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Short Communication

In vitro Antioxidant Activity of Methanolic and Ethanolic Extracts of seeds of *Macrotyloma uniflorum*

Subramani Parasuraman*, Vanishya A/P Raipan

ABSTRACT

Objectives: To study the antioxidant activity of methanolic and ethanolic extracts of seeds of *Macrotyloma uniflorum*. **Methods:** The seeds of *M. uniflorum* were extracted with methanol and ethanol and used for the phytochemical analysis and determination of antioxidant activity. The *in vitro* antioxidant activity was studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging methods. **Result:** Ethanolic extracts of *M. uniflorum* showed more phenolic content (63.48 mg GAE/g) than methanolic extract of *M. uniflorum* (45.84 mg GAE/g). In total flavonoid content analysis, methanolic and ethanolic extracts of *M. uniflorum* showed the presence of flavonoid content of 15.31 mg Rutin/g and 15.44 mg Rutin/g, respectively. In DPPH assay, methanolic and ethanolic extracts of *M. uniflorum* exhibited 50% free radical scavenging activity at 797.71 ± 34.38 µg/mL and 938.80 ± 66.05 µg/mL (mean ± standard deviation; *n* = 3), respectively. In hydroxyl radical scavenging assay, methanolic and ethanolic extracts of *M. uniflorum* exhibited 50% fee radical scavenging activity at 770.27 ± 11.64 µg/mL and 844.94 ± 35.12 µg/mL (mean ± standard deviation; *n* = 3), respectively. Ascorbic acid exhibited potent free radical scavenging with IC_{50} value of 60.54 ± 5.23 µg/mL in DPPH method and 207.98 ± 14.26 µg/mL (mean ± standard deviation; *n* = 3) in hydroxyl radical scavenging method. **Conclusion:** Ethanolic extracts of *M. uniflorum* showed more phenolic content than methanolic extract of *M. uniflorum*. In both, DPPH and hydroxyl radical scavenging assay, methanolic and ethanolic extracts of *M. uniflorum* exhibited antioxidant activity at higher concentration.

Key words: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Free radical, Hydrogen peroxide (H_2O_2).

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INTRODUCTION

The plants are mankind and many drugs are isolated from the plant which includes atropine, digoxin, etc. In 19th century many of the drugs are isolated from the plant which is now used for therapeutic purposes. In Ayurveda, Siddha and Traditional Chinese Medicine (TCM) herbs are the main ingredients for the formulation.[1]

Macrotyloma uniflorum (horse gram) belongs to the family Fabaceae and widely distributed throughout Asia, Africa and Australia.[2] *M. uniflorum* is the one of the important herbs which is used as food grain and rich in protein. It is lesser-known beans and is normally used to feed horses. In Ayurveda, the seeds of *M. uniflorum* are used to treat jaundice or water retention and as part of a weight-loss diet.[3] Different parts of this plant are used for the treatment of asthma, bronchitis, leukoderma, urinary discharges and for the treatment of kidney stones.[4] It is considered an excellent source of dietary fibre, molybdenum, iron, calcium and other micronutrients. In the classical Indian texts like Charak Samhita and Sushruta Samhita about traditional Indian medicinal system, the seeds of *M. uniflorum* are known to cure abdominal lump, bronchial asthma, hiccup, piles and also in regulating or stopping excessive perspiration.[5] The antioxidant properties of extracts of seeds of *M. uniflorum* are not well studied. Hence the present study is to plan to study the antioxidant properties of methanolic and ethanolic extracts of seeds of *M. uniflorum*.

MATERIALS AND METHODS

Collection of seeds of *M. uniflorum*

The seeds of *M. uniflorum* were purchased in the local market in Sungai Petani, Malaysia.

Extraction

The dried seeds of *M. uniflorum* were powdered and was packed in a Soxhlet extractor and extracted with methanol 65±5°C and 95% ethanol at 75±5°C. The extraction was carried out for 72 hr or for 3–4 cycles. The methanolic extract of seeds of *M. uniflorum* (MEMU) and ethanolic extract of seeds of *M. uniflorum* (EEMU) was filtered was concentrated to a dry mass by evaporation under reduced pressure

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using a rotary evaporator (Rotavapor® R-210, BUCHI Corporation). The MEMU and EEMU were stored at room temperature until use.

