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Comparative Assessment of Vitamin Contents and Antifungal Activity of Seeds and Leaves of Unripe Pawpaw (*Carica papaya L.*)

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Abstract

The results of a comparative study on vitamin compositions of unripe *C. papaya* showed that vitamin A and C contents of aqueous and ethanol leaves and seeds extracts respectively, was higher than that of vitamin E. However, vitamin A and C contents in the aqueous leaves and seeds extracts respectively, was significantly ($p < 0.05$) higher than that of ethanol leaves and seeds extracts. The *in vitro* antifungal activity was examined against *Candida albicans, Aspergillus niger, Penicilliumnotatum and Rhizopus stolonifera* by using the surface plate method. The aqueous leaves extract observation of the fungi plates after 48 h of incubation showed clear zones of inhibition at higher concentrations and little or no zone of inhibition observed in *P. notatum*and *R. stolonifer* at lower concentrations of aqueous leaves and seeds extracts. Therefore, aqueous leaves extract is of great importance in the discovery of new drugs for the pharmaceutical industry.

Keywords

Antifungal activity, *Carica papaya*, Leaf extract, Seed extract, Vitamins

1. Introduction

Medicinal plants have been extensively used as sources of medicine for the treatment of various diseases and disorders [1]. These plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes fats, oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenol, glycosides, tannins, saponins, and polyphenols [2]. Bioactive compounds produced during secondary metabolism are implicated in plants' biological activities [2]. In recent times, antimicrobial activities of numerous plants have been carried out to confirm the use of these plants in the treatment of several diseases traditionally [3].

Carica papaya Linn belonging to the family Caricaceae is commonly known as pawpaw in English, Ibepe in Yoruba, or Okroegbe in Igbo (Nigeria). *C. papaya* is an herbaceous plant that originated from Costa Rica and Southern India [2]. Its fruits contain bioactive compounds that ensure their pharmacologic importance [4]. The seeds possess several pharmacological activities including anthelmintic, antifertility, contraceptive, anti-inflammatory, analgesic and antimicrobial properties [5]. The seeds and latex are for the treatment of gastrointestinal nematode infections with anthelmintic activity [6]. The unripe seeds and fruits have inhibitory activity against human enteric pathogens [7]. The *C. papaya* fruits and seeds had been shown to exert an antimicrobial effect on the potentially dangerous microorganism. The seed extract exerts antimicrobial activity against *Trichomonas vaginalis* trophozoites [8]. The seeds and pulp had been reported to show bacteriostatic properties against several enteropathogens like *Bacillus subtilis, Salmonella typhi*, and *Staphylococcus aureus*, and crushed pawpaw seeds were also found to exhibit clinical potential on Conjugal R plasmid transfer

from *Salmonella typhimurium*to *Escherichia coli in vitro* and *in vivo* of genotobiotic mice [9]. However, there is a dearth of information on unripe *C. papaya* leaves and seeds aqueous and ethanol extracts on vitamin contents and anti-fungal activity. Therefore, this study aimed to investigate antifungal activity and vitamin contents in unripe *C. papaya* leaves and seeds aqueous and ethanol extracts.

2. Materials and Methods

2.1. Plant materials

The *C. papaya* seeds and leaves were collected as a fresh sample from several and the same papaya plants at Amina-way Road, University of Ibadan, Ibadan, Nigeria. The seeds of unripe papaya were removed from the unripe fruits by cutting open the fruit with a clean sharp knife. The stalks were removed from the leaves. The leaves were then chopped and sliced into tiny pieces to increase surface area and aid drying of the leaves. The seeds and leaves were air-dried for the duration of four and two weeks respectively, in the Nutritional and Industrial Laboratory, Biochemistry Department, University of Ibadan. The leaves were ground to a fine powder using a blender, while the seeds were ground by a clean and dry commercial milling machine to a smooth powder. The seeds and leaves powders were weighed using an electronic weighing balance, and stored in an airtight container at room temperature.

