Antioxidant, Antimicrobial and Antimutogenic Potential of 4 Iranian Medicinal Plants

Ali Mirzaei[1], Mehdi Akbartabar Toori[2*], Nooshin Mirzaei[1], Reza Ghafarian Shirazi[1, 3]

[1]Medicinal plant research center, Yasuj University of Medical Sciences, Yasuj, Iran
[2]*Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[3]Department of Chemistry, University of Eastern Finland, Joensuu, Finland.
*Corresponding Author: m__akbartabat@yahoo. co. uk

Abstracts: Introduction: Traditional medicine still plays a key role in prevention and treatment of many diseases in Iran. The aim of this study was to evaluate antibacterial, antioxidant and antimutogenic properties of 4 Iranian medicinal plants (Teucrium polium, Origanum Vulgare, Menthe piperita and Matricaria recutica). **Materials and Methods:** The extracts were prepared by maceration method which was assessed for their antioxidant, antimicrobial, antimutogenic properties and phytochemical components analyses. For determination of antimicrobial activity against 3 gram-positive and 2 gram-negative bacteria, agar diffusion method was used. Antioxidant activity was assessed by two methods, trolox equivalent antioxidant activity (TEAC) and dipheny-picrylhydrazyl (DPPH) radical assays. For their antimutogenic potential the Ames Salmonella/microsome mutagenicity assay was carried out. **Results:** Among five human pathogens, Staphylococcus aureus tended to be the most sensitive compared to the other bacteria. The gram-positive bacteria revealed more sensitivity to the plant extracts compared to gram-negative bacteria. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) range of the plant extracts against the test organisms were 6. 25-50 and 12. 5-100 mg/ml respectively. The range of free radical scavenging potential in DPPH and ABTS tests showed 1525-11945 and 345-855 µmole trolox in gram (plant dry weight) respectively. Antimutogenic activity of Menthe piperita (54. 2)Matricaria recutica (43. 6)Teucrium polium(36. 5) and Origanum Vulgare (28. 8) were determined by Ames test in the presence of S9 mixture(+S9). The phytochemical screening of the extracts demonstrated the presence of showed total phenol, flavonoids, tannins, alkaloids, saponine and glycosides, which could be responsible for their potential activities which observed in this study. **Conclusion:** The results of this study showed that the highest antibacterial and antioxidant activities were belong to Matricaria recutica and Origanum Vulgare respectively. However, Menthe piperita was exhibited the highest protection against the mutagenicity.
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1. Introduction

Infectious diseases are major health problem in developing countries and are responsible for high rate of mortality in vulnerable groups in these regions. Resistances of bacteria to synthetic antibiotics cause various problems for treatment of infectious diseases. Resistance of bacteria may be caused by overuse and indiscriminate use of commercial antimicrobial drugs which utilized for treatment of infectious disease. Nowadays, this resistance among the microbial pathogens establishes a global problem and great concern [1]. Nowadays, control of infectious diseases is a major problem worldwide due to increase in the number of antibiotic-resistant microorganism [2]. Due to increase in drug- resistant bacteria, a novel and natural antimicrobial agents are necessary for eradication of pathogen organisms [3]. The traditional medicine still plays a key role in the primary health care in both developed and developing world. Many plant species with biological potential including antimicrobial, antioxidant and Anti mutogenic activities are available in a great volume of literature. Up to 2600 plant species in world, 700 are useful for medicinal potential. They used for treatment of infectious disease and ailments by the ancient people [4]. Screening of medicinal plants for search of novel drug is necessary for due to increased resistance of many bacteria to antibiotics. Medicinal plants constitute an essential source of biological compounds with antioxidant and anti-infectious agents as a medicine. Plant Phytochemicals have shown great potential for treatment of human bacterial and viral diseases [5]. There are a numerous reports on the efficiency of medicinal plants against gram positive and gram negative bacteria, and therefore; plants are still known, as the base for new medicine to treat infectious diseases [6]. Medicinal plants are used as the most common source of many potent drugs with antimicrobial activity. Treatments of bacterial diseases with medicinal plants have minimal side effect in compare to synthetic

