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THE EFFECT OF AQUEOUS AND ALCOHOLIC EXTRACTS OF SOME PLANTS FROM CRUCIFEREA FAMILY AGAINST MICROORGANISMS GROWTH

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ABSTRACT
The research aimed the current test of natural bioactivity of some plants of Cruciferae family aqueous and alcoholic extracts to assess their ability to inhibit the growth of bacteria *Escherichia coli , Salmonella typhimurum , Proteus vulgaris, Pseudomonas aeruginosa, Shigella sonnie & Bacillus cereus* , using the agar well diffusion method, the aqueous the results showed that more effective in inhibiting growth of bacteria than alcoholic extracts, The highest inhibition rate were to extracts of *Raphanus raphanistrum* and *R. sativus* followed by *Brassica oleracea var. acephala* and *Brassica oleracea var. botrytis* finally *Brassica oleracea var.* + on the other side the best alcoholic extracts was *Brassica oleracea var. botrytis* and Red cabbage then Broccoli compared to the diameters of inhibition of alcoholic extracts which was a maximum at. When we check out the sensitivity of bacteria to antibiotics was the most influential Nalidixic acid.

KEYWORDS: bioactivity, Cruciferae family, growth of bacteria, Red cabbage, Broccoli.

INTRODUCTION

Due to the significance of the plant kingdom including richness of the plant kingdom that its natural components can be benefited and extracted and studying their efficiency in preservation of food as well as in treating many diseases[1] as WHO indicated that about 80% of world population depend mainly on the traditional medicine; particularly, the extracts of crude plants or their active compounds; therefore, the need requires developing researches in the developing countries and to take care of them as being medication source[2]. The Cruciferae family is considered as the richest plant one of the active compounds such as Glucosinolates compounds, vitamins and mineral salts[3] as the cruiceferae family is considered as alternative treatments and the most important of which are: cabbages, cauliflower, broccoli, watercress and radish. As they contain many active compounds including: Indol, Sulforaphane and Phenolic acids and they are the most important compounds that work on preventing formation of cancerous diseases resulting from the damage accruing in DNA as well as the organic sulfate compounds which also fights cancer and resists clotting, reduce cholesterol and hypertension and it has anti germ effect[4] so the current study aims to prepare the raw aqueous and alcoholic extracts of six types of the cruiceferae family vegetables to know their impactanti germ growth effects and they were: *R. raphanistrum, Raphamus sativus, Brassica oleracea , Brassica oleracea* var. botrytis, *Brassica oleracea* var. capitata, *Brassica oleracea* var. broccoli compared with use of chemical antibiotics, as they are the most consuming vegetables for eating as food starters around the years especially, in winter.

MATERIALS & METHODS

Plant Sampling

The cruiceferae family plants: *R. raphanistrum, Raphamus sativus, Brassica oleracea, Brassica oleracea* var. botrytis, *Brassica oleracea* var. capitata, *Brassica oleracea* var. broccoli samples were collected from the local vegetables markets and they were well washed with the water to prepare them for extraction process where the soft plant parts were peeled by using sterilized and sharp scratcher.

Preparation of Raw Aqueous and Alcoholic Extracts

The *R. raphanistrum, Raphamus sativus, Brassica oleracea , Brassica oleracea* var. botrytis, Brassica *oleracea* var. capitata , *Brassica oleracea* var. broccoli were exposed to the two extraction processes of distilled water and ethyl Alcohol (99.9%) apart for each plant, where the aqueous extracts were prepared by taking 100g of the said plants parts in 200 ml of the distilled sterilized water in boiled water bath for half an hour and then the mixture is filtered through several layers of gauze and then centrifuged at 300 rpm for fifteen minutes and then the extracts were sterilized by passing them through membrane filters 0.2 micrometer in diameter[5]. As for the alcoholic extracts, they were prepared by taking 100g of each plant apart and extracted in 200 ml of ethyl alcohol (99.9%) and then the flasks in vibrating incubator at 37ºC for six hours. After that, they were centrifuged at 300 rpm for fifteen minutes and then the extracts were sterilized through 0.2 micrometer in diameter membrane filters [6].

