



## Research Article

### QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES IN AQUEOUS EXTRACT OF *CLERODENDRUM SERRATUM*

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#### ABSTRACT

The aim of this study was to quantify the secondary metabolites of aqueous extract of root of *Clerodendrum serratum*. The root of *Clerodendrum serratum* was extracted by using water. The yield of aqueous extract was 10%w/w. The total phenolic, flavonoid, saponin and alkaloidal content determined by spectrophotometric method. The amount of total phenolic in extracts was determined in comparison with the gallic acid, whereas total flavonoid, saponin, alkaloid content was estimated corresponding to rutin, diosgenin and atropine respectively. The phenolic, flavonoid, saponin and alkaloidal content of aqueous root extract of *Clerodendrum serratum* were found to be 134.4mg/g, 67.67mg/g, 38.2mg/g and 12.67mg/g respectively. The presence of such important metabolites in *C. serratum* indicates its therapeutic importance in man and animal.

**Keywords:** *Clerodendrum serratum*, Phenolic, Saponin, flavonoid and alkaloid

#### INTRODUCTION

World plant biodiversity is the largest source of herbal medicine and still about 60–80 % world population relies on plant based medicines which are being used since the ancient ages as traditional health care system. Plants are biosynthesizing large amount of secondary metabolites through various metabolic pathways. These are very complex and having unique carbon skeleton structures and many of them of high interest to the pharmaceutical and chemical industry. Secondary metabolites derived from primary metabolites showing several biological activities which totally depend on the complex structure of the metabolite and also on chirality.<sup>1</sup> Such natural compounds isolated from the plant sources provide a great degree of broad spectrum of the modern drugs as we use today. Plant derived pharmaceuticals represent a large market value; about 25% of today's pharmaceuticals contain at least one active ingredient of plant origin.<sup>2</sup>

Out of all the secondary metabolites phenols are the major group metabolites comprising of both medicinal and nutritional properties.<sup>3</sup> This class of metabolite responsible for chemopreventive properties due to its antioxidant activity, anticarcinogenic and anti-inflammatory activity.<sup>4</sup> They are also associated with the inhibition of atherosclerosis. It is also reported that the anti cancer activity is associated with inhibition of growth factor and signalling pathways.<sup>5</sup> Flavonoids, another class of secondary metabolites having a benzo- $\gamma$ -pyrone ring consider under the polyphenolic group. These compounds possess greater biological activity solely depends on its chemical nature, degree of hydroxylation, polymerization or substitutions or conjugations etc.<sup>6</sup> Due to its antioxidant activity flavonoids are having vital role as dietary supplement and as well as medicinal.<sup>7,8</sup> Studies proven the antioxidant activity of flavonoid even more effective than the vitamin C, E and

carotenoids.<sup>9</sup> The metabolites saponins categorize under glycosidic group of compound comprising of either triterpene or steroid as aglycon part and hexose and/or uronic acid as glycon part. Saponins are popular for its haemolytic activity and foaming properties. The one of the vital importance of saponin is steroidal hormone synthesis and also possess significant anti-cancer, anti-tumorigenic activity.<sup>10</sup> Alkaloids are another important class of secondary metabolites having minimum one nitrogen atom either within the heterocyclic ring or outside the ring. Alkaloids are having wide range of physiological effects and pharmacological effects, including antibiotic,<sup>11</sup> antifungal, anti bacterial, anticancer,<sup>12-14</sup> narcotics, poisons, stimulants,<sup>15</sup> antimicrobial, anti-HIV and antiparasitic activities etc.<sup>16</sup> The other important metabolites present in plants are terpenoids, glycosides, lignin, coumarins, anthraquinones which also shows versatile therapeutic activity.

