



# ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL EVALUATION OF *GREWIA ABUTILIFOLIA*'S LEAVES EXTRACTS

Dolly Rani<sup>1</sup>, G.T. Kulkarni<sup>2</sup>, Nitin Rai<sup>3</sup> and Harsha Kharkwal<sup>4\*</sup>

<sup>1,2</sup>Amity Institute of Pharmacy, Amity University, Noida, India.

<sup>3</sup>Pharmacopoeia Commission for Indian Medicine & Homoeopathy, Ministry of Ayush, Govt. of India, Ghaziabad, India.

<sup>4\*</sup>Amity Institute of Phytochemistry and Phytomedicine, Amity University, Noida, India.

## Abstract

*Grewia abutilifolia* is a threatened medicinal plant having immense unexplored therapeutic potential. The aspiration of this study was to establish the antimicrobial and phytochemical constitution of this natural product in order to perpetuate its unexplored potential. Extraction was carried out such as water, methanol, n-Hexane and Ethyl Acetate. The antimicrobial activities of different extracts were assessed by the Disk diffusion method against four bacterial and two fungal strains. All the extracts have demonstrated an equivalent effect against four bacterial strains at different concentrations. The aqueous extract was found to be more effective against *E. coli* while extract of ethyl Acetate exhibited almost equal activity against all four bacterial strains. N-hexane's extract displayed more activity against by cold maceration and Hot percolation (Soxhlet apparatus) process using various solvents *B. Subtilis*, *P. aeruginosa* and *E. coli*. Almost similar antimicrobial activities pattern was shown by cold macerated extracts. The Minimum Inhibitory Concentration (MIC) of the plant extracts ranged from 50 to 250 µg/ml. Some extracts exhibited anti-microbial potency comparable commercially antimicrobial agents. All these observations thus contribute to a strengthening of the utilization of plants in the cosmeceutical and pharmaceutical industries.

**Key words:** Minimum Inhibitory Concentration (MIC), Therapeutic potential, Percolation, Maceration, Disk diffusion.

## Introduction

Microbial infection is considered as one of the major causes of bereavement in emerging nations (Mostafa, A.A. *et al.*, 2018). Emerging resistance against antibiotics is a global concern in the public health protection and a major confrontation all over the world. As per National data submitted to WHO regarding Antibiotic consumption surveillance (2016-2018), oral administrated antibiotic consumption has risen from 50 to nearby 100% in countries. Medicine such as penicillin and  $\beta$ -lactamase is the most favored oral antibiotics, Economically emerging and underdeveloped nations are at the forefront of this increase (Reta, A. *et al.*, 2019). *Escherichia coli*, *Pseudomonas aeruginosa*, *streptococcus aureus* and *Bacillus cerus* are among the common pathogenic bacterial strains causing significant mortality and morbidity (Mostafa, A.A. *et al.*, 2018; Fair, R.J. & Tor, Y., 2014). As per the study carried out by Reta *et al.*,

2019 antibiotic resistance varied from 0 to 100% in gram-negative and gram-positive strains (Reta, A. *et al.*, 2019). The increasing incidences of MDR (Multi Drug Resistant) microbial strains against the synthetic drugs are limiting their clinical effectiveness as well as also reducing the treatment options. Since ancient times, India is entrenching for its copious biodiversity medicinal plants, due to diverse climatic conditions. *Grewia abutilifolia* is a shrub belonging to the family Tiliaceae and known for its diverse medicinal values (Khasim, S.M. *et al.*, 2020) *Grewia abutilifolia* experiences seasonal flowering and fruiting and it is distributed in moist deciduous forests in the central and southern parts of India. Ethnobotany studies divulges its various uses like a cooling agent, refreshing drink, Anti-inflammatory, Anti-rheumatism, demulcent and anti-diabetic (Quattrocchi Umberto 2012) As per time plant genus is widened in its medicinal properties instead of its traditional use as a fruit and cooling drink (Mall T.P. & Tripathi S.C. 2018) *Grewia abutilifolia* is one of the threatened species recorded in the Indian Biodiversity

\*Author for correspondence : E-mail : hkharkwal@amity.edu

Organization. The present review highlights the possible Phytopharmacological properties of plants with existing ethnopharmacological importance. This study gives scientific evidence for the antimicrobial action of the drug.

