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Research Article

Comparative Evaluation of Various Flower Extracts of *Grewia asiatica* for Phyto-Compounds and Anti-Bacterial Efficacy

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ABSTRACT:

Grewia asiatica is known traditionally for the treatment of various human ailments. Present study was carried out to evaluate the effect of various polarities of solvents on extraction and their bioactivity. Among all twelve extracts methanol, aqueous, ethanol, chloroform and acetone extracts showed the moderate to higher presence of all phytocompounds. Whereas, maximum phenolic (22.19±0.42 µg/mg equivalent to Gallic acid) and Flavonoid content (52.31±0.1 µg/mg equivalent to quercetin) were observed in methanolic extract followed by aqueous and other extracts. Interestingly, the antibacterial assay was also identified methanolic extract as a potential bacterial growth inhibitor which showed significant zone of inhibition against *Pseudomonas aeruginosa* (3.3±0.04 cm), *Vibrio cholera* (3.0±0.08 cm) and *Salmonella abony* (3.0±0.05 cm) at concentration 20 mg/ml and best minimum inhibitory concentration was observed against *Escherichia coli* (0.5±0.03 mg/ml). Furthermore, the potential methanolic extract of flower was identified via Gas chromatography and mass spectrometry (GC-MS) analysis revealed compound 3,4-Altrosan having maximum (35.78%) percent area in the spectrum. The present investigation showed that methanolic extract of *Grewia asiatica* flower showed significant antibacterial inhibition compared with other solvents extracts.

Keywords: Phytochemical; Gas chromatography and mass spectrometry; anti-bacterial; minimum inhibitory concentration

[I] NTRODUCTION

Over the last decades, there has been a lot of attention in the search of natural product as sources of new antioxidant and antimicrobial agents [1-2]. According to the World Health Organization (WHO), medicinal plants would be the greatest resource to find a range of drugs and bioactive compound. As a result, such plants should be investigated to better understand their properties, protection and effectiveness. The traditional system of medicine namely Ayurvedic, Siddha and Unani has been in existence from millions of years. These medicine systems supports necessitate of more than 70% of population live in the rural areas. Besides the demands made by traditional medicinal systems of their raw materials, the requirements of medicinally important plants made by the present pharmaceutical industries have also increased manifold [3]. Plants have been a priceless foundation of natural products for maintaining human health and various disease controls, as, microbial infections cause a serious health

problem worldwide, and plants are a promising source of antioxidant and antimicrobial agents [4]. Moreover, plants posses significant clusters of secondary metabolites for example flavonoids, phenols, alkaloids, saponins, steroids, terpenoids, tannins and polysaccharides that are naturally contributing to diverse biological activities in traditional and modern system of medicine [5]. Commonly used medicinal plants have posse's effective biological activity to manage a range of human ailments [6]. The basis for discovery the natural plant products and their separation using various individual low polar to high polar solvents is significant for pulling out of effective bioactive compounds [7]. Even though slowly increasing solvent polarity in sequential extraction such as ethyl acetate, ethanol, chloroform, water and methanol works out well in the separation of efficient bioactive components, overall qualitatively and quantitatively extraction for secondary metabolites from plant species greatly depends on solvent system used [8].

There is current focus is towards natural antioxidant and antibacterial activity of plant extracts. It is important to discover the medicinal properties of natural plant extracts especially that plant used in folk medicine systems. *Grewia asiatica* is used in folk medicines as to be beneficial for heart, blood and liver disorders, indigestion, thirst, stomatitis, asthma, toxemia, anorexia, fevers and diarrhea. Leaves are reported to have wound and cut healing property to the skin and to relieve irritation, painful rashes. Flowers are used in neurological disorders, rheumatism and urinary tract problems as described in traditional system [9-12]. Numerous investigations have been carried out from all parts of *G. asiatica* but in case of flower no sufficient analysis traced out till date. Therefore, the study provides first time description of phytochemical investigation and antibacterial approach of *Grewia asiatica* flower extracts. Study will help to researchers for the *in-vitro* and *in-vivo* investigations to trace out potential compounds for various inhibitory activities.

