Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 9, 2015

Short Communication

PHYTOCHEMICAL SCREENING, ANTIOXIDANT PROPERTIES IN VARIOUS EXTRACTS FROM THE LEAVES OF *FLAGELLARIA INDICA* L. FROM SABAH, MALAYSIA

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Received: 15 Jun 2015 Revised and Accepted: 11 Jul 2015

ABSTRACT

Objective: This study was conducted to evaluate the antioxidant capacities of the leaves of *Flagellaria indica* L. (FI) and its phytochemical constituents in six different extracts.

Methods: The assessment was done via a 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay for the antioxidant test, the Folin-ciocalteau method for total phenolic content, Willet's method for total flavonoid content and several other qualitative phytochemical tests carried out on all extracts.

Results: The results show the highest values of radical scavenging in the following order of extracts: butanol>ethyl acetate>aqueous>chloroform>methanol>hexane. The total phenolic content is the highest in the ethyl acetate (e. acetate) extract (153.28 mg/g) followed by butanol (134.78 mg/g), aqueous extract (65.88 mg/g), chloroform (55.28 mg/g), methanol (45.98 mg/g) and hexane (22.78 mg/g), expressed as gallic acid equivalents. The total flavonoids content was also the highest in e. acetate extract (38.96 mg/g) followed by butanol (28.45 mg/g), aqueous (21.18 mg/g), chloroform (12.9 mg/g), methanol (10.78 mg/g) and hexane extract (4.92 mg/g) using cathechin equivalents.

Conclusion: The antioxidant and radical scavenging activities of FI might be due to the strong presence of phenolic constituents, flavonoids and several other bioactive compounds. Thus, further research can be conducted to elucidate the potential of this plant for pharmacological importance.

Keywords: *Flagellaria indica*, Phenolic, Flavonoids, Radical scavenging activity, Bioactive compounds.

Antioxidants can be found widely in plants and they are proven to be scavenging the free radicals in human body and protect our body from oxidative stress that lead to chronic heart failure and diabetes mellitus [1, 2]. Antioxidants serve to reduce and control the levels of free radicals, assessing them to perform useful biological functions by repairing damage done by free radicals [3]. Plant polyphenols, a diverse group of phenolic compounds such as flavanols, flavonols, anthocyanins, and phenolic acids possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron and from their potential to chelate metal ions [4, 5].

Flagellaria indica L. (Flagellariaceae) (FI) is a climbing plant found in many of the tropical and subtropical regions of India, South East Asia, Polynesia and Australia. It is more commonly known in Sabah, Malaysia as *rotan tikus* or false rattan. The leaves of this plant are claimed by people native to the regions above to possess medicinal value, such as a cure for cough and vomiting, diuretic properties, wound-healing properties, decoction of boiled leaves taken as a health tonic, and so on. The native Murut tribe of Sabah claims that FI has medicinal properties that are able to cure semi-paralytic conditions when the whole plant is boiled and the water from the boiling process is used to bathe the patient [6]. However, there have been no reports on the antioxidative properties of FI, except for a study of a DPPH test on several seashore plants, including FI, without reference to the specific properties of this plant [7]. Therefore, this study was conducted to evaluate the antioxidative properties and phytochemical constituents of FI that might be contributing its claimed medicinal values.

The whole plant samples were freshly collected in April 2012 from an herbal farm in the area of Papar, Sabah, Malaysia. Mr. Johnny Gisil, Botanist from Institute of Tropical Biology Conservation, Universiti Malaysia Sabah, identified the plant species based on the morphological characteristics of the plant; a sample was deposited at the university herbarium with collection number CG004. The leaves were separated and washed thoroughly with tap water and air dried. The dried leaves were ground to coarse powder using a heavy duty blender and kept at room temperature prior to further analysis.

