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Antimicrobial and Anti diabetes potential of some plant extracts

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***Abstract*— This work aimed to detect the therapeutic potential of garlic *(Allium sativum)*, olive (*Olea europaea*) and Ginger (*Zingiber officinale roscoe*) plants. There are differences among them in susceptibility. The effect of garlic has been tested on some microorganism. Bacteria such as gram positive *Staphylococcus aureus*, gram negative *Escherichia coli*, yeast like *Candida albicans* and *Aspergillus niger*,. *Aspergillus flavus* uses as samples. The garlic extract makes a value of inhibition in both concentration 50 and 100% (6 mm) and (12 mm), (5 mm) and (10 mm) on *Staphylococcus aureus* and *E. coli* respectively. Also *C.albicans* gave (25 mm) and (29 mm) for the same concentration. (*Aspergillus niger, Aspergillus flavus*) show a high inhibitory action appears as the concentration of garlic extract increased. The olive and was employed as an anti diabetes and antibacterial agent. It gives the response against diabetes when used on mice, and antibacterial on different typed of germs used in this study.**

***Index Terms*— Olive, Ginger, Garlic,Antimicrobial, anti-diabetics**

I. INTRODUCTION

Allium sativum, commonly known as garlic is a species in the onion family Alliaceae and belongs to the plant order liliales [1]. Other members of the garlic family include *Allium cepa* (onion), *Allium ascalanicum* (shallot) and Allium porrum (Heeks). Of all the Allium species, garlic is the most important [2]. Garlic is commonly called 'Tantanwa' in Hausa. Garlic (*Allium sativum*) has been used medicinally since before the time of the Sumerian civilization (2600–2100 BC), by when it was already widely cultivated in India and China [3]. Therapeutic applications of garlic have been known for many ages. The plants are broadly used as antibiotics and are effective against diabetes, atherosclerosis and cancer [4]. This plant is also known to reduce blood plasma cholesterol and blood pressure. It also inhibits platelet mass formation [5] had revealed that garlic stimulates the activity of the defensive cells of the body such as the lymphocytes and macrophages. These blood cells protect us from pathogens. They are also able to destroy cancerous cells in the initial stage of cancer formation. Garlic is currently used with some degree of success, as a complement in the treatment of AIDS [6].

The olive tree date back to Biblical and Roman times and to Greek mythology. Historically, the products of *Olea europaea* have been used as aphrodisiacs, emollients, laxatives, nutritive, sedatives, and tonics. Specific conditions traditionally treated include colic, alopecia, paralysis, rheumatic pain, sciatica, and hypertension [7]. In the latter part of the 20th century, Keys et al conducted the seven countries study, which revealed the Mediterranean diet is linked to a reduced incidence of degenerative diseases, particularly coronary heart disease (CHD) and cancers of the breast, skin, and colon [8] [9]. This study inspired much research into the Mediterranean diet. In addition to olive oil, the Mediterranean diet is rich in healthful fiber, fish, fruits, and vegetables [9]. Since olive oil is the major energy source in the Mediterranean diet, recent research has focused on the contribution it makes to reported health benefits of the diet. Compared to diets of other countries, the Mediterranean diet has a relatively high fat content; however, as the diet is associated with low incidence of cancer and CHD, despite the high fat intake, it has been suggested the type of fat is more important than the total amount consumed [9]. To manufacture olive oil, olives are crushed to create a pomace, which is then homogenized before being pressed to produce oil. The first oil extracted is the highest quality extra virgin olive oil – produced using centrifugation and water only. The pomace can then be processed again to yield; the lower quality refined virgin olive oil. Further extraction with organic solvents can be undertaken to produce low quality refined husk oil [10].

