Journal of Applied Life Sciences International
7(1): 1-9, 2016; Article no.JALSI.26748
ISSN: 2394-1103

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Evaluation of Antimicrobial and Antioxidant Profile of *Grewia* L. Root Extracts

Chetna Sharma[1*], Manasi Malgaonkar[1], S. G. Sangvikar[1], S. N. Murthy[1] and S. D. Pawar[1]

[1]National Research Institute of Basic Ayurvedic Sciences, Pune, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors CS and MM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MM managed the literature searches, analyses of the study and author CS managed the experimental process performed the spectroscopy analysis. Authors SGS and MM identified the species of plant. Authors SGS, SNM and SDP managed the analyses. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2016/26748
Editor(s):
(1) Palanisamy Arulselvan, Institute of Bioscience, Universiti Putra Malaysia, Malaysia.
Reviewers:
(1) Nyoman Kertia, Medicine Gadjah Mada University, Yogyakarta, Indonesia.
(2) Mindaugas Liaudanskas, Lithuanian University of Health Sciences, Lithuania.
(3) Anonymous, Universidade Federal da Grande Dourados (UFGD), Brazil.
(4) Anonymous, University of Novi Sad, Serbia.
Complete Peer review History: http://www.sciencedomain.org/review-history/15668

Original Research Article

Received 30th April 2016
Accepted 25th July 2016
Published 5th August 2016

ABSTRACT

Aims: To analyze and compare the *in vitro* antimicrobial activity and antioxidant profile of *Grewia asiatica* L.; *Grewia tiliifolia* Vahl.; *Grewia tenax* (Forsk.) Fiori.
Study Design: This study was designed to evaluate and compare the antimicrobial activity and antioxidant profile of different extracts of *Grewia* which are commonly used in Ayurvedic drug preparations.
Place and Duration of Study: The work was done in National research institute of Basic Ayurvedic Sciences during the month of August to December 2015.
Methodology: All the three *Grewia* species were subjected to screen the antioxidant potential by various methods, such as FRAP, DPPH, ABTS and NO radical scavenging assay. Further, to assess the *in vitro* antimicrobial activity of *Grewia* species by using Broth microdilution technique by CLSI guidelines against different microrganisms.
Results: Total phenolics and flavonoids were highest in *G. tenax* as compared to other species.

**Corresponding author: E-mail: chetna.sh18@gmail.com;*

Highest antioxidant activity was seen in *G. tenax* while lowest in *G. tiliifolia.* All extracts showed significant antimicrobial activity, hydroalcoholic extract of *G. asiatica* had most effective antibacterial activity against *K. pneumoniae* (MIC – 3.90 µg/ml) as well as same extract showed effective antifungal potential against *C. albicans* and *A. fumigatus* (MIC – 31.2 µg/ml). *G. tenax possesses* remarkable antibacterial activity against *B. subtilis* and *G. tiliifolia* showed the same activity against *E. coli.*

Conclusion: The species which exhibited well marked antioxidant and antimicrobial activity was rich in secondary metabolite contents (flavonoids and phenols). Antioxidant results of present investigation provided supportive scientific evidence for *G. tenax*. Findings of this study supported the traditional use of *G. asiatica* and *G. tenax* in the treatment of some microbial infections.

Keywords: Tiliaceae; antioxidant activity; total phenolic content; flavonoids; antimicrobial activity.

1. INTRODUCTION

Grewia, (Family: Tiliaceae) is an imperative medicinal plant. Ayurveda, the ancient Indian treatise on medicine, mentions the use of different plant parts of *Grewia* to cure inflammation, burning sensation, fever, blood disorders, wound healing, ulcerative colitis, heavy menstrual flow and diabetes [1-3].

Grewia asiatica L. (Phalsa in hindi and Parushaka in Sanskrit) possess pharmacological and medicinal properties. Fruits are known to cure inflammation and are also administered in respiratory, cardiac, and blood disorders. The root bark of *G. asiatica* is used in treating rheumatism [4].

Grewia tenax (Forsk.) Fiori (Gangeruki in Sanskrit) is multipurpose plant species which serves as the source of food, fodder, fiber, fuel wood, timber and a range of traditional medicines that cure various diseases and have antibiotic properties [5]. The plant preparations of *G. tenax* are used as an important component of folk medicine for the treatment of trachoma, tonsillitis [6-7].

