e-ISSN 2320 –7876 www.ijfans.org
Vol.11, Iss.7, 2022
 UGC CARE Listed (Group -I) Journal

A REVIEW ARTICLE ON PRESENCE OF BENZYL-ISOTHIOCYNATE IN PAPAYA SEEDS

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Abstract

Different solvent and extraction method are used to extract phytochemicals from the seeds of Carica papaya. The GC/MS analysis of MeOH extract identified potential antibacterial compounds such as isothiocyanatomethyl benzene, 9-octadenoic acid, hexadecenoic acid and Beta-sitole. Different extraction method is by extraction, distillation, maceration, solvent extraction. Aqueous extracts prepared from heattreated seeds had no anthelmintic activity or benzyl isothiocyanate content although both appeared when these extracts were incubated with a myrosinase-containing fraction prepared from papaya seeds. A 10 h incubation of crude seed extracts at room temperature led to a decrease in anthelmintic activity and fractionated samples showed a lower benzyl isothiocyanate content relative to non-incubated controls. Benzyl thiocyanate, benzyl cyanide, and benzonitrile were not detected in any preparations and cyanogenic glucosides, which were present, could not account for the anthelmintic activity detected. Thus, our results are best explained if benzyl isothiocyanate is the predominant or sole anthelmintic agent in papaya seed extracts regardless of how seeds are extract regardless of how seeds are extracted.

Keywords: -Carica papaya, benzyl isothiocyanate, solvent extraction, glucosinolate, antibacterial properties, Anthelmintic, nematode, myrosinase,

Introduction

In South America (Roig y Mesa, 1974), India (Lal et al., 1976), and other parts of the world for centuries, papaya seeds have been utilized as a vermifuge (Werner, 1992). Human clinical studies have been resulted in seems at odds outcomes with Robinson (1958). Fernando claims that papaya seeds are efficient claimed they are not in 1959.

In vitro and in infected animals, seeds can efficiently destroy helminths (krishnakumari and Majumder, 1960; Dar. Unknown as of yet are the number and type of anthelmintic substances found in papaya seeds. Studies conducted in the past have demonstrated that seeds may be pulverized and extracted that have anthelmintic activity and include bioactive substances like benzyl isothiocyanate (BITC). To concentrate the bioactive material, diethyl ether could be utilized. Principles derived from water-soluble seed extracts, yet, while substances that was separated into diethyl ether or organic solvent layer's anthelmintic properties were demonstrated. Fractions formed by the initial extraction of the seeds with water or that partitioned to the water layer, which both create BITC, were reportedly never evaluated for anthelmintic activity.

Papaya seed extracts made by steam distillation were used to determine the effectiveness of this fraction against helminths (quoted in Ettinger and Hodgkins,1956). A volatile currency with little water solubility is BITC. It is not surprising that this molecule may be extracted from an aqueous solution at a fairly low temperature. However, the steam distillation that these researchers performed would not have identified any additional promising bioactive potentially heat-sensitive compounds, as a result various extraction methods and solvent have evidence that BITC can make extracts with bioactive qualities that are effective against helminths. Several alkaloids, such as carpaine and carpasemine, have been implicated in the anthelmintic properties of papaya seeds. BITC, which Panse and Paranjpe later recognized in 1943 as benzyl thiourea (Krishnakumari and Majumder, 1960; Tang, 1971). Ettlinger and Hodgkins (1956) reported that benzyl thiourea, a byproduct created during the purification of the bioactive component, was present in seed preparations at a concentration one-tenth that of BITC. Dar et al. (1965) then examined the bioactivity of BITC and benzyl thiourea separately and discovered that BITC was roughly 20 times more poisonous to Ascaris lumbricoides than benzyl thiourea. This study falls short of demonstrating that BITC is the only active anthelmintic ingredient in papaya seed extracts since it only tested chemicals that were already known to be present.(1-5)

Procedure for the papaya extracts

Process 1.

