Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1241
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1241*
Original Research Article

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTI-OXIDANT ACTIVITIES OF SALVADORA PERSICA AND CALOTROPIS GIGANTEA EXTRACTS AGAINST SELECTED PATHOGENS

HUSSAIN A[1], BANO SA[1], REHMAN ZU[2], WAHEED F[3], BASHIR T[2], ALAM S[1], NAWAZ U[2]

[1]*Department of Microbiology, the University of Haripur Khyber Pakhtunkhwa, Pakistan*
[2]*Department of Microbiology, Abbottabad University of Science and Technology Abbottabad, Pakistan*
[3]*Department of Microbiology, and Molecular Genetics, University of Punjab Lahore, Pakistan*
**Corresponding author`s email address:* asma_baano@yahoo.com

(Received, 04th June 2024, Revised 05th July 2024, Published XXX 2024)

Abstract: *Multiple diseases are treated using medicinal plants nowadays. Salvadora persica and Calotropis Gigantea are beneficial herbs for traditional herbal medicine. The aim of the study was to Screen phytochemicals, antibacterial and antioxidant properties of Salvadora persica and Calotropis Gigantea against Staph. Aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhimurium.* ***Methods;*** *Phytochemicals of both Salvadora persica and Calotropis Gigantea were screened by GC-MS analyzer. Antibacterial and antioxidant activity of ethnolic and Methonolic extracts of screened phytochemicals was tested using Agar Well Diffusion and DPPH assay respectively. Salvadora persica leaf extracts (100g/ml) inhibited Staph. Aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhimurium. Density was used to establish the minimal inhibitory concentration of Salvadora persica and Calotropis Gigantea extracts.* ***Results:*** *Both plant extracts were rich in over 250 detected chemicals via GC-MS analyzer, with 52.5% of these having antibacterial and antioxidant properties Salvadora persica extracts were characterized by 1, 8-Cineole polyphenols (45%) and cyclic ethylene mercaptole (55%), demonstrating antibacterial and antioxidant effects. Meanwhile, Calotropis Gigantea extracts contained major components Campestrol (69%) and β-tocopherol (4%). Salvadora persica and Calotropis Gigantea extracts exhibited inhibition against Pseudomonas aeruginosa at 3.12 mg/ml. Antioxidant activity, assessed via the DPPH test, revealed 35% activity with 200µl of Methanolic extracts. Both Salvadora persica and Calotropis Gigantea leaf extracts exhibited antibacterial activity against Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Notably, Calotropis Gigantea was effective against all chosen pathogens, while Salvadora persica exclusively targeted Staphylococcus aureus. Although Calotropis Gigantea had fewer antioxidants compared to Salvadora persica, the latter's extracts held potential for mitigating oxidative stress, aging, and related disorders. Previous studies highlighted 1, 8-Cineole polyphenols (45%) and cyclic ethylene mercaptole (55%) as antibacterial and antifungal agents against Klebsiella pneumonia and Aspergillus nigar.* ***Conclusion:*** *These findings underscore the promising applications of Salvadora persica and Calotropis Gigantea extracts in both antimicrobial and antioxidant interventions. And can be effected against different diseases such as Typhoid, skin diseases, and disease caused by Escherichia coli and staphylococcus aureus. Further exploration of their specific mechanisms of action and potential therapeutic applications of different metabolites and chemical compounds are warranted to harness the benefits offered by these natural extracts.*

Keywords: Phytochemicals, Extracts, Antimicrobial and antioxidant activity

Introduction

In recent years, antibiotic resistance has become a global concern. Resistant microorganisms produce new illnesses, which increase mortality and toxicity(1, 2). In 2003, 3.5 billion health-related fatalities were reported in European Union, causing 50,000 deaths(3). Microbial fatal diseases are still a global public problem which are caused by different bacterial strains acquiring and spreading resistance genes. These Gene spread and microbial strain acquisition cause health crises(4).

In recent years, excessive use of antimicrobial medicines and vaccinations has improved control and treatment of deadly illnesses (5). So, a solution must be found. Plants' composition and elements make them an alternative drug. Bioactive substances, natural products, primary and secondary metabolites are found in plant extracts. These metabolites and chemicals treat body infections (5)

80% of the world's population uses medicinal plants, including plant extracts, for basic health care (6). Many plant species with biological uses remain undiscovered. Traditional remedies are more compatible with the body, more culturally acceptable, and have fewer negative effects. More than 35,000 plant species are used for medicinal purposes worldwide. Plants have been used to treat a wide spectrum of illnesses for thousands of years (7).

Apocynaceae comprises six Calotropis Gigantea species(8). C. gigantea is a non-cultivable plant found in Africa and Asia. Hindi calls it " Madar" Wasteland plant with thick oblong leaves and odorless purple blossoms(9) (Fig-1). Incised or injured plant leaves or stalks exude milky white pungent fluid. The extracts of Calotropis Gigantea is used for the remedy of snack biting (10),and Scorpian biting(11). Calotroxin, uscharin, uschridin, and proceroside are glycosides, alkaloids, flavones, tannins, and other phytoconstituents of C. gigantea (9). Also detected are cardenolides, flavonoids, terpenes, pregnanes, and nonprotein amino acids(12). Most sap, roots, and/or leaf components tested are cytotoxins (Kumar et al., 2013a). Before penicillin, the milky sap was thought to treat syphilis(13); the plant was called "vegetable mercury.

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

Ayurvedic, Chinese, and homoeopathic medicine employ Calotropis Gigantea to treat fever(14), indigestion(15), diarrhea(16), cold(17), dyspepsia (18), cough(17), asthma(17), antipyretic (19), rheumatism(17), eczema(15), leprosy(20), and leukoderma(15). 1-4 sap components have been studied for anti-inflammatory and anticancer properties (Kumar et al., 2013b). Pharmacological actions of Calotropis Gigantea includes antimalarial(21), anti-inflammatory(22), (23), anti-diarrheal(24), anticonvulsant(25), hepatoprotective (26), antitumor (27), ant nociceptive(19).

