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Full Length Research

Physico-chemical and antibacteria evaluation of oil extracted from ripe and unripe pawpaw seed (*carica papaya)*

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ABSTRACT: Crude oil samples extracted from seeds of ripe and unripe pawpaw **(***Carica papaya*) were evaluated for their physicochemical characteristics and antibacterial activities using standard methods. The chemical characteristics evaluated for oil from ripe and unripe pawpaw seed were Iodine value (g/100g) 52.44 ± 0.02 and 47.62 ± 0.01, Acid value (mg/g) 9.27 ± 0.01 and 13.30 ± 0.01, Free fatty acid (Oleic) (mg/g) 2.61 ± 0.01 and 3.80 ± 0.02, Peroxide value (mmol/kg) 5.40 ± 0.02 and 2.00 ± 0.02, Saponification value (mg/g) 121.90 ± 0.02 and 111.80 ± 0.02 respectively. The physical characteristics evaluated were Specific gravity 0.920 ± 0.01 and 0.923 ± 0.02, Viscosity (cp) 57.355 ± 0.01 and 58.760 ± 0.01, Refractive index 1.462 ± 0.01 and 1.463±0.01, Smoke point (^{0}C) 50.00±0.02 and 60.00±0.01, Flash point (^{0}C) 235.00 ± 0.01 and 240.00 ± 0.01, Fire point (^{0}C) 273.00 ± 0.01 and 284.00 ± 0.01 and %Oil yield 40.00 ± 0.02 and 43.50±0.02 respectively. Antibacterial activity evaluated were *Proteus mirabilis* with zones of inhibition of 0.50 ± 0.01mm and 0.40 ± 0.01 mm, *Klebsiella pneumonia* zones of inhibition with 0.60 ± 0.01 mm and 0.70 ± 0.01 mm, *Staphylococcus aureus* had 0.20 ± 0.01 mm for ripe pawpaw and no zone for oil from unripe pawpaw seed, *E. coli* (mm) and *Pseudomonas aeruginosa* (mm) were not susceptible to the oil. The results indicated good values for ripe pawpaw seed oil due to susceptibility of *Staphylococcus aureus* to the oil to unripe pawpaw seed oil and higher zone of inhibition in *Proteus mirabilis* to unripe pawpaw seed oil.

Key words: Antibacterial activities, oil, pawpaw seeds (*Carica papaya*), ripe, unripe.

INTRODUCTION

Seeds have nutritive and calorific values, which make them necessary in diets. They are also good sources of edible oils and fats (Odoemelam, 2005). In addition, seed oils were found to be of nutritional, industrial and pharmaceutical importance (Nzikou et al., 2010). Various vegetable oils are usually obtained from various sources. These include the common seed/vegetable oils such as soybean oil, castor oil, cottonseed oil, peanuts oil and sunflower oil. They also include oils such as palm oil, palm kernel oil, coconut oil, castor oil, rapeseed oil, etc. other less common but equally important vegetable oils include rice bran oil, tiger nut oil, patua oil, kome oil, niger seed oil, piririma oil and many others. The utilization of oil in various applications is largely determined by the yield, composition, physical and chemical properties of the oil (Aluyor et al., 2008). In Nigeria, the major sources of edible oil are peanut (Arachis hypogoea) and oil palm (Eloesis guineensis). These oils are used mainly as cooking oils, for the production of soap, margarine, and cosmetics (Ong et al., 1995). Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). These purposes vary from the use of rosewood and cedarwood in perfumery, to flavouring drinks with lime, fennel or juniper berry oil (Lawless, 1995), and the application of lemongrass oil for the preservation of stored food crops (Mishra and Dubey, 1994). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharma-ceuticals, alternative medicine and natural therapies

