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Qualitative and Quantitative Analysis of Phytochemicals and Pharmacological Value of Some Dye Yielding Medicinal Plants

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ABSTRACT

The attractive colours and fragrance produced by the plants is due to specific phytochemicals present in them. They may be tannins, flavonoids, glycosides, saponins, steroids and alkaloids. *Emblica officinalis*, *Acacia catechu*, *Acacia concina* and *Hibiscus rosa-sinensis*, are medicinal dye yielding plants belong to different families. The qualitative analysis carried out for these plants showed that tannins, saponins, flavonoids, terpenoids and alkaloids are present in all the plants except phlobatannins that is only present in *Acacia catechu*. The pet ether and chloroform extract of *Emblica officinalis* does not show potential for oil and fat components where as all the extract of *Emblica officinalis* showed positive test for carbohydrates. The identification of colouring chemical constituents of natural products together with their therapeutic properties is discussed.

Key words: Medicinal dye yielding plants, phytochemical constituents.

INTRODUCTION

The worldwide demand for natural dyes is nowadays of great interest due to the increased awareness on therapeutic properties of natural dyes in public. Natural dyes are derived from naturally occurring sources such as plants, insects, animals and minerals. Organic pigments are large and often complex organic molecules responsible for the different colours of plants and foods. Besides giving the vegetable their characteristic colour, they are also responsible for critical plant functions. The different variations of colours are due to combinations of pigments. A spectrum of beautiful natural colours ranging from yellow to black exists in the above sources. These colours are exhibited by various organic and inorganic molecules (pigments) and their mixtures are due to the absorption of light in the visible region of 400-800 nm. This absorption of light depends on the structure or constituents of the colouring pigment/ molecules contain various chromophores present in the dye yielding plant to display the plethora of colours[1]. The use of natural products together with their therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs[2].Among the all natural dyes, plant-based

pigments have wide range of medicinal values. Many of the plants used for dye extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity. Natural dyes are not only used to impart colour to an infinite variety of materials such as textiles, paper, wood etc. but also they are widely used in cosmetic, food and pharmaceutical industry. They have wide range of medicinal importance in pharmaceutical industry.

MATERIALS AND METHODS

Collection of plant samples

The flowers of *Hibiscus rosa sinensis* are collected from the university campus and pods of *Acacia concina, Emblica officinalis* and *Acacia catechu* was taken from the Phagwara market.

Processing of plant samples

The dried pods of *Acacia concina* and *Emblica officinalis* were taken from the market, crushed to powder form, and then taken in the soxhlet apparatus for hot extraction with organic solvents. The fresh flowers of *Hibiscus rosa sinensis,* collected from the university campus, were shade dried and soxhlated with methanol, and then separated with different organic solvents according to their polarity gradient. The powdered form of *Acacia catechu* was poured into the two separate beakers containing methanol and pet ether, for cold extraction.

Preparation of aqueous extract of plant sample

The aqueous extract of each plant sample is prepared by soaking10 g of powdered samples in 200 ml of distilled water for 12 h. The extracts are then filtered using filter paper or Whattman filter paper.

Preparation of Organic extract of plant sample

The selected plants are dried in air. The dried and crushed material of these plants was extracted with the help of different solvents of varied polarity like petroleum ether, chloroform, ethyl acetate and alcohols at their boiling points successively. The various crude extracts so obtained were analyzed qualitatively for various components..

Phytochemical analysis

Chemical tests are performed on different organic and aqueous extracts of each plants with standard methods for various secondary metabolites

Qualitative analysis of phytochemical constituents

Test for Alkaloids

Take some petroleum ether extract of *Acacia catechu* in a test tube and add 2-3 drops of Dragendroff's reagent (potassium bismuth iodide solution) appearance of pale yellow colour indicates that absence of alkaloids in this extract. Again perform the same experiment with chloroform, methanol and water extract in another test tube appearance of pink colour indicates that absence of alkaloids in these extracts. Appearance of brown colour indicates that presence of alkaloids.