Miswak, or Salvadora persica, is a Salvadoraceae plant(28) (Fig-2). It grows in dry and subtropical Africa, the Middle East, Pakistan and India. Traditional herbal treatment uses fresh leaves, twigs, and roots to treat asthma (29), scurvy(30), cough(31), rheumatism(32), and dental hygiene(33). Furthermore *Salvadora persica* was used to clean teeth(34) and promote oral health(35) in Pre-Islamic and Islamic times. In addition to its pharmacological active components, SP's mechanical action when used for brushing contributes to tooth and oral health(35). Tannins inhibit glucosyltransferase to decrease plaque and gum disease, and resins prevent cavities (36). SP's antibacterial, anti-inflammatory, and antioxidant qualities are linked to potassium, sodium chloride, *Salvadorian*, vitamin C, *Salvadorian,* silica, saponins, and other minerals in its natural extracts(35). Hence because of these minerals and compounds, it is antibacterial, anti-infectious, and antioxidant (36).Further pharmacological actions of *Salvadoran persica* includes anti-fungal (37),anti-inflammatory (38), anti-cancerous(39), Analgesic (40),Anti-ulcer (41), anti-convulsant (42),Anti-fertility(43),anthelmintic(44),wound healing(45),ant-coccoidal (46),antidepressant (34).

Methodology

Study Pathogens

Four Salmonella typhi. Pseudomonas aeruginosa, Staph. Aureus and Escherichia coli Pathogens were collected from NESCOM Hospital Islamabad and transported to laboratory of microbiology university of Haripur.

Sample processing and Inoculation

Bacterial strains maintained at -20°C were activated by culturing in Nutrient broth for one day at 37°C. 0.5 McFarland bacterial turbidity was obtained from an overnight broth culture in distilled water (47).

***Plant Collection* and Extraction Preparation**

Both Calotropis gigantea and Salvadora persica leaves were procured from the local Haripur region. The collected leaves were subsequently placed in sterilized bags for preservation within the laboratory premises, intended for subsequent applications as depicted in Figure 3. The preparation of plant extracts was conducted through the modification of a conventional procedure as referenced by [52]. To elaborate, 250g of dried plant powder for each species was immersed in distilled water or specific solvents such as Ethanol and Methanol. This immersion was sustained for a duration of 24 hours at ambient temperature. Following this period, filtration was carried out using No. 1 Whitman filter paper. Subsequent to filtration, the resulting extracts underwent evaporation and concentration processes conducted at 40°C. The concentrated solution was subjected to a one-hour duration at 40°C for drying purposes. The ensuing dried residue was then meticulously stored within glass vials, which were maintained at a temperature of 4°C, as visually indicated in Figure 01 [53].

Figure 01: Plant Collection and Extraction Preparation

Phytochemical Screening of Extracts (GC–MS)

Salvadora persica and *Calotropis Gigantea* were studied using GC-MS Analyzer. The analyzer was set to 50°C for 0.5 minutes before being tuned to 300°C at 5°C/min up to 230°C. 220 and 250 °C were selected for the injectors. Injecting 100:1 split into 1 L of dilute sample (in chloroform). Helium is the carrier gas (2 mL/min). Individual compounds were identified by comparing their retention times and mass spectra to genuine reference compounds in the NIST library database. Chemical components in extracts were detected using GC–MS (48)

.

Antimicrobial activity assay:

Extracts were evaluated against four *Salmonella typhi, Pseudomonas aeruginosa, S. aureus,* and *E. coli.* Antibacterial sensitivity was tested using agar well diffusion, and the lowest inhibitory concentration was determined using MH broth (49). Antibacterial activity of *Salvadora persica* and *Calotropis Gigantea* extracts was investigated using agar well diffusion. Extracts were evaluated against four Bacterial strains *Salmonella typhi, Pseudomonas aeruginosa, S. aureus,* and *E. coli.* Antibacterial sensitivity was tested using agar well diffusion, and the lowest inhibitory concentration was determined using MH broth (MIC). *Salvadora persica* and *Calotropis Gigantea* extracts were evaluated for antibacterial activity using agar well diffusion (50).

Antioxidant activity Assay:

DPPH free radical scavenging tested plant extracts' antioxidant properties (1,1-diphenyle-2-picrylehydrazyle). 0.004% DPPH was generated in 95% ethanol. 1000g/ml-3 g/ml dilution. Control: 5 ml DPPH in 5 ml solvent. Ascorbic acid was compared. Vertexing and holding at 37°C in the dark for 30 minutes. Antioxidant absorption was measured at 517 nm (51).

DPPH Scavenging % inhibition= standard –sample ×/100

Minimum Inhibitory Concentration:

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

The Minimum Inhibitory Concentrations (MICs) were determined through a serial dilution technique in a broth medium. The MIC corresponds to the lowest concentration of an extract that effectively prevents the growth of microorganisms within a 24-hour period. Our approach involved using broth dilution to calculate the MIC. For this process, *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* were cultured on nutritive agar at 37 degrees Celsius for 24 hours. A 0.5 McFarland standard was obtained by suspending the cultured samples in normal saline. The McFarland standard was then mixed with 9950 normal saline to create a diluted solution. The actual MIC determination was performed using 96-well microtiter plates. In these plates, the first column served as control for the extracts, while the second through twelfth columns contained Mueller Hinton broth. The eleventh column was designated for bacterial growth control, while the twelfth column assessed the sterility of the MH broth medium. The microtiter plate was subsequently placed in an incubator set at 37°C for a period of 24 hours, and the readings were taken the following day. Ensured the sterility of the medium and having a control for bacterial growth were vital components for the success of the experiment. The resulting MIC values indicate the lowest concentration of the extract at which bacterial growth in the broth was entirely inhibited. Further, for the determination of the extract's MICs, 20ml of the clear wells containing MICs were plated on Mueller-Hinton agar (49).

Results

Phytochemical Screening of Extracts by GC-MS:

More than 45 (27 *C.* Gigantea and 21 *in S. Persica*) different Compounds were detected during GC-MS analysis as shown in table 1 &2.

