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Phytochemical Composition and Antioxidant Properties of *Tambourissa ficus*, a Mauritian Endemic Fruit

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Abstract: Until now, the Mauritian endemic fruit *Tambourissa ficus* of the Monimiaceae family has remained unexplored. The study's goal was to look into the phytochemical composition and antioxidant properties of different solvent extracts of the fruit. The presence of phenolics, flavonoids, terpenes, coumarins, alkaloids, and tannins was discovered through qualitative screening. The highest total polyphenol content (TPC = 9.78 ± 0.18 mg GAE/g dw) and the highest total flavonoid content (TFC = 8.84 ± 0.07 mg QE/g dw) was observed in ethanolic extract, while the highest total terpenoid content (TTC = 587.9 ± 0.72 mg linalool/g dw) was found in the acetone extract. The antioxidant activity vs. ABTS was the highest (4.71 ± 0.18 mg TE/g dw) in the ethanol extract. All three groups—TPC, TFC, and TTC revealed a moderate correlation with ABTS antioxidant activity, being 0.754, 0.778, and 0.774 on average, respectively. Ultraviolet–visible (UV-Vis) spectroscopy spectrophotometry and Fourier transform infrared spectroscopy (FTIR) spectroscopy confirmed the presence of polyphenolic compounds. Individual noteworthy phytochemicals, including the alkaloids chelidonine, protopine, and brevicarine, which are potential antioxidant compounds, were also discovered in the fruit through liquid chromatography–mass spectrometry (LC-MS) screening. The overall antioxidant activity of the fruit can, therefore, be attributed to the synergistic effects of the multiple chemical components in the extracts.

Keywords: Mauritius; *Tambourissa ficus*; endemic fruit; phytochemicals; antioxidant activity

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1. Introduction

In recent years, there has been a lot of interest in the field of free radicals' chemistry since these compounds are capable of causing a variety of human diseases [1]. Free radicals are highly reactive molecules with unpaired electrons in their outer orbitals. They oxidize or, in some cases, reduce other atoms by accepting or donating electrons [2]. The electron transport chain of the mitochondria during aerobic respiration is the primary source of free radicals in the human body [3]. External sources of free radicals include cigarette smoke, environmental pollutants, radiation, and industrial solvents [4].

Free radicals react with macromolecules like proteins and nucleic acids, causing oxidative stress in the body. Diseases like diabetes, heart disease, atherosclerosis, and liver disease become more prominent. Free radicals cause irreversible damage to essential macromolecular targets like DNA, which can lead to cancer [5]. As a result, free radical concentrations are regulated by a number of defense mechanisms, including detoxifying enzymes and antioxidants [6].

An antioxidant is a molecule that is stable enough to donate an electron to a free radical and neutralize it, reducing its ability to cause damage. A healthy balance of free radicals and

antioxidants is required for proper physiological function. Antioxidants can interact with free radicals safely and stop the chain reaction before vital molecules are harmed [7]. Some of these antioxidants, which include glutathione, ubiquinol, and uric acid, are produced by the body during normal metabolism. Although the body has several enzyme systems that scavenge free radicals, using antioxidants from outside sources can also help in dealing with oxidative stress [8]. Antioxidants, both synthetic and natural, are widely used in food and medicine. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are two common examples of synthetic antioxidants. However, some physical properties of BHT and BHA, such as their high volatility and instability at elevated temperatures, strict legislation on the use of synthetic food additives, the carcinogenic nature of some synthetic antioxidants, and consumer preferences, have shifted manufacturers' attention from synthetic antioxidants to natural antioxidants [9].

Plants are the primary source of natural antioxidants as they contain a variety of enzymatic systems that catalyze antioxidant reactions. Polyphenols, flavonoids, and terpenes are examples of phytochemicals that can act as reducing agents [10]. As a result, the demand and search for new plants high in these natural antioxidants, especially as an alternative to synthetic antioxidants, is ongoing worldwide.

Mauritius harbours a plethora of endemic plants that have received little to no attention in terms of their antioxidant potential. There are currently about 600 plant species, with 277 being endemic to the island and 147 exclusively found in the Mascarene Islands. The high demand for antioxidants coupled with the increasing interest in Mauritian endemic plants makes the exploration of the island's flora for potential antioxidants a quest of much importance.

The Monimiaceae family has long been known for its traditional medicine applications, and it has proven to be a rich source of chemically diverse specialized metabolites with numerous potent biological and therapeutical properties. Their phytochemistry and pharmacology have recently expanded, albeit to varying degrees. According to previous research, there are approximately 93 components, 35 of which are undescribed compounds such as phenolics, flavonoids, terpenoids, and homogentisic acid derivatives. Furthermore, anticancer, antioxidant, antiparasitic, antiviral, and antibacterial activities of pure isolated compounds have been demonstrated [11].

Aside from providing new and important perspectives for the management of various diseases, the chemical and biological diversity of the isolated compounds opens up promising avenues of research and contributes to renewed interest in this family. The genus *Tambourissa* is represented by approximately 43 species that are exclusively distributed in the Indian Ocean islands, including 26 species in Madagascar, 10 species in Mauritius, 5 species in Comoros, and 2 species in La Reunion. These species are highly endemic, with each being restricted to a single island.

