Journal Pre-proof

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PII: S2214-7861(24)00001-9

DOI: https://doi.org/10.1016/j.jarmap.2024.100528

Reference: JARMAP100528

To appear in: *Journal of Applied Research on Medicinal and Aromatic Plants*

Received date: 23 June 2023
Revised date: 16 November 2023
Accepted date: 2 January 2024

Please cite this article as: Kumanan N Govaichelvan, Nazimah Hamid, Kevin Kantono, Khanom Simarani and Jamilah Syafawati Yaacob, Unleashing the Power of Nature: Investigating the Effects of Storage on Plant-Based Pigments and Bioactivities in Tropical *Ficus* spp. Extracts, *Journal of Applied Research on Medicinal and Aromatic Plants,* (2024) doi:https://doi.org/10.1016/j.jarmap.2024.100528

Unleashing the Power of Nature: Investigating the Effects of Storage on Plant-Based Pigments and Bioactivities in Tropical *Ficus* spp. Extracts

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Declarations:

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

Competing interests: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Funding: This research was funded by Universiti Malaya (SATU Joint Research ST009-2021).

Author Contributions: J.S.Y. conceived and designed the experiments; K.N.G. performed the experiments; K.N.G. and J.S.Y. analyzed the data; N.H. and K.K. advised on the statistical analysis; J.S.Y. and K.S. contributed reagents/materials/ analysis tools; K.N.G. wrote the first draft of the paper; J.S.Y., K.S. and N.H. revised the paper.

Acknowledgements: The authors thank Universiti Malaya, Malaysia for the experimental facilities and financial support (SATU Joint Research ST009-2021) provided.

Abstract

The phytochemical composition and antioxidant activities of tropical *Ficus* trees: *Ficus benjamina* (FB), *Ficus racemosa* (FRa) and *Ficus religiosa* (FRe) were analysed in this study. The bark and leaf samples of these species were subjected to solvent extraction using absolute methanol and analysed for their total chlorophyll (TCC), total carotenoid (TXc) and total anthocyanin (TAC) contents. The TPC, TFC and antioxidant potential of the extracts were also determined. The stability of the extracts during storage at different temperatures (4°C, -20°C and -80°C) was investigated at weeks 0, 4 and 8. The results showed that the bark of *F. racemosa* and leaves of *F. benjamina* contained the highest amounts of phenolic content, with significantly higher antioxidant properties. PLSR analysis revealed that the secondary metabolite composition strongly influenced the antioxidant activities differently with *Ficus* trees. VIP scores were also computed to determine the most important factors that contributed to the ABTS and FRAP activities in the extracts from each species. Specifically, TPC, TFC, TXc, TCC, chlorophyll a, and chlorophyll b contents were the most important variables for *F. benjamina*. Meanwhile, TPC, TAC, TFC and chlorophyll b were the most important factors for *F. racemosa*, and only TAC, TPC and chlorophyll b were the most important factors for *F. religiosa*. Long term storage (8 weeks) of the extracts at 4°C was observed to cause the highest percentage of metabolite degradation (up to 88.56% in TXc, 66.86% in TPC and 81.93% in TFC). Storage at -80°C was found to be the most suitable for retaining the secondary metabolites content and bioactivities of the samples. Taken together, *F. religiosa* leaf was identified as the best source of pigments and antioxidants. The findings of this study highlight the huge potential of plant extracts as both natural pigments and antioxidants in the food industry. These extracts can serve as a source of colorants while also improving the nutritional quality of food products, which aligns with the growing demand for clean-label and sustainable food options that can replace synthetic food additives.

Keywords: *Ficus* trees; phenolics compound; antioxidant; storage stability

1. Introduction

Malaysia ranks 12th globally in being one of the most bio-diverse country (Kamarudin, 2010). However, rapid socio-economic growth in the country has led to ecosystem destruction and contributed to the lack of bioresources, including useful medicinal plants (Hezri and Nordin Hasan, 2006). Noteworthy, medicinal plants are usually endemic species. This is because their medicinal properties are due to the presence of secondary metabolites produced in response to the stimuli in their natural environments that may not be expressed under culture conditions (Figueiredo and Grelle, 2009). Therefore, global efforts on in situ conservation to protect indigenous plants focus on establishing protected areas and taking an approach that is ecosystem-oriented, rather than species-oriented (Chen et al., 2016). The amount of biodiversity conserved can be increased via in situ conservation (Forest et al., 2007). Thus, an understanding of the bioactivities in medicinal plants is essential for their conservation and to further explore their utilisation to improve health (Madharia and Jahan, 2015).

At present, the use of organic pigments such as anthocyanins have garnered an increasing interest among the scientific community, to serve as a healthier alternative to synthetic dyes in the food and textile industries (Jackman et al., 1987). As such, the stability of anthocyanin pigments during processing and storage as well as its mechanism of degradation are still researched for its sustainable utilization in various industries (Liu et al., 2018). In addition, the discovery of reactive oxygen species (ROS) that are responsible in inducing oxidative stress in the human body has increased the need for novel antioxidant sources. This is because, diseases such as neurodegenerative disorders, coronary heart diseases, diabetes, lung damage, arthritis, inflammation and cancer are the results of oxidative stress (Stadtman, 1992).

Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO•) are examples of ROS that contain radical and non-radical oxygen species formed by the partial reduction of oxygen. Endogenous ROS are produced in cells via mitochondrial oxidative phosphorylation, or they may be generated due to the interaction with exogenous factors like xenobiotic compounds. Cellular antioxidant defense system can be overwhelmed when ROS level increases or cellular antioxidant capacity decreases, thus resulting in oxidative stress. Oxidative stress causes ROS-mediated damage of nucleic acids, proteins and lipids, and has been shown to be involved in carcinogenesis (Trachootham et al., 2009). Besides that, ROS has been claimed to induce gene activation that promotes tumour metastasis (Ishikawa et al., 2008). Meanwhile, studies have shown that plant compounds such as carotenoids, tetrapyrrole, phenolics, flavonoids and

alkaloids can be an effective antioxidant source due to their free radical scavenging abilities (Sakuta, 2014). The *Ficus* genus includes over 1400 species and are classified into about 40 genera, where most are found in the tropics or subtropics and only a few with edible fruits (Mehraj et al., 2013). *Ficus* spp. provides fundamental genetic resources as it possesses high economic and nutritional values and also contributes to the biodiversity in the rainforest ecosystem (Rønsted et al., 2007). *F. religiosa* (locally known as Ara Suci), *F. racemosa* (Ara Cedung) and *F. benjamina* (Beringin) are native to the South Asian tropical climates. They have been widely used as traditional medicines by the Indians and Chinese in this region (Devanesan et al., 2018; Paarakh, 2009; Sirisha et al., 2010). Previous phytochemical investigations on these selected species have shown the presence of tannins, saponins, flavonoids, steroids, terpenoids and other useful phenolic compounds in their bark extracts. Moreover, modern researches have revealed the antioxidant, antibacterial and other pharmaceutical properties from the bioactive extracts obtained from leaves and barks these species (Al-Yousuf, 2012; Deep et al., 2013; Sandeep et al., 2018).

In this study, the content of secondary metabolites and antioxidant properties in different aerial organs of the three Ficus trees (*F. benjamina, F. religiosa, F. racemosa*) were compared. The stability of the extracts when stored at various temperatures was further evaluated. The outcomes of this study provide valuable information on the medicinal properties of several *Ficus* species, which currently are still underutilized. This study further adds to the knowledge on the stability of bioactive extracts during storage, with potential to aid in the development of storage strategies for plant-based pigments.

2. Materials and Methods

2.1 Plant Materials and Sample Preparation

Leaf and bark samples of *F. benjamina, F. racemosa* and *F. religiosa* were collected from Universiti Malaya, Malaysia. The samples were cut into small pieces and oven-dried at 45°C for 24 hours. To prepare the plant extract, 6 g of dried samples were soaked in 180 mL of 70% (v/v) methanol for 48 hours and incubated at 4°C in airtight jars wrapped with aluminum foil. For chlorophyll and carotenoid quantification, absolute methanol was used for the extraction instead. The solvent was then filtered using a filter paper (No. 1, Whatman filter paper, United States). The filtrate was used in the determination of chlorophyll and carotenoid contents. For measurement of

phenolic and flavonoid contents, the filtrate was evaporated to dryness using a rotary evaporator (Rotavapor® R-3, Büchi Labortechnik AG, Flawil, Switzerland) at 45°C. Then absolute methanol was added to adjust the stock concentration to 20 mg/mL. The extracts were stored at three different temperatures: 4°C, -20°C and -80°C for further analysis. All assays were carried out in triplicates.

2.2 Total Chlorophyll and Carotenoid Contents

The total chlorophyll and carotenoid pigments content of the extracts were measured based on a previously described protocol (Sumanta et al., 2014). The absorbance values at 665.2 nm, 652.4 nm and 470 nm were obtained using a UV-200-RS spectrophotometer. Chlorophyll and carotenoid contents were calculated using the formula shown below (Lichtenthaler and Buschmann, 2001):

$$\text{Chlorophyll a } (C_a, \mu g/mL) = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$\text{Chlorophyll b } (C_b, \mu g/mL) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$\text{Xanthophylls + Carotene } (C_{x+c}, \mu g/mL) = (1000\ A_{470} - 1.63\ C_a - 104.96\ C_b)/221$$

2.3 Total Phenolic Content

The total phenolic content (TPC) of extracts was measured based on the method described by Sun et al. (2007), with minor modifications. Briefly, 20 µL of the methanolic extract was added to 150 µL of diluted Folin-Ciocalteu reagent and incubated in the dark for 10 minutes at ambient temperature. Then, 150 µL of 2% (w/v) sodium carbonate, Na_2CO_3 solution was added into the mixture and kept under dark conditions for 45 minutes. The absorbance was then read using a UV-200-RS spectrophotometer (MRC Ltd., Holon, Israel) at 765 nm. The TPC of samples was evaluated using the calibration curve plotted with a series of gallic acid standards. Results were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g DW) of the extracts.