[II] MATERIALS AND METHODS

2.1. Collection of plant and authentication

Flower samples were collected from the vicinity of Integral University, Lucknow and the specimens were identified and authenticated as *Grewia asiatica* L**.** in Birbal Sahni Institute of Palaeobotany, Lucknow, India (Reference sample submitted in the herbarium of BSIP as specimen). The dried fine powder sample was used for the extraction from different individual solvents [13]

2.2. Phytochemical estimation

Qualitative estimation

Qualitative estimation of all extracts has been performed by following protocols of Adetuyi and Popoola, Trease and Evans [14-15].

Determination of total phenol content (TPC)

Total phenols in test samples were estimated by using Folin–Ciocalteu method given by McDonald and coworkers [16].

Plant extract of all solvents were dissolved in Methanol (1 mg/ml concentration). The assay mixture (5 ml) contained 0.5ml of extract or standard (Gallic acid), 2ml aqueous (1N) Na_2CO_3 and 2.5ml of (2 N) Folin–Ciocalteu reagent (Sigma-Aldrich, Germany). Samples were incubated at room temperature for 15 minutes and absorbance at 765 nm was measured by spectrophotometer.

The standard curve was prepared using 1, 10,100 and 200 mg/ml solution of Gallic acid (GA) in methanol, however, blank contained 500µl of double distilled water, Na_2CO_3 and the Folin–Ciocalteu reagent.

Determination of total flavonoid content (TFC)

The quantitative flavonoid content was estimated by the aluminum chloride method by using spectrophotometer [17]. Flower extracts were dissolved in methanol (1 mg/ml concentration). Aliquots of 0.5 ml of each sample were mixed with methanol (1.5 ml), 10 % Aluminium Chloride (0.1 ml), 1M Potassium acetate (0.1 ml) and distilled water (2.8 ml) and kept for 30 minutes at room temperature.

The absorbance of the reaction mixture was taken at 415 nm. Quercetin was used as a standard.

2.3. Anti-bacterial assay

Bacterial cultures of six test organisms, namely, *E. coli* (NCIM 2277), *V. cholerae* (NCIM 5316), *S. aureus* (NCIM 2079), *B. subtilis* (NCIM 2097), *S. abony* (NCIM 2257), *P. aeruginosa* (NCIM 2036) were procured from National Chemical Laboratory, Pune. Pure cultures were maintained on nutrient agar media slants and stored at 4º C. The test culture was prepared by transferring a loop full culture to the freshly prepared sterile broth (50 ml) and incubating for 24 hours at 37º C.

Well diffusion method

Antibacterial activity was carried out by well diffusion method [18]. The test sample of extracts for the antibacterial activity was prepared by dissolving extracts in DMSO (Dimethyl sulfoxide) in 5, 10, 15, 20 mg/ml concentration and DMSO used as a negative control while Ampicillin (10µg/ml) was used as positive control. Freshly prepared microbial broth culture was observed under spectrophotometer at 600 nm wavelength for determination of colony forming units (cfu) and culture was maintained at the turbidity of 1×10^8 CFU/ml. Fresh culture broth of *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Vibrio cholerae, Salmonella abony, Pseudomonas aeruginosa* suspension (about 0.1 ml) was spread over the Nutrient agar media using L-shaped sterilized glass spreader separately. The wells were made in each plate with the help of borer of 8 mm diameter. In these wells, about 50 µl of each extract was loaded individually. Petri plates were incubated for 24 hours at 37ºC. After incubation, clear zone of inhibition was measured.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of extracts has been evaluated against six bacterial strains following standard protocol of Islam and their coworker [19]. Overnight incubated bacterial culture (turbidity equivalent to McFarland solution) having 1 to 2×10^8 cfu/ml was used. Different test concentrations (0.1-5 mg/ml) of extracts and drug (Ampicillin) were used for MIC determination. The test was performed on 96 well microtiter plates.