antibiotics [7, 8]. The active components of plant – derived drugs are belonging to secondary metabolites. Therefore, screening of plant extract for their important phytochemical constituent is very essential. According to much result study, there are many bioactive compounds present in medicinal plant such as phenolic compounds, flavonoids, alkaloids, tannins, essential oils and unsaturated long chain aldehydes with antibacterial potential. These compounds responsible for the plants antioxidant, antimicrobial anti-inflammatory, antihypertensive and antidiabetic in litrature[9, 10]. Researchers are more focusing in folk medicine for develops a new drug against cancer, microbial and viral infections. Although hundreds of the plants samples have been examined for their antimicrobial activities, most of them have not yet been well qualified. Teucrium polium belong to the family of (Lamiaceae) that used for anti-inflammatory, anti-bacterial, anti-hypertensive, anti-hyperlipedemic and anti-hyperglycemic potential in traditional medicine [11, 12]. Origanum vulgare is a member of the to the Labiatae family, have used for antimicrobial, antioxidant and antimutagenic activities. It may have a major property for industrial applications. Over the years, it also used as flavouring agents in various food products [13, 14]. Mentha piperita (Labiate) is mostly found in the tropics and subtropical regions. The leaves and other parts are used as antibacterial, antiviral and fungicidal. It used also for relief of pain and blood flow to the affected area. [15]. Matricaria recutica L. belongs to the family Asteraceae. The flowers of the plant are used widely by the Iranian as tea preparation. The anti-inflammatory, antispasmodic, antibacterial properties were reported in literature for centuries [16].

2. Materials and methods

2. 1. Plant materials and extracts preparation:

The plants were collected from different localities of Iran in July 2012 and identified by the botanist. The plants were dried in shade and then grounded. The grounded materials were individually extracted with, 70% (v/v) ethanol, using maceration method for 24h. The extracts were collected and dried using rotary evaporator (Hyedolph model 4000, Germany) and remained at freeze for study until used. The voucher of these plants was deposited at the Biochemistry laboratory in medical college. Antibacterial activity of the extracts was tested againstfive human pathogens3 species of gram-positive (Lysteria monocytogenous, Staphylococcus aureus and Bacillus cereus and 2 species of gram-negative bacteria (Pseudomonas aeruginosa and salmonella typhi. Test organisms were collected in clinical samples obtained from patients, attending in government hospital Vali– e-Aser Iran. The bacteria were isolated in nutrient agar medium and identified by biochemical and standard antibiogram tests. For the antimicrobial evaluation, the microorganisms were cultured overnight at 37 ºC in nutrient agar in Mueller Hinton Broth (MHB) (Oxoid, England) were adjusted to 107cfu mL-1 with sterile saline.

Media Preparation and Antibacterial Activity of plant extracts. The antimicrobial potential was determined by agar disc diffusion. The molten Mueller Hinton agar was inoculated with 100 µl of the suspension containing 108 colony-forming units (cfu/ml) of bacteria cells poured into the Petri plate (Hi-media). For agar disc diffusion method, the sterile disc (6mm) Whatman paper disc No. 1) were separately impregnated with 50 µl of the each plant extracts, allowed to dry at 37°C and was introduced on the upper layer of the previously inoculated agar surface in triplicate. Plates were kept for 2 h in refrigerator to enable prediffusion of the extracts into the agar. Then, the plates were incubated overnight (18 h) at370C. Ciprofloxacin (5 mcg), and nalidixic acid (10mcg) were used as standard antibiotics. Discs without extracts but loaded with 100 µl of organic solvents were applied as a negative Control.

Table 1. properties of medicinal plants collected for biological activity.

