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Phytochemical and Spectrophotometric Analyses of the Hill Toon, *Cedrela Serrata* Royle Methanolic Leaves Extract and Its Fractions

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***Abstract* – The present research was conducted to analyze the phytochemicals and spectrophotometry of the hill toon, *Cedrela serrata* Royle methanolic leaves extract and its fractions. Various phytochemicals such as alkaloids, carbohydrates (monosaccharides and disaccharides), flavonoids, steroids (phytosterols), saponins, tannins, phlobatannins, terpenoids, cardiac glycosides and anthraquinones were present in the methanolic extract (ME) as well as in fractions. Whereas monosaccharides and phlobatannins were weakly present in ME and aqueous fraction (AQF), while they were absent in case of n-butanol fraction (NBF) and ethyl acetate fraction (EAF). ME contained 5 compounds with first 2 compounds were present in high concentration while the remaining 3 were present in negligible concentration. They were absent in both AQF and EAF, whereas NBF showed the same compound with high concentration. In overall, the spectrophotometric analysis confirmed that good separation of the compounds had occurred among different fractions. The results concluded that the *C. serrata* extracts showed significant phytochemicals and spectrophotometric properties. It is suggested that further research should be needed on *C. serrata* to find out its active biological component.**

***Keywords* – *Cedrela Serrate*, Fractions, Leaves Extract, Phytochemicals, Spectrophotometric Analysis.**

I. INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications (Baris et al., 2006). It has been estimated that 14-28% of higher plant species are used as medicine. About 74% of pharmacologically active plant derived components were discovered after following up on ethno-medicinal use of the plants. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, the most notably synergistic effects and poly-pharmacological application of plant extracts (Gibbons, 2003). Scientists are on their way to achieve some plants derived compounds to control of diseases. Natural plant products are biodegradable, exhibit structural diversity and complexity and rarely contain halogenated atoms (Duke et al., 2000). Plant extracts containing compounds such as terpenes, steroids, alkaloids, phenolic and cardiac glycosides (Duke, 1990) are known to affect insect behaviour and can function as deterrents to insect pests (Mancebo et al., 2000).

Plants are potent biochemists and have been components of phyto-medicine since times immemorial. Plant based natural constituents can be derived from any part of the plant (Gordon and David, 2001). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (Wink, 1999). Hence, there is a constant need for new and effective therapeutic agents. The research on the medicinal plants should be extended with the identification of the active principles in the plants. Scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. It is after such evaluations that they can be approved for use in the primary health care. Such research activities could also lead to the development of more effective and new drugs (Parekh et al., 2006).

Phytochemicals, alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin, cardiac glycoside and phenolic compounds were reported by Edeoga et al. (2005) in African cabbage, *Cleome rutidosperma* DC.; ivory, *Emilia coccinea* (Sims.); Maxican fireplant, *Euphorbia heterophylla* L.; cutleaf ground cherry, *Physalis angulata* L.; licorice weed, *Scopania dulcis* L.; common wire weed, *Sida acuta* Burm; pinkroot, *Spigelia anthelmia* L.; rough leaf false vervain, *Stachytarpheta cayennensis* (Rich.) and coatbuttons, *Tridax procumbens* L.; Uma (2009) in the barks of peepul tree, *Ficus religiosa* L. and bargad, *Ficus bengalensis* L.; Majaw and Moirangthem (2009) in the mata ajam, *Clerodendron colebrookianum* Walp; leaves and ginger, *Zingiber cassumunar* Roxb. Rhizomes; EL-Kamali (2009) in the tropical white weed, *Ageratum conyzoides* L.; sessile joyweed, *Alternanthera nodiflora* Br.; mimosa, *Ambrosia maritime* L.; balloon vine, *Cardiospermum halicacabum* L.; false daisy, *Eclipta prostrate* L.; denseflower knot weed, *Polygonum glabrum* L.; pulicaria crispa, *Pulicaria undulate* (L); soja bean, *Solanum dubium* F.; white potato, *Sonchus cornatus* Hochst and sow thistle, *Sonchus oleraceus* L. Perveen et al. (2012) in the hill toon, *Cedrela serrata* Royle used for the treatment of various diseases, insecticidal, antibacterial, antioxidant, anti-inflammatory and DNA protection activities etc.