Carica papaya seeds were collected from mature fruits, cleaned to remove pulp content, shade dried and crushed to a fine powder, powdered dry seeds (100) g. At 27 degrees Celsius, it was soaked in petroleum ether (hexane fraction). Overnight at 1C, the solution was extracted several times. In vacuo, concentration the seed powder, which had been defatted. The MeOH solution

was concentration under vacuum after being extracted with 80 percent MeOH at room temperature. Steam distilled oil from fresh/dry seed was fractioned on a silica gel column chromatography with 5% EtOAC in petroleum ether, followed by preparation TLC (silica gel 0.5mm) in hexane: MeOH (99:01), By exposed the reference to the iodine vapour chromatogram. This is equivalent using a combination of methods, ands from the other set were retrieved. A ratio 1:1 ratio of MeOH:Et20 On PE- Wax and PE-1 capillary column, reactive compound was analyzed y GC. 60C for 4 minutes, then 60C to 200C in 5Cmin-1 increments, 10 psi nitrogen, 250C FID detected, injection 200C, 1:100 split ratio, and injection volume between .2 and 0.5µl. The data from its UV.IR and GCMS sensors was recorded. With a UV max of -255nm, it showed as a pale-yellow oil. Methanol extracts of the seeds had higher nematicide activity than petroleum ether expects, while steam distilled oil from both fresh dry seeds had the highest activity by far. When mortality was measured after 24 hours, steam distilled oil was 100% fatal to C. elegans percent in 50 rpm, compared to 5.2 percent in the untreated control. Steam distilled oil made up 0.2 to 1% of the dried seeds. Bioassay guided column chromatography and preparative TLC led to the isolation of a chemical that showed a single peak on GC (both polar and non-polar capillary columns) wit Rts 29.4 and 17.5 minutes, respectively. (6)

Process 2.

BITC could be extracted from papaya tissue, according to Brown et al (27). In test, hexane was found to the most effective solvent for extracting BITC from papaya fruit. The tissue from the peel, pulp, and seed samples were crushed in liquid N2 with a mortal and pestle. 1ml hexane was added to 1.5ml microcentrifuge tubes containing 125mg powered tissue. The BITC was extracted for one hour at room temperature with agitation. The supernatants were filtered via 0.45m membrane into the injection vials after centrifugation (10000g for 10 min at 25 C).

A gas chromatography (Helwlett-Packard model 5890) with an SPB-50 column (supelco, 30mts 0.25 m film thickness) was used to inject aliquots of1 liter of each extract. A ramp temperature program was conducted at 250 C while the port was held at that temperature. 70C (at first), then 4C min-1 to 150C. The sample were taken from. The helium carrier gas flow was 1ml, and the injection was spitless min-1. The detection was carried out utilizing a mass selective detector and a 70 eV ion source. The computation was carried out using a calibration curve with standard BITC solution ranging from 10 to 500 mL-1.(8)

Process 3

10g fresh papaya peel, pulp, and seeds were each added individually to 50mL of 0.002% (w/v) tween 80 aqueous solution. Before being transferred to 10mL volumetric flasks, the solution was homogenized using a JY-C92D.The inside wall of the juicer was cleaned three times and the moistening solution were combined in volumetric flasks. To make sample solutions, the foresides solution was diluted with the appropriate amount of .002% tween 80 solution. The needed volumes of standard were dissolved in a 0.2 percent (w/v) tween 80 aqueous solution to

produce a standard stock solution containing 3.2 mg/mL of BITC. The solution was kept at 4 degrees Celsius in the fridge. A consistent volume was achieved by placing 1 mLof the stock solution in the 10 ml volumetric flask and adding water. Then, at a concentration of 32 g/mL, a standard solution containing BITC was produced (with 0.002 percent tween 80).(9-11)

Process 4

The mucilaginous layer on papaya seeds was removed before extracting them. Aqueous extract was made by grinding seeds in a mortar and pestle with distilled water (1g seeds to 10mL water) art room temperature, then centrifuging at 16,000 g at 4C for 30 minutes to remove the debris. Seeds were pulverized in a mortar and pestle without any added solvent, then transferred to a thimble and extracted in a Soxhlet extractor by refluxing 10 times in 8 volumes of pentane. At 38C, the final pentane was concentrated in vaccum followed by Soxhlet extractor, a typical pf 14g of seeds generated about 0.9mL crude oil.

