Borneo Journal of Resource Science and Technology (2023), 13(2): 67-78
DOI: https:doi.org/10.33736/bjrst.5591.2023

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

SENTY VUN-SANG & MOHAMMAD IQBAL*

Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
*Corresponding authors: miqbal@ums.edu.my; driqbalmohammad@gmail.com
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 IC_{50} value. The IC_{50} 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 IC_{50} 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 derivative known as 13,27-cycloursan-3ß-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 ficuseptine and ficuseptine 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)-ß-D-gluco pyranoside, the coumarins umbelliferone and esculin, the flavonoids genistein and kaempferitrin, 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, β-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 ficusseptine, ficuseptine C, seco-dehydroantofine and ficuseptine 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 (Na_2CO_3), sodium chloride (NaCl), sodium nitrate ($NaNO_3$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), 2,2-diphenyl-2-picrylhadrazyl (DPPH), gallic acid, catechol, and ferric chloride ($FeCl_3$) 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.

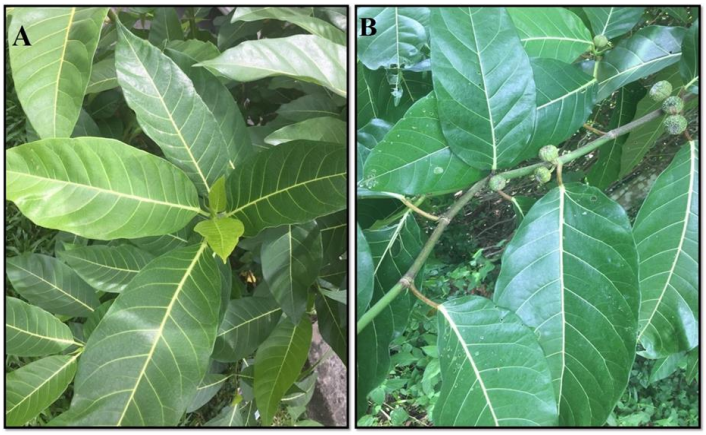

Figure 1. *Ficus septica* Burm. f. (A) leaves, (B) leaves with fruits

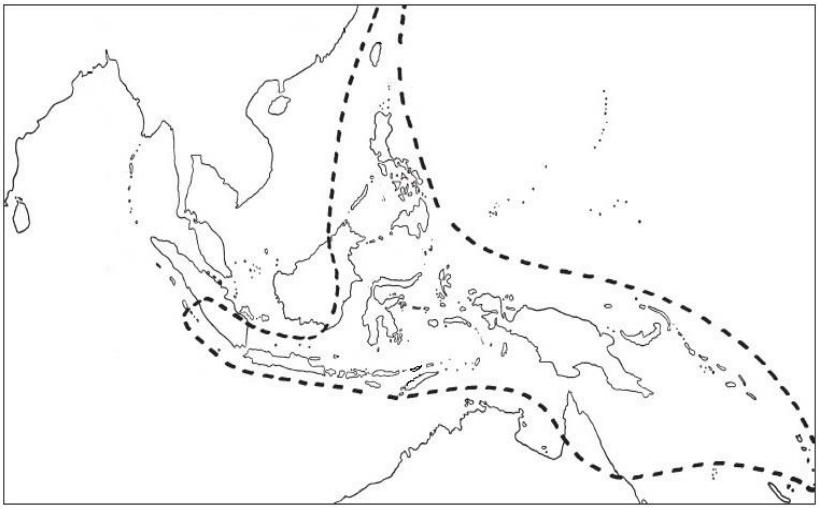

Figure 2. Distribution of *Ficus septica* Burm. f. (Berg & Corner, 2005)

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_2CO_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 1. Qualitative phytochemical screening

Phytochemical Test	Procedures	Reference
Alkaloids	Wagner's test: 2 ml sample + 2 ml Wagner's reagent + 1 ml Hydrochloric acid Presence result: Formation of reddish brown precipitate	Vimalkumar *et al*. (2014)

