



## Phytochemical and Pharmacological Analysis for Seeds of Two Varieties of Iraqi *Raphanus sativus*

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### ABSTRACT

This study aimed to assess the antioxidant and the antimicrobial activity of seeds extract of two varieties (red and white) of Iraqi *Raphanus sativus*. Preliminary qualitative analysis of various secondary metabolites by specific chemical tests was conducted on the ethanol seeds extract. The plant's seeds extract was assessed for its in vitro antioxidant and antibacterial activity. Preliminary phytochemical investigation of both white and red radish seeds indicated the presence of various chemical constituents like alkaloids, Glycosides, steroids, Tannins, Carbohydrates and flavonoids. The results obtained from the present study reveal that *R. sativus* seeds extract of both varieties exhibits valuable antibacterial activity against most of tested bacteria and exhibited significant ( $P < 0.01$ ) antioxidant activity especially in concentrations of 150 and 200 mg/ml which are comparable to that of ascorbic acid. In conclusion the presence and diversity of secondary metabolites may be responsible for the observed antibacterial and significant antioxidant activity of red and white radish.

**Keywords:** Antibacterial, Antioxidant, Phytochemical Analysis, *Raphanus sativus*.

### INTRODUCTION

The use of natural products that have medicinal activities is as ancient as human civilization. Plants have provided human with all his requirements in terms of shelter, clothing, food, flavours, and perfumes; and important vast number of medicines. Herbal medicine is the oldest type of healthcare product known to humankind and was used by all communities throughout history<sup>1</sup>. Thus, in recent years, there has been increasing interest in finding alternative therapeutic uses of natural products, especially those derived from plants<sup>2, 3</sup>. Medicinal activities of plants have long been related to the production of secondary metabolites which include tannins, terpenoids, coumarins, alkaloids and flavonoids. However, the antioxidant, antimicrobial and other medicinal properties are widely exploited for the benefit regarding human healthcare<sup>4</sup>. Resistance to anti-infective drugs by bacteria is a growing problem. Thus, reduce the use of antibiotics, better understanding the mechanisms of bacterial resistance and create new drugs wither synthetic or natural are needed<sup>5,6</sup>. The antimicrobial agents from plant sources may act through diverse mechanisms, and could therefore be of therapeutic importance in the treatment of bacterial infections<sup>7,8</sup>. Oxidative stress results in an increased production of reactive oxygen species and lead to unlimited oxidation of proteins and membrane lipids or may cause DNA damage<sup>9,10</sup> and other serious diseases like ageing, cancer, diabetes, atherosclerosis and other neurodegenerative diseases<sup>11</sup>. In certain circumstances protective mechanism carried out by defense system can be exhausted, that's lead to elevated levels of peroxidation products. In such conditions external supplement of antioxidants is required to decrease the Exacerbating

conditions<sup>12</sup>. *Raphanus sativus* L. (Radish) is a member of the Brassicaceae family. It is an essential vegetable that have various medicinal activities including anticancer<sup>13</sup>, antimicrobial, antidiabetic<sup>14</sup>, diuretic<sup>15</sup>, antifertility<sup>16, 17</sup>, hypertensive, antimicrobial<sup>18</sup>, nephroprotective<sup>18, 19</sup>, gastroprotective<sup>20</sup> and hepatoprotective<sup>21</sup>. *Raphanus sativus* was selected for the present study for its antimicrobial and antibacterial potential<sup>22</sup>. *Raphanus sativus* L. seeds and leaves contain 'raphanin' which has been shows antibacterial and antifungal properties<sup>24</sup>. Many workers have also studied the antioxidant ability and phytoconstituents of different extracts of radish leaf and aerial parts and it's gave significant antioxidant activity<sup>25</sup>.

### MATERIALS AND METHODS

#### Plant material

The dried seeds of white and red radish of the family Brassicaceae were collected from local market in Baghdad, and authenticated by the National Herbarium at Abu-Graib, Baghdad.

#### Chemicals

The compound 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich.

#### Experimental work

The experimental work is divided into extraction of active constituents, Preliminary phytochemical analysis of various secondary metabolites like alkaloids, Glycosides, steroids, Tannins, Carbohydrates, fats, oils and flavonoids and finally investigation of antioxidant and antibacterial activity of the ethanolic extract of seeds.



