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CHARACTERIZATION OF PHYTOCHEMICAL CONSTITUENTS AND EVALUATION OF *IN VITRO* ANTIMICROBIAL ACTIVITY OF *GREWIA ASIATICA* L. LEAF EXTRACT

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Grewia asiatica L. is a medicinal plant, used for various health benefits and disease treatment. The leaves of the plant has been widely used due to its potential antimicrobial and anti-irritant properties The current research focuses on investigating the phytochemical composition of the methanolic extract of *G. asiatica* L. leaves using GC-MS. The methanolic extract of leaves were also analyzed for its antibacterial potential against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* and potential antifungal activity against *Alternaria alternata, Aspergillus niger, Candida albicans* and *Fusarium solani* using well diffusion assay. The chemical composition of leaf extract revealed presence of total 44 compounds namely n-Hexadecanoic acid, Octadecanoic acid, 2,3-dihydro-Benzofuran and Heneicosane. Leaf extract has shown potent antimicrobial activity against *Staphylococcus aureus* (IZ=16±1.88mm), *Pseudomonas aeruginosa* (15±2.23mm), *Fusarium solani* (16±0.35mm) and *Aspergillus niger* (13±0.34mm). The conducted research and the results observed has expanded the presence of significant antimicrobial potential in the methanolic leaf extract *Grewia asiatica* L., that further substantiate the use of the tested leaf extract as an effective source of natural antimicrobial compound drug and its commercial application.

Keywords: Antibacterial activity, Antifungal activity, GC-MS, *Grewia asiatica* L., Methanol extract.

Introduction:

Grewia genus (Family: Tiliaceae) is a vital medicinal plant and ayurveda mentions its use to cure inflammation, wound healing, fever, blood disorders, heavy menstrual flow and diabetes. This genus comprises approximately 150 species, small trees or shrubs, due to their salt and drought tolerance capacity, these species are majorly distributed in arid and semiarid areas around the world including India, Pakistan, Africa, the Himalaya, Madagascar, China, Bangladesh, and Thailand[1,2]. *Grewia asiatica* L. is one such fruit bearing member of this genus and commonly known as Phalsa.

Grewia asiatica L.has two different varieties viz. tall and short (dwarf) that are found in India. Its medicinal value has been attributed to the presence of variety of metabolites including coumarins, saponins and anthraquinone[3,4]. The ripe fruit of this plant has reportedly been used in treating conditions like jaundice upset stomach, diarrhea, intestinal infection and cough.

Phalsa roots are used in treating rheumatism and barks are mainly known for the treatment of remedies such as wound healing and osteoporosis.Major scientific studies have been performed on fruit of phalsa, however due to its shorter shelf-life it is currently suitable only for local marketing. Although the fruit of the plant has been well-explored, there is an urgent need to shift the focus on investigating pharmacological potential of other plant parts of *G. asiatica*[5-7].

Many effective strategies for combating common microbial infections have been developed, despite this the ever-increasing incidences of infections caused by multidrug-resistant (MDR) bacteria is increasing which is intimidating[8]. In the past few decades, the search for new anti-infection agents has engaged many research groups from the field of ethnopharmacology. An earlier study reported that the methanol leaf extract of *G.asiatica* was most potent against *Candida albicans, Aspergillus thiogenitalis, Penicillium notatum, Penicillium citrinum* and *Aspergillus niger* in the decreasing order of sensitivity[9]. In an another study the bark and fruit of *G. asiatica* was reported to possess antibacterial activity against bacterial strains (namely *Proteus vulgaris, Salmonella typhi, Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Shigella dysenteries*)[10]. Among the primary and secondary metabolites present in plant parts of *G. asiatica,* carbohydrates and proteins, tannins, flavonoids and phenols respectively were identifiedand their antioxidant potential was also reported in a recent study[11]. In an effort to broaden the range of antimicrobial agents from natural resources, this study was conducted with the purpose of finding out the efficacy of *G. asiatica* leaves as antimicrobial agents for commercial purposes.

