A COMPARISON OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF EDIBLE *FICUS AURICULATA* AND NON-EDIBLE *FICUS RACEMOSE*

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Abstract

Ethnobotanically important plants possess a wide array of biologically active phytochemicals. Among these, phenolics, and flavonoids have shown notable pharmacological properties. The present study was conducted to compare contents of total flavonoids (TF) and total phenolics (TP) of two fig species namely *Ficus racemose* and *Ficus auriculata*. *Ficus racemose* is a non-edible fig whereas *Ficus auriculata* is a commonly consumed plant by locals. Additionally, reactive oxygen species (ROS) scavenging potential of the selected *Ficus* species was also estimated using the spectrophotometric method. Fresh fruit samples of selected *Ficus* species were collected from their native habitat in District Abbottabad. Half of the samples were shade-dried and extracted with 70% methanol. Antioxidant, TF, TP of the methanolic extracts were quantified using 2,2-diphenyl-1picrylhydrazyl (DPPH), aluminium chloride ($AlCl_3$), and folin ciocalteau reagent assays respectively. Results revealed that the phenolics were detected at all the selected wavelengths. 370 nm proved an optimal wavelength for quantification of total phenolics in *Ficus* species. Dried fruit of *Ficus racemosa* was recorded for the highest TPC. The same fruits showed the highest total flavonoid content at 517 nm. On the contrary, percent antioxidant activity was found highest in the fresh fruits of *Ficus racemosa*. The study gave a deeper insight into the phytochemicals and antioxidant potential of two *Ficus* species which can further be exploited to get maximum ethnobotanical benefits.

Keywords: *Ficus recemosa*; *Ficus auriculata*; folin ciocalteau; phenolic content; antioxidant activity