(Reynolds, 1996; Lis-Balchin and Deans, 1997). While some of the oils used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity, there are few published data for many others (Morris et al., 1979; Ross et al., 1980; Yousef and Tawil, 1980; Deans and Ritchie, 1987; Hili et al., 1997). Carica papaya Linn is commonly known for its food and nutritional values throughout the world. The genus Carica papaya Linn is the most widely cultivated and best known species of the four genera that belongs to a small family Caricaceae (Krishna et al., 2008). One of the most important fruits cultivated throughout the tropical and subtropical regions of the world (Anonymous, 2000). Papaya known by different names in the world viz. Arabic (fafay, babaya); Burmese (thimbaw); Creole (papayer, papaya); English (bisexual pawpaw, pawpaw tree, melon tree, papaya) etc. Green papaya fruit must be cooked (often boiled) prior to consumption to denature the papain in the latex (Odu et al., 2006). Ripe papaya fruits and papaya products are consumed by humans for their flavour and nutritional value (Saran, 2010). Unripe papaya fruits are consumed both as a cooked vegetable and processed products (Morton, 1987). The seeds are numerous, small, black, round and covered with gelatinous aril. Medical research in animals has confirmed the contraceptive and abortifacient capability of papaya. Its seeds have contraceptive effects in adult male Langur Monkeys, possibly in adult male humans (Oderinde et al., 2002). The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. The seed being consumed offers a cheap, natural, harmless, readily available monotherapy and preventive strategy against intestinal parasitosis (Okeniyi et al., 2007). Benzylisothiocynate present in seeds is the chief or sole antihelminthic (Kermanshai et al., 2001). There is increasing interest in the *C. papaya* seed due to its medicinal value. The seed has been shown to be a good source of oil (25.6%) that may be useful for medicinal, biofuel, and industrial purposes (Afolabi et al., 2011). The physicochemical properties of oils determine their quality and whether they are suitable for consumption (Fokou et al., 2009). Various parts of the plant have been used in traditional medicine in different parts of the world. The efficacy of treatment with *C. papaya* is dependent on the quality of the different compounds in the preparation. The quantity of the compound is also different in the fruit, latex, leaves and roots and varies with the extraction method, age of plant part, and the cultivation and sex of tree (Morton, 1987). In West and Central Africa, the fruit extracts are used by traditional healers for the treatment of hypertension and for the prevention of miscarriages in women (personal communication), and is known to produce uterine relaxation in the rat (reviewed by Eno et al., 2000). In the Northern Nigeria, a cold water decoction of the ripe fruit is used to control and calm mentally agitated individuals (Gupta et al., 1990). The unripe papaya has been shown to have antimicrobial and antioxidant activities (Osato et al., 1993). Moreover, the methanol extract of unripen fruit depressed the blood pressure and heart rate in rats (Eno et al., 2000). Many reports have shown the pharmacological properties of papaya latex with anthelmintic activity, antifungal action and bacteriostatic effects on a number of infectious organisms (Govindachari et al., 1991; Charoensiri et al., 2009). The latex has many applications in folk medicine. It is used as a styptic and vermifuge, an anti-chigger application and remedies for freckles, warts, corns, ringworm, infected wounds, malignant tumors and the pain of burns. Small dose of latex and sugar are taken as a digestive, emmenagogue and for whooping cough. In India and Malaya, the latex is smeared on the mouth of the uterus to induce abortion (reviewed by Morton, 1997). Cherian (2000) has shown that the crude papaya latex contains an uterotonic principle which can evoke sustained contraction of the uterus. Crushed leaves and seeds have been used for anthelmintic purpose and fever. In Ibo land and Ghana, the yellow red of the dried leaves is used to treat gastric problems (Gupta et al., 1990). Intraperitoneal injection of alcohol extract of papaya leaf was shown to be effective as sedative, central muscle relaxation and anti-convulsant property in rat (Gupta et al., 1990). Papaya seeds are used against intestinal parasite in humans and farm animals in India, Central and South America and elsewhere. The ethanolic extracts of papaya seeds have been shown to be effective against helminthes *in-vitro* (inside) of the infected animals (Wilson et al., 2002). Studies with the chloroform alcoholic and benzene extract of papaya seeds have shown their antifertility effects in male rats, mice and rabbits. The reversal of fertility occurred within 15 days to a month, and the compound was free of side effect (Lohiya et al., 2008).

Furthermore, the pentane extract of papaya seed caused relaxation of mammalian vascular smooth muscle (Wilson et al., 2002). Sripanidkulchai et al. (2001) reported that the decoction of papaya root is employed by practitioners of Northeast of Thailand for treatment of dysuria. A similar extract also showed diuretic activity in rats. The diuretic activity of *C. papaya* may be due to the high salt content of its extract (Sripanidkulchai et al., 2001) The aim of this research is to determine the physicochemical characteristics and antibacterial activity of the crude oil extracted from ripe and unripe seeds of papaya obtained from Owo in Owo local Government Area in Ondo State and Ootunja town in Ikole local Government Area in Ekiti State, and the objectives of this study are to investigate the potential use of this seed oil from pawpaw in food production and pharmaceutical products, to certify which oil from the two samples (Ripe and unripe seed oil) have good antimicrobial effect against the selected microorganism for curative purposes and also to serve as eye opener to the consumption of oil from seeds of pawpaw for its health benefits.

