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Phytochemical constituent, percentage yield and phenolic content estimation of different solvent system of Carica papaya leaves.

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Abstract

Carica papaya is an important medicinal plant used in the management of different disease conditions. Phytochemical screening was carried out using different chemical test, Percentage yield and total phenolic content was evaluated using Folin Ciocalteu method in different solvent system; methanol, ethanol, ethyl acetate, n-butanol and n-hexane respectively. The phytochemical screening of the studies showed the presence of flavonoids, saponins, tannins, terpenoids, glycosides, steroids, carbonhydrate, anthraquinone and alkaloids. The percentage yield of crude extract and total polyphenol content was high in methanol, ethanol and ethyl acetate when compared with n-butanol and n-hexane. The studies show that Carica papaya leave extracts is a potent source of secondary metabolites. The use of the plant in the management of diseases is justified.

Keywords: Carica papaya, Phytochemicals, Percentage, Total Polyphenol.

1. Introduction

Medicinal plants constitute the main source of new pharmaceuticals and healthcare products (Edeoga, Okwu, & Mbaebie, 2005). In industrialized countries, these plants have been extracted and used in the development of new drugs (Shrikumar & Ravi, 2007). They constitute the basis of health care throughout the world since the ancient days and are still widely used and have considerable importance in international trade (Patrick, Atangwho, Eyong, & Egbung, 2008). Approximately 20% of the world plants have been subjected to pharmacological or biological test and it could be said that natural products of plant origin are important source of constituents that could be developed into drugs, dyes, fragrance and pesticides (Hamburger & Hostattman, 1991). These plants contain secondary metabolites (alkaloids, flavanoids, saponins, steroids, tannins and phenolic compounds), vitamins and minerals which are bioactive compounds. The bioactive compounds have been shown to have antimicrobial properties, medicinal importance (Edeoga, et al., 2005) and physiological effect in animals (Edeoga and Eriata, 2001). They are widely distributed among different plant species (Tedong, Dimo, Dzeufiet, Asongalem, & Sokeng, 2006).

Pawpaw is native to tropical America, from Southern Mexico through the Andes of South America. It was spread to the south by Indians, and throughout the Caribbean with Spanish exploration. Carica papaya (pawpaw) belongs to the family of Caricaceae. Pawpaw is not a tree but herbaceous succulent plants that posses self-supporting stem (Dick, 2003). Pawpaw is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20years. The plants are male, hermaphrodite, or female (Bruce & Peter, 2008).The male trees are uncommon, but sometimes occur when home owners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self pollinated. The plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides (McLanghlin, 1992). The high level of natural self-defense compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1991). Papaya is rich in antioxidant nutrients such as carotenes, flavonoids and vitamin C as well as vitamin B (folate and pantothenic acid). It is also a good source of fiber and minerals such as magnesium.

Polyphenols are natural organic chemicals characterized by the presence of large number of phenol structural units (Quideau, 2011). Researchers and food manufacturers have become increasingly interested in polyphenols due to the recognition of their antioxidant property, abundance in our diet and their probable role in the prevention of various diseases associated with oxidative stress such as cardiovascular, cancer and neurodegenerative diseases. Polyphenols can be extracted from fresh, frozen or dried plant samples. Usually before extraction plant samples are treated by milling, grinding and homogenization, which may be preceded by air-drying or freeze-drying. Generally, freeze-drying retains higher levels of phenolics content in plant samples than air-drying. Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples. The solubility of phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used. Depending on the solvent system used during exaction, a mixture of phenolics soluble in the solvent will be extracted from plant materials.

2. Materials and Methods

2.1. Materials

Collection of Plant Samples

The present study was conducted between May and July, 2017 in Biochemistry Department, Kogi State University, Anyigba, in Kogi state, Nigeria. Fresh green leaves of pawpaw (Carica papaya) were obtained from the premises of kogi state university, Anyigba, Kogi State. The leaves was cut into smaller portion, sun dried for three weeks at room temperature and reduced to coarse powder using hand blender. The sample was packed into an air tight container before storage until required for further analysis.

