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Impact of Medicinal Plants Phytocomponents against Antibiotic Resistant Bacteria

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ABSTRACT

The flavonoids and essential oils contained in five medicinal plants Rheum palmatum (Rp), Cassia angustifolia (Ca), Glycyrrhiza glabra (Gg), Cichorium intybus (Ci) and Matricaria chamomilla (Mc) from different families were extracted, screened against different antibiotic resistant bacterial strains (ARB) (six obtained from pharmaceutical products and twelve from hospital drains) and identified based on GC/MS chromatographic technique. The results showed that only Rp, Ca, and Gg extracts exhibited antibacterial activity against two bacterial strains out of six (33.33%) and three out of twelve (25%). The combinations were done between the three extracts and synergism action was obtained. Different concentrations exhibited slight inhibitory effect at 10% as compared with great inhibitory effect at 30%. The flavonoids and volatile oils contents were (2.88/0.2%); (1.6/0.1%); (2.68/0.25%) for Rp, Gg and Ca extracts, respectively.

Keywords: Medicinal plants, Flavonoids, Essential oils, Extraction, Antibacterial activity.

INTRODUCTION

A medicinal plant is any plant in which one or more of its organ contains substances that can be used for therapeutic purposes on which are precursors for the synthesis of useful drugs. Medicinal plants contain biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have preventive and curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants and are useful for humanity [1]. Medicinal herbs have been used in one form or another under indigenous systems of medicine. Dubey *et al* [2] mentioned that the complete phytochemical investigations of medicinal plants of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant. Number of plants were screened for primary and secondary metabolites for their medicinal values *Clitoria ternatea*, *Guazuma ulmifolia* and *Madhula indica* [3], *Maytenus emarginata* [4], *Artemisia annua* [5], *Nardostachys jatamamsi* [6], *Thymus vulgaris* [7], *Allium giganteum* [8], *Cephalotaxus koreana* [9], *Boswellia ovalifoliolata* [10], *Nerium oleander* and *Momordica charantia* [11] and *Jatropha* [12]. Many studies have shown that natural antioxidants from plant sources can effectively inhibit oxidation of food and reduce the risk of age-dependent diseases [13,14]. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds [15]. The number of reports on the isolation of natural antioxidants, mainly of plant origin, has increased immensely during the last decade [16]. Polyphenolic compounds are commonly found in both edible and inedible plants, they have multiple applications in food, cosmetic and pharmaceutical industries [17]. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen

quenchers or metal chelators. In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects [18]. Flavonoids, abundant in fruits, vegetables, teas, medicinal plants, have attracted the greatest attention and have been studied extensively, because they are a kind of highly effective antioxidants with a lower toxicity than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [19].

The aim of this study was to evaluate flavonoids and essential oils contained in different five medicinal plants used (*Rheum palmatum, Cassia angustifolia, Glycyrrhiza glabra, Cichorium intybus and Matricaria chamomilla*) as a potential source of natural antioxidants and phenolic compounds were extracted and screened against different antibiotic resistant bacterial strains (ARB).

EXPERIMENTAL SECTION

Medicinal Plants and solvents used

Different five medicinal plants were used viz: *Rheum palmatum* (Sorrel Rhubarb) (Polygonaceae), *Cassia angustifolia* (Indian Senna) (Caesalpinaceae), *Glycyrrhiza glabra* (Gan Cao or Iriqsus) (Fabaceae), *Cichorium intybus* (Chicory) (Asteraceae), and *Matricaria chamomilla* (Chamomile) (Compositae), these plants were collected from Cairo, Egypt and extracted in different solvents viz: (Ethyl acetate, Chloroform, Ethanol, Methanol, hot and cold water). All chemicals and solvents used were of analytical grades.

Preparation of plant extracts

The fresh aerial parts of the medicinal plants used were spread in a laboratory tray and dried in a moisture extraction oven at 65°C for 3 hours. They were separately ground and sieved through a 1mm test sieve to obtain powdered processed plant materials. About 60g was boiled in 1.9 L of distilled water and the final volume was reduced to 240 ml by gentle boiling over 4h and freeze dried. A stock solution was prepared at a concentration of 1000µg/ml from hot water extract by dissolving 10 mg of the extract in 10 ml of distilled water and sterilized by autoclaving at 110°C for 10 min and stored at -20°C until use. To prepare cold water extract, the plant materials were macerated with distilled water (500 ml) and kept for 48h at room temperature (28-30°C). The extract was filtered and the stock solution prepared. Also, extraction from other different solvents (i.e. ethyl acetate, chloroform, ethanol, and methanol) was obtained by Soxhlet extraction (6h). The types of extract were evaporated to dryness in a rotary evaporator at 37°C and stored in desiccators prior to further analysis.

Media and test organisms used

Three media to collect bacterial samples other than selective merdia were used: Peptone tween water (1gm**:** 10ml to 1000ml deionized water) in pharmaceutical drugs and peptone water (1 gm to 1000ml deionized water) in water dilutions; Tryptic Soya agar (TSA) general media for bacterial count; Muller-Hinton agar for determination of antibiotic resistant bacteria. Six antibiotic resistant bacterial strains (ARB) as Gram-positive (G+ve) *Micrococcus sp* (DFR8D), *Staphylococcus xylosus* (ASP13D), *Staphylococcus sciuri* (CER15D), *Staphylococcus sciuri* (UNI21D), *Micrococcus sp* (DFR37D) and Gram-negative (G-ve) *Alcalignes xylosoxidan*s (UNO9D), isolated and identified before from different pharmaceutical products in Cairo, Egypt (Dafrex, Unocron MR, Aspico, Cervitam, Unimax and Dafrex, respectively) according to API 20 Test Kit (BioMerieux) were selected in this study. Also, about twelve ARB strains as Gram-positive (G+ve) *Staphylococcus aureus* (HD1S), *Staphylococcus aureus* (HD5S), *Staphylococcus aureus* (HD10S) and Gram-negative (G-ve) *Pseudomonas aeruginosa* (HD8S), *Enterobacter cloacae* (HD2S), *Klebsiella pneumonia* (HD3S), *Enterobacter cloacae* (HD4S), *Proteus mirabilis* (HD6S), *Klebsiella pneumonia* (HD7S), *Klebsiella pneumonia* (HD9S), *E. coli* (HD11S), *Enterobacter cloacae* (HD12S), isolated and identified before from different hospital drains (HD) [20] were selected for the same purpose.

