Journal of Pharmaceutical Research International

Volume 35, Issue 36, Page 21-28, 2023; Article no.JPRI.111076
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Assessment of Secondary Metabolites and Thin-Layer Chromatographic Analysis of *Carica papaya* (Caricaceae) Leaves Ethanolic Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2023/v35i367489

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111076

Original Research Article

Received: 17/10/2023
Accepted: 21/12/2023
Published: 30/12/2023

ABSTRACT

Carica papaya, also referred to as pawpaw, is a tropical fruit-bearing tree in the Caricaceae family. Papaya is one of the world's most extensively grown crops. Its fruits, seeds, and leaves are widely utilized in cooking and traditional medicine. Papaya leaf extract, tea, and juices are extensively used to cure ailments and promote health. The study aimed to quantify the presence of potential chemical compounds in *Carica papaya* leaves using Thin-Layer Chromatographic analysis (TLC).

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Standard qualitative phytochemical tests were performed on the leaves of *C. papaya* to determine the presence of various phytochemicals, and TLC analysis to ascertain the presence of potential chemical constituents. Extract of *Carica papaya* leaves in ethanol showed the presence of tannins, alkaloids, glycosides steroids, proteins, flavonoids, carbohydrates, etc., after the phytochemical screening, all of which are useful chemical ingredients responsible for a variety of pharmacological effects. The lack of color change noticed when the heated sample was treated with Fehling's solution revealed the absence of reducing sugar. TLC analysis using several solvent systems revealed the presence of potential chemical constituents with varying Rf values. This variance in the Rf values showed the phytochemicals and potential chemical constituents' polarity and this gave a hint on the type of solvent systems suitable for the analysis and separation of pure components. Hence, *Carica papaya* leaf extract contains a high concentration of both primary and secondary plant metabolites. TLC profiling yielded a good result, suggesting the existence of numerous possible chemical ingredients in the plant leaves used for the analysis.

Keywords: Carica papaya; phytochemical analysis; secondary metabolites; thin layer chromatography.

1. INTRODUCTION

Traditional herbal medicine development and utilization can be traced back to the Stone Age [1]. Traditional healing and magic or incantations appear to be significantly more common than Western medicine [2]. Traditional medicine in Africa is an accepted form of medical care, with some components in herbalism, divination, and spirituality [3,4].

Herbal medicine is the utilization of herbal products, herbal supplements, or finished herbal products including whole plants, plant parts, such as leaves, bark, fruits and vegetable flowers, and roots, or their extracted active ingredients intended for human or animal medicinal use, according to the World Health Organization (WHO). According to reports, almost 80% of Africans rely on medicinal herbs for health care [5]. Herbal medicine is becoming more popular in Nigeria and throughout West Africa due to its cost-effectiveness, accessibility, acceptance, and minimal toxicity [6]. Globally, there is a reintroduction of traditional medicine due to its user-friendliness and the inherent adverse effects of contemporary drugs [7]. Scientific research has become increasingly interested in long-standing traditional medical systems to examine the potential for producing novel phyto-therapeutic compounds [7]. Health problems are becoming more prevalent, and seemingly simple meals and food condiments may increase the effects of existing prescription medications. As a result of food scarcity, there is a growing quest for a scientific basis to employ plants and plant components as foods and pharmaceuticals, forcing a shift in the search to food and fruit waste. Furthermore, repurposing food and fruit waste as diets and therapies has the potential to reduce both environmental waste and associated public health problems [8,9]. *C. papaya* is regarded as a nutraceutical fruit plant due to its many nutritional and health benefits [10].

Fig. 1. The Leaves, fruit, and Stem of *Carica papaya (C. papaya)*

The qualities of *C. papaya* fruit and other plant parts are well-known in traditional medicine. Over the last few decades, significant progress has been made in the biological activity and therapeutic applications of *C. papaya*. *C. papaya* has enormous therapeutic potential for a variety of diseases [10]. The medicinal effects of the *C. papaya* plant's leaves, seeds, latex, and fruit have been reported in previous studies. Latex can be found in abundance in *C. papaya* stems, leaves, and fruits [11]. Many active ingredients found in *C. papaya* leaves, including papain, flavonoids, cystatin chymopapain, tocopherol, ascorbic acid, cyanogenic glucosides, etc., have been shown to increase the total antioxidant properties of the plant [12].