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	Hossain *et al*. (2013)
Tannins	Braymer's test: 2 ml sample + 1 ml 10% ferric chloride Presence result: Formation of blue or greenish colour solution	Ugochukwu *et al*. (2013)
Saponin	Foam test: 1 ml sample + 5 ml distilled water – shake vigorously Presence result: Formation of copious lather	Firdouse and Alam (2011)
Phenols	Ferric chloride test: 1 ml sample + 2 ml distilled water + few drops 10% ferric chloride Presence result: Formation of blue or green colour	Philip *et al*. (2011)
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	Vimalkumar *et al*. (2014)
Anthraquinones	5 ml sample + 2 ml diluted sulphuric acid + 2 ml benzene + 2 ml diluted ammonia solution Presence result: Rose pink colour appearance	Harborne (1998)
Phytosterols	Sulphuric acid test: 1 ml sample + 1 ml chloroform + few drops sulphuric acid Presence result: Formation of bluish green colour	Philip *et al*. (2011)
Triterpenoids	Salkowki's test: 2 ml sample + 1 ml chloroform + few drops sulphuric acid Presence result: Reddish brown precipitate produce immediately	Ugochukwu *et al*. (2013)

The percentage inhibition of radical scavenging activities (%RSA) was then calculated using Eq. (1):

$$\% \text{ RSA} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad \text{Eq. (1)}$$

where; A_{Sample} is absorbance values of the extracted sample and $A_{Control}$ is absorbance values of the control sample.

The 50% inhibitory concentration of the extract (IC_{50}) was calculated using a plot representing %RSA against extract concentration. The values (x) were calculated using the slope of the linear regression obtained by replacing y by 50 in the linear regression equation y = mx + c. The IC_{50} value of the sample is represented by the value of x.

RESULTS

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

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).

Table 2. Total phenolic and total flavonoid contents of aqueous leaves extract of *F. septica*

	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CAE/g)
Aqueous crude	27.32 ± 0.03	12.65 ± 0.00

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*

Phytochemical Test	Results
Alkaloids (Wagner's test)	+
Flavonoids (Alkaline reagent test)	+
Tannins (Braymer's test)	+
Saponins (Foam test)	+
Phenols (Ferric chloride test)	+
Steroids (Liebermann-Burchard test)	+
Anthraquinones	-
Phytosterols	-
Triterpenoids (Salkowki's test)	+

+ = 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 IC_{50} (y = 12.201x - 4.3551, R^2 = 0.9668) of *F. septica* was found to be 4.45 µg/mL. Meanwhile, the IC_{50} (y = 16.009x + 23.48, R^2 = 0.9438) of ascorbic acid was found to be 1.66 µg/mL.

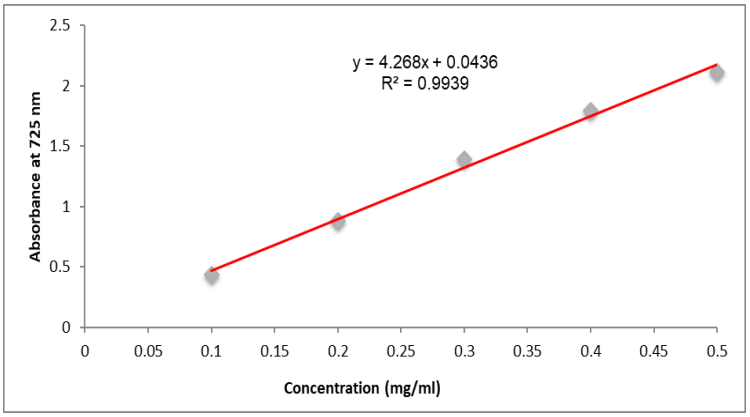

Figure 3. Linear curve of gallic acid concentration vs. absorbance for determination of total phenolic content

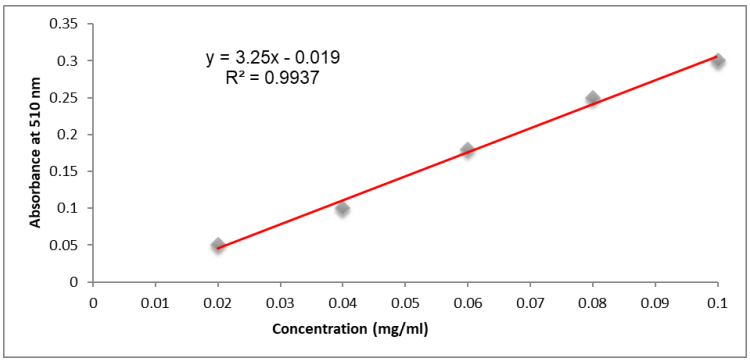

Figure 4. Linear curve of catechin concentration vs. absorbance for determination of total flavonoid content

