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Screening for phytochemicals and antioxidant activity of flowers of *Madhuca indica* J.F. Gmel.

Mandakini Raj [1], Chandrasekhar Bhoi[2] and Sanhita Padhi[Ψ]
Department of Botany and Biotechnology
Ravenshaw University, Cuttack-757003

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ABSTRACT

Madhuca indica J. F. Gmel. [Syn. *Madhuca longifolia* var. *latifolia* (Roxb.) A.Chev], commonly known as "Mahua" is an economically important plant and known for its medicinal and ethnobotanical uses as well as for its edible flowers having high nutritional values. However, due to lack of scientific knowledge on its food, nutritional properties and industrial applications, the species has not been exploited to the extent possible. Hence, the present study is aimed at screening of phytochemicals, estimation of reducing power and determination of the physicochemical properties of Mahua flowers with various solvent concentrations. The chloroform, acetone, methanol, ethanol and aqueous extracts of the flowers were investigated for its phytochemical activity. Mahua flower shows higher reducing power in aqueous extracts as compared to acetone, methanol and ethanol extracts.

1. Introduction

Since time immemorial, medicinal plants have played a very significant role in curing human diseases especially in traditional system of medicine and pharmaceutical drug formulations (Chaudhary *et al.,* 2015). The large population of the world depends on the medicinal plants for their health care needs in view of their easy availability and having no side effects. The high cost of modern allopathic medicines also compels them to resort to plant-based traditional medicines (Togboto and Towson, 2005). The world health organisation (WHO) estimated that 80% of the population of developing countries relay on traditional medicines, mostly plant drugs, for their primary health care needs (Prabakaran *et al*., 2011). India has one of the oldest, richest and most diverse cultural traditions in the field of utilization of medicinal plants (Mehta *et al*., 2013). Medicinal plant has been providing modern medicine with numerous plant derived therapeutic agent (Evans, 2000).

Madhuca indica (Syn. *Madhuca longifolia* var. *latifolia*), belonging to family Sapotaceae, is one of the multi-purpose wild medicinal plants having great economic importance as almost all parts of the plant are used by human beings for some purpose or other (Banerji and Mitra, 1996) such as producing country liquor from succulent corollas and oil from the seeds (Boral *et al.*, 1999). The plant is known to possess various therapeutic properties and has been one of the noteworthy plants mentioned in various medicinal systems (Chidrewar *et al.,* 2010). *M. indica* is considered as a stimulant, demulcent, emollient, heating and astringent (Awashthi and Mitra, 1967). Thus, the present study aimed to determine the physicochemical parameters, preliminary phytochemical screening and reducing power of flowers.

2. Material and methods

2.1 Collection of plant materials

The fresh flowers of *M. indica* were collected in early

[Ψ] *Corresponding author, Email:* san_puri9828@rediffmail.com

morning during the month of March, 2017 from the village Patolokota, Keonjhar District, Odisha. The collected flowers were cleaned manually to remove all foreign materials and damaged flowers. The samples were then washed carefully in tap water followed by distilled water to remove remaining unwanted materials. Then the samples were air-dried under shade at room temp to avoid direct loss of phyto-constituents of flowers by sunlight. The anther parts were removed from dried flowers and then the samples were transferred to air tight jars and stored in freeze at 4^0C for further use.

2.2 Preparation of plant extract

The flowers were cut in to small pieces and kept in thimble for extraction. The samples were extracted with different organic solvents (methanol, ethanol and acetone) with different concentrations (≈100%, 75% and 50%) and in aqueous solution by using Soxhlet Apparatus at a temperature not exceeding the boiling points of respective solvents (Pandey and Tripathy, 2014). The liquid extracts were evaporated and stored in a refrigerator at 4^0C in small sterile glass vials for further experimental work.

2.3 Organoleptic characters

The organoleptic character like colour, odour, taste, physical appearance and surface characteristics were studied (Katiyar *et al.,* 2011; Patel *et al.,* 2012; Sanmugarajah *et al.,* 2013).

