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Research article

In Vitro Assessment of Antioxidant activity and Qualitative Phytochemical Screening of *Ficus religiosa* L.

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Abstract

Ficus religiosa is a commonly occurring plant in South Asia which has been widely used in traditional medicine to treat inflammation, microbial infections, and metabolic ailments. The present study aims to investigate the phytochemical composition of *F. religiosa* and evaluate its antioxidant activity through phytochemical screening and in vitro antioxidant assays. Qualitative phytochemical screening was performed on the methanolic extracts of *F. religiosa* leaves and stems to identify the secondary metabolites. The methanolic extracts were obtained by extracting 10 g of the respective powdered material with methanol using the maceration method. The concentrated extracts were subsequently used for qualitative phytochemical analysis and biological assays at the required experimental concentrations. Their phytochemicals and antioxidant activities are correlated. This comparative approach helps to give insight into the therapeutic potential of *F. religiosa* and help determine which part of the plant has a maximum antioxidant activity, which can help the rational use of the plant in the formulation of an antioxidant. The *F. religiosa* contains phytochemicals and exhibits antioxidant activity, suggesting its potential as a natural source of bioactive compounds that may contribute to protection against oxidative stress. However, further quantitative analyses and in vivo studies are required to validate its therapeutic applications.

Keywords: *Ficus religiosa*, phytochemicals, antioxidant activity, DPPH assay, phenolic compounds, pharmacology

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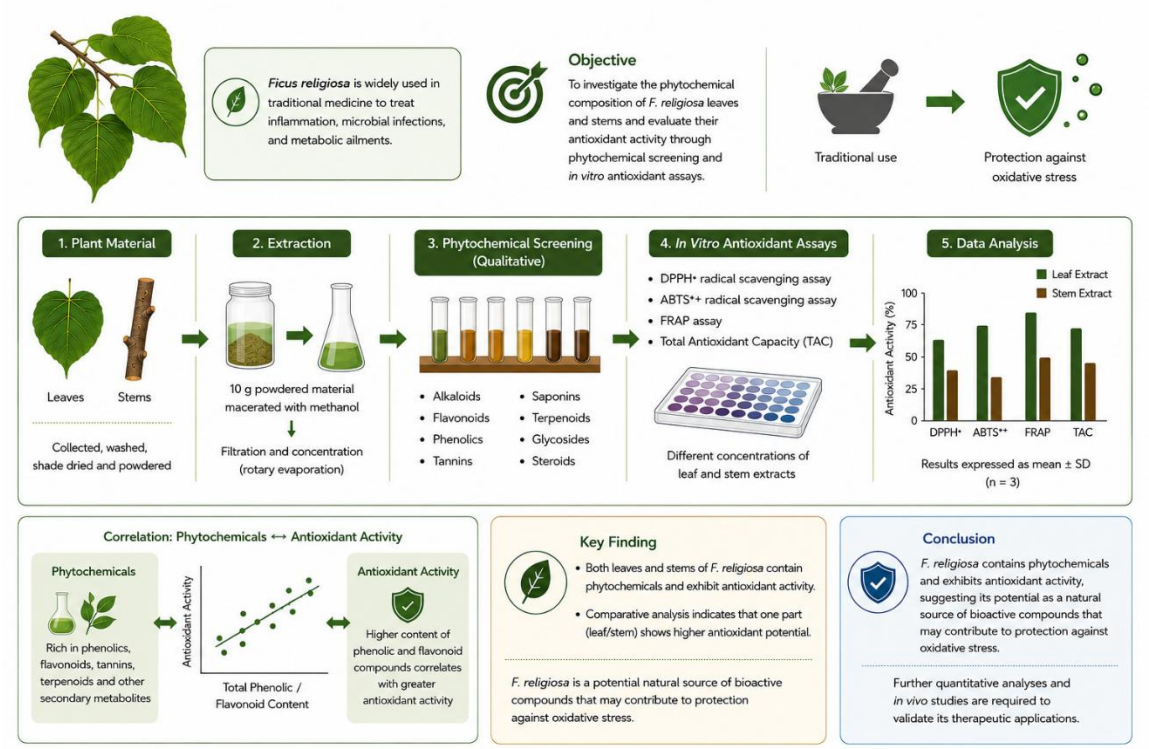
Graphical Abstract



