Phytochemical Analysis and Antioxidant Activity of Aqueous Extract of
Ficus septica Leaves from Sabah, Malaysia

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Received: 3 April 2023  Accepted: 20 July 2023  Published: 31 December 2023

ABSTRACT

Medicinal plants have long been used as primary antidotes for a variety of ailments, including tuberculosis, heart diseases, cancer, wound healing, asthma, diabetes mellitus, hypertension, pharyngitis, etc. Medicinal plant of Ficus septica Burm. f. (Moraceae) is a subtropical tree commonly known as the ivory fig, septic fig or white-veined fig. The present work aims to investigate the antioxidant activity, phenolic and flavonoid content, and qualitative screening of various phytochemicals in aqueous extracts of F. septica leaves. Total phenol and flavonoid contents were calculated using Folin-Ciocalteau and aluminium chloride reagents. The antioxidative effect of F. septica was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Ficus septica was found to contain 27.32 ± 0.03 mg/g total phenolics expressed as gallic acid equivalent and 12.65 ± 0.00 mg/g total flavonoid expressed as catechin equivalent. In addition, the leaf extracts were found to contain various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids. The ability of F. septica to scavenge the DPPH radical was determined by its IC50 value. The IC50 value of F. septica was 4.45 µg/mL. Inferred from the presence of phytochemicals, total phenolic and flavonoid content, and antioxidant activity of the plant, F. septica could be a potential addition to pharmaceutical products to improve human health by participating in the antioxidant defence system against the production of free radicals.

Keywords: Antioxidant activity, Ficus septica, leaves extract, Moraceae, phytochemical constituents

INTRODUCTION

Traditional and complementary medicine (TCM) is reportedly used by 80% of the population in developing countries and is integrated to varying degrees into their national health systems (WHO, 2018). World Health Organization (WHO) (2019) reports that out of a population of 30 million Malaysians, 9 million have used or are using TCM to prevent or treat medical conditions.

The biodiversity of Malaysia is the 15th largest in the world, including medicinal plants (Butler, 2016). One of the Malaysian medicinal plants is Ficus septica Burm. f. (Moraceae), commonly known as 'Ara' (Malay), 'Litotobau' (Sabah), and 'Uok' (Sarawak) (MyBIS, 2023). Another common name for this plant is ivory fig, septic fig or white-veined fig, due to the conspicuous white veins on the upper surface of the leaf (Figure 1).

These plants are distributed from northeast India to northern Australia (Queensland), the Solomon Islands, Taiwan, the Ryukyu Islands and throughout insular Southeast Asia (Figure 2) (Berg & Corner, 2005; Rodriguez et al., 2017). In Indonesia, this plant is also known as 'Libho' or 'Awar-Awar' (Nugroho et al., 2015; Nurhidayati et al., 2021; Yamin et al., 2022), while in the Philippines it is known as 'Hauili' or 'liwliw' tree (Fugaban-Hizon, 2021).

In traditional medicine, the leaves of this plant are used to cure colds and fevers, neutralise venom derived from poisonous animals, treat skin diseases, shortness of breath, abscesses, appendicitis, gastrointestinal complaints and treat fungal and bacterial infections (Damu et al., 2005; Ueda et al., 2009; Sudirga et al., 2014; Kubo et al., 2016; Fugaban-Hizon, 2022). In the Dusun tribal community of Sabah, this plant is known as 'Sitotobau Topurak’ and the roots of this plant are mainly used during puerperal delivery (Kulip, 2007) and to treat headaches and stomachaches (Awang-Kanak et al., 2021).
Nugroho et al. (2011) reported that this plant’s ethanolic extract has a cytotoxic effect on MCF-7 and T47D cells with IC50 values of 13 and 6 g/mL, respectively. In combination with doxorubicin (3.75 nM), the extract displayed a synergistic effect. In MCF-7 breast cancer cells, the extract also induced apoptosis and suppressed the expression of the Bcl-2 protein (Sekti et al., 2010). The extract (750 mg/kg BW) was able to induce apoptosis via a p53-independent pathway in 7,12-dimethyl benz[a]anthracene-induced liver carcinoma in rats (Septhea et al., 2011). Nastiti et al. (2014), discovered that the ethyl acetate fraction of the ethanolic extract of *F. septica* modulated macrophage phagocytosis and lymphocyte proliferation in Balb/c mice. The ethanolic extract of *F. septica* was discovered to be useful in the biosynthesis of silver nanoparticles (AgNPs) (Fugaban-Hizon, 2021). The methanol extracts of the fruit, heartwood, leaves and stem of *F. septica* had a promising anti-DENV-1 and DENV-2 effect (Huang et al., 2017).

