

QUALITATIVE AND QUANTITATIVE ANALYSIS OF BIOACTIVE NATURAL COMPOUNDS OF HIBISCUS ROSA-SINENSIS, EMBLICA OFFICINALIS AND ACACIA CATECHU

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Cite this article:

Hussain A, Shaikh S, Ayaz S, Ahmad T, Ishaque M, Remhan M et al. Qualitative and Quantitative Analysis of Bioactive Natural Compounds of Hibiscus Rosa – Sinesis, Emblica Officinalis and Acacia Catechu. AJMAHS. 2023; 1(1):32-39.

ABSTRACT

Objective: To evaluate the qualitative and quantitative analysis of chemical constituents of *Hibiscus rosa-sinensis*, *Embllica officinalis* and *Acacia catechu*.

Methodology: All plants were dried separately under the shade. The dried materials were crushed by using mortar and pestle. Each plant material was soaked in aqueous and organic solvents (Petroleum ether & chloroform) separately for extract preparation.

Results: Quantitative and qualitative results show that all these plants contain various phytochemical substances.

Conclusion: Medicinal plants contain potential phytochemicals used for curing various ailments. The current article explain the phytochemical analysis of *Hibiscus rosa-sinensis*, *Embllica officinalis* and *Acacia catechu*. These medicinal plants have potential therapeutic effects against different diseases. The qualitative analysis of *Hibiscus rosa-sinensis* shows the presence of alkaloids, saponins, flavonoids, fats and fixed oil, sterol and triterpenoids for aqueous extract. Sterol and triterpenoids are present in petroleum ether. The phytochemical studies of *Embllica officinalis* explain the positive results for carbohydrate in all the three extract (Petroleum ether, chloroform and aqueous), whereas sterol and triterpenoids, alkaloids and fats and fixed oil are present in the aqueous extract. The water extract of *Acacia catechu* contains alkaloids, sterol and triterpenoids, carbohydrate and flavonoids. Spectrophotometer technique explains the quantitative analysis of alkaloids, phenol, tannins, saponins and flavonoids.

Key words: Medicinal plants, Phytochemical analysis, *Emblica officinalis*, *Acacia catechu*, *Hibiscus rosa-sinensis*

Abbreviations: TLC (Thin layer chromatography), HPLC (High performance liquid chromatography), FTIR (Fourier transform infrared spectroscopy), NMR (Nuclear magnetic resonance), DW (Distilled water), HCL (Hydrochloric acid), NaCl (Sodium chloride)

INTRODUCTION

The main sources of drugs are plants, animals and minerals used from the ancient times for curing various diseases¹. Approximately 5000 year oldest written evidences have been found from Nagpur. It is composed of twelve recipes for preparation of more than 250 medicinal plants. Mandrake, poppy and henbane are some of the plants used as therapeutic medicine². The father of medicine, Hippocrates (459–370 BC) classified medicinal plants on the basis of physiological actions. According to his classification the Wormwood, garlic, asparagus, oak and opium are used for fever, intestine parasites, diuretics, astringents and narcotics respectively^{3,4}. A Chinese book “Pen T’Sao,” written in 2500 BC contains 365 medicinal plants. *Datura stramonium*, *Rhei rhisoma*, *Cinnamomum camphora*, *Podophyllum*, *Theae folium*, *ephedra gerardiana*, *Cinnamomum verum* and *Panax quinquefolius* are some medicinal plants mentioned in “Pen T’Sao”^{5,6}.

700 species of medicinal plants were mentioned in the Ebers Papyrus, written by circa in 1550 BC. Some medicinal plants of this collection used for various therapies are coriander, pomegranate, aloe vera, fig, castor oil plant, onion, juniper, garlic and senna^{7,8}. The isolation and identification of various chemical constituents

from the medicinal plants play an important role in the field of medicine. Furthermore, the modern techniques such as TLC, HPLC, FTIR, NMR and Infrared spectroscopy are used for the qualitative and quantitative analysis⁹. Whereas the medicinal plants contain bioactive chemical constituents such as Silymarin, Glycyrrhizin, tannins, glycoside, β -sitosterol, picrosides, flavonoids, phenol, sterol, terpenes, terpinoids, alkaloids and vitamins having various pharmacological action against diseases⁹⁻¹¹.

