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Research Article

Qualitative and quantitative determination of phytochemicals from flowers of Spanish Cherry tree

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ABSTRACT

The present enquiry was intended to analyze the phytochemicals qualitatively and quantitatively from flowers of Spanish cherry tree. Flower powder was extracted using polar and nonpolar solvent by soxhlet apparatus. Percentage yield of crude extracts was determined and further the extracts were subjected to analyze the phytochemicals qualitatively and quantitatively by standard procedure. Qualitative analysis showed the absence of alkaloids while presences of tannins, saponins, terpenoids, steroids, glycosides, flavonoids, phenols. Quantitative estimation of phytochemicals was determined using standard curve. Result revealed that the tannin content was 4.3 ± 0.01 (mgTAE /gm), flavonols content was 0.28 ± 0.05 (mgQE/gm). Saponins content was 3.6 ± 0.7 % and terpenoids content was 1.47 ± 0.37 %. A well conducted studies on phytochemicals revealed that they are vital for humans because they provide protection against a variety of ailments. Therefore, the present study is aimed to analyze phytochemicals qualitatively and quantitatively.

Keywords: Phytochemicals, Tannins, Saponins, Flavanols, Terpenoids

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INTRODUCTION

Phytochemicals are bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases¹. Dietary intake of phytochemicals may promote health benefits, protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. Majority of foods, such as whole grains, beans, fruits, vegetables and herbs contain phytonutrients and phytochemicals. These phytochemicals, either alone and/or in combination, have tremendous therapeutic potential in curing various ailments. Some of the benefits of phytochemical are there low toxicity, low cost, easy availability and their availability to prevent some chronic diseases²⁻³. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds⁴.

Mimosa pudica L. is one of the most used therapeutic plant by tribal peoples. The water distilled from the flowers is used as a stimulant medicine, calm anxiety, panic attacks and as a brain tonic by the traditional practitioners of Southern India⁵. According to Unani system of medicine

flowers of this drug are considered as expectorant, cures biliousness, liver complaints, disease of nose headache and their smoke is considered good for asthma. It is also used for preparing lotion for wound and ulcers⁶. Therefore, the objective of this study was to determine the phytochemicals qualitatively and quantitatively.

MATERIALS AND METHODS

Collection of flowers

Fresh flowers were collected from the campus of DDU Gorakhpur University. The flowers were rinsed and dehydrated. The dehydrated flowers were then pulverized with the help of mechanical grinder and kept in sealed container at 4 °C temperature in refrigerator.

Preparation of flowers extracts

Sohxlet apparatus was used for extraction. Dried powder of flowers was subjected for extraction with different solvent (methanol, methanol 80%, ethanol, ethanol 80%, acetone, chloroform, ethyl acetate and water). After effective extraction solvents were concentrated using rotatory evaporator under reduced pressure. The crude

extract was weighed and its percentage yield was determined.

Qualitative analysis of phytochemicals

Standard procedure was followed for qualitative analysis of phytochemicals of flower extracts as described by Trease and Evans 1989⁷, Harborne 1973⁸, and Sofowara 1993⁹.

Test for alkaloids

Mayer's reagent (KI + Hg₂Cl₂ solutions): Few drops of Mayer's reagent was added to the extract, appearance of cream colored precipitate indicates the presence of alkaloids.

Dragendorff's reagent (excess of KI + BiNO₃ solutions): Few drops of Dragendorff's reagent was added to the extract, reddish brown colored precipitate appeared.

Hager's reagent (Picric acid): Few drops of Hager's reagent was added to the extract, appearance of yellow colored precipitate indicates the presence of alkaloids.

Test for glycosides

Keller-Killiani test: 1.0 ml of glacial acetic acid containing traces of ferric chloride and 1.0 ml of concentrated sulphuric acid were added to the extract. A reddish brown colour formed at the junction of the 2 layers and the upper layer turned bluish green indicating the presence of glycosides.

Borntrager's test: 1.0 ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract, appearance of a reddish pink color indicates glycosides.

Test for flavonoids

Alkaline reagent test: Few drops of NaOH solution were added to the extract. Formation of intense yellow color occur which disappear when concentrated HCl is added.

Test for saponins

Foam Test: 2.0 ml of distilled water was added to the extract and shaken vigorously for 15 minutes. If foam produced persists for ten minutes it indicates the presence of saponins

Test for tannins and phenols

Ferric chloride test: 1.0 ml of ferric chloride solution was added. Bluish-black color appeared confirmed the presence of tannins.

