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# Pharmacognostic and phytochemical evaluation of leaves of *Onosma bracteatum*

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**Abstract**
The name *Onosma* was making known to into contemporary botanical vocabulary by Linnaeus, it was derived from a Latin word “osma” coined from a Greek word, “osma” (smell). The Boraginaceae is the family name for *Onosma bracteatum* Wall. It is recognised as Gaozaban in the ancient unani system of medicine and as Sedge in the Middle East. *O. bracteatum* is commonly used as a demulcent, alterative, diuretic, immunity enhancer and spasmolytic and as a major constituent of various ayurvedic formulations for the treatment of hypertension, leprosy, rheumatism and asthma. The Pharmacognostic investigations carried out on plant till date are not sufficient. Thus the present study includes detailed investigation of leaves of *Onosma bracteatum* using macroscopy, microscopy, physiochemical, phytochemical parameters and fluorescence analysis. The study also involves qualitative and quantitative estimation of some metabolites. The overall outcome of this detailed study will be helpful in proper identification, evaluation and grounding of a monograph of *O. bracteatum*.

**Keywords:** *Onosma bracteatum, boraginaceae,* microscopical, phytochemical, fluorescence, evaluation

## Introduction

Linnaeus coined the name *Onosma* for this species, which is taken from the Latin word "osma," which means "smell." Numerous species of *Onosma* L. are found throughout Asia, Eurasia, the Mediterranean region and Europe, primarily in Iran, Syria, Turkey, China, Pakistan, India and Sri Lanka. All species grow in sunny, dry or damp climates, mainly in rock crevices and are commonly referred to as rock garden plants. [1]

The *Boraginaceae* family includes *Onosma bracteatum Wall.* In the Unani system of medicine, it is known as Gaozaban, while in the Middle East, it is known as Sedge. *O. bracteatum* is a common demulcent, alterative, diuretic, immunity enhancer, and spasmolytic used in ayurvedic formulations for hypertension, leprosy, rheumatism and asthma.[2]. Native practitioners see it as a tonic and alterative and it is frequently used as a decoction in the treatment of rheumatism, syphilis, hypochondriasis, renal disorders and leprosy.

It's a good refrigerant and demulcent. When compared to other few drugs, it has almost same efficacy in reducing excessive thirst and restlessness caused by febrile excitation. It also helps with functional heart palpitation, stomach and bladder discomfort and strangury. It's utilised in the form of an infusion made with either cold or hot water in a 1 to 20 ratio. [3, 4]

Lycopsamine and supindine viridiflorate are the most common unsaturated pyrrolizidine alkaloids found in *Onosma bracteatum* leaves. Potassium and calcium are also present along with mineral acids in this medication. The fresh juice contains 30% nitrate of potash, while the dried herb contains only 3%. Much saline mucilage is found in the stems and leaves. Borage's wholesome revitalising benefits are primarily due to saline mucilage. Borage gives beverages a nice flavour and a cooling effect. Borage has a high concentration of ascorbic acid (38 mg/100 g). Choline, glucose, fructose, amino acids and tannin are all found in flowers (about 3 percent). Proteins (21.9%) as well as oil (38.3 percent) are present in seeds.

One of the most important source of gamma-linoleic acid and linoleic acid is seed oil. Internally, only unsaturated pyrrolizidine alkaloids (UPA) free oil is used. [5, 6]

The uses of plant bio-actives act as potent medicines for curing ailments, fighting infections and healing that predominates worldwide. The biologically active plant metabolites contain useful compounds having medicinal value that are utilized for treating various disorders affecting mankind. The botanical medicines due to their structural diversity and wide availability in nature are one of oldest disciplines found within healing practice for various ailments. [7]

Taking into consideration the various ethno medical applications of this plant, there is a strong urge for its detailed and systematic standardisation of *O. bracteatum* Linn. As a result, the current study's goal is to analyse pharmacognostic and physiochemical characteristics for plant evaluations.

## Materials and Methods

### Plant Material Collection and Authentication

The leaves of *Onosma bracteatum* Wall. were collected from Nashik district of Maharashtra, India. The plant material was cleaned thoroughly. The Plant sample was authenticated by K.L.E Society’s Raja Lakhamagouda Science Institute, Belgaum, India. The specimen voucher number is OBLAU14.

