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Research Article

Pharmacognostical, Physicochemical and Preliminary Phytochemical Standardization of Aerial Parts of *Onosma bracteatum* Wall

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ABSTRACT
Objective: The present study was undertaken to develop the standardization parameters of powdered aerial parts of *Onosma bracteatum* Wall, family Boraginaceae.
Methods: Different parameters such as pharmacognostical, physicochemical, preliminary phytochemical evaluation along with thin layer chromatography for identification of phytoconstituents were studied.
Results: On microscopical examination of the aerial parts it showed the presence of oval to polygonal thin walled straight epidermal cells; spiral vessels, a few fibres elongated with blunt tips, long warty, tubercle based unicellular hairs and paracytic stomata. On physicochemical evaluation it was found to contain more amount of polar constituents as the ethanol extractive value was found to be more. Total ash value and acid insoluble ash indicated the presence of inorganic acids and silicaceous matter respectively. Foaming index and swelling index were indicative of saponins and mucilaginous matter present in the aerial parts. On preliminary phytochemical screening and thin layer chromatographic studies it revealed the presence of saponins, flavonoids, phenolic compounds and mucilage.
Conclusion: The present work carried out can serve as a purpose for identification, authentication and standardization of the crude drug.

Keywords: *Onosma bracteatum*, aerial parts, Pharmacognostical, physicochemical, phytochemical, thin layer chromatography.

INTRODUCTION

Herbal medicine is one of the most diverse fields of complementary medicine with a very long history and many different approaches originating in different countries[1,2]. Although herbs are supposed to be safe but many unsafe and fatal side effects including direct toxic effects, allergic reactions, effects from contaminants and/or interactions with drugs and other herbs have been reported[3,4,5]. Moreover, evidence based studies on the efficacy and safety of traditional Indian medicines is limited. Therefore, there is a need that experience based empirical knowledge if coupled with elucidation of the exact chemical in plant responsible for therapeutic action could provide a scientific basis to the herbal drugs and increase their acceptability. In light of the above mentioned facts, the present study was undertaken to standardize ethanopharmacologically useful plant Gojihva consisting of aerial parts of *Onosma bracteatum* Wall, family Boraginaceae. The aerial part of *Onosma bracteatum* is known traditionally for the treatment of asthma and bronchitis and is a deciduous perennial herb, 38 cm, erect, stout and patently hispid which is a habitat of East Asia-Himalayas from Uttar Pradesh to Central Nepal, commonly found in rocky slopes in dry areas, 3300-5000 meters. The leaves, flowers and seeds of this plant are acrid, cooling and has been reported to be used in the treatment of asthma, bronchitis, stomatitis, throat troubles, diseases of the chest and lungs, ophthalmic, stomatitis, gingivitis, insanity, gonorrhea, lumbago, leprosy, allay thirst. It is also useful in relieving functional palpitation of the heart, irritation of the stomach and bladder and strangury. As reported in the literature, plant of *Onosma bracteatum* contains tannins and sugars[6-9].

MATERIALS AND METHODS

Identification and storage of plant materials

Dried aerial parts of *O. bracteatum* were obtained from a commercial supplier in Ahmedabad. The plant was identified and authenticated by Dr. Minoo Parabia, Head and Professor, Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat. A voucher specimen of the plants was deposited in the herbarium of the Department of Pharmacognosy, Anand Pharmacy College, Anand, Gujarat (APC/2007/01). Dried aerial parts of *O. bracteatum* were separately milled into powder with the aid of an electrical grinder, passed through sieve no. 60 and finally stored in airtight bottles in a dry and dark place before analysis.