Phytochemical Screening of *Calotropis Gigantea* Extracts:

Ethyl acetate was employed as the solvent for the phytochemical screening of *Calotropis gigantea* extract. Through GCMS analysis, a total of twenty-seven (27) distinct peaks, each with varying percentages, were identified as shown in Fig-01. This analysis unveiled the presence of bioactive compounds with diverse medicinal properties. The chromatogram of the extract exhibited these peaks, providing insight into the assortment of functional groups and bioactive compounds within the *Calotropis gigantea* extract, as depicted in Fig-02. The outcomes of the GC-MS analysis culminated in the determination of the quantity and nature of bioactive compounds and secondary metabolites present in the ethyl acetate extract of *Calotropis gigantea*. Notably, Tocopherol and Campestrol emerged as the predominant chemical compounds, exhibiting the highest percentages (69% and 4%, respectively). Following closely were compounds such as Methyl 2-O-Benzyl-D-Arabinofuranoside at 1% concentration. This insight provides valuable information about the composition of the extract and its bioactive constituents as shown in table-01.

Phytochemical Screening of *Salvadora persica* Extracts:

Phytochemical screening of *Salvadora persica* extract was performed in ethyl acetate solvent. Twenty-one (21) different peaks with different percentages were detected by GCMS Analysis which reveals about bioactive compounds with different medicinal properties. Chromatogram (Fig-03) of *Salvadora persica* extract shows characteristic peaks at RT values varying from 3 to 20 which detects and reveals about the presence of different functional groups and bioactive compounds with different percentages, in the extract of *Salvadora persica* (table-2). Results of GC-MS finalized about number of bioactive compounds and secondary metabolites in ethyl acetate extract of *Salvadora persica.* 1, 8-cineole polyphenols and cyclic ethylene mercaptole were the chemical compounds that were detected with highest (45 % and 55 %) percentage followed by 4- ketopentanoic acid methyl ester (12%). According to previous study, *Paranthaman et al, 2020*, detected presence of 27 bioactive metabolites in Ethanolic extracts of *Salvadora persica* leaves by GC-MS analysis. Antimicrobial and antioxidant agents detected were 1-8-cineole polyphenols and cyclic ethylene mercaptole with some new constituents and metabolites as shown in table-02. Hence composition of extracts of *Salvadora persica* with different metabolites reveals uses of *Salvadora persica* by traditional herbalists and practitioners' treatment of different diseases.

Antimicrobial Activities of Extracts:

Antimicrobial activities of Methonolic extracts of *Calotropis Gigantea* (28 mm, 26 mm) showed the highest activity against *Escherichia coli and Staphs. aureus*, followed by Methanolic extracts of *Salvadora persica* (20 mm) against *Staph.aureus* as shown in table-03 and Fig-05.

A= Zones of inhibition by Calotropis Gigantea (GC) and Salvadora persica (S p) against Salmonella typhi

B= Zones of inhibition by Calotropis Gigantea (GC) and Salvadora persica (S p) against Escherichia coli

C= Zones of inhibition by Calotropis Gigantea (GC) and Salvadora persica (S p) against Pseudomonas aeruginosa

D= Zones of inhibition by Calotropis Gigantea (GC) and Salvadora persica (S p) against Staphylococcus aureus.

Minimum Inhibitory Concentration (MIC):

Each plant extract's minimum inhibitory concentration (MIC) against all specified diseases *Staph.aureus Pseudomonas aeruginosa, E. coli and Salmonella were* also determined as shown in Table-4. Salvadora persica (Fig-05) has best inhibition than *Calotropis Gigantea* against *Staph.aureus* and *Salmonella typhi*. Highest inhibition was approximately at 12.5 ug/ml by Ethanolic extracts of *Salvadora persica* by Ethanolic and Methanolic extracts of *Calotropis gigantea* (Fig-06)

Salvadora persica against selected pathogens while Lowest MIC value observed was 0.78 ug/ml that is Methanolic extract of *Calotropis gigantea* against *Staphylococcus aureus.* Ethanolic and Methanolic extracts of *Salvadora persica* Shown almost same (12.5 µg/mL) values and inhibition against *Pseudomonas aeruginosa* and *Salmonella typhi* followed by Ethanolic extracts (12.5 µg/mL) of *Calotropis gigantea* against *Staphylococcus* as shown fig-05 and fig-06.

Anti-oxidant activities of extracts:

Ethanolic and Methanolic extracts of *Salvadora persica* and *Calotropis Gigantea* were tested at four different concentrations (50ul, 100ul, and 200ul) and showed variable percentages of inhibition. Surprisingly, the scavenging activity of each extract increased with concentration. *Salvadora persica* showed comparatively strong antioxidant activity than Calotropis gigantea. The

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

200 ul extract of *showed* the best antioxidant activity as shown in Fig-07 and Fig-08.
where among them, the Methanolic extract of *Salvadora persica* has the highest (35%) anti-oxidant activity (Fig-08), followed by Ethanolic extract of *Calotropis gigantea (29%)* as shown in Fig-07. The lowest (3 %) antioxidant activity was showed by Methanolic extract of *Calotropis gigantea* followed by Ethanolic extract (3 %) of *Salvadora persica* at 50µl concentration. 200 µl Methanolic extracts of *Salvadora persica* showed the highest (30%) activity but it reduces to half (15%) in Methanolic extract of *Calotropis gigantea* at same concentration as shown in table-5..

Table-1: Phytochemical Screening of Extracts of Calotropis gigantea by GC-MS

Compound name	RT(min)	Molecular Weight	Peak area %
METHYL 2-O-BENZYL-D-ARABINOFUR	3.018	605924.625	1.000951965
2- BUTANOL, 1-BENZYLOXY-3-METHY	3.038	295878.031	0.488773165
PENTANOIC ACID, 3-METHYL-, ETH	3.314	528034.75	0.872282457
STRYCHANE, 1-ACETYL-20.ALPHA.-	3.384	320132.313	0.528839817
B –TOCOPHEROL	3.454	2516657.25	4.157370265
CAMPESTEROL	13.178	48203236	69.62892048
1-[(TRIMETHYLSILYL)ROPAN-	17.074	1101013.75	1.81881018
DIETHYLENE GLYCO, TMS DERIVATI	19.045	142106.688	0.234751919
ACETIC ACID, TMS DERIVATIVE	19.185	64722.492	0.106917763
XYLO-HEXOS-5-ULOSE, 2,3,4,6-TE	22.952	2678.18	0.004424196
GLYCEROL, 3TMS DERIVATIVE	23.202	1468.821	0.002426406
SILANE, (BUTOXYMETHYL)TRIMETHY	23.247	1907.366	0.003150857