2.4 Total Anthocyanin Content

The pH differential method was employed in determining the total anthocyanin contents (TAC) of the samples following a previously described method (Yusof et al., 2018). The pH of methanolic extracts of samples were adjusted using 0.025 M potassium chloride at pH 1.0 and 0.4

M sodium acetate at pH 4.5. The absorbance was read at 520 nm and 700 nm using a UV-200-RS spectrophotometer (MRC Ltd., Holon, Israel). The following formula was used to determine the concentration of the anthocyanin pigment:

$$\text{Anthocyanin pigment content (mg/l)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)}$$

where $A = (Abs_{520} - Abs_{700})_{pH1.0} - (Abs_{520} - Abs_{700})_{pH4.5}$

MW of cyanidin-3-glucoside = 449.2 g/mol

DF = dilution factor

ε = 26, 900

2.5 Total Flavonoid Content

Aluminium chloride colorimetric method as described by Yusof et al. (2018) was used to quantify the total flavonoid content (TFC) of the methanolic extracts. A 25 µL aliquot of each extract was mixed with 150 µL of 70% (v/v) methanol, 10 µL of aluminium chloride, $AlCl_3$ ($AlCl_3 \cdot 6H_2O$), 10 µL of sodium acetate, ($NaC_2H_3O_2 \cdot 3H_2O$) (1 M) and 2.80 mL of autoclaved milli-Q water. The mixture was incubated for 40 minutes. Then, using a microplate reader, the absorbance was measured at 415 nm. A calibration curve using quercetin as the standard was then plotted to calculate the flavonoid concentrations. The TFC was expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW) of extracts.

2.6 Evaluation of Antioxidant Properties

2.6.1 ABTS Radical Scavenging Activity Assay

The ABTS assay was conducted following the method described by Yusof et al. (2018). Briefly, 0.0054 g of potassium persulfate, $K_2S_2O_8$ was dissolved into 10 mL of autoclaved milli-Q water to prepare 2.6mM $K_2S_2O_8$ solution. Next, 0.0381 g of ABTS was added to 10 mL of autoclaved milli-Q water to obtain a 7.4mM ABTS solution. The two solutions were combined and left to incubate at room temperature in a dark environment for a duration of 12 hours. Following that, the mixture was diluted using autoclaved milli-Q water, and the absorbance was measured at 734 nm. This process was repeated until the absorbance reading reached 0.70 ± 0.02

at 734 nm. Next, 20 µL of methanolic extracts at five distinct concentrations were introduced to 200 µL of the diluted ABTS mixture and allowed to stand for 30 minutes. Methanol was used as the blank. The percentage of inhibition was expressed using the formula described below:

$$\text{Percentage of inhibition (\%)} = (Abs_{blank} - Abs_{sample}) / (Abs_{blank}) \times 100\%$$

2.6.2 Ferric-reducing Antioxidant Power Assay

Ferric-reducing Antioxidant Power (FRAP) reagent was prepared by mixing 25 mL of 30mM acetate buffer with 2.5 mL of 10mM TPTZ and 2.5 mL of 20mM of iron (III) chloride at a volume ratio of 10:1:1 (Chan et al., 2007). The mixture was incubated for 15 minutes at 37°C before use. Then, 10 µL of the extract was diluted at five different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0mg/mL). FRAP reagent (300 µL) was further added to the extract and incubated at 37°C for 30 minutes under dark conditions. A calibration curve was plotted using iron (II) sulphate. Absorbance was measured at 593 nm. Autoclaved milli-Q water was used as the blank. The antioxidant potential of the extract was expressed as milligrams of iron (II) sulphate equivalent ($FeSO_4$) per gram of dried sample.

2.7 Storage and Stability Assessment

The extracts were stored at 4°C, -20°C, -80°C and the secondary metabolite content and antioxidant properties of the extracts were assessed at day 0 as well as after 4 and 8 weeks of storage.

2.8 Statistical Analysis

Data was analyzed using either an independent t-test or Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) as the post-hoc analysis on SPSS (IBM, USA). The relationships between the secondary metabolites content and antioxidant activities were determined using a multivariate approach, namely the Partial Least Squares Regression (PLSR) using XLSTAT version 2021.1 (Addinsoft Inc., Paris, France). The scores for Variable Importance in Projection (VIP) were calculated to evaluate the importance of the secondary metabolites content to the resulting antioxidant potential. A VIP score of more than 0.8 (90% confidence interval; CI) was deemed as important, whereas a VIP score of more than 1 (95% CI) was deemed

as the most important. The relationships between the secondary metabolites content and antioxidant potential were then illustrated using a correlation plot. Additionally, Hotelling–Lawley multivariate analysis of variance (MANOVA) tests were carried out to determine if there are significant differences ($p<0.05$) between the different *Ficus* species, source of extract (organ type), storage temperature and storage duration in terms of the bioactive secondary metabolites content and antioxidant potential.

3. Results

3.1 Bioactive Pigments Content

The absorption spectrum of extracts between 400 to 700 nm was recorded (Fig. 1). Based on the absorption spectra, it can be observed that the extract of *F. racemosa* leaves (FRaL) exhibited the highest peaks compared to other extracts. Following that, the bioactive pigments such as total chlorophyll, carotenoid, and anthocyanin content in the extracts of bark and leaves of the three *Ficus* species were quantified and compared. Among the bark extracts of the three *Ficus* species (Fig. 2a), the *F. benjamina* bark (FBB) extract was observed to possess the least amount of total chlorophyll, carotenoid, and anthocyanin pigments. In contrast, *F. religiosa* bark (FReB) extract contained significantly the highest amounts of total chlorophyll (TCC) and carotenoid (TXc) pigments. However, no significant difference was observed between its anthocyanin content (TAC) and that found in *F. racemosa* bark (FRaB), but both were significantly higher than TAC of *F. benjamina* bark (FBB). The chlorophyll a content (Ca) of the bark extracts from all three species also showed insignificant differences. FReB was found to contain significantly the highest amount of chlorophyll b (Cb), but no significant difference was observed between the Cb in FBB and FRaB extracts.

Results for the leaf extracts of the three *Ficus* species are summarised in Fig. 2b, *F. religiosa* leaf (FReL) extract also had significantly the highest amounts of TCC, Ca, TXc and TAC pigments. It was noted that the TCC, Ca, and Cb values of both *F. benjamina* (FBL) and *F. racemosa* (FraL) leaves showed no significant differences between them. Nevertheless, FBL exhibited a significantly higher TXc compared to FRaL, whereas FRaL demonstrated a significantly higher TAC than FBL.

The ratio of chlorophyll a to chlorophyll b (a/b) in the six samples was also evaluated (Table 1). Data showed that the chlorophyll a/b ratio of FBB was significantly the highest among the bark extracts, while FReL was significantly the highest among the leaf extracts. In contrast, FReB had significantly the lowest chlorophyll a/b ratio among the bark samples. Interestingly, the bark extracts of *F. benjamina* and *F. racemosa* had higher chlorophyll a/b ratio than leaf extracts. However for *F. religiosa*, the leaf extract had a higher chlorophyll a/b ratio than bark extract.

3.2 Phenolics and Flavonoids Quantification

The TPC of extract was expressed as mg gallic acid equivalent per gram dry weight of sample (mg GAE/g DW), while the TFC was expressed as mg quercetin equivalent per gram dry weight of sample (mg QE/g DW). Among the bark extracts, FRaB had significantly the highest TPC and TFC, with TPC of 561.663 ± 4.583 mg GAE/g DW and TFC of 173.264 ± 0.865 mg QE/g DW, respectively (Fig. 3a). In contrast, FBB extract was found to contain significantly the least amount of TPC (39.823 ± 0.406 mg GAE/g DW) and TFC (33.213 ± 0.723 mg QE/g DW) (Fig. 3a). The leaf extract from *F. racemosa* (FRaL) also contained significantly the highest amount of TFC (171.455 ± 0.642 mg QE/g DW) (Fig. 3b). On the other hand, FBL was observed to contain significantly the highest TPC among all samples, with a TPC value of 472.754 ± 5.001 mg GAE/g DW (Fig. 3b).

3.3 Evaluation of Antioxidant Potential

3.3.1 ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of extracts was expressed as IC_{50}, which describes the minimum concentration of extract required to inhibit 50% of the ABTS free radicals. As seen in Table 2, the bark extract of *F. racemosa* (FRaB) exhibited significantly the lowest IC_{50} value (0.087 ± 0.002 mg/mL), indicating that it possessed the highest antioxidant potential against ABTS radicals among all bark samples. On the other hand, the bark sample of *F. benjamina* that had significantly the highest IC_{50} value, had the least ABTS antioxidant potential. In terms of the leaf extract, FBL had significantly the lowest IC_{50} value had the highest ABTS antioxidant potential compared to other leaf samples. Meanwhile, both FRaL and FReL extracts showed similar ABTS radical scavenging properties (Table 2).