Test for steroids and terpenoids

Liebermann Burchard test: 1.0 ml of anhydrous acetic acid and 1ml chloroform was added to the extract and cooled at 0 °C. Then 1.0 drop of concentrated sulphuric acid was added from the side of the test tube. At the junction a brown ring appear between two layers. Formation of lower deep red color indicates the presences of terpenoids and the upper layer turns green which show the presence of steroids.

Salkowski test: 1.0 ml of chloroform and 1.0 ml of sulphuric acid was added. Reddish brown color lower layer showed the presence of steroids while yellow color upper layer indicated the presence of terpenoids.

Test for proteins

Biuret test: 1 ml of 40 % NaOH solution and two drops of one percent CuSO₄ solution were added. Appearance of proteins indicated by violet color.

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) were added. Appearance of amino acids indicated by purple colour.

Test for carbohydrate

Fehling's test: Fehling's solution was added to the extract and boiled in water bath. Presence of carbohydrates is indicated by appearance brick red precipitate

Benedict's reagent: Benedict's solution was added and boiled in water bath. The presence of carbohydrates is detected by red precipitate.

Quantitative analysis of phytochemicals

Determination of tannins

Tannins content was determined by the procedure of Van-Burden and Robinson 1981¹⁰. Sample (500 mg) was weighed into a plastic bottle (50 ml). 50 ml of distilled water was added and shaken for 1 h. in a mechanical shaker after that it was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of it was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ prepared in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured within 10 min at 605 nm.

Determination of saponins

Procedure of Obadoni and Ochuko 2001¹¹ was followed to determine the saponins content. The samples were ground and 20 gm of each were put into a conical flask and 100 Cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h. with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage.

$$\text{Percentage of Saponin} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of total flavonols

Total flavonols content was determined using the method of Kumaran and Karunakaran 2007¹². 2 ml of the plant extract (1 mg/ml) was mixed with 2 ml of AlCl₃ prepared in ethanol and 3 ml of 50 gm/l sodium acetate solution. The mixture was incubated at 20 °C for 2.5 hrs. after which the absorption was measured at 440 nm. Total flavonols content was calculated in terms of quercetin (mg/gm) using the calibration curve.

Determination of terpenoids

Terpenoids content was determined by Ferguson 1956¹³. 10 gm of dried flower powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids.

RESULT AND DISCUSSION

The present work revealed the percentage yield of crude flower extracts which was higher in ethanol (28.4 ± 0.68) followed by ethanol 80 % (20.7 ± 0.99), methanol (19.0 ± 0.44), aqueous (17.8 ± 0.76), methanol 80 % (15.7 ± 0.27), acetone (8.1 ± 0.3), chloroform (5.8 ± 0.86), ethyl acetate (4.8 ± 0.39) (Table 1). The crude drug extracted in different solvents was subjected to various chemical tests for the detection of phytochemicals. The result revealed the presence of tannins, saponins, terpenoids, steroids, phenols, carbohydrates, flavanoids and glycosides while absences of alkaloids were reported from flowers. However, their intensity of appearance differ in different solvents (Table 2). The quantitative determination of phytochemicals showed that tannins content was 4.3 ± 0.01 (mg/gm TAE) which was determined using standard curve equation derived from standard curve of tannic acid¹⁴. Tannins are potential antioxidants. They have been considered to be cardioprotective, anti-inflammatory, anti-carcinogenic and anti-mutagenic, among others. Tannins enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of noninsulin-dependent diabetes mellitus. Tannins can improve the pathological oxidative state of a diabetic situation¹⁵. Flavanols content was 2.8 ± 0.05 (mg/gm QE) which was determined using standard curve of quercitin. Flavanols and their related oligomers can act as potent antioxidants,

reduce platelet activation, promote endothelium-dependent vasorelaxation, and modulate eicosanoid production¹⁶. Saponins content was 3.6 ± 0.7 % and terpenoids content was 1.47 ± 0.37 %. Saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning¹⁷. (Table 3)

Table 1: Percentage Yield of Different Extract of *Mimusops elengi* L. Flower

Solvents	Flower
Methanol	19.0 ± 0.44
Methanol 80 %	15.7 ± 0.27
Ethanol	28.4 ± 0.68
Ethanol 80 %	20.7 ± 0.99
Chloroform	5.8 ± 0.86
Acetone	8.1 ± 0.3
Aqueous	17.8 ± 0.76
Ethyl Acetate	4.8 ± 0.39

