

## Proximate and Spectroscopic Analysis of *Centrosema pubescens* Benth

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### ABSTRACT

*Centrosema pubescens* commonly known as 'Butterfly pea' is a perennial herb under the family of Fabaceae. The plant is used as forage, manure and used as one of the key ingredients in broiler chicken feed as it contains rich of proteins and minerals. As the plant has not been explored much for their detailed chemical characteristics and medicinal value, the present study scientifically documents the complete proximate analysis, physico-chemical nature, phyto-chemical characteristics and spectroscopic analysis of various solvent fractions of *C. pubescens*. The study gives the insight of chemical constituents of plant and recommends the researchers for taking biopotency studies to explore its medicinal value.

**Key Words:** *Centrosema pubescens*, butterfly pea, proximate analysis, spectroscopy studies

### INTRODUCTION

*Centrosema pubescens* commonly known as 'Butterfly pea' is a legume in the family of Fabaceae. This plant is native to Central and South America and cultivated in tropical regions as forage for livestock [1]. *C. pubescens* is a perennial herb that can reach a height of 45 cm. The root system can reach up to 30 cm in depth, frequently in association with Rhizobium, nitrogen-fixing bacteria. Leaves are trifoliolate, leaflets up to 6.5 cm long, elliptic or oblong-lanceolate 1-7 cm long, 0.5-4.5 cm wide, rounded at the base and mostly acuminate at the apex, slightly hairy, especially on the lower surface. Inflorescences several-flowered, in leaf-axils; flower cluster stalk 2.5-5.5 cm long. Standard petal is up to 4 x 3.5 cm, mauve-purple with a white or yellowish central band often flanked by darker purple lines, velvet hairy outside with a short spur outside just above the claw. Seeds transversely oblong to squarish with rounded corners, 4-5 mm x 3-4 mm x 2

mm in size, brownish-black, mostly with dark mottles [2-4].

*Centrosema pubescens* is grown as a cover crop as it naturally suppresses weeds and widely used as forage. The plant is well adapted to tropical conditions and altitudes below 600m from sea level. Due to richness of protein and minerals the *C. pubescens* leaves are used as one of the ingredients in cattle diet and broiler chickens. This plant is also used as green manure crop in rubber, coconut and oil palm plantation [5]. Like other N-fixing legumes, Centro is soil improver and the amount of nitrogen fixed by Centro is average of 259 kg/ha with ranged from 126 – 395 kg/ha as reported [6-8].

### TAXONOMY [2,3]

Kingdom : Plantae  
Unranked : Angiosperms  
Unranked : Eudicots  
Unranked : Rosids  
Division : Tracheophyta

Order : Fabales  
Family : Fabaceae  
Tribe : Phaseoleae  
Genus : Centrosema  
Species : *C. pubescens*  
Binomial name: *Centrosema pubescens*  
Benth.  
Common name: Butterfly pea  
Synonyms: *Centrosemamolle*, *Bradburya pubescens* (Benth.) Kuntz,  
*Centrosemaferrugineum* A. Rich

## MATERIALS AND METHODS

### Plant Collection and Authentication

The fresh plant materials were collected from the Western ghat regions of Palakkad District, Kerala, India. The collected plant materials were authenticated at Pharmacy Department of National Ayurveda Research Institute for Panchakarma (NARIP) Cheruthuruthy. The plant material was processed and herbarium was prepared as per the standard guidelines. Voucher specimen is maintained in the Department of Biochemistry and Pathology, NARIP for ready reference.

### Extraction [9]

The air-dried, powdered plant leaf material was extracted by various methods using different solvents. The hot solvent extraction for 8 hours was done by Soxhlet method using ethanol. Methanolic extract was prepared by cold extraction method. The plant material was soaked in methanol for overnight 10-12 hours and filtered through Whatman filter paper No. 1. The aqueous extract was prepared as per the protocol prescribed in Ayurvedic pharmacopeia of India. The solvent was removed under reduced pressure at suitable temperature using rotovac equipment. The solvent free extract was aliquoted and stored in refrigerated condition for ready to use form.

### Physicochemical characteristics [10,11]

Physico-chemical analysis such as moisture content, total ash, water soluble

ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive were carried out as per the standard protocols developed and standardized at NARIP was used.