Grewia tiliifolia Vahl. (Dhanu vriksha in Sanskrit), bark is exploited in treating burning sensation, cough, skin diseases, wounds, ulcers, diarrhoea, haemorrhage, seminal weakness, general debility [8-10], cardiac diseases, disorders of blood, and diseases of nose, in opium poisoning and as aphrodisiac as well as tonic.

Reactive oxygen species formation are crucial part of the defense mechanisms against infection, but excessive generation of free oxygen radicals may damage tissue and cause tissue injury. Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids has been play major role in the pathogenesis of atherosclerosis, aging, cancer, diabetes, cardiovascular diseases, and rheumatoid arthritis [11]. Hence, recently, research all over the world focused on finding naturally occurring antioxidants from plants. Several members of the species *Grewia* are being used traditionally as a source of antioxidant, possess sufficient antioxidant capacity that they can be used in the battle against cellular damage and disease [12]. *G. asiatica* and *G. tiliifolia* also possess high content of antioxidants like vitamin C, total phenolics, flavonoids, tannins and anthocyanins [13-14]. These species have noteworthy antibacterial and antifungal potential. *G. asiatica* leaves possess antimicrobial potential and are therefore used to treat skin rashes and pustular eruptions.

An attempt was made in the present study to investigate and compare the antimicrobial, total flavonoids, total phenolic content as well as antioxidant potential of *Grewia*.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Materials

The plant part (Roots) of all the three species of *Grewia* (*Grewia asiatica* L., *Grewia tiliifolia* Vahl., *Grewia tenax* (Forsk.) Fiori.) were collected from Pune region of Western Ghats, India. Specifically *G. asiatica* was collected from Sinhgad location of Pune region (18.3663°N, 73.7559°E), *G. tiliifolia* from Mulshi area (18.5011°N, 73.5138°E) and *G. tenax* from Pirangut (18.5120°N, 73.6944°E). Weather parameters were almost similar for the species collected, which can be mentioned as 25-30°C average temperature, precipitation up to 1000 mm per year and plants were growing in the red / reddish brown soil. Collected roots of all the species were thoroughly washed under running tap water

followed by distilled water. Roots were shade dried at room temperature and dried plant roots were powdered (40 gms) with the help of grinder, which was further stored and used for the experimental purpose. Part of collected specimens were deposited in herbarium section of National Research Institute of Basic Ayurvedic Sciences, Pune with voucher number 257 (*Grewia asiatica* L.), 258 (*Grewia tiliifolia* Vahl.), and 261 (*Grewia tenax* (Forsk.) Fiori.).

2.2 Preparation of Plant Extract

The successive extracts of the powdered roots were prepared in different solvents such as water, methanol and hydro alcoholic in predetermined proportion (1:8). This mixture was macerated overnight. Different extracts obtained was then filtered using whatmann no. 42 (125 mm) filter paper and lyophilized using lyophilizer (labconco free zone 4.5) at -50°c and 0.020 mbar pressure for 3-4 days. The lyophilized material was stored at 4°C in air tight container till further use. The final extract yield was 10 gm powder per species, per solvent.

2.3 Antioxidant Analysis

2.3.1 Ferric reducing power assay (FRAP)

The FRAP assay was analyzed according to the method described by Oyaizu, [15]. 200 mg of plant extract was mixed with phosphate buffer and potassium ferricyanide [K_3 Fe $(CN)_6$]. The reaction mixture was incubated and a portion of trichloroacetic acid was added. The upper layer of the solution was mixed with distilled water and Ferric chloride ($FeCl_3$). The reading of Absorbance was taken at 700 nm. Butylated Hydroxy Toluene (BHT) was used as the reference standard.

2.3.2 Inhibition of DPPH radical method

0.1 mM solution of 1, 1-diphenyl-2-picrylhydrazil (DPPH) dissolved in methanol and solution was prepared. 1ml of this solution was added to various concentration of plant sample. After half an hour incubation, absorbance was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as the reference standard for calculation. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples [16-17].

2.3.3 Nitric oxide radical scavenging assay

Nitric Oxide Radical test was carried out on the basis of Griess reaction [18,19]. The reaction mixture containing sodium nitroprusside [Na_2 $[Fe(CN)_5NO]$] in phosphate buffered saline and plant extracts and the reference compound in different concentrations was incubated. After incubation, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent was added. The absorbance was measured at 546 nm. Ascorbic acid served as a positive control for the test.