A spectrophotometer is the specific device, which measures the absorption of a monochromatic light beam by a sample and added reagent. The objective of this laboratory exercise is to become familiar with a typical spectrophotometric analysis and examine the effect of an interfering substance. The inorganic analyte being considered in this particular analysis is phosphate and the interfering substance is arsenic (Kool et al., 2007).

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Cedrela serrata belongs to family Meliaceae, comprising about 50 genera and 1400 species, forms a large botanical family of mostly pantropical distribution. The genus *Cedrela* is included in the tribe Cedreleae of the sub-family Swietenioideae. The specific name, *serrata* comes from serra (a saw), referring to the toothed leaf-margins (Ram et al., 2000). In the present research , *C. serrata* has been used to determine phytochemicals and spectrophotometric analyses.

II. General Experimental Procedures

The present research was carried out in the Molecular Biology Laboratory, Department of Biochemistry, Hazara University, Mansehra and Quaid-i-Azam University, Islamabad, Pakistan.

Preparation of extract of Cedrela serrata

The hill toon, *Cedrela serrate* Royle leaves were collected from Balakot, Mansehra, Pakistan and identified by the experts, rinsed and kept at 25±2 °C under shade until drying. Such weight of leaves was ground by an electric blender to obtained 350 g fine powder which was dissolved and homogenized in methanol (80%; 2 L). It was kept for evaporating methanol for 4 week, filtered twice and concentrated on rotary evaporator to obtained methanolic extract (ME) in the form of dark green gummy residue (30 g) (Naqvi and Perveen, 199 and 1993; Perveen et al., 2012). Its 25 g was fully dissolved in d-H_2O. Then it was partitioned in their respective solvent based on increasing polarity, i.e., n-butanol (NBF), ethyl acetate (EAF) and aqueous (AQF) fractions (Rashid et al., 2009). They were used for the phytochemicals and spectrophotometric analyses.

Phytochemical analysis

The methanolic extract and its fractions were screened for the presence of phytochemicals using standard methods of analysis. Following tests were performed (Harborne, 1993; Sofowara, 1993; Trease and Evans, 2002; Edeoga et al., 2005 and Parekh et al., 2006):

1. Alkaloids

The following reagents were used for alkaloids test: a) Mayer's reagent was prepared when 0.355 g of magnesium chloride ($MgCl_2$) in 60 ml of distilled water (d-H_2O) and 5 g of potassium iodide (KI) in 20 ml of d-H_2O, two solutions were mixed and volume was made up to 100 ml with d-H_2O; b) Dragendorff's reagent was prepared when solution A was made up of 1.7 g of basic bismuth nitrate [$Bi(NO_3)_3$] and 20 g of tartaric acid [$HO_2CCH(OH)CH(OH)CO_2H$] in 80 ml of d-H_2O and solution B was made up of 16 g of potassium iodide (KI) in 40 ml of d-H_2O. Solutions A and B were mixed in ratio of 1:1. Each plant extract/fraction (0.5-0.6 ml) was mixed with 8 ml of 1% hydrochloric acid (HCl), warmed and filtered. Two ml sample of the filtrate was treated separately with (a) Mayer's reagent and (b) Dragendorff's reagent. Turbidity or precipitation indicated the presence of alkaloids.

