THE QUALITATIVE PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF EIGHT LIBYAN MEDICINAL PLANTS AGAINST PATHOGENIC BACTERIA

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Abstract

The purpose of this study was to investigate the phytochemical and antibacterial activities of eight Libyan medicinal plants including *Petroselinum crispum*, *Eruca sativa*, *Rosmarinus officinalis*, *Apium graveolens*, *Mentha piperita*, *Thymus capitatus*, *Punica granatum* and *Ocimum basilicum*. These plants were locally available from different regions in Libya. The leaves of the selected medicinal plants were washed, air dried and then powdered then dissolves in three different solvents which represent the aqueous, methanol and ethyl acetate, the phytochemical screening of samples are showed the presence of the important phytochemical compounds such as Alkaloids, Phenols, flavonoids, saponins, carbohydrate and tannins within most all the plants selected. The antibacterial activities of these plants were evaluated using the agar diffusion method against *Staphylococcus aureus*, *Bacillus subtilis* as gram-positive and *Escherichia coli*, *Pseudomonas aeruginosa* as gram-negative. The results obtained revealed that not all of the plants were active against the bacteria tested. Over all, the methanolic extract of *Punica granatum* displayed the greatest antibacterial activity than others. This increased activity may due to the presence of the most active compounds in this plant.

Inhibition zone values of the methanol extracts were within the ranges of 9.00 - 36.00 as significantly compared to the ethyl acetate extracts were 9.00-26.00 mm ($P < 0.05$) and the water extracts which not displayed any activity.

Keywords: *Punica granatum,* Ethyl acetate, antibacterial, Erythromycin

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Abbreviation: EA (ethyl acetate extract). Me (Methanol extract) and Aq (Aqueous extract).

Introduction:

The contribution of medicinal plants in the worldwide spectrum of healthcare has been invaluable. These natural products have been formed the basis of complex traditional systems of medicine existence since thousands of years (Butler and Newman, 2008), Ancient different cultures of the world had plants as an integral part of medical use and their importance. To addition, these plants have also form the establishments of modern medicine where a number of drugs have been developed from lead compounds discovered from medicinal plants.

Few examples being: morphine, aspirin, salicylic acid, taxol which classified as a plant alkaloid and vinblastine that obtained from the Madagascar periwinkle plant. Most of these plant derived drugs were originally discovered through the study of traditional cures and folk know of native people and some of these could not be substituted despite the enormous advancements in synthetic chemistry[1,2]. Plant based medicine gives priority to nutritive, tonic approach over therapeutic intervention, thus leading to a safe and natural curing. However, in due course of time the search for strong medicine led to an increasing emphasis on potent, toxic botanicals like opiates, henbane, belladonna and a gradual abandonment of more gentle, safer remedies that had once been the mainstay of herbal medicine.

The toxicity and ineffectiveness of herbal medicinal products increased and led to a decline in the practice as well as belief in herbalism to a significant level, particularly in the west countries[3]. Looking to the present, the time wheel has taken a full turn and after a century or so, the herbal medicine are becoming increasingly clear; in contrast to the natural, holistic benefits of the herbal approach. In the last two decades there was a dramatic back in the interest in medicinal plants and their products in the worldwide spectrum of healthcare[4]. In the present study, the plants selected approved to have a bioactive components which strongly support the previous finding reported by others. For example, ***Parsley*** (*Apiaceae*), is a remedy against common arthritis, cold, acne and liver diseases, and which mainly contain flavonoid, tannin, phytochemical compounds, vitamin K, C, and A, with some other materials, and has antimicrobial and antioxidant activities[5]. Plant selected also have shown credible antimicrobial, anti-inflammation and antioxidants activities in various studies[6]. The other different seven plants were extensively found to be a remedy for various disease such as extensively used in arthritis, anemia, diabetes, hepatitis, indigestion diseases. The plant contains phytochemical, rich with Vitamins, material and fibers, and have antifungal, antimicrobial and antioxidant activities of through many searches and studies[7,8,9,10,11,12].

Collection of plant samples

Eight edible plants (Table 1) were collected from different regions in Libya. Efforts have been made to collect these plants in quite conditions for the correct botanical identification. Healthy and disease free edible plant part were collected for phytochemical and antimicrobial activity.