Chemicals and reagents

Chemicals and reagents used were all of analytical grade. Folin ciocalteu phenol Reagent and Gallic Acid were obtained from Okey Medicals Kaduna, Kaduna State.

2.2. Methods

Extraction

Ten (10) gram of the grounded leaves sample was weighed into different conical flasks containing 100 ml of the extractants (methanol, ethanol, ethyl acetate, n-butanol and n-hexane). The contents of the different flasks were shaken and the tops was covered with aluminium foil and kept at room temperature for 48 hours (2 days) and filtered off using Whatman filter paper (Cat no 1001 125) of pore size 125mm. The filtrate was concentrated by drying in a water bath maintained at a temperature of 45^oC until a brownish black residue was obtained. These were kept in sealed containers and refrigerated at $2\text{-}4^oC$ until required.

Phytochemical Screening of the Extracts

The extracts obtained were subjected to phytochemical test screening using standard methods of (Trease & Evans, 1989; Odebiyi & Sofowora, 1978).

Percentage yield

The percentage of the extracts were determined as percentage of the weight of the extracts to the original weight of the dried sample used, using the formula;

$$\text{Percentage yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of sample}}$$

Total Phenolic

The total phenolic content was determined following the method of Amin, Zamaliah, and Chin (2004). The Folin-Ciocalteu reagent was diluted 10 times (2.5 ml) and mixed with 2 ml of saturated sodium carbonate (75 g/litre) and 6050 µl of sample and homogenized for 10 seconds and heated for 30 minutes at 45°C. The absorbance was measured at 765 nm after cooling at room temperature. The data obtained was calculated by comparison between a standard curve (212- 1062 µmol Gallic acid/litre) and the absorbance of each sample. The data obtained were expressed as mg Gallic acid equivalents per gram of extract.

Statistical Analysis

The results are presented as means ± Standard deviations. Differences between means were assessed using Analysis of variance (ANOVA) and post test using Dunnett multiple comparison test (Mead & Currow, 1982).

3. Results

3.1. Phytochemical Studies

The phytochemical studies reveals a remarkable presence of flavonoids, saponins, carbonhydrate, tannins, terpernoids, glycosides and steroids in methanol, ethanol and ethyl acetate extracts when compared with n-butanol and n-hexane extracts in which the bioactive compounds were only present in low concentartions. Anthraquinones and alkaloids were present in low concentrations in all the extracts (Table 1)

Table 1: Phytochemical Constituent of Different Solvent System of carica papaya leaves.

Phytochemical	Test	Methanol	Ethanol	Ethyl acetate	n-butanol	n-hexane
Flavonoids	Sodium hydroxide	+++	+++	++	+	+
	Lead acetate	+++	++	+	+	+
	Ferric chloride	++	++	+	+	+
Saponins	Frothing	+++	+++	++	+	+
Tannins	Ferric chloride	++	++	++	+	-
	Chlorogenic	++	+	+	-	-
	Formaldehyde	++	++	+	-	-
Terpernoids	Salkowski	++	++	++	+	-

Glycosides	General Test	++	++	+	+	+
Steroids	Salkowski's	++	+	+	+	-
	Lieberman Buchard's	++	++	+	+	-
Carbohydrates	Free reducing sugar	+++	+++	+++	+	+
	Molisch's	++	+++	+	+	+
Anthraquinones	Free and combined anthraquinones	+	+	+	+	+
Alkaloids	Mayer's	+	+	+	+	+
	Dragendorff's	+	+	+	-	-

Key: - Absent, + Present in low concentration, ++ Present in moderate concentration, +++ Present in high concentration.

3.2. Percentage Yield and Quantitative Estimation of Phenolic Compounds

The extraction yield of methanol and ethanol extracts was significantly (P<0.05) higher when compared to n-butanol and n-hexane extracts. The quantitative estimation of phenolic compounds measured as gallic acid equivalent showed a significantly (P<0.05) higher concentration of total polyphenol in methanol and ethanol extracts when compared to n-butanol and n-hexane extracts (Table 2).

