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Phenolic content and antioxidant activity of solvent extracts of mahua (*Madhuca longifolia*) flowers and fruit

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ABSTRACT

Background: The aim of this study was to investigate the antioxidant activity of ethanol, acetone, methanol and water extracts of fresh mahua (*Madhuca longifolia*) flowers and fruit. Antioxidant potential was examined by measuring total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity using the 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging assay and the Trolox equivalent antioxidant capacity (TEAC) assay.

Results: The highest and lowest phenol and flavonoid contents were found in the acetone and water extracts, respectively. The antioxidant activity of mahua flowers and fruit measured by different assays were higher in the acetone extract compared to the other solvent extracts (acetone extract>methanol extract>ethanol extract>water extract).

Conclusion: The results indicate that the flowers and fruit of mahua are good sources of polyphenols and natural antioxidants and could be useful as functional food ingredients beneficial for human health.

Keywords

Madhuca longifolia
Mahua flower
Antioxidant
Phenols
Flavonoids
Polyphenols

Introduction

The buttercup or mahua (*Madhuca longifolia* (Koenig), synonym *Madhuca indica* J. Gmelin) is a large, shady, deciduous tree, found both wild and cultivated, which dots much of the landscape of central India. The plant is economically important as liquor is made from the flowers, which can also be eaten, and oil is obtained from the seeds [1, 2]. The flowers are a rich source of sugars, vitamin A, ascorbic acid, thiamine, riboflavin, calcium, phosphorus, iron, magnesium, copper, anthocyanins, betalains, salts of malic and salts of succinic acid [3]. Previous studies have shown that the flowers and fruit of mahua contain various nutritional components, including phenolic compounds with high antioxidant activity [4, 5]. Mahua flowers have expectorant properties and so are used to treat chest problems such as bronchitis. They are also taken to increase the production of breast milk. The distilled juice of the flowers is considered a tonic which is both nutritional and helps to reduce fever. The pharmacological use in traditional medicine of various parts of the plant for a wide variety of illnesses, such as epilepsy, inflammation, diabetes mellitus, pain, hydrocoele, stomach ache, skin diseases, chronic bronchitis, Cushing's disease and ulcers has been evaluated [2].

Phenolic compounds are the main class of natural antioxidants present in plant foods and may function as reducing agents, free radical scavengers, singlet oxygen quenchers and potential complexers of prooxidants [6]. They also confer protection against biological macromolecular damage, notably preventing a decrease in antioxidant enzyme activity in the aging brain and liver, decreasing brain and liver malondialdehyde levels and carbonyl content, and improving total antioxidant activity in the organism [7]. The discovery of new and safe antioxidants from plant sources is important for the development of functional foods and nutraceuticals. Screening for phytochemicals is one of the methods that have been used to explore antioxidant compounds in plants.

Extraction is the initial step in the isolation of bioactive components from plant material. The aim of an extraction process

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is to obtain the maximum concentration of target compounds and extracts with the highest antioxidant activity. Solvent extraction has been widely used to extract bioactive components from plants. The solvent system for extraction is selected according to whether the extraction is intended for a preparation or for analysis, the nature of the components of interest, the physicochemical properties of the matrix, the availability of reagents and equipment, the cost and safety concerns [8]. Commonly used solvents for extracting antioxidants are methanol, ethanol and acetone either singly or in combination with an aqueous system [9]. The polarities of the different organic solvents greatly influence the selection of a specific solvent for the extraction of a specific group of bioactive compounds. Antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent. Polar solvents are frequently used for extracting polyphenols from plant matrices. Turkmen *et al*. reported that most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone and ethyl acetate for the extraction of polyphenols from complex food substrates [10]. Ethanol is a good solvent for polyphenol extraction and is safe for human consumption. Methanol is more efficient for extracting lower molecular weight polyphenols, while aqueous acetone is good for extracting higher molecular weight flavanols [11]. The maximum total phenolic content was obtained from barley flour by extraction using a mixture of ethanol and acetone. Extracts with the greatest antioxidant activity were obtained in mate tea and black tea by using 50% aqueous ethanol and 50% aqueous acetone, respectively [10].

Information on the antioxidant profiles of various solvent extracts of mahua flowers and fruit is scarce in the literature. However, a few reports are available on the phenolic content and contribution of phenolic compounds to the overall antioxidant activities of mahua flowers and fruit. Thus, the objective of this study was to evaluate the phenolic composition and antioxidant activity of mahua flower and fruit extracts obtained using various solvent systems.

Materials and methods

Chemicals

Folin–Ciocalteu's phenol reagent, (+)-catechin, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, citric acid, potassium peroxydisulfate ($K_2S_2O_8$), sodium carbonate (Na_2Co_3), sodium acetate trihydrate, hydrochloric acid (HCl), anhydrous ferric chloride ($FeCl_3$), anhydrous aluminium chloride, methanol, ethanol and acetone were obtained from Merck (Mumbai, India). All chemicals were of analytical grade and all water used was deionized.

