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Phytochemical Screening and *In Vitro* Biological Activities of Ethanolic Leaf Extract of *Grewia asiatica* L.

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Abstract

Grewia asiatica is an evergreen, woody tree belonging to the Malvaceae. Its Tamil name is *Palicamaram*. Quantitative Phytochemical analysis of ethanolic leaf extract of *G.asiatica* revealed the biochemical signature of the plant. The antioxidant activity of the ethanolic leaf extract was assessed using DPPH (2,2'-diphenyl 1-picryl hydrazyl) radical scavenging activity assay and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay. The ethanolic extract exhibited 76.87% DPPH free radical scavenging activity. ABTS free radical scavenging activity of the ethanolic leaf extract exhibited 72.44% when compared to control (Ascorbic acid). Antibacterial activity of ethanolic extract of *G.asiatic*a was tested against *Shigella flexineris, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* using Gentamycin, Bacitracin, Amoxyclav and Vancomycin as positive controls and ethanol as negative control. Ethanolic leaf extract of *G.asiatica* showed good inhibition potential against *E. coli, S. aureus* and *P.aeruginosa,* when compared to the controls. It is concluded that the leaf of *G. asiatica* possess good biological properties such as antioxidant and antibacterial activities.

Key words: Antibacterial, Antimicrobial, Antioxidant, Free radical scavanging activity, Phytochemicals.

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INTRODUCTION

Higher plants produce a vast number of secondary metabolites, in addition to primary metabolites, *via* complex pathways, which are regulated in highly sophisticated manners. Many such secondary metabolites of them show strong biological activities (Yazaki, 2006). Bioactive secondary metabolites have been, therefore, utilized as natural medicines and often such plants containing those compounds have been used as medicinal plants and prescribed in many recipes as forms of crude drugs, inhibition of DNA and protein synthesis, inhibition of the nervous system, cardiac activity, modulation of microtuble structure etc,. In most cases these bioactive natural compounds are found in particular organs, which are called medicinal part in pharmacognosy, and their contents in such organs are often seasonally regulated (Karuppusamy, 2009). A vast knowledge of how to use the plants against different illnessesmay be expected to have accumulated in areas where the use of plant is still of great importance. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Rural communities, in particular tribal people, depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, for fire and shade (Hills, 2010). Traditional healers claim that their medicine is cheaper and more effective than modern medicine (Kassaye *et al.*, 2006). The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. There are a few reports on the use of plants in traditional healing by either tribal people or indigenous communities of Tamil Nadu (Kloucek *et al.*, 2005). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use (Sibanda and Okoh, 2007). The aim of the present study was to explore the phytochemical constituents and antioxidant and antibacterial potentials of the ethanolic leaf extract of *Grewia asiatica* L.

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MATERIALS AND METHODS

Plant collection

G. asiatica L. is a potentially new source for natural medicine. Plant sample (leaf) were collected from Siriya Kalvarayan hills, Kallakurichi district, Tamil Nadu, India and was authenticated in Rapinat herbarium, St. Joseph College (Autonomous), Tiruchirappalli, India.

Solvent extraction

The leaf powder was weighed (45 g) and packed using thimbles made from alpha cellulose cotton fibre by Whatmann. The plant powder was kept in Soxhlet apparatus and solvent (Ethanol) was used to extract the phytochemicals at 65°C for 8 hours. The resulting extract was poured into glass Petri dishes and kept for evaporation of excess solvent.

Total phenol content

Estimation of total phenolics was done by Folin-Ciocalteau method described by Parimelazhagan (2016). Fifty mg/ml of ethanolic plant extract was taken into the serious of test tubes like 0.2, 0.4, 0.6, 0.8 and 1.0 ml and all the tubes were made up to 1 ml with distilled water. Another test tube was marked 'B' with 1ml of distilled water served as the blank. The analyses were performed in triplicates. Then added 0.5 ml of 1 N Folin-Ciocalteau reagent to all the test tubes as well as to blank. The gallic acid was used as standard. Then all the tubes were vortexed and allowed to stand for 5 minutes at room temperature. After that 2.5 ml of 5 % sodium carbonate was added to all the test tubes including blank. All the tubes were vortexed, incubated in the dark at room temperature for 40 minutes and then the OD value was measured at 725 nm using spectrophotometer. The standard graph was plotted with concentration of tannic acid against respective absorbance. The amount of total phenol in the sample was expressed as mg Gallic acid Equivalents/g of sample.

