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Original Article

INVESTIGATION OF NEW ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *BRASSICA RAPA* L

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ABSTRACT

The study was carried out to assess the antimicrobial & antioxidant activities of extracts from roots and aerial parts of Brassica rapa L. Determination of the minimum inhibitory concentration of the effective extracts and fractions against the tested microbes together with the antioxidant activities base on EC50 values of the valuable ones are goals of this study. The air-dried powder of *Brassica rapa* L (turnip) both roots and turnip green were separately extracted successfully with70% ethanol followed by fractionation with light petroleum, chloroform and ethylacetate. Also, aqueous extracts of both organs were done and used in this work using another turnip samples. The susceptibility of six micro-organisms covering gram positive bacteria, gram negative bacteria and two fungi to the extracts and fractions of this plant was measured using cut plug method and the results compared with standard antibiotic gentamycin and the standard antifungal fluconazole. The antioxidant potential was also performed by means of the DPPH radical scavenging assay. All the tested fractions and crude extracts revealed positive inhibitory effects against *Candida albicans, Pseudomonas aeruginosa* and *Bacillus subtilis.*MIC of the two aqueous extracts as well as the ethylacetate fraction of turnip roots against *Brassica rapa* L were separately determined and calculated as 25mg /ml, 25 mg /ml and 12.5 mg /ml respectively. Light petroleum fraction of roots showed somewhat strong antifungal activity against *Candida albicans* with MIC calculated as 12.5 mg/ml. It was interested to know that the aquous extracts of both roots and aerial parts were found to posses the strongest DPPH radical scavenging antioxidant activity (5.39 & 8.07 respectively) that can be considered slightly less potent than vitamin C. The spectrum of activity observed in the present study may provide a new plant source for safe antimicrobial and antioxidant drugs.

Keywords: *Brassica rapa*, Antimicrobial, Antioxidant, Roots, Turnip green, Aqueous, Alcoholic fractions.

INTRODUCTION

Plants have formed the basis of traditional system of medicine that have been in existence from ancient years and continue to provide mankind with new remedies[1]. Plant productsas a part of food and botanical portions have been used to cure and prevent diseases throughout history[2]. Nowadays, the need to use natural products as antimicrobial agents became of great importance. The main reason for this is the increased resistance of micro-organisms to the actually known chemical antibiotics and also to decrease the reported side effects of them on human beings. The increase of microbial resistance to antibiotics threatens public health and increase morbidity and mortality. Food antioxidants augments the body's natural resistance to oxidative damage.

Plants of the genus Brassica belong to the family *Brassicaceae* and include several of the most commonly consumed vegetables all over the world. Among them 'turnip' *Brassica rapa* L. that is commonly consumed species locally known in Egypt as Lifft or Shaljam, which is highly consumed as edible vegetable. It is known in the Unani and Arab traditional medicine for its use in chronic gastritis, constipation, cholecystitis, cholecystolithiasis and in liver diseases[3].The antibacterial activities of turnip roots have long been purposed in folk medicine and in traditionally cure for common cold. Turnip, also known as *Brassica rapa*is considered as a weed[4].Also, in folk medicine, the powdered seed is said to be a remedy for cancer and breast tumors, while a salve derived from the roots can help to treat skin cancer[5]. Turnip edible parts are consumed as a raw, boiled and/or fermented vegetable all over the world. They contain a variety of organic compounds with biological activity such as glucosinolates, phenylpropanoids, flavonoids, phenolics and organic acids [6]. The antimicrobial activity of turnip was previously tested [5].The highest antimicrobial activity was observed by methanolic extracts on Micrococcus species while mold was resistant to this extract. Other alcoholic extracts also showed such higher activity. Another research tried to examine the antibacterial effect of crude extracts of turnip species grown in northern Iran showed a significant antimicrobial activity of *Brassica napus*L. against *pseudomonas aeruginosa*[7]. Other valuable biological effects of *Brassica rapa L.* includes hepatoprotection and anti hepatotoxicity [8]. Also, the ethanolic extract of turnip roots exerted anti diabetic activity [9] in all oxanized diabetic rats. Many of the proven biological activities of turnip can be related to antioxidant potential [10]. Other research revealed that the ethanol extract of the roots possessed antioxidant potentials such as free radical scavenging, nitrite scavenging, and lipid peroxidation inhibitory activities as well as reducing power and lipid peroxidation inhibition [11]. In this communication, the present research investigated and comparethe antimicrobial activities of turnip aqueous and ethanolic extracts and ethanolic sub fractions of both roots and turnip greens separately. Another goal of this work is to determine MIC of the significantly positive extracts and fractions of both organs. Also, the anti oxidant capacity was evaluated by the DPPH radical scavenging assay.

