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Effect of Aqueous and Alcoholic Plant Extracts on Inhibition of Some Types of Microbes and Causing Spoilage of Food

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Abstract

The current study included the preparation of the aqueous extract and alcohol leaves Ziziphus (*Ziziphus spina-christi*), Eucalyptus plant (*Eucalyptus camaldulensis*) and assessed the antimicrobial its against reference strains of bacteria and mold, through chemical compound detection leaves Ziziphus plant results showed contain the aqueous extract and alcohol at all effective compounds that have been detected except composite resins in aqueous extract and resins in the alcoholic extract. As the leaves of Eucalyptus plant have aqueous extract and alcohol contained resins, tannins, phenols. It tested the effectiveness of inhibitory extract plant against five isolates bacterial and five isolates of molds. It uses the concentration of 50 and 100 mg/ml of each extract, Similarly maximum zone of inhibition through ethanol extract was obtained against both bacterial and molds isolates as well as increased efficiency with increasing of concentration.

Keyword: Ziziphus; Eucalyptus plant extracts; Inhibition

Introduction

Plants are the fundamental to existence on globe as they directly or indirectly resource around 70-80% of human energy and protein consumption, the rest being resulting from visceral products. They are sparingly significant to man due to their numerous applications, such as antibiotics, analgesic, flavors, perfumes, insecticides, dyes, food additives, poisons etc. [1].

Medicinal plants are gifts of nature to cure a number of diseases among human beings. A large number of plants in different location around the world have been extracted, semi-purified to investigate individually their antimicrobial activity. However, very little information is available on such activity of medicinal plants and out of the 400,000 plant species on earth, only a small amount has been systematically investigated for their antimicrobial activities [2]. Their extracts have gained importance as potential antibacterial agents. Secondary metabolites of plants, including the tannins, flavonoids and alkaloids have been found to possess antimicrobial properties *in vitro* [3].

The Ziziphus and Eucalyptus plants that have received wide attention in the field of folk medicine since the Ziziphus goes back to the plant family Cedria Rhamanaceae and spreads widely in areas with moderate temperatures and dry land areas Hemisphere warm climates, including Iraq [4]. Eucalyptus is one of such medicinal plants belonging to Myrtaceae family, native of Australia. It's spread in many countries, including Iraq. The present study aimed to know the chemical components in plant extracts as well as evaluating the antimicrobial activity of plant extracts including Ziziphus and Eucalyptus against some pathogenic bacteria and molds.

Materials and Methods

Sample collection

The Fresh leaves of the Plant (Ziziphus and Eucalyptus leaves) were collected from Altnoma and Abu Kasib in Basrah province of Iraq-Basra at January-November 2014 and placed in polyethylene bags and transported to the Biotechnology laboratory of the Food Science department /Faculty of Agriculture.

Plants grind

Ziziphus and Eucalyptus leaves were carefully washed using tap water to remove the dusts and then dried in an oven at 60°C for 8 h. The dried leaves were milled separately in a small electric mill (High-Speed Grinder, China), The powdered leaves of these plants were transferred to a glass sealed cans and placed in the refrigerator before the extraction process.

Extract preparation

The aqueous extract of dried plant leaves was made in the distilled water. About 5 grams of each plant leaves powder (Ziziphus and Eucalyptus) were taken and mixed in 50 ml of distilled water. The mixture was taken into 250 ml sterile conical flasks, plugged with sterile cotton and kept in Shaking Incubator (Kottermann, Germany) with the 200 rpm for 24 h. The solution was filtered through muslin cloth, This process was repeated three times after which a clear aqueous extract of the plant was taken.

Hot water extract: 10 g of the weighed plant leaves powder was soaked in 100 ml of boiled hot water. That mixture was boiled for thirty minutes into a conical flask and put for 24 h. The extract was filtered using filter paper and evaporated.

Ethanol extract: The ethanol extract of dried plant leaves was also prepared. The ethanol extract was prepared through the same protocol followed for that of cold water extraction [1].

Detection and chemical solutions used in the study

Wagner reagent: Prepare by the method of Harborn, (1984) were

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dissolved 2 g of KI in 5 ml of distilled water and add a 1.27 g of iodine and blend until completely soluble then complete the volume with distilled water up to 100 ml.