The Mauritian endemic plant *Tambourissa ficus* of the Monimiaceae family is a tree with a mottled grey-brown stem, simple leaves, distinctive flowers, and fruits borne on its trunk. The fruits are brown pots. When ripe, the walls split to reveal orange seeds. The phytochemical composition and pharmacological properties of the fruit are unexplored. Fresh fruits are a great way for people to get natural nutrients. Natural pigments found in fruits are secondary metabolites with important biological activities. These pigments have potent antioxidant properties and numerous health benefits, including anti-aging, anti-atherogenicity, anticancer, and anti-inflammation [12].

T. ficus fruits have been consumed locally for decades, and no adverse effects have been reported as of yet. Furthermore, in a study conducted on a fruit of a similar genus and endemic to Mauritius known as *Tambourissa peltata*, HepG2 and HT29 cell lines treated with *T. peltata* aqueous and methanolic extracts demonstrated nontoxicity up to 250 g/mL after 48 h of treatment [13].

To the best of our knowledge, this is the first work exploring the *T. ficus* edible fruit antioxidant and phytochemical potential. The aim of the research was to analyze

the phytochemical and antioxidant properties of the different solvent extracts of the *T. ficus* fruit.

2. Materials and Methods

2.1. Fruit Sampling

The Native Plant Propagation Centre of Curepipe, which is situated in the central region of Mauritius, benefitting from a mild tropical maritime climate throughout the year, was the area of plant collection (latitude 20.3484, longitude 57.5522, altitude 151 m). The arboretum is home to over 5000 plants, 600 of which are rare Mauritian species. The climate is tropical-rainy, with dry summers and a rainy season that starts in November and ends in May. Initial visits were made for plant recognition and to present the intentions and goals of the research. Prior to visits, authorization was obtained from the National Parks and Conservation Service of the Ministry of Agro Industry, Food Production and Security of Mauritius. Sampling was performed during the month of February 2023, which is a summer month in Mauritius.

2.2. Reagents and Chemicals

The reagents lead acetate $Pb(C_2H_3O_2)_2$, ammonia NH_3, chloroform $CHCl_3$, sulfuric acid H_2SO_4, ferric chloride $FeCl_3$, sodium hydroxide NaOH and iodine and potassium iodide (Wagner's reagent) for the general qualitative analysis of bioactive groups present in the extracts were supplied by Acculab Ltd. (Nouvelle France, Grand Port, Mauritius). The following standards and reagents were used for the spectrophotometric methods: gallic acid, linalool, quercetin, iron (II) sulfate ($FeSO_4$), Trolox (6-hydroxyl-2,5,7,8–tetramethylchromo-2-carboxylic acid), Folin–Ciocalteu reagent, calcium carbonate $CaCO_3$, sodium nitrate $NaNO_3$, aluminum chloride $AlCl_3$, 2,2-diphenyl–1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS) and TPTZ (2,3,5-triphenyltetrazolium chloride) and methanol, ethanol, propanol and acetone for extracts (Pol-Aura, Zabrze, Poland).

2.3. Phytochemical Extraction

Healthy mature fruits were selected and plucked (Figure 1). The seeds were separated from the flesh, which was finely cut into pieces and dried in a drier (Vindon Scientific, Rochdale, UK) at a temperature of 50 °C for 48 h. The dried fruit material was ground and sieved into a fine powder (<500 mm) (Figure S1). The moisture content of the powder was measured with a moisture analysis balance (MA-30, Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany). Water, methanol, ethanol, propanol, and acetone were used as solvents to extract metabolites from the fruits. In a 10 mL test tube, 1 g of dried fruit powder was placed, followed by 10 mL of solvent. The tubes were sealed and agitated on the rotary shaker (06-MX-RD-PRO, Chemland, Stargard, Poland) at 70 rpm. After 24 h, the tubes were centrifuged at 5000 rpm for 15 min at 15 °C (MPW-260, MPW MED. INSTRUMENTS, Warsaw, Poland). The supernatant was collected, the volume and weight recorded, and samples stored at 4 °C.

2.4. Qualitative Phytochemical Screening

General qualitative analysis of bioactive groups present in the extracts was performed according to [14].

Specifically, for phenols, few drops of $Pb(C_2H_3O_2)_2$ were added to a few drops of extract. A positive result is the appearance of a white precipitate; for flavonoids, few drops of 1% NH_3 were added to a few drops of extract. A positive result is the appearance of a yellow coloration; for steroids and terpenes, few drops of extract followed by few drops of $CHCl_3$ and H_2SO_4 were added to the side of a test tube. A positive result for steroids is the formation of a reddish-brown ring at the interface, and a positive result for terpenes is the formation of yellow precipitate; for saponins, distilled water was added to a few drops of extract, and the mixture was shaken vigorously. A positive result is the formation of a froth,

which remains for 15–30 min; for alkaloids to a few drops of extract, few drops of Wagner's reagent were added. A positive result is the appearance of a reddish-brown precipitate; for tannins, few drops of 5% $FeCl_3$ were added to a few drops of extract. A positive result is the appearance of a blue-black or brownish-green precipitate; for coumarins, to a few drops of extract, a few drops of NaOH were added. A positive result is the formation of a yellow colour.

Figure 1. Trunk-borne *T. ficus* fruit pots.