Oil (0.45ml) was poured into the matrix without any additional preparation in this case. Sample were eluted from the column at a rate of .7ml min 1 with successive 100ml volumes of 100 percent hexane, 85 percent hexane/15 percent dichloromethane of either aqueous or oil extracts. The residues were produced to a final volume of 500ml with hexane and stored at 18C after the eluted were evaporated in vaccum at42 C. To determine the potency of each fraction, aliquots (10-10ml) were evaporated to dryness and the residue diluted to 10 ml DMSO before being employed in the nematode killing bioassay.(12)

Process 5

BITC was also isolated from steam distillates of water-soluble papaya seeds extracts, and its anthelmintic efficacy was proven. Because BITC is both volatile and water insoluble, it's not unexcepted that this molecule can be distilled from an aqueous solution at a low temperature. These research's stream distillation, on the other hand, was successful. There is little convincing evidence that BITC is effective in producing extracts with bioactive activities against helminthics. Only one helminthics principle can be extracted. Papaya seed are used to make dish. Benzyl glucosinates, the predominant or probably only glucosinate present in papaya seeds, produces BITC. Many plants seeds contain glycosesinotes, which are metabolized to create isothiocynates y enzymes known as myronases(thio glucoside hydrolases), which are brought into touch with their substances(s) when seeds are damaged. Although a tiny fraction of substances and enzymes reside together in the embryo, myrosinase and glucosinates are found in different compartments of the papaya seeds (the endosperm and sarcotesta of the seeds, respecctive. As a result, papaya seeds must smash or otherwise broken in order to provide significant levels of the antibiotic BITC.(13)

Process 6

Ten-gram papaya seeds or pulp samples were homogenized in ceramic mortar with 10ml distilled water or 2 percent HCL-methanol. After 30 min incubation period, bacteria are ready to be tested. A total of 15ml of methanol was used to extract the residue twice, distilled water is also required. The filtrates, as well as the methanol and water extract, were investigate, then blend and fill with water to a volume of 100ml. 6microlliters 50 microliters. To quench w extract sample solution, 1 M HCL was added to 0.3ml OF IT. Previous to analyzing the content of myrosinase activity, isothiocyanates' (1 and 4methyl isothiocynates,4) and isothiocyanate (1 and 4 – methylthio-3 butenyl isothiocyanrte,4).

Three times using 150liters of n.hexane, the solution was extracted. The n-hexane extract was mixed, and the resulting extract was subjected to reverse phase HPLC to determine the concentration of isothiocynates in each sample, as well as cell tests to determined inhibitory activity.(14)

Conclusion and results

Process 1

Table I,shows the activity of BITC extracted from C. papaya seeds at various doses (5 to 50 ppm) after 24 hours towards juveniles of C. elegal1S and M. incognitt; its activity was also compared with carbofuran (25 and 50 ppm), a synthetic nematicide that is used commercially. The level of mortality varied with BITC concentration, and 25 ppm was entirely fatal to both species of nema todes, according to the results. These results were used to estimate its LD 50 value at 24 hours to be 17 ppm. When used at 25 ppm, carbofuran caused a minimal level of mortality. However, at 5 0 ppm it resulted in 100% and 75% death of juvenile M. incognita and C. elegans, respectively.
The aforementioned findings clearly show that carbofuran and BITC are both less effective nema ticides against both nematode species when used in vitro. The nematode mortality was determine d every one and a half hours after treatment up to five hours, then at 24 hours and 48 hours. The exposure to carbofuran at 50, 100, and 200 ppm and carbofuran at 25, 50, and 200 ppm as functi ons of their exposure periods were also evaluated. BITC needed 30 minutes at 50 parts per millio n and 2.5 hours at 25 parts per million to completely kill C. elegans, whereas it needed significan tly longer exposure times—2 hours and 3.5 hours at 50 and 25 parts per million, respectively— to completely kill M. incognita.
These findings support the assertion that, in vitro, BITC is a more effective nematicidal agent tha n carbofuran. When exposed to carbofuran, it was observed that the nematodes first lost their abil ity to move, but they remained coillike. Thus, treatment for mortality with carbofuran was slow a s opposed to BITC, where the death was abrupt as seen by nematodes instantly going straight. Th e results of the current research show that benzyl isothiocyanate is responsible for the excellent n ematicidal activity of C. papaya seeds toward both saprophytic and plant nematodes.
Typically, the hydrolysis of glucosinolates yields isothiocyanates in plants (Daxenbichler et at, 1

