Brief Communication

Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth)

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**Abstract**

Objectives: This study quantitatively determined the total phenolic contents in ethanolic and aqueous extracts of Gul-e-Zoofa (flowers of *Nepeta bracteata* Benth) using a spectrophotometric method. We also performed a spectral study (UV and IR) of the ethanolic extract and a fluorescence study of the powdered drug and successive extracts to identify and characterize the genuine herbal drug, which has not been previously performed.

Methods: The total phenolic content was determined quantitatively using the Folin Ciocalteu reagent, with Gallic acid as the standard. The fluorescence characteristics of the powdered drug and successive extracts with and without chemical treatment during the day and under a UV light were recorded. The UV and IR spectra of the alcoholic extract of Gul-e-Zoofa were recorded using a spectrometer.

Results: The total phenolic contents of the alcoholic and aqueous extracts were found to be 326.28 and 319.14 mg/g of the Gallic acid equivalent (GAE), respectively. The wavelength of the maximum absorption in the UV spectrum was 320 nm, and the characteristic frequencies in the IR spectrum were 3465.31, 3220.07, 2927.3, 2856.1, 1709.07, 1610.19, 1404.5, 1250.2, 1056.42, 823.04, 775.58, 577.81, and 463.10 cm\(^{-1}\). The fluorescence characteristics of the powdered drug were also observed.

Conclusion: This spectral and fluorescence study of the drug will be helpful for confirming the identity and purity of the genuine drug. The total phenolic content will be helpful for developing new drugs and standardizing the drug. The presence of a high total phenolic content shows that the flowers of *N. bracteata* Benth may possess antioxidant properties, which could lead to a new field of research in the future.

Keywords: Fluorescence study; Gul-e-Zoofa; Spectrophotometer; Total phenolic content; UV and IR spectral study

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Introduction

Gul-e-Zoofa belongs to the Lamiaceae family and is indigenous to Iran.1–4 It is a brightly coloured shrub or sub shrub that ranges from 30 to 100 cm in height. It is found in the western temperate Himalayas from Garhwal to Kashmir at an altitude of 1800–2400 m. The leaves are ovate-obtuse. During summer, the plant produces bunches of pink, blue and, more rarely, white fragrant flowers. The flowers are
bisexual, zygomorphic, rarely sub-actinomorphic and bracteolate.\textsuperscript{5,6} The flower is described as a stimulant, demulcent, expectorant and resolvent and an effective emmenagogue, cleanser, laxative, antihelminthic, anti-inflammatory and diaphoretic drug.\textsuperscript{7,14} It has been claimed that the plant material can cure chronic cough, chronic bronchitis,\textsuperscript{15} sore throat, asthma,\textsuperscript{16} toothaches, uterine or vesicle infections, and indurations of the liver or spleen.\textsuperscript{5} It has been medicinally used for pneumonia, influenza, diphtheria, eye ailments, diarrhoea and sciatica.\textsuperscript{9–14,17} Gul-e-Zoofa is easily available at affordable prices; thus, the physicochemical standardization of this drug has already been performed in our previous work in which preliminary phytochemical screening showed the presence of carbohydrates, flavonoids, glycosides, proteins, phenols and sterols.\textsuperscript{18} According to the literature, there is a positive relationship between total phenolic content and antioxidant potential of a compound.\textsuperscript{19–24} Thus, due to this and the medicinal importance of \textit{Nepeta bracteata} Benth, to continue our previous work, the current study was undertaken to estimate the total phenolic content of the ethanolic and aqueous extracts of \textit{N. bracteata} flowers with expected antioxidant activity. Additionally, a UV, IR, and fluorescence study of the drug were performed to provide a standard for the correct identification, authentication and quality of the genuine drug.

**Materials and Methods**

**Plant material**

The flowers of Gul-e-Zoofa (\textit{N. bracteata} Benth) were procured from the local market in Baradari, Aligarh, UP, India, and identified by the National Institute of Science Communication and Information Resources (NISCAIR) (Reference No. NISCAIR/RHMD/Consult/2011-12/1931/23).