2.5. Quantitative Phytochemical Analysis

2.5.1. Total Phenolic Content (TPC) Determination

Stock gallic acid solution was prepared by dissolving 0.01 g gallic acid in 10 mL of distilled water. Standard solutions of gallic acid were prepared by serial dilutions using distilled water (0–1000 µg/mL). To 20 µL of gallic acid solutions and extracts in a test tube, 1580 µL of distilled water was added, followed by 100 µL of Folin–Ciocalteu reagent, and the mixture vortexed. The mixture was incubated for 6 min at room temperature (25 °C). 300 µL of saturated $CaCO_3$ solution was then added to the tube and vortexed thoroughly until a permanent blue color was obtained. The resulting solution was incubated at 38 °C for 30 min in the dark, and the absorbance was measured at 765 nm (SEMKO, Warsaw, Poland). The TPC concentration was expressed as mg gallic acid equivalent (GAE)/g of dried plant material [15,16].

2.5.2. Total Flavonoid Content (TFC) Determination

Stock quercetin solution was prepared by dissolving 5 mg of quercetin in 1 mL methanol. Standard solutions of quercetin were prepared by serial dilutions using methanol (0–200 µg/mL). To 1 mL diluted standard quercetin solutions and 1 mL extracts, 4 mL of distilled water was added. Moreover, 0.3 mL of 5% $NaNO_3$ was then added. After 5 min, 0.3 mL 10% $AlCl_3$ was added. After 6 min 1 mL of 1 M NaOH was added, and the total volume made up to 10 mL using distilled water and mixed carefully. The mixture was vortexed for 20 min. The absorbance of the reaction mixtures was measured against a blank at a wavelength of 510 nm. The TFC concentration was expressed as mg quercetin equivalent (QE)/g of dried plant material [17].

2.5.3. Total Terpenoid Content (TTC) Determination

Standard linalool solution with concentrations of 0–200 µg/mL was prepared. To 1 mL of standard solutions and 1 mL of extracts, 2 mL of chloroform was added. The sample mixture was vortexed thoroughly and left for 3 min. 200 µL of concentrated sulfuric acid was poured into the mixture and incubated at room temperature for 2 h in

the dark. A reddish-brown precipitate was formed in the mixture during incubation. The supernatant was carefully decanted without disturbing the precipitation. Moreover, 3 mL of absolute methanol was added, and the mixture vortexed well to completely dissolve the precipitate. The absorbance of the reaction mixtures was measured against a blank at a wavelength of 538 nm. The concentration of terpenoid was expressed as mg linalool equivalent (linalool)/g of dried plant material [18].

2.6. Antioxidant and Oxidoreductive Activity Determination

2.6.1. Ferric Reducing Antioxidant Power (FRAP) Assay

Stock $FeSO_4$ solution with concentration of 1 mM was prepared by dissolving 0.0015 g of $FeSO_4$ in 10 mL distilled water. Standard solutions of iron sulfate were prepared by serial dilutions using distilled water (25–200 µmol/dm^3). Moreover, 1000 µL of FRAP working solution was added to a tube. Additionally, 35 µL of the test sample was added, and the mixture was thoroughly mixed and incubated for 15 min at 36 °C. The absorbance of the reaction mixtures was measured against a blank at a wavelength of 593 nm. The reducing activity of the test samples was expressed as µmol of iron sulfate ($FeSO_4$)/g of dried plant material [15,16].

2.6.2. ABTS Assay

Stock Trolox solution was prepared by dissolving 0.05 g Trolox in 5 mL distilled water. Standard solutions of Trolox were prepared by serial dilutions using distilled water (100–600 µM). 1000 µL of ABTS working solution was added to a cuvette. Moreover, 20.4 µL of the test sample was added, the stopwatch was turned on, and the mixture was thoroughly mixed for exactly 10 s. The absorbance of the reaction mixtures was measured against a blank at a wavelength of 734 nm using a spectrophotometer. The free radical cations scavenging capacity of the test samples was expressed as mg Trolox equivalent (TE)/g of dried plant material [15,16].

2.6.3. DPPH Assay

Stock Trolox solution was prepared by dissolving 0.06 g of Trolox in 250 mL of distilled water. Standard solutions of Trolox were prepared by serial dilutions using distilled water (0–400 mg/mL). Moreover, 1000 µL of DPPH working solution was added to a tube. Additionally, 34.5 µL of the test sample was added, thoroughly mixed, and incubated for 20 min in the dark at room temperature. The absorbance of the reaction mixtures was measured against a blank at a wavelength of 517 nm. The reducing activity of the test samples was expressed as mg Trolox equivalent (TE)/g of dried plant material [15,16].

2.7. Ultraviolet–Visible (UV-Vis) Spectroscopy

The extracts (3 mL) were introduced into a quartz cuvette, and the sample was scanned between the wavelength of 200–700 nm using Ultrospec 2000 UV-Vis (Pharmacia Biotech, Uppsala, Sweden). The peak values were recorded [19].

2.8. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Finely ground freeze-dried plant powder and ethanol extract were used for the FTIR analysis. IR spectra were measured using a Nicolet iS50 FTIR (Thermo Scientific, Waltham, MA, USA) spectrometer equipped with an automated beam splitter exchange system (iS50 ABX containing a DLaTGS KBr detector and a DLaTGS solid substrate detector for mid-IR and far IR regions, respectively). Built-in all-reflective diamond ATR module (iS50 ATR), ThermoScientific Polaris™, and HeNe laser as an IR radiation source. Polycrystalline mid-IR spectra were collected in the 4000–400 cm^{-1} range in the sample directly.

