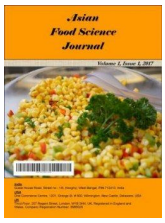

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Effect of Extracting Solvents on the Phyto-chemical Properties of Fermented Pawpaw (*Carica papaya L.*) Seed

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Authors' contributions

This work was carried out in collaboration between both authors. Author EAA designed and supervised the study while author MOA performed the laboratory and statistical analysis and also wrote the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Fruit seeds contain several phytochemical compounds which have been reported to show antiviral, anti-allergic, anti-inflammatory, anti tumor and antioxidant activities. Due to the varied chemical properties of these seeds, different solvents are used in the extraction. Therefore, this research was aimed at evaluating the effect of extracting solvents on the yield, phytochemical compounds and selected chemical properties of fermented pawpaw (*Carica papaya*) seed. Extracts from fermented T-solo variety of pawpaw seeds were obtained using petroleum ether, n-hexane and hot aqueous solvents using standard method. Analysis was carried out to determine some qualitative and quantitative phyto-chemical and some selected chemical composition (Peroxide Value PV and Thiobarbituric Acid Value TBA. Extracts' yield were 26.00, 23.30 and 19.51% for petroleum ether, n-hexane and hot aqueous solvent, respectively. The result of quantitative analysis shows that all the phytochemicals were more abundant in petroleum ether extract. The PV and TBA of n-hexane, petroleum ether and hot water extract were 5.32, 4.52, 0.00 meq/kg and 0.87, 0.16, 0.09 µg/kg

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respectively. The PV and TBA obtained for petroleum ether extract were relatively low and this implied that the extract will undergo little or no autoxidation or oxidative deterioration when used.

Keywords: Phytochemical; polyphenols; antioxidant; extraction; fermentation; autoxidation; seed.

1. INTRODUCTION

Fruits contain antioxidants of different types. They are naturally occurring and so are noted for their ability to protect the plants from various foreign attacks [1]. Antioxidants are compounds that protect foods against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. However, recent studies have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants. Like alpha-tocopherol (vitamin E), flavonoids contain chemical structural elements that may be responsible for their antioxidant activities [1]. There are several examples of antioxidants in fruits. These include polyphenols, phenolic acids, flavonoids and others. Polyphenols share the common phenolic feature, due to the structural diversity, these phytochemicals vary significantly in their physicochemical properties. Owing to the chemical complexity and the frequent occurrence of polyphenols in plants, extraction, separation, identification and analysis of polyphenols remain as challenging as ever, despite the recent advances in new instrumentation [2]. The challenge is multiplied when the complex glycosylation and polymerization patterns and the various food matrices are considered. While it is nearly impossible to develop a protocol for all polyphenols, there are some general approaches to these important aspects of polyphenol research [3].

Before polyphenols are extracted, samples containing these compounds must be collected, reserved and prepared properly. It is generally understood that samples (e.g., plants, foods, biological fluids) collected must represent the actual pool. Care must be taken to minimize the loss of compounds of interest during transportation and preservation of the samples. To avoid degradation of native polyphenols, samples are often dried or frozen before extraction because high moisture or water content aids enzyme activities [4]. Heating and exposure to light and oxygen may affect the polyphenolic composition in many cases; therefore high-temperature drying should be avoided as much as possible [3]. Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity [5]. Therefore, this work was aimed at evaluating the effect of extracting solvents on the yield, phytochemical compounds and selected chemical properties of pawpaw seeds;

2. MATERIALS AND METHODS

2.1 Materials

Matured ripe fruits of *Carica papaya* (variety T. solo) were obtained from Akintola Farm in Ogbomoso. The fruits were identified and authenticated at Department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso. The chemicals and reagents used were of analytical standard. The experiment was carried out in Food Science and Engineering Lipid Laboratory, LAUTECH, Ogbomoso,

2.2 Methods

2.2.1 Preparation of *Carica papaya* seeds

The methods of many researchers [6,7] were adopted with some modifications. The raw *Carica papaya* fruit was cut into two longitudinal halves. The seeds were removed and dehulled manually, the hulls were allowed to float and decanted to obtain the seed kernel. The seed kernels were pre-dried in the oven (Model, Uniscope SM9053) at 50°C for 20 h. The seeds were incubated at 37°C in a dark room, allowed to ferment for 72 h and dried in the oven (Model, Uniscope SM9053) at 120°C for 10 h. It was milled and packaged [7].

