PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON SOME MEDICINAL PLANTS

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ABSTRACT

Biological studies on thirteen ethanolic plant extracts (*Rosmarinus officinalis ,Ocimum basilicum , Moringa oleifera, Zingiber officinale, Curcuma longa, Nigella sativa ,Cinnamomum verum ,Salvia officinalis, Lepidium sativum, Foeniculum vulgare, Anethum graveolens, Ficus benghalensis* and *Cinnamomum camphora*) revealed that four of them (*Rosmarinus officinalis , Zingiber officinale, Cinnamomum verum* and *Cinnamomum camphora*) were the best active against two bacterial species ; *E. coli , S .aureus* and one fungus species *C. albicans*. Also, their synergistic effects against *E. coli, S. aureus* and *C. albicans* were studied .So, the phytochemical studies were completed on these four plants . This study aimed to evaluate the chemical composition of the best active plant extracts. The chemical major content of *Rosmarinus officinalis* was eucalyptol (7.48%) , *Zingiber officinale* was gingerol (12.73%) , *Cinnamomum verum* was (E)- cinnamaldehyde (25.55 %) and *Cinnamomum camphora* was eugenol (27 .35%) . The minimum inhibitory concentration (MIC) values varied from 0.625 to 2.5 mg/ml, for the *S. aureus* (gram positive bacteria) affected by *Rosmarinus officinali, Zingiber officinale, Cinnamomum verum* and *Cinnamomum camphora.* Respectively. *C. albicans* was the most effective microorganism by *Cinnamomum verum* and the least effective microorganism by *Rosmarinus officinalis* and *Cinnamomum camphora.* ethanolic extracts, as MIC ranged from 0.15 to 1.25 mg/ml.

Keywords: *Escherichia coli, Staphylococcus aureus***,** *Candida albicans***,** *Rosmarinus officinalis , Zingiber officinale, Cinnamomum verum, Cinnamomum camphora,* antimicrobial activity, Minimum inhibitory concentration (MIC) .

Introduction

Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediaries and chemical entities for synthetic drugs **(Ncube *et al.*, 2008)**. Phytochemicals are non-nutritive plant secondary metabolites that have protective or disease preventive properties. Plants produce these chemicals to protect themselves but recent research demonstrates that many of these phytochemicals can protect humans and animals against diseases **(Kumar *et al.*, 2009).**

Materials and Methods

Plant materials

In this study, some of the plant materials, leaves of (*Rosmarinus officinalis, Ocimum basilicum, Moringa oleifera, Ficus benghalensis* and *Cinnamomum camphora*) were collected from the agricultural research Station, Sakha, Kafr El-sheikh and the other plant materials, the dried rhizomes of (*Zingiber officinale* and *Curcuma longa*), seeds of (*Nigella sativa, Lepidium sativum, Foeniculum vulgare* and *anethum graveolens*), bark of (*Cinnamomum verum* and *Cinnamomum camphora*) and leaves of (*Salvia officinalis*) were purchased from a local herbal shop (Abuo Shelib, Tanta). Plant materials were compared with samples from Desert Institute Herbarium and Cairo University Herbarium.

Test microorganisms

For the *in vitro* antimicrobial activity, three identified clinical isolates were obtained from **the Regional Center for Mycology & Biotechnology-Al-Azhar University**. These standard strains were two bacterial strains *Escherichia coli* (RCMB 010052) ATCC25955 and *Staphylococcus aureus* (RCMB010010) and one fungal strain *Candida albicans* RCMB 005003 (1) ATCC 10231. *E,coli* and *S.aureus* were subculture on nutrient agar slant and *C.albicans* was subcultured on potato dextrose agar and all were stored at 4°C in a refrigerator until need.

Antibiotics:

Tetracycline and Erythromycin were used as positive control for *E.coli* and *S.aureus*

Antifungal:

Nystatine was used as positive control for *C.albicans*

Culture media:

The Nutrient broth (Oxoid Ltd., London) formed the basis of most media used in microbiological studies. Nutrient agar (Oxoid Ltd., London) was used to prepare enriched culture media and was used for all antibacterial sensitivity tests for plant

extracts evaluation. Potato dextrose agar was used as enriched culture media for *C.albicans*

DMSO (dimethylsulfoxide):

was used as negative control for antimicrobial activity and for preparation of the extracts.

Extraction

The plant materials were collected, washed with running tape water then with distilled water and then allowed to air dry. The plant materials were ground to fine powder. The extraction process was carried out by using ethanol 96% as reported by **Moustafa *et al*. (2014)** with slight modifications. One hundred gram. of air dried powder of plant materials were accurately weighted and then were placed with one liter of ethyl alcohol 96% then fully extracted separately by percolation at ambient temperature; flasks plugged with cotton wool and then kept on a rotary shaker for 72 hours. The extracts were filtered using Whatmann filter paper No. 1. The solvent was evaporated by air convection oven at 38°C. The weight of resulted crude extracts was measured by grams and the crude extracts were preserved in sterilized dishes at refrigerator.

1-Phytochemical studies

A-The Preliminary phytochemical screening

Include testing for tannins, terpenes and/or sterols, flavonoids, alkaloids, carbohydrates and/ or glycosides, saponins, resins and anthraquinones.

B- Gas Chromatography /Mass Spectrum (GC/Mass) of the plants ethanol extracts.

The prepared ethanol extracts of *Rosmarinus officinalis* (leaves), *Zingiber officinale* (rhizome), *Cinnamomum verum* (bark) and *Cinnamomum camphora* (leaves) were subjected to GC/MS analysis using Thermo Scientific TRACE 1310 Gas Chromatograph attached with ISQ LT single quadrupole Mass Spectrometer. Column: DB5-MS, 30 m; 0.25 mm ID (J&W Scientific). Ionization mode: EI (70 ev). Temperature program: 40 ºC (3 min)- 280 ºC (5 min) at 5 ºC/ min- 290 ºC (1 min) at 7.5 º C/ min. Detector temperature 300 ºC. Injector temperature 200 ºC. Carrier gas: Helium (flow rate 1 ml). Searched library: Wiley and Nist mass spectral data base.

2-Biological studies:-

A-Antimicrobial activities of the ethanolic extract of the studied plants

Antimicrobial activities of all ethanolic plant extracts were estimated by means of agar-well diffusion method.

B-Preparation of the standard bacterial suspensions (Adam *et al.*, 2014)

The tested microorganisms were separately cultured on nutrient agar at 37^0C for 24 hrs. This was achieved by streaking the inoculating loop containing the bacteria at the top end of the agar plate moving in a zigzag horizontal pattern until 1/3 of the plate was covered. Then, three to five well-isolated overnight cultured colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a sterile fixed wire-loop and the growth was transferred into a screw-capped tube containing 10ml of nutrient broth (NB). The broth culture (test tubes) was incubated without agitation for 24 hr. at 37^0C, to produce a suspension containing about 10^8 -10^9 colony forming units per ml (cfu/ml). The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (**Collee *et al*. 1996**).

C-Antibacterial activity

Determination of antibacterial activity was performed by well diffusion method. Well diffusion technique **(Akrayi and Abdullrahman 2013).**

D-Preparation of the standard fungal suspensions

Preparation of the fungal suspension of *Candida albicans* was carried out by the same method of preparation of the bacterial suspension but Potato Dextrose Agar (PDA) media (Potato extract 200 ml, Dextrose 20 gL, Agar 16 gL, pH: 5.6) was used instead of nutrient agar medium and Potato dextrose broth was used instead of nutrient broth. The culture was allowed to reach the concentration of 10^8 -10^9 cfu/ml by means of the surface viable counting technique.