Botanical name	Family	Local Name	Part of plant used
Menthe piperita	Labiate	Peppermint	Leaves
Matricariarecutica	Asteraceae	Chamomile	Flowers
Teucriumpolium	Lamiaceae	Germarder	Flowers
OriganumVulgare	Labiatae	Oregano	aerial

At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones(diameter of inhibition zone plus diameter of the disc (including disc diameter of 6 mm). Minimum inhibitory concentration determination: For minimum inhibitory concentration (MIC), a micro-dilution broth susceptibility assay was carried out [17]. For solubilization of extracts dimethylsulfoxide (10% DMSO) was used and then diluted in culture media for use. A volume of 100 µl of 200mg/ ml (w/v) herbs extracts were diluted with 100 µl Mueller Hinton broth (MHB) in twofold dilutions into first row of 96- well micro plates. The serial dilution was made in range of to 0. 10 to 200 mg mL-1. One hundred µL of bacteria suspension of 5 x 105cfu /ml by 0. 5 McFarland turbidity standards

was added to each well which containing 100 µl extracts. The mixture was incubated at 37 ºC for 24 h. The lowest concentration which completely inhibited the microbial growth (no turbidity) was recorded asMIC (mg mL-1). The turbidity was measured by ELISA micro plate reader (Bio-tech USA) at 540 nm and the growth was indicated if OD value that increased twice its initial value. Results were the mean of three experiments. MBC was estimated by sub-culturing the treated broth culture from well which is not display any visible growth in MIC assay, on new sterile Mueller Hinton Agar (MHA) plates and incubated further for 18-24 h[18]. The maximum dilution of the extract that inhibits bacterial colony formation was considered as MBC [17].

2. 2. Antioxidant activity of Dipheny-picrylhydrazyl (DPPH)

The antioxidant activity of extract assessed with some modification [19]. One ml of DPPH (6×10-5-M) in ethanol as working solution was added to 25 µl of the extract (1mg/ml) or Trolox standard and mixed. Mixtures were incubated in dark at room temperature for 20 min. The absorbance of samples and control were measured using spectrophotometer (Pharmacia LKB UV/VIS-Spectrophotometer NovaSpec II) at 517 nm. DPPH working solution and ethanol were used as control and blank respectively. All measurements were performed in three repetitions. Trolox was used as antioxidant standard and results were calculated from standard curved and expressed trolox equivalent/ g plant weight dry(y =24. 023X, R2 = 0. 9996). % Inhibition = [(A0 - A1)/A0] ×100. A0 is the absorbance of control and A1 is the absorbance of the plant extracts. Azino bisethy lbenzothiazoline sulfonic acid (ABTS) or Trolox equivalent antioxidant activity (TEAC). The antioxidant activity was measured using ABTS based on Arnao[20] method. Radical cation was produced by reacting 7mM ABTS solution with 2. 45 mM potassium persulphate in dark room temperature for 12-16 hours before use. Stability of radical is more than 2 days at dark room temperature. ABTS solution was diluted with ethanol to an absorbance 0. 7±0. 02 at 734 nm as a working solution. Two ml ofABTSworking solutionwas added to 20µl of trolox standard or each extract with concentration of 1 mg/ml and mixed. Samples were incubated in 37 for 6 min and decrease in absorbance was measured against ethanol at 734 nm by the spectrophotometer (Pharmacia LKB UV/VIS-Spectrophotometer NovaSpec II). ABTS Working solution and ethanol were used as control and blank respectively. All measurements were performed in three repetitions. Trolox was used as antioxidant weight dry(y =2227x, R2 = 0. 9996).

2. 3. Phytochemical analyses

Plants extract was used for preliminary qualitative screening of phytochemicals as standard and results were calculated from standard curved and expressed trolox equivalent/ g plant described by [21]. The crude extract was diluted with ethanol to the concentration of 1 mg/ml for presence of total phenols, flavonoids, glycosides, alkaloids, saponins and tannins.