## Pharmacognostic studies

### Macroscopy

The leaves of *Onosma bracteatum* were evaluated with the help of sensory organs for organoleptic properties. Its colour, odour, shape, size, margin and other diagnostic parameters were observed and recorded. [8]

### Microscopy

The leaf of *O. bracteatum* was microscopically studied by making freehand slices going through the midrib, clearing with 5 percent KOH solution, staining with conc. hydrochloric acid:phloroglucinol (1:1) and mounting with 50 percent glycerine solution. Photographs of the slides were taken. [8,9]

### Powder microscopy

For powder microscopy, the dried powdered material of leaves of *O. Bracteatum* was cleared with sodium hydroxide and treated with staining reagent phloroglucinol and HCL in 1:1 proportion. The treated powdered materials were mounted on a glass slide in a glycerine medium. Slide was seen under microscope. [9]

### Quantitative Microscopy

Standard techniques were used to determine the stomatal number, stomatal index, vein islet number and vein termination number on fresh leaves. To differentiate between some closely related species that are difficult to characterise using conventional microscopy, a number of readings are taken. [9, 10]

### Determination of Leaf Constants

**1. Stomatal Number and Stomatal Index**: The leaf's upper epidermis was peeled between the midrib and the lamina, and the transparent portion was cleared with clearing solution before being mounted on a glass slide. Using a prism type camera lucida at high power, the stomata and epidermal cells were traced on a black sheet measuring 0.2 mm square (45x). Each drawn square was counted for epidermal cells and stomata according to a guideline. The experiment was performed five times, with the stomatal number calculated directly. The formula for calculating the stomatal index was used.
The average values were calculated, and the findings were expressed in square millimetres. [9, 10]

I= S/S+E

Where, I= Stomatal Index, S= Number of stomata per mm square, E= Number of epidermal cells per mm square

**2. Vein-Islet and Vein Termination Numbers**

Between the midrib and the margin of the leaf, it was macerated in a strong chloral hydrate solution for 24 hours and then bleached (5 percent calcium chloro-hypochlorite). The cleared lamina section was put on a glass slide, and vein islets and vein terminations were traced on a black sheet measuring 0.5 mm square with a low power microscope (5x). The values per sq.mm of leaf area between midrib and margin were calculated by repeating the procedure. [8, 9]

### Evaluation of physical constants

Total Ash value, acid insoluble ash, water soluble ash, sulphated ash, Water soluble extractive value, Alcohol soluble extractive value, swelling index and foaming index were among the physicochemical tests performed on powdered *O.bracteatum*. The moisture content and foreign organic matter of powdered drug was also performed.

### Determination of foreign organic matter

A thin layer of five grammes of air-dried coarsely powdered drug was spread. The sample was examined using either the naked eye or a 6X lens. The alien organic matter was manually isolated as thoroughly as feasible. The weight of the drug taken was used to calculate the proportion of foreign organic materials in the sample. [9, 11]

### Determination of Moisture content

1 g of leaf powder was accurately weighed and placed in a china dish, which was then dried in an oven at 100 °C for an hour. The powder was weighed once more and compared to its original weight. The following expression was used to compute the loss due to drying. [9, 11]

Percentage loss on drying = Weight loss (g)/Weight of sample (g) × 100.

### Ash Value

Quality and purity assessment of a crude drug is done by Ash value. Inorganic radicals such as phosphates, carbonates, and silicates of sodium, potassium, magnesium, and calcium are found in ash. Because these are present in a specific amount in a crude drug, quantitative determination in terms of various ash values aids in standardisation. Inorganic factors in the crude drug, such as calcium oxalate, silica and carbonate concentration, might alter the 'Total ash value.' After that, such factors are removed using acid, and the acid insoluble ash value.
eg. Rhubarb, Liquorice.

