The Pharmaceutical and Chemical Journal, 2022, 9(5):11-19

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Study of antimicrobial activity from plant extracts of *Grewia abutilifolia*

Hitesh Kothari, Pushpendra Singh Naruka

Faculty of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India 313001
Email: mail.hiteshkothari@gmail.com

Abstract *Grewia abutilifolia* is a threatened medicinal plant having immense unexplored therapeutic activities. Four different types of extracts (Ethanolic extract, Petroleum ether soluble fraction, Chloroform soluble fraction and aqueous soluble fraction) of *Grewia abutilifolia* plant were prepared and evaluated for Preliminary phytochemical constituents. All the extracts were screened for antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis*, *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis*, *Trichophyton rubrum, Aspergillus flavus and Aspergillus niger*. Ethanolic extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Enterococcus faecalis* pathogen whereas petroleum extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa and Salmonella enteritidis.*

Keywords *Grewia abutilifolia,* antimicrobial activity, antifungal, antibacterial activity

Introduction

Microbial strains that are multi-drug resistant and those that have decreased sensitivity to antibiotics are both on the rise [1-6]. For several kinds of antibiotics, the development of bacterial resistance has turned into a very important clinical issue [7-10]. The mortality, morbidity, and expense of protracted treatments all significantly rise when infections are brought on by bacteria that are resistant to medication [11-16]. The indiscriminate use of broad-spectrum antibiotics, immunosuppressive drugs, intravenous catheters, organ transplantation, and continuous HIV infection outbreaks have all been linked to this rise [17-23]. Additionally, synthetic medications are frequently adulterated and have negative effects in underdeveloped nations in addition to being expensive and ineffective for the treatment of ailments [24-28]. Therefore, in order to manage microbial infections, it is necessary to look for novel infection-fighting techniques [29-31].

Due to its different climatic circumstances, India has long been known for its abundant variety of medicinal plants [32-33]. The Tiliaceae family plant *Grewia abutilifolia* is well-known for its many therapeutic benefits. Seasonal blooming and fruiting are characteristics of *Grewia abutilifolia*, which is found in wet deciduous forests in central and southern India [34-36]. Its many benefits, such as a cooling agent, refreshing drink, anti-inflammatory, anti-rheumatic, demulcent, and anti-diabetic, are revealed by ethnobotany investigations. The traditional uses of a plant genus as a fruit and a cooling beverage are being replaced with its expanded therapeutic capabilities over time. One of the vulnerable species listed by the Indian Biodiversity Organization is *Grewia abutilifolia* [34-40].

The aim of this study was to evaluate the antimicrobial activity of extracts of Grewia abutilifolia against microbial pathogens: *Bacillus cereus, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Trichophyton rubrum, Aspergillus flavus and Aspergillus niger*.

Experimental Work

Preliminary phytochemical evaluation of plant materials

The plant *Grewia abutilifolia* was collected and cleaned to remove dust using tap water then dried in shade for a few weeks. The dried plant was then mechanically ground to fine powder. Four different types of extracts (Ethanolic extract, Petroleum ether soluble fraction, Chloroform soluble fraction and aqueous soluble fraction) were prepared. These four extracts were screened for the presence of phytochemical constituents such as carbohydrates, tannins, flavonoids, saponins, cardiac glycosides, alkaloids etc. and phytochemical constituents have been detected qualitatively by the characteristic colour changes and precipitation occurring upon treatment with specific reagents as mentioned below:

Test for Carbohydrate

- Molisch test- 2ml of extract was subjected to Molisch's reagent in a test tube. Observed that violet ring was formed.
- Fehling's test- 1ml of extract was treated with Fehling A and Fehling B solutions. The mixture was heated for 5 minutes observed that red precipitate was formed.
- Benedict's test- To 1ml of extract and 1ml of Benedict's reagent was added in a test tube. This mixture was heated at water bath for 7 minutes observed that red colour was formed.
- Barfoed's test- 2ml of extract was added to 1ml of Barfoed's reagent, observed that a red precipitate was formed.