## Material and Method

### Plant material

The *Grewia abutilifolia* leaves were collected during July-August of 2018 in and around Pune, Maharashtra, India. Plant material was washed properly with the help of Tap water and shade dried. Dried plant material was pulverized and screened through mesh size 100mm and authenticated in NISCAIR (voucher no Ref No: NISCAIR/RHMD 3290/91). 100.

### Extraction Procedure

Dried powder was subjected to cold and hot extraction. The extraction was carried out with different solvents with increasing order of polarity, like n-Hexane, methanol, Ethyl Acetate and Aqueous. Extract was filtered through Whatman filter paper (41) to accomplish filtrate. All extracts were concentrated with the help of rotary evaporator. The yield was weighted and stored in a small bottle inside the fridge at (8°C). The percentage yield was deliberated with the help of acknowledged method:

Percentage Yield (%) =  $E/R \times 100$  (E = wt. of Extracted residue, R = wt. of Raw material)

The dried extracts were dissolved in Dimethyl Sulphoxide (DMSO) and taken for antimicrobial evaluation

### Anti-microbial activity

#### Strains of Bacteria and Fungus

The antimicrobial study of various extracts of *Grewia abutilifolia* was assessed against four bacterial and two fungal species, the bacterial species used for the test were *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 11778), and *Staphylococcus aureus* (ATCC 25923). The two fungal strains used were *Penicillium* sp. (MTCC 1995) and *Aspergillus niger* (ATCC 16404). All microbial strains were obtained from Rivpra Formulation Pvt. Ltd.

#### Preparation of Inoculums

For culturing bacterial stains Himedia's Nutrient agar were used and for effective culturing of fungal strains Potato Dextrose Agar by Himedia, India were used. All bacterial species were cultured and inoculated in nutrient broth for the time of 72 hours at 35°C temperature. Fungal inoculums were made by using PDA (Potato dextrose

agar) plate for the period of 96 hours at optimum temperature (37°C) and then fungal spores were collected with the help of sterile cotton swab and conveyed to a sterile plate with fresh Potato dextrose solution (20ml).

### Antibacterial activity of Plant extract (Determination of Zone of Inhibition)

The antimicrobial activity of each plant extract was evaluated by the Disk Diffusion method. The Plant extract was liquefied by Dimethyl sulphoxide (DMSO) and sterilized by Millipore filter having size 0.22 µm and then disinfected paper discs (6mm size) were used to load the desired concentrations 250µg/ml of plant extracts. To achieve the required growth of all bacteria, the streaking method was followed after pouring 25ml of Muller-Hilton agar medium into disinfected dishes. Disinfected paper discs loaded along with plant extracts with various concentrations were positioned on the Agar Plate's top. To permit Plant extract's diffusion, it was incubated at 35°C for 24hrs. Vernier caliper measurements were used as record and were considered as a marker for the activity. All these experiments were done in triplicate (Mostafa, A.A. *et al.*, 2018).

### Anti-fungal activity of plant extracts (Determination of Zone of Inhibition)

The antifungal activity of each plant extract was evaluated by the Disk Diffusion method. The Plant extract were liquified by Dimethyl sulphoxide (DMSO) and sterilized by Millipore filter having size 0.22µm and then used sterile filter paper discs (6mm diameter) to get concentrations 250µg/ml of plant extracts. In Petri dishes, it was repleted with (PDA) Potato dextrose agar and strewed by fungal spores. Filter paper disks of 6 mm size were implanted. The disc, drench concentrations (125µg/ml) of test compounds were investigated by liquifying in dimethyl sulfoxide (DMSO). Zones of inhibition were measured after the time period 96 hrs at temperature 37°C [37°C Temperature need to verify]. Vernier caliper measurement were used as record and considered as marker for the activity. All these experiments were done in triplicate (Bajpai, V.K. *et al.*, 2007).