2.3.4 ABTS radical cation decolourisation assay

According to Re et al. [20] the oxidant is generated by persulfate oxidation of 2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid)-(ABTS2-). ABTS radical cations (ABTS+) are produced by reacting ABTS. Plant extract was added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1ml. The absorbance was calculated at 745 nm.

2.3.5 Total Phenolic Content (TPC)

Total phenolic content (TPC) from plant samples were identified using Folin - Ciocalteu's method [21]. 100 µl of 1:4 diluted Folin - Ciocalteu's phenol reagent, in distilled water was added to 20 µl of lyophilized plant extracts and standard Gallic acid dissolved in distilled water. After incubation at room temperature, 80 µl of sodium carbonate were added and incubated for 30 min at room temperature in the darkness. The absorbance was measured at 735 nm.

2.3.6 Total flavonoids

The total flavonoid content of the plant extracts was determined by aluminum chloride test. 0.1 ml $AlCl_3$ (10%), 0.1 ml Na-K tartarate and 2.8 ml distilled water was added in plant extracts sequentially. The test solution was shaken vigorously. Absorbance at 415 nm was recorded. A standard calibration plot was generated at 415 nm using known concentrations of quercetin [22].

2.4 Antimicrobial Activity

Antibacterial activity of plant extract was determined by Tetrazolium Microplate Microbial viability Assay [23]. Different plant extracts were tested against pathogenic strains of microrganisms: *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* for determining the Minimum Inhibitory Concentration (MIC) and results were calculated.

Equal amount of drug and bacterial culture was mixed in nutrient broth and then inoculated in the 96 well plates. Serial Dilutions were performed according to the standard protocol and kept for incubation at 37°C for 8-10 hours. After incubation, cold 20% Tetrazolium solution was added to each well. The colour change was observed and noted down the MIC value of respective drug against the bacterial cultures. The bacterial growth was corresponded with the colour change to pink from the original colour of the respective drug and in absence of growth the colour remained the same.

Antifungal activity of *Grewia* species, against *Candida albicans* and *Aspergillus fumigatus* were also determined. The drug sample and the fungal culture was mixed in the Sabouraud dextrose broth and added in the 96 well plates. Dilutions were performed according to the protocol and kept for incubation at 37°C for 5-7 days. Fungal growth was observed to determine the MIC value of respective drug.

Positive controls were used during the analysis were Tetracyclin for bacteria and Amphotericin B for fungi.

3. RESULTS AND DISCUSSION

Table 1 shows the antioxidant values of used extracts in the study and Table 2 states the total phenolic contents (TPC) and total flavonoids of the extracts. Extracts having maximum TPC and flavonoids were considered for the evaluation of antioxidant activity as both are known to play major in possessing antioxidant activity. Antioxidant potential was highest in *G. tenax* (99.8%) while lowest in *G. tiliifolia* (53.6%) (Table 1). All the four methods used to analyse antioxidant potential of *Grewia* species were found to reliable in nature. According to Zia-ul-haq et al. [24] *Grewia asiatica* has a high content of antioxidants like vitamin C, total phenolics, flavonoids, tannins and anthocyanins but as per the present study *G. tenax* showed the promising antioxidant activity of the fractions obtained from the *G. tenax* were the consequences of their reducing power potential and the capacity to inhibit free radicals. As evident from other studies also, other species of *Grewia* exhibited good antioxidant profile but from current study *G. tenax* possesses the highest reducing potential as well as Antioxidant capacity. Antioxidant values obtained in this study were similar with the earlier studies carried out by Gupta et al. Siddiqi et al. and Sharma et al. [25-27]. *G. tenax* also possess maximum amount of Phenolic content in aqueous extract therefore only aqueous extract was considered for the antioxidant evaluation as from the literature the highest antioxidant activity may be attributed due to the presence of high phenolic content and flavonoids in the species. From present findings, Table 2 show the total phenolic content and total flavonoids of various plant extract in which total phenolics was highest in the aqueous extract of *G. tenax* (10.67 µg/ml) and flavonoids were found to be maximum (32.7 µg/ml) in hydroalcoholic extract of the same species, as compared to other two species. The findings from antioxidant, phenolic constituents and flavonoids revealed that these species can be used for therapeutic usage at a very lower cost and with minimum side effects in comparison to other commercial drugs available in the market Basri et al. [28].