3.3.2 Ferric-reducing Antioxidant Power (FRAP)

Results for the FRAP antioxidant assay of extracts are summarised in Table 3. Bark extracts of *F. racemosa* and *F. religiosa* exhibited similar FRAP antioxidant potential that were significantly higher than *F. benjamina* bark (FBB) extract. These are in direct contrast to that observed in the leaf extracts, where FBL exhibited significantly higher FRAP potential (1.551 ± 0.129 mg $FeSO_4$/g DW) than both *F. racemosa* and *F. religiosa* leaves.

3.4 Stability of *Ficus* Extract during Storage

The leaf and bark extracts of the three *Ficus* species were stored for 8 weeks at three different temperatures (4°C, -20°C and -80°C). The content of pigments and other secondary metabolites, as well as their antioxidant properties were evaluated after 4 and 8 weeks of storage and compared with the Day 0 sample. The storage stability of extracts would allow identification of the most suitable temperature with minimal degradation of bioactive compounds during storage.

3.4.1 Stability of Total Carotenoid Content during Storage of Extracts

As shown in Fig. 4 and Supplementary Table S1, the carotenoid content in both the bark and leaf extracts of all three *Ficus* species were found to be significantly reduced after 4 weeks of storage at 4°C and continued to degrade even further after 8 weeks. In contrast, significantly lower reduction of carotenoid contents was observed from the extracts stored at -20°C and -80°C after 8 weeks, compared to storage at 4°C. These effects were more apparent in FBB extracts, where the concentration of carotenoid compounds was observed to be stable throughout 8 weeks of storage at-20°C and -80°C, compared to 4°C (Fig. 4a and Supplementary Table S1).

3.4.2 Stability of Total Anthocyanin, Phenolic and Flavonoid Contents during Storage of Extracts

Data revealed that anthocyanin content of both the bark and leaf extracts of all three *Ficus* species increased during storage (Fig. 5 and Supplementary Table S2). The greatest increase was observed when the extracts were stored at 4°C, compared to the other two storage temperatures. This can clearly be observed in FRaL extracts which showed an initial TAC content of 0.1075 ± 0.0017 mg/g DW but increased significantly to 0.2654 ± 0.0221 mg/g DW, 0.2307 ± 0.0280 mg/g

DW and 0.2183 ± 0.0130 mg/g DW when stored at 4°C, -20°C and -80°C, for 8 weeks, respectively (Supplementary Table S2). Similarly, the TAC of FReL extracts also increased significantly from 0.1377 ± 0.0009 mg/g DW to 0.3073 ± 0.0250 mg/g DW, 0.2588 ± 0.0175 mg/g DW and 0.2552 ± 0.0150 mg/g DW after 8 weeks of storage at 4°C, -20°C and -80°C, respectively (Supplementary Table S2).

The stability of phenolic content of the extracts during storage is shown in Fig. 6 and Supplementary Table S3. Overall, it can be observed that the TPC of the extracts significantly decreased over time during storage, whereby the storage at 4°C was observed to result in the most degradation for all extracts (Supplementary Table S3). The TPC of *F. racemosa* bark (FRaB) and leaf (FRaL) showed the least degradation during storage at all tested temperatures, with percentage of degradation of 9.99 – 43.27% (bark) and 15.36 – 33.84% (leaf), respectively. On the other hand, the leaf extracts of *F. benjamina* (FBL) showed the highest degradation after 8 weeks (at all tested temperatures) with percentages of degradation of 59.66%, 63.94% and 66.86% when stored at -80°C, -20°C and 4°C, respectively. Interestingly the bark extracts of this species were observed to be more stable during storage, which showed 17.12%, 23.02% and 66.49% of degradation after 8 weeks when stored at -80°C, -20°C and 4°C, respectively.

The TFC of extracts decreased during storage (Fig. 7). All extracts stored at 4°C resulted in the highest TFC reduction after 8 weeks compared to samples stored at -20°C and -80°C (Supplementary Table S4). FBB extract showed significantly the highest reduction of TFC (81.93%) after 8 weeks storage at 4°C, while FReB extract stored at -80°C showed the least TFC degradation (6.91%) after 8 weeks (Supplementary Table S4).

3.4.3 Effects of Extract Storage on Antioxidant Potential

Fig. 8 and Fig. 9 illustrate the effect of storage on the antioxidant activity of the extracts at three different temperatures. Overall, the IC_{50} values increased significantly until 8 weeks of storage at all storage temperatures, except for FBB extract stored at -80°C, where the increase of IC_{50} was not significant (Supplementary Table S5). The FRaB extracts displayed the most substantial decline in antioxidant potential against ABTS radicals. They exhibited the highest percentage of increase in ABTS IC_{50} after 8 weeks of storage at -80°C, -20°C, and 4°C, with increments of 242.02%, 271.99%, and 271.64%, respectively (Supplementary Table S5).

Meanwhile, the FRAP values of the six extracts were observed to significantly decrease up to 8 weeks of storage (Supplementary Table S6). Generally, storage at 4°C led to the highest decrease of FRAP reducing activity, as shown by the highest percentage of degradation of FRAP values compared to extracts stored at -80°C and -20°C. For example, FRaL extract was observed to yield the highest reduction in FRAP values (64.97%) after 8 weeks of storage at 4°C (Supplementary Table S6). In contrast, the bark extracts of this species (FRaB) seemed to be more stable, with 26.59%, 29.48% and 33.97% of FRAP values reduction after 8 weeks of storage at -80°C, -20°C and 4°C, respectively.

3.5 Relationships Between Antioxidant Activity with Secondary Metabolites Content

The correlation between the secondary metabolites content and the antioxidant activities were analyzed using PLSR. As observed from the PLSR correlation biplot of *F. benjamina* (Fig. 10a), the leaf extracts were separated from the bark extracts. Along Dimension 1, the leaf extracts had negative scores, while bark extracts had positive scores. Meanwhile, most of the X variables such as the total phenolics (TPC), total flavonoids (TFC), total carotenoid (TXc), total chlorophyll (TCC), chlorophyll a and chlorophyll b contents, as well as the ratio of chlorophyll a to chlorophyll b were negatively loaded along Dimension 1. Only total anthocyanin (TAC) was positively loaded along Dimension 1 (Fig. 10a). These imply that the leaves of *F. benjamina* contained higher amounts of TPC, TFC, TXc, TCC, chlorophyll a and chlorophyll b than bark samples (Fig. 2, Fig. 3, Fig. 10a). However, these bioactivities would reduce with storage (Figs. 4, 6 and 7; Supplementary Tables S1, S3 and S4). On the other hand, bark contained significantly higher TAC than leaves (Fig. 2, Fig. 10a). However, TAC in the bark extracts would increase with storage (Fig. 5, Supplementary Table S2). These findings further confirmed earlier results of ANOVA.

In addition, the TFC, TAC, TPC, TXc, ratio of chlorophyll a to chlorophyll b and chlorophyll b content of *F. benjamina* extracts were found to be negatively correlated to the ABTS results (Fig. 10b). These indicate that the increase in these secondary metabolites content would significantly reduce the concentrations of extracts required to scavenge against the ABTS radicals, i.e. increase the antioxidant potency of the extracts. On the other hand, FRAP was observed to be positively correlated to TFC, TAC, TPC, TXc, chlorophyll b, ratio of chlorophyll a to chlorophyll b and TCC (Fig. 10c). These relationships were further confirmed by the PLSR correlation biplot shown in Fig. 10a. However, based on the computed VIP values (Supplementary Table S7), only

TPC, TFC, TXc, TCC, chlorophyll a, and chlorophyll b contents recorded VIP scores of >1.0, implying that these six bioactive metabolites were the most important variables that contributed to the antioxidant activities of *Ficus benjamina* extracts.

The PLSR correlation biplot for *F. racemosa* (Fig. 11a) further showed that the bark extracts were separated from the leaf extracts. Along Dimension 1, the bark extracts had negative scores, while leaf extracts had positive scores. The TPC, TFC and ratio of chlorophyll a to chlorophyll b were negatively loaded along Dimension 1, while the other X variables such as TAC, TXc, TCC, chlorophyll a and chlorophyll b were positively loaded along Dimension 1. *F. racemosa* bark extracts contained significantly higher amounts of TPC and TFC (Fig. 3, Fig. 11a), that reduced with storage (Supplementary Tables S3 and S4). Meanwhile, *F. racemosa* leaf extracts contained significantly higher TXc, TCC, chlorophyll a, chlorophyll b and TAC than bark extracts (Fig. 2, Fig. 11a). However, their contents significantly decreased with storage and increased with storage temperature (Figs. 4 and 5; Supplementary Tables S1 and S2). On the other hand, longer storage duration and at higher temperatures were observed to increase the TAC of the leaf samples (Fig. 5 and Supplementary Table S2).