### Determination of Minimum inhibitory concentrations (MIC's) of effective plant extract

The minimum inhibitory concentration of crude extracts of the *Grewia abutilifolia* was executed by mean of Disk diffusion method. MIC demonstrates least possible concentration of antimicrobial agents that prevents microbial growth within 24hrs and optimum condition of 37 degree Celsius. The plant's extract residues were dissolved in Dimethyl sulphoxide (DMSO) and sterilized by Millipore filter having size 0.22µm and

then sterile filter paper discs of 6mm size were used, to achieve the desired concentrations 1000 µg/ml to 31.5 µg/ml serial dilutions of plant extracts. To achieve the required growth of all bacteria, the streaking method was followed after pouring 25ml of Muller-Hilton agar medium into sterile Petri dishes. Disinfected filter paper discs mounted along with plant extracts with various concentrations were positioned on Agar Plate's Top. Disc mounted with 5µg of Ampicillin was treated as control. To permit Plant extract diffusion, incubate at 35°C for 24hrs. Vernier caliper measurement were used as record and considered as marker for the activity. All these experiments were done in triplicate (Mostafa, A.A. *et al.*, 2018).

### Preliminary Phytochemicals screening

As per various reports, *i.e.* Ali and Hossain, 2015; Weli A.M. *et al.*, 2018 (Ali, S.H.J., Hossain, M.A., 2015; Weli, A.M. *et al.*, 2018) disparate polarity of *Grewia abutilifolia* extracts were appraised their Phytoconstituent for estimation of assorted constituents (Elumalai, E. *et al.*, 2011) Below acknowledge tests were executed on different polarity plant extracts to analyze their presence.

### Alkaloids Observation

Primary characterization was accomplished by Mayer's, Wagner's and Dragendorff's tests. For Mayer's and Wagner's test, 15mg of chloridic extract and 1% HCl (5ml) were prepared, heated for the time period of 5min on a water bath. It was Filtered with the help of filter paper. To the both test tube containing settled filtrate, few drops of Mayer's reagent and Wagner's reagent consecutively were added. Yellowish-white precipitate and Brown precipitate appeared in the Mayer's and Wagner's test, respectively, denotes the presence of alkaloids (Kachkoul, R. *et al.*, 2018). The existence of Dragendroff's test was countered by 2ml acidic solution in another test tube of 10 percent ammonium mixture. Turbidity or precipitate observation indicates the positive Dragendroff's test and presence of Alkaloids (Auwal, M., S. *et al.*, 2014).

### Detection of Saponins

According to research carried out by Weli A.M. *et al.*, 2018 saponin detection was accomplished by taking 5mg of residue diluted with 20ml distilled water and solution was quivered for 20 sec and left for a few minutes at the steady situation. Formation of foam depicts the appearance of saponins, which are known for their foam formation property (Weli, A.M. *et al.*, 2018).

### Detection of Glycosides

As per the published report by Ezeonu, C.S., & Ejikeme, C.M., 2016 Glycoside test was accomplished with 2.00 g from every sample, added 15 ml of 1.0 M Sulphuric acid, after constant heating for period of 5 minutes with the help of water bath. Filtered with Whatman paper, acknowledge test were accomplished with resultant filtrate.

(a) Fehling's solutions (0.2cm<sup>3</sup>), x and y were assorted with filtrate (5 cm<sup>3</sup>) till it became alkalinescent (litmus evaluation). Brick-red colouration on warming depict successful outcome (Ezeonu, C.S., & Ejikeme, C.M. 2016).