The results presented in Table 3 are the mean of triplicates carried out to check the antibacterial and antifungal activity. Least minimal inhibitory concentration (3.90 µg/ml MIC) was seen in the hydroalcoholic extract of *G. asiatica* against *K. pneumoniae*. Hydroalcoholic extract of *G. tenax* showed noticeable activity against *K. pneumoniae* and *S. aureus* with 7.81 µg/ml concentration. Similary hydroalcoholic extract of *G. asiatica* also showed lower MIC values (7.81 µg/ml) for *B. subtilis* and *P. aeruginosa*. Methanolic extract of *G. tenax* exhibited a well marked antibacterial activity followed by aqueous and hydro-alcoholic extract. This data is in agreement with previous reports observed by Saadabi and Moglad as well as Kapoor et al. [29,30]. A study by Kumari et al. [31] *G. asiatica* showed potent antifungal activity against *Candida albicans* which is approximately similar with our findings. *Grewia* species possess antimicrobial potential and are therefore used to treat skin rashes and pustular eruptions [32,33]. Due to well marked antimicrobial properties of these species, there is a constant demand for the investigation of new antimicrobial agents that will help in increasing the easy understanding of health problems and also have access to modern health care system.

In general, among the three extracts antimicrobial activity was found to be highest in hydro-alcoholic extract and less effective in aqueous extract and among the species *G. asiatica* showed good antimicrobial activity indicating the presence of promising antimicrobial compounds.

Table 1. Antioxidant analysis

Species	FRAP % inhibition	DPPH % inhibition	NO % inhibition	ABTS % inhibition
G. tilliaefolia	86.6±2.2	76.11±1.77	53.65±4.34	92.18±1.4
G. tenax	99.1±1.69	85.49±2.68	62.78±2.29	99.8±5.66
G. asiatica	82.53±3.16	82.5±5.66	89.95±3.87	96.41±2.17
Positive controls	82.9%±4.98	88%±5.32	65.29%±6.28	86.32%±2.87

(Positive controls- as mentioned in materials and methods)