Test for Tannins

Take some petroleum ether extract of this plant and add few drops of ferric chloride solution in it, pale yellow colour appears, in chloroform extract yellow colour appears in methanol and water extract appearance of brownish black colour indicates the absence of tannins in all these extracts.

Test for Flavonoids

Take some petroleum ether extract in a test tube then add few fragments of magnesium ribbon and after this add concentrated hydrochloric acid drop wise, absence of colour means absence of flavonoids in this extract, same test is repeated with chloroform extract of *Acacia* and also absence of colour. In methanol and water extract there is appearance of reddish colour shows the presence of flavonoids in these extracts.

Test for Sterols and Triterpenoids

Treat the petroleum ether and chloroform extract of *Acacia* with few drops of conc. sulphuric acid then shake well, no colour appears in these two indicates disappearance of sterols and triterpenoids. Further same treatment of methanol and water extract was done and appearance of reddish colour in the lower layer means presence of steroids appearance of yellow colour in the lower layer indicates presence of triterpenoids.

Test for Carbohydrates

Treat the all four extract with Benedict's

reagent (alkaline solution of cupric citrate complex) absence of red precipitates boiling on water bath indicates the absence of carbohydrates.

Test for Fats and Oils

Treat all the four extract with 0.5N alcoholic potassium hydroxide with a drop of phenolphthalein separately and heat on water bath for 1-2 hours. There is formation of soap or partial neutralization means fixed oils and fats are present in these extracts.

Test for Glycosides

Take all the four extract of this plant separately then add dilute sulphuric acid into it. The solution was boiled and filtered. The filtrate was cooled, and then adds 2-3 drops of benzene. The solution was shaken well, organic layer got separated. After this add equal volume of ammonia solution to the organic layer, ammonical layer did not turn pink, which indicates absence of glycosides in these extracts.

Test for Saponins

Take all the four extracts separately in test tubes and add some water into them and shake well no persistent foam is formed which indicates absence of saponins**.**

RESULTS AND DISCUSSION

Quantitative analysis on phytochemical constituents

Phenols

The quantity of phenols is determined using the spectrophotometer method. The plant sample is boiled with 50 ml of $(CH_3CH_2)_2O$ for 15 min. 5 ml of the boiled sample is then taken into 50 ml flask, and 10 ml of distilled water is added. After the addition of distilled water, 2 ml of NH_4OH solution

Table 1(1): Test for Phytoconstituents for different extract of *Emblica officinalis*

Test for various secondary metabolites	Pet. ether extract	Chloroform extract	Methanol extract	Water extract
Test for alkaloids: (Dragendroff's test)	Absent	Absent	Present	Present
Test for tannins: (Ferric chloride test)	Absent	Absent	Absent	Absent
Test for flavanoids: (Shinoda test)	Absent	Absent	Present	Absent
Test for steroids and terpenoids: (Salkowaski test)	Absent	Absent	Present	Present
Test for carbo-hydrates: (Benedict reagent test)	Present	Present	Present	Present
Test for glycosides:(Borntrager'stest)	Absent	Absent	Absent	Absent
Test for oils and fats:	Absent	Absent	Present	Present
Test for saponins	Absent	Absent	Absent	Absent
Test for sugars(Bromine water test)	Absent	Absent	Absent	Absent

Table 1(2): Test for Phytoconstituents for different extract of *Acacia catechu*

Test for various secondary metabolites	Pet. ether extract	Chloroform extract	Methanol extract	Water extract
Test for alkaloids: (Draggendorff's test)	Absent	Absent	Present	Present
Test for tannins:(Ferric chloride test)	Absent	Absent	Absent	Absent
Test for flavonoids: (Shinoda test)	Absent	Absent	Present	Present
Test for fats and fixed oils:(Saponification test)	Absent	Absent	Absent	Absent
Test for steroids and terpenoids: (Salkowaski test)	Absent	Absent	Present	Present
Test for carbo-hydrates: (Benedict reagent test)	Absent	Absent	Present	Present
Test for glycosides:(Borntrager'stest)	Absent	Absent	Absent	Absent
Test for saponins	Absent	Absent	Absent	Absent
Test for free sugars (Bromine water test)	Absent	Absent	Absent	Absent