Antibacterial activity test

Antibacterial activity was determined by the disk diffusion method according to the National committee for clinical laboratory standards (NCCLS) [21]. Petri plates (100 mm diameter) containing 20 ml of Mueller-Hinton agar medium were seeded with 24 h culture of the bacterial strain (one microorganism per Petri dish). Soaked disks of plant extracts were tested onto the surface of seeded agar media. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony-forming units (CFU)/ml. Incubation was performed at 37ºC for 24 h. The assessment of antibacterial activity was based on measurement of inhibition zone diameter formed around the disk. A negative control was applied by a disk loaded with only the solvent that used free plant extract at the same

environmental conditions. The combined action effect of the sensitive extracts against resistant bacteria also checked as a trial to the combined action of the most sensitive medicinal plant extracts against the most potent antibiotic resistant bacterial strains were selected in this study. The combinations were done between the extracts of *Rheum palmatum* (*Rp*) and *Glycyrrhiza glabra* (*Gg*) which represented as (*Rp/Gg*); *Rheum palmatum* and *Cassia angustifolia* (*Ca*) which represented as (*Rp/Ca*); *Cassia angustifolia* and *Glycyrrhiza glabra* and represented as (*Ca/Gg*) and final combination between the three extracts together of *Rheum palmatum, Cassia angustifolia and Glycyrrhiza glabra* which represented by symbol (*Rp/Ca/Gg*). After the determination of the combined action effect, different concentrations (10 and 30%) of the plant extracts were studied as well.

Determination of total volatile oils by hydrodistillation

The essential oils were prepared from the plant powder under investigation by hydrodistillation using the Egyptian pharmacopoeia [22]; the distillated were extracts with distilled ether after saturation with sodium chloride. The ether extracted was dehydrated over anhydrous sodium sulfate; solvent was removed under reduced pressure at 20°C. The volatile constituents was packed in a dark container and kept in refrigerator till analysis.

Identification of volatile oil constituents using GC/MS:

This method used by SHIMADZU Q p5050A, GC/MS-5989B. Gas chromatography mass with the following conditions: Searched library: Wiley 275. LIB. Column: DBI, 30 m, 0.53 mm ID, 1.5 µm film. Carrier gas: Helium (flow rate 1ml/min). Ionization mode: EL (70eV). Temperature program: 40°C (static for 2 min) then gradually increasing (160°C at a rate of 2°C/min) up to 250°C (static for 7.5 min); detector and injector temperature at 250°C. After stabilization of the chromatographic conditions, the samples were injected and determined the mass spectrum for each peak and from the G1035A Wiley PBM Library (Probability Based Matching) of Gas chromatography mass conclude the equivalent compound name, molecular weight and structure. Qualitative identification of the essential oils was achieved by library searched data base Willey 275LIB and by comparing their retention index and mass fragmentation patterns with those of the available references and with published data, the percentage composition of volatile oil components was determined by computerized peak area measurements.

Determination of total flavonoids

Determination of the total flavonoid contents in the methanolic extract of *Glycyrrhiza glabra, Rheum palmatum and Cassia angustifolil* were done colorimetrically by using aluminum chloride ($AlCl_3$) solution. Standard curve was done using different concentrations of rutin in methanol (six serials 2 fold dilution to give 100 – 3.2 µg /ml). 100 µl of each extract previously prepared were added to 96 Micro well plates and then 100 µl of 2% $AlCl_3$ solution in methanol added. After 10 min, their absorbance was measured using HP spectrophotometer at 415 nm using methanol as blank and the concentration of total flavonoids was calculated [23,24,25]. From the powder we can also estimate the flavonoid content using one gram of defatted air-dried powdered plant accurately weighed and extracted with methanol till exhaustion. The methanolic extract was transferred to a measuring flask 100 ml and completes the volume with methanol; from the methanolic extract 0.5 ml was transferred into a test tube and evaporated to dryness. To the residue 5ml of $AlCl_3$ were added and the procedure continued as mentioned before. The flavonoid content of powder plant calculated as rutin was deduced from the established standard calibration curve [23].

Flavonoid (%) calculated as rutin in the powders:

$$\text{Flavonoid (\%)} = \frac{X}{5000} \text{ x } 100$$

X: amount of rutin in µg obtained from standard curve for the sample.

Calibration Curve

Different aliquots of methanolic solution of apigenin equivalent to (20, 40, 60, 80, 100, 120, 140, 160 and 180 µg) were separately introduced into test tubes; evaporate to dryness on a hot water bath (40 - 60°C) then five ml of 0.1M $AlCl_3$ reagent was added. The intensity of the developed yellow color was measured at λ_{max}=415 nm (wavelength of maximum absorbency) against a blank prepared in the same way but replacing rutin solution by methanol, using the spectrophotometer. Three for each concentration duplicate, determinations were carried out and absorbencies were plotter versus concentration [23].

RESULTS

Antibacterial activity of medicinal plant extracts

About six antibiotic resistant bacterial strains (ARB) obtained from different pharmaceutical products mentioned before were tested against five medicinal plant extracts namely (*Rheum palmatum, Cassia angustifolia, Glycyrrhiza glabra, Cichorium intybus and Matricaria chamomilla*). Only three extracts of *Rheum palmatum, Cassia angustifolia and Glycyrrhiza glabra* having antibacterial activity against two ARB G-ve and G+ve *Alcalignes xylosoxidan*s (UNO9D) and *Staphylococcus xylosus* (ASP13D), respectively, while, the rest extracts exhibited no inhibitory effect on ARB (*i.e. Cichorium intybus* and *Matricaria chamomilla*). About 33.33% ARB strains were sensitive to medicinal plant extracts as recorded in Table (1).

Table (1): Antibacterial activity of medicinal plant extracts against antibiotic resistant bacteria isolated from pharmaceutical products

Plant code	*Plant Name*	*Solvent*	*Inhibition Clear zone Diameter (mm)*					
			DFR8D	*UNO9D*	*ASP13D*	*CER15D*	*UNI21D*	*DFR37D*
Rp	*Rheum palmatum*	**CW**	-	-	-	-	-	-
		HW	-	-	-	-	-	-
		Eth96%	-	20.63±0.11	13.00±0.00	-	-	-
		Meth	-	26.45±0.35	14.14±0.39	-	-	-
		EA	-	20.00±0.00	13.55±0.28	-	-	-
		CF	-	20.33±0.24	13.00±0.00	-	-	-
Ca	*Cassia angustifolia*	**CW**	-	-	-	-	-	-
		HW	-	12.05±0.12	-	-	-	-
		Eth96%	-	-	-	-	-	-
		Meth	-	14.66±0.34	10.00±0.00	-	-	-
		EA	-	12.87±0.22	-	-	-	-
		CF	-	13.11±0.34	-	-	-	-
Gg	*Glycyrrhiza glabra*	**CW**	-	-	-	-	-	-
		HW	-	-	-	-	-	-
		Eth96%	-	21.00±0.00	10.00±0.00	-	-	-
		Meth	-	20.55±0.65	11.23±0.46	-	-	-
		EA	-	-	-	-	-	-
		CF	-	-	-	-	-	-
Ci	*Chichorium intybus*	**CW**	-	-	-	-	-	-
		HW	-	-	-	-	-	-
		Eth96%	-	-	-	-	-	-
		Meth	-	-	-	-	-	-
		EA	-	-	-	-	-	-
		CF	-	-	-	-	-	-
Mc	*Matricaria chamomilla*	**CW**	-	-	-	-	-	-
		HW	-	-	-	-	-	-
		Eth96%	-	-	-	-	-	-
		Meth	-	-	-	-	-	-
		EA	-	-	-	-	-	-
		CF	-	-	-	-	-	-

(-): Negative; CW: Cold water; HW: Hot water; Eth: Ethanol; Meth: Methanol; EA: Ethyl acetate; CF: Chloroform.