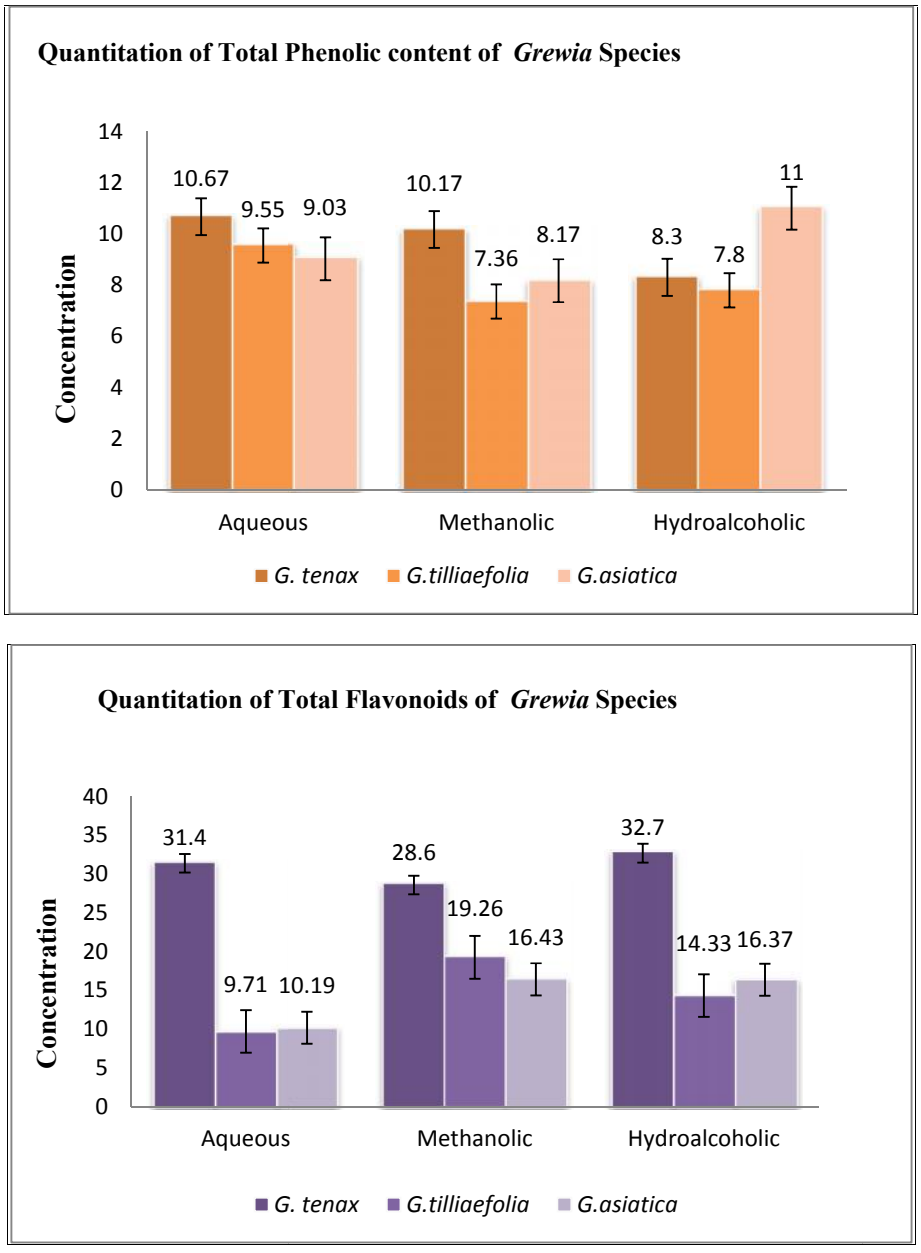

Fig. 1. Total phenolic content and total flavonoids of *Grewia* species

Table 2. Total phenolic content and total flavonoids

Phyto-constituents	*G. tenax* (Forsk.) Fiori (Conc. in µg/ml)			*G. tiliifolia* Vahl. (Conc. in µg/ml)			*G. asiatica* L. (Conc. in µg/ml)		
	A*	M*	H*	A*	M*	H*	A*	M*	H*
TPC	10.67±5.91	10.17±3.17	8.3±3.76	9.55±2.92	7.36±4.21	7.8±3.76	9.03±2.56	8.17±1.98	11±4.38
TF	31.4±2.78	28.6±1.89	32.7±4.32	9.71±3. 22	19.26±5.22	14.33±5.43	10.19±3.98	16.43±3.56	16.37±6.43

(A: Aqueous extract; M*: Methanolic extract; H*: Hydroalcoholic extract)*

Table 3. MIC values of *Grewia* against microorganisms

Test organisms	*G. tenax* (Forsk.) Fiori (Conc. in µg/ml)			*G. tiliifolia* Vahl. (Conc. in µg/ml)			*G. asiatica* L. (Conc. in µg/ml)			Positive control
	A*	M*	H*	A*	M*	H*	A*	M*	H*	(Conc. in µg/ml)
Bacillus subtilis	15.62	15.62	15.62	250	62.5	62.5	62.5	31.2	**7.81**	7.81
Escherichia coli	62.5	125	31.2	31.2	15.62	15.62	125	125	31.2	31.2
Klebsiella pneumoniae	31.2	31.2	**7.81**	31.2	31.2	15.62	62.5	125	**3.90**	31.2
Pseudomonas aeruginosa	62.5	31.2	62.5	61.2	15.62	31.2	31.2	31.2	**7.81**	7.81
Staphylococcus aureus	31.2	31.2	**7.81**	500	125	31.2	125	125	62.5	0.97
Candida albicans	125	125	62.5	62.5	15.62	15.62	250	125	31.2	62.5
Aspergillus fumigatus	62.5	125	-	125	62.5	62.5	31.2	62.5	31.2	62.5

(Positive controls: Tetracyclin for bacteria and Amphotericin B for fungi)