MATERIALS AND METHODS

General

Solvents used were of analytical grade

RotavaporHeidolphvv2000

Spectrophotometer (Optima SP-300, Japan)

Lyophilyser (Hosokawa, England)

Micro-organisms were obtained from American type culture collection (ATCC, Rockville, Maryland) or from Northern regional research laboratories (NRRL, Peoria, Illinois).

DPPH was purchased from Sigma Chemical Co. (St. Louis, USA).

Preparation of turnip extracts

Plant samples were harvested from Egypt' markets in February 2013. The plant samples were kindly verified by professors of botany in Faculty of science, Tanta University. Roots then were separated from turnip greens, each cut into small slices and dried in

shade till complete drying. The plant material was pulverized and sieved using sieve No.4. The powdered drug of each organ was separately stored in amber-colored glass well closed containers.

The dried roots (380 g) and turnip greens (100g) were separately extracted with 70% ethanol till complete exhaustion at room temperature. The collected alcoholic extracts were firstly filtered over Buchner funnel then concentrated under reduced pressure at 50^0C. The brown sticky residue (50 g) of roots (I) and green residue (10 g)of turnip greens (II) were separately suspended in 50% ethanol and fractionated with light petroleum, chloroform and ethyl acetate respectively three times for each. The collected six fractions of both plant organs concerning light petroleum of roots (III) and light petroleum of turnip greens (IV), chloroformic fraction of roots (V) and chloroform of turnip greens (VI) and ethyl acetate fractions of roots (VII) and of turnip greens (VIII) were separately concentrated under reduced pressure till complete dryness then dried in desiccator over anhydrous sodium acetate. Also, Aqueous extracts of both roots and turnip greens were prepared by macerating powdered roots (100g) and powdered turnip greens (27g) with cold water at room temperature three times till complete exhaustion. The two collected aqueous extracts, of roots (IX) and of turnip greens (X), were then lyophilized and kept in refrigerator.

Antimicrobial activity

Antimicrobial activity assay using cut plug method

Two gram negative bacteria (*Pseudomonas aeruginosa* ATCC 11921 and *Escherichia coli* ATCC 29425) and two gram positive bacteria (*Bacillus subtilis* ATCC 11562 and *Staphylococcus aureus* ATCC 6538) were selected for antibacterial activity assay. Gentamycin was used as the positive antibacterial control. Two fungi (*Candida albicans Lab* isolated and *Aspergillusniger* NRRL599) were selected for the antifungal activity assay. Fluconazole was used as the positive antifungal control.

Cut plug method recorded by Pridham, et al[12]was employed to determine the antimicrobial activity of the prepared polymers as following: Freshly prepared spore suspension of different tested microorganisms (0.5 mL of about 10^6 cells/mL) was mixed with 9.5 mL of melting sterile Sabouraud's dextrose medium (for fungi)or nutrient agar medium (for bacteria) at 45°C, poured on sterile Petri dishes, and left to solidify at room temperature. Regular wells were made in the inoculated agar plates by a sterile cork borer of 0.7 mm diameter. Each well was filled with 20 mg of each tested sample. Three replicates were made for each test, and all plates were incubated at 27°C for 72 h for fungi, and at 32°C for 24 h for bacteria. Then the average diameters of the inhibition zones were recorded in millimetres (mm), and compared for all plates.