2.2.2 *Carica papaya* seed extract preparation

The extracts were prepared using three solvents: petroleum ether, n-hexane and aqueous solution.

2.2.3 Solvent extraction process

The method of AOCS [8] was adopted with little modification. Fermented ground *Carica papaya* seed (150 g) was placed in a cellulose paper cone

and placed in Soxhlet extractor. Petroleum ether (1.5l) was poured into the extractor. The heating mantle was set at 60°C, the extraction was for 6 h. This same process was repeated using n-hexane, as solvent. The extracts were concentrated by distillation and evaporated to dryness in rotary evaporator at 40°C. Yield of each extract was determined.

$$\% \text{ Yield} = \frac{weight\ of\ extract}{weight\ of\ sample} x\,100 \quad (1)$$

2.2.4 Aqueous extraction

The method of Puangsri et al. [9] was adopted with some modifications. Fermented ground *Carica papaya* seed (15 g) was poured into 250 ml conical flask and 150 ml of distilled water was added to give a ratio of 1:10 (w/v). It was stirred to form a mixture. The mixture was boiled for 10 minutes on a heating mantle and cooled immediately to room temperature in an ice water bath. It was filtered with Whatman paper. The filtrate was kept in refrigerator at 4°C until it was required for analysis.

2.2.5 Qualitative determination of some phytochemicals in the crude extracts

The presence of some phytochemicals such as alkaloids, tannins, saponnins, flavonoids, carotenoids, terpenoids, steroids and phenolics in the crude extracts was determined.

2.2.6 Alkaloids

One (1) g of each powdered sample was separately boiled with water and acidified with 5 ml of 1% HC1 on a steam bath. The solution obtained was filtered, then treated with 2 ml of the filtrate with few drops of the following reagents separately in different test tubes and observed. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicates the presence of alkaloids in the extract [10].

2.2.7 Tannins

One (1) g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. One (1) ml of cool filtrate was distilled to 5 ml with distilled water and two drops of 10% ferric chloride was added and observed for any formation of precipitates and any colour change. The reaction mixture was observed for a brownish green or blue-black colouration for the confirmation of the presence of tannins [10].

2.2.8 Saponin

One (1) g of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10 mins. The mixture was filtered while hot and allowed to cool. Two and half **(**2.5) ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 min; formation of froth which is stable for some minutes indicated the presence of saponin in the filtrate [10].

2.2.9 Flavonoids

One (1) g of each sample was separately boiled in 20 ml of water and then filtered. Five (5) ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4. A yellow coloration indicated of the presence of flavonoid [10].

2.2.10 Steroids

Two (2) g of each portion of the powdered sample was dissolved in 2 ml of chloroform.0.2 ml of concentrated was carefully added to form a layer. Reddish-brown colour at the interface between the layers indicates the deoxy-sugar characteristics of cadenolides which indicated the presence of steroid [10].

2.2.11 Anthraquinones

Five (5) ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5 min after which it was filtered. The filtrate was shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of anthraquinone [10].

2.2.12 Glycosides

One (1) mg of sample was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicated the presence of glycoside [10].

2.2.13 Terpenoids

Five (5) ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was added to form a layer. A reddish-brown precipitate coloration at the interface formed indicated the presence of terpenoids [10].

2.2.14 Carotenoids

One (1) g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids [10].

2.2.15 Phenolics

Half (0.5) g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 min brown coloration indicated the presence of phenol [10].

2.2.16 Oxalate

One (1) ml of sample was mixed with 2 ml of 2N H_2SO_4. A drop of an aqueous solution of 5.0 % (w/v) $MnSO_4$ was added, it was heated to between 70 and 80°C, it was titrated rapidly with standard 0.01 N $KMnO_4$ till persistent pink colour end point was achieved [10].

2.2.17 Phytic acid

Two grams (2 g) of sample was weighed in 250 ml conical flask. 100 ml of 2% concentrated hydrochloric acid was used to soak each sample in a conical flask for 3 hours. This was filtered through a double layer of filter paper, 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case to give proper acidity. Ten millilitres (10 ml) of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00195 g iron per ml. The end point was observed to be yellow and persisted for 5 min indicating the presence of phytic acid [10].

2.3 Quantitative Determination of Some Phytochemicals in the Crude Extract

2.3.1 Phenolics

One hundred (100) mg of the extract of the sample accurately weighed and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data was used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid [10].