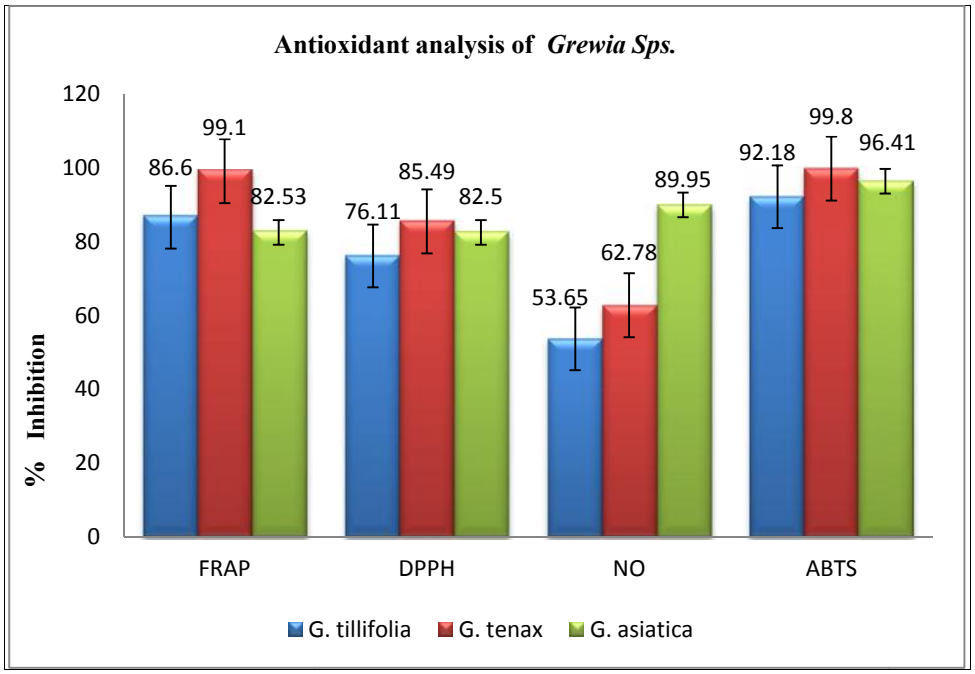

Fig. 2. Antioxidant profile of *Grewia* species

4. CONCLUSION

Wild plants have continuously been used to meet the growing commercial demand for its fruits. In terms of their socio-economic value, the plant is described as prime candidate for domestication and commercialization as new crops in semi arid and arid zones of country Sharma and Patni [32]. In decisive remarks it can be said that, total phenolic content and total flavonoids have positive effect on antioxidant potential, which was seen in the present study as *Grewia tenax* showed optimum activity antioxidant activity and also highest values of total phenolic content and total flavonoids. Due to presence of secondary metabolites such as flavonoids and phenols, which could be responsible for noticeable antimicrobial activity. All extracts of *Grewia* showed effective antibacterial and antifungal activity against microorganisms used under study. It was noted that hydroalcoholic extract of *G. asiatica* and *G. tenax* could be better choice as antimicrobial agent. These herbal *Grewia* species can be used further in drug designing and pharmaceutical industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lavekar GS. Database on medicinal plants used in ayurveda & siddha. Central Council for Research in Ayurveda & Siddha: New Delhi India; 2008.
2. Mishra RK, Patel SP. Srivastava A. Vashistha RK, Singh A, Puskar AK. Ethnomedicinally important plants of Pachmarhi region. Nat. Sci: Madhya Pradesh, India. 2012;10:22–26.
3. Zia-Ul-Haq M, Stanković MS, Rizwan K, De Feo V. *Grewia asiatica* L., A Food Plant with Multiple Uses Molecules. 2013;18: 2663-2682.
4. Parveen A. Irfan M. Mohammad F, Antihyperglycemic activity in *Grewia asiatica*; A comparative investigation. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(1):210-213.
5. Sharma N. Patni V. *Grewia tenax* (Frosk.) Fiori. – A traditional medicinal plant with enormous economic prospective. Asian Journal of Pharmaceutical and Clinical Research. 2012;5(3):28-32.
6. El Ghazali GEB. El Tohami MS. El Egami AAB. Medicinal plants of the Sudan. In: Medicinal plants of the White Nile provinces. Khartoum University Press, Khartoum; 1994.