**Preparation of the extract**

The aqueous and alcoholic extracts of the drug were obtained by refluxing 10 g of powdered drug with 150 ml of distilled water and absolute alcohol for six hours and then filtered and freeze dried in a lyophilizer. Stock solutions were prepared at a concentration of 1 gm/100 ml and subjected to spectrophotometric measurements to determine the total phenolic content.

**Determination of the total phenolic content**

The total phenolic contents of the aqueous and ethanolic extracts of Gul-e-Zoofa were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi.\textsuperscript{25} The calibration curve (Figure 1) was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 \( \mu g/ml \) Gallic acid solutions with 5.0 ml of Folin Ciocalteau reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 30 min at 765 nm. For both of the aqueous and ethanolic extracts (1 gm/100 ml), 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 1 h, the absorbance was measured to determine the total phenolic contents in both extracts separately using the formula,

\[
C = C_1 \times \frac{V}{m}
\]

where \( C = \text{total phenolic content in mg/g, in GAE (Gallic acid equivalent)} \), \( C_1 = \text{concentration of Gallic acid established from the calibration curve in mg/ml} \), \( V = \text{volume of extract in ml} \), and \( m = \text{the weight of the plant extract in g} \).

**UV spectral study**

The UV spectrum of the alcoholic extract was recorded using a Hitachi Ratio Beam U-1800 spectrometer, EVISA. The UV spectrum of the alcoholic extract of Gul-e-Zoofa was plotted according to the light absorbed as a function of the wavelength, and the drug showed a maximum absorption (\( \lambda_{\text{max}} \)), which is characteristic of a particular drug and aids the identification of herbal drugs.

**IR spectral study**

The IR spectrum of the alcoholic extract of Gul-e-Zoofa was determined in Nujol with a Perkin Elmer 1600 FTIR spectrometer, Spectro lab. The IR spectrum of the drug was recorded, and the major bands were noted.\textsuperscript{26}

**Fluorescence study**

The fluorescence characteristics of the powdered drug and successive extracts with and without chemical treatment under day light and under UV light at short (253 nm) and long (360 nm) wavelengths\textsuperscript{27–29} were observed on UV Fluorescence Analysis Cabinet, S.M. Scientific Industries Ltd, India according to the method described by Kokoski et al.\textsuperscript{30,31}

**Results**

**Total phenolic content**

Phytochemical screening of the ethanolic and aqueous extracts of Gul-e-Zoofa was conducted, and both the extracts showed the presence of phenolics.\textsuperscript{15} The total phenolic contents were determined using the Folin Ciocalteu method.

![Figure 1: Standard curve of Gallic acid.](image-url)
in terms of the Gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in Figure 1, and the standard curve equation was \( y = 0.007x + 0.186 \), where \( R^2 = 0.992 \). The total phenolic contents (Gallic acid equivalents, mg/g) in the ethanolic and aqueous extracts were calculated to be 326.28 and 319.14 mg/g, respectively.

**UV spectral study**

The UV spectrum of the ethanolic extract of Gul-e-Zooaf was plotted in terms of the light absorbed as a function of the wavelength, and the drug showed a characteristic wavelength for the maximum absorption (\( \lambda_{\text{max}} \)) at 320 nm (Table 1).

**IR spectral study**

FTIR spectral analysis confirmed the presence of various chemical functional groups in the ethanolic extract. The major bands were observed at 3465.31, 3220.07, 2927.36, 2856.16, 1709.07, 1610.19, 1404.50, 1250.24, 1056.42, 823.04, 775.58, 577.81, and 463.10 cm\(^{-1}\) (Table 1).