The *Hibiscus rosa-sinensis* family Malvaceae is traditionally used for Gonorrhoea, painful menstruation, diarrhea, cough, flu, bronchitis, stomach pain, fever and dysentery¹². The *Emblica officinalis* is easily available in Pakistan, China, Sri Lanka, Malaysia, Uzbekistan and South East Asia. It belongs to the family Euphorbiaceae. It contains numerous amount of biochemical compounds which plays a vital role in the treatment of various ailments¹³. *Acacia catechu* commonly known as catechu/Katha is an important medicinal plant belongs to the family leguminosae. Because of its abundant phytochemical constituents, it is used against various pathogens that cause severe illnesses¹⁴.

MATERIALS AND METHODS

Collection of plant samples

The aerial parts of *Hibiscus rosa sinensis*, *Emblica officinalis* and *Acacia catechu* were collected from the market of Quetta, Balochistan, Pakistan. The plant specimens were identified by Dr. Saad Ullah Leghari, Associate professor University of Balochistan, Pakistan. The voucher number Dept.Bot.399 was preserved in the Herbarium University of Balochistan.

Processing of plant samples

The dried aerial parts of *Hibiscus rosa sinensis*, *Emblica officinalis* and *Acacia catechu* were crushed into powder form.

Preparation of plant extract

Aqueous extract

200gm of all the three plants (*Hibiscus rosa sinensis*, *Emblica officinalis* and *Acacia catechu*) were taken in separate flask of 200ml and dissolved in 200ml of distilled water (DW) for 24 hours. The extracts were filtered to a volumetric flask by using Whattman filter paper. The solution of each plant was extracted with the help of rotary apparatus.

Organic extract

200gm crushed plant materials of all the three plants were added to 200ml of chloroform and petroleum ether. The solution of each plant was taken into soxhlet apparatus for extraction.

The obtained extracts were analyzed qualitatively for various chemical constituents.

Phytochemical analysis

For various secondary metabolites, chemical tests were performed for each plant extract.

Qualitative analysis of phytochemical constituents

Test for Alkaloids

Take all extract of *Acacia catechu* in separate test tube and added 2-3 drops of potassium bismuth iodide solution (Dragendroff's reagent). Appearance of brown color indicates presence of alkaloids.

Test for Flavonoids

Take all three extracts of petroleum ether, chloroform and water extract. The extracts were treated with magnesium ribbon, then add some drops of concentrated hydrochloric acid. For petroleum ether and chloroform extracts the color was absent which indicates the absence of flavonoids. Presence of reddish color indicates the presence of flavonoids.

Test for Tannins

Treat all the three extracts of this plant in separate test tube and react with 2-3 drops of ferric chloride solution. Appearance of pale yellow color, yellow color and brownish black color for petroleum ether, chloroform and water extracts respectively indicate the absence of tannin.

Test for Sterols and Triterpenoids

Take all extracts (petroleum ether, chloroform and water) in separate test tubes and treat it with few drops of concentrated sulphuric acid (H₂SO₄) and shake well. Absence of color indicates the absence of sterols and triterpenoids. Presence of reddish color indicates sterols and triterpenoids in water extracts.

Test for Carbohydrates

All three extracts of plants were taken in separate test tubes and react with benedict's reagents.

After that all the test tubes were put in the boiling water bath for 3-5 minutes. For *Emblica officinalis* and *Acacia catechu* red precipitates were absent which indicates absence of carbohydrates. Presence of red precipitates for *Hibiscus rosa sinensis* indicates presence of carbohydrates.

Test for Glycosides

All the three extracts were treated with dilute sulphuric acid in separate test tube. The solution were boiled in water bath and filtered. The filtrate was cooled and added few drops of benzene and shaken well. Organic layers were separated from the solution. Equal volume of ammonia was added to the organic layer of each test tube. Ammonical layer did not turn pink in each test tube indicates absence of glycosides.

Test for Saponins

Take all extracts of three medicinal plants in separate test tubes and add some water and shake well. Absence of foam formation indicates absence of saponins.

Test for Fats and Oils

All three extracts were treated with 0.5N alcoholic potassium hydroxide and few drops of phenolphthalein in separate test tubes, then heated in water bath for 1-2 hours. Soap formation indicates fats and fixed oils.

