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ISOLATION AND PHYTOCHEMICAL CHARACTERIZATION OF ALKALOIDS FROM *CLEOME GYNANDRA LINN.* AERIAL PARTS

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ABSTRACT

The plant *Cleome gynandra L*. (Cappariaceae) is commonly known as "*Hurhur", "Tilvan"* in Marathi. Traditionally the whole plant is use in the treatment of Tumor, anti-inflammatory, malaria, piles, and rheumatism. The presence of alkaloid was tested qualitatively by dragon drops method. Then it was subjected to quantitative estimation by UV-spectroscopy method. The concentration of 20, 40, 60, 80, 100µg/ml. The absorbance at UV- spectroscopy was observed in 470 nm. Then the isolated alkaloids were subjected for IR spectroscopy. The ranges of IR 3233.23 (N-H st), 2943.49 (C-H st), 2806.78 (C-H st), 2148.88(C-D st), 1644.57(C=C st), 1440.43(CH_3 sy), 1346.97(CH_2 st), 1193.15(C-H st). The total alkaloids were isolated from methanol extract of *Cleome gynandra*. The preliminary phytochemical screening was performed and shows the presence of alkaloid, Flavonoid, phenols, carbohydrate and tannins. The isolated alkaloids confirmed by the preliminary phytochemical test of alkaloid. Further the concentration of alkaloid was determined using UV-spectroscopy method and the Total flavonoid and phenolic content was also determined.

KEYWORDS: Cleome gynandra Linn, Isolation of alkaloids, Alkaloid content, Flavonoid content and Phenolic content.

INTRODUCTION

Cleome gynandra Linn. Is used as a medicinal plant and can be found in all over world .It grows in road sides and in open grass lands. In India it is never cultivated but grows spontaneously. Different species of *Cleome* can be found in all states of India. The medicinal application of this plant is also described in Medicinal plant of India. According to WHO

more than 20,000 species has been used by word population. The plant is use the poor classes as a vegetable, leaves contain high amount of vitamin a and c with calcium and iron. The medicinal property of the plant is because of presence of bioactive substances in the plant and most important of include Alkaloids, Flavonoids, phenols. *C. gynandra linn.* Has proven many medicinal properties such as antimicrobial, anticancer, antioxidant, anti-inflammatory, immunomodulatory activity.

The purpose of study was identifying and quantifying phytochemicals and isolation of the alkaloids from the extract of *Cleome gynandra linn.*

2. MATERIALS AND METHODS

2.1. Plant material

In the present study, the plant of *Cleome gynandra linn.* Were collected from Gunjalwadi, Sangamner, Maharashtra. India in the month of July 2019 and Plant would be authenticated from Botany Department, in-Sangamner Nagarpalika Arts, D.J.Malpani Commerce and B.N. Sarda Science College Sangamner.

2.2. Preparation of extract

200gm dried *Cleome Gynandra linn* plant powder was taken. That defatted with petroleum ether with soxhlet extraction method. Then extraction with 2000ml methanol by using soxhlet extraction method up to the three cycles. Further extraction the Methanolic extract was filter through double filter paper or what Mann filter paper. The filtrate was collected in a n sterile flask, and the extracts were concentrated and then dried by using rotary rotary evaporator.

2.3. Preliminary phytochemical screening

The phytochemical screening of secondary metabolites present in Methanolic extract of Ariel part of *C. gynandra Linn*. Was examined by standard method.

2.4 Quantitative analysis phytochemicals

2.4.1. Estimation of alkaloids

Preparation of solutions: Bromocresol green solution was prepared by heating 69.8 mg Bromocresol green with 3 ml of2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric

acid in 1 L distilled water). Atropine standard solution was made by dissolving 1mg pure atropine, in 10 ml distilled water.

Preparation of standard curve: Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separatory funnels. Then, add 5 ml pH 4.7 phosphate buffers and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

2.4.2. Estimation of flavonoids

The total flavonoid content was determined using calibration curve of Quercetin. The solution of Quercetin 100 µg/ml concentrations were prepared in methanol and further dilutions of (20, 40, 60, 80, 100 µg/ml) were prepared in methanol. The Stock solution of plant extracts was prepared by dissolving 10 mg of the extract transferred to 10 ml volumetric flask and made up the volume with methanol. The 10% aluminium chloride and 1M potassium acetate were prepared using distilled water. The assay was determined using 0.5 ml of each extract stock solution and each dilution of standard Quercetin taken separately in test tubes. To each test tube 1.5 ml methanol, 0.1ml aluminium chloride solution, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. The absorbance of this reaction mixture was recorded at 415 nm on UV spectrophotometer against the blank containing water instead of sample. The TFC was calculated using standard calibration curve of Quercetin. The total flavonoid content was calculated as Quercetin equivalents (mg/g) of dried extract.

