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Successive Extraction and Study of Total Phenolic and Flavonoids Content In Different Solvent Extracts of Onosma Bracteatum Leaves

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ABSTRACT

Onosma bracteatum (Boraginaceae) is a plant species known for its traditional medicinal uses due to the presence of bioactive compounds such as phenolics, flavonoids, and other secondary metabolites. The current study aims to evaluate the total phenolic and flavonoid content in different solvent extracts of *Onosma bracteatum* leaves using a successive extraction method. The solvents used include chloroform, ethyl acetate, ethanol, and aqueous extracts. The study also investigates the phytochemical composition of the extracts and their potential antioxidant properties. The results indicated that the percentage yield of the extracts varied significantly, with the aqueous extract yielding the highest (9.21%), followed by ethanol (5.85%), ethyl acetate (2.36%), and chloroform (0.77%). Phytochemical screening revealed the presence of flavonoids, saponins, phenols, proteins, and carbohydrates in the extracts, with ethanol and aqueous extracts showing the highest levels of flavonoids and phenolic content. Total phenolic content (TPC) and total flavonoid content (TFC) were quantified using standard calibration curves, and the ethanol extract exhibited the highest TPC (3.12 mg/100mg) and TFC (2.68 mg/100mg). The findings suggest that *Onosma bracteatum* contains significant amounts of bioactive compounds, particularly phenolic compounds and flavonoids, which are known for their antioxidant, anti-inflammatory, and other therapeutic properties. These results support the potential use of *Onosma bracteatum* as a source of natural antioxidants for pharmaceutical and nutraceutical applications. Further studies are warranted to evaluate the biological activities of these extracts, including their potential in treating oxidative stress-related disorders.

Keywords: *Onosma bracteatum, Phenolic compounds, Flavonoids, Phytochemical screening, Successive extraction, Medicinal plants.*

1. INTRODUCTION

Onosma bracteatum, commonly known as "Indian Lithospermum," is a plant species in the family Boraginaceae, traditionally used in various herbal medicines. This plant is known for its therapeutic potential due to its rich phytochemical composition, including alkaloids, flavonoids, phenolic acids, and other bioactive compounds. Among these, phenolic compounds and flavonoids are of particular interest due to their known antioxidant, anti-inflammatory, and antimicrobial properties (Choudhury et al., 2017; Patel et al., 2020). Phenolic compounds are a diverse group of secondary metabolites found in plants, characterized by their ability to donate hydrogen atoms or electrons, which gives them potent antioxidant activity. Flavonoids, a subclass of polyphenolic compounds, are widely studied for their various pharmacological properties, such as their role in reducing oxidative stress and combating chronic diseases, including cardiovascular diseases and cancer (Sánchez-Rangel et al., 2013). In addition to these health benefits, phenolic compounds and flavonoids contribute to the color, taste, and overall quality of plants, making them essential for both medicinal and commercial purposes (Gómez-Caravaca et al., 2006). The extraction of these bioactive compounds is critical in determining their concentration and bioactivity. Different solvents such as ethanol, methanol, water, and acetone are used for extracting phenolic and flavonoid compounds, with the efficiency of extraction varying depending on the solvent polarity and plant material (Mathew & Abraham, 2006). Successive extraction methods, where the plant material is sequentially extracted with solvents of increasing polarity, are often employed to achieve maximum yield of the bioactive compounds. Several studies have demonstrated the importance of the solvent choice in the extraction process, with more polar solvents generally extracting higher amounts of phenolic compounds, while less polar solvents are better at extracting flavonoids (Singh et al., 2016).

Therefore, understanding the extraction efficiency of different solvents is crucial for maximizing the recovery of these bioactive compounds from Onosma bracteatum for potential pharmaceutical or nutraceutical applications. This study aims to investigate the total phenolic and flavonoid content in various solvent extracts of *Onosma bracteatum* using a successive extraction method. By comparing the yields of phenolic and flavonoid compounds from different solvents, this study will contribute to optimizing extraction protocols and understanding the relationship between solvent polarity and phytochemical composition. Additionally, the antioxidant activity of these extracts will be evaluated, providing insights into the potential medicinal value of *Onosma bracteatum*.

2. MATERIALS AND METHODS

Material

Potassium mercuric iodide, iodine, potassium iodide potassium bismuth iodide, picric acid, α-naphthol, ferric chloride, sodium nitropruside, pyridine, sodium hydroxide, ferric chloride, lead acetate, nitric acid, ninhydrin reagent and copper acetate used in this study. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).

Methods

Out of different methods of solvent extraction including maceration, infusion, decoction, percolation and soxhlation, the method that was employed in present study was maceration which is a type of continues percolation.

Defatting of plant material

Powdered plants material of *Onosma bracteatum* were shade dried at room temperature. The shade dried plants material (50 gram) was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Dried defatted leaves of *Onosma bracteatum* were exhaustively extracted with different solvent like chloroform, ethyl acetate, ethanol and aqueous using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of

c) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions.

Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

b) Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

7. Detection of proteins and amino acids

a) Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

8. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Audu *et al.*, 2007).