Ginger (*Zingiberofficinale roscoe*, *Zingiberaceae*) was widely used around the world in foods as a spice. Native to tropical Asia, ginger was a perennial cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States. Ginger is a herb which contains a chemical component named Zingiberen. It has anti-inflammatory, analgesic, antipyretic, antimicrobial, hypoglycemic, anti-migraine, anti-schistosomal, anti-motion sickness, anti-oxidant, hepato-protective and anti-thermic properties[11].Ginger rhizome has a long history of use in Chinese and Ayurveda medicine as an antiemetic, antipyretic, and anti-inflammatory agent [12].Ginger was primarily used to treat nausea, but it was also as an anti-inflammatory, a pain remedy and a cholesterol-lowering herb. Because of its effect on platelet aggregation, some herbalists suggest caution for patients taking anticoagulant; on the other hand, no clinically significant anticoagulant effects have been documented [13]. Ginger plant was generally 1-3 ft. in height and having different chemical constituents like an aldehyde, gingerol, shogaol, and paradol etc. it has some tremendous beneficial effect to human body to cure various types of diseases. Ginger consists of fresh or dried roots of Zingiberofficinale. The ginger family consisting of more 1200 plant species in 53 genera. The genus Zingiber includes about 85 species of aromatic herbs from East Asia and tropical Australia [14].

II. MATERIALS AND METHODS.

Preparation of garlic extract.

Fresh garlic (*Allium sativum* L.) bulbs were purchased from local markets. The bulbs were peeled, weighed (100 gm) and cleaned. Cleaned cloves were surface-sterilized by immersing them into 70% (v/v) ethanol for 60s [15]. Residual ethanol on

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the surface was evaporated in the sterile laminar airflow chamber followed by homogenizing aseptically in sterile mortar and pestle. The homogenized mixture was filtered through sterile cheesecloth. This extract was considered as the 100% concentration of the extract. The concentrated mother extract was further diluted to 50% and 75% by mixing with appropriate sterile distill water [16].

Preparation of alcohol extract of olive oil for antimicrobial activity

The alcohol extract was done via soxhlet. 300 g of olive was used in three rounds, 100 g for each round. A quantity of 100 g of olive was put in a filter paper and placed in a cold organic solvent (hexane) for 16 hours. Then the sample was placed in the soxhlet extraction tube and the solvent was placed in the flask placed on a heat source 50-60 C, while the upper part of the soxhlet is a condenser tube. After 30-60 minutes for one round the solvent and the sample were collected and separated by an evaporator then the oil extract is collected [17].

Preparation of garlic extract

Fresh roots of ginger were obtained from the local market. The roots then grinned. The suspension is made by mixing 25g of powder of ginger with 250ml of distill water in a vial, then put the vial in deep freezer under -50 C^o overnight, then put the vial in lyophilizer apparatus for to get a dry material. Then 2g of powder are dissolving it in 10ml of distill water.

Sterilization of extracts.

Plant extracts were further sterilized using a Millipore filter in a laminar air flow cabinet. Garlic extracts, then used for detection the antibacterial and antifungal activity, while olive and ginger extracts used for detecting its antibacterial activity and its activity in the treatment of type II Diabetes in mice.

Antibiotic disc test.

Disc diffusion method was used to test the antibiotic sensitivity of the selected isolates.Results were compared with National community laboratory standard, add new publishers of NCCLS 2002[18]. A sterile cotton swab was dipped into fresh culture and the entire surface of the Muller-Hinton agar plates were swabbed three times by rotating the plate approximately 60C^o between streaking, then incubated at 37C^o and the inhibition zone was observed overnight. We are using three broad spectrum antibiotic discs (Chloramphenicol 10µg, Amoxicillin 25µg, gentamycin 10µg).

Preparation of media for bacterial culture.