2.4 Physico-chemical investigation

The flower samples were subjected to physicochemical analyses such as dry biobass, water soluble extractives, alcohol soluble extractives, pH in 1% w/v solution, pH in 10% w/v solution (Sanmugarajah *et al.,* 2013; Katiyar *et al.,* 2011)

2.5 Preliminary phytochemical screening

Phytochemical analysis in chloroform, acetone, methanol, ethanol and aqueous extracts of flowers of *M. longifolia* var. *latifolia* were carried out using standard procedures to identify the possible bioactive compounds (Harborne, 1998; Trease and Evans, 1989).

2.6 Reducing power

The reducing power of different solvent extracts (methanol, ethanol, acetone and aqueous) of Mahua flowers was determined according to the method of Oyaizu (1986). Different concentrations of extracts were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50^0c for 20 minutes. After incubation, 2.5 ml of 10% TCA was added to the reaction mixture, which was then centrifuged at 3000g for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ solution. Then absorbency was measured at 700 nm against blank prepared by replacing sample with 1.0 ml distilled water. All the analyses are performed in triplicate and result were averaged. Ascorbic acid was used as standard. Increasing absorbance of the reaction mixture indicated increasing reducing power (Indu and Annika, 2014).

3. Results and discussion

3.1. Organolaptic properties

Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. This is again necessary because once the plant is dried and made into powder, it loses its morphological features and becomes prone to adulteration (Chanda, 2014).

Table 1
Organoleptic properties of Mahua flowers

Parameters	Organoleptic characters
Colour	White creamy (Fresh), Brown (Dry)
Odour	Sweet
Taste	Sweet
Surface	Longitudinal

3.2. Physicochemical characters

The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Yi Zeng*et al.,* 2004). The water and alcohol soluble extractives of Mahua flowers were 1.28% and 0.53% respectively. A similar result was obtained by Katiyar *et al.* (2011), where they found the percentage of water and alcohol soluble extractives as 0.664% and 0.680% respectively. The percentage of water loss on drying of flowers was 11.62% and it is recommended as the minimum level to discourage the growth of bacteria, yeast or fungi during storage (Soni *et al.,* 2011) (Table-2).

3.3 Phytochemical screening

Alkaloids, flavonoids, glycosides, carbohydrates and lipids were found to be present in aqueous, ethanol, methanol and acetone extracts of Mahua flowers. Quinones, oxalates and amino acids were absent in all these extracts. Tannins and terpenoides were absent in acetone and aqueous extracts,

Table 2
Physico-chemical characteristics of Mahua flowers

Parameters	Values
Water soluble extractives	1.28%
Alcohol soluble extractives	0.53%
Loss on drying	11.62%
pH 1% w/v solution	6.16
pH 10% w/v solution	6.23

whereas they were present in ethanol and methanol extracts. Saponins were reported only in ethanolic extract and absent in others extracts. Terpenoids, lipids and carbohydrates were also found to be present in chloroform extracts of flowers (Table 3).

Flavonoids and tannins are major groups of compounds that act as primary antioxidant free radical scavenger and antioxidant activity (Polterait, 1997). The qualitative analysis of the extracts confirmed the presence of saponins in the flowers of Mahua. The saponins have the property of precipitating and coagulating red blood cells in humans and plants containing this compound are responsible for stimulating activity (Sodipo *et al.,* 2000).

Plants containing alkaloids are used in medicine as anaesthetic agent (Herourat *et al.,* 1998) and are likely to have antibacterial properties (Okwu, 2004). The presence of alkaloids in these extracts indicated that it can be used as an antibacterial agent. The presence of higher terpenoids in flower extracts that have carboxylic acid groups could also be responsible for the activity of organic extracts (Murugesen and Muthysamy, 2011).

Table 3
Phytochemical screening of Mahua flowers

Phytochemicals	Tests	Aqueous extract	Ethanol extract	Methanol extract	Acetone extract	Chloroform extract
Alkaloids	Wagner's test	+	+	+	+	-
Flavonoids	Alkaline reagent test	+	+	+	+	-
Phenols	Ferric chloride test	+	+	+	+	-
Saponins	Foam test	-	+	-	-	-
Tannins	Braymer's test	-	+	+	-	-
Terpenoides	Salkowki's test	-	+	+	-	+
Quinones	Acid test	-	-	-	-	-
Oxalates	Acid test	-	-	-	-	-
Glycosides	Keller-Kiliani's Test	+	+	+	+	-
Carbohydrates	Fehling test	+	+	+	+	+
Proteins	Millon test	+	+	+	-	-
Lipids	Sudan red test	+	+	+	+	+
Amino acids	Ninhydrin test	-	-	-	-	-

3.5. Reducing power

The reducing ability of compound generally depends on the presence of reductants which have been exhibited antioxidant potential by breaking the free radical chains, donating a hydrogen atom.