E-Antifungal activities

Potato Dextrose Agar (PDA) medium was prepared for antifungal test. The concentration of fungal suspensions were adjusted to 10^8 cells/ml. Fungal cultures were spread on PDA plates. In the plates, wells (8 mm diameter) were made using cork borer. Ethanolic plant extracts (100 µl) were introduced in wells. Antifungal agent Nystatin (50 µl), having a concentration of 1 mg/ml, were introduced in well, which served as positive control. DMSO (dimethylsulfoxide) served as negative control which was poured in wells of petri plates. The plates were held for 1 hr. at room temperature for diffusion of extract into the agar and then incubated for 48 hours at 30°C. after incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm)

F- Antimicrobial activities and synergistic effects of the selected best plant extracts.

The best four plant extracts that have the best antimicrobial activities on the tested microorganisms are A= *Rosmarinus officinalis* (leaves), B= *Zingiber officinale* (rhizome), C= *Cinnamomum verum* (bark) and D= *Cinnamomum camphora* (leaves) ,these four plant extracts were allowed to combine with each other and with antibiotics T=*Tetracyclines* and E=*Erythromycine* (positive controls) in case of *Escherichia coli*

and *Staphylococcus aureus* .Also, plant extracts allow to combine with each other and with antifungal N=Nystatinein (positive control) in case of *Candida albicans*. The antimicrobial activities of all the combined plant extracts were determined by agar well diffusion method. The plates were seeded with 0.1 ml of the inoculums of each tested organism that has a concentration of 10^8 colony forming units per ml (cfu/ml). The inoculums were spread evenly over the plates with sterilized cotton swab. A standard cork borer of 8-mm diameter was used to cut uniform wells on the surface of the plate. The four plant extacts were mixed with each other and with the antimicrobial agents T=Tetracyclines , E=Erythromycine and N=Nystatinein and then the combined plant extracts were introduced in the well. The plates were held for 1 hr at room temperature for diffusion of extract into the agar and then incubated for 24 hours at 37°C in case of bacteria (*Escherichia coli, Staphylococcus aureus*) and incubated for 48 hours at 30°C in case of *Candida Albicans* after incubation, the diameters of the zones of inhibition were measured to the nearest millimeter (mm). four plant extracts and their combination were A, B, C, D, T, E, N, AB, AC, AD, AT, AE, AN, BC, BD, BT, BE, BN, CD, CT, CE, CN, DT, DE, DN.

G- Determination of the Minimum Inhibitory Concentration (MIC mg/ml).

MIC is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism.To determine MIC of the plant extracts, where each ethanolic plant extract was dissolved in DMSO before use. The process was carried out as reported by (**Wiegand *et al.*, 2008)**.

Results and Discussion

The Preliminary phytochemical screening of ethanolic extracts (96%) of the plants tabulated in **Table 1** showed that *Rosmaris officinalis* contains tannins flavonoids, terpenoids, alkaloids and carbohydrates but it is free from resins, anthraquinons and saponins .The present results in agree with that of **Andrade *et al* .(2018)** as they reported that *Rosmaris officinalis* ethanolic extract contains tannins, polyphenol ,flavonol ,terpenoid and alkaloid. On the other hand , *Zingiber officinale* contains resins, terpenoids ,flavonoids, alkaloids and carbohydrates, but free from tannins, anthraquinons and saponins .The present results in agree with the study of **El-Swaify and Abd El-Kawy. (2014)** as they reported that *Zingiber officinale* contains traces of flavonoid, carbohydrates, tannins, steroles or terpenoids, but free from alkaloids. *Cinnamomum Verum* contains tannins, anthraquinons , terpenoids , flavonoids and carbohydrates, but free from resins , alkaloids and saponins. The present results were similar to that of **Mazimba *et al*. (2015) ,** as they found presence of flavanoids, steroids, tannins, triterpenoids in *Cinnamomum Verum* methanolic extract, but disagree for the presence of alkaloids and saponins. *Cinnamomum Camphora* contains tannins, terpenoids , flavonoids and carbohydrates but it free from resins , anthraquinons , alkaloids and saponins .the present data were similar to that of **Ankita *et al*. (2014)** as they reported the presence of alkaloids, tannins and carbohydrate.

Table 1: Preliminary phytochemical screening of the ethanolic plant extracts.

No	Chemical constituents	*Rosmaris officinalis* (A)	*Zingiber officinale* (B)	*Cinnamomum Verum* (C)	*Cinnamomum Camphora* (D)
1	Resin	-	+	-	-
2	Tannins	++	-	++	++
3	Anthraquinones	-	-	+	-
4	Trepenoids	+	+	++	+
5	Flavonoids	+	+	++	+++
6	Alkaloids	+	+	-	-
7	Carbohydrates	+	+	+	+
8	Saponin	-	-	-	-

Separation and identification the main active chemical compounds of the plants ethanolic extracts.

1- Gas Chromatography /Mass Spectrum (GC/Mass) of the plants ethanol extracts.

The gas liquid chromatography results for *Rosmaris officinalis* ethanolic extract represented in **Table 2** and **Fig.1** which showed that *R. officinalis* contains fifty seven compounds mainly flavonoids, terpenoids and some acids. the most abundant compounds are bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- (18.71%), bicyclo [2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (11.48%) , n-hexadecanoic acid (13.57%). Gas liquid chromatography analysis revealed the presence of some active constituents ; eucalyptol (7.48%) , 3-Pinanone (0.60 %) , terpinen-4-ol (0.76%) , m-cymen-8-ol (0.35%) , 5-caranol (1.22 %) , caryophyllene (0.87 %) , caryophyllene oxide (2.03 %), humulene (0.18%) , α-copaene (0.85 %), (+)-α-funebrene (0.80 %) , α-gingerol (0.48%) and epibuphanisine (0.91 %).

The present results agree with **Begum *et al.*, (2013) where as** they reported that *R. officinalis* constituents include flavonoids, 6-methoxygenkwanine, apigenine, diosmetine, diosmine, genkwanine, hispiduline, Luteoline, Sinensetine. Di- and triterpenoids. Carnosolic acid, picrosalvine, rosmariquinone, oleanolic acid, ursolic acid (has anti-inflamation effect) and Monoterpenoids. The present results agree with **Satyal *et al.*, (2017)** as they studied the chemical compositions of six *Rosmarinus officinalis* essential oils. α-Pinene and 1,8-cineole dominated the essential oils.

