Devagiri Journal of Science 3(1), 20-28
© 2017 St. Joseph's College (Autonomous), Devagiri
www.devagirijournals.com
ISSN 2454-2091

Screening of antioxidant potential and antimicrobial studies of *Grewia tiliifolia* Vahl. (Tiliaceae)

[1]Renjini Haridas, [1]Sangeeth Thekkan, [1]Sumathi, P. & [2]Binu Thomas*

[1]PG & Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore- 641 029, Tamil Nadu, India.

[2]Department of Botany, Centre for Post Graduate studies & Research, St. Joseph's College (Autonomous), Devagiri, Kozhikode – 673008, Kerala, India.

Received: 14.09.2017

Revised and Accepted: 16.10.2017

Key Words: *Grewia tiliifolia* Vahl., Antioxidant activity, Antimicrobial activity

Abstract

Aims: This study was aimed to evaluate the antimicrobial activity, and antioxidant profile of different extracts of tender leaf part of *Grewia tiliifolia* Vahl. which are commonly used in Ayurveda drug preparations. Methodology: Tender leaf part of *G.tiliifolia* subjected to analyze the antioxidant potential by various methods, such as DPPH, ABTS and Ferrous ion chelating assay further, to assess the *in vitro* antimicrobial activity of *Grewia* species by using disc diffusion method against different microorganisms. Results: The results of the present investigation revealed that higher levels of chelating ability for ferrous ions was observed in the crude ethyl acetate extract of tender leaves of *G.tiliifolia.* Methanol extract also exhibited maximum antioxidant activity in DPPH and $ABTS^{\bullet+}$ assays. The antimicrobial activity showed the highest zone of inhibition in chloroform extract. Conclusion: Tender leaves of the species which exhibited well marked antioxidant and antimicrobial activity.

1. Introduction

Plants have rich content of bioactive substances and traditional medicinal plants are used for the treatment of infectious diseases (Divya *et al*., 2011). Recently, researchers all over the world focused on finding naturally occurring medicines from plants. The genus *Grewia*, (Family: Tiliaceae) is an important medicinal plant. In India nearly 40 species of genus are found and some of which are well known for their medicinal value (Praveen, 2012; Muhammad, 2013). Different plant parts of *Grewia* use to cure inflammation, burning sensation, fever, blood disorders, wound healing, ulcerative colitis, heavy menstrual flow and diabetes etc. (Chetna *et al.*, 2016). *Grewia tiliifolia* Vahl is a medium sized tree up to 20 m in height, leaves simple, alternate, ovate with oblique base, crenate-denate, acuminate, upper surface minutely stellately hairy. It is useful in vitiated conditions of kapha and pitta,

*Corresponding author
E-mail: binuthomasct@gmail.com

burning sensation, hyperdipsia, rhinopathy, ulcers, skin diseases, haematemesis and general debility (Nataraja *et al*., 2010). Therefore, considering the traditional use of the plant, the present study has been designed to evaluate phytochemical and pharmacological investigation of different solvent extract of *G. tiliifolia* in tender leaf part.

2. Materials and Methods

2.1. Preparation of extract: Tender leaf part of *G tiliifolia* was collected from the Western Ghats region of Malappuram district, Kerala India. Plant materials (tender leaf part of plant) was collected and washed with distilled water and shade dried for a week. The dried sample were manually ground to fine powder using pulverizer and passed through 40 mesh sieve and stored in air tight containers. The coarsely powdered plant material was weighed to 50g and Soxhlet extracted with petroleum ether, chloroform, ethyl acetate and methanol separately for 12 hours. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator and the solid mass obtained was stored at 4^{o}C until further use. The stored filtrate was used for the various phytochemical and pharmacological studies.

2.2. *In vitro* antioxidant activity

2.2.1. Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) method (Shimada *et al*., 1992).

Reagents

- DPPH$^{\bullet}$ solution: 22mg of DPPH$^{\bullet}$ (2, 2-diphenyl-1-picryl hydrazyl) was accurately weighed and dissolved in methanol. The volume was made up to 100ml. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100µM DPPH$^{\bullet}$ solutions.
- Standard solution: 105mg of ascorbic acid was weighed separately and dissolved in 5.0ml DMSO to get 21mg/ml solution. This was serially diluted with DMSO to get lower dilutions.
- DMSO, distilled.
- Methanol, distilled.