### Preparation of extract

The powdered seeds of both varieties (15 g for each) were extracted with 180 ml of 80% ethanol using Soxhlet apparatus until exhaustion. Alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40°C to yield a dark-brown residue designated as a crude extract. The percentage yield of extract for different solvents was calculated using the formula:

$$\text{The yield \%} = \frac{\text{Weight of final extract}}{\text{Weight of powdered sample}} * 100$$

### Preliminary phytochemical screening

Chemical tests were carried out using the ethanolic extracts of white and red radish seeds using standard procedures to identify the active constituents<sup>[26, 27]</sup>. The results are reported in (Table 1).

#### Test for alkaloids

Two alcoholic quantities of both extracts (10 ml for each) were mixed with 5 ml of 1% HCL on a steam bath. Mayer's and Wagner's reagents were added to yield white and reddish brown colour precipitate respectively indicates the presence of alkaloids.

#### Tests for flavonoids

##### (i) Lead acetate test

Few drops of lead acetate solution added for both extracts. Formation of yellow precipitate may indicate the presence of flavonoids.

##### (ii) Shinoda test

To the extracts, 5 ml (95%) of ethanol was added for each then each mixture was treated with few fragments of magnesium turning, followed by addition concentrated hydrochloric acid drop wise. Formation of pink colour indicates the presence of flavonoids.

#### Tests for steroids

##### (i) Liebermann-Burchard test

3 ml of each variety extract was treated with chloroform then acetic anhydride and drops of sulphuric acid were added. The positive result for steroids presence is dark pink or red color.

##### (ii) H<sub>2</sub>SO<sub>4</sub> test

The development of a greenish colour was regarded as an indication for the presence of steroids, when the organic extract (2 ml) was treated with sulphuric and acetic.

#### Test for tannins

10mg of powdered seeds in 10ml distilled water was filtered, and then the 3 ml filtrate mixed with 3ml of FeCl<sub>3</sub> solution (5%w/v). Dark green or blue black precipitate was considered an indication for the presence of tannins.

### Tests for anthraquinones

Borntrager's test used for identification of anthraquinones using 3ml of alcoholic extract that shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken well to develop pink, red or violet color in the ammonia phase that's indicating the presence of free anthraquinones.

#### Test for terpenoids

2 ml of alcoholic extracts were mixed with chloroform 2ml and evaporated to dryness. Then 2 ml of concentrate sulphuric acid was added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

#### Test for cardiac glycoside

Keller-kiliani test used by addition of Alcoholic extracts 2ml to 1ml glacial acetic acid, FeCl<sub>3</sub> and concentrated sulphuric acid. Formation of green-blue colour indicates the presence of cardiac glycoside.

#### Measurement of antioxidant activity (DPPH radical scavenging assay)

Estimation of the antioxidant effectiveness involved UV Spectrophotometric detection<sup>[28]</sup>. Two solutions of sample and control (DPPH with methanol) were prepared. Different solutions (0.025, 0.05, 0.1, 0.15 and 0.2 mg/ml) of ascorbic acid were prepared in methanol. 3 ml of each solution of ascorbic acid were mixed with 1 ml of 4×10<sup>-2</sup> μ/ml solution and incubated for 30 min at 37 °C temperature in dark. Absorbance of each solution was taken against blank at 517 nm. Different solutions of the given sample and control were prepared to give concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 mg/ml). 3 ml of each solution of given sample was mixed with 1 ml of 4×10<sup>-2</sup> μ/ml solution and incubated at 37 °C in dark. Absorbance of each solution of given sample was measured against blank at 517 nm. Percentage antioxidant activity of plant extract and Ascorbic acid was calculated by using formula:

$$\text{Scavenging activity \%} = \frac{AC - AS}{AC} * 100$$

**AC:** absorbance of control (methanol and 4×10<sup>-2</sup> μ/ml solution).

**AS:** absorbance of different concentrations of ascorbic acid and given sample with DPPH solution.

#### Antibacterial analysis

Bacterial strains were subculture and streaked out on Mueller Hinton Agar plates (MH), in order to determine the activity of both seeds extracts against Gram-negative and Gram-positive bacteria. Well diffusion assay was performed. All plates were left to set and incubated at 37 °C for 24 hr. The clear zone around each well refers to the inhibition zone, and the bacterial activity was expressed in term of average diameter of this zone in (mm). Control