RESULTS AND DISCUSSION

Quantitative analysis of phytochemical constituents

Phenols

Spectrophotometer method was used for determine the quantity of phenols. All three

plants samples were mixed with the 50ml of $(\text{CH}_3\text{CH}_2)_2\text{O}$ in separate beaker and boiled for 15 minutes. 10ml of distilled water was taken in 50ml of flask separately and added 5ml of boiled sample, then added 2ml and 5ml of NH_4OH and conc. $\text{CH}_3(\text{CH}_2)_3\text{CH}_2\text{OH}$ respectively in each flask. Now the solutions were passed through the spectrophotometer at 505nm wavelength.

Alkaloids

5gm samples of all plants were taken in 500ml of volumetric flask and added 200 ml of 10% $\text{CH}_3\text{CO}_2\text{H}$ and $\text{C}_2\text{H}_5\text{OH}$ respectively. The mixtures were covered with aluminum file and stood for 4 hours at room temperature. The mixtures were filtered with filter paper and allowed to heat in water bath to become concentrated till it reaches to 1/3 of its original volume. Now the concentrated NH_4OH is added to form precipitation. The precipitates were collected and washed with dilute NH_4OH and filtered. The precipitates/residues of alkaloids were dried and weighed.

Tannins

Spectrophotometer method was used for the determination of quantity of tannin. 10gm plant samples of *Hibiscus rosa sinensis*, *Emblica officinalis* and *Acacia catechu* were mixed in 50ml of distilled water in separate beaker and filtered in 50ml conical flasks respectively. Then, 5ml filtered solution of each plant was mixed with 2ml of 0.1M FeCl_3 , 0.1M HCl and 0.008M $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ in test tubes. The absorbance of each sample was measured at 395nm wavelength by using spectrophotometer.

Saponins

20gm of each tested samples (plants) were mixed with 100ml of 20% ethanol in a volumetric flask and heated in water bath for 3 hours. The mixtures were filtered and re-extracted with 20% ethyl acetate. The extracts were heated in a water bath at 90°C and reduced up to 40ml. Now each solution (40ml) was put into 250ml separating funnels. Then 20 ml of (CH₃CH₂)₂O was added to each funnel and was shaken well. The (CH₃CH₂)₂O layer was disposed while the aqueous layer was recovered. This purification process was repeated again and again. Now the purified solutions were shifted to clean separating funnels and reacted with 60 ml of n-C₄H₉OH and these combined solutions were washed twice with 20ml of 5%NaCl. Now the solutions were heated in water bath till the whole solvents were evaporated. After that each sample was dried in the oven and weighted.

Flavonoids

10gm samples of all selected plants were reacted with methanol and aqueous (100ml) at room temperature. After the filtration of the samples, all these samples were transferred to a water bath in test tubes and the solvents were evaporated till dryness. All the dry materials of each sample were collected and weighted.

Pharmacological properties of *Hibiscus rosa-sinensis*, *Emblica officinalis* and *Acacia catechu*

Hibiscus rosa sinensis

Hibiscus rosa sinensis also known as rose mallow, shoeblack plant, China rose, Chinese hibiscus and Hawaiian hibiscus contain anthocyanin, anthocyanidine, gentisic acid,

lauric acid, niacin, malvalic acid, glycoside, quercetin and riboflavin¹⁵. This plant possess antioxidant¹⁶, anti-inflammatory¹⁵, cardioprotective, anticancer, anti-diabetic and hepatoprotective activity¹⁷.

Emblica officinalis

Emblica officinalis synonyms *Dichelactina nodicaulis* Hance, *Emblica arborea* Raf, *Phyllanthus emblica* Linn, *Phyllanthus glomeratus* Wall and *Cicca emblica* Kurz also known as amla, emblic, emblic myrobalan, Indian gooseberry, myrobalan, amalaki and Malacca tree is one the most important medicinal plant having various therapeutic effects due to its bioactive natural chemicals. These bioactive natural chemicals are emblicanin A, emblicanin B, punigluconin, pedunculagin³, tannins, emblicol, rutin, phyllembelic acid, curcuminoides and phyllembelin¹⁸ having antioxidants¹⁹, antitumor, cardiac tonic, diuretic, astringent, diuretic, refrigerant, laxative, diuretic, liver tonic, hair tonic, cardiac tonic, stomachic, antitussive, antipyretic, and anti-inflammatory activities^{20, 21}. Traditionally the plant is used for the treatment of dysentery, diarrhea, stomachic, warts and eczema²².

