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THERAPEUTIC EFFECTS OF *SALVADORA PERSICA* EXTRACTS AGAINST *SARCOPTES SCABIEI VAR. HOMINIS* AND THEIR SECONDARY INFECTIONS

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Abstract
Salvadora persica, commonly recognized as the Miswak tree, happens in shrub savannah. From northwestern areas. The current evaluation gives a complete summary of the chemical materials and organic results (antibacterial and anti-parasitic specially scabies) of this species. To examine the qualitative and quantitative analyses of methanolic and ethanolic extracts to screen the phytochemical contents, sulfur containing compound present in *Salvadora persica* against scabies and secondary bacterial infection. Numerous compounds were identified using GCMS analysis, biological activities, Molecular docking and anti-bacterial activity of stem extracts. It contributes to widespread disease brought on by secondary bacterial infections and post-infectious conditions such acute post-*streptococcal* glomerulonephritis *Sarcoptic* scabies mites' variant *Hominis* can be killed by sulfur-containing compounds. The extract of *Salvadora persica* have an effect against scabies and the subsequent bacterial infection inside the single dose. They can protect against secondary bacterial scabies infections. The potential interaction and binding affinity between ligands *2-(2 Methyl vinyl) thiophene, Benzene, (Isothiocyanatomethyl)* and *Benzyl Nitrile* and protein (3h7t) were analyzed by Auto dockvina. The ligand tended to bind with the binding energy -4.3kcal/mol, -5.4kcal/mol and -5.7kcal/mol respectively.

Keywords: *Salvadora Persica*, Secondary infections, Scabies, *Sarcoptes scabie var. Hominis.*

Introduction

Medicinal herbs play a vital role in the health and therapy of human beings. These natural bioactive compounds show fewer side effects than those found in synthetic drugs, and their antioxidant properties are the cause of their different therapeutic properties, which can be attributed to their bioactive components [1]. Medicine has been derived from plants for thousands of years to treat a variety of diseases. Plants have been used as medicine in Indian, Egyptian, Chinese, Greek, Roman, and Greek-Roman systems of medicine for a long time [2]. Around 80% of the developing world's population relies on traditional medicines for their primary health care, according to the World Health Organization (WHO). Bioactive compounds found in medicinal plants have a variety of therapeutic properties. Plants possess a wide range of therapeutic properties, including anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic effects [3]. *S. persica* is additionally called as Arak in Arabic language and Peelu in Urdu. The phytochemical examination of its different parts yielded diverse classification of secondary metabolites like glycosides, flavonoids, sterols, terpenes alkaloids and carbohydrates. Organic sulfur containing compounds are showed. Additionally, tremendous investigate is being done on its natural potential and mechanical applications [4].

Numerous pharmacological exercises have been detailed tentatively, counting antimicrobial, antioxidant, pain relieving, anti-inflammatory, anthelmintic, antiulcer, and narcotic, anticonvulsant, osteoporotic, anti-diabetic, hypolipidemic, in expansion to wound healing, antitumor, and scabies. [5]. According to GC-MS analysis of the volatile compounds extracted from *Salvadora persica* L. leaves, we detected the presence of benzyl nitrile, eugenol, thymol, isothymol, eucalyptol, isoterpinolene, and beta-caryophyllene. Those bioactive constituents play a significant role in preventing tooth decay as well as being an effective natural tool for teeth cleaning . The plant extract can also be used as an analgesic for toothache [6] . *S. persica* is a type of woody stick and its properties have shown to be very favorable and pharmacological very important plant with numerous important pharmacologically activities for oral hygiene that protects, analgesic, bacterial, fungal [7] and different diseases [8]. The bioactive components that protect the bacterial infection from oral cavity [9]. In reported studies, agents have been shown to have to prevent from physiological reaction and risk of cancer [10]. The widespread relationship between cancer protective agent and antioxidant substance has been observed that antioxidant composition is huge proportion of cell reinforcement efficiency in *S. persica* [11].Plaque Progress can be treated using these remedies for both bacterial infections and gingivitis [12]. *S. persica additionally* shows inhibitory activities and indicates cariogenic *S. mutans* contrast and control fluctuation [13]. Basic cariogenic microorganisms in human viridian *streptococci*, for example, *Staphylococcus mitis and Staphylococcus mutans*, are resistant to anti-microbes [14]. Pharmacological studies show that *S. persica* had the ability to cure anti-inflammatory, anti-microbial, aphrodisiac, analgesic, anti-plaque, alexiteric, diuretic, anti-pyretic and bitter stomach performance [15]. Troubles in nose, scabies, piles, leukoderma, gonorrhea, boils, scurvy, venereal disease, pain in tooth, hook worm, rheumatism, cough, asthma, high cholesterol plasma levels, stress induced ulcer, issues related to spleen functioning, epilepsy, skin diseases along with pain in joints [16]. Scabies, a human skin infection caused by invasion with in humans, the mite *Sarcoptes scabiei* critical morbidity and mortality through coordinate impacts and secondary bacterial infection [17]. The condition of scabies is frequently left out from global health plans and its substantial impact on health is inadequately recognized, potentially leading to its complete neglect. [18]. Global efforts are needed to control this common pathogenic mite in order to be successful. The complications and secondary effects of scabies remain undervalued, despite the fact that they have a major threat to the public's health. Bacterial skin infections are commonly caused by the invasion of *P. aeruginosa* and *S. aureus.* These bacterial skin infections tend to have both true suppurative and non-suppurative aftereffects. [19]. Scabies infestation gives a vital entrance of section for microbes, and complement inhibitors from scabies mites advance bacterial development in vitro [20]. Skin exposure by bacteria increases the risk of sepsis and invasive infections. Skin infection cause by *P. aeruginos* can to lead to the nonsuppurative complications of intense post *streptococcal* glomerulonephritis (APSGN) and conceivably intense rheumatic fever [21]. Skin disease is dependable for around fifty percentage of

APSGN in tropical settings World Health Organization (2005), evaluated more than 470,000 cases per year [22].A current study was conducted in order to investigate the bio potential of valuable biologically active phytoconstituents present in the medicinal plant *S. persica*. To cure scabies caused by microscopic mites, as well as secondary bacterial infections caused by *Staphylococcus aureus* and *Pseudomonas auriginosa*, and to reduce the inflammation caused by scabies infection to identify secondary metabolites in medicinal plants that may serve as candidate drug molecules.

MATERIALS AND METHODS

Plant collection Fresh stem of *S. persica* was obtained from the Vehari City of Pakistan. Identifying and authenticating the specimens were Dr. Muhammad Naeem (Taxonomist), Department of Botany, Government College University, Faisalabad, Pakistan

Extraction and fractionation *S. persica* was washed with distilled water and then treated with liquid nitrogen for crushing and drying. They were then pulverized with a pestle and mortar. An electric grinder was used to make a fine powder. The methanolic and ethanolic extract was made by dissolving 100 g of powder in 400 mL of 100% methanol and ethanol. It was shaken for 72 h at 37 °C on an orbital shaker. After the plant materials were settled, the supernatant solution containing plant compounds was collected and filtered. The crude methanolic extract was produced by rotational evaporation of the isolated and purified supernatant [32].