### Phytochemical Analysis [12,13]

The phytochemical analysis was carried out as per the standard protocols that has been standardised in NARIP. It comprised of various tests including Froth's test, Libermann Burchard test, Fehling's test, Salkowski test, Dragendorff's test, Keller Killani test and Ellagic acid test.

### Spectrophotometric Analysis [14,15]

Spectrophotometric characteristics were analysed for understanding the basic chemical profiling of study plant and to compare the proposition of major compounds among different solvent extracts. The ethanolic extract, methanolic extract, chloroform extract, petroleum ether extract and aqueous extract of *C. pubescens* leaf at different concentrations were used for spectroscopic analysis using the concerned solvent as blank. The spectrum characteristics were measured after standardizing the procedures and analysis was confirmed by carrying out triplicate analysis at 200-800 nm.

### Chemicals and Reagents

The chemicals and reagents of AR grade purchased from make of Spectrum, Nice and HiMedia were used for the present study.

### Instruments

UV Spectrophotometer of Agilent- Cary 60 was used for spectroscopic analysis.

## RESULTS AND DISCUSSION

*Centrosemapubescens* was authenticated at NARIP, Cheruthuruthy. The proximate composition study of shade dried leaf showed the total ash content 7.11% (Table 1). Phytochemical study showed the highly presence of tannins, saponins,

flavonoids, cardiac glycosides in the polar solvent extracts including ethanol, methanol and water when compared with non-polar solvent extracts of chloroform and petroleum ether.

The phytochemical analysis was done for the ethanolic extract, methanolic extract, chloroform extract, petroleum ether extract and aqueous extract of shade dried leaf. The presence of saponins, flavonoids, terpenoids and carbohydrates compounds in the extracts was documented (Table 2).

Spectrophotometric analysis showed various prominent peaks in the spectrum of 200-800 nm representing the presence of compounds like alkaloids, steroids and flavonoids. The spectroscopy study

documented the data of ethanolic extract, methanolic extract, chloroform extract, petroleum ether extract and aqueous extract at different concentration levels and the study was confirmed by triplicate analysis. Methanolic extract showed high presence of flavonoids, alkaloids in the extract. Ethanolic extract showed presence of tannins, and flavonoids, cardiac glycosides and alkaloids. Petroleum ether extract showed the presence of phenolic compounds and flavonoids. Aqueous extract contained all the above phytochemicals at different concentrations. Spectroscopic studies confirmed the presence of various group of compounds even at higher dilutions (Figure 1-7 and Table 3-9).

**Table 1. Physico Chemical Characteristics of *Centrosema pubescens* Shade Dried Leaf**

S No	Proximate Compounds	<i>Centrosema pubescens</i> (Shade dried Leaf)
1	Moisture content (%)	8.192 %
2	Total Ash (%)	7.110 %
3	Water Soluble Ash (%)	1.160 %
4	Acid Insoluble Ash (%)	2.924 %
5	Water Soluble Extractive (%)	15.379 %
6	Alcohol Soluble Extractive (%)	8.521 %

**Table 2. Phytochemical Characteristics of *Centrosema pubescens* (Shade Dried Leaf).**

Phytochemical Compounds	Method	<i>Centrosema pubescens</i> Shade dried Leaf				
		Ethanolic Extract	Methanolic Extract	Aqueous Extract	Chloroform Extract	Petroleum Ether Extract
Tannins	Ferric Chloride Test	++	++	+++	+	-
	Silver Nitrate Test	++	++	+++	+	-
Saponins	Froth's Test	+	+	+++	-	-
Terpenoids	Salkowsky's Test	+	++	++	-	-
Cardiac Glycosides	Keller-Killani Test	+	+	++	+	-
Flavonoids	Shinoda Test	-	+	++	-	-
	Lead acetate Test	++	+++	+++	-	-
	Alkaline Reagent Test	++	++	+	-	-

Carbohydrates	Fehling 's Test	++	+	++	+	+
	Molisch Test	++	++	+++	-	-
	Benedict's Test	+	+	++	++	-
Phytosterols	Liebermann Burchad Test	+	+	+	-	-
Proteins	Millon's Test	-	-	-	-	-
	Biuret Test	-	-	+	-	-
Alkaloids	Dragendorff's Test	+++	+++	+	-	-
	Mayer's Test	++	++	+++	-	-
	Hager's Test	++	+++	+++	-	-
	Wagner's Test	++	++	+	-	-
Anthraquinones	Modified Brontrager's Test	-	-	-	-	-