2.2. Preparation of extracts

Two solvents were used to extract the biochemical constituents of interest in *C. papaya*. The two solvents were polar solvents with a relative difference in polarity. The selection of two solvents also allows for comparison and better selection. The solvents used were: aqueous solvent (distilled water) which is more polar and absolute ethanol (a less polar solvent).

2.3. Extraction Process

2.3.1. Aqueous extract

250 g of *C. papaya* leaves powder was soaked in 2,250 ml of distilled water in an airtight container, the ratio of mass to volume was 1:9. A 200 g of *C. papaya* seed powder was soaked in 1400 ml of distilled water in an airtight container, the ratio of mass to volume was 1:7

2.3.2. Ethanol extract

250 g of *C. papaya* leaves powder was soaked in 750 ml of absolute ethanol in an airtight container, the ratio of mass to volume was 1:3. A 200 g of *C. papaya* seed powder was soaked in 1000 ml of absolute ethanol in an airtight container; the ratio of mass to volume was 1:5. The mixtures obtained were subjected to intermittent agitation to allow proper mixing. The airtight containers containing the mixtures were placed in a dark room for three days. The extracts were filtered through What man filter paper no. 1, and the filtrates were concentrated under a rotary evaporator at a specific temperature (15-20°C). The extracts were stored at 4°C for further experimental use.

2.4. Vitamin Analysis

2.4.1. Vitamin A (Retinol) content determination

The method of Saleh et al. [10] was used to determine the vitamin A content of C. papaya. Briefly, 1 g of each leaf and seed powder was weighed and extracted with 5 ml of cold Acetone and 5 ml ethanol, until the total loss of pigmentation. Then 3 ml of distilled water was added which was later partitioned with 10 ml petroleum ether. The ether phase was passed through Neutral Alumina (activity III) packed column. The column was eluted with petroleum ether and the first band was pooled into a 25 ml volumetric flask. The absorbance was taken at 620 nm at an interval of 15 seconds and 30 seconds to determine vitamin A content.

2.4.2. Vitamin E (Tocopherol) content determination

The vitamin E content of the extract was determined according to the method described by [11]. A reaction mixture contained 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid in ratio1:1:1 respectively. A 0.3 mL of sample extracts were mixed with 3 ml of the reagent solution. At 95°C, the mixtures were incubated for 1 h after which the absorbance of the green phosphomolybdenum complex formed was determined at 695 nm against a blank. A mixture containing 0.3 mL methanol and a 3 mL reagent solution was used as a blank. Vitamin E was applied as the standard used at concentrations of 10-50 μg/ml. Each concentration was prepared in triplicates.

2.4.3. Vitamin C (Ascorbic acid) content determination

The amount of vitamin C in the analysed sample was determined by titration using the method described by Achikanu et al. [12]. About 0.5 g of the sample extracts were weighed and soaked with 0.4% oxalic acid (10 ml) for 10 min. in a test tube. Then centrifuged at 10,000 rpm for 5 min, and the solution was filtered. A 1 ml filtrate was transferred in

triplicates into a dry test tube, followed by the addition of 9 ml of 2,6-dichlorophenol indophenol. The absorbance was taken at 520 nm at an interval of 15 seconds and 30 seconds to determine vitamin C content.

2.5. Antifungal activity

2.5.1. Fungal strains

The fungi used in this study represent pathogenic species commonly associated with nosocomial infections. The fungi were maintained in the Pharmaceutical Laboratory at Pharmaceutical Microbiology Department, University of Ibadan. These include *Candida albicans, Aspergillus niger, Penicilliumnotatum and Rhizopusstolonifer*. All the fungi strains were sub-cultured from the original culture, stored at −70°C and maintained on Sabouraud Dextrose Agar plates at 4°C, and grown at 37°C when required [13].

2.5.2. Preparation of graded concentration of the sample

1.0 g of the sample was weighed and dissolved into 5 ml of the solvent of extraction for proper dissolution, from which 2.5 ml was taken into another 2.5 ml of the solvent until the 6^{th} test tube which was the last tube for the extract. The 7^{th} and 8^{th} test tubes were negative and positive control (solvent and tioconazole) for fungi serves as the control experiment.