On the other hand, in a trial to check about twelve ARB strains, isolated and identified before from different hospital drains were tested against the same medicinal plant extracts. The same results were obtained above, that the same three extracts showed antibacterial effect only against three G+ve ARB *Staphylococcus aureus* (HD1S); *Staphylococcus aureus* (HD5S); and *Staphylococcus aureus* (HD10S) the same organism from different localities. About (25%) of ARB strains were sensitive to medicinal plant extracts, Table (2). The results obtained obviously that the three extracts showed no inhibitory affect against G-ve ARB strains obtained from hospital drains.

Table (2): Antibacterial activity of medicinal plant extracts against antibiotic resistant bacteria isolated from hospital drains

Plant Code	Plant Name	Solvent	*Inhibition Clear Zone Diameter (mm)* (mean±SD)											
			HD1S	*HD2S*	*HD3S*	*HD4S*	*HD5S*	*HD6S*	*HD7S*	*HD8S*	*HD9S*	*HD10S*	*HD11S*	*HD12S*
Rp	*Rheum palmatum*	**CW**	14.33±0.58	-	-	-	13.36±0.45	-	-	-	-	13.55±0.77	-	-
		HW	13.46±0.39	-	-	-	13.77±0.45	-	-	-	-	13.23±0.47	-	-
		Eth96%	-	-	-	-	-	-	-	-	-	-	-	-
		Meth	19.53±0.76	-	-	-	18.33±0.67	-	-	-	-	17.65±0.47	-	-
		EA	18.36±0.57	-	-	-	16.55±0.78	-	-	-	-	15.55±0.19	-	-
		CF	13.23±0.66	-	-	-	13.77±0.46	-	-	-	-	12.00±0.00	-	-
Ca	*Cassia angustifolia*	**CW**	13.50±0.55	-	-	-	12.55±0.28	-	-	-	-	11.00±0.00	-	-
		HW	12.76±0.73	-	-	-	-	-	-	-	-	11.54±0.11	-	-
		Eth96%	-	-	-	-	12.66±0.65	-	-	-	-	11.98±0.65	-	-
		Meth	13.98±0.56	-	-	-	14.35±0.66	-	-	-	-	12.00±0.00	-	-
		EA	-	-	-	-	13.98±0.89	-	-	-	-	-	-	-
		CF	12.31±0.33	-	-	-	12.36±0.67	-	-	-	-	11.65±0.22	-	-
Gg	*Glycyrrhiza glabra*	**CW**	-	-	-	-	-	-	-	-	-	-	-	-
		HW	-	-	-	-	-	-	-	-	-	-	-	-
		Eth96%	19.88±0.45	-	-	-	13.14±0.44	-	-	-	-	13.00±0.00	-	-
		Meth	18.65±0.36	-	-	-	13.76±0.87	-	-	-	-	13.29±0.72	-	-
		EA	19.59±0.44	-	-	-	13.87±0.36	-	-	-	-	12.54±0.46	-	-
		CF	-	-	-	-	-	-	-	-	-	13.88±0.44	-	-
Ci	*Chichorium intybus*	**CW**	-	-	-	-	-	-	-	-	-	-	-	-
		HW	-	-	-	-	-	-	-	-	-	-	-	-
		Eth96%	-	-	-	-	-	-	-	-	-	-	-	-
		Meth	-	-	-	-	-	-	-	-	-	-	-	-
		EA	-	-	-	-	-	-	-	-	-	-	-	-
		CF	-	-	-	-	-	-	-	-	-	-	-	-
Mc	*Matricaria chamomilla*	**CW**	-	-	-	-	-	-	-	-	-	-	-	-
		HW	-	-	-	-	-	-	-	-	-	-	-	-
		Eth96%	-	-	-	-	-	-	-	-	-	-	-	-
		Meth	-	-	-	-	-	-	-	-	-	-	-	-
		EA	-	-	-	-	-	-	-	-	-	-	-	-
		CF	-	-	-	-	-	-	-	-	-	-	-	-

(-): *Negative; CW: Cold water; HW: Hot water; Eth: Ethanol; Meth: Methanol; EA: Ethyl acetate; CF: Chloroform.*

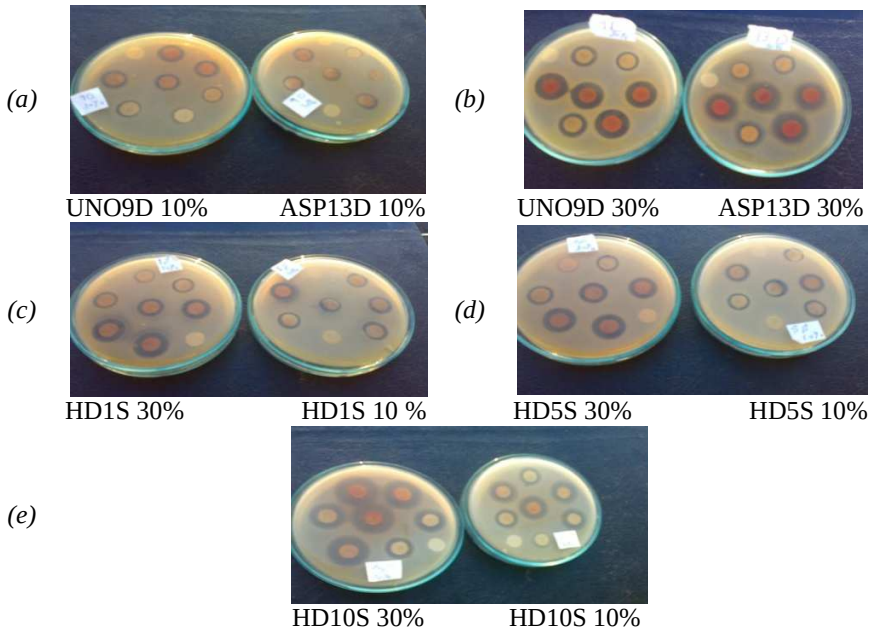


Plate (1): Synergism action obtained at different concentrations (10 and 30%) of combined plant extracts when applied against *(a) Alcalignes xylosoxidan*s (UNO9D) & *Staphylococcus xylosus* (ASP13D); *(b) Alcalignes xylosoxidan*s (UNO9D) & *Staphylococcus xylosus* (ASP13D); *(c) Staphylococcus aureus* (HD1S); *(d) Staphylococcus aureus* (HD5S); and *(e) Staphylococcus aureus* (HD10S).

