Parasiticidal activity of *Brassical oleracea var. botrytis (Cauliflower) on an ascaridae model*

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Abstract - Since ancient times up to present, herbal plants have been used in the treatment of various human diseases and infections. Ascariasis has been widely prevalent and remains a constant problem among humans especially in children. Parasitic worms or helminthes, such as Ascaris lumbricoides, is one of those organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease. This parasite is characteristically related to Ascaris suum which infects pigs and also humans. With this research determined the parasiticidal activity of Brassica oleracea var. botrytis (cauliflower) against Ascaris suum as a model. Extracts from the plant were obtained and were used tested against the test parasite in vitro. In addition, it was compared with pyrantel embonate solutions of different concentrations and was used as a standard. Results revealed the approximate lethal concentration of the plant at 0.00400 g/mL. The median lethal concentration of the extract is less than 0.00800 g/mL with a mortality rate of 53.33 percent. Statistical analysis of the experimental data revealed that there is no significant difference between the parasiticidal effect of cauliflower extract and pyrantel embonate against A. suum indicating the feasibility of the said plant extract as an anti-parasitic drug.

Keywords- antiparasitic, isothiocyanate, Ascaris suum, approximate lethal concentration, median lethal concentration, cauliflower

INTRODUCTION

Worm infestation causes consequences in relation to underprivileged management of the illness (Paterson et al., 2002). This is associated with the health of the youth particularly of the children (Ramasubramaniaraja and Niranjan Babu, 2010). Parasitic disease (Szewczuk et al., 2003) is a constant problem in the Philippines but it does not yet have a definitive control plan (Baldo et al., 2004).

Ascaris lumbricoides is one of the major causes of infection. Ascariasis affects 0.8 to 1.2 billion people worldwide and is a main cause of disease burden especially in developing countries with an estimated loss of from 1.2 to 10.5 disability-adjusted life years per infected person. The efficient support of public health interventions to eradicate the disease burden is worth attention and interest (Chun-Hai Fung and Cairncross, 2009). Anthelminthics is used to drive out parasitic worms from the body by killing them. Herbal or medicinal herbs are used to produce a similar effect as expected (Ramasubramaniaraja and Niranjan

Babu, 2010; Fahmy, et al., 2009). In addition, crops such as vegetables have also been common in the medical management of such infections (Volden et al., 2009a). They are also notable for their high carbohydrate, vitamin and mineral contents (Hanif et al., 2006).

A role in this process is taken part by the famous cruciferous vegetables (Sikora et al, 2008; Ayaz, 2008). The largest and most commonly utilized group of edible plants within the family Cruciferae is the vegetables under the genus of Brassica. The Brassica vegetables comprise cabbage, Brussels sprouts, broccoli, cauliflower, and the like (McNaughton and Marks, 2003). They are widely distinguished for their involvement to human nourishment and for other health benefits (Singh et al., 2007).

Cauliflower (Brassica oleracea var. botrytis) is a crucifer vegetable grown and consumed globally (Pedras et al., 2006). The edible part of cauliflower is a bud (Scalzo et al., 2007). Consumption of cauliflower is revealed to reduce the risks from a number of cancers (Hodges et al., 2006). The vegetable has also been proven to have antifungal activity particularly against C. albicans (Sisti et al., 2003). Moreover, antioxidant properties (Volden et al., 2009b) are confirmed to be present in cauliflowers (Scalzo et al., 2008; Ayaz et al., 2008). Furthermore, it is also an effective antimicrobial agent against a range of microorganisms (Hu et al., 2004; Luciano and Holley, 2009).

Glucosinolates (Kliebenstein et al., 2001) and flavonoids (Volden et al., 2009b) have obtained a large amount of interest due to their potentiality for reducing the risk of certain cancers and cardiovascular disorders. Glucosinolates (Song et al., 2005) are b-thioglucoside N-hydroxysulfates almost established to be a component of Brassicaceae plants (Volden et al., 2009b). Flavonoids, on the other hand, specifically refer to those phenolic substances which act typically as antioxidants, antimicrobials, and also demonstrate biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions (Pietta, 2000).