2.9. Liquid Chromatography–Mass Spectrometry (LC–MS) Screening

LC-MS was used to detect potential bioactive compounds based on their molecular weight and retention time. For this purpose, the methanol extract was used. Analyses were

performed on a Thermo Scientific system consisting of a 'Vanquish Flex' U-HPLC-PDA and an 'Orbitrap Fusion' mass spectrometer fitted with an electrospray source (Thermo Scientific, Waltham, MA, USA). Chromatography was performed on 5 µL sample injections onto a 150 mm × 3 mm, 3 µm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400 µL/min mobile phase gradient of $H_2O/CH_3OH/CH_3CN$ + 1% HCOOH: 90:0:10 (0 min), 0:90:10 (60 min), 0:90:10 (70 min), 90:0:10 (71 min), 90:0:10 (75 min). The ESI source was operated under standard conditions, and the mass spectrometer was set to record high-resolution (60 k resolution) MS1 spectra (m/z 125–2000) in both positive and negative modes using the orbitrap and data-dependent MS2 and MS3 spectra in both modes using the linear ion trap. Detected compounds were assigned using the approach described by [20] and were by comparison of accurate mass (ppm).

2.10. Statistical Analysis and Correlation

All experimental data were processed and analyzed using Microsoft Excel (Microsoft Office 2019) and expressed as mean ± standard deviation of three analytical triplicates. Standard curves were plotted using linear regression to obtain a line of best fit. Significant differences between the variables were obtained through Completely Randomized Design (One-Way ANOVA). A p-value < 0.05 was considered statistically significant. Correlation between polyphenol, flavonoid, and terpene content and antioxidant activity was established by Pearson correlation analysis.

3. Results and Discussion

3.1. Qualitative and Quantitative Profile of Phytochemicals

Methanol, ethanol, and distilled water are common solvents used by researchers [21,22]. Water is currently encouraged as a solvent as it is less expensive and more environmentally friendly. Water is a highly polar solvent with a high surface tension, allowing it to wash hydrophilic compounds containing -COO or -OH groups away from plant tissues [23]. Water extracts not only small bioactive molecules but also polysaccharides and proteins. Organic solvents, on the other hand, have a certain level of solubility that is useful for optimizing both hydrophilic and hydrophobic compound extraction [24].

The results obtained from the qualitative analysis of the extracts are presented in Table 1. The metabolites analyzed include phenolics, flavonoids, terpenes, steroids, saponins, coumarins, alkaloids, and tannins. Phenols were detected in all organic solvent extracts, while flavonoids were abundant in ethanol and acetone fractions. Terpenes were found to be present in all extracts but in higher amounts in the acetone extract. Steroids were only found in propanol and acetone extracts. Saponins were only noticed in the water extract. Coumarins, tannins, and alkaloids, on the other hand, were prolific in all extracts. Plants produce phytochemicals during secondary metabolism, which are usually responsible for their extensive pharmacological properties [25]. The phytochemicals detected are consistent with previous studies on the phytochemical content of fruits [26].

Table 1. Qualitative phytochemical composition of extracts.

Phytochemicals	Water	Ethanol	Methanol	Propanol	Acetone
Phenols	−	+	+	+	+
Flavonoids	−	++	+	+	++
Terpenes	+	+	+	+	++
Steroids	−	−	−	+	+
Saponins	+	−	−	−	−
Coumarins	+	++	++	++	++
Alkaloids	+	++	++	++	++
Tannins	+	++	++	++	++

++: Highly Present, +: Low, −: Absent.

The TPC of the different solvent extracts ranged from 2 to 10 mg GAE/g dw (dry weight), as shown in Table 2. The ethanol fraction yielded the highest (p < 0.05) TPC

value of 9.78 ± 0.18 mg GAE/g dw. Extracts in methanol, acetone, and propanol also yielded significant amounts of phenolic compounds. In contrast, the aqueous fraction had the lowest ($p < 0.05$) TPC value of 2.08 ± 0.06 mg GAE/g dw. Khadija [27] found that the TPC of the ethanolic extract of Tambourissa comorensis, a Tambourissa species of the Monimiaceae family from the Comoros islands, was the highest with a value of 0.896 ± 0.06 mg GAE/g dw, and the aqueous extracts had the lowest TPC value of 0.538 ± 0.17 mg GAE/g dw. The nature of the sample matrix and the chemical properties of the phenolics, which include concentration, polarity, molecular structure, number of aromatic rings, and hydroxyl groups, are the primary determinants of phenolic compound extraction from plant materials [28]. Ethanol and methanol yielded higher ($p < 0.05$) TPC values due to their higher polarity and better solubility for phenolic components found in plant materials. The chemical structures of phenolic compounds, ranging from simple to polymerized, can also affect solubility [29]. TPC values obtained in this study are higher than those obtained in previous studies on fruits from the same family, making *T. ficus* a fruit with a remarkable amount of phenolics.

Table 2. Total flavonoid content (TFC), total polyphenols content (TPC), and total terpenoid content (TTC) of extracts. Different lower-case letters mean statistically different values ($p = 0.05$).