Table 2. Percentage yields and total polyphenol of different extracts of carica papaya leaves.

Extracts	**Percentage yield (%)**	**Total polyphenol (Mg gallic acid/extract)**
Methanol	15.00^{b}	30.05 ± 1.20^{b}
Ethanol	14.50^{b}	29.59 ± 1.33^{b}
Ethyl acetate	12.00^{ab}	24.45 ± 1.24^{ab}
n-butanol	7.50^{a}	19.65 ± 1.33^{a}
n-hexane	6.00^{a}	18.95 ± 1.22^{a}

Values are expressed as mean ± SD of triplicate determination. Values in the same column with different letter subscripts are significantly different p<0.05

4. Discussion

The medicinal effect of plants in the management of diseases is attributed to the presence of the bioactive substances in them. The bioactive substances include flavonoids, saponins, tannins, terpernoids, glycosides, steroids, carbonhydrate, anthraquinone and alkaloids. These bioactive substances are known for their anti-inflammatory, anti-diabetic, anti-microbial, anti-atherosclerotic and anti-carcinogenic properties (Chukwuka, Ikheloa, Okonko, Moody, & Mankinde, 2011). The phytochemical present in the extracts may be responsible for the use of carica papaya in traditional medicine. Terpernoids are attributed for analgesic and anti-inflammatory activities and flavonoids have

been reported to possess many useful properties including anti-inflammatory, estrogenic, enzyme inhibition, antimicrobial, anti-allergic, anti-oxidant, vascular and cytotoxic anti-tumour activity (Harborne & Williams, 2000). Glycosides are known for their beneficial medical use in the treatments of congestive heart failure and cardiac arrhythmias (Makkar, 2007; Firn, 2010). Flavonoids and alkaloids have hypoglycaemic activity. Tanins isolated from Solanum trilobatum linn exhibited anti-microbial activity against Streptococcus pyrogens, Salmonella typhi, Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa (Doss, Mohammed, & Dhanabalan, 2009). Traditionally saponins have been used extensively as detergents, pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effect. Saponins have been reported to possess anti-hypercholesterolemic activity (Messina, 1999).
The differences in extraction yield are due to the polarities of different compounds present in the plant; such differences have been reported in literature concerning Vietnamese medicinal plants (Quang-Vinh & Jong-Bang, 2011). The highest yield was obtained with the most polar solvent (methanol) in an increasing order of polarities from n-hexane, n-butanol, ethyl acetae, ethanol and methanol respectively.
Phenols has structural requirement that is implicated in scarvenging free radicals and acting as food antioxidant (Kasote, 2013). In this studies, total polyphenol content of carica papaya is expressed in gallic acid equivalent/gram of extracts (Chaouche, Haddouchi, & Ksouri, 2013) since gallic acid is one of the major phenolic compounds that occurs in plants. Any plant sample containing high amount of gallic acid may be able to scarvenge free radicals (Gopakumar, Cherupally, & Krishnan, 2013). The result shows that solvent influences the extraction index of phenol compounds. The phenolic extracts of plant are a mixture of different classes of phenols, which are soluble in the solvents. Alcohol provides a good result for the extraction process (Zohra, 2011). Alcohol solutions are the best solvent for the extraction of polyphenols from carica papaya leaves; n-butanol and n-hexane are inefficient for the extraction of total polyphenol from the leaves of carica papaya.
In order to promote herbal drugs, there is an urgent need to evaluate the therapeutic worldwide drug sales based on natural products (WHO, 2000). Phytochemical screening of medicinal plants is very important to identify new sources of therapeutic and industrial important metabolites. It is important to initiate urgent steps for screening of Plants for secondary metabolites.

5. Conclusion

Carica papaya is used in traditional medicine to combat and cure ailments, and it is found to be rich in secondary metabolites. The presence of secondary metabolites may be attributed to their curative properties.

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