WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Evaluation of Secondary Metabolites and *in Vitro* Antioxidant Activity of *Ficus Dalhousiae (Miq.) Miq.* and *Ficus Hispida* L. Fruit

Vijeyabharathi, R.S and Kannan, R.

Department of Botany, Chikkaiah Naicker College, Erode – 4
mail ID: drrkannancnc@gmail.com

ABSTRACT

The present study aims to determine the chemical composition of *Ficus dalhousiae* (Miq.) Miq. and *Ficus hispida* L. Fruit (Nilgiris), have a secondary metabolite, evaluate its antioxidant activity. Phenolic, tannin, and flavonoid secondary metabolites; DPPH and ABTS antioxidant activities; phosphomolybdenum superoxide scavenging activity; and FRAP tests. As a result, we know that the ethanol extract is high in tannin (401.74 mg GAE/ g), total phenols (414.32 mg GAE/ g), and the flavonoid ethyl acetate (76.24 mg RE/ g). The DPPH, ABTS, phosphomolybdenum, and superoxide were all effectively neutralized by the fruit ethanol extract (37.03 µg/L, 74305.6 µM TE/g extracts, 274.73 mg AAE/g extract, etc.). These findings point to the fruit extracts of *Ficus dalhousiae* and *Ficus hispida*, which are rich in antioxidants, as having promising antibacterial properties, especially the ethanol and ethyl acetate extracts.

Keywords: Antioxidant, *Ficus dalhousiae* and *Ficus hispida*

Introduction

Medicinal plants found in nature have long been considered a potential replacement for synthetic bioactive substances; in fact, there is substantial evidence that many pharmaceuticals have their origins in nature (Patel et al., 2014). For decades, people have relied on plant remedies without understanding the science underlying them. Based on their observations, they determined that therapeutic doses are necessary for the maximum efficacy of some medicinal herbs (Baranski et al., 2014). Rural residents of many developing nations rely heavily on traditional medicine for their basic healthcare needs (Aarland et al., 2017). When compared to synthetic or modern treatments, these are often safer and less expensive. Knowledge and research have led to the isolation of many modern pharmaceuticals from natural resources, which continue to serve as a foundational source of beneficial agents. Plants play an essential role in both the food and medicinal industries as main producers. Plants and humans have a long and storied history of mutual aid, with medicinal uses dating back thousands of years (Prince et al., 2011).

Active phytoconstituents from certain plants have been found, but they are generally found in small amounts that aren't enough to heal wounds. Some medicinal plants can help wounds heal by killing germs, protecting cells, and reducing inflammation. In poor countries, skin infections are common because people don't take care of themselves (Kumar et al., 2006). When you get hurt, your skin breaks. The wound has to heal in order to recover the body's structure and function. Wounds heal in three stages: inflammatory, multiply, and remodeling. The remodeling stage determines how strong and how the healed tissue looks (Sumitra et al., 2007). For hundreds of years, medical plants have been used to treat skin and dermatological problems, such as burns, cuts, and wounds (Kumar et al., 2007).

Antioxidants are very important for stopping oxidation because they stop the creation of free radicals, clean up free radicals, and stop the production of hydrogen peroxide and other peroxides (Umapathy et al., 2010). ROS can be made by the body itself or by outside sources. The main places where ROS come from inside cells are mitochondria, peroxisomes, and the endoplasmic reticulum. These are places where the body uses a lot of oxygen (Jayaprakasha and Rao, 2000). The antioxidant system that cells have created, however, is well-balanced and can function either through non-enzymatic antioxidants (such ascorbic acid and tocopherol) or enzymatic antioxidants (like Superoxide Dismutase [SOD], Catalase [CAT], and Glutathione Peroxidases [GPxs]). Extra ROS can be eliminated by these systems.

When ROS levels are low, they can start biological processes by activating signaling pathways (Sudhakar et al., 2016).

The tropical and subtropical parts of the globe are home to the 735 species of Ficus L. (Moraceae) spread among 6 subgenera: Urostigma, Pharmacosycea, Sycomorus, Sycidium, Synoecia, and Ficus itself (Chaudhar et al., 2012). Among the 91 species and 24 infra-specific taxa found in India, 10 are endemic to the country and may be found primarily in the northeastern states, peninsular region, and Andaman & Nicobar Islands (Hamed, 2011). A well-known species of Ficus is the fig (Hamed, 2011). Protecting and repairing themselves from physical attacks, fig species have latex-like substance within their vasculatures. Isolated and identified phytoconstituents from various Ficus species include triterpenes and sterols. The fruit of Ficus dalhousiae and Ficus hispida was studied for its antioxidant and anti-inflammatory qualities, as well as its Secondary Metabolites, due to its potential medical uses.

Materials and Methods

Plant Collection and Identification

A collection of *Ficus dalhousiae* and *Ficus hispida* was made in May 2023 in the Nilgiris district of Tamil Nadu, India, straight from the trees' native environment. Researchers in India, the Southern Circle, Coimbatore, and Tamil Nadu were able to confirm the taxonomic identity of the plant. The newly harvested plant components were washed under running water to eliminate any surface dust or dirt, and then left to dry naturally in the shade. Blending the two together yielded a fine powder that could be utilized in the tests that followed.