Liquid/liquid extraction method was done according to Akkol *et al.* (2012) [8], with some modifications. The dried leaves powder (50 g) was extracted with 500 ml of methanol (99.6%) at room temperature for three d and the residue was re-extracted the same way for maximum yield. The extract was evaporated by a vacuum rotary evaporator (Buchi Labortech AG, model 1, R-215). The methanol extract was (6.3 g) diluted by 700 ml water and extracted successively with 300 ml hexane, 300 ml ethyl acetate, 200 ml chloroform and 200 ml butanol to give hexane (0.32 g), ethyl acetate (0.38 g), chloroform (0.28 g), and butanol (0.53 g) and residual methanol extract fractions (0.58g), respectively. The extract was filtered using Whatman No.1 filter paper to obtain a particle free extract. The residue was re-extracted twice by solvent and filtered. The filtrates were pooled and then concentrated and dried under vacuum pressure. The same extraction procedure was followed for the other solvents. All the products were lyophilised and kept at-20^{o}C till further analysis.

Powdered leaves (50 g) were boiled with distilled water (500 ml) for 10 min. Each of decoctions was removed and allowed to cool at room temperature for 1 h. Thereafter, the aqueous extracts were filtered using a tea strainer to remove coarse residues then filtered again using Whatman No.1 filter paper. The pure filtrate was frozen at-80 ^{o}C and freeze dried. The extract (6.8 g) obtained was kept at-20 ^{o}C until further analysis.

The total phenolic content of FI was determined according to the Folin-Ciocalteau method as described by Velioglu *et al.* [9]. Gallic acid was used as a standard for determining the phenol content. The absorbance was read at 725 nm against blank using spectrophotometer. The results were expressed as gallic acid equivalents (GAE) per g of extract. All the measurements were taken in triplicate and means and standard deviation values were calculated.

The determination of antioxidant activity was done according to the method used by Hatano *et al.* [10]. The reduction of the DPPH radical was determined by reading the absorption at 517 nm using spectrophotometer. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ RSA } = \left[\frac{(\text{Control } - \text{Sample})}{\text{Control}}\right] \times 100$$

Total flavonoids content of FI was determined by using the aluminium chloride colorimetric method as described by Willet [11], with some modifications. 1 mg/ml crude extracts (0.25 ml), 1M sodium nitrate (0.75 ml), incubated for 6 min, and then 10% aluminium chloride (0.15 ml), 4% sodium hydroxide (0.5 ml) and distilled water (1.53 ml) were mixed. After incubation at room temperature for 5 min, the absorbance was measured at 510 nm using a Spectrophotometer (Thermo Fisher Scientific, model 4001/4). Cathechin was used to make the calibration curve. The calculation of total flavonoids content in the extracts was carried out in triplicate and the results were averaged.

One mg/ml of stock solution of all different extracts was prepared. The obtained crude extracts were subjected to preliminary phytochemical screening following the methodology of Harborne [12], Kokate [13] and Gomathi *et al.* [14].

Experimental results were mean±standard error of mean (SEM) of three parallel measurements and analysed by SPSS 17 (SPSS Inc., Chicago, IL). Differences between means were determined using Tukey's multiple comparisons and least signi ficant difference (LSD).

In this study, various solvent extracts viz., methanol, hexane, ethyl acetate, chloroform, butanol and also aqueous extract of FI were analysed together. The amount of phenolic compounds, flavonoid contents, radical scavenging effects and phytochemical properties varied in each extract. The yields from methanol, hexane, ethyl acetate, chloroform, butanol and aqueous extracts from the leaves of FI were 12.6%, 5.08%, 6.03%, 4.44%, 8.41% and 13.6% respectively.

The total phenolic content of all the extracts, reported as gallic acid equivalents per gram of extract, are presented in table 1. It is shown that the highest phenolic content was found in the ethyl acetate extract (153.28±0.71 mg/g), followed by the butanol, aqueous, chloroform, methanol and hexane (22.78±0.28 mg/g) extracts. Results for the total flavonoid content of the various extracts of FI are shown in table 1. The highest amount of flavonoid content was found in the e. acetate extract (38.96±2.43 mg/g) and the lowest amount was found in the hexane extract (4.92±0.35 mg/g), expressed as catechin equivalents per gram of extract.