### Determination of Total Ash

2g of air-dried crude drug was accurately weighed and placed in a tared silica dish, which was then burned at a temperature not exceeding 450 $^0$C until free of carbon, cooled in a desiccator, and the weight was taken. The technique was repeated until the weight remained consistent. The proportion of ash was estimated using air-dried drug as a reference. [9, 11]

### Water Soluble Ash

The ash obtained using the foregoing procedure was heated for 5 minutes with 25 mL of water, filtered, and the insoluble matter was collected in a Gooch crucible, rinsed with hot water, and ignited for 15 minutes at a temperature not exceeding 450 $^0$C before being weighed. The difference in weight shows the water-soluble ash after subtracting the weight of the insoluble stuff from the weight of the ash. The percentage of water-soluble ash was estimated using air-dried drug as a reference. [9, 11]

### Acid Insoluble Ash

The ash obtained in Total ash procedure was heated for 5 minutes in 25 mL of 2 M hydrochloric acid, filtered and the insoluble matter was collected in a Gooch crucible or on ash-free filter paper, rinsed with hot water, ignited, and weighed. The proportion of acid-insoluble ash was estimated using the air-dried drug as a reference. [9, 11]

**Sulphated Ash**
The silica crucible was heated to redness for 10 minutes and then weighed after cooling in a desiccator. 1 gram of air-dried drug was weighed and gently ignited till it was scorched cold. 1 mL Sulphuric acid was used to moisten the residue. It was slowly heated until no more white fumes emerged, then ignited at 800°C ± 25°C until all black particles had vanished. Ignition took place in a location that was free of air currents. A few drops of Sulphuric acid were added to the crucible, which was then fired. After that, it was allowed to cool before being weighed. [9, 11]

**Extractive values**
**Determination of water-soluble extractive value**
In a closed flask, five grams of air dried coarsely powdered drug was macerated with 100 mL chloroform water for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. The filtrate was then filtered, and 25 mL of it was evaporated on a flat shallow dish before being dried at 105°C and weighed. With reference to air-dried drug, the percentage of water-soluble extractive value was calculated. [9, 11]

**Determination of Alcohol-soluble extractive value**
In a closed flask, five grams of air-dried coarsely powdered drug was macerated for 24 hours with 100 mL of ethanol of prescribed strength, shaken regularly for the first 6 hours and allowed to stand for 18 hours. After that, it was filtered to prevent ethanol loss, and 25 mL of the filtrate was evaporated in a flat shallow dish and dried at 105°C before being weighed. With reference to air-dried drug, the percentage of ethanol soluble extractive value was determined. [9, 11]

**Fluorescence analysis**
For observing characteristic colour presentation, usinf fluorescence analysis, leaf powder was treated with various chemicals and observed exclusively to different wavelengths of UV (254 nm and 365 nm) and visible light. [12]

**Extraction**
The extraction of *O.bracteatum* was carried out using successive solvent extraction scheme. The powder of the air dried leaves of *O.bracteatum* was loaded in thimble of soxhlet apparatus and was extracted with different solvents of increasing polarity like petroleum ether, ethyl acetate, ethanol and water. The coarsely powdered leaf sample was firstly extracted with Petroleum ether (40ºC–60ºC). The extract was concentrated on water bath and transferred to a preweighed china dish and dried in a vacuum desiccator. The marc obtained was then air dried and used for further extraction with ethyl acetate followed by ethanol. Finally the marc was refluxed with distilled water to obtain aqueous extract. The extract was filtered and concentrated extract was air-dried. [13]

**Preliminary phytochemical evaluation of extracts**
As described in the standard methods, the preliminary phytochemical screening was qualitatively assessed for the presence of phytochemicals. [9]

**Quantitative estimation of secondary metabolites**
**Quantitative Analysis**
The leaves of *Onosma bracteatum* was subjected to quantitative determination (amount & percentage) of phytochemicals such as alkaloids, flavonoids, and saponin was carried out.

**Alkaloid determination using Harborne (1973) method**
In a 250 mL beaker, 5 g of the sample was weighed, then 200 mL of 10% acetic acid in ethanol was added, capped, and left to stand for 4 hours. This was filtered, and the extract was concentrated to one-quarter of its original volume in a water bath. Drops of concentrated ammonium hydroxide were added to the extract until it was completely precipitated. The entire solution was allowed to settle before being collected and rinsed with weak ammonium hydroxide before being filtered. The residue is the alkaloid, which was dried and weighed. [14]

**Flavonoids determination by the method of Bohm and Kocipai- Abyazan (1994)**
At room temperature, 10 g of the plant material was extracted several times with 100 mL of 80 percent aqueous methanol. Whatman filter paper No 42 (125 mm) was used to filter the entire solution. The filtrate was then transferred to a crucible and dried over a water bath before being weighed at a constant weight. [15]