Preparation of Plant Extracts

A soxhlet apparatus was used to extract the leaf powder from a mixture of thimbles containing progressively more polar organic solvents. Ethyl acetate, chloroform, petroleum ether, and ethanol were among these solvents. It was standard practice to let the thimble air dry between solvent extractions. The extract water was filtered after maceration in water for 24 hours with continuous stirring. The concentrates were subjected to additional testing after being extracted with different solvents, air dried, and condensed using a rotary vacuum evaporator.

Quantification assays

Phenolic content

To determine leaf extract phenolic content, Singleton and Rossi (1965) employed the Folin-Ciocalteu technique. To the 100-fold diluted sample extract, add 2.0 mL folin-ciocalteu. The incubation lasted 60 minutes after 5 minutes at room temperature. It was then mixed with 1.6 mL of 2.5% w/v sodium carbonate solution. Thermo Scientific's UV-visible spectrophotometer (Genesys 50, USA) measures 765 nm absorbance accurately. We created a calibration curve using a known gallic acid content. Every information is in gallic acid equivalents (GAE) per gramme.

Tannins content

Tannin and non-tannin are total phenolics. To quantify tannins, subtract total phenolics (including tannin-free). Makkar (2003) used a 2-mL eppendorf tube to incubate 500µL, 100 mg PVPP, and 500µL distilled water at 4º C for four hours in order to assess the total non-tannin phenolic content in plant materials. Eppendorf tubes were centrifuged at 4º C for 10 minutes at 4000 rpm following incubation. PVPP and tannins were removed by precipitation, leaving only non-tannin phenolics. Total and non-tannin phenolics were measured in the supernatant. Following three analyses, the results were gallic acid equivalents. The tannins in each plant were examined twice: Tannins are equal to total phenolics minus non-tannins.

Flavonoid content

Aluminum chloride quantified sample extract flavonoids. 50 mL sample extract, 150 mL 5% sodium nitrite, and 2.5 mL distilled water were combined. Add 300 mL of 10% (w/v) aluminum chloride, wait 5 minutes at room temperature, and mix for 6 minutes. After adding 550 mL distilled water, 1 mL 1 M sodium hydroxide was added. After mixing, a UV-visible spectrophotometer (Thermo Scientific, Genesys 50, USA) measured solution absorbance at 510 nm quickly. Liu et al. (2008) measured flavonoids in Rutin equivalents (RE) per 100 g dry sample.

In vitro Antioxidant Activity

DPPH radical scavenging assay

Using the stable radical DPPH, Gursory et al. (2009) assessed the extracts hydrogen-donating or radical-scavenging capacity. Extract amounts were 20-100 µL, with methanol increasing the final quantity. Methanolic DPPH (0.004%) was added to BHT and Rutin sample and reference aliquots after thorough mixing. A negative control was prepared by adding 100 µL methanol to 3 mL methanolic DPPH solution. Tubes were 27°C for 30 minutes. To separate test materials from controls, absorbance values were collected at 517 nm against methanol blank. We calculated each sample's IC50, or concentration needed to inhibit 50% of DPPH, to quantify radical scavenging.

ABTS radical cation scavenging activity

ABTS radical cation scavenging assay by Re et al. (1999). Monitor antioxidant activity. For 12-16 hours in the dark at room temperature, a stable radical ABTS was incubated with 2.4 mM potassium persulfate aqueous solution to form an ABTS radical cation. Diluting the ABTS solution in ethanol (1:89 v/v) before testing yielded an absorbance of 0.700 ± 0.02 at 734 nm. Mixture consisted of 30 µL sample solution, 10 µL Trolox solution in ethanol (final concentration 0–15 M), and 1 mL diluted ABTS solution. Negative control test tubes contained 30 microliters of ethanol and 1 milliliter of diluted ABTS. Each tube incubated for 30 minutes at room temperature after vigorously swirling. Following incubation, BHT and Rutin were detected at 734 nm vs ethanol blank. In micromoles per gram of sample extracts, trolox antioxidant activity was evaluated.

Superoxide radical scavenging activity

The experiment assumed the extracts might scavenge superoxide radicals (Beauchamp and Fridovich, 1971). Mix 100 µL sample solution, BHT, and rutin in a 3 mL reaction mixture with 50 mM sodium phosphate buffer (pH-7.6), 20 g riboflavin, 12 mM EDTA, and 0.1 mg NBT. Light was

applied to samples and reaction mixture for 90 seconds before the reaction began. The lighting reaction mixture's unlabeled counterpart was tested. The absorbance at 590 nm after illumination was compared to a blank, an unlit reaction mixture without material.

Phosphomolybdenum assay

A green phosphomolybdenum complex assay determined the sample's antioxidant activity (Prieto et al., 1999). A 300 µL methanol blank was added to the test tube. After adding 300 µL methanol to test tubes, 100 µL organic solvent samples (1 mg/mL) and standards (BHT and rutin) were added. Each test tube needs three milliliters of reagent solution with 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Mix well. Foil sealed the test tubes after 90 minutes in a 95-degree Celsius incubator. We compared the samples' absorbance at 695 nm to a blank reagent after cooling to room temperature. AAE per milligram of extract was utilized to express all results.