*Clerodendrum serratum* belonging to family Verbenaceae an Ayurvedic plant to Indian system which is rich in such metabolites. It is a deciduous shrub widely distributed in Western ghats.<sup>17</sup> In Ayurveda, the plant is well known as Bharangi (Sanskrit) and commonly known as Blue Glory (English). As per the traditional claims the root is the potential source of drug for ailments such as asthma, body ache, bronchitis, cholera, dropsy, eye diseases, fever, inflammations, malaria, ophthalmia, rheumatism, snakebite, tuberculosis, ulcers and wounds.<sup>18-20</sup> Quantification of metabolites becoming an urgent need which scientifically demonstrates the use of any plant traditionally. The current focus of the current study was to ascertain secondary metabolites of aqueous extract of the root of *Clerodendrum serratum* such as total phenolic, flavonoid, saponin and alkaloids, which has not been investigated yet.

## MATERIALS AND METHODS

### Collection and authentication of plant material

Roots of *Clerodendrum serratum* L. (Verbenaceae) was purchased from the local market of Faizabad Uttar Pradesh, India. The taxonomical recognition was done from Department of Agronomy, Acharya Narendra Dev Agriculture Technical University, Dist. Faizabad, Uttar Pradesh.

### Preparation of extract

The collected brown colored roots (100gm) were extracted (decoction) with water (500ml) at a temperature not exceeding 110°C for 2hrs. The extract was allowed to cool and filtered & the filtrate was lyophilized. The yield of obtained dried extract was 10%w/w.

### Chemicals

Total phenolic content, total flavonoids content, saponin content and alkaloid content were determined by using several chemicals/reagents which has been mentioned in appropriate section of materials and methods. All the chemicals were analytical grade and obtained from Sigma Aldrich Co., St. Louis, USA. Before doing the following specific quantification tests the extract was evaluated for the presence of preliminary phytochemicals.<sup>21</sup>

### Spectrometric Quantification of Total Phenolic Content

Folin Ciocalteu reagent was used to evaluate the amount of total phenolic content. Gallic acid was used as a standard for the determination of phenolic content and it was expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 1mg/ml of plant extract was prepared in methanol and 0.5ml of sample was placed in to test and it is mixed with 2.5ml of Folin Ciocalteu reagent. The Folin Ciocalteu reagent was previously diluted 10 fold. The mixture is added to 2ml of 7.5% sodium carbonate. The tubes were allowed to stand for 30 minutes covered with parafilm at room temperature before taking the absorbance 760 nm. Reading was taken in triplicate. Folin-Ciocalteu reagent is reactive to reducing substance including polyphenols. This is basically a colour reaction produces blue coloration which was measured spectrophotometrically.<sup>22</sup>

### Spectrophotometric Quantification of Total Flavonoid Content

Total flavonoids were measured by a colorimetric assay.<sup>23,24</sup> An aliquot of sample (1mg/ml) or standard solutions (10-50µg/ml) of rutin was added to a 75 µl of sodium nitrite (NaNO<sub>2</sub>, 5%)

solution, and mixed properly. After 5 – 6 minute, 0.15 mL aluminium chloride (AlCl<sub>3</sub>, 100 g/L) was added. Again, after waiting a couple of times (approximately 5 min), 0.5 mL of sodium hydroxide (NaOH, 4%) was added. The final volume was kept at 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All samples were analyzed in three replications.

### Spectrometric Quantification of Total Saponin Content

To prepare standard curve 50, 62.5, 75, 87.5, 100, 112.5 and 125.5 µg/ml of the standard saponin solutions was placed into test tubes and the volumes were made up with aqueous methanol (80%, 0.25 mL). Stock standard solution of diosgenin was prepared by dissolving 10 mg of it in a mixture of 16mL methanol and 4 mL of distilled water. To the each aliquot vanillin reagent (8%, 0.25 mL) and sulphuric acid (72% v/v, 2.5 mL) were added. After properly mixing the tubes were transferred to a 60°C water bath. After 10 mins, the tubes were cool in the ice cold water bath for 3 – 4 mins. The absorbance was measured at 544 nm against the reagent blank. The sample was prepared by dissolving 0.1 g of freeze dried sample in aqueous methanol (80%, 0.1 mL). From that 0.25 mL of aliquot was taken for spectrophotometric determination at 544 nm.<sup>25</sup>

### Spectrometric Quantification of Total Alkaloid Content

For the standard curve of atropine, total 5 different concentrations were used. 100 ppm solution was initially prepared from atropine (1 mg in 10 ml of distilled water). From this stock solution, exactly 0.5, 1, 1.5, 2 and 2.5 ml of atropine solutions was transferred to five different separating funnel. To each and individual funnels, 5 ml of phosphate buffer (pH4.7) and 5ml of Bromocresol green (BCG) solution was added and mixed vigorously. The formed complex mixture is extracted with chloroform. The chloroform fraction was collected in a 10ml of volumetric flask and make up the volume with chloroform. Absorption at a wavelength of 470 nm of each flask was measured and calibration graph was drawn.