2. 4Anti mutagenicity test

The Bacteria species strain such as TA 100 was checked regularly for ultraviolet-light sensitivity, crystal-violet sensitivity, ampicillin resistance, histidin requirement and spontaneous reversion rate. The medium was prepared according to Maron and Ames protocols [22]. In a sterile capped tubes 0. 5 ml S9 mixture or phosphate buffer for presence or absence of metabolic activation, 0. 1 ml of different concentration extract, 0. 1 ml of bacteria suspension from an overnight culture and 0. 1ml Sodium azide were added and incubated in an incubator for 30 min at 370C. After incubation period, the mixture was added to 2 ml melted top agar preincubated in 450Cand vortex-mixed. The entire mixture was poured on glucose minimal agar plate, After solidification, the plates were inverted and was incubated at 37O C for 48-72 h. The revertant colonies were counted on each plate. The inhibition rate of mutagenicity (%) was calculated. Samples were tested on triplicate plates in two independent parallel experiments. The plant extract were replaced with the same amount of 100 µLDimetylesulfoxide %10(DMSO) for a negative control. Sodium azide (Sigma Ltd.)10µg /ml were used as positive control. The % inhibition was estimated as follow:

% inhibition = [1-T /M] ×100 where T is No. of revertants per plate in presence of mutagen and test sample and M is No. of revertants per plate in positive control. The antimutagenic effects of extracts were graded according to % the inhibitory effect by extracts. When % inhibition of mutagen by plants extracts were more than 40%, 25-40 and less than 25%, The antimutagenic effect considered strong, moderate and weak respectively[22].

Statistical analysis: All results are expressed as mean ± standard deviation (SD). Analysis was estimated by one way analysis

of variance (ANOVA). Statistical significance was set at $P < 0.05$.

3. Results

3. 1. Antimicrobial activity

The profile of four medicinal plants used in this research is depicted in (Table 1). The results of the antimicrobial activity of the investigated extracts are shown in (Table 2). The evaluation of antimicrobial potential by disc diffusion method indicated that all the bacterial tested showed growth

inhibition toward the plant extract, however, with differing sensitivity. Among the bacterial pathogens, S. aureusis more sensitive compared to other bacteria. Gram-positive bacteria were exhibited more sensitive to plant extracts compare to Gram-negative bacteria. The highest antimicrobial potential against S. aureus

Table 2. Antibacterial activity of hydro-alcoholic of four plants extract (50 mg/ml on different bacterial strains by disc diffusion method.

	S. typhi	P. aeruginosa	L. monocytogenous	S. aureus	B. cereus
Menthe piperita	9	7	14	16	15
Matricariarecutica	14 8	9	24	13	
Teucriumpolium	12	6	12	21	10
OriganumVulgare	10	8	12	18	11
Plant extract (50 mg/ml) Zone of inhibition(mm) Hydro alcoholic extract					
For each plant extract, four concentration (5, 10, 25 and 50 mg/ml) was managed. Ciprofloxacin (5 µg) and nalidixic acid (10µg) used as standards or positive control. Dimetylesulfoxide(DMSO)was used as anegative control.					

Table 3. MIC and MBC of four hydro-alcoholic extractswere estimated ondifferent bacterial strains. MIC and MBC (mg/ml) Hydro alcoholic extract

Plant S. typhi	P. aeruginosa	L. monocytogenous	S. aureus	B. cereus
Menthe piperita	25(50) 25(50)	12. 5(25)	12. 5(25)	12. 25(25)
Matricariarecutica	12. 5(25)	50(50) 25(25)	6. 25 (12. 5)	12. 5(50)
Teucriumpolium	12. 5(50)	50(100)	25(50) 6. 25 (12. 5)	25 (50)
OriganumVulgare	25(25)	50(100)	25(50) 12. 5(25)	25(50)
For each plant 5 twofold dilution (3. 12, 6. 25, 12. 5, 25 and 50 mg/ml) were carried out. MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration				

Table 5. Antimutagenic of fourhydro-alcoholic plant extracts using s. typhomorium test with AT100 with or without metabolic activation against sodium azide

Experiment	Revertants (CFU/plate)		Inhibition (%)	
	-S9	+ S9	-S9	+ S9
Menthe piperita	230	274	44. 6	45. 2
Matricaria recutica	260	285	38. 1	43. 6
Teucrium polium	296	321	29. 5	36. 5
OriganumVulgare	331	360	21. 2	28. 8
Sodium azide	420	505	-	-
DMSO	73	95	-	-
Sodium azide and DMSO as a positive and negative controls was used respectively.				

bacteria was belonging to Matricaria recutica with 24 mm in diameter at 50 mg/ml concentration. The lowest zone of growth inhibition (6mm) was observed at 50 mg/ml concentration of the extracts against P. aeruginosa bacteria.