The TPC, TXc, TFC, ratio of chlorophyll a to chlorophyll b, chlorophyll a and TAC were negatively correlated to the ABTS IC_{50} values of *F. racemosa* extracts (Fig. 11b), while FRAP was positively correlated to TPC, TFC, ratio of chlorophyll a to chlorophyll b and TXc (Fig. 11c). Nevertheless, among these variables, only TPC, TAC, TFC and chlorophyll b were the most important factors that contributed to the antioxidant potential of *F. racemosa* samples, with VIP scores of >1.0 (Supplementary Table S7). However, TCC was also found to be an important factor in influencing the antioxidant potential of *F. racemosa* (VIP score >0.8)

A PLSR analysis was also performed for *F. religiosa*. Based on the correlation biplot (Fig. 12a), it can be observed that the bark extracts were also separated from the leaf extracts. Along Dimension 1, the bark samples had negative scores while leaf samples had positive scores. Only TPC was observed to be negatively loaded along Dimension 1, while the other X variabes (TFC, ratio of chlorophyll a to chlorophyll b, chlorophyll a, TCC, chlorophyll b, TXc and TAC) were positively loaded along Dimension 1. *F. religiosa* leaf extracts contained significantly higher TFC, ratio of chlorophyll a to chlorophyll b, chlorophyll a, TCC, chlorophyll b, TXc and TAC than the bark extracts (Fig. 2, Fig. 12a). However, TPC was significantly higher in the bark than leaf samples (Fig. 3, Fig. 12a). It was also observed that the TFC, ratio of chlorophyll a to chlorophyll

b, chlorophyll a, TCC, chlorophyll b and TXc in *F. religiosa* leaf extracts would reduce with storage duration and storage temperature (Figs. 4 and 7; Supplementary Tables S1 and S4). Similarly, the TPC in *F. religiosa* bark extracts decreased with storage duration and storage temperature (Fig. 6, Supplementary Table S3). In contrast, TAC amount was observed to higher in the leaf extracts when stored at higher temperatures for a longer period of time (Fig. 5, Supplementary Table S2). Moreover, the ABTS IC_{50} values of *F. religiosa* extracts were negatively correlated to the TPC, TCC, TFC and chlorophyll b contents (Fig. 12b), while FRAP potential of the extracts was positively correlated to the TPC, ratio of chlorophyll a to chlorophyll b and TFC (Fig. 12c). However, based on the computed VIP values (Supplementary Table S7), only TAC, TPC and chlorophyll b were identified as the most important (VIP score > 1.0), while TXc and TCC were deemed as important (VIP score > 0.8) factors that influenced the antioxidant potential of *F. religiosa* extracts (Supplementary Table S7).

4. Discussion

4.1 Metabolite Content and Antioxidant Activity

Through pigment quantification using spectroscopy, it was identified that FReB and FReL contained the highest chlorophyll and anthocyanin contents among the bark and leaf extracts, respectively. The pigment composition in plants depends on host plant variety and surrounding environmental factors, which includes growing season, temperature, period of sun-light exposure, type of soil, soil moisture content and also method of leaf harvest (Rath et al., 2017). Noteworthy, methanolic extracts of all bark samples contained lesser chlorophyll and anthocyanins compared to their corresponding leaf samples. This is because, the photosynthetic activity in the bark is lesser than leaves. Chloroplast synthesis is influenced by sunlight exposure. Observation on thylakoid structure and frequency between leaves and bark revealed that bark chloroplasts contain lesser number of thylakoids, with longer shape and irregular arrangements compared to leaf chloroplasts (Pilarski, 1999a). In addition, the outer surface of bark has more chloroplast than the inner surface. The deeper the inner surface of the bark, the lesser the photosynthetic pigments are distributed due to reduction in transmittance of sunlight. However, if barks and leaves are equally sun-exposed, they can have similar amount of chlorophyll. Moreover, genetic factor and maturity can also

influence photosynthetic pigment composition in plants (Pilarski, 1999b; Pilarski and Tokarz, 2006).

Anthocyanins are found to be higher in the leaf extracts than bark samples. Previous studies have reported that anthocyanins are found in photosynthetic vacuoles and can enhance photosynthetic efficiency (Gould et al., 2010; Gould et al., 2000). Anthocyanins serve as a photoprotective agent for shade adapted chlorophylls during high light intensity. Hence the authors inferred that leaves possess anthocyanins as an adaptive mechanism to enhance photosynthesis.

For pigment content analysis, absolute methanol extract was used without any thermal process due to their heat-sensitivity. Chlorophylls are sensitive to light and oxygen, which can cause immediate degradation (Özkan and Bilek, 2015). Loss of central magnesium atoms due to the substitution of hydrogen ion in the chlorophyll structure degrades chlorophyll. Sometimes, chlorophyll can transform into pheophytin, resulting in the extract turning darker or brownish (Delgado-Vargas, 2003). The inevitable exposure to light and oxygen may have degraded the chlorophyll in the methanolic extract in this study. Hence, the actual chlorophyll content may not be accurate when using measurements based on the absorbance values. In this study, all extracts exhibited significantly higher amounts of chlorophyll b than chlorophyll a. This could be due to rapid degradation of chlorophyll a, which occurred at a faster rate than chlorophyll b. Chlorophyll could either deteriorate on standing due air exposure, allomerization or plant acid, which can be neutralized by suspending magnesium carbonate before extraction (Bruuinsma, 1963).

Among the bark extracts, FRaB extract showed significantly the highest ABTS radical scavenging activity compared to FBB and FReB. FRaB extract also contained significantly the highest amounts of phenolics and flavonoids. Meanwhile, among the leaf extracts, FBL extract exhibited significantly the highest ABTS radical scavenging activity compared to FRaL and FReL extracts. FBL also contained significantly the highest amount of phenolics. These showed that the high phenolics content in FRaB and FBL extracts correlated with their high antioxidant potential. This is in line with the findings reported in literature which showed that the accumulation of secondary metabolite composition influences antioxidant activity (Lopez et al., 2016). Nevertheless, the synthesis and accumulation of secondary metabolites in plants can be affected by endogenous genetic and morphological factors environmental stresses (Yang et al., 2018), which explained the different observations recorded by the different *Ficus* extracts. Environmental stresses include pathogen attack, high light, drought and nutrient deficiencies. Wounding reported

to increase accumulation of phenylpropanoids. Nutrient stress has been reported to increase phenolic content in plants (Akula and Ravishankar, 2011). Meanwhile, the increase in biosynthesis of flavonoids play an essential protective role during stress by drought or toxic metals (Winkel-Shirley, 2001). In this study, the collected plant samples belonged to the same geographical region (within 100 m radius). According to Hahn et al. (2013), the activation of secondary metabolites biosynthesis pathways are genetically controlled. Thus, although the plants were exposed to stresses of similar intensities, their responses were coordinated by their innate genetic makeup.

4.2 Storage Stability Assessment

Generally, the total carotenoid, phenolic and flavonoid contents showed a decreasing trend except anthocyanin. Correspondingly, antioxidant activities reduced significantly over 8 weeks of storage. Extracts stored at 4°C showed the least antioxidant activity and the highest degradation of metabolites. Greater degradation of phenolic compounds has been reported. However only few studies investigated degradation of phenolics at -20°C and -80°C storage temperature (Deng et al., 2018). Retention of metabolites at low temperature corresponds to its antioxidant activity (Ali et al., 2018), as observed in this study. For example, the carotenoid content in FBB extract remained high after 8 weeks of storage at -80°C (not significantly different compared to the initial value, with only 8.87% of degradation), which corresponded to its stable antioxidant capacity against ABTS radicals after 8 weeks storage.

In this study, higher amounts of anthocyanin were observed after extract storage, with the highest percentage of increments showed by the *Ficus* extracts which were stored at 4°C. This may potentially be due to overestimation of the anthocyanin concentration in the extracts. It has been established that at high concentrations, anthocyanins do not follow Beer's law due to the stacking effect that is caused by either self-association or intermolecular co-pigmentation. Stacking effect causes bathochromic and hyperchromic shift. Bathochromic shift can alter the transmittance, absorbance and reflectance properties thus result in overestimation of anthocyanin contents. On the other hand, other metabolites content was observed to decrease with storage, with the highest degradation shown by the *Ficus* extracts stored at 4°C compared to those stored at -80°C and -20°C. It has been reported that at lower temperature, chemical reactions would occur slowly, thereby explaining the highest degradation percentages observed in the extracts stored at 4°C (Dangles and Fenger, 2018; Samad et al., 2016).

5. Conclusion

Results revealed that the leaves of *F. benjamina* contained significantly higher amounts of phenolic content compared to its bark extracts. In contrast, both *F. racemosa* and *F. religiosa* bark extracts contained significantly higher phenolic contentsthan their leaves. The high metabolites content of the extracts was observed to be correlated with their antioxidant capacities. Storage at 4°C resulted in the highest degradation of carotenoid, phenolic and flavonoid contents. In addition, longer term storage of up to 8 weeks was observed to cause significant degradation of these metabolites (at all temperatures tested), except for carotenoid contents in *F. benjamina* bark and *F. religiosa* leaf extracts stored at -80°C and -20°C where the decrease was insignificant. Extract stored at very low temperature (-80°C) is the most suitable for preserving the secondary metabolites content and bioactive properties, however storage at freezing temperature (-20°C) also yielded satisfactory outcomes, which could be a more economically feasible option. Taken together, storage at 4°C was found to cause the most degradation of metabolite content and antioxidative capacity of the extracts. The obtained results underscore the significant potential of plant-derived pigments, especially those from the *Ficus* species for use in the food industry as a sustainable source of colorants and antioxidants. This finding addresses the escalating demand for environmentally-friendly and clean-label options that can replace synthetic food additives.

6. References

Akula, R., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant signaling & behavior 6, 1720-1731.

Al-Yousuf, H.H.H., 2012. Antibacterial activity of *Ficus carica L.* extract against six bacterial strains. International Journal of Drug Development and Research 4, 307 - 310.

Ali, A., Chong, C., Mah, S., Abdullah, L., Choong, T., Chua, B., 2018. Impact of storage conditions on the stability of predominant phenolic constituents and antioxidant activity of dried Piper betle extracts. Molecules 23, 484.

Bruuinsma, J., 1963. The quantitative analysis of chlorophylls a and b in plant extracts. Photochemistry and photobiology 2, 241-249.

