Original Research Article

Quantitative analysis of phenols, flavonoids and antioxidant activity in aegle marmelos and chrysanthemum morifolium from temple waste

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Abstract

The present work was to screen wastes collected from temple in various solvent extracts A detailed study was performed on the leaves of *Aegle marmelos* and flowers of *Chrysanthemum morifolium* in different successively extracted with different solvents like petroleum ether,ethyl acetate,ethanol and water. The phytochemical screening, total phenol contents (TPC) and total flavonoid contents (TFC) also determined. Results of quantitative phytochemical screening in *Aegle marmelos* showed the total flavanoids content (TFC) in ethanolic and aqueous extract was found to be 3.371 mg/100mg and 2.445 mg/100mg respectively and total phenol content (TPC) in aqueous extract was found to be 1.138 mg/100mg while total flavonoid contents in *Chrysanthemum morifolium* was found to be 1.665 mg/100mg and 1.386 mg/100mg in ethanol and ethyl acetate extract respectively.The IC_{50} value for *Chrysanthemum morifolium* in ethanol and ethyl acetate was found to be 43.86 µg/ml and 52.35 µg/ml respectively and for aqueous extract of *Aegle marmelous* was found to be 11.97 µg/ml. When compare to standard the *A.marmelos* showed the high antioxidant activity than *C.morifolium.*Hence, waste collected from temple waste represents a source of potential antioxidants that could be used in pharmaceutical and food industries.

Key Words: *Aegle marmelos*, *Chrysanthemum morifolium*, Total flavonoid contents(TFC), Total phenolic contents (TPC), Temple waste.

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INTRODUCTION

Natural compounds have been used for cure of symptoms originate by diseases since very long time. Beside this the great advancement have been found in novel drugs in recent decades, plants have an important significance to health protection[1]. Polyphenol compound have been regarded as an important group of secondary metabolites and bioactive compounds of plants[2]. Herbal medicines are more efficiently appreciated worldwide due to the fact that herbal plants continue to play important role in health protection and maintenance of maximum population of the world. Indeed there are various medicinal plants are being used frequently and naturally in the prevention and treatment of several diseases.The medicinal plants are great source of secondary metabolites like alkaloids, glycosides, steroids and flavonoids, these are high potential source of drugs[3]. Phytochemicals are the secondary metabolities present in trace amount in higher plants and they include the alkaloids, Steroids, flavonoids, terpenoids, tannins and many others[4]. Most of the herbal drugs having free radical scavengers are known for their curative and medicinal activity[5]. At present, there is a common mode to obtain naturally occurring antioxidants, which are sufficient and free from danger, to supplement processed food or pharmaceuticals and synthetic antioxidants are not preferred due to the harmful effects such as human toxicity and environmental pollution[6]. Plants are one of the crucial sources of valuable anti-oxidants.Natural antioxidants,extracted from plants, are secondary metabolites, mainly plant phenolics[7] such as phenolic acids, flavonoids and carotenoids, these are amongst various antioxidants

produced by plants for their nourishment[8]. The research work of this study was to determine the total phenolic content(TPC) total flavonoid content(TFC)and antioxidant activity in various extracts of leaves of *Aegle marmelos and* flowers of *Chrysanthemum morifolium* temple waste collected from temples of Vidisha using spectrophotometric methods, as well as to examine antioxidant activity of waste extracts using *DPPH* (2, 2-dipheny-l-picrylhydrazyl) method.*Chrysanthemum morifolium* belonging to the asteraceae family is commonly known as Sewanti and *Aegle marmelos* belonging to family *Rutaceae* is commonly known as *Belpatra.*

MATERIAL AND METHODS

Material: The reagents and solvents used for the extraction, phytochemical analyses and antioxidant activity profiling were analytical grade reagents.

Collection of plant material: Leaves of *Aegle Marmelos and* flowers of *Chrysanthemum morifolium* was collected from temple of Vidisha (M.P.), The plant material was dried under shade at room temperature for about 15 days. The dried plant sample was powdered by mechanical grinder and sieved to give particle size 40-100 mm. The powder was stored in polythene bags at room temperature before extraction.

Preparation of extract: *Aegle marmelos* and flowers of *Chrysanthemum morifolium* dried and powdered plant material was extracted with hot continuous percolation method (Soxhlet extraction). The temperature was maintained at 50°C. The extraction was carried out using(500ml) petroleum ether,ethyle acetate,ethanol and water for 24 hours as a solvent. The extract was filtered through a paper filter (Whatman, No.1) and evaporated to dryness under reduced pressure by the rotary evaporator. The obtained crude extract was stored in dark glass bottles for further processing.

Extractive yield value = Weight of concentrated extract/Weight of dried powder × 100

Qualitative phytochemical analysis of extract: The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The extract was examined for the presence of various phytoconstituents such as carbohydrate, alkaloids, glycosides, saponins, phenolic compound, and flavonoids.