Materials

Mahua flowers and fruit (ripe and unripe) were collected in April and August 2013, respectively, from the Allahabad area, Uttar Pradesh, India. The species *Madhuca longifolia* was identified at the Department of Botany, University of Allahabad, and a herbarium specimen was deposited at the department (Voucher No. 01). Flowers were collected in the morning on polythene sheets laid under trees, placed in a clean polythene bag and brought to the laboratory under hygienic conditions. Seeds were removed from fruit and the seedless fruit was then comminuted in a high-speed mixer, packed in an airtight polythene bag and stored in a refrigerator. Comminuted whole flowers were used for the antioxidant study.

Preparation of flower and fruit extracts

Mahua flowers and fruit were washed with tap water to remove soil and dirt, comminuted in a high-speed mixer, and milled into coarse particles about 2 mm in diameter. Then 15 g of coarse powder was extracted with 100 ml of solvent. The solution was stirred using an orbital shaker at 120 rpm for 24 h at room temperature. The extracts were then filtered, transferred into a flask and dried using a rotatory evaporator (RV10; IKA, Staufen, Germany) at 40°C. Extracts were stored at 4°C to avoid compound degradation before chemical analysis and use in experiments. The extraction process was carried out in triplicate. The solvents were Millipore water, and methanol, ethanol and acetone in different concentrations (50%, 70% and 100%) in distilled water; two solvent systems (methanol and ethanol) in a 1:1 ratio were used. All tests were performed at ambient temperature (25–27°C).

Determination of polyphenol content

Total phenolic content

The total phenolic content (TPC) of each extract was determined using the Folin–Ciocalteu micro-method [12]. Briefly, 20 µl of extract solution were mixed with 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu reagent, followed by the addition of 300 µl of Na_2CO_3 solution (20%) after 1 min but before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorb-

ance was measured at 760 nm. Gallic acid was used as the standard for the calibration curve. Results were expressed as milligrams of gallic acid equivalents per gram of fresh sample (mg GAE/g FW).

Total flavonoid content

The total flavonoid content (TFC) of each extract was investigated using the aluminium chloride colorimetry method described by Chang *et al*. with slight modifications [13]. In brief, the extract sample was diluted with methanol to 100 mg/ml. The calibration curve was prepared by diluting quercetin in methanol (0–100 mg/ml). The diluted extract or quercetin (2.0 ml) was mixed with 0.1 ml of 10% (w/v) aluminium chloride solution and 0.1 ml of 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 min. The maximum absorbance of the mixture was then measured at 415 nm using a UV–Vis spectrophotometer. TFC was expressed as milligrams of quercetin equivalent per gram mahua (mg QCE/g).

Antioxidant activity assays

Total antioxidant capacity

The assay was based on the reduction of molybdate (Mo) (VI) to Mo(V) by the sample and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH [14]. An aliquot of 0.1 ml of sample solution (containing 100–500 µg of dried extract in the solvent) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent as used in the sample and was incubated under the same conditions as the other samples. Ascorbic acid (1–10 mg/ml) was used as the standard. Total antioxidant capacity (TAC) was expressed as ascorbic acid equivalent.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was calculated according to the procedure described by Benzie and Strain [15]. The FRAP reagent included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in the ratio 10:1:1 (v/v/v). A 3 ml aliquot of the FRAP reagent was mixed with 100 µl of the sample extract in a test tube and vortexed in the incubator at 37°C for 30 min in a water bath. Reduction of ferric-tripyridyltriazine to the ferrous complex produced an intense blue colour which was measured using a UV–Vis spectrophotometer at 593 nm after 4 min. Results were expressed in µmol Trolox/g.

DPPH radical scavenging activity

The free radical scavenging activity of the extracts was measured using the slightly modified method of Alothman *et al*. [16]. The antioxidant capacity of the solvent extracts was determined through evaluation of the free radical scavenging effect on the DPPH radical. An aliquot (100 µl) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was then thoroughly vortexed and kept in the dark for 30 min. Absorbance was measured later at 515 nm against a blank of methanol and ascorbic acid as standard. Results were expressed as percentage of inhibition of the DPPH radical.

Free radical scavenging activity using ABTS

A modified procedure using ABTS as described by Re *et al*. was used [17]. The ABTS+ stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidizing agent. The working solution of ABTS+ was obtained by diluting the stock solution in ethanol to give an absorption of 0.70±0.02 at 734 nm. Sample extracts (10 µl) were added to 90 µl of ABTS+ solution and absorbance was read at 734 nm at 30°C exactly 10 min after initial mixing. The percentage inhibition of ABTS+ of the test sample and known solutions of Trolox was calculated using the following formula: % inhibition=100(A0–A)/A0, where A0 is the first absorbance at 734 nm, obtained by measuring the same volume of solvent, and A is the final absorbance of the test sample at 734 nm. The calibration curve between % inhibition and known solutions of Trolox (100–2000 µM) was then established.