Due to the important pharmacological properties and uses of *F. septica*, several scientific studies have reported the phytochemical composition of the extracts of this plant. In previous research, de Padua et al. (1999) reported that the leaves and roots of *F. septica* contain saponins and flavonoids, the roots contain polyphenols, and the fruits contain alkaloids and tannins. Another study documented the discovery of seven triterpenes, along with a unique triterpene known as 13,27-cycloarsan-3β-yl acetate, and two lignans from the non-alkaloidal fractions of the stem of this plant (Kuo et al., 2002). A diverse range of phenanthroindolizidine alkaloids, namely fucisepentine and fucisepentine A-N, have been obtained through the extraction of phytochemicals from the stems of *F. septica* (Damu et al., 2005). Furthermore, Wu et al. (2002) reported the isolation of several compounds from the leaves, including vanillic acid, (5-acetyl-2-hydroxy phenyl)-β-D-glucopyranoside, the coumarins umbelliferone and esculin, the flavonoids genistein and kaempferitin, squalene and uracil.

A previous study on nuclear magnetic resonance spectroscopy of dichloromethane extracts of *F. septica* twigs led to the isolation of β-sitosteryl-3β-glucopyranoside-6′-O-fatty acid esters, α-amyrin fatty acid esters, and a mixture of β-sitosterol and stigmasterol. In comparison, α-amyrin and long-chain saturated fatty alcohols were identified from the leaves (Ragasa et al., 2016). The methanol leaf extract of *F. septica* was subjected to gas chromatography-mass spectrometry (GC-MS) analysis, which revealed the presence of eight compounds exhibiting antifungal properties. The compounds mentioned in the study conducted by Sudirga and Ginantra (2017) include 2,3,5-trimethyl heptane, sulphurous acid cyclohexyl methyl hexadecyl ester, dodecanoic acid methyl ester, 3-deoxy-D-mannonic acid, hexadecanoic acid methyl ester, octadecamethyl-cyclononasiloxane, 1-heptacosanol and 1,2-benzene dicarboxylic acid mono(2-ethylhexyl) ester. In a recent study conducted by Deli et al. (2022), the authors examined the liquid chromatography-mass spectrometry (LC-MS) analysis of *F. septica* exudate. The study identified several significant components, including ficuspteptine, ficuspteptine C, seco-dehydroantofine and ficuspteptine D. Several isomers of caffeoylgalactaric acid and sinapoylgalactaric acid were also isolated.

Many studies have been conducted on the medicinal potential of solvent extraction of *F. septica*. However, the study on aqueous extraction was limited. Therefore, in this study, aqueous extracts of leaves of *F. septica* from Sabah, Malaysia, were qualitatively screened for phytochemicals, their antioxidant activity, and the determination of their phenolic and flavonoid content using standard assays.