Table 4. Phytochemicalsresults of the fourhydro-alcoholic plant extracts (1mg/ml)

plant	Flavonoids	Tannins	glycosides	Saponins	Alkaloids	Total phenol
Menthe piperita	+	+	-	-	-	+
Matricariarecutica	+	+	+	+	-	-
Teucriumpolium	+	+	+	_	+	+
OriganumVulgare	+	+	+	_	_	+
Key: + present; - absent						

The MIC and MBC range were 6. 25-50 and12. 5-100mg/ml, respectively. In this study, most of the extracts (755%) were active against both Gram-positive and Gram-negative bacteria, while all extracts (100%) were active against Gram-positive bacteria only (Table 3). All these plants were bacteriostatic at lower concentrations and bactericidal at higher concentrations as depicted in (Table 2 and Table 3).

3. 2. Antioxidant Activity

All plant extracts in the ABTS assay demonstrated a remarkable antioxidant activity. The free radical scavenging effect ranged in ABTS and DPPH between1525-11945 and 345-855 µmole trolox in gram dry weight respectively. The antioxidant activity measured by TEAC was higher than DPPH for all plant extracts (Fig. 1). In ABTS O. Vulgare exhibiting the highest activity however, in

DPPH assay M. piperita with highest activity was seen. There was a strong relationship between the DPPH and TEAC assays (Fig. 1). There was significantly different antioxidant activity between DPPH and ABTS methods in each plant extracts. According to results, there was high correlation between two antioxidant methods (R=0. 89).

3. 3. Phytochemical Screening

The results of the phytochemical analysis showed the presence of different types of active constituents like total phenol, flavonoids, tannins, alkaloids, saponine and glycosides. The first three ones were found in all plant extracts (Table 4).

3. 4. Anti mutagenicity potential by Ames test

All plant extracts exhibited different level of protection against the mutagenicity induced by sodium azide in TA100 strain organism. Antimutaginicity of M. piperita(54. 2), M. recutica(43. 6), T. polium(36. 5) and O. Vulgare(28. 8) were reported by Ames test in the presence of S9 mixture(+S9). In Absence of S9 mixture (-S9) antimutaginicity activity all plant extracts were decreased when compare to(+S9) (Table 5).

4. Discussion

The medicinal plants have been main source for drugs over many centuries in either developed and developing world. The aim of antibacterial susceptibility is to obtain in vitro information for getting appropriate, adequate and optimal treatment in living organisms. Resistance of bacteria to antibiotics is associated with recurrence of infectious diseases which makes a big problem for population health [23]. Gram negative bacteria are impermeable to some external agents, due to presence of lipopolysacharid layer in their outer membranes. However, while in outer layer of gram-positive bacteria peptidoglycan compound is present. [24]. The presence of peptidoglycan in outer layer of gram-positive bacteria is an inefficient permeability barrier and made difference in component and thickness of bacteria cell wall [25]. This is one of the most important cause and why the gram positives bacteria is much more sensitive to biologic and toxic substances than the gram-negatives bacteria. [26]. In recent investigation the gram-positive bacteria were exhibited more sensitive to gram-negative bacteria and S. aureus was the most sensitive bacteria reported. Studies of other researchers showed that susceptibility of gam positive bacteria to total phenols is more than to gram negative bacteria. Antibacterial activity of plant extracts was screened on basis of zone of inhibition (ZOI). ZOI less than 12, 12-16 and more than 16 mm were considered low, moderately and highly active respectively[27].

