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RESEARCH ARTICLE

MEDICINAL HERBS AS A SOURCE OF ANTIMICROBIAL SUBSTANCES.

Michael R.Gohar¹, Ensaf E. Daood¹, Refeea M .Naseef², Mohamed A.Alwakeel².

1. Agricultural Microbiology, Fac. Agric, Cairo University, Giza.
2. Pharmacognosy Department, Fac. Pharmacy , Misr University for Science and Technology.

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*Corresponding Author

Michael R. Gohar.

Abstract

This study aimed to test a variety of naturally occurring some medicinal herbs and their extracts for their antimicrobial potential against a group of some bacterial and fungal pathogens. A total of 6 herbs (*Thymus vulgaris*, *Foeniculum vulgare*, *Datura stramonium*, *Matricaria chamomilla*, *Ammi visnaga linne* and *Cassia angustifolia*) collected from famous Egyptian plant dealers. The antimicrobial activity of aqueous, Methanol and Petroleum ether extracts were assisted by measuring the inhibition zone by agar well diffusion method and minimum inhibitory concentration (MIC) in order to increase the reliability and precision of the study. A number of 5 bacterial, 3 fungal (Molds) and 1(Yeast) species were chosen: **G+ Bacteria:** *Staphylococcus aureus* (RCMB 010027), *Bacillus subtilis* (RCMB 000101(5)). **G- Bacteria:** *Pseudomonas aeruginosa* (RCMB 000102(3)), *Escherichia coli* (RCMB 000103(9)), *Klebsiella pneumonia* (RCMB 0010093(12)). **Fungi (molds):** *Aspergillus fumigatus* (RCMB 02564), *Penicillium italicum* (RCMB 001003), *Syncephalastrum racemosum* (RCMB 005003), and **(yeast):** *Candida albicans* (RCMB 05035). The three different solvents gave markedly varied abilities to extract the antimicrobial compounds active against versatile groups including G+, G- bacteria and fungi. The active antagonism of crude methanolic extract can be descendingly arranged as thyme>senna>fennel>Datura>chamomil. The extract from *Ammi vesnaga* gave negative results. The hyphal fungi, *Penicillium italicum* was less antagonized (11.1 – 21.9 mm IZ) than *Aspergillus fumigatus* (12.3 – 22.6 mm IZ), and *Syncephalastrum racemosum* (12.6 – 20.9 mm IZ), whereas *Candida albicans* was not affected by any methanolic extract of 5 herb types and the sixth (thyme) gave relatively moderate activity (20.9 mm IZ). Methanolic extracts gave in MICs of 0.015-7.81 µg/ml (Thyme), 0.49 – 125 µg/ml (Senna) and 31.25 – 500 µg/ml (Fennel) compared to MICs of 3.9 – 500 µg/ml, 31.25 – 500 and 15.63 – 500 µg/ml for those of ether extracts respectively in the same order of herbs. Essential oil and extracts of two herbs (*Thymus vulgaris*, *Foeniculum vulgare*), were analysis by Gas chromatography/mass spectrometry analysis revealed the presence of 12 peaks in fennel essential oil which indicating the presence of 12 phytochemical constituents representing 100% of the oil, while GC-MS analysis revealed the presence of 18 peaks in Thyme essential oil which indicating the presence of 14 phytochemical constituents representing 99.98% of the oil. The major constituents of the essential oil in fennel were Anethol (63.37%), D-Limonene (20.18%), L-Fenchone (7.44%) and Estragole (3.40%). The major constituents of the essential oil in thyme were Thymol (62.16%), o-Cymene (9.75%), Caryophyllene (7.30%), δ-Terpinene (5.66%), Butylated hydroxyl anisole (5.39%).

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Introduction:-

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey *et al.*, 2011). 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 (Kamali *et al.*, 2010). The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind.

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents (Kroschwitz *et al.*, 1992). Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th Century that had effectiveness against serious bacterial infections.

A wide range of medicinal plant parts is used for extracting raw drugs as they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Uniyal, 2006). In recent years drug resistance of human pathogenic bacteria has been commonly reported from all over the world. With the continuous use of antibiotics, microorganism have become resistant. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, immunosuppressant and allergic reactions (Lopez., 2001). The antimicrobial effect of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies.

Thus, innovative scientific methods for the discovery and validation of multicomponent botanical therapeutics are important for the development of medicine and both the standardization of extracts and the identification of the efficient chemical and biological, properties. Since 1990s there has been a growing shift in interest towards plants as significant sources for new pharmaceuticals. Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs. Essential oil of Thyme was evaluated for its antibacterial activities against six Gram-positive and Gram negative pathogenic bacteria: *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus sp.*, *Pantoea sp.* and *Escherichia coli* (Jianu *et al.*, 2012). The essential oil extracted from the fruits of *F. vulgare* exhibited antibacterial effect against food borne pathogens such as *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus aureus* (Mohsenzadeh., 2007), *Listeria monocytogenes* and *S. aureus* (Dadalioglu and Evrendilek., 2004 and Cantore *et al.*, 2004).

The methanol extracts of *D. stramonium* and *Datura innoxia* showed antagonistic activity against Gram positive bacteria in a dose dependent manner. Little or no antimicrobial activity was found against *Escherichia coli* and *Pseudomonas aeruginosa* (Takhi and Ouinten., 2011). The essential oil and methanol extract of *Matricaria chamomilla* L. were tested against bacterial and fungal strains using a broth microdilution method. The results suggest that *M. chamomilla*, oil and methanol extract have significant antimicrobial activity (Fatouma *et al.*, 2011).

Materials and methods:-

Herbal materials:-

All herbal species were identified at the Pharmacognosy Department –Faculty of Pharmacy - Misr University for Science and Technology. The specimens were bought from famous Egyptian plant dealers. The collected fresh herbal materials were washed thoroughly under running tap water to remove all debris contaminants, then cut into small pieces and air-dried in the shade for 15 days and each of the selected parts of each herb was ground in a grinding machine (Pharmacognosy Department laboratory mill) to make the samples smooth and then homogenized to fine powder before being kept in dry and dark place for further work. Among the most widely applied as folk medicine and/or for pharmacological drug industries 6 samples were selected, and the effective portions of the herbs were collected, which include: *Thymus vulgaris*, *Foeniculum vulgare*, *Datura stramonium*, *Matricaria chamomilla*, *Ammi visnaga* Linne and *Cassia angustifolia*.

