PHYTOCHEMICAL, ANTIMICROBIAL, RADICAL SCAVENGING AND *In-Vitro* BIOLOGICAL ACTIVITIES OF *Teucrium stocksianum* LEAVES

GUL RAHIM [a], RAHMATULLAH QURESHI [b], ALI HAZRAT [a], BASHIR AHMAD [c], AYAZ ALI KHAN [d], TARIQ AZIZ [e], METAB ALHARBI [f] AND ABDULRAHMAN ALSHAMMARI [f]*

[a]*Department of Botany, University of Malakand Chakdara, 18800, Pakistan.*
[b]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.*
[c]*Department of Zoology, University of Malakand, Chakdara, 18800, Pakistan.*
[d]*Department of Biotechnology, University of Malakand Chakdara, 18800, Pakistan.*
[e]*Department of Agriculture, University of Ioannina, 47100 Arta, Greece.*
[f]*Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.*

ABSTRACT

Background: *Teucrium stocksianum* is locally used as home remedy in different parts of the world for the treatment of digestive problems as well as microbial infections. The present study was designed to screen phytochemical constituents and in vitro antimicrobial as well as radical scavenging activities of the leaves extracts from the *Teucrium stocksianum.* **Methods:** Plant samples of the *Teucrium stocksianum* were collected from the growing locality and four different solvents viz., n-hexane, methanol, ethanol, and water were used for the preparation of plant extracts. The in vitro biological activities were investigated against eight human pathogens such as like Bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogen, Escherichia coli, Klebsiella pneumoniae and Pseusdomonas aeruginosa and Fungi: Aspergillus niger and Aspergillus fumigatus using agar well diffusion method and minimum inhibitory concentration. The antioxidant properties were carried out by DPPH and reducing power assays using various concentrations. **Results**: Results revealed that all pathogens were highly susceptible and inhibited by all solvent extracts. The methanolic and ethanolic leaves extracts exhibited promising antimicrobial activity and inhibited the growth of pathogens at par with the standard drugs viz., chloramphenicol for Bacteria and Fluconazole for fungi. Moreover, performance of methanolic and ethanolic extracts was the best amongst all solvents. In terms of antioxidant properties, both methods i.e., DPPH free radicals and reducing power resulted significant activity. **Conclusion:** The Phytochemical screening and biological activities of *Teucrium stocksianum* leaves extracts is reported for the first time. The ethanol, methanol, n-hexane and aqueous extracts of *Teucrium stocksianum* possess significant inhibitory effect against tested pathogens. The present investigation *Teucrium stocksianum* leaves extract contain potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. These results denote in-depth analysis of methanolic and ethanolic extracts to search potential compound responsible for antimicrobial activity. The results of the study support the folklore claim along with the development of new antimicrobial drugs from the plant. The results showed that *Teucrium stocksianum* leaves extracts possess multiple essential phytochemicals. Presence of phenolic compounds in plant extract demonstrates antioxidant activity. Both tests for antioxidant properties exhibited marked effects and can be employed as a potential natural antioxidant agent which may be used for mitigating oxidative stress. Nevertheless, there is need to explore mechanism involved for such kind of activity. The current findings hold up the ethno-pharmacological utilization of plant in the treatment of microbial infections and embrace great perception in the development of unusual antimicrobial and antioxidant agents.

Keywords: *Teucrium stocksianum*, phytochemical constituents, antimicrobial, antioxidant properties.

1. INTRODUCTION

Globally, many herbal plants products have attracted the attention of scientists for exploring new phytochemical compounds and their antioxidant properties. Different plants parts have been examined for their biological activities and in some cases, active substances have been identified and isolated. Common Mountain Germendar (*Teucrium stocksianum Boiss*.) is a member of *Lamiaceae* family. The plant is widely distributed in mountainous regions of Saudi Arabia, Oman [1-2], Iran [3] and Pakistan [2]. This species possessed 15–30 cm size with diffusely branched stem having lush green leaves. The plant is high medicinal value leaves are reported to cure diseases like digestive disorder and inflammatory conditions and diabetes [4] another study reported its use in diabetes and burning feet syndrome [5]. Various plant extracts have been experimentally tested anti-ulcerogenic and cytoprotective properties [6]. In addition, the plant extract is also used for analgesic and anti-inflammatory purposes [4]. Other studies reported its uses for blood purification, hypertension and as well as fits and relived throat disease [7]. Since antiquity, many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which is therefore, should be utilized to combat the disease-causing pathogens [8-10].

With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [11]. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products [12]. This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics [13-14] Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants [15-16]. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens [17].

Medicinal properties of plant extracts have been claimed to lie in their phytoconstituents components comprise alkaloids, flavonoids, phenolic compounds tannins, which produce a proper physiological action on human body. Phytochemicals are the natural bioactive compounds mostly found in medicinal plants. These phytochemicals work with fibers and nutrients to form a part of defense system against stress conditions and various diseases. Phytoconstituents are of two principal groups according to their function i.e., primary, and secondary constituents. Primary constituents include amino acid, proteins, common sugars, and chlorophyll while secondary constituents comprise of alkaloids, saponins, terpenoids, flavonoids, phenolic compounds, tannins and so on [18]. *Teucrium stocksianum* is a medicinal plant that is locally used by the peoples of Malakand Division Pakistan to cure digestive problems as well as infectious diseases. In order to validate ethnobotanical use, present study was carried out for screening phytochemical constituents and antimicrobial as well as antioxidant activities from the leaves of the said plant.

2. MATERIALS AND METHODS

2.1. Plant material:

The plant samples of *Teucrium stocksianum* were collected from Talash (Gumbatka) at latitude 34°46.375 N, longitude 071°56.081 E at the vegetative stage of the plant from the growing localities, Dir (L) Malakand Division, Pakistan in the months of May and June 2011. From the fresh collection Voucher specimen was prepared and identified by Dr. Rahmatullah Qureshi and confirmed through the Flora of Pakistan [19]. The determined voucher was

**Corresponding author email: iwockd@gmail.com*

deposited in the department of Botany, Pir Mehr Ali Shah Arid Agriculture University, and Rawalpindi for reference.