Table 1: Selected medicinal plants for phytochemical and antimicrobial screening.

Local name	Scientific name	Family	Habitat
Gerger	*Eruca sativa.*	*Brassicaceae*	Wild
Nana	*Mentha piperita L.*	*Lamiaceae*	Farm
Krafiss	*Apium graveolens L.*	*Apiaceae*	Wild
Madanose	*Petroselinum crispum*	*Apiaceae*	Wild
Habaq	*Ocimum basilicum L.*	*Lamiaceae*	Wild
Romman	*Punica granatum L.*	*Punicaceae*	Wild
Eklil El-Gabal	*Rosmarinus officinalis L.*	*Lamiaceae*	Farm
Zatar	*Thimys capitatus L.*	*Lamiaceae*	Farm

Preparation of plant samples

Fresh plants were cleaned with distilled water and external moisture wiped out with a dry cloth. The edible portion was separated, dried under shade place for several days. The dried plant portions have been grinded to obtain the powder samples and then stored in cleaned polyethylene container until to be used.

Chemicals

Methanol, Ethyl acetate, Chloroform, Wagner, Dragendroff, Hager, Benedict, Fehling A&B, Biuret, Ninhydrin, Barfoed, α-naphthol, Ferric Chloride, Sulphuric acid, Hydrochloric acid, Gelatin, Sodium Hydroxide, Lead acetate, Glacial acetic acid, Muller Hinton Agar, Nutrient Broth, were obtained from the chemistry department, University of Tripoli. Tetracycline was provided by the microbiology department.

Preparation of plant extracts

Organic extracts

Ten grams of each grounded sample were separately dissolved in a flask containing 100 ml of 95% ethyl acetate and other flask containing 96% methanol for 72 hr. The samples were filtrated using Whatman No1 filter paper. The obtained samples were collected and evaporated until dryness at 40 °C under reduced pressure. The residue was kept in refrigerator at 4 °C until to be.

Aqueous extract

10 grms of each powdered sample was dissolved in 100 ml of distilled water and boiled for 15-20 min on slow heat and filtrated using Whatman No 1 filter paper. The filtrates were collected and evaporated until dryness at 40 °C under reduced pressure. The residue was kept in the refrigerator at 4 °C until to be used.

Phytochemical screening

The methanolic, ethyl acetate and aqueous extracts were used for phytoche-mical screening using standard methods carried out by[13,14,15] in order to determine alkaloids, carbohydrates, glycosides, Saponins, Terpenoids & Steroids, phenols, tannins, flavonoids, Proteins and amino acids:

Preliminary qualitative phytochemical analysis
Alkaloids

Wagner, Dragendorff and Hager tests were used for the detection of alkaloids. First extracts were dissolved individually in dilute Hydrochloric acid and filtered. Formation of brown, red and yellow precipitation was observed which indicate the presence of alkaloids.

Carbohydrates

Prepared samples were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Molisch's Test (General test for carbohydrates)

Prepared samples were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test (for reducing sugar)

The obtained samples were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehiling's Test (reducing sugar)

Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with

Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Barfoed's Test (for Monosaccharide)

1ml of Barfoed's reagent was added to extract and boiled on the water bath. The solution was observed for the color change reaction[16].

Glycosides:

Keller-Killani´s Test

0.5 grm of the extract was shaken with distilled water 5 ml. To this, glacial acetic acid 2 ml containing a few drops of ferric chloride was added, followed by H_2SO_4 1ml along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring[17].

Saponins (Foam Test)

0.5 gm of each extract was shaken with 2 ml of water. Foam was produced persists after ten minutes to indicates the presence of saponins.

Terpenoids / Steroids

For the detection of Terpenoids, Salkowski's test was used, extracts obtained were treated with chloroform and then filtered. The filtrates were treated with few drops of Conc. Sulphuric acid and shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes. However, for the detection of steroids the extracts were treated with chloroform and then filtered. The obtained sample then were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring was observed at the junction which indicates the presence of steroids.

Phenols (Ferric Chloride Test)

Samples prepared were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour was observed to detect the presence of phenols.