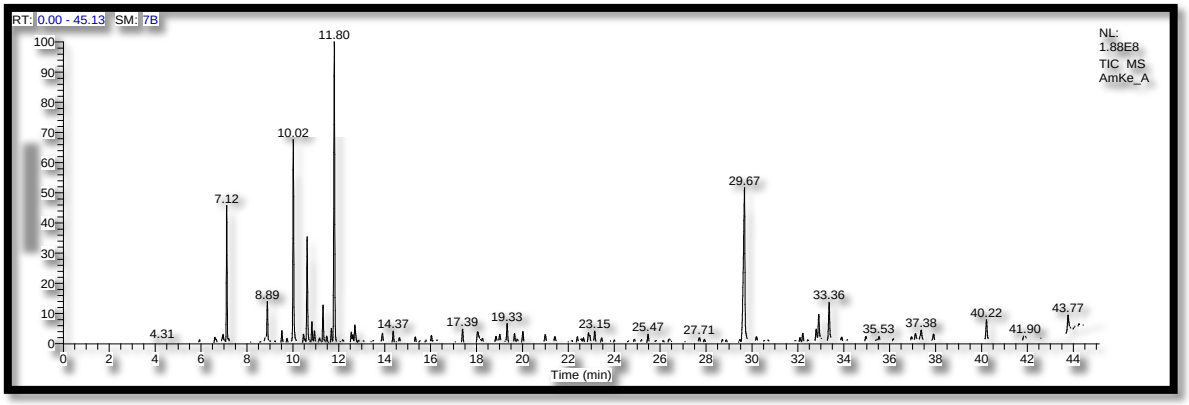

Fig.1 . GC/Mass of *Rosmaris officinalis* (A) ethanolic extract.

Table 2: GC/Mass of *Rosmaris officinalis* (A) ethanolic extract.

No.	Rt.	%	Name	Molecular Formula	Molecular Weight
1	6.60	0.5	Octanal	C8H16O	128
2	6.96	7.48	Eucalyptol	C10H18O	154
3	8.89	2.89	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	C10H18O	154
4	9.52	0.75	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl-	C10H14O	150
5	9.74	0.24	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	C10H18O2	170
6	10.02	11.48	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	C10H16O	152
7	10.46	0.60	3-Pinanone	C10H16O	152
8	10.62	6.45	endo-Borneol	C10H18O	154
9	10.83	1.23	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	C10H18	138
10	10.94	0.76	Terpinen-4-ol	C10H18O	154
11	11.16	0.35	m-Cymen-8-ol	C10H14O	150
12	11.31	2.22	3-CYCLOHEXENE-1-METHANOL, à,à,4-TRIMETHYL-	C10H18O	154
13	11.47	0.43	BICYCLO[3.1.1]HEPT-2-ENE-2-METHANOL, 6,6-DIMETHYL-	C10H16O	152
14	11.68	0.69	1,7,7-TRIMETHYL-BICYCLO[2.2.1]HEPTAN-2-OL	C10H18O	154
15	11.80	18.71	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	C10H14O	150
16	12.18	0.27	3-(2-HYDROXYPHENYL)ACRYLIC ACID	C9H8O3	120
17	12.54	0.52	BICYCLO[4.1.0]HEPTAN-3-OL, 4,7,7-TRIMETHYL-, (1à,3à,4à,6à)-	C10H18O	154
18	12.61	0.33	Benzaldehyde, 4-(1-methylethyl)-	C10H12O	148
19	12.70	1.22	5-Caranol, (1S,3R,5S,6R)-(-)-	C10H18O	154
20	13.90	0.79	BICYCLO[2.2.1]HEPTAN-2-OL, 1,7,7-TRIMETHYL-, ACETATE, (1S-ENDO)-	C12H20O2	196
21	14.37	0.68	Phenol, 2-methyl-5-(1-methylethyl)-	C10H14O	150
22	14.64	0.30	2-Methoxy-4-vinylphenol	C9H10O2	150
23	15.33	0.42	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethylidene)-	C10H14O	150
24	16.04	0.50	2,3-DIMETHYL-1,4-THIAZANE S,S-DIOXIDE	C6H13NO2S	163
25	17.39	0.87	Caryophyllene	C15H24	204
26	18.05	1.17	2,6-CRESOTALDEHYDE	C8H8O2	136
27	18.26	0.18	Humulene	C15H24	204
28	18.85	0.85	á-copaene	C15H24	204
29	19.01	0.73	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202

30	**19.33**	**1.31**	**1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-**	**C15H24**	**204**
31	**19.66**	**0.62**	**2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL-**	**C15H26O**	**222**
32	**20.02**	**0.80**	**(+)-á-FUNEBRENE**	**C15H24**	**204**
33	**20.99**	**0.62**	**3-Hydroxymethylene-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one**	**C11H16O2**	**180**
34	**21.41**	**2.03**	**Caryophyllene oxide**	**C15H24O**	**220**
35	**22.40**	**0.34**	**1,3-BENZODIOXOLE, 4,5-DIMETHOXY-6-(2-PROPENYL)-**	**C12H14O4**	**222**
36	**22.56**	**0.36**	**7-epi-cis-sesquisabinene hydrate**	**C15H26O**	**222**
37	**22.65**	**0.32**	**Caryophylla-4(12),8(13)-dien-5à-ol**	**C15H24O**	**220**
38	**22.88**	**0.97**	**2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-**	**C11H14O3**	**194**
39	**24.88**	**0.29**	**4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol**	**C10H12O3**	**180**
40	**25.47**	**0.59**	**Tetradecanoic acid**	**C14H28O2**	**228**
41	**27.71**	**0.40**	**4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol**	**C15H26O2**	**238**
42	**27.93**	**0.47**	**1-Hexadecanol**	**C16H34O**	**242**
43	**28.88**	**0.24**	**PENTADECANOIC ACID, 14-METHYL-, METHYL ESTER**	**C17H34O2**	**270**
44	**29.67**	**13.57**	**n-Hexadecanoic acid**	**C16H32O2**	**256**
45	**30.20**	**0.29**	**1-EICOSANOL**	**C20H42O**	**298**
46	**32.09**	**1.60**	**8,11-Octadecadienoic acid, methyl ester**	**C19H34O2**	**294**
47	**32.91**	**1.95**	**Oleic Acid**	**C18H34O2**	**282**
48	**33.36**	**3.16**	**Octadecanoic acid**	**C18H36O2**	**284**
49	**33.90**	**0.29**	**1-DOCOSANOL**	**C22H46O**	**326**
50	**34.96**	**0.43**	**Morphinan, N-formyl-5,6-didehydro-3,4,6-trimethoxy-**	**C20H25NO4**	**343**
51	**35.53**	**0.41**	**1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one**	**C17H24O3**	**276**
52	**36.95**	**0.24**	**Villosin**	**C20H28O2**	**300**
53	**37.12**	**0.48**	**Gingerol**	**C17H26O4**	**294**
54	**37.38**	**0.91**	**Epibuphanisine**	**C17H19NO3**	**285**
55	**37.91**	**0.52**	**Podocarpa-5,8,11,13-tetraen-7-one, 13-hydroxy-14-isopropyl-**	**C20H26O2**	**298**
56	**40.22**	**1.54**	**9-ANTHRACENOL, 1,4,8-TRIMETHOXY-**	**C17H16O4**	**284**
57	**43.77**	**1.54**	**á-Sitosterol**	**C29H50O**	**414**

Data for the *Zingiber officinale ethanolic* extract gas liquid chromatography in **Table 3** and **Fig. 2** showed that it contains sixty compounds included flavonoids, terpenoids and hydrocarbons. The major compounds are gingerol (12.73%), 1, 3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl (10.68 %), sesquiphellandrene (7.26 %). Also there are active constituents present in minor percent; eucalyptol (0.24%), levomenthol (0.195%), à-terpineol (0.25 %) , vanillin (0.24 %), aromandendrene (1.52 %), (E)-á-famesene (6.08 %) , cubenol (0.30 %) , α-acorenol (0.49 %) ,globulol (1.23 %), , villosin (0.48 %), corymbolone (0.61 %). **Hassan *et al.* (2012)** identified components from the terpene family, most of them were sesquiterpene hydrocarbons among them zingiberene (9%, 6%), β- bisabolene (4%, 5%), α-farnesne (11%, 7%), β-sesquiphellandrene (9%, 13%), monoterpene hydrocarbons which is α-curcumene (14%, 0%) and phenolic compounds which are gingerol (25%, 23%) and shogaol (18%, 25%) in methanol and n-hexane respectively. Also **Jiang *et al.*, (2006)** separated some compounds from *Zingiber officinale* , particularly regarding the content of [6]-, [8]-, and [10]-gingerols, the most active anti-inflammatory components in this species .their results agree with the present results .