2.2. Preparation of Extracts

Fresh and disease-free leaves were sorted from the plants or undesirable material or plant parts and properly washed 2 -3 times in tap water and once rinsed with distilled water to remove the attached impurity of the plant and then dried in shade. The dried leaves were crushed to powder (80 mesh) by using a grinding mill and kept in refrigerator until use. The resultant powder leaves were then macerated in n-hexane, methanol, ethanol, and water with continuous agitating for 24 hours periods at 37°C. The macerate was filtered with the help of Whatman filter paper No. l. All these selective solvent filtrates were pooled and concentrated at 40°C under reduced pressure by using rotary evaporator until the menstruum evaporated and rendered a greenish color concentrate. These concentrates were designated as crude extracts of the selective solvents. The final traces of solvents were evaporated through water bath. The obtained extracts were then used for phytochemical screening and antioxidant activities

The percentage of crude extracts yield was calculated using the following formula [20]:

$$\text{Yield\%} = \frac{\text{Weight of lyophilized extract}}{\text{Weight of dried flower}} \times 100$$

2.3. Phytochemicals Screening Test:

The phytochemical investigation was carried out on organic (n-hexane, methanol, ethanol) and water extracts, already prepared using standard procedures for the presence of plant constituents such as, alkaloids, tannins, anthraquinone, glycoside, reducing sugar, saponins, flavonoids, phlobatannin, steroid, and terpenoid by following the works of [21-23]. The inferences of the tests were expressed as positive (P) or negative (N) qualitatively.

2.4. Antimicrobial assay

2.4.1 Microorganisms and culture media

Six bacteria strains viz., *Staphylococcus aureus* (ATCC 6538), *S. epidermidis* (ATCC12228), *Streptococcus pyogen* (ATCC BAA-946), *Escherichia coli* (ATCC15224), *Klebsiella pneumoniae* (ATCCUC57) and *Pseusdomonas aeruginosa* (ATCC7221) and two fungi species *(Aspergillus niger* 0198, *A. fumigatus* 0064) were used to study antimicrobial activity. Antimicrobial susceptibility was tested on solid (Agar-agar) media in Petri plates. For bacterial assay nutrient agar (40 gm/L) and for fungus Potato Dextrose Agar (39 gm/L) used for surface colony growth. The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were determined by serial micro dilution test. For bacterial cells growth, suspension culture was prepared at 2% Lauria Broth (LB), and for fungus cells growth, 2.4% Potato dextrose broth (PDB) was used for assessment.

2.4.2. Antibacterial Activity

Antibacterial activity was determined by Agar-well diffusion assay [24]. For this purpose, Lauria Bertini agar media was prepared and autoclaved at 121°C for I5 minutes which was then cooled and poured in autoclaved Petri plates under sterilized conditions of the safety chamber [25]. Wells of 6mm diameter were bored in each plate by a sterile borer. Bacterial inoculums were prepared from overnight grown cultures (24 h) in Luria broth and the turbidity was adjusted equivalent to approximately 1.2x108 CFU/ml [26]. Each bacterial suspension (30 µl) was spread uniformly over the surface of Lauria-Bertini agar [25] plates with a glass spreader containing 4 wells of 6 mm diameter. The wells were filled with 75 µl each of the extracts. The extracts were allowed to diffuse in to the medium at room temperature. The plates were incubated at 37°C for 24 h. this method depends on the diffusion of various extracts from a cavity through the solidified agar layer of petri plate to an extent, so that the growth of the inoculated microorgisms is prevented entirely in circular area or Zone around the cavity containing the extracts. The results were expressed in terms of the diameter of the inhibition zone [24]. DMSO was used as negative control, and chloramphenicol as positive control. All the assays were done in triplicate and the results were given in mean ± S.D.

2.4.3. Antifungal Activity

Antifungal activity was determined by agar tube dilution method [27]. Plant extracts dissolved in DMSO were diluted in 1.5 ml of sterile Sabouraud dextrose agar, and allowed to solidify in slanting positions. Control containing the solvent alone and positive control with Flucanazole were applied. Test fungal cultures were inoculated on the slanting position of the media in the test tubes and the test tubes were incubated between 28-30°C. Fungal growth was examined for 48 h [24]. The diameter of the fungal growth was compared with that of the control. Percentage inhibition was calculated with reference to negative and positive controls.

2.5. Preparation of microbial inoculum

2.5.1. Test for antibacterial activity

The antibacterial assay was carried out by micro dilution method in order to determine the antibacterial activity of compounds tested against the pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 X 107 CFU/ml. The inocula were prepared and stored at 4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. All experiments were performed three times.

2.5.2. Test for antifungal activity

In order to investigate the antifungal activity of the extracts, a modified micro dilution technique was used. The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 – 107 in a final volume of 100 µl per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid potato dextrose agar to verify the absence of contamination and to check the validity of the inoculum.

2.6. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentrations (MMC):

2.6.1. Determination of MIC

The minimum inhibitory concentrations (MIC) for minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were performed by a serial dilution technique using 96-well microtiter plates [28]. The different plant extracts viz. Methanol, Ethanol, n-hexane, Aqueous was taken (1 mg/ml) and serial dilution of the extract with luria broth for bacterial culture and for fungus, potato dextrose broth medium with respective inoculum were used. The microplates were incubated for 24 hours at 37°C for bacteria and 28°C for 72 hours for fungal species. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

2.6.2 Determination of Minimum Bactericidal Concentration (MBC):

The MBCs were determined by serial sub-cultivation of 2 µl into microtitre plates containing 100 µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate reader and compared with the standards chloramphenicol for Bacteria as the positive control. All experiments were performed in duplicate and repeated three times.

2.6.3. Determination of Minimum Fungicidal Concentration (MFC):

The fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 µl into microtiter plates containing 100 µl of broth per well and further incubation 72 hours at 28°C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. A commercial standard (Flucanazole, Sigma) was used as positive controls (1–2000 µg/ml) for fungi. All the assays were done in triplicate and the results were given in mean ± S.D.

2.7. Antioxidant activity

2.7.1 Preparation of Stock Solution:

Stock solution for each extract (n-hexane, methanol, ethanol, and water) was freshly prepared by dissolving 0.02 g of the plant extract in 20 ml of methanol to make a concentration of I mg/ml. Six concentrations of Plant Extract 10 ,20 ,30 ,40 ,50 and 60 µg/mL were prepared from this stock solution to give the primary standards. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as standard antioxidants.

2.7.2. DPPH Antioxidant bioassay

The DPPH activity was carried out by following the method of Blois [29] and Yildirm *et al.*, [30] with some modification. For this activity, 1mM solution of DPPH radical solution in methanol was freshly prepared. This solution in 1ml was about 0.3 mM of I, l-diphenyl-2-picrylhydrazy (DPPH) in MeOH was also prepared and kept in the dark. For all the different concentrations, 2 ml of the plant extract was mixed with I ml of DPPH; for the blank I ml of ethanol was used instead of DPPH and for the control, ethanol was used instead of the extract Triplicates solutions were prepared. The reaction mixtures were kept for 30 min in a dark room and the absorbance were taken at 517nm. The equation below was used to calculate the percentage scavenging activities (AA) of each extract.

$$\% \text{ DPPH Scavenging capacity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

The tests were done in triplicate. Ascorbic acid and Butylated hydroxytoluene (BHT) were used as standard antioxidants.