Combined action effect of sensitive extracts against resistant bacteria

A trial to check the combined action of the three sensitive medicinal plant extracts against the five antibiotic resistant bacterial strains (two from pharmaceutical products and three from hospital drains) were selected for these combinations. Four combinations were done between the extracts of (*Rp/Gg*); (*Rp/Ca*); (*Ca/Gg*) and final combination was mixture of the three extracts together (*Rp/Ca/Gg*). As shown in Table (3), the results obtained revealed that the combination action officiated to synergism between the mixture of plant extracts (*Rp/Ca/Gg*) gave high antimicrobial activity than others. Also, at different concentrations, the plant extracts of (*Rp/Ca/Gg*) exhibited slight inhibitory effect at concentration of 10% as compared with great inhibitory effect at 30% concentration, and synergism action was obtained between plant extractions when tested in combinations Plate ($1_{(a-e)}$).

Table (3): Combination action of sensitive medicinal plant extracts

Plant Code	*Solvent*	*Inhibition clear zone Diameter (mm)*				
		UNO9D	*ASP13D*	*HD1S*	*HD5S*	*HD10S*
Rheum palmatum (Rp)	**Meth**	19.46±0.55	16.63±0.12	17.00±0.00	15.37±0.66	16.00±0.00
Glycyrrhiza glabra (Gg)	**Eth 96%**	16.00±0.00	15.00±0.00	15.00±0.00	13.00±0.00	14.22±0.34
Cassia angustifolia (Ca)	**Meth**	18.25±1.06	16.66±0.53	16.11±0.39	15.11±1.01	15.24±0.44
Rp/Ca		22.25±0.59	20.87±0.26	19.87±0.55	20.00±0.00	19.55±0.87
Rp/Gg		21.00±0.00	18.33±0.98	18.00±0.00	18.00±0.00	19.00±0.00
Ca/Gg		20.00±0.00	17.45±0.49	17.11±0.24	16.55±0.69	18.00±0.00
Rp/Gg/Ca		24.33±1.03	21.00±0.00	20.34±0.38	20.13±0.38	20.00±0.00

Table (4): Total Volatile Oils and Flavonoids in Medicinal Plants

Medicinal Plant	*Total volatile oil (%)*	*Flavonoids (%)*
Rheum palmatum	0.2	2.88
Glycyrrhiza glabra	0.1	1.6
Cassia angustifolia	0.25	2.68

Table (5): GC/MS analysis of volatile oils of medicinal plants

Peak #	***Rt* (min)**	*Glycyrrhiza glabra (%)*	*Rheum palmatum (%)*	*Cassia angustifolia (%)*	**Compound name**
1	4.0	-	4	-	***α–thugen***
2	4.13	4	-	1	***α-pinene***
3	4.3	2	-	2	***β-pinene***
4	6.11	5	10	7	***Octanol***
5	7.23	12	-	-	***γ-terpinene***
6	7.6	-	-	9	***γ-terpinolene***
7	7.8	10	9	8	***Stragole***
8	8.1	-	12	-	***Trans-ocimene***
9	8.92	-		25	***Cis-limonene oxide***
10	9.1	-	8	10.5	***Trans-anethole***
11	9.6	15		-	***Isofenchon***
12	11.11	-	25	-	***Eugenol***
13	11.4	-	8	-	***α-copaene***
14	13.22	-	6	-	***α-farnesene***
15	13.5	-	6	-	***δ-cadinene***
16	13.55	8	-	4.5	***β-caryophyllene***
17	14.03	4	-	-	***Citronellyl acetate***
18	14.33	5	-	15	***Caryophyllene oxide***
19	14.88	35	-	10	***Geranylhexanolate***
20	15	-	12	8	***palmitic acid***
Total	--	100	100	100	

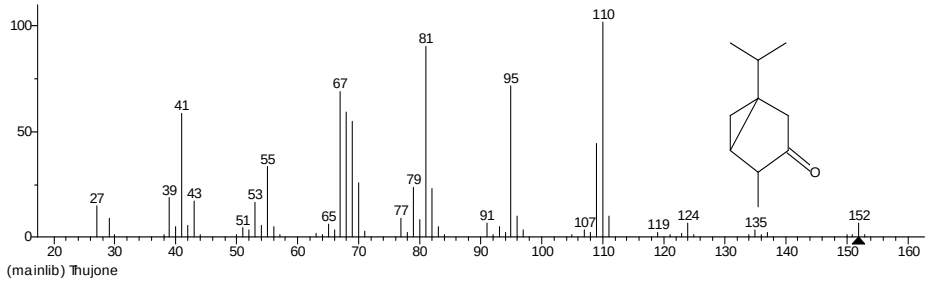
Rt: Retention time.

Identification of essential oils and flavonoids in medicinal plants

As shown in Table (4) the total volatile content of *Glycyrrhiza glabra* 0.1%, *Rheum palmatum* 0.2% and *Cassia angustifolia* 0.25% was obtained. Also, determination of total flavonoids was done. It has been recognized that flavonoids showed antibacterial activity and their effects on human nutrition and health are considerable. Each value in the Table (4) was obtained by calculating the mean average of three replicates experiments. The results showed the total Flavonoids content of *Glycyrrhiza glabra* was 1.6 % while in case of *Rheum palmatum* was 2.88%, and

