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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 and alcoholic leaves extract , Ziziphus (*Ziziphus spina-christi*) and Eucalyptus plant (*Eucalyptus camaldulensis*) and assessed its antimicrobial activity against reference strains of bacteria and mold. Through chemical compound detection Ziziphus leaves results showed that the aqueous extract and alcohol contain all effective compounds that have been detected except composite resins in aqueous extract and resins in the alcoholic extract. The Eucalyptus leaves aqueous and alcohol extract contained resins, tannins, phenols. It was tested the effectiveness of the inhibitory plant extract against five isolates of bacteria and five isolates of molds. We used a concentration of 50 and 100 mg/ml of each extract. A similar maximum zone of inhibition through the ethanol extract was obtained against both bacteria and mold as well as increased efficiency as dose dependent .

Keyword: *Ziziphus*, *Eucalyptus*, extracts, inhibition

INTRODUCTION

Plants are fundamental to existence on Earth as they are direct or indirect resource of around 70-80% of human energy and protein consumption, the rest being result of animal products. They are, immensely significant to man due to their numerous applications, such as in antibiotics, analgesics, flavors, perfumes, insecticides, dyes, food additives, poisons etc. (Zamin *et al.,* 2014). Medicinal plants are gifts of nature helping us cure a number of diseases among humans. A large number of plants in different locations around the world have been extracted, semi-purified to individually investigate their antimicrobial activity. However, very little information is available about 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 (Varahalarao & Chandrashekhar 2010). Their extracts have gained importance as potential antibacterial agents. Secondary metabolites of plants, including tannins, flavonoids and alkaloids have been found to possess antimicrobial properties *in vitro* (Dahanukar *et al*., 2000).

The Ziziphus and Eucalyptus plants have received wide attention in the field of folk medicine since Ziziphus goes back to the Rhamnaceae family, which spreads widely in areas with moderate temperatures and dry land areas in the world, including Iraq (Sudhersan & Hussain, 2003). Eucalyptus is one of such medicinal plants belonging to Myrtaceae family, native to Australia. It has spread in many countries, including Iraq. The present study aimed to find out the chemical components in plant extracts as well as evaluate the antimicrobial activity of plant extracts, Ziziphus and Eucalyptus, against some pathogenic bacteria and molds.

MATERIALS AND METHODS

Sample collection

The fresh leaves of plants (Ziziphus and Eucalyptus) were collected from Altnoma and Abu Kasib in Basrah province of Iraq from November 2013 to January 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 cleaned using tap water. 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 put into 250 ml sterile conical flasks, plugged with sterile cotton and kept in Shaking Incubator (Kottermann, Germany) on 200 rpm for 24 hours. 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 in a conical flask and

put away for 24 hrs. The extract was filtered using filter paper and evaporated. Ethanol Extract: The ethanol extract of dried plant leaves was also prepared. It was prepared through the same protocol followed by that of cold water extraction (Zamin *et al*. 2014).

Detection and chemical solutions used in study

Wagner reagent

1.27 g of iodine and 2 g of KI was dissolved in 100 mL of water to make the reagent (Harborn, 1984)**.**

Fehling reagent

a - Copper sulfate solution. Dissolved 34.66 g of $CuSO_4$ $5H_2O$ in water and diluted to 500 ml; b - Alkaline tartrate solution. Dissolved 173 g of potassium sodium tartrate (Rochelle salt, $KNaC_4$ H_4 O_6 $4H_2O$) and 50 g NaOH in water and diluted when cooled to 500 ml. Mixed with equal volumes of two solutions at the time of using (Harborn, 1984)**.**

Lead acetate 1% solution

Dissolved 1 g of lead acetate and volume completed to 100 ml (Harborn, 1984).

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

Resins

0.5 g of powdered extract was mixed with 5 ml of 95% ethanol, the solution was left for two minutes in a water bath (100 ºC), the solution was filtered and added to 10 ml of an aqueous solution of 4% hydrochloric acid; the appearance of turbidity indicates the presence of resins (Mason & Wasserman, 1987).

Tannins

Tannin content of crude extracts was estimated according to the method of Mason & Wasserman (1987) including boiling 0.5 g of extracts in 2.5 ml of distilled water, mix was filtered, then filtrate was divided after cooling into two parts; the first section was added to 1% lead acetate solution. This is indicated by the presence of white sediment gelatinous textures on the existence of tannins when the second part was added to a concentration of ferric chloride solution (1%), the appearance of bluish green colour indicated the positive test.