Table 3: Gas liquid chromatography of *Zingiber officinale* ethanolic extract .

No.	Rt.	%	Name	Molecular Formula	Molecular Weight
1.	7.11	0.24	Eucalyptol	C10H18O	154
2.	10.62	0.50	endo-Borneol	C10H18O	154
3.	10.82	0.19	Levomenthol	C10H20O	156
4.	11.31	0.25	α-Terpineol	C10H18O	154
5.	11.79	0.45	DECANAL	C10H20O	156
6.	12.37	0.19	6-OCTEN-1-OL, 3,7-DIMETHYL-	C10H20O	156
7.	13.09	12. 73	Geraniol	C10H18O	154
8.	14.37	0.27	Phenol, 2-methyl-5-(1-methylethyl)-	C10H14O	150
9.	16.30	0.27	n-Decanoic acid	C10H20O2	172
10.	16.83	0.24	Vanillin	C8H8O3	152
11.	17.99	0.28	4-Methyl-5H-furan-2-one	C5H6O2	98
12.	18.38	6.18	(E)-á-Famesene	C15H24	204
13.	18.44	1.52	Aromandendrene	C15H24	204
14.	19.05	5.18	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	C15H22	202
15.	19.26	0.22	5á,10à-EUDESMA-4(14),11-DIENE	C15H24	204
16.	19.40	10.68	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-	C15H24	204
17.	19.85	0.30	Cubenol	C15H26O	222
18.	20.08	7.26	α-SESQUIPHELLANDRENE	C15H24	204
19.	20.24	0.34	2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL-	C15H26O	222
20.	20.37	0.40	Eudesma-4(15),7-dien-1á -ol	C15H24O	220
21.	20.46	0.55	Caryophyllene oxide	C15H24O	220
22.	20.64	0.95	Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]-	C15H26O	222
23.	20.75	1.77	trans-Sesquisabinene hydrate	C15H26O	222
24.	20.82	0.54	Aromandendrene oxide	C15H24O	220
25.	21.00	1.62	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	C15H26O	222
26.	21.23	0.70	Dodecanoic acid	C12H24O2	200
27.	22.19	2.12	ZINGIBERENOL	C15H26O	222
28.	22.30	0.51	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-à,à,4a,8-tetramethyl-, (2R-cis)-	C15H26O	222
29.	22.45	1.23	Globulol	C15H26O	222
30.	22.58	1.74	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2-yl)cyclohex-2-enol	C15H26O	222
31.	22.98	4.87	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	C11H14O3	194
32.	23.09	0.49	α-acorenol	C15H26O	222
33.	23.51	0.34	7-Hydroxyfarnesen	C15H24O	220
34.	23.90	3.06	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1à,2à,3á,6à)]-	C15H26O	222

35.	24.05	2.95	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trimethyl-6-methylene-	C15H24O	220
36.	24.51	0.12	(-)-Spathulenol	C15H24O	220
37.	24.61	1.07	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C28H48O	400
38.	24.70	0.25	6-(p-Tolyl)-2-methyl-2-heptenol, trans-	C15H22O	218
39.	24.89	0.20	BETA-CEDREN-9-ALPHA-OL	C15H24O	220
40.	25.03	0.22	α-(4-Hydroxy-3-methoxyphenyl)propionic acid	C10H12O4	196
41.	25.20	0.31	Bergamotol, Z-à-trans-	C15H24O	220
42.	25.27	0.39	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-	C15H22O	218
43.	25.58	0.60	TETRADECANOIC ACID	C14H28O2	228
44.	26.10	0.60	cis-Z-à-Bisabolene epoxide	C15H24O	220
45.	26.49	6.49	Diepicedrene-1-oxide	C15H24O	220
46.	26.60	1.31	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	C15H24O2	236
47.	26.85	0.21	Diepicedrene-1-oxide	C15H24O	220
48.	28.20	0.61	Corymbolone	C15H24O2	236
49.	29.44	0.76	(E)-1-(6,10-Dimethylundeca-5,9-dien-2-yl)-4-methylbenzene	C15H22	202
50.	29.77	2.92	n-Hexadecanoic acid	C16H32O2	256
51.	29.98	0.27	geranyl-à-terpinene	C20H32	272
52.	30.89	0.89	trans-Geranylgeraniol	C20H34O	290
53.	31.86	1.17	1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one	C15H20O3	248
54.	32.38	0.48	Villosin	C20H28O2	300
55.	32.84	1.54	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280
56.	32.98	0.94	cis-Vaccenic acid	C18H34O2	282
57.	33.11	0.63	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	C20H34O	290
58.	33.40	0.73	Octadecanoic acid	C18H36O2	284
59.	34.33	7.07	(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one	C17H24O3	276
60.	38.65	0.90	(3R,5S)-1-(4-Hydroxy-3-methoxyphenyl)decane-3,5-diyl diacetate	C21H32O6	380

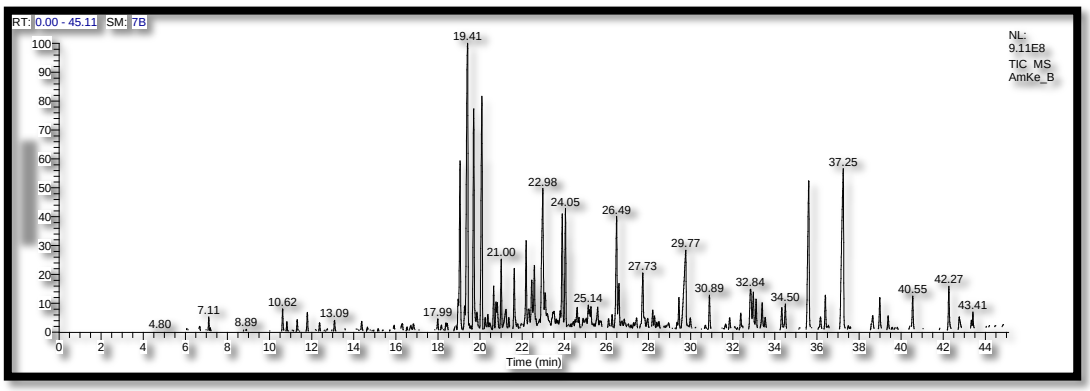

Fig.2: GC/Mass of *Zingiber officinale* ethanolic extract.