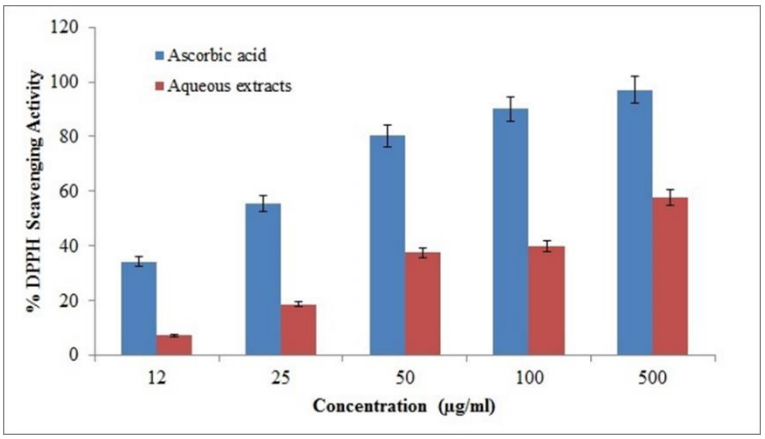

Figure 5. Radical scavenging activity of *Ficus septica* aqueous extracts by DPPH method. Data presented as mean ± standard error of mean (SEM) values of three replicates

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 *Myracrodruon 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, anti-angiogenic (Sultana & Ata, 2008), anti-cancer, anti-inflammatory and anti-arthritic (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.

REFERENCES

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. & Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 48(4): 487-491. DOI: 10.1093/jac/48.4.487.

Awang-Kanak, F., Matawali, A., Jumat, N.R. & Bakri, S.N.S. (2021). A preliminary survey on edibles and medicinal plants used by Dusun of Kampung Pinolobu, Kadamaian, Kota Belud, Sabah, Malaysia. *Journal of Tropical Biology and Conservation*, 18: 21-30. DOI: 10.51200/ jtbc.v18i.3440.

Baumgartner, B., Erdelmeier, C.A.J., Wright, A.D., Rali, T. & Sticher, O. (1990). An antimicrobial alkaloid from *Ficus septica*. *Phytochemistry*, 29(10): 3327-3330. DOI: 10.1016/0031-9422(90)80209-Y

Berg, C.C. & Corner, E.J.H. (2005). *Moraceae: Ficeae*. Flora Malesiana, Series 1, Volume 17/Part 2 pp. 1-70.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1): 25-30. DOI: 10.1016/S0023-6438(95)80008-5.

Butler, R.A. (2016). The top 10 most biodiverse countries. Retrieved February 11, 2023, from https://news.mongabay.com/2016/05/top-10-bio diverse-count ries/amp/?print

Cseke, L.J., Kirakosyan, A., Kaufman, P.B., Warber, S.L., Duke, J.A. & Brielmann, H.L. (2006). *Natural product from plants*. Second Edition. Boca Raton: CRC Press.

Damu, A.G., Kuo, P.C., Shi, L.S., Li, C.Y., Kuoh, C.S., Wu, P.L. & Wu, T.S. (2005). Phenanthroindolizidine alkaloids from the stems of *Ficus septica*. *Journal of Natural Products*, 68(7): 1071-1075. DOI: 10.1021/np050095o.

Damu, A., Kuo, P.C., Shi, L.S., Li, C.Y., Su, C.R. & Wu, T.S. (2009). Cytotoxic phenanthro-indolizidine alkaloids from the roots of *Ficus septica*. *Planta Medica*, 75(10): 1152-1156. DOI: 10.1055/s-0029-1185483.

Deli, J., González-Beiras, C., Guldan, G.S., Moses, R.L., Dally, J., Moseley, R., Lundy, F.T., Corbacho-Monne, M., Walker, S.L., Cazorla, M.U., Ouchi, D., Fang, R., Briggs, M., Kiapranis, R., Yahimbu, M., Mitjá, O. & Prescott, T.A.K. (2022). *Ficus septica* exudate, a traditional medicine used in Papua New Guinea for treating infected cutaneous ulcers: *in vitro* evaluation and clinical efficacy assessment by cluster randomised trial. *Phytomedicine*, 99: 154026. DOI: 10.1016/j.phymed.2022.154026.

de Padua, L.S., Bunyapraphatsara, N. & Lermens, R.H.M.J. (Eds.) (1999). PROSEA: Plant Resources of South – East Asia No. 12(1), Medicinal and Poisonous Plants 1. Leiden: Backhuys Publishers. pp. 277-289.

Firdouse, S. & Alam, P. (2011). Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. *International Journal of Phytomedicine*, 3(1): 32-35.

