PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF CARICA PAPAYA.L

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Abstract

The extract of the plant's (Carica papaya) leaves was screened by using phyotchemical analysis. The plant produces quninone, alkaloids, carbohydrates, amino acids, glycosides, polyphenols, tannins, saponins, steroids, terpenoids, and glycosides by colouring technique. The plant extract was made using solvent extraction with ethanol. Carica papaya has numerous biological qualities, including antioxidant, antibacterial, and anti-diabetic properties. Antioxidant Activity (In-vitro) assessed by Free radical scavenging method. Antibacterial activity assessed against Bacillus subtilis and E.Coli by Disc Diffusion method. The extracts produced by batch technique from Carica papapya leaves were employed in the study.

Keywords: *Carica papaya*.L, Phytochemical Screening, Antioxidant, Antibacterial activity.

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1. Introduction

Plants are thought to be an excellent source for the investigation and discovery of new pharmaceutical chemicals and medicines that could be prospective drugs for humans since they act as intermediates in the manufacture of beneficial drugs **Makkar, H.P.S (2009)**. About 80% of the extracted active substances from higher plants that are used in modern medicine have a positive correlation between their traditional applications and the current therapeutic uses. Traditional medicine, which harnesses the pharmacological effectiveness of natural substances present in herbal preparations to treat human ailments, is based on biological activity **Saxena, M et al (2013)** . We are fully aware of the value of plants. The plant kingdom is a treasure trove of potential medications, and there has been a growing understanding of the relevance of medicinal plants in recent years. Plant-based drugs are widely available, less expensive, more cost-effective, and have less adverse effects. When considering the current hunt for therapeutically effective novel medications such as anticancer treatments, the plants that have been selected for medicinal usage for over 1000 years form the most obvious choice **Dewick, P.M. (1996)**. Indigenous medical knowledge is being passed down orally from generation to generation around the world **Verma, S. (2016)**. The World Health Organisation (WHO) published the Global Report on Traditional and Complementary Medicine in 2019. It was stated that the safety of herbal medications is frequently required to be thoroughly reviewed in most countries, using techniques similar to those used for conventional medicine, such as postmarketing surveillances **World Health Organization, (2019)**. Medical plants have lately gained popularity since their usage in ethnomedicine to cure common diseases such as colds and fevers, as well as other medical claims, is now supported by solid scientific data. The medicinal plant study began with extraction processes, which are crucial to the extraction outcomes (e.g., yield and phytochemical content) as well as the subsequent assays performed. Nowadays, a large range of technologies with various extraction processes are accessible. As a result, the purpose of this review is to define and analyse the most often used methods based on their principles, strengths, and limitations in order to aid in determining the applicability and economic feasibility of the approaches **Azwanida, N.N. (2015)**. Ayurveda, the Indian system of medicine, is almost as old as Indian civilisation and is part of India's rich past. The term 'Ayurveda' literally means "science of life." The inclusion of ayurveda principles in the hymns of the Vedas, the earliest record of human intellect, proves Ayurveda's exceptional antiquity and uniqueness. There is nothing in the cosmos that is non-medicinal and cannot be used for a variety of reasons and in a variety of ways **Jain, R (2017)**. Natural metabolites present in the leaves, bark, and twigs have medicinal benefit due to the presence of carotenoids, polyphenols, and conventional antioxidant vitamins C **Halliwell, B et al (1995)**. They protect plants from disease and harm while also adding colour, scent, and flavour to the plants. Phytochemicals are plant compounds that protect plant cells from environmental threats such as pollution, stress, dehydration, UV exposure, and pathogenic attack **Gibson, E.L et al (1998)**. Supplementary phytochemicals are also available; however, there is no evidence that they give the same health advantages as dietary phytochemicals **Saxena, M (2013)**. There are thousands of known and undiscovered phytochemicals. Plants create these substances to protect themselves, but the current study shows that many phytochemicals can also protect humans from the disease **Rao, B.N. (2003)**. Chemicals known as secondary metabolites, which are produced to help plants survive by interacting with diseases, herbivorous insects, and the environment, are not essential for the plant's immediate existence **Jackson, P.A (2015)**. Alkaloids, glycosides, flavonoids, steroids, saponins, and terpenenoids are examples of secondary metabolites that are crucial in protecting plants from environmental stressors, pathogen attacks, and insect and pest infestations **Monyela, S. (2021)**.