(b) In place of aqua, 1.0 M Sulphuric acid (15cm<sup>3</sup>) was acclimate replicate the evaluation and amount of precipitate acquired with x. Excessive precipitate overcome with a positive outcome of glycoside although low outcome reflects the negative outcome (Ezeonu, C.S., & Ejikeme, C.M. (2016).

### Detection of Cardiac Glycosides:

#### Keller-Killiani test (Cardiac glycoside):

5ml distilled water was quivered with 0.5 gm of extract, then few drops of ferric chloride and glacial acetic Acid (2ml) were added. Further, H<sub>2</sub>SO<sub>4</sub> (1ml) was added along the wall of the test tube, the brown band depicted presence of cardiac glycosides (Iqbal, E. *et al.*, 2015).

### Detection of terpenoids:

**Salkowski test:** 100mg crude concentrate was individually quivered with 2ml chloroform, followed by addition of 2ml concentrated H<sub>2</sub>SO<sub>4</sub> along the test tube sides. Reddish Brown coloration depicts successful outcome of terpenoids (Iqbal, E. *et al.*, 2015).

### Test for reducing sugars:

**Fehling test:** 1ml of ethanol adjoined with 10mg of extract, afterwards 2ml distilled water was add, further 4 drops of (10%w/v) aqueous ferric chloride were added. Blue green colour depicts positive outcome for reducing sugar (Ismail, A.M. *et al.*, 2016).

### Test for carbohydrates:

**Molisch test:** 2-3 ml of extract was taken, then two drops of Alpha naphthol mixture in Ethanol, were added and followed by the addition of few drops of Conc H<sub>2</sub>SO<sub>4</sub> at the edges of the test tube. Violet ring showed successful outcome (Ismail, A.M. *et al.*, 2016).

### Tannin's Identification:

10 mg of every concentrate was combined in 1mL of ethanol, then 2 mL of distilled water was added, then 4 drops of ferric chloride aqueous solution (10% w/v) were added. The appearance of a blue color depicted the

presence of polyphenols.

5ml of 45% methanol was boiled with 2gms of extracts. The blend was refrigerated and filtered. The filtrate was subjected to the acknowledged tests:

(1) Lead sub-acetate test: three drops of the lead sub-acetate solution were mixed to 1 ml of the filtrate solvent. The appearance of tannins was observed when a cream gelatinous precipitate formed.

#### Detection for Phenols:

**Ellagic Acid Test:** Two drops of 5% w/v Gallic Acid and 5% w/v NaNO<sub>2</sub> mixture was combined with test. Validation of phenols accomplished by the absence of muddy or Niger Gray, otherwise vice versa.

## Results

### Plant extraction yield

The percentage yield of various extracts was demonstrated in table 1. The concentrate of 50g of shrivel plant material with various solvent capitulated concentrate stretch from 1.56 to 9.43% of range. The highest capitulate of plant extract, appeared in water (6.99%) followed by methanol (7.58%) and Ethyl acetate (1.93%). Although n-Hexane (0.71%) establishes the lowest extract yield, to cold extraction percentage yield for water (5.83%) followed by methanol (5.67%), n-hexane (0.69%) and ethyl acetate (1.83%), respectively.

### Antibacterial activity of plant extracts

The 6 plant extracts were investigated to assess their antimicrobial activity against four bacterial and two fungal species. Out of four bacterial species two were gram-positive (*B.subtilis* and *S.aureus*) and two were gram-

**Table 1:** Percentage yield of various extracts of leaves of *Grewiaabutillifolia*.

Plant Extract	Percentage Yield(w/w)
HEX	0.71%
MET	7.58%
ETA	1.93%
AQA	6.99%
cHEX	0.69%
cMET	5.67%
cETA	1.88%
cAQA	5.83%

\*\* HEX- Hexane Extract(Hot Extraction), MET-Methanol Extract(Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction), AQA- Aqueous Extract( Hot Extraction), cHEX- Hexane Extract(Cold Extraction), cMET-Methanol Extract(Cold Extraction), cETA- Ethyl Acetate Extract (Cold Extraction), cAQA- Aqueous Extract( Cold Extraction).