Qualitative Screening of phytochemicals

Extract of plant in different solvents were subjected to different qualitative test in order to detect the various Phyto constituents by using following standard protocol including Benedict`s Test, Hager`s Test and Wagner's tests etc**.** (Raaman *et al*., 2006, Singh *et al*., 2017), (Silva GO *et al*., 2017, Raaman *et al*., 2006), (Tiwari *et al*., 2011), (Audu *et al*., 2007, Singh *et al*., 2017).

Results were expressed as:

+	++	+++	++++	_
GOOD	VERY GOOD	V.V GOOD	Excellent	Absent

Quantitative Analysis

GC-MS analysis

Metabolite identification by GC-MS analysis Metabolite profile of *S. persica* leaf extract analyzed by GC-MS. The Instrument used, Agilent Technologies GC systems with GC-7890A/MS-5975C model with DB-5MS column (30 m in length x 0.25 um in diameter x 250 um in thickness. The oven temperature was kept at 50 °C for 1 minute and the temperature steadily increased to 25 °C/min to 120 °C for 5 min and luL sample was introduced for analysis (Fig. 3.4). Helium gas 99.9 % was used as the carrier gas, the flow rate of carrier gas was 1 mL/minute sample injected temperature was upheld at 230 °C and the split ratio is 20 during the experiment period. The ionization mass was done with 70 eV. The mass spectra were recorded for the mass range 55-416 m/z for 26 minutes. The compounds appearing with different peaks were identified by comparing their mass spectra. During elution through the column they distinguished based on production of electronic signals, specific for each compound appearing in our sample. The calibration of mass to charge ratio was done by comparing it with mass spectrum (fingerprint) obtained for each molecule. Finally, the mass spectra obtained for each compound was compared with the PubChem and NIST library [33] **[34]**.

Anti-Diabetic Activity

The protocol given by Dessalegn et al.[35] was used for the antidiabetic activity by the DNSA assay, and 200 µL methanol and ethanol crude extract were mixed with 200 µL of α-amylase solution. The reaction mixture was incubated for 10 min at 25 °C. Following the incubation, 200 µL of 1% starch solution was added and further incubated for 10 min. After this, 400 µL of DNSA solution were

added, and the absorbance was checked by ELISA at 630 nm. Distilled water was used as a negative control, and metformin as the standard.
% Inhibition = Abs control − Abs sample/Abs control × 100.

Anti-oxidant Activity
The antioxidant potential of *S. persica* was measured using the method described. Through a microassay with some modifications, S. persica whole fruit hydro-methanolic extract was tested for its antioxidant potential using DPPH radical. The assay volume was adjusted to 100 µL. Totals of 90 µL of DPPH solution and 10 µL of different extract concentrations were placed in wells of a 96-well microplate. DPPH with methanol was taken as a negative control, while ascorbic acid was taken as positive control [36].

Anti-Inflammatory Activity
The approach described by Williams et al.[37] was modified to test the impact of the anti-inflammatory activities. Different concentrations of fractions 12, 6, 3, 1.5, 0.75, and 0.37 mg/mL were prepared. 100 µL of the sample was taken inside centrifuged tubes, and 0.5 mL of bovine serum albumin BSA was added. The mixture was incubated at 37° C for 20 min and then kept in a water bath for 10 min at 70 °C. The tubes were cooled down, and absorbance was checked in an ELISA reader at 630 nm in ELISA plates. The percentage inhibition was checked by the given formula [37].
% BSA inhibition = (Abs Control − Abs sample/Abs control) × 100
Anti-microbial activity of different fractions of plant extract was determined against different microbes by using well diffusion method.

Bacterial Strains
Anti-bacterial activity multiple bacterial strains were used to find the anti-bacterial activity of the sample by disc diffusion method, as explain below. Chosen bacterial includes: *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. For preparation of culture, nutrient broth was used. Nutrient broth was prepared by dissolving 13 g of nutrient broth in 1 L distilled water and mixing properly to form a yellow-colored solution. Autoclaved the broth and poured equally in 4 conical flasks and labeled accordingly by picking colony of selective bacterial strains by using sterile loop and transferring it into respective labeled flasks in sterilized laminar flow. Incubate the broth for 24 h at 37 °C and turbidity was observed, which indicates bacterial growth . For preparing agar plates to promote bacterial growth, 23 g of nutrient agar was dissolved in 1000 mL of distilled water in a conical flask and autoclaved. Transferred the agar in sterile petri plates in a laminar flow and left for solidification overnight. Witnessed for contamination, if any, and stored the plates for further testing [32].

Well diffusion susceptibility method
Nutrient agar medium plates were seeded with 18-24-hour-old cultures of microbial inocula (a standardized inoculum of 1-2 × 107 CFU ml-1 0.5 McFarland Standard). Four wells (8 mm in diameter) were cut into the agar media with a sterilized cork borer and then plant extracts in 24, 30 and 36 µl volumes containing 4, 5 and 6 mg were poured into the wells. An antibiotic (24 µl per well) and DMSO (24 µl per well) were also poured into one well each as a positive and negative control, respectively. Inoculated plates were then incubated at 37ºC for 24 hrs and zones of inhibition were measured in mm. Three replicates were prepared for each microorganism [32].

***In-silico* Analysis**
Due to presence of sulfur compound in *S. persica* extract biactive components through GCMS, , molecular docking study was carried out to check the possible interaction and binding affinity between the ligands i.e.

Protein and ligands preparation

Structure of protein and its detail was determined by the databank which is known as PDB. (https://www.rcsb.org.search). The ID of protein is protease paralogue S-D1 (3h7t). This protein is Crystal structure of scabies mite inactivated prot ease paralogue *S-D1*. Above mentioned ligands are used due to presence of sulfur and because of its advantageous biological activities. The ligands Structure were obtained from the PubChem. It is the data base which contains all the information about chemical structures and compounds. The link of PubChem is (https://pubchem.ncbi.nlm.nih.gov/).Receptor ligand interaction was done by using auto dock vina (https://vina.scripps.edu/) and check by the discovery studio (https://discover.3ds.com/discovery-studio-visualizer-download .The interaction visualization of receptor and ligand done by the pymol (https://pymol.org/2/),

Docking Studio

Receptor ligand interaction was done by using auto dock vina (https://vina.scripps.edu/) and check by the discovery studio (https://discover.3ds.com/discovery-studio-visualizer-download

Visualization

The interaction visualization of receptor and ligand done by the pymol (https://pymol.org/2/),

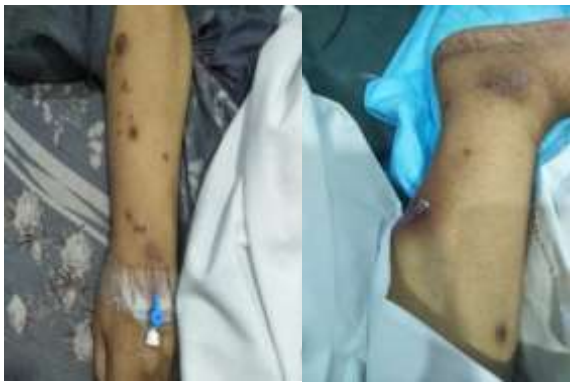
A popular tool for 3D visualization of macromolecules is pymol, a cross-platform molecular graphics program.