2.7.3. Reducing power assay

The assay was conducted by following the method investigated by Oyaizu [31]. The test solution was taken in 2 ml and phosphate buffer (pH 6.6, 0.2 M, 2ml) as well as potassium ferricyanide (10 mg/ml, 2 ml) mixed together and then incubated for 20 min at 50°C Trichloroacetic acid (100 mg/l ,2 ml) was also added to the whole mixture. In a test tube from each of the above mixtures an amount of 2 ml was combined with 0.4 ml of 0.1% (w/v) ferric chloride and 2 ml distilled water, after a period of 10 minute the absorbance was taken spectrophtometrically at 700 nm. A high reducing power correspond indicated increased absorbance of the reaction mixture. The tests were done in triplicate. Butylated hydroxytoluene (BHT) and Ascorbic acid were used as reference standard antioxidants.

2.8. Statistical analysis

The standard deviation is expressed as mean ± SD from three separate observations. The analysis was done for means and Standard Error of the Mean (SEM) for biological activities by using the following formula:

$$SEM = \frac{s}{\sqrt{n}}$$

Where:

s = sample standard deviation and n= size (number of samples)

3. RESULTS

3.1. Physical properties

Most of solvents expressed blackish color with some exception such as blackish to light greenish black (**Table 1**). Some of these extracts were pilular and non-stick recorded from ethanol and water however some pilular (semisolid) and sticky were also recorded from n-hexane and methanol. The highest weight of raw material (150g) was used for n-hexane, followed by water (140g), methanol (130g each), and ethanol (120g). With location of extract's weight, n-hexane and methanol yielded maximum crude extract's weight (18.5g), (17.3g) respectively, followed by water (16.0g), and ethanol (11.4g). Percentage yield of crude extract was ranged from 9.5 to 13.30 with the highest value obtained from methanol (13.30 %). It was followed by n-hexane (12.3%), water (11.42%) and ethanol (9.5% interestingly the least values were detected from ethanol (9.5%).

Table 1: Color, consistency and %age yield of leaves extracts from different solvents.

Solvents	Color of the extracts	Consistency	Weight of raw material (gm)	Weight of the extract (gm)	% Yield
n-hexane	Blackish	Pilular and sticky	150	18.5	12.3
Methanol	Blackish	Pilular and sticky	130	17.3	13.30
ethanol	Greenish Black	Pilular and non-sticky	120	11.4	9.5
Water	Blackish	Pilular and non-sticky	140	16.0	11.42

3.2. Phytochemical constituents

Table 2 depicts the presence of various phytochemicals in the leaves extracts. The highest phytochemicals were traced out by methanol and ethanol (8 each), water (6) and n-hexane (2). Alkaloids and flavonoids were detected by all solvents, followed by terpenoid, saponins, reducing sugar which is detected by all solvents except n-hexane, anthraquinone are detected by methanol and water and steroids are detected by methanol, whereas glycoside as well as phlobatannin is detected by ethanol only.

Table 2: Presence of various phytochemical in *T. stocksianum* leaves by different solvents.

Plant constituents	n-hexane	Methanol	Ethanol	Water
Alkaloids	*	*	*	*
Tannin	–	*	*	*
Saponins	–	*	*	*
Anthraquinone	–	*	–	*
Steroid	–	*	–	–
Phlobatannin	–	–	*	–
Terpenoid	–	*	*	*
Flavonoids	*	*	*	*
Glycoside	–	*	*	–
Reducing sugar	–	–	*	–

Legend: * = Detected and - = Not detected

3. 3. Antimicrobial Assay

In the current study, the inhibitory effect of different extracts (viz. Methanol, Ethanol, n-hexane, Aqueous) of leaves from *Teucrium stocksianum* were evaluated against both fungicidal and bacterial strains. The results of antimicrobial activity are provided in **Table 3**. The **Table 3** depicts the inhibition of zone (IZ) and their activity index (AI).

Table 3: Antimicrobial activity of different extracts of *Teucrium stocksianum*.

Pathogens/Accession No		n-hexane	Methanol	Ethanol	water	Chloramphenicol
Echerichia coli (ATCC 15224)	I.Z A.I	10.3±0.26 0.455	21.5±0.32 0.951	16.5±0.44 0.730	11.5±0.24 0.508	22.6
Staphylococcus aureus (ATCC 6538)	I.Z A.I	8.2±0.40 0.383	17.3±0.34 0.808	20.4±0.46 0.953	8.9±0.32 0.415	21.4
Staphylococcus epidermidis (ATCC 12228)	I.Z A.I	6.4±0.44 0.365	14.1±0.62 0.805	16.8±0.48 0.96	8.4±0.54 0.48	17..5
Pseudomonas aeruginosa (ATCC 7221)	I.Z A.I	9.3±0.22 0.502	18.4±0.32 0.994	21.6±0.42 1.167	11.3±0.22 0.610	18.5
Streptococcus pyogen (ATCC BAA-946)	I.Z A.I	6.3±0.46 0.5	12.5±0.56 0.992	13.6±0.62 1.079	5.6±0.28 0.444	12.6
Klebsella pneumonia (ATCC UC57)	I.Z A.I	11.4±0.32 0.690	16.22±0.68 0.983	15.4±0.63 0.933	13.6±0.83 0.824	16.5
Aspergillus niger (0198)	I.Z A.I	11.4±0.34 0.527	19.3±0.45 0.892	20.4±0.42 0.943	12.6±0.41	21.63
A.fumigatus. (0064)	I.Z A.I	10.3±0.22 0.570	17.4±0.45 0.962	19.6±0.62 1.084	11.5±0.42 0.636	18.07

AI-activity index=IZ of the test sample/ IZ of the standard, each value is represented as mean±SD, IZ= inhibition zones.

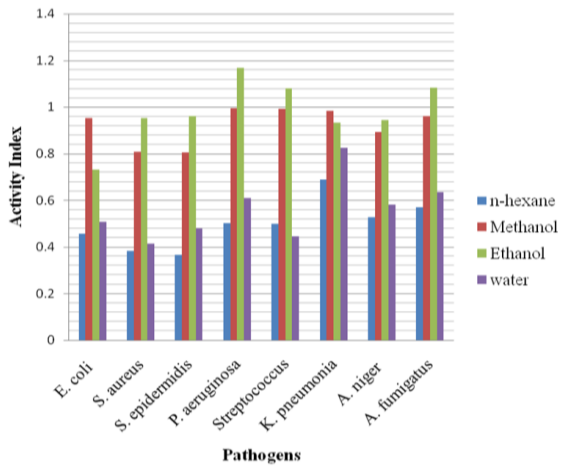

Figure 1: Activity index against various microorganisms.