Extracts	TFC (mg QE/g dw)	TPC (mg GAE/g dw)	TTC (mg linalool/g dw)
Water	0.64 ± 0.01 ^{a}	2.08 ± 0.06 ^{a}	47.3 ± 0.3 ^{a}
Ethanol	8.85 ± 0.03 ^{e}	9.78 ± 0.18 ^{e}	427.7 ± 2.6 ^{c}
Methanol	5.90 ± 0.02 ^{b}	9.33 ± 0.28 ^{d}	384.0 ± 0.4 ^{b}
Propanol	6.18 ± 0.03 ^{c}	8.27 ± 0.18 ^{b}	429.6 ± 1.8 ^{c}
Acetone	7.87 ± 0.05 ^{d}	8.74 ± 0.19 ^{c}	587.9 ± 0.7 ^{d}

Table 2 shows that water is not particularly efficient at extracting flavonoids in this study ($p > 0.05$). Water is usually better for extracting sterols, saponins, and polysaccharides. For example, the flavonoid rutin, which is theoretically quite polar based on its partition coefficient (Kow ≈ −0.47), is only marginally soluble in water (S ≈ 130–150 mg/L) [30]. Ranghoo-Sanmukhiya et al. [31] showed that TFC values for six Mauritian endemic species ranged from 0.2 to 0.7 mg QE/g dw. In contrast, the current study reveals significant TFC values of the endemic T. ficus. The TTC of the extracts was strikingly high ($p < 0.05$) when compared to TPC and TFC. Unlike the TPC and TFC, the acetone fraction produced the highest ($p < 0.05$) TTC value of 587.9 ± 0.72 mg linalool/g dw, followed by the propanol fraction. Among organic solvents, methanol had the lowest ($p < 0.05$) TTC value, while water had the least amount of TTC.

Using a similar terpenoid quantification technique, Truong et al. [18] discovered that the TTC of *S. buxifolia* bark extracts ranged from 453.70 to 842.59 mg linalool/g dw. Terpenes are the largest and most diverse plant secondary metabolites in nature, according to [32], particularly in higher plants such as citrus, conifers, and eucalyptus. Terpenes are the informative and defensive vehicles used by plants for antagonistic and mutualistic interactions. Plants produce terpenes on a regular basis to combat biotic (pathogenic microbes, herbivore pests, and weeds) and abiotic (water, temperature, light, and salt) stress [33]. Terpenes differ due to the arrangement of their carbon skeletons, the layering of functional groups, and the substituents on their backbone. Using organic solvents of different polarity, the specific compounds can be extracted, which impacts further bioactivity of extracts [34]. Hydrocarbons, aldehydes, oxygenated hydroxyl or carbonyl derivatives, and esters are among [34]. Dirar et al. [35] also concluded that acetone is a superior solvent for extracting some terpenes, which the current study supports.

3.2. Antioxidant Activity

The antioxidant and oxidoreductive characteristics of the extracts are presented in Table 3.

Table 3. Antioxidant properties of extracts. Different lower-case letters mean statistically different values ($p = 0.05$).

Extracts	FRAP (uM (Fe^{2+}/g dw)	ABTS (mg TE/g dw)	DPPH (mg TE/g dw)
Water	0.33 ± 0.02^{a}	0.97 ± 0.05^{a}	0.95 ± 0.01^{a}
Ethanol	1.22 ± 0.02^{b}	4.71 ± 0.18^{d}	4.46 ± 0.02^{e}
Methanol	1.22 ± 0.01^{b}	4.02 ± 0.15^{b}	3.93 ± 0.06^{d}
Propanol	1.25 ± 0.02^{c}	4.64 ± 0.08^{cd}	1.43 ± 0.07^{b}
Acetone	3.38 ± 0.02^{d}	4.41 ± 0.21^{c}	3.12 ± 0.10^{c}

The acetone fraction had the highest ($p < 0.05$) FRAP value, and the lowest ($p < 0.05$) was with the water fraction. The FRAP values of the remaining fractions were nearly identical. These findings are consistent with previous research on the antioxidant activity of plant extracts. Attanayake and Jayatilaka [36] reported that the FRAP values of most of the plants in their study were less than 2 uM after evaluating the antioxidant properties of 20 medicinal plant extracts traditionally used in Ayurvedic medicine in Sri Lanka. A recent study by Abubakar et al. [37] provided the antioxidant results of Apocynum hendersonii extracts quite similar to our results, especially for FRAP.

Extracts obtained from organic solvents were found to have higher ($p < 0.05$) antioxidant power than water-based extracts. Sim and Nyam [38] found that the water-based extract of Hibiscus cannabinus leaf extracts had the lowest reducing power, which was attributed to the low solubility of the antioxidant constituents in water, and this corresponds to the ABTS and DPPH results obtained in this study. Except for the water extract, which had an ABTS value of 0.97 ± 0.05 mg TE/g dw ($p < 0.05$), all of the ABTS values were in the 4 mg TE/g dw range ($p > 0.05$). This was also the case for DPPH, with the water extract having a DPPH value of 0.95 ± 0.01 mg TE/g ($p < 0.05$).

The ethanol extract outperformed the methanol and acetone extracts in terms of DPPH scavenging activity. According to Rajurkar and Hande [39], studying 11 medicinal plants, all plant extracts had DPPH values less than 2 mg TE/g, and 9 of the plant extracts had values less than 4 mg TE/g. In fact, our study demonstrates higher antioxidant activity than some previous analyses on medicinal plants.

3.3. Correlation between Phytochemical Contents and Antioxidant Properties of Extract

As shown in Table 4, significant positive correlations were observed between the studied phytochemical contents and antioxidant results, indicating that these compounds played a significant role in the antioxidant assays. Because the TFC and TTC values have higher R values (especially ABTS), this indicates that these two phytochemicals have greater antioxidant capacity than the phenolic compounds in the extracts.

Table 4. Correlation between phytochemical contents and antioxidant properties of extract.