Case Study of Immune Compromised Patient

A patient at Lahore Care Hospital had HCV for 11 years, diabetes for 11 years, and high blood pressure for 5 years. She suffered scabies prior to 6 months and three to four time's secondary infections. Her immune system was severely weakened. When a patient's pus sample is taken and cultured. Clusters of gram-positive *cocci* with high *S.aureus* proliferation are its defining characteristic. To diagnose the infection and carry out antimicrobial activity against that bacterium.

Microbiology report

- **SPECIMEN** = PUS
- **Gram Strain**: Pus cells---... + HPF.
- **Gram positive *cocci* in clusters ----+*+/HPF**
- Heavy growth of *S. aureus* isolate.



Results

Phytochemical Screening,

A qualitative study of *Salvadora persica* extracts revealed the presence of a good profile of phytoconstituents i.e., flavonoids, phenolics, tannins, carbohydrates, steroids, terpenoids, and glycosidessuch as, Alkaloids, Flavonoids, Carbohydrates and Saponins. Steroids was noticed to be absent in all fractions of plant extract.

Quantitative analysis

Quantitative analysis of compounds in fraction of methanol and ethanol extract of *S. persica* was determined by GCMS as shown in the table 3 and 4. There are many compounds are separated and identified by GC-MS in methanol fraction and ethanol fraction as following. There are 2 Sulfur containing compounds are identified i.e.,*2-(2-Methylvinyl) thiophene* and *Benzene, (Isothio-cyanato-methyl)* by methanolic fraction, before there is only one compound identified Sulfur containing that is Antioxidant Sulphur-Containing Imidazoline Alkaloid from *S. persica* roots [23].

Biological Activities

Numerous biological activities of *S. persica* were performed by using different methods.

Antioxidant Activity

Antioxidant potential of different fractions of methanolic and ethanolic extract of *S. persica* was evaluated. In DPPH Scavenging activity, continuous variation in results was shown on the table5. The methanol extract 96% ± 0.0011 3 mg/100 ml, Ethanolic extract showed 95% ±0.0087 at 1.8 mg/60 ml. Ascorbic acid as standard was run too and showed maximum 89% ± 0.063 at 3 mg/100 ml. The concentration at which a drug exerts half of its maximum inhibitory action is known as the IC_{50} an antagonist of a biological process is often described by this value. In which methanol, ethanol and Ascorbic acid vale of IC_{50} are 7.7994, 3.0474 and 1.107 respectively (Table 2, Graph 1).

Anti-Diabetic Activity

Anti-diabetic potential of methanolic extract and ethanolic extract with different concentration of *S. persica* were analyzed by ELIZA. Methanolic extract showed 89%± 0.114 at 3 mg/100 ml, Ethanolic extract showed 88% ± 0.0063 0.9 mg/30 ml and standards were run too that is metformin which showed 69% ±0.0378 at 1.8 mg/60 ml. In which methanol, ethanol and Ascorbic acid vale of IC_{50} are 3.6741, 2.096 and 3.4208 respectively (Table 3, Grapg 2).

Anti-Inflammatory Activity

Anti-inflammatory potential of methanolic extract and ethanolic extract with different concentration of *S. persica* were analyzed by ELIZA. Methanolic extract showed 76%±0.109
3 mg/100 ml, Ethanolic extract showed 61%±0.00251 at 1.8 mg/60 ml and standard was run too that is NSAID which showed 91%±0.119 at 0.9 mg/30 ml. These values were analyzed at the wavelength of 630 nm. In which methanol, ethanol and Ascorbic acid vale of IC_{50} are 1.7296, 0.7861and 0.7477 respectively(Table 4).

At wavelength 490 nm

At the wave length of 490 nm the methanolic extract showed 61%±0.0025 at 3 mg/100 ml, Ethanolic extract showed 52%±0.0005 at 0.9 mg/30 ml and standard that is NSAID it showed maximum activity 90% ±0.0020 at 0.9 mg/30 ml. In which methanol, ethanol and Ascorbic acid vale of IC_{50} are 1.6271, 3.1012 and 1.0935 respectively (Table 5).

Antimicrobial activity

Antimicrobial activity against *S. aureus and P. aeruginosa* with methanolic extract and ethanolic extract, concentrations are (5 µg into 10 ml distil water and 3 µg into 10 ml distil water) Control is DMSO which are in central Position which show no any activity. In Fig 5a) Petri plate contain *S.aureus*. Methanolic extract with 5 µg into 10 ml distil water show good activity as compare to 5 µg Ethanolic Extract against *S.aureusand 3* µg Methanolic extract show more activity as compare to the sample 4 which is 3 µg into 10 ml distil water. In Fig 5b) Petri plate contain *P. aeruginosa* . Methanolic extract with 5 µg into 10 ml distil water show good activity as compare to 5 µg Ethanolic Extract against *P. aeruginosa and 3* µg Methanolic extract show more activity as compare to the sample 4 which is 3 µg into 10 ml distil water (Fig. 3).

Serial No.	Test Name	Results	M	E
01	**Benedict`s Test (Reducing Carbohydrates)**	Dark brown color indicates the presence of Reducing Carbohydrates	++++	++
02	**Hager`s Test**	Light Yellow Color Indicates the Presence of Alkaloids.	+++	+++
03	**Wagner`s Test**	Reddish brown color indicates the presence of alkaloids.	+++	+++
04	**Foam Test (Saponins Test)**	Foam indicates the presence of saponins	+++	++
05	**Steroid Test**	No Presence of lower reddish Brown +layer / No results	-	-
06	**Flavonoids test**	Intense yellow to become colorless solution indicate the presence of flavonoids	++++	++++

Table 1: Phytochemical analysis and their indications showing the presence of plant Compounds mentioned as above.