7. El Ghazali GEB. El Tohami MS. El Egami AAB. Abdalla WS. Mohammed MG. Medicinal plants of the Sudan. In: Medicinal plants of northern Kordofan. Omdurman Islamic University Printing and Publishing House, Omdurman; 1997.
8. Chopra RN. Indigenous Drugs of India – Their Medical and Economic Aspects. The Art Press. Calcutta, India. 1933;550.
9. Asolkar LV. Kakkar KK. Chakre OJ. Second supplement of glossary of indian medicinal plants with active principles, publication and information directorate. CSIR, New Delhi. Part-I. 1992;339-340.
10. Raghunathaiyar S. Indian Medicinal Plants. Orient Longman Ltd. Hyderabad, India. 1996;3:104-105
11. Hellwell B, Gutteridge JMC. Free radicals in biology and medicine. Clarendon Press, Oxford, UK 2nd edn; 1989.
12. Kirtikar KR, Basu BD. Indian Medicinal Plants. International Book distributors, Book Sellers and Publishers. Dehra Dun, India; 1987.
13. Asghar MN. Khan IU. Sherin L. Ashfaq M. Evaluation of antioxidant activity of Grewia asiatica berry using 2, 2-azinobis-(3-ethylbenzoline-6-sulphonic acid) and N, N-dimethyl-pphenylenediamine radical cations decolourazation assays. Asian. J. Chem. 2008;20:5123–5132.
14. Yadav VR. Pandit V. Vijayan P. Antioxidant, antimicrobial and cytotoxicity properties of the methanolic extract from Grewia tiliaefolia Vahl. Pharmacognosy magazine. 2008;4(16):329-334.
15. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition. 1986; 44:307-315.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-1200.
17. Gomez-Alonso S. Fregapane G. Salvador MD. Gordon MH. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. J Agric Food Chem. 2003;51:667-672.
18. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR. Analysis of nitrate, nitrite and $_{15}$N in biological fluids. Anal Biochem. 1982;126: 131-136.
19. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging property of Ginkgo biloba extract HGb 761. Biochem Biophys Res Commum. 1994; 201:748-755.
20. Re R. Pellegrini N. Protoggenete A. Pannala A. Yang M. Rice-Evans C. Antioxidnat activity applying an improved ABTS radical cation decoloration assay. Free Radic Biol Med. 1999;26:1231-1237.
21. Slinkard K, Singleton VL. Total phenol analyses; automation and comparision with manual methods. Am J Enol Vitic. 1977; 28:49-55.
22. Khatiwora E, Adsul VB, Kulkarni MM, Deshpande NR, Kashalkar RV, Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea. International Journal of Chem Tech Research. 2010;2-3:1698-1701.
23. Perumal S, Pillai S, Cai LW, Mahmud R, Ramanathan S. Determination of minimum inhibitory concentration of *Euphorbia hirta* (L.) extracts by Tetrazolium Microplate Assay. Journal of Natural Products. 2012; 568-76.
24. Zia-Ul-Haq M, Stanković MS, Rizwan K, De Feo V. *Grewia asiatica* L., a food plant with Multiple Uses. Molecules. 2013;18: 2663-2682.
25. Gupta MK, Lagarkha R, Sharma DK, Sharma PK, Singh R, Ansari HS. Antioxidant activity of the successive extracts of *Grewia asiatica* leave. Asian Journal of Chemistry. 2007;19(5):3417-3420.
26. Siddiqi R, Naz S, Sayeed SA, Ishteyaque S, Haider MS, Tarar OM Antioxidant potential of the polyphenolics in *Grewia asiatica*, *Eugenia jambolana* and *Carissa carandas*. Journal of Agricultural Science. 2013;5(3):217-223.
27. Sharma N, Patni V. Comparative analysis of total flavonoids, quercetin content and antioxidant activity of in vivo and *in vitro* plant parts of *Grewia asiatica* Mast. International Journal of Pharmacy and Pharmaceutical Science. 2013;5(3):464-469.
28. Basri TSJ, Reddy GVS, Jayaveera KN. A study on phytochemical and antioxidant activity of *G. tenax*. International Journal of Pharmaceutical Research and Bio-science. 2014;3(4):703-710.
29. Saadabi AMA, Moglad EH. Experimental evaluation of certain sudanese plants used in folkloric medicine for their antibacterial activity (*In Vitro* Tests). Journal of Applied Sciences Research. 2011;7(3):253-256.

30. Kapoor BBS, Mishra R, Acharya S, Lakhera S, Purohit V. Antimicrobial screening of some herbal plants of the Rajasthan desert: An overview. Unique Journal of Engineering and Advanced Sciences. 2013;1(1):38-40.
31. Kumari S, Mazumder A, Pahwa S, Jaju S. Studies of the antifungal and antiviral activity of methanolic extract of leaves of *Grewia asiatica*. Pharmacognosy Journal. 2009;1(3).
32. Sharma N, Patni V. *Grewia tenax* (Frosk.) Fiori. – Atraditional medicinal plant with enormous economic prospective. Asian Journal of Pharmaceutical and Clinical Research. 2012;5(3):28-32.
33. Zia-Ul-Haq M, Shahid SA, Muhammed S, Qayum M, Khan I, Ahmad S. Antimalarial, antiemetic and antidiabetic potential of *Grewia asiatica* L. leaves. J. Med. Plants Res. 2012;6:3213–3216.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15668