Acacia catechu

Acacia catechu family *fabaceae* is restorative medicinal plant. Phytochemical constituents of this plant are catechin, catechutannic acid, epicatechin, Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercitrin and quercetin. These chemical constituents have various pharmacological effects like antibacterial, antiviral, antioxidant, anti-fungal, antipyretic,

anti-inflammatory, hypoglycaemic, antidiarrhoeal, haemostatic, astringent, digestive and hepatoprotective activity^{22, 23}. It is used for cough, ulcers, boils, bleeding piles, uterine hemorrhages, dyspepsia, chronic bronchitis, gingivitis, sore throat, hoarseness of voice and tonsillitis. (20-21)

Table 2: Phytochemicals of *Emblica Officinalis* in different Extracts

S. No	Test for phytochemicals	Petroleum ether extract	Chloroform extract	Water/Aqueous extract
1	Alkaloids (Dragendroff's test)	-	-	+
2	Flavonoids (Shinoda test)	-	-	-
3	Tannins (Ferric chloride test)	-	-	-
4	Sterol and triterpenoids (Salkowaski test)	-	-	+
5	Carbohydrate (Benedict reagent test)	+	+	+
6	Glycosides (Borntrager's test)	-	-	-
7	Saponin	-	-	-
8	Fat and oils (Saponification test)	-	-	+

[Present (+), Absent (-)]

Table 3: Phytochemicals of *Acacia catechu* in different Extracts

S. No	Test for phytochemicals	Petroleum ether extract	Chloroform extract	Water/Aqueous extract
1	Alkaloids	-	-	+
2	Carbohydrate	-	-	+
3	Glycosides	-	-	-
4	Sterol and triterpenoids	-	-	+
5	Flavonoids	-	-	+
6	Tannins	-	-	-
7	Saponin	-	-	-
8	Fat and fixed oils	-	-	-

[Present (+), Absent (-)]

Table 4: Phytochemicals of *Hibiscus rosa sinensis* in different Extracts

S. No	Test for phytochemicals	Petroleum ether extract	Chloroform extract	Aqueous extract
1	Flavonoids	-	-	+
2	Fat and fixed oils	-	-	+
3	Alkaloids	-	-	+
4	Sterol and triterpenoids	+	-	+
5	Saponin	-	-	+
6	Tannins	-	-	-
7	Carbohydrate	-	-	-
8	Glycosides	-	-	-

[Present (+), Absent (-)]

Table 5: Quantitative analysis of chemical constituents of *H.rosa-sinensis*, *E.officinalis* and *Acacia catechu*

S. No	Botanical name	Alkaloids	Saponins	Phenols	Flavonoids	Tannins
1	<i>H.rosa-sinensis</i>	8.3±0.18	0.39±0.13	0.50±0.14	0.675±0.09	2.4±0.10
2	<i>E.officinalis</i>	10.2±0.14	0.50±0.12	0.21±0.02	0.035±0.17	1.4±0.07
3	<i>Acacia catechu</i>	12.03±0.16	0.51±0.18	0.27±0.05	0.715±0.20	2.01±0.10

CONCLUSION

Biologically active compounds of medicinal plants have promising effects against various ailments. Attention of research on the medicinal plants increases day by day because of development of new molecules having best therapeutic effects and least side effects. The current study reveals that *Hibiscus rosa-sinensis*, *Embllica officinalis* and *Acacia catechu* contain various phytochemicals alkaloids, gentisic acid, phenols, anthocyanidine, tannins, pedunculaginrutin, niacin, flavonoids, saponins, phyllembelin, emblicanin B, emblicol, gossypetin, catechutannic acid, anthocyanin, lauric acid, malvalic acid, glycoside, quercetin, epicatechin, phyllembelic acid, emblicanin A, punigluconin, curcuminoides, catechin tetramer, catechin, quercitrin, Atzelchin, dicatechin, gallochin, phlobatannin, kaempferol and quercitin with a wide range of pharmacological activities against different diseases.

Acknowledgement

We are extremely thankful to Dr Aadil Ameer Ali for providing chemicals and laboratory facilities for this research work.

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