THE ANTIMICROBIAL ACTIVITIES OF SOME PLANTS EXTRACTS AGAINST CANDIDA ALBICANS SSP. AND STAPHYLOCOCCUS AUREUS SSP.

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Abstract

This study was conducted to determine the effect of hot and cold water, Ethanol, Methanol, Acetone, Petroleum Ether and Hexane extracts of Allium Sativum, ZingiberOfficinale and Nigella sativa against Staphylococcus aureussp. and Candida albicans sp. after 24 or 48 hrs of incubation where the inhibition zones on Nutrient Agar and Potato Dextrose agar appeared. The results showed that the hot water extract was not effective at all compared other extracts. Also, results indicated that both Candida albicans sp. was more sensitive toward all plants extracts except water extracts compared with Staphylococcus aureussp. which was higher resistance toward almost extracts.

Introduction:

Many microbial diseases are controlled by antibiotics. However, continuous use of antibiotics leads to drug resistance and thereby to a reduced efficacy of the drugs. Antibiotics accumulate in the environment, and pose a potential risk to consumers and to the environment (¹). Therefore, there has been an increasing awareness about the importance of medicinal plants. Drugs from these plants are inexpensive, safe, efficient, and rarely accompanied by side effects (²).

Plant derived antimicrobials are, among others, in the focus of research because of their easy accessibility and wide antimicrobial spectrum. Usually several ingredients with different target sites are responsible for the antimicrobial properties which decreases the possibility of development of microbial resistance (³). Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintic, antibacterial and insecticides (³,⁴).

In this study, the antifungal activities of Allium Sativum, ZingiberOfficinale and Nigella sativa were investigated against Candida albicans sp. and Staphylococcus aureussp.
Material and Methods:-
Selected Pathogenic microorganism:-
Candida albicans ssp. was isolated from clinical oral sample which identified in the microbiological lab, University College of Umluj, and Staphylococcus aureus ssp. was isolated from clinical skin sample which identifies in the microbiological lab, Al-Hawraa General Hospital in Umluj. These two isolates have been used in this study. Candida albicans ssp. was grown on Sabouraud Dextrose Agar (P.D.A.) plates for 48 hours and Staphylococcus aureus ssp. was grown on Nutrient Agar (N.A.) plates for 24 hours.

Antibiotics Drugs:-
Nine drugs were evaluated for their activities against Candida albicans ssp. and Staphylococcus aureus ssp. (Table 1).

Table 1: Antibiotics drugs names, codes, weight and manufactured company.

| Drug Name         | Drug Code | Weight | Manufactured By                      |
|-------------------|-----------|--------|--------------------------------------|
| Penicillin        | PG        | 10µg   | Mast Group Ltd, Merseyside, UK       |
| Erythromycin      | E         | 15µg   |                                      |
| Ampicillin        | AP        | 10µg   |                                      |
| Cephalothin       | KF        | 30µg   |                                      |
| Clindamycin       | CD        | 2µg    |                                      |
| Augmentin         | AUG       | 30µg   |                                      |
| Cotrimoxazole     | TS        | 25µg   |                                      |
| Gentamicin        | GM        | 10µg   |                                      |
| Cefoxitin         | FOX       | 30µg   |                                      |

Plant extracts preparation:-
Garlic cloves (Allium Sativum), Ginger rhizomes (Zingiber Officinale) and Black seeds (Nigella sativa) were used in this study. The fresh plant materials were collected and washed with distilled water. Then approximately 20 grams of fresh plant materials were separately crashed.

After that, they have been extracted with 150 mL of (hot water, cold water, ethanol, methanol, hexane, petroleum ether and acetone respectively). Extracts were filtered through Whatman No. 1 filter paper under vacuum and concentrated to dryness at 37. Then, 100 mg of the dry residue was dissolved in 1 mL of sterile distilled water.

Antimicrobial Tests:-
For antimicrobial tests, desk diffusion method was used to test the antimicrobial activities of nine drugs against Candida albicans ssp. and Staphylococcus aureus ssp. Then, antimicrobial activities of plants extracts were determined on Candida albicans ssp. and Staphylococcus aureus ssp. The extracts were dissolved in dimethyl sulfoxide (DMSO, 15 µL). Sterile filter paper discs (5 mm in diameter) were placed onto P.D.A. for Candida albicans ssp. and N. A. for Staphylococcus aureus ssp. The Petri dishes were incubated at 28 °C for 48 hours for Candida albicans ssp. and at 37 °C for 24 hours for Staphylococcus aureus ssp. Inhibition zones around filter papers were measured. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

Result and Discussion:-
Antibiotics Drugs Test:-
Nine of the represented drugs were tested against Candida albicans ssp. and Staphylococcus aureus ssp. (Table 2). The results of Candida albicans ssp. in Fig. 1 showed that widest range of inhibition zone was with 25 mm for AUG. Then followed by KF and FOX with inhibition zones 24 and 23 mm respectively. On the other hand, PG, E, AP, CD and TS have no effects on Candida albicans ssp. Therefore, AUG had been used as a positive control in plants extracts antimicrobial activities.