Results and Discussion

Quantification assays

Quantification of total phenolics, tannin and flavonoid

The Folin-Ciocalteu reagent was used to measure the total phenolics of all the fruit extracts in this investigation. The results are displayed in Table 1. The standard curve's linear regression equation ($y = 0.0114x - 0.001$, $R^2 = 0.9945$) was used to determine the total phenol content, which was reported in gallic acid equivalents (GAE). The ethanol and ethyl acetate fruit extracts of *F. dalhousiae* yielded the largest amounts of total phenolic, at 414.32 and 262.07 mg GAE/g extract, respectively. A considerable amount of total phenolics (205.84 mg GAE/g extract) were discovered in the ethyl acetate extracts of *F. hispida* fruit. The results showed that out of all the extracts, the *F. dalhousiae* ethanol extract had the best antioxidant capability due to its high total phenolic content. Phenolic compounds, which are believed to be the most significant antioxidant component, are found in all plants (Djeridane et al., 2006). According to scientific research, consuming a wide range of phenolic compounds contained in natural foods on a regular basis reduces the risk of serious health conditions due to their antioxidant capacity (Surh, 2002). The bark acetone extract of *Ficus amplissima* contains a substantial concentration of phenolic chemicals at 50 µg/mL, according to Arunachalam et al. (2014). According to research, there is a significant amount of phenolic content in the methanol extract of F. bengalensis bark (Ramasamy et al., 2015).

Quantification of total tannin

Tannin concentration was determined by analyzing solvent extracts generated from the soxhlet apparatus and maceration of fruit extracts of *F. dalhousiae* and *F. hispida* (Table 1). Both the ethanol and ethyl acetate extracts of *F. dalhousiae* fruit samples had higher tannin content, with 401.74 and 223.42 mg GAE/g extract, respectively. next came F. hispida ethyl acetate extract (173.86 mg GAE/g extract). There are more tannins in the ethanol extract of fruit from *F. dalhousiae* and *F. hispida* because the polyphenolic components are polymerized more effectively. Free radicals can be scavenged more effectively by tannins and other high molecular weight phenolics, according to research. Due to their astringent properties, tannins are found in several medications. They relieve a variety of ailments, such as leucorrhea, hemorrhoids, diarrhea, and neck pain (Flieger et al., 2021). Due to its low tannin content and other anti-nutritional elements, this fruit was completely safe to consume. Additionally, the tannins present in *F. dalhousiae* and *F. hispida* fruits may improve the extracts' antioxidant qualities.

Quantification of total flavonoid

The fruit extracts of *F. hispida* and *F. dalhousiae* were tested for flavonoid content (Table 1). A linear regression equation of the standard curve was used to compute the total flavonoid content, which was expressed in Rutin equivalents (RE) ($y = 0.0098x + 0.0066$, $R^2 = 0.998$). Both the *F. hispida* (50.87 mg RE/g) and *F. dalhousiae* (76.24 mg RE/g) ethyl acetate extracts had significant amounts of flavonoids, out of all the extracts examined. The data showed that the *F. dalhousiae* ethyl acetate extract had the highest total flavonoid content. According to studies, the chloroform extract of the fruits of *F. religiosa*, *F. benghalensis*, and *F. glomerata* had a high concentration of flavonoids, ranging from 93.96 to 361.45 mg QE/g (Abusufyan et al., 2018). Ruqaya et al. (2017) revealed that the methanol extract of *F. religiosa* fruit had a high flavonoid concentration (113 mg RE/g), which effectively contributed to the scavenging of free radicals. Fruit extracts from *F. dalhousiae* and *F. hispida* contain a lot of flavonoids, which aid in their antioxidant activity and ability to scavenge free radicals.

In vitro antioxidant studies

DPPH scavenging activity

The DPPH radical scavenging activity of several fruit extracts from *F. dalhousiae* and *F. hispida* is illustrated in Fig. 3. With each extract, we calculated their IC_{50} value, which showed how much of the extract was needed to scavenge half of the DPPH free radicals. An antioxidant with a low IC_{50} value has promising antioxidant properties. The 37.03 µg/mL IC_{50} value was determined for the ethyl acetate extract of *F. dalhousiae*. According to the results, the ethanol extract of F. guttata had more antioxidant activity than the 55.52 µg/mL fruit of *F. hispida*. Ethyl acetate and ethanol extracts, in comparison to aqueous, chloroform, and petroleum ether extracts, demonstrated possible antioxidant action in the fruit. The fruit samples that were investigated display significant free radical scavenging activity, comparable to that of the natural antioxidant rutin (6.35 µg/mL) and the synthetic antioxidant BHT (7.93 µg/mL). Relative Ficus species have also been found to exhibit strong DPPH radical scavenging capabilities, according to some publications. DPPH has a long history of use as a free radical to research reducing chemicals and is a great reagent for investigating the free radical

scavenging properties of plant components (Duan et al., 2006). Yadav et al. (2011) reported that the DPPH• scavenging activity of *F. benghalensis* latex was good, with an IC_{50} of 28.63 µg/mL. Research by Asokumar et al. (2009) revealed that the DPPH scavenging activity of *F. microcarpa* leaf extracts was high, with an IC_{50} value of 28.63 µg/mL. *Ficus religiosa* extracts had DPPH scavenging capabilities ranging from 62 to 68 µg/mL, per Ruqaya et al. (2017). Every part of *F. microcarpa*, including the leaves, stems, and fruit, has a strong DPPH scavenging activity that ranges from 7.3 to 21.4 µg/mL, according to Changwei et al. (2018).