Material and methods:

- *Plant collection and preparation:*

G. asiatica L. leaves were collected during the month of January 2022 and authenticated and submitted in the Herbarium, Department of Botany, University of Rajasthan, Jaipur (*Voucher no. RUBL-21374*). Plant material was collected, thoroughly washed and dried. Dried leaves were crushed, fine powdered and 20g of this powder was extracted in methanol solvent using Soxhlet apparatus. Prepared extract was filtered using Whatmann filter paper no. 4 and dried using rotary evaporator. Later reconstituted in methanol and stored at 4ºCuntil further use.

- *Gas Chromatography-Mass Spectroscopic (GC-MS) analysis of Grewia asiatica L. leaf extract:*

GC-MS analysis of the methanol leaf extract was performed using the equipment Shimadzu GCMS – QP2020 (Kyoto, Japan). The parameters of the equipment have been listed in the Table 1. Theidentification of components was based on comparison of their retention indices and data available in Wiley and NIST (National Institute Standard and Technology, USA) library. The phytoconstituents were identified after comparison with the NIST 17 library and the results obtained have been listed. The findings were also verified using published research articles and the PubChem online database for bioactivities of compound and their respective chemical structures.

- *Antimicrobial activity:*

Test organism: The microbial strains utilized for this investigation were two gram-positive bacteria: *Bacillus subtilis* (MTCC441), *Staphylococcus aureus* (MTCC3160) and two gram-negative bacteria: *Escherichia coli* (MTCC1652) and *Pseudomonas aeruginosa* (MTCC740). Fungal strains used were *Alternaria alternate* (MTCC2060), *Aspergillus niger* (MTCC282) *Candida albicans* (MTCC3958) and *Fusarium solani*

(MTCC3004). These strains were collected from S.M.S. Medical College, Jaipur, Rajasthan, India and maintained in sterile nutrient agar (HiMedia) slants at 4°C.

- *Test for antibacterial activity:*

The *in vitro* antibacterial assay of the leaf extracts was conducted by the agar well-diffusion method[12]. Bacterial strains were incubated at 30 °C for 24 h by inoculation in nutrient broth and diluting the microbial culture to achieve a concentration of 10^8 CFU/ml. Muller-Hinton Agar was sterilized and poured into sterilized petri dish and solidified. These plates were inoculated with 200µl of the bacterial culture media and wells were created using a sterile cork borer. Dried methanol leaf extract was dissolved in DMSO (100%) and 50 µl of the prepared extract (2mg/ml) was pipette into these wells. The prepared petri plates were kept for 1-2 h at 4 °C and then incubated at 35°C for 18-24 h. The inhibition zones (IZ) formed on the media was measured in millimeters. The common antibiotic streptomycin (1mg/ml) was used as the standard and 100% (v/v) DMSO as negative control.

- *Test for antifungal activity:*

  Petri plates were poured with Potato Dextrose Agar (PDA) and inoculated with fungal species. 50µL each of the pre prepared extracts were poured into the wells using a pipette. Ketoconazole (1mg/ml) was used as the positive control and DMSO as a negative control in a separate plate. The petri dishes were then incubated for 42 h at 35 °C. The zone of inhibition (in mm) was measured after incubation and their respective Activity Indices (AI) was also calculated.

- *Statistical Analysis:*

  Each experiment was carried out in replicates (n=3) and data is represented as Mean ± Standard deviations (SD).

Results and Discussion:

The GC-MS analysis of *G. asiatica* L. leafextract revealed presence of total 44 compounds consisting of fatty acids, phenols, fatty acyl esters etc. (Fig. 1 and Table 2). The compounds such as n-Hexadecanoic acid (13.74%), Octadecanoic acid (6.80%), 2-methyl-Hexadecane (2.50%), Hexadecanoic acid, methyl ester (2.20%), 2,3-dihydro-Benzofuran (2.17%), Hexadecanol (0.92%) and 2-Methoxy-4-vinylphenol (0.59%) were identified as phytoconstituents present in major quantities.

