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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

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

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bisexual, zygomorphic, rarely sub-actinomorphic and bracteolate.[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.[7–14] It has been claimed that the plant material can cure chronic cough, chronic bronchitis,[15] sore throat, asthma,[16] tooth aches, uterine or vesicle infections, and indurations of the liver or spleen.[5] It has been medicinally used for pneumonia, influenza, diphtheria, eye ailments, diarrhoea and sciatica.[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.[18] According to the literature, there is a positive relationship between total phenolic content and antioxidant potential of a compound.[19–24] Thus, due to this and the medicinal importance of *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 *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 (*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.[25] The calibration curve (Figure 1) was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/ml Gallic acid solutions with 5.0 ml of Folin Ciocalteu 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,

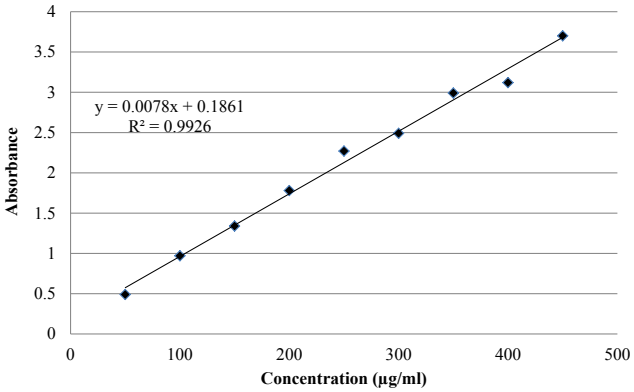

Figure 1: Standard curve of Gallic acid.

$$C = C_1 \times V/m$$

where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), C_1 = concentration of Gallic acid established from the calibration curve in mg/ml, V = volume of extract in ml, and m = 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 (λ_{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.[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[27–29] were observed on UV Fluorescence Analysis Cabinet, S.M. Scientific Industries Ltd, India according to the method described by Kokoski et al.[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.[18] The total phenolic contents were determined using the Folin Ciocalteu method

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-Zoofa 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 (λ_{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, 2927.3, 2856.1, 1709.07, 1610.19, 1404.5, 1250.2, 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 (λ_{max})	320 nm
2.	In IR spectral region (ν, 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	Orange	Light green	Dark blue
4	Iodine solution (2%)	Brown	Dark green	Black
5	Ferric chloride solution (5%)	Brownish green	black	Dark green
6	Sodium hydroxide solution (10%)	Yellow	Grey	Black
7	Acetic acid + Sulphuric acid	Dark brown	Dark green	Bluish black
8	10% 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

Table 3: Fluorescence analysis of successive extracts of *Nepeta bracteata* Benth.

S. No	Extracts	Day light	Short UV	Long UV
1	Pet. ether	Green	Dull green	Green
2	Diethyl ether	Straw colour	Purple	Light green
3	Ethyl acetate	Transparent	Transparent	Transparent
4	Chloroform	Transparent	Transparent	Transparent
5	Alcohol	Transparent	Transparent	Transparent
6	Water	Transparent	Transparent	Transparent

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.[32] Phenolic compounds in plants are also very important because their groups have scavenging abilities. For Gul-e-Zoofa, we determined that the total phenolic content was slightly more in the ethanolic extract compared to the aqueous extract. Thus, Gul-e-Zoofa 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 (λ_{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 section related to Unani medicine was examined by AL and AR.