For the preparation of the sample the plant extract (1mg/ml) was dissolved in 2N HCl and then filtered. The pH of the extract was adjusted to neutral with 0.1 N NaOH. One ml of this solution transferred to a separating funnel and to that mixture 5 ml of Bromocresol green solution along with 5 ml of phosphate buffer was added and mixed properly. The formed mixture was extracted further with chloroform (5ml) and transferred to 10 ml of volumetric flask and make up the volume with the same solvent. The absorbance of the complex in chloroform was measured at 470 nm.<sup>26,27</sup>

Table 1: Standard gallic acid (concentration vs absorbance)

S. No.	Conc. (µg/ml)	Absorbance Gallic acid
1.	10	0.110
2.	20	0.176
3.	30	0.247
4.	40	0.298
5.	50	0.326

Table 2: Standard rutin (concentration vs absorbance)

S. No.	Conc. (µg/ml)	Absorbance Rutin
1.	10	0.135
2.	20	0.151
3.	30	0.165
4.	40	0.177
5.	50	0.201

Table 3: Standard diosgenin (concentration vs absorbance)

S. No.	Conc. (µg/ml)	Absorbance
		Diosgenin
1.	50	0.172
2.	62.5	0.189
3.	75	0.207
4.	87.5	0.279
5.	100	0.321
6.	112.5	0.384
7.	125.5	0.418

Table 4: Standard atropine (concentration vs absorbance)

