Stress response and health affecting compounds in Brassicaceae

Jahangir, M.

Chapter 8

Study of different biological activities of *Raphanus sativus* L. (red radish)

Muhammad Jahangir

Division of Pharmacognosy, Section Metabolomics, Institute of Biology,
Leiden University, Leiden, The Netherlands

Abstract

Brassicaceae vegetables are a good source of food around the world. Previously a diverse range of metabolites have been reported from this genus with regard to human nutrition. Extensive data are available on the biological activities of primary and secondary metabolites of *Brassica* plants, such as antioxidant, anticancer, and antimicrobial activity. For the active compounds include among others are phenolics, glucosinolates, and sterols. Among other vegetables *Raphanus sativus* L. (red radish) is an important member of Brassicaceae, well known as food and as a model system for plant research. Red radish extracts were evaluated for antibacterial, acetylcholine esterase inhibition, CB1 and Adenosine A1 receptor binding activity. Although showing to be little active, it is concluded that radish extract did not show significant activity for any of aforementioned assays. We can conclude that radish can be used as a food, rather than considering as a medicinal plant with higher pharmacological value.

1 Introduction

Several species belonging to the *Brassica* family have been used as food since time immemorial. In recent years it has been shown that these plants have a high content of diverse health-promoting antioxidant metabolites such as polyphenols [58], cinnamic acid derivatives,[354] flavonoids, vitamins etc.[55, 56, 89, 190] A part from nutritional value to human and animal populations, these phytochemicals have a role in the plant response to different stress factors.[19] Throughout the course of growth and development, plants are exposed to various environmental factors, both of biotic and abiotic character,[18, 27] which ultimately results in the quantitative and/or qualitative change in various primary and secondary metabolites.[19]

There is currently much interest in identifying phytochemicals with health promoting biological activity in food.[60] Glucosinolates and their hydrolysis products, common in cruciferous plants (Brassicaceae family) including *Brassica* species, were found to possess antitumor activities.[167, 223] Also phenolics as antioxidants and antimicrobial compounds are now well-known,[29, 173, 392] while antifungal activity of *Brassica* phytoalexins was also reported.[341, 346]

Radish (*Raphanus sativus* L.) contains such health promoting (poly)-phenolics, flavonoids and glucosinolates. In the present study radish leaves and roots extracts were tested for some biological activities with general interest, acetylcholine esterase inhibition in connection with treating the symptoms of alzheimer disease, CB1 and adenosine A1 receptor binding in connection with possible affects on appetite, obesity, anorexia, bulimia and also antimicrobial activity which has several potential uses. The aim of present work is to investigate the potential of bioactive compounds present in radish, for different biological activities.

2 Materials and methods

2.1 Preparation of plant material

Red radish (*Raphanus sativus* L.) plants were purchased from local market for acetylcholine esterase assay, while for the other bioactivities, the plant material was grown in green house conditions, until six week plant age. Fresh and healthy plants were selected and washed thoroughly with de-ionized water and kept in open air at room temperature for half an hour to remove water from plant surface. Roots as commonly consumed food were used for further fractionation.

2.2 Extraction of plant material

The dried plant material was extracted 3 times with 100% MeOH by ultrasonication at room temperature for 30 min. Extract was dried by rotary evaporator, resulting in 148 g of dry methanol extract. It was suspended in deionized water. This was partitioned successively with *n*-hexane and chloroform. The resulting fractions were taken to dryness. For different bioactivity assays, fresh plant material was produced every time. In case of acetycholinesterase inhibitors isolation a large scale extraction was done, with plant material purchased from the market. Leave and root extracts (chloroform and *n*-hexane) were analyzed for adenosine A1 and CB1 receptor binding activities.

2.3 Acetylcholine esterase inhibitory activity

Acetylcholine esterase inhibitory activity was evaluated by using the Ellman's reagent in combination with thin layer chromatography (TLC) as previously reported.[435] To determine the false positive activity the same method with a modification is used as previously reported.[436] Microplate reader (HTS 7000 Bio Assay Reader, Perkin Elmer, USA) was used to measure the absorbance at 405 nm for the enzyme reaction in the microplate assay as previously reported.[437] Fluorimetric method is used for the confirmation of the activity.[438]

2.3.1 Bioassay guided fractionation for acetylcholine esterase inhibitory activity.

The chloroform fraction was sub-fractioned on a silica gel column by a stepwise gradient of chloroform – ethyl acetate (1: 0 → 0:1), and final elution with methanol in the end for chloroform fraction. For the *n*-hexane fraction the eluent was a gradient of *n*-hexane – ethyl acetate (1:0 → 0:1), and final elution with methanol. Chloroform extract resulted in 142 sub-fractions, *n*-hexane extract provided 45 sub-fractions. These fractions were pooled according to evaluation with analytical TLC. The fractions showing AChE inhibition in the TLC method were further processed by preparative TLC. After each step an analytical TLC analysis was performed to check the activity including a test for false positives.

