained from various determinations are compiled in Table no 6 & 7

Table no 6. Physico-chemical parameters of powder of O. corymbosa.

Sr. No.	Parameters	Values (% w/w)
1	Loss on drying	8.00%
	Ash value	
2	Total ash	12.25%
3	Acid insoluble ash	2.00%
4	Water soluble ash	5.50%

5	Extractive value	
6	Water soluble extractive	10.00%
7	Foaming Index	4.5%

Table no 7. Physico-chemical parameters of powder of *O. corymbosa*.

The values given here are expressed as percentage of air-dried material. Each value is average of three determinations.

Sr. No	Parameter	Mean \pm SD (%)		
		Summer	Monsoon	Winter
1.	“Total Ash Content”	11.66 \pm 0.63	11.57 \pm 0.54	11.09 \pm 0.81
2.	“Acid Insoluble Ash content”	2.39 \pm 0.59	2.99 \pm 0.83	2.78 \pm 0.52
3.	“Water Soluble Ash content”	6.38 \pm 0.34	6.95 \pm 0.91	6.35 \pm 0.65
4.	“Sulphated Ash content”	16.40 \pm 0.34	15.26 \pm 0.74	15.72 \pm 0.87
5.	“Ethanol soluble extractive”	6.7 \pm 0.12	6.12 \pm 0.31	6.41 \pm 0.83
6.	“Water soluble extractive”	13.16 \pm 0.20	12.68 \pm 0.33	12.96 \pm 0.27
7.	“Moisture content”	5.79 \pm 0.53	5.93 \pm 0.43	5.24 \pm 0.19

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