The radical scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox/g).

Statistical analysis

All tests were performed in triplicate, and the data were presented as means±standard deviation. The data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple-range test ($p<0.05$), using the SPSS Statistics 17 for Windows (SPSS Statistical Software, Chicago, IL, USA) software package. Pearson correlation analysis was performed to establish the relationship between antioxidant activity and total phenolic content.

Results and discussion

Extraction yield

Extraction is the main step for recovering and isolating phytochemicals from plant material. The extraction yield depends on the solvents, length of time and temperature of extraction, as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of the sample are the two most important factors affecting extraction yield. The efficiency of extraction is affected by the chemical nature of phytochemicals as well as the presence of interfering substances [18]. The percentage yields for the different solvent extracts of the flowers and fruit of *M. longifolia* are shown in Table 1. The extraction yields varied from 1.853±0.129% to 16.337±0.716% with yields descending in the order 50% aqueous acetone>50% aqueous ethanol>70% aqueous methanol>50% aqueous methanol>70% aqueous acetone>70% aqueous ethanol>100% methanol>ethanol and methanol mixture 1:1>water>100% ethanol>100% acetone.

The extraction yield of pure methanol is higher than that of pure ethanol or pure acetone for the flowers and ripe and unripe fruit. This might be due to the higher polarity of methanol compared to the other solvents [19]. The yield of the flowers and unripe fruit in the water extract is only slightly higher than that of the pure methanol extract, whereas the yield of aqueous solvent extract (ranging from 16.337±0.716% to 7.362±0.187% for 50% aqueous acetone) is higher than that of the pure solvent extracts (ranging from 1.853±0.129% to 2.157±0.254% for pure acetone). These results indicate that increasing the amount of water in the solvent enhances extraction yield. However, compounds other than phenolics may have been extracted and contributed to the higher yield in aqueous solutions of solvents. This may be the reason why the yields of aqueous methanol, ethanol and acetone extracts are higher than the yields of water, methanol, ethanol and acetone extracts. Liu *et al*. reported similar findings where the antioxidant activities of lychee flower extracts for all assays were in the order: acetone extract>methanol extract>water extract [20].

Solvent (%)	Flowers	Fruit	
		Unripe	Ripe
Water			
100	3.600±0.528^{bc}	2.287±0.150^{a}	5.663±0.647^{b}
Acetone:water			
100	2.157±0.254^{a}	1.853±0.129^{a}	2.030±0.332^{a}
70:30	4.883±0.775^{d}	4.987±0.620^{c}	7.440±0.785^{c}
50:50	10.340±0.66^{f}	7.362±0.187^{e}	16.337±0.716^{g}
Methanol:water			
100	3.483±0.465^{bc}	2.257±0.350^{a}	6.390±0.272^{b}
70:30	7.007±0.378^{e}	5.600±0.420^{dc}	11.880±0.510^{e}
50:50	5.007±0.660^{d}	5.406±0.457^{c}	9.873±0.237^{d}
Ethanol:water			
100	2.757±0.060^{a}	1.863±0.119^{a}	2.597±0.464^{a}
70:30	3.787±0.780^{c}	3.623±0.476^{b}	5.92±0.71^{b}
50:50	7.436±0.386^{e}	6.190±0.684^{d}	13.130±0.327^{f}
Methanol:ethanol			
50:50	3.580±0.434b^{c}	2.543±0.599^{a}	6.337±0.716^{b}

Values are means±standard deviations from three independent experiments. Different superscripts in the same column indicate significant differences (p<0.05)

Table 1 - Extraction yield from flowers and fruit using different solvent extraction systems

Total polyphenol content

Table 2 shows the TPC of the sample extracts measured using Folin–Ciocalteu's colorimetric method. The TPC of mahua samples extracted by different solvents ranged from 8.352±0.831 GAE/g FW to 25.385±1.018 GAE/g FW for flowers, from 6.886±0.084 GAE/g FW to 15.890±1.018 GAE/g FW for unripe fruit, and from 2.857±0.831 GAE/g FW to 14.396±1.018 GAE/g FW for ripe fruit. Therefore, flower extracts had higher polyphenol contents than the fruit. The TPC of mahua extracts obtained using different extraction solvents differed significantly (p<0.05) and was in the following order (from high to low): 50% acetone>70% acetone>50% methanol for flowers and ripe fruit, and 50% acetone>70% acetone for unripe fruit. However, the differences between methanol/ethanol (1:1), 50% ethanol and 50% methanol solvent were not significant (p=0.05).

