Phytochemical Analysis and Antioxidant Activity of *Ficus lepicarpa* Leaves from Sabah, Malaysia

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ABSTRACT *Ficus lepicarpa* belongs to family Moraceae and commonly known as 'Saraca fig'. It has been used by local people as vegetable dish, as a tonic and to treat aliments such as fever and ringworm. The present study was aimed at evaluating the phytochemical constituents and antioxidant activity of methanol extract of *F. lepicarpa* leaves. The antioxidant activity of *F. lepicarpa* leaves extracts were estimated using FRAP assay (ferric reducing antioxidant potential) method. The test of phytochemical screening showed the presence of flavonoids, saponins, steroids, phytosterols, and absence of alkaloids, tannins, phenols, anthraquinones and triterpenoids. The result obtained from this study provides information that *F. lepicarpa* has antioxidant activity and possesses the potential to be used to treat or prevent degenerative diseases where oxidative stress is implicated. Further studies are in progress to evaluate the *in vivo* potential of *F. lepicarpa* in animal model of carbon tetrachloride mediated oxidative tissue injury.

KEYWORDS: Phytochemical constituents, Antioxidant activity, *Ficus lepicarpa*, Moraceae, Leaves Extract.

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INTRODUCTION

Antioxidants are compounds that inhibit the oxidation of other molecules by inhibiting the oxidizing chain reactions by free radicals that causes damage and death to the cell (Azlim Almey *et al*., 2010). In recent years, there is an intense interest in the measurement and use of plant antioxidants for scientific research as well as industrial (dietary, pharmaceutical and cosmetic) purposes (Rahim & Khan, 2006; Suhaj, 2006). This is mainly due to synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have been scrutinised for possible toxic and carcinogenic effects (Azlim Almey *et al*., 2010). Therefore, it is considered to be an important task in evaluating plant antioxidant activity and their free radical quenching ability that are from natural sources, healthier and safer than synthetic ones and more acceptable to the modern consumers.

Ficus lepicarpa (*F. lepicarpa*) belongs to the family *Moraceae* which consist of more than 750 species, which most of them found in the tropics (for example, Borneo rainforests contain more than hundred fig species) (Corner, 1975; Grison-Pige *et al*., 2002). *F. lepicarpa* is a wild growing tree that grows from five to 15 meter tall. It is found in humid forest, typically on rocky banks of rivers, up to 1700 m altitude. *F. lepicarpa* commonly known as Saraca fig (Corner, 1975; Jansen *et al*., 2016) and locally known as 'Kelupang Gajah' (Malay) (Milow *et al*., 2014), 'Ombuwasak' (Rungus) or 'Tombuwasak' (Dusun) (Ahmad & Holdsworth, 1995) and 'Litotobow' (Murut) (Kulip, 2003). Traditionally the *F. lepicarpa* leaves are used to treat ringworm (Faridah Hanum & Hamzah, 1999) and as vegetable (Kulip, 2013; Jansen *et al*., 2016) while its root is used for tonic drink and treating fever (Ahmad & Holdsworth, 1995). The objective of this research was to evaluate the phytochemical constituents and antioxidant activity of methanol extract of *F. lepicarpa* extracts *in vitro*.

MATERIALS AND METHODS

Plant material

Ficus lepicarpa (Figure 1) plant samples were collected from the village of Morion, Tandek, Kota Marudu, Sabah, Malaysia, in May 2016. Species of the *Ficus* was authenticated by Mr. Julius Kulip and Mr. Johnny Gisil from the Institute for Tropical Biology and Conservation (IBTP), Universiti Malaysia Sabah. Voucher specimen number was issued for the plant sample (SVS: 001). The plant sample was kept at the Institute for Tropical Biology and Conservation herbarium for future reference.

Figure 1. Photograph of *F. lepicarpa* plant leaves.