Results of **Table 4** and **Fig.** 3 for *Cinnamomum verum* ethanolic GC/Mass analysis revealed that it contains thirty eight compounds included flavonoids and terpenes .The major compounds are (E)- cinnamaldehyde, (25.55 %) , 9-methoxybicyclo[6.1.0]nona-2,4,6-triene (21.10%) , eucalyptol (6.68 %) 2-Propenal, 3-(2-methoxyphenyl)- (5.53%) , levomenthol (3.91 %) phenol, 2-methyl-5-(1-methylethyl)- (1.81%) apiol (2.67 %) .while some compounds record a minor quantities á-copaene ,(0.24%) ,α-cadinol (0.58%), ylangenal (0.32%) and curcumenol (0.32 %) . The present results were similar to those of **Batiha *et al*. (2020**) as they reported that the main chemical components of *Cinnamomum verum* detected were (*E*)-cinnamaldehyde (52.87%), chromen-2-one (10.63%), *o*-methoxycinnamaldehyde, (5.04%), γ-muurolene (4.92%), cadina-1(10),4-diene (4.64%) and acetic acid cinnamyl ester (4.35%), while EAECV was found to possess 26 compounds and the main chemical components identified were (*E*)-cinnamaldehyde (53.81%), coumarin (9.92%), γ-muurolene (5.37%), *p*-methoxycinnamaldehyde, (4.91%), acetic acid cinnamyl ester (4.83%), cadina-1(10),4-diene (4.78%) and cinnamyl alcohol (4.27%).

Table 4: Gas liquid chromatography of *Cinnamomum verum* ethanolic extract .

No.	RT.	%	Compound name	Molecular Formula(M. F.)	Molecular Weight (M. W.)
1.	5.70	0.49	BICYCLO[3.1.0]HEXANE, 4-METHYLENE-1-(1-METHYLETHYL)-	C10H16	136
2.	6.08	0.39	á-Myrcene	C10H16	136
3.	6.93	1.80	D-Limonene	C10H16	136
4.	7.09	6.68	Eucalyptol	C10H18O	154
5.	7.17	0.56	Benzyl alcohol	C7H8O	108
6.	8.70	0.44	Benzoic acid, methyl ester	C8H8O2	136
7.	8.81	0.45	Undecane	C11H24	156
8.	10.81	3.91	Levomenthol	C10H20O	156
9.	11.15	0.31	2-Cyclohexen-1-one, 4-(1-methylethyl)-	C9H14O	138
10.	12.37	0.45	3-Phenylpropanol	C9H12O	136
11.	12.73	0.69	(-)-Carvone	C10H14O	150
12.	13.45	25.55	Cinnamaldehyde, (E)-	C9H8O	132
13.	14.37	1.81	Phenol, 2-methyl-5-(1-methylethyl)-	C10H14O	150
14.	16.67	0.22	Germacrene D	C15H24	204

15.	16.90	21.10	9-Methoxybicyclo[6.1.0]nona-2,4,6-triene	C10H12O	148
16.	17.74	6.28	Coumarin	C9H6O2	146
17.	17.85	1.62	2-Propenoic acid, 3-phenyl-	C9H8O2	148
18.	18.49	0.37	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aà,3bá,4á,7à,7aS*)]-	C15H24	204
19.	18.81	0.56	1-(2,4-Dimethoxyphenyl)-propan-2-one	C11H14O3	194
20.	19.22	0.24	á-copaene	C15H24	204
21.	20.12	5.53	2-Propenal, 3-(2-methoxyphenyl)-	C10H10O2	162
22.	21.28	0.69	(-)-Spathulenol	C15H24O	220
23.	21.96	1.52	Levodopa	C9H11NO4	197
24.	22.39	2.67	Apiol	C12H14O4	222
25.	22.51	2.74	1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	C12H16O3	208
26.	22.79	0.58	à-Cadinol	C15H26O	222
27.	22.89	0.55	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1à,4á,4aá,8aá)]-	C15H26O	222
28.	23.18	0.21	BENZALDEHYDE, 4-HYDROXY-3,5-DIMETHOXY-	C9H10O4	182
29.	23.61	0.32	Ylangenal	C15H22O	218
30.	24.87	0.46	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	C10H12O3	180
31.	25.47	0.33	TETRADECANOIC ACID	C14H28O2	228
32.	25.81	0.32	Curcumenol	C15H22O2	234
33.	28.87	0.26	HEXADECANOIC ACID, METHYL ESTER	C17H34O2	270
34.	29.63	3.92	n-Hexadecanoic acid	C16H32O2	256
35.	30.12	1.41	Octasiloxane, hexadecamethyl-	C16H50O7Si8	578
36.	32.21	0.82	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296
37.	32.81	1.20	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280
38.	32.92	2.56	Oleic Acid	C18H34O2	282

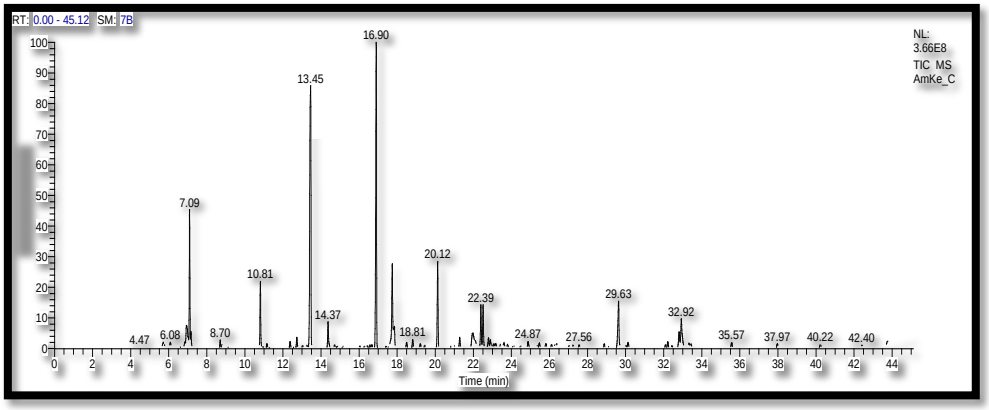

Fig. 3: Gas liquid chromatography of *Cinnamomum verum* ethanolic extract .

Results in **Table** 5 and **Fig.4** for *Cinnamomum Camphora* ethanolic GC/Mass analysis revealed that *Cinnamomum Camphora* contains thirty eight compounds , the major compounds are eugenol (27 .35%) , Levomenthol (12.38%) , spathulenol (16. 24) , D-limonene (3.82%) and n-hexadecanoic acid (3.95%) , bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- (4.25%). On the other hand, terpinen-4-ol, p-cymen-7-ol , aromandendrene compounds are present in minor quantities (0.71 %, 1.41% ,0.64%) respectively .

The present data were in accordance with those of **Guo *et al.* (2016**) as they reported that the composition of *Cinnamomum Camphora* extract was determined by gas chromatography/mass spectrometric (GC-MS) analyses. D-camphor (51.3%), 1,8-cineole (4.3%), and;-terpineol (3.8%), while D-camphor (28.1%), linalool (22.9%), and 1,8-cineole (5.3%) were the main constituents of its extract. Also the present data agree with study of **Frizzo *et al.*** (**2000**) as they found that the composition of *Cinnamomum camphora* extract was determined by gas chromatography/mass spectrometric (GC-MS) analyses. The composition is made by monoterpenes and 2% by sesquiterpenes oxygenated terpenes represented 81% of the total, camphor being the main component (68%) and linalool the second most important (9%). The essential oil of *Cinnamomum camphora* was reported to have antimicrobial (**Narayanan *et al,* (1980) Dubey and Mishra, (1990)**, fungi toxic **Tiwari *et al,* (1994**), nematicidal **Nakamura *et al,*** (**1990)** and leech repelling **Nath *et al,* (1986**) activities.