For Staphylococcus aureus ssp., all tested antibiotics drugs have different effects on it. The width inhibition zone was with 35 mm for KF, which followed by both of CD and E with equal inhibition zone of 34 mm. Hence, KF had been used as a positive control in plants extracts antimicrobial activities.
Table 2: Diameter of inhibition zone (mm) from Antibiotics drugs on Candida albicans ssp. and Staphylococcus aureus ssp.

| Zone of inhibition (mm) for candida albicans ssp. | Drug Code | Zone of inhibition (mm) for Staphylococcus aureus ssp. |
|-----------------------------------------------|----------|-----------------------------------------------------|
| 0 Cont.                                      |          | 0                                                   |
| 0 PG                                         | 14       |                                                     |
| 0 E                                          | 34       |                                                     |
| 0 AP                                         | 25       |                                                     |
| 24 KF                                        | 35       |                                                     |
| 0 CD                                         | 34       |                                                     |
| 25 AUG                                       | 28       |                                                     |
| 0 TS                                         | 31       |                                                     |
| 8 GM                                         | 17       |                                                     |
| 23 FOX                                       | 19       |                                                     |

Antimicrobial of Plants Extracts Tests:

Medicinal plants are commonly available resources, have less if no side effects, economic and have antimicrobial properties. The majority of these plants used in this study are applied in traditional medicine. Recent studies have revealed that medicinal plants from various parts of the world can provide a rich source of antibacterial activities.

The antifungal activities of the plant extracts in (Table 3) indicated the variable antifungal activities of it against Candida albicans ssp. The diameter of inhibition zone for AUG had been used in this test as a positive control to compare between antifungal activity of antibiotic drug and plants extracts. The results in Fig. 1 showed that the extract of Nigella sativa showed the highest activity against Candida albicans ssp. The extract of Allium Sativum by Acetone had the width inhibition zone with 43 mm. and the extract of ZingiberOfficinale by Petroleum Ether had the width inhibition zone with 33 mm. All tested plants extracted by hot or cold water did not show any antifungal activity against Candida albicans ssp. the results of Candida albicans ssp. sensitivity were in agreement with who mentioned that Allium sativum, Zingiberofficinale and Nigella sativa gave the highest percentage of suppression of biomass for it.

Table 3: Diameter of inhibition zone (mm) from seven extracts of Allium Sativum, ZingiberOfficinale and Nigella sativa on Candida albicans ssp.

| Plant Name          | Positive Cont. | Extracted By | Diameter of inhibition zone (mm) |
|---------------------|----------------|--------------|---------------------------------|
|                     |                | H.W. C.W. A E H M PE |                               |
| Allium Sativum      | 25             | 0 0 43 20 29 6 38 |                               |
| ZingiberOfficinale   | 25             | 0 0 30 28 13 25 33 |                               |
| Nigella sativa      | 25             | 0 0 49 28 40 25 48 |                               |
For Staphylococcus aureus ssp., the three plants which have been extracted by seven ways, had been tested for their antimicrobial activities by zones of inhibition ranged from 0-20 mm (Table 4). All the three plants extracts by hot and cold water had no antimicrobial activities against Staphylococcus aureus ssp. (Fig. 2). As a matter of fact, almost five of each plant extracts had no antimicrobial activities against Staphylococcus aureus ssp. Only Acetone and Ethanol extracts of Allium Sativum had antimicrobial activities against Staphylococcus aureus ssp. with 12 and 20 mm respectively while Acetone and Petroleum Ether extracts of Zingiber Officinale had approximately size of inhibition zone with 11 and 10 mm respectively. With close results, Nigella sativa Hexane extract had antimicrobial activity with 13 mm and Methanol extract had a close result by inhibition zones 12 mm. The results showed that it was less effective against Staphylococcus aureus ssp. and were agreed with (16-17).

**Table 4:** Diameter of inhibition zone (mm) from seven extracts of Allium Sativum, Zingiber Officinale and Nigella sativa on Staphylococcus aureus ssp.

| Plant Name       | Positive Cont. | Extracted By Diameter of inhibition zone (m) |
|------------------|----------------|---------------------------------------------|
|                  |                | H.W. | C.W. | A  | E  | H  | M  | PE |
| Allium Sativum   | 35             | 0    | 0    | 12 | 20 | 0  | 0  | 0  |
| Zingiber Officinale | 35           | 0    | 0    | 0  | 11 | 0  | 0  | 10 |
| Nigella sativa   | 35             | 0    | 0    | 0  | 0  | 13 | 12 | 0  |

**Figure 1:** Diameter of inhibition zone (mm) from seven extracts of Allium Sativum, Zingiber Officinale and Nigella sativa on Candida albicans ssp.

**Figure 2:** Diameter of inhibition zone (mm) from seven extracts of Allium Sativum, Zingiber Officinale and Nigella sativa on Staphylococcus aureus ssp.
Studies should investigate the synergistic capacity of plant extracts or other natural products, independent of the antimicrobial activity they have. Therefore, the results of the present study seem to be promising and may enhance the natural products uses, showing the potential of these plants in the treatment of infectious diseases caused by Candida albicans spp. and Staphylococcus aureus spp.

**Conclusion:-**
This study results suggest that Allium sativum, Zingiber officinale and Nigella sativa extracts should be analyzed further, as it might provide a new compound effective against pathogenic microbes specially Candida albicans spp. Such simple and inexpensive alternatives to conventional treatment of bacterial infections may be worthy of further rigorous investigation. Future studies on the chemical characteristics of extracts and active components should be carried out for each plant and antimicrobial property.

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