Cauliflower also produces glucosinolates, and when the plant material has been incorporated into the soil, myrosinase enzymes hydrolyze glucosinates into various volatile hydrolysis products (Spitz et al., 2000; Wittstock and Halkie, 2002). The most widely involved hydrolytic products are the isothiocyanates (Zasada et al., 2009). In the study of the anthelminthic effect of the aqueous extract of Carica papaya against Ascaris lumbricoides and Ascardia galli, benzyl isothiocyanate (von Weymarn et al., 2006) was responsible for such action. The metabolic and carbohydrate pathways as well as the neuromuscular coordination are targeted by this isothiocyanate causing paralysis of the agent (Mali et al., 2008).

The large roundworm Ascaris suum (Takamiya et al., 2009) in pigs can be used as a model (Dawson et al., 2005) for A. lumbricoides infections in humans to the following reasons. Firstly, both roundworms are very closely related ascarid species (Frontera et al., 2003). Secondly, both of them constitute natural host - parasite relationships, with migratory cycles that are thought to be similar since

there are no existing data that unequivocally demonstrate the contrary. Thirdly, the pig has been extensively used as a model in biochemical research because of the anatomical, physiological, immunological, metabolic and nutritional similarities between humans and pigs (Boes et al., 1998). Pyrantel pamoate (Hennessy et al., 2000) was recently used as a reference in experiments concerning parasitism. Ascaris suum was recently proven to cause infections not only to pigs but also to humans.

The rationale of this study is to determine the effectivity of cauliflower as a potential anthelminthic agent against the model, Ascaris suum. Ascaris suum was used as a representation of human ascariasis.

MATERIALS AND METHODS

Plant material

Brassica oleracea var. botrytis or cauliflowers were bought from a supermarket in Batangas City. Taxonomic identification was performed by botanists from the Herbarium of University of Santo Tomas, España Manila, where a voucher specimen has been deposited.

Preparation of plant extracts

One hundred grams of cauliflower in a frozen state were pulverized. The pulverized frozen cauliflower was placed in a sealed vessel made of glass and allowed to stand at 25° Celsius for three hours to effect enzymatic reaction. Then, 300 milliliters of hexane were then added thereto, and extraction was carried out for one hour while gently stirring. The hexane was removed thereafter by centrifugation. Then, three hundred milliliters of hexane were then added to the residue, and extraction and centrifugation were carried out in the same manner. The resulting residue was distilled under reduced pressure by means of an evaporator to remove the remaining hexane. Further, the residue was further freeze-dried to obtain the dry product (Murata et al., 2003).

Calculation of Percent Yield

Percent yield was calculated as follows:

$$\% \text{ yield} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

The Test Organisms

The pig roundworm, Ascaris suum, was used for evaluating the antiparasitic activity of the extract using a reference substance for comparison. The parasite was collected from Batangas City Slaughterhouse and Lipa City Slaughterhouse and was confirmed by a parasitologist from Research Institute for Tropical Medicine (RITM) in Alabang, Makati City.

In Vitro Preparation

Baldwin and Moyle Solution (BaMS) was used as the medium to sustain the pig roundworm in vitro. The rationale for this is that the Baldwin and Moyle solution appear to best mimic the conditions in the porcine intestine.

One hundred fifty one grams of NaCl (sodium chloride), 28.4 grams KCl (potassium chloride), 13.4 grams CaCl2 (calcium chloride), and 20.4 grams MgCl2 (magnesium chloride) were dissolved in two liters of distilled water, then, 27.2 grams of KH2PO4 (potassium phosphoric acid) in one liter of distilled water. One volume of KH2PO4 solution and four volumes of NaCl-KCl-CaCl2-MgCl2 solution were mixed and diluted with 35 volumes of distilled water. The pH was adjusted to 7.2 with 1M sodium bicarbonate (Austria and Villapando, 2006).