Table-2: Phytochemical Screening of Extracts of Salvadora persica by GC-MS

Compound name	RT(min)	Molecular Weight	Peak area %
4- KETOPENTANOIC ACID METHYL ESTER C6H10O3	3.018	11921376	12.19575937
3 ,5-DITHIAHEXANOL 5,5-DIOXIDE	3.434	34728984	35.52830914
EICOSYNE	5.204	50183.84	0.051338875
EICOSENE	5.249	34283.969	0.035073052
HEXADECANOIC ACID	17.384	3556.458	0.003638314
ISOMER OF 9-OCTADECENOIC ACID METHYL ESTER	17.519	421852.125	0.431561508
FURANE AND THEIR DERIVATIVES	17.694	10838271	11.08772553
HEPTADECENE	18.04	3054063.5	3.124356075
BICYCLO[7.2.0]UNDEC-4-ENE, 4,1	18.635	13042852	13.34304735
1 ,8-CINEOLE POLYPHENOLS	19.26	1025272.875	45.45669
CYCLIC ETHYLENE MERCAPTOLE	20.13	5679019.5	55.809728277
2 ,4,4-TRIMETHYL-3-HYDROXYMETHY	20.346	2649753.75	2.710740699

Table-3: Zone of inhibitions by Extracts

Pathogens	Calotropis Gigantea		Salvadora persica	
	Methanolic	Ethanolic	Methanolic	Ethanolic
Escherichia coli	28 mm	12.6 mm	6.7 mm	8.5 mm
Staph. aureus	26 mm	25 mm	22 mm	18.3 mm
Pseudomonas aeruginosa	20 mm	23 mm	6.8 mm	9.5 mm
Salmonella typhimurium	18 mm	22 mm	6.5 mm	13 mm

Table-4; MIC Values ranges from of Ethanolic and Methonolic extracts of Calotropis gigantea and Salvadora persica

S.N	Bacterial strains	Calotropis gigantea		Salvadora persica	
		Ethanolic	Methanolic	Ethanolic	Methanolic
1	Escherichia coli	6.25 µg/mL	1.56µg/mL	1.56µg/ml	0.78µg/mL
2	Staphylococcus aureus	12.5µg/mL	0.78µg/mL	6.25µg/mL	1.56µg/mL
3	Salmonella typhimurium	3.12 µg/mL	3.12 µg/mL	12.5µg/mL	6.25 µg/mL
4	Pseudomonas aeruginosa	1.56µg/mL	1.56µg/mL	6.25 µg/mL	12.5µg/mL

Table-05: Anti-oxidant activities of Ethanolic and Methanolic Extracts of Salvadora persica and Calotropis gigantea

Concentration Of Extracts	Salvadora persica		Calotropis Gigantea	
	Ethanolic extract	Methanolic extract	Ethanolic extract	Methanolic extract
50 µg/ml	5 %	8 %	9 %	3 %
100 µg/ml	10 %	22 %	20 %	6 %
200 µg/ml	20 %	35 %	29 %	12 %

