GC-MS analysis and antioxidant activity of methanolic extract of *Grewia asiatica* L.

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Abstract

Grewia asiatica L. (Tiliaceae) is a perennial shrub, indigenously known as Phalsa and endemic to India, Pakistan, Sri Lanka and African countries. Its fruit juice is useful in diabetes, diabetic nephropathy and in the treatment of coronary heart diseases. The different parts possess anti-inflammatory, antimicrobial, anticancer and antioxidant properties. The present study was conducted to analyze the phytochemical composition of methanolic extract of different parts of Grewia asiatica. The different extracts prepared were evaluated for their antioxidant activity. The phytochemical investigation was done using gas chromatography–mass spectrophotometry (GC-MS). The antioxidant activity of methanol extract of different parts was evaluated using DPPH and FRAP assays using ascorbic acid as standard.

GC-MS analysis reveals stigmasterol (7.47%), α-tocopherol-β-D-mannoside (4.75%), oleic acid (3.47%) from root, 2-(hydroxymethyl)-2-nitro-1,3-propanediol (6.33%), phytol (2.51%), neophytadiene (2.28%) from stem, n-hexadecanoic acid (12.46%),5-hydroxymethylfurfural (4.93%), octadecanoic acid (2.14%) from flower and α -tocopherol, 2(5H)-furanone in fruits. G. asiatica root methanolic extract showed maximum DPPH free radical-scavenging activity (87.32 ± 0.15%) at 500 µg/ml and AEAC (557.9 ± 0.65 µM) for FRAP assay at 1000 µg/ml concentration. Observed results reveal bioactive compounds in G. asiatica which could be used as a source of natural antioxidants.

Keywords: *Grewia asiatica* L., Bioactive compounds, GC-MS, DPPH, FRAP, Antioxidant activity.

Introduction

Grewia is the only genus in the family Tiliaceae to bear edible fruits and consists of around 150 species distributed throughout the world. *G. asiatica* is a perennial shrub, 4 m high and commercially cultivated for its fruit production[11,25]. It is widely cultivated in India, Pakistan, Nepal, Bangladesh, Vietnam, Philippines and Sri Lanka[15]. As per Ayurveda, its fruit juice is used in treating diabetes, diabetic nephropathy and coronary heart diseases. Its processed product as a carbonated drink is useful in relieving heat stroke[6]. The fruit and bark of *G. asiatica* are demulcent, febrifuge and antidiarrheal. The leaves are used in treating pustular eruptions by tribal communities from Singrauli, India[5]. The bark is also used in treating certain urinary infections and alleviating burning sensation in the vagina[10].

Various phytochemicals are present in *G. asiatica viz*. Flavonoids (anthocyanins, isoflavonoids, flavanol), phenolic acids, phytosterols (β-sitosterol, stigmasterol and campesterol) and triterpenes[13,17]. Phytochemical analysis of seeds reveals the presence of palmitic, stearic, oleic and linolenic acids[18]. *G. asiatica* possesses antimicrobial[24], antioxidant[26], anticancerous[14], radioprotective[20], antihyperglycemic[12] and hepatoprotective[21] activities.

Free radicals are unstable and highly reactive molecules or atoms due to the presence of unpaired electrons in their outermost orbit. These unstable molecules cause damage to DNA and proteins by oxidizing them[19]. To prevent this damage, antioxidant compounds are used[8]. Considering the recent scenario, there is an ever-growing need for antioxidants to be administered for better protection against radical damage. There is a rise in research on antioxidants from natural sources due to their inexpensive, cosmopolitan distribution and no side effects[23]. The present study was conducted to elucidate the phytochemical constituents of different parts of *G. asiatica* using GC-MS and their antioxidant potential was analyzed using DPPH and FRAP assay.

Material and Methods

Plant Materials: *Grewia asiatica* L. was collected (April 2020) from the Banseli region near Pushkar, Rajasthan, India. The taxonomic identification was performed by Professor Amit Kotia, Department of Botany, University of Rajasthan, Jaipur. The voucher specimen was deposited in the Herbarium of the above-mentioned department (RUBL-21374).

Extraction of Plant Materials: Shade-dried and powdered plant materials (50 g each) were Soxhlet extracted in methanol (250 ml) for 48h, filtered and dried *in vacuo* using rotary evaporator. Each prepared extract was stored (at 4 °C) and used for subsequent experiments. Yield (%) was calculated using the formula given below.