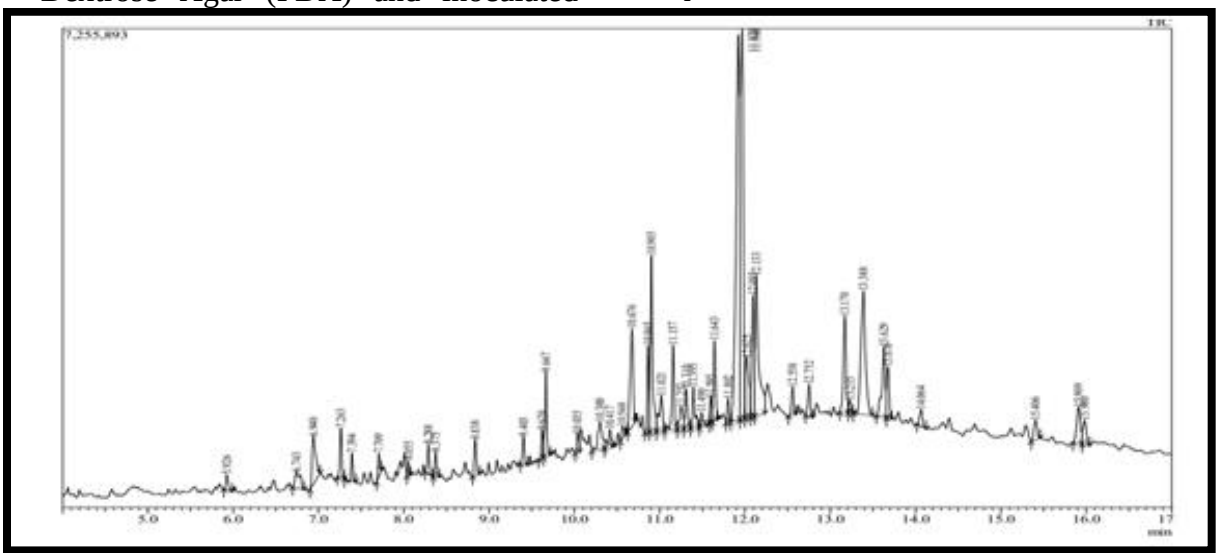

Fig. 1: GC-MS chromatogram of *G. asiatica* L. methanol leaf extract.

Benzaldehyde and heptadecane have earlier been isolated in *Grewia tenax* fruit peel and are said to possess antitumor and herbicidal properties[13]. Alternative form of decenal (aldehyde), Cyclohexadiene (ketone), Octadecane and Triacontane (alkane) were earlier reported in *G. lasiocarpa*[14]. Palmitic acid (*n*-Hexadecanoic acid) identified in extractis said to possess antitumor, antimicrobial and antioxidant activities and Isopropyl myristate is used in pharmaceutical preparation[15,16]. Tributylacetylcitrate listed in Table 2 is used in pharmaceutical coating of solid oral tablets or capsules[17]. Bis-(2-methylpropyl)-1,2-Benzenedicarboxylic acid and 4,8,12,16-Tetramethylheptadecan-4-olide are ester earlier reported in *Crotalaria ramosissima* leaf extract and *Moringa oleifera* seed and leaf extract respectively[18,19]. These have not been reported earlier in *G. asiatica* plant.