Procedure

Various concentrations of sample (4ml) were mixed with 1ml of methanolic solution containing DPPH$^{\bullet}$ radicals, resulting in the final concentration of DPPH$^{\bullet}$ being 0.2mM. The mixture was shaken vigorously and left to stand for 30min, and the absorbance was measured at 517nm. The percentage inhibition was calculated according to the formula:

$$\% \text{ inhibition} = [(A_0-A_1)/A_0] \times 100,$$

Where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

2.2.2. $ABTS^{\bullet+}$ radical cation scavenging activity (Roberta *et al.*, 1999).

Principle

$ABTS^{\bullet+}$ decolourization assay involves the generation of the $ABTS^{\bullet+}$ chromophore by the oxidation of $ABTS^{\bullet+}$ with ammonium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the plant extracts on $ABTS^{\bullet+}$ radical cation were measured at 734nm.

Reagents

- 7mM $ABTS^{\bullet+}$
- 2.45mM Ammonium persulphate
- Ethanol
- $ABTS^{\bullet+}$ solution**:** 7mM $ABTS^{\bullet+}$ was mixed with 2.45mM Ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hrs before use. $ABTS^{\bullet+}$ solution were diluted to an absorbance of 0.7±0.05 with ethanol at 734nm.

Procedure

The reaction was initiated by the addition of 1ml of diluted $ABTS^{\bullet+}$ to 10µl of different concentration of plant extract of sample and 10µl of methanol as control. The absorbance was read at 734nm and the percentage inhibition was calculated by the following equation.

$$\% \text{ inhibition} = [(A_0-A_1)/A_o] \times 100,$$

Where A_0 is the absorbance of control, A_1 is the absorbance of test compound.

2.2.3. Ferrous ion chelating activity (Dinis *et al.*, 1994).

Principle

Iron (II) chelating activity was measured by the inhibition of the formation of iron (II) ferrozine complex after preincubation of the samples. The Fe^{2+} was monitored by measuring the formation of ferrous iron-ferrozine complex against methanol blanks at 562nm.

Reagents

- 2mM Ferric chloride
- Methanol
- 5mM Ferrozine

Procedure

The reaction mixture contained 1ml of various concentrations of the extract, 0.1ml of 2mM ferric chloride and 3.7ml of methanol. The control contained all the reaction reagents except sample. The reaction was initiated by the addition of 0.2ml of 5mM ferrozine. After 10 mins at room temperature the absorbance of the mixture was determined at 562nm against blank. A lower absorbance of

the reaction mixture was indicated a higher Fe^{2+} chelating ability. The capacity to cheated the ferrous ion was calculated by

% chelating = $[(A_0-A_1)/A_0] \times 100$,

Where A_0 was the absorbance of the control, and A_1 of the mixture containing the extract or the absorbance of a standard solution. IC_{50} value (µg extract/ml) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. EDTA was used for standard.

2.3. *In vitro* antimicrobial activity

Antimicrobial activity of the various solvent extracts of tender leaf part of *G. tiliifolia was* determined by the disc diffusion method (Bauer *et al.*, 1966). All petri dishes were plated with nutrient agar and potato dextrose agar medium prepared according to the manufacturer's manual given below. The microbiological assay was done by comparing the inhibition of the growth by measured concentration of the antibiotics.

2.3.1. Microbial stains

The bacterial and fungal strains which are taken for this study. The bacterial strains include *Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumoniae.*and the fungal strains are *Aspergillus flavus, Trichophyton rubrum, Candida albicans, Candida tropicalis* and *Cryptococcus neoformans.*

2.3.2. Preparation and standardization of inoculums

All the bacterial and fungal cultures were transferred into 100ml of nutrient broth (NB). The inoculated broths were incubated at 37° C for 24 hours and at 27° C for 72 hours in the case of bacteria and fungi respectively.

2.3.3. Antibacterial activity

Nutrient agar medium was prepared and transferred into sterile petri plates. 25ml of the standardization bacterial inoculum was spread on agar medium using sterile cotton swab. The discs impregnated in extracts were placed on the inoculated agar medium. Ampicillin 10µg/disc was used as standard to determine the sensitivity of each microbial species. All the petri plates were incubated at 37° C for 24 hours. After the incubation period, diameter of zone of inhibition was measured.

