

PHYTOCHEMICAL SCREENING OF SELECTED PLANTS *BOSWELLIA OVALIFOLIOLATA*, AND *MEMECYLON EDULE*

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ABSTRACT

Preliminary Phytochemical screening: The n-hexane, chloroform, alcohol and ethyl acetate extracts of selected plants were screened qualitatively for the presence various phytoconstituents and total phenolic content of the alcoholic and ethyl acetate extracts were determined. *Boswellia ovalifoliolata*: Qualitative phytochemical screening of ethyl acetate extract of *boswellia ovalifoliolata* bark revealed the presence of steroids, terpenoids, alkaloids, tannins, saponins, flavonoids, phenols and oils. Alcoholic extract of *boswellia ovalifoliolata* bark revealed the presence of steroids, terpenoids, glycosides, alkaloids, tannins, saponins flavonoids, phenols, oils and proteins and carbohydrates.

The total phenolic content in alcoholic and ethyl acetate extracts of *boswellia ovalifoliolata* bark was found to be 20 ± 2.5 mg/g and 34.9 ± 4.26 mg/g respectively.

Memecylon edule :Qualitative phytochemical screening of ethyl acetate extract of *memecylon edule* leaves revealed the presence of steroids, terpenoids, alkaloids, tannins, flavonoids, phenols and oils. Alcoholic extract of *boswellia ovalifoliolata* bark revealed the presence of steroids, terpenoids, glycosides, alkaloids, tannins, saponins, flavonoids, phenols, oils and proteins and carbohydrates. The total phenolic content in alcoholic and ethyl acetate extracts of *boswellia ovalifoliolata* bark was found to be 80 ± 1.9 mg/g and 150.53 ± 2.87 mg/g respectively.

Kew Word :- Phytochemical, *Boswellia ovalifoliolata*, *Memecylon edule*

INTRODUCTION:

Medicinal plants are an important therapeutic aid for various ailments and those are a source for generating novel drug compounds for curing many diseases. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants (Dorman H.J. and Deans S.G., 2000). Accordingly, in the present investigation, the qualitative and quantitative phytochemical analysis of selected medicinal plants (*Boswellia ovalifoliolata*, and *Memecylon edule*) was carried out and determined the total phenolic contents.

Materials and Methods:

Collection of plant material:

The leaves of *Memecylon edule* and bark of *Boswellia ovalifoliolata* was collected from Kumau hills near Haldwani, forest region, Nainital, Haldwani District, Uttarakhand, India. The plant species was authenticated by Dr. Gulfishan Assistant Professor, Department of Botany, Glocal University, Saharanpur, Uttar Pradesh,

India. The leaves and bark were collected in the month of January-February. The voucher specimens (GU-HS-05/02/2019; GUO-BS- 09/12/2021 ;) were deposited in the institutional museum, Glocal University, Saharanpur, Uttar Pradesh, India.

Preparation of Extracts:

The collected plant materials of *Boswellia ovalifoliolata* and *Memecylon edule* were immediately sprayed with alcohol to cease the enzymatic degradation of secondary metabolites. The plant material was kept under shade for drying for about seven days and it was pulverized to obtain a coarse powder and packed in air tight container. The air dried powdered plant material was extracted in Soxhlet assembly successively with petroleum ether, chloroform, ethyl acetate and ethanol. Each time before extracting with the next solvent the powdered material is dried in hot air oven below 50⁰C. Each liquid extract is filtered, concentrated by evaporation under reduced pressure below 40⁰ C. in a rotary flash evaporator until a soft mass is obtained The extract obtained with each solvent is weighed. The percentage is calculated in terms of air dried weight of the plant material. The colour and consistence of the extract were noted. (Kokate et.al).

Preliminary Qualitative Phytochemical Screening:

The n-hexane, chloroform, ethyl acetate and ethanol extracts were subjected to the following chemical tests to identify chemical constituents of the plants (Wagner 1984; Kokate C.K. *et al.*, 1995; Wallis 1995; Peach and Tracey, 1955).

Tests for Alkaloids

About 50 mg of solvent – free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with alkaloidal reagents as follows.

Dragendorff's Test

To a 1 ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent reddish brown precipitate indicates positive test.

Mayer's Test

To a 1ml of filtrate, two drops of Mayer's reagent was added along with the sides of the test tube. If the test is positive, it gives white or creamy precipitate.

Wagner's Test

To a 1 ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

Hager's Test

To a 1 ml of filtrate, 1 or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

Tests for Carbohydrates

About 100mg of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to the following tests.

Molisch's Test

To 2 ml of filtrate, two drops of alcoholic solution of α – naphthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates.

Fehling's Test

1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

Barfoed's Test

To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugar.