Table 1(3): Test for Phytoconstituents for different extract of *Acacia concina*

Test for various secondary metabolites	Pet. ether extract	Chloroform extract	Methanol extract	Water extract
Test for alkaloids: (Dragendroff's test)	Absent	Absent	Present	Present
Test for tannins: (Ferric chloride test)	Absent	Absent	Absent	Absent
Test for flavonoids: (Shinoda test)	Absent	Absent	Present	Present
Test for steroids and terpenoids: (Salkowaski test)	Absent	Absent	Absent	Absent
Test for carboo-hydrates: (Benedict reagent test)	Absent	Absent	Absent	Absent
Test for glycosides: (Borntrager'stest)	Absent	Absent	Absent	Absent
Test for oils and fats:	Absent	Absent	Present	Present
Test for saponins	Absent	Absent	Absent	Absent
Test for sugars: (Bromine water test)	Absent	Absent	Absent	Absent

Table 1(4): Test for Phytoconstituents for different extract of *Hibiscus rosa-sinensis*

Test for various secondary metabolites	Pet. ether extract	Chloroform extract	Methanol extract	Water extract
Test for alkaloids: (Dragendroff's test)	Absent	Absent	Present	Present
Test for tannins: (Ferric chloride test)	Absent	Absent	Absent	Absent
Test for flavonoids: (Shinoda test)	Absent	Absent	Present	Present
Test for steroids and terpenoids: (Salkowaski test)	Present	Absent	Present	Present
Test for carboo-hydrates: (Benedict reagent test)	Absent	Absent	Absent	Absent
Test for glycosides: (Borntrager'stest)	Absent	Absent	Absent	Absent
Test for oils and fats:	Absent	Absent	Present	Present
Test for saponins	Absent	Absent	Present	Present
Test for sugars:(Bromine water test)	Present	Present	Present	Present

Table 2: Quantitative analysis of phytochemical constituents (%)

S.No	Plants	Phenols	Alkaloids	Tannins	Saponins	Flavonoids
1	*E.officinalis*	0.23±0.03	11.2±0.16	1.1±0.05	0.55±0.13	0.037±0.19
2	*Acacia catechu*	0.30±0.06	11.3±0.15	2.1±0.11	0.53±0.20	0.718±0.23
3	*Acacia concina*	0.53±0.12	10.2±0.15	2.3±0.11	0.51±0.20	0.719±0.23
4	*H.rosa-sinensis*	0.52±0.16	8.5±0.21	2.0±0.09	0.41±0.15	0.680±0.10

and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ is added to the mixture. The sample is made up to the mark and left for 30 min to react for colour development and measured at 505 nm wavelength using a spectrophotometer.

Alkaloids

5 g of the plant sample is prepared in a beaker and 200 ml of 10% CH_3CO_2H in C_2H_5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture then filtered and the extract is allowed to become concentrated in a water bath til it reaches 1/4 of the original volume. Concentrated NH_4OH is added until the precipitation is complete. The whole solution is allowed to settle and the precipitate is collected and washed with dilute NH_4OH and then filtered. The residue is alkaloid, which is then dried and weighed.

Tannins

Quantity of tannins is determined by using the spectrophotometer method. 0.5 g of plant sample is weighed into a 50 ml plastic bottle. 50 ml of distilled is added and stirred for 1 h. The sample is filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtered sample is then pipetted out into test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)_6.3H_2O$. The absorbance of the sample is measured with a spectrophotometer at 395 nm wavelength within 10 min.

Saponins

The plant samples were ground and 20 g of each plant sample is put into a conical flask and 100 ml of 20% C_2H_5OH is added to the plant sample. The sample is heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts are reduced to 40 ml over a water bath at about 90°C. The concentrated is then transferred into a 250 ml separating funnel and 20 ml of $(CH_3CH_2)_2O$ is added to the extract and vigorously shaken. The aqueous layer is recovered while the $(CH_3CH_2)_2O$ layer is discarded and the purification process is repeated. 60 ml of n-C_4H_9OH is added and the combined n-C_4H_9OH extracts is washed twice with 10 ml of 5% NaCl. The remaining solution is then heated in a water bath and after evaporation; the samples are dried in the oven to a constant weight.