**MATERIALS AND METHODS**

**Chemicals**

Folin-Ciocalteu reagent (FCR), sodium carbonate (Na2CO3), sodium chloride (NaCl), sodium nitrate (NaNO3), aluminium chloride (AlCl3), sodium hydroxide (NaOH), 2,2-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, catechol, and ferric chloride (FeCl3) were purchased from Sigma Aldrich (St. Louis, MO, USA). Chemicals of analytical or gas chromatography (GC) quality were purchased from Fisher Scientific (Hampton, New Hampshire, USA) and J.T. Baker® (Phillipsburg, New Jersey, USA), respectively.
Collection of Plant Materials

In November 2016, leaves of *F. septica* were collected from Tandek (6.5312° N, 116.8467° E), Kota Marudu, Sabah, Malaysia, and transported in polythene bags to the Biotechnology Research Institute (BRI), Universiti Malaysia Sabah (UMS), where the study was conducted. The plant was identified and authenticated by Julius Kulip and Johnny Gisil, botanists from the Institute for Tropical Biology and Conservation (IBTP). Their references can be found in BORNEENSIS, IBTP (BORH 80).

Plant Extracts Preparation

Fresh leaves of *F. septica* were washed completely three times with tap water to remove dirt and then washed once with distilled water. The washed leaves were left on a sieve for a while to remove excess water before being dried in an oven at 40 °C for five days. The dried leaves were ground into a powder using a blender and stored in an airtight container at -80 °C before extraction. The leaves were subjected to hot aqueous extraction.

**Hot Aqueous Extraction**

The aqueous extracts of dried ground leaf powder were prepared using the hot aqueous extraction method according to Cseke *et al.* (2006) with slight modifications. Leaf powders were extracted with sterile Milli-Q water in a 1:10 ratio. Hot aqueous extracts were prepared by adding 100 g of the leaf powder to 1 L of sterile Milli-Q water in a sterile Erlenmeyer flask and heating at 80 °C for 10 minutes on a hot plate, with constant stirring using a glass rod. The decoction was then allowed to cool at room temperature for one hour before being sieved into a sterile flask with a sieve to remove the extract residue. The filtrate was filtered into a
sterile flask using Whatman No. 1 filter paper (Whatman, Maidstone, Kent, UK) before being transferred to a 50 mL Falcon tube and frozen at -80 °C. The frozen samples were then freeze-dried using a freeze dryer. The freeze-dried extracts were stored at -80 °C in an airtight Falcon tube until they were dissolved into solution for further testing.

**Determination of Total Phenolic Content (TPC)**

The aqueous extracts of *F. septica* were analysed for TPC using gallic acid (0.1 – 0.5 mg/mL) as a standard by the modified method of the Folin-Ciocalteu assay, as described by Vun-Sang et al. (2022). The extracts (0.2 mL) were reacted with 1.5 mL of Folin-Ciocalteu reagent and incubated for five minutes. Sodium carbonate (Na$_2$CO$_3$) (60 g/L) was added to the mixtures and allowed to stand in the dark at room temperature for 90 minutes and measured with a UV-Vis spectrophotometer at 725 nm. Quantification of total phenolic content was done in milligrams of gallic acid equivalents (GAE) per gram of extract, using distilled water instead of extract for the blank. The analysis was performed three times and the average absorbance value was recorded.

**Determination of Total Flavonoid Content (TFC)**

The flavonoid content of *F. septica* was determined using the modified colorimetric method of Vun-Sang et al. (2022). It is estimated by the colorimetric method with AlCl$_3$ at 510 nm using catechin dilutions (0.01 – 0.1 mg/mL) as a reference standard. Briefly, 0.25 mL of either the extracts or the catechin was mixed with 1.25 mL of distilled water and 0.075 mL of 5% NaNO$_3$ and then incubated for six minutes in the dark. Then, 0.15 mL of 10% AlCl$_3$ was added and mixed for five minutes at room temperature. Finally, 0.5 mL of NaOH and 0.3 mL of distilled water were added to a final volume of about 2.5 mL. The TFC determination in the extracts was performed in triplicate, and the results were averaged. The TFC of the extract was expressed in milligrams of catechin equivalents (CAE) per gram.

**Phytochemical Screening**

The stock solution of the aqueous extract (1 mg/mL) of *F. septica* was prepared, and qualitative phytochemical screening was carried out to determine the presence and absence of various phytochemical compounds using published standard methods, which are briefly listed in Table 1.