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

Gas Chromatography-Mass Spectrophotometry (GC-

MS): The GC-MS analysis of methanolic extract of *G. asiatica* was carried out using Shimadzu GCMS – QP2020 (Kyoto, Japan), fitted with a capillary column (30 m × 0.25 mm (ID) × 0.25 µm film thickness of RXi5 Sil MS; 5% phenylmethylsiloxane). The initial temperature was adjusted to 50 °C with a hold time of 2 min and then increased to 200 °C at an 8 °C/min rate. The final temperature was increased to 280 °C at the rate of 10 °C/min with a hold time of 2 min. Injector and Interference temperatures were adjusted at 280 °C and 250 °C and injection was in split mode. The helium flow rate was also adjusted to 1.18 mL/min, the mass spectral scan mode range was 40 – 550 m/z with a total running time of 37.75 min.

Prepared samples (2µL) were used for GC-MS analysis after filtering with a 0.22 µm membrane filter. Interpretation of the result was based on the values of retention time and peak area (%). The data was compared with the Mass Spectral search program database present in NIST 17 (National Institute Standard and Technology, USA).

DPPH Free Radical Scavenging Activity: The free radical scavenging activity was determined using the protocol given by Blois[4]. Different concentration (150µg/ml-1000 µg/ml) of the earlier prepared extract was added to 195 µl of 0.2 mM DPPH solution. The microplate was incubated for 30 min at room temperature and absorbance was recorded at 517 nm (Thermo Scientific Multitaskin GO Microplate Spectrophotometer, USA). Ascorbic acid was used as a standard. The scavenging activity (% inhibition) was calculated using the following equation:

$$\text{DPPH Scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of control and A_1 is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) assay: The FRAP activity was evaluated by using the method of Benzie and Strain[3] with slight modifications. Prepared extracts (50-1000 µg/ml) were added to 0.4 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide solution. The reaction mixture was incubated at 50 °C for 20 min, later 0.5 ml of 10% TCA was added to the mixture and centrifuged at 5,000 rpm for 10 min. The supernatant was collected (100 µl) and added to $FeCl_3$ solution (100 µl of distilled water and 50 µl of 0.1% $FeCl_3$). The absorbance was recorded at 655 nm (Thermo Scientific Multitaskin GO Microplate Spectrophotometer, USA) against the blank after 10 min. The result was expressed as µM AEAC (Antioxidant Equivalent Ascorbic Acid Content).

Results and Discussion

The yield (%) of methanol extract of different parts is given in table 1. The highest yield (%) was recorded in root extract (4.64 %) out of all the parts studied (Table 1). This implies that there are more polar compounds in the root as compared to other plant parts. GC-MS analysis of the methanolic extract of *G. asiatica* revealed the presence of various phytochemicals in all the plant parts studied (Fig.1). A total of 35 compounds were isolated and identified from the methanol extract of root out of which oleic acid (3.47%), n-hexadecanoic acid (2.07%), tetradecanoic (1.02%), 9,12-octadecenoic acid (Z)-, methyl ester (0.27%) are major fatty acids and stigmasterol (7.74%), γ-sitosterol (0.55%) are two phytosterols (Table 2). Neophytadienen (2.28%), hexadecane (1.97%), dodecane (1.95%), phytol (0.57%) and 2,3-dihydro-benzofuran (0.28%) were present in the stem extract (Table 2). n-hexadecanoic acid (12.46%) and 5-hydroxymethylfurfural (4.93%) were isolated from flower extract. α-tocopherol (0.64%) was found in the fruit of *G. asiatica* which is a strong free radical scavenging antioxidant compound (Table 2). Dimethoxy methyl- silane (0.20%) and 24-Norursa-3,12-diene (0.83%) are the compounds that have not been reported in *G. asiatica* earlier.

Table 1
Plant yield of methanolic extract of *G. asiatica*

Plant part	Dried extract yield (g)	Percentage yield (%)
Root	2.32	4.64
Stem	1.40	2.80
Flower	1.27	2.54
Fruit	0.60	1.20

The analysis performed revealed the presence of fatty acids namely n-hexadecanoic acid, 9,11-conjugated linoleic acid, stearic and palmitic acids in all the plant parts studied. Saturated fatty acids were found to be lower in terms of concentration as compared to polyunsaturated fatty acids. Present study is in agreement with earlier studies where stigmasterol, hexadecane and phytol have been reported in the stem bark of *G. lasiocarpa*[1]. Zia-Ul-Haq et al[27] reported presence of γ-sitosterol in *G. lasiocarpa* and *G. nervosa*, this compound possesses antioxidant activity. Phytol, betulin and lupeol were earlier identified in stem bark extract of G. *asiatica*[2]. Earlier research on *G. asiatica* is focused primarily on the fruit reporting various phenols, flavanol and anthocyanins.