Table 1: Total phenolic and flavonoid contents in various extracts of FI leaves

Extracts	Total phenolic (gallic acid equivalent)(mg/g)	Total flavonoid (catechin equivalent)(mg/g)
Methanol extract	45.98±0.57	10.78±0.70
Hexane extract	22.78±0.28	4.92±0.35
Ethyl Acetate extract	153.28±0.71	38.96±2.43
Chloroform extract	55.28±0.14	12.90±0.63
Butanol extract	134.78±0.28	28.45±1.69
Aqueous extract	65.88±1.84	21.18±2.89

Data are presented as mean±standard error of mean (SEM) of three replicates

FI possessed good radical scavenging activity in all the extracts as exhibited by the results for the *in-vitro* (DPPH) studies. The leaf extracts of FI reduced DPPH in a concentration-dependent manner, whereas decolourization increased with increasing concentrations of plant extracts. The scavenging of free radicals is a major antioxidant mechanism that inhibits the chain reaction of free radical generation in tissues, which leads to lipid peroxidation [15].

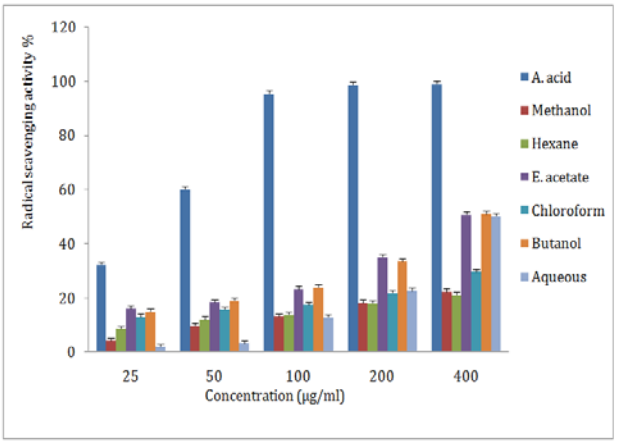

Fig. 1: DPPH free radical scavenging activity in various extracts of FI leaves, Data presented as mean±standard error of mean (SEM) values of three replicates

The radical scavenging activity of FI extracts implies that it could be beneficial in pathological alterations in tissues, attributable to free radicals that cause lipid peroxidation.

The data is shown in fig. 1. Ascorbic acid, a commercial antioxidant, was used as a standard comparison for DPPH radical scavenging activity. The highest free radical scavenging activity at 400 µg/ml extract concentration is shown by the butanol extract (51.07%), followed by the ethyl acetate (50.83%), aqueous (50.35%), chloroform (29.69%), methanol (22.33%) and hexane extracts (21.14%).

Several qualitative phyto chemical screenings were conducted on the various extracts of FI, and it was found that bioactive constituents such as flavonoids, tannins, saponins, steroids, triterpenoids, alkaloids and phytosterols were present in the extracts of FI. Anthraquinones and anthraquinone glycosides were not found in any of the extracts. The results are shown in table 2. Most of the bioactive constituents were found in the extracts of methanol extract, which might be due to polarity of the solvent.

From this study, we can say that the ethno-botanical claims and medicinal values of FI are mainly due to its antioxidative properties from phenolic, flavonoid compounds and the presence of several phytochemical constituents. This study could serve as a constructive reference to allow further *in-vivo* studies, which can be conducted to evaluate the extent of protective effects of FI against chemically induced cellular injuries. If further tests on lethal toxicity levels are conducted, this plant could provide a good source of antioxidants with pharmacological value.

Table 2: The phytochemical analysis in different solvent extracts of FI leaves

Bioactive compounds	Extracts					
	Methanol	Hexane	E. Acetate	Chloroform	Butanol	Aqueous
Flavonoids	+	+	++	++	++	++
Tannins	++	–	–	–	–	++
Saponins	++	++	++	++	++	++
Steroids	+	+	+	–	++	++
Triterpenoids	++	–	++	+	+	++
Alkaloids	+	+	++	++	++	++
Anthraquinones	–	–	–	–	–	–
A. glycosides	–	–	–	–	–	–
Phytosterols	+	+	++	+	++	–

++= Strong presence; += Presence;–= Absence

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest. This research is financially supported by Grant-in-Aid for Research Priority Area Scheme, Universiti Malaysia Sabah (SBK0027-SKK-2012).

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