These results indicate that 50% acetone gave the highest yields among the 11 solvents for extracting total phenolics from mahua.

Total flavonoid content

Flavonoids are widespread plant secondary metabolites, and include flavones, flavanols and condensed tannins. Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress. In vitro, flavonoids from several plant sources have shown free radical scavenging activity and protection against oxidative stress [21]. In order to estimate the potential effect of flavonoids on the antioxidant activity of mahua flowers and fruit, the TFC of the extracts was analyzed; the results are presented

in Table 1. The TFC in flowers ranged from 2.03±0.332 to 13.130±0.327 mg QCE/g FW, from 5.431±0.248 to 21.010±1.000 mg QCE/g FW for unripe fruit, and from 4.287±0.150 to 21.923±1.75 mg QCE/g FW for ripe fruit. Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruit and vegetables. Plant-derived polyphenols display characteristic inhibitory patterns towards the oxidative reaction in vitro and in vivo [22]. Ho *et al*. reported that longan (*Dimocarpus longan* Lour.) flowers contained large amounts of total phenols and total flavonoids with levels varying depending on the solvent used for extraction [23]. Barreira *et al*. also found the same result with chestnut flowers, which contained considerable amounts of polyphenols and flavonoids [24].

Effect of the solvent system

The extraction of phenolic compounds is influenced by the polarity of the extracting solvents and the solubility of the compound in the solvent used [16, 25]. Therefore, it is hard to select an appropriate solvent for the extraction of phenolic compounds from all samples. In this study, phenolic compounds were extracted from mahua using H_2O and nine solvents: pure acetone, acetone/H_2O (70:30, v/v), acetone/H_2O (50:30, v/v), pure methanol, methanol/H_2O (70:50, v/v), methanol/H_2O (50:50, v/v), pure ethanol, ethanol/H_2O (70:50, v/v) and ethanol/H_2O (50:50, v/v). The recovery of total phenolic compounds varied greatly among the different solvents (Table 2). The most efficient solvent was 50% acetone which yielded from 14.396±1.018 to 25.385±1.018 mg GAE/g FW total phenolics from mahua flowers, and ripe and unripe fruit, while pure ethanol and pure water gave the lowest recoveries with 2.857±0.831 to 4.451±0.190 mg GAE/g FW.

Several earlier studies measured the effect of different solvents on total phenolic compounds and antioxidant activity. Zhou and Yu demonstrated that 50% acetone extract contained the highest levels of total phenolic contents for two varieties of wheat bran samples and mentioned that ethanol was a less effective solvent [26]. Sulaiman *et al*. reported that acetone is an efficient solvent for extracting phenolic compounds from different types of vegetable, with the lowest total phenolic content found in water extract [25]. Furthermore, Liu *et al*. reported that higher levels of phenolic compounds from lychee flowers were found in acetone extract than in methanol extract and water extract [20]. Similarly, Rebey *et al*. reported that acetone extract contained the highest total phenolic contents for mature green cumin seeds and also mentioned that distilled water was an inefficient solvent [27].

Ho *et al.* reported that different solvent extracts of longan flowers, which have high levels of total phenols and total flavonoids, exhibited good antioxidant ability [23]. For chestnut water extracts, the flower extract had a higher poly-

Solvent (%)	Flowers		Fruit			
			Unripe		Ripe	
	TPC (mg GAE/g FW)	TFC (mg QCE/g)	TPC (mg GAE/g FW)	TFC (mg QCE/g)	TPC (mg GAE/g FW)	TFC (mg QCE/g)
Water						
100	8.352±0.831^{a}	2.03±0.332^{a}	6.886±0.084^{a}	9.90±0.907c	2.857±0.831a	12.05±1.055^{c}
Acetone:water						
100	16.905±0.412^{e}	9.100±0.1^{d}	11.410±0.412^{c}	14.587±1.039^{f}	5.916±0.412^{bc}	12.210±1.08^{c}
70:30	20.723±0.401^{f}	11.773±1.16^{e}	14.130±0.235^{e}	17.343±1.553^{g}	13.643±0.530^{f}	21.923±1.75^{f}
50:50	25.385±1.018^{g}	13.130±0.32^{f}	15.890±1.018^{f}	12.620±1.091^{d}	14.396±1.018^{f}	14.320±0.70^{d}
Methanol:water						
100	10.495±0.343^{b}	11.880±0.51^{e}	7.564±0.467^{a}	7.090±0.630^{b}	5.000±0.343^{b}	18.920±1.010^{e}
70:30	11.722±0.530^{c}	9.873±0.237^{d}	8.293±0.186^{b}	5.431±0.248^{a}	11.743±0.626^{e}	18.627±1.2^{e}
50:50	15.055±1.618^{d}	6.337±0.716^{b}	12.611±0.194^{d}	7.276±0.139^{b}	9.560±1.618^{d}	4.287±0.150^{a}
Ethanol:water						
100	9.945±0.190^{b}	2.597±0.464^{a}	7.015±0.127^{a}	15.453±0.768^{ef}	4.451±0.190^{ab}	9.100±0.419^{b}
70:30	12.263±0.119^{c}	7.44±0.78^{c}	8.813±0.418^{b}	13.643±0.530^{d}	7.007±0.378^{c}	8.380±0.433^{b}
50:50	14.597±0.499^{d}	5.663±0.647^{b}	12.399±0.193^{d}	11.010±1.000^{c}	8.919±0.678^{d}	9.953±0.947^{b}
50:50	17.436±0.386^{e}	13.130±0.32^{f}	12.491±0.168^{d}	21.010±1.000^{h}	9.560±2.598^{d}	18.815±0.411^{e}
50:50	17.436±0.386^{e}	13.130±0.32^{f}	12.491±0.168^{d}	21.010±1.000^{h}	9.560±2.598^{d}	18.815±0.411^{e}