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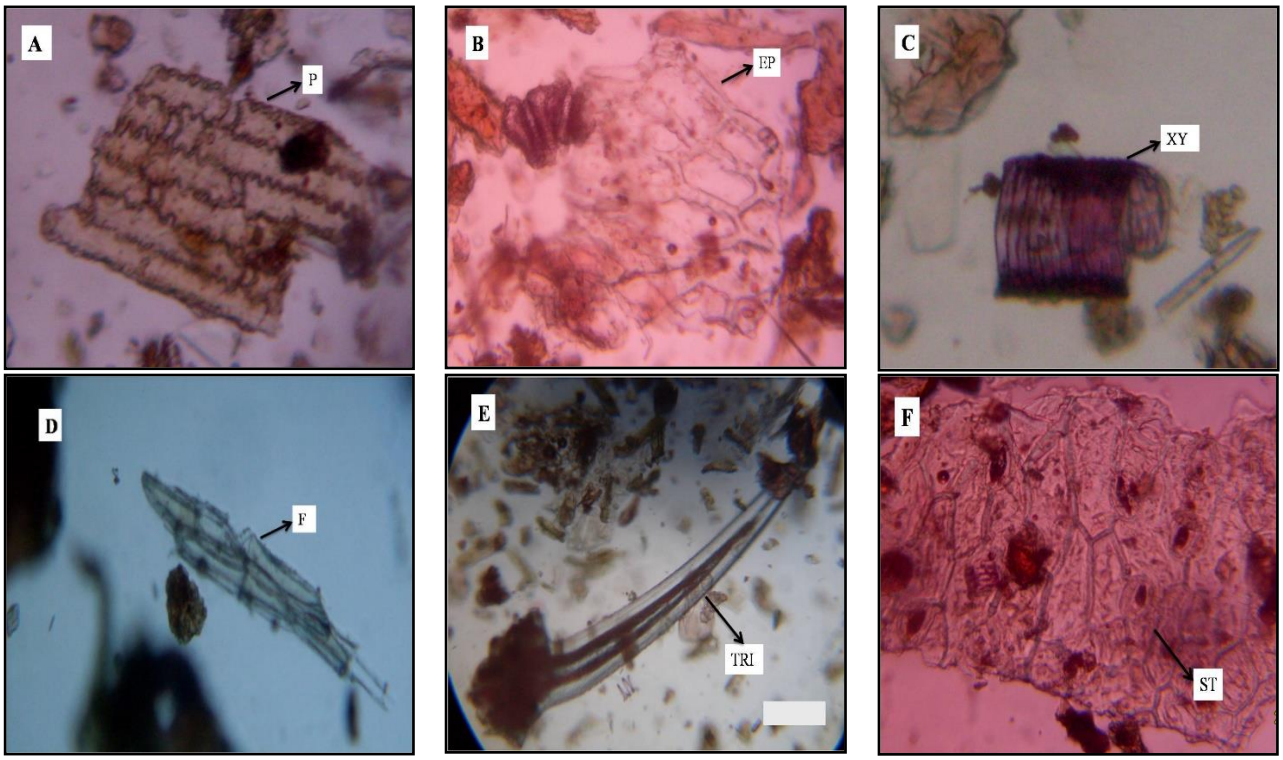

Figure 1(A-F): Powder characteristics of *O. bracteatum*, P-Palisade cells, EP-Epidermal Cell, XY-Xylem Vessel, F-Fibre, TRI- Trichome, ST-Paracytic Stomata.

Table 1: Fluorescent properties of aerial parts of *O. bracteatum.*

Sr. no.	Reagent	Daylight	U.V. (254 nm)	U.V. (365 nm)
1	Methanol	Green	-	Green
2	1 M NaOH in methanol	Greenish brown	-	Greenish brown
3	1 M NaOH in water	Brown	-	Green
4	1 M HCl in methanol	Brown	-	Green
5	1 M HCl in water	Brown	-	Green
6	50% HNO_3	Brown	-	Green
7	50% H_2SO_4	Brownish green	Purple	Green
8	HNO_3	Orange	-	Green
9	CH_3COOH	Brown	-	Greenish brown
10	1% picric acid	Brown	-	Green
11	10 % $K_2Cr_2O_7$	Brown	-	Green
12	5% I_2	Brown	-	Brown
13	Dilute NH_3	Green	-	Green
14	5% $FeCl_3$	Green	Purple	Green

Reagents and chemicals

All the reagents and chemicals used for the evaluation purpose were of analytical grade procured from SD Fine chemicals (Mumbai, India) and Merck Ltd. (Mumbai, India).

Macroscopic and microscopic examination

The macroscopic examination was carried out with the help of naked eyes and simple hand lens for the evaluation of shape, size, color, and fracture. For powder microscopy, the aerial parts were finely powdered and screened for the presence of its own and foreign vegetative matters (other than the organ selected for the research studies). The powder was passed through sieve No.180 and sieve No.125 (Jayant make), separately, to obtain fine and very fine powder respectively and then subjected for microscopic examination using binocular microscope (Radical PRM-12A). The sample was treated with various reagents like 50% glycerin as temporary mountant; 2% phloroglucinol in ethanol (90%) and concentrated hydrochloric acid (1:1) for lignin; 5% of alcoholic ferric chloride for phenolic compounds; 2% Iodine solution for starch grains; and Ruthenium red (0.08%) in 10% lead acetate for mucilage and studied for their components of diagnostic value[10,11,12].