2.3.2 Phytic acid

Two grams (2 g) of sample was weighed in 250 ml conical flask. 100 ml of 2% concentrated hydrochloric acid was used to soak each sample in a conical flask for 3 h. This was filtered through a double layer of filter paper, 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case to give proper acidity. Ten millilitres (10 ml) of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00195 g iron per ml. The end point was determined [10].

The percentage phytic acid was calculated as below

$$\% \text{ Phytic acid} = \frac{X \, x \, 1.19}{2} x \, 100 \quad (2)$$

Where X = Titre value x 0.00195

2.3.3 Saponins

The [10] procedure was used in saponins determination. Soxhlet extractor and two different organic solvents were used. The first solvent extracted lipids and interfering pigments while the second solvent extracted saponins proper. Five grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. Some quantity of petroleum spirit (boiling point 40–60°C) enough to cause a reflux was put into the flask. Extraction will continue for 3 h, to obtain the lipids and interfering pigments. The defatted material in the thimble was used for the second extraction for saponins. A fresh preweighed flask was fitted into the soxhlet apparatus (which will bear the thimble containing defatted sample) and methanol was put in the flask. The quantity of methanol was enough to reflux and flush for 3 h. The saponins were exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content were removed and the methanol recovered, saponins and little quantity of methanol was left in the flask. It was taken to an oven and kept at slanting position at a temperature of 70°C to evaporate the residual methanol. The flask and content was weighed and the difference between

the flask plus saponin and flask alone is the mass of saponins extract. Calculation

$$\% \text{ Saponins} = \frac{Mass\ of\ Saponnin\ in\ mg}{Mass\ of\ Sample} x\,100 \quad (3)$$

2.3.4 Tannins

Ten grams of each sample was weighed into a 100 ml conical flask and 50 ml of methanol was added. The flask was stoppered, shaken and left for 24 h. The content of the flask was shaken and the solid particles were allowed to settle. After filteration, the volume of the extract was measured. To 1 ml portion of the extract, 5 ml of fresh vanillin – HCl was added and the solution was left to develop colour in 20 min. The absorbance was measured at 500 nm against a reagent blank using corning 253 spectrophotometer. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions. The result of tannin is expressed in terms of tannic acid in mg/ml of extract [10].

2.3.5 Oxalate

The AOAC [11] procedure was used in oxalate determination. Five grams of the sample was weighed into a 100 ml beaker, 20 ml of 0.30 M HCl was added and warmed to (40 – 50°C) using magnetic hot plate and stirred for one hour. It was extracted three times with 20 ml of 0.30 M HCl and filtered into a 100 ml volumetric flask. The combined extract was diluted to 100 ml mark of a volumetric flask. The oxalate was estimated by pipetting 5 ml of the extract into a conical flask and made alkaline with a 1.0 ml of 5 M ammonium hydroxide. A little indicator paper was placed in the conical flask to enable us know the alkaline regions. It was acidified (2 or 3 drops of phenolphtalein indicator was added, excess acid decolourizes solution) by dropwise addition of glacial acetic acid. 1.0 ml of 5% $CaCl_2$ was then added and the mixture was allowed to stand for 3 h after which it was then centrifuged at 3000 rpm for 15 min. The supernatants were discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. Two milliliters of 3 M of $H_2SO_4$was added to each tube and the precipitate dissolved by warming in a water bath (70 – 80°C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.01 M $KMnO_4$ at room temperature until the first pink color appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was warmed to 70 – 80°C and titrated until a permanent pink colour that persisted for at least 30 seconds was attained.

2.3.6 Calculation

1 mL 0.05 M KMnO4 = 2.2 mg Oxalate (4)

2.3.7 Alkaloids

Five grams of the sample was dispersed in 10% acetic acid solution in ethanol to form 1:10 w/v dispersion. The sample was stirred every 30 min for 4 h. The mixture was filtered using Whatman filter paper. The filtrate was concentrated by evaporation over a water bath until it remained ¼ of the original volume. Concentrated ammonia solution was added in drops and the alkaloid in the filtrate was precipitated. It was filtered using a pre-weighed filter paper (Whatman). The filter paper and the precipitate was dried in the oven at the temperature of 60°C, cooled in a desicator and reweighed. The difference between the mass of filter paper plus the precipitate and the mass of filter paper alone gave the mass of the Alkaloids [10].

$$\% \text{ Alkaloids} = \frac{Mass\ of\ alkaloid}{Mass\ of\ sample} x\,100 \quad (5)$$

2.3.8 Flavonoids

The method was based on the formation of the flavonoids - aluminium complex which has a maximum absorptivity at 415 nm. 100 µl of the extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank sample was prepared from 100 ml of extracts and a drop of acetic acid, and then diluted to 5 ml with methanol. The absorption of standard rut in solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates [12].

