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Short Communication

Phytochemical Screening and Antioxidant Activity of *Ficus carica* Leaf

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ABSTRACT

Medicinal plants are a source of phytochemical compounds that play a significant role in human healthcare. The potent effects of these plants are mainly due to the presence of secondary metabolites. This study aimed to identify various phytochemicals and quantify the total phenolic and flavonoid content in the methanolic extract of *Ficus carica* leaves, as well as to measure their antioxidant properties.

Alkaloids, glycosides, steroids, flavonoids, saponins, and tannins were all detected in the raw powdered extract of *F. carica* leaves. However, alkaloids were absent in the methanolic extract. In the quantitative analysis, the total flavonoid content was significantly higher than the total phenolic content. The result of the study highlighted the potent phenol and flavonoid contents and antioxidant properties in the extract, indicating its potential for developing new drugs. This study also supports some of the traditional medicinal properties of the plant.

Keywords: *F. carica,* Phytochemicals, Antioxidant, Methanol.

INTRODUCTION

Medicinal plants have been used for health therapy and beauty enhancement for centuries. Historically, plants were the primary form of therapy, but in recent decades, they have become alternative or complementary products. The painful side effects of synthetic drugs have forced researchers to consider plants as safe therapies for treating illness as well as promoting health and beauty. Alkaloids, terpenoids, and phenolic compounds are three major phytochemical compounds, in which the presence of phenolic compounds is mostly studied among these three major classes of phytoconstituents.[1] This class of phytochemicals is known for numerous biological and physiological activities, including antioxidants.[2,3] Because of the solubility of phenolic compounds, the polarity of the solvent affects the amount obtained from the extraction of this compound.[4] So, this is important to know for biological activity, which solvent extraction provides the best value of yield in phytoconstituents. Most phenolic compounds are soluble in polar solvents like methanol and ethanol.[5] However, their usage varies due to differing toxicity levels. Methanol extracts are primarily formulated for topical applications, and deemed safer than oral dosages.

This study utilized *Ficus carica (F. carica)* as the plant sample for methanolic extraction. In tropical forests, *Ficus*, commonly known as "Fig," holds a pivotal role in the Moraceae family, playing a fundamental role in the ecosystem. *Ficus* encompasses a diverse genus of woody trees, shrubs, and vines distributed worldwide, predominantly in subtropical and tropical regions.[6] It thrives across India, from southern to northern regions, reaching heights of approximately 2,000 meters in the Himalayas. With nearly 800 species and 2,000 varieties, *Ficus* ranks among the largest genera of angiosperms.[7]

Ficus carica leaves are empirically used to treat various disease such as antispasmodic, cardiovascular, anti-inflammatory, and respiratory conditions.[8] Many researchers have investigated the biological properties of these leaves and identified numerous benefits, including antimicrobial, antioxidative, anticancer, antimutagenic, hepatoprotective, hypoglycemic, anti-hyperlipidemic, and antipyretic effects.[9-15] Analysis of the leaf extract has revealed the presence of three hydroxycinnamic acids (5- and 3-O-caffeoylquinic acids and ferulic acid), two furanocoumarins (psoralen and bergapten), and one flavonoid glycoside (quercetin 3-O-rutinoside).[16,17] Additionally, chlorogenic acid has been identified in *Ficus carica* leaves.[18]

Additionally, some of the reports indicated that antioxidant activity of the leaf of *Ficus carica* depends on the solvent used for extraction.[19-21] The purpose of this study was to determine the qualitative phytochemical components of *F. carica* leaves, using methanol as a solvent. Thereafter, total phenolic content (TPC), Total Flavonoid content (TFC), and the antioxidant activity of the methanolic extract were evaluated using the most reliable assay, Ferric reducing/Antioxidant power **(**FRAP) method.

MATERIAL AND METHODS

The plant sample used in this investigation was *Ficus carica* Linn leaves were collected from Patna, Bihar, India. For the extraction, a polar solvent, methanol was used.

Preparation of plant sample

Fresh and healthy leaves of *F. carica* were washed with tap water followed by distilled water to remove dust and unwanted materials, and then left to dry in shades at room temperature for 15 days to reduce water content to prevent microbial growth. Afterward, the dried leaves were grind with the help of a grinder into a fine powder and stored in an airtight container for further analysis.