Preparation of Unresolved Bacteria:

The unresolved bacteria was prepared in concentration of 10^5 in 1 bacteria cell/ ml by Macferland method as prepared by Jabbar (2007) where 0.05 of BACL2 was added to 9.95 ml of H2SO4 and the density of unresolved germs prepared with Macferland was compared. The unresolved bacteria was prepared as aforesaid of the

following types of bacteria: *Escherichia coli, Salmonella typhimurum, Proteus vulgaris Pseudomonas aeruginosa, Shigella sonnie* & Bacillus, which were obtained from the microorganisms laboratory of the center of market researches and consumer protection/ Baghdad University where they were diagnosed after conducting the biochemical tests and they were activated on the feeding papers.

Assessment of the Inhibiting Efficiency of the Plant Extracts against the Microorganisms

Agar well diffusion method including preparation of agar feeding medium was used as per the suppler' instructions and poured in Petri dishes and then the medium was vaccinated with 0.1 ml of the unresolved bacteria previously prepared of each type of the tested bacteria and the unresolved bacteria was diffused by using piece of sterilized cotton and the dishes were left for fifteen minutes and then (3-4) wells in diameter of 10 mm were made by using cork drill and 100 micro liter of each type of the pre- prepared raw aqueous and alcoholic extracts were added by using the micro- pipettes on basis of couple for each type of bacteria of all extracts and then the dishes were incubated at 37ºC for 24 hours and the inhibiting diameter was measured from which the well diameter is subtracted[7] and then obtained results were compared with four types of commonly used and wide spectrum antibiotics as positive control parameter and: Nalidixic acid (NA, 30 mcg), Cefotaxime (Ctx, 30 mcg) Doxycycline (Do, 30 mcg) & Azithromycin (Azm, 30 mcg) were for conducting the test of bacteria sensitivity to the antibiotics.

Statistical Analysis

The findings of the study of anti- plant extracts activity of the cruiceferae family in the growth of the testing bacteria by using the statistical analysis system (SAS) and the significant differences were compared among the averages of the inhibition area diameters by least significant difference (LSD) under probability level ($P<0.05$) [14,15].

RESULTS & DISCUSSION

The Table (1) shows the superiority of the raw aqueous level of the *R. raphanistrum* and *Raphamus sativus* in its inhibiting action towards the bacteria *B. cereus, W. coli* at average diameters of (20-20) mm of the R. raphanistrum and (15-24) mm of *Raphamus sativus* respectively and followed by the extracts of the *Brassica oleracea* and *Brassica oleracea* var. botrytis, then *Brassica oleracea* var. capitata and at last the *Brassica oleracea* var. broccoli which revealed effect towards the negative bacteria of gram pigment, they are *E. coli*, Sh. Sonnie, while bacteria *P. aeruginosa, S. typhimrium, P. vulgaris* shows no sensitivity towards any of the aqueous extracts towards the isolates and the maximum was *R. raphanistrum* and *Brassica oleracea* and followed by the *Raphamus sativus*. As Al Hadithi and others indicated (2013) that the radish contains high rates of glycosides and titans and titans are multi ring phenol compounds found nearly in all parts of plant and their antimicrobials efficiency is due to its ability of inhibiting the adhesion proteins and enzymes and some conveying protons existing in the cellular membrane[8] and the titans work is due to the ability of their interception into the cellular membrane and can be effective on the activity of some enzymes [9] in addition to the phenol compounds including the phenol acids and phlaphynodes and Hydroxycinnamic acids the cruiceferae family has high content of them. Such as cabbages, cauliflower and broccoli[6,12]. When our results are compared with previous study results, we see they are in accord with what Al Janabi and others reached (2014) which concluded that the difference of the inhibiting effect of other plants types of the cruiceferae family such as: watercress, jenebra and hwera and showed that the watercress showed anti action to *E. coli, S. typhimrium* and *B. subtilus* in average diameters (15.9-8-4- 7.7) mm respectively. That confirms the difference in the living creature's sensitivity towards the extract itself. The reason of that is due to what each plant owns of active sub- metabolism compounds which are different in their rates from one plant to another.