**Determination of Antioxidant Activity (DPPH Assay)**

To assess their ability to scavenge free radicals, the antioxidant activity of the extracts was determined using a slightly modified DPPH radical method, following the protocol of Brand-Williams et al. (1995) as described by Vun-Sang et al. (2022). The DPPH assay is considered a valid, accurate, simple and cost-effective method for determining the radical scavenging activity of antioxidants because the radical compound is stable and does not require generation (Kedare & Singh, 2011). A stock solution (1 mg/ml) of the aqueous extract of *F. septica* and ascorbic acid was prepared. In this test, different concentrations of the extracts or the standard (ascorbic acid) were added to 2.0 mL of DPPH solution in methanol (3.94 mg/100 mL). The resulting mixture was vigorously mixed and incubated for 10 minutes at room temperature in the dark before absorbance was measured at 517 nm using a spectrophotometer.

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Procedures</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Wagner’s test: 2 ml sample + 2 ml Wagner’s reagent + 1 ml Hydrochloric acid Presence result: Formation of reddish brown precipitate</td>
<td>Vimalkumar et al. (2014)</td>
</tr>
</tbody>
</table>
**Flavonoids**

Alkaline reagent test:
1 ml sample + few drops diluted sodium hydroxide + few drops diluted sulphuric acid
Presence result: Yellow colour disappear when sulphuric acid were added

**Tannins**

Braymer’s test:
2 ml sample + 1 ml 10% ferric chloride
Presence result: Formation of blue or greenish colour solution

**Saponin**

Foam test:
1 ml sample + 5 ml distilled water – shake vigorously
Presence result: Formation of copious lather

**Phenols**

Ferric chloride test:
1 ml sample + 2 ml distilled water + few drops 10% ferric chloride
Presence result: Formation of blue or green colour

**Steroids**

Liebermann-Burchard test:
1 ml sample + 2 ml chloroform + 10 drops acetic acid + 5 drops sulphuric acid
Presence result: The change of red colour from blue to green

**Anthraquinones**

5 ml sample + 2 ml diluted sulphuric acid + 2 ml benzene + 2 ml diluted ammonia solution
Presence result: Rose pink colour appearance

**Phytosterols**

Sulphuric acid test: 1 ml sample + 1 ml chloroform + few drops sulphuric acid
Presence result: Formation of bluish green colour

**Triterpenoids**

Salkowki’s test:
2 ml sample + 1 ml chloroform + few drops sulphuric acid
Presence result: Reddish brown precipitate produce immediately

Table 2 shows the results of the analysis of the total phenolic and total flavonoid content of the aqueous extracts from the leaves of *F. septica*. The total phenolic concentration in the aqueous extracts of *F. septica* was determined using a series of gallic acid concentrations to which the absorbance values were plotted to obtain a linear calibration curve ($y = 4.268x + 0.0436$) with a coefficient ($R^2$) of 0.9939 (Figure 3). The flavonoid content was estimated from a linear calibration curve for a range of catechin concentrations ($y = 3.25x - 0.019$) with a coefficient ($R^2$) of 0.9937 (Figure 4).

**RESULTS**

**Total Phenol and Total Flavonoid Contents of Ficus septica Aqueous Extract**

<table>
<thead>
<tr>
<th></th>
<th>Total phenolic content (mg GAE/g)</th>
<th>Total flavonoid content (mg CAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous crude</td>
<td>27.32 ± 0.03</td>
<td>12.65 ± 0.00</td>
</tr>
</tbody>
</table>

Results are express as mean ± SD (n = 3)
Phytochemical Analysis of *Ficus septica* Aqueous Extracts

Table 3 shows the results of the qualitative phytochemical analysis of the aqueous extracts of *F. septica*, which revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids as bioactive components. In contrast, anthraquinones and phytosterols were not detected in the extracts.

