Original Article

Phytochemical Analysis and Evaluation of *In-Vitro* Antioxidant Activity of Bark Extracts from *Madhuca longifolia* (Madhu) and *Ficus racemosa* (Attikka) Grown in Sri Lanka

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Abstract

Madhuca longifolia (Maddhu) and Ficus racemosa (Attikka) are well known medicinal plants which have been valued for decades in Sri Lankan Ayurvedic medicine. This research was aimed to study the phytochemical analysis and evaluate *in-vitro* antioxidant activity of two solvent extracts (70% aqueous acetone and 80% aqueous methanol) obtained from each bark of selected plants. The crude extracts were prepared by steeping the dried powder in each solvent overnight in the dark conditions. Phytochemical screening of crude extracts was performed. *In-vitro* radical scavenging activity and antioxidant activity were evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. The results of phytochemical analysis revealed the presence of alkaloids, phenolics, flavonoids, carbohydrates, proteins and saponins in both extracts obtained from *M. longifolia* while revealing the presence of phenolics, flavonoids, carbohydrates, alkaloids, tannins in both bark extracts obtained from *F. racemosa*. Radical scavenging activity of 80% aq. methanol and 70% aq. acetone extracts obtained from bark of *M. longifolia* were determined as 25.4±0.1 and 23.1±0.4 mmol Trolox equivalents/100 mg dry weight of bark of *M. longifolia* (DW) and antioxidant capacity of 80% aq. methanol and 70% aq. acetone extracts were 42.6 ± 0.7 and 38.0 ± 1.1 mmol Fe (ll) equivalents/100 mg DW of the bark. Radical scavenging activity for two different extracts obtained from bark of *F.racemosa* were as 18.6 ± 0.3 (80% aqueous methanol) 20.2 ± 0.7 (70% aq. acetone) mmol Trolox equivalents/100 g DW of bark. Antioxidant capacity by FRAP assay for the two different extracts of *F. racemosa* were 28.5 ± 0.4 (80% aq. methanol) and 32.8 ± 1.6 (70% aq. acetone) mmol Fe (II) equivalents/100 g DW of the bark. It was concluded that the presence of remarkable *in-vitro* antioxidant activity of two different plant extracts were observed while significant antioxidant activity was showed by 80% aq. methanol extract of *M. longifolia*.

Keywords- Antioxidant activity, Ficus racemosa, Madhuca longifolia, phytochemical analysis,

Introduction

Nowadays, attention is being focused on the development of herbal products using natural biomaterials, which can overcome the apparent drawbacks of synthetic chemicals. Even though various synthetic chemicals are being used in many formulations as antioxidants, they have limited use due to their potential toxicity and association with multistage process of carcinogenesis in humans [1]. Researches have been shown that these active synthetic molecules may adversely affects the human skin through self-inducing reactive oxygen species. Hence, to overcome those side effects, researches are focused on formulation of novel products using natural biomaterials such as naturally occurring antioxidants namely, ascorbic acid, alpha carotene, vitamin E and A, flavanone and flavones etc, which have the ability to donate electrons thereby prevent free radical chain reactions. Therefore, various compounds present in plant extracts with ability to reduce oxidative damage can also be helpful in improving the efficacy relating to continuous action of herbs on human skin [2].

Many research studies have been successfully carried out worldwide in order to investigate the potential agents from various medicinal plants and as well as to formulate novel herbal products using natural products [1]. Medicinal plants are being effective source of both traditional and modern medicines which are useful for primary healthcare,

as they have potent pharmacological activity since prehistoric times. Medicinal plants synthesize different bioactive compounds for functions including defense against insects, fungi diseases and herbivorous mammals and rich source of medicines [3]. Because of the less side effects, efficacy and safety and the origin of medicinal plants, herbal medicine is being developed both in developing and developed countries since few decades.