Muller Hinton agar medium was prepared by Suspension 38g of Muller Hinton agar medium in 1000ml sterilized D.W, heat till boiling to dissolve the medium completely. Sterilized by autoclaving at 15Ibs pressure and 121^{o}C for 15min, mix well before pouring. Then the media were poured into a disposable Petri dishes in a depth of 3 to 4mm, the final pH the medium was adjusted to 6.8 by using a pH - meter. After solidification, all prepared plates containing medium were kept at 4^{o}C till use. To provide a firm surface for wells, making which were filled with 25microletterof garlic extract at 50, 75 and 100% concentration

Measurement of bacterial inoculum concentration

Bacterial isolates were supplemented from bacterial isolates bank in Microbiology Lab., Postgraduate laboratories, College of Applied Science, Al-Nahrain university, at which single colonies from cultures grown on nutrient agar for 18-24 hrs were transferred to test tubes containing 5ml of normal saline and mixed well by vortex, then bacterial growth was compared with McFarland tube No.0.5 turbidity standard solution, which was equivalent to a bacterial inoculums concentration of $1.5*10^8$ cell/ml

Determination of inhibition zones of treatments

By using a cotton swab, a touch of bacterial culture from normal saline was transferred to Muller Hinton agar medium and streaked three times by rotating the plate approximately 60^o between streaking. To ensure even distribution of the inoculums, the inoculated plates was placed at room temperature for 10 min to allow absorption of excess. Then, using sterilized pauster pipette for making wells (the wells were arranged so as to avoid the development of overlapping of inhibition zones) which were then filled with 25µl of 50, 75 and 100% concentration of garlic extract. The plates were incubated at 37^{o}C for 18-24 hrs. After incubation, inhibition zones were measured using a ruler for determination their diameters in millimeters. Inhibition zones were measured by ruler also and the results were compared with the standards as in NCCLs, 2007 [19].

Antifungi test for yeast

For *Candida albicans* the test is similar to the bacterial test. The yeast was cultured on PDA at 28C^o for 72 hrs and 0.5 McFarland of yeast suspension was prepared. *Candida albicans* are cultured by sterile cotton swab by dipping it into yeast suspension and the entire surface of PDAplates that swabbed three times by rotating the plate approximately 60C^o between streaking. The plates were incubated at 28C^o overnight and the inhibition zone were measured.

Preparation of media for fungi culture.

Potato dextrose agar (PDA) medium was prepared by Suspension 39 g of Potato dextrose agar medium in 1000 ml sterilized D.W, heat till boiling to dissolve the medium completely. Sterilized by autoclaving at 15Ibs pressure and 121C^o for 15min, mix well before pouring. Then the media were poured into a disposable Petri dishes in a depth of 3 to 4mm,. After solidification, all prepared plates containing medium were kept at 4C^o till use.

Preparation of fungal inoculum

Aspergillus niger and *Aspergillus flavus* are cultured in petri dishes contains PDA media for 7 day's temperature 28C^o. Then the spores were harvested by adding 5ml of sterile DW on the plate. The spore suspension was then centrifuged at 3000 RPM for 5 min. The supernatant was removed and the spores were washed twice by re-suspending in sterile DW and further centrifuged. Then 1ml of sterile DW was added to the supernatant and mixed vigorously. Spores were counted according to Sambrook and Russel, (2001) by hemocytometer. The hemocytometer was set up by placing it flat on the surface with the glass coverslip on top of the grids. The spore suspension was vortex for 5 Sec, 10 microliter of the suspension was delivered to the divot on the hemocytometer. The hemocytometer was placed on the microscope and the spores were to settle for about 30 Sec. The total number of spores was counted in each of the five squares. The following equation was used to estimate the number of spores per ml in original suspension

Spores/ml= (average No.of spores/5) (25) (10^4) (dilution factor).

III. Experimental animals

Albino mice (22-29g) age of 8 weeks were obtained from the Department of medicines and medical equipment.

Experimentally induce diabetes in mice

Seven albino mice obtained with (20-29g) and treated with Alloxan 100 mg/kg of body weight was used to induce diabetes. Mice were injected intraperitoneally. The dose was prepared by dissolving 0.2ml alloxan in 0.9 NaCl solutions. A solution of 5% glucose was administered orally to combat could occur. Blood glucose was observed 20 hrs after alloxanistion [20]

IV. Results and Discussions

1- Antibctirail and antifungal activity of garlic.