Tannins (Gelatin Test)

The sample obtained, 1% gelatin solution containing sodium chloride was added. White precipitate was found which indicates the presence of tannins.

Flavonoids

Two different tests were used for the detection of flavonoid alkaline reagent and lead acetate tests. First extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, that becomes colour less on addition of dilute acid, indicates the presence of flavonoids. Secondly the samples few drops of lead acetate solution were added. Yellow colour precipitate has been formed indicates the presence of flavonoids.

Detection of Proteins and Amino acids

Biuret Test (proteins)

To 0.5ml of each extract, was mixed with 2ml of Biuret reagent was added and the reaction mixture observed for the formation of violet colour solution[16].

Ninhydrin Test (Amino acids)

To each extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Preparation of Medicinal plants for *in vitro* Antimicrobial Assay

Four bacterial species obtained from the faculty of Medicinal, university of Tripoli (Libya) were selected for testing. Representative strains of Gram-positive bacteria were *S. aureus* and *B. subtilis* (Isolated from Tripoli Medical Center). Representative strains of Gram-negative bacteria were *E. coli* and *P. aeruginosa*. For agar well diffusion tests, the samples were initially diluted in distilled water to obtain concentration of 500, 250, 150 and 75mg/ml.

The plates were prepared using 50ml of sterile Muller Hinton Agar. The surface of the plates was aseptically inoculated using a 100µl of suspension of bacteria grown overnight at 37ºC in Muller Hinton. Agar cylinders of 6mm in diameter were cut from the culture media using a sterile glass derhum tube; and then filled with the dilutions of extract samples. The plates were then incubated at 37ºC for and the inhibition zones were measured .

Results

The phytochemical screenings of eight different Libyan plants were investigated under different conditions including three extracts of aqueous, methanolic and ethyl acetate. From table (2, 3, 4 and 5), it is demonstrated that most of the plants contain carbohydrates, phenols, tannins, flavonoids, saponins, Steroids, alkaloids, and tannins. Furthermore, as indicated by the ethyl acetate, methanol and aqueous extracts, there were no saponins in *E sativa* and *M piperita*, and no glycosides and phenols in *T. capitatus*. From the result obtained, this work found that, the absence of terpenes and steroids in *E. sativa* which was different, with the ethyl acetate, methanol and aqueous extracts being the most contain of alkaloids in all the eight plants tested. Significant differences were found between Biuret and Ninhydrin tests for the detection of amino acids and proteins in the extracts of *R. officinalis*.

Table 2: The phytochemical screening of *M. piperita* , *T. capitatus* , *P. granatum* and *O. basilicum.*

	Tests	Extract	*M. piperita*	*T. capitatus*	*P. granatum*	*O. basilicum*
Alkaloids	**Wagner**	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
	Dragendroff	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
	Hager	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
Carbohydrate	**Molisch**	EA	-	+	+	-
		Me	-	-	-	-
		Aq	+	+	-	+
	Benedict	EA	-	+	-	-
		Me	+	+	+	+
		Aq	+	+	+	+
	Fehling	EA	-	+	-	-
		Me	+	+	+	+
		Aq	+	+	+	+
	Barfoed	EA	+	+	+	-
		Me	+	+	+	-
		Aq	+	+	+	-
Glycosides	**Killer-Killiani**	EA	-	-	-	-
		Me	-	-	-	-
		Aq	+	-	+	+
Saponins	**Foam**	EA	-	-	-	-
		Me	-	-	-	+
		Aq	-	+	+	+

+ = Positive results, - = negative results (not found)

Table 3: The phytochemical screening of *M. piperita* , *T. capitatus* , *P. granatum* and *O. basilicum.*

	Tests	Extract	*M. piperita*	*T. capitatus*	*P. granatum*	*O. basilicum*
Terpenes & Steroids	**Salkowski**	EA	+	+	+	-
		Me	+	-	+	-
		Aq	-	+	+	+
	Liberman Burchard	EA	+	+	+	+
		Me	+	-	+	-
		Aq	+	+	+	+
Phenols	**Ferric Chloride**	EA	-	-	-	-
		Me	-	-	+	-
		Aq	+	-	+	-
Tannin	**Gelatin**	EA	+	+	+	+
		Me	-	+	+	+
		Aq	+	+	+	+
Flavonoid	**Alkaline**	EA	+	+	+	+
		Me	+	+	-	+
		Aq	+	+	+	+
	Lead acetate	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
Protein & Amino acid	**Biuret**	EA	+	-	-	+
		Me	+	-	-	+
		Aq	+	+	-	+
	Ninhydrin	EA	+	+	-	-
		Me	+	+	+	-
		Aq	+	+	-	+