In present study the extracts were active against both gram-negative and gram-positive bacteria may specify a wide spectrum of activity. The low MIC value observed for S. aureusis a good clue of high efficiency against this bacterium. High MIC may be a clue of low efficiency or that the organisms have the capacity for increasing resistance to the biological compounds. The MIC and MBC of extracts have been found different values from plant extract. According to MIC and MBC results, plant extracts in lower concentration was bacteriostatic however, in higher concentrations bactericidal activity were considered. In a study similar to recent research leaf ethyl acetate of Mentha piperita had potent antibacterial activity against B. cereus, P. aerogenosa, s. aureus [15]. Antibacterial and antioxidant activities of O. vulgare in this study was confirmed by other researchers [14]. Among the polyphenols in recent plant extracts, flavonoid and tannin compounds are most important. Tannin compounds are known antimicrobial agents because, able to penetrate in the bacteria cell membranes and probably produce membrane disintegrate and result in inhibited the microbial growth [28, 29]. Sediment of microbial proteins by tannin compounds impedes bacteria growth and consequently the nutritional proteins will not be available for bacteria. Tannins may be blocking the bacteria proliferation by different mechanism such as: iron-trapping, reaction with enzymes and inhibition of reverse transcriptase enzyme activity [29]. Radical scavenging potential is very important to the survival of organisms, due to the damaging role of free radicals in diet and living organism. Routinely, plant antioxidant activity is determined by DPPH and TEAC free radical scavenging methods. The present research results revealed that all plant extracts have potent activity by inhibiting free radicals. Present results were comparable to those obtained by Ogunmoyole and Muanda [30-31] with methanol extract. The antioxidant potentials obtained in this study probably due to the presence of total phenols, flavonoids and tannins. The antioxidant activity of medicinal plants could be associated to the levels of their phenolic compounds which include phenolic acids, flavonoids and tannins [32]. Therefore, recent investigated plants could be comprise compounds with potent radical scavenging ability which have efficient therapeutic effects for protection of cancer, cardiovascular and inflammatory disease. There was also significantly different antioxidant activity between DPPH and ABTS methods in each plant extracts. The DPPH method revealed the capability of the extract to transfer electrons or hydrogen atoms, although the ABTS assay specified the hydrogen donating and the chain-breaking ability of the extract to free radical [33]. Furthermore, it was differences in solubility DPPH and ABTS assays. In general, DPPH

could assess hydrophilic substances, whereas ABTS can be used to study both hydrophilic and lipophilic substances [20]. Similarly, several secondary metabolites with antimicrobial and antioxidant activities, were reported in literature [34, 35]. The phytochemicals derived from root, stem, leaves, fruits, flowers and seeds of medicinal plants include total phenol compounds, essentials oils, proteins and tannin. Their interaction in plant produce bioactive agent [36]. The inhibitory activities or antibacterial properties of plants were associated with the presence of tannins, alkaloids, flavonoids, terpenoids or essential oils. The tannins exhibited antibacterial activity against S. aureus, and, E. coli in literature [37]. Presence of tannins, alkaloids and glycosides were induce antibacterial and antioxidant activitis in Eugenia uniflora, Dichrostachyscinerea which similar with present study [38, 39]. Phytochemical compounds such as polyphenols and flavonoids are responsible for the plants antioxidant activity and presented antimicrobial anti-inflammatory, antihypertensive and antidiabetic[40). Ames test (Salmonella/microsome) has been widely used to detect mutagenic activity of biological and chemical compounds. All plant extracts displayed different level of protective effect against mutagenicity that induced by sodium azide in S. typhimurium bacteria with or without metabolic activation. The Ames test without S9 (-S9) used for detect of direct mutagens while, in the presence of S9 (+S9) applicable for the recognition of indirect mutagens. The rationale screening of antimutagenicity assay is that anti-mutagenic compounds can possibly be anticarcinogens. In this study, M. piperita and M. recutica have reported strong antimutagenic effect however; T. polium and O. Vulgare were recognized with moderate and weak antimutagenic effect respectively, in presence and absence of S9mixture.