Benedict's test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

Tests for Glycosides

For detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hrs on a water bath, filtered and the hydrolysate was subjected to the following tests.

Borntrager's Test

To 2 ml of filtrate hydrolysate, 3ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthroquinone glycosides.

Legal's Test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink color.

Tests for Saponins**Foam or Froth Test**

A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

Tests for Phytosterols and Triterpenoids**Libermann – Burchard's test**

The extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroids / triterpenoids and their glycosides.

Salkowski test

Few drops of concentrated sulphuric acid were added to the chloroform extract shaken on standing. Red color in the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

Test for Terpenes (Knollar's test):

5 mg of extract is treated with 2ml of 0.1% anhydrous stannic chloride in pure thionyl chloride. A deep

purple color that changes to red indicates the presence of terpenes.

Tests for Phenolic Compounds and Tannins

Ferric chloride test

About 50 mg of extract was dissolved in distilled water and to this a few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

Gelatin test

A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

Lead acetate test

A small quantity of extract was dissolved in distilled water and to this 3 ml of 10% lead acetate solution was added to this. A bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagents

An aqueous solution of extract was treated with 10% ammonium hydroxide solution – yellow fluorescence indicates the presence of flavonoids.

Shinoda test or Magnesium – Hydrochloric acid reduction

A little quantity of the extract was dissolved in alcohol and a few fragments of magnesium turnings and conc. hydrochloric acid (drop wise) were added. If any pink or crimson red color develops. The presence of flavonolglycoside is confirmed.

Quantitative estimation of total phenolic content:

Total phenolic content

The total phenol content was determined by following the method of **Scalbert A et al (2005)** with slight modifications. Briefly, 0.1 mL of the extracts were mixed with 0.2 mL of Folin-Ciocalteu reagent 0.75 mL of 7.5% sodium carbonate solution and the reaction mixture was incubated for 30 min and the absorbance was read at 765 nm. The mean of three readings was used and the total phenolic content was expressed as milligram of gallic acid equivalents (GAE)/gram of dry extract.

Results and Discussion:***Boswellia ovalifoliolata:***

The physical nature and percentage yield of successive extraction of *boswellia ovalifoliolata* bark is tabulated in Table 3.1, Qualitative phytochemical screening of alcoholic extracts of *Boswellia ovalifoliolata* revealed the presence of alkaloids, steroids, terpenoids, carbohydrates, flavonoids, glycosides, proteins whereas ethyl acetate extracts shows alkaloids, steroids, flavonoids, saponins, fixed oils and phenolic compounds. The results of phytochemical screening are summarized in the Table 3.5.

Memecylon edule:

The physical nature and percentage yield of successive extraction of *Memecylon edule* leaves is tabulated in Table 3.1.

Alcoholic extract revealed the presence of alkaloids, glycosides, steroids, tannins, saponins, sugars, gums, fixed oils, phenolic compounds terpenoids, carbohydrates, flavonoids, proteins whereas ethyl acetate extracts shows alkaloids, steroids, flavonoids, fixed oils and phenolic compounds. The results of phytochemical screening are summarized in the Table 3.6.

Table 3.1 Physical nature and Percentage Yield of the different extracts of *Boswellia ovalifoliolata* bark

S.No.	Name of the extract	Method of extraction	Physical Nature	Colour	% yield of extract (%w/w) in gms
1.	n-hexane	Successive solvent extraction in soxhlet apparatus	Greasy Sticky	Greenish	0.6
2	Chloroform		Sticky	Greenish	1.4
3.	Ethyl acetate		Sticky	Greenish brown	2.52
4.	Alcohol		solid	Reddish brown	9.5

Table 3.2: Physical nature and Percentage Yield of the different extracts of Memecylon edule leaves

S.No.	Name of the extract	Method of extraction	Physical Nature	Colour	% yield of extract (%w/w) in gms
1.	n-hexane	Successive solvent extraction in soxhlet apparatus	Sticky	Greenish	2.1
2	Chloroform		Sticky	Greenish	2.6
3.	Ethyl acetate		Sticky	Greenish brown	7.47
4.	Alcohol		Semi solid viscous	Greenish black	13.3

Table 3.3 Preliminary Phytochemical Screening of different extracts of *boswellia ovalifoliolata* bark

Constituents	Test	n-hexane extract	Chloroform extract	Ethyl acetate extract	Alcoholic extract
Alkaloids	Mayer's reagent	-	+	+	+
	Dragendorff's reagent	-	+	+	+
	Hager's reagent	-	+	+	+