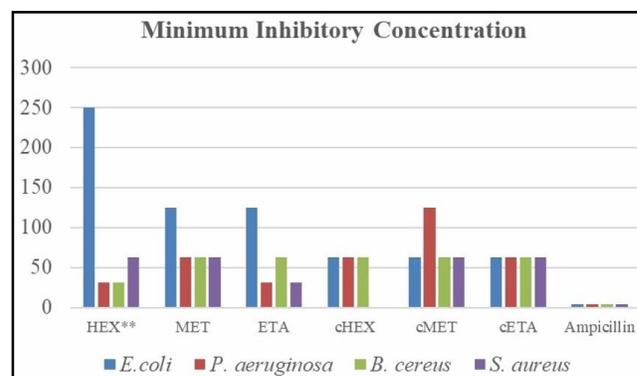
negative bacteria (*E.coli* and *P.aeruginosa*). The result showing antibacterial activity depicted in table 2, asserted that each extract with varying concentrations displayed equivalent antibacterial activity to standards. Methanol, Ethyl acetate and n-Hexane have evident finer activity than the standard counter four microorganisms. The aqueous extract was additional effectual counter to *E. coli*. Ethyl acetate extract was found to be almost equally effective against the all four bacterial microorganisms. N-Hexane was effectual against *B.subtilis*, *P.aeruginosa* and *E.coli*. Almost similar antibacterial activities were shown against cold macerated extracts. The presence of Phytochemicals was shown in table 3. Different extracts did not manifest significant antifungal activity.

The results of the antibacterial activity of extracts revealed that all strains were found to be susceptible to the methanol Extract, ethyl acetate and n-Hexane extracts. However, water extract was found to be active against the *E. coli*. All results collectively suggest that methanol, n-hexane and Ethyl acetate have the most effective results and manifest as an antibacterial agent.

Experiments were performed to determine their Minimum inhibitory concentration of extracts against all 4 susceptible bacterial stains.

### Minimum inhibitory concentration (MIC's) of the effective plant extracts

The minimum inhibitory concentration of the most effective extracts was employed by the Disk Diffusion method to assess their bactericidal and bacteriostatic properties. The upshot depending on concentration of Plant extract were acknowledged in table 4 and embellished with Fig. 1. The reticence effect of methanolic extract (Cold extraction) was found to be 62.5µg/ml counter *E. coli*, 62.5µg/ml counter *S.aureus*, 62.5µg/ml



**Fig 1:** Minimum Inhibitory Concentration of various extracts of leaves of *Grewiaabutillifolia* against Microorganism

\*\*HEX- Hexane Extract (Hot Extraction), MET-Methanol Extract (Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction), cHEX- Hexane Extract (Cold Extraction), cMET-Methanol Extract (Cold Extraction), cETA- Ethyl Acetate Extract (Cold Extraction).