Carica papaya (Fig. 1) grows in all tropical and subtropical countries across the world, with a lifespan of 5 to 10 years. *C. papaya* leaf is about 75 cm broad on long, hollow petioles, with the blades separated into 5 – 9 main segments [13]. The fruit is usually long or spherical and contains about 1000 seeds [14]. Natural chemicals found in plant leaf bark and twig tissues exhibit anti-tumor and pesticidal capabilities [15]. Soil waterlogging usually leads to the plant death within 3-4 days [13]. *C. papaya* thrives in well-aerated, well-drained, organic matter-rich soil with a pH of 5.5 to 6.7. Plants used for therapeutic purposes are frequently inexpensive and have high concentrations of pharmacologically active chemicals with high bacterial resistance [16]. The edible component of *C. papaya* fruit contains Na, K, Ca, Mg, Fe, Cu, Zn, and Mn [12]. The plant contains carotenoids and several vitamins [17,18]. The leaves of *C. papaya* have many active phytochemicals and have been shown to increase the total antioxidant properties and reduce lipid peroxidation in the blood [10,19,20]. Pharmaceutical companies and academic organizations are always looking for new antibiotics to combat emerging and re-emerging resistant pathogenic organisms [21,22].

Thin-layer chromatography (TLC) is a chromatographic analytical method that separates mixtures, using an aluminum foil sheet impregnated with silica gel, or other suitable materials that function as stationary phase, and a suitable solvent system that forms the mobile phase. Different analytes ascend the TLC plate, resulting in sample separation [23]. TLC can be used to track a reaction's progress, identify chemicals in a test sample, and determine compound purity [24]. Phytochemical screening and TLC were used to determine the chemical composition of *C. papaya* leaves ethanolic extract.

2. MATERIALS AND METHODS

2.1 Plant Collection, and Extraction

Fresh leaves of *Carica papaya* Caricaceae were obtained in the wild, identified, and verified at the Department of Pharmacognosy and Phytochemistry, College of Pharmacy, Madonna University Nigeria. The leaves were properly washed, dried, and mashed with an electric blender to fine granules. About 250 g of the leaf fine granules were macerated in 600 ml of 100% Ethanol (analytical grade) for 72 hours before being filtered. The marc was then rewashed and filtered until all of the extractable components were removed. Both filtrates were mixed and concentrated using a rotary evaporator.

2.2 Phytochemical Analysis

The tests were conducted following previously reported procedures [25,26].

Alkaloids: approximately 20 ml of 3% sulphuric acid in 50% ethanol was added to 2g of extract and cooked in a boiling water bath for 10 minutes before being cooled and filtered, and then 2ml of the filtrate was poured into four test tubes labeled T1 - T4. A milky precipitate was detected after a few drops of Mayer's reagent were introduced to T1. A few drops of Dragendroff reagent were added to T2 filtrate, and a brick-red precipitate formed. A reddish-brown precipitate in the Wagner reagent indicates the presence of an alkaloid, but a yellow precipitate in Picric acid solution tests indicates the presence of an alkaloid.

Glycosides: 0.5g of powdered plant extract in a test tube was dissolved in 10 ml of 1% sulphuric acid and boiled for 15 mins, allowed to cool and neutralized with 20% KOH before adding 10 ml of the resultant mixture to an equivalent amount of Fehling solutions 1 and 2 and boiling for another 5 mins. A brick-red precipitate observed near the tube demonstrated the presence of glycosides.

Tannins: 0.5g of the powdered plant extract was dissolved in in 20 ml of distilled water, and filtered. A few drops of ferric chloride were added to 3ml of filtrate, resulting in a greenish-black precipitate. A precipitate formed after a few drops of lead acetate solution were added to 3ml of filtrate. These reactions suggest the presence of tannins.

Flavonoids: 0.2g of extract of the powdered plant extract was dissolved in 10 ml of ethyl acetate, boiled for 3 minutes, and filtered. 4 mL of the filtrate was mixed with 1 mL of ammonia solution, the layers were allowed to separate, and a yellow coloration in the ammonia layer indicated the presence of flavonoids. In addition, 4ml of the filtrate was agitated with 1ml of 1% aluminum chloride solution before the layers separated. The formation of a yellow coloration in the aluminum chloride layer showed the presence of flavonoids in the sample.

Reducing Sugar: Fehling Solution Test: 5 mL mixture of the same proportion Fehling solutions 1 and 2 were added to 5 mL of extract and heated for 5 minutes in a water bath. There was no color change, indicating that decreasing sugar was not present.