Flavonoids

10 g of plant sample is repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until a constant weight

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

Emblica officinalis (*Phyllanthus emblica* Linn.), also known as amla, the foremost plants utilized from ancient time to date, has been used in Ayurveda the ancient Indian system of medicine. According to the Ayurveda, *Charak Samhita* and *Sushrut Samhita*, amla is regarded as the "best among rejuvenative herbs", and the "best among the sour fruits"[3]. It is distributed in tropical and subtropical areas of china, India, Indonesia and the Malay Peninsula. It is highly vulnerable due to its magnificient vitamin C content[4].The fruits act as antioxidants[5] immunomodulatory agents[6], cytoprotective against chromium[7], protects against oxidative stress in ischemic reperfusion injury[8] etc. The fruits of emblica contain a wide variety of phenolic compounds, such as tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides and emblicol[9]. The fruit have a broad range of therapeutic effects including antitumour[10] and induction of apoptosis[11]. The root, bark and leaves are also used for the treatment of indigestion, diarrhea, dysentery, eczema and warts[12]. Traditionally, the fruit is beneficial as an astringent, cardiac tonic, diuretic, laxative, liver tonic, diuretic, refrigerant, stomachic, restorative, antipyretic, anti inflammatory, hair tonic and digestive medicine[13-14].

Acacia catechu Willd (Family: *Fabaceae* and subfamily: *Mimosoideae*.) is highly valuable plant, useful internally as well as externally. The bark, wood, fruits, gum and flowering tops, all parts of *Acacia catechu* are used for medicinal purpose[15]. It is used to cure bleeding in gums for its powerful astringent and antioxidant activities[16-17]. The decoction of resin is an effective gargle in case of sore throat, cough and hoarseness of voice and tonsillitis[19]. The paste is beneficial, externally, in skin diseases and wounds[20]. The bath of its decoction is an effective panacea for various skin affection. In stomatitis, halitosis, dental caries and cavities, halitosis, dental caries and cavities, khadira (*Acacia catechu* in Sanskrit is known) is used with great benefit, due to vitiation of kapha doshas. It dries up the mucous secretions and regains the taste sensation.The extracts of *Acacia catechu* exhibits various pharmacological effects like antipyretic, antiinflammatory, antidiarrhoeal, hypoglycaemic, hepatoprotective, antioxidant and antimicrobial activities[21-22].

Shikakai

Acacia concinna is an important medicinal plant belonging to family Acaciaceae, grown commercially in India and Far East Asia. The drugs

used in indigenous system of medicines like Ayurveda in India has about 18,000 species of angiosperms, of which about 3,000 species are considered as important sources of medicinal and aromatic chemical compounds. *Acacia concinna'*s fruits have been used for hair care in India for centuries. The plant parts used as dry powder or the extract of the bark, leaves or pods. It is a common shrub found in jungles throughout India[23]. The bark contains high levels of Saponins, which are foaming agents that are found in several other plant species[24]. Some of the important medicinal plants having antimicrobial activity and also possess secondary metabolites[25]. The saponin of the bark has spermicidal activity against human semen[26]. In aqueous state it has a low pH.

Hibiscus rosa sinensis

Hibiscus a genus of family Malvaceae is herbs, shrubs and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to posses various medicinal properties. *Hibiscus rosa sinensis* (Malvaceae) is widely cultivated in the tropics as an ornamental plant. Chinese hibiscus is the English name of *Hibiscus rosa sinensis*[27]. The plant has been used as powerful antioxidant[28], anti-inflamatory[29], cardioprotective[30], antidiabetic, hepatoprotective and anticancer[31] agent. *H. rosa sinensis* contain numerous phytochemicals such as quercetin glycoside, riboflavin, niacin, anthocianin, anthocianidine, malvalic acid, gentisic acid and lauric acid[29].

CONCLUSION

The secondary metabolites are different in various organic extracts of all the four plants. Their qualitative analysis revealed their appearance where as their quantitative analysis give almost approximate idea for their quantity present. The pharmacological study showed their application part.

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