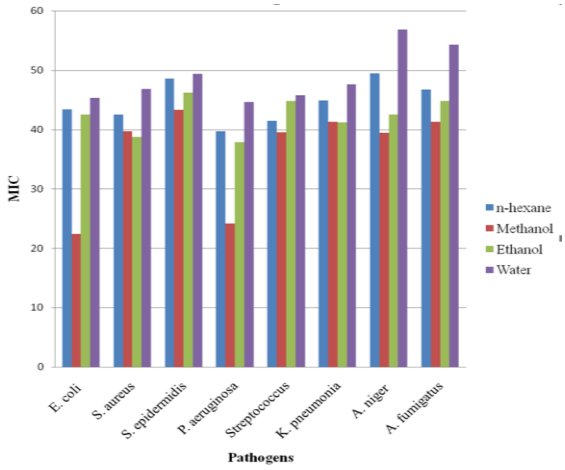

Figure 2: MIC against various microorganisms.

The antimicrobial potential of *Teucrium stocksianum* was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, viz., chloramphenicol, Fluconazole. The results revealed that all the extracts are potent antimicrobials against the tested microorganisms. Among solvents extracts, methanol and ethanol showed high degree of inhibition followed by aqueous and n-hexane extract. In Ethanol extract, maximum inhibition zone diameter was observed in *P. aeruginosa* and in *S. aureus* with diameter 22.3±0.42 mm 19.5±0.52 mm, respectively. Similarly, Methanol extract showed maximum inhibition zone with diameter of 21.5±0.32 mm in *E. coli* and 18.4±0.32 mm *P. aeruginosa*. The n-hexane (6.3-11.4 mm) and aqueous extract (5.6-13.6 mm) showed minimum activity, respectively (Table 3, Fig. 1). For the antifungal activity, *Aspergillus niger* (20.4±0.42 mm) and *Aspergillus fumigatus.* (19.6±0.62mm) showed efficient antifungal activity for ethanol plant extract and for methanolic extract. *Aspergillus niger* (18.3±0.45mm) and *Aspergillus fumigatus.* (17.4±0.45mm) showed proficient antifungal activity. n-hexane and aqueous extract showed lowest inhibition zone with diameter ranging between 10-11 mm and 11-12 mm against the two pathogenic fungal strains, respectively (**Table 3**, **Figure 1**).

Table 4: Table MIC (µg / ml), MBC and MFC performance of different extracts of Teucrium stocksianum against pathogenic organisms.

Pathogens/Accession No		**n-hexane**	**Methanol**	**Ethanol**	**Water**
Echerichia coli (ATCC 15224)	MIC MBC	43.4 86.3	22.4 44.7	42.5 83.7	45.3 85.8
Staphylococcus aureus (ATCC 6538)	MIC MBC	42.5 84.4	39.7 77.5	38.7 78.6	46.8 94.3
Staphylococcus epidermidis (ATCC 12228)	MIC MBC	48.6 97.4	43.3 85.3	46.2 87.5	49.4 98.4
Pseudomonas aeruginosa (ATCC 7221)	MIC MBC	39.7 78.6	24.2 48.4	37.9 75.2	44.6 97.8
Streptococcus pyogen (ATCC BAA-945)	MIC MBC	41.5 82.2	39.5 78.1	44.8 89.2	45.8 86.3
Klebsella pneumonia (ATCC UC57)	MIC MBC	44.9 87.4	41.3 83.7	41.2 77.4	47.6 93.5
Aspergillus niger (0198)	MIC MFC	49.5 98.4	39.4 79.5	42.5 86.3	56.8 109.5
A.fumigatus. (0064)	MIC MFC	46.7 93.6	41.3 84.7	44.8 87.5	54.3 113.6

MIC=minimum inhibitory concentration,
MBC= minimum bactericidal concentration
MFC= minimum fungicidal concentration.

3. 4. Determination of MIC, MBC and MFC values

Minimum Inhibitory Concentration (MIC) is defined as the least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of micro-organism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC and MFC was determined by subculturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the Bacteria and fungi was taken as MBC and MFC, respectively. Moreover, it was noted that most of the antimicrobial properties in different plant part extractions shows, MBC value that is almost two fold higher than there corresponding MICs [32]. Methanol extract of *Teucrium stocksianum* showed least MIC value 22.4 µg/ml against *Pseudomonas aeruginosa* while ethanol extract 37.9 µg/ml against *E. coli*. While the rest of the pathogen showed comparatively efficient MIC value (**Table 4**, **Figure 2**). *Aspergillus niger* was proved to have highest activity 39.4 µg/ml and 42.5 µg/ml in methanol and ethanol extract respectively. comparatively high activity at 41.3 µg/ml and 44.8 µg/ml of *Aspergillus fumigatus* was observed in methanol and ethanol extract, respectively. The least MBC and MFC value 22.4µg/ml and 39.4 µg/ml was observed in methanolic and ethanol extracts against *Echerichia coli* and *Aspergillus niger* respectively.

Table 5: Shows the %age DPPH scavenging activity of leaves extracts and with the standard.

Plant Extract	**10 µg/mL**	**20 µg/mL**	**30 µg/mL**	**40 µg/mL**	**50 µg/mL**	**60 µg/mL**
n-hexane	21.26±0.256	27.76±0.411	43.89±0.089	.52.56±0.123	68.18±0.0437	71.3±0.0234
Methanol	23.86±0.453	28.31±0.214	48.12±0.572	.52.1 6±0.016	68.18±0.232	86.4±0.065
Ethanol	17.76±0.123	31.56±0.114	38.82±0.025	.47.52±0.036	61.28±0.639	83.8±0.036
water	25.86±0.824	39.31±0.316	44.72±0.224	.62.86±0.233	68.23±0.035	78.7±0.723
Ascorbic acid	45.06±0.067	58.41±0.618	88.68±0.0014	91.12±0.0062	94.13±0.00043	96.36±0.00063
BHT	76.87±0.032	85.37±0.54	81.41±0.054	91.77±0.0061	94.63±0.0077	97.41±0.001

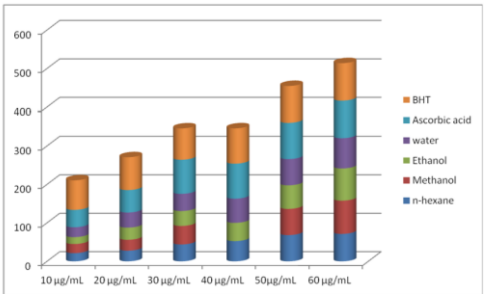

Figure 3: Scavenging activity of the extracts against 1, I-diphenyl-2-picrylhydrazy (DPPH) radical with ascorbic acid and BHT as standard antioxidants.