Test for Protein and Amino Acids

- Biuret's test- 1ml of extract was mixed with 1ml of 10% sodium hydroxide solution in a test tube and heated. Further 0.7% copper sulphate solution was added to above solution. Observed that formation of violet colour, that indicated presence of protein.
- Millon's test-3ml of extract was treated with 5ml of Milion's reagent. A white precipitate was formed. On heating the solution, it was turned to brick red colour that indicated presence of protein.
- Ninhydrin test- 2ml of extract was treated with 4-5 drops of 5% ninhydrin solution and heated. Observed that formation of blue colour. Concluded the presence of amino acid.

Test for Glycoside

- Borntrager's test- Dilute sulphuric acid was added to 3ml of extract, boiled for 5-6 minutes and filtered. Filtrate was cooled and 3ml of benzene was added and stirred the solution. Few ml of dilute ammonia solution was added. Observed that a pink to red coloured layer was formed indicated the presence of anthraquinone glycosides.
- Keller-Kiliani test- 2ml of extract was mixed with 3ml of glacial acetic acid and a drop of 5% ferric chloride in a test tube, followed by addition of 2-5 drops of concentrated sulphuric acid at the side of test tube, observed that blue colour was formed in acetic layers. Result that cardiac glycoside is present.

Test for Saponin

- Froth test- 1ml of extract diluted with distilled water and shake regularly in graduated cylinder for 20 minutes. No change observed in this extract. Result that forth tests is negative.

Test for Alkaloids

- Mayer's test- Few drops of Mayer's reagent were added to 2ml of extract. A white creamy precipitate formed.
- Dragendroff's test- Few drops of Dragendorff's reagent were added to 2ml of extract added in a test tube. Red precipitate formed.
- Hager's test- To 3ml of extract, few drops of Hager reagent were added in a test tube. Yellow precipitate was formed. Result that alkaloid is present.
- Wagner's test- Few drops of Wagner's reagent were added to 2ml of extract in a test tube. A reddish-brown precipitate was formed. Result that alkaloid is present.

Test for Flavonoids

- Shinoda's test- 1 ml of extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were added to the filtrate followed by introducing few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.
- Alkaline reagent test- 2 ml of extract was treated with few drops of sodium hydroxide (NaOH) in test tube yellow colour is formed. In this mixture few drops of Conc. H_2SO_4 were added if the mixture becomes colourless. Results indicated that flavonoids are present.

Test for Triterpenoid and terpenoids

- Salkowski's test- 2ml of extract was treated with chloroform and filtered. To this filtrate, few drops of Conc. H_2SO_4 were added by shaking and allow to stand for few minutes. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids. In bottom yellow colour resulted that Triterpenoid are present.
- Liebermann-Burchard Test–2ml of extract was treated with chloroform and filtered. To this filtrate, few drops of acetic anhydride were added, boiled and cooled. Few drops of concentrated sulphuric acid were added through side of test tube. Observe that brown ring at junction of two layers was turned green indicating that a steroid is present. Another layer red colour showed resulted that Triterpenoid are present.

Test for Tannins and phenolic compounds

- Ferric chloride test- 2ml of extract was dissolved in distilled water. To this solution, 2ml of 5% ferric chloride was added. Violet colour was observed. Results revealed that phenolic compound is present.
- Lead acetate test- 2ml of extract was dissolved in distilled water. Then few drop of lead acetate solution were added. White precipitate was observed. Result that phenolic compound is present.
- Dilute iodine test- To 2ml of extract, dilute iodine solution was added. Red colour was observed. Result that phenolic compound is present.

Test for Coumarins: 1ml of extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Quinones: 1ml of the extract was treated with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for Anthocyanins: 2 mL of the extract was treated with 2 ml of 2N HCl. The appearance of a pink-red color that turns purplish blue after addition of ammonia indicates the presence anthocyanins [41-50].

Extraction of *Capparis decidua*

Four different types of extracts were prepared using dried fine powder of *Grewia abutilifolia* and different solvents viz: ethanol, petroleum ether, chloroform and water so as to get 250µg/ml.

***In vitro* Anti-microbial activity**

Strains of Bacteria and Fungus

The antimicrobial study of various extracts of *Grewia abutilifolia* was assessed against three Gram positive, three Gram negative and three fungal species. The Gram-positive bacteria species used for the test were *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 23521). The Gram-negative bacterial species used for the test were *Escherichia coli* (ATCC 25762), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella enteritidis* (ATCC 24579). The fungal strains used were *Trichophyton rubrum* (MTCC 1889), *Aspergillus flavus* (ATCC 27541) *and Aspergillus niger* (ATCC 16412).