+ = Positive results, - = negative results (not found)

Table 4: The phytochemical screening of *P. crispum*, *E. sativa* , *R. officinalis* and *A. graveolens L.*

	Tests	Extract	*P. crispum*	*E. sativa*	*R. officinalis*	*A. graveolens*
Alkaloids	**Wagner**	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
	Dragendroff	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
	Hager	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
Carbohydrate	**Molisch**	EA	-	-	-	-
		Me	+	+	+	+
		Aq	+	+	+	+
	Benedict	EA	-	-	-	-
		Me	+	+	+	+
		Aq	+	+	+	+
	Fehling	EA	-	-	-	-
		Me	+	+	+	+
		Aq	+	+	+	+
	Barfoed	EA	+	+	-	+
		Me	+	+	-	+
		Aq	+	+	+	+
Glycosides	**Killer-Killiani**	EA	-	-	-	-
		Me	-	+	+	+
		Aq	-	+	-	+
Saponins	**Foam**	EA	-	-	-	-
		Me	+	-	+	+
		Aq	+	-	+/	+

+ = Positive results, - = negative results (not found)

Table 5 The phytochemical screening of *P. crispum*, *E. sativa* , *R. officinalis* and *A. graveolens* .

	Tests	Extract	*P. crispum*	*E. sativa*	*R. officinalis*	*A. graveolens*
Terpenes & Steroids	**Salkowski**	EA	+	-	+	+
		Me	+	-	-	+
		Aq	+	-	-	+
	Liberman Burchard	EA	+	-	+	+
		Me	+	-	+	+
		Aq	+	-	-	+
Phenols	**Ferric Chloride**	EA	-	-	-	-
		Me	-	-	+	-
		Aq	+	+	+	+
Tannin	**Gelatin**	EA	+	+	+	+
		Me	+	-	+	+
		Aq	+	-	+	+
Flavonoid	**Alkaline**	EA	+	+	+	+
		Me	+	+	+	+
		Aq	+	+	+	+
	Lead acetate	EA	+	+	+	+
		Me	-	+	+	+
		Aq	+	+	+	+
Protein & Amino acid	**Biuret**	EA	+	+	-	-
		Me	+	+	-	+
		Aq	+	-	-	-
	Ninhydrin	EA	+	-	+	-
		Me	-	+	+	+
		Aq	-	+	-	+

+ = Positive results, - = negative results (not found)

Bacterial strains were tested in 75, 150, 250 and 500 mg/ml methanol, ethyl acetate and aqueous extracts for 24h. It was observed that all the extracts showed different growth inhibition against the gram positive and gram negative bacterial strains. For the gram positive and gram negative bacteria, the extracts became more active with increasing the extract concentration which improved the antibacterial activities of most the plants tested.

The antibacterial activities of the plants studied were assessed using the agar will diffusion method by measuring the diameter of growth inhibition zone showed in table (6, 7, 8, 9 and 10).

Table 6:Antimicrobial activity of the Methanol leaf extract of *P. granatum* against different bacteria.

Organisms / Concentration	*S. aureus*	*B. subtillis*	*E. coli*	*P. aeruginosa*
500mg	26.00±1.00	35.00±1.00	36.00±1.00	30.00±1.00
250mg	21.00±1.15	29.00±0.57	30.00±1.00	27.00±1.00
150mg	15.00±0.58	21.00±1.15	25.00±1.00	23.00±0.58
75mg	10.00±0.58	14.00±1.00	14.00±1.00	13.00±1.53

*Data are mean ±standard deviation. Means within a row are significantly different at $P < 0.05$

Table 7: Antimicrobial activity of the Ethyl Acetate leaf extract of *P. granatum* against *B. subtillis*:

Organisms / Concentration	*B. subtillis*
500mg	25.00±0.58
250mg	23.00±1.00
150mg	16.00±0.58
75mg	9.00±0.58

*Data are mean ± standard deviation.