DPPH is one of the most commonly used methods for the evaluation of antioxidant activity. It is based on the ability of antioxidant compounds to reduce DPPH free radicals causing the decolorization of solution from purple color to pale yellow. The highest percentage of inhibition for DPPH assay was exhibited by root (87.32 ± 0.15%) at 500 µg/ml and the lowest by fruit (17.61 ± 2.15%) at 1000 µg/ml whereas stem (58.27 ± 1.70%) and flower (85.65 ± 0.13%) showed the highest inhibition at 1000 µg/ml (fig. 2). Mesaik et al[16] also cited the strong antioxidant potential of *G. asiatica* extract showing 85% inhibition of DPPH stable-free radicals. IC50 value of the methanolic extract of fruit was reported to be 1287 ± 0.05% which was supported by an earlier study[9] which states that fresh fruit of *G. asiatica* shows the best antioxidant potential.

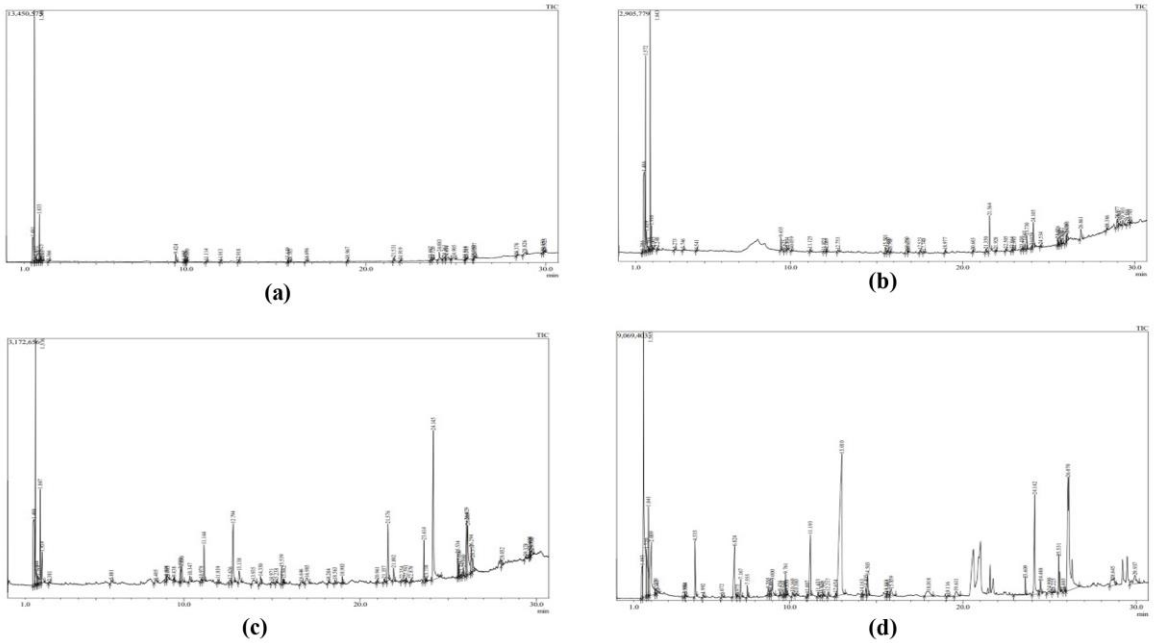

Fig. 1: GC-MS chromatogram (a) Root extract (b) Stem extract (c) Flower extract (d) Fruit extract

Table 2
The phytochemical compounds detected in the methanol extract of *G. asiatica* using GC-MS