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 was used. All bacterial species were cultured and inoculated in nutrient broth for

the time of 72 hours at 35ºC temperature. Fungal inoculums were made by using PDA (Potato dextrose agar) plate for the period of 96 hours at optimum temperature (37ºC) 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 extracts

The antimicrobial activity of each plant extracts 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 35ºC 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.

Anti-fungal activity of plant extracts

The antifungal activity of each plant extract was evaluated by the Disk Diffusion method. The Plant extracts were liquified by Dimethylsulphoxide (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 48 hrs at temperature 24ºC. Vernier calliper measurement were used as record and considered as marker for the activity. All these experiments were done in triplicate [51-52].

Results and Discussion

All the four extracts were evaluated for the presence of the phytochemical constituents. The results showed that EEGA and AEGA contains Saponins, Tannins, Flavonoids, Terpenoids, Steroids, Phlobatannins, Carbohydrates, Coumarines, Alkaloids, Proteins and Emodins whereas PEGA and CFGA contains Saponins, Tannins, Flavonoids, Steroids, Coumarines, Alkaloids, Proteins and Emodins as shown in Table 1.

Table 1: Phytochemical test results of different extracts of *Grewia abutilifolia*

Phytochemical tests	Ethanol Extract fraction	Petroleum ether soluble fraction	Chloroform soluble fraction	Aqueous soluble fraction
Saponins	++	-	++	+
Tannins	+++	+	+	++
Flavonoids	+	+	++	+
Terpenoids	+	-	-	+
Steroids	+++	+++	+++	++
Phlobatannins	++	-	-	++
Carbohydrates	+	-	-	-
Coumarines	+++	-	+	++
Alkaloids	+++	++	-	+
Proteins	+	+	-	-
Emodins	+	-	+++	++
Anthraquinones	-	-	-	-
Anthocyanins	-	-	-	-

- = Absent, + = Mildly present, ++ = Moderately present, +++ = Highly present

Anti-microbial activity

The antimicrobial study was measured as in terms of Zone of Inhibition of various extracts of *Grewia abutilifolia* was assessed against three Gram positive, three Gram negative and three fungal species. The Gram-positive bacterial species used for the test were *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923) and

Enterococcus faecalis (ATCC 23521). The Gram-negative bacterial species used for the test were *Escherichia coli* (ATCC 25762), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella enteritidis* (ATCC 24579). The fungal strains used were *Trichophyton rubrum* (MTCC 1889), *Aspergillus flavus* (ATCC 27541) and *Aspergillus niger* (ATCC 16412) by Disk Diffusion method.

Ethanolic extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Enterococcus faecalis* pathogen. Petroleum extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa and Salmonella enteritidis.*

Chloroform soluble fraction of *Grewia abutilifolia* showed potent antifungal activity against *Aspergillus flavus* pathogen. All the four extracts of *Grewia abutilifolia* showed mild to moderate antifungal activity against the fungal strains used for the study.

Table 2: Antibacterial activity of different extracts of *Grewia abutilifolia*

	Diameter of inhibition zone (mm) (Mean ± S.D)					
Bacterial Species	**Ethanol extract**	**Petroleum ether soluble fraction**	**Chloroform soluble fraction**	**Aqueous soluble fraction**	**Gentamycin**	**DMSO**
Bacillus cereus (ATCC 11778)	10.8±0.45	12.7±0.49	12.0±0.56	9.2±0.63	13.8±0.78	NI
Staphylococcus aureus (ATCC 25923)	9.3±0.14	22.3±1.23	14.1±0.45	13.6±0.56	25.7±0.45	NI
Enterococcus faecalis (ATCC 23521)	14.0±0.76	12.8±0.69	10.9±0.66	12.9±0.44	14.7±0.34	NI
Escherichia coli (ATCC 25762)	13.9±0.97	20.3±1.09	12.8±	14.3±	20.3±1.33	NI
Pseudomonas aeruginosa (ATCC 27853)	16.7±0.76	22.5±1.45	20.0±1.21	15.8±	22.8±1.34	NI
Salmonella enteritidis(ATCC 24579)	11.7±0.45	15.3±0.33	10.8±0.65	13.7±0.57	15.5±0.64	NI