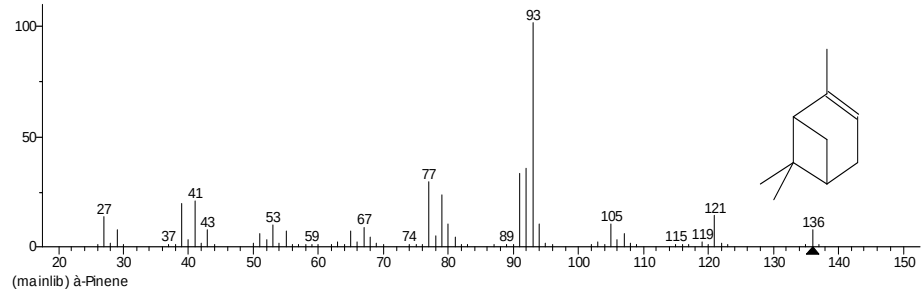
Cassia angustifolia was 2.68%. From these results, revealed that the following; *(i)* The GC/Mass chormatography of *Glycyrrhiza glabra* revealed that the presence of the following compounds (α–pinene, β-pinene, Octanol, γ–terpinene, Stragole, Isofenchon, β-caryophyllene, Citronellyl acetate, Caryophyllene oxide and Geranyl hexanolate), it is obovious that (Geranyl hexanolate) represented the highest percentage up to (35%) while (β-pinene) was the lowest one up to (2%); *(ii)* The GC/Mass chormatography of *Rheum palmatum* revealed that the precence of the following compounds (α–thugen, Octanol, γ–Stragole, Trans-ocimene, Trans-anethole, Eugenol, α-copaene, α-farnesene, δ-cadinene and palmitic acid), it is obvious that (Eugenol) represented the highest percentage up to (25%) while (α–thugen) was the lowest one up to (4%); *(iii)* The GC/Mass chormatography of *Cassia angustifolial* exhibited the following compounds (α–pinene, β-pinene, Octanol, γ-terpinolene, Stragole, Cis-limonene oxide, Trans-anethole, β-caryophyllene, Caryophyllene oxide, Geranyl hexanolate and palmitic acid), it is obvious that (Cis-limonene oxide) represented the highest percentage up to (25%) while (α-pinene) was the lowest one up to (1%) Table (5) (Fig. $1_{(a-t)}$).

(a)



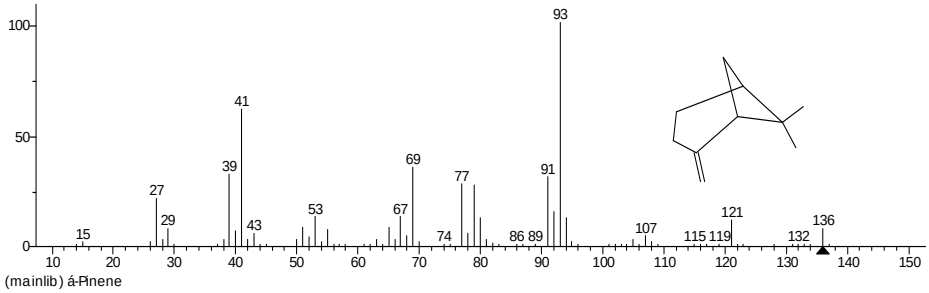
α–thugen MW 152

(b)



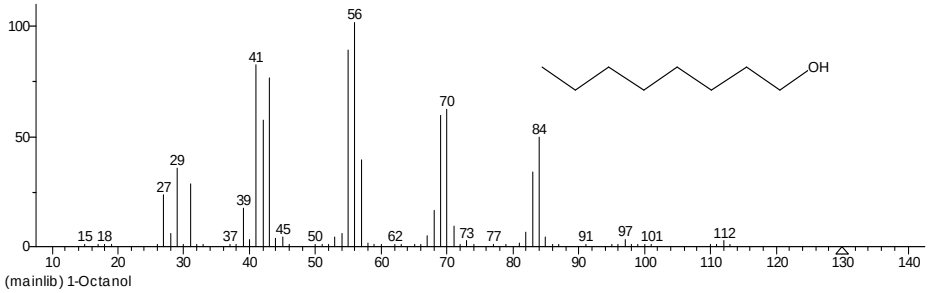
136 α–pinene MW

(c)



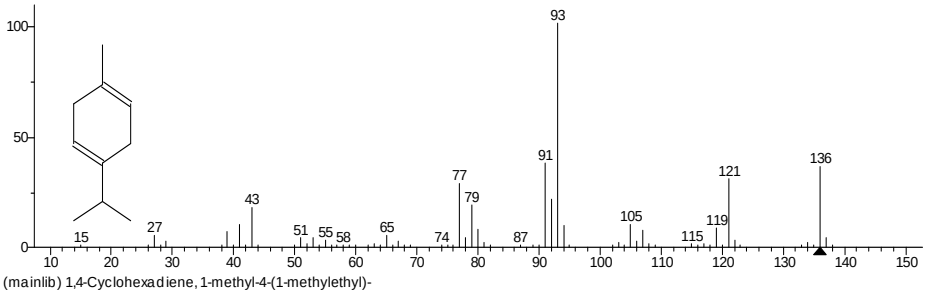
β-pinene MW 136

(d)



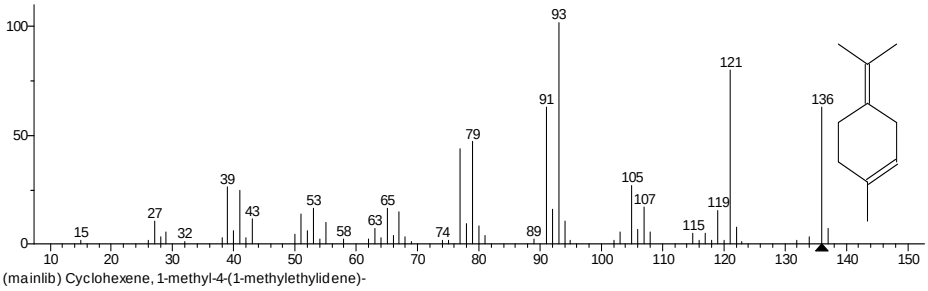
Octanol MW 130

(e)



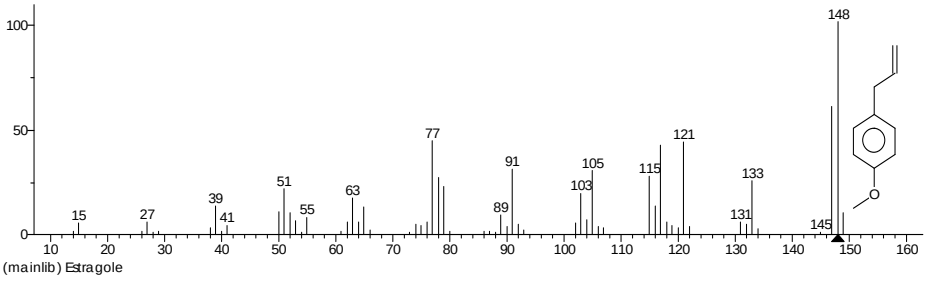
γ–terpinene MW 136

(f)



