Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2024; 13(1): 230-232
Received: 13-11-2023
Accepted: 26-12-2023

Kiran Khangarot
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Ashmita Mishra
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Richa Bhardwaj
Department of Botany, IIS (Deemed to be University), Jaipur, Rajasthan, India

RA Sharma
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Corresponding Author:
Kiran Khangarot
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Phytochemical and *in vitro* antioxidant potential screening of *Grewia asiatica* L.

Kiran Khangarot, Ashmita Mishra, Richa Bhardwaj and RA Sharma

DOI: https://doi.org/10.22271/phyto.2024.v13.i1c.14831

Abstract
Grewia asiatica L. is a fruit-bearing shrub, commonly known as *phalsa* through most parts of India and is well known for its Phyto medicinal importance. In this study, we aimed to perform a preliminary phytochemical and antioxidant activity analysis of *G. asiatica* by ABTS (2,2-azino- bis -3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay using ascorbic acid as standard. The result concluded that in all the extracts of different plant parts, carbohydrates and proteins, tannins, flavonoids and phenols, are among the primary and secondary metabolites confirmed to be present. The leaf of *G. asiatica* showed the highest antioxidant activity that is 0.197±0.01 µg AAE/ml for ABTS assay among all the plant parts studied. The tests performed conclude that the plant of *G. asiatica* is rich in phytochemicals and exhibits significant antioxidant properties and thus can be utilized as a source of natural antioxidants.

Keywords: ABTS, antioxidant activity, *Grewia asiatica* L., phytochemistry

Introduction
Free radicals are highly reactive molecules due to the presence of unpaired electrons in their outermost orbit, which oxidizes various molecules resulting in their damage (Rakesh *et al*., 2010) [9]. To prevent this phenomenon from occurring certain compounds are utilized that delay or prevent oxidization of these oxidizable compounds even when they are present in low concentrations, these compounds are known as antioxidants (Halliwell & Gutteridge, 1999) [3]. Endogenous antioxidants are generated inside the body and play a major part in rescuing cells from damage due to free radicals and exogenous antioxidants are procured from outer sources such as diet (Bouayed and Bohn, 2010) [2]. Considering the recent scenario, there is an ever-growing need for exogenous antioxidants to be administered for better protection against radical damage. It is in turn leading to a rise in research on antioxidants from natural sources due to their inexpensive nature, wide distribution and no side effects (Tadhani *et al*., 2007) [13]. *G. asiatica*, is a seasonal summer plant and a member of the family Tiliaceae. Traditionally used as a refreshing drink, anti-inflammatory agent and for the treatment of some urological disorders. It is found distributed in central and south India, also seen in the western Himalayas and Northern plains up to the height of 3000 ft. (Shukla *et al*., 2016) [11]. It possesses various polyphenolic compounds namely tannins, anthocyanins, and flavonoids (Zia-Ul-Haq *et al*., 2013) [15]. Its fruit is a rich source of vitamins and minerals and contains various bioactive compounds, like anthocyanins, tannins, phenolics, and flavonoids (Qamar *et al*., 2020) [6]. It has been shown to possess various properties namely antioxidant, antimicrobial, anticancer and anti-inflammatory properties (Qamar *et al*., 2021) [7]. The present study has been undertaken to analyze preliminary phytochemical distribution in *G. asiatica* plant parts and the antioxidant activity of different plant parts by ABTS and hydrogen peroxide assay.

Materials and Methods
Plant collection
G. asiatica was collected from the Banseli region near Pushkar, Rajasthan (Lat. 26.51˚, Long. 74.55˚) in the month of April 2020. The specimen was identified and submitted to the Herbarium, Department of Botany, University of Rajasthan, Jaipur (RUBL-21374).

Extract preparation
Plant parts were washed, dried and ground to fine powder form. The powdered material (50 g) was extracted in various solvents in order of their increasing polarity using Soxhlet extraction and dried in vacuo.

The prepared extract was used for further analysis and Yield (%) was calculated using the formula given below.

$$\text{Plant Yield (\%)} = \frac{\text{weight of extract (g)}}{\text{weight of plant material taken (g)}} \times 100$$

Qualitative estimation

Phytochemical screening of the extracts was carried out using the standard qualitative method given by Harborne, 1973; Trease and Evans, 1985; Sofowora, 1993 [4, 14, 12].