**Fluorescence study**

The powder and successive extracts of *N. bracteata* Benth were subjected to fluorescence analysis using different reagents.

| Table 1: UV and IR spectral data of the ethanolic extract of *Nepeta bracteata* Benth. |
|-----------------------------|
| 1. In UV spectral region (\( \lambda_{\text{max}} \)) | 320 nm |
| 2. In IR spectral region (\( \nu \), cm\(^{-1}\)) | (3465.31), (3220.07), (2927.36), (2856.16), (1709.07), (1610.19), (1404.50), (1250.24), (1056.42), (823.04), (775.58), (577.81), (463.10) |

| Table 2: Fluorescence analysis of the powdered drug (Nepeta bracteata Benth) with various chemical reagents. |
|-----------------------------|
| S. No. | Chemical reagent | Day light | Short UV | Long UV |
| 1 | Conc. sulphuric acid | Brown | Black | Black |
| 2 | Conc. hydrochloric acid | Dark brown | Dark green | Dark blue |
| 3 | Conc. nitric acid (2%) | Orange | Light green | Dark blue |
| 4 | Iodine solution (5%) | Brown | Dark green | Black |
| 5 | Ferric chloride solution (10%) | Brownish green | Black | Dark green |
| 6 | Sodium hydroxide solution (10%) | Yellow | Grey | Black |
| 7 | Acetic acid + Sulphuric acid (10%) | Dark brown | Dark green | Bluish black |
| 8 | NaOH + CuSO\(_4\) sol. | Bluish green | Blackish green | Green |
| 9 | 10% NaOH + Lead acetate sol. | Yellowish white | Grey | Off white |
| 10 | Acetic acid | Straw colour | Light green | Dark green |

The behaviour of the drug was observed under day light, UV light at 254 nm and UV light at 360 nm as per the standard procedure, and the results are reported Tables 2 and 3.

**Discussion**

Currently, plant materials rich in phenolics are used in the food industry because they decrease the oxidative degradation of lipids and maintain the quality and nutritional value of food.\(^2\) Phenolic compounds in plants are also very important because their groups have scavenging abilities. For Gul-e-Zooaf, we determined that the total phenolic content was slightly more in the ethanolic extract compared to the aqueous extract. Thus, Gul-e-Zooaf could be a potent source of natural antioxidants.

There are a number of parameters given by WHO and AYUSH to check for genuine drugs. Here, we have conducted a UV and IR spectral study to standardize *N. bracteata* Benth. Every drug has its own characteristic wavelength for the maximum absorption (\( \lambda_{\text{max}} \)) in the UV spectrum and characteristic frequencies in the IR spectrum, and the finger print region of the IR spectrum is especially different for each drug species and even for different extracts of the same species. Thus, this UV and IR spectral study of the alcoholic and aqueous extracts will be helpful for authentication and identification of the genuine drug of *N. bracteata* Benth (Table 1).

The powdered drug and successive extracts of the drug were screened qualitatively under day light and under a mercury vapour lamp at UV wavelengths of 254 and 360 nm to determine the fluorescence characteristics using different acids and reagents. The results are provided in Tables 2 and 3, which provide further information for identification of this herbal drug.

**Conclusion**

This spectral study of *N. bracteata* Benth will be helpful for confirming the identity and purity of the drug. By observing the powder and extracts under UV light, contamination may be detected. The colour of the drug in powder form and upon treatment with different chemicals under day light and UV light is a helpful diagnostic feature for the identification of the genuine drug. The total phenolic content will be helpful for developing new drugs and standardization of the drug. In addition, our results show that the flowers of *N. bracteata* Benth may possess antioxidant properties, which could lead to a new field of research in future.
Conflict of interest

The authors have no conflict of interest to declare.

Authors’ contributions

The design of the study, experiments, interpretation of the data, writing of the paper, and all correspondences and revisions were performed by NS. The literature review and experiments were performed by ZM under the guidance and supervision of NS. The drug was suggested by AL, and the experiments were performed by NS. The design of the study, experiments, interpretation of the data, writing of the paper, and all correspondences and revisions were performed by AM under the guidance and supervision of NS. The drug was suggested by AL, and the experiments were performed by ZM under the guidance and supervision of NS.

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