Plant part of *G. asiatica*	Compound name	PA (%)	RT
Root	Stigmasterol	7.74	28.826
	α-Tocopherol-*β*-D-mannoside	4.75	29.850
	Oleic Acid	3.47	26.037
	Tetradecanoic acid	1.02	21.532
	γ-Sitosterol	0.55	24.905
	9,12-Octadecadienoic acid, methyl ester	0.27	25.514
	9,11-Conjugated linoleic acid	0.24	25.980
Stem	2-(hydroxymethyl)-2-nitro-1,3-Propanediol	6.33	16.750
	Hexadecanoic acid, methyl ester	2.97	23.530
	Neophytadiene	2.28	22.505
	Tetradecanoic acid	2.24	21.564
	Dodecane	1.95	12.089
	Hexadecane	1.97	15.798
	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	1.85	25.526
	Phytol	0.57	25.722
	2,3-dihydro-benzofuran	0.28	12.753
Flower	n-Hexadecanoic acid	12.46	24.145
	5-Hydroxymethylfurfural	4.93	12.794
	9,11-Conjugated linoleic acid	2.70	26.010
	Phytol	2.51	25.722
	Octadecanoic acid	2.15	26.294
	Butanedioic acid, monomethyl ester	0.51	10.347
	1-Tridecene	0.43	15.662
Fruit	5-Hydroxymethylfurfural	10.97	13.010
	9,12-Octadecadienoic acid	8.44	26.070
	n-Hexadecanoic acid	7.43	24.142
	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	2.9	25.531
	α-Tocopherol	0.64	29.937
	3-Heptanol	0.51	14.163

PA = peak area; RT = retention time

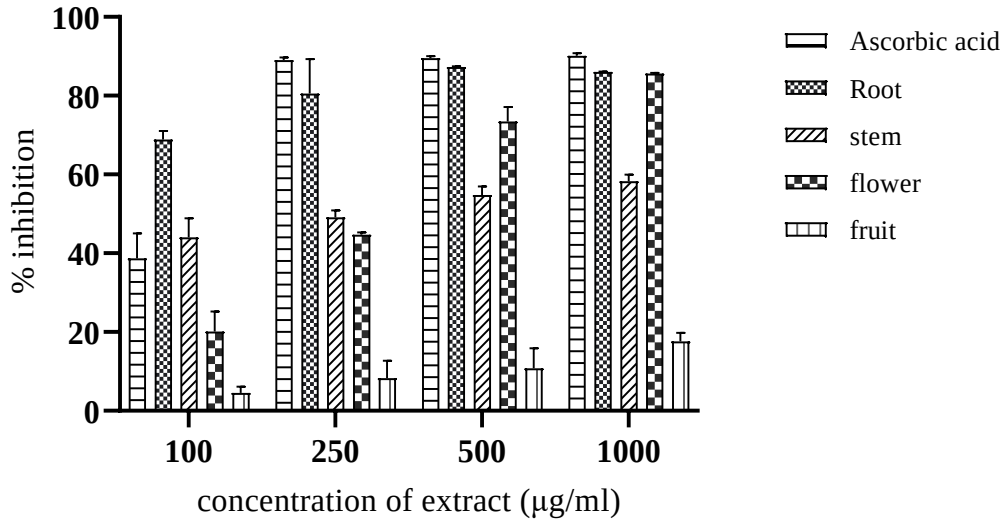

Fig. 2: DPPH free radical scavenging activity.

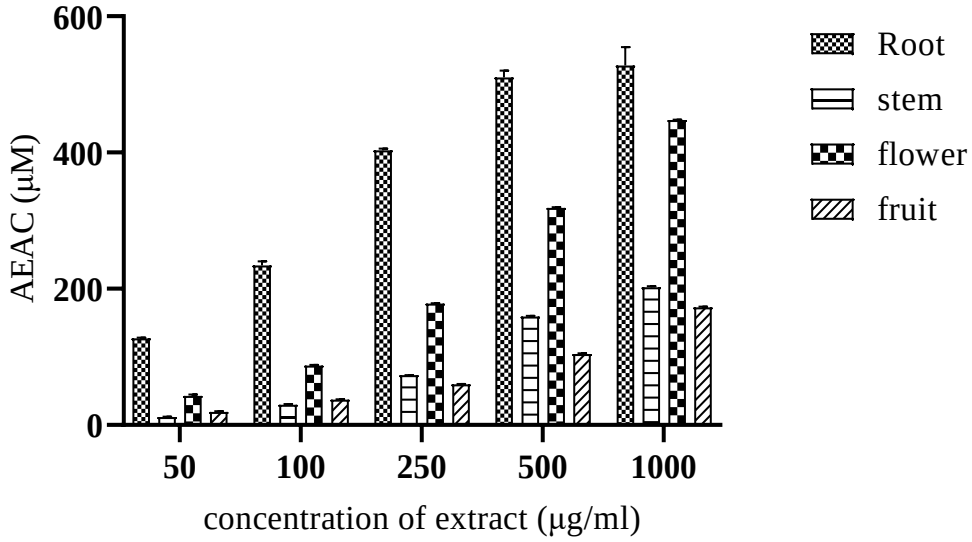

Fig. 3: FRAP assay.