Table 5: Gas liquid chromatography of *Cinnamomum camphora* ethanolic extract

No.	RT.	%	Compound name	M. F.	M. W.
1.	5.69	2.26	BICYCLO[3.1.0]HEXANE, 4-METHYLENE-1-(1-METHYLETHYL)-	C10H16	136
2.	6.08	1.18	á-Pinene	C10H16	136
3.	6.63	0.61	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	C10H16	136
4.	6.81	0.33	1,3,8-p-Menthatriene	C10H14	134
5.	6.90	3.82	D-Limonene	C10H16	136
6.	6.98	27.35	Eugenol	C10H18O	154
7.	8.51	0.50	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	C10H16	136
8.	10.84	12.38	Levomenthol	C10H20O	156
9.	10.94	0.71	Terpinen-4-ol	C10H18O	154
10.	11.16	3.86	2-Cyclohexen-1-one, 4-(1-methylethyl)-	C9H14O	138
11.	12.61	0.66	Benzaldehyde, 4-(1-methylethyl)-	C10H12O	148
12.	13.00	0.66	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	C10H16O2	154
13.	14.03	1.41	p-Cymen-7-ol	C10H14O	150
14.	14.38	1.07	Phenol, 2-methyl-5-(1-methylethyl)-	C10H14O	150
15.	14.75	4.25	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	C10H18O2	170
16.	15.15	0.47	2(3H)-Benzofuranone, hexahydro-3-methylene-	C9H12O2	152
17.	15.25	0.45	LIMONENE DIOXIDE 4	C10H16O2	168
18.	15.92	1.46	5-Iodo-2,7-dioxa-tricyclo[4.3.1.0(3,8)]decane	C8H11IO2	266
19.	16.31	0.89	7-Oxo-2-oxa-7-thiatricyclo[4.4.0.0(3,8)]decan-4-ol	C8H12O3S	188
20.	17.34	1.08	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-	C10H16O2	168

21.	17.91	1.30	7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)-	C10H16O 2	168
22.	18.44	0.64	Aromandendrene	C15H24	204
23.	19.20	0.75	Dodeca-1,6-dien-12-ol, 6,10-dimethyl-	C14H26O	210
24.	19.46	0.59	4-HYDROXY-4-METHYL-HEX-5-ENOIC ACID TERT-BUTYL ESTER	C11H20O 3	200
25.	20.62	0.66	2-Cyclohexen-1-one, 3-(hydroxymethyl)-6-(1-methylethyl)-	C10H16O 2	168
26.	21.32	16. 24	(-)-Spathulenol	C15H24O	220
27.	24.61	0.62	α-acorenol	C15H26O	222
28.	24.90	1.65	1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulene-4,7-diol	C15H26O 2	238
29.	26.09	0.69	Aromadendrene oxide-(2)	C15H24O	220
30.	26.58	1.13	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	C15H24O	220
31.	27.00	0.62	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	C12H20O	180
32.	28.26	0.42	2,5-Octadecadiynoic acid, methyl ester	C19H30O 2	290
33.	28.60	0.41	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)-2,3,4,4a,5,6-hexahydronaphthalen-1(8aH)-one	C15H22O 2	234
34.	29.66	3.95	n-Hexadecanoic acid	C16H32O 2	256
35.	32.44	1.14	Phytol	C20H40O	296
36.	32.93	1.78	9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31Cl O	298
37.	33.36	0.95	Octadecanoic acid	C18H36O 2	284
38.	33.43	0.56	Ethyl Oleate	C20H38O 2	310

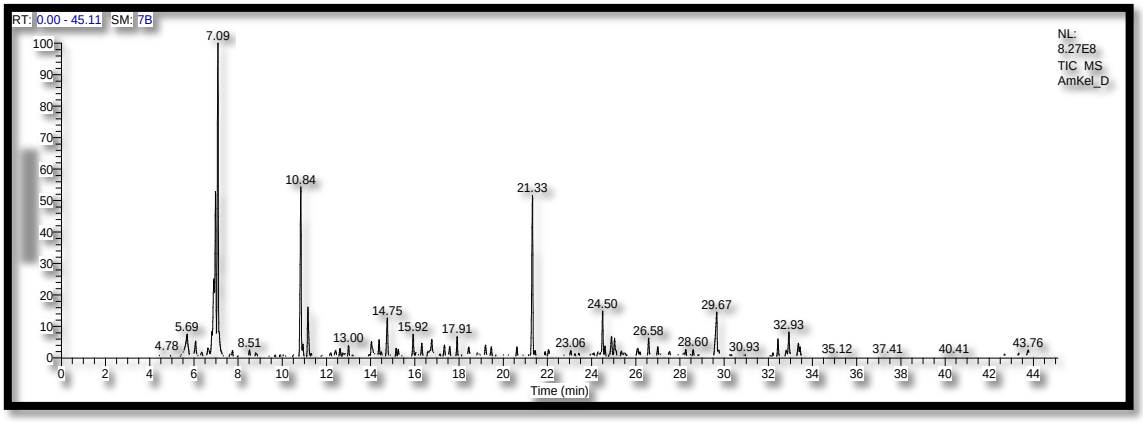

Fig. 4: Gas liquid chromatography of *Cinnamomum camphora* ethanolic extract .

2-Biological Activity

I-Antibacterial activity of the best four plant extracts and their synergistic effects against *E. coli* and *S. aureus*

Results of **Table 6** showed the antibacterial activity of the best four plant extracts and their synergistic effects against *E. coli and S. aureus.* The combination between *Rosmarinus officinalis* and *Zingiber officinale* showed clear zone diameters 15 and 25 mm. for *E. coli.* and *S. aureus,* respectively . Synergistic between *Rosmarinus officinalis* and *Cinnamomum verum* showed clear zone diameters 25 and 28mm. for *E. coli.* and *S. aureus,* respectively . Synergistic between *Rosmarinus officinalis* and *Cinnamomum camphora* showed clear zone diameters 15 and 30 mm. for *E. Coli.* and *S. aureus,* respectively . Synergistic between *Rosmarinus officinalis* and *Tetracycline* gave clear zone diameters 43 and 51mm. for *E. coli.* and *S. aureus* ,respectively . Where *Tetracycline* showed clear zone diameters 42 and 52mm for *E. coli.* and *S.aureus* ,respectively .Also , Synergistic between *Rosmarinus officinalis* and Erythromycin obtained clear zone diameter 30 and 45mm. for *E. coli.* and *S. aureus* ,respectively .where Erythromycin showed clear zone diameters 37 and 49 mm. for *E. coli* .and *S. aureus*.

On the other hand, the synergistic between *Zingiber officinale* with *Cinnamomum verum Cinnamomum camphora,* Tetracycline and Erythromycin showed clear zone diameters 20,20,42 and 22 mm. respectively for *E. coli* and 28 , 34, 53 and 45 mm. for *S. aureus* ,respectively. Where, the synergistic between and *Cinnamomum verum* with *Cinnamomum camphora,* Tetracycline and Erythromycin showed clear zone diameters 32, 37and 27mm. for *E. Coli.* Respectively and 30, 51and 44 mm. for *S. aureus,* respectively. Also, synergistic between *Cinnamomum camphora* with Tetracycline and Erythromycin showed clear zone diameters 41 and 21mm. respectively for *E. coli.* and 51and 45 mm. for *S. aureus,* respectively.