Table 1:- The herbal names and their plant families, common names, Arabic names, effective part and therapeutic application.

Plant species	Family	Common name	Arabic name	Part used	Therapeutic use
<i>Thymus vulgaris</i>	Lameaceae	Thyme	Zaatar	Leaves and flowers	Expectorant, Bronchodilator, Antibacterial, Antifungal
<i>Foeniculum vulgare</i>	Umbelliferae	Fennel	Shammer	Fruits	Antiinflammatory, Antimicrobial and Antiviral, antipyretic, antispasmodic
<i>Datura stramonium</i>	Salanaceae	Datura	Dautra	Leaves	narcotic, anti-spasmodic, anodyne, antimicrobial activity
<i>Matricaria Chamomilla</i>	Asteraceae	Chamomile	Sheeh papooning	Flowers	Tonic, stomachic, anodyne, and antispasmodic
<i>Ammi visnaga linne</i>	Umbelliferae	Ammi visnaga, khella	Khella ballady	Fruit	Dermatologic, and respiratory symptoms, antimicrobial activity and inhibits certain mutagens.
<i>Cassia angustifoli</i>	Leguminosae	Senna	Senna	Dried leaflets	As a laxative, diuretics cause diarrhea, Powerful cathartic, stimulation of intestinal peristalsis.

Test microorganisms:-

A number of 5 bacterial, 3 fungal (Molds) and 1 (Yeast) species were chosen: **G+ Bacteria:** *Staphylococcus aureus* (RCMB 010027), *Bacillus subtilis* (RCMB 000101(5)). **G- Bacteria:** *Pseudomonas aeruginosa* (RCMB 000102(3)), *Escherichia coli* (RCMB 000103(9)), *Klebsiella pneumonia* (RCMB 0010093(12)). **Fungi (molds):** *Aspergillus fumigatus* (RCMB 02564), *Penicillium italicum* (RCMB 001003), *Syncephalastrum racemosum* (RCMB 005003), and **(yeast):** *Candida albicans* (RCMB 05035). All microorganisms obtained from Microbiology Department – Faculty of Pharmacy - Misr University for Science and Technology and The Regional Center for Mycology and Biotechnology (RCMB).

Preparation of herbal (crude) extract:-

Methanol, Petroleum ether and distilled water were used in succession as low and high polar organic solvents to extract the active ingredients in the selected tissues (Harborne., 1973), and (Balbaa *et al.*, 1976). The extracts were prepared according to the methods described by Erturk *et al.*, (2003) and Holopainen *et al.*, (1988).

For solvent extraction:-

Thirty g portions of the air-dried powdered herb were taken in 300 ml of each organic solvent (methanol or petroleum ether) in a 500 ml conical flask plugged with cotton wool and kept on a rotary shaker at 200 rpm for 5 days (Essam ., 2006). After this period, the supernatant was collected and the solvent was filtered by using cotton and cloth and followed by filter paper (Qiong Wu *et al.*, 2005) to ensure there are no deposits in the filtrate then under vacuum pump to extract all the remaining. Then, evaporating the filtrate on rotary evaporator (at 45°C) took place till dryness (taking the recovered solvent back on the merc). Adding another fresh 300 ml solvent on the merc reaming suspension filtration.

The previous steps were repeated twice, through application of the specific solvent, filtration and evaporation to obtain a pure herb extract. The solvents were evaporated by rotary evaporator of the various extract samples (at 45°C) to make the final volume (10-20%) of the original volume (Parekh *et al.*, 2005) then were autoclaved at 121°C temperature (15 lbs pressure) for 15 min and stored at 4°C in airtight bottles in Refrigerator.

For aqueous extraction:-

Thirty g portions of the air-dried powdered herb were taken in 300 ml of distilled water in a 500 ml conical flask plugged with cotton wool and then kept on a rotary shaker at 200 rpm for only 3 days. The extract was then treated as

previously mentioned in methanol and petroleum ether extracts with some modification, e.g. solvent evaporation occurred at 60°C until (10-20%) of the initial volume. The collected solvents (methanol and petroleum ether and aqueous) extracts were lyophilized by using (model Free Zone 6 plus 12L LABCONCO) to ensure the complete dryness. A sample of 1 gm. from each herbal extract was taken and put in screw capped weighed vials then kept in a refrigerator before testing antimicrobial activity and the designed chemical analysis (Sahin *et al.*, 2003). Samples were aseptically taken for testing the various biological and biochemical parameters (Bradly., 1992).

Antimicrobial activity and media used:-

Media used:-

Nutrient agar (NA) (Shiriling and Gottlieb., 1966) was used to cultivate and maintain the examined bacterial species whereas that of Saboured's Dextrose agar with antibiotic (Midgled *et al.*, 1997) was applied to deal with pathogenic mold and yeast. Both media were prepared and sterilized before being applied as slants in test tubes or in bulk to be poured in Petri dishes.

Antimicrobial activity:-

The screening of antimicrobial activity of the six selected herb extracts was carried out using the agar well diffusion method as described by Holder and Boyce (1994). Negative control was prepared with respective solvents used for extraction. The dry extract was weighed (1gm) and dissolved in sterile 5% dimethylsulfoxide (DMSO) as a carrier and used as a sample extract (Beni *et al.*, 2014). The tested organisms were subcultured in the suitable media i.e. nutrient agar medium for bacteria and Saboured's dextrose agar for molds and yeast. Ampicillin and Gentamycin were used as a standard antibacterial substance to be compared with Gram positive and negative bacteria respectively and Amphotericin B was used as a comparable agent for fungi (mold and yeast) (Agwa *et al.*, 2000). The bacterial strains were inoculated into nutrient agar medium for bacterial cultures and incubated at 37°C for 24 h, while the fungal cultures (mold and yeast) in Sabouroud's dextrose agar and were incubated at (25-30°C) for 3-7 days before being assayed for antimicrobial activity.

Preparation of the essential oil sample:-

Three-hundred grams of the air dried powder from each of thyme or fennel were added to 1L distilled water and used the Clavenger distillation apparatus for hydrodistillation to isolate the essential oil (2 ml for each) . It was taken to Cairo University Research Park at the Faculty of Agriculture for the test of GC/MS.