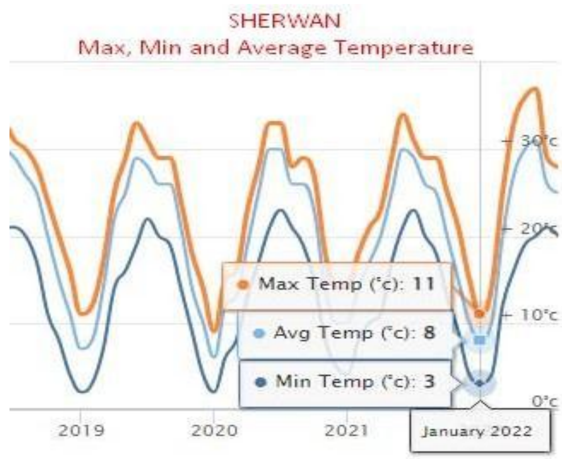
Introduction

Medicinal plants possess many important biochemical constituents and are beneficial in the field of medicine and pharmaceutical sciences. Determining antioxidant molecules from natural sources is a burning issue. The use of medicinal plants in the preparation of traditional medicines is reported across the globe, however, unsuitable exercise of traditional medicines can have harmful effects and additional investigation is required to find out the safety and efficacy of such practices (Chen et al., 2023). Modern procedures are now being utilized for the extraction of active chemicals from plants and their utilization in the formation of drugs (Bitwell et al., 2023). Among thousands of medicinal plants, present studies were focused to determine free radical scavengers especially phenolic compounds, flavonoid contents, and ascorbic acid present in *Ficus racemosa* (syn. *Ficus glomerata*) and *Ficus auriculata* (syn. *Ficus roxburghii*) of Fig family because of their medicinal importance. *Ficus* is a diverse group of commercially important plants with significant genetic and phytochemical diversity among its members known for their remarkable pharmacological activities (Salehi et al., 2021). *Ficus auriculata* is traditionally used for treating wounds, vomiting, dysentery, diarrhea, cholera and mumps (Mehra and Tandon, 2021). Gastrointestinal problems are cured by use of juice extracted from fresh leaves of *Ficus racemosa* (Pahari et al., 2022). Similarly, *Ficus racemosa* is part of herbal remedies against diabetes, dysentery, chronic wounds, glandular swelling, cervical adenitis, gynecological disorders, infection of bronchi, Asthma, piles, and diseases of the kidney and spleen (Deep et al., 2013). Constituents of plant sources mostly used for traditional purposes show their action against the reactive chlorine species, reactive nitrogen species, and reactive oxygen species. These species under physiological and pathological situations are manufactured in humans and other animals (Mansoor et al., 2022). These species are very reactive and are usually unstable and called free radicals because the outer orbit of these species comprises an unpaired electron. There are “two faces” of free radicals in biology. At physiologic levels, they support signaling and regulatory molecules on the other hand these free radical species are extremely harmful and toxic oxidants to cells at pathologic levels (Mittler et al., 2022). Free radicals which are of no use and may cause damage to cells are neutralized by enzymatic and non-enzymatic reactions through antioxidants. These antioxidants form networking in the living system and work together to get rid of all oxidative stresses. Humans cannot produce phenolic compounds and thus rely on its external sources. Functional food development and their supplementation with conventional food is necessary and need of the time (Zheng et al., 2021). Previous experimentation shows that this group of phenolic compounds has anticancer, antiviral, anti-inflammatory, anti-allergic, and antibacterial activities (Weli et al, 2014). Due to the frequent use of *Ficus* species in traditional medicines, researchers are now interested to find out its pharmacological and phytochemical properties. The leaf and fruit parts of *Ficus auriculata* were reported to contain anti-inflammatory and antioxidant activities (El-Fishawy et al., 2011). The fruit of *Ficus glomerata* contains phenolic compounds which is why it shows free radical scavenging activity against gastric mucosal damage in ulcer patients (Rao et al., 2008). Though previous reported showed the phytochemical composition of species of fig, no comparison between its edible and non-edible species has been made so far. Therefore, the current study aimed to quantify the total flavonoids, total phenolics, and total antioxidant potential of selected edible and non-edible figs in both fresh and dried form. Based on this, we hypothesized that shade drying does not alter the phytochemical profile of the specimen. Moreover, the antioxidant potential of the selected species in its fresh and dried forms has been made for the first time.

Materials and Methods

2.1. Study Area Description

Two species of the fig genus were selected and identified using the flora of Pakistan. The plants identified were authenticated by Professor Dr Manzoor Hussain, taxonomist and chairman Department of Botany Hazara University, Mansehra. Herbarium sheets with the specific voucher number consisting of leaf and fruit samples were deposited in herbarium department of botany, Hazara University Mansehra. Fruits of both Ficus species were collected from the hilly area of Bachaa Sherwan KPK Pakistan with GPS location 34°10'35.3"N 73°03'05.1" E and Altitude: 1072.00m/3517.06 ft. The average Temperature and rainfall of this area are shown in the figure. 1 a and b respectively. The local community of this area is mainly linked with agriculture. The reason for the selection of this medicinal plant is that the local community use this plant fruit as a remedy for the treatment of various type of pimples and pustule.



Figures. 1 (a) Average Temperature of Sherwan region from 2019 to 2022 (Source: World Weather Online)

Figures. 1 (b) Rainfall amount of Sherwan region from 2019 to 2022 (Source: World

Weather Online)

2.2. Chemicals and reagents

All the analytical grade chemicals and reagents (sigma) were used in this study. Reagents and chemicals that are used in this study are Methanol (99%), ascorbic acid, 2,2-diphenyl- 1-picrylhydrazyl (DPPH), Rutin, Gallic acid, Sodium hydroxide, Folin-Ciocalteu reagent, Catechin, anhydrous aluminum chloride, sodium nitrite, and anhydrous sodium carbonate. All the listed chemicals were purchased from Worldwide Scientific (Islamabad, Pakistan).

2.3. Fruit Extract Preparation

A fresh form of fruit samples was washed with distilled water and sliced using a surgical blade while dry form Sample fruits were washed with distilled water and sliced in thin slices through a sterilized blade and kept in shade for drying at room temperature for 40 days. Fruit tissues of both fresh and dried form with a weight of 1 g were grounded with mortar and pestle. Homogenate was prepared in 5 ml of 10% acidified Methanol and added in labeled centrifuge tubes, vortexed to mix properly, and incubated in ice for 30 min. After 30 min samples were vortexed again and centrifuged (Eppendorf 5415 R) at 12000 rpm for 30 min at 4 $^{\circ}$C. Supernatant was collected in a clean labeled tube and pellets were discarded. The supernatant was stored at -21 $^{\circ}$C (Dawlance) until further analysis. All samples were prepared in triplicates.