2.4. Gas Chromatography-Mass Spectrometry Analysis

The dried powder of methanol extract was dissolved in the respective solvent and GC-MS analysis of sample was carried out by following process in the GC-MS machine. The sample (1 µl) was injected into a RTX-5 column of GC-MS (model GC-MS-QP-2010 plus, Shimadzu Make). Helium, a carrier gas, was used at a constant column flow of 1.2 ml/min. The temperature was set between 100°C to 200°C with constant rise of 5°C/min and then held isothermal at 200°C for 6 min; further temperature was elevated orderly 10°C/min up to 290°C and again held iso-thermal at 290°C for 10 min. The ion source and injector temperatures were 250°C and 270°C, respectively. Mass spectra were taken at fragments from 40 to 950 Dalton and scan at interval of 0.5s at 70 eV. The constituents were finally confirmed by computer matching of the mass peaks of spectra with Wiley and National Institute Standard and Technology (NIST) libraries mass spectral database.

[III]RESULTS

3.1. Phytochemical analysis

Preliminary phytochemical analysis of all extracts of flower showed the presence of variety of phytochemicals such as phenol, flavonoid, alkaloid, tannin and saponin (Table 1). Present results identified that methanol, aqueous, ethanol, chloroform and acetone extracts showed the moderate to higher presence of all phytocompounds, however, other extracts were showed either low or absence of phytocompounds.

Extracts	PH	FLV	AL	TN	SP
n-hexane	-	-	++	+	+
Dichloromethane	-	-	+	+	-
Methanol	+++	++	+++	+	+++
Aqueous	+	++	+	++	+
Ethanol	+	+	-	+	++
Butanol	-	-	+	+	+
Chloroform	++	+++	+	+	++

Acetone	+	+	+	+	+
Ethyl acetate	-	+	-	-	+
Cyclohexane	+	++	-	-	-
2-propanol	+	+	+	-	-
Benzene	+	+	-	-	-

Table: 1. (-) = not detected, (+) = low presence, (++)=moderate presence, (+++) = strong presence. (PH-Phenols, FLV-Flavonoids, AL-Alkaloids, TN-Tannins, SP-Saponins)

3.2. Estimation of Percent yield, total phenolic and flavonoid contents:

The results of percent yield, total phenolics and flavonoids estimation showed great variation in different solvents. The maximum yield was obtained by methanol extract (3.90%) followed by aqueous (2.13%), ethanol (2.11), benzene (2.30%), 2-propanol (2.25%) and other extracts. Higher phenolic content was estimated in methanol 22.19±0.42 µg/mg of extract equivalent to Gallic acid followed by aqueous (16.0±0.32 µg/mg) and other extracts (Table 2). Analogous to phenolic content higher flavonoid also estimated in methanolic extract which was 52.31±0.1 µg/mg of extract equivalent to Quercetin followed by aqueous 35.0±0.12 µg/mg and other extracts.

Extracts	**Percent yield**	**TPC (µg/mg)**	**TFC (µg/mg)**
n-hexane	0.65	NA	NA
Dichloromethane	0.50	NA	NA
Methanol	3.90	22.19±0.42	52.31±0.1
Aqueous	3.13	16.0±0.32	35.0±0.12
Ethanol	2.80	10.2±0.11	15.1±0.56
Butanol	1.50	NA	NA
Chloroform	1.32	5.3±0.31	4.91±0.21
Acetone	1.25	7.3±0.44	14.5±0.36
Ethyl acetate	1.90	NA	8.0±0.23
Cyclohexane	1.40	3.22±0.32	9.8±0.20
2-propanol	2.25	4.21±0.51	5.70±0.45
Benzene	2.30	3.16±0.11	4.50±0.31

Table: 2. Results of percent yield, TPC and TFC. All estimation was performed in triplicate manner and expressed as mean value of ±S.D, n=3.