Madhuca longifolia commonly known as Mahua in Sinhala, belongs to the family Sapotaceae is a medium to large sized deciduous tree (Figure 1a). They are distributed in south Asian countries such as India, Sri Lanka and Nepal. Mahua grows widely under dry topical and sub topical climate conditions and can be found in forests, revenues and private lands. A various parts of *M. longifolia* are used in traditional and folklore system of medicine and known as universal panacea of ayurvedic medicine. The bark of Mahua is used to treat diabetics, ulcers, rheumatism, tonsillitis and bleedings. Mahua oil is traditionally used in skin diseases, rheumatism, headache and laxative, [4]. The flowers of Mahua are used as cooling agent, astringent and also it is used to treat acute and chronic tonsillitis, aphrodisiac, pharyngitis and bronchitis [4]. The leaves are the major component of Mahua tree that can be used as wound healing hepatoprotective, antimicrobial, astringent, and used for bronchitis, cephalgia and Cushing's disease. The roots of Mahua are traditionally used as anti-inflammatory, antioxidant, antipyretic and also root powder is used for diarrhoea and other chronic fluxes [5].

(a)

(b)

Figure 1: Images of (a) *Madhuca longifolia* plant (b) *Ficus racemosa* plant

Ficus racemosa plant which is usually known as cluster fig tree belongs to the family Moracea. It is commonly known in Sri Lanka as "attikka" which is grown all over the country. It is one of the members of the sacred trees to be planted around temples. This plant is used in traditional medicine systems such as Ayurveda, Unani, Sidda and also homeopathy medicine system for the treatment of various disorders [6]. *F. racemosa* is an ever green, moderate to large sized spreading tree with smooth, grey bark and it is about 20 m tall often with areal roots (Figure 1b**)** [7]. Though there are scientific reports found in the similar work carried out internationally, a very few research studies carried out in Sri Lanka were found in the literature. Therefore, this research was aimed to study the phytochemical analysis, total phenolic contents and evaluate *in-vitro* antioxidant activity of different solvent extracts obtained from bark of *F. racemose* and *M. longifolia.*

Materials And Methods

Plant materials and chemicals

Bark of *F. racemosa* was collected from Kamburupitiya in Matara district and the bark of *M. longifolia* was collected from Beliattha in Hambanthota district (Southern province, in Sri Lanka) in 2020. The plant material of *F. racemosa* was authenticated at the National Herbarium, Peradeniya**,** in Sri Lanka while the plant material of *M. longifolia* was authenticated at Pinnaduwa herbarium in Galle Sri Lanka.

The chemicals of 2-2-diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tripyridyl-s-triazine), Trolox, conc. HCl, conc. H_2SO_4, $FeCl_3.6H_2O$,

$Fe(II)SO_4.7H_2O$, ethanol, methanol, acetone and hexane were purchased from Sigma Aldrich local agencies in Sri Lanka.

Preparation of the plant extracts

The crude extracts of 70% aqueous acetone and 80% aqueous methanol of each plant were prepared according to the published method [8] with slight modifications. Briefly, ground powder of oven dried barks was steeped in the solvent (300 mL) separately in Scott Duran bottles with occasional shaking (GEMMYCO shaker, Lab shaking incubator, model:IN-666) for 24 hours in the dark conditions at room temperature. After 24 hours, these extracts were filtered using four layers of muslin cloth and were concentrated under the vacuum using the rotary evaporator (HAHN HS-2005S-N) below 65 ℃. They were subjected to freeze drying (freeze dryer-BIOBASE BK-FD10PT) until a constant weight was gained.

Qualitative testing for phytochemical analysis

The bark extracts obtained from selected plants were screened qualitatively according to the method published [3], [9] to detect the presence of phytochemicals having interest in therapeutically and pharmacological applications such as carbohydrates, proteins, alkaloids flavonoids, phenolic compounds, saponins and terpenes.

Determination of total phenolic content

The total phenolic contents of the bark extracts were determined by Folin-Ciocalteu assay colorimetric method [8] and the results were expressed as mg Gallic Acid Equivalent (GAE) to 100 g dry weight of bark.