Phytochemical analysis

One gram of MEMU and EEMU was dissolved in 100 ml of its own mother solvent and a stock of concentration 1% w/v was obtained and tested for the presence of carbohydrates, proteins, sterols, alkaloids, tannins, glycosides, flavonoids, phenolic compounds, and saponins.[6]

Determination of total phenolic content

The total phenolic content in MEMU and EEMU was determined by the Folin-Ciocalteu method and gallic acid was used as a standard equivalent (in mg GAE/g). Approximately, 500µl of Folin–Ciocalteu reagent were mixed to 100µl diluted *M. uniflorum* extracts. After 5 min, 400µl 7.5% sodium carbonate was added to the mixture and stand for 30 min at room temperature. The absorbance was measured spectrophotometrically at 765 nm (Model UV 1800, Shimadzu, Japan).[7]

Determination of total flavonoid content

The total flavonoid content of the crude extract was determined by the aluminum chloride colourimetric method. 0.5 ml of each extract was mixed with 0.5 ml aluminum chloride (2%), and then 3 ml potassium acetate (5%) was added. The solution was left for 40 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was plotted by different concentration of rutin equivalents (in mg Rutin/ g).[8,9]

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity: Extracts (0.2 ml) at different concentrations (400–1000 µg/ml) was mixed with 0.8 ml of tris hydrochloric acid (HCl) buffer (100 mM; pH 7.4). One millilitre DPPH (500 mM in 1.0 ml ethanol or methanol) solution was added to the mixture. The mixture was shaken vigorously and incubated for 30 min at room temperature. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Vitamin C was used as a standard antioxidant in this method. Percentage (%) of DPPH scavenging activity was determined using [(Absorbance of control – Absorbance of test sample)/ Absorbance of control) × 100]).[10,11] All the determinations were carried out in triplicate.

Hydroxyl radical scavenging assay: A solution of hydrogen peroxide (H_2O_2) (40 mM) was prepared in phosphate buffer, pH 7.4. The extracts (400-1000 µg/ml) in Dimethyl sulfoxide (DMSO) was added to a hydrogen peroxide solution and at 230 nm, the absorbance was determined after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard.[12] Percentage inhibition was determined using [(Absorbance of control – Absorbance of test sample)/ Absorbance of control) × 100]. All the determinations were carried out in triplicate.

RESULTS

Phytochemical screening

MEMU showed the presence of alkaloid, amino acids, flavonoid, glycoside, mucilage, saponin, steroid, tannins and phenolic compounds, and EEMU showed the presence of alkaloid, amino acids, flavonoid, saponin, steroid, tannins and phenolic compounds.

Total phenolic content

Gallic acid is used as the standard for total phenolic content analysis. The standard concentration course was constructed using 100, 200, 400, 600, 800 and 1000 µg/ mL concentrations. Ethanolic extracts of *M. uniflorum* showed more phenolic content than methanolic extract of *M. uniflorum* (Table 1).

Table 1: Total phenolic and flavonoid content.

Analysis	Extract	Content of phytoconstituents
Total phenolic content	Methanolic extract	45.84 mg GAE/g
	Ethanolic extract	63.48 mg GAE/g
Total flavonoid content	Methanolic extract	15.31 mg Rutin/g
	Ethanolic extract	15.44 mg Rutin/g

Total flavonoid content

Rutin is used as the standard for total flavonoid content analysis. The standard concentration course was constructed using 100, 200, 400, 600, 800 and 1000 µg/ mL concentrations. Methanolic and ethanolic extracts of *M. uniflorum* showed the presence of flavonoid content of 15.31 mg Rutin/g and 15.44 mg Rutin/g, respectively (Table 1).

Antioxidant Activity

In the DPPH method, ascorbic acid exhibited potent free radical scavenging property than methanolic and ethanolic extracts of *M. uniflorum* with IC_{50} value of 60.54 ± 5.23 µg/mL (mean ± standard deviation; n = 3). Methanolic and ethanolic extracts of *M. uniflorum* exhibited 50% free radical scavenging activity at 797.71 ± 34.38 µg/mL and 938.80 ± 66.05 µg/mL (mean ± standard deviation; n = 3), respectively.