Table 3. Phytochemical screening of aqueous leaves extract of *F. septica*

<table>
<thead>
<tr>
<th>Phytochemical Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids (Wagner’s test)</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids (Alkaline reagent test)</td>
<td>+</td>
</tr>
<tr>
<td>Tannins (Braymer’s test)</td>
<td>+</td>
</tr>
<tr>
<td>Saponins (Foam test)</td>
<td>+</td>
</tr>
<tr>
<td>Phenols (Ferric chloride test)</td>
<td>+</td>
</tr>
<tr>
<td>Steroids (Liebermann-Burchard test)</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids (Salkowki’s test)</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present; - = Absent

DPPH Scavenging Activity of *Ficus septica* Aqueous Extracts

The DPPH radical scavenging activity of the aqueous extracts of *F. septica* was estimated at different concentrations from 12 to 500 µg/mL. The DPPH scavenging activity increased with the concentration of the extract samples (Figure 5). The IC₅₀ (y = 12.201x - 4.3551, R² = 0.9668) of *F. septica* was found to be 4.45 µg/mL. Meanwhile, the IC₅₀ (y = 16.009x + 23.48, R² = 0.9438) of ascorbic acid was found to be 1.66 µg/mL.

**Figure 3.** Linear curve of gallic acid concentration vs. absorbance for determination of total phenolic content

**Figure 4.** Linear curve of catechin concentration vs. absorbance for determination of total flavonoid content
DISCUSSION

The aim of this study was to evaluate the phytochemical profile, total phenolic and flavonoid content, and antioxidant activity of the aqueous extracts of the leaves of *Ficus septica*. The aqueous extracts of *F. septica* show the presence of phenolic and flavonoid compounds. These results are in agreement with those of Yamin *et al.* (2022), who demonstrate the presence of total phenolic and flavonoid content in methanol extracts and their fractions of *F. septica* fruits.

Phenolic compounds are considered secondary metabolites known to be potent chain-breaking antioxidants and are among the most important plant constituents. Their radical scavenging activity is related to their hydroxyl groups (Hossain & Nagooru, 2011). Phenolic phytochemicals synthesised from phenylalanine and tyrosine is abundant and diverse in plants. They have been found to possess various biological functions, such as antioxidant and anti-inflammatory properties (Naczk & Shahidi, 2004). Phenolic compounds have been shown to protect plants from microbes and herbivores. This could explain why the leaves and stems of the plant contain more phenolic compounds than the rhizome (Jing *et al.*, 2010).

Flavonoids are a group of phytochemicals that occur naturally in plants and are known for their potential health benefits. Derivatives of flavonoids have been shown to possess various properties such as anti-allergic, anti-inflammatory, antibacterial, antiviral, antioxidant and anticarcinogenic activities (Yao *et al.*, 2004; Saeed *et al.*, 2012). Similar to phenolic compounds, flavonoids are highly effective in scavenging various oxidants, including singlet oxygen and other free radicals associated with various diseases (Jing *et al.*, 2010; Saeed *et al.*, 2012).

An initial phytochemical screening of the leaves of *F. septica* revealed that the leaf extract contained alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids. These results are in agreement with those of Damu *et al.* (2005; 2009), who found eight alkaloids in methanol extracts from the stem and roots of *F. septica*, and with a study by Baumgartner *et al.* (1990), who found phenanthroindolizidine alkaloid and antofine in the leaves of *F. septica*.

Alkaloids are considered as strong antioxidants and anti-inflammatory compounds (Macáková *et al.*, 2019). Some of the important plant-derived alkaloids used in modern medicines include morphine (analgesics), caffeine (stimulant), quinine (antimalarial) and ephedrine (anti-asthma) (Kurek, 2019).

Saponins are glucosidic plant compounds that possess forming properties. Saponins isolated from numerous plants have demonstrated hypoglycemic (Lee *et al.*, 2000) and antioxidant (Hu *et al.*, 2002) properties. In addition, saponins have also been reported to have antifungal and anti-viral activities (Mengoni *et al.*, 2002).