+++ Highly present ++ Medium + Low - Nil

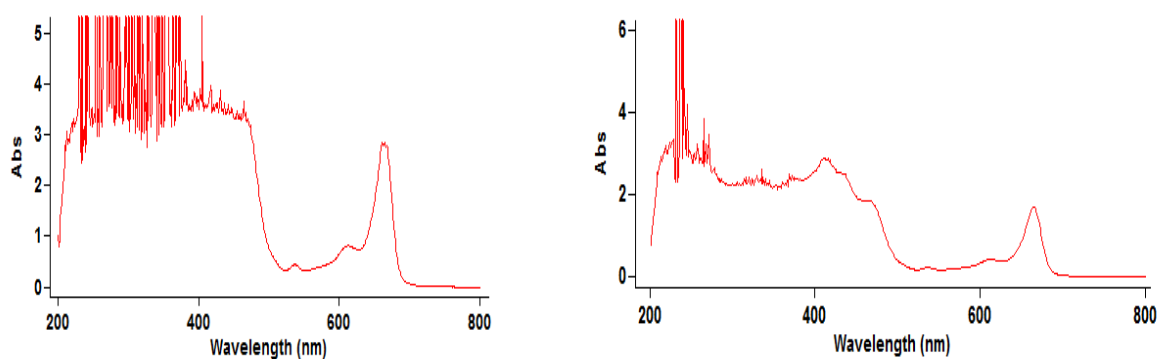


Fig.1. UV Spectroscopic analysis of Ethanolic extract of *Centrosema pubescens* shade dried leaf at different concentrations.

**Table 3. Comparative data of Spectroscopic analysis of Ethanolic extract of *Centrosema pubescens* shade dried leaf at different concentrations**

<i>Centrosema pubescens</i> Leaf Ethanol Concentration 1.25% 200-800 nm		<i>Centrosema pubescens</i> Leaf Ethanol extract Concentration 0.625% 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance
665.0	2.843	665.0	1.698
295.0	5.128	345.0	2.366
291.0	3.608	330.0	2.448
288.0	5.328	271.0	3.467
257.0	3.504	257.0	3.236

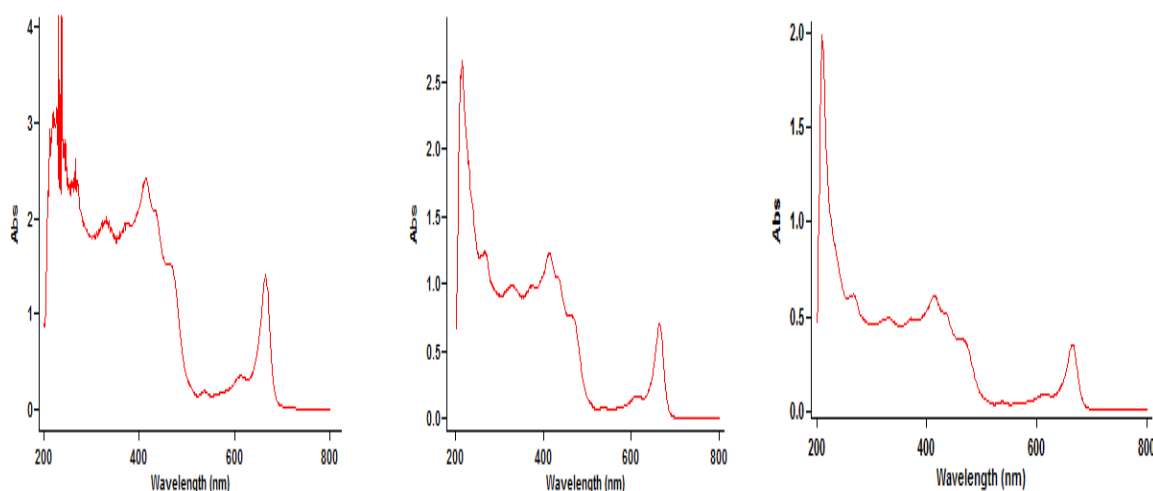


Fig.2. UV Spectroscopic analysis of Ethanol extract of *Centrosema pubescens* shade dried leaf at different concentrations.