2.5.3. Surface plate method (fungi)

A sterile Sabouraud Dextrose Agar (62 g/l) was prepared accordingly and aseptically poured into the sterile plates in duplicates and allowed to set properly. From the diluted organism (10^{-2}) 0.2 ml was taken into the prepared sterile nutrient agar using a sterile spreader to cover all the surface of the agar. The wells were made using a sterile cork borer of 8 mm diameter. In each well, the graded concentrations of the extract were introduced into wells including the controls. The plates were left on the bench for 120 min. to allow the extract to diffuse properly into the agar i.e. Pre-diffusion. The plates were incubated uprightly in the incubator for 48 h at 26-28°C.

2.5.4. Determination of minimum fungicidal concentration (MFC)

To verify that the extract was able to kill the fungi cells (fungicidal effect) the plates were also evaluated for MFC. Briefly, aliquots from each well from susceptibility testing assays were transferred to plates containing Sabouraud (SDA), which were then incubated at 37°C for 48 h. Results were evaluated by analysing the presence or absence of growth in the SDA [14].

2.6. Statistical Analysis

All experiments were conducted in a completely randomized design with three replicates for each treatment. The results are expressed as mean ± SD. using the Analysis of Variance (ANOVA) SPSS 20 statistical software. The data were considered significantly different at $P< 0.05$.

3. Results

3.1. Vitamins composition

The results of vitamins composition of unripe *C. papaya* seeds and leaves aqueous and ethanol extracts are shown (Tables 1, 2, and 3). The aqueous leaves extract has the highest content of vitamin A (63.94 ± 1.71 mg/100 ml), while the least vitamin A content was observed in ethanol seeds extract (5.36 ± 0.30 mg/100 ml). The aqueous leaf extract has a higher vitamin A content than ethanol leaf extract. Similarly, the aqueous seed extract (Table 1). The same trend was also observed in Vitamin C and E contents of *C. papaya* seeds and leaves aqueous and ethanol extracts (Tables 2 and 3). Comparatively, vitamin A content of *C. papaya* was observed to be highest in aqueous and ethanol leaves extracts than Vitamin C and E, while vitamin C content was highest in aqueous and ethanol seeds extracts. However, the least content of vitamin E was observed in leaves and seeds of aqueous and ethanol extracts as compared to vitamin A and C (Table 4).

Table 1. Vitamin A content of the C. papaya seeds and leaves aqueous and ethanol extracts

Sample	Vitamin A (mg/100 ml)
Aqueous leaf extract	63.94* ± 1.71
Ethanol leaf extract	60.94 ± 0.62
Aqueous seed extract	6.38** ± 0.14
Ethanol seed extract	5.36 ± 0.30

Values are mean ± SD, n = 3. *' ** Significant differences at $P < 0.05$ as compared to the ethanol leaf extract and ethanol seed extract respectively.

Table 2. Vitamin C content of the C. papaya seeds and leaves aqueous and ethanol extracts

Sample	Vitamin C (mg/100 ml)
Aqueous leaf extract	31.63* ± 1.00
Ethanol leaf extract	25.40 ± 1.29
Aqueous seed extract	11.30** ± 0.56
Ethanol seed extract	9.55 ± 0.48

Values are mean ± SD, n = 3. *' ** Significant differences at P < 0.05 as compared to the ethanol leaf extract and ethanol seed extract respectively.

Table 3. Vitamin E content of the C. papaya seeds and leaves aqueous and ethanol extracts

Sample	Vitamin E (mg/100ml)
Aqueous leaf extract	1.00* ± 0.08
Ethanol leaf extract	0.87 ± 0.06
Aqueous seed extract	7.41** ± 0.46
Ethanol seed extract	6.74 ± 0.17

Values are mean ± SD, n = 3. *' ** Significant differences at P < 0.05 as compared to the ethanol leaf extract and ethanol seed extract respectively.