The standard disk diffusion method was used to determine the spread of the *E. coli* and *S.aureus* to different antibiotics, results in table 1.

Table (1): Antibiotic disk spread of *E.coli* and S.*aureus*

Antibiotic	Concentration (µg)	*E.coli*	*S.aureus*
Amoxicillin	25	R	R
Gentamycin	10	18 mm turbid	12 mm
Chloramphenicol	10	18 mm	24 mm

R= resistant

Both species show resistance to Amoxicillin. *S.aurues* are sensitive to Gentamycin. The inhibition zone was 12mm and *E.coli* give a zone 18mm but with turbidity and not clear zone. Both species show sensitivity to Chloramphenicol *E.coli* zone was 24mm and *S.aureus* 18mm. So the suitable antibiotics work as a control is Chloramphenicol and that's what it was used in further tests. Chloramphenicol solubility 33.3 mg/ml of ethanol [21]. The stock solution of an antibiotic prepared by adding 333 mg of Chloramphenicol in 10 ml of 95% ethanol and then the stock was diluted to 50 and 75% by ratio v/v (1-1) and (3-1). Ethanol is a common antiseptic compound so we made a well on the plate that contain 95% of ethanol to exclude the antiseptic activity of it with the antibiotic. So it was used with the concentration of 50, 75 and 100% of stock antibiotic and 50,75 and 100% of garlic extract to taste. The results listed in table 2.

Table 2. The effect of Garlic extract on *E. coli* with compare to Chloramphenicol as a control

Concentration %	(A)	(C)	C-A	G
50	18 mm	36 mm	36-18=18	5 mm
75	18 mm	38 mm	38-18=20	8 mm
100	18 mm	45 mm	45-18=27	10 mm

A= Alcohol inhibition zone, C=Chloramphenicol inhibition zone

C-A: Inhibition zone of alcohol excluded ,G: Garlic extract

Table 3. The effect of Garlic extract on *S. aureus* with compare to Chloramphenicol as a control

Con.	(A)	(C)	C-A	G
50%	25 mm	43 mm	43-25=18	6 mm
75%	25 mm	46 mm	46-25=21	9 mm
100%	25 mm	50 mm	50-25=25	12 mm

A. Alcohol inhibition zone, C. Chloramphenicol inhibition zone

C-A. Inhibition zone of alcohol excluded,G: Garlic extract

Also garlic contains PDA media was inoculated with *C.albecans* suspension 0.5 McFerland by cotton swab in 3 directions by rotating the plate 60C^{o} and then 3 wells are made with a Pasteur pipte and add 50µl of each concentration of garlic extract. The plate incubated at 28C^{o} for 24hour. Inhibition zone, then measured by a ruler. Show Table (4) and the followed picture

Table 4. The effect of Garlic extract on *C. albicans*

Concentration of garlic extract %	*C.albicans* zome
25	25 mm
75	27 mm
100	29 mm

As mentioned before, plates contain PDA media was used. Extract 100µl put on the plates and spread it using L shape spreader and let it absorb. The plates inoculated by the spore suspended in the center of the plates and incubated at 28C^{o} for 48 hrs and the radial extension measured in the plates by ruler. The result mention in table (5).

Table 5. The effect of Garlic extract on *A. niger* . and *A. flavus*

Concentration of garlic extract %	Zone of growth *A. niger*	The growth Zone of *A. flavus*
50	Non groth	7 mm
75	Non groth	4 mm
100	Non groth	2 mm
Control	13 mm	12 mm

A.niger shows high sensitivity to garlic extract than *A. flavus*. It is preventing spore germination in all concentrations of the garlic.

The garlic inhibition activity is very clear to define the highest concentration of garlic extract produce high inhibitory action against *A.flavus*.