2.3.9 Steroids

One hundred (100) ml water was added to 5 g of the sample. 0.1 M Ammonium Hydroxide was added to take the pH to 9.1. Two (2) ml of Petroleum Ether, 3 ml of Acetic Anhydride and conc. H_2SO_4 were also added. The absorbance was read at 420 nm. A calibration curve was

constructed using catechol solutions as standard and steroid content of the extract was expressed in terms of mg of catechol per gram of dry weight and standard graph [13].

2.3.10 Glycosides

One (1) g sample was extracted with 40 ml water and placed in the oven 100°C for 15 min. One (1) ml of the extract and 5 ml of water was added to 2 ml Glacial Acetic Acid and one drop of $FeCl_3$ together with 1 ml of Conc. H_2SO_4. Absorbance of the resulting solution was read at 410 nm. A calibration curve was constructed using catechol solutions as standard and steroid content of the extract was expressed in terms of mg of catechol per gram of dry weight and standard graph [13].

2.3.11 Anthraquinones

One (1) g of the ground sample was mixed with 25 ml water and boiled with 10 ml of sulphuric acid. Filter while hot and shake the filtrate with 5 ml of chloroform. Pipette the chloroform layer into another test tube. Add 1 ml of dilute ammonia. Absorbance of the resulting solution was read at 530 nm. A calibration curve was constructed using catechol solutions as standard and steroid content of the extract was expressed in terms of mg of catechol per gram of dry weight and standard graph [13].

2.3.12 Terpenoids

One (1) g of sample was weighed and mixed with 10 ml Petroleum Ether. It was extracted for 15 min and filtered. The Absorbance was read at a wavelength of 420 nm. A calibration curve was constructed using catechol solutions as standard and terpenoid content of the extract was expressed in terms of mg of catechol per gram of dry weight and standard graph [13].

2.3.13 Carotenoids

One (1) g of the sample was weighed into 20 ml Acetone. It was left for 1 h and filtered. 10 ml water was added to the filtrate. The filtrate was poured into a separating funnel. Five (5) ml of Petroleum Ether was added to the funnel allowing it to flow into it by the side of the funnel and left for some minutes to separate. The lower layer was discarded. The Absorbance was measured at 440 nm. A calibration curve was constructed using catechol solutions as standard and steroid content of the extract was expressed in terms of mg of catechol per gram of dry weight and standard graph [13].

2.4 Determination of Some Chemical Properties of the Crude Extract

2.4.1 Determination of peroxide value

Five (5) g of oil or fat was weighed into clean dry 250 ml glass-stoppered Erlenmeyer flask or conical flask stoppered. Thirty (30) ml of the acetic acid chloroform solution was added. The flask was swirled until the sample was dissolved in the solution and 0.5 ml saturated KI solutions was added. The solution was allowed to stand with occasional shaking for 1 min and 30 ml of distilled water was added. It was titrated with 0.1 N Na_2SO_3 until yellow colour almost disappeared. 0.5ml starch indicator solution was added while shaking the titration flask vigorously near the end point. Na_2SO_3 was added drop wisely until the blue colour disappeared [10].

2.4.2 Calculation

$$PV\ (m{-}.cq.of\ peroxide.100\ g) = \qquad (6)$$

Where

S = titration of sample (ml)

N = Normality of sodium thiosulphate

2.4.3 Determination of thiobarbituric acid value

Ten (10) g of the sample was macerated with 50 ml water for 2 min then it was transferred to distillation flask. 2.5 ml 4 N HCl was added to the mixture. Distillation was done at a rate that 50 ml of distillate was collected 10 min from the time boiling commenced. A 5 ml of distillate was pipetted into a glass stoppered tube. A 5 ml TBA reagent (0.2883 g in 100 ml of 90% glacial acetatic acid) was added, it was shaken and heated in boiling water for 35 min. A blank sample was prepared using 5 ml water for 35 min to cool the sample and blank tubes. Absorbance of the sample was measured against blank at 538 nm using 1 cm cells [10].