Chan, E., Lim, Y., Omar, M., 2007. Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. Food chemistry 104, 1586-1593.

Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F., Steinmetz, A., 2016. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chinese medicine 11, 37.

Dangles, O., Fenger, J.-A., 2018. The chemical reactivity of anthocyanins and its consequences in food science and nutrition. Molecules 23, 1970.

Deep, P., Singh, K., Ansari, M.T., Raghav, P., 2013. Pharmacological potentials of *Ficus racemosa*—a review. International Journal of Pharmaceutical Sciences Review and Research 22, 29-34.

Delgado-Vargas, F., 2003. O. Paredes-Lo pez. Natural Colorants for Food and Nutraceutical Uses.

Deng, M., Deng, Y., Dong, L., Ma, Y., Liu, L., Huang, F., Wei, Z., Zhang, Y., Zhang, M., Zhang, R., 2018. Effect of Storage Conditions on Phenolic Profiles and Antioxidant Activity of Litchi Pericarp. Molecules 23, 2276.

Devanesan, E.B., Anand, A.V., Kumar, P.S., Vinayagamoorthy, P., Basavaraju, P., 2018. Phytochemistry and Pharmacology of *Ficus religiosa*. Systematic Reviews in Pharmacy 9, 45-48.

Figueiredo, M.S., Grelle, C.E.V., 2009. Predicting global abundance of a threatened species from its occurrence: implications for conservation planning. Diversity and Distributions 15, 117-121.

Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Procheş, Ş., van der Bank, M., 2007. Preserving the evolutionary potential of floras in biodiversity hotspots. Nature 445, 757 - 760.

Gould, K.S., Dudle, D.A., Neufeld, H.S., 2010. Why some stems are red: cauline anthocyanins shield photosystem II against high light stress. Journal of Experimental Botany 61, 2707-2717.

Gould, K.S., Markham, K.R., Smith, R.H., Goris, J.J., 2000. Functional role of anthocyanins in the leaves of Quintinia serrata A. Cunn. Journal of experimental botany 51, 1107-1115.

Hahn, A., Kilian, J., Mohrholz, A., Ladwig, F., Peschke, F., Dautel, R., Harter, K., Berendzen, K., Wanke, D., 2013. Plant core environmental stress response genes are systemically coordinated during abiotic stresses. International journal of molecular sciences 14, 7617-7641.

Hezri, A.A., Nordin Hasan, M., 2006. Towards sustainable development? The evolution of environmental policy in Malaysia, Natural Resources Forum. Wiley Online Library, pp. 37-50.

Ishikawa, K., Takenaga, K., Akimoto, M., Koshikawa, N., Yamaguchi, A., Imanishi, H., Nakada, K., Honma, Y., Hayashi, J.-I., 2008. ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science 320, 661-664.

Jackman, R.L., Yada, R.Y., Tung, M.A., Speers, R.A., 1987. Anthocyanins as food colorants—a review. Journal of food biochemistry 11, 201-247.

Kamarudin, K.R., 2010. An Update on Diversity of Sea Cucumber (Echinodermata: Holothuroidea) in Malaysia.

Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current protocols in food analytical chemistry 1, F4. 3.1-F4. 3.8.

Liu, Y., Tikunov, Y., Schouten, R.E., Marcelis, L.F., Visser, R.G., Bovy, A., 2018. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: a review. Frontiers in chemistry 6, 52.

Lopez, T., Corbin, C., Falguieres, A., Doussot, J., Montguillon, J., Hagège, D., Hano, C., Lainé, É., 2016. Secondary metabolite accumulation, antibacterial and antioxidant properties of in vitro propagated Clidemia hirta L. extracts are influenced by the basal culture medium. Comptes Rendus Chimie 19, 1071-1076.

Madharia, P., Jahan, A., 2015. Ethnomedicinal plants and their conservation in Chhattisgarh State: Review and Perspectives. IOSR J Environ Sci Toxicol Food Technol 1, 46-50.

Mehraj, H., Sikder, R., Haider, M., Hussain, M., Uddin, A.J., 2013. Fig (*Ficus carica L.*): A new fruit crop in Bangladesh. International Journal of Business, Social and Scientific Research.

Özkan, G., Bilek, S.E., 2015. Enzyme-assisted extraction of stabilized chlorophyll from spinach. Food chemistry 176, 152-157.

Paarakh, P.M., 2009. *Ficus racemosa Linn*.–an overview. Indian Journal of Natural Products and Resources 8.

Pilarski, J., 1999a. Gradient of photosynthetic pigments in the bark and leaves of lilac (Syringa vulgaris L.). Acta Physiologiae Plantarum 21, 365-373.

Pilarski, J., 1999b. Gradient of photosynthetic pigments in the bark and leaves of lilac (Syringa vulgaris L.). Acta Physiologiae Plantarum 21, 365-373.

Pilarski, J., Tokarz, K., 2006. Chlorophyll distribution in the stems and trunk of beech trees. Acta Physiologiae Plantarum 28, 233-236.

Rath, S., Chhatria, C., Sahoo, S., 2017. Comparative studies of pigment and nutrient contents of various castor genotypes. Journal of Tropical Agriculture and Food Science 45, 121-129.

Rønsted, N., Salvo, G., Savolainen, V., 2007. Biogeographical and phylogenetic origins of African fig species (Ficus section Galoglychia). Molecular phylogenetics and evolution 43, 190-201.

Sakuta, M., 2014. Diversity in plant red pigments: anthocyanins and betacyanins. Plant biotechnology reports 8, 37-48.

Samad, M., Hashim, S., Simarani, K., Yaacob, J., 2016. Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (Phoenix dactylifera) cultivars. Molecules 21, 419.

Sandeep, Kumar, A., Tomer, V., Gat, Y., Kumar, V., 2018. Ficus religiosa: A wholesome medicinal tree. Journal of Pharmacognosy and Phytochemistry 7, 32-37.

Sirisha, N., Sreenivasulu, M., Sangeeta, K., Chetty, C.M., 2010. Antioxidant properties of Ficus species–a review. Int J PharmTech Res 2, 2174-2182.

Stadtman, E.R., 1992. Protein oxidation and aging. Science 257, 1220-1224.

Sumanta, N., Haque, C.I., Nishika, J., Suprakash, R., 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences ISSN 2231, 606X.

Sun, T., Powers, J.R., Tang, J., 2007. Effect of enzymatic macerate treatment on rutin content, antioxidant activity, yield, and physical properties of asparagus juice. Journal of food science 72, S267-S271.

Trachootham, D., Alexandre, J., Huang, P., 2009. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nature reviews Drug discovery 8, 579.

Winkel-Shirley, B., 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant physiology 126, 485-493.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., Wang, Q., 2018. Response of plant secondary metabolites to environmental factors. Molecules 23, 762.

Yusof, Z., Ramasamy, S., Mahmood, N., Yaacob, J., 2018. Vermicompost Supplementation Improves the Stability of Bioactive Anthocyanin and Phenolic Compounds in Clinacanthus nutans Lindau. Molecules 23, 1345.

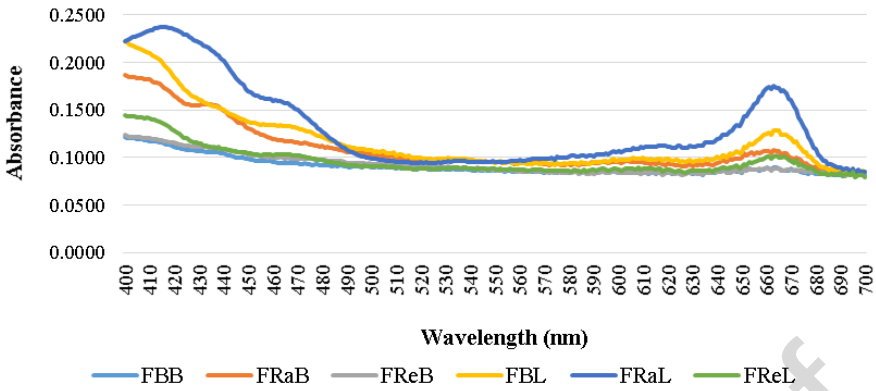

Fig. 1 UV-vis absorption spectra of methanolic extract obtained from leaves and barks of *Ficus* spp. FB: *F. benjamina;* Fra: *F. racemosa* and FRe: *F. religiosa.* Symbols: B at the end: bark; L at the end: leaf.