Total tannin content

One mg/ml of ethanolic plant extract was taken in eppendorf tubes. From the stock solution, 500ml of extract was taken and mixed with 500ml of distilled water. Then 100 mg of polyvinyl polypyrrolidone (PVPP) was added into the test tubes and incubated for 4 hours at 4°C. After the incubation, the tubes were centrifuged at 3000 rpm for 10 min at 4°C. The supernatant contains only the non-tannin phenolics. Then, 100 ml (50mg/ml) of non-tannin phenolics was taken into serious of test tubes. Then all the test tubes were made up to 1 ml with the distilled water. A test tube marked 'B' with 1 ml of distilled water served as the blank. Then added 0.5 ml of 1 N Folin-Ciocalteau reagent to all the tubes including blank. Tannic acid was used as the standard. All the tubes were vortexed well and allowed to stand for 5 minutes at room temperature. After that 2.5 ml of 5% sodium carbonate was added to all the tubes including blank. The test tubes were vortexed again and incubated in the dark at room temperature for 40 minutes and then the OD value was measured at 725 nm using spectrophotometer. The standard graph was plotted with concentration of tannic acid against respective absorbance. Finally, the amount of non-tannin phenolics and total phenolics in the sample were expressed as mg Tannic acid Equivalents/g sample. The tannin in the sample was calculated by the following equation Tannins (g) = Total phenolics (g) – Non tannin phenolics (g).

Antioxidant activity assays

2,2′-Di–Phenyl Picryl Hydrazyl (DPPH) radical scavenging activity

The very popular method of Brand-Williams *et al.* (1995), which involves the reaction of chemical compounds in the test solution with DPPH radical (2,2′-diphenyl 1-picryl hydrazyl) was adopted. One ml of 0.2 mM DPPH radical solution was combined with 1 ml of the sample (prepared using methanol and DMSO solvents) at varying concentrations (100-500 ìg/ml). Corresponding blank solutions with L-ascorbic acid (of equal concentrations as the test solutions, in the range of 20-100 ìg/ml) were used as a positive control, as it is an antioxidant known to rapidly scavenge free radicals. A mixture of 1 ml methanol with 1 ml DPPH was used as control. The disappearance of DPPH radical was monitored at 517 nm using a spectrophotometer, after incubation at room temperature in the dark. Per cent inhibition was calculated.

2,2′- Azino-Bis 3-ethylbenzoThiazoline-6-Sulfonic acid (ABTS) assay

ABTS was dissolved in water so as to get 7 mM concentration. By reaction with 2.45 mM potassium persulfate (1:1), ABTS radical cation (ABTS•+) was produced and this reaction was allowed to proceed in the dark at room temperature for 12-16 hours prior to use. After incubation, ABTS•+ solution was diluted with double distilled water to adjust the absorbance to 0.7 at 734 nm. Then, 1 ml of freshly prepared ABTS•+ solution was added with 1ml of the sample (10-100 mg/ml). The absorbance was measured at 734 nm using a spectrophotometer. Ascorbic acid was used as a positive control.

Microorganisms

The antibacterial activity of the ethanolic extract of *G.asiatica* by impregnating five chosen concentrations

of the obtained extracts, namely, 1 mg, 2 mg, 3 mg, 4 mg, and 5 mg per 1ml of solvent to treat the different bacterial strains *Viz*, *Salmonella typhi, Shigella flexineris, Staphylococcus aureus* and *E. coli* by well diffusion method. Standard antibiotics such as vancomycin, bacitracin, amoxyclav and gentamicin were used as positive controls. The cultures were obtained from K.A.P. Viswanathan Govt. Medical College, Trichy, India. The bacterial species were sub-cultured on nutrient broth 29 and incubated at 37 °C for 18-24 h for reviving the organisms. Later, fresh overnight cultures were used for the experiment.

Antibacterial activity assay -Well diffusion method

The well diffusion method with few modifications was used to evaluate anti-microbial activity. Wells were cut using sterile, autoclaved steel borers and filled with extract (at a wide range of concentrations) prepared using different solvents (50 ìl). Muller-Hinton agar dispersion plates were inoculated with bacterial cultures after wells were cut. Sterile antibiotic discs (positive controls) were employed to obtain the zones of inhibition. Control wells contained equal amounts (50 ìl) of the solvent. Standard antibiotics such as vancomycin, gentamicin, bacitracin and amoxicillin-clavulanate (HIMEDIA) were used as reference or positive control. Agar plates containing bacteria were incubated at 37 °C for 24 hours. Inhibition zones were recorded as the diameter of growth-free zones, including the diameter of the disc in mm, at the end of the incubation period.