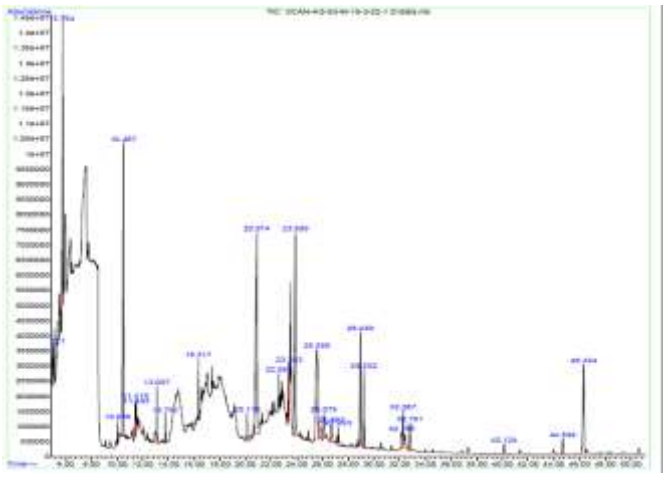


Figure 1: GC-MS Methanolic Components Graph

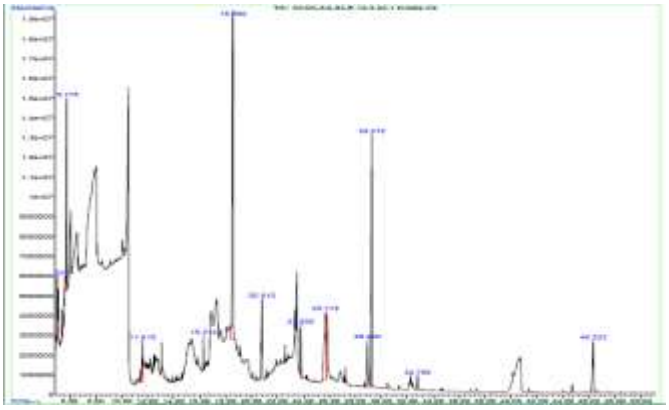


Figure 2: GC-MS Ethanolic Components Graph

Extracts	Concentration	Percentage/

		Standard Deviation	IC_{50}
Methanol	3 mg/100 ml	96 ±0.0011	
	1.8 mg/60 ml	95± 0.0015	7.7994
	0.9 mg/30 ml	96±0.0011	
Ethanol	3 mg/100 ml	94±0.021	
	1.8 mg/60 ml	95±0.0087	3.0474
	0.9 mg/30 ml	95±0.0005	
Ascorbic acid			
(Standard)	3 mg/100 ml	89± 0.063	
	1.8 mg/60 ml	89± 0.026	1.107
	0.9 mg/30 ml	86± 0.0320	

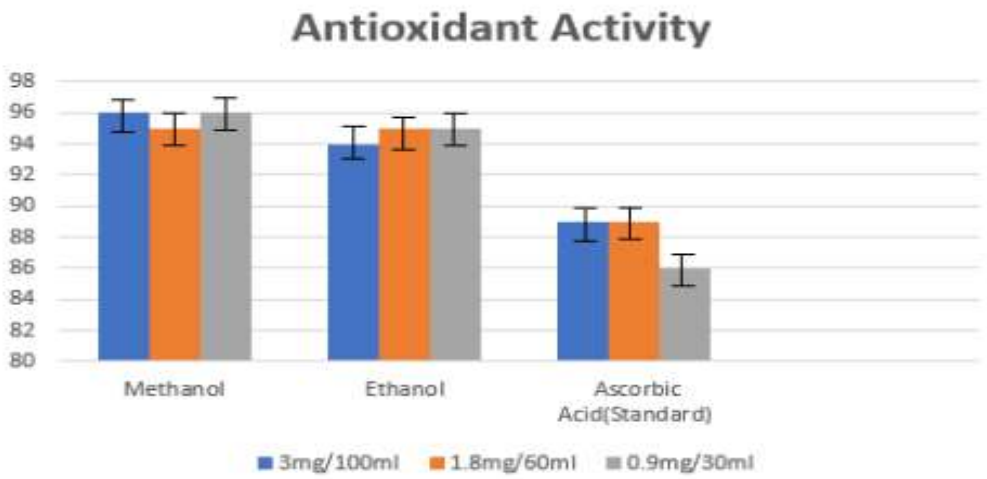
Table 2: Anti-oxidant activity results with different concentration, Percentage and their standard deviation

Extracts	Concentration	Percentage/ Standard Deviation	IC_{50}
Methanol	3 mg/100 ml	89± 0.114	
	1.8 mg/60 ml	87±0.0005	3.6741
	0.9 mg/30 ml	88±0.0015	
Ethanol	3 mg/100 ml	81±0.0274	
	1.8 mg/60 ml	87±0.0199	2.096
	0.9 mg/30 ml	88±0.0063	
Metformin			
(Standard)	3 mg/100 ml	63 ±0.2973	
	1.8 mg/60 ml	69 ±0.0378	3.4208
	0.9 mg/30 ml	65±0.0049	

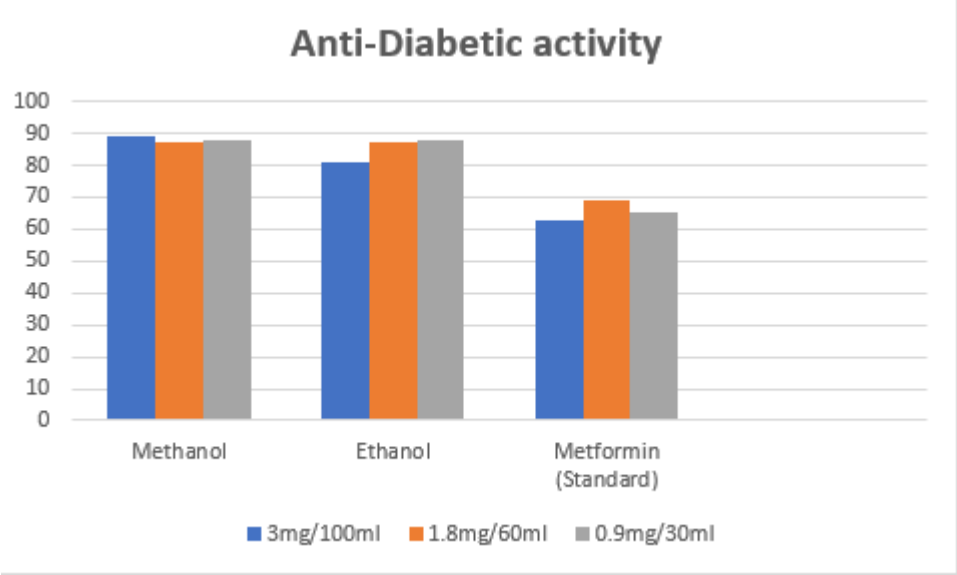
Table 3: Anti-Diabetic activity results with different concentration, Percentage and their standard deviation

Extracts	Concentration	Percentage/ Standard Deviation	IC_{50}
Methanol	3 mg/100 ml	76 ±0.109	
	1.8 mg/60 ml	69 ±0.0135	1.7296
	0.9 mg/30 ml	58 ±0.054	
Ethanol	3 mg/100 ml	56 ±0.0096	
	1.8 mg/60 ml	61 ±0.00251	0.7861
	0.9 mg/30 ml	54 ±0.0035	
NSAIDS			
(Standard)	3 mg/100 ml	88 ±0.054	
	1.8 mg/60 ml	87 ±0.0282	0.7477
	0.9 mg/30 ml	91 ±0.119	