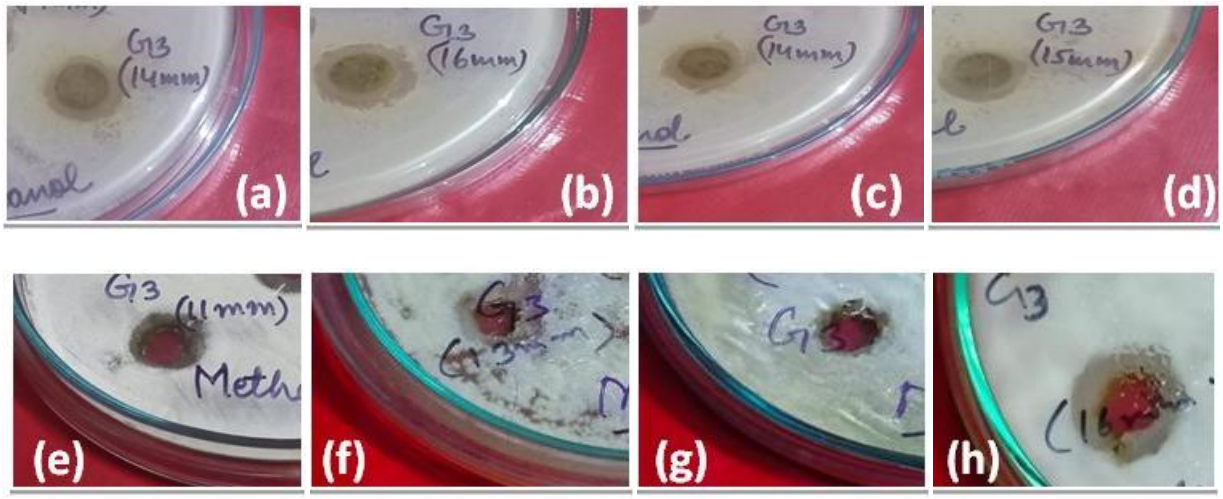

Fig. 2: Antimicrobial activity of methanol leaf extract of *G. asiatica* by agar well diffusion method with measured IZ in mm against pathogen: (a) *B. subtilis* (b) *S. aureus* (c) *E. coli* (d) *P. aeruginosa* (e) *A. alternate* (f) *A.niger* (g) *C. albicans* (h) *F. Solani*

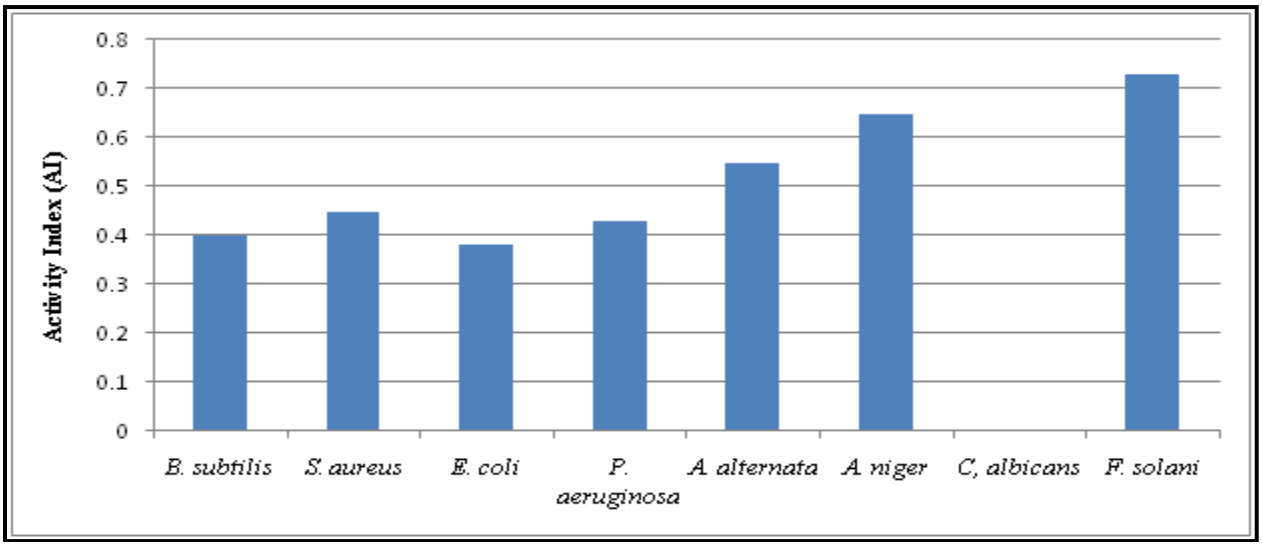

Fig. 3: Activity index of *G. asiatica* L. leaf against microbial strains.

Antimicrobial activity analyzed by well diffusion assay revealed that the methanol leaf extract possess more potent antifungal potential as compared to antibacterial potential (Fig. 2). The methanol leafextract showed significant growth inhibition of both Gram positive and gram negative bacteria (Table 3). Highest activity was shown against *S. aureus* with AI of 0.43 and least activity against *E. coli* with AI of 0.38. In general, Gram-positive bacteria are more sensitive than Gram-negative bacteria because of their difference in cell wall components this trend is also in accordance with present findings[20]. In case of antifungal activity highest inhibition of growth was recorded against *F. Solani* (16±0.35mm) and least growth inhibition was recorded against *A.alternate* (11±0.09 mm), with activity index of 0.73 and 0.55 respectively (Table 4). No significant activity was recorded against *C. albicans*. Fig. 3 illustrates Activity Indices of leaf extract against different bacterial and fungal strains.

