Original Research Article

EFFECT OF DIFFERENT SOLVENTS ON PHYTOCHEMICAL EXTRACTION POTENTIAL AND ACUTE TOXICITY OF *CARICA PAPAYA* SEED

Comment [BE1]: Shouldn't the topic rather be; Acute toxicity studies and Phytochemical constituents of different solvents extract of Carica papaya

ABSTRACT

Aim: To investigate the effect of five extraction solvents of varying polarity, namely aqueous, methanol, ethyl acetate, chloroform and n-hexane on phytochemicals yield and composition of *Carica papaya* seed. The acute toxicity test of each solvent fraction was also carried out and the average weight of rats in each group was measured before and after the experiment.

Methodology: The phytochemical screening, both qualitative and quantitative was carried out using standard methods and procedures. Acute toxicity study was conducted by determining the LD_{50} of each extract.

Place and Duration of Study: Department of Biochemistry Laboratory, Faculty of Basic Medical Sciences, Bayero University Kano, Nigeria, from April 2018 to August 2018.

Results: The results shows that the higher the solvent polarity, the better the yield of extract thus the extract yield was higher in aqueous followed by methanol, ethyl acetate, chloroform and n-hexane in that order. Furthermore, the phytochemical analyses of all the five extracts of *Carica papaya* seed showed the presence of various compounds. The phytochemicals include flavonoids, alkaloids, tannins, saponins and cardiac glycoside in varying amounts. Anthraquinones was not detected in all the five extracts. The LD_{50} of the aqueous, methanol, ethyl acetate, chloroform and n- hexane extracts of *Carica papaya* seed in rat was greater than 5000 mg /kg body weight while the results of the weight changes shows that there is no statistically significant ($p>0.05$) difference in weight gain or weight loss in rats administered with either of the five extracts of *Carica papaya* seed as compared with the control rats.

Conclusion: It was concluded that *Carica papaya* seed contain bioactive phytochemicals which yield is highest when extracted with water and that the plant material could have clinical potential with safe therapeutic application.

Keywords: Extraction, Phytochemical, Acute toxicity, *Carica papaya* seed

1. INTRODUCTION

In Nigeria today, most rural communities depend on plant based products for phytochemicals to satisfy medicinal requirements. Plant products are generally considered safe and proven to be effective against various human ailments [1]. The world Health Organization (WHO) reported that more than 80% of the world's populations are believed to be dependent mainly on traditional medicine, which is largely obtained from plant [2]. This upsurge in the use of traditional medicine has given the practice a significant place in healthcare delivery particularly in developing countries and thus has led the WHO to advocate the application of scientific criteria and methods for proof of safety and efficacy of medicinal plants. The two resolutions of the WHO on essential drugs for member states from Africa namely resolutions AFR/RC49/R5 and AFR/RC50/R5 are classical examples aimed at encouraging medicinal plant research and promotion of its use in health care delivery systems [3]. Despite this, and many other interventions, data regarding phytochemical characteristics, safety and efficacy of medicinal plants are only available for few plants and these has limited its potentials in drug development by pharmaceutical companies [4].

Phytochemical analyses and acute toxicity test are important processes aimed at understanding the nature and safety of medicinal plants and information obtained from these techniques can provide data about plant that have shown to be efficacious. Phytochemicals are extracted from plants using different solvents in processes that are dependent on solvent polarity and solubility of bioactive compounds in the plant material (5). However the safety of the crude extracts cannot be ascertained since plants are known to produce toxic compounds as well [2] thus the need for toxicity analyses. In acute toxicity test, a single oral dose of the test substance is administered to animals to determine the gross behavior and the dose that can cause the death of 50% of the animals, called the $LD_{50.}$ It is usually expressed as the amount of chemical administered (e.g. Milligrams) per 100 g (for small

animals) or per kilogram (for bigger subjects) of the body weight of the test animal [6]. LD_{50} is the first step in the assessment and evaluation of the toxic characteristics of a substance [7].