2.4.3. Estimation of phenolic compounds

The total phenolic contents of the Methanolic extract of Cleome gynandra linn. was estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi.25 The calibration curve was plotted by mixing 1 ml aliquots of 5, 10, 15, 20, 25, and 35 mg/ml Gallic acid solutions with 5.0 ml of Folin Ciocalteau reagent and 4.0 ml of sodium carbonate solution . The absorbance was measured after 30 min at 765 nm. For the Methanolic extracts, 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 1 h, the absorbance was measured to determine the total phenolic contents in extract using the formula,

$C = C1 \times V/m$

Where,

C = total phenolic content in mg/g, in GAE (Gallic acid equivalent),

C1 =concentration of Gallic acid established from the calibration curve in mg/ml,

V= volume of extract in ml,

m = the weight of the plant extract in g.

2.5 Isolation of alkaloids

100gm dried Methanolic extract of *Cleome gynandra linn*. Was taken in that extract add the tartaric acid solution (Acidification) up to the pH 5. Then the acidified extract was taken in that add the chloroform (natural compound) was separated. Then the mixture basify with sodium carbonate up to the pH 9-10. Then add the chloroform then separation of the two fractions. One is aqueous fraction and second is chloroform fraction in that chloroform fraction 1º, 2º, 3º alkaloids was present and the aqueous fraction Quaternary amine and alkaloid N-oxide was present.

2.5.1 UV spectral study

The UV spectrum of the Methanolic extract was recorded using a Shimadzu spectrometer. The UV spectrum of the Methanolic extract of *Cleome gynandra linn*. was plotted according to the light absorbed as a function of the wavelength, and the drug showed a maximum absorption (l max), which is characteristic of a particular drug and aids the identification of herbal drugs.

2.5.2 IR spectral study

The IR spectrum of the Methanolic extract of *Cleome gynandra linn*. Was determined in FTIR spectrometer, Spectro lab. The IR spectrum of the drug was recorded, and the major bands were noted.

3. RESULT AND DISCUSSION

3.1 Preliminary phytochemical screening

The preliminary phytochemical was done to detect presence of various chemical constituents by performing chemical organic confirmatory tests for alkaloids, glycosides, tannins and phenolic compound, flavonoids, proteins.

Table 1: Preliminary Phytochemical Screening of Methanolic Extract.

Name of the phytoconstituents	Methanolic extract
Carbohydrates	+
Amino acid	+
Proteins	+
Steroids	_
Alkaloids	+
Saponin glycosides	+
Triterpenoids saponin	+
Flavonoids	+
Phenol/ Tannins	+
Phytosterols & triterpenoids	+
Coumarins	_

3.2 Quantitative analysis of phytochemicals

The alkaloids, flavonoids and phenols content of Methanolic extract of *C. gynandra Linn.* The total alkaloid content was calculated with the help of the graph shown in **Figure 1**, and the standard curve equation was y =0.0013x - 0.006, where R2 =0.9957. The total alkaloid contents in the Methanolic extracts were calculated to be 356.28 mg/g.