**Table 4. Comparative Data of Spectroscopic Analysis of Ethanol Extract of *Centrosema pubescens* Shade Dried Leaf at Different Concentrations.**

<i>Centrosema pubescens</i> Leaf Ethanol Concentration 0.5% 200-800 nm		<i>C. pubescens</i> Leaf Ethanol Concentration 0.25% 200-800 nm		<i>C. pubescens</i> Leaf Ethanol Concentration 0.125% 200-800	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
665.0	1.400	665.0	0.707	665.0	0.350
339.0	1.894	414.0	1.232	413.0	0.610
322.0	1.960	329.0	0.989	331.0	0.494
310.0	1.845	265.0	1.243	266.0	0.616
271.0	2.405	212.0	2.558		

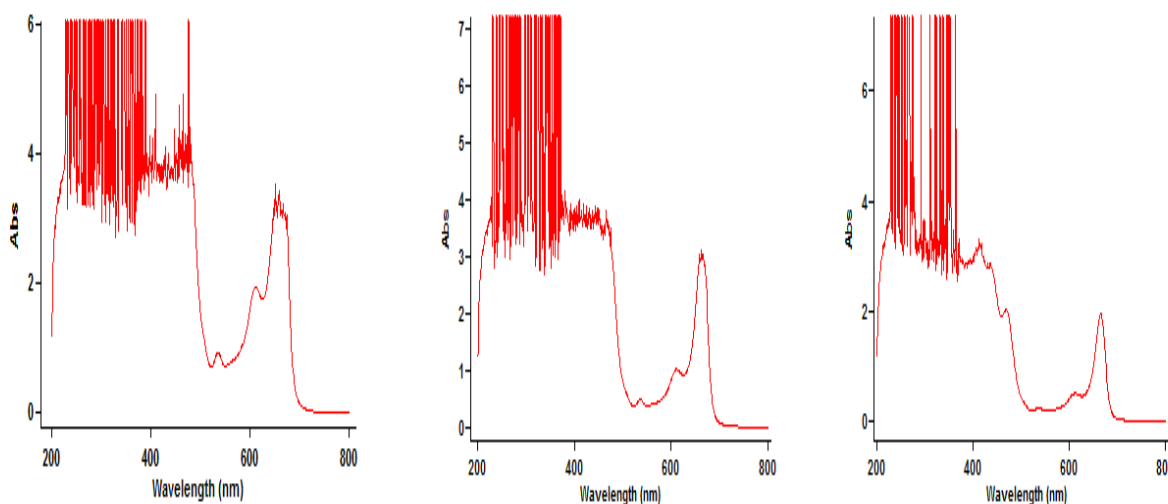
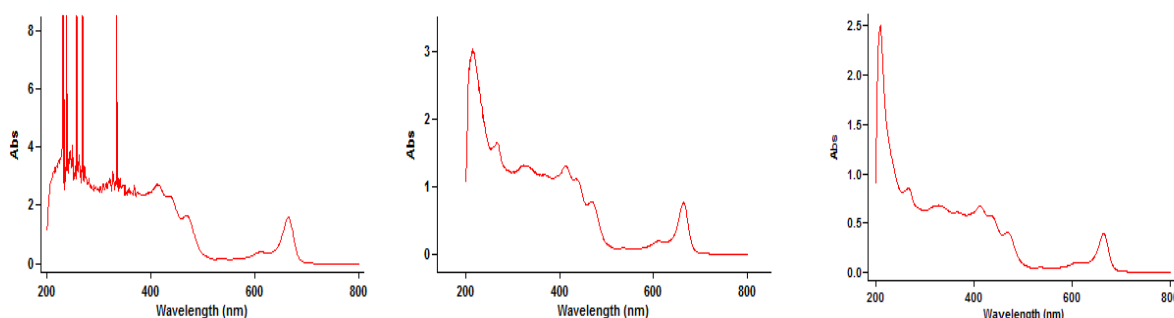


Fig.3. UV Spectroscopic analysis of Methanol extract of *Centrosema pubescens* shade dried leaf at different concentrations.