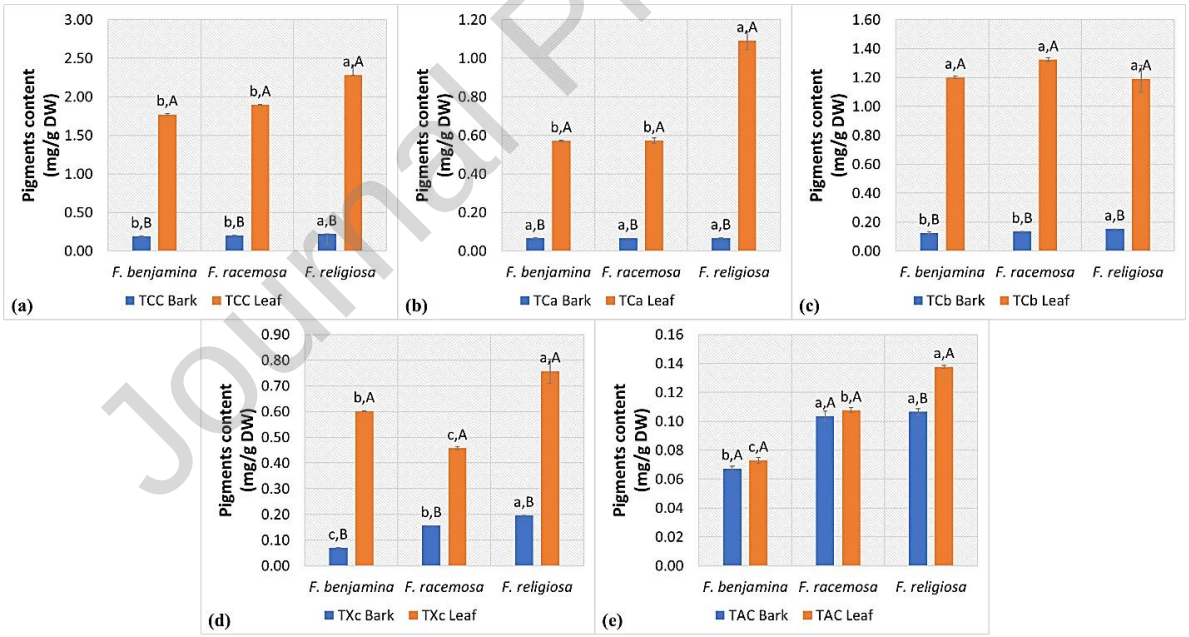

Fig.2 Amount of bioactive pigments; (a) total chlorophyll, (b) chlrophyll a, (c) chlorophyll b, (d) total carotenoid and (e) total anthocyanin in the extracts from the barks and leaves of *Ficus* spp. Different small letters indicate significant difference (at $p < 0.05$) between *Ficus* species, while different capital letters indicate significant difference (at $p < 0.05$) between organ type.

TCC: total chlorophyll content, TCa: Total chlorophyll a, TCb: Total chlorophyll b, TXc: Total carotenoid (xanthophyll + carotene), TAC: Total anthocyanin content.

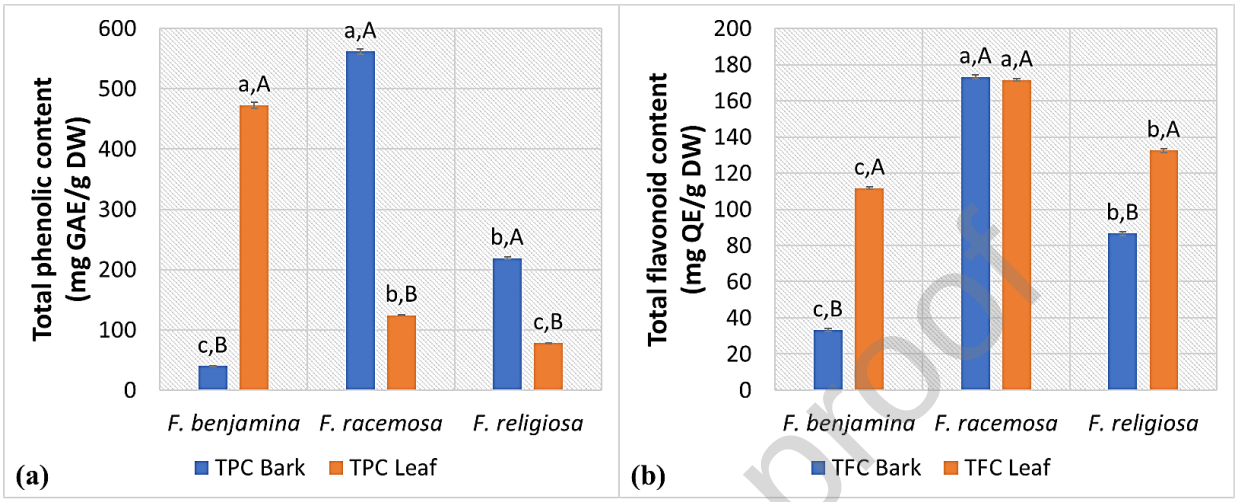

Fig.3 (a) Total phenolic and (b) flavonoid contents in the extracts from the barks and leaves of *Ficus* spp. Different small letters indicate significant difference (at $p < 0.05$) between *Ficus* species, while different capital letters indicate significant difference (at $p < 0.05$) between organ type.

TPC: total phenolic content, TFC: Total flavonoid content.

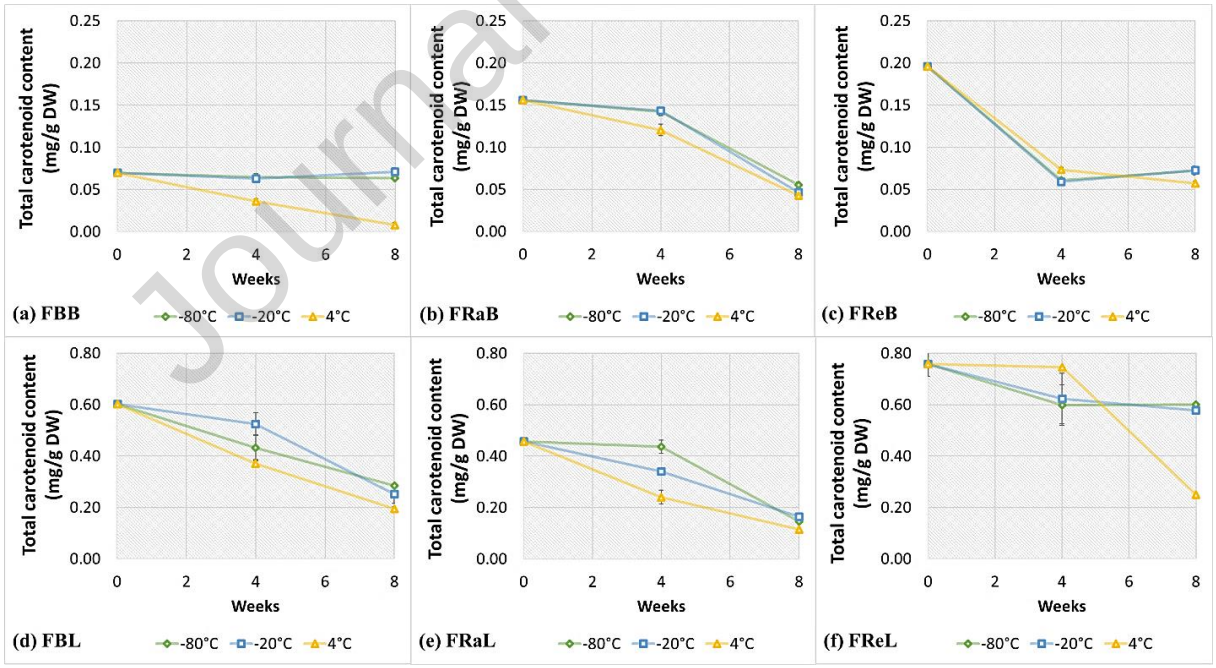

Fig. 4 Storage effects of *Ficus* bark and leaf extracts at different temperatures on total carotenoid content in of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

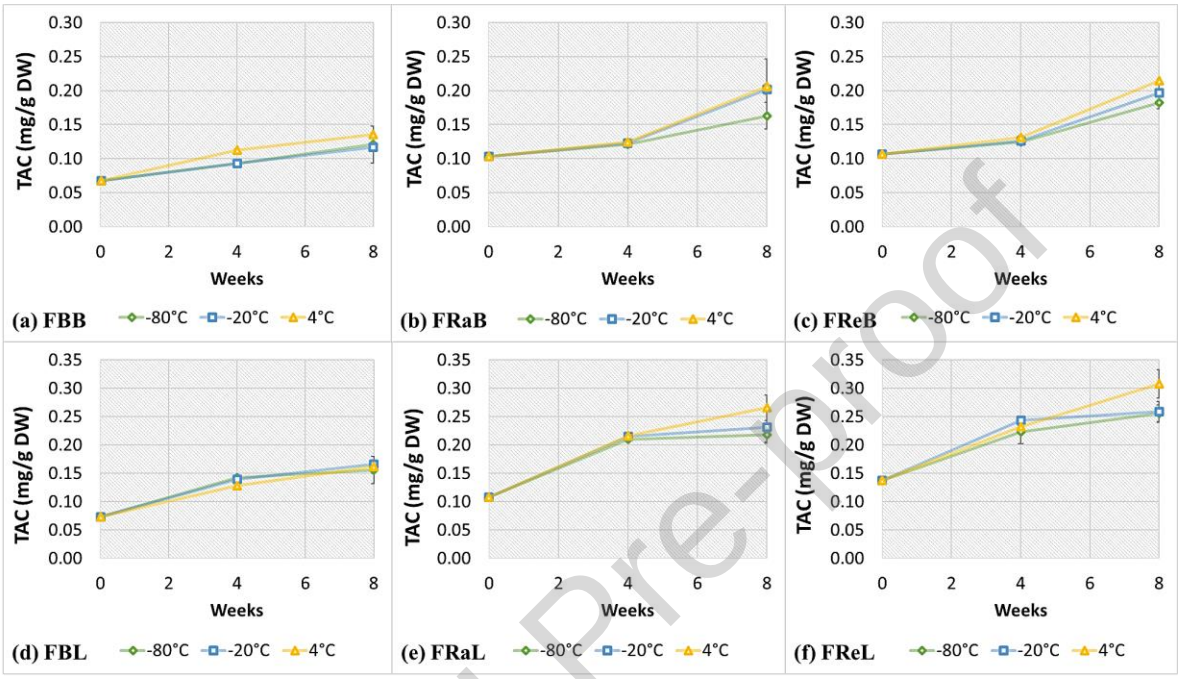

Fig. 5 Storage effects of *Ficus* bark and leaf extracts at different temperatures on total anthocyanin content of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