2.4.4 Calculation

TBA value (mg MA per kg sample)= 7.8 x A (7)

Where

A = absorbance of sample vs blank

MA = malonaldehyde

3. RESULTS AND DISCUSSION

The results of the yields, qualitative, quantitative phytochemical composition and some selected chemical properties of crude extracts of *Carica papaya* seed are discussed below.

Table 1. Effect of Solvent extraction on the yields of *Carica papaya* seed extracts

Solvent	Yield (%)
Hot Aqueous	19.51
N-hexane	23.30
Petroleum ether	26.00

3.1 Effect of Solvent Extraction on the Yields of *Carica papaya* Seed Extracts

Data on the effect of solvent extraction on the yields of *Carica papaya* seed extract is as shown in Table1. Solvent extraction process was carried out on seeds of T-solo Elongated yellow variety of *Carica papaya*. Three solvents were used for the extraction processes which were hot aqueous, n-hexane and petroleum ether. Petroleum ether gave the highest yield of 26.00% while n-hexane gave a yield of 23.30% and hot aqueous gave the least yield of 19.51%. This indicates that to obtain the best yield from T-solo Elongated yellow variety of *Carica papaya* seed, petroleum ether solvent is preferable.

3.2 Qualitative Results of the Phytochemical Compounds of *Carica papaya* seed

The preliminary result of phytochemical analyses of three extracts of *Carica papaya* using three solvents (hot water, n-hexane and petroleum ether) presented on Table2. The table shows that hot water extract did not contain tannin while n-hexane and petroleum ether extract contain tannin. Saponins were only found in hot water extract, flavonoid, phytic acid, oxalate, glycosides, steroids, terpenoids, phenolics, alkaloids, strobosteroids, carotenoids, anthraquinones and phlobatannins occurred in the three extracts. Carotenoids were detected in n-hexane and hot water extract. This is similar to work of Adeneye et al. [14] who did a preliminary phytochemical analysis of *Carica papaya* seed extract and detected the presence of alkaloids, flavonoids, saponins and tannins.

3.3 Quantitative Composition of the Phytochemical Compounds of *Carica papaya* seed

The quantitative phytochemical composition of crude extracts of *Carica papaya* seed as affected by different extracting solvents is as shown on Table 3. The tannin content of n-hexane extract was 0.33% while that of petroleum ether was 0.36%, this implies that petroleum ether extract has higher tannin yield which will be more preferable in terms of its ability to act as an antioxidant but in terms of its antinutritional property, n-hexane extract will be preferred because it contains lesser tannin. Tannins form complexes with protein to produce toxins which reduce digestibility and palatability [15]. The saponin content of hot water extract is 0.04% but it was 0.00% in other extracts.

The flavonoid content of hot water extract is 0.95% and was the least. There was a significant difference between the value of the flavonoid content of hot water extract and that of n-hexane

Table 2. Qualitative phytochemical composition of crude extracts of *Carica papaya* seeds

Phytochemicals	Hot aqueous extract	n-hexane extract	Petroleum ether extract
Tannins	-	+	+
Saponins	+	-	-
Flavonoids	+	+	+
Phytic acid	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Phenolics	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	+
Oxalate	+	+	+
Strobosteroids	+	+	+
Carotenoids	+	+	+
Anthraquinones	+	+	+
Phlobatannins	+	+	+

Key: + = present; - = absent

Table 3. Phytochemical composition of crude extracts of *Carica papaya* seeds

Phytochemicals	Aqueous extract	n-hexane extract	Petroleum ether extract
Tannin (%)	-	0.33±0.00	0.36±0.00
Saponins (%)	0.04±0.00	0.00±0.00	0.00±0.00
Flavonoids (%)	0.95±0.05	3.62±0.01	3.66±0.01
Alkaloids (%)	6.76±0.02	6.90±0.04	7.25±0.02
Phytic acid (%)	0.07±0.00	0.12±0.01	0.15±0.02
Steroids (%)	0.24±0.01	0.43±0.01	0.49±0.01
Terpenoids (%)	0.92±0.00	2.08±0.05	1.33±0.02
Oxalate (%)	1.29±0.04	2.51±0.00	2.33±0.08
Phenolics (%)	8.74±0.02	14.69±0.01	17.14±0.01
Glycosides (%)	3.11±0.04	6.40±0.01	9.17±0.03
Strobosteroids (%)	0.13± 0.01	0.18±0.03	0.28±0.05
Carotenoids (%)	17.5± 0.02	19.30±0.00	19.75± 0.02
Anthraquinones (%)	10.5± 0.01	19.50±0.00	21.50±0.04
Phlobatannins (%)	0.11±0.04	0.25± 0.00	0.38± 0.02