Collection and Selection of the Test Organisms

Worms were transported to the laboratory in a wide-mouthed container containing Baldwin and Moyle solution and were subjected to testing on the same day. Screening and sorting of the worms were done according to length and motility. Fifteen to forty centimeters-long worms were used in the study which corresponded to mature ascarids. For motility, adult worms exhibiting vigorous undulating reaction to the heat sensitivity test were used. The worms were placed in a dissecting pan, then, the posterior end of the worm was touched with the heated wire loop. A reaction or movement suggested that the worm was still alive. Only one person performed the heat sensitivity test to limit possible variations and error in data gathering.

Pig roundworms that met the criteria for length and motility were transferred to fresh Baldwin and Moyle solution and were incubated at 37° C for 30 minutes to establish the norms and body temperature in an artificial environment. The worms were again subjected after thirty minutes to the heat sensitivity test (Austria and Villapando, 2006).

Determination of Approximate Lethal Concentration

Twenty rectangular microwaveable containers were prepared, 10 for the cauliflower extract and 10 for the pyrantel pamoate. The preparation of the concentrations was done.

In preparing the concentrations for the extract, residues of 0.36 grams, 0.42 grams, 0.49 grams, 0.54 grams, 0.60 grams, 1.20 grams, 1.80 grams, 2.40 grams, 3.00 grams, and 3.60 grams were separately dissolved with 10 milliliters of Baldwin and Moyle solution and were further diluted to make 300 milliliters each of 0.0012 grams/milliliter, 0.0014 grams/milliliter, 0.0016 grams/milliliter, 0.0018 grams/milliliter, 0.0020 grams/milliliter, 0.0040 grams/milliliter, 0.0060 grams/milliliter, 0.0080 grams/milliliter, 0.0100 grams/milliliter and 0.0120 grams/milliliter solutions respectively using a volumetric flask.

The same procedure was done for the positive control but with different concentrations of 0.02 grams, 0.04 grams, 0.06 grams, 0.08 grams, 0.10 grams, 0.12 grams, 0.14 grams, 0.16 grams, 0.18 grams, and 0.20 grams which resulted to 0.00007 grams/milliliter, 0.00013 grams/milliliter, 0.00020 grams/milliliter, 0.00027 grams/milliliter, 0.00033 grams/milliliter, 0.00040 grams/milliliter, 0.00047 grams/milliliter, 0.00053 grams/milliliter, 0.00060 grams/milliliter, and 0.00067 grams/milliliter respectively.

The ten varying concentrations were placed separately in each container. Five pig roundworms were placed in each prepared concentrations.

These were observed for 36 hours.

The approximate lethal concentration was determined from the container that caused one death of the total number of worms (Austria and Villapando, 2006).

Determination of Median Lethal Concentration (LC50)

The determination of the approximate lethal concentration of cauliflower extract served as the basis for obtaining the median lethal concentration, two higher concentrations and two lower concentrations than that of the approximate lethal concentration were prepared.

A total of 33 rectangular microwaveable containers were used, 15 for the cauliflower extract, 15 for the positive control and 3 for the negative control. In the negative control, 300 milliliters of Baldwin and Moyle solution were placed. There were three replications per concentration. These were observed for 36 hours.

Median lethal concentration was determined from the container which caused 50 percent of the death of the total number of worms (Austria and Villapando, 2006).

Statistical Analysis

Calculation of Median Lethal Concentration

The analysis of the results of the toxicity of the experiment was based on the logistic concentration-response relationship.

Uncorrelated t-test

To test the validity of the null hypothesis, uncorrelated t-test was used. The data was programmed and subjected to STATISTICA version 5.0 (Austria and Villapando, 2006).

RESULTS AND DISCUSSIONS

Infections with gastrointestinal nematode parasites are extremely widespread and the most prevalent parasitic helminth in humans is Ascaris lumbricoides (Paterson et al., 2002). Herbal medicines are being studied to help reduce the inconvenience ascariasis brings.

The cauliflower is considered as a food of high nutritional value (Scalzo et al., 2007). Flavonoids and glucosinolates have been the focus of this research due to their potential as health-promoting phytochemicals.

Cauliflower Extract

One hundred grams of residue were obtained during the initial extraction which resulted to a brownish, rough powder weighing 8 grams.