2.4. Determination of Total Phenolic contents

Spectrophotometer (UV-Vis spectrophotometer TG80+) was used for the determination of total phenolic content. 0.5 ml of folin calteaue reagent (1:10 dilution) + 2.5 ml of 20% Na_2CO_3 was mixed in a quartz cuvette and incubated for 30 min at room temperature which was then used as blank while the same solution was used in another quartz cuvette for calibration. For sample analysis 0.1 ml of each plant extract was added in 0.5 ml of folin reagent (1:10 dilution) in a cuvette and topped up by adding 2.4 ml of Na_2CO_3 (20%). All these extracts were Incubated at room temp for 30 min. Absorbance was measured in a 1 cm quartz cuvette at 280 nm, 370 nm, 460 nm, 517 nm, 570 nm, 630 nm, and 760 nm. Gallic acid was used as positive control prepared in 80% methanol and linear regression curves were drawn (Dirar et al., 2019).

2.5. Determination of Total Flavonoid contents

Total flavonoid contents were determined by using an aluminum chloride assay (Seifu et al, 2017). Sample extract 0.5 mL was added in 2 ml distilled water in a 10 ml test tube. In each tube, 5% $NaNO_2$ with an amount of 0.15 ml was added and placed for 5 min incubation. After incubation, 0.15 ml $AlCl_3$ (10%) was added and provided 1 min time for the reaction to settle down. After this procedure, 1 M NaOH solution of volume 1 ml was added, and adjusted the total volume with distilled water up to 5 ml. The absorbance of the resulting solution was analyzed after 10 min at 517 nm in Spectrophotometer (UV- Vis spectrophotometer TG80+) Rutin is used as a standard to compare the total flavonoid contents of samples.

2.6. Determination of Antioxidant activity

The antioxidant capacity of the fresh and dry forms of both *Ficus* species was investigated through DPPH assay. This assay was performed with a small modification in the method described by Alam et al., (2019). The absorbance of 80% methanol was considered blank while the negative control (DPPH solution) was also run simultaneously. DPPH solution was prepared by dissolving 4 mg in 125 ml 80% methanol. 0.2 ml of sample extracts was added to 3 ml cuvette in 2.8 ml of DPPH solution and absorbance was measured at 517 nm on a UV-Vis TG80+ spectrophotometer. Ascorbic acid was used as positive control prepared in 80% methanol and linear regression curves were drawn.

2.7. Data analysis

Three biological and three technical replicates were used in the study. Results were tested statistically by Analysis of Variance (ANOVA) at $p \leq 0.05$. Significant differences among the means were calculated by multiple comparison Tukey HSD test (SPSS 26.0).

Results

3.1. Systematic Position of Selected Figs

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Rosales

Family: Moraceae

Genus: *Ficus*

Species: *Ficus auriculata*/ *Ficus racemosa*

3.2. Total Phenolics Content

Three-way ANOVA followed by Tukey HSD test showed significant results for the three factors i.e., fruit type, species, and wavelengths at $p \leq 0.05$. However, the interaction of all three factors was found non-significant. Among the main factors, dried fruits contained significantly higher concentration (0.09 mg/g GAE) of total phenolics than fresh fruits (0.06 mg/g GAE). Similarly, the total phenolics in *F. racemosa* were higher (0.09 mg/g GAE) than in *F. auriculata* (0.06 mg/g GAE). Among the wavelengths, 280 nm (0.11 mg/g GAE) and 370 nm (0.10 mg/g GAE) recorded significantly higher concentrations of total phenolics than the rest of the wavelengths i.e., 460 nm (0.07 mg/g GAE), 517 nm (0.06 mg/g GAE), 760 nm (0.06 mg/g GAE), 570 nm (0.06 mg/g GAE) and 630 nm (0.06 mg/g GAE). Interaction of the three factors revealed the highest value of total phenolics (0.18 mg/g GAE) at 370 nm in dried fruits of *F. racemosa* whereas the lowest concentration (0.04 mg/g GAE) was observed in fresh fruits of *F. auriculata* at 630 nm. Furthermore, it was observed that *F. racemosa* showed the highest concentration of total phenolic in its dried fruits at all wavelengths. A similar pattern for a concentration of total phenolics was observed for fresh fruits of *F. racemosa* whose concentration followed the concentration of total phenolics in dried fruits of *F. racemosa* at all wavelengths. (Figure 2)