Table 4. Comparison of vitamin contents in C. papaya seeds and leaves aqueous and ethanol extracts

Vitamin Composition	ASE	ESE	ALE	ELE
Vitamin C	11.30**** ± 0.56	9.55*** ± 0.48	31.63** ± 1.00	25.40* ± 1.29
Vitamin E	7.41 ± 0.46	6.74 ± 0.17	1.00 ± 0.08	0.87 ± 0.06
Vitamin A	6.38 ± 0.14	5.36 ± 0.30	63.94** ± 1.71	60.94* ± 0.62

Values are mean ± SD, n = 3. *' **'***'**** Significant differences at P < 0.05 as compared to the ethanol leaf extract, aqueous leaf extract, ethanol seed extract, and aqueous seed extract respectively. Where; ALE = Aqueous leaf extract; ELE = Ethanol leaf extract; ASE = Aqueous seed extract; ESE = Ethanol seed extract.

3.2. Antifungal activity

The inhibitory effect of aqueous extract of *C. papaya* seeds and leaves were evaluated against different fungal strains (*Candida albicans, Aspergillus niger, Penicilliumnotatum and Rhizopus stolonifera*). A well diffusion method was used to determine the antimicrobial activity of aqueous extract of *C. papaya* leaves (Figure 1). The activity of *C. papaya* aqueous seeds and leaves extract (100, 50, 25, 12.5, and 6.25 mg/l,) were quantitatively assessed based on inhibition zone, and results were compared with the activity of standard drug tioconazole and control (aqueous). High inhibition zones were observed in *C. albicans*, *A. niger*, *P. notatum*, and *R. stolonifera*, which was concentration-dependent. However, at low concentrations of the sample, no inhibition zone was observed in *P. notatum* and *R. stolonifera*. Little or no inhibition zone was observed in the aqueous extract of *C. papaya seed*s. The inhibition zones found in *C. papaya* aqueous leaves extract was higher compared to that of aqueous seeds extract (Tables 5 and 6).

Table 5. Antifungal activity of aqueous extract of C. papaya leaves

Treatment (mg/ml)	*C. albicans* (mm)	A. *niger* (mm)	*P. notatum* (mm)	*R. stolonifer* (mm)
100	18	16	14	12
50	16	14	12	10
25	14	12	10	-
12.5	12	10	-	-
6.25	10	-	-	-
Negative (aqueous)	-	-	-	-
Positive (tioconazole 10ug/ml)	38	40	38	40

Table 6. Antifungal activity of aqueous extract of C. papaya seeds

Treatment (mg/ml)	*C. albicans* (mm	A. *niger* (mm)	*P. notatum* (mm)	*R. stolonifer* (mm)
100	0.2	0.4	0.6	-
50	0.1	0.1	0.2	-
25	0.5	0.3	0.3	-
12.5	0.3	0.4	-	-
6.25	0.3	0.1	-	-
Negative (aqueous)	-	-	-	-
Positive (tioconazole10ug/ml)	38	40	38	40