**Saponin determination by method of Nahapetian and Bassiri (1974)**
In 200 mL of 20% ethanol, 20 g of plant sample was dispersed. At about 55 °C, the suspension was heated for 4 hours over a hot water bath with constant stirring. After filtration the residue was re-extracted with 200 mL of 20 percent ethanol. Over a water bath at around 90 °C, the mixed extracts were reduced to 40 mL. The concentrate was placed to a 250 mL separating funnel, which was then filled with 20 mL of diethyl ether and vigorously agitated. The aqueous layer was preserved, while the ether layer was thrown away. The purifying procedure was carried out once more using n-butanol (60 mL). 10 mL of 5% aqueous sodium chloride was used to wash the mixed n-butanol extracts twice. In a water bath, the residual solution was heated. The samples were dried in the oven to a consistent weight after evaporation. The saponin content was calculated as a percentage of total saponin. [16]

**Results and Discussion**
**Macroscopic Characters**
The leaves of *O.bracteatum* are cluster of radical leaves having features as mentioned in Table I:

**Table 1:** Morphological characteristic of Leaf of *O.bracteatum*

| Sr. No | Parameter | Observation |
|---|---|---|
| 1 | Colour | Green to yellowish green |
| 2 | Base | Decurrent |
| 3 | Shape | Lanceolate to ovate lanceolate |
| 4 | Leaf Margin | Uneven margin with somewhat Dentate or crenate type |
| 5 | Leaf Apex | Subacute |
| 6 | Type of leaf | Cluster of radical leaves |
| 7 | Leaf Surface /Texture | Smooth |
| 8 | Venation | Prominent veins and reticulate type of venation |
| 9 | Size | 10-25 cm long and 2-3.5 cm broad |
| 10 | Odour | Odourless |

**Microscopic Characters: (Fig. 1 and 2)**
A microscopical character reveals the dorsiventral nature of leaf surface. The cells of upper epidermis are polygonal thin

walled straight. The epidermis, consisting of a single layer of cells, coated on the outside with cuticle. The upper epidermis is followed by Mesophyll layer which consist of palisade and spongy parenchyma. Trichomes are simple single-celled hairs. Palisade cells are having columnar shape arranged in one layer. The vascular bundles show the arrangement of xylem and phloem tissue. The vascular bundle is open, forming a flattened arch in the "V" shape bundles located dorsally in the cortical parenchyma. The anatomical arrangements of leaf with its magnified view are shown in figure 1 and 2 respectively.