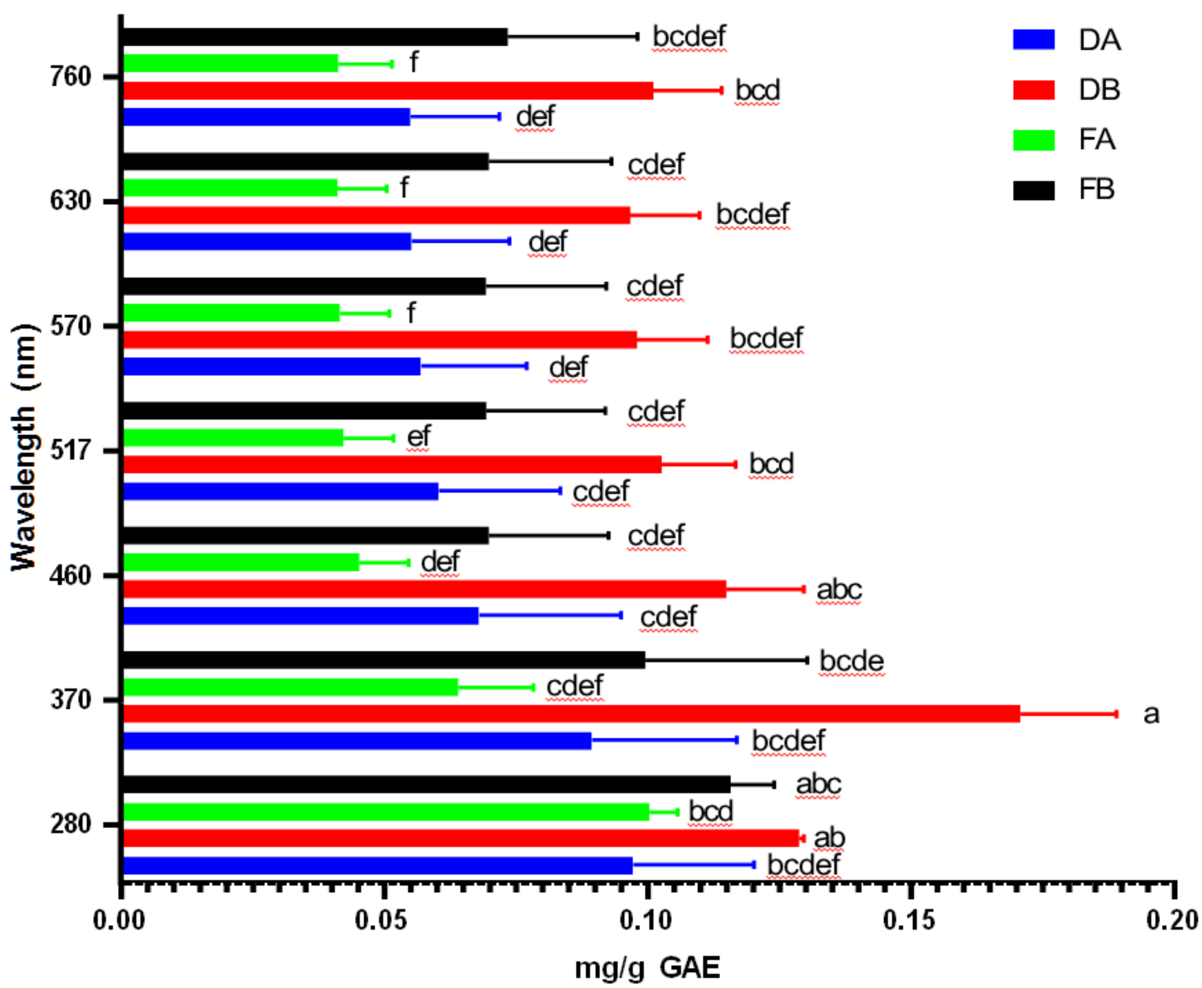


Figure 2. The concentration of Total Phenolics observed in dried and Fresh fruits of *F. auriculate* and *F. racemose* at seven wavelengths (280 nm, 370 nm, 460 nm, 517 nm, 570 nm, 630 nm, and 760 nm). Three-way ANOVA results for interaction (Fruit type*species*wavelengths) are presented as main ± SD. Different letters show significant differences at $p \leq 0.05$.

