

Isolation And Purification Of Flavonoids From The Leaves Of Locally Produced Carica Papaya

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ABSTRACT: The leaves of *Carica papaya* (150g) was defatted with N-Hexane and extracted with Methanol. The N-Hexane extract showed the presence of Flavonoid, Saponin, Tannin, Glycoside, Anthraquinone, Resin and Steroid while Methanolic extract showed the presence of Flavonoid, Saponin and Resins. 6g of the Methanolic extract was chromatographed using Column chromatography over Silica gel of column (200g:60-200 mesh) and eluted with the solvent mixture of CH₂Cl₂/CH₃OH /H₂O in the ratio of (70:30:1). The yield of the isolated Flavonoid was 0.23%.

Key word: Extraction, Isolation, Phytochemical screening, Purification, Flavonoids, *Carica papaya*

INTRODUCTION

Cacarica papaya PLANT

Pawpaw is the fruit of the *Carica papaya*. It is a large tree like plant with a single stem growing from 5 to 10m tall. It grows rapidly within three years and is frost sensitive. *Carica papaya* (papaya, papaw, pawpaw) is a species of flowering plant in the Caricaceae family, as the sole member of the *Carica* genus. It is endemic to the tropics of the Americas, and was first cultivated in Mexico several centuries before the Mesoamerican classic cultures emerged [1]. It is a tree-like plant, growing on a single stem 16 to 33 feet tall, with leaves spirally arranged at the top. The lower part of the trunk is conspicuously scarred where the leaves and fruit are borne. The plant is usually unbranched if unlopped. The shape of the flowers is similar to the *Plumeria*, but are much smaller and wax-like. These appear on the axils of the leaves, and will eventually mature into a fruit, 5.9 to 18 inches long, and 3.9 to 12 inches in diameter. The fruit is ripe when it feels soft, and its skin is coloured amber to orange [2].

CHEMICAL COMPOUNDS

Carica papaya papaya contains many biologically active compounds. Two important compounds are the chymopapain and papain which aid digestion. The level of the compound varies in the fruit latex, leaves, and roots. In addition, plants part from male and female trees differ in the quantity of the compound [3], [4]. For example phenolic compounds tend to be higher in male trees than female trees. The quantity of fresh papaya latex and dry latex (crude papain) also vary with the sex of the tree and age of the tree. Female and hermaphrodite trees yield more papaya latex than younger fruits. However, the activity of the papain the primary and secondary metabolite compounds in the fruit of one cultivar studied were linalool oxide respectively in another cultivar, the primary and secondary volatile compounds were as linalool oxide and linalool respectively [6].

With the exception of infertility, the leaves and root of *Carica papaya* contain cyanenic glycosides in the form cynana. The leaves also contains tannins. Both of these compound at high concentration can cause adverse reaction. Also, inhaling papaya powder (which has high papain and chymopapain enzymes) can induce allergies. In addition to decreasing infertility, papain might cause abortion shortly after conception. The papain apparently dissolves the proteins responsible for adhering the newly fertilized egg to the wall of the uterus [7].

AIM OF THIS STUDY

The aim of this research work is to extract and isolate and purify flavonoids from the leaves of *Carica papaya*.

MATERIALS AND METHOD

METHODS

Sample Preparation

The leaves of *Carica papaya* (pawpaw) were collected from Federal Polytechnic Bauchi quarters, Bauchi state, Nigeria. The leaves were dried for the period of six days in a shade and grounded into fine powder and sieved using a laboratory sieve of 212µm aperture.

Extraction

Procedure

Carica papaya prepared sample (150g) was defatted with n-Hexane (250ml) in batches of 30g using Soxhlet extractor. The extraction was carried out for about six hours at temperature range between 65°C and 70°C. After the extraction, all the thimbles were dried in an oven at 50°C. The extracted Marc was further extracted with Methanol solvent (250ml). The extract was then evaporated using water bath to remove the solvent completely.

Preliminary purification

The Methanol extract was adjusted with water to give 90% and then partitioned with t-Butylmethyl ether and n-hexane mixture (9:1). It was shaken very well and the Methanol portion separated and evaporated [8], [9].

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Phytochemical screening

The extracts were phytochemically screened in order to determine the presence of naturally occurring substances or secondary metabolite in the leaves of the *Carica papaya* plant [10], [11], [12], [13].

TEST FOR FLAVONOID [14]

The plants extracts (2ml) was acidified with 1% HCl and dissolve in 20% NaOH. A carnyary yellow indicated the presence of Flavonoid.

TEST FOR SAPONIN [15]

The extract (2ml) in a test tube was vigorously shaken for about 2 minute. A Persistent foaming showed the presence of Saponins.

TEST FOR TANNIN [15]**FRONTHING TEST**

FeCl₂ (2drops) was added to the extract (1ml). A green precipitate indicated the presence of Tannins.

TEST FOR STEROID [14]

Concentrated H₂SO₄ (2ml) was added to the extract (2ml). It was examined for the appearance of effervescence and a clear reddish brown colour at the interface which indicated the presence of Steroids.

TEST FOR ANTHRAQUINONE [16]

The extract (2ml) was treated with 5ml of benzene and observed for 2 layers. The colourless upper layer was pipette and the organic layer was treated with 3ml of 10% Ammonia solution (3ml). A Change in colour from rose pink to red indicated the presence of Anthraquinones.

TEST FOR CARDIAC GLYCOSIDE [15]

Extract (2ml) was added in succession to 3ml, 3.5% Iron (III) Chloride 3ml and Ethanoic acid (3ml). A dark green precipitate was formed. Finally concentrated H₂SO₄ was carefully poured down the side of the test tube and observed for formation of brownish red layer in the interface which indicated the presence of Cardiac Glycoside.

TEST FOR ALKALOIDS [17]

The leaf extract (2g) was warmed with 1% tetraoxosulphate (VI) acid (20ml) in cornical flask and place on a water bath for 2 minute. It was intermittently shaken and centrifuged to obtain the supernatant. A drop of Meyer reagent was added to the supernatant (0.1ml) in a test tube and observed. A cream precipitate indicated the presence of Alkaloid.

ISOLATION OF FLAVONOID BY COLUMN CHROMATOGRAPHY COLUMN CHROMATOGRAPHY

The Methanolic extract (6g) was chromatographed over Silica gel column (200g:60-200 mesh) and eluded with solvent mixture of CH₂Cl₂/CH₃OH/H₂O (70:30:1.V/V) 350ml, 150ml, and 5ml of water respectively. The fractions were further screened for Flavonoids.

RESULT

The two extractions yield two different extract as shown in table 1 below. The result for the column chromatography is obtained as shown in table 2.

TABLE 1*Result for the phytochemical screening*

Component	N-Hexane extracts	Methanol extracts
Flavonoid	+	+
Tannin	+	+
Saponin	+	-
Anthraquinone	+	-
Steroid	+	-
Alkaloid	+	-
Cardiac Glycoside	+	-
Resins	+	+

*Hints "+” indicate presence “-” indicate absence***TABLE 2***Pytochemical screening of the isolated extracts*

Properties	Flavonoid
Extract 1	-
Extract 2	-
Extract 3	+

*Hints: "+” indicate presence and “-” indicate absence***CONCLUSION**

Flavonoid (0.35g) was successfully isolated from the leaves of *Carica papaya* using the methods adopted and a yield of 0.23% was obtained. Although, the yield was very low, it is still appreciable because it is an important antioxidant and is highly effective even at low concentrations.

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