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

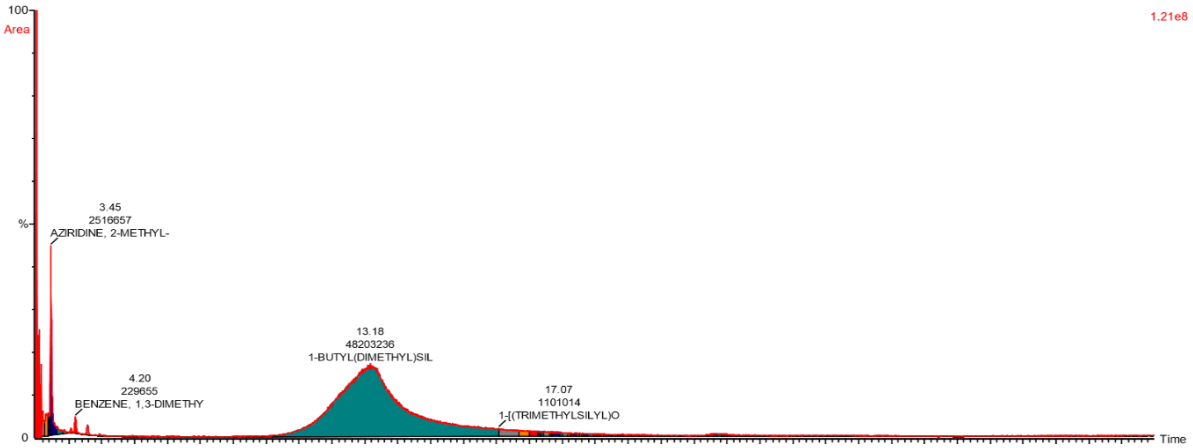

Fig-02: Chromatogram of Extracts of Calotropis Gigantea by GC-MS

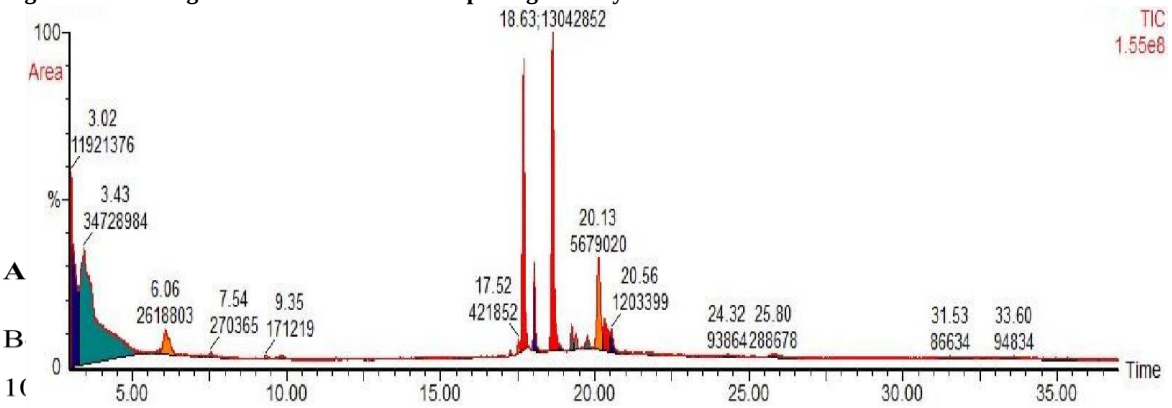

Fig-03: Chromatogram of Extracts of Salvadora persica by GC-MS

Fig-04: Zone of inhibition by different extracts against selected Pathogens

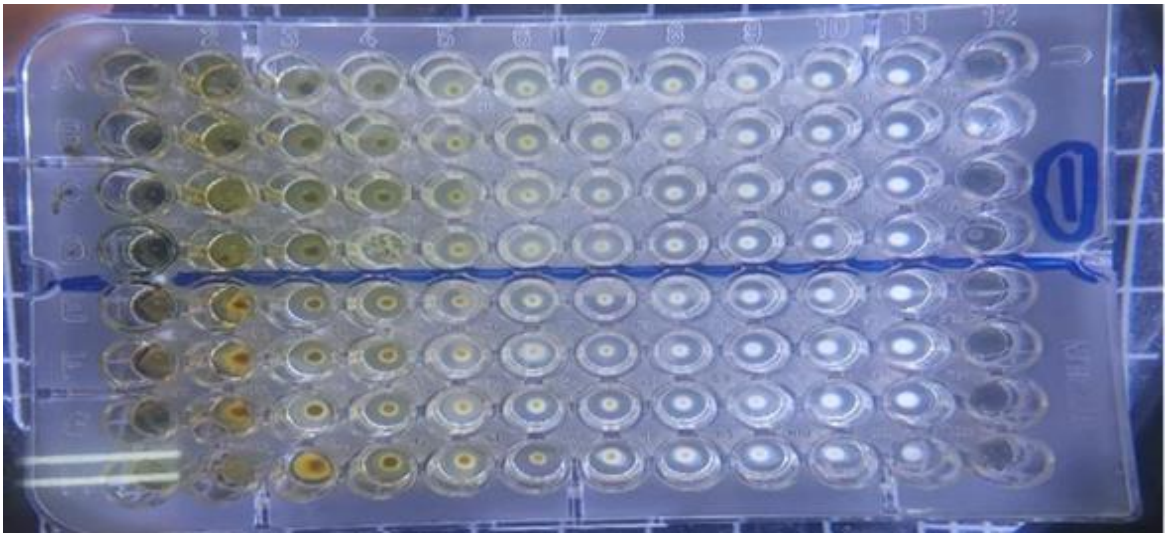

Fig-5; MIC of Ethanolic and Methonolic extracts of Salvadora persica

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

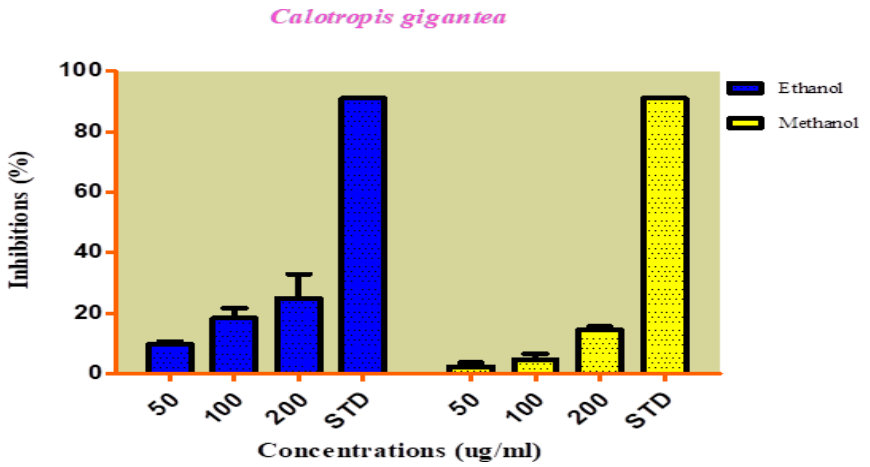

Fig-6; MIC of Ethanolic and Methonolic extracts of Salvadora persica

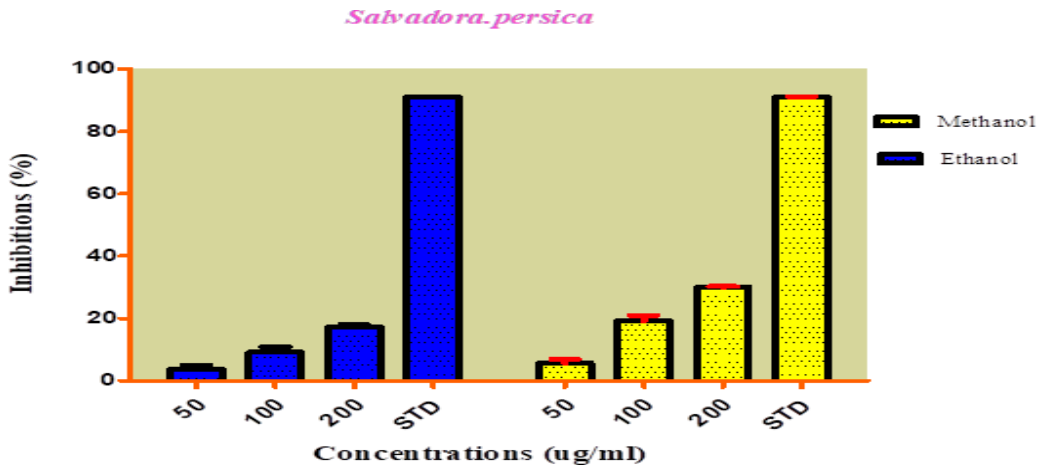

Fig-07: Anti-oxidant activities of Ethanolic and Methanolic Extracts of Calotropis Gigantea