3.3. Total Flavonoid content analysis

Two-way ANOVA results for total flavonoids in *Ficus* sp. were significant for both factors whereas the interaction was non-significant at $p \leq 0.05$. Interestingly, both species have statistically similar total flavonoid content in their fresh and dried fruits. However, the dried fruits of *F. racemosa* showed significantly higher total flavonoids content (0.15 mg/g QE) than flavonoids in dried (0.13 mg/g QE) and fresh fruits (0.12 mg/g QE) of *F. auriculata* (Figure 3).

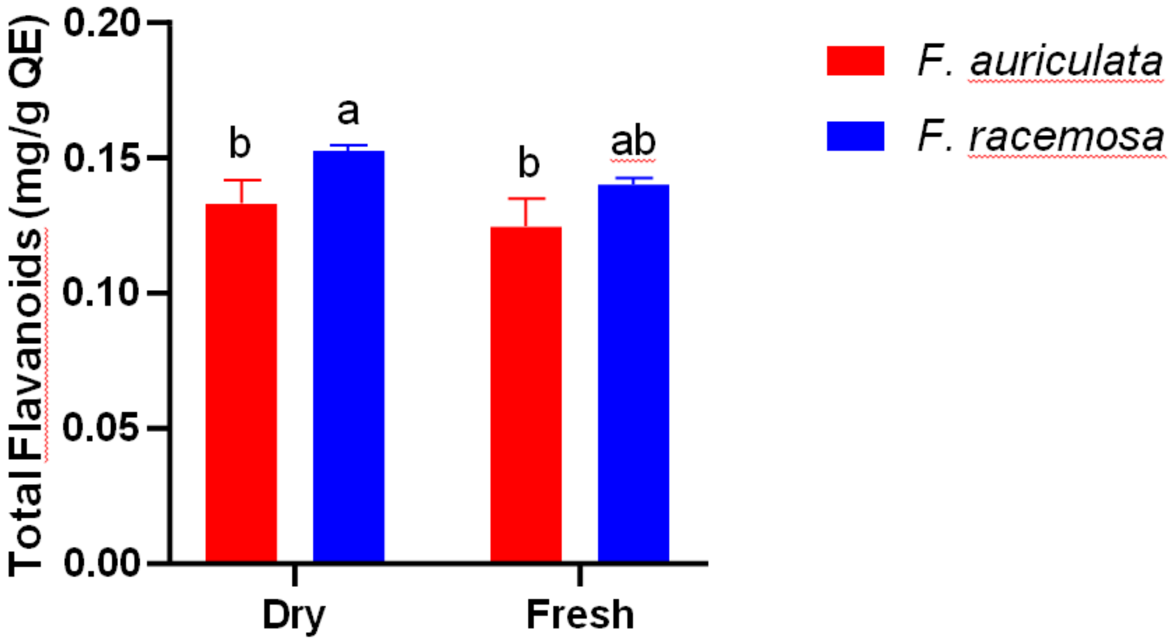


Figure 3. Total Flavonoids were recorded in dried and fresh fruits of *F. auriculata* and *F. racemosa* at 517 nm. Two-way ANOVA results are presented as mean ± SD. Different letters show significant differences at $p \leq 0.05$.

3.4. Determination of Antioxidant activity

Two-way ANOVA revealed significant results for the percent antioxidant activity of *Ficus* sp. for only one factor i.e., fruit type at $p \leq 0.05$. The results for the second factor i.e., Species and the interaction (Fruit type*species) were found non-significant. The highest antioxidant activity (13.21%) was observed in fresh fruits of *F. racemosa* whereas dried fruits of *F. auriculata* showed the least antioxidant activity (5.18%). No significant difference was recorded in the antioxidant activity of fresh and dried fruits of a single species (Figure 4).

