WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 10, 399-406. Research Article ISSN 2277– 7105

BIOCHEMICAL ESTIMATION OF PRIMARY METABOLITES AND ANTIOXIDANT ACTIVITY OF MADHUCA LONGIFOLIA L.

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Article Received on
30 March 2018,

Revised on 20 April 2018,
Accepted on 10 May 2018,

DOI: 10.20959/wjpr201810-11767

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ABSTRACT

The present study was aimed for isolation and quantification of Primary Metabolites from selected plant species. Antioxidant activity of plants was also carried out by using FRAP, Catalase and Peroxidase assay. In *Madhuca longifolia* L. the maximum amount of primary metabolites was protein (150 mg/gdwt) and the minimum was TSS (4 mg/gdwt) in leaves. Thus, the leaves of *Madhuca longifolia* L. were the rich source of protein. In the case of *Madhuca longifolia* L. leaves was found to be better antioxidant when observed by Peroxidase and Catalase assay. When different samples were analyzed by FRAP assay it was observed that seeds had better antioxidant activity as compared to leaves. The present results showed potential of this medicinal plant which can be used as herbal drug as therapeutic ventures in future aspects.

KEYWORDS: Primary Metabolites; Antioxidant activity; FRAP assay; Catalase assay; Peroxidase assay; Madhuca longifolia L.

INTRODUCTION

Most plants are edible and contain different amount of vitamins, protein or carbohydrates etc., these helps the body to replace worn out cells or tissues, digest food and combat ailments among other health related problems.[1] The potential of these plants depends upon the presence of phytochemicals inside those may be primary metabolites or secondary metabolites that are secreted by plants during life. Primary metabolites are those organic substances which are synthesized during photosynthesis and these organic compounds are essential for plant life, growth and development.[2] These are widely distributed in nature, occurring in one form or another in virtually all organisms. They are like chlorophyll, amino

acids, nucleotides and carbohydrates have a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as industrial raw material and food additives.[3] *Madhuca longifolia* L. has been evaluated for their composition of primary metabolites. Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants can also protect the human body from free radicals and ROS (Reactive Oxygen Species) effects.[4] The FRAP assay is based on the ability of plant extract to reduce Fe^{3+} to Fe^{2+} ions.[5] Peroxidases are group of enzymes containing phorphyrin in their structure and catalyzing redox reactions. They belong to one of the most significant enzymes in bioanalytical chemistry with broad possibility of application in the both detection and as supporting enzymes.[6] Catalase is a ubiquitous enzyme found in aerobic organisms. It efficiently catalyzes the decomposition of hydrogen peroxide to oxygen and water together with other enzyme systems, protects cells against the harmful effects of reactive oxygen species such as superoxide anions, hydrogen peroxide, and hydroxyl redicals.[7] *Madhuca longifolia* L. commonly known as the Butter nut tree is a medium to large sized deciduous tree distributed in Nepal, India and Sri Lanka. Mahua seeds are of economic importance as they are good source of edible fats[8] *Madhuca longifolia* L. belongs to the family of Sapotaceae. It is both wild and cultivated.[9] Ethnomedical uses say to possess significant antipyretic, hepatoprotective, anti-inflammatory, analgesic, antitumour, antiprogestational, antiestrogenic and wound healing activity. Traditionally *Madhuca longifolia* L. bark is used in rheumatism, ulcers, bleedings and tonsillitis.[10]

MATERIALS AND METHODS

Collection of plant Material

Plant parts of *Madhuca longifolia* L. (leaves and seeds) were collected from the fields at Jaipur and authenticated. The voucher (RUBL* No. 211564 for *Madhuca longifolia* L.) of experimental plant was deposited in the Herbarium of Department of Botany, University of Rajasthan, Jaipur. The plant parts were separated and washed thoroughly 2-3 times with running tap water and then air dried under shade after complete shade drying the plant material was powdered and used for phytochemical analysis.

Quantification of Primary Metabolites

Quantification of Primary Metabolites was carried out by using following methods of carbohydrates, proteins, lipids and phenols.

Carbohydrates

Total Soluble Sugars

Extraction

The dried experimental plant material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the method of.[11]

Starch

Extraction

The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5 mL of 52% perchloric acid.[12] Later, 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes.

Quantitative Estimation

1mL of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid.[13]

Proteins

Extraction

The test sample (50mg each) were separately homogenized in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^0C for 24 hours. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 mL of 5% TCA and heated at 80^0 on a water bath for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature.[14]

Quantitative Estimation

Each of the above samples (1 mL) was taken and the total protein content was estimated using the spectrophotometer.[15]

Lipids

Extraction and Quantification

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol (2/1:v/v).[16] The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. Two layers were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

Phenols

Extraction

The deproteinized test materials (200mg each) were macerated with 10 mL of 80% ethanol for 2 hours, and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol.