NI=No Inhibition, DMSO=Dimethylsulphoxide

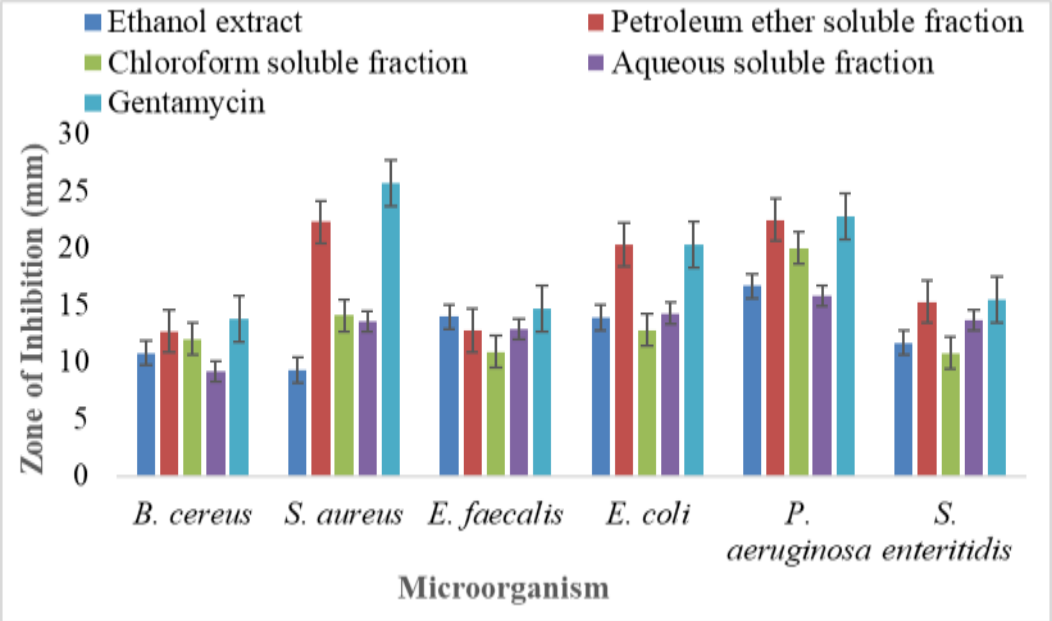

Figure 1: Graphical representation of Antibacterial activity of different extracts of Grewia abutilifolia

Table 3: Antifungal activity of different extracts of *Grewia abutilifolia*

	Diameter of inhibition zone (mm) (Mean ± S.D)					
Fungal Species	**Ethanol extract**	**Petroleum ether soluble fraction**	**Chloroform soluble fraction**	**Aqueous soluble fraction**	**Fluconazole**	**DMSO**
Trichophyton rubrum (MTCC 1889)	10.3±0.35	11.6±0.67	13.1 ± 0.34	9.83±0.84	15.9 ± 0.45	NI
Aspergillus flavus (ATCC 27541)	9.7 ±0.29	16.3±0.34	21.7±0.44	17.7±0.76	19.7±0.48	NI
Aspergillus niger (ATCC 16412)	10.4±0.75	12.9±0.86	12.3±0.57	11.5±0.34	14.3±0.56	NI