Fluorescence analysis

The aerial parts of the plant was subjected to fluorescence analysis by treating separately with different reagents and was observed immediately in visible light and UV lights (254 and 366 nm) using UV cabinet (Durga Scientific) for fluorescence behavior[13,14].

Table 2: Standardization parameters of aerial parts of *O. bracteatum.*

Sr. No.	Standardization parameters	*O. bracteatum* (%)
1	Moisture content	10.00±0.30
2	Total solids	90.00±0.30
3	Solvent Extractive Value	
	Water soluble extractive	-
	Ethanol soluble extractive	2.00±0.10
	Methanol soluble extractive	1.00±0.10
	Ether soluble extractive	1.20±0.02
4	Ash Value	
	Total ash	25.40±1.11
	Acid-insoluble ash	2.80±0.05
	Water-soluble ash	16.37±0.15
5	Foaming index	Less than 100
6	Swelling index	1.56±0.07

Table 3: Elemental analysis of aerial parts of *O. bracteatum.*

Sr. No.	Minerals	*O. bracteatum* (%)
1	Calcium	36.300±1.23
2	Iron	0.694±0.12
3	Potassium	22.332±1.12
4	Magnesium	4.708±0.21
5	Sodium	3.140±0.19
6	Phosphorus	0.408±0.05
7	Sulphur	1.467±1.00
8	Zinc	0.033±0.002

Physicochemical evaluation

The aerial parts were subjected to determination of different physicochemical constants such as moisture content, total solids, solvent extractive values (alcohol, methanol, ether), foaming index, swelling index and ash values (total ash, acid insoluble ash, water soluble ash)[15-19].

Elemental analysis

The aerial parts of the plant was subjected to analysis of major elements such as calcium, potassium, sulphur and trace elements (iron, copper and zinc) according to the method of Shahidi et al. The mineral content was determined using inductively coupled plasma atomic emission spectrophotometer (Perkin Elmer, USA, 3300RL). The concentration of each element in the sample was calculated as the percentage of dry matter[20].

Preliminary phytochemical screening

50 g of the powdered aerial parts of *O. bracteatum* was extracted successively with petroleum ether (60-80°C b.p.), toluene, chloroform, methanol using soxhlet apparatus[21,22]. The extraction with each solvent was carried out until the solvent was colorless for 24 h. Finally, the marc left was extracted with water by digesting on a boiling water bath. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The extracts were taken in a tarred porcelain dish and evaporated to dryness on a water bath and dried at 105ºC to a constant weight. The percentage extractives were calculated with reference to air dried drug. The presence or absence of the primary (carbohydrates, proteins and amino acids) and secondary phytoconstituents (glycosides, alkaloids, saponins, tannins, steroids, coumarins etc.) was detected by various qualitative chemical tests[23,24].

Determination of Total phenolic content

The total phenolic content in the aerial parts (50 mg/ml in methanol) was determined by modified folin ciocalteau method using gallic acid (100 µg/ml) as a standard in the concentration range of 2 to 10 µg/ml. The absorbance was recorded at 760 nm with a Shimadzu double beam UV/Visible spectrophotometer 1650 PC. The calibration curve was plotted with gallic acid, and the results were expressed as grams of gallic acid equivalents (GAE) per gram of dry weight of aerial parts of *O. bracteatum*[25].

Determination of Total flavonoid content

Total flavonoid content in the aerial parts (0.5 ml of extract, 50 mg/ml in methanol) was determined according to colorimetric aluminum chloride method reported by Woisky and Salatino, 1998. The content was determined using quercetin as a standard solution (100 µg/ml) in the concentration range of 2 to 10 µg/ml. The absorbance of the reaction mixture was measured at 415 nm with a Shimadzu double beam UV/Visible spectrophotometer 1650 PC. Total flavonoid content was calculated from calibration curve and reported as quercetin equivalent (% w/w)[26].