Table 1: GC- MS Instrumentation Parameters:

Instrument	**GC-MS Shimadzu QP 2020. (Kyoto, Japan)**
GC Condition	
Carrier Gas	Helium; Constant flow
Column Dimension	Column SH-RXI-5silMS (30×0.25mm×0.25µm)
Column Oven temperature	50ºC, with gradual increase up to 280ºC
Injection Temperature	200ºC
Injection Mode	Split
Split Ratio	1:50
MS parameters	
Transfer line Temperature	250ºC
Ionization Mode	Electron impact (EI) mode
MS Voltage	0.96Kv
Scan Range	35-500 m/z

In previous study leaves of the phalsa showed potent antifungal activity against *S. cerevisiae*, gram-positive bacteria and gram-negative bacteria[21]. In a study performed by Ateeb *et al*., (2023), hydroethnol leaves extract and silver nanoparticles (AgNPs) showed growth inhibition against *E. coli* with inhibition zone of 14mm and 15mm respectively, which is comparable to current findings. Whereas both showed no activity against *P. aeruginosa*, however, in present experiment conducted methanol leaf extract showed potent inhibition activity against bacterial pathogen *P. aeruginosa* (15±2.23mm)[22].

Dawar *et al*., (2020) reported in their experiment that, 100% concentration of aqueous leaves extract reported growth inhibition (*in vitro*) against root rot causing fungi *M. phaseolina*, *F. Oxysporum* and *R. solani* and *in vivo*, 1.0 % leaves powder suppressed the colonization of *M. phaseolina*, *Fusarium* species and *R. solani* notably[23].

Table2: Compounds detected by GC-MS in methanol leaf extract of *G. asiatica* L.:

Peak no.	Peak Area %	Retention time	Compound Name
1	0.47	5.926	Nonanal
2	1.03	6.743	2,3-dihydro-Benzofuran
3	1.15	7.263	2-Decenal
4	0.56	7.394	2-Nitrocumene
5	0.59	7.709	2-Methoxy-4-vinylphenol
6	0.27	8.055	2-Undecenal
7	0.68	8.288	Tetradecane
8	0.59	8.375	3-Methoxy-4-[3-oxo-3-(pyrrolidin-1 yl)propoxy]benzaldehyde
9	0.94	8.838	1,4-dione,2,6-bis(1,1-dimethylethyl)-2,5 Cyclohexadiene
10	0.70	9.403	Dodecanoic acid
11	0.69	9.620	1-Hexadecanol
12	2.07	9.667	Octadecane
13	0.53	10.035	Benzophenone
14	1.47	10.300	Heptadecane
15	0.72	10.417	Pentadecanal
16	0.44	10.560	Triacontane
17	0.44	10.676	Tetradecanoic acid
18	1.90	10.865	1-Nonadecene
19	4.83	10.903	Octadecane
20	1.59	11.021	Isopropyl myristate
21	2.35	11.157	6,10,14-trimethyl-2-Pentadecanone
22	0.95	11.252	Pentadecanoic acid
23	1.10	11.310	hept-3-yl isobutyl ester -Phthalic acid,
24	1.30	11.395	1-Hexadecanol
25	0.36	11.490	Heneicosane
26	0.62	11.595	bis(2-methylpropyl)-1,2-Benzenedicarboxylic acid
27	2.20	11.643	methyl ester -Hexadecanoic acid,
28	0.53	11.802	Isophytol
29	17.15	11.920	Dibutyl phthalate
30	13.74	11.966	n-Hexadecanoic acid
31	2.47	12.025	Di-sec-butyl phthalate
32	3.50	12.093	1-Eicosene
33	6.14	12.133	Heneicosane
34	0.81	12.558	butyl hept-3-yl ester- Phthalic acid
35	0.92	12.752	1-Hexadecanol
36	3.24	13.170	Bis(2-ethylhexyl) maleate
37	0.38	13.235	5-methyl-Heneicosane
38	6.80	13.388	Octadecanoic acid
39	2.88	13.629	1-Hexacosanol
40	1.50	13.676	2-methyl-Hexadecane
41	0.56	14.064	Tributylacetylcitrate
42	0.80	15.406	4,8,12,16-Tetramethylheptadecan-4-olide
43	1.70	15.909	1-Hexacosanol
44	1.01	15.980	Tetracosane

Antifungal potential of various species of *Grewia* genus namely, *G. optiva*, *G. mollis* and *G. flava* has also been recorded in previous studies[24-26]. This antimicrobial potential is attributed to phenols present in plant extract. The mechanism responsible for phenolic toxicity in microorganisms is through enzyme inhibition leading to protein inactivation and ultimately death of bacteria[27].