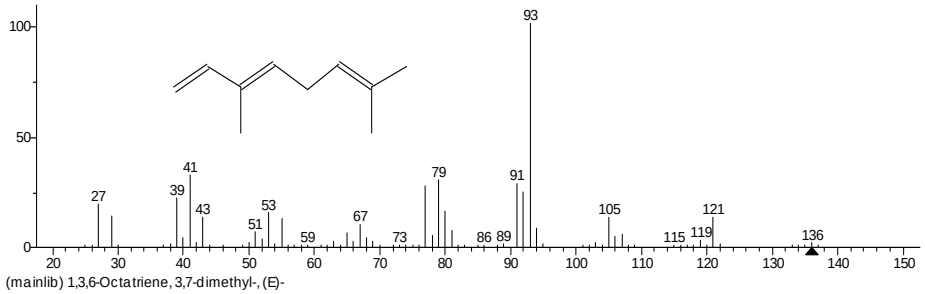
α–terpinolene MW 136

(g)



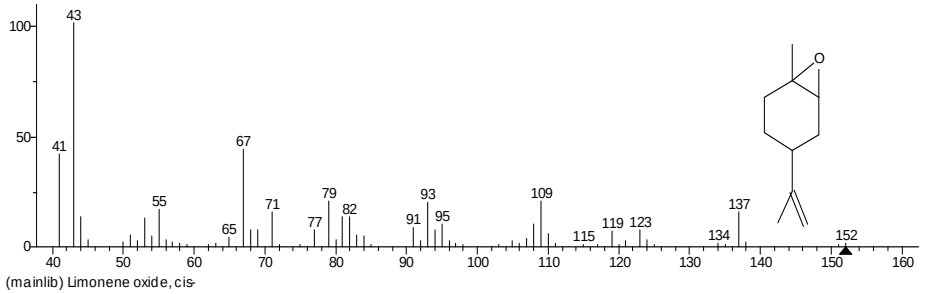
Stragole MW 148

(h)



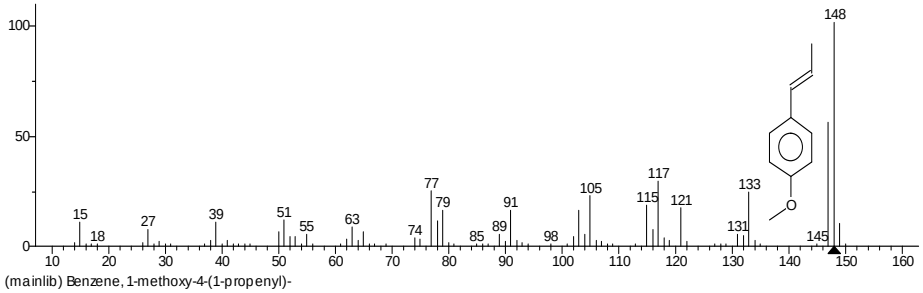
Trans-ocimene MW 136

(i)



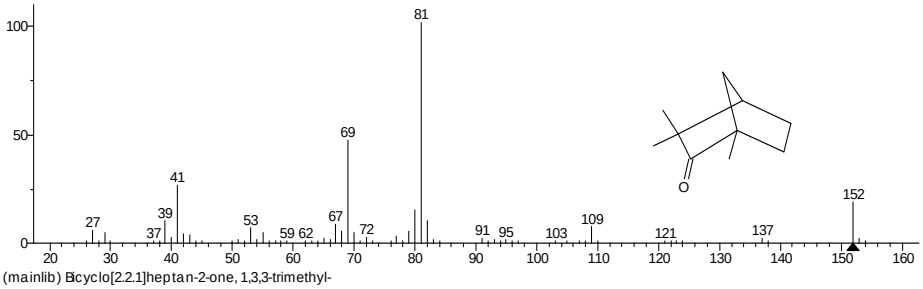
Cis-limonene oxide MW152

(j)



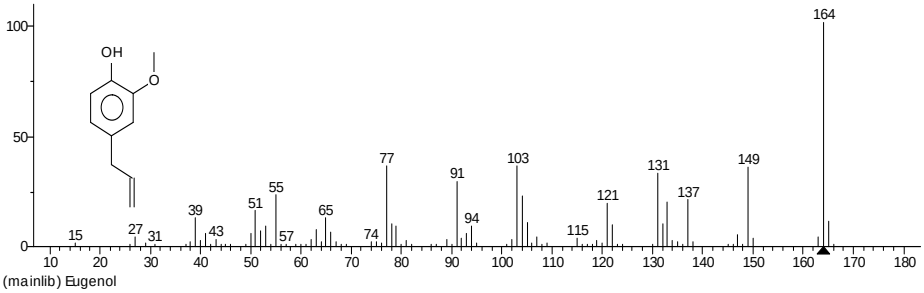
Trans-anethole MW 148

(k)



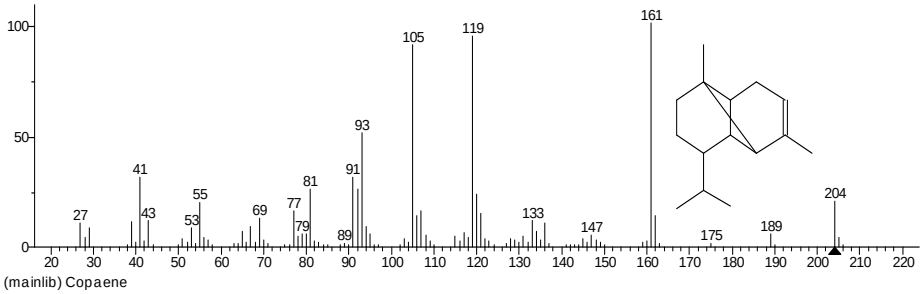
Iso-fenchon MW 152

(l)



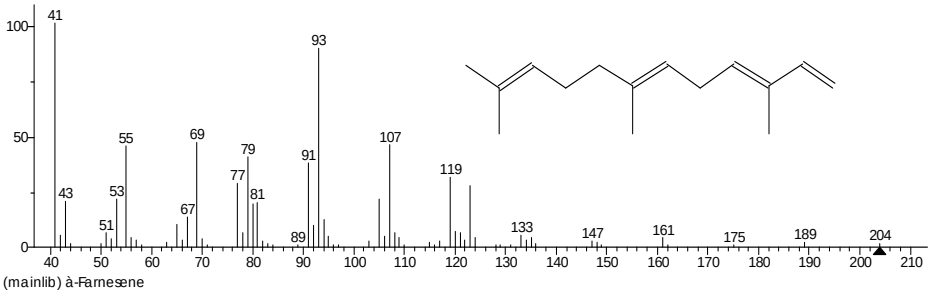
Eugenol MW 164

(m)