RESULTS AND DISCUSSION

The total phenol content of *G. asiatica* was found to be 25.15 mg/g and Tannin to be 19.28 mg/g. Each herb typically contains distinct phenolic compounds with differing levels of antioxidant activity. Differentiating all the compounds and assessing their antioxidant activities is therefore problematic, due to the scope and complexity of the natural combinations of phenolic compounds in the plant extracts. The intake of antioxidant phenolic compounds from foods is an important factor for protecting health. Therefore, the determination of phenolic contents in plants is crucial in order to find new sources of phenolic compounds.

Antioxidant activity

Nowadays, the antioxidative properties of plant extracts are becoming of increasing interest. This is particularly due to their potential as natural additives, and the use of them as natural antioxidants is gradually becoming the preferred choice for the pharmacological and food industry *in lieu* of synthetic ones. There is evidence of strong antioxidant activity in many plants belonging to the *Nepeta* and *Teucrium* genera (Kaska *et al*., 2019). Therefore, in this study, antioxidant capacities of the ethanolic extract from *G. asiatica* have been evaluated using different methods (DPPH and ABTS). Free radicals, which are highly reactive, are produced in normal cellular function in the body and due to external factors such as pollution and radiation (Negi and Jayaprakasha, 2003). Owing to the injurious effects, that the free radicals can have on foods and biological systems, radical scavenging activity is vital. It is possible for radical scavengers to react directly with peroxide radicals to terminate the peroxidation chain reaction, thereby extending the quality of food products (Gulcin *et al*., 2010). The ABTS and DPPH radical scavenging methods, used in this study, are commonly used to assess the determination of potential radical scavenging activities of the extract investigated. The extract was able to reduce the stable radical as yellow-colored diphenyl picrylhydrazine. Transition metals in the reagent, which contain unpaired electrons that react quickly with peroxides to form alkoxyl radicals, so that they can provide the enzymic activity. Moreover, reductones can also react with certain precursors of peroxide, which in this way prevent peroxide formation (Lee *et al*., 2013) and the reducing capacity of a plant is an important mechanism

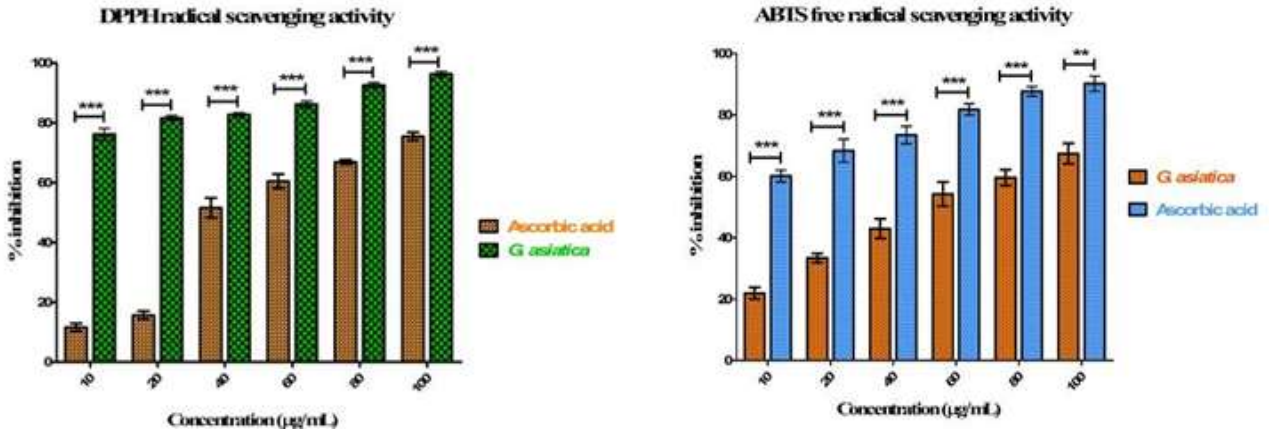

Fig. 1. DPPH and ABTS radical scavenging activity of ethanolic leaf extract of *G. asiatica*