Table 3:Antibacterial activity of *G. asiatica* L. methanol leaf extract:

Extract	Inhibition zones (mm)			
	Gram (+ ve) pathogenic bacteria		Gram (− ve) pathogenic bacteria	
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
***G. asiatica* L. leaf**	14±0.45	16±1.88	14±2.21	15±2.23
Streptomycin	35±1.20	35±1.20	35±1.20	35±1.20

Table 4: Antifungal activity of *G. asiatica* L. methanol leaf extract:

Extract	Inhibition zones (mm)			
	Alternaria alternata	***Aspergillus niger***	***Candida albicans***	***Fusarium solani***
***G. asiatica* L. leaf**	11±0.09	13±0.34	0±0.0	16±0.35
Ketoconazole	20±0.21	20±0.21	20±0.21	20±0.21

Conclusion

Grewia asiatica L. is a medicinal and commercial valued plant and modern pharmacological studies have confirmed its pharmacological potential attributed to variety of chemical compounds and their extracts. In present study 44 total compounds were identified in methanol extract of leaf using GC-MS technique out of which dominant phytochemicals were fatty acids viz. Octadecanoic acid, n-Hexadecanoic acid, , fatty acid ester such as Hexadecanoic acid, methyl ester and various phenols namely, Hexadecanol and 2-Methoxy-4-vinyl phenol. Extract was also tested against various microbial strains and significant antimicrobial activity was reported.

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Reference:

1. Bayer C and Kubitzki K 2003, Malvaceae. In: *Flowering Plants· Dicotyledons: Malvales, Capparales and Non-betalain Caryophyllales.* (Ed.) Kubitzki K, Springer Berlin Heidelberg. **Vol. 5** pp. 225-311.
2. Dev R, Kannan V, Kumar MS, Dayal D and Patel R 2019, Grewia species: diversity, distribution, traditional knowledge and utilization. In: *Wild Fruits: Composition, Nutritional Value and Products.* (Ed.) Mariod AA, Springer Nature, Switzerland. Pp. 395-426.
3. Zia-Ul-Haq M, Stanković MS, Rizwan K and De Feo V 2013, *Grewia asiatica* L., a food plant with multiple uses. *Molecules.***18**(3) 2663-82.
4. Wani TA, Pandith SA, Rana S, Bhat WW, Dhar N, Razdan S, Chandra S, Kitchlu S, Sharma N and Lattoo SK 2015, Promiscuous breeding behaviour