Fehling reagent: Prepare the detector by the method of Harborn [5] and through the preparation of solutions, (a) dissolving 35 grams of copper sulphate in the amount of distilled water and dilute the solution with distilled water up to 500 ml (b) The solution was attended by dissolving 7 g of NaOH and 175 g of Rochelle's Salt in distilled water and he finished size with distilled water to 500 ml and when to use mixed in equal volumes of two solutions.

Ferric chloride 1%solution: Prepare the solution according to Harborn [5] as the weight of 1 g of ferric chloride and put into a volumetric flask and completed the volume to 100 ml.

Lead acetate 1% solution: Prepare the weight of 1 g of lead acetate and placed in a glass beaker and he finished size of 100 ml [5].

Detection of groups and effective compounds found plant leaves extracts under study

Resins: According to method Mason and Wasserman [6] add 5 ml of ethyl alcohol concentration (95%) to 0.5 g of leaves extracts after leaving in a water bath to a boil for two minutes nominated and then add to the filtrate 10 ml of distilled water acidified hydrochloric acid concentrate, where inferred the existence of resin materials emergence of turbidity.

Tannins: According to Mason and Wasserman [6] included a boiling 0.5 g of leaves extracts in 2.5 ml of distilled water and was filtered mix, then filtrate divides after the cold into two parts first section of it was added to 1% lead acetate solution. This is indicated by the presence of white sediment gelatinous textures on the existence of dragons either the second section was added to a concentration of ferric chloride solution (1%), the appearance of bluish green colour proof of the positive test.

Phenols: According to Harborne [5] by adding 0.1 ml of the leaves extracts to 0.06 ml of 1% ferric chloride solution, the appearance of bluish green colour indicates the presence of phenols.

Alkaloids: Add several drops of Wagner reagent to 1.00 ml of an aqueous extract and alcohol and that the appearance of brown precipitate a sign of positive detection.

Glycosides: 10 ml of 50% H_2SO_4 was added to 1 ml of the leaves extracts and the mixture heated in boiling water for 15 min. 10 ml of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmed in the presence of glycosides.

pH determination: The pH was measured by Electronic digital(Sartorius, Germany), It has been taking 1 g of plant leaves powder and blending with 10 ml of distilled water and left in a magnetic stirrer for 10 minutes.

Preparation concentrations of plant extract: For the purpose of preparation of the solution inventories (Stock solution) of aqueous extracts taken 1 g of the leaves powder extract (Ziziphus and Eucalyptus separately) and dissolved in 10 ml sterile distilled water became our stock solution concentration of 100 mg/ml. The solution sterility by filtration using membrane filters (Millipore filters) especially with a diameter of 0.22 µm. This solution, as a source of work concentrations (50,100) mg/ml. Alcoholic extract may take 1 gram to 3 ml ethyl alcohol and completed the volume to 10 ml with distilled water. The concentration of the solution was 100 mg/ml and used (50,100) mg/ml in microbial inhibition.

Bacterial isolates: Bacterial isolates: *Bacillus subtilus, Escherichia coli, Pseudomonas erogenous, Staphylococcus aures* and *Streptococcus* sp. were obtained from Food Science Dept., Agriculture college, Basrah University and grow on Nutrient broth ((Himedia Labs.) 37°C for 18 h. The turbidity of activity growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1×10^7 (cfu/ml). The grown suspension was used for further testing.

Fungal isolates: Five mold isolates were obtained from Microscopic Biology laboratory in the Marine Science center, Basrah university. It's *Aspergillus niger*, *A. flavus, Penicillium notatum, Mucor* sp. and *Geotrachium* sp. were maintained in Potato Dextrose Agar and incubated at 30°C for 3-5 days.

Assay for antimicrobial activity: Bacterial activity was determined using the agar well diffusion method [7], 5 wells (0.6 cm) made into previously seeded Mueller Hinton agar plates containing 1×10^7 cfu/ml of each of the test organism were filled with 0.1 ml of each extract, The concentration of extracts employed was 50 and 100 mg/ml . Ethanol (70% v/v) and sterile distilled water were used as controls. The plates left in the refrigerator for two hours and incubated at 37°C for 24-48 h. After incubation the diameter of inhibitory zones formed around each wells was measured in mm and recorded. The test was carried out by triplicate.