NI=No Inhibition, DMSO=Dimethylsulphoxide

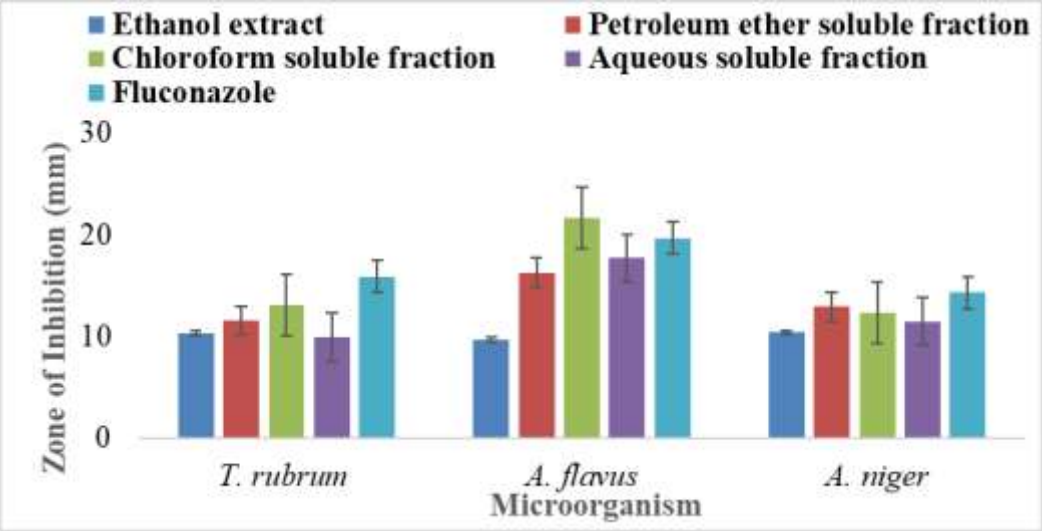

Figure 3: Graphical representation of Antifungal activity of different extracts of Grewia abutilifolia

Conclusion

All the four extracts i.e. ethanol extract fraction, petroleum ether soluble fraction, chloroform soluble fraction and aqueous soluble fraction of *Grewia abutilifolia* were evaluated for the antimicrobial activity against three Gram positive, three Gram negative and three fungal species. The Gram-positive bacterial species used for the test were *Bacillus cereus*), *Staphylococcus aureus* and *Enterococcus faecalis*. The Gram-negative bacterial species used for the test were *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. The fungal strains used were *Trichophyton rubrum*, *Aspergillus flavus* and *Aspergillus niger*.

Ethanolic extract of *Capparis decidua* showed potent antimicrobial activity against *Bacillus cereus* whereas significant activity was observed against *Enterococcus faecalis, Escherichia coli and Salmonella enteritidis* pathogens. Petroleum extract of *Capparis decidua* was found to potent antimicrobial activity against *Escherichia coli and Salmonella enteritidis.* All the four extracts of *Capparis decidua* showed mild to moderate antifungal activity against the fungal strains used for the study.

Ethanolic extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Enterococcus faecalis* pathogen whereas petroleum extract of *Grewia abutilifolia* showed potent antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa and Salmonella enteritidis.* All the four extracts of *Grewia abutilifolia* showed mild to moderate antifungal activity against the fungal strains used for the study.

References

[1]. Meade, E., Slattery, M. A., & Garvey, M. (2020). Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: resistance is futile?. *Antibiotics*, *9*(1), 32.

[2]. Morris, S., & Cerceo, E. (2020). Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized setting. *Antibiotics*, *9*(4), 196.

[3]. Dahiya, P., & Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian journal of pharmaceutical sciences*, *74*(5), 443.

[4]. Lin, Y., Li, W., Sun, L., Lin, Z., Jiang, Y., Ling, Y., & Lin, X. (2019). Comparative metabolomics shows the metabolic profiles fluctuate in multi-drug resistant Escherichia coli strains. *Journal of proteomics*, *207*, 103468.

[5]. Aleksic, V., Mimica-Dukic, N., Simin, N., Nedeljkovic, N. S., & Knezevic, P. (2014). Synergistic effect of Myrtus communis L. essential oils and conventional antibiotics against multi-drug resistant Acinetobacter baumannii wound isolates. *Phytomedicine*, *21*(12), 1666-1674.

[6]. Karim, A.M., Maha, A., Khalid, M.S. (2017). GC-MS analysis and Antimicrobial Activity of Sudanese *Jatropha curcas* L. (Eupharbiaceae) Fixed Oil. *The Pharmaceutical and Chemical Journal*, *4*(6):114-120.

[7]. Kumar, N., & Chauhan, L. S. (2015). Antimicrobial potential of hydrazide-hydrazone derivatives: a review. *Int J Pharm Clini Res*, *7*(2), 154-161.

[8]. Kumar, N., Chauhan, L. S., Dashora, N., & Sharma, C. S. (2014). Anticonvulant potential of Hydrazone derivatives: A Review. *Sch. Acad. J. Pharm*, *3*(5), 366-373.

[9]. Alanis, A. J. (2005). Resistance to antibiotics: are we in the post-antibiotic era?. *Archives of medical research*, *36*(6), 697-705.