Gas Chromatography / Mass Spectra (GC/MS) analysis:-

The phytochemical investigation of Thyme and fennel essential oils were performed on a GC-MS equipment. Experimental conditions of GC-MS system as follows: Instrument (HP 6890 Series Gas Chromatograph System with an HP 5973 Mass selective detector. Used Gas: Helium (1.5 ml/min). With capillary column: TR-FAME (Thermo 260 M124 P) (30m, 0.25mm ID, 0.25µm Film) (70% Cyanopropyle – Polysilphenylenesiloxane). Injector Temperature: 200°C, Temperature transfer line: 250°C. The amount of sample injected was about 1 µL (5 µl/1 ml solvent).

Results and Discussion:-

Screening of antimicrobial compounds in herbs:

The three different solvents gave markedly varied abilities to extract the antimicrobial compounds active against versatile groups including G+, G- bacteria (antibacterial) and fungi (molds and yeast – antifungal). Collectively under the examined environmental conditions, methanol was the most active solvent for extracting the antimicrobial compounds embedded in tissues of the herbs either as antibacterial or antifungal, followed by Petroleum ether solvent, mean while the aqueous solution extraction was the poorest.

The active antagonism of crude methanolic extracts shown in Table(2) can be descendingly arranged as **thyme>senna>fennel>Datura>chamomile** using molds, G+ and G- bacteria. However, the extract from *Ammi vesnaga* gave negative results either for antibacterial and antifungal influences, in addition to certain cases e.g. *Candida albicans* and *Pseudomonas aeruginosa* were not antagonized by any methanolic extracts, except that of thyme which gave active antagonism against both of them i.e. inhibition zone of 20.9 and 20.3 mm beyond well diameter respectively.

Staphylococcus aureus and *B. subtilis* (G+) were more susceptible to methanolic extracts i.e. 13.6 - 29.6 mm and 13.9 - 28.2 mm IZ respectively, which are higher than those of *E. coli* (13.2 – 19.9 mm IZ) and *Klebsiella pneumonia* (13.6 – 25.9 mm IZ)(G-). A behavior that was expected *P. aeruginosa* gave no response to various methanolic

extracts except to that of thyme (20.3 mm IZ). Among the hyphal fungi, *Penicillium italicum* was less antagonized (11.1 – 21.9 mm IZ) than *Aspergillus fumigatus* (12.3 – 22.6 mm IZ), and *Syncephalastrum racemosum* (12.6 – 20.9 mm IZ), whereas *Candida albicans* was not affected by any methanolic extract of 5 herbs types and the sixth (thyme) gave relatively moderate activity (20.9 mm IZ).

An overall evaluation of the antimicrobial activities against the examined test fungi or bacteria of the various medicinal herbs extracted by methanol, it can be seen that those of thyme were comparably equal to those the concerned standard antibiotics, whereas those of the remainder methanolic extracts were lower or markedly beyond those of the standards which gave inhibition zones of 19.5 -22.9 mm for Amphotericin B against fungi 28.3 -28.9 mm for Ampicillin against G+ bacteria and 20.3 – 25.3 mm for Gentamycin against G– bacteria (see Table 2).

Table 2:- Antimicrobial activities against some fungi and bacteria compared to standard antibiotics as inhibition zones in mm beyond well diameter (6 mm) of the 6 medicinal herbs extracted by methanol. (NA: No activity, IZ: Inhibition zone, AI: Activity index)

Test organism	Herb	Datura	Fennel	Chamomile	Thyme	Senna	Ammives naga	Standard antimicrobial
Fungi (mold)								Amphotericin B
<i>Aspergillus fumigatus</i>	IZ	14.3±0.37	16.2±0.44	12.3±0.37	22.6±0.44	18.3±0.44	NA	22.9±0.44
	AI	0.624	0.707	0.537	0.986	0.799	---	
<i>Penicillium italicum</i>	IZ	12.3±0.58	13.1±0.25	11.1±0.37	21.9±0.19	15.4±0.25	NA	21.3±0.37
	AI	0.577	0.615	0.521	1.028	0.723	---	
<i>Syncephalastrum racemosum</i>	IZ	13.1±0.44	16.1±0.37	12.6±0.63	20.9±0.44	17.9±0.37	NA	19.5±0.55
	AI	0.671	0.825	0.646	1.071	0.917	---	
Fungi (yeast)								
<i>Candida albicans</i>	IZ	NA	NA	NA	20.9±0.25	NA	NA	21.4±0.25
	AI	---	---	---	0.976	---	---	
Gram Positive bacteria								Ampicillin
<i>Staphylococcus aureus</i>	IZ	17.2±0.44	19.6±0.58	13.6±0.63	29.6±0.37	20.6±0.28	NA	28.9±0.14
	AI	0.595	0.678	0.470	1.024	0.712	---	
<i>Bacillus subtilis</i>	IZ	16.3±0.58	20.9±0.25	13.9±0.25	28.2±0.25	23.1±0.37	NA	28.3±0.37
	AI	0.575	0.738	0.491	0.996	0.816	---	
Gram Negative bacteria								Gentamycin
<i>Pseudomonas aeruginosa</i>	IZ	NA	NA	NA	20.3±0.37	NA	NA	20.3±0.37
	AI	---	---	---	1	---	---	
<i>Escherichia coli</i>	IZ	13.2±0.37	15.2±0.58	11.3±0.37	19.9±0.58	16.3±0.63	NA	21.4±0.25
	AI	0.616	0.710	0.528	0.929	0.761	---	
<i>Klebsiella pneumoniae</i>	IZ	16.3±0.44	17.1±0.25	13.6±0.25	25.9±0.44	19.8±0.44	NA	25.3±0.63
	AI	0.644	0.675	0.537	1.023	0.782	---	

The second powerful solvent extractor of antimicrobial compound from the 6 examined herbs was Petroleum ether, however, that of Chamomile had no antifungal activity and a restricted antibacterial effect not exceeding 12.4 mm IZ, e.g 10.6 – 12.4 mm against G+ and nil 11.3 mm IZ for G–bacteria. Also, *Candida albicans* and *Pseudomonas aeruginosa* were not antagonized except in case of Thyme extract which gave 17.8 and 12.2 mm respectively. Also, it had higher antagonistic activity against the other microorganisms except in cases of *E.coli* and *Klebsiella pneumonia* where that of Datura ether extract being more active, giving 16.4 and 17.8 mm compared to only 14.6 and 16.3 mm for Thyme, which recorded inhibition zones ranging from 18.2 – 20.3 mm when molds were the test organisms (Table 3).