MIC determination for the most efficient extracts and fractions against the most susceptible microorganisms

Half-fold serial dilutions were made for selected extracts and fractions in order to prepare concentrations of 6.25, 12.5, 25, 50 and 100 mg/mL in distilled water, zero concentration was considered as a negative control. A previously prepared pure spore suspension of the most susceptible microorganism (0.5 mL of about 10^6 cells/mL) was mixed with 9.5 mL of each concentration in sterile test tubes, incubated at 27°C for 72 h for fungi, and at 32°C for 24 h for bacteria, then the optical density of growth was measured by spectrophotometer (Optima SP-300, Japan) at 620 nm for each incubated mixture.The growth-inhibiting effect was quantitatively determined by percentage of the surviving cells (% Optical density). Results were represented graphically, and the minimum inhibitory concentration (MIC) was recorded for each tested material [13].The MIC value was determined as the lowest concentration of the sample at which the tested micro-organisms did not demonstrate any visible growth after incubation.

Antioxidant activity assay using DPPH radical scavenging method

The antioxidant activity of the extracts were assessed on the basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical with using ascorbic acid as a standard. The radical scavenging effects of antioxidants on DPPH are due to their hydrogen donating ability which causes an absorbance drop at 517 nm. Serial dilutions (25-100 µg/ml) of each extract or fraction were measured by the same assay to obtain EC50 (the effective concentration at which the DPPH scavenging activity being half its maximal activity). In the DPPH radical scavenging, antioxidants react with the DPPH radical, which is a stable free radical and exists naturally in deep violet color, to turn into a yellow colored (diphenylpicryl hydrazine). The degree of discoloration indicates the radical scavenging potential of the antioxidant [14, 15]. Different extracts or fractions were prepared in a concentration (100 µg/ 1ml DMSO) using ascorbic acid (reference compound). A sample solution (250 µl) of each extract or fraction was added to 1 ml DPPH/DMSO solution (6 mg/50 ml) and the total volume was adjusted to 3 ml with DMSO. An equal amount Of DMSO was used as a control. The mixture was vortexes for 15 seconds then incubated for 30 min in dark at room temperature. Absorbance was measured using a Jenway spectrophotometer at 517 nm. Activity of scavenging (%) was calculated using the following formula:

DPPH radical scavenging % = [1 – (A sample/ A control)] x 100.

The $EC_{50\%}$ values (mg /ml) which are the effective concentration at which the DPPH scavenging effect being 50% was obtained by interpolating from linear regression analysis. All the aforementioned experiments were conducted in triplicate trials. Data were expressed as mean ± standard deviation (SD). Data were analyzed by using one-way ANOVA followed by Duncan's multiple range tests using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Preliminary screening of the antimicrobial activities of ethanolic and aqueous extracts and several ethanolic fractions of *Brassica alba*L. roots and turnip greens showed positive inhibitory effects against most of the tested human pathogenic organisms. *Aspergillusniger* was resistant against all of them. The results are listed in Table1. It is obviously cleared that the tested Gram- positive bacteria (*Bacillus subtilis* and Staphylococcus aureus) exhibited more susceptibility to extracts and fractions than Gram- negative ones. This had been represented by higher inhibition zones. These results coincided with the results obtained by Tenore et al (2012) on testing seed related to the genus Brassica [16]. It is noteworthy that all the tested extracts and fractions showed their most powerful inhibitory effects against *bacillussubtilis*. Also, *Candida albicans* was the sole susceptible fungus.

A valuable result obtained is that the inhibition zones of both the light petroleum fraction of the ethanolic extract of roots and its ethylacetate fraction (14mm) equal to that of the well-known antifungal fluconazole. Another significant result observed is that all the tested extracts and fractions of both roots and turnip greens were more powerful against *Bacillus subtilis*than the aminoglycoside antibiotic gentamycin.MIC values are listed in Table 2 and shown in Figures 1, 2,3 and 4. *Pseudomonas aeruginos* that was susceptible to the both aqueous extracts. This result agreed with previously mentioned antipseudomonal activity of another species *Brassica Napus*L[7].