2. Experimental

Material and Methods:

Collection, Identification and Authentication of Plant materials

The Carica papaya plant species was gathered in and around the Villupuram District of Tamil Nadu, India.

Preparation of Plant powder

The plant leaves were allowed to air dry in the shade for 10 to 15 days. The dried material was then ground into a fine powder using an electrical grinder and stored in airtight containers. Then the powdered substance was used for further analysis.

Preparation of the Ethanolic extract

As per the methodology of Indian pharmacopoeia (Anonymous, 1996) the ethanolic extract was prepared. The powdered leaves were subjected to batch extraction individually and successively with 140 ml of ethanol and 60 ml of distilled water. These extract was filtered by Whatmann filter paper. The extract was then stored in an airtight container.

Preliminary Phytochemical Analysis
Following the established procedure, a preliminary phytochemical analysis of the Carica papaya plant as a whole was conducted. A preliminary phytochemical study of the extracts is prepared.

Qualitative analysis of phytochemical:
The leaf extract was examined for the presence of bioactive substances using the procedures outlined below.

Detection of Carbohydrates
Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test for the Absence of carbohydrates.

Benedict`s test
Filtrates were treated with Benedict`s reagent and heated on water bath for few minutes and then cooled. Orange-red precipitate indicates the presence of carbohydrates.

Detection of Alkaloids
1% of conc.HCl is added to the plant extracts. Filtered and warmed. The filter is now used for the presence of alkaloids

Mayer`s Test
Filtrates were treated reagent (Potassium mercuric iodide). Formation of yellow cream precipitate indicates the presence with Mayer`s of alkaloids**.**

Detection of Phenols
a. Ferric chloride test
To 1ml of plant extract, add 2ml of distilled water followed by few drops of 10% $FeCl_3$. Formation of dark green color indicates presence of phenols

Detection of Flavonoids
To 1ml of plant extract, 2.5 ml ammonia solution and conc. H_2SO_4 were added. Formation of yellow color indicates presence of Flavonoids**.**

Detection of Proteins
a. Biuret test; The extract were treated with 1ml of 10% NaOH and heated. To this a drop of CuSO4 solution was added. Formation of purple violet color indicates presence ofproteins**.**

Detection of Glycosides
Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides. To 1ml of plant extract, 1ml of acetic acid and few drops of $FeCl_2$ and concentrated H_2SO_4 were added. Formation of brown ring indicates presence of glycosides.

Detection of Quinones
To 1ml of plant extract, 1ml of conc. H_2SO_4 was added. Formation of yellow precipitate indicates presence of Quinones.

Detection of Anthocyanins
To 1ml of plant extract, 2ml of conc. Hcl and 1ml of NH_3 were added. The color changes from pink red to blue violet. It indicates the presence of Anthocyanins.

Detection of Anthroquinones
To 1ml of plant extract, 2ml of conc. H_2SO_4 and 1ml of NH_3 were added.Formation of rose pink color indicates presence of Anthroquinones

Detection of Steroids
To 1ml of plant extract, equal volume of chloroform added. And fewdrops of conc. H_2SO_4 is added in side of the tube. Formation of brown ring indicates presence of steroids.

Detection of Saponins
To 1ml of plant extract, 2ml of H_2O, 8ml of olive oil were added and shake vigorously. Formation of emulsion indicates presence of saponins.

Detection of Phlobatannins
To 2ml of plant extract were hydrolyzed with 1 ml Hcl and the mixture was boiled for a few minutes. The deposition of red precipitate indicates the presence of phlobatannins.

Detection of Terpenoid
To 0.5ml of plant extract, add 2ml of chloroform and conc. H_2SO_4 was added carefully alone the side of test tube. Formation of reddish brown color layer indicates presence of terpenoids.

Detection of Oxalate
To 1ml of plant extract, glacial acetic acid is added. There will be a Greenish black colouration indicates the presence of oxalate.

Detection of Quinine
1ml of NaoH is thoroughly combined with 1 ml of the plant extract. Quinine presence is indicated by the formation of blue, green, or red.

