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 officinalis, 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 °C (3 min)- 280 °C (5 min) at 5 °C/min- 290 °C (1 min) at 7.5 °C/ min. Detector temperature 300 °C. Injector temperature 200 °C. 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°C 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°C, to produce a suspension containing about 10⁸ - 10⁹ 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 10⁸ - 10⁹ 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 10⁸ 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=Erythromycin (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 extracts were mixed with each other and with the antimicrobial agents T=Tetracyclines, E=Erythromycin 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 the presence of alkaloids, tannins and carbohydrates in *Zingiber officinale* extract, but disagree for the presence of 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, dioxime, genkwanine, hispiduline, Luteoline, Sinensetine. Di- and triterpenoids. Carnosolic acid, picrosalvine, rosmariquinone, oleanolic acid, ursolic acid (has anti-inflammation 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- | C10H18O           | 150             |
| 5   | 9.74 | 0.24 | Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endol,5-exo)- | C10H18O2         | 170             |
| 6   | 10.02| 11.4 | 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-   | C10H18O           | 138             |
| 10  | 10.94| 0.76 | Terpinen-4-ol                             | C10H18O           | 154             |
| 11  | 11.16| 0.35 | m-Cymen-8-ol                              | C10H18O           | 150             |
| 12  | 11.31| 2.22 | 3-CYCLOHEXENE-1-METHANOL, 3,4,4-TRIMETHYL- | C10H18O           | 154             |
| 13  | 11.47| 0.43 | BICYclo[3.1.1]HEPT-2-ENE-2-METHANOL, 6,6-DIMETHYL- | C10H18O           | 152             |
| 14  | 11.68| 0.69 | 1,7,7-TRIMETHYL-BICYclo[2.2.1]HEPTAN-2-OL  | C10H18O           | 150             |
| 15  | 11.80| 18.7 | Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl- | C10H18O           | 150             |
| 16  | 12.18| 0.27 | 3-(2-HYDROXYPHENYL)ACRYLIC ACID           | C10H18O2          | 120             |
| 17  | 12.54| 0.52 | BICYclo[4.1.0]HEPTAN-3-OL, 4,7,7-TRIMETHYL-, (1a,3a,4a,6a)- | C10H18O          | 154             |
| 18  | 12.61| 0.33 | Benzaldehyde, 4-(1-methylthyl)-            | 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-methylthyl)-         | C10H18O           | 150             |
| 22  | 14.64| 0.30 | 2-Methoxy-4-vinylphenol                   | C9H16O2           | 150             |
| 23  | 15.33| 0.42 | 2-Cyclohexen-1-one, 3-methyl-6-(1-methylthylidene)- | C10H18O           | 150             |
| 24  | 16.04| 0.50 | 2,3-DIMETHYL-1,4-THIAZANE SS-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             |
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-[S-(R*,S*)]- C15H24 204
1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-[S-(R*,S*)]- C15H26O 222
2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL- C15H24 204
(+)-α-FUNEBRENE C15H24 204
3-Hydroxymethylen-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one C11H16O2 180
Caryophyllene oxide C15H24O 220
1,3-BENZODIOXOLE, 4,5-DIMETHOXY-6-(2-PROPENYL)- C12H14O4 222
7-epi-cis-sesquisabinene hydrate C15H26O 222
Caryophylla-4(12),8(13)-dien-5α-ol C15H24O 220
2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- C11H14O3 194
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol C10H12O3 180
Tetradecanoic acid C14H28O2 228
4,4,8,Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol C15H26O2 238
1-Hexadecanol C16H34O 242
PENTADECANOIC ACID, 14-METHYL- , METHYL ESTER C17H34O2 270
n-Hexadecanoic acid C16H32O2 256
1-EICOSANOL C20H42O 298
8,11-Octadecadienoic acid, methyl ester C19H34O2 294
Oleic Acid C18H34O2 282
Octadecanoic acid C18H36O2 284
1-DOCOSANOL C22H46O 326
Morphinan, N-formyl-5,6-dihydroxy-3,4,6-trimethoxy- C20H25NO4 343
1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one C17H24O3 276
Villosin C20H28O2 300
Gingerol C17H26O4 294
Epibuphanisine C17H19NO3 285
Podocarpa-5,8,11,13-tetraen-7-one, 13-hydroxy-14-isopropyl- C20H26O2 298
9-ANTHRACENOL, 1,4,8-TRIMETHOXY- C17H16O4 284
á-Sitosterol C29H50O 414