Molds activity was determined according to Dixit et al. [8] to add 1ml from leaves extracts (concentration of 50 and 100 mg/ml) to Potato Dextrose Agar media (45°C) and take 0.5 cm of old molds disc in the center of media. The dishes were incubated at 25°C for 4-6 days with a control sample without extracts. Percent mold inhibition was measured after growth by by the formula:

Growth Inhibition (%) = [Dc-De/Dc] × 100

Where, Dc: Diameter of colony in the control (mm), De: Diameter of the colony with extracts (mm).

Results and Discussion

Chemical detection initial of effective compounds of aqueous and alcoholic extracts Ziziphus and Eucalyptus in the light of the results of our study have bio-efficacy groups on some fungal and bacterial isolates. Water and alcohol extracts of Ziziphus and Eucalyptus were in nature characterized as strength viscous green, dark color and aromatic smell. Table 1 occurs the effective chemical croups in leaves extracts of Ziziphus and Eucalyptus plants. pH values of extracts were 4.34-5.91.

Effects of aqueous and alcoholic extract of plants against bacterial and fungal isolates

The observation of antibacterial activity alcoholic extracts of the tow plant extract on pathogenic bacteria using agar well diffusion method showed that the extract of Eucalyptus showed maximum zone of inhibition against *Bacillus subtilis* (19 mm) , *Staphylococcus aureus* (17 mm) and *Pseudomonas* , E.coli (16 mm). The ethanol extract of Ziziphus leaves showed the minimum rate of antibacterial activity on all the five pathogenic bacteria when compare to Eucalyptus extract activity. Effect of concentration on antimicrobial activity showed that the trend was similar for all extracts as higher concentrations (100 mg/ml) produced wider zone of inhibition. These results similar to

Active substances	Aqueous extract of Ziziphus	Alcoholic extract of Ziziphus	Aqueous extract of Ecalyptus	Aqueous extract of Ecalyptus
Resins	-	-	+	+
Tannins	+	+	+	+
Alkaloids	+	+	-	-
Phenols	+	+	+	+
Glycosides	+	+	+	+
pH	5.91	4.73	5.30	4.34

(-)Negative detection , (+)Positive detected.

Table 1: Some active groups of Eucalyptus and Ziziphus leaves extracts.

Bacteria test	Aqueous extract of Ziziphus		Aqueous extract of eucalyptus		Alcoholic extract of Ziziphus		Alcoholic extract of eucalyptus	
	50	100	50	100	50	100	50	100
Pseudomonas	10	12	9	12	13	14	14	16
Streptococcus	11	14	11	14	15	18	11	15
Staphylococcus	8	11	13	16	12	17	14	17
E.coli	12	15	10	13	10	12	12	16
Bacillus subtilis	11	13	12	16	13	16	16	19

Table 2: Effect of alcohol and aqueous extract of Eucalyptus and Ziziphus leaves against bacteria test (diameter inhibition mm).

Molds test	Diameter growth for control sample (mm)	Aqueous extract of Ziziphus		Aqueous extract of eucalyptus		Alcoholic extract of Ziziphus		Alcoholic extract of eucalyptus	
		50	100	50	100	50	100	50	100
Aspergillus niger	85	49.4	64.4	52.7	66.5	59.8	70.7	56.7	74.1
A. flavus	80	24.8	41.6	30.1	42.3	29.9	47.3	44.3	51.8
Penicillium notatum	85	47.6	59.4	38.7	52.9	46.9	64.2	52.9	62.2
Mucor sp.	76	33.8	48.8	41.7	60.5	45.7	57.8	42.6	59.6
Geotrachium sp.	75	28	45.2	34.9	54.1	30.1	49.1	48.7	56.9

Table 3: Effect of alcohol and aqueous extract of Eucalyptus and Ziziphus leaves against mold test (growth inhibition %).

the results of many who have studied the Eucalyptus and Ziziphus leaves extracts in inhibiting the growth of many microorganisms [9,10] (Table 2).