Tannins are high-molecular-weight phenolic compounds found naturally in a variety of plant products. Tannins are divided into two categories: hydrolysable tannins (esters of phenolic acids) and non-hydrolysable or...
condensed tannins. Some of the common tannins are aflavins, daidezein, genistein and glycitein. Tannins have been reported to have anti-HIV-1 properties (Lü et al., 2004), anti-bacterial properties against Staphylococcus aureus (Akiyama et al., 2001) and anti-parasitic properties (Kolodziej & Kiderlen, 2005). In addition, Souza et al. (2007) found that tannin-rich fractions from the stem bark of the Myrcrodroton urundeuva plant possess antioxidant, anti-inflammatory and anti-ulcer properties in mice.

Triterpenoids are the largest group of phytochemical compounds, and over 20,000 triterpenoids have been identified in nature (Liby et al., 2007). Triterpenoids have been reported to have several biological properties, such as antioxidant, anti-microbial, anti-viral, antiangiogenic (Sultana & Ata, 2008), anti-cancer, anti-inflammatory and anti-arthritis (Shah et al., 2009). Triterpenoids exhibit cytotoxicity against a variety of cancer cells without causing toxicity in normal cells (Laszczyk, 2009). According to the literature, all current secondary metabolic molecules have potential health-promoting effects.

The high antioxidant activity of F. septica may be attributed to the presence of these secondary metabolites. The IC$_{50}$ of F. septica and the ascorbic acid standard were 4.45 µg/mL and 1.66 µg/mL, respectively. When the IC$_{50}$ value is lower than 50 µg/mL, the antioxidant activity is categorised as being very strong. The level of antioxidant strength is divided into four levels: very strong (IC$_{50}$ <50 µg/mL), strong (IC$_{50}$: 50 – 100 µg/mL), moderate (IC$_{50}$:101 – 150 µg/mL), and weak (IC$_{50}$: 250 – 500 µg/mL) (Setha et al., 2013; Jumina et al., 2019). The DPPH spectrophotometric assay is a popular method for determining the antioxidant activity of medicinal plants. The test was developed based on the idea that DPPH obtains hydrogen from antioxidant chemicals. The ability of antioxidants to release hydrogen is a measure of their ability to scavenge free radicals (Saeed et al., 2012; Fugaban-Hizon, 2022). The DPPH assay suggests that the extract of F. septica contains phytochemical elements capable of releasing hydrogen as a free radical to scavenge potential damage. The DPPH findings in this study are in agreement with those of Fugaban-Hizon (2022), who investigated the antioxidant effect of an ethanolic extract of F. septica leaves using the DPPH assay.

Ficus septica has been found to contain various phytochemicals and secondary metabolites that exhibit various biological activities. These include antioxidant effects (Yamin et al., 2022), antimicrobial properties (Vital et al., 2010) and anti-cancer activity against T47D lineage breast cancer cells (Nugroho et al., 2015). Additionally, it has demonstrated anti-angiogenic effects on chorioallantoic membrane (CAM) of chicken embryos induced by basic fibroblast growth factor (bFGF) (Nurhidayati et al., 2021), antiprotozoal activity against Trichomonas vaginalis and Entamoeba histolytica parasitic infections (Vital et al., 2010), anti-malarial activity (Kubo et al., 2016) and even biological control against fungal infections by Colletotrichum acutatum in chilli peppers and Carica papaya (Sudirga et al., 2014; Sudirga & Parwanayoni, 2022).

CONCLUSION

Our current data suggest that the aqueous extract of Ficus septica possesses remarkable antioxidant activities and vital phytochemicals with antioxidant, antitumor, antimicrobial and anti-inflammatory activities. This work may serve as a useful reference for future in vivo studies to evaluate the degree of protective properties of F. septica against chemically induced cellular damage. Ficus septica is a plant with phytopharmaceutical potential.

ACKNOWLEDGEMENTS

This research was supported by the Ministry of Higher Education and the Government of Malaysia under the Fundamental Research Grant Scheme FRG0411-SG-1/2015. The authors would like to thank Professor Dr. Lee Ping Chin, Director of the Biotechnology Research Institute, Universiti Malaysia Sabah, for her support and encouragement.

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Nature Reviews Cancer, 7(5): 357-369. DOI: 10.1038/nrc2129.