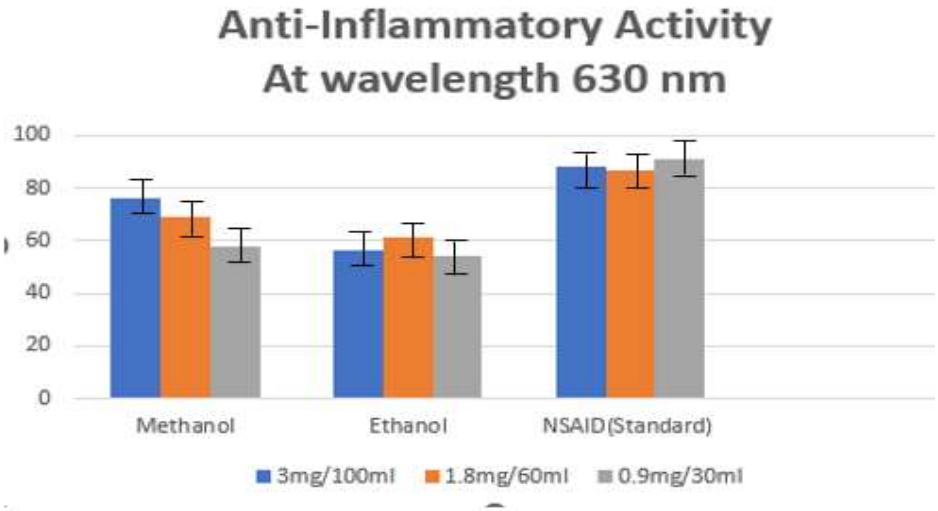
Table 4: Anti-Inflammatory activity results with different concentration, Percentage and their standard deviation at the wave length of 630



Graph 1: Percentage of Anti-oxidant activity of Methanolic, Ethanolic extract and Ascorbic acid



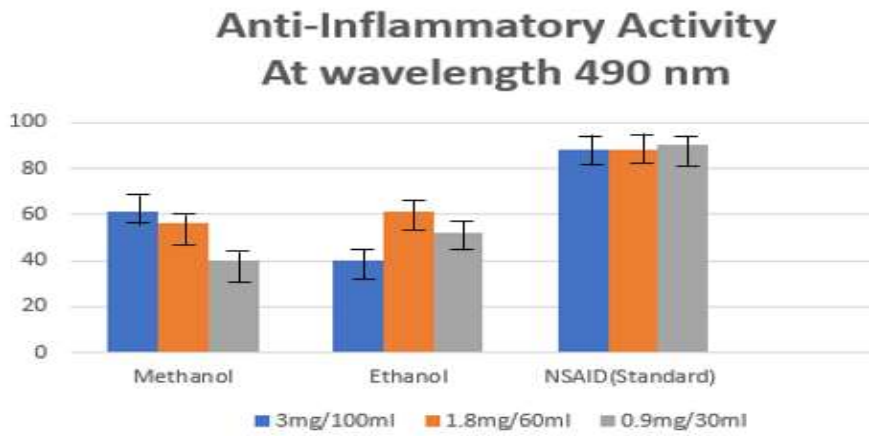
Graph 2: Percentage of Anti diabetic activity of Methanolic, Ethanolic extract and Metformin.



Graph 3: Percentage of Anti-Inflammatory activity of Methanolic, Ethanolic extract and NSAID at the wavelength of 630nm.

Extracts	Concentration (mg/ ml)	Percentage/ Standard Deviation	IC_{50}
Methanol	3 mg/100 ml	61 ±0.0025	
	1.8 mg/60 ml	56 ±0.0147	1.6271
	0.9 mg/30 ml	40 ±0.008	
Ethanol	3 mg/100 ml	40 ±0.0136	
	1.8 mg/60 ml	61 ±0.0285	3.1012
	0.9 mg/30 ml	52 ±0.0005	
NSAID(Standard)490nm	3 mg/100 ml	88 ±0.0381	
	1.8 mg/60 ml	88 ±0.0121	1.0935
	0.9 mg/30 ml	90±0.0020	

Table 5: Anti-Inflammatory activity results with different concentration, Percentage and their standard deviation at the wavelength of 490nm



Graph 4: Percentage of Anti-Inflammatory activity of Methanol, Ethanolic extract and NSAID at the wavelength of 490nm

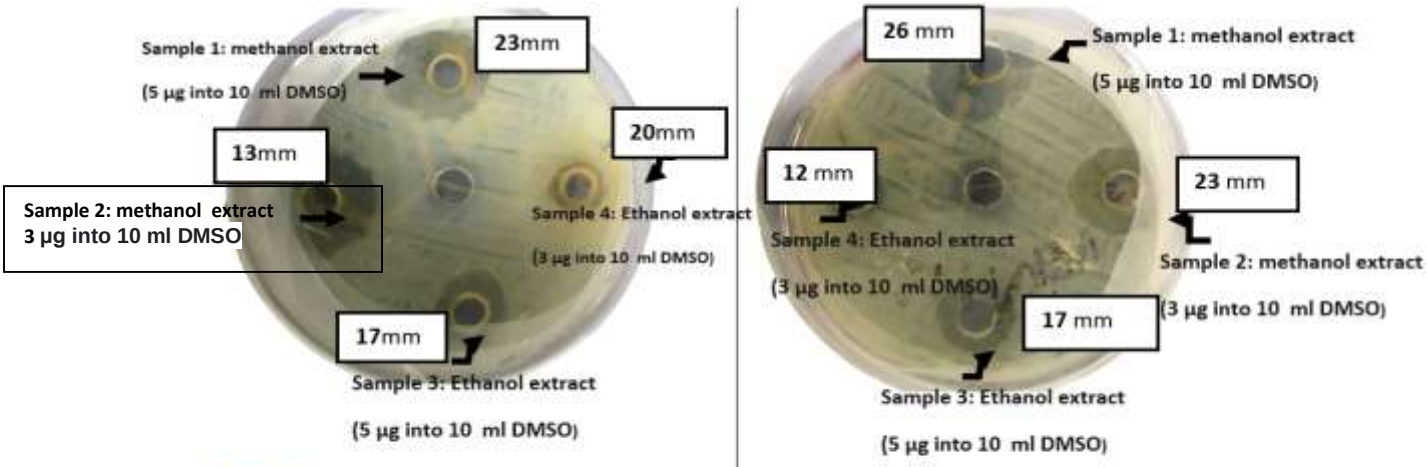


Figure a) Antimicrobial Activity against *S.aureus* with methanolic Extract and Ethanolic extract with the concentration of (5 µg into 10 ml DMSO and 3 µg into 10 ml DMSO). Control is DMSO which is in central Position.

Figure b) Antimicrobial Activity against *P. aeruginosa* with Methanolic extract and Ethanolic Extract with concentration of (5 µg into 10 ml DMSO and 3 µg into 10 ml DMSO). Control is DMSO which is in central Position.