The four different solvents such as n-hexane, methanol, ethanol, and water detected the plant phyto-constiteunts and taken for further antioxidant assay. different dilutions were prepared from the leaves extract and used to evaluate antioxidant activities. DPPH was used for the assessment of antioxidant experiment. Results of the assay is shown in Figure 3. The leaves extract of *Teucrium stocksianum* were diluted in the range of 10µg to 60µg. In all the extracts, there was an increase in absorbance with increasing concentration, which was also supported by the color intensity of the samples used during the experiment; the more intense the color, the higher the absorbance.

While looking at **Figure 3,** methanol and ethanol showed increasing absorbance, but n-hexane show a constant trend in the absorbance pattern. If one looks at the MeOH, EtOH and the two standards, they have absorbance that is directly proportional to the concentration; that is, as the concentration of the extracts increases, the absorbances also increase. There could be a number of reasons for this behavior. One could assume that the extract with this constant behavior does contain compounds that possess antioxidant activities but in very small quantities, whereas those that showed high antioxidant behaviors, have high concentration. In the DPPH free radical scavenging method, all the extracts show good scavenging activities with methanol extracts having the highest activity followed by EtOH, water, and n-hexane. The n-hexane extract shows a constant scavenging activity, even at high concentrations. It should be noted that for a compound to be considered an antioxidant, it must be able to donate an electron; such an electron usually come from the hydroxyl group or an electron-rich compound. Since n-hexane a non-polar solvent, it could be assumed that there are no electron-donating species present in the hexane extract; these accounts also for the outcome obtained.

Table 6: Reducing power assay of *Teucrium stocksianum* leaves extracts with the standard.

Plant Extract	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50µg/mL	60 µg/mL
n-hexane	0.315±0.071	0.327±0.084	0.331±0.067	0.329±0.035	0.341±0.066	0.347±0.024
Methanol	0.522±0.014	0.576±0.078	0.634±0.043	0.854±0.071	1.135±0.055	2.38±0.042
Ethanol	0.301±0.043	0.291±0.023	0.342±0.083	0.450±0.034	0.721±0.097	0.965±0.073
water	0.211±0.049	0.223±0.097	0.241±0.021	0.273±0.045	0.289±0.062	0.409±0.087
Ascorbic acid	0.628±0.0056	0.648±0.032	0.842±0.094	1.82±0.073	1.56±0.077	2.64±0.068
BHT	0.423 ±0.027	0.439±0.055	0.758±0.046	1.22±0.028	1.44±0.053	2.11±0.064

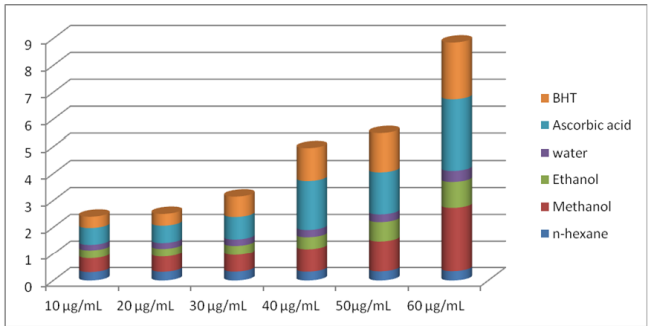

Figure 4: Reducing power of the extracts and the standard antioxidants (BHT and ascorbic acid)

Table 4 shows the reducing power of the extracts represented as mean ± SO (n = 3). The extracts showed increasing reducing power with increasing concentration (**Figure 4**). From graphical representation of values in **Table 6**, the methanol extract showed the highest activity, which is even comparable to the standard antioxidants; this is closely followed by the ethanol, water and n-hexane extract. The other extracts showed activities which are less than BHT and ascorbic acid standard antioxidant one could infer that the other extracts do have the antioxidant properties but may be the concentration of the compounds responsible for such activities, are in trace amounts.

4. DISCUSSION

This study revealed the presence of various important phytoconstituent in the leaves extracts such as like alkaloids, flavonoids, tannin, anthraquinone, terpenoid, phlobatannin, saponins, steroid, glycoside and reducing sugar (**Table 2**). These phytochemicals were detected by different solvents. Various studies reported that all reported phytochemicals are responsible for medicinal effects of various kinds. For example, due to astringent properties, alkaloids possessed anti-diarrheal activity that would affect on intestinal tract and may exhibit antimicrobial effects as well as antihypertensive antifungal, antifibrogenic and anti-inflammatory properties [33]. In response to microbial infection Flavonoid are also synthesized by medicinal plants [34]. Almost half of the 200 phytoalexins characterized until now mostly belong to the flavonoids [35]. Another study also describes the importance of some alkaloids against intestinal and as well as HIV infection which is mostly related with AIDS [36].

Terpenes are a large group of compounds which derive aroma in plants due to presence of essential oil. They are manufactured from isoprenoid units, resembling in fatty acids in its property [37]. Saponins are isolated from leaves extracts and various studies reported that cardiac depressant, antihyper-cholesterol, and anti-inflammatory properties are governed by such chemicals [38-39]. Results show that leaves extracts of *Teucrium stocksianum* also have flavonoid. These compounds mitigate the process of carcinogenesis and prevent oxidative cell from damage resulting into anticancer properties and various other biological activities [40-41]. Such chemicals possess antimicrobial and anti-inflammatory activities [41]. This study discovered the presence of steroids in the leaves extracts. These phytochemicals enhance sexual desire and are of potential pharmaceutical importance [42]. Besides, these phytochemicals inhibit the production of various microbes such as viruses, bacteria, yeast, and fungi [43]. Tannin, a nontoxic phytochemical is responsible for physiological stimuli in animals after consuming plants containing them [43]. This phytochemical acts as antidiarrheal, antifungal, anti-hemorrhoidal and antioxidant agents [44]. In addition, the bitter taste of drinks and foods are due to tannin [45].