**Table 2:** Zone of Inhibition of various extracts of leaves of *Grewiaabutilifolia* against Microorganism.

Micro organism	Concentration (µg/ml)	HEX	MET	ETA	AQA	cHEX	cMET	cETA	cAQA
<i>E. coli</i>	1000	17±0.1	13±0.2	15±0.1	11±0.1	13±0.2	15±0.1	13±0.1	11±0.4
	500	12±0.25	11±0.1	12±0.1	10±0.2	12±0.1	12±0.3	12±0.2	9±1.0
	250	7±0.2	9±0.25	9±0.2	8±0.2	10±0.2	10±0.1	10±0.3	7±0.2
	125	0	7±0.2	7±0.1	7±0.3	8±0.3	9±0.21	8±0.1	6±0.7
	62.5	0	6±0.2	6±0.3	6±0.4	7±0.1	7±0.2	7±0.3	0
	31.25	0	0	0	0	0	0	0	0
<i>P. aeruginosa</i>	1000	14±0.13	12±0.2	16±0.6	0	11±0.1	13±0.2	15±0.2	0
	500	13±0.1	11±0.13	15±0.3	0	10±0.01	11±0.4	13±0.1	0
	250	12±0.4	9±0.2	13±0.1	0	9±0.02	9±0.1	11±0.2	0
	125	9±0.2	8±0.32	11±0.3	0	8±0.1	7±0.1	9±0.1	0
	62.5	8±0.8	7±0.14	9±0.3	0	7±0.2	0	7±0.4	0
	31.25	7±0.1	0	7±0.2	0	0	0	0	0
<i>B. cereus</i>	1000	15±0.3	14±0.16	12±0.1	0	14±0.25	12±0.4	14±0.3	0
	500	13±0.1	13±0.1	11±0.2	0	12±0.1	11±0.12	12±0.1	0
	250	11±0.3	10±0.2	9±0.2	0	10±0.2	10±0.1	11±0.2	0
	125	10±0.2	8±0.15	8±0.1	0	8±0.2	8±0.2	9±0.2	0
	62.5	9±0.25	7±0.1	7±0.2	0	7±0.1	7±0.1	7±0.2	0
	31.25	7±0.5	0	0	0	0	0	0	0
<i>S. aureus</i>	1000	12±0.2	13±0.1	15±0.1	0	0	13±0.8	14±0.2	0
	500	10±0.1	11±0.0	13±0.2	0	0	12±0.1	13±0.4	0
	250	9±0.3	10±0.1	12±0.3	0	0	10±0.2	10±0.3	0
	125	8±0.2	8±0.2	10±0.2	0	0	8±0.3	8±0.1	0
	62.5	7±0.25	7±0.0	8±0.1	0	0	7±0.5	7±0.1	0
	31.25	0	0	7±0.4	0	0	0	0	0
<i>Panicillium frequentans</i>	1000	0	0	0	0	0	10±0.1	0	0
	500	0	0	0	0	0	9±0.3	0	0
	250	0	0	0	0	0	8±0.5	0	0
	125	0	0	0	0	0	7±0.1	0	0
	62.5	0	0	0	0	0	6±0.2	0	0
	31.25	0	0	0	0	0	0	0	0
<i>Aspergillus niger</i>	1000	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0	0
	125	0	0	0	0	0	0	0	0
	62.5	0	0	0	0	0	0	0	0
	31.25	0	0	0	0	0	0	0	0

\*\*HEX- Hexane Extract(Hot Extraction), MET-Methanol Extract(Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction), AQA- Aqueous Extract( Hot Extraction), cHEX- Hexane Extract(Cold Extraction), cMET-Methanol Extract(Cold Extraction), cETA- Ethyl Acetate Extract (Cold Extraction), cAQA- Aqueous Extract( Cold Extraction).

for *Paeruginosa* and 125µg/ml for bacillus species, While the inhibitory effect of Ethyl acetate extract(cold extraction) was found to be 62.5µg/ml counter *E.coli*, 62.5µg/ml counter *S.aureus*, 62.5µg/ml for *Paeruginosa* and 62.5µg/ml for *B.cerus* species. Ethyl acetate extract's (Hot extraction) inhibitory effect was found to be 62.5µg/ml for *E. coli*, 31.25µg/ml for *S.aureus*

and 62.5µg/ml for *Paeruginosa* and 125µg/ml for Bacillus species. N-Hexane extract (Hot extraction) show inhibitory effect at 250µg/ml counter *E. coli*, 62.5µg/ml counter *S.aureus*, 31.25µg/ml for *Paeruginosa* and *B.cerus* species. While methanol (Hot Extract) shows an inhibitory effect on 125 µg/ml counter *E.coli*, 62.5 µg/ml counter *P. aeruginosa*, *B. cerus* and *S. aureus*. N-