ABTS cation radical scavenging activity

ABTS radical decolorization assay was used to determine the TEAC (Trolox Equivalents Antioxidant Capacity). As µM Trolox Equivalents/g of extract, the results were presented. In Figure 5, we can see the outcomes of the ABTS cation radical scavenging activities of the fruit extracts of *F. hispida* and *F. dalhousiae*. Radical scavenging activity was highest in the fruit's ethanol extracts. After extracting the *F. dalhousiae*, the following solvents are used: ethyl acetate, water, petroleum ether, chloroform, and 74305.6µM TE/g. The results showed that the synthetic antioxidant BHT had a concentration of 95347.2 µM TE/g extract, while the conventional natural antioxidant rutin had 94166.7 µM TE/g sample.

Similar to this, a lot of study has been done on the ABTS cation radical scavenging activity of many Ficus species. According to earlier research by Arunachalam et al. (2013), Ramasamy et al. (2015), and Changwei et al. (2018), the distinct species are *F. amplissima*, *F. bengalensis*, and *F. macrocarpa*, respectively. All of these experiments show that Ficus species are effective at scavenging ABTS radicals. According to these findings, fruit extracts of *F. dalhousiae* and *F. hispida* may have significant antioxidant and free radical scavenging properties when taken with nutrients because of their high capacity to donate hydrogen.

Superoxide scavenging activity

Superoxide radicals, which are precursors to more reactive oxygen species, are notoriously damaging to biological components. The production of superoxide radical in living organisms can lead to the dismutation reaction, which in turn forms hydrogen peroxide. Figure 6 shows the findings of studying the fruit extracts of *F. hispida* and *F. dalhousiae* for their ability to scavenge superoxide anion. Results showed that the extracts effectively neutralized the superoxide radicals produced in the riboflavin-NBT-light system in laboratory tests. A 55.22% scavenging activity was observed in the ethanol extract of *F. dalhousiae*, with F. hispida coming in second at 49.42%. Comparable to BHT and BHA, the ethyl acetate extracts from both samples exhibited strong free radical scavenging activity. There was moderate scavenging activity observed in all of the *F. dalhousiae* fruit extracts. Ramasamy et al. (2015) found that a methanol extract of *F. bengalensis* bark at 800µg/mL had the highest superoxide scavenging activity, 143.91%. *F. amplissima* bark, fruit, and leaf acetone extracts yielded 47.90%, 50.81%, and 48.49%, respectively, according to a study by Arunachalam et al. (2013). *F. dalhousiae* fruit ethyl acetate extract showed a good percentage of superoxide radical scavenging activity, making it an encouraging antioxidant.

Phosphomolybdenum assay

A green phosphate/Mo (V) combination was detected at its peak absorbance at 695 nm, using the phosphomolybdneum technique, which relies on the antioxidant component reducing Mo (VI) to Mo (V). Table Fig. 7 displays the results of an analysis of the total antioxidant capacity of several fruit extracts of *F. dalhousiae* and *F. hispida*. Extracts of *F. dalhousiae* in ethanol and ethyl acetate, respectively, showed the highest antioxidant capacity, at 274.73 and 163.41 mg AAE/g, from the plant. Additionally, the antioxidant activity of the other extracts was quite good. According to research published by Murugan et al. (2012), a significant antioxidant capacity was observed in an acetone extract of *F. amplissima* bark (958.80 mg AAE/g extract). According to Changwei et al. (2018) and Yadav et al. (2011), the latex of *F. benghalensis* demonstrated the greatest activity in scavenging phospho-molybdenum. The fruit acetone extract of *F. amplissima* exhibits significant antioxidant capacity (277.15 mg AAE/g extract), according to a study (Paudel et al., 2020). The antioxidant capacity of the ethyl acetate extract was higher than that of the other solvent extracts tested. Free radical scavenging activity is thus associated with the antioxidant capacity seen in *F. dalhousiae* extracts.

The current research emphasizes the impressive antioxidant capabilities of chosen species of Ficus, with the adaptable *F. dalhousiae* serving as the most abundant source of compounds with a wide range of chemical structures. For the first time, our study has also shown that *F. dalhousiae* has better antioxidant benefits than *F. hispida*. There is an immediate need to create considerably less harmful medications from *F. dalhousiae* for inflammation-related illnesses, given the shifting worldwide trend towards the use of safe herbal remedies. As the need for medicine continues to rise, this finding should further motivate researchers to look for other medicinal characteristics in these species.