Introduction

Plant based medicine has continued to be popular especially in underdeveloped nations where modern medicine might not be readily available. It is claimed that up to 80 percent of the world population is being dependent on traditional herbal remedies to meet the primary healthcare needs (World Health Organization, 2013). A significant number of the contemporary pharmaceuticals can be obtained directly or indirectly through plant sources, which demonstrates the relevance of ethnobotanical knowledge to the development of medical science (Newman and Cragg, 2020). Moreover, the growing rate of chronic diseases and the restrictions of synthetic antioxidants have heightened the need to seek natural alternatives that have fewer side effects (Liu, 2013). Therefore, medicinal plants still remain as a source of new therapeutic agents.

F. religiosa or Peepal tree is a member of the family of Moraceae and is widely spread in the Indian subcontinent including Pakistan, India, Nepal, and Sri Lanka (Kirtikar and Basu, 2006). Conventionally, it has massively been used in Ayurvedic and folk systems of medicine to treat a diversity of diseases. Different components of the plant such as leaf, bark, root, and fruits have been reported to have medicinal properties. As an example, the anti-inflammatory and antimicrobial properties of the bark are widely used, whereas the leaves are used in asthma and wound treatment (Verma and Gupta, 2018).

Phytochemicals are naturally secondary metabolites synthesized as a result of the plant defense mechanisms, and are of importance to their medicinal values. (Kedare er al., 2011). In example to these are flavonoids and phenolic compounds that are quite familiar in

terms of antioxidant properties. The phytochemicals that make medicinal plants possess a pharmacological potential and it is based on these phytochemicals that they were used traditionally. The vast percentage of contemporary medications are based on the plant-derived compounds or their synthetic equivalents, which highlights the relevance of phytochemical screening to the discovery of biologically active agents (Newman and Cragg, 2020). Free radicals (superoxide anions and hydroxyl radicals) are very reactive substances with the power to cause harm to cellular elements, like lipids, proteins, and DNA (Halliwell and Gutteridge, 2015).

This oxidative injury has been found to contribute to the pathogenesis in a wide range of chronic diseases, such as cancer, cardiovascular diseases, diabetes and neurodegenerative diseases. Antioxidants are very important in alleviating oxidative stress through the neutralization of the free radicals and avoiding injury of cells. Although synthetic antioxidants can be found, issues of their possible toxicity, and side effects have prompted more interest in natural antioxidants that are made out of plants (Liu, 2013). The medicinal plants, and especially those abundant in phenolic and flavonoid compounds have shown to have a high level of antioxidant activity and thus they are worth considering in the development of therapeutic uses. Since *F. religiosa* has been reported to contain bioactive compounds, it is postulated that it has a high antioxidant capability, which therefore adds on to its pharmacological effects.

Although some of the studies have reported that bioactive compounds and antioxidant activity is present, there are varying results because of varied approaches to extraction, parts of plant used and experimental conditions. More studies that combine phytochemical screening and pharmacological evaluation are required to relate the chemical constituents of *F. religiosa* and their biological activities. These studies will assist in confirming its therapeutic efficacy and provide some basis for standardization of its medicinal applications. The study focuses on analyzing the phytochemical profile of the plant extracts to identify the presence of key secondary metabolites such as flavonoids, tannins, and alkaloids. Furthermore, it seeks to evaluate the antioxidant potential of the plant using established in vitro assays and to examine the pharmacological activities associated with its bioactive compounds.