2.3.4. Antifungal activity

Potato dextrose medium was prepared and transferred into sterile petri plates. 200µl of the standardized fungal inoculum was spread on agar medium using sterile cotton swab. The discs impregnated in extracts were placed on the inoculated agar medium. Tetracycline 10µg/disc was used as reference standard to determine the sensitivity of each microbial species

tested. All the petri plates were incubated at 27° C for 72 hours. After the incubation period, diameter of zone of inhibition was measured. Growth inhibition was determined as the diameter of the inhibition zones around the discs. The growth inhibition, diameter was an average of 4 measurements, taken at four different directions. All the tests were performed a triplicate.

3. Result and Discussion

The present study investigate *in vitro* anti-microbial and anti-oxidant activity of tender leaf part of *G. tiliifolia*.

3.1. *In vitro* antioxidant activity

Different extracts like petroleum ether, chloroform, ethyl acetate and methanol extracts of the tender leaf part of *G. tiliifolia* was evaluated for its antioxidant activity on different *in vitro* models like DPPH, $ABTS^{\bullet+}$ and ferrous ion chelating assay.

3.1.1 DPPH radical scavenging activity

The percentage of scavenging effect on the DPPH radical was accelerated with the increase in the concentrations of the extracts from 50-250 µg/ml. Methanol extract showed maximum (80.88%) scavenging activity and petroleum ether least (57.12%) scavenging activity (Table 1).The result confirmed that the tender leaf plant part *G. tiliifolia* has exhibited the highest ability to quench the DPPH radical.

3.1.2 Ferrous ion chelating assay

The metal chelating activity was determined and it increases with the increase in the concentration of extract from 50-250µg/ml. The percentage of inhibition of the metal chelation maximum in ethyl acetate (40.05%) and least activity in methanol extract (32.52%) (Table 2). Therefore it is apparent that the tender leaf plant part extract of *G. tiliifolia* exhibited excellent chelating ability for ferrous ions and might afford protection against oxidative damage.

3.1.3 $ABTS^{\bullet+}$ radical scavenging activity

Different solvent extracts like petroleum ether, chloroform, ethyl acetate and methanol extracts of the tender leaf plant part extract of *G. tiliifolia* was taken to examine the antioxidant activity. Methanol extract exhibited the maximum antioxidant activity (72.72%) and least activity was exhibited by petroleum ether extract (56.18%) (Table 3).

From the results obtained for DPPH, $ABTS^{\bullet+}$ and ferrous ion chelating assay the tender leaf plant part extract of *G. tiliifolia* can be considered as a potential radical scavenging activity.

3.2 Antimicrobial activity

Chloroform extract of the tender leaf plant part extract of *G. tiliifolia* showed maximum

antibacterial and antifungal activity when compared to other solvent extracts. *Staphylococcus aureus* (27mm) exhibited maximum activity, Moderate activity was shown against *Pseudomonas aeruginosa* (23mm) and *Acinetobacter baumannii* (22mm). The least activity was shown against *Salmonella typhi* (18mm) and *Klebsiella pneumoniae* (6mm) (Table 4). In the case of fungal strains Chloroform ether extract showed maximum antifungal activity against *Aspergillus flavus* (35mm). Moderate activity was shown against *Cryptococcus neoformans* (20mm). *Candida tropicalis* showed least activity (5mm) in ethyl acetate extract. No activity was shown against *Trichophyton rubrum* and *Candida albicans* (Table 5).

Table: 1. DPPH scavenging activity of different solvent extract of tender leaf plant part of *G. tiliifolia*

S. No.	Solvents	Concentration (µg/ml)	% of inhibition
1.	Petroleum ether	50	30.68
		100	47.62
		150	49.27
		200	56.09
		250	57.12
2.	Chloroform	50	28.51
		100	30.68
		150	38.94
		200	51.75
		250	62.7
3.	Ethyl acetate	50	27.7
		100	33.57
		150	34.09
		200	42.45
		250	63.11
4.	Methanol	50	61.15
		100	65.7
		150	78.71
		200	79.44
		250	80.88