Values are the means±standard deviations from three independent experiments. Different superscripts in the same column indicate significant differences (p<0.05)
TFC total flavonoid content, *TPC* total phenolic content

Table 2 - Total phenolic content and total flavonoid content of mahua extracts obtained using different solvent extraction systems

phenol and flavonoid content than leaf or fruit extracts; the flower extract also showed a higher antioxidant activity than the other two extracts [24]. These findings are in agreement with our results and suggest that a mixed polarity solvent (acetone/water mixtures) is a good solvent for the extraction of phenolic compounds. Indeed, the addition of up to 50% water to acetone increased the extraction of total phenolic compounds [16].

Antioxidant activity assays

Total antioxidant capacity

The TAC of different solvent extracts of mahua was measured using the phosphomolybdenum method which is based on formation of phosphomolybdenum (V). This activity was measured spectrophotometrically at 695 nm [14] and expressed as equivalents of ascorbic acid. A significant difference (p<0.05) in total antioxidant capacity was observed between the different solvents for all mahua extracts (Table 3). These results indicated that the extracting solvent affected the total antioxidant capacity of the extracts. Extraction into acetone/H_2O (50:50, v/v) gave the highest total antioxidant capacity (33.293±1.509 mg equivalents of ascorbic acid/g FW) for mahua flower extracts, while methanol/H_2O (50:50, v/v) gave the highest TAC values of 16.337±0.72 and 9.562±0.17 mg equivalents of ascorbic acid/g FW, respectively, for unripe and ripe fruit. On the other hand, 70% ethanol yielded the lowest antioxidant capacity among the solvents (2.030±0.332 to 2.543±0.294 mg equivalents of ascorbic acid/g FW) for unripe and ripe fruit, while absolute methanol was the most inefficient solvent for flowers (7.00±0.378 mg equivalents of ascorbic acid/g FW).

Many previous studies have measured the effects of different solvents on antioxidant capacity using various methods. Zhou and Yu stated that extraction into 70% methanol extract gave the highest antioxidant capacity [26]. However, Al-Farsi *et al*. indicated that acetone/H_2O (70:30, v/v) exhibited strong antioxidant capacity measured by different methods compared with other solvents, while methanol/H_2O (50:50, v/v) afforded the lowest [28]. On the other hand, Liu *et al*. reported that lychee flowers showed higher activity in antioxidant assays in acetone extracts than in methanol or water extracts [20]. These significant variations indicated that a change in solvent polarity might significantly influence antioxidant activity. Extraction conditions and procedures may also alter antioxidant capacity) [25].

Ferric reducing/antioxidant power

The FRAP assay was used to evaluate the antioxidant potential of mahua flowers, and unripe and ripe fruit. The FRAP assay treats antioxidants in the sample as a reductant in a redox-linked colorimetric reaction. The antioxidant capacity of fruit extracts is determined by the ability of the antioxidants in these extracts to reduce ferric iron to ferrous iron in the FRAP reagent, which consists of TPTZ prepared in sodium acetate buffer, pH 3.6. The reduction of ferric iron in the FRAP reagent results in the formation of a blue ferrous–TPTZ complex [29], with maximum absorbance at 593 nm. Antioxidant compounds that act as reducing agents exert their effect by donating a hydrogen atom to the ferric complex, thus breaking the radical chain reaction. In the present study, flower extracts demonstrated the greatest reducing power among the extracts (Table 3). Significant differences (p<0.05) in FRAP values were found among all extracts. The ferric reducing ability of the extracts revealed that all demonstrated good FRAP activity (20.719±1.332 to 101.679±0.879 µmol Fe (II)/g FW extract). Among all solvents, the highest activity was seen for 50% acetone (87.290±0.879 to 101.679±0.879 µmol Fe (II)/g FW extract) followed by 70% acetone (80.703±0.520 to 97.746±0.81 µmol Fe (II)/g FW extract) for mahua fruit and flower extracts. The FRAP assay has been used by several authors to assess the antioxidant activity of various food product samples [30].