It has also been proposed that tannin toxicity would be related to molecular size (Mr), since the larger the molecule the more effectively it binds to proteins. This has been observed in many cases; dimeric ellagitannins have been found to be more astringent than related monomers [46]. Besides, in some situation the toxicity of tannins was found to be no higher than that of catechins [47]. Tannins are a vast group of polyphenolic compounds which have received attention in recent years due to their claimed ability to cure a variety of diseases. Tannins are subdivided into two principal groups: hydrolysable tannins and proantocyanidins (condensed tannins), Proantohocyanidins are polymers of flavan-3-ols (for example catechin) and flavan-3, 4-diols linked through an interflavan bond that is not susceptible to hydrolysis [48] the tannin compounds exhibit a multiple action on infectious disease [49].

The present study also exhibited the existence of cardiac glycosides in the plant extract. This group of phytochemicals works as stimuli during cardiac failure [50]. The Anthraquinones, the biggest group of [51] have been found to possess antibacterial effects by inhibiting nucleic acid synthesis. The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [52, 17]. These compounds have significant therapeutic application against human pathogens including bacteria, fungi, or virus. Plentiful studies have been conducted with the extracts of different plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [53-54]. In the current study, various extracts of *Teucrium stocksianum* was assessed for screening of their antimicrobial activity in certain Gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram positive (*Staphylococcus aureus*, *S. epidermidis*, *Streptococcus*) bacteria as well as fungus pathogens (*Aspergillus niger* and *Aspergillus fumigatus*) which was regarded as human pathogenic microorganism. Susceptibility of the plant extracts was tested by serial microdilution method (i.e. MIC) and agar well diffusion method was determined. Our preliminary inquiry showed that all solvent extracts of *Teucrium stocksianum* were active against the human pathogens like *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogen*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseusdomonas aeruginosa* and fungal pathogens i.e., *Aspergillus niger* and *Aspergillus fumigatus*. This scrutiny of using several extracts to study the usefulness of plant for antimicrobial activity has also been understand by many scientists in various plant species like *Adhatoda zeylanica* [55], *Trianthema decandra* [56], *Argemone mexicana* [57], *Tinospora cordifolia* and *Cassia fistula* [58].

The organic (methanol and ethanol) extracts of *Teucrium stocksianum* showed significant antimicrobial activity against multi-drug resistant clinically isolated pathogens (**Figure 1-2**). Although, the mechanism of the action of these plant constituents is not yet fully known the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteriostatic abilities [37] mention that most of the antibiotic compounds previously identified in plants are apparently aromatic or saturated organic molecules which can easily solubilized in organic solvents. Various other studies revealed that the organic extracts possess the best antimicrobial activity in *Leucas aspera* [11] and *Holarrhena antidysenterica* [59]. The study also revealed that n-hexane extract shows moderate activity and aqueous extract exhibited minimum antimicrobial activity. However, Murugesan is of opinion based on his work in different alcoholic extracts (i.e. ethanol and methanol) that n-hexane extract of *Memecylon umbellatum* showed significant antimicrobial activity [60]. Besides, water extract from leaves of *Pterospermum acerifolium* had been reported to have well-known antimicrobial activity against a number of gram positive and gram negative human pathogenic bacteria [61].

The antimicrobial investigation using the agar well diffusion method and MIC value has been used by many researchers [62-64]. In the current investigation, MIC values of the plant extracts in this study were lower than the MBC values (**Table 3-4, Fig. 1-2**) signifying that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration [64]. For screening antioxidant properties, both methods (i.e. DPPH free radicals and reducing power) resulted significant activity Although the crude extract of this plant showed a relatively low activity compared to that of reference material, however, this may either be due to low concentration of this compound used in the extract or to an opposed effect with other phytochemicals of the extract. The methanol extract showed the highest activity, which is even comparable to the standard antioxidants. It was closely followed by the ethanol, water and n-hexane extract. The other extracts showed activities which were less than BHT and ascorbic acid standard antioxidant. This may be attributed either due to inference other active compounds in the extracts or trace amount of compound in plant extract [65-67].

CONCLUSIONS

It can be concluded that *Teucrium stocksianum* leaves contain potential antimicrobial, antioxidant and phytochemical components that may be used in drug development by the pharmaceutical industry as a therapy against various diseases. The ethanol, methanol, n-hexane and aqueous extracts of *Teucrium stocksianum* possess significant inhibitory effect against tested pathogens. The results of the study endorse the folklore claims along with the development of new antimicrobial drug from the plant**.** Presence of phenolic compounds in plant extract demonstrates antioxidant activity. For screening antioxidant properties, both methods (i.e. DPPH free radicals and reducing power) resulted significant activity. Both tests of plant extracts exhibited that tested plant part can be employed as a potential natural antioxidant agent which may be used for mitigating oxidative stress. Nevertheless, there is need to explore mechanism involved for such kind of activity. Hence, there is need to isolate and identify, characterize the structure of the active compound found in plant extract.

AUTHOR CONTRIBUTION

Conceptualization, G.R, R.Q, A.H, B.A, A.A.K and A.S; Original draft, B.A and A.S.; Methodology G.R, R.Q, A.H, B.A, and A.A.K; Data curation: A.S.; Writing- review & editing, T.A. Visualization, A.S.A and M.A; Resources, T.A.; Project administration, M.A, ;Funding acquisition, A.S.A and M.A.; Validation, T.A; Investigation, T.A; Formal analysis, A.A.K.; Supervision, T.A .

FUNDING

No external funding was received.