Protein: 0.5g of extract was combined with 20 ml of distilled water before being filtered. The filtrate was employed in further research. In a test tube, two drops of Million's reagents were added to a small volume of filtrate. The formation of a white precipitate suggests the presence of proteins. 5 mL filtrate was heated with 3 drops of strong nitric acid (Xanthoproteic reagent), which produced a yellow hue that turned orange when diluted with NaOH, indicating the presence of protein.

Steroids and Terpenoids: 1g of the powdered plant extract were dissolved in 9 ml of absolute ethanol, refluxed for 5 minutes, and filtered. 5 mL of hot distilled water was added after condensing the filtrate to 2.5 mL. After 1 hour, the mixture was filtered to remove waxy particles. The filtrate was extracted with 2.5 ml of chloroform with a separating funnel. 1 mL of conc. sulphuric acid was carefully added to 0.5 mL of chloroform extract in a test tube; a reddish-brown interface indicated the presence of steroids. Another 0.5 ml of chloroform extract was dried in a water bath before being heated for 10 minutes with 3 ml of conc. sulphuric acid; a grey coloration indicated the presence of terpenoids.

Saponins: 0.5 g of the powdered plant extract was dissolved in 20 ml of distilled water, boiled for 2 mins, and filtered. 5 mL of filtrate and 15 mL of distilled water were quickly mixed. Saponin's presence was indicated by the development of a stable foam. About 2 drops of olive oil were added to the foamy solution and swirled thoroughly. The formation of an emulsion showed the presence of saponin.

2.3 Thin-Layer Chromatographic Analysis

Chromatogram preparation: Following the extraction method, the extract is placed in a beaker and dissolved in dichloromethane. For analysis, a TLC chamber was employed. A filter paper was cut and placed within the chamber to saturate the chamber with vapor, preventing the eluent from easily evaporating when it rose on the plate. After pouring a portion of the solvent into the chamber, the chamber is tilted such that the solvent wets the filter paper. Close the chamber lid or place the watch glass on the beaker. Using a pencil, a straight line was drawn on the white surface 3cm from the bottom of the TLC plate and the locations where the samples were to be placed. The indicated regions should be 1cm away from the plate's edge and 1cm apart.

Spotting the TLC plate with the sample: A very small spot was placed on the line drawn on the TLC plate, allowed to dry thoroughly, and placed in the TLC chamber carefully, using forceps. The chamber lid was closed, the spotted sample was allowed to move close to solvent fonts, and the chromatogram was carefully removed from the chamber. Visible spots were determined and others were quantified using the UV lamb, all Rf values were carefully recorded.

3. RESULTS AND DISCUSSION

The results obtained are presented in the tables below.

Table 1. The percentage yield of the extract

Variable	Quantity
Weight of dried leaves (W1)	250 g
Volume of ethanol	600 ml
Weight of dried extract (W2)	21.5 g
Percentage yield = W2/W1 × 100	8.6 %

3.1 Phytochemical screening of Carica papaya leaves

The phytochemicals analysis of the ethanol extract of *Carica papaya* leaves was carried out and the test results were as seen in Table 2.

3.2 Thin Layer Chromatography Analysis

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent front}}$$

The plant extract was obtained at a low yield of 8.6% (Table 1). All of the reagents tested positive for alkaloids in the phytochemical screening, including Wagner's reagent, Mayer's reagent, Dragendoff's reagent, and picric acid solution.

Table 2. Phytochemical analysis of the ethanol extract of *Carica papaya* leaves

Test	Observation	Result	Inference
Alkaloids			
Mayer's reagent	Milky precipitate	+	Alkaloid present
Wagner's reagent	Reddish brown precipitate	+	Alkaloid present
Drangedoff's	Brick red precipitate	+	Alkaloid present
Picric acid solution	Yellowish precipitate	+	Alkaloid present
Carbohydrates			
Molisch's test	Purple ring formation	+	Carbohydrate present
Glycoside			
Fehling's solution	A brick-red precipitate	+	Glycoside present
Tannin			
Ferric chloride test	Greenish black precipitate	+	Tannin present
Lead acetate test	Formation of precipitate	+	Tannin present
Flavonoids			
Ammonia test	Yellow coloration	+	Flavonoid present
1% ammonium chloride solution	Yellow coloration	+	Flavonoid present
Reducing Sugar			
Fehling solution test	No color change	-	Reducing sugar absent
Protein			
Million's test	White precipitate	+	Protein present
Xanthoproteic test	A yellow color which changed to orange with the addition of alkali	+	Protein present
Steroids			
	Reddish brown interface	+	Steroid present
Terpenoids			
	A grey coloration	+	Terpenoids present
Saponins			
Frothing test	Froth formation	+	Saponin present
Emulsion test	Emulsion formed	+	Saponin present