Preparation of extracts

The plants leaves were dried under shade at room temperature for three weeks. The dried leaves were manually ground to a fine powder and stored in airtight container for extraction. The methanol plant extracts were prepared by the method described by (Jantan *et al*., 2014) with some modification. Each dried plant material (100 g) were ground and macerated in methanol at the ratio of 1:10 (w/v) for seven days at room temperature with occasionally stirring. The extract was filtered through Whatman filter paper No.1 and the entire extraction process was repeated thrice on the residue with fresh methanol solvent. The filtrates were combined and methanol were removed under reduced pressure and air-dried overnight. Thereafter, the air dried samples were dissolved in distilled water to a concentration of 1 mg/ml for use in the subsequent assays.

Phytochemical screening

Phytochemical screening is qualitative assay which were estimated based on the methods given respectively for alkaloids (Vimalkumar *et al*., 2014), flavonoids (Hossain *et al*., 2013), tannins (Ugochukwu *et al*., 2013), saponins (Firdouse & Alam, 2011), phenols (Philips *et al*., 2011), steroids (Vimalkumar *et al*., 2014), anthraquinones (Harborne, 1998), phytosterols (Philip *et al*., 2011) and triterpenoids (Ugochukwu *et al*., 2013).

Total phenolic content (TPC)

Total phenolic content in the methanol extract was estimated by the Folin Ciocalteu's method following the method described by (Velioglu *et al*., 1998). The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard garlic acid. The data for total phenolic contents were expressed as mg of garlic acid equivalent weight per gram of plant extract.

Total flavonoid content (TFC)

Total flavonoid content was estimated by aluminum chloride colourimetric method as described by (Zou *et al*., 2004). The calculation of total flavonoids in the extracts was done in triplicates and the results were averaged. The calibration curve was plotted using standard catechin. The total flavonoid content was expressed in mg of catechin equivalents per gram of plant extract.

Ferric Reducing Antioxidant Potential (FRAP)

The ferric-reducing antioxidant power (FRAP) assay estimated as the method described by (Chan *et al*., 2007). The calibration curve was plotted using standard ascorbic acid. The assay was performed in triplicates and the results were averaged.

RESULT AND DISCUSSION

The phytochemical screening of *F. lepicarpa* leaves extract revealed the presence of flavonoids, saponins, steroids and phytosterols as presented in Table 1.

Table 1. Phytochemical screening of methanol leaves extract of *F. lepicarpa*

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 (Sulphuric acid test)	+
Triterpenoids (Salkowki's test)	-

+ = Present; ++ = Strong present; - = Absent

The concentration of total phenolic content of *F. lepicarpa* methanol leaves extracts (Table 2) were determined according to the equation (y = 4.268x + 0.036, R^2 = 0.9939) as garlic acid equivalent (mg/g extract) clarified by standard curve of garlic acid in Figure 2.

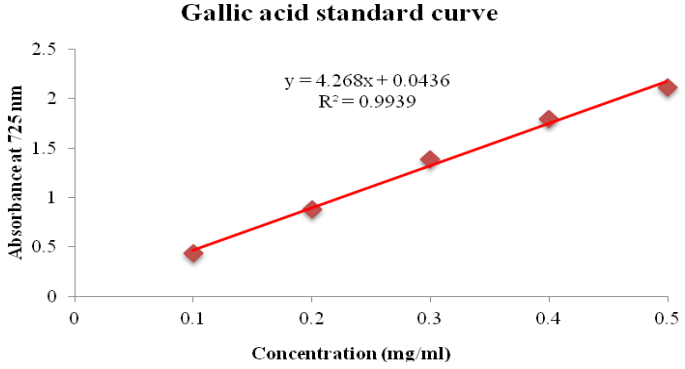

Figure 2. Total phenolic content for standard gallic acid. R^2 values represent mean data set of n = 3.

A linear calibration curve of catechin with r2 value of 0.9937 was obtained (Figure 3). Table 2 shows mean total flavonoid content of the plants' leave extracts measured using the equation of y = 3.25x + 0.019 (R^2 = 0.9937), whereby y = absorbance at 510 nm and x = concentration of total flavonoid compounds in mg per g of the extract.