991). A glucoside of benzyl isothiocyanate from L. sativum, known as gluctoropeolin, was found to be toxic to these nematodes after 48 hours at 5000 ppm in the presence of the enzyme myrosi nase in a study on the nematicidal activity of specific glucosinolates from cruciferae towards the sugarbeet cyst nematode, Heterodera The results of the current study, however, indicate that BIT C is 200 times more harmful to M. incognita than gluctoropeolin was to H. schachtii. According to estimates, the waste produced by the papaya puree business makes around 22% of the total.

Process 2.

All three tissues examined during the development of the papaya experienced a general reduction in BG levels. But only in the fruit pulp did the BITC buildup brought on by the breakdown of the BG precursor take place. Overall, BITC was far less prevalent than BG during the ripening process. The substantial difference between the levels of precursor and product in the tissues may be explained by the fact that BITC is a volatile chemical. It would be straightforward for tissues to release BITC because myrosinase activity produces it.

There was a general decline in BG levels for all three tissues analyzed during papaya development. However, the accumulation of BITC due to the BG precursor's breakdown only occurred in the fruit pulp. Overall, during the ripening process, there was significantly less BITC than BG. The fact that BITC is a volatile substance may help to explain the significant discrepancy between the amounts of precursor and product in the tissues. Since myrosinase action causes the production of BITC, it would be simple for tissues to release it.

We believe that this is the first time the quantity of BITC in the papaya fruit's interior cavity has been measured. At least a portion of the overall BITC that may shield the fruit's from a fruit fly infection would come from the volatile BITC thar originated from the, and this source of BITC might be separate from the BITC made by the pulp or peel. The concept of using papaya fruit as an intriguing dietary source of benzyl glucosinolate and isothiocyanates is strengthened by the fact that BG levels in the pulp did not decline throughout ripening and that no thermal treatment is required before consumption.

Process 3

A papaya's 1 and 4 measurements. In order to inactivate myrosinase, we first identified the aqueo us preparations that produced the highest level of glucosinolate content after being microwaved f or three minutes. 1269 mol (564 mg)/100 g of fresh papaya seeds were present. This quantity in papaya seeds is comparable to that found in Karami daikon (1272 mol (579 mg)/100 g), a traditio nal wild strain of the Japanese white radish that has the spiciest flavor and the strongest antimuta genic properties of all the daikon strains. It is also significantly higher than that found in Brussels sprouts, as was previously reported.

The quantity of 1 in the whole papaya fruit was then determined after myrosinase was inactivate d with HCl. 100 grams of papaya seed had just 1.43 moles (213 grams) of 1. The enhanced 1 lev el in grated seeds that had not been subjected to HCl treatment was 461 mol (68.7 mg)/100 g, wh ich is close to the 3 level in grated Karami daikon [421 mol (66.9 mg)/100 g]. In the papaya seed preparations, no further hydrolysis products or hydrolysis product metabolites were found. The p apaya seed preparation also shown a strong hydrolysis activity toward 5 (4600 units/100 g), as w as predicted.
Compared to Karami daikon (541 units/100 g), this activity is substantially greater. These findin gs showed that the papaya seed is a more reliable source of glucosinolate, an isothiocyanate prec ursor as well as myrosinase. The grated papaya seed contains one as much myrosinase as the grat ed Karami daikon despite having ten times as much myrosinase. This suggests that 4 could be les s effective as a myrosinase substrate than 5 or 6. In contrast to the papaya seeds, the papaya pulp' s 1 and 4 contents and myrosinase activity were below the detection level, whether or not myrosi nase had been inactivated.