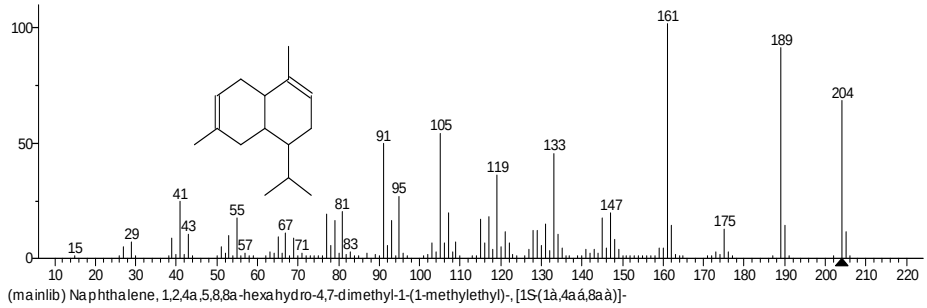
α-copaene MW 204

(n)



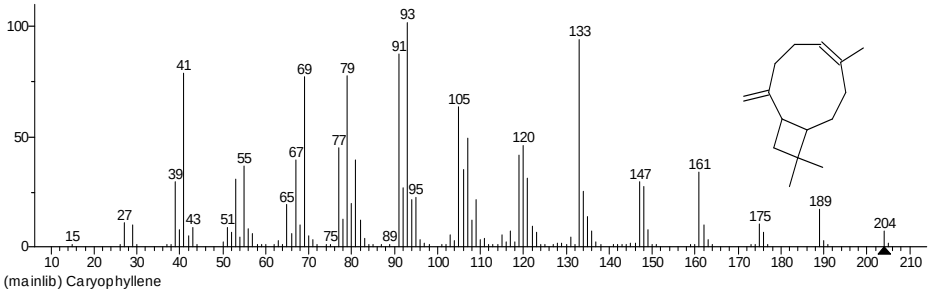
α-farnesene MW 204

(o)



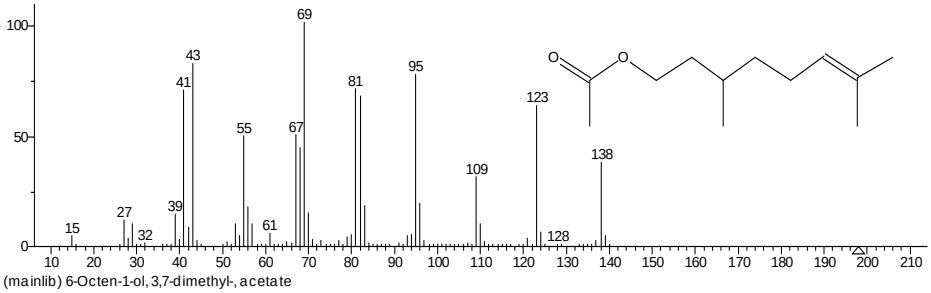
γ-Cadinene MW 204

(p)



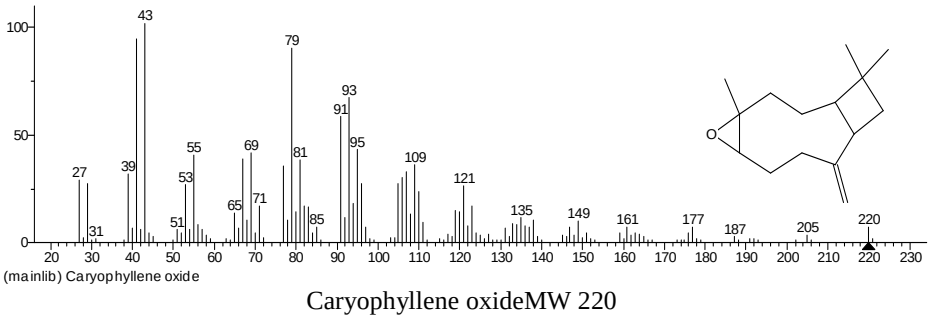
β-caryophyllene MW 204

(q)



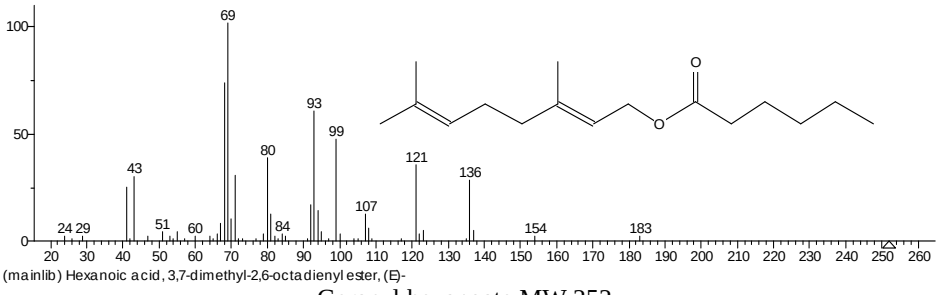
Citroneyllyl acetate MW 198

(r)



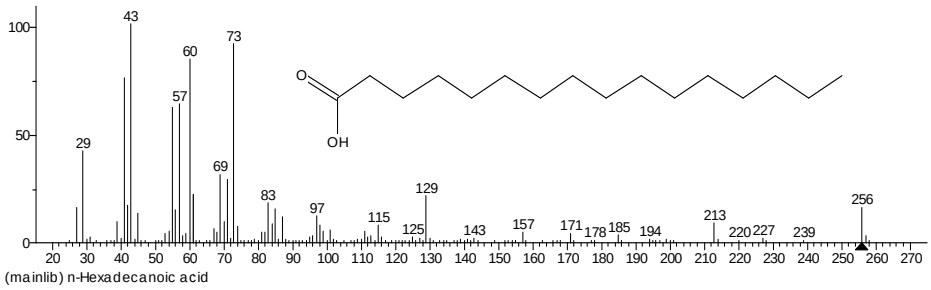
Caryophyllene oxideMW 220

(s)



Geranyl hexanoate MW 252

(t)



Palmitic acid MW 256

Fig. (1): GC/MS fragmentation and structural of medicinal plants essential oils.