Preparation of Extracts

The dried powder of *F. carica* leaves was macerated with methanol solvent in a 1:1 (powder: solvent) ratio. Approximately 20 grams of dried powder of *F. carica* Linn leaves and 20 ml of solvent were used. The extract was shaken for 24 hours on shaker at 120 rpm at room temperature. Afterward, the mixture was filtered using Whatman No. 1 filter paper. To improve the filtration quality, all samples were centrifuged to obtain a better-quality filtrate. Extracts were stored at low temperatures for further analysis.

Phytochemical screening

Qualitative phytochemical analysis

Phytochemical analysis was done using the standard protocol of Harborne (1973) and Sofowora (1993) to detect the presence of Alkaloids, Glycosides, Steroids, flavonoids, Saponins, and tannins.[22,23]

Quantitative phytochemical analysis

- *Total phenolic content (TPC)*

The total phenolic content was determined by the Folin-Ciocalteu method using the procedure explained by Singleton et al. with some modifications.[24] The calibration curve and maximum wavelength have been calculated using gallic acid as the standard compound. An aliquot of diluted sample in methanol was taken and added with 1.0 ml distilled water, then 0.5 ml of Folin- Ciocalteu reagent was mixed with the solution in the dark and shaken well. After that, the mixture present in the test tube was allowed to stand for 3 minutes then 2.0 ml of 20% sodium carbonate (Na_2CO_3) was added and then incubated in the dark for 60 minutes. The sample was measured at 650 nm wavelength using a double beam UV-Vis spectrophotometer against the blank and the result of total phenol content was calculated as milligram gallic acid equivalent (mg GAE)/gram. All the experiments were done in a triplicate manner.

- *Total flavonoid content (TFC)*

Total flavonoid contents were determined by the aluminium chloride colorimetric method.[25] Rutin was used as the standard compound to determine the maximum wavelength and to establish the calibration curve. An aliquot of diluted sample and a standard solution of rutin (1 mg/mL) was added to the test tube with methanol to make up the volume of 1 ml and then 4 ml of distilled water was added. After that, 300 µl of 5% Sodium nitrite was added to the mixture and incubated for 5 minutes in the dark. 0.3 ml of 10% Aluminium chloride solution was added and again incubated for 6 minutes in the dark. Now 2ml of 1M sodium hydroxide was added to the mixture. The final volume was adjusted to 8 ml with distilled water and mixed well. The mixture present in the test tube was allowed to stand for 15 minutes, and then the sample was measured at 510 nm in a double-beam UV-VIS spectrophotometer against the blank. The total flavonoid content was expressed as milligram rutin equivalent (mg RE)/gram. All the experiments were done in three replicates.

Table 1: Phytochemical screening of *F. carica* leaves

Secondary metabolites	*Raw leaves*	*Methanolic extracts*
Alkaloids	+	-
Glycosides	+	+
Triterpenes/Steroids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+

ANTIOXIDANT ACTIVITY

Ferric Reducing/Antioxidant Power (FRAP) Method

Antioxidant redox colorimetric reactions depend on the FRAP reagent as a reducing agent. The FRAP assay was carried out with a minor modification following the previously published protocol.[26,27] An aliquot of 0.1 ml of sample was added with 3.8 ml of FRAP reagent. FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM Iron (III) chloride hexahydrate in a ratio of 10:1:1. The sample was mixed and incubated for 30 min at 37°C. After incubation, the sample was measured at 593 nm in a double-beam UV-VIS spectrophotometer. The blank was prepared by substituting the same amount of diluted extract and standard with solvent. The calibration curve and maximum wavelength were calculated using Iron (II) sulfate as standard. All the experiments were done in triplicates.

Statistical Analysis

The statistical analysis was performed by using GraphPad Prism 5.0.

RESULT

Phytochemical Screening

Alkaloids, glycosides, steroids/triterpenes, flavonoids, saponins, and tannins were found in the powder material of *F. carica* leaves, according to the results of the phytochemical screening. However, the alkaloids determine a negative result from the methanol extract shown in Table 1. This suggests that the component extracted in the solvent will be less affected as compared to raw material. Comparing the methanol extract to the raw material, only one component was absent.