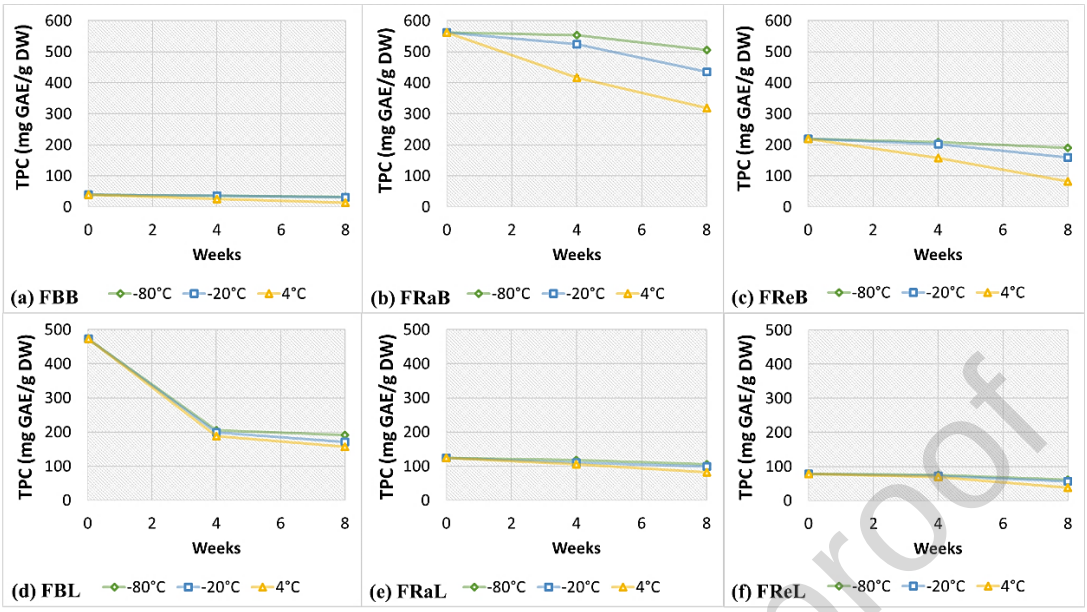

Fig. 6 Storage effects of *Ficus* bark and leaf extracts at different temperatures on total phenolic content of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

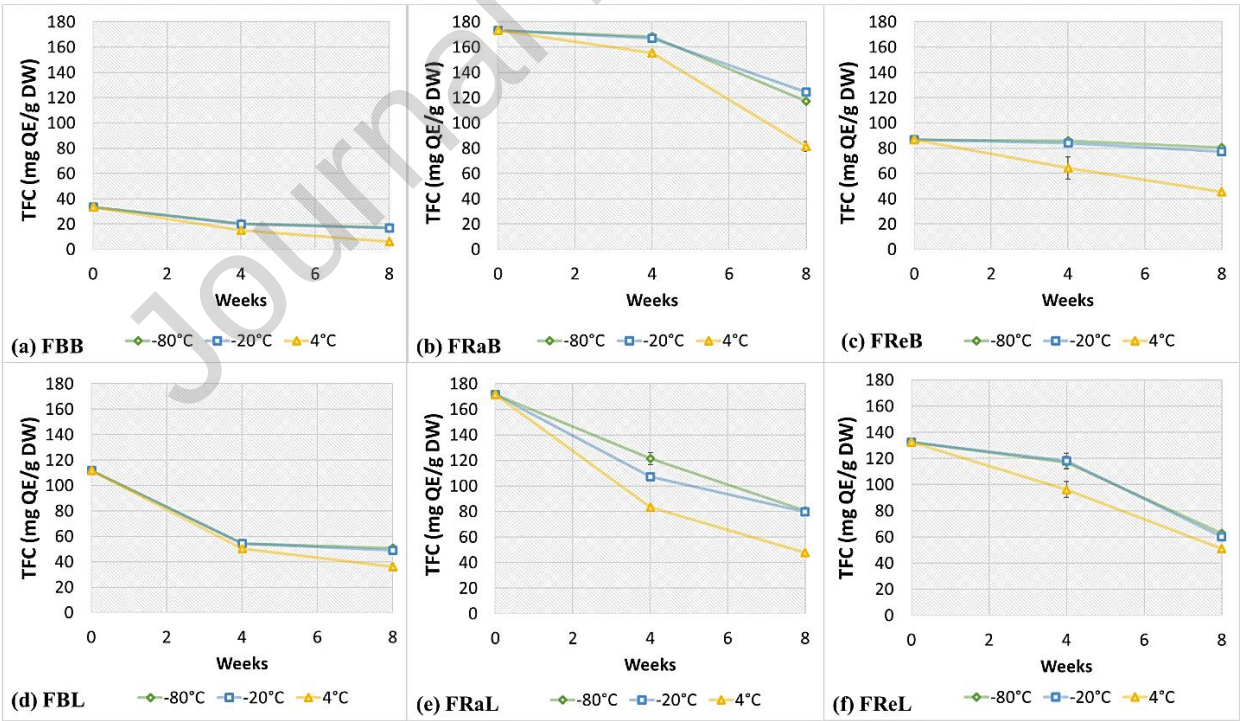

Fig. 7 Storage effects of *Ficus* bark and leaf extracts on total flavonoid content of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

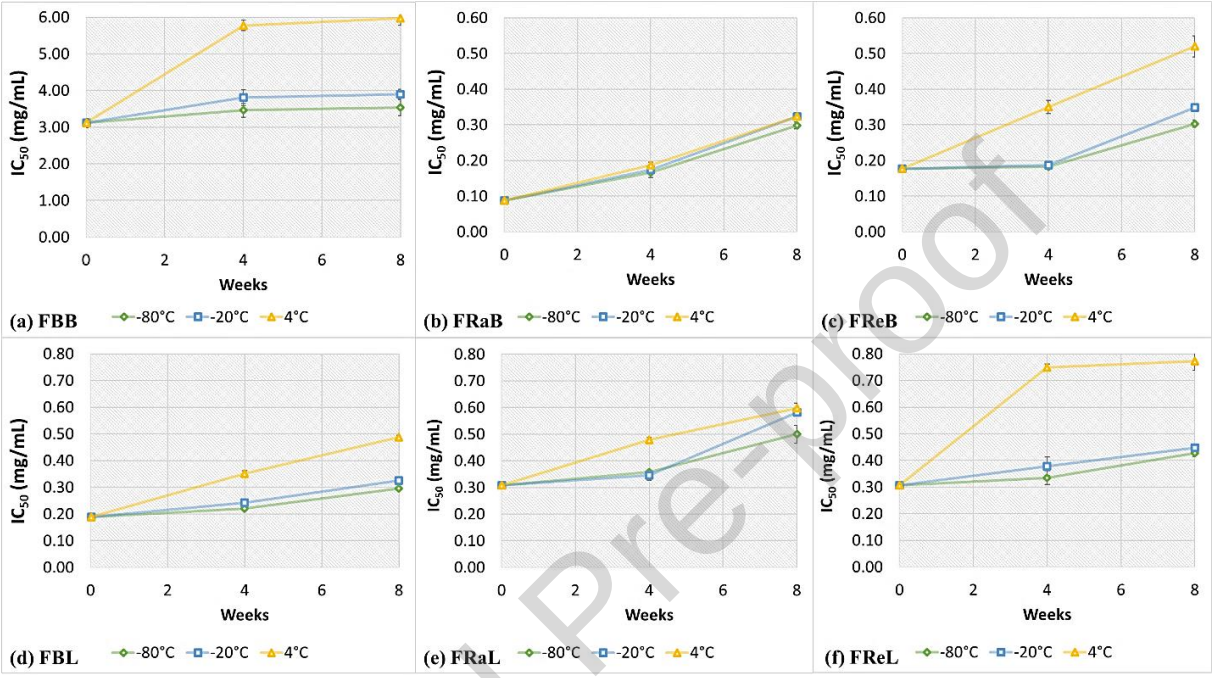

Fig. 8 Storage effects of *Ficus* bark and leaf extracts at different temperatures on scavenging activities against ABTS radicals of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