is containing sterile distilled water with 0.1 % DMSO. The strains including gram positive (*Staphylococcus aureus*) and gram negative (*E.coli*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Salmonella typhi*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Salmonella paratyphi*) bacteria were selected to test their susceptibility against seeds extracts of red and white radish. The strains were obtained from collage of sciences / University of Baghdad. They were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 40C. Fresh inoculums were taken for the test. The results were compared with the standard antibiotics, Amoxiclav (10 mg/ml), Cefuroxime (10 mg/ml), Cefixime (10 mg/ml) and Cefpodoxime (10 mg/ml). While extracts concentrations were (25, 50, 75, and 100 mg/ml) for both varieties extracts.

## RESULTS

### Percentage of yield

The percentage yield for extract from seeds shows comparable percentage for both varieties (3.6 %) for red radish and (3.8 %) for white radish.

### Phytochemical analysis

Phytochemical analysis indicated the presence of alkaloids, flavonoids, saponins, anthraquinones, tannins, steroids, terpenoids, and cardiac glycosides in both seeds extract (Table 1).

### Antioxidant activity

Antioxidant assay showed that the ethanol extracts of two varieties have potent antioxidant ability and they can act as radical scavengers for free radicals especially in concentrations of 100, 150 and 200 mg/ml at these concentrations red and white radish seeds extracts have non-significant difference from that of ascorbic acid with scavenging activity of 100%. Antioxidant activity is highly correlated with increasing of concentration.

DPPH is a stable free radical has colour changes from violet to yellow by reduction by either the process of hydrogen- or electron- donation [29]. One of the most important properties of phenolic compounds and flavonoids are their ability of protection against oxidative stress<sup>30</sup>. Presence of these compounds in the seeds contributed to its antioxidant activity. Both red and white radish show similar pattern of scavenging activity with an EC50 (39.84 ± 3.513) for red radish and (43.84 ± 3.523), (The EC50 is the concentration of a drug that gives half-maximal response) however both extract show scavenger activity similar to ascorbic acid at concentration of 150 mg/ml and above (p value >0.05) as illustrated in (Table 2) and (Figure 1).

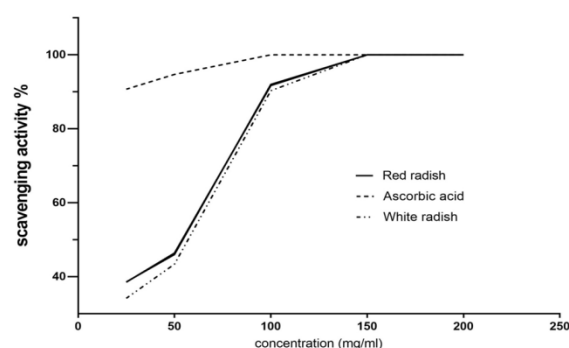
**Table 1:** Qualitative phytochemical analysis of *Raphanussativus* L seeds extract.

Tested Component	Type of Test	Radish seeds extract	
		Red radish	White radish
Alkaloids	Mayer	+Ve	+Ve
	Wagner	+Ve	+Ve
Flavonoids	Lead acetate	+Ve	+Ve
	Shinoda	+Ve	+Ve
Steroids	Liebermann-Burchard	+Ve	+Ve
	H2SO4	+Ve	+Ve
Tannins	FeCl3	+Ve	+Ve
Anthraquinones	Borntrager	+Ve	+Ve
Terpenoids	H2SO4	+Ve	+Ve
Cardiac glycoside	Keller-kiliani	-Ve	-Ve

**Table 2:** Effect of concentration of radish seeds extracts on scavenging activity % compared to ascorbic acid.

Concentration (mg/ml)	Red radish	White radish	Ascorbic acid	P value
25	38.50 ± 1.58	38.57 ± 1.42	90.70 ± 0.39	<0.001 [Sig.]
50	46.37 ± 0.27	45.98 ± 0.28	94.70 ± 0.42	<0.001 [Sig.]
100	91.70 ± 0.43	92.03 ± 0.41	100 ± 0.00	<0.01 [Sig.]
150	100 ± 0.00	100 ± 0.00	100 ± 0.00	>0.05 [NS]
200	100 ± 0.00	100 ± 0.00	100 ± 0.00	>0.05 [NS]
P value	<0.001 [Sig.]	<0.001 [Sig.]	<0.001 [Sig.]	