Phenols

The detector of ferric chloride was prepared by melting 1 g of ferric chloride in 100 ml of distilled water. The filter paper was moistened in the plant extract, drops of reagent Wohlen or ferric chloride were added to the filter paper, and the paper was exposed to ammonia vapor. The appearance of blue colour indicates phenols (Harborne, 1984).

Alkaloids

2 drops of Wagner reagent were added to 1 ml of the extract. A brown precipitate was an indication of the presence of alkaloids.

Glycosides

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

pH measurement

pH of the Plant Extract One half grams of the leaves extracts were mixed with 2.50 ml of distilled water and left in a magnetic mixer for 10 minutes. The pH of the solutions was noted using a pH meter(Pyeunicam, England) ..

Bacterial isolates

Bacterial isolates: *Bacillus subtilis, Escherichia coli, Pseudomonas erogenous, Staphylococcus aureus* and *Streptococcus* sp. were obtained from the Food Science Dept., Agriculture College, Basrah University and grown on the nutrient broth (Hi-media Labs.) at 37 ºC for 18 hours. The turbidity of activity of 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 of 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 the Microscopic Biology laboratory in the Marine Science center, Basrah university. The *Aspergillus niger, A. flavus, Penicillium notatum, Mucor* sp. and *Geotrachium* sp. were maintained in Potato Dextrose Agar and incubated at 30 ºC for 3-5 days.

Assay for antimicrobial activity

Bacterial activity was determined using the agar well diffusion method (Gupta, 1994), 5 wells (0.6 cm) were made in Mueller Hinton agar plates and streaked 1×10^7 cfu/ml of each of bacteria test. 0.1 ml of each extract was added to wells . The concentration of each plant extract was prepared in 50 and 100 mg/ml. Ethanol (70% v/v) and sterile distilled water were used as controls. The plates were allowed to diffuse in the refrigerator for 2 hours and were incubated at 37 °C for 24-48 h. After incubation the diameter of inhibitory zones formed around each

well was measured in mm and recorded. The test was carried out by triplicate. The antifungal tests were conducted in solid medium testing at concentration 50 and 100 mg/ml. The obtained solutions were dispensed into Petri plates. Pathogen grown on PDA without any extract was used as a control. Three plates for each concentration were prepared and inoculated aseptically with 5 mm diameter disks of the test fungus taken from actively growing edge of one week old culture and incubated at 25 °C for seven days (Dixit *et al*., 1976). The percent mycelial growth inhibition (PI) was calculated using the following formula:

$$PI\ (\%) = [Dc\text{-}De/Dc] \times 100$$

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

RESULTS AND DISCUSSION

The chemical tests of effective compounds in 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. In nature, Water and alcohol extracts of Ziziphus and Eucalyptus were characterized as strong viscous green, dark color and aromatic smell. Tab. 1 shows effective chemical croups in leaves extracts of Ziziphus and Eucalyptus plants. pH values of extracts were 4.34-5.91.

The growth of all isolates was found to be inhibited, though to varying degrees, with gram-positive more susceptible than gram-negative bacteria. The observation of antibacterial activity of alcoholic extracts of the two plant extract on pathogenic bacteria using the agar well diffusion method showed that the extract of Eucalyptus to have a maximum zone of inhibition against *Bacillus subtilis* (19 mm), *Staphylococcus aureus* (17 mm) and *Pseudomonas* and *E. coli* (16 mm). The ethanol extract of Ziziphus leaves showed decrease rate of antibacterial activity of all five pathogenic bacteria when compared to Eucalyptus extract activity. Ethanol extracts of each plant were reported as more effective, producing larger zones of growth inhibition sizes. 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 are similar to those of many who studied the Eucalyptus and Ziziphus leaves extracts in inhibiting the growth of many microorganisms (Babayi *et al*., 2004; Mahesh & Satish, 2008).

The effectiveness of plant extracts sometimes changed after separation and purification process so it can be said that the effectiveness of Eucalyptus and Ziziphus extracts. The types of natural active compound extracts were dependent on the solvent used in the extraction and the method of extraction. Normally, The phenolic compounds are the main component in extracts, the alkaloid compounds comes in second class. The inhibitory activity of Eucalyptus and Ziziphus extracts against different types of bacteria because of the effect on the permeability of cell membrane, protein cells and DNA (Harborne, 1984).