**Table 5. Comparative Data of Spectroscopic Analysis of Methanolic Extract of *Centrosema pubescens* Shade Dried Leaf at Different Concentrations**

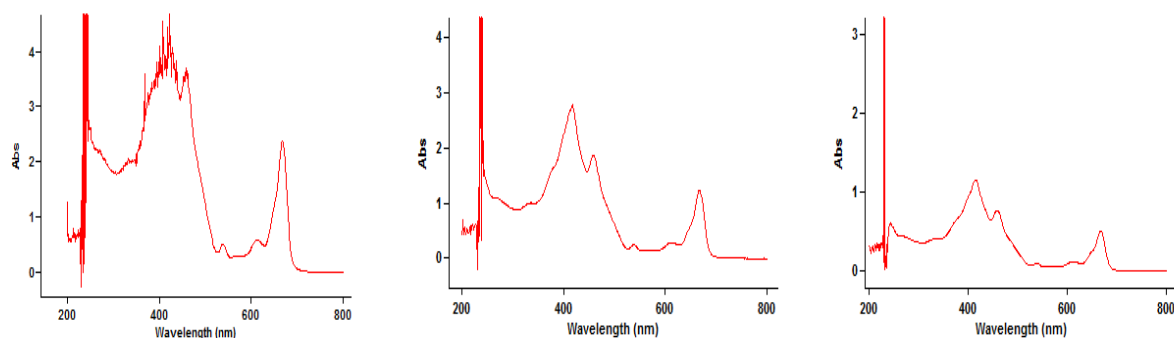
<i>C. pubescens</i> Leaf Methanol Concentration 2.5% 200-800 nm		<i>C. pubescens</i> Leaf Methanol Concentration 1.25% 200-800 nm		<i>C. pubescens</i> Leaf Methanol Concentration 0.625% 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
665.0	3.131	667.0	3.052	665.0	1.958
392.0	4.021	255.0	4.883	399.0	2.974
254.0	3.806	244.0	4.185	289.0	3.367
220.0	3.611	224.0	3.663	255.0	3.171
270.0	3.250	235.0	3.293	271.0	4.684



*Fig.4. UV Spectroscopic analysis of Methanolic extract of Centrosema pubescens shade dried leaf at different concentrations.*

**Table 6. Comparative Data of Spectroscopic Analysis of Methanolic Extract of *Centrosema pubescens* shade Dried Leaf at Different Concentrations.**

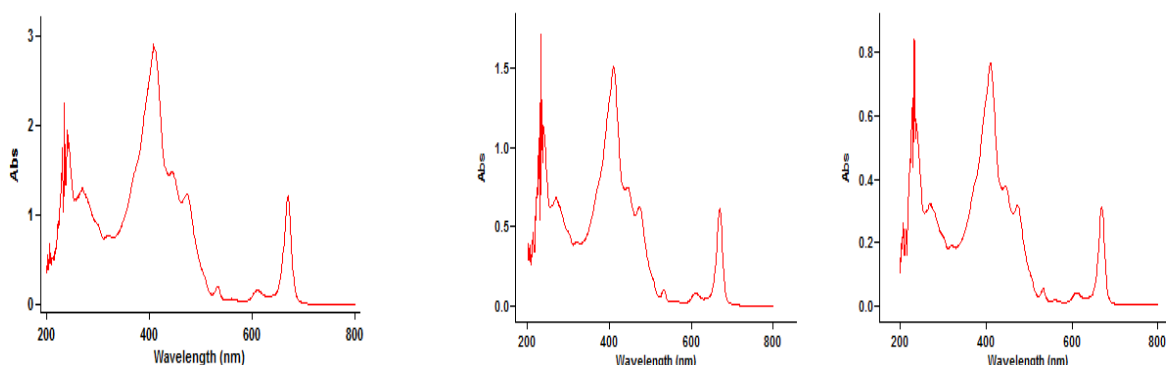
<i>C. pubescens</i> Leaf Methanol Concentration 0.5% 200-800 nm		<i>C. pubescens</i> Leaf Methanol Concentration 0.25% 200-800 nm		<i>C. pubescens</i> Leaf Methanol Concentration 0.125% 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
665.0	1.601	665.0	0.763	665.0	0.397
369.0	2.570	269.0	1.632	535.0	0.056
254.0	3.146	213.0	2.995	328.0	0.682
223.0	3.521	269.0	1.632		
266.0	3.208				



*Fig.5. UV Spectroscopic Analysis of Chloroform Extract of Centrosema pubescens Shade Dried Leaf AT Different Concentrations.*

