Original Research Article

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

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 microorganisms.

Results: Total phenolics and flavonoids were highest in G. tenax as compared to other species. 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

The genus Grewia, belonging to family Tiliaceae (Phalsa family), is an important well established medicinal plant. According to Ayurvedic research, the ancient Indian treatise on medicine, different plant parts of Grewia are used to cure inflammation, burning sensation, fever, blood disorders, wound healing, ulcerative colitis, heavy menstrual flow and diabetes[1-3].

Grewia asiatica L., known as ‘Phalsa in hindi and Parushaka in sanskrit’, possess pharmacological and medicinal properties, its fruit (Phalsa) cure inflammation and is also administered in respiratory, cardiac, and blood disorders. The root bark of G. asiatica is used in treating rheumatism [4].

Gangeruki (Sanskrit name), scientifically called as Grewia tenax (Forsk.) Fiori is also used as multipurpose plant species which is 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 used 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, in recent time 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 significant 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. MATERIAL 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 (18.3663º N, 73.7559ºE), G. tiliifolia from Mulshi area (18.5011ºN, 73.5138ºE) and G. tenax from Pirangut (18.5120ºN, 73.6944ºE). Weather parameters were almost similar for the species collected, which can be mentioned as 25-30ºC 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 (labconcofreezone 4.5) at -50ºc and 0.020 mbar pressure for 3-4 days. The lyophilized material was stored at 4ºC 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 [15]. 200 mg of plant extract in 1ml of distilled water was mixed with phosphate buffer and potassium ferricyanide \([K_3Fe(CN)_6]\). 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 (FeCl₃). The reading of Absorbance was taken at 700nm. Butylated Hydroxy Toluene (BHT) was used as the reference standard.

2.3.2 INHIBITION OF DPPH RADICAL METHOD

0.1mM 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

NO Radical test was carried out on the basis of Griess reaction [18,19]. The reaction mixture containing sodium nitroprusside [Na$_2$[Fe(CN)$_5$NO]] 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. (1999) [20] the oxidant is generated by persulfate oxidation of 2, 2-azinobis-(3-ethylbenzoline-6-sulfonic acid)-(ABTS$^2-$). ABTS radical cation (ABTS$^+$) are produced by reacting ABTS. Plant extract at various concentration was added to 0.3ml 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. 0.1ml AlCl$_3$ (10%), 0.1ml 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 microorganisms: 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 Antioxidant potential was highest in *G. tenax* (99.8%) while lowest in *G. tiliifolia* (53.6%) (Table 2).

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.* (2013) [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* possess the highest reducing potential as well as Antioxidant capacity. Antioxidant values obtained in this study were similar with the earlier studies carried out (Gupta *et al.*, 2007, Siddiqi *et al.*, 2013, Sharma *et al.*, 2013) [25-27]. As a result, *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.*, 2014) [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 (2011) as well as Kapoor *et al.*, (2013) [29, 30]. A study by Kumari *et al.*, (2009) [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>
<thead>
<tr>
<th>Species</th>
<th>FRAP % inhibition</th>
<th>DPPH % inhibition</th>
<th>NO % inhibition</th>
<th>ABTS % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. tiliifolia</em></td>
<td>86.6 ± 2.2</td>
<td>76.11 ± 1.77</td>
<td>53.65 ± 4.34</td>
<td>92.18 ± 1.4</td>
</tr>
<tr>
<td><em>G. tenax</em></td>
<td>99.1 ± 1.69</td>
<td>85.49 ± 2.68</td>
<td>62.78 ± 2.29</td>
<td>99.8 ± 5.66</td>
</tr>
<tr>
<td><em>G. asiatica</em></td>
<td>82.53 ± 3.16</td>
<td>82.5 ± 5.66</td>
<td>89.95 ± 3.87</td>
<td>96.41 ± 2.17</td>
</tr>
<tr>
<td>Positive controls</td>
<td>82.9% ± 4.98</td>
<td>88%± 5.32</td>
<td>65.29%± 6.28</td>
<td>86.32% ± 2.87</td>
</tr>
</tbody>
</table>

(Positive controls- as mentioned in materials and methods)
**Table 2. Total Phenolic Content and Total Flavonoids**

<table>
<thead>
<tr>
<th>Phyto-constituents</th>
<th>G. tenax (Forsk.) Fiori (Conc. in µg/ml)</th>
<th>G. tiliifolia Vahl. (Conc. in µg/ml)</th>
<th>G. asiatica L. (Conc. in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TPC</strong></td>
<td>10.67 ± 5.91</td>
<td>10.17 ± 3.17</td>
<td>8.3 ± 3.76</td>
</tr>
<tr>
<td><strong>TF</strong></td>
<td>31.4 ± 2.78</td>
<td>28.6 ± 1.89</td>
<td>32.7 ± 4.32</td>
</tr>
</tbody>
</table>

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

**Table 3. MIC values of Grewia against microorganisms**

<table>
<thead>
<tr>
<th>Test Organisms</th>
<th>G. tenax (Forsk.) Fiori (Conc. in µg/ml)</th>
<th>G. tiliifolia Vahl. (Conc. in µg/ml)</th>
<th>G. asiatica L. (Conc. in µg/ml)</th>
<th>Positive Control (Conc. in µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus subtilis</strong></td>
<td>15.62</td>
<td>15.62</td>
<td>15.62</td>
<td>250</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>62.5</td>
<td>125</td>
<td>31.2</td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td>31.2</td>
<td>31.2</td>
<td><strong>7.81</strong></td>
<td>31.2</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>62.5</td>
<td>31.2</td>
<td>62.5</td>
<td>61.2</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>31.2</td>
<td>31.2</td>
<td><strong>7.81</strong></td>
<td>500</td>
</tr>
<tr>
<td><strong>Candida albicans</strong></td>
<td>125</td>
<td>125</td>
<td>62.5</td>
<td>62.5</td>
</tr>
<tr>
<td><strong>Aspergillus fumigatus</strong></td>
<td>62.5</td>
<td>125</td>
<td>-</td>
<td>125</td>
</tr>
</tbody>
</table>

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

**Fig 1. Total Phenolic content and Total Flavonoids of Grewia Species**
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, 2012). 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.
REFERENCES