3.3. Anti-bacterial analysis

The inhibition of bacterial growth by all flower extracts of *G. asiatica* was evaluated against six pathogenic bacteria. The results revealed that methanol flower extract showed maximum activity against *P. aeruginosa* (3.3±0.04 cm), *V. cholera* (3.0±0.08 cm) and *S. abony* (3.0±0.05 cm) at maximum concentration 20 mg/ml and minimum activity against *S. aureus* (2.4±0.06 cm) at 10 mg/ml concentration (table 3). The antibacterial activity was varied against bacterial strains by other extracts. Dichloromethane, n-hexane, cyclohexane and butanol extracts did not show any satisfactory activity against all bacterial strains, however, aqueous extract shown moderate antibacterial activity. Among all the extracts methanol extracts potentially inhibited the bacterial growth which was comparable with that of standard antibiotic Ampicillin.

Minimum inhibitory concentration estimation:

The most potent methanolic extract was assayed for MIC determination and the minimum MIC values were observed against *E. coli* (0.5±0.03 mg/ml), followed by *V. cholerae* (1.5±0.02 mg/l), *S. aureus* (1.5±0.05 mg/ml), *S. abony* (1.5±0.07 mg/ml), *P. aeruginosa* (2.0±0.06 mg/ml), and *B. subtilis* (2.5±0.04 mg/ml). The standard antibiotic ampicillin showed minimum MIC value (0.5 mg/ml) *against S. abony and E. coli* (Table 4).

3.4. GC-MS analysis

Among all the twelve extract, methanolic extract was found to retain higher amount of phytocompounds which possessed most potent antibacterial activity. Accordingly, this most potent extract was subjected GC-MS analysis. The analysis spectrum of methanolic extract showed six outstanding peaks occupying higher percent area (fig. 1). The major components were 3,4-Altrosan with 35.78% peak area; 4H-pyran-4-one, 2-hydroxy-3-methyl with 20.79% peak area; Lupenone with 8.10% peak area; Stigmast-5-EN-3-ol, 3 beta with 4.34% peak area; Hexadecanoic acid with 6.29% peak area and Tetradecanoic acid with 3.36% peak area (Table 5). The identified

compounds were popularly known for various biological activities.

Extracts	(mg/ml)	Zone of inhibition (in cm)					
		Pa	***Bs.***	***Ec***	***Vc.***	***Sa.***	***Sab***
n-hexane	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	-	-	-	-	-
	20	-	-	0.8±0.04	-	-	0.6±0.06
Dichloromethane	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	0.4±0.06	0.5±0.03	1.2±0.06	1.0±0.08	0.6±0.07	-
	20	0.7±0.07	1.0±0.06	1.6±0.05	1.3±0.06	1.9±0.09	0.9±0.06
Methanol	5	0.8±0.06	0.6±0.04	1.0±0.07	1.0±0.05	1.1±0.06	1.0±0.05
	10	1.3±0.04	1.2±0.03	1.5±0.05	1.8±0.03	1.6±0.08	2.0±0.09
	15	2.0±0.05	2.0±0.04	2.2±0.06	2.1±0.04	1.8±0.05	2.5±0.04
	20	3.3±0.04	2.8±0.06	2.7±0.09	3.0±0.08	2.4±0.06	3.0±0.05
Aqueous	5	-	-	0.4±0.06	-	0.4±0.05	_
	10	_	0.5±0.06	0.4±0.08	0.5±0.05	0.8±0.09	-
	15	0.5±0.03	1.2±0.05	0.9±0.05	0.8±0.08	1.3±0.04	-
	20	1.2±0.05	1.6±0.07	1.3±0.05	1.0.0±0.06	2.1±0.07	1.0±0.06
Ethanol	5	-	-	-	-	-	-
	10	0.3±0.08	-	-	-	-	-
	15	0.5±0.04	0.5±0.07	-	-	0.8±0.05	00
	20	0.8±0.07	1.1±0.05	0.8±0.07	0.6±0.05	1.3±0.08	0.6±0.04
Butanol	5	-	-	-	-	-	-
	10	-	0.3±0.08	-	-	-	-
	15	-	0.6±0.05	-	-	-	-
	20	0.6±0.05	1.0±0.04	-	-	-	-
Chloroform	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	0.8±0.09	-	-	0.5±0.07	-	-
	20	1.1±0.06	0.8±0.04	-	1.0±0.06	1.1±0.05	1.2±0.07
Acetone	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	-	-	-	-	-
	20	-	0.8±0.05	-	-	-	-
Ethyl acetate	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	0.7±0.05	0.2±0.04	-	0.5±0.03	-	-
	20	1.2±0.06	0.4±0.07	0.8±0.05	1.0±0.06	1.1±0.04	-
Cyclohexane	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	-	-	-	-	-
	20	1.2±0.05	-	1.0±0.07	-	-	-
2-propanol	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	0.7±0.04	-	-	-	-	-
	20	1.0±0.08	-	-	-	-	-
Benzene	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	15	-	-	-	-	-	-
	20	0.9±0.07	-	-	-	-	-
Standard Ampicillin	10µg/ml	3.3±0.04	2.6±0.05	2.9±0.03	2.4±0.02	2.2±0.04	2.8±0.05
Control		00	00	00	00	00	00