DPPH radical-scavenging activity

DPPH is a very stable organic free radical able to accept an electron or hydrogen radical. Consequently, reduction of DPPH by antioxidants results in loss of absorbance. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances [31]. The DPPH method is an easy and rapid way to evaluate antioxidant activity. The DPPH values of the antioxidant extracts are presented in Table 4. Mahua extracts from the different extraction solvents differed significantly (p<0.05) in their DPPH values. The values for flowers ranged from 89.442±0.124% to 71.419±0.774% inhibition, for unripe fruit from 74.225±1.080% to 88.581±0.134% inhibition, and for ripe fruit from 71.96±1.18% to 88.262±0.138% inhibition. The DPPH value was affected by the extracting solvents in the following order (from high to low): acetone>ethanol:methanol>methanol>ethanol for all mahua extracts. The highest DPPH antioxidant activity was seen in 50% acetone extracts for flowers, and unripe and ripe fruit. Kchaou *et al*. also reported similar results with date varieties [32]. These results show that the concentration of phenolic compounds and the degree of hydroxylation and polymerisation can affect radical scavenging activity [33, 34].

Solvent (%)	Flowers		Fruit			
			Unripe		Ripe	
	FRAP (μmol Fe (II)/g FW)	TAC (μmol ascorbic acid equiv/g extract)	FRAP (μmol Fe (II)/g FW)	TAC (μmol ascorbic acid equiv/g extract)	FRAP (μmol Fe (II)/g FW)	TAC (μmol ascorbic acid equiv/g extract)
Water						
100	36.947±1.29^{b}	10.216±1.08^{b}	30.086±0.769^{b}	7.44±0.785^{c}	21.225±1.913^{a}	4.576±0.960^{c}
Acetone:water						
100	87.746±0.818^{i}	20.193±0.33^{d}	81.751±0.219^{g}	9.100±0.1^{ef}	77.010±1.440^{h}	7.097±0.298^{e}
70	97.746±0.818^{j}	20.230±1.38^{d}	90.600±0.529^{h}	13.130±0.326^{h}	80.703±0.520^{i}	8.540±0.367^{f}
50	101.679±0.879^{k}	33.293±1.509^{f}	94.484±0.879^{i}	7.827±0.871^{cd}	87.290±0.879^{j}	4.877± 0.305^{c}
Methanol:water						
100	62.110±0.762^{f}	7.00±0.378^{a}	54.916±0.762^{e}	11.88±0.510^{g}	47.722±0.762^{e}	7.766±0.208^{e}
70:30	65.108±1.33^{g}	12.720±1.56^{c}	55.133±1.006^{e}	9.873±0.236^{f}	50.937±0.605^{f}	7.402±0.205^{e}
50:50	69.480±1.53^{h}	11.257±1.22^{bc}	64.952±1.981^{f}	16.337±0.72^{i}	54.758±1.195^{g}	9.562±0.17^{g}
Ethanol:water						
100	35.108±1.332^{a}	13.293±1.51^{c}	27.914±1.332^{a}	2.597±0.464^{a}	20.719±1.332^{a}	3.327±0.266^{c}
70:30	52.902±0.599^{e}	19.493±0.69^{d}	29.700±0.427^{b}	2.030±0.332^{a}	23.239±0.602^{b}	2.543±0.294^{a}
50:50	42.902±0.599^{c}	18.890±1.16^{d}	35.707±0.599^{c}	5.663±0.647^{b}	28.513±0.599^{c}	3.087±0.061^{ab}
Methanol:ethanol						
50:50	47.242±0.88^{d}	24.193±0.85^{e}	40.048±0.887^{d}	8.536±0.396^{de}	32.854±0.887^{d}	5.607±0.432^{d}

Values are means±standard deviations from three independent experiments. Different superscripts in the same column indicate significant difference (p<0.05)
FRAP ferric reducing antioxidant power, *TAC* total antioxidant capacity

Table 3 - Antioxidant activities of mahua extracts obtained using different solvent extraction systems

ABTS radical scavenging activity

ABTS+, which is generated from the oxidation of ABTS, is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants [17]. The ABTS values of the antioxidant extracts are presented in Table 3. Mahua extracts from different extraction solvents differed significantly (p<0.05) in their ABTS values. The ABTS radical scavenging abilities of mahua flower extracts are ranked as follows: acetone>methanol>ethanol:methanol>water>et