ABTS Free Radical Scavenging Activity Assay

ABTS radicles were prepared by mixing Ammonium per sulfate (2.5 mM) and ABTS (7 mM) solution (Re *et al*. 1999) [10], which was diluted to prepare ABTS free radical reagent. 5 µl of standard (0 to 5 µg/ml) and samples were added to the 200 µl of ABTS free radical reagent in 96 well plates and incubated for 10 min in the dark. After incubation, absorbance was measured of the decolorization at 750 nm using a microplate reader (Thermo Scientific Multitaskin GO Microplate Spectrophotometer, USA). A standard curve of Ascorbic acid (0 to 5 µg/ml) was prepared to calculate the microgram Ascorbic Acid equivalence per milliliter (µg AAE/ml) of the extract under study.

Results and Discussion

The maximum yield (%) was obtained in the methanol extract of all of the plant parts studied as compared with other solvent extracts. Out of all the plant parts, crude extract of root showed a higher percentage yield (Table 1).

Table 1: Plant yield (%) of crude extract of *G. asiatica*

Extract	Plant part	Yield (%)
n-Hexane	Root	2.31
	Stem	1.8
	Leaves	1.37
	Flower	2.09
	Fruit	1.66
Chloroform	Root	2.89
	Stem	0.68
	Leaves	0.93
	Flower	1.62
	fruit	1.02
Ethanol	Root	4.24
	Stem	1.01
	Leaves	0.8
	Flower	1.88
	fruit	1.06
Methanol	Root	4.64
	Stem	3.12
	Leaves	3.94
	Flower	2.05
	fruit	1.32

A large variety of secondary metabolites are present in *G. asiatica* and among primary metabolites, carbohydrates and proteins are present in all extracts. (Table 2). ABTS assay is applicable for both lipophilic and hydrophilic compounds, for this assay, microgram ascorbic acid equivalence per milliliter (µg AAE/ml) was calculated and the standard curve of ascorbic acid plotted in a graph (fig. 1) and it was found to be highest in leaf (0.197±0.01 µg AAE/ml) followed by flower, stem, root and lastly fruit (0.101±0.03 µg AAE/ml) (Table 3).

Table 2: Qualitative Phytochemical Screening of crude extract of *G. asiatica*

Extract	n-Hexane Extract					Chl. Extract					Methanol Extract				
Plant part	R	S	L	Fl	Fr	R	S	L	Fl	Fr	R	S	L	Fl	Fr
Carbohydrate	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+
Tannins	-	+	-	-	-	-	+	-	-	-	+	++	+	+	+++
Flavonoids	-	+	-	-	-	+	+	+	-	-	+	++	++	+	+
Alkaloids	-	-	-	-	-	-	+	+	-	-	++	++	+	+	+
Phenols	+	-	-	-	+	+	-	-	-	+	+	+	+	+	+
Mucilage	++	+	-	++	+	++	+	+	++	+	+	-	-	+	-

R = Root, L = Leaves, S = Stem, Fl = Flower, Fr = Fruit, (+) = present, (-) = absent

Table 3: ABTS radical scavenging activity

Plant Part	Concentration	AAE (µg/ml)
Root	5 µl	0.101±0.03
Stem	5 µl	0.166±0.02
Leaves	5 µl	0.197±0.01
Flower	5 µl	0.192±0.01
Fruit	5 µl	0.102±0.02

Data expressed as mean ± SEM (n = 3)