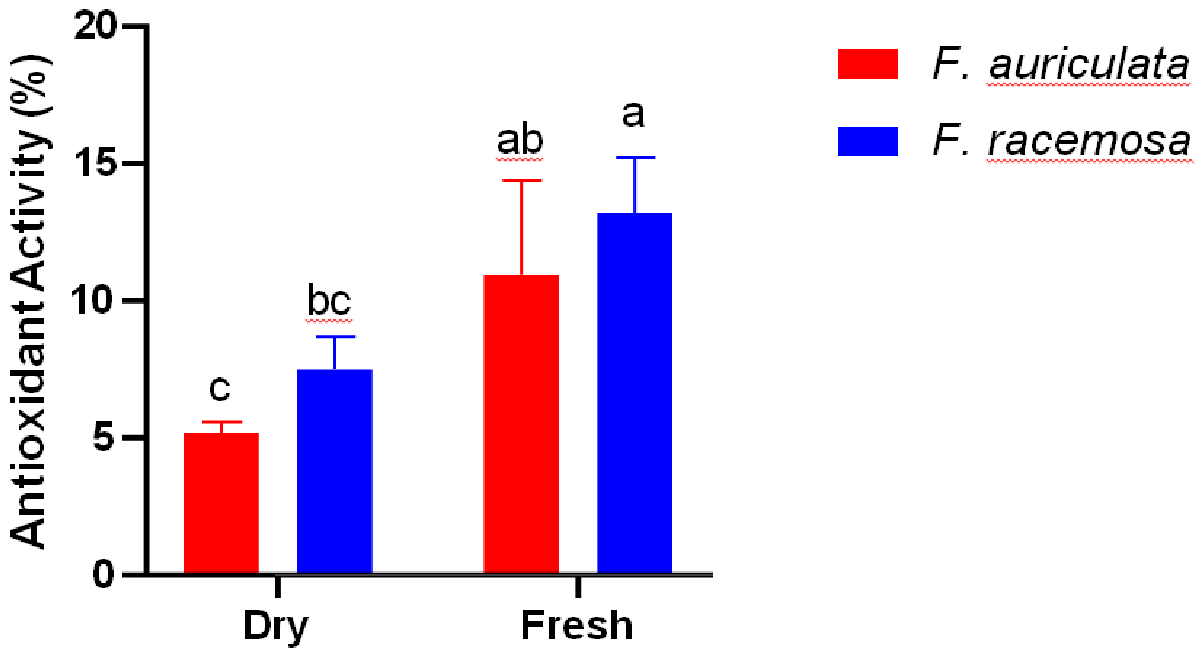


Figure 4. Percent Antioxidant Activity in dried and fresh fruits of *F. auriculata* and *F. racemosa* at 517 nm. Two-way ANOVA results are presented as mean $\pm$ SD. Different letters show significant differences at $p \leq 0.05$.

Discussion

Most researchers today emphasize determining different pharmacological properties present inside natural sources, especially in plants. Among the hidden secrets of natural medicinal plants are the

most useful ones that could heal or ameliorate diseases or sufferings. Pharmaceutical industries pay attention to find out active components present inside these kinds of medicinal plants and other natural sources to use them in antibiotics,

drugs, and other nutritional products. This research aimed to dig out some important biochemical constituents of two species of *the Ficus* genus with a focus on the dry and fresh form of extract preparations that may be beneficial in the field of medicinal and pharmaceutical sciences. Regular use of *Ficus* species for the preparation of traditional medicines brings the attention of researchers to explore its pharmacological and phytochemical properties. New methods are operated for the withdrawal of active chemicals from these plantsand to find out their antioxidant potential and other biological function (Venkatesh and Chauhan, 2008). Gaire and colleagues found that *Ficus auriculata* exhibited possible antioxidant activity (Gaire et al., 2011). After investigation, it was concluded by researchers that *Ficus auriculata* is commercially significant and shows inspiring pharmacological properties due to the richness of antioxidants in leaves and fruits (El-Fishawy et al, 2011). It was also observed that antioxidant activity is higher in fruits than leaves extract (El-Fishawy et al, 2011).