Total Contents of Phenolics and Flavonoids

The total phenolic and flavonoid contents were determined to calculate the approximate value of phenolic and flavonoid compounds in the methanolic extract of *F. carica* leaves. The phenolic content is 11.7 mg GAE/g and the flavonoid content is 56.72 mg RE/g and the standard graph is shown in Fig 1. in the leaves of methanolic extracts of *F. carica*.

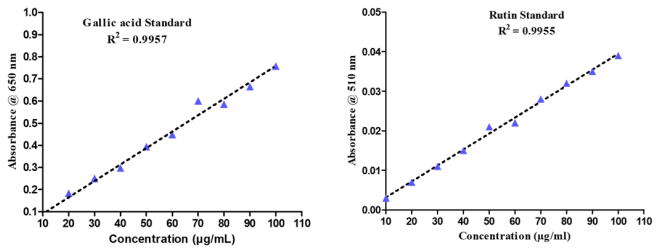

Fig 1: Standard graph of gallic acid in total phenolic content and rutin in total flavonoid content

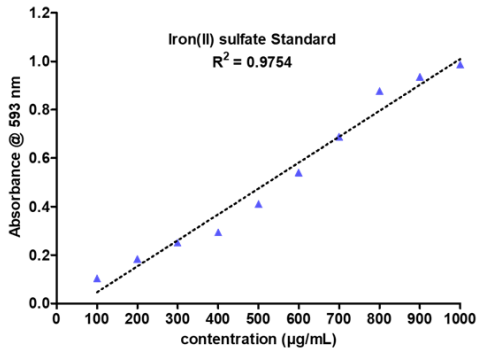

Fig 2: Standard graph of Iron(II) sulfate in FRAP

Antioxidant Activity:

The FRAP Method was used to calculate the antioxidant potential of the leaf of *F. carica* in methanolic extract. In this study Iron (II) sulfate were chosen as standard for determining the antioxidant potential. The FRAP method shows 427 mg $FeSO_4$/g and the standard graph is shown in Fig. 2.

Based on the result of antioxidant potential in leaves, it is suggested that the methanol extract of *F. carica* Linn was suitable as a source of antioxidants.

DISCUSSION

Phytochemical analysis of medicinal plants is crucial for identifying diverse bioactive compounds that serve as new sources for medicinal and industrial purposes, potentially leading to drug development. The leaves of *Ficus carica* are known to harbor a rich reservoir of bioactive compounds, particularly phenolic compounds, with flavonoids typically categorized among them. Consequently, flavonoid content in methanol extracts is generally higher than total phenolic content. The total phenolic content among the three vegetal materials of fig varies significantly, with the order being leaves > peels > pulps.[16,28] In this study, Gallic acid and rutin were employed as standard compounds to quantify phenolic and flavonoid contents in the extracts, respectively. Compounds structurally like rutin were identified as flavonoids, while those resembling gallic acid were classified as phenolic compounds.

There is a growing interest among researchers in food science, medicine, and health regarding antioxidants.[29] Antioxidant properties of *F. carica* pulps, peels, and leaves were evaluated.[30,16] In the FRAP assay, antioxidants present in the extracts reduce the Fe^{3+}–TPTZ complex, forming a blue Fe^{2+}–TPTZ compound. The change in absorbance at 593 nm correlates directly with the FRAP value of antioxidants in the sample.[31] Leaves consistently exhibited superior antioxidant activity, likely attributable to their higher phenolic content.[16]

CONCLUSION

Therefore, the result of the present study indicated that leaves of *Ficus carica* contain effective substances like glycosides, steroids/ triterpenes, flavonoids, saponins, and tannins and these plant secondary metabolites are more effective and used as drugs against various harmful diseases. The total phenolic and flavonoid content show effective results in methanol for drug discovery and improvement. Although the methanol extract of *F. carica* leaves contains fewer phytochemical compounds, it exhibits higher antioxidant activity. Further studies are required to identify the specific compounds responsible for the antioxidant activity and to formulate the extract into a dosage form.

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CONFLICTS OF INTEREST

All the authors declare no conflict of interest.

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