Thin layer chromatographic studies (TLC)

Various extracts obtained in successive solvent extraction were subjected to TLC studies using Silica gel 60 F_{254} TLC plate. Saturation time for the mobile phase chamber was 20 min. The plates after development were first viewed through UV-fluorescence cabinet (254 and 365 nm) and the R_f values of the fluorescing spots were noted. Further derivatization using different spraying reagents was carried out[27]. The colour and R_f of various spots were noted. These results were compared with the results obtained in qualitative chemical tests. TLC development

Table 4: Successive solvent extraction of aerial parts of *O. bracteatum.*

Sr. no.	Solvent	Colour of extract	Consistency of extract	Extractive value (%w/w)
1	Petroleum ether	Greenish brown	Semisolid	0.10
2	Toluene	Dark green	Semisolid	0.56
3	Chloroform	Dark green	Semisolid	0.45
4	Acetone	Green	Semisolid	0.68
5	Methanol	Light green	Semisolid	1.15

Table 5: Qualitative chemical test of different extracts of aerial parts of *O. bracteatum.*

Sr. No.	Phytoconstituents	P	T	C	A	M	W
1	Steroids	-	-	-	-	-	-
2	Triterpenoids	-	-	-	-	+	-
3	Reducing sugars	-	-	-	-	-	-
4	Carbohydrates	-	-	-	-	-	-
5	Alkaloids	-	-	-	-	-	-
6	Phenolic compounds	-	-	-	-	+	-
7	Anthraquinones	-	-	-	-	-	-
8	Saponins	-	-	-	-	+	-
9	Tannins	-	-	-	-	-	-
10	Flavonoids	-	-	-	-	+	-
11	Mucilage	-	-	-	-	-	+

P - Petroleum ether, T - Toluene, C - Chloroform, A - Acetone, M - Methanol, W – Water; + present, - absent

Table 6: TLC profile of various extracts of aerial parts of *O. bracteatum* obtained by successive solvent extraction.

Solvent system	No. of spots (R_f) Petroleum ether	Toluene	Chloroform	Acetone
Ethyl acetate: Methanol: Water (100:13.5:10)	-	-	-	5 in short UV (0.43,0.51, 0.60,0.73, 0.82) 3 in long UV (0.45,0.49, 0.54)
Toluene:Ether (1:1) saturated with 10% acetic acid	2 in short UV (0.32, 0.58) 2 in long UV (0.32, 0.39)	-	-	3 in short UV (0.22,0.40, 0.67) 1 in long UV (0.40)
Toluene : Ethyl acetate (93: 7)	5 in short UV (0.03,0.08, 0.16,0.23, 0.30) 6 in long UV (0.03,0.08, 0.16,0.23, 0.30, 0.38)	5 in short UV (0.18,0.26, 0.33,0.44, 0.56) 3 in long UV (0.18,0.33, 0.44)	-	4 in short UV (0.24,0.29, 0.39,0.58) 5 in long UV (0.34,0.39, 0.46,0.59, 0.73)
Chlorofrom: Ethyl acetate (70:30)	-	-	2 in short UV (0.32,0.67)	-

was carried out in different mobile phase as listed in Table 6 and 7.

RESULTS AND DISCUSSION

Macroscopic and microscopic examination

Stem was 5.1-8.8 cm long and 3.4 to 4.5 cm in diameter, flattened, erect, stout; rough due to white, hard, hispid hairs and longitudinal wrinkles; colour was greenish yellow; fracture short, odour and taste was not characteristic. Leaves were lanceolate to ovate lanceolate, 12.5-28.5 cm long, 1.6 to 3.3 cm broad, acuminate tubercle based hispid hairs present on both the surfaces, greenish to light yellow on top and white beneath.

Powder study showed group of oval to polygonal thin walled straight epidermal cells; spiral vessels, a few fibres elongated with blunt tips, long warty, tubercle based unicellular hairs and paracytic stomata. The characters of powder study are as shown in Figure 1 (A-F).

Fluorescence analysis

The results of the fluorescent properties of the powder of aerial parts of *O. bracteatum* obtained on treatment with several reagents are as presented in Table 1. Fluorescence analysis revealed the presence of starch and phenolic compounds in aerial parts of *O. bracteatum*. Reaction with acid and alkali showed fluorescence indicating that phenolic compounds like flavonoids, flavones and coumarins may be present.

Physicochemical evaluation

Percent content of total ash for aerial parts of *O. bracteatum* (25.4%, standard-NMT 26%) was found to be higher indicating the percentage of inorganic acids. Acid insoluble ash value was found to be 2.8% (standard-NMT 4%) due to the presence of silicaceous matter. Foaming index was found to be less than 100. Swelling index was found to be 1.56 indicating the presence of mucilage content. Results for standardization parameters are as presented in Table 2.