Solvent (%)	Flowers		Fruit			
			Unripe		Ripe	
	% DPPH inhibition	ABTS (TEAC) (μmol Trolox/g)	% DPPH inhibition	ABTS (TEAC) (μmol Trolox/g)	% DPPH inhibition	ABTS (TEAC) (μmol Trolox/g)
Water						
100	71.419±0.774^{a}	30.566±0.93^{d}	75.753±1.616^{c}	45.566±0.389^{g}	71.96±1.18^{a}	25.846±0.781^{c}
Acetone:water						
100	86.725±0.384^{f}	44.533±1.208^{f}	82.271±0.513^{e}	29.826±0.205^{b}	81.655±0.530^{f}	37.983±1.826^{f}
70:30	83.130±0.327^{de}	67.3633±2.04^{h}	87.040±0.94^{f}	43.526±0.030^{f}	80.223±0.106^{e}	44.92±0.327^{g}
50:50	89.442±0.124^{g}	74.18±1.155^{e}	88.581±0.134^{g}	59.1±0.1^{h}	88.262±0.138^{g}	45.22±1.105^{g}
Methanol:water						
100	78.915±0.353^{c}	38.403±0.859^{e}	76.572±0.393^{c}	20.196±0.332^{a}	75.670±0.408^{dc}	31.44±1.512^{e}
70:30	76.337±0.716^{b}	56.5633±1.66^{f}	75.370±0.471^{bc}	32.08±1.34^{c}	76.523± 0.389d	30.75±1.25^{d}
50:50	82.624±1.105^{de}	63.5533±2.18^{g}	79.833±0.861^{d}	44.846±0.166^{fg}	75.862±0.172cd	33.146±1.790^{e}
Ethanol:water						
100	76.115±1.001^{b}	12.243±0.745^{a}	74.225±1.080^{b}	35.06±1.060^{d}	73.163±1.125b	15.453±0.767^{a}
70:30	82.597±0.464^{de}	16.653±1.502^{b}	65.733±0.453^{a}	31.993±1.323^{c}	74.913±0.332^{c}	13.64±0.529^{a}
50:50	81.834±0.808^{d}	22.223±1.12^{c}	75.300±1.099^{bc}	29.21±0.709^{b}	73.385±1.184^{b}	21.01±1.00^{b}
Methanol:ethanol						
50:50	83.949±0.317^{e}	58.403±0.859^{f}	83.081±0.334^{e}	38.65±1.31^{e}	82.611±0.343^{f}	22.18±0.747^{b}

Values are means±standard deviations from three independent experiments. Different superscripts in the same column indicate significant difference (p<0.05)
ABTS 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), *DPPH* 2,2-diphenyl-2-picrylhydrazyl hydrate, *TEAC* Trolox equivalent antioxidant capacity

Table 4 - DPPH and ABTS activity of mahua extracts obtained using different solvent extraction systems

	Correlation coefficient (r)				
	TPC	FRAP	DPPH	TAC	ABTS
100% water extract (N=9)					
TPC	1	0.977**	0.183	0.940**	0.448
FRAP		1	0.992	0.957**	0.288
DPPH			1	0.985	0.814**
TAC				1	0.575
100% acetone extract (N=9)					
TPC	1	0.974**	0.904**	0.924**	0.443
FRAP		1	0.901**	0.934**	0.474
DPPH			1	0.988**	0.766*
TAC				1	0.738*
70% acetone extract (N=9)					
TPC	1	0.843**	–0.021	0.923**	0.981**
FRAP		1	0.491	0.958**	0.777*
DPPH			1	0.306	0.730
TAC				1	0.894**
50% acetone extract (N=9)					
TPC	1	0.968**	0.922**	0.891**	0.971**
FRAP		1	0.962**	0.905**	0.993**
DPPH			1	0.969**	0.949**
TAC				1	0.914**
100% methanol extract (N=9)					
TPC	1	0.993**	0.923**	–0.171	0.398
FRAP		1	0.915**	0.733	0.372
DPPH			1	–0.393	0.584
TAC				1	–0.956**
70% methanol extract (N=9)					
TPC	1	0.206	0.772*	0.999	0.440
FRAP		1	0.782	0.891**	0.953**
DPPH			1	0.529	0.544
TAC				1	0.848**
50% methanol extract (N=9)					
TPC	1	0.896**	0.842**	0.586	0.867**
FRAP		1	0.930**	0.417	0.925**
DPPH			1	0.303	0.929**
TAC				1	0.763
100% ethanol extract (N=9)					
TPC	1	0.986**	0.792*	0.839**	0.667
FRAP		1	0.685*	0.804**	0.750
DPPH			1	0.743*	0.540
TAC				1	–0.649
70% ethanol extract (N=9)					
TPC	1	0.982**	0.600	0.923**	0.931
FRAP		1	0.707*	0.972**	0.680
DPPH			1	0.850**	–0.801**
TAC				1	–0.384
50% ethanol extract (N=9)					
TPC	1	0.979**	0.844**	0.867**	0.528
FRAP		1	0.908**	0.927**	0.731
DPPH			1	0.957**	0.717
TAC				1	0.546
Ethanol:methanol extract (1:1) (N=9)					
TPC	1	0.894**	0.926**	0.903**	0.928**
FRAP		1	0.824**	0.920**	0.988**
DPPH			1	0.880**	0.895**
TAC				1	0.945**