Table: 3. Results of anti-bacterial activity at different concentrations of various extracts. Results were expressed as mean ± S.D., n=3. (-) indicated no activity.

MIC values against different bacterial strains (mg/ml)						
Test Sample	***Pa***	***Bs***	***Ec***	***Vc***	***S a***	***S ab***
Methanol	2.0±0.06	2.5±0.04	0.5±0.03	1.5±0.02	1.5±0.05	1.5±0.07
Ampicillin	1.0±0.066	0.5±0.05	0.5±0.02	1.0±0.072	1.0±0.09	1.5±0.06

Table: 4. MIC experiment was performed in triplicates and all the data was expressed as mean ±S.D, n=3. [*Ec=Escherichia coli, Sa=Staphylococcus aureus, Bs=Bacillus subtilis, Vc=Vibrio cholerae, Sab=Salmonella abony, Pa=Pseudomonas aeruginosa*]

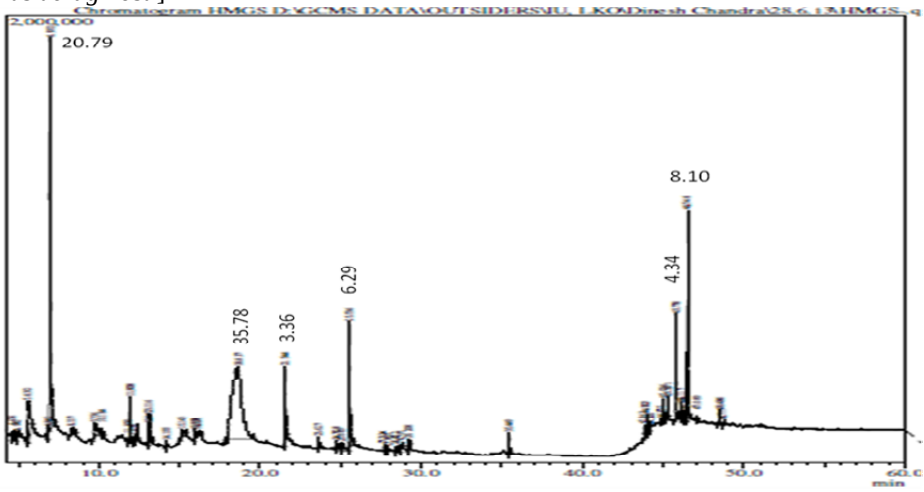

Fig: 1. GC-MS graph of methanolic extracts

[IV] DISCUSSION

In our study, results confirmed that methanol extract is most effective among all other extracts it may be due to better solubility of phenolics and flavonoids in polar solvents [20]. Comparing our results with others study, the values found to be higher for total phenol content than other studies obtained in flower plants such as *Moringa oleifera, Okra* Flowers [21-23]. Phenolics and flavonoids are secondary metabolites which present in all plants. These compounds are well known for various biological and protective activities [24].