2. Carbohydrates

Fehling's test was performed for detection of carbohydrates**.** Half ml of each extract/fraction was mixed in d-H_2O and filtered it. One ml of each Fehling A (7% copper sulphate ($CuSO_4$) in d-H_2O) and B [25% sodium hydroxide (NaOH) solution containing 35% potassium-sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) were mixed them and boiled on spirit lamp for 2 min (no change in color). Then 1 ml of each extract/fraction was added and boiled again for 1 min. Red precipitate indicated the presence of carbohydrates.

a) Monosaccharides

One ml of the extract/fraction was mixed with 2 ml d-H_2O and then filtered. Three ml of the filtrate and 2 ml Bardford reagent [13.3 g of copper-acetate {$Cu(C_2H_3O_2)$}] in 200 ml of d-H_2O plus 1.8 ml glacial acetic acid (CH_3CO_2H) were mixed and heated in water bath for 3-5 min. Red precipitate indicated the presence of monosaccharides (glucose and galactose).

b) Disaccharides

Three ml of filtrate mixed with 2 ml of Bardford reagent and heat for 7-12 min in water bath. Red color precipitate indicated the presence of disaccharides.

3. Flavonoids

Two methods were performed for flavonoids: a) Half ml of extract/fractions were suspended in d-H_2O and mixed with heat and filtered. In filtrate, 5 ml of dilute nitric acid (HNO_3) solution was added followed by addition of drops of concentrated sulphuric acid (H_2SO_4), yellow color indicated presence of flavonoids; b) Half g of extract was suspended in ethyl acetate ($H_8C_4O_2$) and heated over water bath and filtered. One ml dilute HNO_3 solution was added in the filtrate and shaken. A yellow color indicated the presence of flavonoids.

4. Steroids

Half ml of each extract/fraction was shaken with few drops of petroleum ether ($CH_3CH_2OCH_2CH_3$), to remove the coloring materials. The residue was extracted with 10 ml chloroform ($CHCl_3$) and dried the $CHCl_3$ layer over anhydrous sodium sulfate [$Na_2(SO_4)$]. Five ml of $CHCl_3$ layer was mixed with 0.25 ml of acetic anhydride $(CH_3CO)_2O$ and then 2 drops of concentrated H_2SO_4 was added. Different colors indicated the presence of sterols [sterol (green to pink) and phytosterol (pink to purple)] in each extract/fraction.

5. Saponins

Two tests were performed for saponins: a) Frothing: each extract/fraction (0.1 ml) and 5 ml of d-H_2O were boiled and filtered, then shaken. Appearance of froth was indicated the presence of saponins; b) Emulsion: each extract/fraction (0.1 ml) was suspended in d-H_2O and filtered. Olive oil was added in it, then emulsion appeared which indicated the presence of saponins.

6. Tannins

Each extract/fraction (0.25 ml) was dissolved in d-H_2O (10 ml) and filtered, then aqueous ferric chloride ($FeCl_3$) (1%) was added in it. An intense green, purple, blue or black color indicated the presence of tannins.

7. Phlobatanins

Half ml of the each extract/fraction was suspended in d-H_2O, boiled and filtered. Aqueous HCl (1%) was added in it. Red precipitate indicated the presence of phlobatanins.

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8. Terpenoids

Terpenoids were tested through Salkowski's test. Five ml of each extract/fraction was mixed in 2 ml of $CHCl_3$, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

9. Cardiac glycosides

For the presence of cardiac glycosides, the Keller-Killani's test was performed. Five ml of aqueous suspension of each extract/fraction was treated with 2 ml glacial acetic acid ($C_2H_4O_2$) and added 1 drop of $FeCl_3$. A brown ring of interface was indicated the presence of deoxy-sugar characteristics of cardenolides. A violet ring observed below the brown ring, while in $C_2H_4O_2$ layer a greenish ring observed just gradually throughout thin layer.

10. Anthraquinones

Half ml of each extract/fraction was shaken with 10 ml of benzene (C_6H_{12}) and filtered. Then 10% ammonia solution (NH_3+H_2O; 0.5 ml) was added in it and shaken well. Violet color in the layer indicated the presence of anthraquinones.