*p<0.05 (two-tailed); **p<0.01 level (two-tailed)
ABTS 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), *DPPH* 2,2-diphenyl-2-picrylhydrazyl hydrate, *FRAP* ferric reducing antioxidant power, *TAC* total antioxidant capacity, *TPC* total phenolic content

Table 5 - Effects of different extraction solvents on antioxidant activity of mahua

hanol fraction. The ABTS values ranged from 12.243±0.745 to 74.18±1.155 µmol Trolox/g FW for mahua flowers, from 20.196±0.332 to 59.1±0.1 µmol Trolox/g FW for unripe fruit, and from 13.64±0.529 to 45.22±1.105 µmol Trolox/g FW for ripe fruit. The results suggested that 50% acetone showed highest value of ABTS antioxidant assay for mahua flowers, and unripe and ripe fruit.

Correlation analyses between phenolic compositions and antioxidant activities

Correlation analyses (Table 5) between the phenolic content and antioxidant activity of all mahua extracts were performed. High to weak correlations were observed when the 11 different solvent extracts of mahua flowers and fruit were separately analyzed. In the literature, some authors have suggested correlations between all these parameters, while others have found no such relationships [35]. The associations between the TPC (mg GAE/g FW) and FRAP (mg GAE/g FW) of the 11 different solvent extractions were calculated using linear regression analysis, and the correlation coefficients (r) between these two parameters were in the range 0.206–0.993 (p<0.01), indicating that there are significant and moderate correlations between the TPC and FRAP of the solvent extractions. The correlations between TPC and DPPH inhibition are shown in Table 5; the correlation coefficients were lower than those of TPC and FRAP (r –0.021 to 0.957) and not statistically significant (p>0.05).

TPC and TAC, and TPC and the ABTS parameter showed high to weak correlations, with the correlation coefficients (r) ranging from –0.171 to –0.940, and from 0.398 to 0.981, respectively. FRAP and DPPH assays showed the same trends. This is proved by the significant

correlations between FRAP values and DPPH values for all extracts in the current study. Correlation values were 0.491–0.992 for all solvents (significant at $p<0.01$). The highest r found from correlation analyses was between the phenolic contents and antioxidant activity (Table 5) of various mahua extracts with 50% acetone. Significant ($p<0.01$) linear correlations existed between TPC and DPPH, TPC and FRAP, and TPC and TAC (r=0.922, r=0.968 and r=0.891, respectively), between TPC and ABTS, and FRAP and TAC (r=0.971 and r=0.905, respectively), and between FRAP and ABTS, and DPPH and ABTS (r=0.993 and r=0.949, respectively). The lowest r was mostly found with the ethanolic extracts. Barreira *et al*. found that the phenol and flavonoid content of chestnut water extract correlated with its DPPH radical scavenging activity and reducing power [24]. Marimuthu *et al*. observed that the antioxidant activity (TEAC value) of ethanolic extract from the bark of *Chamaecyparis obtuse* var. *Formosana* correlated with its phenolic content [36]. However, some authors have mentioned that there is no direct correlation between phenolic content and antioxidant activity [37]. Our results show that phenolic components may affect the antioxidant capacity of mahua flower extracts.

Conclusion

This study revealed that extracts of mahua flowers and fruit have significant antioxidant activity depending upon the type of solvent used for extraction. An aqueous solution of 50% acetone was the most efficient solvent for the extraction of antioxidants from mahua flowers. All assays showed that this extract contained the highest amount of phenolic compounds and exhibited the strongest antioxidant activity, while water was the most inefficient solvent for the extraction of phenolic compounds. The highest correlation coefficient between various assays was found for 50% acetone, indicating it was the best solvent. The result indicated that flowers had higher polyphenolic content and antioxidant activity than fruit in all extracts, while unripe fruit showed more antioxidant activity in all assays than ripe fruit. The flowers and fruit of *M. longifolia*, already consumed as a foodstuff in different parts of India, could be used as an accessible source of natural antioxidants with consequent health benefits. Further studies using HPLC/LC–MS to identify the main phenolic compounds in *M. longifolia* fruit responsible for its antioxidant activity are in progress.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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