Carica papaya is a fast growing tree-like herbaceous plant in the family *caricaceae* with four genera. The genus *Carica linn* is the most common and is the most widely cultivated species [8]. *Carica papaya* is believed to have originated from the lowlands of East-Central America [1] but presently grown in all tropical countries and many sub-tropical regions of the world including Nigeria. *Carica papaya* is a soft wooden perennial plant that has a life span of about 5-10 years, although commercial plantations are usually replanted sooner. Researchers have reported that *Carica papaya* seed has several therapeutic uses and is being used for centuries in folk medicine across Nigeria. Previous studies have documented anthelmintic activity of *Carica papaya* seed. Sapaat and co-workers reported that over 90% efficacy percentage against *Hymenolepis diminuta* in rats was observed following administration of1.2g/kg body weight of *Carica papaya* seed [9]. Aqueous extract of *Carica papaya* seed at 100mg/ml concentration was reported to have significantly inhibited bacterial activity against *Salmonella typhi* and other bacteria [10]. This research aimed to investigate the effect of different solvents on phytochemicals yield and composition and also determine the LD_{50} of each extract of *Carica papaya* seed. This is important as it will provide data on the type of bioactive compounds available in the plant material and the most desirable solvent for its extraction. It will also provide toxicity and/or safety information on the plant material

Comment [BE2]: *Carica papaya* may appear once and first time. Subsequently, *C. papaya* may subserve

Comment [BE3]: Same as BE2 above

Comment [BE4]: Same as BE2 above

2. MATERIALS AND METHODS

2.1 Plant Sample and Collection

Forty five matured unripe *Carica papaya* was bought in April, 2016 from Na'ibawa fruit market Kano, Nigeria. Taxonomic authentication of the plant was done by the department of Plant Biology, Bayero University Kano, Nigeria and was given accession number BUKHAN 0012.

Comment [BE5]: mature

2.2 Experimental Animal

Sixty six apparently healthy young male Wistar rats, each weighing between 120-150g were used for the study. The study was carried out at the animal house unit of the department of Biological Sciences, Bayero University Kano, Nigeria. All animal procedures were performed according to the guide for the care and use of laboratory animals of the National Institute of Health as well as the Animal Welfare Act. Prior to the experiment, the animals were acclimatized in the laboratory for one week and were maintained on standard pellet rat diet with free access to water.

2.3 Sample Preparation and Extraction

Each of the *Carica papaya*, was cut to remove the seeds which was washed with tap water, shade-dried and ground into fine powder with an electric blender. Maceration as described by Azwanida [11] was used. 500 g of the powdered dried *Carica papaya seed* was suspended in 1500 ml of each of the five solvents namely n- hexane, chloroform, ethyl acetate, methanol and water for 24 hours and shaken at regular intervals. Each of the extract was then sieved first with cheese cloth and then with Watman filter paper No 1. The filtrate in each case was concentrated to dryness in a water bath preset at 45^{o}C and was kept in the refrigerator at 4^{o}C until required.

2.4 Phytochemical Analyses

Qualitative phytochemical analyses of aqueous, methanol, ethyl acetate, chloroform and n-hexane extracts of *Carica papaya* seed were carried out using standard methods to detect which phytochemical is present (results not shown). In each case, where the presence of a given phytochemical was established, then the amount of that phytochemical was determined quantitatively. The tests are as shown below;

(i) Test for flavonoids

1.0g of each of the five extracts of *Carica papaya* seed was diluted with 200µL of distilled water separately followed by the addition of 150 µL of sodium nitrate (5%) solution. The mixture was then incubated for 5 minutes and 150 µL of ammonium chloride (10%) solution was added and made up to 5ml with distilled water. The mixture was shaken well and left for 15 minutes at room temperature. The absorbance was measured at 510nm. The total flavonoids were expressed as rutin equivalent (mg RE)/g extract on a dry weight basis using the standard curve [12].