R. Time	% area	Compound name	Compound nature	Activity**
6.952	20.79	4H-pyran-4-one, 2-hydroxy-3-methyl	Flavonoid	Anti-inflammatory, Antifungal, antioxidant
18.637	35.78	3,4-Altrosan	Polyhydroxy compound	Bactericide, Fungicide
21.544	3.36	Tetradecanoic acid (Myristic acid)	Fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor
25.536	6.29	Hexadecanoic acid (Palmitic acid)	Fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-Alpha reductase inhibitor
45.778	4.34	Stigmast-5-EN-3-ol, 3 beta	Steroid	Antioxidant, antibacterial activity,antiinflammatory, antiarthritic, antiasthma, diuretic
46.546	8.10	Lupenone	Terpenoid	Antioxidant, Anti-microbial

Table: 5. GC-MS identified compounds of methanolic extract. **activity was confirmed by Dr. Dukes medicinal plant compounds reference book

Previous reports on *G. asiatica* were also identified methanol solvent for extraction of various phytocompounds having vast biological activities [11-12]. These classes of phytochemicals are well known for medicinal properties against various types of pathogenic attacks and consequently could recommend for the treatment of various ailments [25]. The *in-vitro* anti-bacterial analysis results presented in table 3 showed maximum activity by methanolic extracts as compare to other extracts which was also comparable with standard ampicillin. The highest activity was found in methanolic extracts against *P. aeruginosa* followed by *V. cholera* and *S. abony*, which confirmed methanolic extract antibacterial potential against both grams positive as well as gram negative bacterial strains. However, it is recommended that plant extracts posses greater or equivalent than 10mm diameter zone of inhibition considered as active [26-28]. Interestingly, the MIC results identified methanolic extract for broad spectrum bacterial inhibition against gram negative bacteria *E.coli* which was 0.5 mg/ml similar to the ampicillin. The extract also has appreciable MIC value against other gram positive strain which was comparable with studies performed on other species of *G. asiatica* [10, 20 and 29].

The findings revealed that maximum types of phytocompounds and bioactivities were reported in methanolic extract, which was further analyzed by GC-MS analysis for identification of compound on the basis of similarity index and molecular weight. Normally, phytocompounds are highly complexes, so for their analysis by GC-MS method well appropriate due to its sensitivity and selectivity [30-31]. Numerous studies have been reported which identified compounds by GC-MS analysis [32]. The GC-MS analysis was confirmed various components in methanolic extract but major identified compounds are 3,4-Altrosan; 4H-pyran-4-one, 2-hydroxy-3-methy; Lupenone; Stigmast-5-EN-3-ol, 3 betarea; Hexadecanoic acid and Tetradecanoic acid. These compounds were previously studied and well known for diversity of biological activities such as anti-oxidant, anti-microbial, anti-inflammatory etc. [33]. The overall study revealed that methanolic flower extract of *G. asiatica* has more potentiality to minimize the disease like conditions. Our study validated that *G. asiatica* is a medicinal plant having diverse ethno-pharmacological approach in which flower extracts also have wide verities of secondary metabolites with potential antibacterial activities. The study can be further preceded for separation and identification of bioactive compounds and *in vitro, in vivo* evaluation for novel natural drug discovery.

[V] CONCLUSION

The overall study concluded that *G. asiatica* methanolic flower extracts have significant amount of TPC and TFC which have potential to inhibit the growth of infectious micro-organism. The study also concluded that this extract contained various compounds for diverse activity which will be a good healthcare and alternative formulation of synthetic drugs for human beings.

FINANCIAL DISCLOSURE

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