Table 1. Physicochemical characteristics of ripe and unripe pawpaw seed oil.

Parameters	A	B
Iodine value (g/100g)	52.44±0.02	47.62±0.01
Acid value (mg/g)	9.27±0-01	13.30±0.01
Free fatty acid (Oleic) (%)	2.61±0.01	3.80±0.02
Peroxide value (mmol/kg)	5.40±0.02	2.00±0.02
Saponification value (mg/g)	121.90±0.02	111.80±0.02
Specific gravity	0.920±0.01	0.923±0.02
Viscosity (cp)	57.355±0.01	58.760±0.01
Refractive index	1.462±0.01	1.463±0.01
Smoke point (^{0}C)	50.00±0.02	60.00±0.01
Flash point (^{0}C)	235.000.01	240.00±0.01
Fire point (^{0}C)	273±0.01	284.00±0.01
Oil yield (%)	40.00±0.02	43.50±0.02

± mean of triplicate results. A=Ripe pawpaw seed oil; B= Uunripe pawpaw seed

MATERIALS AND METHODS

Collection and identification of plant material

Fresh Pawpaw seeds from ripe and Unripe pawpaw were harvested randomly from different locations in Owo, Owo Local Government Area, Ondo state and Ootunja town, Ikole Local Government Area in Ekiti State Owo town to have a good representation of the samples in western part of Nigeria (Figure 1).

Preparation of the sample

The matured seeds from ripe and unripe Pawpaw seed (*carica papaya*) were removed freshly and the seeds separated manually. The particles were removed in order to have a clean seed. The seeds were Sundried for three days and milled mechanically by small milling machine in the market, and stored in a clean bottle for extraction process.

Extraction Procedure

A pre oven dried thimble was weighed with analytical weighing balance (w1). 10g of the powdered sample was added to it (w2), and put inside the soxhlet apparatus. 500ml boiling flask was filled to 2/3 with solvent (Hexane) and fitted to the soxhlet carrying the condenser to cool the solvent. It was heated gently and allowed to siphon after soaking the sample for 5 to 6 hours. After the extraction, the thimble with the extracted sample were removed and allow to dry at low temperature in an oven for few minutes, cool and weighed (w3). % crude oil was determined from each stage. The process was done up to five times until a required quantity of the oil has been collected. The oil was stored at room temperature until it is required for analysis.

Characterization of the Extracted Oil

The physical and chemical characteristics of the oil were determined by AOAC (2000) method. Figure 2 revealed the colour of the oil extracted from both samples

Source of Bacteria

Escherichia coli, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhii* and *Klebsiella pneumonia* were collected from department of microbiology, Federal Medical Centre Owo, Ondo state of Nigeria.

Antibacterial Screening Test of Pawpaw (*Carica papaya*)

The extracts were tested for their antibacterial properties using the agar – well technique (Pelczer and Black, 1993). The assay for antibacterial activities was carried out with *E. coli, S. aureus, Pseudomonas aeruginosa, Proteus mirabils, and K. pneumonia* respectively.

RESULTS AND DISCUSSION

The results of the physicochemical analysis of the oil (ripe and unripe pawpaw seed oil) were presented in Table1. The Papaya seed oils had low iodine value (52.44 g/100g and 47.62 g/100g) for the ripe seed and unripe seed oil respectively. These values were higher than the results reported by Abdulhamid et al. (2014), for pawpaw seed oil (24.87 g/100g), and sweet orange seed oils (37.08 g/100g).

The Papaya seed oil had 9.27 mg/g and 13.30 mg/g (ripe seed and unripe seed oil respectively) for Acid value, 2.61 mg/g (for ripe seed oil) and 3.80 mg/g (for unripe seed oil) for free fatty acids respectively. The value for the ripe oil seed compared favorably with values obtained by Abdulhamid et al. (2014) for pawpaw seed oil (9.46 mg/g) and sweet orange seed oil (7.59 mg/g) while that from the unripe pawpaw seed oil is higher than both reported respectively. The peroxide values in mmol/kg for ripe and unripe pawpaw seed oils were 5.40 and 2.00 respectively. The Peroxide value for ripe are relatively high compared to the Peroxide values for pawpaw seed (3.12) and sweet orange seed oil (2.21) as reported by Abdulhamid et al. (2014) but higher than the value obtained for unripe pawpaw seed. The free fatty acids and the peroxide values are valuable measures of oil quality and oil rancidity.