Similarly, extracts obtained from *Ficus racemosa* are a powerful chemopreventive agent and suppress $KBrO_3$-facilitated nephrotoxicity in rats (Khan and Sultana, 2005). Potassium bromate ($KBrO_3$) acts as a carcinogen in rats and is liable for the generation of glutathione content and antioxidant enzymes (Khan and Sultana, 2005). This *Ficus racemosa* extract also significantly recovered the antioxidant enzymes and glutathione content in rats (Khan and Sultana, 2005). During the finding of phytochemical properties of *Ficus racemosa*, it is revealed that its trunk bark, fruits, leaves, stem bark, and roots contain many phytochemicals (Devaraj et al., 2008). Ethanolic extract of *Ficus racemosa* has the potency to act as an antioxidant (Veerapur et al, 2009). The fruit of *Ficus glomerata* contains phenolic compounds which is why it shows free radical scavenging activity against gastric mucosal damage in ulcer patients (Rao et al., 2008). During the comparative studies between the ethanolic and water extracts of *Ficus racemosa* through stopped-flow spectrophotometric analyses and nanosecond pulse radiolysis it is revealed that 20 µg/ml of ethanol extract exhibit higher steady state antioxidant activity than any other extract of ethanol and water (Veerapur et al., 2009). The literature cited demonstrates that *Ficus auriculata* and *Ficus racemosa* possess phenolic and flavonoid compounds and have high antioxidant activity. However, no single study was done to analyze the comparative antioxidant activity, and total phenolic and flavonoid contents between both *Ficus auriculata* and *Ficus racemosa* and in between their fresh and dry forms. These results obtained from different research papers favor my research results. It was observed that all the selected wavelengths showed results for total phenolic contents in fresh and dried fruits of both species. The results suggest that phenolics with a wide range of absorption were present in the Methanolic extracts of Ficus species. The highest TPC was observed at 370 nm which suggests that the species contain phenolics with their maximum absorbance in the UV region of the spectrum. Though it contradicts the previous reports in which 765 nm was used as the optimal

wavelength for measuring TPC, it also points to the need of revisiting the standard protocol of TPC determination currently in practice. (Negi et al., 2018; Patle et al., 2020). Unlike phenolic where a large difference was observed in their content depending upon the type of fruit and specie. The difference in the flavonoid content was limited to species only. This may be attributed to differential regulatory patterns of flavonoid biosynthesis among the two species. For instance, six genes were reported to show a differential expression pattern in four species of Ficus (Chen et al., 2022). Expression profiling of genes involved in flavonoid biosynthesis can provide further insights into the differential concentration of flavonoids observed. Percent Antioxidant activity was found higher in dried fruits of Ficus species. The process of drying may likely be the reason behind the lower percent antioxidant activity in dried fruits as demonstrated in the research article of Thamburaj et al in 2022. It has been reported that the process of drying affects the activity

of polyphenols which reduces the percent antioxidant activity of Ficus species (Slatnar et al., 2011; Karam et al., 2016). The phytochemistry of the above-discussed Ficus species may reveal potential bioactive compounds. Therefore, we recommend investigating these biochemical compounds using HPLC, LCMS, GCMS, and for structural elucidation NMR.

Conclusion

Based on these results it was concluded that *Ficus racemosa* showed higher TPC, TFC, and percent antioxidant activity. The selected phytochemicals were found higher in dried fruits whereas antioxidant activity was higher in the fresh form which suggests that the process of drying reduces the antioxidant activity. Though the taste of *Ficus racemosa* is not good, it possesses higher bioactive phytochemicals and antioxidants than *Ficus auriculata and* may prove an important addition to the ethnobotanical recipes currently in practice. Based on these results, it was concluded that different species of fig show differential phytochemicals and antioxidant profile. Furthermore, it also suggests that an exploration of non-edible figs may lead to the discovery of higher antioxidant-containing species. The study recommends a through investigation of pharmacologically important constituents of figs using sensitive analytical techniques. Moreover, a structure-activity relationship may reveal the potential mechanism of action of fig phytochemicals.

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