Quantitative Estimation

Total phenol content in each sample was estimated by spectrophotometer.[17]

FRAP Assay (Ferric reducing ability of Plasma)

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH.[18]

Reagents

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x H_2O and 16 mL conc. Acetic acid per 1 mL of buffer solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$ x 6 H_2O in distilled water

Catalase (CAT) assay

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Reagents and Test sample solutions

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (50M, pH- 7)
- H_2O_2 (24 mM)
- Na_2EDTA (0.1mM)
- PVP (1%)

Procedure

Plant sample (200mg) was homogenized with 5mL of phosphate buffer with Na_2EDTA and refrigerated centrifuged at 10000rpm for 20 minutes at 4°C. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of[19] with the following modifications. 2.0mL of phosphate buffer, 0.8mL of H_2O_2 was added and finally 0.2 mL enzyme extracts then immediately absorbance taken at 240nm.

Peroxidase Assay (POXA)

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20°C.

$$\text{Pyrogallol} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Purpurogallin}$$

Reagents and Test sample solutions:

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (125µmol, pH- 6.8)
- Pyrogallol (50µmol)
- H_2O_2 (30%)

Procedure

Plant sample (200mg) was homogenized with 10mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 minutes. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of[20] with the following modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H_2O_2 was added. The amount of purpurogallin formed was determined by taking the absorbency at 420nm immediately after adding 0.1mL enzyme extract.

RESULTS AND DISCUSSION

Quantification of Primary metabolites

In the present study, quantification of primary metabolites in leaves and seeds of *Madhuca longifolia* L. have been under taken, the results are present in Table 1.

Table 1: Phytochemical costituents from Leaves and Seeds of *Madhuca longifolia* L.

Phytochemical constituents		*Madhuca longifolia* L.	
		Leaves (mg/gdwt)	Seeds (mg/gdwt)
Proteins		316±3.29	272±2.87
Lipids		4±0.02	10±0.08
Phenols		4.85±0.02	3±0.02
Carbohydrates	TSS	1.5±0.009	8.5±0.07
	Starch	6±0.04	5±0.03

Values are the mean ± SEM (n = 3 variable in each group). *P < 0.05; **P < 0.001 compared with the control; P < 0. 001

Determination of antioxidant activity

The antioxidant activity of leaves and seeds of *Madhuca longifolia* L. findout by using FRAP, Catalase and Peroxidase methods. The results of antioxidant activities of *Madhuca longifolia* L. are present in Table 2.

Table 2: Antioxidant activity of *Madhuca longifolia* L.

Antioxidant assay	*Madhuca longifolia* L.	
	Leaves	Seeds
FRAP (mM/g/fresh wt)	0.679±0.001	1.918±0.009
Catalase (mM/lit/g/fresh wt)	0.56±0.001	1.44±0.007
Peroxidase (mM/lit/g/fresh wt)	0.4256±0.001	0.8344±0.003

Values are the mean ± SEM (n = 3 variable in each group). *P < 0.05; **P < 0.001 compared with the control; P < 0. 001

The maximum amount of primary metabolite is protein and minimum is TSS in leaves of *Madhuca longifolia* L. Conclusion is that the leaves of *Madhuca longifolia* L. are the rich source of protein.

Antioxidant activity was carried out using FRAP, Catalase and Peroxidase methods. *Madhuca longifolia* L. leaves was found to be better antioxidant when observed by Peroxidase and Catalase. When different samples were analyzed by FRAP assay it was observed that seeds had better antioxidant activity as compared to leaves. The present results

showed potential of this medicinal plant which can be used as herbal drug as therapeutic ventures in future aspects.

CONCLUSION

In the present study, *Madhuca longifolia* L. contains many primary metabolites like protein, phenol, carbohydrates etc. The highest amount of primary metabolite is protein in the leaves of *Madhuca longifolia* L. Primary metabolites analysis is necessary for knowing the nutritional potential of plants. The highest antioxidant capacity observed in leaves of *Madhuca longifolia* L. when observed by Peroxidase and Catalase while FRAP assay shows seed of *Madhuca longifolia* L. as a better antioxidant. Thus, Leaves and seeds of *Madhuca longifolia* L. may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases.

ACKNOWLEDGMENTS

The authors are thankful to Head, Department of Botany, University of Rajasthan, Jaipur for providing necessary facilities and CSIR New Delhi for providing fellowship to one of the authors Mrs. Priyanka Kumari.

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