Table 1: Phenolic, Tannin and flavonoids content of *F. dalhousiae* and *F. hispida* fruit

Extracts		**Phenolic GAE/g extract**	**Tannin GAE/g extract**	**Flavonoids RE/100 g**
F. dalhousiae	**Petroleum ether**	32.16 ± 0.5	34.42 ± 0.68	24. 61 ± 0.47
	Chloroform	28.07 ± 0.87	24.47 ± 0.67	22.5 ± 0.77
	Ethyl acetate	262.07 ± 1.33	223.42 ± 0.87	76.24 ± 0.2
	Ethanol	414.32 ± 1.01	401.74 ± 0.72	47.19 ± 0.71

	Hot Water	47.95 ± 0.5	44.66 ± 0.9	33.18 ± 0.4
F. hispida	**Petroleum ether**	13.45 ± 0.5	10.35 ± 0.78	8.35 ± 0.47
	Chloroform	33.33 ± 0.87	33.87 ± 0.77	11.75 ± 0.61
	Ethyl acetate	205.84 ± 0.5	173.86 ± 0.46	50.87 ± 0.65
	Ethanol	68.71 ± 0.5	70.67 ± 0.33	12.97 ± 0.88
	Hot Water	51.16 ± 0.5	52.92 ± 0.59	7.67 ± 0.2

Values are mean of triplicate determination (n=3) ± standard deviation, statistically significant at $p < 0.05$ where $^{a}>^{b}>^{c}>^{d}$ in each column

Fig. 1: DPPH scavenging activity of *F. dalhousiae* and *F. hispida* fruit

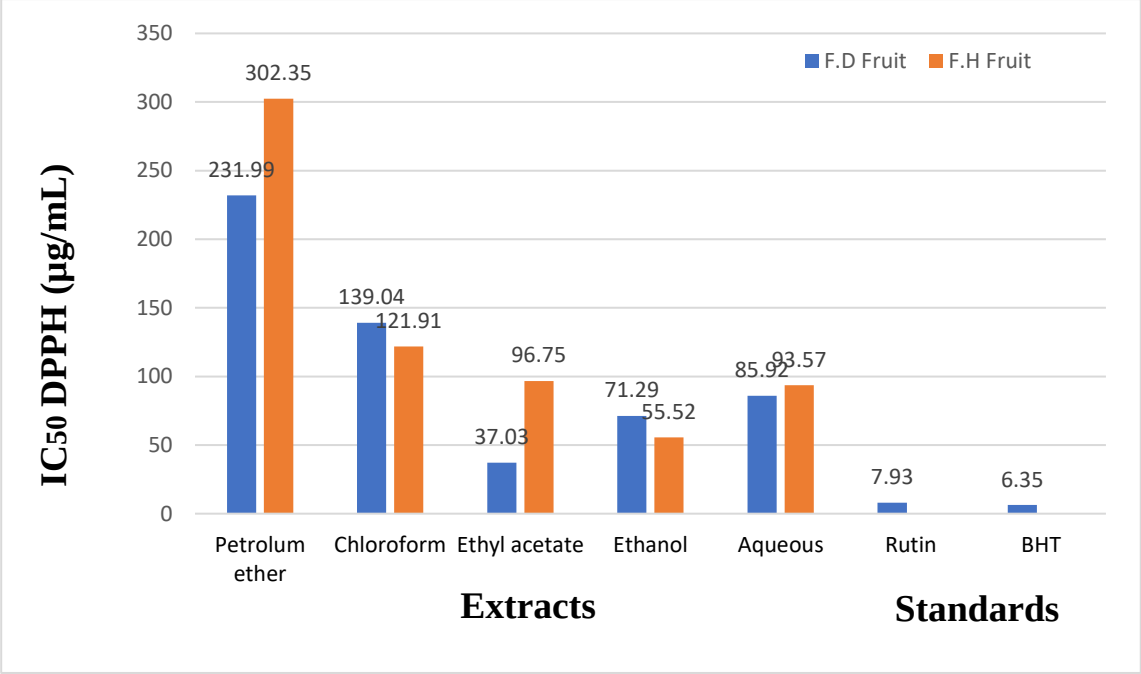


Fig. 2. ABTS scavenging activity and Superoxide radical of *F. dalhousiae* and *F. hispida* fruit