Detection of Tannins
To 1ml of plant extract, 5ml of water was added. Then boiled for few minutes and filtered to the filtrate few drops of 0.1% $FeCl_2$ were added. Formation of brownish green indicates the presence of tannins.

In Vitro Antioxidant Activity
A number of methods may be necessary to accurately analyse the in vitro antioxidant activity of a particular molecule or the antioxidant capacity of a biological fluid. The extract from Carica

papaya was examined for its antioxidant properties using conventional methods. The Carica papaya Extract and standard solutions had respective concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 g mL-1. To avoid agglomeration of extract Carica papaya, a dilute solution of extract Carica papaya was sonicated for 30 minutes at room temperature using a sonicator bath. Spectrophotometry was used to compare absorbance to equivalent blank solutions. Using the following formula, the % inhibition was calculated.

Radical scavenging activity % = $\frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$

DPPH Assay

The experiment was carried out in a 96-well microtiter plate. 10 l of each sample or standard solution was added individually to 200 l of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) solution in each well of the microtiter plate. After 30 minutes of incubation at 37 °C, the absorbance of each solution was measured at 490 nm.

Superoxide Radical Scavenging Assay (Alkaline DMSO Method)

To the reaction mixture containing 1 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL water) and 0.3 mL of the sample in freshly distilled DMSO at various concentrations, 0.1 mL of nitro blue tetrazolium (NBT;1 mg mL−1) was added to make a final volume of 1.4 mL. At 560nm, the absorbance was measured.

Hydrogen Peroxide Radical Scavenging Assay

A solution of hydrogen peroxide (20 mM) was prepared in phosphate-buffered saline (pH 7.4). At varied concentrations, 1 mL of the samples and standard were added to 2 mL of hydrogen peroxide solution in PBS. After 10 minutes, the absorbance was measured at 230 nm.

Antibacterial Activity

Preparation of nutrient agar medium

Peptone	-	0.150mg
NaCl	-	0.150mg
Beef extract	-	0.45mg
Yeast extract	-	0.045mg
Agar agar	-	0.450mg
Water	-	30ml

Therefore, said components were dissolved in 30ml of distilled water and autocleaved for 15 minutes at 121℃. The medium's pH was maintained at 7.2. After fully combining, pour into sterile petri plates.

Antibacterial activity of Carica papaya

An ethanolic extract of Carica papaya was tested for antibacterial activity using the disc diffusion method. By reconstituting the extracts with water, different concentrations (50, 100, and 150g/ml) were obtained. As a result, the components were dissolved in 30mL of distilled water and autoclaved at 121°C for 15 minutes. The pH of the medium was kept constant at 7.2. After fully mixing, pour into sterilised Petri plates. Pseudomonas and E.coli were seeded into the appropriate medium using a 10l spread plate method (106cells/ml) and bacteria cultures grown in nutritional broth for 24 hours. Following the solidification of the extracts, sterile filter paper discs (6mm in diameter) soaked in them were placed on test organism plates. Gentamycin (20 g/ml) is a common antibiotic. The anti assay plates were incubated at 37 degrees Celsius for 24 hours. The diameters of the inhibitory zones were measured in millimetres.

Measurement of zone of inhibition

The mean diameter of the inhibitory zone surrounding the disc was used to calculate the antibacterial potential of the test chemicals in millimetres. The zones of inhibition of the examined microorganisms by the extracts were measured on a millimetre scale.

3. Result and Discussion

PHYTOCHEMICAL TEST: The phytochemical analysis of the Carica papaya leaves investigated and summarized in the **table.1**. The leaves from Carica papaya were examined for their phytochemical composition, which revealed the presence of carbohydrates, alkaloids, phenols, flavanoids, glycosides, steroids, saponins, oxalate, qninine, and tannins.