Conclusion: The antibacterial, antioxidant and antimutagenicity activities of hydroethanolic extracts in present study provides the scientific basis for the consumption of these plants in folk medicine. Medicinal plants with high polyphenols have multipurpose tool for the treatment of disease in indigenous medicine. These results encourage reserachers to carry out some study for extract of active compound with biological activity such as antibacterial, antioxidant and antimutagenic capacity.

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References

1. Brankica D. Antibiotic Susceptibility of Salmonella Spp.: A. Gordana Mijovic, Bogdanka Andric, Dragica Terzic Milena Lopicic Comparison Of Two Surveys With A 5 Years Interval. Journal of IMAB Annual Proceeding (Scientific Papers) 2012; 18:(1): 216 -219
2. Singer RS, Finch R, Wegener HC, BywaterR, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. Lancet Infect Dis. 2003; 3: 47-51.
3. Rawat RBS, Uniyal RC. National Medicinal plants Board committed for overall development of the sector. Agro Bios Med Plants 2003; 1: 12-16.
4. Molly M R. Classifications, Terminology and Standards WHO, Geneva: Xiaorui Zhang Traditional Medicines, WHO. Traditional medicines: global situation, issues and challenges. Pharmacol. 2011; 43: 393-397
5. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005; 100:80-84.
6. Evans CE, Banso A, Samuel OA. Efficacy of some nupe medicinal plants against Salmonella typhi: an in vitro study. Journal of Ethnopharmacology 2002; 80:21-24
7. Kubmarawa D G, joku AA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. African Journal of Biotechnology 2007;6 (14):1690-1696.
8. Punitha SMJ, BabuMM, Sivaram V, Shankar VS, Dhas SAet al. Immunostimulating influence of herbal biomedicines on nonspecific immunity in grouper Epinephelustauvina juvenile against Vibrio harveyi infection. Aquaculture Int. 2008; 16: 511-523.
9. Duraipandiyan V, Ayyanar M, IgnacimuthuS. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement. Altern Med. 2006; 6:35-35.
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4:685-688.
11. Abdollahi M, Karimpour H, Monsef-Esfehani HR. Antinociceptive effects of Teucrium polium L total extract and essential oil in mouse writhing test. Pharmacol Res. 2003; 48:31–35.
12. Esmaeili MA, Yazdanparast R. Hypoglycaemic effect of Teucriumpolium: studies with rat pancreatic islets. J Ethnopharmacol. 2004; 95: 27–30.
13. Cassiano B, Altemir J M, Maria R, Alves R, Rogério L C, José Vladimir de O. Evaluation of Origanumvulgareessential oil as antimicrobial agent in sausage. Braz J Microbiol. 2007; 38(4).
14. Govaris A, botsoglou E, sergelidis E, chatzopoulou PS. Antibacterial activity of oregano and thyme essential oils against Listeria monocytogenes and Escherichia coli O157:H7 in feta cheese packaged under modified atmosphere. In LWT - Food Science and Technology 2011; 44:1240-1244.
15. Bupesh G, Amutha C, Nandagopals, Ganeshkumara, Sureshkumarp, Saravanak Murali. Antibacterial activity of Menthapiperita L. (peppermint) from leaf