B. Antibacterial and anti diabetic activity of olive and ginger extracts.

Results display in table (6) indicates that both stock and diluted alcohol extracts has no activity on *klebsiella spp.* and *Pseudomonasspp*. and the diluted alcohol extract has a slight activity on *E .coli* and *S. aureus*.

Table 6. Diameter of inhibition zone caused by the olive alcohol extract against *E.coli*, *Klebsiella spp.*, *S. aureus*and *Pseudomonas* spp.

Type of Sample	Diameter of inhibition zone (CM)			
	E.coli	*Klebsiella spp*	*S.aureus*	*Pseudomonas spp*
Stock	00	00	00	00
Diluted	1	00	1.7	00

The no effect of the alcohol extract on some of microorganism may be because of the method of extraction. At the same time it has been found that some dilutions gave an effect on *E. coli* and *S.aureus,* but the stock did not do. We have no explanation and it needs further study.

Result display in table (7) indicates that both stock and diluted crush extracts had been working on each of *E.coli*, *Klebsiella spp.*, *S.aureus* and *Pseudomonas spp.*

Table 7. Diameter of inhibition zone caused by the olive crush extract against *E.coli*, *Klebsiella spp.*,*S.aureus* and *Pseudomonas spp.*

Type of Sample	Diameter of inhibition zone (mm)			
	E.coli	*Klebsiella spp*	*S.aureus*	*Pseudomonas*
Stock	16	13	18	18
Diluted	10	12	11	15

The effects are very clear on all types of the organisms under study. There are big differences between the use of oil produced by alcohol extract as anti-bacterial and the crushed extract. That may be because the second one contains some active compounds which are doing the job. If we compare the stock with the diluted we found that the stock containing more active compound than the dilute. On the other hand the method of extraction plays the main action in the results of the treatment.

Table 8. The level of glucose in blood before and after injection alloxain and after treating with olive extracts

Mice No.	g\ld before Alloxan	g/ld after Alloxan	g/ld after olive treated	The reducing value extracts
1	113	280	126	154
2	97	315	177	138
3	110	220	130	90
4	108	246	95	151
5	121	297	120	177
6	111	350	115	235
7	90	357	118	239

From table (8) show that the animal used in this experiment has a normal level of blood glucose. After treating with alloxan, the blood level of glucose has at the treated mice. So of mice had been sick with diabetes . From same table can clearly see that the level of glucose in the blood of treated mice has been dropping down to be in the normal level after treated with olive extracts .

C. Genger affect

The results listed in the table (9) show that the effects of active compounds on *Klebseilla spp*. And *Pseudomonas spp.* The inhibitory action looks like more than on *Klebseilla spp*. Than *Pseudomonas spp..*, with different dilution but the stock solution in both cases, gave, the more inhibition than the other concentration. That may due to the concentration of the active compounds present. The other results show no effects on the other microorganisms, that may because of the specify of the active compounds present in the extract .

Table 9. The inhibition effect of active compound of ginger on *Klebseilla spp, Pseoudomonas spp , Staphylococcus aureasa and E. coli.*

No	Treatment	Diameter of inhibition zone in mm *Klebseilla spp*	Diameter of inhibition zone in mm *Pseoudomonas spp*	Diameter of inhibition zone in mm *Staphylococcus aureasa*	Diameter of inhibition zone in mm *E. coli.*
1	Stock	21mm	20 mm	On action	On action
2	Dilution 10^{-1}	19 mm	16 mm	On action	On action
3	Dilution 10^{-2}	15 mm	10 mm	On action	On action
4	Dilution 10^{-3}	15 mm	12 mm	On action	On action
5	Dilution 10^{-4}	14 mm	10 mm	On action	On action

V. Conclusion

It has found that the extract of garlic, olive and ginger have different effect on some microorganism as anti germs and also after the used olive as anti diabetes, it gives high response. Also it is found that the method of extraction in the case of olive has an effect.

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