Process 4

The greatest concentrations of BITC were found in the seeds (238.0–683.0 g/g), followed by the peel (1.4–31.0 g/g), and the lowest quantities were found in the pulp (0.2–2.3 g/g). The statistical analysis was performed using SPSS 17.0 (IBM Corp., Chicago, IL, USA). Statistics showed that the levels of BITC in the three papaya fruit segments were statistically significant. The BITC concentrations in the pulp and peel were noticeably greater than those of other papayas, while the BITC content in the seeds was the lowest, when the seeds of immature papaya (S1) were white and not plump. The BITC concentration in the pulp and peel marginally rose as the papaya fruit grew, but the BITC level in the seeds increased. At this stage, the seeds were still white but plumper (S2)

As the seeds grew older and eventually became black, the BITC content in the seeds significantly increased while the BITC concentration in the seeds decreased (S3-S6)..BITC levels were highest and increased throughout fruit ripening in seeds, whereas they were lowest and decreased during frit growth and ripening in the pulp. This study's findings showed how BITC patterns varied during the course of three papaya fruit segments at varying stages of development. They mostly corresponded with the developing tendences outlined in prior research.

Process 5

Furthermore, a recent review, with the objective of developing a database for the glucosinolate content of cruciferous vegetables from 18 published studies providing 140 estimates for 42 items (25), demonstrated that the highest glucosinolate values were for cress (389 mg/100 g), whereas the lowest values were for Pe-tsai Chinese cabbage (20 mg/100 g). These data suggested that papaya seed is an equivalent or richer source of glucosinolate compared to cruciferous

vegetables. Next, we measured the amount of 1 in the intact papaya fruit with HCl treatment to inactivate myrosinase. With enzyme inactivation, 100 g of the papaya seed contained only 1.43 µmol (213 µg) of 1. In contrast, grated seeds without HCl treatment had an increased 1 level of 461 µmol (68.7 mg)/100 g, also comparable to the 3 level in grated Karami daikon [421 µmol (66.9 mg)/100 g].

The papaya seed preparations contained no additional hydrolysis products or hydrolysis product metabolites. Additionally, as predicted, the papaya seed preparation had strong hydrolysis activit y toward 5 (4600 units/100 g). Compared to Karami daikon (541 units/100 g), its activity is signi ficantly more potent. These findings showed that the papaya seed is a stronger source of myrosin ase as well as glucosinolate, an isothiocyanate precursor.

Process 6

During column chromatography, BITC copurifies with the anthelmintic activity of papaya seed e xtracts made with water or pentane. Furthermore, the bioactivity and BITC in the diethyl ether fr action are fully recovered with repeated extraction of aqueous extracts with diethyl ether. We obs erved that variations in preparations' nematode,
killing capacity were accompanied by variations in their BITC concentration. For instance, BITC content rises along with an increase in anthelmintic activity when heattreated seed extracts are c ombined with a soluble protein fraction that has myrosinase activity. Additionally, after 10 hours at room temperature, extracts lose some of their killing ability, which is accompanied by a drop i n BITC concentration.

There aren't enough cyanogenic glucosides in papaya seed aqueous extracts to be poisonous to n ematodes, and the fraction with the anthelmintic activity lacks any measurable BTC, BC, or BN. All these findings suggest that BITC is the primary and maybe the sole effective anthelmintic in t he papaya seed preparations we studied when taken into consideration with the prior research. Th is finding offers a more extensive analysis of potential candidate bioactive chemicals naturally pr esent in papaya seed extract and validates the prior hypothesis by Dar et al.(1965).

Preparation of papaya seeds for toxicity Our findings show that in our 500 ml experiment, it take s the equivalent of 1.2–2.4 mg of seed to kill >90% of the C. elegans in 4-
5 hours, and to eradicate all nematodes still present from the assay mixture in 12 hours. 3.1–
6.2 g of papaya seeds is an efficient dose for treating C. These in vitro viability results plus the pr esumption that the average adult human small intestine is 1.3 l in volume would be used to estim ate the viability of elegans in our assay (Masoro, 1973).
Despite the speculative nature of this dosage, mice and rats were found to not die from it (Chinoy et al., 1994), while children were shown to be effective against Ascaris at a dose that was 5–
10 times lower (Robinson, 1958).

Table 1.

Incubation mixture		Dosage tested in nematode viability test	
Main component[b]	Addition[c]	Volume (µl)	Nematodes killed (%)
Buffer	VoG50[d]	100	0
Extract of heat-treated seed in buffer	Buffer[e]	100	0
	VoG50	50	100
		100	100
	Boiled VoG50	100	0

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