Figure 3: Results of Antimicrobial Activity against *S. aureus* and *P. aeruginosa* with methanolic Extract and Ethanolic extract with the concentration

Sr. no	Bacterial Strains	Antibacterial Activity	
	Methanolic Extract	**5 µg**	**3µg**
1.	*S. aureus*	+++	+
2.	*P. aeruginosa*	+++	+

Sr. no	Bacterial Strains	Antibacterial Activity	
	Ethanolic Extract	**5 µg**	**3µg**
1.	*S. aureus*	+++	+
2.	*P. aeruginosa*	+++	+

+++ = Very good Activity	+ = Less Activity

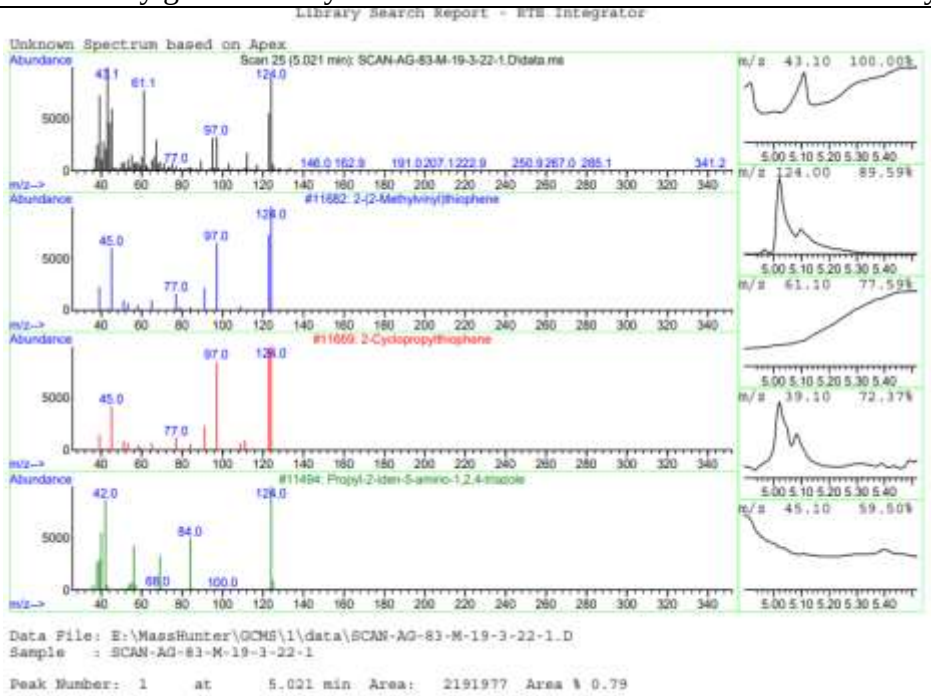


Figure 4: *Methyl-vinyl-thiophene* identified by the GC-MS

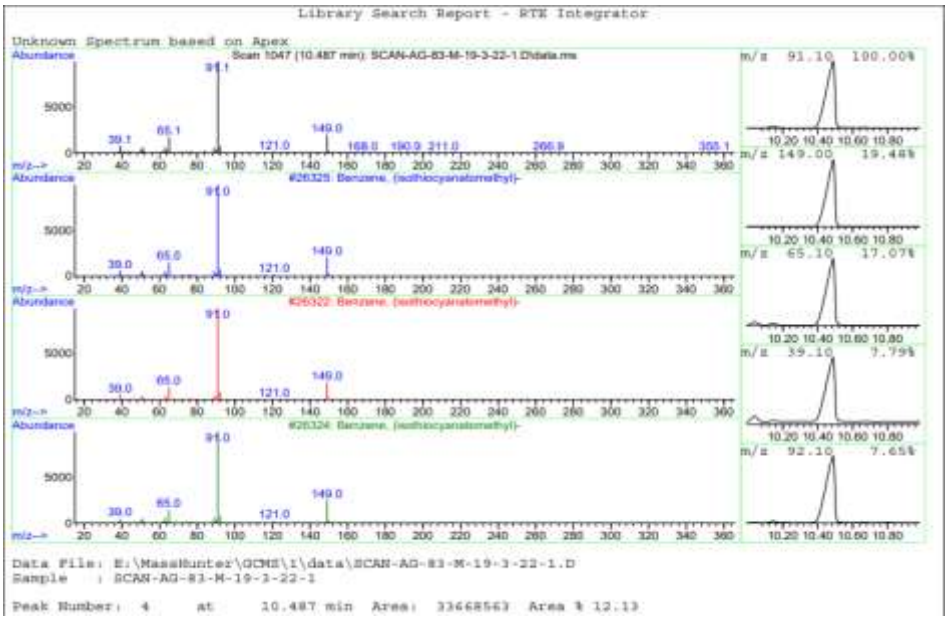


Figure 5: *Benzene, (Isothio-cyanato-methyl)* identified by the GC-MS

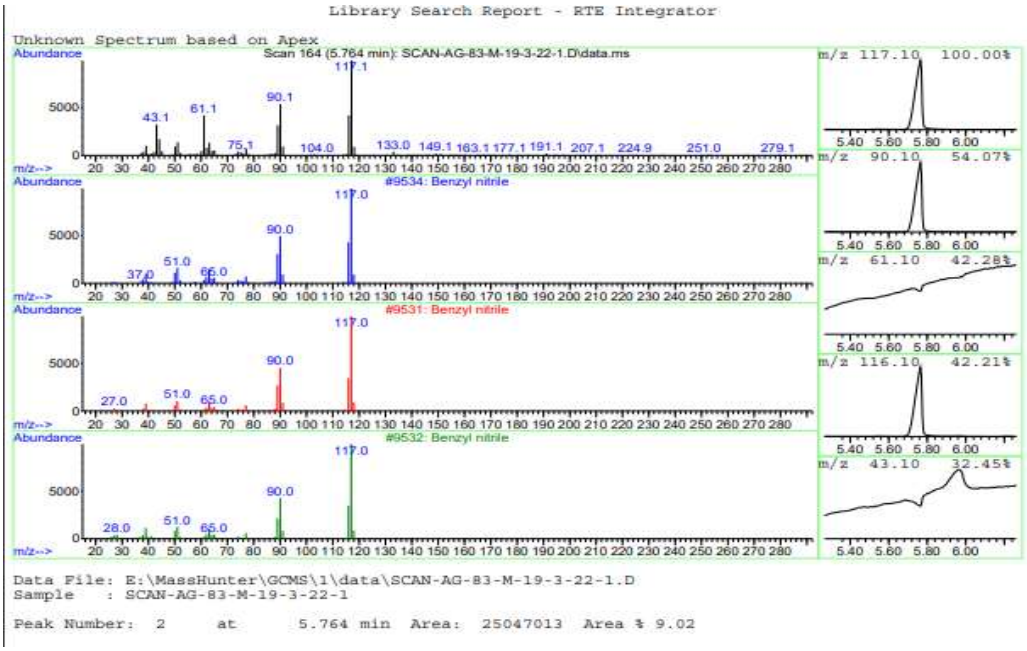


Figure 6: *Benzyl Nitrile* identified by the GC-MS

In silico Analysis

Molecular docking of compounds with protease paralogue S-D1 was performed and results show that the Benzyl Nitrile (5.7), Isothio-cyanato-methyl (5.4)show highest binding energy and their intraction are present in figure ..1