Spectrophotometric analysis

Spectrophotometric analysis of *C. serrata* methanolic leaves extract and its fractions was carried out using UV-VIS spectrophotometer (200-600 nm; DAD 8453, Agilent, Tokyo, Japan). In this experiment, stock solution of test samples were prepared by dissolving 5 mg of each extract/fraction in 5 ml methanol. Further dilution was prepared as 50 µl/5 ml CH_3OH. This dilution was tested for spectrophotometric analysis. Spectrophotometer was calibrated by standard curve using methanol as a blank. Wavelength was adjusted in UV-visible range in order to get peaks and valleys of different compounds present in each extract/fraction. Based on these peaks, different color lines of each extract/fraction showed different composition with excellent separation from ME. Hence, spectrophotometric analysis measured the concentration of a compound present in each extract with different combinations to separate each compound from ME and its fractions (Skoog et al., 2007).

III. RESULTS

Methanolic extract (ME) of hill toon, *Cedrela serrata* Royle was prepared and partitioned into 3 fractions, i.e, n-butanol (NBF), ethyl acetate fraction (EAF) and aqueous fraction (AQF). They were tested for the phytochemical and spectrophotometric analyses.

Phytochemicals analysis:

Phytochemicals analysis of the *C. sarrata* showed the presence of different classes of the compounds in ME and its fractions (Table 1). Alkaloids test by Dragendorff's and Mayer's reagents indicated strong presence (+++) in ME and all the fractions of *C. sarrata*. Fehling test indicated that the presence of carbohydrate(s) in the extract and all fractions. Bardford's test for monosaccharides showed that ME and AQF contained significant (+++) and weak presence (-), respectively, while absent in case of EAF and NBF. The same test for disaccharides indicated that ME as well as EAF, NBF and AQF had the highest (+++) contents. The ME and all fractions contained strong presence (+++) of flavonoids as well as steroids. Saponins test by frothing and emulsion clearly indicate their strong presence (+++) in ME and all the fractions of *C. sarrata*. Tannins were also strongly present (+++) in ME and all the fractions of *C. sarrata*, whereas phlobatannins were weakly present in ME while absent (-) in case of EAF and NBF, but AQF showed moderate (++) presence. Terpenoids, cardiac glycosoids, phytosterols and anthroquinones indicated their strong presence (+++) in ME and all fractions of *C. serrata*.

Table 1: Phytochemical analysis of methanolic extract (ME) and fractions of *Cedrela serrata* Royle.

S.No.	Phytochemicals	Tests	Extract or fractions ME*	EAF*	NBF*	AQF*
1.	Alkaloids	Dragendorff's	+++*	+++	+++	+++
		Mayer's	+++	+++	+++	+++
2.	Carbohydrates	Fehling test*1	+++	+++	+++	+++
		Bardford's test*2	++*	-*	-	+*
		Bardford's test*3	+++	+++	+++	+++
3.	Flavonoids		+++	+++	+++	+++
4.	Steriods (phytosterols)		+++	+++	+++	+++
5.	Saponins	Frothing test	+++	+++	+++	+++
		Emulsion test	+++	+++	+++	+++
6.	Tannins	FeCl3 test	+++	+++	+++	+++

7.	Phlobatannins	+	-	-	++
8.	Terpenoids	+++	+++	+++	+++
9.	Cardiac glycosides				
	Keller-Kiliani test	+++	+++	+++	+++
10.	Anthraquinones	+++	+++	+++	+++

*+++: strongly; ++: moderately; -: absent; +: weakly positive; NBF: n-butanol fraction; EAF: ethyl acetate fraction; AQF: aqueous fraction; Fehling test*1: for the presence of carbohydrates; Bardford's test*2: for monosaccharides; Bardford's test*3: disaccharides.

Spectrophotometric analysis

Spectrophotometric analysis of ME and its fractions of *C. serrata* confirmed the purity of fractions from ME. The solvent system used was methanol. Methanol was also used as blank for finding of spectrum in UV-visible range (200-600 nm). Different peaks and valleys showed different composition of compounds in same volume. The presence of 5 compounds at 284, 327, 464, 485 and 582 nm in ME, with first 2 compounds showing maximum absorbance (Figure 1), which means that they were present at high concentration while the remaining 3 were present in negligible concentration as they showed very low absorbance. The spectrum of the fractions showed that the compounds which gave high absorbance at 284 and 327 nm were absent in both AQF and EAF whereas, NBF showed the same compound in high concentration. The EAF also gave high absorbance at 410 nm whereas, no absorbance was observed in ME, which means that there were certain compounds of ME, which were now trying to concentrate in EAF. In overall, the spectrophotometric analysis confirmed that good separation of the compounds had occurred among different fractions (Figure 1).