Table 8: Antimicrobial activity of the Methanol leaf extract of *T. capitatus* against different bacteria**.**

Organisms / Concentration	*S. aureus*	*B. subtillis*
500mg	27.00±0.58	29.00±0.58
250mg	23.00±1.00	25.00±1.00
150mg	19.00±1.00	22.00±0.58
75mg	13.00±1.00	10.00±0.58

*Data are mean ± standard deviation. Means within a row are significantly different at $P < 0.05$.

Table9: Antimicrobial activity of the Methanol leaf extract of *M. piperita* against different bacteria**.**

Organisms / Concentration	*S. aureus*	*B. subtilis*
500mg	32.00±1.00	27.00±1.00
250mg	30.00±1.00	21.00±1.00
150mg	25.00±0.57	16.00±1.00
75mg	13.00±1.53	9.00±1.00

*Data are mean ± standard deviation. Means within a row are significantly different at P < 0.05

Table 10: Antimicrobial activity of the Ethyl Acetate leaf extract of *R. officinalis* against *S. aureus*.

Organisms / Concentration	*S. aureus*
500mg	27.00±2.08
250mg	25.00±1.00
150mg	12.00±1.15
75mg	8.00±0.57

*Data are mean ± standard deviation.

Among all the extracts, the methanol leaf extracts showed the highest activity (9.00 - 36.00mm) when compared to the ethyl acetate extracts (9.00 - 27.00mm). With regard to the aqueous extracts obtained of all the plants tested, it has been observed that it was inactive against the organisms screened. The methanol extracts of *P. granatum* showed the highest activity against *E. coli.* The ethyl acetate leaf extracts of *R. officinalis* and *P. granatum* showed a less activity on *S. aureus* and *B. subtillis* (9.00mm).

The obtained results of the leaf extracts are comparable with standard antibiotic Tetracycline. *B. subtillis* and *E. coli* are highly sensitive to the Methanol leaf extract of *P. granatum* than the standard Tetracycline antibiotic.

Discussion

Carbohydrates, phenols, tannins, flavonoids, saponins, steroids, alkaloids, and tannins in eight different plants belonging to different families and regions were assessed and compared. The significance of the plants in traditional medicine and the importance of the distribution of these chemical constituents were studied and discussed with their role and effect in many reports[14].

It should be noted that plant constituents such as steroidal compounds are of important and interest in pharmacy due to their relationship with such compound and six hormones[18]. The phytochemical analysis of *E. sativa* revealed that contain important secondary metabolite such as flavonoids, alkaloids, tannins and phenols[19], which was similar to our finding work. In contrast, the aqueous extract of *O. basilicum* showed the presence of flavonoids and alkaloids that different from the study reported by[20]. The differences could be attributed to many factors. It could be due to the cultivation regions. It has been reported that some plant spices that grow in different areas may contain different phytochemical contents[20]. Another fact it should be noted that, the solvent used for the estimation of flavonoids levels might have been too low to give positive results in qualitative test used which probably had a different profile of chemical and compounds.

Reports from other studies also indicated the absence of alkaloids, steroids or terpenes and flavonoids[20]. This is contrary to what was obtained in this work. In most cases tannins are usually associated with flavonoids, which are their monomeric precursor. The positive results obtained for tannins and flavonoids confirmed this fact.

The antibacterial activities of eight different extract plants including *P. granatum*, *M. piperita*, *T. capitatus*. *Thymus capitatus, Petroselinum crispum, Apium graveolens*, *Eruca sativa* and *R. officinalis* were determined against four pathogenic bacteria strains. Those are becoming clinical problem in hospital patients. *S. aureus* is known to cause series disease such as urinary tract infection, pulmonary tract infection and other blood infection[21]. The results obtained in this work had provided different level of activity against in *E. coli*, *S. aureus*, *P. aeruginosa* and *B. subtillis* due to the different extracts