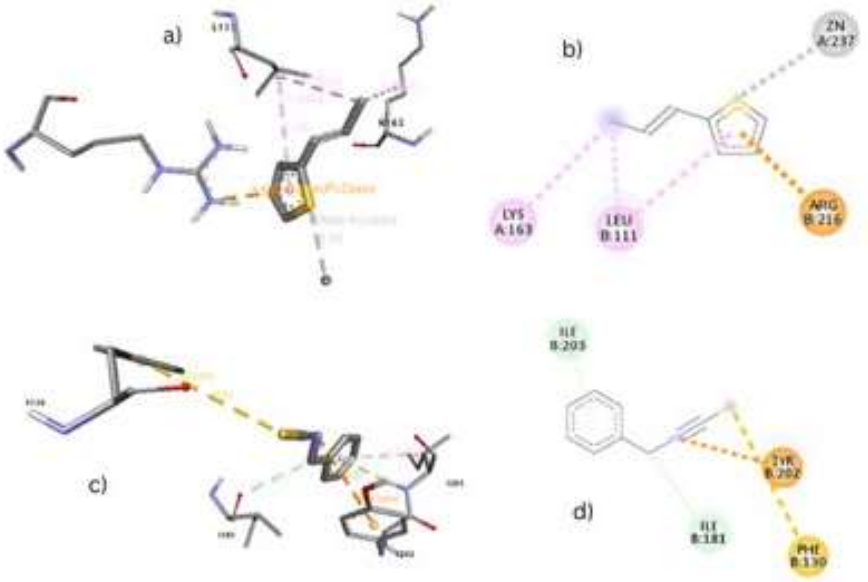


Figure 7. Molecular interaction of protein protease paralogue S-D1 (3h7t). with bioactive components with bioactive compounds (a) show the the 3D image Benzyl Nitrile of(3h7t). (b) showed of 2d image of Benzyl Nitrile of (3h7t). (c) 3D Isothio-cyanato-methyl 3D image Isothio-cyanato-methyl with (3h7t). (d)2d image Isothio-cyanato-methyl of (3h7t).

Discussion

From beginning of mankind, nature is the most potent source of medicines**.** Living organisms produce bioactive compounds. This study assessed the phyto-constituents of *Salvadora persica* fractions through quantitative and qualitative methods. Selected fractions were investigated to find out bio-potential of secondary metabolites through in vitro anti-inflammatory, antidiabetic, antioxidant and antimicrobial activity.

Various biological activities of *S. persica* have been recorded using, in addition to the oil, different extracts of each part of the plant and their parts. Traditionally, as previously indicated, several portions of *S. persica* have been utilized as a primitive medicine for a variety of ailments for centuries. Numerous studies have been conducted in order to comprehend the chemically based action mechanism of these activities.

Numerous pharmacological actions, such as those that are antibacterial, antihelminthic, anti-osteoporosis, anti-inflammatory, healing, antioxidant, anticonvulsant, sedative, hypolipidemic, anti-diabetic, antidepressant, and anticancer, had been experimentally tested.

An overview of the pharmacological actions of the plant on its traditional and phytochemical uses is provided in this part, along with a comprehensive analysis.

Numerous attempts have been undertaken to verify *S. persica* antibacterial potential against diverse microorganism **[24]**). Studies have proven that antibacterial activity of *S. persica* minerals and compounds against oral infections. It has been demonstrated that these substances prevent cariogenic development and acid generation. Additionally, this herbal compound has abilities to prevent plaque. Different Miswak components were examined by being prepared in various solvents, put to agar plates, or poured into agar. Although miswak demonstrated substantial antibacterial activity against all the microorganisms studied in both situations, the effects of the extract were stronger or comparable to those of miswak added in agar. At minimum inhibitory concentrations [MIC] and minimum bactericidal concentrations [MBC] of 3.12 and 6.25 mg/ ml, respectively, the fruit extract has recently shown selective antibacterial efficacy against mutant *Staphylococcus* isolates (Al Bratty *et al*., 2020). *S. persica* root oils, both dried and fresh, have been investigated **[25]**.

The methanolic extract of *S. persica* has been shown to possess a considerable antibacterial activity compared to isolates of *S. aureus* and *Staphylococcus* species (Fig.3), and it can be used as a good alternative method to control oral infections. Interestingly, *S. persica* provided four substances that were extremely effective against MRSA: luteolin, apigenin, astragalin, and kaempferol-3-orhamnoside (methicillin-resistant *S.aureus*).

This particular form of bacterium is harmful since it is resistant to many medications and can lead to sepsis and pneumonia. With IC_{50} values of 10, 11, 5, 3, and 4.5 mg/ ml respectively, the isolated compounds demonstrated adequate effectiveness against MRSA. But the *S. persica* aqueous extract was less effective than the alcohol extract **[26]**.

persica antibacterial properties could be attributed to a number of different phytochemical components. One of these is the powerful radical compound *benzyl isothiocyanate*, which has a substantial anti-gram-negative bacterial effect. It has electrophilic and lipophilic qualities that may allow it to pass through bacterial outer membranes and harm the membrane potential to block bacterial redox system [27]. Compared to each treatment, a mixture of *S. persica* and antibiotics produced much increased antibacterial activity. Present study presented that Bioactive compounds are useful antioxidants that show scavenging activity against reactive oxygen, superoxide and hydroxyl radicals by single-electron transfer that is comparable with standard ascorbic acid. The radical scavenging activity of the sample is closest to the standard at each concentration. DPPH assay was performed to check scavenging activity against free radicals. Fraction revealed the maximum DPPH The methanol extract (96% ± 0.0011) 3 mg/100 ml, Ethanolic extract showed 95% ±0.0087 at 1.8 mg/60 ml as shown in Table 2. Previous findings show the similar results[28]. [28].The α-amylase enzyme mostly present in saliva and pancreatic juice, is responsible for hydrolyzing, polysaccharides like starch. Inhibition of this enzyme helps in the prevention of high blood glucose level. Results showed that methanolic fractions possessed significant enzyme inhibition which is concentration

dependen. Methanolic extract showed 89%± 0.114 at 3 mg/100 ml, Ethanolic extract showed 88% ± 0.0063 0.9 mg/30 ml and standards were run too that is metformin which showed 69% ±0.0378 at 1.8 mg/60 ml (Table 3). Previous studies showed marked enzyme inhibition by secondary metabolites derived from plant fractions [29]. The antioxidant, antidiabetic and antilipidemic activity of Salvadora persica twig in alloxan diabetic male rats). Present study showed Plant fractions with different concentrations have significant anti-inflammatory potential and depending on the dose concentration as shown in Fig 5b. The suppression of albumin denaturation was used to evaluate *Salvadora persica* anti-inflammatory potential (Table 4). Methanol and ethanol fractions of the medicinal plant which lessen albumin denaturation. The defensive effect of *Salvadora persica* fractions against protein denaturation is just like to NSAIDs which play significant anti-inflammatory role in arthritis. Previous findings show similar results[30].

This mite causes scabies disease, which is a serious public health threat. Sarcoptes scabiei (S. scabiei) is a parasite mite that causes scabies disease. The long-term effects of the scabies disease may include septicemia, acute post-streptococcal glomerulonephritis, heart disease, and secondary infections . The safe and cost-effective alternative treatment strategy is the use of medicinal plants which have beneficial therapeutic potential against variety of diseases due to the presence of many bioactive phytoconstituents with no or minimal side effects**1** . In an insilico analysis, it was shown that benzyl nitrile (5.7), and isothio-cyanato-methyl (5.4) have the highest binding energy (Fig. 7), which suggests that they may be effective against the scabies protein protease paralogue S-D1 (3h7t). These Benzyl Nitrile and Isothio-cyanato-methyl compounds may be applied in the future to drug development. [31]

Conclusion

The methanolic extract of *S. persica* is more efficient than the ethanolic extract. Contains sulfur-containing components, which show good concentration according to GC-MS. Sulfur can directly kill the mites, it can absorb and damage the nervous system of the mites and kill them. *S. persica* has many anti-inflammatory components identified by the GC-MS and also confirms from the phytochemical screening test that it contains a good quantity of flavonoids which are responsible for the activity of anti-inflammatory agent and also contain vitamin C which acts as a skin healing process. On the other hand, the most advantageous point of this plant which prevents secondary bacterial infection which is the possibility of *S.aureus* and *P.aeruginosa* which can influence the scabies infestation. So, a single dose of this plant can prevent multiple problems at once. It is an inexpensive and natural treatment.