Table 6: Antibacterial activity of the best four plant extracts and their synergistic effects against *E. coli.* and *S. aureus* .

No.	Best plant extracts and their combinations	*E. coli* zone of inhibition (mm)	*S. aureus* zone of inhibition (mm)
1	A	22	26
2	B	18	26
3	C	25	40
4	D	20	27
5	T	42	52
6	E	37	49
7	AB	15	25
8	AC	25	28
9	AD	15	30
10	A T	43	51
11	A E	30	45
12	BC	20	28
13	BD	20	34
14	B T	42	53
15	B E	22	45
16	CD	32	30
17	C T	37	51
18	C E	27	44
19	D T	41	51
20	D E	21	45

A=*Rosmarinus officinalis* B= *Zingiber officinale* C= *Cinnamomum verum*
D= *Cinnamomum camphora* T= Tetracycline E= Erythromycin

As antimicrobial agent, ginger (*Z. officinale*) extract exhibited higher antifungal than antibacterial effects *in vitro*, showing anti-*Candida* activity against strains isolated from patients .This finding was related to the high anti-biofilm activity against *C. albicans*, at concentrations ranging from 0.625 mg/mL to 5 mg/mL (**Aghazadeh *et al*** .,**2016**) .Ginger was also effective against other fungal strains, such as *Fusarium* spp., and it inhibited the growth of fungi that were resistant to amphotericin B and ketoconazole **(Wang and Ng**, **2005** and **Ficker *et al*** .,**2003**). Among bacteria, it showed efficacy against *Pseudomona aeruginosa, Staphylococcus aureus, Acinetobacter baumannii* (**Aghazadeh *et al*** .,**2016**) ,*Escherichia coli, Bacillus subtilis* and *Salmonella typhi* (**Rahmani *et al*** .,**2014)** .

Furthermore, 6-gingerol and 12-gingerol showed antibacterial activity against periodontal bacteria (**Rahmani *et al*** .,**2014)**, so that a clinical trial was performed to test a polyherbal mouthwash containing, among the others, the hydroalcoholic extract of *Z. officinale*; it was worth noting that it was effective in reducing gingival and plaque indices similarly to chlorhexidine mouthwash **(Mahyari *et al*** .,**2016**) . On the other hand, the antidiarrheal activity of 6-gingerol has been accredited to its ability to bind to

the toxin produced by *Vibrio cholera*, rather than due to direct antibacterial activity (**Semwal *et al* .,2015).**

The present data agree with **Shreya *et al*. (2015)** as they reported that Cinnamon oil showed a similar or sometimes even larger inhibitory zone than the conventional antibiotic – Streptomycin. The effect of the extracts and oil was studied by their influence on the growth rate of bacteria. It was found that the presence of cinnamon in the medium had a noticeable effect on the log phase of an actively growing culture, i.e. the log phase duration was significant. Thus, Cinnamon spice proves to be a potential antimicrobial agent and must be subjected to further analysis of its properties. *Cinnamomum verum* essential oil is reported to have antimicrobial effects (**Narayanan et al, 1980; Dubey and Mishra, 1990**), fungitoxic effects (**Tiwari *et al*, 1994**).

Karadag *et al.* (2019) found that *Rosmarinus officinalis* L. (rosemary) is a common culinary spice and herbal drug, which is used for centuries all over the world. In their study, a polar to polar fractions of *R. officinalis* flowers were evaluated for their *in vitro* antioxidant, antibacterial, cytotoxic, anti-inflammatory and analgesic activities, respectively. Phytochemical compositions of *R. officinalis* extract fractions were analyzed by GC–MS and LC–MS. The antibacterial potential was determined using the *in vitro* broth microdilution assay against a panel of human pathogens. The constituents of the polar fractions were identified as rosmarinic acid, luteolin, quercetin and apigenin by LC techniques, whereas the n-hexane fraction was analyzed by GC–MS to determine the main volatile components camphor (19.6%), 1,8-cineole (11.7%), verbenone (11.5%), borneol (10.6%), α-pinene (5.8%), and linalool (5.7%). According to the bioactivity results, the polar fraction showed the highest antioxidant activity, whereas n-hexane fraction was found to be most effective against *Staphylococcus aureus* (78 μg/mL). In conclusion, *R. officinalis* flower n-hexane and ethyl acetate fractions exhibited remarkable *in vitro* antibacterial, antioxidant, anti-inflammatory and analgesic activities possibly due to their polyphenol content.

Kumar and Kumari (2019) reported that *C. camphora* (L.) leaf oils have antifungal activity against *Choanephora cucurbitarum* and antibacterial activity against *Pasturella multocida* and *Aspergillus niger*

II-Antifungal activity of the best plant extracts and their synergistic effects against *C.albicans*

Results of **Table 7** showed the antifungal activity of the best four plant extracts and their synergistic effects against *C.albicans.* The combination between *Rosmarinus officinalis* and Zingiber *officinale* show a clear zone diameter 20 mm. Synergistic between *Rosmarinus officinalis* and *Cinnamomum verum* show a clear zone diameter 46 mm. . Synergistic between *Rosmarinus officinalis* and *Cinnamomum camphora* show a clear zone diameter 15 mm. Synergistic between *Rosmarinus officinalis* and Nystatine a clear zone diameter 21mm.

On the other hand the synergistic between *Zingiber officinale* and *Cinnamomum verum Cinnamomum camphora,* and Nystatine show a clear zone diameter (50,18 and 16 mm.),respectively. Where, the synergistic between

Cinnamomum verum and *Cinnamomum camphora,* and Nystatine show a clear zone diameter (50 and 53mm., respectively). Also, synergistic between *Cinnamomum camphora* and Nystatine show a clear zone diameter (21mm.).

Ankita *et al* 2014 reported that the assessment of antifungal activity of *C. camphora* (L.) J. Preslwas performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against *Aspergillus Niger,Scolorotium,Candida Albicans and Rhizopus*.The antibacterial effect was studied by the agar direct contact method using *Bacillus Cerus, Pseudomonas and Escherichia Coli*...Finally, the results of antimicrobial activity of the aqueous extract showed a pronounced antifungal activity against the tested strains. The results revealed that the methanolic extract exhibited significant antimicrobial activity of concentration of 100-500 µ/ml respectively against tested organisms, particularly more effective against *Aspergillus niger,Candida albicans and Escherichia coli* than the other extracts when compared to the standard drug *Chloroamphenicol,Ampicillin and Streptomycin*.