Percent of inhibition of molds results is shown in the Tab. 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 of Ziziphus leaves. The alcoholic and water extract of Eucalyptus leaves was highly 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 alcoholic extract over 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 (Cowan, 1999). Tannins work by stimulation of phagocyte cells, host-mediated tumor activity and a range of anti-infective actions as well as the ability to form complexes with proteins. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cells envelop transport proteins etc. (Haslam, 1996). Tannins can be toxic to filamentous fungi; yeasts and bacteria, Condensed tannins have been reported to bind with cell walls of ruminal bacteria, preventing their growth and protease activity. Several workers have reported antimicrobial activity of tannins (Hori *et al*, 2006; Reddy *et al*. 2007). Eucalyptus leaves containing flavonoids are known to be synthesized by plants in response to microbial infection, The inhibitory activity is due to formation of complexes with extracellular and soluble proteins and bacterial cell wall and disruption of microbial membranes (Newman & Cragg , 2012). The phenolic compound types and pH low in Ziziphus leaves extracts inhibition were lead to an increase of inhibitory activity against mold test, these results are consistent with a study Bukar *et al.* (2015) which shows that the high acidity works on changing the nature of living material, in particular proteins in the cell membrane through the process of deforming proteins that lose their function leading to a crash in the cell membrane of bacteria.

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

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

(-)Negative detection , (+)Positive detected

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

Bacteria test	Ziziphus aqueous extract		Eucalyptus aqueous extract		Ziziphus alcoholic extract		eucalyptus alcoholic extract	
	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 3. Effect of alcohol and aqueous extract of Eucalyptus and Ziziphus leaves against mold test (growth inhibition %)

Molds test	Control sample growth diameter (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
spergillus niger	85	49.41	64.35	52.70	66.47	59.76	70.70	56.70	74.11
A. flavus	80	24.75	41.62	30.12	42.25	29.87	47.25	44.25	51.75
Penicillium notatum	85	47.64	59.41	38.70	52.94	46.94	64.23	52.94	62.23
Mucor sp.	76	33.81	48.81	41.71	60.52	45.65	57.8	42.63	59.60
Geotrachium sp.	75	28	45.2	34.93	54.13	30.13	49.06	48.66	56.93

CONCLUSION

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 active components of the plants of the exact mechanism of action will contribute greatly to the development of new alternative and satisfactory artificial preservatives used in the food industry today.

REFERENCE

1. Babayi, H., Kolo, I, Okogun, J.I. and Ijah, U.J.J. 2004. The antimicrobial activities of methanolic extracts of Eucalyptus camaldulensis and Terminalia catappa against some pathogenic microorganisms. Nigerian Soc Exp Biol. 16 (2): 106 – 111.
2. Bukar, A.M., Kyari ,M.Z., Gwaski ,P.A, M. Gudusu ,M. , Kuburi ,F.S and Y. I. Abadam. 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. J. Pharmaco Phytochem. 3(5): 98-101.
3. Cowan , M. 1999 . Plants products as Anti-microbial agents. Clin. Microbiol . Rev.12(4): 564-582.
4. Dahanukar, S.A., R. A. Kulkarni, and N. N. Rege . 2000. Pharmacology of medicinal plants and natural products. Indian J. Pharmacol. 32: S81-S118.
5. Dixit, S.N. ,Tripthi , S.C. and R.R. Upadhyey . 1976 .The Antifungal Substance of Rose flower (Rosa indica). Economic Bot. 30(4): 371-374.
6. Gupta,S.S.1994 .prospects and perspectives of natural plant products in medicine. Indian J. Pharmacol. 26:1-12.
7. Harborn, J.B. 1984 . Phytochemical methods aguids to modern techniques of plants analysis.2nded. Chapman and Hall, London, New York. 288p.
8. Mason,T.L. and B.P. Wasserman . 1987. Inactivation of red beta Glucan Synthase by native and Oxidized phenolic compounds. Phytochem. 26:2197-2202.
9. Mahesh,B. and S. Satish. 2008 . Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World J. Agri. Sci. 4 (S): 839-843.
10. Newman, D. J. and Cragg, G. M. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat. Prod. 75: 311–335.
11. Sudhersan, C. and J.Hussain . 2003 . In Vitro clonal propagation of a multipurpose tree Ziziphus spina – Christi (L) Desf . Tur. J. Bot. 27: 167-171.
12. Varahalarao, V. and Chandrashekhar, N. 2010. In vitro bioactivity of Indian medicinal plant

Calotropis procera. J. Global Pharma Technol. 2(2): 43-45.

13. Zamin ,I. , Shah , J.A , Khan , I. , Majid , A. , Rehman , M.M. ,Malik , Z. ,Hyder ,H. ,Bibi , J. and B. Naz . 2014 . In-Vitro efficacy of crude extract of Zizipus Jujuba against selected bacterial strains . Int. J. Sci. Res. Pub. 4(2): 1-5.