[10]. Andersson, D. I. (2003). Persistence of antibiotic resistant bacteria. *Current opinion in microbiology*, *6*(5), 452-456.

[11]. Obritsch, M. D., Fish, D. N., MacLaren, R., & Jung, R. (2005). Nosocomial infections due to multidrug-resistant Pseudomonas aeruginosa: epidemiology and treatment options. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *25*(10), 1353-1364.

[12]. Kazanowski, M., Smolarek, S., Kinnarney, F., & Grzebieniak, Z. (2014). Clostridium difficile: epidemiology, diagnostic and therapeutic possibilities—a systematic review. *Techniques in coloproctology*, *18*(3), 223-232.

[13]. Burrowes, B., Harper, D. R., Anderson, J., McConville, M., & Enright, M. C. (2011). Bacteriophage therapy: potential uses in the control of antibiotic-resistant pathogens. *Expert review of anti-infective therapy*, *9*(9), 775-785.

[14]. Holmberg, S. D., Solomon, S. L., & Blake, P. A. (1987). Health and economic impacts of antimicrobial resistance. *Reviews of infectious diseases*, *9*(6), 1065-1078.

[15]. Angulo, F. J., & Mølbak, K. (2005). Human health consequences of antimicrobial drug—resistant salmonella and other foodborne pathogens. *Clinical infectious diseases*, *41*(11), 1613-1620.

[16]. Klastersky, J. (1998). Science and pragmatism in the treatment and prevention of neutropenic infection. *The Journal of antimicrobial chemotherapy*, *41*(suppl_4), 13-24.

[17]. Cespedes, L. D., Fonseca, C. M. Y., & Sanchez, R. V. Y. (2022). Rhodotorula an emergent pathogen. *J Exp Clin Microbiol Vol*, *6*(3).

[18]. Leather, H. L., & Wingard, J. R. (2001). Infections following hematopoietic stem cell transplantation. *Infectious disease clinics of North America*, *15*(2), 483-520.

[19]. Nitha, B., Remashree, A. B., & Balachandran, I. (2012). Antibacterial activity of some selected Indian medicinal plants. *International Journal of Pharmaceutical Sciences and Research*, *3*(7), 2038.

[20]. Chernak, E. D., & DiNubile, M. J. (1994). Infections in the Pharmacologically Immunosuppressed Host. *Acute Rheumatic and Immunologic Disease: Management of the Critically Ill Patient*, 27.

[21]. Banach, D. B., Seville, M. T. A., & Kusne, S. (2016). Infection Prevention and Control Issues After Solid Organ Transplantation. In *Transplant Infections* (pp. 843-867). Springer, Cham.

[22]. Wahab, S., Muzammil, K., Nasir, N., ul Hasan, A., Siddiqui, Z., Aggarwal, P., & Nasir, S. (2021). Agents of mucormycosis outbreak associated with COVID-19.

[23]. Rao, M. L., & Savithramma, N. (2012). Antimicrobial activity of Dasamoola–an ayurvedic drug. *World Journal of Pharmaceutical Research*, *1*(3), 803-12.

[24]. Cordell, G. A., & Colvard, M. D. (2012). Natural products and traditional medicine: turning on a paradigm. *Journal of natural products*, *75*(3), 514-525.

[25]. Sheldon, J. W., Balick, M. J., Laird, S. A., & Milne, G. M. (1997). Medicinal plants: can utilization and conservation coexist?. *Advances in economic botany*, *12*, i-104.

[26]. GPHC, J., & Malai, T. V. (2014). Challenges, Constraints and Opportunities in Herbal Medicines–A Review. *International Journal of Herbal Medicine*, *2*(1): 21-24.

[27]. Kumar, V., & Kumar, V. (2009). An overview of herbal medicine. *Int. J. Ph. Sci*, *1*(1), 1-20.

[28]. Saggar, S., Mir, P. A., Kumar, N., Chawla, A., Uppal, J., & Kaur, A. (2022). Traditional and Herbal Medicines: Opportunities and Challenges. *Pharmacognosy Research*, *14*(2).

[29]. Mookherjee, N., & Hancock, R. E. W. (2007). Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cellular and Molecular Life Sciences*, *64*(7), 922-933.

[30]. Ho, K. (2001). Bacteriophage therapy for bacterial infections: rekindling a memory from the pre-antibiotics era. *Perspectives in Biology and Medicine*, *44*(1), 1-16.