(Table 3): Antimicrobial activities against some fungi and bacteria compared to standard antibiotics as inhibition zones in mm beyond well diameter (6 mm) of the 6 medicinal herbs extracted by petroleum ether. (NA: No activity, IZ: Inhibition zone, AI: Activity index)

Herb Test organism		Datura	Fennel	Chamomile	Thyme	Senna	Ammi vesnaga	Standard antimicrobial
<u>Fungi (mold)</u>								Amphotericin B
<i>Aspergillus fumigates</i>	IZ	13.2±0.44	12.7±0.25	NA	19.6±0.58	13.7±0.25	12.6±0.25	22.9±0.44
	AI	0.576	0.554	---	0.855	0.598	0.550	
<i>Penicillium italicum</i>	IZ	14.7±0.25	12.9±0.37	NA	20.3±0.25	14.9±0.44	13.7±0.37	21.3±0.37
	AI	0.690	0.605	---	0.953	0.699	0.643	
<i>Syncephalastrum racemosum</i>	IZ	15.9±0.63	13.1±0.44	NA	18.2±0.37	15.8±0.37	NA	19.5±0.55
	AI	0.815	0.671	---	0.9333	0.810	---	
<u>Fungi (yeast)</u>								Amphotericin B
<i>Candida albicans</i>	IZ	NA	NA	NA	17.8±0.37	NA	NA	21.4±0.25
	AI	---	---	---	0.831	---	---	
<u>Gram Positive bacteria</u>								Ampicillin
<i>Staphylococcus aureus</i>	IZ	13.6±0.44	15.2±0.63	10.6±0.63	20.6±0.44	16.2±0.44	13.9±0.44	28.9±0.14
	AI	0.470	0.525	0.366	0.712	0.560	0.480	
<i>Bacillus subtilis</i>	IZ	16.2±0.25	15.5±0.58	12.4±0.58	21.4±0.37	17.3±0.37	15.8±0.58	28.3±0.37
	AI	0.572	0.547	0.438	0.756	0.611	0.558	
<u>Gram Negative bacteria</u>								Gentamycin
<i>Pseudomonas aeruginosa</i>	IZ	NA	NA	NA	12.2±0.25	NA	NA	20.3±0.37
	AI	---	---	---	0.600	---	---	
<i>Escherichia coli</i>	IZ	16.4±0.58	13.4±0.44	NA	14.6±0.37	12.3±0.44	12.6±0.37	21.4±0.25
	AI	0.766	0.626	---	0.682	0.574	0.588	
<i>Klebsiella pneumonia</i>	IZ	17.8±0.58	14.9±0.37	11.3±0.63	16.3±0.25	14.2±0.58	15.6±0.44	25.3±0.63
	AI	0.703	0.588	0.446	0.644	0.561	0.616	

The overall antagonistic activities of the various ether herbs extracts can be ascendingly arranged as Chamomile < Fennel < Ammi vesnaga < Senna < Datura < Thyme. The inhibition zones averaged for them: 11.43, 13.96, 14.03, 14.91, 15.4 and 17.89 mm respectively. Generally, the antimicrobial activities of ether extracts from the versatile herbs were apparently lower than those of the standard specific antibiotics which induced inhibition zones ranging from 19.5 – 22.9 mm for Amphotericin B against fungi, 28.3 – 28.9 mm for Ampicillin against G+ bacterial species and 20.3 – 25.3 mm IZ for Gentamycin against G- bacteria. As was to be expected, the extraction of antimicrobial components from the various examined medicinal herbs was the poorest for aqueous solution compared to both methanol and Petroleum ether. The aqueous crude extract of the various herbs hasn't any antifungal activity against *Aspergillus fumigates*, *Penicillium italicum* and *Syncephalastrum racemosum* as well as *Candida albicans*. Meanwhile, the antibacterial activity of the aqueous extract of both herbs Chamomile and *Ammi vesnaga* didn't antagonize either of the examined species of *S. aureus* and *B. subtilis* (G+), *P. aeruginosa*, *E. coli* and *K. pneumonia* (G-) (Table 4).

Table 4:- Antimicrobial activities against some fungi and bacteria compared to standard antibiotics as inhibition zones in mm beyond well diameter (6 mm) of the 6 medicinal herbs extracted by aqueous solution.(NA: No activity, IZ: Inhibition zone, AI: Activity index)

Test organism	Herb	Datura	Fennel	Chamomile	Thyme	Senna	Ammi vesnaga	Standard antimicrobial
<u>Fungi (mold)</u>								Amphotericin B
<i>Aspergillus fumigatus</i>	IZ	NA	NA	NA	NA	NA	NA	22.9±0.44
	AI	---	---	---	---	---	---	
<i>Penicillium italicum</i>	IZ	NA	NA	NA	NA	NA	NA	21.3±0.37
	AI	---	---	---	---	---	---	
<i>Syncephalastrum racemosum</i>	IZ	NA	NA	NA	NA	NA	NA	19.5±0.55
	AI	---	---	---	---	---	---	
<u>Fungi (yeast)</u>								
<i>Candida albicans</i>	IZ	NA	NA	NA	NA	NA	NA	21.4±0.25
	AI	---	---	---	---	---	---	
<u>Gram Positive bacteria</u>								Ampicillin
<i>Staphylococcus aureus</i>	IZ	10.9±0.37	13.1±0.28	NA	17.2±0.28	14.8±0.28	NA	28.9±0.14
	AI	0.377	0.453	---	0.595	0.512	---	
<i>Bacillus subtilis</i>	IZ	12.7±0.58	15.4±0.37	NA	18.3±0.37	15.3±0.37	NA	28.3±0.37
	AI	0.448	0.544	---	0.646	0.540	---	
<u>Gram Negative bacteria</u>								Gentamycin
<i>Pseudomonas aeruginosa</i>	IZ	NA	NA	NA	12.9±0.44	NA	NA	20.3±0.37
	AI	---	---	---	0.635	---	---	
<i>Escherichia coli</i>	IZ	13.9±0.58	11.9±0.58	NA	15.7±0.25	12.9±0.44	NA	21.4±0.25
	AI	0.649	0.556	---	0.733	0.602	---	
<i>Klebsiella pneumoniae</i>	IZ	15.8±0.44	12.8±0.63	NA	17.2±0.37	14.2±0.58	NA	25.3±0.63
	AI	0.624	0.505	---	0.679	0.561	---	

The same behavior was detected when evaluating those aqueous extracts of Datura, Fennel and Senna for their activity against *P.aeruginosa* i.e. that of thyme extract was the only relatively antagonize this species recording 12.9 mm IZ compared to 20.3 for the standard Gentamycin. Regarding the other two G- bacteria, *E.coli* and *K.pneumonia*, it can be observed that both were antagonized with or moderate level i.e. 15.9 and 17.2 mm IZ respectively against 21.4 and 25.3 mm IZ for the same standard antibiotic. Fig (1) shows representatives of IZ of some herbs and reference antibiotics extracted by the 3 solvents.