Hexane Extract (Cold Extraction) showed an inhibitory effect at 62.5 µg/ml against *E. coli*, *P. aeruginosa* and *B. cereus*, while 125 µg/ml for *S. aureus*. For Aqueous Extracts (Cold and hot Extraction) MIC was found to be 250 µg/ml and 125 µg/ml respectively. Penicillin was used as standard and has 4 µg/ml MIC. Variation in the results of various extracts was possibly due to variation in the chemical constituent present within it. Terpenoids, Phenols and Alkaloids are various chemical constituents responsible for effective antimicrobials. The Phytoconstituents generally interact with proteins of the microbial cell wall and cause its turmoil to disseminate a flux of protons towards cell exterior which induces cell death or inhibit enzymes obligatory for amino acid Biosynthesis and also inhibit Efflux pump (Khameneh, B. *et al.*, 2019). As per Lebecque, S. *et al.*, 2019 the hydrophobic nature of some plant extracts enables them to act with proteins of the microbial cell membrane and mitochondrial turmoil their permeability and structure

(Lebecque, S. *et al.*, 2019) This study leads to a conclusion that all extracts of plants having antibacterial activity could be used for some preservative use and Phytomedicine uses or pharmaceutical preparation formation.

## Discussion

The medicinal value of plants lies with various chemical constituent's presence. Phytoconstituents present in the plant extract attribute for their medicinal value. In the current study, some of the plant extracts has potential anti-Bacterial activity against some pathogenic bacteria. The study helps to conclude more options for treatment of pathogenic bacterial species. As bacterial resistance is the most common problem in front of researchers, so extracts can be tried against the drug resistance strains of bacteria. Plants based safer antimicrobial agents could be proved as an alternative of synthetic compounds with less incidence of adverse reaction and associated side effects.

**Table 3:** Phytochemicals present in different extracts of leaves of *Grewiaabutifolia*.

Phytoconstituents	HEX	MET	ETA	AQA	cHEX	cMET	cETA	cAQA
Alkaloids	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Carbohydrates	+	-	+	+	+	-	+	+
Saponins	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+
Sterols	+	+	+	-	+	+	+	-
Phenols	-	+	+	+	-	+	+	+

\*\* HEX- Hexane Extract(Hot Extraction), MET-Methanol Extract(Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction), AQA-Aqueous Extract( Hot Extraction), cHEX- Hexane Extract(Cold Extraction), cMET-Methanol Extract(Cold Extraction), cETA- Ethyl Acetate Extract (Cold Extraction), cAQA- Aqueous Extract( Cold Extraction).

**Table 4:** Minimum Inhibitory Concentration of various extracts of leaves of *Grewiaabutifolia* against Microorganism.

Microorganism	HEX**	MET	ETA	cHEX	cMET	cETA	Ampicillin
<i>E. coli</i>	250*	125	125	62.5	62.5	62.5	4
<i>P. aeruginosa</i>	31.25	62.5	31.25	62.5	125	62.5	4
<i>B. cereus</i>	31.25	62.5	62.5	62.5	62.5	62.5	4
<i>S. aureus</i>	62.5	62.5	31.25	-	62.5	62.5	4

\*All values in µg/ml unit.

\*\*HEX- Hexane Extract(Hot Extraction), MET-Methanol Extract(Hot Extraction), ETA- Ethyl Acetate Extract (Hot Extraction), cHEX- Hexane Extract(Cold Extraction), cMET-Methanol Extract(Cold Extraction), cETA- Ethyl Acetate Extract (Cold Extraction).

## Acknowledgment

The authors are grateful to Dr. Ashok K. Chauhan, Founder President, Ritnand Balved Education Foundation (RBEF) and Amity Group of Institutions and to Dr. Atul Chauhan, Chancellor, Amity University Uttar Pradesh (AUUP) for facilitating this work. Also, we acknowledge our sincere gratitude to all those from AIPP, AUUP and Rivpra Formulations Pvt. Ltd., Haridwar Uttarakhand who extended their kind help and support.