2.4 Adenosine A1 and cannabinoid CB1 receptors binding activities of radish extract.

Radish leaves and roots were evaluated for adenosine A1 (Table-1) and cannabinoid CB1 (Table-2) receptor binding activities. Extraction of plant material was done similar as for acetylcholine esterase inhibitory activity. Adenosine A1 receptor binding activity was done by the method as previously reported by our group,[439] while CB1 activity was done as reported by Horswill and others.[440]

2.5 Antibacterial activity

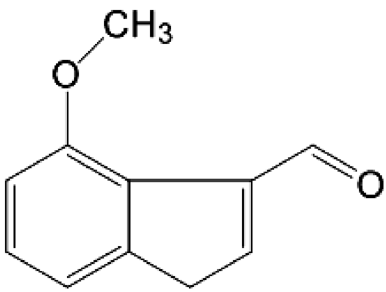
Bacterial growth inhibition by the extract (methanol-water: 1-1) of radish leaves and roots was evaluated by spectrophotometer (HTS 7000 Bio Assay Reader, Perkin Elmer, USA) at 590 nm by using 96 well plates, as reported in literature.[441] Methanol-water extract was evaporated and 80 mg/ml stock solution was prepared in milli-pore water. Series of dilutions (40, 20, 10, 5 and 2.5 mg/ml) were prepared to test the antibacterial activity. Different bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* and *Bacillus subtilis*) were used.

3 Results and Discussion

Brassica vegetables are considered to be a good source of phytochemicals for different bioactivities,[49] including antioxidant,[279] anti-inflammatory and antibacterial properties.[173] In our previous studies we found that particularly radish was rich in plant metabolites (see **chapter 4** and **7**, and **Table 1** in **chapter 8**) comparing to some other *Brassica* species. Therefore this species was chosen for further studies on biological activities.

Acetylcholine esterase inhibitory activity was done on radish roots and leaves. Chloroform and *n*-hexane fractions were found active after fractionation of the methanol (100%) extract of red radish roots. The TLC false positive test was done throughout the fractionation and isolation work. Two spots from chloroform fraction were identified as real positive, while all the spots from *n*-hexane fraction were found to be false positive for acetylcholine esterase inhibitory activity. These two compounds in chloroform fraction were identified as 4-methoxy-1*H*-indole-3-carboxaldehyde (4methoxy-I-3-C) (**Figure 1 A**) and 1*H*-indole-3-carboxaldehyde (I-3-C) (**Figure 1 B**), by using ^{1}H NMR, J-resolved, COSY and HMBC spectra. To validate the method galanthamine was used as reference compound, showing IC50 of 4.55 µM concentration. At the same conditions the IC50 of 4-methoxy-I-3-C was 6.604 mM and of I-3-C was 3.07 µM.

Acetylcholine esterase inhibitory activity is well reported for alkaloids,[442] but the presence of an aldehyde group in these compounds make the results suspicious, so these compounds were further assayed for the confirmation of the acetylcholine esterase activity by using the fluorimetric method as previously reported.[443] The identified compounds didn't show any activity by this method. So it is concluded that the activity assayed by Ellman's method was false positive, which was not detected by the false positive activity test, showing the limitation of Ellman's reagent. So at the end no active compound for acetylcholine esterase inhibitory activity was found in red radish.



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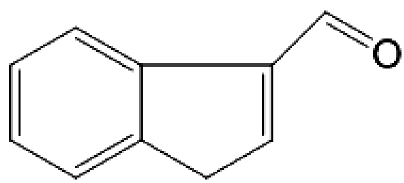

B

Figure 1: 4-methoxy-1*H*-indole-3-carboxaldehyde (A) and 1*H*-indole-3-carboxaldehyde (B)

Table 1 – Dry weight of plant material, methanol as crude extract, and extracts of radish leaves, radish roots, Brussels sprout and broccoli, after fractioned with *n*-hexane, chloroform and water.

	Radish leaves	Radish roots	Brussels sprout	Broccoli
Dry Weight (g)	50	50	50	50
Methanol (crude extract) (g)	12.675	25.1	18.046	14.941
n-Hexane (g)	2.531	0.410	0.536	01.007
Chloroform (g)	0.046	0.271	0.359	0.040
Water (g)	8.364	23.796	15.859	11.228

For the antimicrobial analysis, different concentrations of the radish root and the leave extract (Methanol: water – 1:1) have been evaluated. Positive (ampicillin) and negative (without any extract and ampicillin) controls were used to compare the results. Only a slight decrease in bacterial growth was observed in case of radish root extract, for *Staphylococcus aureus* (**Figure 2**).