2. Materials and Methods

Leaf samples of *F. religiosa* were collected from Patokki area of Punjab, Pakistan during its active vegetative growth to maintain the optimum phytochemical composition. The leaves and stems were harvested and carried separately in sterile conditions. The plant material was confirmed by Dr. Sana Khalid, Department of Botany, Lahore College for Women University, Lahore and a voucher specimen (Voucher No. LCWU-BOT-FR-2014-015) was kept in the herbarium of the college to be used in the future to guarantee the reproducibility and reliability of the study. Plant material that was extracted was thoroughly rinsed with distilled water, shade dried at room temperature to preserve heat labile compounds, and ground into a fine powder to increase the efficiency of the extraction process. (Heinrich et al., 2020). 100 g of finely powdered leaf and stem material was extracted in a series of sequential solvents

with increasing polarity petroleum ether, chloroform, methanol, and distilled water. The powdered material was macerated with petroleum ether for 48 hours, shaken every hour, to extract non-polar compounds like lipids, waxes, and some terpenoids. The extract was filtered on Whatman No. 1 filter paper and dried under reduced pressure by using a rotary evaporator. Residual plant material was air dried to remove any traces of solvent before extracting repeatedly with chloroform under the same conditions to obtain moderately non-polar components. After chloroform extraction, the residue was further extracted in methanol for polar phytochemicals (flavonoids, phenolics, tannins, alkaloids, and glycosides) (Trease and Evans, 2009). Finally, highly polar components like polysaccharides and water-soluble phenolics were extracted from the remaining plant material by using distilled water. Filtrate was concentrated under reduced pressure after each extraction for 48 hours without shaking. The dried extracts were then weighed, placed in airtight containers and kept at 40°C for further phytochemical and biological studies. This sequential extraction approach improves extraction efficiency by breaking down compounds based on their polarity, leading to a detailed analysis of the phytochemical profile and biological activities. (Azwanida, 2015).

The antioxidant activity of the different extracts was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted method for assessing free radical scavenging potential. In this assay, a decrease in absorbance at 695 nm indicated the ability of the extracts to neutralize free radicals, reflecting their antioxidant capacity

The experiment was conducted in triplicate to ensure accuracy, and the results were expressed as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel and SPSS software to assess variability and significance, thereby enhancing the reliability and scientific validity of the findings (Motulsky, 2014)

$$\%\text{Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where a control represents the absorbance of the control solution (without extract) and a sample represents the absorbance in the presence of the extract. The decrease in absorbance indicates the ability of the extract to scavenge free radicals, thereby reflecting its antioxidant activity (Do et al., 2014; Kedare and Singh, 2011).

3. Results and discussion:

The results of above experiments indicated that there was a difference in the phytochemical composition based on the polarity of the extraction solvent with the methanolic extraction and aqueous extraction exhibiting higher phytochemical profile than the non-polar solvents. Alkaloids, flavonoids, tannins, and saponins are present in the polar extracts as other studies have indicated that methanol and water were more effective in extraction of the phenolic and flavonoid compounds (Do et al., 2014.) These molecules are known to have a pharmacological significance especially in antioxidant and antimicrobial functions (Liu, 2013) (Table 1)

Table #1. Qualitative Phytochemical Screening of *F. religiosa* Extracts

1	2	3	4	5
Alkaloids	–	+	+	+
Flavonoids	–	–	+	+
Tannins	–	–	+	+
Saponins	–	–	+	+

Key: 1. Phytochemical, 2. Petroleum ether, 3. Choloroform, 4, Methanol, 5. Aqueous. (+ = Present; – = Absent)

Qualitative phytochemical screening revealed the presence of phenolic compounds and flavonoids that could be responsible for the comparatively higher antioxidant activity found in methanolic extract. The present study did not quantify these metabolites but earlier studies have reported that these phytochemicals have a strong free radical scavenging activity and they help to play significant role in the antioxidant activity of medicinal plants (Liu, 2013).