Evaluation of in-vitro radical scavenging activity

In-vitro radical scavenging activity of each extract was determined using the 2, 2,-diphenyl-1-picrylhydrazyl (DPPH) assay according to a published method [8] and the results were expressed as mmol Trolox equivalents to 100 g dry weight of the bark.

Evaluation of in-vitro antioxidant activity

In-vitro antioxidant activity of each extract was determined using the ferric reducing antioxidant power (FRAP) assay according to a published method [8]. The antioxidant activity was calculated using the standard calibration curve of Trolox and expressed as mmol Fe (II) equivalents/100 g DW of the bark.

Statistical analysis

All experimental measurements were carried out in triplicates and the results were expressed as the mean ± Standard Deviation. The results were analysed by multiple comparison one-way ANOVA at Turkey 95% and independent sample t-test using the SPSS 21 software. At ($p<0.05$), values were considered significantly different at 95% level of confidence.

Result

Phytochemical screening

The results of preliminary phytochemical analysis revealed the presence of alkaloids, phenolics, flavonoids, carbohydrates, proteins and saponins in the both extracts obtained from *M. longifolia* while revealing the presence of phenolics, flavonoids, carbohydrates, alkaloids, tannins in both bark extracts obtained from *F. racemosa*.

Total phenolic content

The 80% aq. methanolic bark extract of *F. racemosa* had total phenolic content of 4414.2 ± 117.4 mg GAE/100g DW of the bark and 70% aqueous acetone bark extract of *F. racemosa* had total phenolic content of 5195.4 ± 132.9 mg GAE/100g DW. The 80% aq. methanolic bark extract of *M. longifolia* had total phenolic content of 7646.9±179.2 mg GAE/100g DW of the bark and 70% aqueous acetone bark extract of *M. longifolia* had total phenolic content of 7551.8±26.6 mg GAE/100g DW (Table 1).

Table 1: Total phenolic contents of different solvent extracts from selected plants.

Solvent Type	TPC (mg GAE/100 g DW ± SD)	
	M. longifolia	*F.racemosa*
70% aq. Acetone	7551.8±26.6[a]	5195.4 ± 132.9[c]
80% aq. Methanol	7646.9±179.2[b]	4414.2 ± 117.4[d]

Results are expressed as M±SD: Mean ± Standard Deviation. Means followed by different letters in a column and a raw are significantly different ($p<0.05$).

In vitro antioxidant activity of the extracts obtained from

Radical scavenging activity of 80% aq. methanol and 70% aq. acetone extracts obtained from bark of *M. longifolia* were determined as 25.4±0.1 and 23.1±0.4 mmol Trolox equivalents/100 mg dry weight of bark of *M. longifolia* (DWM) and antioxidant capacity of 80% aq. methanol and 70% aq. acetone extracts were 42.6 ± 0.7 and 38.0 ± 1.1 mmol Fe (ll) equivalents/100 mg DW of the bark. Radical scavenging activity for the two different extracts obtained from bark of *F.racemosa* were as 18.6 ± 0.3 (80% aqueous methanol) 20.2 ± 0.7 (70% aq. acetone) mmol Trolox equivalents/100 g DWF of bark. Antioxidant capacity by FRAP assay for the two different extracts of *F. racemosa* were 28.5 ± 0.4 (80% aq. methanol) and 32.8 ± 1.6 (70% aq. acetone) mmol Fe (II) equivalents/100 g DW of the bark (Table 2).

Table 2: In-vitro antioxidant activities of different solvent extracts from selected plants.

Solvent Type	Radical Scavenging activity (mmol Trolox/100 g DW of bark)		Antioxidant activity (mmol Fe(II) equivalents/100 g DW of the bark)	
	M. longifolia	*F.racemosa*	*M. longifolia*	*F.racemosa*
70% aq. Acetone	23.1±0.4[a]	20.2 ± 0.7[c]	38.0 ± 1.1[e]	32.8 ± 1.6[g]
80% aq. Methanol	25.4±0.1[b]	18.6 ± 0.3[d]	42.6 ± 0.7[f]	28.5 ± 0.4[h]

Results are expressed as M±SD: Mean ± Standard Deviation. Means followed by different letters in a column and a raw are significantly different ($p<0.05$).