DISCUSSION

The present work was carried out in an attempt to throw light about overcome of antibiotic resistant bacteria by natural plant extracts. Many previous studies reported that the flavonoids have antibacterial activity, also many flavonoids with one or more isoprenoid groups have been isolated from *Glycyrrhiza* species, *Rheum palmatum* and *Cassia angustifolia* [26, 27]. Some reports showed that the *Glycyrrhiza glabra* contain prenyllicoflavone A, licoflavone A, and shinflavanone, which have flavonoids skeleton and have microbial activity [28]. The *Glycyrrhiza glabra* containing (liquiritigenin), two prenylflavanones (isobavachin a flavanone and sigmoidin B), a prenylated coumestan (glycerol), and glabrene [29]. Also, *Glycyrrhiza glabra* containing flavones, flavanones, isoflavones, isoflavans, chalcones and petrocarpans [30]. The appearance of yellow colour of *Glycyrrhiza glabra* is due to the flavonoids content of the plant, which includes prenyllicoflavone A, licoflavone A, shinflavanone, Liquiritin, Isoliquiritin (achalcone) and other compounds, the isoflavones glabridin and hispaglabridin A and B have significant antioxidant activity [31]. The *Rheum palmatum* containing chrysophanol glycosides, along with di-O, C-glucosides of the monomeric reduced forms (rheinosides A–D), and dimeric reduced forms (sennosides A–F) [32]. Also, *Rheum palmatum* containing quercetin 3-O-glucoside, quercetin 3-O-galactoside, quercetin 3-O-rutinoside, quercetin 3-O-arabinoside and quercetin 3-O-[6$^{''}$-(3-hydroxy-3-methylglutaroyl)-glucoside [33]. Isolates from *Senna* species the following flavonoids obtained (Rutin or 5, 7, 3', 4' tetrahydroxy-flavone 3-O-rhamnoglucoside and two flavonoids from aurone group were isolated and identified from the leaves of *Senna italica*) [34]. Some scientists successfully isolated from *Senna surattensis* Quercetin, quercetin 3-O-glucoside 7-O-rahmnoside and rutin [35]. The *Cassia senna* containing flavonoids and glucosides [36]. In relation to the volatile oils, the *Glycyrrhiza glabra* contain volatile oil (0.12%) as cadinene, methyl chavicol and benzaldehyde were the main one of *Glycyrrhiza glabra* [37]. Also, reported that the volatile oils of *Glycyrrhiza glabra* contain terpenoid, octanoic acid, paeonol, octadecane, benzaldehyde, α-terpineol and 4-terpineol [38]. The volatile oil from the rhizomes of *Rheum palmatum* terpenoid, palmitic acid, paeonol, α-copaene, δ-cadinene and methyl eugenol was reported [39]. The antibacterial activity of a hot water extract (HWE) and a cold ethanolic extract (CEE) of *Trichosanthes cucumerina* demonstrated significant ($P \leq 0.05$) antibacterial effects against *Staphylococcus aureus*, *S. pyogenes*, *E. coli* and *P. aeroginosa*. On comparing the two extracts against the tested bacterial strains, the CEE was found to be more effective as an antibacterial agent than the HWE. Thus, a concentration of 56.25 µg/mL of HWE and 28.12 µg/mL of CEE required to completely inhibiting the growth of above tested organisms. Further, *S. aureus* and *S. pyogenes* appear to be more susceptible than *E. coli* and *P. aeroginosa* to the antibacterial actions of the HWE and CEE. Garlic exhibited antibacterial activity against *S. aureus* in 98% ethanolic solvent at 200mg/ml concentration [40]. Also showed activity against *E. coli* at 500mg/ml and 200mg/ml concentrations respectively after 2h decoction. Activity of garlic juice in absolute ethanol was more pronounced against test bacteria than in methanol [41]. Four test organisms *Candida pseudotropicalis*, *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis* were inhibited by methanol extracts of both the leaf and the stem bark of *Ficus sur* (Forssk) and no inhibitory effect obtained against *Pseudomonas aeruginosa* and *Salmonella typhimorium* [42]. South African *Helichrysum* species was mainly observed antimicrobial activity against two Gram-positive bacteria *S. aureus* and *B. cereus* [43]. Antimicrobial activity test was performed on different extracts of *Helichrysum chasmolycicum* and also 3,5-dihydroxy-6,7,8-trimethoxyflavone and kaempferol 3-O-glucoside which were the major flavonoid compounds obtained from the aerial parts by microbroth dilutions technique. The ethanol–ethyl acetate extract showed moderate antimicrobial activity against *Pseudomonas aeruginosa*, petroleum ether–60% ethanol-chloroform extract and 3,5-dihydroxy-6,7,8-trimethoxyflavone showed moderate antifungal activity against *Candida albicans* [44]. *Trigonella foenum*

graceum contains many important phytochemical like Aziridine, 1, 2,3-trimethyl-, trans-, which may prove to be a potent antimicrobial agent [45]. The seed oil of *Euphorbia rothiana* was satisfactory yield and observed that major percentage of unsaturated fatty acid present in seed oil was found to have significant anti-inflammatory and analgesic activity. Further seed oil can be subjected for ulcerogenic activity [46]. The best antibacterial activity as indicated by the minimum inhibitory concentration (MIC) values was obtained by *Phyllanthus amarus* against *Staphylococcus aureus* (G+ve) with a MIC value of 17.7 µg/ml. *Phyllanthus myrtifolius* and *Phyllanthus urinaria* inhibited growth of *Pseudomonas stutzeri* (G-ve) with MIC values of 78 µg/ml and 117 µg/ml, respectively [47]. The crude methanol extract of *Trilepisium madagascariense* enhanced the antimicrobial activity and exhibited that the most sensitive microbes were *Enterococcus faecalis* ATCC 10541 (MIC range of 60–780 µg/ml) for bacteria and *Candida guillermondi* (MIC range of 0.01–190 µg/ml) for yeasts [48]. The aqueous, ethyl acetate, methanolic and Total Oligomer Flavonoids (TOF) enriched extracts, obtained from the aerial parts of *Cyperus rotundus*, were investigated for their contents in phenolic compounds. Significant antibacterial activity against reference strains; *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella enteritidis* and *Salmonella typhimurium*, was detected in the presence of ethyl acetate and TOF enriched extracts [49]. The *Cassia senna* contains volatile oils may have antimicrobial activity [36]. Microorganisms become resistant as a result of genetic mutations or acquisition of pre-existing genes that confer resistance [50], which occur either in DNA of the bacterial chromosomes or in the extra chromosomal transferable DNA called plasmids [51]. The antibacterial activities of the solvent extracts from *Laurencia obtusa* exhibited weak activity against Gram-positive bacteria, only one Gram-negative bacterium, *E. cloaceae* and yeast strain *C. albicans*. However, the essential oil of *L. obtuse* was the most active showing a strong inhibitory effect against all tested Gram-positive bacteria including methicillin-oxacillin resistant *S. aureus* (MRSA), Gram-negative bacterium, *E. cloaceae* and yeast strain. The methanol extract and the essential oil of *L. obtusa* var. *pyramidata* showed neither antibacterial nor antifungal activity [52]. Thus antibiotic resistance can be disseminated to other bacteria by the plasmid during conjugation [53]. The rapid spread of antibiotics resistance genes in bacterial population is due to selective pressures resulting from the intensive and the indiscriminate use of antibiotics in human therapy [54].

CONCLUSION

In general, higher content of flavonoids than essential oils were obtained and only three medicinal plants extracts out of five exhibited antibacterial activity against both G+ve and G-ve bacteria that already resistant to many different types of antibiotics were reported before. The combinations between the three extracts revealed that synergism action was obtained and at different concentrations slight inhibitory effect at 10% as compared with great inhibitory effect at 30% was reported in this study.

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