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QUALITATIVE AND QUANTITATIVE STUDY OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *NOTONIA GRANDIFLORA* LEAVES

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and antioxidant activity of the ethanolic extract of *Notonia grandiflora* leaves. The extraction process yielded 12.5% ethanolic extract, indicating a high content of polar phytoconstituents. Preliminary phytochemical screening confirmed the presence of important bioactive compounds such as alkaloids, flavonoids, phenols, glycosides, saponins, tannins, and proteins. Quantitative analysis revealed that the extract contained 0.52 mg/100 mg of total flavonoids and 0.89 mg/100 mg of total phenols, which are known contributors to antioxidant activity. The antioxidant potential was assessed using the DPPH radical scavenging assay. The ethanolic extract showed dose-dependent inhibition of DPPH radicals with a maximum of 51.87% at 100 µg/ml, and an IC_{50} value of 95.11 µg/ml compared to 18.14 µg/ml for the standard antioxidant, ascorbic acid. These findings suggest that *Notonia grandiflora* leaves are a rich source of antioxidant phytochemicals and may have potential applications in the development of natural antioxidant therapies.

Keywords: *Notonia grandiflora*, phytochemical screening, antioxidant activity, DPPH assay, flavonoids, phenolic content, ethanolic extract, IC_{50}, medicinal plants, radical scavenging.

INTRODUCTION

Medicinal plants have been widely used across traditional healthcare systems for the treatment and prevention of various ailments due to their rich repository of bioactive compounds. These bioactive phytochemicals, including alkaloids, flavonoids, phenolics, glycosides, saponins, and tannins, play vital roles in therapeutic efficacy through their antioxidant, antimicrobial, and anti-inflammatory properties (Kumar & Pandey, 2013). Increasing attention is being directed toward exploring plant-based antioxidants as natural alternatives to synthetic compounds, which are often associated with adverse effects (Phatak & Hule, 2010).

Oxidative stress, caused by an imbalance between free radicals and antioxidants in the body, is linked to the development of numerous chronic diseases including cancer, cardiovascular disorders, neurodegenerative diseases, and skin-related conditions like acne (Lobo *et al.*, 2010). Natural antioxidants, primarily phenolic and flavonoid compounds, have shown the ability to scavenge reactive oxygen species (ROS) and thereby prevent oxidative damage at the cellular level (Rice-Evans *et al.*, 1997). Evaluating the total phenolic and flavonoid contents of plant extracts is therefore crucial in determining their antioxidant potential.

Notonia grandiflora, a lesser-known plant belonging to the family Asteraceae, has traditionally been used in various indigenous healing practices. Despite its ethnomedicinal relevance, limited scientific literature is available regarding its phytochemical composition and pharmacological potential. Investigating the antioxidant activity of its ethanolic leaf extract through qualitative and quantitative analysis of phytochemicals could provide insights into its therapeutic applications.

This study aims to identify and quantify the phytochemical constituents of the ethanolic extract of *Notonia grandiflora* leaves, and to assess its antioxidant activity using the DPPH radical scavenging method. The findings are expected to support the potential of this plant as a natural source of antioxidants and lay the groundwork for further pharmacological investigations.

MATERIALS AND METHODS

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. The leaves of *Notonia grandiflora* were collected from local area of Bhopal in the month of February, 2025. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Notonia grandiflora* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Selection of a suitable extraction technique is also important for the standardization of herbal products. Extraction is used to extract suitable soluble constituents, with the aid of the chosen solvents except those not necessary. The products obtained from the plant were thoroughly washed in tap water and rinsed in purified water. The cool, stable samples obtained from the plants were cut into small pieces and dried under shade for 3 to 4 weeks.

Maceration was a popular and inexpensive homemade technique for the preparation of tonic since a long time. This technique was used for the extraction of essential oils and active compounds from plant materials. Generally, the maceration procedure consists of multiple steps in extraction.

The coarsely powdered crude drug undergoes grinding to increase the surface area for proper mixing of powdered materials with the solvent. This process is done in a closed vessel where an appropriate solvent (menstruum) is added. Next, the solvent is strained off followed by pressing the solid residue of the extraction process known as marc to recover an optimum amount of occluded solution. Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield. Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of plant material

50 gram shade dried leaves were coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction process.

The extraction was continued till the defatting of the material had taken place.

Extraction by soxhlet extraction process

Defatted powdered of *Notonia grandiflora* has been extracted with ethanolic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} x100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

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

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

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

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

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

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

Molisch's Test: Filtrates was 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.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was 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: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides (Khandelwal, 2005).