Determination of Total Phenolic content: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 5-25µg/ml was prepared in methanol.

Preparation of Extract: 10 mg extract of leaves and flowers was dissolved in 10 ml methanol and filtered, two ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 2 ml of each extract (ethanol, ethyle acetate, and aqueous) and standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15second and allowed to stand for 15 minute at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Labindia, 3000+).

Determination of Total flavonoids content: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg extract of leaves and flowers was dissolved in 10 ml methanol and filtered, three ml (1mg/ml)of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract and standard and allowed to stands for 15 minute at room temperature; absorbance was measured at 420 nm.

Determination of Antioxidant activity (DPPH radical scavenging activity): DPPH scavenging activity has been used by various researchers as a rapid, easy and reliable parameter for screening the *in vitro* antioxidant activity of waste extracts. DPPH is a stable free radical and accepts an electron to become a stable diamagnetic molecule. The absorption maximum of a stable DPPH radical in methanol was at 517 nm in U.V spectrophotometer.It was observed that with the increase of concentration, there is decrease of absorbance value. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidants molecules and radical, progresses, which results in the scavenging of the radical by electron donation.

Preparation of standard(Ascorbic acid) and extracts solutions[9]: For DPPH scavenging,0.135mM solution of DPPH were prepared in methanol. Stock solution of extracts and standard were prepared in methanol to achieve the concentrations of µg/ml. Dilution are made to obtain the concentrations of 20,40,60,80,100 µg/ml. 2ml of each diluted solutions of extracts and standard were mixed with 2ml of methanolic solution of DPPH. Control contained DPPH solution and methanol except the extract.the sample extracts and standard were incubated in darkness for 20 minutes.the absorbance was measured at 517nm.Percentage inhibition of sample and standard were calculated using the following equation.

% inhibition=[A0 of control- A of control)/ A0 of control] ×100

Where, A0 is the absorbance of the control solution,A is the absorbance of extract solution. Then, curves were plotted between the percentage of inhibition and concentration in µg/ml. The equation of this curve allowed to calculate the inhibitory concentration 50% (IC_{50}) corresponding to the sample concentration that reduced the initial DPPH absorbance of 50 %. The lower the IC50 value, the
higher is the antioxidant activity.

RESULTS AND DISCUSSION

Calibration Curve of Gallic acidc

Table 1: Preparation of calibration curve of Gallic acid

S. No.	Concentration(in µg/ml)	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

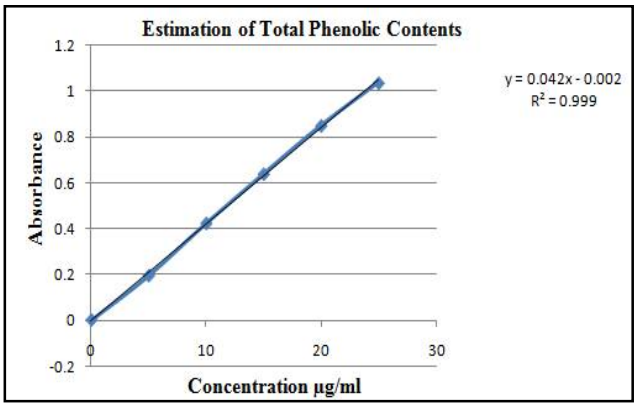

Figure 1: Graph of Estimation of Total Phenolic content

Calibration Curve of Quercetin

Table 2: Preparation of calibration curve of Quercetin

Sr. No.	Concentration(In µg/ml)	Absorbance
0	0	0
1	5	0.352
2	10	0.610
3	15	0.917
4	20	1.215
5	25	1.521

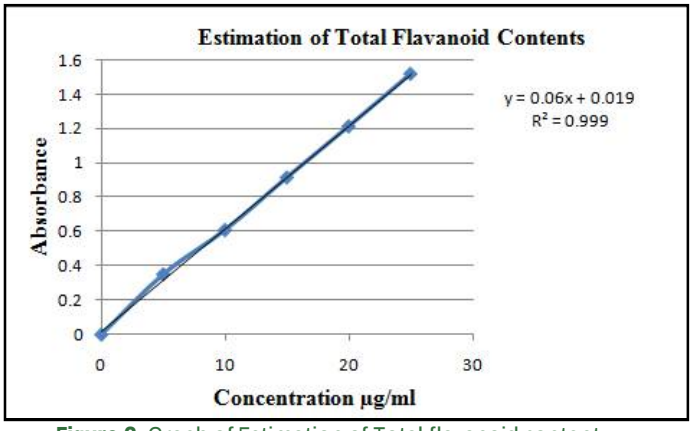

Figure 2: Graph of Estimation of Total flavanoid content

Table 3: Total Phenolic and Total flavanoid content of *Aegle marmelos*

Sr. No.	Extracts	Total Phenol (mg/100mg)	Total flavanoid (mg/100mg)
1	Ethanol	-	3.371
2	Aqueous	1.138	2.445