The antifungal activity of rosemary essential oil was tested against *Candida albicans, Candida dubliniensis, Candida parapsilosis, and Candida krusei* **Gauch *et al*. (2014)**. Such dermatophytes are the mostcommon agents causing topicalmycoses **Jessup *et al*. (2000)**. It was found that an oil concentration of 8% was capable of inhibiting the growth of *Candida* sp. A similar study evaluated the effect of *R. officinalis* hydroalcoholic extract against two dermatophytes, *Microsporum gypseum* and *Trichophyto nrubrum* and showed that a concentration of 10% R.officinalis extract was responsible for 86%inhibition of fungal growth **(Sudan and Singh 2019)**

Table 7: Antifungal activity of the best four plant extracts and their synergistic effects against *C.albicans*

No.	Best plant extracts and their combinations	*C. albicans* (zone of inhibition (mm)
1	A	17
2	B	18
3	C	57
4	D	16
5	N	20
6	AB	20
7	AC	46
8	AD	15
9	AN	21
10	BC	50
11	BD	18
12	BN	16
13	CD	50
14	CN	53
15	DN	21

A=*Rosmarinus officinaliS* **B= *Zingiber officinale*** **C= *Cinnamomum verum*** **D= *Cinnamomum camphora*** **N=Nystatine (Antifungal)**

III-Determination of the Minimum Inhibitory Concentration (MIC).

The (MIC) values varied from 2.5 to 20 mg/ml, respectively for the *E .Coli* affected by *Rosmarinus officinalis , Zingiber officinale , C. verum and C.camphora* ethanolic extracts (**Table** 8). All the three microorganisms used were susceptible to ethanolic extract but the MIC was different. Also, the (MIC) values varied from 0.625 to 2.5 mg/ml for the *S. aureus* (Gram positive bacteria) affected by *Rosmarinus officinalis , Zingiber officinale , C.verum* and *C.camphora.*, respectively . *C. albicans* was the most effective microorganism by *C. verum* and the least effective microorganism by *Rosmarinus officinalis* and *C. camphora.* ethanolic extracts, as MIC ranged from 0.15 to 1.25 mg/ml.

respectively. Disturb cellular function/ metabolism and loss of cellular constituents, leading their death.

The present results disagree with **Maciel *et al*.(2019**) as they reported that The MIC of the *Zingiber officinale* essential oils recorded 21.95 mg/ml. and *Rosmarinus officinalis* 5.55 mg/ml. **Ceylan *et al .(*2014**) reported that the antimicrobial activity of *R. officinalis* essential oil was evaluated *in vitro* against 13 microorganisms which are known to cause human diseases. The results indicated that the *R. officinalis* essential oil showed anti-bacterial activity mainly against the Gram-positive bacteria (*S. aureus and S. epidermidis*), MIC of *S. aureus* ATCC 25923 was 0.312 µl/ml. *R. officinalis* essential oil in MIC concentrations reduced the *S. aureus* ATCC 25923 For *S. aureus* MIC 5 µl/ml. According to the results of antimicrobial activity, the *R. officinalis* essential oil is more active against Gram-positive than Gram negative bacteria.

Hameed *et al*., 2016 reported that Rosmarinus' essential oils are more active against Gram (−ve) bacteria. *R. officinalis* essential oil expressed a strong inhibitory activity against *K. pneumoniae* with an MIC of 2.08 mg/ml, and *S. aureus* with an MIC of 8.35 mg/ml. *E. coli* and *P. aeruginosa* were inhibited with 16.7 mg/ml. *R. officinalis* has also a bactericidal power. Minimal bactericidal concentrations were 4.17 mg/ml for *K. pneumoniae* and 33.4 mg/ml for *E. coli, S. aureus, and P. aeruginosa*. **Yesil Celiktas *et al*. (2007)** worked on *R. officinalis* and found the following MIC: *E. coli* (20 mg/ml), *S. aureus* (10 mg/ml), *P. aeruginosa* (10 mg/ml), and *K. pneumoniae* (20 mg/ml). **Okoh *et al*. (2010)** found that South African sample of *R. officinalis* (oriental region of the Cape) exhibited the following MIC: *E. coli* (7.5 mg/ml), *S. aureus* (3.75 mg/ml), and *K. pneumoniae* (0.94 mg/ml).

Othman et *al*.(2019) reported that the phytochemical compounds found in ginger are paradole, gingerol, zingiberine, zingiberol and bisabolene **,** while rosemary extracts contain carnosic acid and carnosol .These compounds have antibacterial and antifungal properties. Additionally, the strongest antibacterial and antifungal activities of rosemary extract attributed to the peculiar phenolic antioxidant. Finally, the results suggested that the antifungal ability of ethanol extracts from rosemary and ginger may be due to monoterpene, which disrupts fungal membrane integrity.

Table 8: The Minimum inhibitory concentration (MIC) of the best four studied plant extracts.

organism	***Rosmarinus officinalis*** MIC (mg/ml.)	***Zingiber officinale*** MIC (mg/ml.)	***Cinnamomum verum*** MIC (mg/ml.)	***Cinnamomum camphora*** MIC (mg/ml.)
E. coli	2.5	20	5	20
S .aureus	0.3	0.625	2.5	2.5
C. albicans	0.625	0.625	0.15	1.25

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دراسات فيتوكيميائية وبيولوجية على بعض النباتات الطبية

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أوضحت الدراسات البيولوجية علي مستخلص كحولي لثلاثه عشر نبات من النباتات الطبيه وهى (الروزماري *Rosmarinus officinalis*-الريحان *Ocimum basilicum* – المورينجا *Moringa oleifera*- الزنجبيل*Zingiber officinale*- الكركم *Curcuma longa*- حبة البركة *Nigella sativa*- القرفة *Cinnamomum verum* - المريمية*Salvia officinalis* - حب الرشاد*Lepidium sativum*- الشمر*Foeniculum vulgare*- الشبت*Anethum graveolens*- الكافور*Cinnamomum camphora*- التين البنغالي *Ficus benghalensis*) أن أربعة منهم وهم (الروزماري- الزنجبيل- القرفة- الكافور) هؤلاء النباتات الاربعة أوضحت افضل تأثير مضاد للميكروبات علي نوعين من البكتيريا (*E. Coli- S. aurues*) وفطر واحد وهو *C. albicans* كما تمت دراسة التأثيرالتأذري لهذه النباتات الاربعة علي الميكربات المستخدمة. هذه الدراسة تهدف الى تحديد المحتوي الكيميائي للمستخلص الكحولي لأفضل أربع نباتات تأثيرا علي الميكروبات.المكون الكيميائي الأساسي لمستخلص الروزماري كان (7.48%) eucalyptol ولمستخلص الزنجبيل gingerol (12.73%) ولمستخلص القرفة (25.55 %) (E)- cinnamaldehyde ولمستخلص الكافور 27 .35%) (وأيضا تمت دراسة التركيز المثبط الأدني لكل نبات .التركيز المثبط الأدني تراوح من 0.625إلى 2.5 mg/mlلميكروب *S. aureus* والتي تم التأثير عليها بالنباتات الأربعة. و كانت *C.albicans* أكثر ميكروب تأثرا بمستخلص القرفة وأقل ميكروب تأثرا بمستخلص الروزماري والكافوربتركيز مثبط أدني تراوح من 0.15 الى 1.25 mg/ml

يتبين من هذه الدراسه أن هذه النباتات الاربعه تحتوى على مركبات فعاله و لها تاثير بيولوجى على الميكروبات وعلى الخلايا السرطانيه المختلفه وعلى ذلك أنها تعتبرمن النباتات ذات الفائده الطبيه والصيدليه الواعده .

الكلمات المفتاحية: الروزماري- الزنجبيل- القرفة- الكافور- النشاط المضاد للميكروبات- التركيز المثبط الأدني- *Candida albicans-Staphylococcus aureus- Escherichia coli*