Calculation of Percent Yield

The percent yield has been calculated as follows:

Table1.1
Percent Yield

	WEIGHT OF RESIDUE	WEIGHT OF SAMPLE	PERCENT YIELD
Crude Cauliflower extracts	8 grams	100 grams	8%

Approximate Lethal Concentration

The cauliflower extract exhibited toxic effect on one out of five worms in the concentration of 0.00400 grams per milliliter while the standard, pyrantel embonate exhibited the same effect on a lower concentration of about 0.00040 grams per milliliter after 36 hours.

Median Lethal Concentration

From the approximate lethal concentration of the cauliflower extract, two lower concentrations (0.00180 g/mL and 0.00200 g/mL) and two higher concentrations (0.00600 g/mL and 0.00800 g/mL) than the said concentration were used. The median lethal concentration of the cauliflower extract was most probably less than 0.00800 g/mL since 53.33 percent of the worms died at this concentration (Table 1.2).

Table 1.2
LC50 of the Cauliflower Extract against the test organism

CONCENTRATION (g/mL)	NUMBER OF DEAD WORMS WITH CAULIFLOWER EXTRACTS (36 HOURS)			PERCENT (%)
	1	2	3	
0.00180	0	0	0	0
0.00200	0	0	0	0
0.00400	1	1	1	20
0.00600	2	2	2	40
0.00800	3	2	3	53.33

For the standard, two lower concentrations (0.00027 g/mL and 0.00033 g/mL) and two higher concentrations (0.00047 g/mL and 0.00053 g/mL) than the approximate lethal concentration of 0.00040 g/mL, the median lethal concentration could be lesser than 0.00053 g/mL because at this, the researchers obtained 73.33 percent mortality (Table 1.3)

Table 1.3
LC50 of Pyrantel Embonate against the test organism

CONCENTRATION (g/mL)	NUMBER OF DEAD WORMS WITH PYRANTEL EMBONATE (36 HOURS)			PERCENT (%)
	1	2	3	
0.00027	0	0	0	0
0.00033	0	0	0	0
0.00040	1	1	0	13.33
0.00047	2	2	2	40
0.00053	4	3	4	73.33

Table 1.4
Difference of responses on organism using cauliflower extract and pyrantel embonate

Group Statistics

Test	N	Mean	Std. Deviation	Std. Error Mean
Actual cauliflower extract	5	3.4000	3.57771	1.60000
pyrantel embonate		3.8000	4.71169	2.10713

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
actual	Equal variances assumed	.556	.477	-.151	8	.884	-.40000	2.64575	-6.50111	5.70111
	Equal variances not assumed			-.151	7.462	.884	-.40000	2.64575	-6.57857	5.77857

Levene's test for equality of variances was also observed. It was found out that the obtained sig.value of 0.477 is greater than 0.05 which means that the variability in the two conditions is about the same, that the response in one substance does not vary too much more than the response in the second condition. The variability in the two conditions is not significantly different.

As to the result of t-test, the Sig. (2-Tailed) value is 0.884 and this value is greater than 0.05. The researchers therefore conclude that there is no statistically significant difference between the mean number of dead worms with cauliflower extract and pyrantel embonate. Table 1.4 revealed that the mean using cauliflower extract was nearly close when using pyrantel embonate. Researchers found out that the differences between the condition means are likely due to chance and not likely due to the substance used.

CONCLUSION

In the present study, the antihelminthic activity of the extract from Brassica oleracea var. botrytis against Ascaris suum was reported. The observations/ findings indicated the potential activity of the cauliflower extract as an antiparasitic agent against the test organism, Ascaris suum, as it is evident from this study that the said extract has produced acceptable results against the selected parasite. It may be safe to assume based from the findings that the parasiticidal activity of the cauliflower extract increases with an increase in its concentration. The parasites are more susceptible to the extract at a higher concentration.

With all the findings obtained, we therefore conclude that the cauliflower extract can be a favorable antiparasitic agent especially when used at higher concentrations against Ascaris suum.

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