(ii) Test for alkaloids

A total of 100ml of 20% acetic acid was added to 2.5g of each extract of *Carica papaya* seed in a 250ml beaker and covered to stand for 4hours. The mixture was then filtered and the volume reduced to one-quarter using a water bath. A concentrated ammonium hydroxide was then added drop-wise to the sample until the precipitate was complete. The whole solution was then allowed to settle and the precipitate was collected by filtration and weighed [13]. The percentage of total alkaloid content was calculated as;

$$\text{Percentage of total alkaloids (\%)} = \frac{\text{weight of residue (g)} \times 100}{\text{Weight of sample taken}}$$

(iii) Test for saponins

This was estimated based on vanillin-sulphuric acid colorimetric reaction with some modifications. 50µg of each extract was added with 250 µL of distilled water. To this, 250 µL of vanillin (800mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and mixed thoroughly and the solution was kept in a water bath at 60^{o}C for 10 minutes after which it was cooled in ice-cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract derived from the standard curve [12]

(iv) Test for tannins

500 µg of each of the five extract of *Carica papaya* seed were taken in a test tube separately and treated with 100mg of polyvinyl polypyrrolidone and 500 µL of distilled sample was centrifuged at 5000rpm for 5 minutes and 20 µL of the supernatant was taken. This supernatant has only simple phenolics free of tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannin content of the extract was calculated as;

Tannins (mg/g) extract) = total phenolics (mg GAE/g extract) – free phenolics (mg GAE/g extract) [14]

(v) Test for Cardiac glycosides

Cardiac glycosides of each *Carica papaya* seed extract were quantitatively determined according to Solich *et al* and was based on vanillin-sulphuric acid colorimetric reaction with some modifications [14]. 50µg of each extract was added with 250 µL of distilled water. To this, 250 µL of vanillin (800mg of vanillin in 10 ml of 99.5% ethanol) was added. Then 2.5 ml of 72% sulphuric acid was added and mixed thoroughly and the solution was kept in a water bath at 60^{o}C for 10 minutes after which it was cooled in ice-cold water and the absorbance was read at 544nm. The values were expressed as diosgenin equivalents (mg DE/g extract derived from the standard curve [15].

(vi) Test for anthraquinones (qualitative)

Carica papaya seed extracts (0.5 g) were shaken with 5 mL of chloroform. The chloroform layer was filtered and 5.0 cm3 of 10% ammonia solution was added to the filtrate. The mixture was shaken thoroughly and the formation of a pink-violet or red, yellow colour in the ammoniacal phase indicates the presence of anthraquinones [12].

2.5 Experimental Design for the Acute Toxicity

The acute toxicity study was conducted in accordance with Lorke's method [16]. According to the method, LD_{50} is given by the square root of the highest dose that did not kill, multiplied by the lowest dose that killed. The first stage involved the oral administration of three different doses of 10, 100 and 1,000 mg/kg body weight of the crude extract of each of the five extracts, to three different groups of adult male albino rats. In a fourth group, three adult male albino rats were administered with equivalent volume of distilled water to serve as control. All the animals were orally administered the extract using a curved needle to which a catheter had been fixed. The animals were monitored closely every 30 minutes for the first 3 hours after administration of the extracts and hourly for the next 6 hours for any adverse effects. Then the animals were left for 72 hours for further observations.

When no death occurred, the second stage of the method [16] was employed. For this stage, only one animal was required in each group. Groups 1-3 animals for each extract were orally given 1,600, 2,900 and 5,000mg/kg body weight dose of the crude extract while group 4 animal, was administered distilled water. All the animals were left for observation as in stage one.

3. RESULTS

3.1 Extraction and Percentage Yield of Carica papaya Seed

The crude extracts of *Carica papaya* seed from five different solvents of varying polarity were all brownish in colour with an offensive odour; it dissolves partially in distilled water. A total of 2500g of the ground seed powder of *Carica papaya* with 500g for each test was used and the cumulative weight of the extracts was 426.45g. For all the five extracts, polarity index of solvents is proportional to the yield of the extracts thus the extract yield was in the following order; aqueous> methanol > ethyl acetate > chloroform > n-hexane.