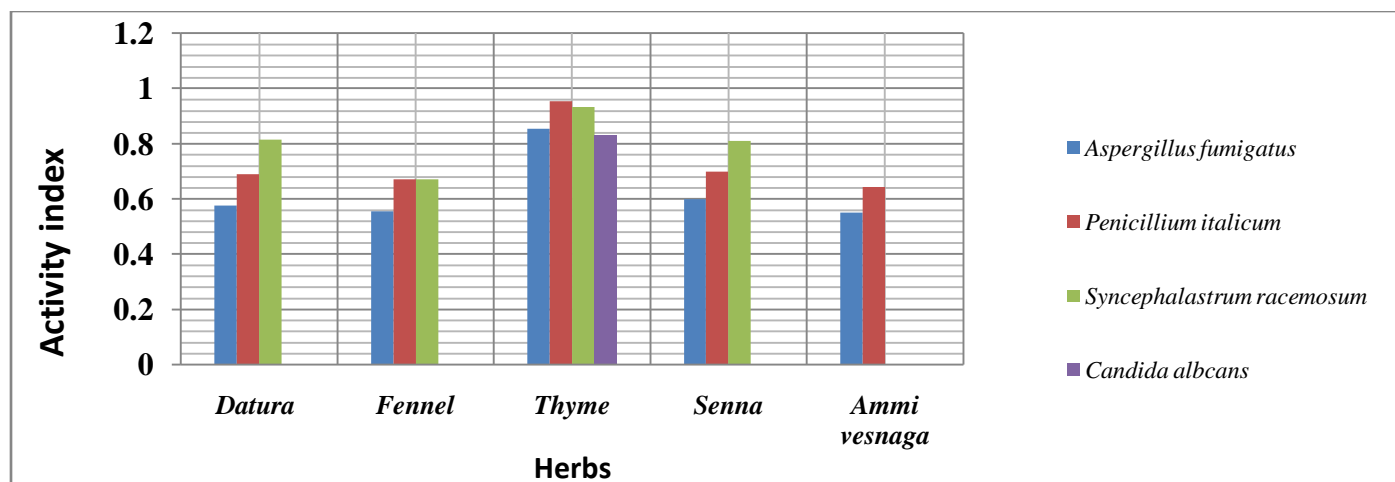
(A) (B) (C) (D)

Fig 1:- Antimicrobial activities against some fungi and bacteria as inhibition zone of the medicinal herbs extracted by methanol, petroleum ether and aqueous solution compared to standard antibiotics.

- Chamomile extract against *Bacillus subtilis*.
- Fennel extract against *Syncephalastrum racemosum*.
- Thyme extract against *Staphylococcus aureus*.
- Datura extract against *Aspergillus fumigates*.

Antimicrobial Activity Indices:-

The antimicrobial activity index either for antifungal or antibacterial antagonism of the 6 herbs appear in Graph (1) and (2). It is clear that their values are a reflection of the antimicrobial mechanisms against both fungi and bacteria, since their figures paralleled the increments or declines in the action of the antagonistic compounds. These attributes indicate the entire dependency of the indices on the type of herb, the solvent, applied and the type of selected test organism and reference antibiotic. Some of the herb's extracts showed no activity against certain tested fungi or bacteria therefore have no AI and will be neglected in neither Fig nor description. As for methanolic extracts and irrespective of the examined species, their Activity indices compared to the reference antibiotics, ranged between 0.521 to 1.071 for the examined molds whereas *candida albicans* was antagonized only with thyme methanol extract with a relatively moderate AI, 0.976 an indicative that this yeast species is not sensitive to the others examined herbs. Again, petroleum ether has relatively lower strength as extraction solvent. Thus almost similar behaviours in AI, shown by methanol extract are repeated with petroleum ether extracts against the examined fungi or bacteria, however with lower levels. As to the aqueous extracts, they have no antifungal against hyphal or unicellular fungi. In case of true bacteria either G- or G+ the calculated AI were either nil or lower or relatively so when compared with those of methanolic or petroleum ether extracts (Graph1, 2). Graph 1: Antibacterial activity index of the 6 medicinal herbs extracted by petroleum ether related to specific standard antibiotic for both G+ and G- bacteria.



Graph 2:- Antifungal activity index of the 5 medicinal herbs extracted by petroleum ether related to specific standard antibiotic. (Chamomile was dropped as it has no activity)

Minimum Inhibitory Concentration (MIC):-

The Minimum Inhibition Concentration is known as the highest dilution or the least concentration of the herb extract that inhibits the growth of an organism. MIC is used to determine the potentiality of new antimicrobial substances against a specific microorganism which can be compared with utilizable ones. MIC is widely applicable in microbiological laboratories to evaluate antagonistic activity of antibiotics either produced by pure cultures or embedded in plant tissues.

Three of the herbs containing active antagonistic compounds extracted by two active solvents: Methanol and Petroleum ether were tested by MIC which inhibited the growth of almost all, except in few cases, of the examined organisms and data are shown in Table (5). MIC values significantly varied depending upon the type of herb, the solvent applied and the test microorganism. It ranged between nil-500 µg/ml compared to 0.49 - 7.81 µg/ml for Amphotericin B, 0.015 -0.03 µg/ml for Ampicillin and 0.12 – 3.9 µg/ml for Gentamycine.

As to the potentiality of the tested solvents ,it can be observed that Methanolic extracts were more active than those of Petroleum ether , therefore and irrespective of the microorganisms, methanolic extracts gave in MICs of 0.015- 7.81 µg/ml (Thyme), 0.49 – 125 µg/ml (Senna) and 31.25 – 500 µg/ml (Fennel) compared to MICs of 3.9 – 500 µg/ml, 31.25 – 500 and 15.63 – 500 µg/ml for those of ether extracts respectively in the same order of herbs. These values confirm that Thyme contained more antagonistic compounds against the tested organisms and fennel had the least.