On overall, methanol of leaf extracts of *P. granatum* at different concentration are very active against all the tested bacterial strains. Antibacterial properties of the leaf extracts against the bacteria tested suggests the leaf can be used for wound healing and septicemia that reported[22]. The presence of secondary metabolites from the leaves such as eudesmanoids[23] and isoflavone glycoside[24] may cause of the antibacterial activity of this plant. Also there are evidence from the literature that, the phenols, tannins, flavonoids are active against a wide range of microbes[25,26,27,28]. Their activity is probably due to their ability to combine with extracellular and sailable protein and to complex with bacterial cell walls. Also more lipophilic flavonoids in leaves may disrupt microbial membranes[28]. Low and high concentration of the methanol and Ethyl Acetate extracts were recorded in the antibacterial activity of *P. granatum*, *T. capitatus*, *M. piperita* and *R. offcinalis*.

The results obtained from *in vitro* studied showed only the ethyl acetate and methanol extracts of *P. granatum* possessed various antibacterial activity against the pathogenic bacteria selected which in different with the study reported[29]. Furthermore, the aqueous extracts were inactive against the bacteria tested. For example, the water extract of *R. officinalis* and *T. capitatus* were showed no activity against *P. aeruginosa* which with agreement to the study conducted by[30].

The study conducted also provide promise results which could enhance the curative process of MRSA in the Libyan hospitals. Due to the results obtained by[31], A total of 569 doctors and nurses from 4 main different hospitals were investigated for MRSA. Isolates from 109 of the 569 subjects (19%) were confirmed as MRSA by polymerase chain reaction assay; the majority (98/109) were from a general hospital. The results provide evidence that Libyan health care workers could serve as MRSA carriers and has a role in the spread of MRSA to the public and other workers. further studies are going in order to isolate, identify and characterize the composition of these plants. The plant studied here can be seen as a potential source of useful phytochemical compounds and elucidate the structure of these compounds.

Conclusion

The methanol extract of *P. granatum* gave significant antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. However, the methanol leaf extract of *T. capitatus* and *M. piperita* were active against *S. aureus* and *B. subtilis*. The ethyl acetate leaf extract of *R. officinalis* was only active against *S. aureus*. The results obtained indicate that these active extracts have the potential to be a source of biological active agents for pathogenic bacteria and can be useful natural chemical product for new drugs as an alternative to the use of antibiotics.

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References

1. Kong JM, Goh NK, Chia LS & Chia, TF, Recent advances in traditional plant drugs and orchids. *Acta Pharm Sin*, 24 (2003) 7.

2. Mans DR, Rocha AB & Schwartsmann G. Plant-based anticancer drug discovery and development. *The Oncol*, 5 (2005) 185.

3. Ernst, E., The rise and fall of complementary medicine. *J. Roy Soc Med,* 91 (1998) 235.

4. Briskin DP, Medicinal plants and phytomedicines linking plant biochemistry and physiology to human health. *Pla Phys,* 124 (2002) 507.

5. Meyer H, Bolarinwa A, Wolfram G & Linseisen J. "Bioavailability of apigenin from apiin-rich parsley in humans". *Ann Nut Metabo.* 50 (2006) 167.

6. Blamey M & Grey-Wilson C. Illustrated Flora of Britain and Northern Europe. (1989) ISBN 0-340-40170-2.

7. Das S, Kaur H & Tyagy AK. Evaluation of taramira oil-cake and reduction of its glucosinolate content by different treatment. *Ind J anim scien* 73 (2003) 687.

8. Peterson RL, Peterson CA & Melville, LH. Teaching Plant Anatomy Through Creative Laboratory Exercises.NRC(2008). *Press*. ISBN 9780660197982.

9. Nakatani, N. Phenolic antioxidants from herbs and spices. *Biofactors* 13 (2000) 141.

10. Singh R, Singh SK & Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auriculiformis A. Cunn. *F Chem Toxicol,* 45 (2007) 1216.

11. Steele J, Perfumeros & the sacred use of fragrance in amazonian shamanis. *The Smell Culture Reader*, edited by Jim Drobnick Berg Publishers. (2006) 23.

12. Bunsawat J, Natalina EE, Kate LH, Sproles, E & Lawrence AA. Phylogenetics of Mentha (*Lamiaceae*). Evidence from Chloroplast DNA Sequences. *Syst. Bot.* 29 (2004) 959.