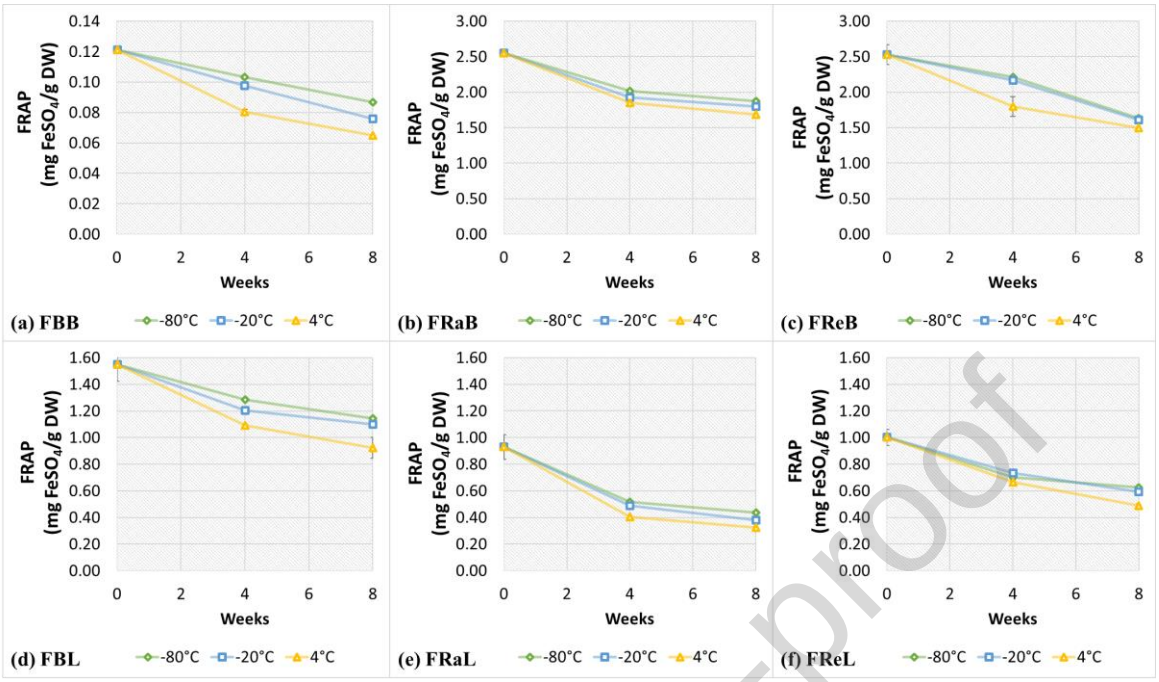

Fig. 9 Storage effects of *Ficus* bark and leaf extracts at different temperatures on FRAP values of: (a, d) *F. benjamina*, (b, e) *F. racemosa* and (c, f) *F. religiosa*. Error bars represent the standard error of mean obtained from three replicates.

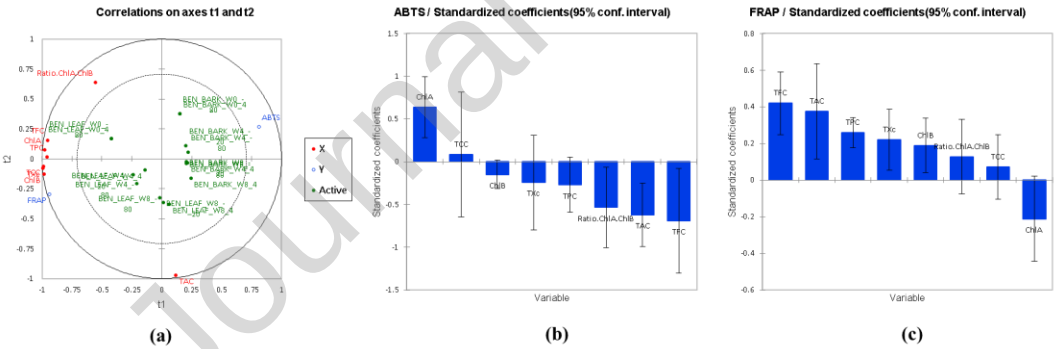

Fig. 10 Partial Least Squares Regression (PLSR) analysis to determine the relationship between the bioactive secondary metabolites content and antioxidant potential of *Ficus benjamina*: (a) PLSR correlation plot, (b) standardized coefficients of variables corresponding to ABTS IC_{50}, and (c) standardized coefficients of variables corresponding to FRAP potential.

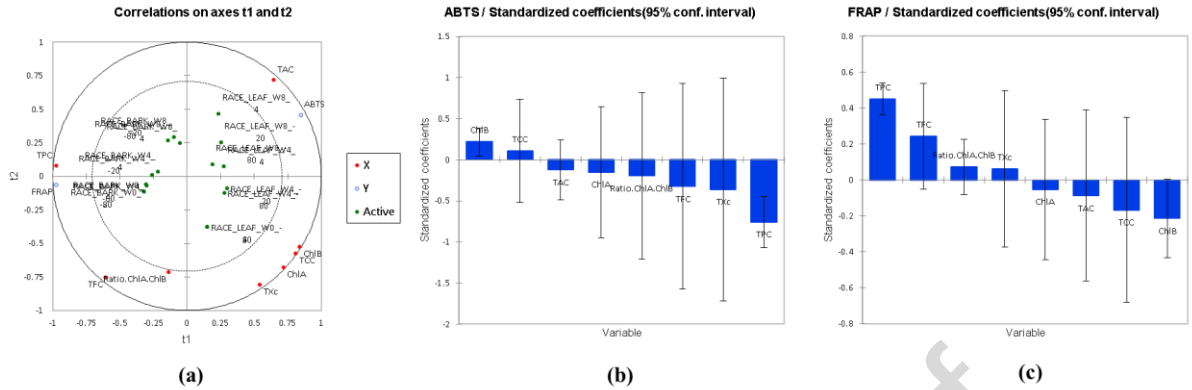

Fig. 11 Partial Least Squares Regression (PLSR) analysis to determine the relationship between the bioactive secondary metabolites content and antioxidant potential of *Ficus racemosa*: (a) PLSR correlation plot, (b) standardized coefficients of variables corresponding to ABTS IC_{50}, and (c) standardized coefficients of variables corresponding to FRAP potential.

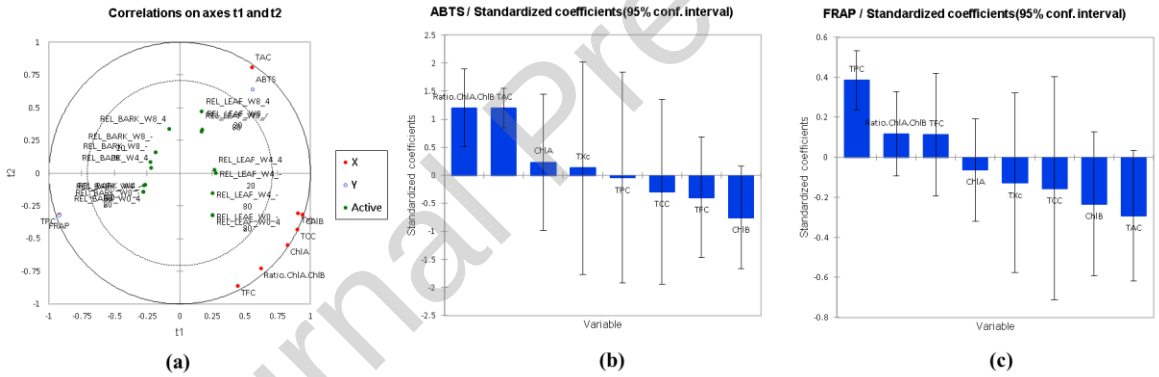

Fig. 12 Partial Least Squares Regression (PLSR) analysis to determine the relationship between the bioactive secondary metabolites content and antioxidant potential of *Ficus religiosa*: (a) PLSR correlation plot, (b) standardized coefficients of variables corresponding to ABTS IC_{50}, and (c) standardized coefficients of variables corresponding to FRAP potential.

Table 1. Chlorophyll a/b ratio in the bark and leaf extracts of *Ficus* spp.

Aerial organ	Species	Sample ID	Chlorophyll a/b ratio
Bark	*F. benjamina*	FBB	$0.527 \pm 0.007^{a,A}$
	F. racemosa	FRaB	$0.492 \pm 0.001^{b,A}$
	F. religiosa	FReB	$0.441 \pm 0.002^{c,B}$
Leaf	*F. benjamina*	FBL	$0.476 \pm 0.001^{b,B}$
	F. racemosa	FRaL	$0.433 \pm 0.015^{b,B}$
	F. religiosa	FReL	$0.922 \pm 0.040^{a,A}$

Values are mean ± standard error of a minimum three samples. Different small letters indicate significant difference (at $p < 0.05$) between *Ficus* species, while different capital letters indicate significant difference (at $p < 0.05$) between organ type.

Table 2. ABTS radical scavenging properties of the bark and leaf extracts of *Ficus* spp.

Aerial organ	Species	Sample ID	ABTS (IC_{50})
Bark	*F. benjamina*	FBB	$3.114 \pm 0.128^{a,A}$
	F. racemosa	FRaB	$0.087 \pm 0.002^{b,B}$
	F. religiosa	FReB	$0.177 \pm 0.008^{b,B}$
Leaf	*F. benjamina*	FBL	$0.189 \pm 0.000^{b,B}$
	F. racemosa	FRaL	$0.308 \pm 0.005^{a,A}$
	F. religiosa	FReL	$0.306 \pm 0.003^{a,A}$

Values are mean ± standard error of a minimum three samples. Different small letters indicate significant difference (at $p < 0.05$) between *Ficus* species, while different capital letters indicate significant difference (at $p < 0.05$) between organ type.

Table 3. FRAP antioxidant potential of the bark and leaf extracts of *Ficus* spp.

Aerial organ	Species	Sample ID	FRAP value (mg $FeSO_4$/g DW sample)
Bark	*F. benjamina*	FBB	$0.121 \pm 0.002^{b,B}$
	F. racemosa	FRaB	$2.555 \pm 0.005^{a,A}$
	F. religiosa	FReB	$2.529 \pm 0.140^{a,A}$
Leaf	*F. benjamina*	FBL	$1.551 \pm 0.129^{a,A}$

F. racemosa	FRaL	$0.930 \pm 0.093^{b,B}$
F. religiosa	FReL	$1.002 \pm 0.060^{b,B}$

Values are mean ± standard error of a minimum three samples. Different small letters indicate significant difference (at $p < 0.05$) between *Ficus* species, while different capital letters indicate significant difference (at $p < 0.05$) between organ type.

Declaration of interest statement: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.