Presence of reducers causes the conversion of the Fe3+ ferricyanide complex used in this method to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe^{3+} ion. As the concentration of flower extract increases, simultaneously antioxidant power also increases. The reducing powers of extracts were very high and it increases as the quantity of samples increase (Saha *et al*., 2010; Indu and Annika, 2014).

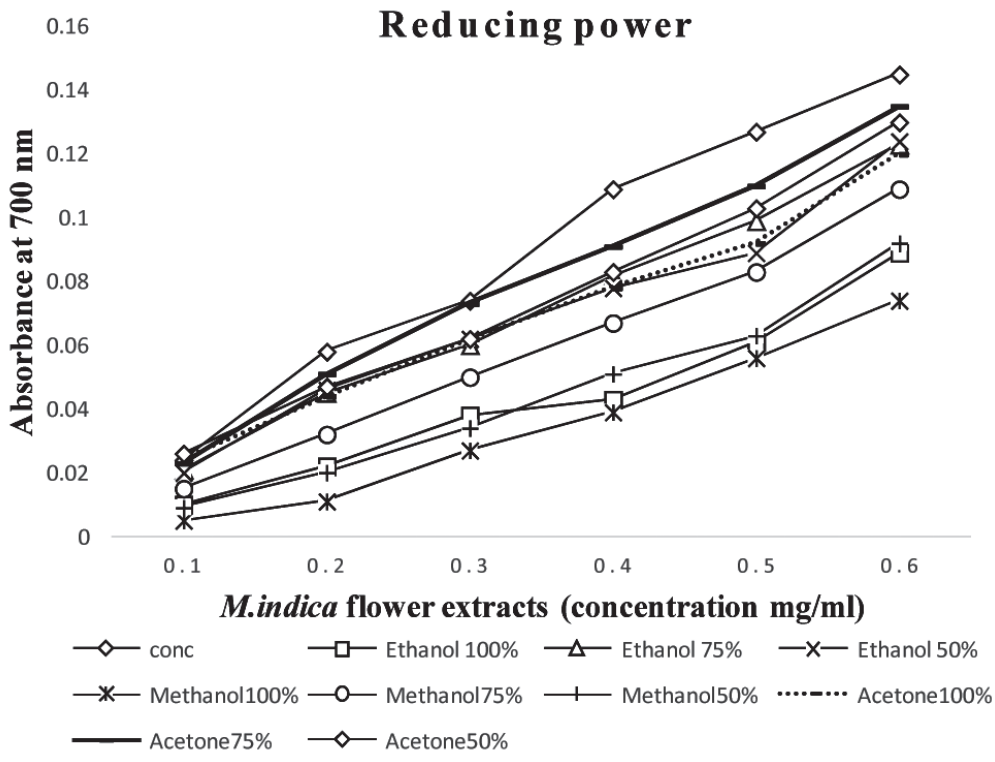

Fig. 1. Graph showing reducing power of *M. longifolia var. latifolia* flowers.

4. Conclusion

Plants are important sources of potentially bioactive constituents for development of new therapeutic agents. *M. indica* - a plant of Indian origin have tremendous medicinal values and wide range of uses but due to lack of knowledge and scientific data, the economic potential of the species has not been fully exploited (Patel *et al.,* 2012).

As the first step towards achieving this goal, flowers of Mahua were analysed for their organoleptic properties, physicochemical characteristics, phytochemical constituents to determine the purity and quality of a crude drug especially in powered form. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industries. The high reducing power of flower extract of *M. indica (longifolia* var. *latifolia)* implies that it is capable of donating hydrogen atom in a dose dependent manner and can be used as antioxidants. The plant is a rich source of various bioactive compounds useful in curing a number of diseases and the medicinal properties of different plant parts of Mahua need to be studied in detail so that this multi-purpose species could be put to more effective use for human welfare.

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