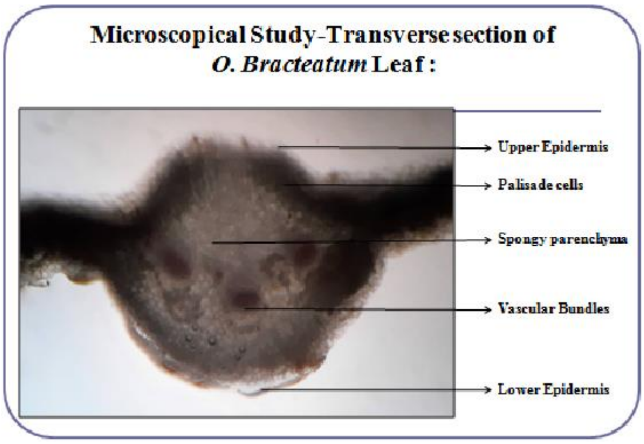


**Fig 1:** Transverse section of Leaf of *O.bracteatum*

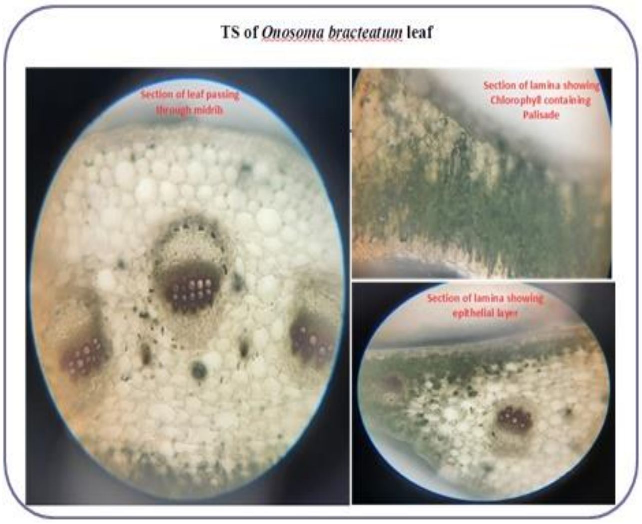


**Fig 2:** Magnified view of Transverse section of Leaf of *O.bracteatum*

**Powder microscopy (Fig. 3)**

The study observed various diagnostic characters like Unicellular covering trichomes, lignified and non-lignified fibers, vessels, collenchymas cell, palisade cells, epidermal cells, calcium oxalate crystals etc. Observed powdered microscopic characters are shown in figure 3.

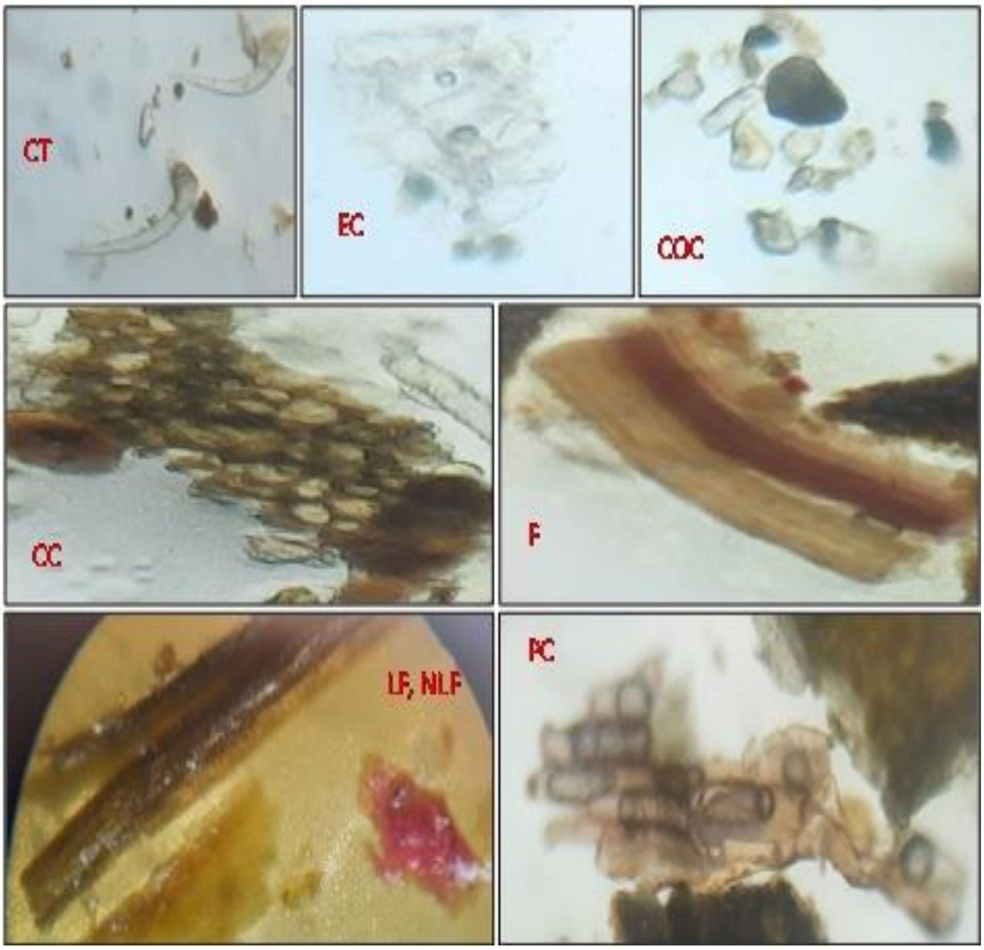


**Fig 3:** Powder characteristics of Leaf of *O.bracteatum*

Where, CT: Covering trichome, EC: Epidermal cells, PC: Palisade cells, COC: Calcium oxalate crystals CC: Collenchyama cells, LF: Lignified fibers, NLF: Nonlignified fibers; F: Fibers.

**Quantitative Microscopy:** Various leaf constants are determined in quantitative microscopy of leaf. These constants are also known as microscopical constants. Quantitative Microscopy helps in standardization of leafy drugs and in finding out any adulterants. The observed leaf constants are as per Figure 4 and Table II.