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References

1. Hamedi SH. Determination of the scientific name of *Zoufa*: a traditional Persian medicinal plant. **Trad Integr Med 2016**; 1(2): 79–81.
2. Amin GR. *Popular medicinal plants of Iran*. Tehran: Iranian Ministry of Health Publications; 1991. pp. 40–44.
3. Joharchi MR, Amiri SM. Taxonomic evaluation of misidentification of crude herbal drugs marketed in Iran. **Avicenna J Phytomed 2012**; 2: 105–112.
4. Faraz M, Nickavar B, Tehrani HH. Essential oil analysis of *Nepetacrispa* and *N. menthoides* from Iran. **Iran J Pharm Sci 2009**; 5(1): 43–46.
5. Kiritikar KR, Basu BD. *Indian medicinal plants*. Dehradun, India: International Book Distributers; 1990.
6. Avicenna. *Kitabul Adviyatul Qalbia*. Aligarh: National Printing Co; 1956.
7. Bhatt J, Qudsia N, Aslam M, Ahmad S, Tanveer S. Pharmacognostical and phytochemical evaluation of Nepetabractaeta and hptlc finger printing of its extracts. **Int J Univers Pharm Life Sci 2012**; 2(1): 147–148.
8. Said HM. *Medicinal herbs*. Hamdard Foundation Pakistan; 1996. pp. 29–130.
9. Nadkarni AK. *Indian materia medica*. India Bombay: Popular book Depot; 1954. p. 673.
10. Baitar Ibn. *Al Jamiul Mufradatul Adviawal Aghzia* (Urdu); 1999. p. 185.
11. Ibn-e-Sina. *Al Qanoon Fit Tibb* (Urdu translation by Ghulam Hussain Kantoori). Lucknow: Matba Nawal Kishore; 1887. p. 288.
12. Hakeem. *Bustanul Mufradat* (Urdu translation). Lucknow, India: Idara Taraqqi Urdu Publication; 1893. p. 188.
13. Lubhaya RH. *Goswami, Bayanul Advia*. Delhi: Goswami Kutub Khana Gali Qasim; 1977. pp. 303–304.
14. Ghani MN. *Khazainul Advia* (Urdu translation). Urdu Bazar Lahore, Pakistan: Sheikh Mohd Bashir and Sons; 1921. p. 768.
15. Vohora SB. Unanijoshandah drugs for common cold, catarrh, cough, and associated fevers. **J Ethnopharmacol 1986**; 16: 201.
16. Wang J, Li F, Pang N, Tian G, Jiang M, Zhang HP, Ding JB. Inhibition of asthma in OVA sensitized mice model by a traditional Uygur herb Nepeta bracteata Benth. **Evid Based Complement Altern Med 2016**; 2016: 85769897.
17. Ahmadi BB, Bahmani M, Tajeddini P, Naghdi N, Kopaei MR. An ethno-medicinal study of medicinal plants used for the treatment of diabetes. **J Nephropathol 2016**; 5(1): 44–50.
18. Latif A, Zeenat, Siddiqui N, Rauf A. Physiochemical standardization of market sample of Gul-e-Zoofa. **Int J Drug Formul Res 2013**; 83 [online].
19. Rahman K. Studies on free radicals, antioxidants, and co-factors. **Clin Interv Aging 2007**; 2(2): 219–236.
20. Piluzza G, Bullitta S. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. **Pharm Boil 2011**; 49(3): 240.
21. Fecka I, Raj D, Krauze-Baranowska M. Quantitative determination of four water-soluble compounds in herbal drug from Lamiaceae using different chromatographic techniques. **Chromatographia 2007**; 66: 87–93.
22. Saeed LN, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Toriliseptophylla. **BMC Complement Altern Med 2012**; 12: 221.
23. Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. **BMC Res Notes 2014**; 7: 560.
24. Esmaeili AK, Taha RM, Mohajer S, Banisalam B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from in vivo and in vitro grown *Trifolium pratense* L. (Red Clover). **BioMed Res Int 2015**: 11643285.
25. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. **Am J Enol Vitic 1965**; 16: 144–158.
26. Jain MK, Sharma SC. *Modern organic chemistry*. Jalandhar, India: Vishal Publishing Co; 2004. pp. 1036–1099.
27. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Pharmacognostic evaluation of leaf and root bark of Olopteleaintegrifolia Roxb. **Asian Pac J Trop Biomed 2012**: 169–175.
28. Arya V, Gupta R, Gupta VK. Pharmacognostic and phytochemical investigations on PyruspashiaBuch.-Ham.ex D. Don stem bark. **J Chem Pharm Res 2011**; 3(3): 447–456.
29. Ansari SH. *Essential of pharmacognosy*. 1st ed. New Delhi: Birla Publications Pvt. Ltd; 2007.
30. Kokoski CJ, Kokoski RJ, Sharma PJ. Fluorescence of powdered vegetable drugs under ultra-violet radiation. **J Am Pharm Assoc 1958**; 47: 715–717.
31. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. **J Am Pharm Assoc 1949**; 38: 324–331.
32. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. **J Agric Food Chem 1999**; 47: 3954–3962.

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