Parameters	FRAP	ABTS	DPPH	TPC	TFC	TTC
FRAP	1					
ABTS	0.54896	1				
DPPH	0.333834	0.622806	1			
TPC	0.512248	0.965626	0.784889	1		
TFC	0.623255	0.948893	0.762314	0.944358	1	
TTC	0.834865	0.917917	0.569816	0.881282	0.915261	1

Zawawi et al. [40] discovered an even stronger relationship between the TFC of honey and FRAP and DPPH, which were 0.888 and 0.899, respectively, compared to the TPC and DPPH, which was 0.597. However, Kim et al. [41] found a stronger relationship between TPC and DPPH than TFC and DPPH, where R was 0.949. The terpene content correlated with the antioxidant activity of the extracts, particularly with ABTS. Terpenes, particularly

triterpenes and tetraterpenes (carotenoids), appear to be important compounds for humans in relation to ROS-mediated oxidative stress [42].

Some structural characteristics of terpenes explain their antioxidant properties. The oxygenated type with a phenolic structure and the hydrocarbon type with a methylene group in its structure has the highest antioxidant activity for the monoterpene group. The allylic alcohol types are the most active antioxidant compounds among sesquiterpene compounds. Triterpenes with dienone–phenol rearrangement have the highest antioxidant activity among the various triterpene structural types and carotenoids, which contain conjugated double bonds and, therefore, act as an extremely efficient antioxidant among tetraterpenes [43].

3.4. UV-Vis Spectroscopy

Figure 2 depicts the UV scan from 200–700 nm for the five different solvent extracts of *T. ficus* fruit flesh. The water extract contained two major peaks at wavelengths of 230 nm and 248 nm. Peaks appeared in the ethanol extract at 282 nm, 304 nm, and 660 nm. Numerous peaks appeared in the methanol extract, ranging from 230 nm to 660 nm. The acetone extract had three significant peaks at 226 nm, 409 nm, and 669 nm, while the propanol extract had two significant peaks at 272 nm and 306 nm.

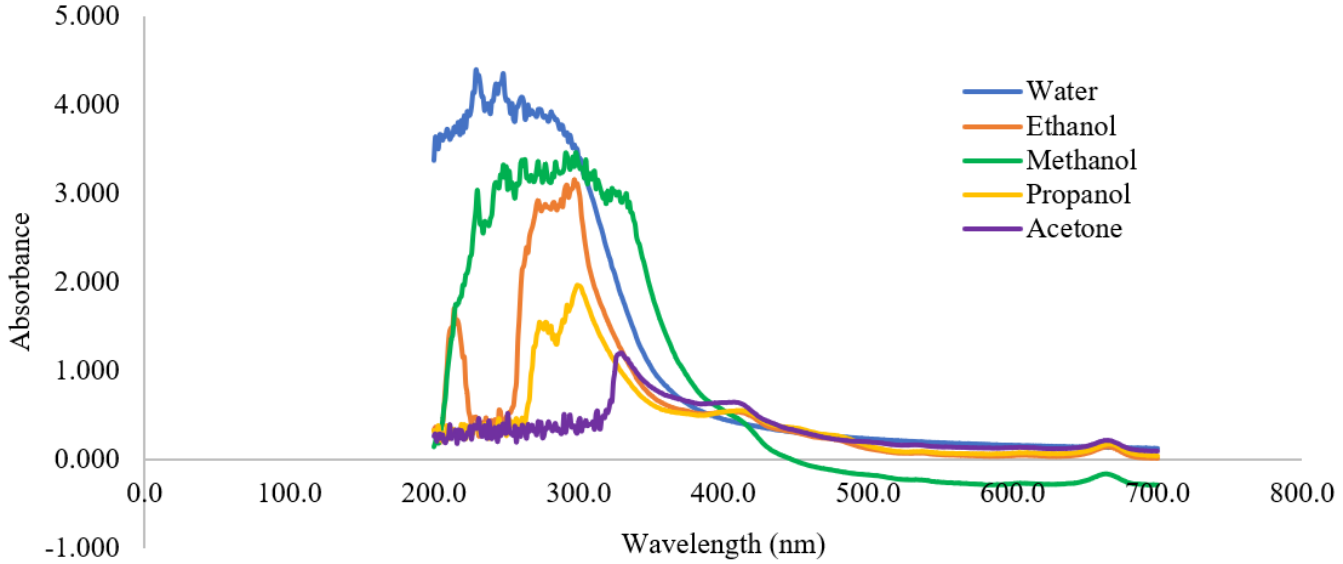

Figure 2. UV scan for the five different solvent extracts of *T. ficus* fruit flesh.

Plants' colors are due to a combination of different compounds, including carotene, a member of the terpenoids class. The appearance of one or more peaks in the UV-VIS spectra between 200 and 400 nm indicates the presence of unsaturated groups and heteroatoms such as S, N, and O. These peaks could be phenolics, flavonoids, alkaloids, polysaccharides, or glycosides (Table S1). The absorption at 278 nm represents the typical peak for flavonoids and their derivatives. The peak at 295 nm is an indication of triterpenes. However, the use of UV-visible spectrophotometry in the analysis of complex media is hampered by the inherent difficulties in assigning absorption peaks to specific constituents. To enable proper extract characterization and constituent identification, UV VIS findings must be supplemented with another analytical technique, such as FTIR [44].