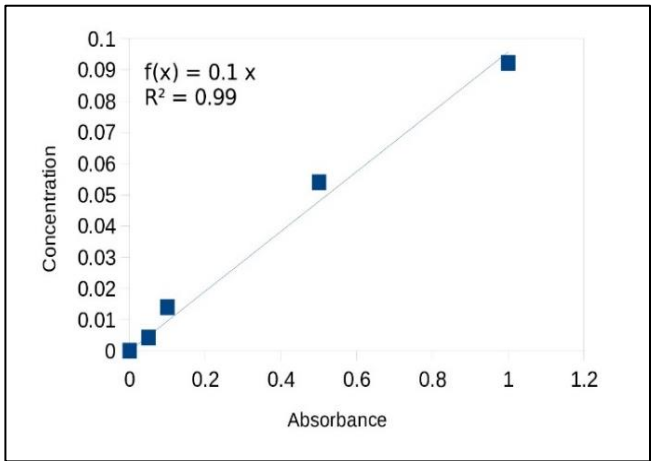

Fig 1: Standard curve of ascorbic acid

The lower antioxidant value of fruit was due to the low shelf life, which is the reason behind conducting this study on all the plant parts of *G. asiatica.* An earlier study performed by Asghar *et al*., 2008 [1] shows 107.2 TEAC µmol/g TEAC (Trolox Equivalent Antioxidant Capacity) and 60.9 TEAC µmol/g of peel and pulp respectively. No study has been reported for ABTS radical scavenging assay for other plant parts.

Conclusion

The results of the present study conducted strongly indicated that *G. asiatica* has strong antioxidant activity, which can be attributed to the presence of phenolics, flavonoids, alkaloids, tannins, terpenoids and saponins. Despite having identified numerous bioactive compounds, its utilization is limited, likely attributed to the short shelf life and small size of phalsa fruit. Thus, there is a need to shift the focus of research from fruit to other plant parts.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Funding Acknowledgment
We acknowledge the financial assistance provided by the EMR Division under HRD group of the Council of Scientific and Industrial Research (CSIR), New Delhi (File no. 09/149(0836)-2020-EMR-I).

References

1. Asghar MN, Khan IU, Sherin L, Ashfaq M. Evaulation of antioxidant activity of *Grewia asiatica* berry using 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) and N, N-dimethyl-p-phenylenediamine radical cations decolourization assays. Asian Journal of Chemistry. 2008 Oct 1;20(7):5123.
2. Bouayed J, Bohn T. Exogenous antioxidants—Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative medicine and cellular longevity. 2010 Jul 1;3(4):228-237.
3. Halliwell B and Gutteridge JMC. Free Radicals in Biology and Medicine. 3rd Edition, Oxford University Press, Oxford; c1999. p. 1-25.
4. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer science & business media; c1973
5. Kumar S, Singh B, Bajpai V. Traditional uses, phytochemistry, quality control and biological activities of genus Grewia. Phytomedicine plus. 2022 Aug 1;2(3):100290.
6. Qamar M, Akhtar S, Ismail T, Sestili P, Tawab A, Ahmed N. Anticancer and anti-inflammatory perspectives of Pakistan's indigenous berry *Grewia asiatica* Linn (Phalsa). Journal of Berry Research. 2020 Jan 1;10(1):115-31.
7. Qamar M, Akhtar S, Ismail T, Wahid M, Barnard RT, Esatbeyoglu T, Ziora ZM. The chemical composition and health-promoting effects of the Grewia species—A systematic review and meta-analysis. Nutrients. 2021 Dec 20;13(12):4565.
8. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989 Jun 1;10(6):1003-1008.
9. Rakesh SU, Patil PR, Mane SR. Use of natural antioxidants to scavenge free radicals: a major cause of diseases. International Journal of PharmTech Research. 2010;2(2):1074-1081.
10. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999 May 1;26(9-10):1231-1237.
11. Shukla R, Sharma DC, H Baig M, Bano S, Roy S, Provazník I, A Kamal M. Antioxidant, antimicrobial activity and medicinal properties of *Grewia asiatica* L. Medicinal Chemistry. 2016 May 1;12(3):211-216.
12. Sofowora A. Phytochemical screening of medicinal plants and traditional medicine in Africa edition, Spectrum Books Ltd Nigeria. 1993;150-156.
13. Tadhani MB, Patel VH, Subhash R. In vitro antioxidant activities of *Stevia rebaudiana* leaves and callus. Journal of food composition and Analysis. 2007 May 1;20(3-4):323-329.
14. Trease GE and Evans WC. Pharmacognosy. Bahiv Tinal, London, 1985;17:149.
15. Zia-Ul-Haq M, Stanković MS, Rizwan K, De Feo V. *Grewia asiatica* L., a food plant with multiple uses. Molecules. 2013 Feb 28;18(3):2663-2682.