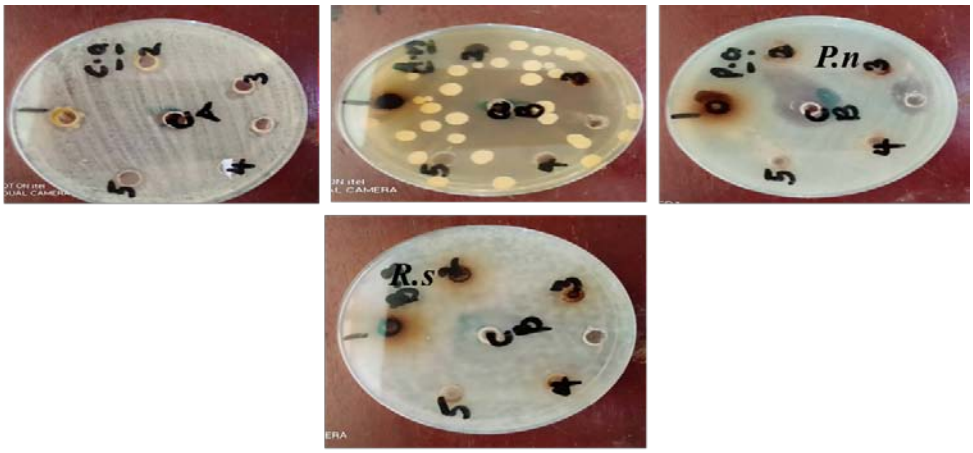

Figure 1. Culture media showing minimal inhibitory antifungal activity of aqueous C. papaya leaves extract.

Where 1, 2, 3, 4, and 5 are 100, 50, 25, 12.5, and 6.25 mg/l respectively, C= control (aqueous), B= (tioconazole).

4. Discussion

The use of plants to heal diseases, including infectious ones, has been extensively applied by people. Data from the literature as well as our results reveal the great potential of plants for treatment. The results of a comparative study on vitamins composition of aqueous and ethanol extracts of leaves and seeds of *C. papaya* showed that vitamin A composition of aqueous and ethanol leaves extract was higher than that of vitamin C and E. However, the vitamin A content in the aqueous leaves extract was significantly ($p < 0.05$) higher than that of ethanol leaves extract (Table 4). Similarly, the vitamin C composition of aqueous and ethanol seeds extracts was higher than that of vitamin A and E. However, the vitamin C content in the aqueous seeds extract was significantly ($p < 0.05$) higher than that of ethanol seeds extract. Meanwhile, the content of vitamin E in aqueous and ethanol leaves and seeds extracts was non-significantly ($p > 0.05$) as compared to vitamin A and C (Table 4). The high concentration of vitamin C in both aqueous and ethanol seeds extracts implies that for an individual with a deficiency of vitamin C upon consumption of *C. papaya* seeds, the vitamin C requirement of the body can be met. Vitamin C inhibits scurvy (a deficiency of vitamin C) a condition that leads to the lesion of the skin and blood vessel [15]. The concentration of vitamin A in the aqueous *C. papaya* leaf extract is higher than that of the ethanol extract but in general, their levels are high in both extracts. Vitamin A has many functions in the body which includes; visual cycle, regulation of gene expression and immune function [16]. Individuals who consume *C. papaya* leaf juice are like to have a high concentration of vitamin A in their body and have good immunity against foreign organisms [16].

The fungi plates were observed after 48 h of incubation, it was observed that there were clear zones of inhibition of some plates of higher concentrations for almost all the tested fungi and no zone of inhibition observed in *P. notatum* and *R. stolonifer* at lower concentrations of plant extracts (Tables 5 and 6). So also, in the aqueous seeds extract, there was little or no zone of inhibition observed in all tested fungi in all the plates at higher and lower concentrations as compared to the standard drug used (Table 6). Therefore, our results revealed the importance of *C. papaya* extracts when associated with antifungals, to control resistant fungi, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antifungals resistant fungi under very low concentration, thus minimizing the possible toxic effects. Even though the *C. papaya* extracts have not been completely investigated. Therefore,

more studies need to be conducted to search for new compounds.

5. Conclusions

Carica. papaya seeds and leaves aqueous and ethanol extracts contain a high amount of vitamins C and A, individuals who are deficient in these vitamins can be supplied with *C. papaya* leaf juice or soup of *C. papaya* seed to prevent the consequences which can be caused by the deficiency of these vitamins.

On the antifungal activity, the overall study furthers our understanding of the antifungal activity of *C. papaya* seeds and leaves extracts. Our results demonstrate and compare its activity against *C. albicans*, *A. niger*, *P. notatum and R. stolonifer*, it displays an even more potent activity against *C. glabrata*, and *A. niger* organisms that frequently show decreased susceptibility to conventional antifungals. The extracts may be used in combination with conventional antifungals, to improve the efficacy of treatment that even against the clinical isolates considered resistant to most conventional drugs commonly used in the treatment of various diseases.

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