Table: 2. Effect of ferrous iron chelation activity in different solvent extract of tender leaf plant part of *G. tiliifolia*

S.NO	Solvents	Concentration	% of inhibition
1	Petroleum ether	50	6.98
		100	23.92
		150	26.34
		200	30.37
		250	33.06
2	Chloroform	50	1.34
		100	11.29
		150	27.41
		200	35.48
		250	38.44
3	Ethyl acetate	50	4.03
		100	10.21
		150	19.62
		200	28.22
		250	40.05
4	Methanol	50	8.33
		100	11.55
		150	13.97
		200	21.5
		250	32.52

Table: 3. Effect on $ABTS^{\bullet+}$ scavenging activity in different solvent extract of tender leaf plant part of *G. tiliifolia*

S. No	Solvents	Concentration(µl)	% of inhibition
1	Petroleum ether	50	56.18
2	Chloroform		59.46
3	Ethyl acetate		68.4
4	Methanol		72.72

Table: 4. Antibactirial activity in different solvent extract of tender leaf plant part of *G. tiliifolia* against different bacterial strains

S. No	Bacteria	Control	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	*Acinetobacter baumannii*	20mm	6mm	22mm	6mm	10mm
2	*Pseudomonas aeruginosa*	20mm	6mm	23mm	15mm	12mm
3	*Staphylococcus aureus*	18mm	10mm	27mm	20mm	12mm
4	*Salmonella typhi*	24mm	5mm	18mm	15mm	11mm
5	*Klebsiella pneumoniae*	10mm	6mm	6mm	6mm	6mm

Table: 5. Antifungal activity in different solvent extract of tender leaf plant part of *G. tiliifolia* against fungal species tested by disc diffusion assay

S. No	Fungus	Control	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	*Aspergillus flavus*	40mm	_	35mm	10mm	15mm
2	*Trichophyton rubrum*	_	_	_	_	_
3	*Candida albicans*	_	_	_	_	_
4	*Candida tropicalis*	_	_	_	5mm	_
5	*Cryptococcus neoformans*	35mm	5mm	20mm	10mm	15mm

4.Conclusion

Grewia. tiliifolia is an important traditional folk medicine. Tender leaves of the species which exhibited well marked antioxidant and antimicrobial. Thus the results obtained from this investigations indicate that tender leaf plant part extract of *G.tiliifolia*, rich in secondary metabolites and confirmed that the tender leaves have the potential source of natural antioxidant and exhibited good antimicrobial potential. That could have great importance as therapeutic agents in preventing diseases.

References

Bauer, A.W, Kirby, W.M., Sherris J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., **45(4):** 493-496.

Chetna, S., Manasi, M.S., Sangvikar, G., Murthy, S.N and Pawar, S.D. (2016). *Invitro* Evaluation of Antimicrobial and Antioxidant Profile of *Grewia* L. Root Extracts. *Int. J. Appl. Life Sci.,* **7(1):** 1-9.

Dinis, T.C., Madeira, V.M. and Almeida, M.L. (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.*, **15:** 161-169.

Divya, N., Mythili S. and Sathiavelu A. (2011). Phytochemical analysis and *In vitro* antimicrobial activity of *Andrographis paniculata* (Acanthaceae) *J. Pharm. Res.,* **4(7):** 2140-2142

Muhammad, Z.U., Milan, S. Stankov, K.R. and Vincenzo, D.F. (2013). *Grewia asiatica* L., a Food Plant with Multiple Uses. *Molecul*., **18:** 2663-2682.

Nataraja, T.S., Vengatakrishnan, V., Murugesan, S. and Damodar Kumar, S. (2010). Free radical scavenging activity of methanolic extract of *Grewia tiliaefolia* bark in various *In-vitro* model systems. *Res. J. Pharmaceut. Biol. Chem. Sci.,* **1(3):** 502-509.

Praveen, K.G. (2012). Phytochemical and pharmacological properties of the genus *Grewia*: A Review. *Int. J. Pharm. Pharmaceut. Sci.,* 4 (4):72-78.

Re Roberta, Nicoletta P., Anna, P., Ananth, P., Min, Y. and Catherine, R.E. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad. Biol. Med.,* **26(9):** 1231–1237.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem*., **40:** 945-948.