Fugaban-Hizon, C. (2021). Morphological characteristics of biosynthesized silver nanoparticles derived from *Ficus septica* leaf ethanolic extract. *Natural Volatiles and Essential Oils (NVEO)*, 8(4): 12858-12866.

Fugaban-Hizon, C. (2022). DPPH scavenging activity of *Ficus septica* leaf ethanolic extract. *Bulletin of Environment, Pharmacology and Life Sciences*, 11(5): 23-26.

Harborne, J.B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. Third Edition. London, UK: Chapman and Hall. pp. 100-101.

Hossain, M.A. & Nagooru, M.R. (2011). Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medical plant *Corydyline terminalis* L. Kunth. *Pharmacognosy Journal*, 3(24): 25-30. DOI: 10. 5530/pj.2011.24.5.

Hossain, M.A., AL-Raqmi, K.A., AL-Mijizy, Z.H., Weli, A.M. & Al-Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pacific Journal of Tropical Biomedicine*, 3(9): 705-710. DOI: 10.1016/S2221-1691(13)60142-2.

Hu, J., Lee, S.O., Hendrich, S. & Murphy, P.A. (2002). Quantification of the group B soya-saponins by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, 50(9): 2587-2594. DOI: 10.1021/jf0114740.

Huang, N.C., Hung, W.T., Tsai, W.L., Lai, F.Y., Lin, Y.S., Huang, M.S., Chen, J.J., Lin, W.Y., Weng, J.R. & Chang, T.H. (2017). *Ficus septica* plant extracts for treating Dengue virus *in vitro*. *Peer J.*, 5: e3448. DOI: 10.7717/peerj.3448.

Jing, L.J., Mohamed, M., Rahmat, A. & Abu Bakar, M.F. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species (*Boesenbergia rotunda*, *Boesenbergia pulchella* var *attenuata* and *Boesenbergia armeniaca*).

Journal of Medicinal Plants Research, 4(1): 027-032. DOI: 10.5897/JMPR09.308.

Jumina, J., Siswanta, D., Zulkarnain, A., Triono, S., Priatmoko, P., Yuanita, E., Fatmasari, N. & Nursalim, I. (2019). Development of C-Arylcalix[4]resorcinarenes and C- Arylcalix[4] pyrogallolarenes as antioxidant and UV-B protector. *Indonesian Journal of Chemistry*, 19(2): 273-284. DOI: 10.22146 /ijc.26868.

Kedare, S.B. & Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4): 412-22. DOI: 10.1007/s13197-011-0251-1.

Kolodziej, H. & Kiderlen, A.F. (2005). Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitized RAW 264.7 cells. *Phytochemistry*, 66(17): 2056-2071. DOI: 10. 1016/j.phytochem.2005.01.011.

Kubo, M., Yatsuzuka, W., Matsushima, S., Harada, K., Inoue, Y., Miyamoto, H. & Fukuyama, Y. (2016). Antimalarial phenanthroindolizine alkaloids from *Ficus septica*. *Chemical and Pharmaceutical Bulletin*, 64(7): 957-960. DOI: 10.1248/cpb.c16-00181.

Kulip, J. (2007). Common medicinal plants of Sabah. *Sepilok Bulletin*, 6: 1-23.

Kuo, P.C., Chm, C.C., Shi, L.S., Li, C.Y., Wu, S.J., Damu, A.G., Wu, P.L., Kuoh, C.S. & Wu, T.S. (2002). Non-alkaloidal constituents from the stem of *Ficus septica*. *Journal of the Chinese Chemical Society*, 49(1): 113-116. DOI: 10.1002/jccs .200200019.

Kurek, J. (2019). *Introductory Chapter: Alkaloids - Their importance in nature and for human life*. IntechOpen. DOI: 10.5772/intechopen.85400.

Laszczyk, M.N. (2009). Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. *Planta Medica*, 75(15): 1549-1560. DOI: 10.1055/s-0029-1186102.

Lee, K.T., Sohn, I.C., Kim, D.H., Choi, J.W., Kwon, S.H. & Park, H.J. (2000). Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their antioxidant activity *in vitro*. *Archives of Pharmacal Research*, 23(5): 461-466. DOI: 10.1007/BF02976573.

Liby, K.T., Yore, M.M. & Sporn, M.B. (2007). Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nature Reviews Cancer*, 7(5): 357-369. DOI: 10.1038/nrc2129.

Lü, L., Liu, S.W., Jiang, S.B. & Wu, S.G. (2004). Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharmacologica Sinica*, 25(2): 213-218.