4. Detection of saponins

Froth Test: Extract was 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.

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

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was 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.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/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 extract 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 765 nm using a spectrophotometer (Mishra *et al.*, 2017).

***In-vitro* antioxidant activity of *Notonia grandiflora* using DPPH method**

Total free radical scavenging capacity of extract from *Tridax procumbens* estimated

according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

RESULTS AND DISCUSSION

The present study investigated the phytochemical composition and antioxidant potential of the ethanolic extract of *Notonia grandiflora* leaves. The percentage yield from the extraction process demonstrated that the ethanolic extract yielded significantly more (12.5%) than the petroleum ether extract (1.1%), indicating that polar compounds were more abundant in the plant material.

Phytochemical screening of the ethanolic extract revealed the presence of several important secondary metabolites, including alkaloids (positive in Dragendorff's and Hager's tests), glycosides, flavonoids, phenols, proteins, saponins, tannins, and some carbohydrates (positive Fehling's test). The absence of diterpenes and certain other carbohydrate indicators suggests selective solubility and extraction of bioactive constituents in ethanol. The presence of flavonoids, phenols, and tannins is particularly significant, as these compounds are well known for their antioxidant and antimicrobial properties (Kumar & Pandey, 2013; Lobo *et al.,* 2010).

Quantitative estimation of total flavonoid and phenol content further supported the extract's potential antioxidant activity. The ethanolic extract contained 0.52 mg/100 mg of flavonoids and 0.89 mg/100 mg of phenolic compounds, indicating a moderate presence of these bioactives. These results align with the qualitative screening and validate the plant's antioxidant capacity.

Antioxidant activity was evaluated using the DPPH radical scavenging method, where the ethanolic extract exhibited a concentration-dependent increase in % inhibition. At the highest concentration (100 µg/ml), the extract showed 51.87% inhibition, compared to 92.56% by ascorbic acid, a known standard. Although the extract's antioxidant activity was lower than that of ascorbic acid, it still showed promising potential, with an IC_{50} value of 95.11 µg/ml versus 18.14 µg/ml for ascorbic acid. This suggests moderate antioxidant efficiency, which may be attributed to the moderate concentration of flavonoids and phenols in the extract.

Table 1: Results of % Yield of *Notonia grandiflora*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	1.10
2.	Ethanolic	12.5

Table 2: Phytochemical screening of extract of *Notonia grandiflora*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	+ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	+ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 3: Estimation of total flavonoids and phenol content of extract of *Notonia grandiflora*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.52	0.89

Table 4: % Inhibition of ascorbic acid and Ethanolic extract of *Notonia grandiflora* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	45.84	20.45
2	20	50.92	26.74
3	40	61.21	36.92
4	60	65.47	40.21
5	80	76.08	42.63
6	100	92.56	51.87
IC 50 value		**18.14**	**95.11**

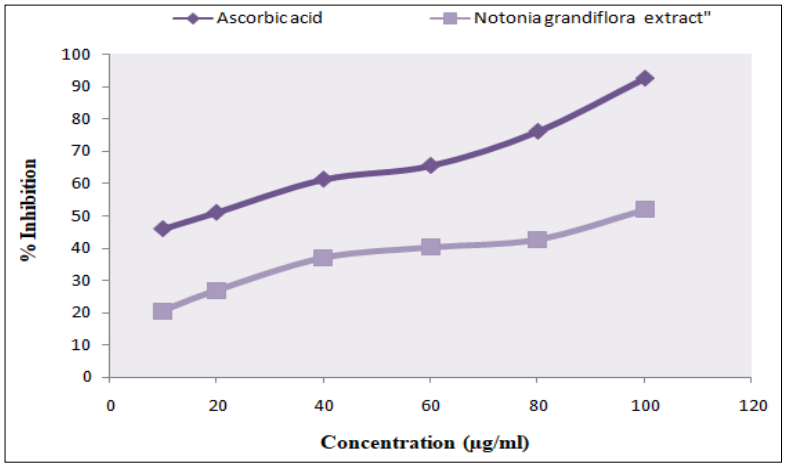

Figure 1: Graph of % inhibition of ascorbic acid and ethanolic extract of *Notonia grandiflora*

CONCLUSION

In conclusion, the ethanolic extract of *Notonia grandiflora* contains a variety of phytochemicals with notable antioxidant properties. The moderate levels of phenolics and flavonoids may contribute to its observed radical scavenging activity. These findings support further investigation into its potential therapeutic applications, particularly in oxidative stress-related disorders and skin conditions such as acne, where antioxidants play a key protective role.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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