As a result, the MIC of turnip green aqueous extract against *Pseudomonasaeruginosa* was determined and calculated as for the first time as 50 mg / ml.MIC of the two aqueous extracts as well as the ethylacetate fraction of turnip roots against *Brassica rapa*L were separately determined and calculated as 25mg /ml, 25 mg /ml and 12.5 mg /ml respectively. Light petroleum fraction of roots showed somewhat strong antifungal activity against *Candida albicans* with MIC calculated as 12.5 mg/ml.

Numerous surveys have highlighted the potential importance of extracts from *Brassica* species as sources of polyphenolics (flavonoids, phenolic acids and related analogues) able to exert antimicrobial effects [17-19].Being one of the oldest cultivated vegetables that have been consumed by human since pre historical times[20], it was been focused on to be studied. Actually, it is well known that phenolic acids are present in ionized form at the buffer pH value (7.0) and are too polarto penetrate the semi permeable bacterial membrane and react with the cytoplasm or cellular proteins [18].This is the same reason why the lipidic wall of Gram-negative pathogens represents a great barrier for most polyphenols and hence only slight inhibition is achieved.

Table 1:antimicrobial activities andinhibition zone diameters (mm) produced by 20 mg of brassica rapa l. Roots and aerial parts different extracts and alcoholic fractions against different bacteria which were maintained on nutrient agar after 24 h and fungi which were kept on sabouroud's agar slopes after 72 h by the cut plug method on nutrient agar at 30°c

Tested extract or fraction	Tested microorganisms representing inhibition zone diameter(mm)					
	E.Coli	*P. aeruginosa*	*Staph. aureus*	*Bacillus subtilis*	*Aspergillusniger*	*Candida albicans*
I	8	8	8	11	--	10
II	9	8	7	12	---	10
III	7	9	7	12	---	14
IV	7	8	7	11	---	12
V	7	8	10	11	---	11
VI	8	8	9	12	---	10
VII	10	9	9	15	---	14
VIII	9	8	10	14	---	10
IX	9	11	11	15	---	12
X	10	12	10	16	---	12
Control	6	6	6	6	6	6
$CK1^+$	---	----	---	---	11	14
$CK2^+$	7	10	8	10	---	---

Note: The positive controls ($CK1^+$, $CK2^+$) on fungi and bacteria were fluconazole and gentamycin sulfate respectively.

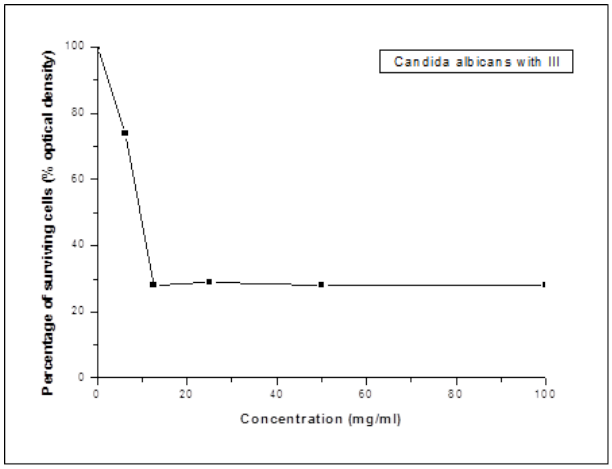
Table 2: MIC of selected Brassica Rapa L. Extracts and fractions against different sensitive micro-organisms

Extract or fraction	Percentage of surviving cells (% Optical density) Concentration (mg/ml)						Microorganism
	0.0	6.25	12.5	25	50	100	
III	100	74	28	29	28	28	*Candida albicans*
VII	100	69	32	30	31	32	*Bacillus subtilis*
IX	100	58	43	30	29	29	*Bacillus subtilis*
X	100	78	48	30	31	30	*Bacillus subtilis*
X	100	66	48	39	31	30	*Pseudomonas aeruginosa*