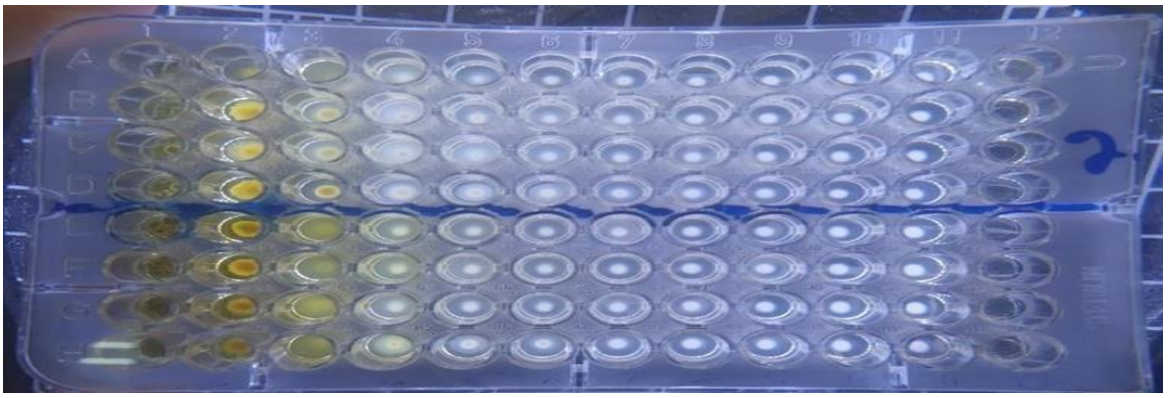

Fig-08:: Anti-oxidant activities of Ethanolic and Methanolic Extracts of Salvadora persica

Discussion

Medicinal plants have therapeutic properties due to the presence of different bioactive compounds. These bioactive compounds are responsible for antimicrobial and antioxidant properties. These plant-derived bioactive chemicals are employed in the pharmaceutical industry as a source of antibacterial characteristics. In phytochemical

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

screening of current study, GC-MS finalized about 21 number of bioactive compounds and secondary metabolites in ethyl acetate extract of *Calotropis gigantea* , TOCOPHEROLE and CAMPESTEROLE were the Chemical compounds that were detected with highest (69% ,4%) percentage followed by METHYL 2-O-BENZYL-D-ARABINOFUR (1 %) Similarly Phytochemical screening of *Salvadora persica* resulted (21) different compounds with different percentages by GCMS Analysis. Results of GC-MS finalized about 27 number of bioactive compounds and secondary metabolites in ethyl acetate extract of *Salvadora persica.* 1 , 8- Cineole Polyphenols And Cyclic Ethylene Mercaptole were the Chemical compounds that were detected with highest (45 % and 55%) percentage followed by 4- Ketopentanoic Acid Methyl Ester (12%). Current study is matching with (52), who reported that BENZYL ISOTHIOCYANATE (52%) is the predominant antibacterial component of *Salvadora persica* and antifungal agents against *Klebsiella pneumonia* and *Aspergillus niger*. Similar finding were also reported by (53)with little change in chemical percentage. There is difference in number and percentages of chemical compounds in current study in comparison to previous studies. These differences are due to solvent used as in current study solvent used is ethyl acetate for GC-MS Analysis and solvents used in others studies were chloroform, ethanol and methanol etc.

In Antibacterial activities, Current study investigated the different zones of inhibitions (ZOI) by Ethanolic and Methanolic extracts of both *Salvadora persica* and *Calotropis gigantea*. Among both selected plants, Methanolic extracts of *Calotropis gigantea* showed the highest (28mm, 26mm) zones of inhibitions against *E. coli* and *Staphylococcus aureus* followed by Ethanolic extract of *Calotropis gigantea* that is 25 mm against *Staph. Aureus.* But Ethanolic extracts of *Calotropis gigantea* was more effective than their Methanolic extracts, against *Pseudomonas aeruginosa* and *Salmonella typhimurium* with 23 mm and 22 mm zones of inhibitions. Similarly in *Salvadora persica* both Ethanolic and Methanolic extracts were effective only against *Staphylococcus aureus* with 22 mm and 18.3 mm zones of inhibitions. They were found a little bit resistant with lower 8 mm, 9.5 mm and 13mm zones of inhibitions against others pathogens like *E. Coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium.* Similar results were (52) reported in 2018 with some exceptions that *Salvadora persica* extracts have no antibacterial impact *on Staphylococcus aureus*. Current investigations are consistent with the findings of (54) who reported that the diameter of zones of growth inhibition in Jordanian *Salvadora persica* extract was approximately 13 mm for Escherichia coli, 12 mm for *Staphylococcus aureus*, 3 mm for *Bacillus subtilis*, and 3.8 mm for *Pseudomonas aeruginosa* strain. There is great difference in our study according to (55) who reported that *Pseudomonas aeruginosa* resisted Methanolic extract of *Salvadora persica*, however in the current investigation, *Salvadora persica* inhibited *Pseudomonas aeruginosa* with a 9.5mm zone of inhibition. So in brief there is a little difference in current results due to use of different methods of agar well diffusion method, solvents and extracts for finding antimicrobial activities against different pathogens.

In antioxidant activities, increase in concentrations of extracts increases scavenging activity. Among both Ethanolic and Methanolic extracts of both selected plants, the Methanolic extract of *Salvadora persica* has highest (35%) scavenging activity than Ethanolic extract *Calotropis gigantea (29%)* at 200µg/ml concentration of extract *s (table-5)* . While Ethanolic and Methanolic extracts of both selected plants have lower scavenging activity (3% and 6%) at lower concentration of 50 µg/ml. Current investigations of regarding antioxidant activities of both selected plants are in accordance with (56) who reported that leaves extract of *Calotropis gigantea* showed 45% and 40% free-radical scavenging (table-5) with some exceptions. Results and outcomes are different with little differences because of differences in polarity between solvents, type of DPPH assay (0.005%) method used, and varying (50 µg/ml ,100 µg/ml and 200 µg/ml) concentration of extracts. Different findings were based on different methods used. Hence based on presented results, it was observed that both *Salvadora persica* and *Calotropis gigantea* extracts has antimicrobial and antioxidant activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* , *Salmonella* and *Escherichia coli due* to the presence of antimicrobial and antioxidant bioactive compounds. Future studies are needed to know the mechanism action and toxicity of *Salvadora persica* and *Calotropis gigantea extracts* against parasites. There is a need to check the antimicrobial and antioxidant activities of new discovered bioactive compounds in this current study.