Table 1: Percentage yield of five extracts of *Carica papaya* Seed

Solvent used	Polarity index	Weight (g)	% Yield
n-Hexane	0.0	60.25	12.05
Chloroform	4.1	80.45	16.09
Ethyl acetate	4.4	90.85	18.17
Methanol	5.1	96.34	19.27
Aqueous	9.0	98.56	19.71

3.2 Phytochemical Contents of Different Solvent Extracts of *Carica papaya* Seed

The crude seed extracts of *Carica papaya* seed extracted using water, methanol, ethyl acetate, chloroform and n- hexane screened for the presence of some classes of phytochemicals (flavonoids, alkaloids, saponins, cardiac glycosides, tannins and anthraquinones) showed that flavonoids, alkaloids and saponins are prominently present in all the extracts in varying composition while other phytochemicals are present in relatively small quantities. Anthraquinones were not detected in all the extracts.

Table 2: Phytochemical Contents of Five Different Solvent Extracts of Crude *Carica papaya* Seed

Solvent	Polarity index	Flavonoids	Alkaloids	Saponins	Tannins	Cardiac glycoside
n-Hexane	0.0	34.04 ±0.08	16.20 ±0.02	26.78±0.04	0.04±0.02	1.97±0.02
Chloroform	4.1	36.76 ±1.02	21.62 ±0.06	36.76 ±1.04	0.14 ±0.60	1.96 ±1.02
Ethyl acetate	4.4	23.50 ±0.04	19.88 ±0.06	23.50 ±0.02	0.091 ±0.02	2.18 ±0.04
Methanol	5.1	38.68 ±0.42	37.62 ±0.24	38.64 ±0.02	0.03 ±0.06	1.20 ±0.04
Aqueous	9.0	35.85 ±1.02	37.26 ±0.04	35.86 ±0.04	0.09 ±1.02	0.84 ±0.60

n = mean of 3 tests±SD;

Anthraquinones = Negative

3.3 Acute Toxicity (LD_{50}) of *Carica papaya* Seed Extracts

All the animals orally administered with the crude extracts of *Carica papaya* seed obtained from the following solvent; n-hexane, chloroform, ethyl acetate, methanol and water up to 5000mg/kg body weight showed no signs of distress and were physically active, even 72 hours post administration. No death of animal was observed throughout the study.

182 Table 3: Determination of LD_{50} dose following oral administration of *Carica papaya* seed extracts

Solvent	Dose (mg/kg bw)	Mortality ratio	% Survival
Aqueous		0/3	100
	10	0/3	100
	100	0/3	100
	100	0/3	100
	1600	0/1	100
	2900	0/1	100
	5000	0/1	100
Methanol	10	0/3	100
	100	0/3	100
	1000	0/3	100
	1600	0/1	100
	2900	0/1	100
	5000	0/1	100
Ethyl acetate	10	0/3	100
	100	0/3	100
	1000	0/3	100
	1600	0/1	100
	2900	0/1	100
	5000	0/1	100
Chloroform	10	0/3	100
	100	0/3	100
	1000	0/3	100
	1600	0/1	100
	2900	0/1	100
	5000	0/1	100
n-hexane	10	0/3	100
	100	0/3	100
	1000	0/3	100
	1600	0/1	100
	2900	0/1	100
	5000	0/1	100

183 **3.4 Weight Changes**

184 Table 4 shows the result of weight changes in rats following the administration of aqueous,
185 methanol, ethyl acetate, chloroform, n- hexane seed extract of *Carica papaya.* Generally, there were
186 increases in weight of the experimental animals at the end of the each experiment

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Table 4: body weight of experimental animals before and after oral acute administration of *Carica papaya* seed extracts