S. No.	Conc. (µg/ml)	Absorbance
		Atropine
1.	50	0.122
2.	100	0.159
3.	150	0.188
4.	200	0.207
5.	250	0.229

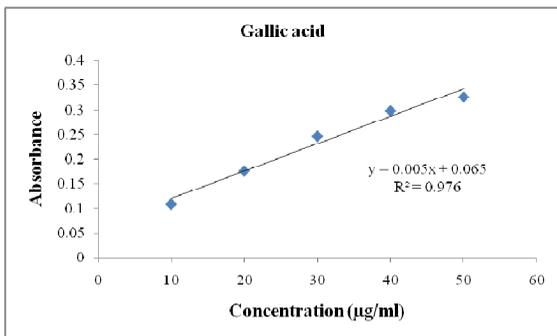


Figure 1: Calibration graph for gallic acid standard

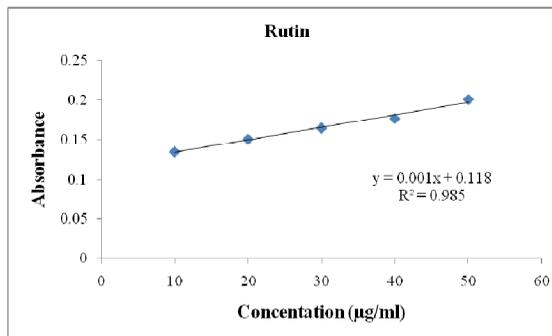


Figure 2: Calibration graph for rutin standard

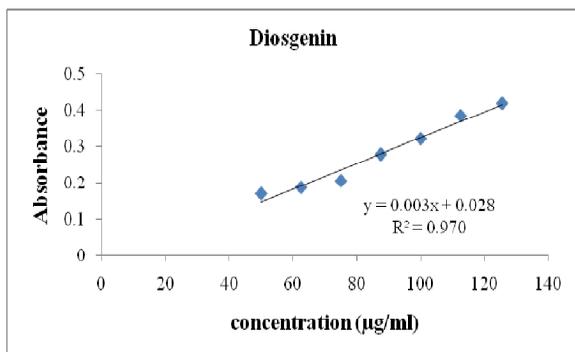


Figure 3: Calibration graph for diosgenin standard

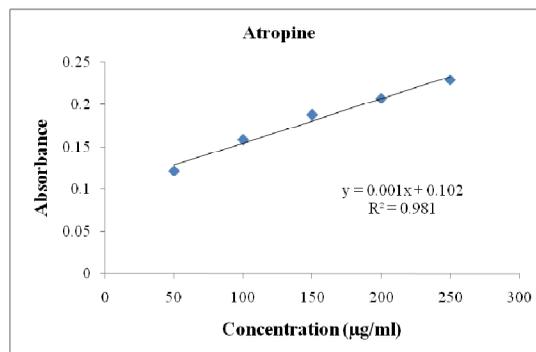


Figure 4: Calibration graph for atropine standard

**RESULTS AND DISCUSSION**

The activity of any kind of phytomedicine is principally depending upon the presence of phytochemicals. In this study, we have quantified the presence of total phenolic, total flavonoid, total saponin and total alkaloidal content. The content of total phenol in the aqueous extract of *C. serratum* was obtained from gallic acid standard curve. The observed absorbance and calibration curve and of gallic acid is tabulated and figured in table no 1 and figure 1 respectively. Line of regression from gallic acid graph was used for estimation of phenol content in the aqueous extract of *C. serratum*. From standard curve of gallic acid line of regression was found to be  $y = 0.005x + 0.065$  and  $R^2 = 0.976$ . The total phenolic content expressed as mg gallic equivalents (GAE) per gram of sample (mgGAE/g). The total phenolic content in extract was  $134.4 \pm 0.917$  in mg/g equivalent of gallic acid.

Flavonoid is one of the important phenolic compounds existing in nature.<sup>28</sup> The total flavonoid content for aqueous extract of *C. serratum* was measured with the aluminium chloride colorimetric assay where rutin was used as standard. The absorbance of rutin is tabulated in table 2. The rutin solution of

concentration ranges from 10 - 50 µg/ml with a regression coefficient ( $R^2$ ) = 0.985 (Figure 2). The slope (m) and intercept found to be 0.001 and 0.118 respectively. The total flavonoid content (mg of rutin equivalent/ g dry material) of the extract was found to be  $67.67 \pm 3.512$ .

In the current study, we also quantify the saponin spectrophotometrically at 544 nm by using diosgenin as standard. Table 3 is shows the absorbance of diosgenin at different concentration. The total saponin content is expressed in mg/g equivalent of diosgenin. The total saponin in the extract was calculated from the regression equation obtained from the calibration curve of diosgenin ( $y = 0.003x + 0.028$ ,  $R^2 = 0.970$ ) (Figure 3). The total saponin in the extract was found to be  $38.2 \pm 1.311$  in mg/g equivalent of diosgenin.

There are several methods reported for quantification of alkaloid like HPLC<sup>29</sup> gas chromatography<sup>30</sup>, electrochromatography<sup>31</sup> fluorimetry<sup>32</sup> etc., but in the current work we used the most easy and rapid, accurate spectroscopic method. The alkaloidal content was examined in plant extracts was expressed in terms of atropine equivalent as mg of AE/g of extract. The absorbance of atropine at different concentrations is showing in table 4. The

standard curve equation  $y = 0.006x - 0.003$ ,  $R^2 = 0.997$  showing in figure 4. The Total Alkaloid content (mg/g equivalent of Atropine) present in the aqueous extract of *C. serratum* was  $12.67 \pm 3.786$ . The presence of these photochemicals indicates its therapeutic importance. The medicinal properties<sup>17-20</sup> of *C. serratum* might be due to above phytochemicals.

## CONCLUSION

Estimation is the foundation which provides prospect to concret the use of certain traditional values. From the current study, we can conclude that aqueous extract of *C. serratum* have a significant amount of secondary metabolites like phenolic compounds, flavonoids, saponins and alkaloids. Further, the presence of these secondary metabolites with proven potential as bioactive class can be further explored for isolation of specific compound for the natural drug development process

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