	Wagner's reagent	-			
Glycosides	Keller-Killiani test	-	-	-	+
	Borntrager's test	-	-	-	+
	Legal's test	-	-	-	-
	Baljet's test	-	-	-	+
Steroids	Liebermann's test	+	+	+	+
	Salkowski reaction	+	+	+	+
	Liebermann-Burchard test	+	+	+	+
Tannins	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	+	+
Sugars &	Molish's reagent	-	-	-	+
Carbohydrates	Fehling's test	-	-	-	+
	Benedict's test	-	-	-	+
	Protein	Millon's test	-	-	-
	Biuret test	-	-	-	+
Amino acid	Ninhydrin test	-	-	-	+
Terpenoids	Knoller's test	+	+	+	+

Flavonoids	Alkaline reagent test	-	-	+	+
	Shinoda test	-	-	+	+
Saponin	Foam test	-	-	+	+
Gums and mucilage	Swelling test	--	-	-	+
Fixed oil and fats	Spot test	+	+	+	+
Phenolic compounds	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	+	+

+ indicates Presence - indicates Absence.

Table 3.4 Preliminary Phytochemical Screening of different extracts of *Memecylon edule* leaves

Constituents	Test	n-hexane extract	Chloroform extract	Ethyl acetate extract	Alcoholic extract
Alkaloids	Mayer's reagent	-	+	-	+
	Dragendorff's reagent	-	+	+	+
	Hager's reagent	-	+	+	+

	Wagner's reagent	-	+	+	+
Glycosides	Keller-Killiani test	-	-	-	+
	Bortrager's test	-	-	-	+
	Legal's test	-	-	-	-
	Baljet's test	-	-	-	+
Steroids	Liebermann's test	+	+	+	+
	Salkowski reaction	+	+	+	+
	Liebermann-Burchard test	+	+	+	+
Tannins	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	+	+
Sugars &	Molish's reagent	-	-	-	+
Carbohydrates	Fehling's test	-	-	-	+
	Benedict's test	-	-	-	+
	Millon's test	-	-	-	+
Protein	Biuret test	-	-	-	+
	Ninhydrin test	-	-	-	+
Amino acid	Ninhydrin test	-	-	-	+
Terpenoids	Knoller's test	+	+	+	+

Flavonoids	Alkaline reagent test	-	-	+	+
	Shinoda test	-	-	+	+
Saponin	Foam test	-	-	-	+
Gums and mucilage	Swelling test	-	-	-	+
Fixed oil and fats	Spot test	+	+	+	+
Phenolic compounds	Ferric chloride test	-	-	+	+
	Lead acetate test	-	-	+	+
	Gelatin solution	-	-	+	+

+ indicates Presence - indicates Absence.

Table 3.5 Total phenolic content of alcoholic extract of *boswellia ovalifoliolata* bark and *memecylon edule* leaves :

Plants	Extract	Total phenolic content (mg/gm of dried extract)
<i>Boswellia ovalifoliolata</i> bark	Ethyl acetate	20±2.5
	Alcohol	34.9±4.26
<i>Memecylon edule</i> leaves	Ethyl acetate	80±1.9
	Alcohol	150.53±2.87

In the present study, the preliminary qualitative and quantitative phytochemical analysis were studied for the selected two medicinal plants. On qualitative phytochemical screening of medicinal plants extracts revealed the presence of different phytochemical constituents like steroids, terpenoids, phenols, flavanoids, alkaloids, glycosides, phenols, tannins, carbohydrates, oils and amino acids where as in quantitative phytochemical analysis shows that the highest yield was in alcoholic extract of *boswellia ovalifoliolata* and

memecylon edule. Many investigations were reported that some medicinal plants contain a wide variety of natural bioactive compounds, such as phenolics, alkaloids, flavonoids and tannins, which possess more potent biological activities (Lamba, S.S *et al.*, 2000; Marles, J. R. and Farnsworth, N. R. 1995; Catapano, A.L. 1997; Rui Hai Liu 2004; Harbone JB and Williams CA 2000) of great value in preventing the onset and/or progression of many human diseases. Results from this study indicates furthermore isolation of plant phytoconstituents (pure Compounds) from selected plants have the advantages in developing the new drug molecules.

CONCLUSIONS:

A systematic pharmacological investigation and evaluation of the selected two plants namely *Boswellia ovalifoliolata* and *Memecylon edule*. Qualitative investigation of alcoholic and ethyl acetate extracts of selected plant drugs showed the presence of bioactive compounds such as Flavonoids, Phenolic compounds, Alkaloids, Glycosides, Steroids and Tannins. There is variability in phenolic content of both alcoholic and ethyl acetate extracts of the selected plants. Between the two selected plants alcoholic extract of *memecylon edule* leaves shows more phenolic than *boswellia ovalifoliolata* bark.

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