Macáková, K., Afonso, R., Saso, L. & Mladěnka, P. (2019). The influence of alkaloids on oxidative stress and related signaling pathways. *Free Radical Biology and Medicine*, 134: 429-444. DOI: 10.1016/j.freeradbiomed.2019.01.026.

Malaysia Biodiversity Information System (MyBIS). (2023). *Ficus septica*. Retrieved February 11, 2023, from https://www.mybis.gov.my/sp/35786.

Mengoni, F., Lichtner, M., Battinelli, L., Marzi, M., Mastroianni, C.M., Vullo, V. & Mazzanti, G. (2002). *In vitro* anti-HIV activity of oleanolic acid on infected human mononuclear cells. *Planta Medica*, 68(2): 111-114. DOI: 10.1055/s-2002-20256.

Naczk, M. & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054(1-2): 95-111. DOI: 10.1016/j.chroma.2004.08.059.

Nastiti, K., Sudarsono & Nugroho, A.E. (2014). Evaluation of *in vitro* immunomodulatory effect of fractions of *Ficus septica* Burm. f. and their total flavonoid and phenolic contents. *International Food Research Journal*, 21(5): 1981-1987.

Nugroho, A.E., Ikawati, M., Hermawan, A., Putri, D.D.P. & Meiyanto, E. (2011). Cytotoxic effect of ethanolic extract fractions of Indonesia plant *Ficus septica* Burm. f. on human breast cancer T47D cell lines. *International Journal of Phytomedicine*, 3(2): 216-226. DOI:10.5138 /ijpm.v3i2.331.

Nugroho, A.E., Akbar, F.F., Wiyani, A. & Sudarsono (2015). Cytotoxic effect and constituent profile of alkaloid fractions from ethanolic extract of *Ficus septica* Burm. F. leaves on T47D breast cancer cells. *Asian Pacific Journal of Cancer Prevention*, 16(16): 7337-7342. DOI: 10.7314/apjcp.2015 .16.16.7337.

Nurhidayati, L.G., Nugroho, A.E., Retnoaji, B., Sudarsono & Fakhrudin, N. (2021). Antiangiogenesis activity of Awar-Awar leaf extract (*Ficus septica* Burm. f.) in chorioallantoic membrane assay. *Indonesian Journal of Pharmacy*, 32(1): 1-9. DOI: 10.22146/ijp.607.

Philip, D., Kaleena, P.K., Valivittan, K. & Girish Kumar, C.P. (2011). Phytochemical screening and antimicrobial activity of *Sansevieria roxburghiana* Schult. and Schult. F. *Middle-East Journal of Scientific Research*, 10(4): 512-518.

Ragasa, C.Y., Macuha, M.R., Reyes, M.M.D.L., Mandia, E.H. & Altena, I.A.V. (2016). Chemical constituents of *Ficus septica* Burm. F. *International Journal of Pharmaceutical and Clinical Research*, 8(11): 1464-1469.

Rodriguez, L.J., Bain, A., Chou, L.S., Conchou, L., Cruaud, A., Gonzales, R., Hossaert-McKey, M., Rasplus, J.Y., Tzeng, H.Y. & Kjellberg, F. (2017). Diversification and spatial structuring in the mutualism between *Ficus septica* and its pollinating wasps in insular South East Asia. *BMC Evolutionary Biology*, 17: 207. DOI: 10.1186/s12862-017-1034-8.

Saeed, N., Khan, M.R. & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*, 12: 221. DOI: 10.1186 /1472-6882-12-221.

Sekti, D.A., Mubarok, M.F., Armandani, I., Junedy, S. & Meiyanto, E. (2010). Awar-awar (*Ficus septica* Burm. f.) leaves ethanolic extract induced apoptosis of MCF-7 cells by downregulation of Bcl-2. *Traditional Medicines Journal*, 15(3): 100-104. DOI: 10.22146/tradmedj.8133.

Septhea, D.B., Anindyajati, Darma, A., Nurzijah, I. & Nugroho, A.E. (2011). *Ficus septica* Burm. f. leaves ethanolic extract induces apoptosis in 7,12-dimethylbenz[a]nthracene-induced rat liver cancer quantitatively. *Indonesian Journal of Cancer Chemoprevention*, 2(2): 242-248. DOI: 10.14499/indonesianjcanchemoprev2iss2pp255-260.

Setha, B., Gaspersz, F., Idris, A.P.S., Rahman, S. & Mailoa, M.N. (2013). Potential of seaweed *Padina* sp. as a source of antioxidant. *International Journal of Scientific and Technology Research*, 2(6): 221-224.