The effectiveness of plant extracts sometimes change after separation and purification process so that it can be said that the effectiveness of Eucalyptus and Ziziphus extracts a nature of the active compounds were obtained according to the user solvent type and method of extraction of the fact that mostly phenolic compounds in the first class and then alkaloids second class differ and that the increase the effectiveness of the aqueous and alcoholic extract of Eucalyptus and Ziziphus may return to the extract on the permeability of the cell membrane and the work of the bacterial cell effect [5].

Percent inhibition of molds results is shown in the Table 3 the alcoholic extract of both plants were preceded in the susceptibility inhibitor of molds and alcoholic extract of Eucalyptus leaves was more influential than with Ziziphus leaves. The alcoholic and water extract of Eucalyptus leaves high inhibitory against *Aspergillus niger*, the diameter growth was 22 mm and 28.5 mm, respectively, the growth diameter of control sample was 85 mm. The alcoholic and water extracts had a lower effect on *Aspergillus flavus*.

The superiority of the alcoholic extract than that of the aqueous one was due to the presence of phenol compound and the absence of this compound in the aqueous solution. This property leads to the decomposition of the membrane of microbes [11].

Newman and Cragg [12] found that Eucalyptus leaves contain flavone as well as contain phenolic compounds which have an important role to discourage the growth of bacteria that work on the inhibition of the enzymes responsible for the metabolic basic interfere interactions in a specialist with proteins leading to the metamorphosis of protein and then the in ability of bacteria to continue while While observed both Mason, and Wasserman [5] that several phenolic compound like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes.

The reason for Ziziphus leaves extracts inhibition was content with the existence of phenolic compound types as well as due to the low acid function, which have been instrumental in increasing the effectiveness and these results are consistent with a study Bukar et al. [13] which shows that the high acid works to change the nature of living material, in particular proteins in the cell membrane through the process and deformed proteins that lose their function leading to a crash in the cell membrane of bacteria.

Conclusion

The results of this study have shown that the aqueous and alcoholic of Ziziphus and eucalyptus leaf extracts have great potential as antimicrobial agents in the treatment of infectious organisms. Further detailed investigation of the active components of the plant for the exact mechanism of action will contribute greatly to the development new alternative and satisfactory artificial preservatives used in the food industry today.

References

1. Zamin I, Shah JA, Khan I, Majid A, Rehman MM, et al. (2014) In-Vitro efficacy of crude extract of Zizipus Jujuba against selected bacterial strains. IJSRP. 4: 1-5.
2. Varahalarao V, Chandrashekhar N (2010) In vitro bioactivity of Indian medicinal plant Calotropis procera. J of global Pharma technology 2: 43-45.
3. Dahanukar SA, Kulkarni RA, Rege NN (2000) Pharmacology of medicinal plants and natural products. Indian J Pharmacol 32: S81-S118.
4. Sudhersan C, Hussain J (2003) In Vitro clonal propagation of a multipurpose tree Ziziphus spina-Christi (L) Desf. Turkish Journal of Botany. 27: 167-171.
5. Harborn JB (1984) Phytochemical methods aguids to modern techniques of plants analysis.2nded. Chapman and Hall, London, New York. p. 288.
6. Mason TL, Wasserman BP (1987) Inactivation of red beta Glucan Synthase by native and oxidized phenolic compounds. Phytochemistry 26: 2197-2202.
7. Gupta SS (1994) Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol. 26: 1-12.
8. Dixit SN, Tripthi SC, Upadhyey RR (1976) The Antifungal Substance of Rose flower (Rosa indica). Economic Botany 30: 371-374.
9. Babayi H, Kolo I, Okogun JI, Ijah UJJ (2004) The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminalia catappa against some pathogenic microorganisms. NSEB 16: 106-111.
10. Mahesh B, Satish S (2008) Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. WJAS 4: 839-843.
11. Cowan M (1999) Plants products as Anti-microbial agents. Clin Microbiol Reviews 12: 564-582.
12. Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. JNP 75: 311-335.
13. Bukar AM, Kyari MZ, Gwaski PA, Gudusu M, Kuburi M, et al. (2015) Evaluation of phytochemical and potential antibacterial activity of Ziziphus spina-christi L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri Borno State, Nigeria. JPP 3: 98-101.