13. Roopashree TS, Raman D, Shobha Rani RH1 & Narendra C. Antibacterial activity of antipsoriatic herbs. *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *Int J Appl Res Nat Prod*. 3 (2008) 20.

14. Obasi NL, Egbuonu ACC, Ukoha PO & Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of Samanea saman (fabaceae or mimosaceae) pods. *Afr J Pur Appl Chem*i. 4 (2010) 206.

15. Sani AA, Ilyas M & Haruna A. Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). *Lif Sci J*. 4 (2007) 75.

16. Ermias R, Tukue M & Bahlbi A., phytochemical screening of Ziziphus spina-christi and Piper nigrum. *J ato mol*, 1 (2011) 41.

17. Ayoola, GA, Coker HB, Adesegun, SA. Adepoju-Bello, AA, Obaweya K, Ezennia EC & Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop J Pharm Res*. 7 (2008) 1019.

18. Hong-Fang Ji, Xue-Juan Li & Hong-Yu Z. Natural products and drug discovery. *EMBO rep*. 10 (2009) 194.

19. Muhammad G, Alia S, Hira T, Muhammad I, Rahmatullah Q & Asyia Z. Phytochemical analysis and antibacterial activity of *Eruca sativa* seeds. *Pak. J. Bot*., 43(2011) 1351.

20. Daniel VN, Danang, IE & Nimyil, ND. Phytochemical Analysis and Mineral Elements Composition of *Ocimum Basilicum* Obtained in JOS Metropolis, Plateau State, Nigeria. *Int J of Eng Techn.*11(2011) 135.99.

21. Ryan, K.J & Ray CG. Sherries medical microbiology: An introduction to infectious diseases (4th ed.), McGraw Hill, New York. 2004, 45.

22. Para S, Thulasi MS, Penchalaneni J & Challagundla VN. Antibacterial Activity and Phytochemical Analysis of *Mentha piperita* L. (*Peppermint*)—An Important Multipurpose Medicinal Plant. *Amer J Plan Sci. 4* (2013), 77.

23. Prasad PP, Sawaikar DO, Rojatkar SR & Nagasampagi BA. Eudesmanoids from Sphaeranthus in- dicus, *Fitoter*, 71 (2000) 264.

24. Shekhani MS, Shah PM, Yasmin A, Siddiqui R, Perveen S, Khan KM, Kazmia US & Atta-Ur-Rahman. An Immunostimulant Sesquiterpene Glycoside from Sphaeran-thus indicus. *Phytochem*. 29 (1990) 2573.

25. Mason TL & Wasserman BP. Inactivation of Red Beet Betaglucan Synthase by Native and Oxidized Phenolic Compounds. *Phytochem*. 26 (1987) 2197.

26. Scalbert A. Antimicrobial Properties of Tannins, *Phytochem*. 30 (1991) 3875.

27. Jones GA, Mc Allister TA, Muir AD & Cheng KJ. Effects of Sainfoin (Onobrychis viciifolia scop.) Condensed Tannins on Growth and Proteolysis by Four Strains of Ruminal Bacteria. *Appl Env Microb*. 60 (1994) 1374.

28. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T & Linuma M, Comparative Study on the Antibacterial Activity of Phytochemical Flavanones against Methicillin-Resistant *Staphylococcus aureus*. *J Ethnoph*. 50 (1996) 27.

29. Ayad IK, Kamaruzaman S, Tavga SR & Khairulmazmi BA. Phytochemical Determination and Antibacterial Activity of *Punica granatum* Peel Extracts against Plant Pathogenic Bacteria. *Am J Plan Sc*. 7 (2016) 159.

30. Bassam A, Ghaleb A, Dahood A, Naser J & Kamel A. Antibacterial Activities of Some Plant Extracts Utilized in Popular Medicine in Palestine. *Turk J Biol*. 28 (2004)

31. Ahmed MO, A.K, Elramalli Amri SG, Abuzweda AR & Abouzeed YM. Isolation and screening of methicillin-resistant Staphylococcus aureus from health care workers in Libyan hospitals. *EMHJ*. 18 (2012) 37.