Shah, B.A., Qazi, G.N. & Taneja, S.C. (2009). Boswellic acids: A group of medicinally important compounds. *Natural Product Report*, 26(1): 72-89. DOI: 10.1039/b809437n.

Souza, S.M., Aquino, L.C., Milach, A.C. Jr, Bandeira, M.A., Nobre, M.E. & Viana, G.S. (2007). Anti-inflammatory and antiulcer properties of tannins from *Myracrodruon urundeuva* Allemão (Anacardiaceae) in rodents. *Phytotherapy Research*, 21(3): 220-225. DOI: 10.1002/ptr.2011.

Sudirga, S.K., Suprapta, D.N., Sudana, I.M. & Wirya, I.G.N.A.S. (2014). Antifungal activity of leaf extracts of *Ficus septica* against *Colletotrichum acutatum* the cause of anthracnose disease on chilli pepper. *Journal of Biology, Agriculture and Healthcare*, 4(28): 27-52.

Sudirga, S.K. & Ginantra, I.K. (2017). Identification of bioactive compounds of *Ficus septica* leaf extract has potential as botanical pesticides to control anthracnose disease on chilli pepper. *Journal of Biological and Chemical Research*, 34(1): 150-159.

Sudirga, S.K. & Parwanayoni, N.M.S. (2022). Use of *Ficus septica* leaf extract for biological control of anthracnose disease in *Carica papaya* caused by *Colletotrichum* spp. *KnE Life Sciences*, 7(3): 469-478. DOI: 10.18502/kls.v7i3.11153.

Sultana, N. & Ata, A. (2008). Oleanolic acid and related derivatives as medicinally important compounds. *Journal of Enzyme Inhibition Medicinal Chemistry*, 23(6): 739-756. DOI: 10.1080/14756360701633187.

Ueda, J., Takagi, M. & Shin-ya, K. (2009). Aminocaprophenone- and pyrrolidine-type alkaloids from the leaves of *Ficus septica*. *Journal of Natural Products*, 72(12): 2181-2183. DOI: 10.1021/np900580f.

Ugochukwu, S.C., Uche, I.A. & Ifeanyi, O. (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. *Asian Journal of Plant Science and Research*, 3(3): 10-13.

Vimalkumar, C.S., Hosagaudar, V.B., Suja, S.R., Vilash, V., Krishnakumar, N.M. & Latha, P.G. (2014). Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb., infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and non-infected plants. *Journal of Pharmacognosy and Phytochemistry*, 3(4): 69-72.

Vital, P.G., Velasco Jr., R.N., Demigillo, J.M. & Rivera, W.L. (2010). Antimicrobial activity, cytotoxicity and phytochemical screening of *Ficus septica* Burm and *Sterculia foetida* L. leaf extracts. *Journal of Medicinal Plants Research*, 4(1): 58-63. DOI: 10.5897/JMPR09.400.

Vun-Sang, S., Rodrigues, K.F., Dsouza, U.J.A. & Iqbal, M. (2022). Suppression of oxidative stress and proinflammatory cytokines is a potential

therapeutic action of *Ficus lepicarpa* B. (Moraceae) against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. *Molecules*, 27: 2593. DOI: 10.3390/molecules27082593.

World Health Organization (WHO). (2018). Pharmacovigilance for traditional medicine products: Why and how? World Health Organization. Regional Office for South-East Asia. Retrieved March 11, 2021, from https://apps.who.int/iris/handle/10665/259854

World Health Organization (WHO). (2019). WHO global report on traditional and complementary medicine 2019. World Health Organization. Retrieved March 1, 2021, from https://apps.who.int/iris/handle/10665/312342

Wu, P., Rao, K.V., Su, C., Kuoh, C. & Wu, T. (2002). Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of *Ficus septica*. *Heterocycles*, 57(12): 2401-2408. DOI: 10.3987/COM-02-9615.

Yamin, Andriani, R., Sabarudin, Haijah, N. & Kasmawati, H. (2022). Antioxidant activity assay and determination of phenolic and flavonoid content of Libho (*Ficus septica* Burm. F.) fruits. *Open Journal of Chemistry*, 8(1): 008-013. DOI: 10.17352/ojc.000029.

Yao, L.H., Jiang, Y.M., Shi, J., Tomás-Barberán, F.A., Datta, N., Singanusong, R. & Chen, S.S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59(3): 113-122. DOI: 10.1007/s11130-004-0049-7.