ACKNOWLEDGMENTS

The authors greatly acknowledge and express their gratitude to the Researchers Supporting Project number (RSP2022R462), King Saud University, Riyadh, Saudi Arabia.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Western AR: The flora of the United Arab Emirates: An introduction. *United Arab Emirates University* **1989.**
2. Nadaf SK, Al-Farsi SM, Al-Hinai V: Germplasm collection of range land forage and medicinal plant species in north Oman. In: Ann. Rep. ICARDA–APRP. *ICARDA–APRP, Dubai* **2003**, 63–64.
3. Mojab F, Javidnia K, Yazdani D and Rustaiynan A: Essential oil of the aerial parts of Teucrium stocksianum Boiss. Subsp. stocksianum (Lamiacea) from Iran. *J. Med. Plants* **2003**, 2: 49-53.
4. Radhakrishnan RM, Zakaria MNM, Islam MW, Kamil M, Ismail A, Chan K, Al-Attas A: Analgesic and anti-inflammatory activities of Teucrium stocksianum. *Pharm. Biol* **2001,** 39: 455-459.
5. Barkatullah IM and Hussain F: Ethnobotanical studies of plants of Charkotli Hill, Batkhala, District, Malakand, Pakistan. *Front. Biol. China* **2009,** 4: 539-548.
6. Islam MW, Zakaria MNM, Radhakrishnan R, Kamil M, Chan KC and Al-Attas A: Effect of Teucrium stocksianum on gastric ulceration and secretion in rats. *Pharm. Biol* **2002**, 40: 216–220
7. Iqbal I and Hamayun M: Studies on the traditional uses of plants of Malam Jabba valley, District Swat, Pakistan. Ethnobotanical Leaflets; *Int. Web Journal* **2004**, 1–30, 12 May 2006.
8. Kamali HH, Amir MYEL EL: Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from Selected Sudanese Medicinal Plants. *Curr. Res. J. of Bio. Sci*. **2010**; 2: 143-146.
9. Lalitha P, Arathi KA, Shubashini K, Sripathi, Hemalatha S, Jayanthi P: Antimicrobial Activity and Phytochemical Screening of an Ornamental Foliage Plant, Pothos aurea (Linden ex Andre). *An Int. J. of Chem*. **2010;** 1: 63-71.
10. Iniaghe OM, Malomo SO and Adebayo JD: Proximate composition and Phytochemical constituents of leaves of some Acalypha species. *J. of Nutrition* **2009**, 8:256-258.
11. Preethi R, Devanathan VV, Loganathan M: Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens. *Adv. in Bio.Res*. **2010.** 4: 122-125.
12. Sharma A: Antibacterial activity of ethanolic extracts of some arid zone plants. *Int. J. of Pharm.Tech. Res*. **2011**; 3:283-286.
13. Enne VI, Livermore DM, Stephens P, Hal LMC: Persistence of sulphonamide resistance in Escherichia coli in the UK despite national prescribing restriction. *The Lancet.* **2001**; 28: 1325-1328.
14. Westh H, Zinn CS, Rosdahl VT: An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. *Microb. Drug Resist*. **2004;** 10: 169-176.
15. Kim H, Park SW, Park JM, Moon KH, Lee CK: Screening and isolation of antibiotic resistance inhibitors from herb material Resistant Inhibition of 21 Korean plants. *Nat. Prod. Sci.* **1995**; 1: 50 - 54.
16. Alagesaboopathi C: Antimicrobial Potential and Phytochemical Screening of Andrographis Affinis Nees an Endemic Medicinal Plant from India. *Int. J. of Pharma and Pharmaceutical Sci*. **2011;** 3: 157- 159.
17. Ahmad I, Beg AZ: Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multiple drug resistant human pathogens. *J. Ethanopharma*. **2001**; 74: 113-123.
18. Hedge, I.C: Flora of Pakistan (Labiatae) edited by Nasir E, Ali SI, 192. *University of Karachi* **1990,**
19. Dellavalle PD, Cabrera1 A, Alem D, Larrañaga P, Ferreira F and Rizza MD:Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp. *Chilean Journal of Agricultural Research* **2011**, 71: 231-239.
20. Sofowora A: Medicinal Plants and Traditional Medicine in Africa. John Wiley and son Ltd **1993**, 150-153.
21. Trease GE and Evans WC: Pharmacognosy. 11th Ed. Brailliar Tiridel and Macmillian Publishers, London1989,
22. Harborne JB: Phytochemical Methods 3rd Ed. Chapman and Hall Ltd., London **1973**, 135-203.
23. Hemaiswarya S, Raja R, Anbazhagan C and Thiagarajan V: Antimicrobial and mutagenic properties of the root tubers of Gloriosa superba Linn. (Kalihari). *Pak. J. Bot* **2009,** 41: 293-299.