## Funding

This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

- Ali, S.H.J. and M.A. Hossain (2015). Phytochemical studies of various polarities leave crude extracts of *Omani Datura metel* L. and evaluation of their antimicrobial potential. *J. Coastal Life Med.*, **3(3)**: 214–219.
- Auwal, M., S., Saka, I.A. Mairiga, K.A. Sanda, A. Shuaibu and A. Ibrahim (2014). Preliminary phytochemical and elemental analysis of

- aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*, **5(2)**: 95–100.
- Bajpai, V.K., A. Rahman and S.C. Kang (2007). Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu. *Industrial Crops and Products*, **26(1)**: 28–35. doi:10.1016/j.indcrop.2006.12.012.
- Elumalai, E., M. Ramachandran, T. Thirumalai and P. Vinothkumar (2011). Antibacterial activity of various leaf extracts of *Merremia emarginata*. *Asian Pacific Journal of Tropical Biomedicine*, **1(5)**: 406–408. doi:10.1016/s2221-1691(11)60089-0.
- Ezeonu, C.S. and C.M. Ejikeme (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science*, 2016, 1–9. doi:10.1155/2016/5601327.
- Fair, R.J. and Y. Tor (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, **6**: PMC.S14459. doi:10.4137/pmc.s14459.
- Iqbal, E., K.A. Salim and L.B.L. Lim (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University - Science*, **27(3)**: 224–232. doi:10.1016/j.jksus.2015.02.003.
- Ismail, A.M., E.A. Mohamed, M.R. Marghany, F.F. Abdel-Motaal, I.B. Abdel-Farid and M.A. El-Sayed (2016). Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts. *Journal of the Saudi Society of Agricultural Sciences*, **15(2)**: 112–117. doi:10.1016/j.jssas.2014.06.002.
- Khameneh, B., M. Iranshahy, V. Soheili and B.S. Fazly Bazzaz (2019). Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrobial Resistance & Infection Control*, **8(1)**: doi:10.1186/s13756-019-0559-6.
- Kachkoul, R., T. Sqalli Houssaini, R. El Habbani, Y. Miyah, M. Mohim and A. Lahrichi (2018). Phytochemical screening and inhibitory activity of oxalocalcic crystallization of *Arbutus unedo* L. leaves. *Heliyon*, **4(12)**: e01011. doi:10.1016/j.heliyon.2018.e01011.
- Khasim, S.M., C. Long, K. Thammasiri and H. Lutken (Eds.). (2020). Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation. doi:10.1007/978-981-15-1636-8.
- Lebecque, S., L. Lins, F.E. Dayan, M.L. Fauconnier and M. Deleu (2019). Interactions Between Natural Herbicides and Lipid Bilayers Mimicking the Plant Plasma Membrane. *Frontiers in Plant Science*, **10**: doi:10.3389/fpls.2019.00329.
- Mall, T.P. and S.C. Tripathi (2018). Anti-Cancer Potential Plants from Bahraich (Uttar Pradesh) India. *World Journal of Pharmaceutical Research*, **7(2)**: 844–894.
- Mall, T.P. and S.C. Tripathi (2018). Anti-Cancer Potential Plants from Bahraich (Uttar Pradesh) India. *World Journal of Pharmaceutical Research*, **7(2)**: 844–894.
- Quattrocchi Umberto (2012). Medicinal and poisonous plants. CRC Press Taylor and Francis Group, 1886.
- Reta, A., A. Bitew Kifilie and A. Mengist (2019). Bacterial Infections and Their Antibiotic Resistance Pattern in Ethiopia: A Systematic Review. *Advances in Preventive Medicine*, 2019, 1–10. doi:10.1155/2019/4380309.
- Weli, A.M., S. Al-Salmi, H. Al Hoqani and M.A. Hossain (2018). Biological and phytochemical studies of different leaves extracts of *Pteropyrum scoparium*. Beni-Suef University *Journal of Basic and Applied Sciences*, doi:10.1016/j.bjbas.2018.05.00.