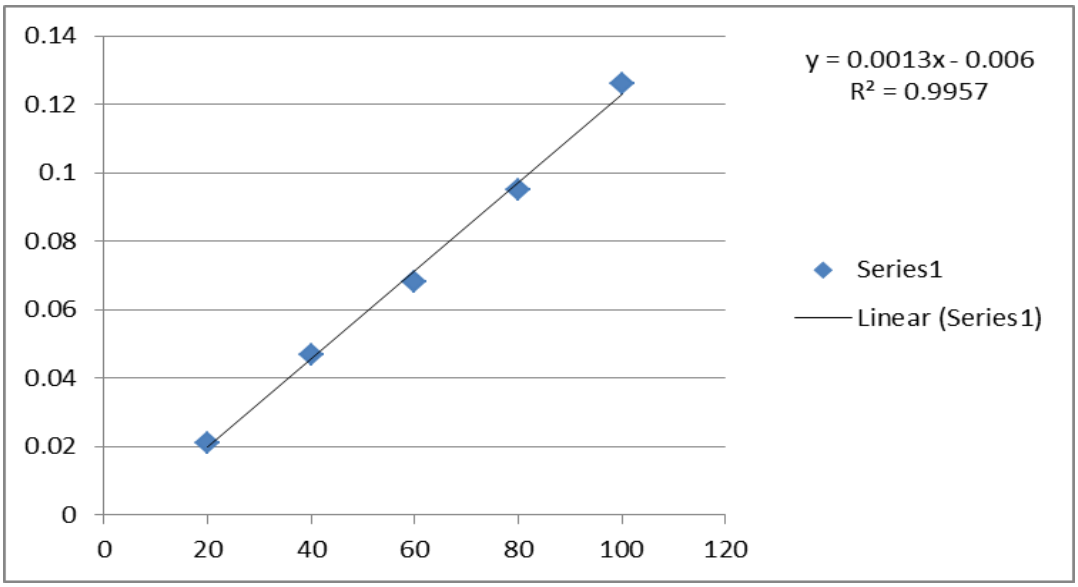

Fig. 1: Standard calibration curve of atropine.

The total flavonoid contents were determined using the Quercetin equivalent in mg/g of the extract. The total flavonoid content was calculated with the help of the graph shown in Figure 2, and the standard curve equation was y =0.0037x - 0.0034, where R2 =0.9934. The total flavonoid contents in the Methanolic extracts were calculated to be 396.28 mg/g.

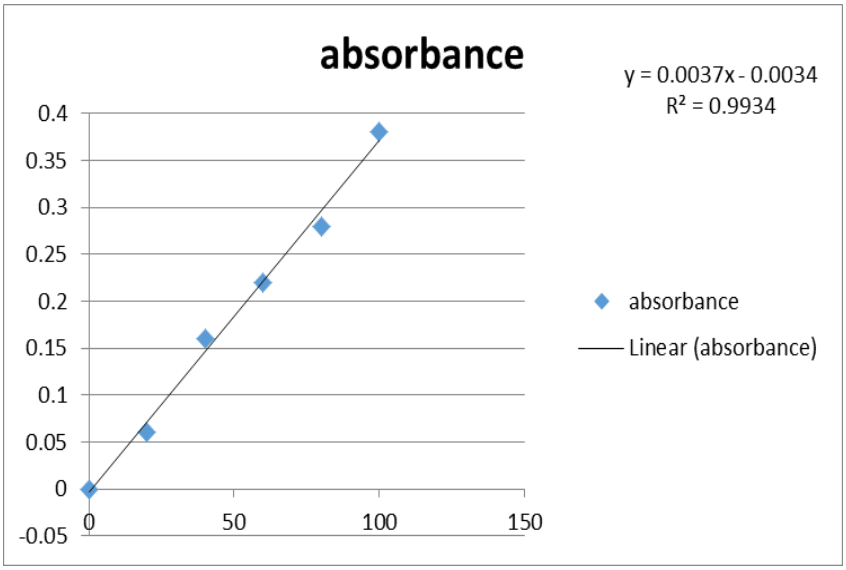

Fig. 2: Standard calibration curve of Quercetin.

The total phenolic contents were determined using the Folin Ciocalteau method and terms of the Gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in **Figure 3**, and the standard curve equation was y =0.0045x+0.0076, where R2 =0.9939. The total phenolic contents in the Methanolic extracts were calculated to be 326.28 mg/g, respectively.

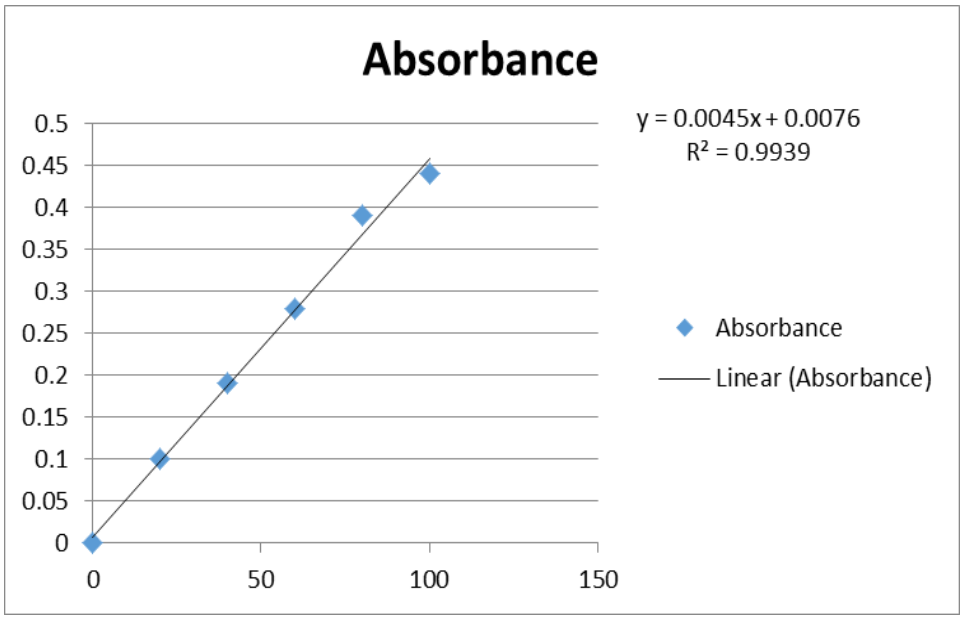

Fig. 3: Standard calibration curve of Gallic acid.