Discussion

With the agreement to the results obtained in this study, it was reported that *M. longifolia* contains sapogenins, steroids, saponins, flavonoids, triterpenoids and glycosides [10]. Folin-Ciocalteu method is most widely used procedure for quantification of phenolic compounds in plant materials. This assay is based on the reduction of phosphomolybdic-phosphotungstic acid (FC) reagent to a blue complex in an alkaline solution occurs in the presence of phenolic compounds [8]. The total phenolic contents of the different extracts were evaluated by using this method with reference to the standard curve equation: $y = 0.001x$, $r^2 = 0.9961$. The results obtained for the concentration of total phenolic contents were expressed as mg Gallic Acid Equivalent (GAE)/100 g dry weight (DW) of bark. *M. longifolia* showed significantly high amount of total phenolics (7646.920±179.230 mg GAE/100 mg DW of bark) than *F. racemosa* extract which may contribute for high antioxidant activity of the plant. *In-vitro* radical scavenging activity of the four different extracts were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for 1 mg/mL concentration of samples with reference to the standard curve equation: y = 0.2635x, r2 = 0.9966. The results obtained for the concentration of radical scavenging activity were expressed as mmol Trolox equivalents/100 g DW of bark. FRAP assay was a simple and speedy method which uses antioxidants as reductants in a redox colorimetric method used to determine antioxidant activity of plant materials. At low pH, ferric tripyridyltriazine complex was reduced to the ferrous (Fe^{2+}) form, which has an intense blue color with an absorption maximum at 593 nm [8]. *In-vitro* antioxidant activity of plant extracts was evaluated by using ferric-reducing antioxidant power activity (FRAP assay) with reference to the standard curve equation: y = 0.6225x, r2 = 0.9976. The results obtained for the concentration of antioxidant contents are expressed as mmol Fe (II) equivalents/100 g DW of the bark. The results of this study revealed that the 80% aq. methanol extract of *M. longifolia* bark had the highest antioxidant value for both DPPH and FRAP assay. A study was conducted to determine the antioxidant activity of methanolic extract of *M. longifolia* bark and it was evaluated by free radical scavenging activity using DPPH assay, reducing

power assay and superoxide scavenging activity compared to ascorbic acid and gallic acid and the results concluded that *M. longifolia* bark was rich in antioxidant activity [11]. A comparative study on the antioxidant activity of methanolic extracts of *Terminalia paniculata* and *Madhuca longifolia* was performed by Agrawal and the team [12]. Results had showed that both the extracts possessed significant antioxidant property but *M. longifolia* exhibited higher activity in case of DPPH and hydrogen peroxide radical scavenging [12]. Our research findings obtained for *F. racemosa* are in the agreement of the results reported in the literature. Methanolic extract of leaves and bark of *F. racemosa* had been screened for antioxidant activity based on 1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging assay. It had been found that both leaf and bark extract of *F. racemosa* contain higher scavenging activity even that of the standard BHT [13]. Another research has been conducted to study the antioxidant activity of *F. racemosa* bark using two solvents such as ethanol and water. According to the results of the research that ethanolic extract exhibited significantly higher antioxidant activity than the water extract [14].

Conclusion

The aim of this study was to evaluate antioxidant activities of different solvent extracts obtained from two plants selected namely, *M. longifolia* and *F. racemosa*. By considering the results obtained for phytochemical analysis, total phenolic, and *in-vitro* antioxidant activity of the two different solvent extracts of both plants tested, 70% aqueous acetone extract of *M. longifolia* showed significantly greatest *in-vitro* antioxidant activities among all the extracts tested.

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Conflict of interests

The authors declare that there is no conflict of interest.

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