24. Bertani G: Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. *Bacteriol.* **2004,** 186: 595-600.
25. Luqman S, Srivastava S, Darokar MP and Khanuja SPS: Detection of antibacterial activity in spent roots of two genotypes of aromatic grass Vetiveria zizanioides. *Pharm. Bio* **2005,** 43: 732–736.
26. Sultanova N, Makhmoor T, Abilov ZA, Parween Z ,Omurkamzinova VB, Rahman A, Choudhary MI:Antioxidant and antimicrobial activities of Tamarix ramosissima. *J. Ethnopharmacol* **2001,** 78: 201–205.
27. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA: Screening of crude extracts of six medicinal plants used in South–West Nigerian unorthodox medicine for antimethicillin resistant S. aureus activity. *BMC Comp. Alt. Med*. **2005**; 5:1-7.
28. Blois M.S: Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, 26: 1199-2000.
29. Yıldırım A, Mavi A and Kara AA: Antioxidant and antimicrobial activities of Polygonum cognatum Meissn extracts. *J. Sci. Food Agric* **2003**, 83: 64-69.
30. Oyaizu M: Studies on product of browing effect reaction prepared from glucose amine. J. Nutr1986, 44: 307-315
31. Omar K, Geronikaki A, Zoumpoulakis P, Camoutsis C, Sokovic M, Ciric A, Glamoclija J: Novel 4-thiazolidinone derivatives as potential antifungal and antibacand antibacterial drugs. *Bioorg. & Med. Chem*. **2010;** 18: 426–432.
32. Ghosal S, Krishna-Prasad BN and Laksmi V: Anti-amoebic activity of Piper longum fruits against Entamoeba histolytica in vivo. *J.Ethnopharmacol* **1996,** 50: 167-170.
33. Dixon RA, Dey PM, Lamb CJ: Phytoalexins: enzymology and molecular biology. *Adv. Enzymol*. **1983**, 55: 1-69.
34. Harborne JB: Flavonoids in the Environment: Structure-activity relationships. In: V. Cody, E. Middleton, Jr., J. B. Harborne, A. Beretz (Eds.), Plant Flavonoids in Biology and Medicine II.Biochemical, Cellular and Medicinal Properties. *Alan R. Liss, New York* **1988,** 17-27.
35. McDevith JT, Schneider DM, Katiyar SK and Edlind FS:Berberina: a candidate for the treatment of diarrhea in AIDS patients abstract No. 175. 36th Interscience conference on Antimicrobial Agents and Chemotherapy. *American Society for Microbiology*, Washington, DC **1996,**
36. Cowan M: Plant products as antimicrobial agents. *Clin. Microbiol. Rev*. **1999**; 12: 564-582.
37. Trease GE and Evans WC: *Pharmacognosy. 11th Edition, Tindal LTD, London* **1985**, 60-75.
38. Lewis WH and Elvin-Lewis MP: Medicinal plants as sources of new therapeutics. *Ann. Mo. Bot. Gard* **1995,** 82: 16-24.
39. Okwu DE: Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *J. Sustain Agric. Environ* **2004,** 6: 30-34.
40. Cushnie TP and Lamb AJ: Antimicrobial activity of flavonoids, *Int.J.Antimicrobial Agents* **2005,** 26: 343-356.
41. Okwu DE. Evaluation of chemical composition spices and flavouring agents. Global *J. Pure Appl. Sci*., **2001,** 7: 455-459.
42. Scalbert A: Antimicrobial properties of tannins. *Phytochemistry* **1991,** 30: 3875- 3882.
43. Asquith TN and Butter LG: Interaction of condensed tannins with selected proteins. *Phytochemistry* **1986**, 25: 1591-1593.
44. Chikezie PC, Agomuo EN and Amadi BA: Biochemistry, Practical/Research Method: A Fundamental Approach. Mega soft publishers **2008,** 2: 51-53.
45. McManus JP, Davis KG, Beart, JE, Gaffney SH., Lilley TH and Haslam E: Polyphenolinteractions. Part 1. Introduction: Some observations on the reversible complexation of polyphenols with proteins and polysackarides. *J. Chem. Soc. Perkin Trans* II **1985**, 9: 1429-1438.
46. Siwaswamy SN, Mahadevan A: Effect of tannins on the growth of Chaetomium cupreum. *Journal of Indian Botanical Society* **1986**, 65: 95-100.
47. Haslam E: Plant Polyphenols- Vegetable Tannins Revisited. *Cambridge University Press, Cambridge,U.K* **1989,**
48. Haslam E: Natural Polyphenols (vegetable tannins) as drugs: possible modes of action. *J. Nat. Prod* **1996,** 59:205-215.
49. Olayinka AO, Onoruvwe O and Lot TY: Cardiovascular effects of Methanolic extract of the stem bark of Khaya senegalensis. *Phytotherapy Research* **1992**, 6: 282-284.
50. Harborne JB, Baxter H, Moss GP: Phytochemical dictionary. *A handbook of bioactive compounds from plants* **1999**, 2nd ed. London, UK. Taylor & Francis.
51. Kelmanson JE, Jager AK and Vaan Staden J: Zulu medicinal plants with antibacterial activity. *J. Ethanopharmacol*. **2000**; 69: 241-246.
52. Guleria S, Kumar A: Antifungal activity of some Himalayan medicinal plants using direct bioautography. *J. Cell Mol. Bio*. **2006**; 5: 95-98.
53. Zakaria Z, Sreenivasan S, Mohamad M: Antimicrobial Activity of Piper ribesoides Root Extract against Staphylococcus aureus. *J. App. Biol. Sci*. **2007,** 1: 87-90.
54. Ilango K, Chitra V, Kanimozhi P, Balaji G: Antidiabetic, Antioxidant and Antibacterial Activities of Leaf extracts of Adhatoda zeylanica. Medic (Acanthaceae). *J. Pharm. Sci. & Res*. **2009;** 2:67-73.
55. Geethalakshmi R, Sarada DVL, Marimuthu P: Evalution of antimicrobial and antioxidant potentials of Trianthema decandra L. *Asian J. of Biotech*. **2010;** 2: 225-231.
56. Rahman MS, Salehin MF, Jamal MA, Pravin HM, Alam A: Antibacterial activity of Argemone mexicana L. against water brone microbes. *Res. J. of Medicinal plant*. **2011;** 5: 621-626.
57. Upadhyay RK, Tripathi R, Ahmad S: Antimicrobial activity of two Indian medicinal plants Tinospora cordifolia (Family: Menispermaceae) and Cassia fistula (Family: Caesalpinaceae) against human pathogenic bacteria. *J. of Pharma. Res.* **2011**; 4:167-170.
58. Seyydnejad SM, Niknejad M, Darabpoor I, Motamedi H: Antibacterial Activity of Hydroalcoholic Extract of Callistemon citrinus and Albizia lebbeck. *American J.of App. Sci.* **2010**; 7: 13-16.
59. Murugesan S, Pannerselvam A, Chanemougame TA: Phytochemical screening and antimicrobial activity of the leaves of Memecylon umbellatum Burm. *F.J. of App. Pharma. Sci*. **2011;** 1: 42-45.
60. Thatoi HN, Panda SK, Rath SK, Dutta SK: Antimicrobial Activity and Ethnomedicinal Uses of Some Medicinal Plants from Similipal Biosphere Reserve, Orissa. *Asian J. of Plant Sci.* **2008;** 7: 260-267.
61. Arora DS, Kaur GJ: Antibacterial activity of some Indian medicinal plants. *J. Nat. Med.* **2007;** 61:313–317.
62. Gurudeeban S, Rajamanickam E, Ramanathan T, Satyavani K: Antimicrobial activity Of Citrullus colocynthis in Gulf of Mannar. *Int. J. of Curr. Res*. **2010**; 2: 078-081.
63. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS: Antibacterial activity of the plant used in Indian herbal medicine. *Int. J. of green pharma*. **2010;** 10: 22-28.
64. Maji S, Dandapat P, Ojha D, Maity C, Halder SK, Das PK, Mohapatra T, Pathak K, Pati BR, Samanta A, Mondal KC: In vitro antimicrobial potentialities of different Solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. *Journal of Phytology.* **2010**; 2: 57–64.
65. Naveed, M.; Batool, H.; Rehman, S.u.; Javed, A.; Makhdoom, S.I.; Aziz, T.; Mohamed, A.A.; Sameeh, M.Y.; Alruways, M.W.; Dablool, A.S.; Almalki, A.A.; Alamri, A.S.; Alhomrani, M. Characterization and Evaluation of the Antioxidant, Antidiabetic, Anti-Inflammatory, and Cytotoxic Activities of Silver Nanoparticles Synthesized Using Brachychiton populneus Leaf Extract. *Processes* **2022,** 10, 1521.
66. Saleem, A.; Afzal, M.; Naveed, M.; Makhdoom, S.I.; Mazhar, M.; Aziz, T.; Khan, A.A.; Kamal, Z.; Shahzad, M.; Alharbi, M.; Alshammari, A. HPLC, FTIR and GC-MS Analyses of Thymus vulgaris Phytochemicals Executing In Vitro and In Vivo Biological Activities and Effects on COX-1, COX-2 and Gastric Cancer Genes Computationally. *Molecules* **2022**, 27, 8512.
67. Naveed, M.; Bukhari, B.; Aziz, T.; Zaib, S.; Mansoor, M.A.; Khan, A.A.; Shahzad, M.; Dablool, A.S.; Alruways, M.W.; Almalki, A.A.; Alamri, A.S.; Alhomrani, M. Green Synthesis of Silver Nanoparticles Using the Plant Extract of Acer oblongifolium and Study of Its Antibacterial and Antiproliferative Activity via Mathematical Approaches. *Molecules* **2022**, 27, 4226.