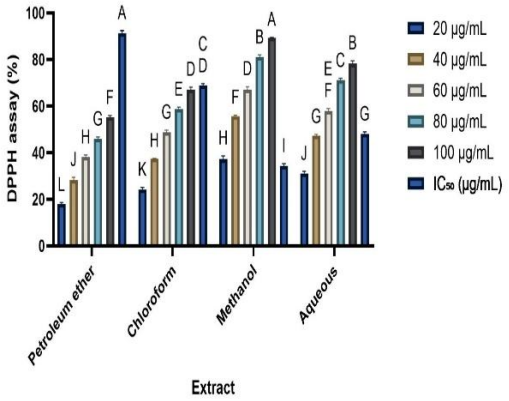


Fig 1: showing the Antioxidant Activity of *F. religiosa* Leaf Extracts Determined by DPPH Assay

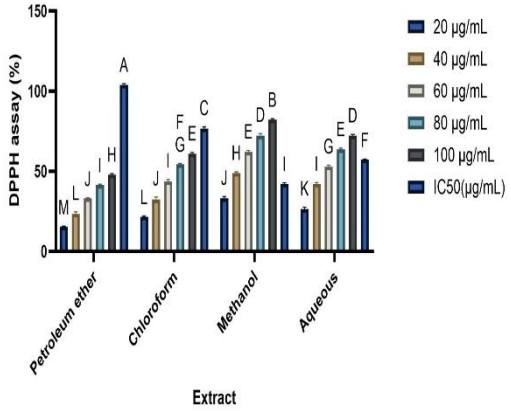


Fig 2. Showing the Antioxidant Activity of *F. religiosa* Stem Extracts Determined by DPPH Assay

The values in fig 1 and 2 the antioxidant activity was observed to be increased with increasing concentration of the extract. Among the plant extracts, the methanolic extract of the leaves had the highest radical scavenging activity and the lowest IC_{50} value, which indicates the highest antioxidant activity.

The antioxidant properties of the leaf extracts were always higher than the stem extracts, indicating that the leaves may produce an effective combination of antioxidant phytochemicals. The extracts using petroleum ether exhibited the least activity for both plant parts and ascorbic acid was used as the positive control with the highest antioxidant activity. This can be explained by the fact that leaves are metabolically more active and tend to accumulate higher levels of secondary metabolites involved in defense against environmental stressors such as pathogens, UV radiation, and herbivory (Patil et al., 2024).This enhanced activity can be attributed to phenolic compounds and flavonoids, which act as hydrogen or electron donors to neutralize reactive oxygen species

(ROS) and prevent oxidative damage (Halliwell and Gutteridge, 2015). Additionally, flavonoids possess hydroxyl groups that enable effective free radical scavenging and metal ion chelation, further strengthening their antioxidant role (Panche et al., 2016).

Despite these promising findings, certain limitations must be acknowledged. Variations in extraction methods, solvent polarity, and experimental conditions may influence the results, and the study was limited to in vitro assays, which may not fully reflect in vivo biological responses. Therefore, further studies involving isolation of active compounds and in vivo investigations are necessary to validate these findings.

Conclusions:

This study emphasizes phytochemical composition and biological activity of *F. religiosa* especially its antioxidant potential. The qualitative phytochemical analysis confirmed the presence of flavonoids, tannins, alkaloids and saponins in the extracts and antioxidant assessments showed measurable biological activities. The current study reports the presence of these phytochemicals, but does not provide a direct causal relationship between each compound and the shown activities, since the metabolites were not isolated nor quantified. Hence, the biological activity observed could be due to the interaction of several bioactive compounds found in the extracts.

Recommendation:

According to the results obtained in this study, it is suggested that further studies are done on quantitative estimation, isolation and structural characterization of bioactive compounds that are present in the plant *F. religiosa* in order to determine the compounds responsible for the biological activities. Moreover, in vivo studies and clinical investigations are suggested to assess the safety, efficacy and therapeutic potential of these compounds. Standardization of extraction procedures and bioactivity-guided fractionation would also make it easier to develop natural antioxidants and antimicrobial agents from *F. religiosa* for pharmaceutical

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Declaration on the Use of Artificial Intelligence (AI):

The authors declare that any artificial intelligence (AI)-assisted technologies used during the preparation of this manuscript were employed solely to improve language, grammar, readability, or formatting. All scientific content, including the study design, data collection, analysis, interpretation of results, and conclusions, was conceived, verified, and approved by the authors. The authors accept full responsibility and accountability for the accuracy, originality, and integrity of the manuscript.

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