Table 4: Total Phenolic and Total flavanoid content of *Chrysanthemum morifolium*

S. No.	Extracts	Total Phenol (mg/100mg)	Total flavanoid (mg/100mg)
1.	Ethanol	-	1.665
2.	Ethyl acetate	-	1.386

Table 5: Result of *in vitro* free radical scavenging activity

Conc. In (µg/ml)	Ascorbic acid	Extracts (% Inhibition)		
	% Inhibition	*C. morifolium Ethanol*	*C. morifolium Ethyl acetate*	*A. marmelos Aqueous*
20	45.90164	36.06557	21.31148	43.60656
40	60.32787	44.2623	52.13115	57.04918
60	67.86885	67.21311	56.39344	66.55738
80	78.68852	70.16393	66.55738	76.06557
100	85.2459	71.80328	78.36066	77.70492
IC 50	2.67843	43.86	52.35	11.97

Absorbance of control (Ao) = 0.305

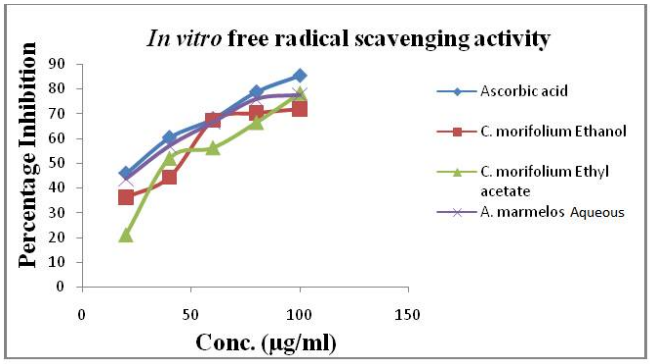

Figure 3: Graph of *in vitro* free radical scavenging activity

Total Phenolic content estimation (TPC): The total phenolic contents (TPC) in the examined waste extracts using folin-ciocalteu's reagent was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.042X+0.002, R^2= 0.999, where Y is the absorbance, X is the gallic acid equivalent (GAE) and R^2 is the regression equation value. The result are shown in table 1.

Total flavanoid content estimation (TFC): Results of standard quercetin are shown in table 2. The total flavanoid contents(TFC) in the examined waste extracts using aluminium chloride was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.06X+0.019, R^2= 0.999, where Y is the absorbance, X is the quercetine equivalent (QE) and R^2 is the regression equation value. Results of total phenolic content (TPC) and total Flavonoid content(TFC) of *A.marmelos* and *C. morifolium* are shown in table 3 and table 4.

Antioxidant activity: The results of DPPH scavenging activity, percentage of different concentration of standard ascorbic acid and extract of *Aegle marmelos, and Chrysanthemum morifolium* are shown in table 5. Inhibitory concentration of 50% (IC_{50}) for standard ascorbic acid was found to be 2.67 µg/ml and for ethanol and Ethyl acetate extract of *C. morifolium* was found to be 43.86 µg/ml, 52.35µg/ml respectively. IC_{50} for aqueous extract of *A. marmelos* was found to be 11.97µg/ml thus the anti-oxidant activity of sample was less than that of standard ascorbic acid. Although ethanol and ethyle acetate both exhibited DPPH free radical scavenging activity. Results of quantitative phytochemical screening in *A.marmelos* showed the total flavanoids content (TFC) in ethanolic and aqueous was found to be 3.371 mg/100mg and 2.445 mg/100mg respectively and total phenolic content (TPC) in aqueous extract was found to be 1.138 mg/100mg while total flavonoid contents in *C.morifolium* was found to be 1.665 mg/100mg and 1.386 mg/100mg in ethanol and ethyl acetate extract respectively. Hence, waste collected from temple waste represents a source of potential antioxidants that could be used in pharmaceutical and food industries. Flavonoids seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Ethanolic extract has the richest content of flavonoids in both the waste, and aqueous extract was the least. In the determination of total flavonoids, the results showed that the ethanolic solvent was having richest content of flavonoids than aqueous solvent which may be explained by its good polarity and solubility for phenolic compounds extracted from plants[10,11].

CONCLUSION

Results of our study suggests the great value of the *C.morifolium* and *A.marmelos* collected from temple wastes for use in pharmaceuticals.On the basis of our study it could be concluded that these wastes are also have potential sources of antioxidant substances of great significance.the present research work suggests that the offered temple waste flowers *C.morifolium* and leaves *A.marmelos* can be used for the health benefits and formation of herbal medicines. The data generated from these experiments have provided the chemical basis for the wide use of these waste as therapeutic agent for treating various ailments. However, there is need to further carry out advanced hyphenated spectroscopic studies in order to elucidate the structure of these compounds.

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