However, as to the specific bacterial groups, it was found that Thyme either methanolic and ether extracts are most active against both *Staphylococcus aureus* and *Bacillus subtilis* (0.015-0.03 and 3.9-1.95 mg/ml) and nil or very weak for Fennel and Senna respectively.

Table 5:- Minimum Inhibition concentration (MIC) in mg /ml of Methanol and Petroleum ether of the active 3 herbs compared to standard specific antibiotics: (NA: no activity)

Solvent /Herbs Test organism	Petroleum ether extract				Methanol extract			Standard antibiotics mg/ml
	Fennel	Thyme	Senna		Fennel	Thyme	Senna	
<u>Fungi (mold)</u>								Amphotericin B
<i>Aspergillus fumigatus</i>	500	7.81	125		62.5	0.98	15.63	0.49
<i>Penicillium italicum</i>	125	3.9	125		500	1.95	125	1.95
<i>Syncephalastrum racemosm</i>	125	15.63	62.5		62.5	3.9	31.25	7.81
<u>Fungi (yeast)</u>								
<i>Candida albicans</i>	NA	31.25	NA		NA	3.9	NA	1.95
<u>Gram Positive bacteria</u>								Ampicillin
<i>Staphylococcus aureus</i>	500	3.9	62.5		31.25	0.015	3.9	0.015
<i>Bacillus subtilis</i>	62.5	1.95	1.25		62.5	0.03	0.49	0.03
<u>Gram Negative bacteria</u>								Gentamycin
<i>Pseudomonas aeruginosa</i>	NA	500	NA		NA	7.81	NA	3.9
<i>Escherichia coli</i>	31.25	125	500		500	7.81	62.5	1.95
<i>Klebsiella pneumoniae</i>	15.63	62.5	62.5		62.5	0.12	7.81	0.12

GC-MS (Gas Chromatography-Mass Spectrometry) analysis:-**Chemical composition of the essential oils by GC-MS:-**

Gas chromatography coupled with mass spectrometry (GC–MS) is a commonly used technique for separating and identifying the components of complex volatile mixtures. GC–MS can be a valuable tool in natural product research assisting in the separation and identification of isolated components.

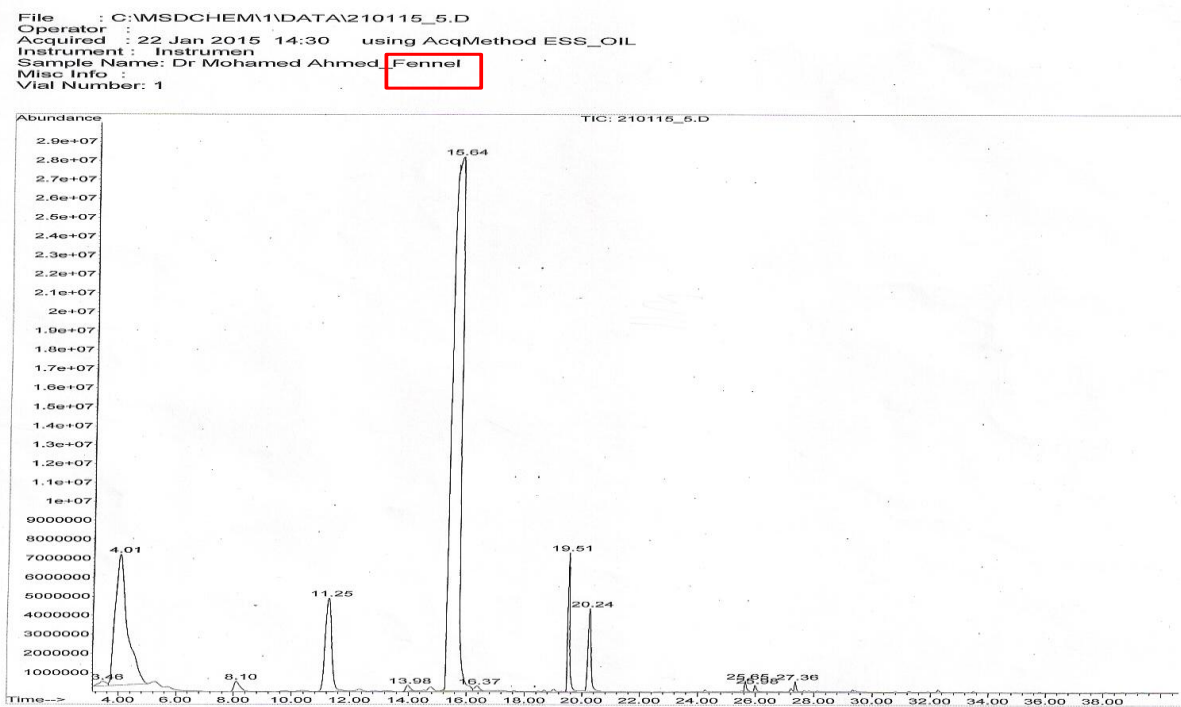
Herbs essential oils are volatile and well suited to GC–MS analysis. GC–MS is known for its high-resolution separation of structurally similar sesquiterpenes, which are the main constituents of herbs essential oils. The identification of the component was based on library searched database, as well as, comparing their mass fragmentation pattern with published data (Adams,. 1989).

The percentage yield determined according to the Egyptian pharmacopoeia and the components of each oil were identified by comparing their mass spectra with the published data. The samples were analyzed and the results of the GC-MS analysis and the percentages of different group constituents are compiled in the Tables (6 and 7) and Graph (3 and 4) for Thyme and Fennel respectively. The result of the chemical composition of Fennel and Thyme essential oils indicate that the overall, GC-MS analysis revealed the presence of 12 peaks in fennel essential oil which indicating the presence of 12 phytochemical constituents representing 100% of the oil. The major constituents of the essential oil tested were Anethol (63.37%), D-Limonene (20.18%), L-Fenchone (7.44%) and Estragole (3.40%). In addition, the tested fennel essential oil also contained considerable amounts of various minor constituents whose contribution was <10% (Table 6).