TABLE 1: Inhibiting Activity of the Aqueous Extracts of cruiceferae family Plant On the Research Bacteria Growth Measured in mm

Bacterial isolates (cells)	*Brassica oleracea* var. capitata	*Brassica oleracea*	*Raphamus sativus*	*R. raphanistrum*	*Brassica oleracea* var. botrytis	var. broccoli	LSD Value
E. coli	11	12	15	20	6	0	5.27*
S. typhimrium	0	0	0	0	0	0	NS
Sh. Sonnie	0	6	0	0	8	8	7.880*
P. vulgaeiss	0	0	0	0	0	0	NS
P. aeruginosa	0	0	0	0	0	0	NS
B. cereus	0	0	24	20	0	0	6.19A*
LSD value	4.75*	4.81*	4.33*	5.09*	3.47*	3.26*	

As Table (2) shows that alcoholic extracts of this study reveals no inhibiting effect towards the bacterial isolates *P. aerugrinosa E. coli*, while S. *typhimrium* reveals sensitivity to alcoholic extracts of the *Brassica oleracea* var. capitata, *Raphamus sativus and Raphamus sativus* at average diameters of (5-7-7) mm respectively and as for *P. vulgaris* gave maximum sensitivity to *Brassica oleracea* var. broccoli then cauliflower and red radish and at last positive *B. cereus* of gram pigments gave maximum inhibiting diameter of the two alcoholic extracts of *R. raphanistrum* and *Raphamus sativus* then followed by cauliflower. The analytic statistics results showed of the alcoholic extracts of *R. raphanistrum* and *Raphamus sativus* then followed by cauliflower extract. The

statistical analysis results showed that there are significant differences for using alcoholic extracts towards the isolates and the highest was for alcoholic extracts of *Brassica oleracea* var. botrytis flowed by *R. raphanistrum* then broccoli.

TABLE 2: The Inhibiting Activity of Alcoholic Extracts of Cruiceferae Family Plant in Bacteria Growth in This Research Measured in mm

Bacterial isolates (cells)	*Brassica oleracea* var. capitata	*Brassica oleracea*	*Raphamus sativus*	*R. raphanistrum*	*Brassica oleracea* var. botrytis	var. broccoli	LSD Value
E. coli	0	0	0	0	0	0	NS
S. typhimrium	7	5	7	7	0	0	3.69*
Sh. Sonnie	0	0	0	0	8	8	NS
P. vulgaeiss	0	0	0	5	7	9	3.72*
P. aeruginosa	0	0	0	0	0	0	NS
B. cereus	0	0	6	6	4	0	2.28*
LSD value	3.41*	2.37*	3.62*	3.70*	2.79*	3.45*	

Table (3) shows the sensitivity of microorganisms to some commonly used antibiotics which indicated to the preference of using the antibiotics Nalidixic Acid (NA) compared with other antibiotics where the pathogenic bacterium of the Research showed resistance to many of them especially E. coli, typhoid and variances in addition to the resistance of all kinds of bacteria to the antibiotic Cefotaxime (CTX) while they showed their sensitivity to some Research extracts which would make them more suitable for use.

TABLE 3: Sensitivity of Research Pathogenic Microorganisms to Some Antibiotics Measured in mm

Bacterial Isolates	NA	AZM	DO	CTX	LSD vaklue
E. coli	15	0	0	0	4.77
S. typhimrium	18	0	0	0	6.02*
Sh. Sonnie	13	20	24	0	7.99*
P. vulgariss	8	0	0	0	4.17*
P. aeruginosa	13	20	9	0	6.81*
B. cereus	16	20	22	0	7.92*
LSD value	5.01*	8.47*	8.79*	0.00 NS*	

Both Tables (1) and (2) show the superiority of the aqueous extract over alcoholic extract at the rates of inhibiting the Research bacteria and the reason of that is attributed to existence of Glucosinolaes in high rates in the cruiceferae family where mitrosinase enzyme dissolve the water in the Glucosinolaes existing in the cell where there is no contact between the enzyme and Glucosinolaes inside the intact cell but when the botanical tissue is mechanically torn when the extracts are prepared by crushing and cutting under humidity, mitrosinase enzyme dissolve Glucosinolaes rapidly into different products such as compounds containing sulfate which showed to them anti germ activity[7&13] in addition to the physiological compounds as their effect is different as per the number of hydroxyl groups in phenol compounds and their positions. More number they are more effected they have and vice versa due to the increase of the negative electrons in the active compounds which would increase their anti- germ activity as they intercept with the germs vital activities including conveying electrons inside the germs and then they interact with the vital nitrogen compounds such as nuclear proteins and acids and then inhibiting the microorganism growth[9] which would encourage in increasing consumption of the vegetables of this family as they are fresh as starters and with salads as they have nutritional value useful to the human health which contains the anti- inflammation activity, enzyme inhibitor and anti- sensitivity and they are exclusively used as diet and anti- cancer as well as their inhibition against germs growth[6].

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