The FRAP assay estimates the electron-donating capacity of antioxidant compounds based on the reduction of ferric ions (Fe^{+3}) into ferrous ions (Fe^{+2}). This caused a change in the colour of the reaction mixture which was monitored using a spectrophotometer. Highest AEAC value was shown by root, 557.9 ± 0.65 AEAC µM (fig. 3). Present findings are in accordance that free radical scavenging activity positively correlated with plant extract concentration[22]. Higher antioxidant activity was shown by root in both DPPH radical scavenging and FRAP assay indicating larger quantity of phenols, flavanoids and n-hexadecanoic. Earlier studies on G. *asiatica* are focused majorly on leaves and fruit[7]. This was an attempt to reveal the antioxidant potential of other plant parts.

Conclusion

The results conclude that the methanolic extract of plant parts of *G. asiatica* possesses antioxidant properties due to the presence of phytochemical constituents identified using GC-MS. The compounds identified include phytosterols viz. stigmasterol, *γ*-sitosterol, fatty acids viz. 9,12-octadecadienoic acid, (Z, Z)-, oleic acid, 9,11-conjugated linoleic acid methyl ester, 9,11-conjugated linoleic acid, n-hexadecanoic acid, terpenes viz. neophytadiene, phytol, organic compound viz. 5-hydroxymethylfurfural and α-tocopherol (Vitamin E). Thus GC-MS proves to be an easy and fast approach for phytochemical analysis even for a small amount of plant material.

The present study indicates the antioxidant potential of the plant and it can be used as a source of natural antioxidants in nutraceuticals for promoting better health. It can play a key role in the prevention of diseases caused by free radicals like cancer, premature aging and diabetes.

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References

1. Akwu N.A., Naidoo Y. and Singh M., Cytogenotoxic and biological evaluation of the aqueous extracts of *Grewia lasiocarpa*: An Allium cepa assay, *South African Journal of Botany*, **125**, 371-380 **(2019)**

2. Asghar M.N., Khan I.U., Sherin L. and Ashfaq M., Evaluation 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*, **20(7),** 5123 **(2008)**

3. Benzie I.F., Strain J.J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Analytical Biochemistry*, **239**, 70–76 **(1996)**

4. Blois M.S., Antioxidant determinations by the use of a stable free radical, *Nature*, **181**, 1199-1200 **(1958)**

5. Chauhan R.S., Studies on Medicinal Plants Used by Tribal Communities in District Singrauli of Madhya Pradesh, *IJSRP*, **7(1)**, 17-19 **(2017)**

6. Choudhary M.I., Siddiqui J., Abbaskhan A., Naheed S., Adhikari A. and Awalia J.A.J., Bio-active antioxidants from plant foods for nutraceutical product development, U.S. Patent **10**, 005, 809 **(2018)**

7. Gupta M.K., Lagarkha R., Sharma D.K., Sharma P.K., Singh R. and Ansari H.S., Antioxidant activity of the successive extracts of *Grewia asiatica* leaves, *Chem. Asian J.*, **19**, 3417 **(2007)**

8. Halliwell B. and Gutteridge J.M.C., Free radicals in biology and medicine, Oxford University Press, USA **(1999)**

9. Hassan H., Amin M., Rajwana I.A., Ullah S., Razzaq K., Faried H.N., Akhtar G., Naeem-Ullah U., Qayyum M.A., Aslam M.M. and Ali K., Nutritional functions and antioxidative enzymes in juice extract from two different maturity stages of low temperature stored phalsa (*Grewia subinaequalis* DC) fruit, *LWT*, **153**, 112552 **(2022)**

10. Khan M.I.C.M. and Hanif W., Ethno veterinary medicinal uses of plants from Samahni valley dist. Bhimber (Azad Kashmir) Pakistan, *Asian J Plant Sci*, **5(2)**, 390-396 **(2006)**