Conclusion

Based on the obtained results, it can be concluded that both Calotropis gigantea and Salvadora persica exhibit antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, and Escherichia coli. Notably, Calotropis gigantea demonstrated the most substantial zone of inhibition against Staphylococcus aureus and Pseudomonas aeruginosa when compared to Salvadora persica. Among the various extracts evaluated, the Methanolic extracts of Salvadora persica and Calotropis gigantea displayed the most potent antimicrobial activity. Moving on to antioxidant activity, it was observed that both plant extracts exhibited levels of antioxidant activity classified as strong, intermediate, and lower. Salvadora persica and Calotropis gigantea particularly demonstrated the highest levels of antioxidant activity. In the context of GC-MS analysis, the most prominent compounds identified were MONOTERPENES, 1-Butyl (Dimethyl) Silyloxypropa, and Octadecadienoic Acid. Salvadora persica extract contained the highest percentage of MONOTERPENES and 1-Butyl (Dimethyl) Silyloxypropa, while Calotropis gigantea extract contained the highest percentage of Octadecadienoic Acid. These findings collectively shed light on the antimicrobial, antioxidant, and chemical composition aspects of Calotropis gigantea and Salvadora persica extracts.

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
Consent for publication
Approved
Funding
Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

Author Contribution

ADNAN HUSSAIN
Coordination of collaborative efforts.
Study Design, Review of Literature.
SYEDA ASMA BANO (Associate Professor)
Conception of Study, Development of Research Methodology Design, Study Design,, Review of manuscript, final approval of manuscript.
Conception of Study, Final approval of manuscript.
ZIA UR REHMAN
Manuscript revisions, critical input.
Coordination of collaborative efforts.
FADIA WAHEED
Data acquisition, analysis.
Manuscript drafting.
TOUSEEF BASHIR
Data entry and Data analysis, drafting article.
SADIA ALAM
Data acquisition, analysis.
Coordination of collaborative efforts.
USMAN NAWAZ
Manuscript revisions, critical input.
Data acquisition, analysis.
Coordination of collaborative efforts.

References

1. Van Leenders GJ, Van Der Kwast TH, Grignon DJ, Evans AJ, Kristiansen G, Kweldam CF, et al. The 2019 International Society of Urological Pathology (ISUP) consensus conference on grading of prostatic carcinoma. The American journal of surgical pathology. 2020;44(8):e87.
2. Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. Journal of Orthopaedic Research®. 2018;36(1):22-32.
3. Wolf T, Lyne K, Sanchez Martinez G, Kendrovski V. The health effects of climate change in the WHO European Region. Climate. 2015;3(4):901-36.
4. Oaks Jr SC, Shope RE, Lederberg J. Emerging infections: microbial threats to health in the United States. 1992.
5. McHugh D, Gil J. Senescence and aging: Causes, consequences, and therapeutic avenues. Journal of Cell Biology. 2018;217(1):65-77.
6. Zaidi MA, Crow Jr SA. Biologically active traditional medicinal herbs from Balochistan, Pakistan. Journal of ethnopharmacology. 2005;96(1-2):331-4.
7. Shagal M, Modibbo U, Liman A. Pharmacological justification for the ethnomedical use of Datura stramonium stem-bark extract in treatment of diseases caused by some pathogenic bacteria. Int Res Pharm Pharmaco. 2012;2(1):16-9.
8. Wong SK, Lim YY, Chan EW. Botany, uses, phytochemistry and pharmacology of selected Apocynaceae species: A review. Pharmacognosy Communications. 2013;3(3).
9. Chan EWC, Wong SK, Chan HT. Apocynaceae species with antiproliferative and/or antiplasmodial properties: a review of ten genera. Journal of Integrative Medicine. 2016;14(4):269-84.
10. Misra MK, Mohanty MK, Das PK. Studies on the method–ethnobotany of Calotropis gigantea and C. procera. Ancient science of life. 1993;13(1-2):40.
11. Singh E, Kamble S, Bipinraj N, Jagtap S. Medicinal plants used by the Thakar tribes of Raigad district, Maharashtra for the treatment of snake-bite and scorpion-bite. Int J Phytother Res. 2012;2(2):26-35.
12. Kumar D, Kumar S. Calotropis gigantea (L.) Dryand-A review update. Indian Journal of Research in Pharmacy and Biotechnology. 2015;3(3):218.
13. Chitme H, Chandra R, Kaushik S. Evaluation of antipyretic activity of Calotropis gigantea (Asclepiadaceae) in experimental animals. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2005;19(5):454-6.
14. Mossa J, Tariq M, Mohsin A, Ageel A, Al-Yahya M, Al-Said M, et al. Pharmacological studies on aerial parts of Calotropis procera. The American journal of Chinese medicine. 1991;19(03n04):223-31.
15. Meena AK, Yadav A, Rao M. Ayurvedic uses and pharmacological activities of Calotropis procera Linn. Asian journal of traditional medicines. 2011;6(2):45-53.
16. Paul A, Kumar A. Review on pharmocological properties of Aaka (Calotropis procera). International Journal of Economic Plants. 2018;5(3):157-62.
17. Singh S, Singh S, Mishra R, Shrivastava MP. Preliminary phytochemical screening of Calotropis gigantea leaf. International Journal of Scientific and Research Publications. 2014;4(2):1-3.
18. Oloumi H. Phytochemistry and ethno-pharmaceutics of Calotropis procera. 2014.
19. Mandal SC. Evaluation of anti-inflammatory and antinociceptive activity of methanol extract of Calotropis gigantea root. International Journal of Green Pharmacy (IJGP). 2017;11(03).
20. Sharma R, Thakur GS, Sanodiya BS, Savita A, Pandey M, Sharma A, et al. Therapeutic potential of Calotropis procera: A giant milkweed. ISOR J Pharm Bio Sci. 2012;4(2):42-57.
21. Sharma P, Sharma J. In-vitro schizonticidal screening of Calotropis procera. Fitoterapia. 2000;71(1):77-9.
22. Arya S, Kumar VL. Antiinflammatory efficacy of extracts of latex of Calotropis procera against different mediators of inflammation. Mediators of inflammation. 2005;2005(4):228-32.
23. Pathak A, Argal A. Analgesic activity of Calotropis gigantea flower. Fitoterapia. 2007;78(1):40-2.
24. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantea R. Br. in experimental animals. J Pharm Pharm Sci. 2004;7(1):70-5.
25. Argal A, Pathak AK. CNS activity of Calotropis gigantea roots. Journal of ethnopharmacology. 2006;106(1):142-5.
26. Lodhi G, KUMAR SINGH H, Pant KK, Hussain Z. Hepatoprotective effects of Calotropis gigantea extract against carbon tetrachloride induced liver injury in rats. Acta Pharmaceutica. 2009;59(1):89-96.
27. Bairagi SM, Ghule P, Gilhotra R. Pharmacology of Natural Products: An recent approach on Calotropis gigantea and Calotropis procera. 2018.
28. Halawany HS. A review on miswak (Salvadora persica) and its effect on various aspects of oral health. The Saudi dental journal. 2012;24(2):63-9.
29. Khatak M, Khatak S, Siddqui A, Vasudeva N, Aggarwal A, Aggarwal P. Salvadora persica. Pharmacognosy reviews. 2010;4(8):209.