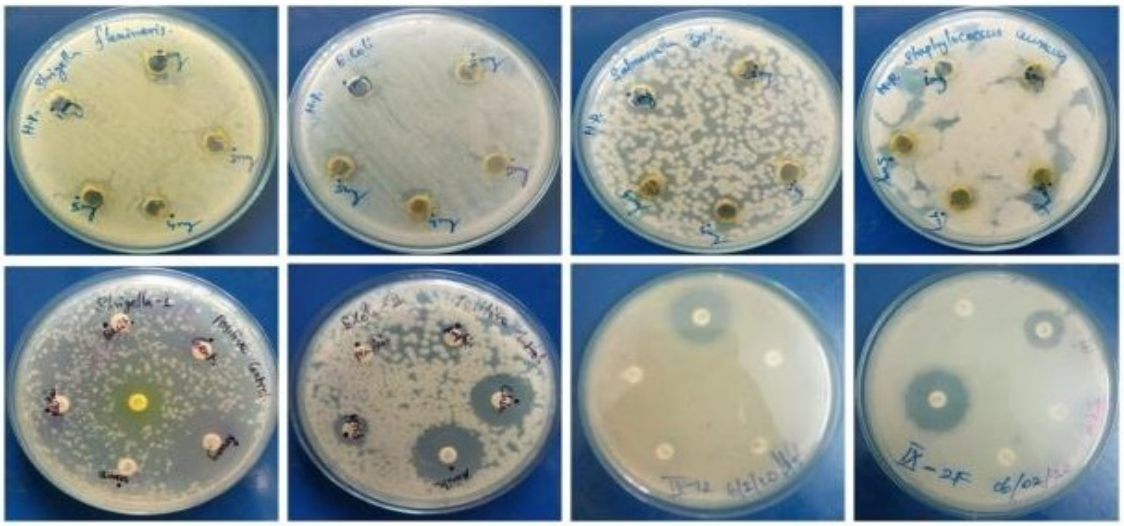

Fig. 2. Zones of inhibition obtained for ethanolic extract of *G. asiatica.*

Table 1. Zones of inhibition obtained for ethanolic leaves extract of *G. asiatica* against different species of bacteria.

Conc. of the plant extract	Species of Bacteria (Diameter of Zone of inhibition in mm)			
	S.flexineris	*E.coli*	*S.typhi*	*S.aureus*
1	3.0±0.133	1.1±0.143	1.0±0.119	0.9±0.087
2	3.0±0.145	1.2±0.124	0.8±0.103	0.7±0.092
3	3.0±0.466	1.1±0.419	0.9±0.272	0.9±0.289
4	1.1±0.632	1.3±0.593	0.7±0.344	0.9±0.386
5	1.3±0.679	1.4±0.602	0.9±0.382	0.8±0.390

of antioxidant action, which may serve as a significant indicator of its potential antioxidant activity. Our results indicate that the extract showed strong reducing antioxidant activity. *G. asiatica* ethanolic leaf extract shows significant DPPH radical scavenging activity of 74% of inhibition in 100 mg/mL concentration and ABTS showed 68% of inhibition in 100 mg/mL concentration (Figure 1).

Table 1 and Figure 2, outlines the antibacterial efficacies of ethanolic leaf extract of selected plant and different commercially used antibiotics (vancomycin, gentamicin, bacitracin and amoxicillin-clavulanate, against various strains of clinically relevant bacterial pathogens. Plant extract showed maximum inhibitory activity against *S.flexineris* followed by *E.coli*, *S.typhi* and *S.aureus.* Amoxyclav (amoxi.cillin-Clavalunic acid) had the greatest efficacy against *E. coli,* while gentamicin showed greater inhibitory properties against *S. typhi, S. aureus, E. coli* and *Shigella flaxineris* (data not shown).

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potentials of medicinal plants used in various traditional systems. Results of the present investigation the phytochemical analysis of *G. asiatica* L. ethanolic leaf extract and its antioxidant and antibacterial activities had clearly established its medicinal potentials of bone fractures.

CONCLUSION

From the results, we conclude that the crude ethanolic extract of *Grewia asiatica* L. exhibited significant antioxidant and antibacterial activity and properties that support its use in folk medicine. The plant extract needs to be characterized using GC-MS and the isolated compounds obtained through other methods such as preparative HPLC need to be studied for antibacterial assays. With that information and with molecular docking and simulation studies (combined with drug target identification in microbes), we can obtain newer antimicrobial compounds.

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