[31]. Brown, K. L., Cosseau, C., Gardy, J. L., & Hancock, R. E. (2007). Complexities of targeting innate immunity to treat infection. *Trends in immunology*, *28*(6), 260-266.

[32]. Kala, C. P., Dhyani, P. P., & Sajwan, B. S. (2006). Developing the medicinal plants sector in northern India: challenges and opportunities. *Journal of Ethnobiology and Ethnomedicine*, *2*(1), 1-15.

[33]. Singh, A., & Singh, P. K. (2009). An ethnobotanical study of medicinal plants in Chandauli District of Uttar Pradesh, India. *Journal of Ethnopharmacology*, *121*(2), 324-329.

[34]. Pundlik, S. P. (2021). The Phytochemical Determination from Leaf Samples of Two Grewia Species. *Journal of Scientific Research*, *65*(7).

[35]. Dev, R., Kannan, V., Kumar, M. S., Dayal, D., & Patel, R. (2019). Grewia Species: Diversity, Distribution, Traditional Knowledge and Utilization. In *Wild Fruits: Composition, Nutritional Value and Products* (pp. 395-426). Springer, Cham.

[36]. Kumar, S., Singh, B., & Bajpai, V. (2022). Traditional uses, phytochemistry, quality control and biological activities of genus Grewia. *Phytomedicine Plus*, 100290.

[37]. Rani, D., Jha, M., Rai, N., & Kharkwal, H. (2021). The in-vitro Sun Protection factor, estimation of Total Flavonoid, Phenol, Alkaloid Content of Leaf extracts of Grewia abutilifolia. *Annals of the Romanian Society for Cell Biology*, *25*(5), 4249-4259.

[38]. Semenya, S. S., & Maroyi, A. (2020). Ethnobotanical survey of plants used to treat respiratory infections and related symptoms in the Limpopo province, South Africa. *Journal of Herbal Medicine*, *24*, 100390.

[39]. Goyal, P. K. (2012). Phytochemical and pharmacological properties of the genus Grewia: a review. *International journal of pharmacy and pharmaceutical sciences*, *4*(4), 72-78.

[40]. Dev, R., Sharma, G. K., Singh, T., Dayal, D., & Sureshkumar, M. (2017). Distribution of Grewia species in Kachchh Gujarat, India: taxonomy, traditional knowledge and economic potentialities.

[41]. Trabelsi, A., El Kaibi, M. A., Abbassi, A., Horchani, A., Chekir-Ghedira, L., & Ghedira, K. (2020). Phytochemical study and antibacterial and antibiotic modulation activity of *Punica granatum* (pomegranate) leaves. *Scientifica*, Article ID 8271203, 1-7.

[42]. Trease GE, Evans WC. Pharmacognosy. 15th ed. Sounders Publishers. London; 2002. p. 42-44, 22-229, 246-249, 304-306, 331-332, 391-393.

[43]. Sofowora, A. (1993). Screening plants for bioactive agents. *Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria*, 134-156.

[44]. Kokate, C. K. Purohit AP & Gokhle S. B., (2006). ". *Text book of pharmacognosy" thirty Forth edition*, 112-121.

[45]. Evans WC (2002). Trease and Evans Pharmacognosy, 15th edition. W.B Sauders Company Ltd, London. pp 137-139, 230-240.

[46]. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

[47]. Kumar, M., Dandapat, S., & Sinha, M. P. (2014). Plant mediated synthesis of silver nanoparticles using *Adhatoda vasica* aqueous leaf extract. *The Ecoscan, 5*, 30-36.

[48]. The Indian Pharmacopoeia (1996). Quality Control Methods for Medicinal Plant Materials, (1)23.

[49]. Vishnoi, N. R. (1979). Advanced practical chemistry. V*ikas Publication House, PVT Ltd., Ghaziabad-India*, 447-449.

[50]. Afzal, M., Kazmi, I., & Anwar, F. (2013). Antineoplastic potential of *Bryophyllum pinnatum* Lam. on chemically induced hepato-carcinogenesis in rats. *Pharmacognosy Research*, *5*(4), 247-253.

[51]. Fair, R.J. and Y. Tor (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, 6: PMC.S14459.

[52]. 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.