3.5. FTIR Spectroscopic Analysis

Figure 3A,B show the FTIR spectrum of the freeze-dried *T. ficus* fruit powder and the ethanol extract. The spectrum is basically a plot of transmitted (or absorbed) frequencies vs. intensity of the transmission (or absorption). The x-axis displays frequencies in inverse centimeters (wavenumbers). Strong bands were observed at absorption peaks 3286 cm^{-1},

2928 cm^{-1}, 2852 cm^{-1}, 1022 cm^{-1}, and 1612 cm^{-1} in the dry powder, while in the ethanol extract, strong bands appeared at 2924 cm^{-1}, 2856 cm^{-1}, and 3313 cm^{-1}.

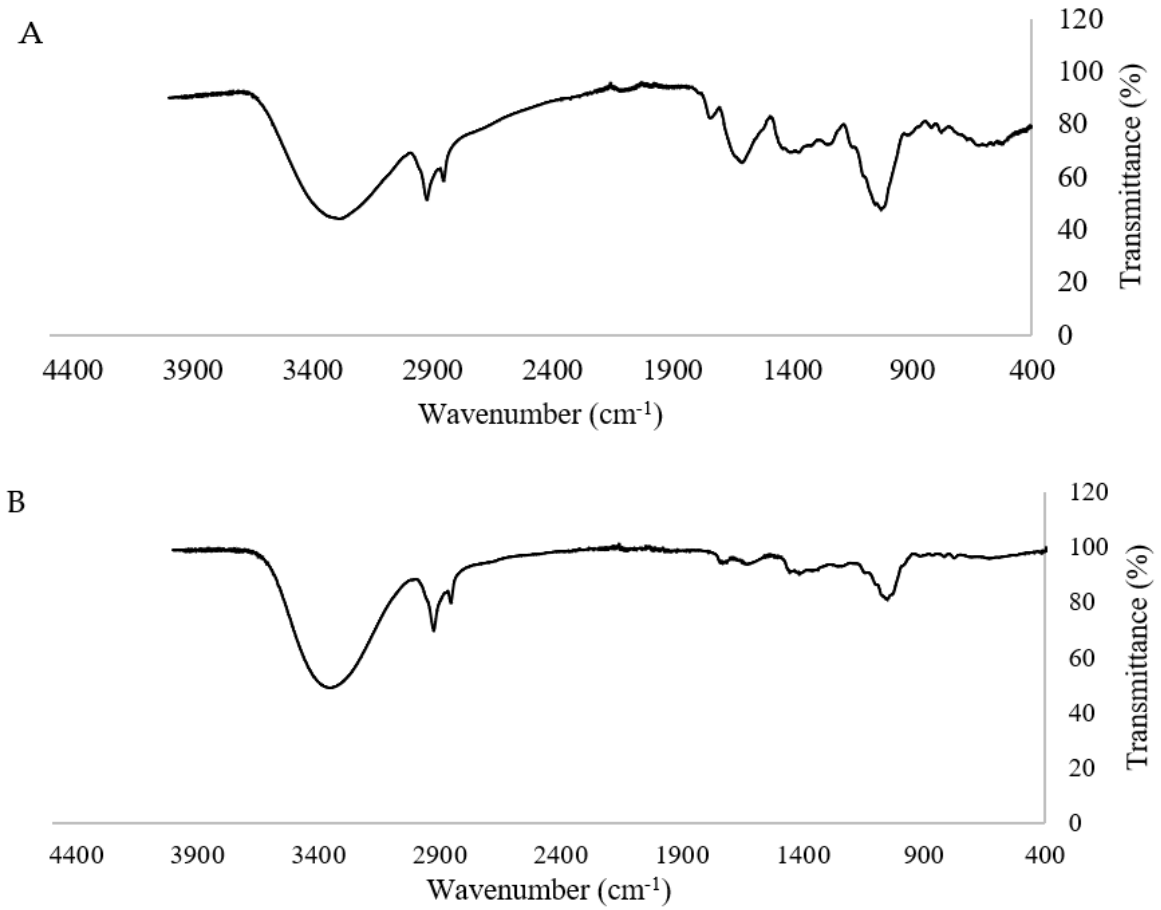

Figure 3. FTIR spectra of (**A**). *T. ficus* freeze-dried powder (**B**). of ethanol extract of *T. ficus* flesh.

The remaining bands ranged from weak to medium in strength, indicating the presence of various organic compounds. Peaks 3286 cm^{-1} and 1022 cm^{-1} are characteristic peaks of OH groups. Peaks 2928 cm^{-1}, 2924 cm^{-1}, 2852 cm^{-1}, and 2856 cm^{-1} corresponds to C-H stretching. Together, these peaks represent the polysaccharide characteristics peak. A ketone and an amine produced notable bands at 1612 cm^{-1} and 3313 cm^{-1}, respectively [45].

As shown in Supplementary Tables S2 and S3, the spectrum profile is indicative of various functional groups such as alcohols, carboxylic acids, alkanes, alkynes, esters, aldehydes, ketones, aromatics, halides, nitro compounds, and proteins. The functional groups present in the dried fruit powder and the ethanol fruit extract of *T. ficus* did not differ significantly. The presence of phenols and flavonoids is indicated by C-H out-of-plane bending (oop bend) vibration for substituted benzene rings. The presence of phenols, flavonoids, and terpenes was detected in the phytochemical screening, which was supported by the identification of benzenoid compounds. C=O may be related to carotenoids and chlorophylls [46].