Table 3: Antioxidant activity of all extracts and fractions using DPPH scavenging assay method

Extract or Fraction	Percentage of scavenging ability on DPPH	EC50 (mg/ml)
I	66.93 ± 1.18	19.27± 0.02
II	52.20± 1.18	38.22± 0.00
III	58.65 ± 1.19	27.18± 0.02
IV	13.70 ± 0.89	161.09± 0.01
V	11.11 ± 0.45	203.87± 0.01
VI	28.16 ± 0.89	80.69± 0.00
VII	32.81 ± 1.19	78.58± 0.01
VIII	47.29 ± 0.77	44.80± 0.01
IX	84.75 ± 0.89	5.39± 0.00
X	83.72 ± 0.78	8.07± 0.01
Reference (Ascorbic acid)	86.30 ± 0.44	4.45± 0.01

Each value is expressed as mean± S.D. (n = 3).



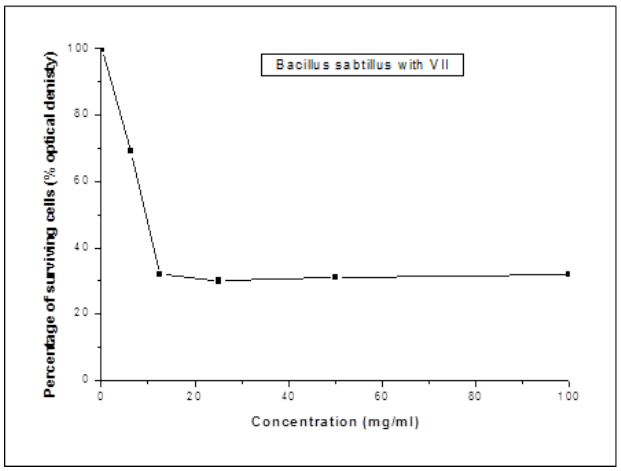


Fig. 1: Growth inhibition of different concentration of fraction (iii) against candida albicans

Fig. 2: Growth inhibition of different concentration of fraction (vii) against bacillus subtilis

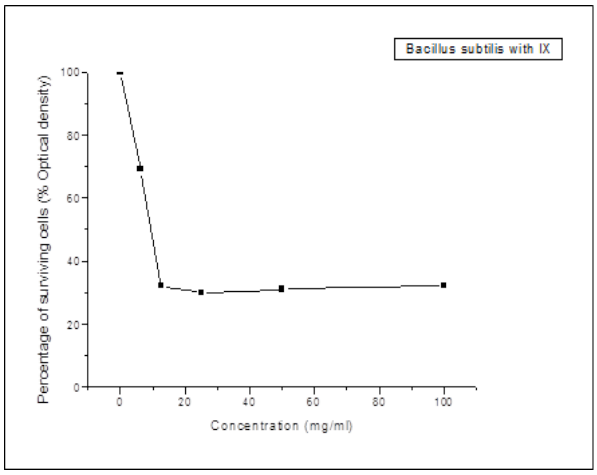


Fig. 3: Growth inhibition of different concentration of fraction (ix) against bacillus subtilis

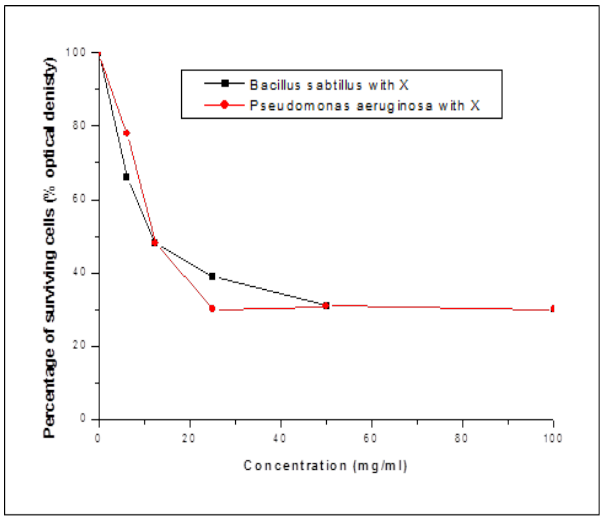


Fig. 4: Growth inhibition of different concentration of fraction (x) against bacillus subtilis and pseudomonas aeruginosa