SE: Standard error, Sig.: significant, and NS: non-significant  
Data presented using (Mean ± SE (%))



**Figure 1:** scavenger activity% of each concentration for the different extracts

### Antibacterial activity

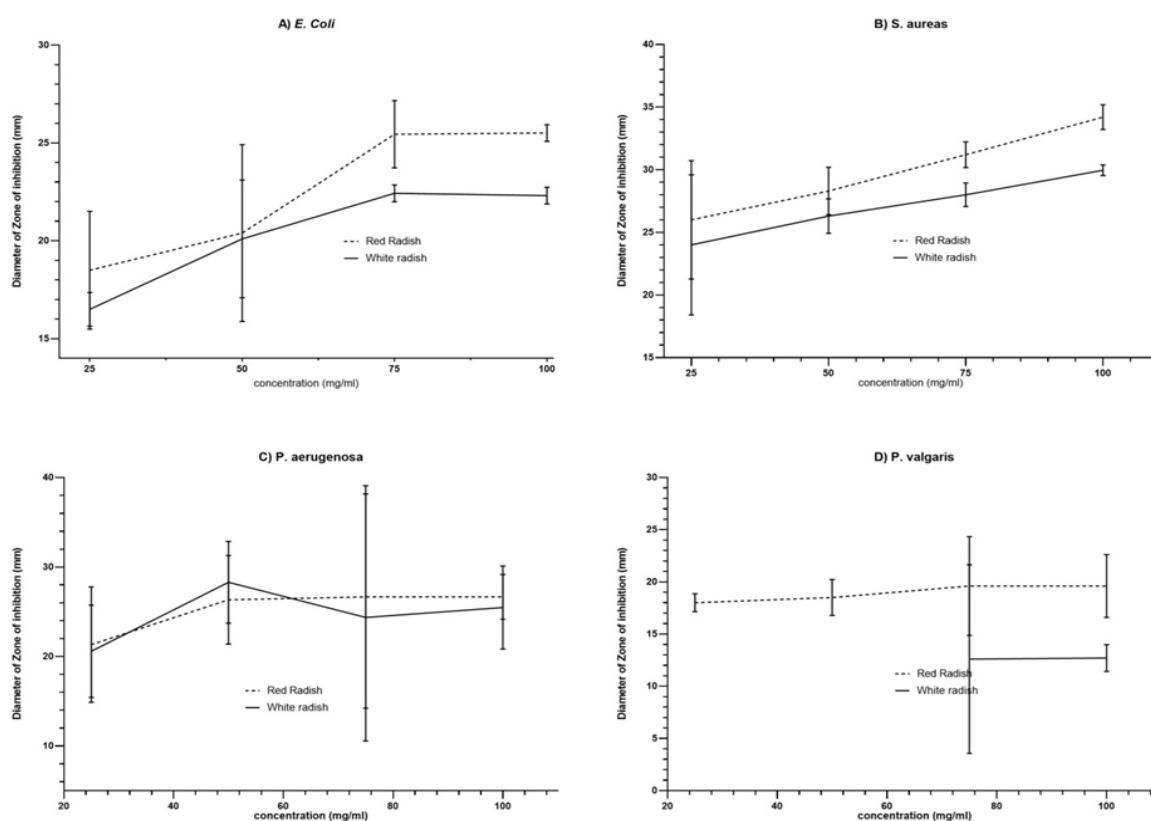
The results obtained for both varieties showed that *R. sativus* seeds extract exhibits a valuable antimicrobial activity against most of the tested microorganisms at a zone of inhibition ranged from 34.2 mm at concentration

100 mg ml<sup>-1</sup> for *S.aureas* to 18 mm at concentration 25 mg ml<sup>-1</sup> for *P. valgaris* where least activity was observed for red radish. For white radish inhibition zone ranged from 12.6 mm at 75 mg ml<sup>-1</sup> for *P. valgaris* to 29.96 mm at 100 mg ml<sup>-1</sup> for *S.aureas* (Table 3). Red radish was better than white reddish by inducing more zone of

inhibition at concentration of 75 and 100 mgml<sup>-1</sup>, the same effect induced by red radish in *S. auras*, in *P. aerogenosa* both red and white reddish had similar effect on zone of inhibition, in *P valugaris* only at concentration 100 mg/ml red reddish was better than white reddish, as illustrated in (Figure 2).