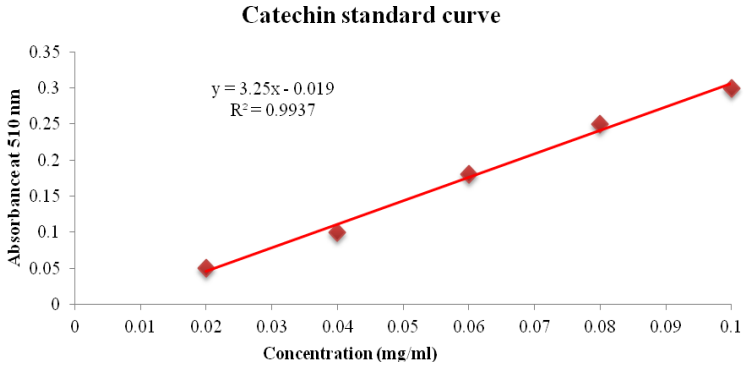

Figure 3. Total flavonoid content for standard catechin. R^2 values represent mean data set of n = 3.

Table 2. Total phenolic content and total flavonoid content of methanol leaves extract of *F. lepicarpa*

Total phenolic content (mg/g)	Total flavonoid content (mg/g)
2.4±0.06	5.0±0.02

Results are express as mean ± standard deviation.

The FRAP assay was employed to estimate the antioxidant capacity of the samples in vitro using ascorbic acid as reference standard. The concentration ranged from 0.02 – 0.1 mg/ml. In this test, the result (Figure 4) revealed that a good linearity of ascorbic acid standard curve was obtained with R^2 = 0.9951. FRAP assay had been used to determine antioxidant activity as it is simple and quick. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants (Hodzic *et al.*, 2009). As shown in Figure 4, a higher absorbance value indicates a stronger reducing power of the standard. *F. lepicarpa* methanol extract showed concentration-dependent reducing power. However, its reducing power was weaker than that of ascorbic acid, which exhibited the strongest reducing power. *F. lepicarpa* methanol leaves extract showed lower antioxidant activity than that of ascorbic acid at the concentrations tested.

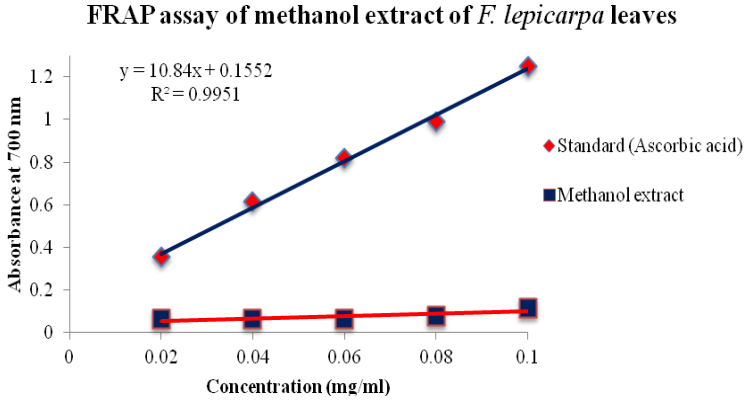

Figure 4. Ferric Reducing Antioxidant Potential (FRAP) assay activity of methanol leaves extract of *F. lepicarpa*. Ascorbic acid was included as positive control. Each value is the mean ± standard deviation.

CONCLUSION

The phytochemical screening showed that the *F. lepicarpa* leaves extract contain a mixture of phytochemicals as flavonoids, saponins, steroids and phytosterols. The quantitative total phenolic and total flavonoids screening indicated that the methanolic leaves extract contain flavonoids and phenols and the results from antioxidant activities reflected by the FRAP assay have demonstrated that the *F. lepicarpa* crude extract possesses a relatively low antioxidant activity compared to the reference standard at the concentrations tested.

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