**Table 7. Comparative Data of Spectroscopic Analysis of Chloroform Extract of *Centrosema pubescens* Shade Dried Leaf at Different Concentrations.**

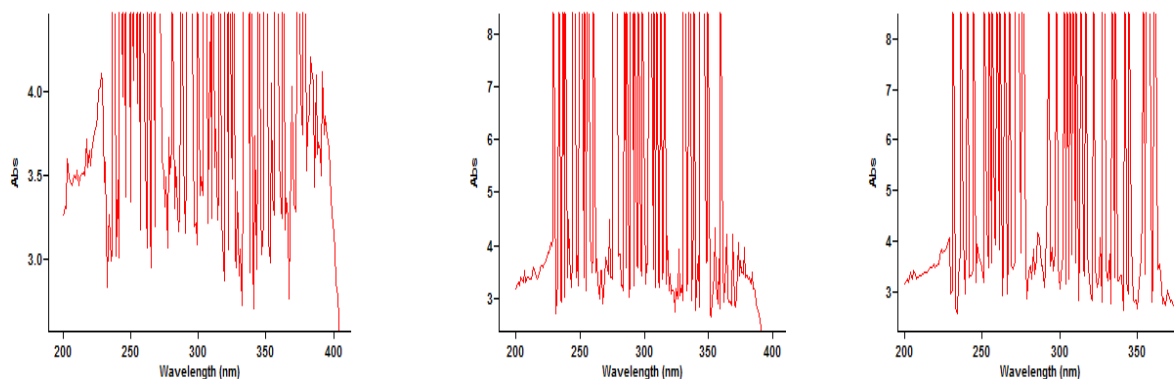
<i>C. pubescens</i> Leaf Chloroform Concentration 1.25% 200-800 nm		<i>C. pubescens</i> Leaf Chloroform Concentration 0.625% 200-800 nm		<i>C. pubescens</i> Leaf Chloroform Concentration 0.25% 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
668.0	2.370	668.0	1.232	668.0	0.511
345.0	2.043	243.0	1.591	237.0	0.329
232.0	2.121	231.0	0.674	230.0	0.590



*Fig.6. UV Spectroscopic Analysis of Petroleum Ether Extract of Centrosema pubescens Shade Dried Leaf at Different Concentrations.*

**Table 8. Comparative Data of Spectroscopic Analysis of Petroleum Ether Extract of *Centrosema pubescens* Shade Dried Leaf at Different Concentrations**

<i>C. pubescens</i> Leaf Petroleum Ether Concentration 2.5% 200-800 nm		<i>C. pubescens</i> Leaf Petroleum Ether Concentration 1.25% 200-800 nm		<i>C. pubescens</i> Leaf Petroleum Ether Concentration 0.625% 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
670.0	1.208	670.0	0.614	670.0	0.313
473.0	1.235	473.0	0.622	473.0	0.316
245.0	1.785	229.0	1.055	229.0	0.651



*Fig.7. UV Spectroscopic analysis of Aqueous extract of Centrosema pubescens Shade Dried Leaf at Different Concentrations*

**Table 9. Comparative Data of Spectroscopic Analysis of Aqueous Extract of *Centrosema pubescens* Shade Dried Leaf at Different Concentrations.**

<i>C. pubescens</i> Leaf Aqueous Concentration 1/2 200-800 nm		<i>C. pubescens</i> Leaf Aqueous Concentration 1/4 200-800 nm		<i>C. pubescens</i> Leaf Aqueous Concentration 1/8 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance	Wavelength	Absorbance
302.0	3.493	325.0	3.174	369.0	3.039
283.0	3.554	281.0	3.831	283.0	3.834
281.0	5.314	273.0	4.489	281.0	3.505
228.0	4.111	227.0	4.051	224.0	3.833

## CONCLUSION

The present study scientifically documented the chemical profiling of the medicinal plant *Centrosema pubescens*. The proximate composition, physico-chemical studies, phytoconstituents and spectroscopic studies revealed the chemical composition and characteristics of polar and non-polar solvent extracts of the plant. The study gives an overall understanding for its medicinal value and provides supporting basic data to the researchers for initiating further studies on biopotency of this plant.

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**CONFLICT OF INTEREST:** Nil

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