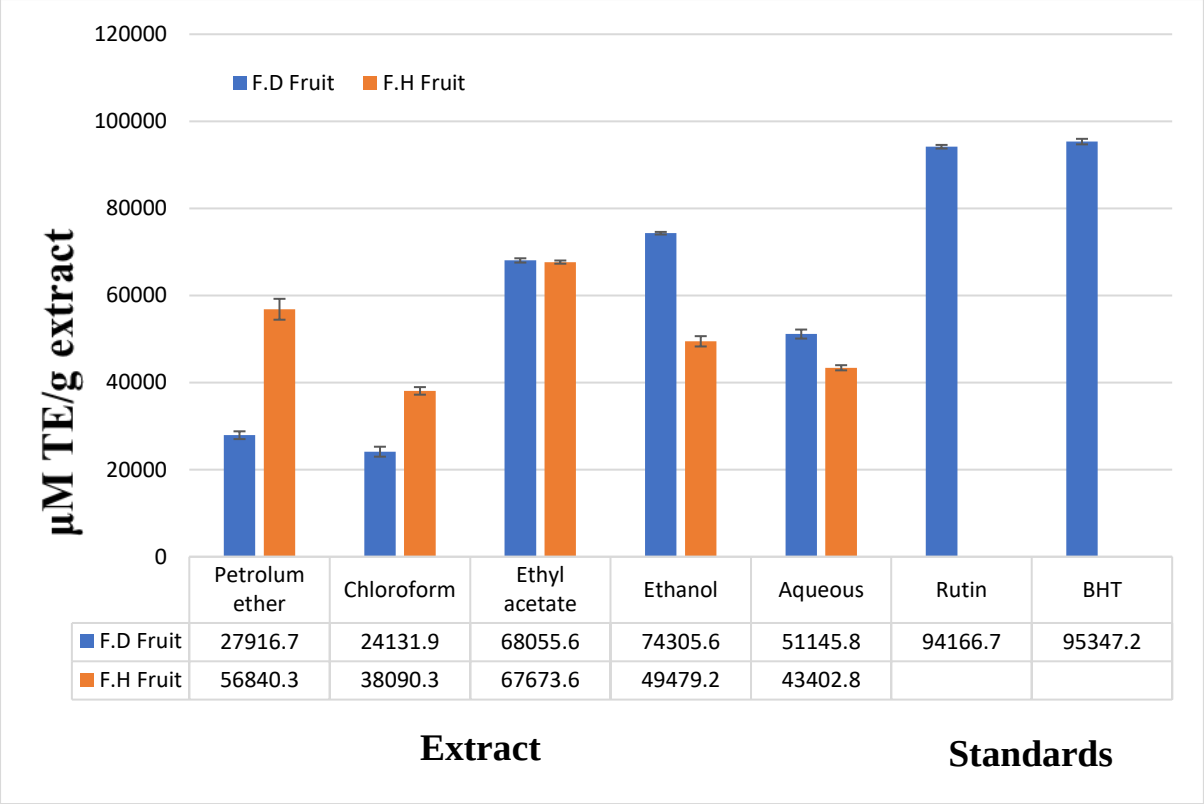


Fig. 3. Superoxide radical of *F. dalhousiae* and *F. hispida* fruit

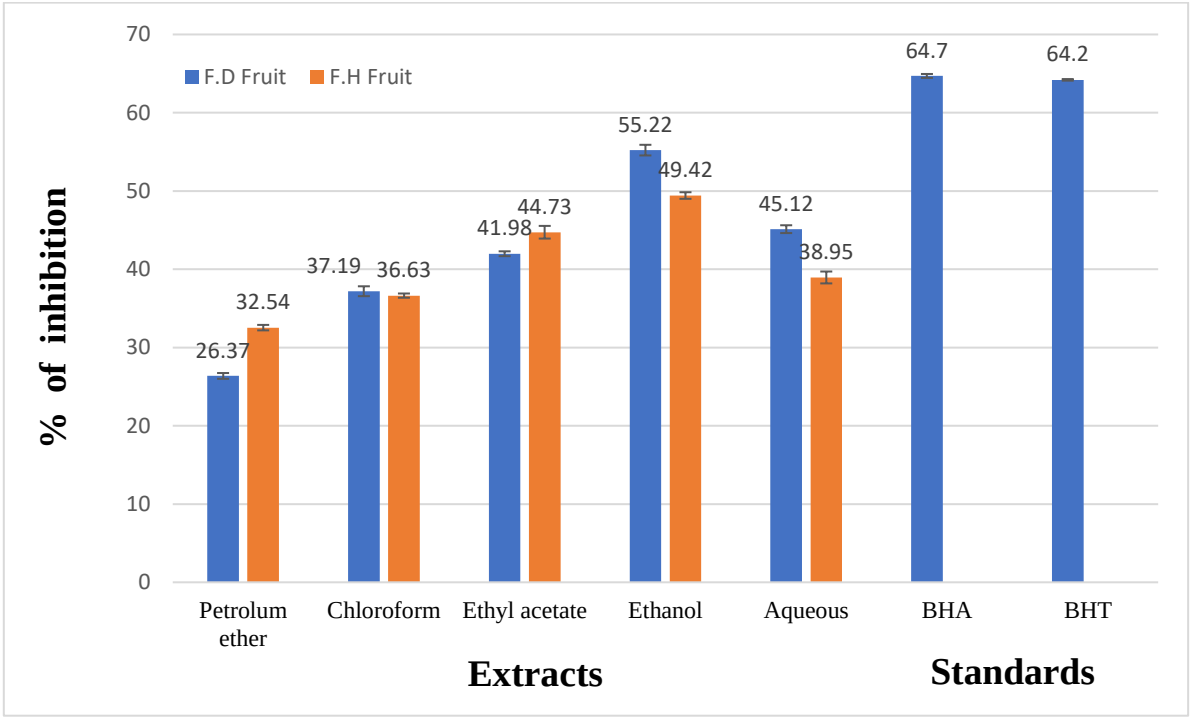
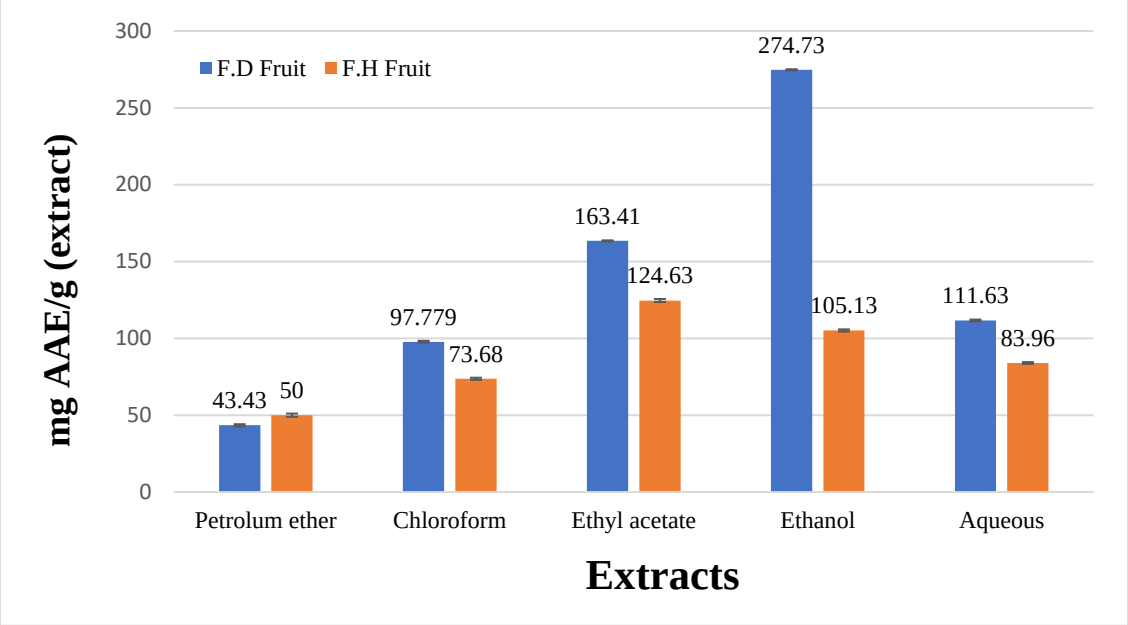