extracts – a medicinal plant. Acta agricultura eSlovenica 2007; 89 (1):73-79.
16. Shafaghat A, Salimi F, RoghayehMahmoodi R. Antioxidant, antimicrobial activity and chemical analysis of the flavonoid from Chaerophyllummacropodum. Journal of Medicinal Plants Research 2012; 6(11): 2111-2116.
17. Koochak H, Seyyednejad SM, Motamedi H. Preliminary study on the antibacterial activity of some medicinal plantsof Khuzestan (Iran). Asian Pacific Journal of Tropical Medicine 2010;3(3)180-184.
18. Shahbudin S, Muhammad T, Deny S, Qaralleh Nurul H, Abdul Rahim AB. Antimicrobial activity of mangrove plant (Lumnitzeralittorea). Asian Pacific Journal of Tropical Medicine 2011; 4(7) 523-52.
19. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zeval-los L, Byrne HD. comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006; 19: 669-675.
20. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 2001; 73: 239–244.
21. Ga Ayoola H, Coker S, Adesegun A, Adepoju B, Obaweya E, Ezennia A. Phytochemical screening and antioxidant activities of some selected medicinal plants used for Malaria therapy in Southwestern Nigeria. Trop J Pharmaceut Res. 20087(3): 1019-1024.
22. Ong T, Wong W, St Wart JD. Chlorophyllin a potent antimutagen against environmental and dietary comp lex mixture, Nutr Res. (1986);173: 111-115.
23. Aqil F, Khan MSA, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of β-lactamase producing methicillin resistant Staphylococcus aureus. J Basic Microbiol 2005; 45:106-114.
24. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol Rev. 1985;49(1):1-32.
25. Yao J, Moellering R. Antibacterial agents. In: Manual of Clinical Microbiology, Murray P, Baron E, Pfaller M, Tenover F, Yolken R (Eds), ASM, Washington DC. 1995; 1281-1290.
26. Scherrer R, Gerhardt P. Molecular sieving by the Bacillus megaterium cell wall and protoplast. J Bacteriol. 1971; 107(3):718-35.
27. Indu MN, hatha AAM, Abirosh C, Harsha U, VivekanadanG. Antimicrobial Activity of Some of The South-Indian Spices Against Serotypes of Escherichia Coli, Salmonella, Listeria Monocytogenes and Aeromonas Hydrophila. Brazilian Journal of Microbiology 2006; 37:153-158.
28. Erasto P, Bojase–MoletaG, Majinda RRT. Antimicrobial and antioxidant flavonoids from the roots wood of Bolusathusspesiosus. Phytochem. 2004; 65: 875-880.
29. Viljoen A, Van Vuuren S, Ernest E, Klepser M, Demirci B, Basser H, Van Wyk BE. Osmitopsisasteriscoides (Asteraceae) – the antimicrobial and essential oil composition of cape Dutch remedy. J Ethnopharmacol; 2003; 88:137-143.
30. Ogunmoyole T, Olalekan OO, Fatai O, Makun J O, Kade IJ. Antioxidant and phytochemical profile of aqueous and ethanolic extract of Garcinia kola. journal of Pharmacognosy and Phytotherapy2012; 4(5): 66-74.
31. Muanda F, Kone D, Dicko A, Soulimani R, Younos C. Phytochemical Composition and Antioxidant Capacity of Three Malian Medicinal Plant Parts. Evid-Based Compl Alt Med. 2011; 8
32. Wong CC, Li HB, Cheng KW, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. 2006; 4:705-711.
33. Perez-Jimenez J, Saura-Calixto F. Literature data may underestimate the actual antioxidant capacity of cereals. J Agric Food Chem. 2005; 53:5036-5040.
34. Khan MA, Inayat H, Khan H, Saeed M, Khan I, Rahman I. Antimicrobial activities of the whole plant of Cestrum nocturnum against pathogenic microorganisms. Afr J Microbiol Res. 2011 5: 612-616.
35. Samiullah BA, Bano A, Naz R, Yasmin H. In vitro inhibition potential of Lespedeza bicolor Turcz against selected bacterial and fungal strains J. Med. Plant. Res. 2011; 5: 3708-3714.
36. Kahkashan P, Najat A. Bokhari, Dina A, SolimanW. Antibacterial activity of Phoenix dactylifera L. leaf and pit extracts against selected Gram negative and Gram positive pathogenic bacteria. Journal of Medicinal Plants Research 2012; 6(2):296-300.
38. Adebajo AO, Adewumi CO, Essein EE. Antiinfective agents of higher plants. International symposium of Medicinal Plants. 5th edn. University of Ife, Nigeria. 1983: 152–158.
39. BansoA, Adeyemo SO. Evaluation of antibacterial properties of tannins isolated from Dichrostachyscinerea. Afr. J. Biotechnol. 2007; 6 (15): 1785-1787. 40. Balch JF, Balch PA. Prescription for Nutritional Healing. New York: A very, Penguin Putnam Inc. 2000; 267-270.