**Table 3:** Antimicrobial sensitivity assay for red and white radish seeds extract compared to the tested antibacterial drugs.

Sample		Diameter of Zone of inhibition(mm)				
		<i>E.coli</i>	<i>S.aureas</i>	<i>P.aerugenosa</i>	<i>P.valgaris</i>	<i>K.pneumoniae</i>
Red radish (mg ml <sup>-1</sup> )	25	18.5±0.7*	26 ± 1.1*	21.33 ± 1.5	18± 0.2	NA
	50	20.4 ± 1.05 *	28.3 ± 0.44	26.33 ± 1.15*	18.5 ± 0.4*	NA
	75	25.45 ± 0.4*	31.2 ± 0.24*	26.66 ± 2.89*	19.6 ± 1.1	NA
	100	25.51 ± 0.1*	34.2 ± 0.23*	26.66 ± 0.58*	19.6 ± 0.7*	NA
White radish (mg ml <sup>-1</sup> )	25	16.5 ± 0.2*	24 ± 1.3*	20.58 ± 1.2*	NA	NA
	50	20.1 ± 0.7 *	26.3 ± 0.32	28.29 ± 1.06*	NA	NA
	75	22.43 ± 0.1*	28 ± 0.22*	24.36 ± 3.21*	12.6 ± 2.1	NA
	100	22.31 ± 0.1*	29.96 ± 0.1*	25.47 ± 1.08*	12.7 ± 0.3	NA
Amoxiclave		74.33	50.66	74.66	35	73.46
Cefuroxime		76.66	64.66	65	36.66	72.88
Cefixime		76	67.66	73.66	28.66	69.30
Cefpodoxime		76.66	66.66	73.33	34.64	71.34



**Figure 2:** Comparison between red and white radish seeds extracts zone of inhibition with concentration

## DISCUSSION

The members of family Brassicaceae are rich in phytochemicals<sup>[31, 32]</sup>, and have potential medicinal roles including antimicrobial, antifungal, antimutagenic, antioxidant and antitumor<sup>[33]</sup>. Detection of chemical constituents of the radish seeds and pharmacological screening may provide us the basis for development of novel compounds.

This study demonstrates that seeds extract of Iraqi varieties revealed significant antioxidant activity especially at concentration of 150 mg/ml and above with p value >0.05. The percentage scavenging was directly proportional with increase in concentration of the seeds extract. These in vitro results should be confirmed in vivo as well as the mechanism by which it exerts its effects remains unknown, so the mechanism as well as chemicals responsible could be isolated and studied in future.

The results of the present study provide promising results and may enhance the use of potential *R. sativus* seeds extract in the treatment of various bacterial infections especially against *E. coli*, *S.aureas* and *P.aerugenosa*. Red radish was better than white radish at concentration of 75 and 100 mg ml<sup>-1</sup>, the same effect induced by red radish in *S. auras*, in *P. aerugenosa* both red and white reddish had similar effect on zone of inhibition, in *P valugaris* only at concentration 100 mg/ml red radish was better than white radish.

Ethanol extracts for both varieties are rich in flavonoids, phenolic compounds, Saponins and other secondary metabolites which are reported to have antibacterial activity against tested organisms<sup>[4]</sup>. More lipophilic flavonoids may disrupt microbial membrane<sup>[34]</sup>. Phenol and polyphenols present are known to be toxic to microorganism<sup>[35]</sup>. Exhibited antibacterial activity of *R. sativus* may be attributed to the diverse active compounds presents in it which either due to their individual or combined action.

## CONCLUSION

Phytochemical investigation of Iraqi *Raphanus sativus* L seeds extracts was performed and the results exhibited the presence of alkaloids, flavonoids, steroids, anthraquinoin and terpenoids in the both red and white varieties seeds and the absence of cardiac glycosides in this plant parts, also this study demonstrates that seeds extract have potent antioxidant and antimicrobial activity that proportionated with concentration of the sample used.

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