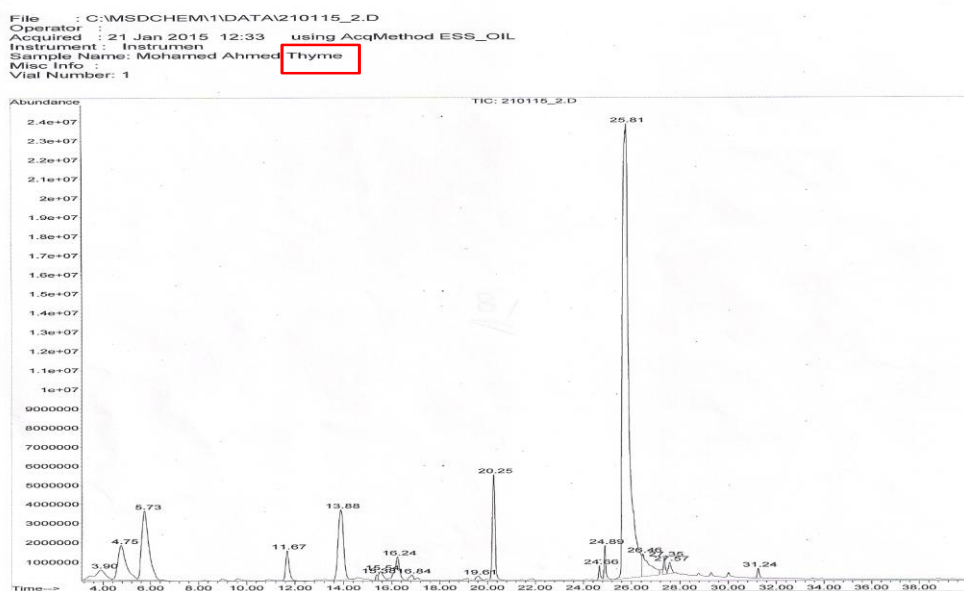
Table 6:- The GC/MS of the essential oil of Fennel. * Rt= retention time in minutes, Relative % = The Percentage of the total compound.

Peak No.	Rt*	Name	Relative %
1	3.46	Beta-Pinene	0.44
2	4.01	D-Limonene	20.18
3	8.10	Trans-alloocimene	0.77
4	11.25	L-Fenchone	7.44
5	13.98	Terpinen-4-ol	0.31
6	15.64	Anethole	63.37
7	16.37	Beta-copaene	0.29
8	19.51	Estragole	3.40
9	20.24	Butylatedhydroxyanisole	3.09
10	25.65	O-cymen-5-ol	0.30
11	25.98	Trans-isoeugenol	0.18
12	17.36	Benzoic acid, 4-ethoxy-ethyl	0.23

Regarding the groups of chemical constituents represented Anethol, Fenchone and Astragol were the main oxygenated monoterpenes, while Limonene was the major monoterpene. A literature search revealed Anethole (62.0%), Fenchone (20.3%), Estragole (4.90%) and Limonene (3.15%) to be the main components of essential oils from wild-growing Fennel seed native to the podgorica region, central south Montenegro (Damjanovic *et al.*, 2005).



Graph 3:- The GC/MS analysis of the antimicrobial compounds in Fennel.



Graph 4:- The GC/MS analysis of the antimicrobial compounds in Thyme.

Mimica-Dukic *et al.*, (2003) also reported Anethol (74.18%), Fenchon (11.32%), Estragol (5.29%), Limonene (2.53%) and α -pinene (2.77%) as the major compounds identified in the essential oil from *F. vulgare* mill. Ozcan *et al.*, (2006) reported Estragole (61.08% and 40.49%), Fenchone (23.46% and 16.90%) and Limonene (8.68% and 17.66%), respectively, as the major constituents in the essential oil of bitter Fennel (*F. vulgare* spp. *piperitum*) grown in Turkey. Such variations in the chemical composition of essential oil across countries might be attributed to the varied agroclimatic (climatic, seasonal, geographical) conditions of the regions, stage of maturity and adaptive metabolism of plants. On a quantitative basis, the amounts of the four main components were: Anethol (63.37%), D-Limonene (20.18%), L-Fenchone (7.44%) and Estragole (3.40%).

The GC-MS analysis revealed the presence of 18 peaks (4 belonged to Thymol) in Thyme essential oil which indicating the presence of 18 phytochemical constituents representing 99.98% of the oil. The major constituents of the essential oil tested were Thymol (62.16%), o-Cymene (9.75%), Caryophyllene (7.30%), δ -Terpinene (5.66%), Butylated hydroxyl anisole (5.39%) Table(7). In addition, the tested Thyme essential oil also contained considerable amounts of various minor constituents whose contribution was < 10%. Generally, the oil was found to be rich in the active monoterpene phenols (thymol and carvacrol) and their corresponding monoterpenes p-cymene and δ -terpinene, which collectively showed synchronized patterns of variation during the different collection periods and in different seasons. Monoterpene phenols (mainly thymol) and their derivatives (mainly carvacrol methyl ether), sesquiterpenes (mainly caryophyllene) and their oxygenated derivatives (e.g. caryophyllene oxide) were found in different levels.

Table 7:- The GC/MS of the essential oil of Thyme. * Rt= retention time in minutes, Relative % = The Percentage of the total compound. * The 4 Thymol peaks were considered as 1 peak.

Peak No.	Rt*	Name	Relative%
1	3.90	Δ^3 -Carene	2.21
2	4.75	Gamma-Terpinene	5.66
3	5.73	O-Cymene	9.75
4	11.67	Humulene	2.08
5	13.88	Caryophyllene	7.30
6	15.38	Estragole	0.20
7	15.54	cis-alpha-Bisabolene	0.77
8	16.24	endo-Borneol	2.18
9	16.84	Curcumene	0.32
10	19.61	Cuminal(P-cumic aldehyde)	0.33
11	20.25	Butylated hydroxy anisole	5.39
12	24.66	Thymol *	62.16
13	27.35	Durenol	0.99
14	27.57	Phenol,2,3,5,6-tetramethyl	0.64

The comparison of our result with literature shows important qualitative and quantitative differences in compositions. The genus *Thymus* has numerous species and varieties and their essential oil composition has been studied earlier (Guillen & Manzanos, 1998, Jordan *et al.*, 2003; Sotomayor *et al.*, 2004). From thyme grown in other countries (Piccaglia *et al.*, 1993), six chemotypes have also been reported, geraniol, linalool, γ -terpineol, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol. Our results differ from those obtained by Ozcan *et al.*, (2004) who studied the oil composition of thyme of the same species sample in Turkey, in which thymol (46.2%), alpha terpinene (14.1%), p-cymene (9.9%) and alpha-pinene (3.0%) were revealed to be dominant. For the Spanish thyme essential oil, the major components quantified were 1,8-cineole, followed by terpenyl acetate, borneol, linalool, beta-pinene, alpha-terpineol and camphor (Jordán *et al.*, 2006). On the contrary carvacrol, which was not detected in our sample was found to be the main component in the previous report (Hudaib, 2002). These probably due to the plant varieties or sites, as well as the time of harvesting.