Elemental Analysis

Calcium was the most abundant macro element present in aerial parts of the plants. This was followed closely by potassium, magnesium, sodium, sulphur and iron. The results are as summarized in the Table 3.

Preliminary phytochemical screening

Extracts obtained by successive solvent extraction were semisolid in consistency. Highest extractive value (1.15%) was found for methanolic extract of *O. bracteatum*

Table 7: TLC profile of ethanolic extract of aerial parts of *O. bracteatum.*

Solvent system/ Spraying reagent	R_f (Colour)		
	254 nm	365 nm	Spraying reagent
Chloroform: Methanol: Water (70:30:4) Anisaldehyde sulphuric acid	0.09 (light blue), 0.53 (white), 0.89 (blue)	0.09 (blue), 0.18 (violet), 0.41 (violet), 0.75 (violet)	0.09(blue),0.16(violet), 0.25(blue), 0.41 (blue), 0.51 (blue), 0.65 (blue) 0.78(yellowish brown)
Ethyl acetate: Methanol: Water (100:13.5:10)	-	-	(0.76)blue
Ethyl acetate: Methanol: Water (100:13.5:10) 10% ethanolic potassium hydroxide	0.05(blue), 0.55(green)	-	0.45(yellow), 0.76(yellow)
Toluene : Ethyl acetate (93: 7) 10 % ethanolic potassium hydroxide	0.09 (pink), 0.23 (red), 0.29 (light blue) 0.38 (red)	0.38 (yellowish green) 0.75 (bluish violet)	0.75 (Blue)
Toluene : Ethyl acetate (93: 7) Vanillin sulphuric acid	0.07 (pink), 0.29 (red), 0.32 (light blue), 0.44 (red)	0.24 (blue), 0.8 (blue)	0.24 (blue), 0.8 (blue)
Ethyl acetate: Methanol (9:1) Vanillin sulphuric acid	0.10 (light blue), 0.21(white), 0.39 (light blue), 0.50(greenish brown), 0.74 (light blue)	0.10 (blue), 0.21 (blue), 0.39 (blue), 0.50 (blue), 0.74 (blue)	0.14 (blue), 0.44(red), 0.50(yellowish brown), 0.58(blue), 0.65 (blue), 0.73 (blue)

indicating the presence of polar constituents. The results revealing the colour, consistency and extractive value of different extracts of aerial parts of *O. bracteatum* obtained by successive solvent extraction are as shown in Table 4.

Preliminary qualitative chemical tests of various extracts obtained by successive solvent extraction revealed the presence of phenolics, flavonoids, saponins in methanolic and aqueous extract of the aerial parts of *O. bracteatum* (Table 5). Additionally, high content of mucilage was found in the aerial parts of *O. bracteatum* indicating the presence of polysaccharide in higher percentage.

Total phenolic content

Total phenolic content has been reported as gallic acid equivalent with reference to standard curve, Y=0.0915X-0.0072, R^2=0.9996. Total phenolic content in aerial parts of *O. bracteatum* was found to be 0.34% w/w calculated as gallic acid equivalent.

Total flavonoid content

The total flavonoid content was calculated as quercetin equivalent with reference to standard curve, Y=0.0961X+0.0114, R^2 = 0.9948. Total flavonoid content in aerial parts of *O. bracteatum* was found to be 0.15% w/w calculated as quercetin equivalent.

Thin layer chromatographic studies

Different solvent systems were found to be effective to obtain maximum number of spots for the extract. From the results obtained by qualitative chemical test, TLC profile of the ethanol extract and of various extracts obtained by successive solvent extraction it may be confirmed that the aerial parts of the *O. bracteatum* shows the presence of phenolics, flavonoids, saponins. The solvent system, spraying reagent, color and the R_f values of different spots recorded are as tabulated in the Table 6 and 7.

CONCLUSION

Standardization parameters developed herewith in the experimental work will be useful for identification and authentication of the plant in herbal formulations as well as for identifying the crude drug. The results obtained from the preliminary phytochemical screening can be used further to explore the phytomarkers present in the aerial parts of the plant. The information provided in the work can further be utilized for therapeutical and pharmacological study of aerial parts of the plant *Onosma bracteatum* Wall.

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