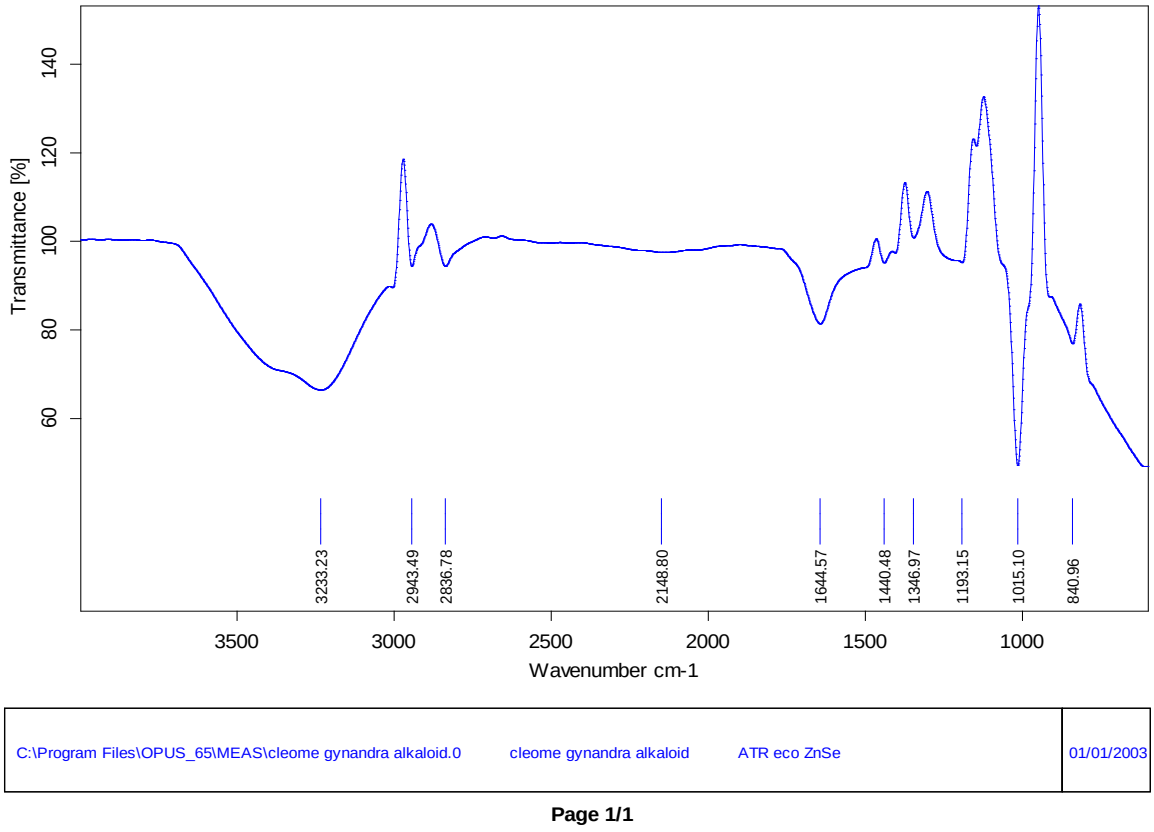

Fig. 4: IR spectral study of isolated alkaloids from *cleome gynandra linn*.

3.3. Isolation of alkaloid

% Isolated alkaloid: 0.5% yield.

3.3.1. UV spectral study

The UV spectrum of the isolated alkaloids of *Cleome gynandra linn*. Plant was plotted in terms of the light absorbed as a function of the wavelength, and the drug showed a characteristic wavelength for the maximum absorption (l max) at 470 nm.

3.3.2 IR spectral study

FTIR spectral analysis confirmed the presence of various chemical functional groups in the isolated alkaloids. The major bands were observed at 3233.23, 2943.49, 2806.78, 2148.88, 1644.57, 1440.43, 1346.97, 1193.15, 1015.10, 840.96.

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