3.6. LC-MS Analysis

Mass spectrometry can perform quantitative metabolite analysis with high sensitivity and selectivity, as well as identify metabolites. The combination of liquid chromatography and mass spectrometry facilitates metabolite identification by reducing sample complexity and allowing metabolite separation prior to detection [47]. On a molecular weight basis, the spectra in the current study corresponded to glycosides, alkaloids, flavonoids, amino acids, esters, phenolics, organic acids, and terpenes (Figure S3 and Table 5).

Table 5. List of compounds identified from LC-MS analysis.

Molecular Mass	Retention Time/min	Corresponding Compounds	Compound Class
257.11	1.82	5-methylcytidine	glycoside
259.13	4.00	1-(4-aminophenyl)-3,3-dichloropiperidin-2-one	alkaloid
252.09	14.41	7-hydroxy-3-methylflavone (3-chlorophenyl) (4-chloropyridin-3-yl) methanone	flavonoid
317.21	26.72	petunidol, petunidin, myrtillidin	anthocyanins flavonoid
202.22	29.40	spermine, ornithine	amino acid
240.23	32.96	6-hydroxyflavanone	flavonoid
353.23	40.18	chelidonine protopine	alkaloid
377.27	58.17	3-hydroxypropyl-glucosinolate	glucosinolate
413.27	61.90	Ile-pro-ile, trimethylsilyl ester	ester
191.02	1.82, 2.49	Protocatechuic acid-3-glucoside	phenolics
333.12	3.80	3-Cyano-4′-iodobenzophenone	phenolics
559.28	22.19	2-[bis[2-[bis(carboxymethyl)amino]ethyl]amino]acetic acid	organic acid
327.22	37.87	guaianolide-(16)	terpene
309.24	59.25	brevicarine	alkaloid
197.81	74.53	2-hydroxybenzophenone	phenolic

The alkaloid piperidine derivative 1-(4-aminophenyl)-3,3-dichloropiperidin-2-one could be a potential antioxidant. Piperidine was found to have a protective effect against oxidative damage in in vitro experiments by inhibiting free radicals, reactive oxygen species, and hydroxyl radicals in a study by [48]. Manjusha et al. [49] also mentions the antioxidant capacity of the alkaloid. Chelidonine, another identified alkaloid, is the primary bioactive ingredient in *Chelidonium majus* and has anti-cancer, anti-inflammatory, and antioxidant properties [50].

Flavonoids 7-hydroxy-3-methylflavone and 6-hydroxyflavanone might also be powerful antioxidants. Flavonoids in plants are mostly found as glycosides, which are hydroxyl groups linked with sugar units and two aromatic rings (A and B). Many connecting sites exist between the basic skeleton C ring and B ring, and A ring and B ring frequently have substituents such as hydroxyl, methoxy, methyl, and isopentyl, resulting in a wide range of derivatives and active functions [51].

Numerous studies have shown that the medicinal value of plants is closely related to the antioxidant activity of flavonoids. In general, flavonoids have high free radical scavenging activity. The mechanism of DPPH scavenging in vitro is that flavonoids' ring substituents, such as hydroxyl and methoxy, react with free radicals [52]. Anthocyanins are a type of water-soluble flavonoids found in a variety of fruits and vegetables. Petunidin, like europinidin and malvidin, is an O-methylated 3-hydroxy anthocyanidin derived from delphinidin.

Petunidin has high antioxidant activity because it is an anthocyanin. Phenolic acids are a type of dietary polyphenol that acts as a natural antioxidant [53]. Protocatechuic acid-3-glucoside has recently been shown to have antioxidant properties in a variety of diseases, making these "old compounds" a potential "new application" for medical therapies [54]. The identification of all these interesting individual phytochemicals indicates that the antioxidant activity of the fruit can be attributed to the synergistic effects of the multiple chemical components in the extracts.

4. Conclusions

Plants will continue to play an important role in the discovery and development of new antioxidants. According to the findings, the Mauritian endemic fruit *T. ficus* contains significant amounts of phytochemicals with significant antioxidant potential. Through positive reaction with the respective test reagent, qualitative phytochemical screening of extracts revealed the presence of phenols, flavonoids, terpenes, coumarins, alkaloids, and tannins.

A significant amount of phenolics and flavonoids were discovered through quantitative phytochemical analysis. However, it was the terpene content that stood out the most. The antioxidant potential of the fruit extracts, particularly the ethanol fraction, was revealed by the ABTS assay. There were strong correlations between flavonoid composition and antioxidant potential.

The UV-VIS and FTIR spectroscopy results corroborated the phytochemical test results. Through LC-MS screening, a few interesting compounds were discovered. In conclusion, the fruit is high in terpenes, but more research is needed, particularly to determine the content of individual terpene compounds. It also has antioxidant potential, which can be attributed to the flavonoids found in the fruit, making it a valuable source of bioactive compounds for the development of novel functional products in a variety of industries, particularly the food and pharmaceutical industries. However, more research is needed to learn about the fruit's toxicity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app131910908/s1, Table S1. Peak information of *T. ficus* fruit flesh extracts; Table S2. FTIR peaks interpretation of freeze-dried powdered *T. ficus* flesh; Table S3. FTIR peaks interpretation of ethanol extract of *T. ficus* flesh; Figure S1. *T. ficus* preparation, A. Dissection picture of *T. ficus* showing yellow flesh and white seed; B. Dried *T. ficus* flesh; C. Dried *T. ficus* powder; D. Picture of rotator used to macerate *T. ficus* powder; Figure S2. LC-MS chromatogram of methanol extract of *T. ficus*.

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