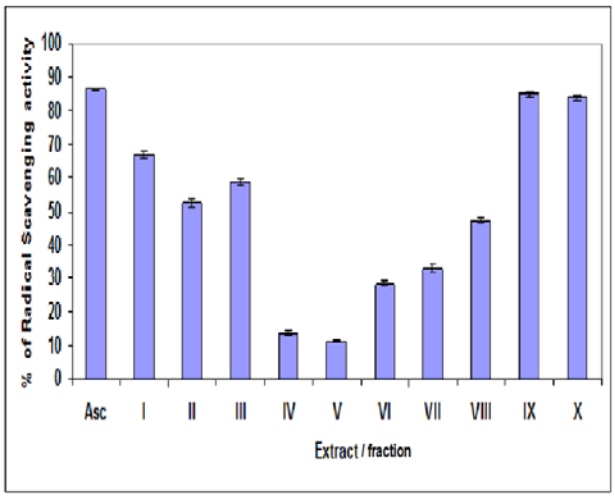


Fig. 5: Comparison between the percentage of scavenging ability of both roots and aerial parts aqueous and ethanolic extracts and ethanolic subfraction using dpph method with ascorbic acid as reference

The antioxidant activity of all the prepared extracts and sub fractions were measured. In this way, serial dilutions (25-100 µg/ml) of each extract or fraction were measured to obtain EC50 (the amount of compound that gives half-maximal response). The results are listed in Table 3. The DPPH radical scavenging activity of all fractions gradually increased in a concentration-dependent manner. Effectiveness in reducing power was in descending order: aqueous extract of root >aqueous extract of turnip green > ethanolic extract of root > light petroleum fraction of root >ethanol and ethylacetate of turnip greens>>> chloroform of root. The aqueous extract of root is superior to that of turnip greens and it gives antioxidant scavenging DPPH potential slightly less than that of ascorbic acid, the standard powerful antioxidant.

CONCLUSION

The results of this investigation clearly prove the antibacterial activity of the roots and turnip greens. Thus, the study ascertains the value of *Brassica rapa* L. and reports for the first time its antimicrobial activity on microorganisms including bacterial (Gram-positive and negative) and fungal species as well as pronounces the MIC of several effective extracts and fractions on the sensitive micro-organisms.

Numerous surveys have highlighted the potential importance of extracts from *Brassica* species as sources of polyphenolics (flavonoids, phenolic acids and related analogues) able to exert antimicrobial effects [13-15]. Actually, it is well known that phenolic acids are present in ionized form at the buffer pH value (7.0) and are too polar to penetrate the semi permeable bacterial membrane and react with the cytoplasm or cellular proteins [16].This is the same reason why the lipidic wall of Gram-negative pathogens represents a great barrier for most polyphenols and hence only slight inhibition is achieved.

The antioxidant activities of four extracts of root and aerial parts of turnip together with six ethanolic sub fractions of both organs were examined for radical-scavenging activity of DPPH. Turnip roots proofed that it exhibithig her antiradical activity in both aqueous and ethanolic extracts. The aqueous extract of root showed wonderful percentage scavenging activity equal to 5.39 knowing that percentage scavenging activity of ascorbic acid is 4.45. Pharmaceutical companies should focus on the natural resources of medicines that should be more reliable and safe in use. Turnip is almost safe and has no need for further safety tests. Our challenge in drug companies will be how to formulate the plant in a suitable, easily administered dosage forms.

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