Values are means and standard deviation of triplicate determination

Table 4. Selected chemical properties of crude extracts of *Carica papaya* seed

Chemical property	Hot Aqueous extract	N-hexane extract	Petroleumether extract
Peroxide value(meq/kg)	0.00 ± 0.00a	5.32±0.05c	4.52 ± 0.16b
ThiobabituricAcid (µg/kg)	0.16 ±0.02a	0.87 ±0.10b	0.09 ±0.01a

Values are means and standard deviation of triplicate determination. Values with different letters within the same row are significantly different (p< 0.05)

and petroleum ether extract. Petroleum ether extract had the highest flavonoid content which is 3.66% while that of n-hexane was 3.62%. Flavonoid has been associated with antioxidative activities [3]. The phytic acid content of the extracts ranged between 0.15% and 0.07% with petroleum ether extract having the highest value while hot aqueous extract has the least value (0.07%). Alkaloid content ranged from 7.25% to 6.76%, petroleum ether extract has the highest value of 7.25%. The value of n-hexane extract is 6.90% while that of hot aqueous extract is 6.76%.

The steroid content ranged from 0.49% to 0.24%. Petroleum ether extract has the highest value while hot aqueous extract has the least and n-hexane has a value of 0.43%. n-hexane extract has the highest terpenoid content of 2.08% while petroleum ether extract has 1.33% and hot aqueous extract has the least value of 0.92%. Similarly, glycoside content ranged from 9.17% to 3.11% with petroleum ether extract having the highest value and hot aqueous extract has the least while that of n-hexane extract was 6.40%. However, in oxalate n-hexane extract had the highest value of 2.51%, petroleum ether extract gave a value of 2.33% while hot aqueous extract was 1.29%. It is evident that petroleum ether extract contains more phytochemical compounds than the other extracts so it is likely to have more antioxidant activities since antioxidant activities are associated with phytochemicals [16].

3.4 Chemical Properties of Crude Extracts of *Carica papaya* Seed

Data on some chemical properties of crude extracts of *Carica papaya* seed is as shown in Table 4. The peroxide and thiobarbituric acid values were determined to ascertain the evidence of rancidity or unsaturation of fats in the seed extracts. These two factors also determine level of oxidation or generation of aldehyde, which reduces the quality of oil [17]. The peroxide value of n-hexane extract of *Carica papaya* seed, was 5.32meq /kg which is much higher and significantly different ($p<0.05$) from that of petroleum ether which was 4.52 meq/kg while hot water extract has a peroxide value of 0.00. The Thiobarbituric Acid Value (TBA) of n-hexane extract was the highest (0.87 µg/kg) this is significantly different ($p< 0.05$) from that of hot water extract which is 0.16 µg/kg while that of petroleum ether extract (0.09 µg/kg) was the least.

Ibeto et al. [5] reported that the peroxide value of Jatropha oil seed was 1.93meq/kg, Shea nut oil 0.28 meq/kg and Arachis hypogaea oil was 22.2

meq/kg while the peroxide values of some non-edible oils were in the range of 4.36-9.82 meq/kg which is within the range of the peroxide values obtained for *Carica papaya* seed extract. This implies that petroleum ether extract can keep for some time without undergoing primary oxidation or autoxidation which cause rancidity. Afolabi and Ofobrukweta [6] recorded that TBA value of *Carica papaya* seed oil obtained from Ogun State was 0.451 µg/kg. Since, the TBA values obtained for the *Carica papaya* seed extracts are low, particularly, petroleum ether extract (0.09 µg/kg), it shows that the extract has a relatively low lipid peroxidation. Hence, it is likely to be rich in antioxidant and will undergo little or no oxidative deterioration during storage.

4. CONCLUSION

The result of the extraction shows that petroleum ether extract had the yield of 26%. The quantitative analysis shows that all the phytochemicals were more abundant in petroleum ether extract. The peroxide value and thiobarbituric acid value obtained for petroleum ether extract were relatively low and this implied that the extract will undergo little or no autoxidation or oxidative deterioration when used. This indicates that the extract can be a good preservative.

5. RECOMMENDATION

Petroleum ether is recommended as the appropriate extraction solvent for optimum yield of elongated T-solo variety. Equally, petroleum ether extract is preferable for the highest phytochemical composition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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