11. Khan R.S. et al, Phalsa (*Grewia asiatica* L.) fruit berry a promising functional food ingredient: A comprehensive review, *Journal of Berry Research*, **9(2)**, 179-193 **(2019)**

12. Khattab H.A., El-Shitany N.A., Abdallah I.Z., Yousef F.M. and Alkreathy H.M., Antihyperglycemic potential of *Grewia asiatica* fruit extract against streptozotocin-induced hyperglycemia in rats: anti-inflammatory and antioxidant mechanisms, *Oxid. Med. Cell. Longev.*, https://doi.org/10.1155/2015/549743 **(2015)**

13. Koley T.K., Khan Z., Oulkar D., Singh B., Bhatt B.P. and Banerjee K., Profiling of polyphenols in phalsa (*Grewia asiatica* L.) fruits based on liquid chromatography high resolution mass spectrometry, *J. Food Sci Technol*, **57**, 606–616 **(2020)**

14. Marya B., Dattani K.H., Patel D.D., Patel P.D., Patel D., Suthar M.P., Patel V.P. and Bothara S.B., *In vitro* cytotoxicity evaluation of aqueous fruit and leaf extracts of *Grewia asiatica* using MTT assay, *Pharm. Chem*, **3**, 282-287 **(2011)**

15. Mehmood A., Ishaq M., Usman M., Zhao L., Ullah A. and Wang C., Nutraceutical perspectives and value addition of phalsa (*Grewia asiatica* L.): A review, *Journal of Food Biochemistry*, **44(7)**, Doi: 10.1111/jfbc.13228 **(2020)**

16. Mesaik M.A., Ahmed A. and Khalid A.S., Effect of *Grewia asiatica* fruit on the glycemic index and phagocytosis tested in healthy human subjects, *Pakistan Journal of Pharmaceutical Sciences*, **26(1)**, 85–89 **(2013)**

17. Morton J.F., Fruits of Warm Climates, Echo Point Books & Media Miami, Florida **(2013)**

18. Qamar M., Akhtar S., Ismail T., Sestili P., Tawab A. and Ahmed N., Anticancer and anti-inflammatory perspectives of Pakistan's indigenous berry *Grewia asiatica* Linn (Phalsa), *Journal of Berry Research*, **10(1)**, 115-131 **(2020)**

19. Rakesh S.U., Patil P.R. and Mane S.R., Use of natural antioxidants to scavenge free radicals: a major cause of diseases, *International Journal of Pharm Tech Research*, **2(2)**, 1074-1081 **(2010)**

20. Sharma K.V. and Sisodia R., Evaluation of the free radical scavenging activity and radioprotective efficacy of *Grewia asiatica* fruit, *Journal of Radiological Protection*, **29(3)**, 429 **(2009)**

21. Sharma K.V. and Sisodia R., Hepatoprotective efficacy of *Grewia asiatica* fruit against oxidative stress in Swiss albino mice. ran, *J. Radiat. Res*., **8(2)**, 75-8 **(2010)**

22. Srivastava J., Kumar S. and Vankar P.S., Correlation of antioxidant activity and phytochemical profile in native plants, *Nutrition & Food Science*, **42(2)**, 71-79 **(2012)**

23. Tadhani M.B., Patel V.H. and Subhash R., *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus, *Journal of food Composition and Analysis*, **20(3-4)**, 323-329 **(2007)**

24. Tripathi S., Chaurey M., Balasubramaniam A. and Balakrishnan N., *Grewia asiatica* Linn. -As a Phytomedicine: A Review, *Research Journal of Pharmacy and Technology*, **3(1)**, 1-3 **(2010)**

25. Ullah W. et al, Ethnic uses, pharmacological and phytochemical profile of genus *Grewia*, *Journal of Asian Natural Products Research*, **14(2)**, 186-195 **(2012)**

26. Zeid A.H.S.A. and Sleem A.A., Anti-hyperlipidemic effect and lipoidal constituents of *Grewia asiatica* L. leaves, *Bulletin of the National Research Centre*, **30**, 557-573 **(2005)**

27. Zia-Ul-Haq M., Ahmad S., Imran I., Ercisli S. and Moga M., Compositional study and antioxidant capacity of *Grewia asiatica* L. seeds grown in Pakistan, *Comptes Rendus Acad. Bulg. Sci*, **68**,191-200 **(2015)**.

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