in relation to reproductive success in *Grewia asiatica* L. (Malvaceae). *Flora-Morphology, Distribution, Functional Ecology of Plants* .**211** 62-71.
5. Tripathi S, Chaurey M, Balasubramaniam A and Balakrishnan N 2010, *Grewia asiatica* Linn.-As a Phytomedicine: A Review. *Research Journal of Pharmacy and Technology*. **3**(1) 1-3.
6. Sharma N and Patni V 2013, In vivo and in vitro qualitative phytochemical screening of *Grewia* species. *Int. J. Biol. Pharm. Res*. **4**(9) 634-9.
7. Hiwale S 2015, *Sustainable horticulture in semiarid dry lands.* Springer India.
8. Hart CA and Kariuki S 1998, Antimicrobial resistance in developing countries. *Bmj.* **317**(7159) 647-50.
9. Sangita K, Avijit M, Shilpa P and Shivkanya J 2013, Studies of the antifungal and antiviral activity of methanolic extract of leaves of *Grewia asiatica*. *Pharmacognosy Journal.***1**(3) 221-3.
10. Naqvi SB, Azhar I, Jabeen S and Hasan SF 2012, Report: studies on antibacterial activity of some traditional medicinal plants used in folk medicine. *Pak J Pharm Sci.* **25**(3) 669-74.
11. Khangarot K, Mishra A, Bhardwaj R and Sharma RA 2024, Phytochemical and in vitro antioxidant potential screening of *Grewia asiatica* L. *Journal of Pharmacognosy and Phytochemistry*. **13**(1) 230-2.
12. Perez C 1990, Antibiotic assay by agar-well diffusion method. *ActaBiol Med Exp*. **15** 113-5.
13. Aboagarib EA, Yang R and Hua X 2015, Physicochemical, nutritional, and functional characteristics of seeds, peel and pulp of *Grewia tenax* (Forssk) Fiori fruits. *Tropical Journal of Pharmaceutical Research.* **14**(12) 2247-54.
14. Akwu NA, Naidoo Y, Singh M, Nundkumar N and Lin J 2019, Phytochemical screening, in vitro evaluation of the antimicrobial, antioxidant and cytotoxicity potentials of Grewia lasiocarpa E. Mey. ex Harv. *South African Journal of Botany*. **123** 180-92.
15. Ganesan T, Subban M, Christopher Leslee DB, Kuppannan SB and Seedevi P 2022, Structural characterization of n-hexadecanoic acid from the leaves of *Ipomoea eriocarpa* and its antioxidant and antibacterial activities. *Biomass Conversion and Biorefinery*. **14** 14547-58.
16. Myristate M 1982, Final report on the safety assessment of myristyl myristate and isopropyl myristate. *Int. J. Toxicol.* **1**(4) 55-80.
17. Kim H, Choi MS, Ji YS, Kim IS, Kim GB, Bae IY, Gye MC and Yoo HH 2018, Pharmacokinetic properties of acetyl tributyl citrate, a pharmaceutical excipient. *Pharmaceutics*. **10(**4) 177.
18. Lanchana HA and Garampalli RH 2024, Analysis of phytochemical constituents, antibacterial, antioxidant and GC-MS profiling of *Crotalaria ramosissima* leaf extracts. *International Journal of Pharmaceutical Sciences and Drug Research*. **16**(3) 426-34.
19. Aja PM, Nwachukwu N, Ibiam UA, Igwenyi IO, Offor CE and Orji UO 2014, Chemical constituents of *Moringa oleifera* leaves and seeds from Abakaliki, Nigeria. *American Journal of Phytomedicine and Clinical Therapeutics*. **2**(3) 310-21.
20. Nikaido H and Vaara M 1985, Molecular basis of bacterial outer membrane permeability. *Microbiological reviews*. **49**(1) 1-32.
21. Shrimanker MV, Patel DD, Modi HK, Patel KP and Dave RM 2013, In-vitro antibacterial and antifungal activity of

Grewia asiatica Linn. leaves. *American Journal of PharmaTech Research*. **3**(1) 38-42.

22. Ateeb M, Asif HM, Ali T, Baig MM, Arif MU, FarooqMI, Kaleem M and Shaukat I 2023, Photocatalytic and Antibacterial activities of bio-synthesised silver nanoparticles (AgNPs) using *Grewia asiatica* leaves extract. *International Journal of Environmental Analytical Chemistry*. **5** 1-9.
23. Dawar SH, Hanif AS and Siddique R 2020, Management of root rot fungi by *Grewia asiatica* L. leaves and on the growth of crop plants. *Pak. J. Bot*. **52**(2) 469-76.
24. Arora S 2011, Antibacterial, antifungal, antioxidant and phytochemical study on the leaves extract of Grewia optiva.*Jouranl of Pharmacy Research* **4**(9) 3130-32.
25. Al-Youssef HM, Amina M and El-Shafae AM 2012, Biological evaluation of constituents from *Grewia mollis*. *Journal of Chemical and pharmaceutical research*. **4**(1) 508-18.
26. Mongalo NI, McGaw LJ, Finnie JF and Van Staden J 2022, Isolation and characterization of antimicrobial and anti-inflammatory triterpenoids from the acetone extract of *Grewia flava* DC. (Malvaceae) roots. *South African Journal of Botany*. **149** 87-95
27. Mason TL 1987, Inactivation of red beet β-glucan synthase by native and oxidized phenolic compounds. *Phytochemistry*. **26**(8) 2197-202.