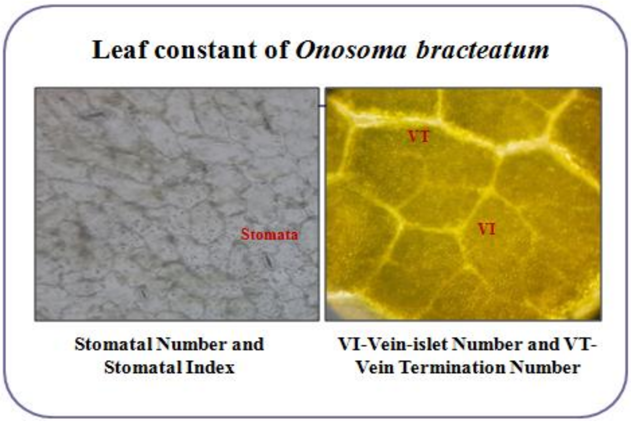


**Fig 4:** Leaf Constants of *Onosma bracteatum* leaf

**Table 2:** Quantitative microscopy of leaf of *Onosma bracteatum*

| Sr. No. | Parameters | Range | Mean |
|---|---|---|---|
| 1 | Stomatal Number (Upper epidermis) | 11-18 | 15 |
| 2 | Stomatal Number (Lower epidermis) | 09-15 | 12 |
| 3 | Stomatal Index | 13-16 | 15 |
| 4 | Vein islet Number | 10-14 | 12 |
| 5 | Vein Termination Number | 08-13 | 11 |

**The physicochemical Study:** The purity and quality of various phytochemicals can be a great source of information and it can be assessed by physiochemical constants. The drug's total ash values give an indication of its inorganic content, such as earthy materials and other contaminants. The ash value was discovered to be very high. The value of acid insoluble ash was determined to be lower than the value of water soluble ash. The extractive values are important for determining the chemical ingredients in a crude drug as well as estimating the solubility of certain constituents in various solvents. The most common and least expensive method of detecting adulterants, exhausted materials and selecting a suitable solvent for extraction of crude powder which yields most soluble elements in high proportion is extractive value determination. The extractive value of water soluble extracts was found to be higher than the extractive value of alcohol soluble extracts. The weight loss on drying is expressed as a percentage w/w loss. It calculates the amount of volatile materials (including water) that can be ejected under the given conditions (desiccators or hot air oven). The drying loss was determined to be 3.33 percent w/w, which appears to be less than what is required to sustain the growth of microorganisms such as mould, bacteria, yeast, and fungal, which cause changes in the chemical compositions of crude pharmaceuticals. The results of other parameters relevant for evaluating crude drugs, such as swelling index and foaming index, are listed in the table below. (Table III)

**Table 3:** Physicochemical parameters of the leaf of *O. bracteatum*

| Sr. No. | Physiochemical parameters | Value (% w/w) |
|---|---|---|
| 1 | Foreign organic matter (FOM) | 2 |
| 2 | Moisture content by loss on drying method (LOD) | 3.33 |
| 3 | Total ash value | 20 |
| 4 | Water-soluble ash value | 15 |
| 5 | Acid-insoluble ash value | 10 |
| 6 | Sulphated ash value | 21 |
| 7 | Water soluble extractive value | 20 |
| 8 | Alcohol soluble extractive value | 10 |
| 9 | Swelling Index | 1.25 |
| 10 | Foaming Index | Less than 100 |

### Fluorescence analysis

Fuorescence is exhibited by several components in the visible range in daylight. Many natural materials that do not fluoresce in daylight exhibit fluorescence when exposed to ultra violet light. By using different reagents, substances that are not fluorescent can often be transformed into fluorescent derivatives or breakdown products. As a result, crude drugs are frequently evaluated qualitatively in this manner, and it is a significant parameter for crude drug pharmacognostic evaluation. [17]

Fluorescence examination of *O.bracteatum* leaf powder was carried out with various reagents, and fluorescence was seen at UV 254 nm and UV 366 nm. The results were compared to their respective visible light observations, which were summarised in Table IV. The qualitative study can be aided by fluorescence analysis of the leaf powder, which can be utilised as a reference data for the identification of adulterants. Various chemical elements found in plant material exhibit fluorescence, which is a noteworthy occurrence. Each compound's fluorescence colour is distinct.