Key: + = positive result, - = negative result

Table 3. Chromatograms of the retention factor (Rf) value of *Carica papaya* leaves extract

Solvent System (ml)	Visually (Daylight)	Ultraviolet Light (245 nm)	Solvent System (ml)	Visually (Daylight)	Ultraviolet Light (245 nm)
	0.55 (g)	0.55 (g)	**n-hexane: ethyl**	0.20 (g)	0.20 (g)
	0.65 (lg)	0.66 (g)	**acetate**	0.29 (y)	0.29 (y)
	0.72 (g)	0.72 (g)	**(7:3)**	-	0.40 (y)
Methanol	0.77 (y)	0.77 (y)		-	0.73 (y)
(10)	0.82 (y)	0.82 (y)		-	0.88 (y)
	0.91 (g)	0.92 (g)			
Benzene:	0.17 (g)	0.17 (g)	**Petroleum**	0.25 (y)	0.25 (y)
ethanol	0.26 (g)	0.26 (g)	**ether: ethyl**	0.35 (y)	0.35 (y)
(9:1)	0.66 (lg)	0.42 (b)	**acetate**	0.39 (g)	0.39 (g)
	0.82 (lg)	0.54 (b)	**(9:1)**	0.49 (g)	0.49 (g)
	0.92 (g)	0.66 (lg)		0.72 (g)	0.60 (lg)
	0.95 (g)	0.82 (lg)		0.81 (lg)	0.72 (g)
	-	0.91 (lg)		-	0.81 (lg)
	-	0.95 (g)			

Key: g = green, lg =light-green, y =yellow

Protein and terpenoids were also found in the results. Tannin, saponin, flavonoids, and glycosides have also been found. During the phytochemical screening of these leaves, the absence of color change when the heated sample was reacted with Fehling's solution revealed the absence of reducing sugar. A basic phytochemical screening of *C. papaya* leaves revealed the existence of chemical components in ethanol extract via direct maceration with ethanol, revealing ethanol as a solvent capable of dissolving a wide range of molecules (Table 2). The existence of these phytochemicals could explain the reported pharmacological benefits of *C. papaya* leaves, such as anti-inflammatory, antibacterial, and anticancer characteristics [12]. According to prior research, these phytochemicals are also responsible for the usage of these leaves as nutritional supplements, prophylactics, and treatments for specific medical disorders [27,28].

The total number of spots obtained was 26. Using ethanol (10 mL) as the mobile phase, the chromatogram yielded 6 spots (3 green, 1 bright green, and 2 yellow) that could be visualized with ultraviolet light and the naked eye to show the presence of various chemicals. Chromatogram results employing petroleum ether: ethyl acetate (9:1) as mobile phase yielded 7 spots (2 light-green, 2 yellow, and 3 green spots) when visualized with ultraviolet light, and 5 spots when observed with naked eyes. This demonstrates the presence of several chemicals (Table 3).

When examined with the normal eye, chromatograms with the solvent system benzene: methanol (9:1) showed 6 spots (four green and two light-green spots), however when visualized with UV lamb, 8 spots were identified, including two brown, three light-green, and three green dots. For hexane: ethyl acetyl (7:3), UV lamb revealed 5 spots (four yellow and one green), and naked eyes revealed two spots (one green and one yellow spot). The distinct spots on the chromatogram and the corresponding Rf values from the various solvent mixtures used in the study demonstrate the presence of several phytochemicals in the plant. This variation in Rf values of phytochemicals provides important information about their polarity and aids in the selection of acceptable solvent systems for pure component separation. It also indicated the chemical elements that *C. papaya* leaves are likely to contain. These constituents have been reported to relieve oxidative stress [29], among other medicinal benefits.

4. CONCLUSION

The ethanolic leaf extract of *Carica papaya* analyzed in this study showed a high level of both primary and secondary metabolites, which may be responsible for the plant's purported pharmacological characteristics. Furthermore, Thin Layer Chromatographic profiling yielded an excellent result with different colors on the chromatogram, indicating the presence of potentially useful chemical constituents. This points to the existence of many phytochemicals and potentially beneficial chemical constituents, which, when quantified, can serve as lead compounds in the design and development of valuable therapeutic agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/111076