		Treatment groups/ doses						
Extract	Weight (g)	Control (g)	10mg/ Kg	100 mg/kg	1000 mg/kg	1600 mg/kg	2900 mg/kg	5000 mg/kg
Aqueous	Before	123±2.45	133±3.22	143±2.47	137±3.44	145±4.21	138±2.11	147±2.67
	After	126±3,22	137±2.17	145±3.01	140±2.13	149±1.55	141±2.11	150±3,17
Methanol	Before	142±3.21	143±2,78	133±2.43	151±2.55	148±1.54	141±2.04	145±1.26
	After	145±2.25	151±2.34	143±2,37	153±2,04	149±1.32	146±2.13	149±2.30
Ethyl acetate	Before	139±2.11	147±2.28	137±1.45	141±2.10	139±2.3.42	143±2.15	143±2.03
	After	142±1.47	151±2.33	142±1.07	146±1.86	140±2.65	145±2.40	145±2.00
Chloroform	Before	145±1.33	145±2.11	143±1.68	151±2.12	137±3.45	141±2.14	151±4.33
	After	148±2.33	138±2.33	147±2.30	147±2.08	141±2.44	145±1.56	147±2.88
n- Hexane	Before	137±1.88	145±4.21	152±2.06	147±3.05	138±3.04	145±2.12	147±2.19
	After	142±2.33	146±2.45	151±2.76	148±2.36	141±2.07	147±3.45	151±3.21

4. DISCUSSION

Carica papaya is a known medicinal plant reported to possess health benefits against many diseases [17]. This health benefits are attributed to the presence of many bioactive phytochemicals in various parts of the plant [1]. In traditional herbal practice, water being a universal and more readily available solvent, is used to extract plant constituents however this practice was found to be scientifically inadequate as it was reported that chemical properties of solvents used in extraction of plant material influences the phytochemical composition of extracts [2]. Solvent polarity plays important role in determining the yield and chemical constituents of extract [4]. In the present study, five solvents namely, water, methanol, ethyl acetate, chloroform and n-hexane were used for the extraction of seed of *Carica papaya* and it was discovered that water gave the highest yield of extract followed by methanol, ethyl acetate and chloroform. Hexane, the most non polar of all the solvents, had the lowest yield. This finding is consistent with Ogbuehi *et al.* [18] who observed a proportional relationship between polarity of extraction solvent and the yield of extracts. Methanol is the best solvent for the extraction of flavonoids, alkaloids and saponins in *Carica papaya* seed however, this is not so with tannins which produced the highest yield when the highly non- polar n- hexane was used. This therefore could provide hint for researchers who wish to choose extraction solvent based on the targeted phytochemical of interest. For example, it has been reported that medicinal plants with nephroprotective properties mediates their protective effect via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids [19]. Therefore if a researcher requires *Carica papaya* seed extract with high amount of these phytochemicals, then the most desirable solvent is methanol.

Previous studies have reported the non-toxic nature of aqueous seed extract of *Carica papaya* with LD_{50} above 5000mg/kg body weight of rat [20]. This is similar to the findings of this research however this study went further to determine the LD_{50} for methanol, ethyl acetate, chloroform and n- hexane extracts which was found to be same as that of the aqueous extract of *Carica papaya* seed. This shows that all the five seed extracts of *Carica papaya* are safe for use in clinical practice.

There were generally slight increases in the calculated body weight of experimental animals before and after administration of extracts in all the treatment groups and control. Losses in body weight of experimental animals following administration of drug or toxicant are regarded adverse effect of drug and chemicals [21]. However, the present study recorded non-significant increases ($P>0.05$) in weight

of animals and this further suggest the safety of *Carica papaya* seed extracts. These slight increases in the weights of rats could be due to normal effect of food on animal since the animals were allowed free access to water and food following administration of the seed extracts of *Carica papaya.*

5. CONCLUSION

This study has provided data on the most efficient solvent for the extraction of *Carica papaya* seed to obtain a higher extract yield or the desired phytochemical for any biological or pharmacological study. It has also determined the LD_{50} of all the solvent extracts studied. This can serve as a prelude for further studies on the sub-acute and chronic toxicity effect of *Carica papaya* seed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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