**Table 4:** Observations of *O.bracteatum* leaf powder under UV-visible light (254 and 366 nm)

| Sr. No | Treatment | Visible light (colour) | UV-254 nm (colour) | UV-366 nm (colour |
|---|---|---|---|---|
| 1 | Powder alone | Faint Green | Dark green | Black |
| 2 | Drug powder + HCl | Light Brown | Dark purple | Black |
| 3 | Drug powder + HNO3 | Brown | Light Blue | Black |
| 4 | Drug powder + H2SO4 | Orange | Blue | Light Blue |
| 5 | Drug powder + Acetic acid | Orange | Light green | Black |
| 6 | Drug powder + Picric acid | Yellow | Dark green | Light yellow |
| 7 | Drug powder + NaOH | Dark red | Green | Black |
| 8 | Drug powder + KOH | Dark brown | Black | Black |
| 9 | Drug powder + Ethanol | Light green | Pink | Black |
| 10 | Drug powder + Methanol | Light green | Baby pink | Black |
| 11 | Drug powder + Ethyl acetate | Light Yellow | Dark Pink | Light pink |
| 12 | Drug powder + CHCl3 | Light green | Red | Black |
| 13 | Drug powder + Pet ether | Colourless | Pink | White |
| 14 | Drug powder + Iodine | Light Orange | Grey | Black |
| 15 | Drug powder + FeCl3 | Red | Black | Black |
| 16 | Drug powder + NH3 | Light Brown | Blue | Black |

**Extraction of plant material:** The colour, odour, consistency (nature), and percent yield of several solvent extracts of the plant were calculated, and the results are listed in Table No.V.

**Table 5:** Percentage extraction yield and characterization of *O.bracteatum* L. Leaf

| Sr. No | Extract | Colour | Odour | Nature | Yield (% w/w) |
|---|---|---|---|---|---|
| 1 | Pet Ether | Greenish brown | Characteristic | Semisolid | 2.8 |
| 2 | Ethyl Acetate | Dark green | Characteristic | Semisolid | 2.32 |
| 3 | Ethanol | Reddish brown | Characteristic | Semisolid | 20.76 |
| 4 | Aqueous | Brown | Characteristic | Semisolid | 8.78 |

**Preliminary phytochemical screening:** Phytochemical study of plants helps us to find out the active constituents responsible for pharmacological activity and hence useful in drug discovery and development. *O.bracteatum* leaves reveals the presence of Carbohydrates, steroids, terpenoids, tannins, alkaloids, saponins, flavonoids, tannins and phenolics in different extracts as shown in Table VI.

**Table 6:** Preliminary Phytochemical screening of various extracts of leaf of *O.bracteatum*

| Sr. No | Chemical test | Pet-ether extract | Ethyl acetate extract | Ethanol extract | Aqueous Extract |
|---|---|---|---|---|---|
| 1. | Carbohydrate | - | - | + | + |
| 2. | Protein/ Amino Acid | - | - | - | - |
| 3. | Steroid | - | + | + | + |
| 4. | Glycoside | - | - | + | - |
| 5. | Alkaloids | + | + | + | - |
| 8. | Test for Saponins | - | - | + | + |
| 7. | Test for Flavonoids | - | + | + | + |
| 9. | Tannin and Phenolic Compounds | - | + | + | + |

+: Present, -: Absent

### Quantitative estimation of secondary metabolites

The amount of secondary metabolites present in leaves of *Onosma bracteatum* is mentioned in Table VII.

**Table 7:** Quantitative estimation of secondary metabolites in leaves of *Onosma bracteatum*

| Sr. No | Total alkaloid content | Total flavonoid content | Total saponin content |
|---|---|---|---|
| 1 | 0.25 mg/g (25 %) | 0.60 mg/g (60 %) | 0.165 mg/g (16.5 %) |

### Conclusions

The current study can provide valuable information on proper identification and standardisation of plant material while analysing organoleptic, microscopic investigations, physicochemical parameters, and phytoconstituents, all of which can aid in the assessment of its potential therapeutic activities. Furthermore, the findings of this study may be valuable in the development of a monograph on O.bracteatum leaf.

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