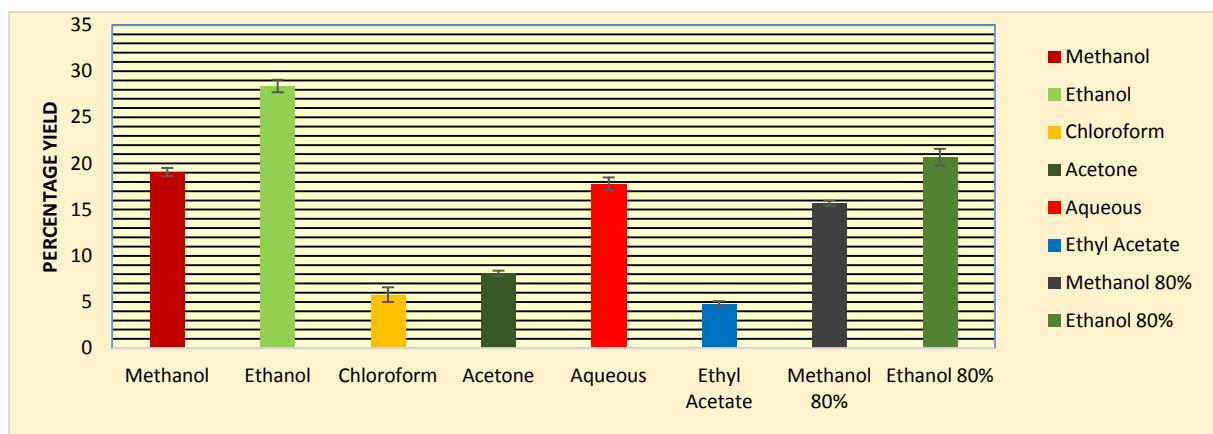
Table 2: Qualitative Analysis of phytochemicals from *Mimusops elengi* L. flowers

Chemical constituent	Tests	Colour	Extracts					
			MFE	EFE	CFE	AFE	AqE	EAE
Alkaloids	Mayer's	Cream	-	-	-	-	-	-
	Hager's	Reddish brown						
	Wagner's	Reddish brown						
Saponins	Foam	Yellow	+	+	-	+	++	-
Glycosides	Keller-killiani	Reddish brown	+	+	-	-	+	-
	Borntragers	Reddish pink	-	-	-	-	-	-
Flavanoids	Alkaline	Yellow	+++	+++	++	++	+++	++
Steroids /Terpenoids	Libermann-Burchard	Green/Red	++	+	+	-	+	-
	Salkowski	Red/Yellow	+	++	+	-	++	-
Tanins/Phenols	Ferric chloride	Blue black	++	++	+	+	++	+
Carbohydrates	Benedict's	Red precipitate	+	-	-	-	+	-
	Fehling's	Brick red	+	+	-	-	+	-
Proteins and amino acids	Ninhydrin	Violet color	+	-	-	-	+	-
	Biuret	Purple	+	-	-	-	+	-

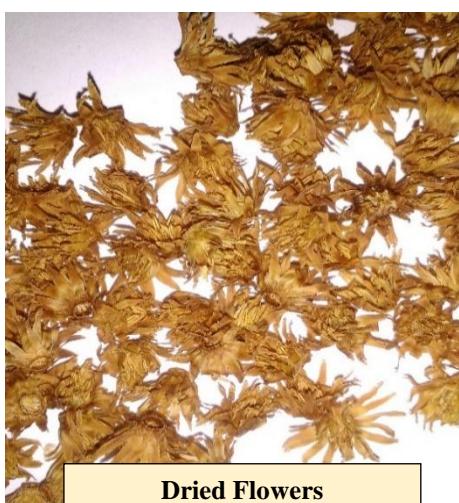
MFE-Methanol Flower extract, EFE- Ethanol Flower extract, CFE- Chloroform Flower extract, AFE- Acetone Flower extract, AqE- Aqueous Flower extract, EAE- Ethyl Acetate extract; +, ++, +++ =Indicate the intensity of appearance

Table 3: Quantitative Analysis of Secondary Metabolites from *Mimusops elengi* L.

Phytochemicals	Flowers
Tannins	4.3 ± 0.01 (mg/gm TAE)
Saponins	3.6 ± 0.7 %
Terpenoids	1.47 ± 0.37 %
Flavonols	0.28 ± 0.05 (mg/gm QE)



Percentage Yield of Different Extracts of Flowers



Dried Flowers



Powder form of Flowers



Flowers Extraction through Soxhlet



Rotatory Evaporator

CONCLUSION

Plants have a noteworthy position in the medicinal field due to their therapeutic properties and prove to be a rich source of drugs. Therapeutic properties are due to the presences of various phytochemicals present in the plant. Plant derived chemical compounds play a key role in the reduction of chronic diseases as well as prominent impact in health benefits. They act as antioxidant, antitumor, anti-

inflammatory, anticancerous, anti-diabetics etc. Therefore, exploration is further required for isolation and characterization of phytochemicals.

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