Also there are active constituents present in minor percent; eucalyptol - Fig. 2, [8]
1,5,9-Trimethyltricyclo[6.3.1.0(1,5)]dodecane
C12H14O4 222
β-curcumene (14%, 0%) and phenolic compounds which are gingerol (25%, 23%) and epi-famesene (6.08 %), cubenol (0.30 %), α-acoreol (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-famesne (11%, 7%), β-sesquiphellandrene (9%, 13%), monoterpenic 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                              | C10H20O           | 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 | Aromandrene                                   | C15H24            | 204              |
| 14  | 19.05| 5.18 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-| C15H22            | 202              |
| 15  | 19.26| 0.22 | 5á,10á-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-ethyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4à)]- | C15H26O | 222 |
| 23  | 20.75| 1.77 | trans-Sesquisabinene hydrate                 | C15H26O           | 222              |
| 24  | 20.82| 0.54 | Aromandrene 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-Hydroxyfarnesene                           | C15H24O           | 220              |
| 34  | 23.90| 3.06 | Cyclohexanol, 3-ethyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1à,2à,3à,6à)]- | C15H26O | 222 |
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%) apiole (2.67 %). While some compounds record a minor quantities á-copaene, (0.24%), α-cadinol (0.58%), ylangenal (0.32 %) and curcumeneol (0.32 %). The present results were similar to those of Batigha 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                      |
| No. | retention time (min) | peak area (a.u.) | compound                                                                 | molecular formula | mass (amu) |
|-----|---------------------|-----------------|--------------------------------------------------------------------------|-------------------|------------|
| 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,4a,4á,7a,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            | á-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-(1a,4a,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            | Curcumeno                                                               | 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%). 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-Menthenatriene                                                       | 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-methylethyldiene)-                                | 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                        | C10H16O 2| 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-                             | C10H18O 2| 170   |
| 16. | 15.15 | 0.47 | 2(3H)-Benzofuranone, hexahydro-3-methylene-                                  | C9H12O2 | 152   |
| 17. | 15.25 | 0.45 | LIMONENE DIOXIDE 4                                                          | C10H16O 2| 168   |
| 18. | 15.92 | 1.46 | 5-Iodo-2,7-dioxatri cyclo[4.3.1.0(3,8)]decane                               | C8H11IO 2| 266   |
| 19. | 16.31 | 0.89 | 7-Oxo-2-oxa-7-thiatricyclo[4.4.0.0(3,8)]decan-4-ol                           | C8H12O3 S| 188   |
| 20. | 17.34 | 1.08 | 2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, trans-            | C10H16O 2| 168   |
|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
| 21 | 17.91 | 1.30 | 7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)- | C10H16O2 | 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 | C11H20O3 | 200 |
| 25 | 20.62 | 0.66 | 2-Cyclohexen-1-one, 3-(hydroxymethyl)-6-(1-methylethyl)- | C10H16O2 | 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-cycloprop[a]azulene-4,7-diol | C15H26O2 | 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 | C19H30O2 | 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-hexahydonaphthalen-1(8aH)-one | C15H22O2 | 234 |
| 34 | 29.66 | 3.95 | n-Hexadecanoic acid | C16H32O2 | 256 |
| 35 | 32.44 | 1.14 | Phytol | C20H40O | 296 |
| 36 | 32.93 | 1.78 | 9,12-Octadecadienoyl chloride, (Z,Z)- | C18H31ClO | 298 |
| 37 | 33.36 | 0.95 | Octadecanoic acid | C18H36O2 | 284 |
| 38 | 33.43 | 0.56 | Ethyl Oleate | C20H38O2 | 310 |
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 28 mm. 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 51 mm. for *E. coli* and *S. aureus*, respectively. Where *Tetracycline* showed clear zone diameters 42 and 52 mm. for *E. coli* and *S. aureus*, respectively. Also, synergistic between *Rosmarinus officinalis* and *Erythromycin* obtained clear zone diameter 30 and 45 mm. 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* and *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, 37 and 27 mm. for *E. coli*. Respectively and 30, 51 and 44 mm. for *S. aureus*, respectively. Also, synergistic between *Cinnamomum camphora* with *Tetracycline* and *Erythromycin* showed clear zone diameters 41 and 21 mm. respectively for *E. coli* and 51 and 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 *Pseudomonas 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 *Pasteurella 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 21 mm.

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. Presl was performed in terms of percentage of radial growth on solid medium (potatoes dextrose agar PDA) against Aspergillus Niger, Scolerotium, 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 most common agents causing topical mycoses 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 rubrum 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 S  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 monoterpenes, which disrupts fungal membrane integrity.
Table 8: The Minimum inhibitory concentration (MIC) of the best four studied plant extracts.

| organism     | Rosmarinus officinalis | Zingiber officinale | Cinnamomum verum | Cinnamomum camphora |
|--------------|------------------------|---------------------|------------------|---------------------|
|              | MIC (mg/ml.)           | MIC (mg/ml.)        | MIC (mg/ml.)     | 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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أُوضحت الدراسات البيولوجية على مستخلص كحولٌي ثلاثنيات عشر نباتًا منها النباتات الطبية وهي:

- Moringa oleifera - Ocimum basilicum - Rosmarinus officinalis
- Nigella sativa - Curcuma longa - Zingiber officinalis
- Lepidium sativum - Salvia officinalis - Cinnamomum verum - Anethum graveolens - Foeniculum vulgare - Ficus benghalensis

وتعد هذه الدراسة الأولى في تحديد التأثيرات الفردية والبرمجية للنباتات المستخدمة. هذه الدراسة

تهدف إلى تحديد المحتوى الكيميائي المستخلص الكحولي لأفضل أربع نباتات تأثيرًا على الميكروبات. المكون الكيميائي الأساسي لمستخلص الروزماري كان (7.48%) eucalyptol (12.73%) gingerol (27.35%) cinnamon aldehyde (25.55%)

(وأيضاً تم تعيين تركيز المثبت الأولي لكل نبات التركيز المثبت الأولي تراوح من 0.625 إلى 2.5 mg/ml)

والميكروبات المثبتية كانت C. albicans S. aureus E. coli. و تأثير تلك النباتات على الميكروبات تأثيرًا مستخلص القرة وأقل ميكروب تأثيرًا مستخلص الكافَرس وتركيز المثبت الأولي تراوح من 0.15 إلى 1.25 mg/ml.

يينتين من هذه الدراسة أن هذه النباتات الأربعة تحتوي على مركبات فعالة ولها تأثير بيولوجي على الميكروبات وعلى الخلايا السرطانية المختلفة وعلى ذلك أنها تعتبر من النباتات ذات القائمة الطبية والصيدلي.

الكلمات المفتاحية: الروزماري- الزنجبيل- الفركه- الكافرس- الكافرس- التركيز المثبت الأولي-

Candida albicans-Staphylococcus aureus- Escherichia coli