References:-

1. **Dubey, R.; Dubey, K.; Sridhar, C. and Jayaveera, K.N. (2011).** Human Vaginal Pathogen Inhibition Studies on Aqueous, Methanolic and Saponins Extracts of Stem Barks of *Ziziphus Mauritiana*. Int. J. Pharm. Sci. Res., 2(3): 659-663.
2. **Kamali, H.H.; EL, Amir, M.Y.E.L. (2010).** Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from Selected Sudanese Medicinal Plants. Curr. Res. J. of Bio. Sci. 2010; 2(2): 143-146.
3. **Kroschwitz, J.I. and M. Howe-Grant, (1992).** Kirk- Othmer encyclopedia of chemical Technology, 2: 893.
4. **Uniyal, S.K.; K.N. Singh, P. Jamwal and B. Lal, (2006).** Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan J. Ethnobiol. Ethnomed., 2: 1-14.
5. **Lopez, A.; Hudson, J.B and Towers, G.H.N, (2001).** Antiviral and antimicrobial activities of Colombian medicinal plants. J. Ethnopharmacology, 77:189-196.
6. **Jianu C, Misca C, Pop G, Rusu LC, Ardelean L, Gruia AT.(2012).** Chemical Composition and Antimicrobial Activity of Essential Oils Obtained from Dill (*Anethum graveolens L.*) Grown in Western Romania. Revista de Chimie (Bucharest); 63(6):641–645.
7. **M. Mohsen zadeh. (2007).** Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococcus aureus* and *Escherichia coli* in nutrient broth medium Pak. J. Biol. Sci., 10 , pp. 3693–3697.
8. **Dadalioglu, G.A. Evrendilek. (2004).** Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas L.*), and fennel (*Foeniculum vulgare*) on common food borne pathogens .J. Agric. Food Chem., 52 , pp. 8255–8260
9. **Takhi D, Ouinen M. (2011).** Study of antimicrobial activity of secondary metabolites extracted from spontaneous plants from the area of Laghouat, Algeria. Adv Environm Biol. ;5(2):469–476.
10. **Fatouma, M.; Abdoul-Latif, L.; Nabil, M.; Prosper Edou , Adwa A. Ali , Samatar O. Djama , Louis-Clément Obame , Ismael H. N. Bassolé and Mamoudou H. Dicko (2011).** Journal of Medicinal Plants Research Vol. 5(9), pp. 1512-1517, 4 May,
11. **Essam Mohamed Abd-el Aziz hoballah (2006)** Department of Agricultural Microbiology, Cairo University, Antimicrobial compounds in some local medicinal plants. Page 13.
12. **Harborne, J. B. (1973).** "Methods of plant analysis." Phytochemical methods. Springer Netherlands,. 1-32.
13. **Balbaa, S. I. (1976).** Investigating the efficacy of three plants species (*sclerocarya birrea*, *Neo carya Mocrophylla* and *Grewia Mollis*) as anti snake venom. Diss. M. sc Dissertation (Unpublished), Usmanu Danfodiyo University Sokoto, 1976.
14. **Ertürk, S. (2003).** Digital image stabilization with sub-image phase correlation based global motion estimation. Consumer Electronics, IEEE Transactions on, 49(4), 1320-1325.
15. **Jantti-Alanko, S., E. Holopainen, and H. Malmberg.(1988).** "Recurrence of nasal polyps after surgical treatment." Rhinology. Supplement 8 : 59-64.
16. **Chen, Guo-Qiang, and Qiong Wu.(2005).** "The application of polyhydroxyalkanoates as tissue engineering materials." Biomaterials 26(3)3 : 6565-6578.
17. **Parekh, A. B., & Putney, J. W. (2005).** Store-operated calcium channels. Physiological reviews, 85(2), 757-810.
18. **Efron, Bradley.** Bootstrap methods: another look at the jackknife. Springer New York, 1992.
19. **Karaman, I., Şahin, F., Gulluce, M., Ogutçu, H., Şengul, M., & Adıguzel, A. (2003).** Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus L.* Journal of ethnopharmacology, 85(2), 231-235.
20. **Hollenstein, E., Davis, M., Damjanovic, D., & Setter, N. (2005).** Piezoelectric properties of Li-and Ta-modified (K_{0.5}Na_{0.5})NbO₃ ceramics. Applied Physics Letters, 87(18), 182905.
21. **Mimica-Dukić, N., Bozin, B., Soković, M., Mihajlović, B., & Matavulj, M. (2003).** Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Plantamedica, 69(5), 413-419.
22. **Piccaglia, R., Marotti, M., Giovanelli, E., Deans, S. G., & Eaglesham, E. (1993).** Antibacterial and antioxidant properties of Mediterranean aromatic plants. Industrial crops and Products, 2(1), 47-50.
23. **Ozcan, U., Cao, Q., Yilmaz, E., Lee, A. H., Iwakoshi, N. N., Ozdelen, E., & Hotamisligil, G. S. (2004).** Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science, 306(5695), 457-461.
24. **Vogel, V. G., Costantino, J. P., Wickerham, D. L., Cronin, W. M., Cecchini, R. S., Atkins, J. N., & Robidoux, A. (2006).** Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. Jama, 295(23), 2727-2741.
25. **Hudaib, M., Speroni, E., Di Pietra, A. M., & Cavrini, V. (2002).** GC/MS evaluation of thyme (*Thymus vulgaris L.*) oil composition and variations during the vegetative cycle. Journal of pharmaceutical and biomedical analysis, 29(4), 691-700.
26. **Piccaglia, R., Marotti, M., Giovanelli, E., Deans, S. G., & Eaglesham, E. (1993).** Antibacterial and antioxidant properties of Mediterranean aromatic plants. Industrial crops and Products, 2(1), 47-50.