Fig. 4. Phosphomolybdenum assay of *F. dalhousiae* and *F. hispida* fruit



Conclusion

Ficus callosa is a well-known medicinal plant used around the world to treat various illnesses. It has scientifically proven antibacterial, anticancer, antifungal, and anti-inflammatory properties. However, there is no scientific proof of some traditional uses, like wound healing and easing menstrual pain. Different parts of *F. callosa* are used in various regions for different health issues, showing the need for more research. Identifying specific compounds that provide these benefits can be difficult, as most studies look at crude extracts. More research is needed to link these compounds to their health effects and to prove *F. callosa* potential in modern medicine. The Ficus genus is known for its healing properties, and studying further may validate its traditional uses. This research also looks at the nutritional value of *F. callosa* for humans and emphasizes its importance in both traditional medicine and cooking. There are 132 Ficus species used for food and 78 known for their health benefits, making the Ficus genus an important source of nutrients. Studies confirm that Ficus species can have health advantages. By examining their traditional uses in diets and medicine, we can better understand their contributions to nutrition and health, potentially sparking more interest in ethnopharmacological research. Future studies should conduct thorough ethnobotanical surveys. These should focus on nutritional and health uses while considering growth stages, cultural practices, and regional food preferences.

ACKNOWLEGEMENT

In order to express my appreciation to everyone who assisted and contributed, I would like to thank each and every author who worked on this study. In addition to Sasi Bioprospecting Laboratory in Coimbatore, Tamil Nadu, India.

Reference

1. Aarland, R.C., Bañuelos-Hernández, A.E., Fragoso-Serrano, M., Sierra-Palacios, E.D., Díaz de León-Sánchez, F., Pérez-Flores, L.J., Rivera-Cabrera, F., & Mendoza-Espinoza, J.A. (2017). Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of *Echinacea purpurea* and *Echinacea angustifolia* extracts. *Pharm Biol., 55*(1), 649-656. doi: 10.1080/13880209.2016.1265989.
2. Abusufyan, S., Ibrahim, M., & Mohib, K. (2018). Comparative *in vitro* Antidiabetic and Antioxidant Activity of Various Extracts of *Ficus* Species. *Pharmacognosy Journal, 10*, 349-354.
3. Arunachalam, K., Iniyavan, M., & Parimelazhagan, T. (2013). A HPTLC Method for the Identification of Potential Therapeutic Compound of Kaempferol from *Ficus amplissima* Smith. *International Journal of Pharmaceutical Sciences Review and Research, 22*, 166-171.
4. Arunachalam, K., Murugan, R., & Parimelazhagan, T. (2014).Evaluation of Antioxidant Activity, and Nutritional and Chemical Composition of *Ficus amplissima* Smith Fruit. *International Journal of Food Properties, 17*, 452-463.
5. Asokumar, K., Umamaheswari, M., Bahrudeen, A., Sivashanmugam, A.T., Subhadradevi, V., & Ravi, T.K. (2009). Antioxidant and Hepatoprotective activities of the fractions of *Ficus microcarpa* using *in vitro ex vivi* models. *Food Chemistry, 10*, 125-162.
6. Barański, M., Srednicka-Tober, D., Volakakis, N., Seal, C., Sanderson, R., Stewart, G.B., Benbrook, C., Biavati, B., Markellou, E., Giotis, C., romadzka-Ostrowska, J., Rembiałkowska, E., Skwarło-Sońta, K., Tahvonen, R., Janovská, D., Niggli, U., Nicot, P., & Leifert, C.

(2014). Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *Br J Nutr.*, *112*(5), 794-811. doi: 10.1017/S0007114514001366.

7. Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*, 276–277.

8. Changwei, A., Anping, L., Abdelnase, A., Elzaawely, T., & Xuan, S.T. (2018). Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. Extract. *Food Control, 19*, 940–948.