The saponification values for ripe pawpaw seed and unripe pawpaw seed oil were found to be 121.9 mg/g and 111.80 mg/g respectively. The saponification value for the two oils are higher than the saponification value of 24.31mg/g in pawpaw seed oil, but compared favorably with 106.30 mg/g in sweet orange oil (Abdulhamid et al (2014). This indicated that both oils could be used in soap making since their saponification values are high. Higher saponification value justifies the usage of fat or oil for soap production.

There is no significant difference in the refractive index of ripe and unripe pawpaw seed oil as indicated in the values (1.462 and 1.463).

The percentage oil yield of ripe pawpaw seed and unripe pawpaw seed oil were 40.00% and 43.50% respectively. The values are closely similar to that of percentage oil yield of pawpaw seed oil (40.10%) and sweet orange seed oil (43.10%) reported by Abdulhamid et al. (2014). However, the oil contents of the seed oil in the present study are higher than that of some conventional oil seed crops: Cotton seeds (15.0-24.0%), Soybean (17.0-21.0%) and Mustard seeds (24.0-40.0%) (Pritchard et al., 1991). Such variation in oil content across species and locations might be attributed to the environmental and geological conditions of varied regions (Manzoor et al., 2007). With this relative high percentage oil yields in the present study, the processing of oils for industrial, primarily soap production, as well as edible purposes would be viable.

The result for the antibacterial analysis of the seed oils (ripe and unripe Pawpaw seed oil) is presented in Table2. It is a measure of the extent to which the oils are susceptible to the bacterial tested, or whether they were resistant to the oils. Growth of *Proteus* was inhibited by the ripe seed oil up to a distance of ±0.50 mm while the unripe seed oil was ±0.40 mm. This showed that the antibacterial strength of ripe pawpaw seed oil is greater than that of the unripe pawpaw seed oil. The ripening nature may have influence on the chemical nature of the oil positively. *Klebsiella* has ±0.60 mm and ±0.70 mm for ripe and unripe seed oil respectively, indicating that both oil are resistant to *Klebsiella* invasion, but the unripe nature of the pawpaw may probably influence the effective chemical nature in unripe pawpaw seed oil over

Table 2. Antibacteria activity of oil extracted from ripe and unripe pawpaw seed

ORGANISMS	ZONE OF INHIBITION	
	A	B
Proteus mirabilis (mm)	0.50±0.01	0.40±0.01
Klebsiella pneumonia (mm)	0.60±0.01	0.70±0.01
Staphylococcus aureus (mm)	0.20±0.01	no zone
E. coli (mm)	no zone	no zone
Pseudomonas aeruginosa (mm)	no zone	no zone

± mean of triplicate results. A=Ripe pawpaw seed oil; B= Uunripe pawpaw seed.

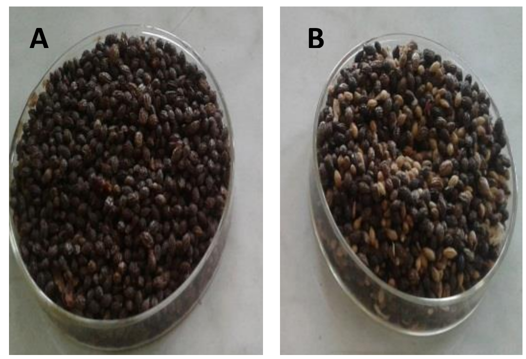

Figure 1. A, Ripe and **B,** Unripe Pawpaw seeds.

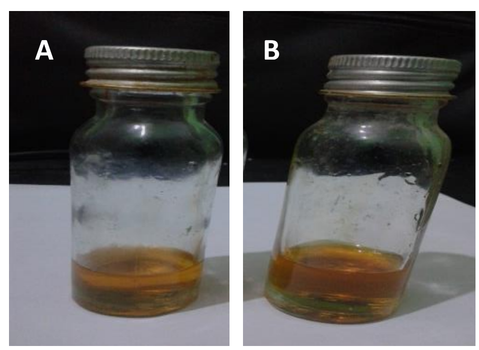

Figure 2. A, Ripe and **B,** Unripe Pawpaw seeds oil.

ripe pawpaw seed oil as indicated by its higher zone of inhibition. The ripe seed oil is resistant to *Staphylococcus* invasion while the unripe seed oil is not. The ripening nature may probably strengthen the chemical nature against *Staphylococcus aureus. B*oth oils have no resistance against *Escherichia coli* and *Pseudomonas.*

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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