Table 1 Phytochemical Analysis of Carica papaya

S.NO	TESTS	OBSERVATION
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Phenols	+
4.	Flavanoids	+
5.	Proteins	-
6.	Glycosides	+
7.	Quinones	-
8.	Anthocyanins	-
9.	Anthroquinones	-
10.	Steroids	+
11.	Saponins	+
12.	Phlobatannins	-
13.	Terpenoid	-
14.	Oxalate	+
15.	Qninine	+
16.	Tannins	+

(+) PRESENCE **(-)** ABSENCE

Antioxidant Activity of Carica Papaya

The ability of antioxidants to absorb and neutralise free radicals, quench singlet and triplet oxygen, and degrade peroxides is critical. As a result of this process, the antioxidants are oxidised. This demands the replenishment of antioxidants on a regular basis. The DPPH test was performed to evaluate Carica papaya's antioxidant capacity. The antioxidant activity, as measured by the percentage of inhibition, was 40% when the aqueous extract was consumed in varied concentrations ranging from 10 to 60 gmL.

In DPPH, H_2O_2, O_2-, NO, and OH radical scavenging assays were used to investigate the extract Carica papaya antioxidant activity *in vitro* **(See Table 2)**. The ABTS test is a relatively new one that screens complex antioxidant combinations such as plant extracts, beverages, and biological fluids using a more potent, chemically generated radical. Researchers were interested in employing ABTS•+ to measure antioxidant activity because of its solubility in both organic and aqueous settings, as well as its stability over a wide pH range. When the DPPH-free radical reacts with hydrogen donors, a matching hydrazine is formed. When it reacts with hydrogen donors, the DPPH radical changes color from purple to yellow. It is a discoloration test in which the antioxidant is added to a DPPH solution in ethanol or methanol and the absorbance at 490 nm is measured. Most human diseases, including cardiovascular problems and cancer, appear to be characterised by free radical participation, particularly increased production. A superoxide scavenger capable of interacting prevents the formation of a red dye formazan. In the body, many oxidase enzymes create hydrogen peroxide. There is accumulating evidence that hydrogen peroxide, either directly or indirectly through its reduction product, the hydroxyl radical (OH•), causes substantial injury to biological systems.The presence of phytochemicals such as alkaloids, sugars, flavonoids, gums and mucilages, phenolic compounds, saponins, tannins, and terpenoids contribute to the higher antioxidant activity of Carica papaya extract. The extract Carica papaya chemical process has the lowest antioxidant activity, with 67 percent. All of the Carica papaya extracts exhibit excellent antioxidant activity when compared to common antioxidants like ascorbic acid. The antioxidant activity order was the same for all of the assessed methods. Carica papaya extract has good antioxidant function because it contains a large amount of phytochemicals like alkaloids, flavonoids, phenolic compounds, and terpenoids.

Table 2 shows the percentage of DPPH radical, Hydrogen peroxide radical, Superoxide radical, Nitric oxide radical and Hydroxyl radical scavenging activity in extract Carica papaya compared with standard

Compound	Free radical Scavenging activity (%)				
	DPPH	H_2O_2	O_2^-	NO	OH
Extract Carica papaya	67.2	63.4	61.1	65.8	65.1
AscorbicAcid	70.8	76.1	72.0	70.2	75.0