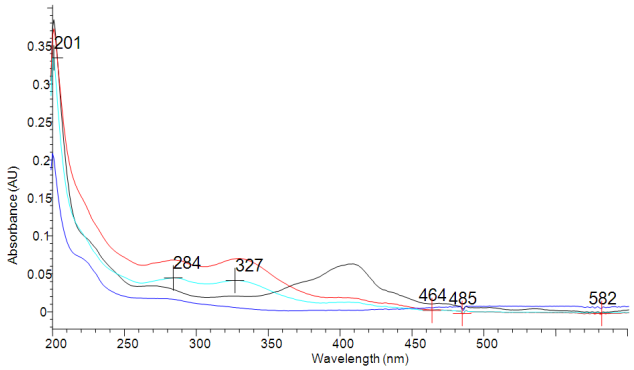

Fig.2. Spectrophotometric analysis of the hill toon, *Cedrela serrata* Royle: methanolic extract (ME: light blue line) shows 284 and 327 nm: high absorbance; 464, 485 and 582 nm: low absorbance, ethyl acetate fraction (EAF: black) shows 268 and 410 nm: high absorbance; n-butanol fraction (NBF: red) shows 284 and 327 nm: high absorbance and aqueous fraction (AQF: dark blue) 229 and 270 nm: high absorbance.

IV. DISCUSSION

The bio-products are more significant for pest control and as herbal medicines due to their less harmful effects as compared to synthetic drugs in the laboratory. Methanol was used as solvent for leaves extract of the hill toon, *Cedrela serrata* Royle. The NBF, EAF and AQF of *C. serrata* leaves extract were studied for antioxidant and DNA protection activities (the DPPH free radical scavenging and free radicals induced DNA damage assays) (Perveen et al., 2012) as well as for possible insecticidal assays like toxicity and residual effects (unpublished), for repellency of red flour beetle, *Tribolium castaneum* (unpublished) and for phytochemical and spectrophotometric properties of active biological component(s) (unpublished) (at the present). Phytochemical and spectrophotometric properties of *C. serrata* were analyzed for 10 different classes of phytochemicals (Table 1). The results showed that strong presence of alkaloids, carbohydrates (monosaccharides and disaccharides), flavonoids, steroids (sterols and phytosterols), saponins, tannins, phlobatannins, terpenoids, cardiac glycosides and anthraquinones in the ME as well as in fractions. Whereas phlobatannins and monosaccharides were weakly present in ME and AQF, while absent in case of EAF and NBF (at the present).

Flavonoids are a group of phytochemical found in varying amounts in foods and medicinal plants, which showed to exert potent antioxidant activity against the superoxide radical. The highest antioxidant and DNA protection activities of ME and NBF of *C. serrata* might be due to the presence of flavonoids in these fractions. The presence of flavonoids is evident that this plant is full of antioxidant compound and it is true from the results of antioxidant assay. Saponins had been found to be potentially useful for the treatment of hypercholesterolemia, which suggested that saponins might be acting by interfering with intestinal absorption of cholesterol reported by Malinow et al. (1977a and b). Trease and Evans (1978) reported that tannins had been widely used as an application to sprains, bruises and superficial wounds. Steroids were present in all fractions of *C. serrate*, as steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones reported by Okwu (2001). The presence of terpenoids in *C. serrata* is reported in the present research and it also reported by other researchers and this plant was widely used in herbal medicine by Hayashi et al. (1993). The presence of cardiac glycosides in *C. serrata* thus gave clear indications that these plants can be used in the treatment of hypertension as reported by Olaleye and Mary (2007).