9. Chaudhar, L.B., Sudhakar, J.V., Anoop, K., Omesh, B., Rinkey, T., & Murthy, G.V.S. (2012). Synopsis of the Genus *Ficus* L. (Moraceae) in India. *Taiwania, 57*, 193-216.

10. Djeridane, A., Yousfi, M., Nadjemi, K., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plant extracts containing phenolic compounds. *Food Chemistry, 97*, 654-660.

11. Duan, X.J., Zhang, W.W., Li, X.M., & Wang, B.G. (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chemistry, 95*, 37-43.

12. Flieger, J., Flieger, W., Baj, J., & Maciejewski, R. (2021). Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. *Materials* (Basel), *14*(15), 4135.

13. Gursoy, N., Sarikurkcu, C., Cengiz, M., & Solak, M.H. (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. *Food Chem Toxicol. 47*(9), 2381-2388.

14. Hamed, M.A. (2011). Beneficial effect of *Ficus religiosa* Linn. on high fat- induced hypercholesterolemia in rats. *Food Chemistry, 129*, 162-170.

15. Jayaprakasha, G.K. & Rao, L. (2000). Chemical composition of the flower oil of *Cinnamomum zeylanicum* blume. *Journal of Agricultural and Food Chemistry*, *48*, 4294-4298. doi: 10.1021/jf991395c.

16. Kumar, A., Robert, D., Wood, K.E., Light, B., Parrillo, J.E., Sharma, S., Suppes, R., Feinstein, D., Zanotti, S., Taiberg, L., Gurka, D., Kumar, A., & Cheang, M. (2006). Homocysteine- and cysteine-mediated growth defect is not associated with induction of oxidative stress response genes in yeast. *Biochemical Journal, 396*, 61-69. doi: 10.1042/BJ20051411.

17. Kumar, B., Vijayakuma, M., Govindarajan, R., & Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing exploring medicinal plants of India. *Journal of Ethnopharmacology, 114*, 103–113. doi: 10.1016/j.jep.2007.08.010.

18. Liu, J., Jiang, B., Zhang, T., Mu, W and Li, Y. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry, 106*(2), 444–450.

19. Makkar, H.P.S. (2003). Quantification of tannins in tree and shrub foliage: A laboratory Mannual. Dondrecht. The Netherlands: Kluwer academic publishers.

20. Murugan, R., Arunachalam, K., & Thangaraj Parimelazhagan,T. (2012). Antioxidant, Anti- inflammatory Activity, and Phytochemical Constituents of Ficus (*Ficus amplissima* Smith) Bark. *Food Science Biotechnolology, 1*, 59-67.

21. Patel, S., Showers, D., P., Tzeng, T.R., Qian S., & Xuan, X. (2014). Microfluidic separation of live and dead yeast cells using reservoir-based dielectrophoresis. *Biomicrofluidics, 6*, 34 -102. doi:10.1063/1.4732800.

22. Paudel, M. R., Joshi, P. R., Chand, K., Sah, A. K., Acharya, S., Pant, B., & Pant, B. (2020). Antioxidant, anticancer and antimicrobial effects of In vitro developed protocorms of *Dendrobium longicornu*. *Biotechnology Reports*, 28, e00527.doi:10.1016/j.btre.2020.e00527.

23. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry, 269*, 337–341.

24. Prince, L., & Prabakaran, P. (2011). Antifungal activity of medicinal plants against plant pathogenic fungus *Colletotrichum falcatum*. *Asian Journal of Plant Science and Research, 1*, 84-87.

25. Ramasamy, M.V., Nishanthini, A., & Sakthi Devi, G. (2015). Evaluation of Antioxidant Properties of *Ficus bengalensis* Bark. *International Journal of Pharmacognosy and Phytochemical Research, 7*,758-763.

26. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice, E.C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free radical biology and Medicine, 26*(1), 1231-1237

27. Ruqaya, M., Al-Ezzy1 Bushra, H., & Saleh1 Rafal, S. (2017). Assessments of Total Flavonoids, Anti-oxidant and Antibacterial Activity of *Ficus religiosa* Methanolic Extract *in vitro*. *International Journal of Pharmaceutical Sciences Review and Research, 45*, 6-10.

28. Singleton, V.L. & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic., 16*, 144-158.

29. Sudhakar, J.V. & Murthy, G.V.S. (2016). Identity of *Ficus amplocarpa* and *F. guttata* (Moraceae), the two closely allied south Indian endemic species and their conservation status. *Rheedea, 26*, 69–73.

30. Sumitra, C., & Parekh, J. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research, 10*, 175 – 181.

31. Surh, Y.J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. *Food and Chemical Toxicology, 40*, 1091-1097.

32. Umapathy, E., Ndebia, E.J., Meeme, A., Adam, B., Menziura, P., Nkeh-Chungag, B.N., & Iputo, J.E. (2010). An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *Journal of Medicinal Plants Research, 4* (5), 789-795.

33. Yadav, Y.C., Srivastava, D.N., Vipin, S., Sarita, S., Seth, A.K., & Sharad, K. (2011). *In-Vitro* Antioxidant Activity of Methanolic Extraction of *Ficus benghalensis L.* Latex. *Pharmacologyonline, 1*, 140-148.