30. Farooqi M, Srivastava J. The tooth-brush tree (Salvadora persica). Quarterly Journal of Crude Drug Research. 1968;8(4):1297-9.
31. Ahmad H, Rajagopal K. Biological activities of Salvadora persica L. Meswak) Med Aromat Plants. 2013;2(4):1-5.
32. Gautam RK, Sharma S, Sharma K. Comparative evaluation of anti-arthritic activity of Salvadora persica linn. and Asparagus racemosus willd: an in vitro study. Indo Am J Pharmaceut Res. 2013.
33. Kamal SA, Mohammed GJ, Hameed IH. Antimicrobial, Anti-inflammatory, Analgesic Potential and Cytotoxic Activity of Salvadora persica: A review. Indian Journal of Public Health Research and Development. 2018;9(3):393-8.
34. Ramadan KS, Farid HE, Almarashi RM. Antidepressant-like effects of aqueous extract of Salvadora persica in rat model of depression. Journal of Diabetes and Metabolism. 2016;7(8):697.
35. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi medical journal. 2015;36(5):530.
36. Kumar M, Saurabh V, Tomar M, Hasan M, Changan S, Sasi M, et al. Mango (Mangifera indica L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants. 2021;10(2):299.
37. Naeini A, Naderi NJ, Shokri H. Analysis and in vitro anti-Candida antifungal activity of Cuminum cyminum and Salvadora persica herbs extracts against pathogenic Candida strains. Journal de Mycologie Médicale. 2014;24(1):13-8.
38. Ahmad M, Imran H, Yaqeen Z, Rehman Z-U, Fatima N, Sohail T. Pharmacological profile of Salvadora persica. Pakistan journal of pharmaceutical sciences. 2011;24(3).
39. Kumar D, Sharma PK. Traditional use, phytochemicals and pharmacological activity of Salvadora persica: A Review. Current Nutrition & Food Science. 2021;17(3):302-9.
40. HOOR T, AHMED M, SHAIKH JM, REHMAN AB. ANALGESIC ACTIVITY OF SALVADORA PERSICA IN MICE. Medical channel. 2011;17(4).
41. Verma R, Purohit S, Bhandari A, Kumar B, Priyanka P. Salvadora persica L (tooth brush tree): a review. Journal of Pharmacy Research. 2009;2(12):1809-12.
42. Mustafa A, Sohaib AU, Hafeez K, Sohail R, Saleem I. International Journal of Pharmacy & Integrated Health Sciences (ISSN: 2789-2840).
43. Iwanowicz-Palus G, Polska P, Polski P, Świst D, Zarajczyk M, Bień A. Herbal medicine as an element that supports new technologies in medicine. EJMT. 2019;2:23.
44. Dkhil MA, Thagfan FA, Abdel-moniem SH, Al-Shaebi EM, Abdel-Gaber R, Al-Quraishy S. Anthelmintic, anticoccidial and antioxidant activity of Salvadora persica root extracts. Saudi Journal of Biological Sciences. 2019;26(6):1223-6.
45. Fatima N, Iqbal W, Yaqeen SS. Evaluation of wound healing effects between Salvadora persica ointment and Solcoseryl jelly in animal model. Pak J Pharm Sci. 2015;28(5):1777-80.
46. Abdel-Baki A-AS, Almalki E, Mansour L, Al-Quarishy S. In vitro scolicidal effects of Salvadora persica root extract against protoscolices of Echinococcus granulosus. The Korean journal of parasitology. 2016;54(1):61.
47. Ashraf K, Halim H, Lim SM, Ramasamy K, Sultan S. In vitro antioxidant, antimicrobial and antiproliferative studies of four different extracts of Orthosiphon stamineus, Gynura procumbens and Ficus deltoidea. Saudi journal of biological sciences. 2020;27(1):417-32.
48. Tyagi T, Agarwal M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of Pistia stratiotes L. and Eichhornia crassipes (Mart.) solms. Journal of Pharmacognosy and phytochemistry. 2017;6(1):195-206.
49. Masoumian M, Zandi M. Antimicrobial activity of some medicinal plant extracts against multidrug resistant bacteria. Zahedan Journal of Research in Medical Sciences. 2017;19(11).
50. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016;6(2):71-9.
51. Moharram H, Youssef M. Methods for determining the antioxidant activity: a review. Alexandria Journal of Food Science and Technology. 2014;11(1):31-42.
52. Fouda A, Saad E, Salem SS, Shaheen TI. In-Vitro cytotoxicity, antibacterial, and UV protection properties of the biosynthesized Zinc oxide nanoparticles for medical textile applications. Microbial pathogenesis. 2018;125:252-61.
53. Salem MZ, Behiry SI, Salem AZ. Effectiveness of root-bark extract from Salvadora persica against the growth of certain molecularly identified pathogenic bacteria. Microbial pathogenesis. 2018;117:320-6.
54. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. Frontiers in microbiology. 2019;10:911.
55. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. Food chemistry. 2013;136(1):237-44.
56. Joo SY. Antioxidant activities of medicinal plant extracts. Journal of the Korean society of food science and nutrition. 2013;42(4):512-9.

[Citation Hussain, A., Bano, S.A., Rehman, Z.U., Waheed, F., Bashir, T., Alam, S., Nawaz, U. (2024 Phytochemical screening, antimicrobial and anti-oxidant activities of salvadora persica and calotropis gigantea extracts against selected pathogens. *Biol. Clin. Sci. Res. J.*, **2024***: 1241*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1241]