In H_2O_2 method, ascorbic acid exhibited potent free radical scavenging property than methanolic and ethanolic extracts of *M. uniflorum* with IC_{50} value of 207.98 ± 14.26 (mean ± standard deviation; n = 3). Methanolic and ethanolic extracts of *M. uniflorum* exhibited 50% free radical scavenging activity at 770.27 ± 11.64 µg/mL and 844.94 ± 35.12 µg/mL (mean ± standard deviation; n = 3), respectively.

In both, DPPH and hydroxyl radical scavenging assay, Vitamin C showed a potent antioxidant effect whereas methanolic and ethanolic extracts of *M. uniflorum* exhibited antioxidant activity at higher concentration.

DISCUSSION

The methanolic and ethanolic extracts of *M. uniflorum* exhibited antioxidant activity in DPPH and hydroxyl radical scavenging assay at higher concentration. Thippeswamy *et al.* reported the antioxidant activity (studied using the DPPH method) of *M. uniflorum* and estimated ascorbic acid content by dinitrophenylhydrazine (DNPH) method.[13] The results showed ascorbic acid content found to be 20.80mg, 20.88 mg, 30.08 mg and 30.27 mg /100g at 6, 12, 18 and 24 h. The changes in the ascorbic acid content were further monitored calorimetrically in the extracts of germinating seeds after germination for 2 to 7 days and were found to be 45.08, 46.94, 52.77, 38.05, 34.52 and 30.75 mg /100g seeds respectively. The ascorbic acid and total antioxidant activity were estimated and the results showed a good amount of ascorbic acid and total antioxidant activity in germinating seeds.[13] Ascorbic acid is a known antioxidant and this may be responsible for the antioxidant activity of the seeds of *M. uniflorum*. In the present study, the antioxidant properties of *M. uniflorum* were studied using its dry seeds. Further studies are required to compare the antioxidant properties of dry and germinated seed extracts of *M. uniflorum*.

Singh *et al.*, studied the *in vitro* antioxidant activities of methanol extract of *Dolichos biflorus* (Synonym of *M. uniflorum*) dal commonly edible food from central Himalayans. Total phenolic and flavonoid contents of methanolic extract of *Dolichos biflorus* dal was 92.10 ± 8.11 mg/ml GAE per 100 mg plant extract and 139.5 ± 55.09 mg/ml (mean ± standard

deviation) quercetin equivalent per 100 mg plant extract respectively, and it showed 4 times greater free radical scavenging activity than that of the synthetic antioxidant ascorbic acid. The study showed that *Dolichos biflorus* exhibited antioxidant activity against DPPH and H_2O_2 induced free radicals. [14]

The seeds of *M. uniflorum* have antioxidant, antihyperlipidemic, antimicrobial and diuretic activities and used for the management of coronary heart diseases.[15,16] Varicola *et al.,* studied the antioxidant and anthelmintic activities of various extract of *M. uniflorum* and reported that the methanolic extract has significant anthelmintic activities with IC_{50} values of 3.86 µg/ml.[3] Pritha *et al.,* studied the antimicrobial and cytotoxic effects of methanol and ethanol extracts of *M. uniflorum* extract and the results showed significant antimicrobial and antioxidant activity and anticancer activity against MG-63 cell line.[17] The extracts of *M. uniflorum* also exhibited significant analgesic, anti-inflammatory, diuretic hepatoprotective and cardioprotective activities.[18-20] These activities may be mediated through the antioxidant properties of seeds of *M. uniflorum*.

CONCLUSION

Ethanolic extracts of *M. uniflorum* showed more phenolic content than methanolic extract of *M. uniflorum*. In total flavonoid content analysis, both methanolic and ethanolic extract exhibited an equal amount of flavonoid content. In both, DPPH and hydroxyl radical scavenging assay, vitamin C showed a potent antioxidant effect whereas methanolic and ethanolic extracts of *M. uniflorum* exhibited antioxidant activity at higher concentration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DNPH: Dinitrophenylhydrazine; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EEMU:** Ethanolic extract of seeds of *M. uniflorum;* **g:** Gram; **GAE:** Gallic acid; **H_2O_2:** Hydrogen peroxide; **HCl:** Hydrochloric acid; **MEMU:** Methanolic extract of seeds of *M. uniflorum;* **mg:** Milligram; **TCM:** Traditional Chinese Medicine.

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