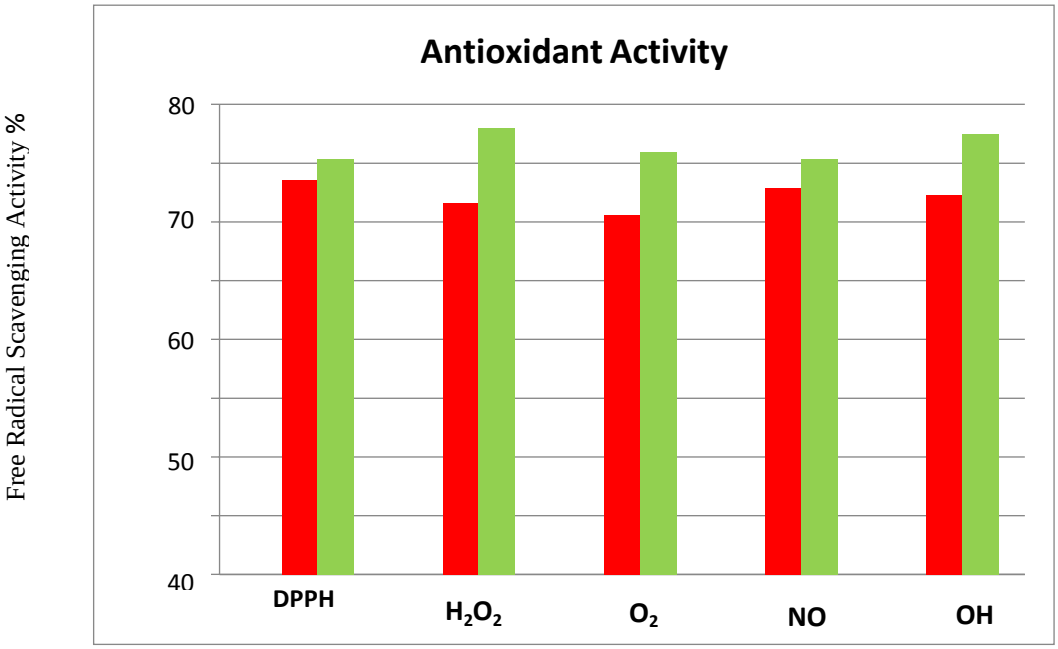

Fig. 1. In vitro antioxidant activity various free radical assay methodcompared with Standard ascorbic acid

H_2O_2radical scavenging assay

In this method, a scavenger is incubated with hydrogen peroxide, and the amount of hydrogen peroxide that is lost or decays can be detected spectrophotometrically at 230 nm. It was decided to reevaluate the antioxidant activity of extract Carica papaya using the 35 H2O2 radical scavenging assay method. Table 2 compares the anti-oxidant activity of extract Carica papaya equal concentrations of 100nM measured at 230nm to standard ascorbic acidwith a 63 percent increase.

Superoxide radical scavenging assay

The antioxidant activity of Carica papaya extract was assessed again using the superoxide scavenging test technique. The superoxide scavenging activity of Extract Carica papaya at an identical concentration of 100 nM was evaluated at 560 nm with a 61% increase when compared to standard ascorbic acid. **(Table 2)**.

Nitric Oxide radical scavenging assay

The decay or loss of hydrogen peroxide can be monitored spectrophotometrically at 230 nm when a scavenger is incubated with hydrogen peroxide. The antioxidant activity of Carica papaya extract was investigated again utilising the 35 H2O2 radical scavenging test technique**.**

Table 3 demonstrates that extract Carica papaya at 100 nM concentrations measured at 230nm has a 63 percent better anti-oxidant activity than standard ascorbic acid.

Antibacterial Activity of carica papaya

An ethanolic extract of Carica papaya was tested for antibacterial efficacy against the pathogenic pathogens Bacillus subtilis and Escherichia coli. The bacterial growth inhibition zone was used to assess the antibacterial activity of ethanolic extracts. The antibacterial result has prompted interest in the development of new antimicrobial

drugs with minimal side effects for the treatment of infectious diseases. By disc diffusion method, Carica papaya leaf extract has high activity against Bacillus subtilis with a zone of inhibition of 16 mm and E. coli with a zone of inhibition of 17 mm, indicating that Carica papaya has better antibacterial activity at l;l concentrations and that the zone of inhibition increases with concentration.

Table 3 Zone of inhibition of the extract Carica papaya

S. No	Positive and negative Pathogen	Zone of inhibition (diameter in mm)				Standard (Gentamicin)
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
1	**Bacillus Subtilis**	8	11	12	16	18
2	**Escherichia coli**	9	12	14	17	21
3	**Control (DMSO)**	NI	NI	NI	NI	NI

NI: No Inhibition

Fig. 2. Antibacterial activity of the Carica papaya against Bacillus Subtilis and Escherichia coli

4. Conclusion

The ethanolic extract of Carica Papaya was examined Phytochemical screening, it showed the presence of biologically active phytochemical Alkaloids, Flavonoids, Tannin, Glycosides, Carbohydrates, Saponins, Steroids. The antioxidant activity of Carica papaya extract was examined utilising several free radical test techniques such as DPPH, H2O2, O2-, NO, and OH. The results demonstrate that Carica papaya extract has high antioxidant activity. Antibacterial activity of ethanolic extract of Carica papaya was evaluated using disc diffusion method. The ethanolic extract of Carica papaya was tested for antibacterial activity against Bacillus Subtilis and Escherichia coli. The results revealed that Carica papaya extract was more efficient against bacteria. Based on the findings of this investigation, it was established that an ethanolic extract of Carica papaya has powerful antibacterial properties.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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