Conflict of interest:

The authors declare no conflict of interest

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REFERENCES

[1] A. A. Mohamed, B. M. Alotaibi, *Journal of Umm Al-Qura University for Applied Sciences* **2023**, *9*, 40-49.
[2] G. B. Smita, in *Natural Medicinal Plants* (Ed.: A. E.-S. Hany), IntechOpen, Rijeka, **2021**, p. Ch. 12.
[3] M. M. Aye, H. T. Aung, M. M. Sein, C. Armijos, *Molecules (Basel, Switzerland)* **2019**, *24*.
[4] F. Şahin, I. Karaman, M. Güllüce, H. Öğütçü, M. Şengül, A. Adıgüzel, S. Öztürk, R. Kotan, *Journal of ethnopharmacology* **2003**, *87*, 61-65.
[5] M. Farag, W. M. Abdel-Mageed, A. A. El Gamal, O. A. Basudan, *Saudi Pharmaceutical Journal* **2021**, *29*, 751-763.
[6] F. Alali, T. Al-Lafi, *Natural product research* **2003**, *17*, 189-194.
[7] aH. R. Poureslami, A. Makarem, F. Mojab, *Dental Research Journal* **2008**, *4*, 106-110; bV. Rajesh, P. Suresh, B. Anil, P. Purohit, *International Journal of Pharmaceutical & Biological Archives* **2010**, *6*, 9-13.
[8] A. Gupta, S. Verma, P. Kushwaha, S. Srivastava, A. Rawat, *Indian J Pharm Educ Res* **2015**, *49*, 71-75.
[9] A. A. o. Pediatrics, *Peter G, editor* **1997**.
[10] A. Hassan, J. Usman, F. Kaleem, M. Omair, A. Khalid, M. Iqbal, *Malays J Microbiol* **2011**, *7*, 57-60.
[11] J. E. Stoeken, S. Paraskevas, G. A. Van Der Weijden, *Journal of periodontology* **2007**, *78*, 1218-1228.
[12] M. Arora, A. A. Siddiqui, S. Paliwal, *Int J Pharm Pharm Sci* **2013**, *5*, 207-210.
[13] D. Stojković, J. Petrović, M. Soković, J. Glamočlija, J. Kukić-Marković, S. Petrović, *Journal of the Science of Food Agriculture* **2013**, *93*, 3205-3208.
[14] M. Khalil, F. Sonbol, *Nigerian Journal of Clinical Practice* **2014**, *17*, 776-784.
[15] G. C. Galletti, G. Chiavari, Y. D. Kahie, *Rapid communications in mass spectrometry* **1993**, *7*, 651-655.
[16] E. Noumi, M. Snoussi, N. Trabelsi, H. Hajlaoui, R. Ksouri, E. Valentin, A. Bakhrouf, *Journal of Medicinal Plants Research* **2011**, *5*, 4138-4146.
[17] A. A. M. Al-Ghamdi, M. El-Zohri, *African Journal of Biotechnology* **2017**, *16*, 517-527.
[18] M. Thompson, D. Engelman, K. Gholam, L. Fuller, A. Steer, *Journal of Clinical Experimental Dermatology* **2017**, *42*, 481-487.
[19] A. Sofrata, E. M. Santangelo, M. Azeem, A.-K. Borg-Karlson, A. Gustafsson, K. Pütsep, *PLoS One* **2011**, *6*, e23045.
[20] A. Mika, S. L. Reynolds, D. Pickering, D. McMillan, K. S. Sriprakash, D. J. Kemp, K. Fischer, *PLoS neglected tropical diseases* **2012**, *6*, e1563.
[21] A. Hussain, M. Ali, N. Javed, *International Journal of Pharmacy* **2011**, *2*, 103-105.
[22] J. R. Carapetis, A. C. Steer, E. K. Mulholland, M. Weber, *The Lancet infectious diseases* **2005**, *5*, 685-694.
[23] M. Farag, W. M. Abdel-Mageed, O. Basudan, A. El-Gamal, *Molecules (Basel, Switzerland)* **2018**, *23*, 483.
[24] M. Z. Aumeeruddy, G. Zengin, M. F. Mahomoodally, *Journal of ethnopharmacology* **2018**, *213*, 409-444.
[25] M. Khan, H. Z. Alkhathlan, S. T. Khan, *Pathogens* **2020**, *9*, 66.
[26] M. Khalil, M. El-Sabbagh, E. El Naggar, R. El-Erian, *Nigerian journal of clinical practice* **2019**, *22*, 1378-1387.
[27] M. M. Ghoneim, W. M. Afifi, M. Ibrahim, M. Elagawany, M. T. Khayat, M. H. Aboutaleb, A. M. Metwaly, *Pharmacognosy Magazine* **2019**, *15*, 232-237.
[28] S. A. Mohamed, J. A. Khan, *BMC complementary and alternative medicine* **2013**, *13*, 1-6.
[29] H. A. El Rabey, F. M. Almutairi, A. I. Al-Sieni, M. N. Al-Seeni, M. A. Al-Duais, M. I. Sakran, M. A. Elbakry, **2017**.

[30] T. Hoor, R. Farooqu, J. M. Shaikh, N. Karim, *The Professional Medical Journal* **2014**, *21*, 070-074.
[31] M. Petran, D. Dragos, M. Gilca, *Journal of ethnobiology and ethnomedicine* **2020**, *16*, 1-33.
[32] M. Afzal, A. S. Khan, B. Zeshan, M. Riaz, U. Ejaz, A. Saleem, R. Zaineb, H. A. Sindhu, C. Y. Yean, N. Ahmed, *Molecules (Basel, Switzerland)* **2023**, *28*, 1743.
[33] F. P. Casuga, A. L. Castillo, M. J.-A. T. Corpuz, *Asian Pacific Journal of Tropical Biomedicine* **2016**, *6*, 957-961.
[34] A. Nazeer, N. George, *Saudi J Med Pharm Sci* **2017**, *3*, 868-872.
[35] R. Perumalsamy, L. Krishnadhas, *Applied Biochemistry and Biotechnology* **2022**, *194*, 1136-1148.
[36] M. Bourhia, K. Bouothmany, H. Bakrim, S. Hadrach, A. M. Salamatullah, A. Alzahrani, H. Khalil Alyahya, N. A. Albadr, S. Gmouh, A. Laglaoui, *Separations* **2021**, *8*, 114.
[37] A. A. Adedapo, O. O. Falayi, A. A. Oyagbemi, *Journal of basic and clinical physiology and pharmacology* **2015**, *26*, 491-499.