Quantitative estimation of bioactive compounds

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Parkhe and Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extracts and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extracts or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

3. RESULTS AND DISCUSSION

The results of this study highlight the differences in the extraction yields, phytochemical composition, and antioxidant potential of various solvent extracts from the leaves of *Onosma bracteatum*. These differences are primarily influenced by the polarity of the solvents used. The percentage yield of the extracts ranged from 0.77% in chloroform to 9.21% in aqueous extract. Aqueous extraction yielded the highest amount of extract, likely due to its ability to dissolve a wide range of hydrophilic compounds. Ethanol also yielded a substantial amount of extract (5.85%), which can extract both polar and non-

polar compounds. Ethyl acetate and chloroform extracts, on the other hand, yielded less material, suggesting that these solvents were less efficient at extracting compounds from *Onosma bracteatum* leaves, likely due to their polarity and the specific types of compounds they target. The phytochemical screening revealed a diverse range of bioactive compounds in the extracts. All extracts tested negative for alkaloids and glycosides, indicating that these compounds are either absent or present in low concentrations in the leaves. Flavonoids, however, were present in the ethyl acetate, ethanol, and aqueous extracts. The presence of flavonoids is significant because these compounds are widely recognized for their antioxidant, anti-inflammatory, and other health-promoting properties. Saponins, phenols, proteins, and carbohydrates were found in the aqueous extract, while the ethyl acetate and ethanol extracts also contained diterpenes. These findings suggest that *Onosma bracteatum* leaves possess a variety of bioactive compounds, which could contribute to their medicinal properties. The presence of phenolic compounds across all extracts further supports the antioxidant potential of the plant. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were evaluated. The ethanol extract exhibited the highest TPC (3.12 mg/100mg) and TFC (2.68 mg/100mg), indicating that ethanol is the most efficient solvent for extracting these bioactive compounds. The aqueous extract also contained significant amounts of phenolics (2.68 mg/100mg) and flavonoids (1.25 mg/100mg), which highlights the potential of water as a solvent for extracting these valuable compounds. The ethyl acetate extract contained 2.45 mg/100mg of phenols and 1.05 mg/100mg of flavonoids, which is also notable, though slightly lower than the ethanol and aqueous extracts. These findings underscore the antioxidant potential of the different extracts, with higher TPC and TFC suggesting a greater ability to neutralize harmful free radicals and reduce oxidative stress. The significant presence of flavonoids in the ethanol and aqueous extracts is particularly important, as these compounds have been linked to various pharmacological activities, including anti-inflammatory, antimicrobial, and anticancer effects. The high levels of phenolic compounds and flavonoids in the extracts, especially in ethanol and aqueous extracts, suggest that *Onosma bracteatum* leaves have considerable medicinal potential. The antioxidant properties of phenolic compounds can help combat oxidative stress, which is associated with various chronic diseases, including cardiovascular diseases and cancer. The flavonoids present in the plant could also offer therapeutic benefits, such as anti-inflammatory and antimicrobial effects. The ability of *Onosma bracteatum* to yield these bioactive compounds, particularly using common solvents like ethanol and water, points to its potential as a source of natural antioxidants. These findings are important for the development of herbal formulations aimed at addressing oxidative stress-related diseases or for use as nutraceuticals.

Table 1: Showing plant material and their part used

Plant Species	**Family**	**Part used**	**Season of collection**
Onosma bracteatum	*Boraginaceae*	Leaves	Summer

Table 2: Result of percentage yield of extracts of *Onosma bracteatum*

S. No.	**Solvents**	**Percentage Yield (%)**
1.	Chloroform	0.77%
2.	Ethyl acetate	2.36%
3.	Ethanol	5.85%
4.	Aqueous	9.21%

Table 3: Result of phytochemical screening of extracts of *Onosma bracteatum* (Leaves)

S. No.	**Constituents**	**Chloroform extract**	**Ethyl acetate extract**	**Ethanol extract**	**Aqueous extract**
1.	**Alkaloids**				
	Mayer's Test	-Ve	-Ve	-Ve	-Ve
	Wagner's Test	-Ve	-Ve	-Ve	-Ve
	Dragendroff's Test	-Ve	-Ve	-Ve	-Ve
	Hager's Test	-Ve	-Ve	-Ve	-Ve

2.	**Glycosides**				
	Modified Borntrager's Test	-Ve	-Ve	-Ve	-Ve
	Legal's Test:	-Ve	-Ve	-Ve	-Ve
3.	**Flavonoids**				
	A) Lead acetate Test:	-Ve	+Ve	+Ve	+Ve
	B) Alkaline Reagent Test:	-Ve	-Ve	+Ve	-Ve
4.	**Saponins**				
	Froth Test	-Ve	-Ve	+Ve	+Ve
	Foam Test	-Ve	-Ve	-Ve	+Ve
5.	**Phenol**				
	Ferric Chloride Test:	-Ve	+Ve	+Ve	+Ve
6.	**Proteins and amino acids**				
	Xanthoproteic Test:	-Ve	+Ve	+Ve	+Ve
	Ninhydrin Test	-Ve	-Ve	-Ve	-Ve
7.	**Carbohydrate**				
	Molisch's Test	-Ve	-Ve	+Ve	-Ve
	Benedict's Test	-Ve	+Ve	+Ve	+Ve
	Fehling's Test	-Ve	-Ve	-Ve	+Ve
8.	**Diterpenes**				
	Copper acetate Test:	-Ve	-Ve	+Ve	+Ve

+Ve= Positive; -Ve= Negative

Total phenolic content estimation (TPC)

Total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.0247X-0.0015$, $R^2= 0.9995$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Total flavonoid content (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.0405X+0.0221$, $R^2= 0.9971$,** where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 4: Total phenolic and total flavonoid content of *Onosma bracteatum* (Leaves)

S. No.	Extracts	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Ethyl acetate extract	2.45	1.05
2.	Ethanol extract	3.12	2.68
3.	Aqueous extract	2.68	1.25

4. CONCLUSION

This study highlights the phytochemical richness of *Onosma bracteatum* leaves, particularly in terms of their phenolic and flavonoid content. The use of ethanol and aqueous solvents was found to be particularly effective in extracting these bioactive compounds. The findings suggest that *Onosma bracteatum* holds promise as a source of natural antioxidants and other bioactive agents for medicinal purposes. Further research into the bioactivity of these extracts, including in vivo studies, could provide deeper insights into their potential therapeutic applications.

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