Adeloye (2007) reported after preliminary phytochemical screening of the crude extract of caesarweed, *Urena lobata* L. that the presence of phenolic compounds (as shown by strong reaction with ferric chloride) but showed no reaction with Draggendoff's reagent indicating the absence of alkaloids in the plant extract. At the present, it showed that alkaloids were present in significant amount in methanolic extract of *C. serrata*. The difference may be due to difference in compound present in 2 different plants, which were used in both studies.

Kumaraswamy et al. (2008) showed that phytochemical analysis of Hamalayan birch, *Betula utilis* Don revealed the presence of carbohydrates and alkaloids in petroleum ether, chloroform, methanol, ethanol and water extract. Whereas glycosides and steroids were present in methanol and petroleum ether, respectively, while flavonoids, tannins, saponins, triterpenoids, protein, resins, fixed oils and fats were absent in all exracts of *B. utilis*. In the present research, phytochemicals screening of *C. serrata* reported that the significant presence of phenolic compounds, alkaloids, flavonoids, tannins, saponins, terpenoids, phytosterols and anthraquinones in ME, NBF, EAF and AQF whereas phlobatannins were absent from NBF and EAF.

Uma et al. (2009) reported preliminary phytochemical analysis of ME of the barks of banyan tree, *Ficus religiosa* L.; *F. bengalensis* L. and showed the presence of carbohydrates, flavonoids, amino acids, steroids, sopanins and tannins. In the present research, ME of *C. serrata* leaves exhibited flavonoids, phytosterols, steroids, sopanins, tannins, carbohydrates and alkaloids. This indicated almost similar results.

Majaw and Moirangthem (2009) showed that *Z. cassumunar* Roxb. rhizomes and East Indian Glory Bower, *Clerodendrum colebrookianum* Walp leaves possessed almost all the important secondary metabolites. *Z. cassumunar* rhizomes showed positive results for all the constituents analyzed, except for one, i.e., anthraquinones while *C. colebrookianum* leaves showed positive results in the constituents analyzed except for glycosides, phlobatannins and anthraquinones. In present research, *C. serrata* leaves showed positive results for all the constituents analyzed, except for phlobatannins and monosaccharides. This indicated that 3 plants used in both studies contains significant amount of the most constituents.

Spectrophotometric analysis of ME and fractions of *C. serrata* confirms the purity of fractions from crude extract. The solvent system used was methanol. Methanol was also used as blank for finding of spectrum in UV-visible range (200-600 nm). Different peaks and valleys showed different composition of compounds in same volume. The presence of 5compounds at, 284, 327, 464, 485 and 582 nm in ME, with the first 2 compounds showed maximum absorbance, which means that they were present at high concentration while the remaining 3 were present in negligible concentration as they showed very low absorbance. The spectrums of the fractions showed that the compounds gave high absorbance at 284 nm and 327 nm. Which are absent from both AQF and EAF, whereas NBF showed the same compounds at further higher conc. EAF also gave high absorbance at 410 nm, whereas no absorbance was observed in ME which means that there are certain compounds of ME which were trying to concentrate in EAF. In overall, the spectrophotometric analysis confirmed that good separation of the compounds had occurred among the different fractions.

Aromdee et al. (2005) performed spectrophotometric determination of total lactones in king of bitters, *Andrographis paniculata* Nees by using dinitrobenzoic acid (C7H4O6N2) and potassium hydroxide (KOH) solutions as colour forming agents. The absorbance of the solution was determined at 536 nm. The result revealed that method was suitable for determination of total lactones in *A. paniculata* especially for the local herbal industries for a rapid result. In the present research, spectrophotometric analysis of ME and its fractions of *C. serrata* was prepared by methanol as blank for finding of spectrum in UV-visible range (200-600 nm). Results confirm the purity of fractions from ME. Differences lie due to different methods used.

V. Conclusion and Recommendation

It is concluded from above tests and assays that *C. serrata* contains certain phytochemicals, which proved to be biologically safe and human friendly. It showed broad-spectrum significant action. It could be used as pharmacologically active medicine and drug discovery purposes.

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