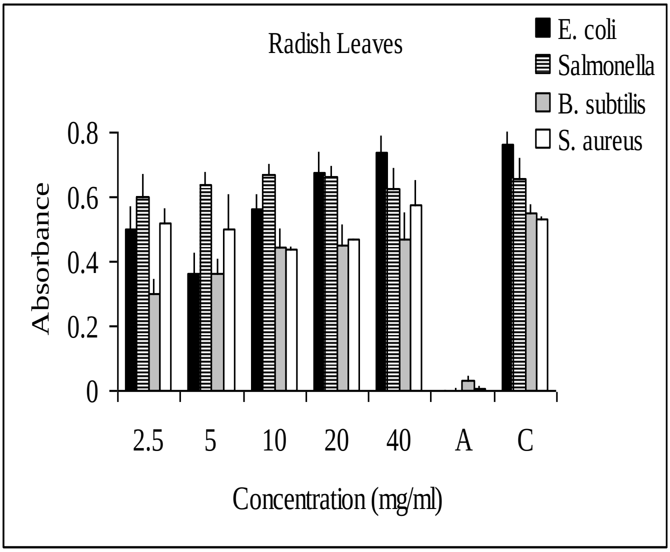

Figure 2 A: Antibacterial activity of *Raphanus sativus* (red radish) leaves, for four different strains of bacteria; *Escherichia coli*; *Salmonella typhimurium*; *Bacillus subtilis*; *Staphylococcus aureus*.

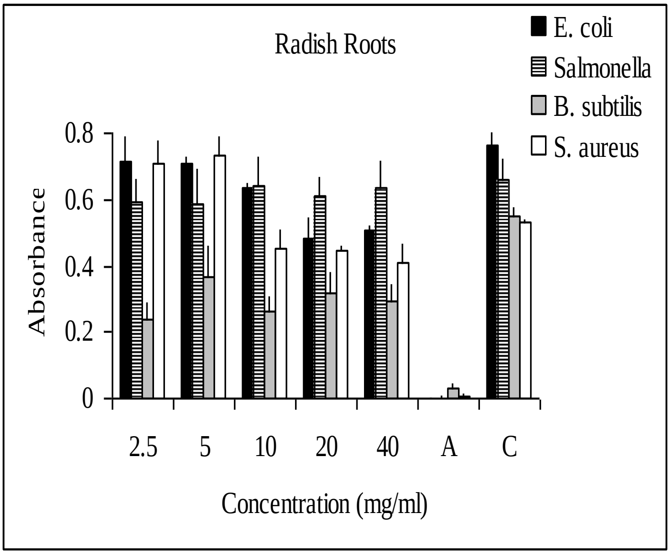

Figure 2 B: Antibacterial activity of *Raphanus sativus* (red radish) roots, for four different strains of bacteria; *Escherichia coli*; *Salmonella typhimurium*; *Bacillus subtilis*; *Staphylococcus aureus*.

The reduced growth as compared with negative control is not considered as significant as still at 40 mg/ml concentration bacterial growth was observed. Even in some cases, an increase in bacterial growth is observed for different extracts. May be a pure isolated compound or a group of certain compounds may show an antibacterial activity, but from these results it can be concluded that red radish is not a good source of antibacterial compounds.

Possible CB1 and adenosine receptor binding activities were evaluated as these activities are related with regulation of food intake and thus with obesity, and other food related diseases. Although some activity was observed in methanol crude extracts. The factions obtained by liquid-liquid partitioning showed relatively higher activities, with a relatively high activity in the *n*-hexane fraction. But from the ^{1}H NMR spectra of the *n*-hexane fraction it is concluded that it contains a high amount of unsaturated fatty acids, that may be responsible for the high activity of this fraction, as unsaturated fatty acids can bind to membranes, showing false positive results in receptor binding assays (**Table 2**).[444]

Table 2 – Adenosine (A1) and CB1 receptor inhibition activity for different fractions of radish leaves and roots.

	Adenosine A1 inhibition (%)		CB1 inhibition (%)	
	Radish leaves	Radish roots	Radish leaves	Radish roots
Methanol (crude extract)	8.5	9.3	3.1	13.1
n-Hexane	76.7	73.4	74.6	42.2
Chloroform	25.8	7.7	34	35
Water	28.2	36.1	48	10.6

4 Conclusion

It is concluded that although radish may have some bioactive compounds, quantitatively these compounds are not present in a major concentration. Either these compounds may not be present in sufficient amounts to ascribe radish for any medicinal use. But radish may affect human health by providing a good source of vitamins, sugars, amino acids and glucosinolates (**chapter 4**).

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