Research Article

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EXTRACTION, PHYTOCHEMICAL SCREENING, SEPARATION AND CHARACTERISATION OF BIOACTIVE COMPOUNDS FROM LEAVES EXTRACTS OF *CLITORIA TERNATEA* LINN. (APARAJITA)

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ABSTRACT

Clitoria ternatea L. (Family: Fabaceae) a perennial twing herb, it is also known as Aparajita. Traditionally it is used as a nerves tonic, leaves and roots are utilized to treat various disorders such as body aches, infections, urogenital disorders, eradication of intestinal worms and as an antidote to animal stings. Extraction by different solvents, phytochemical screening, separation and characterisation of bioactive compound carried out in present study. The analysis of bioactive compounds present in the extracts involving the applications of common phytochemical screening assays, chromatographic techniques and also characterisation of compounds by Fourier transform infrared (FTIR) spectroscopy. The results of present study are very helpful to identify the isolated compound from the plant and the data is also used as a monograph for the *Clitoria ternatea* Linn.(Aparajita).

Keywords: Fabaceae, Aparajita, *Clitoria ternatea*

INTRODUCTION

Clitoria ternatea L. (Family: Fabaceae) a perennial twing herb, steams are terete, more or less pubescent. The Ayurvedic drug 'Aparajita', which is used as a 'tonic to the nerves', alterative and laxative consists of roots and seeds of *Clitoria ternatea*. The leaves and roots are utilized to treat various disorders such as body aches, infections, urogenital disorders, eradication of intestinal worms (anthelmintic) and as an antidote to animal stings[1]. Leaves consist of five to seven leaflets, 6–13 cm long. The leaflets are ovate to oblong, 2–5 cm long and subcoriaceous, rubiaceous stomata with wavy cell walls are found on both upper and lower epidermis of the leaflets. Multicellular trichomes have two basal cells smaller than the terminal cells[2-5]. Extractive value of crude drugs is useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by other means[6]. In present study the extraction done by different solvents according to their polarity. Qualitative chemical tests were conducted for all the extracts of *Clitoria ternatea* Linn. leaves to identify the various phytoconstituents. Thin layer chromatography (TLC) is a very convenient and effective technique for the separation and identification of bioactive compounds. Chromatography represents a group of techniques for separation of compounds of mixtures by their continuous distribution between two phases, one of which is moving past the other[7]. Characterizations were done by Fourier-Transform Infrared (FTIR).

In the present study attempt has been made to extract, separate and characterize the bioactive compounds by using different phytochemical screening, thin layer chromatograpy and spectroscopy techniques.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Clitoria ternatea* Linn.(Aparajita) collected from the garden of Dravyaguna Department, IMS B.H.U. The specimens identified at Department of Botany by Professor N. K. Dubey and voucher specimen no. Feb/2013/16 was deposited.

Preparation of Extracts

The leaves sample was dried for 1-2 weeks and makes coarse powder using mortar and pestle. 100 gm of powder was subjected to successive hot continuous soxhlet extraction with eleven solvents according to its polarity. Each time before extracting with the next solvent the powdered material will be air dried in hot air oven below 50^0C. After the effective extraction, the solvent was distilled off, the extract was then concentrated on water bath and the extract obtained with each solvent will be weighed. Its percentage will be calculated in terms of air-dried weight of plant material of the extracts will be noted[8,9].

Phytochemical screening of extracts

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are carbohydrates, flavonoids, alkaloids, glycosides, steroids and fats, tannins and phenolic compounds[10].

Alkaloid (Wagner's test): Acidify 1ml of alcoholic extract of the drug with 1.5% of HCl and add a few drops of Wagner's reagent. A brown precipitate indicates positive test for alkaloids.

Flavonoid: In a test tube containing 0.5ml of the alcoholic extract of the drug, add 5-10 drops of dilute HCl followed by a small piece of magnesium. Boil the solution for a few minutes.

In the presence of flavonoids a pink, reddish pink or brown color is produced.

Glycoside: Dissolve a small amount of alcoholic extract of the drug in 1ml of water and add 1N NaOH solution. A yellow color indicates the presence of glycosides.

Phenols ($FeCl_3$ test): Dissolve a small quantity of alcoholic extract of the drug in 2ml of distilled water and a few drops of 10% ferric chloride solution. A blue or green color is produced indicates the presence of phenols.

Saponin: Dissolve a small quantity of alcoholic extract of the drug in 5ml of distilled water, shake the mixture vigorously and leave for 3 min. Honeycomb like froth indicates the presence of saponins.

Resin: Dissolve a small quantity of ethanolic extract of the drug in 5ml of acetic anhydride by means of gentle heat, cool and add a drop of sulphuric acid. A bright purplish red color indicates the presence of resins.

Steroid (Liebermann - Burchard's test): To the ethanolic extract of the drug in $CHCl_3$, add acetic anhydride followed by 1ml of concentrated sulphuric acid. A reddish brown ring is formed at the juncture of two layers indicates the presence of steroids.

Tannin: To the ethanolic extract of the drug, add a few drops of 5% aqueous ferric chloride solution. A bluish black color indicates the presence of tannins[11].

Separation of Phytoconstituents by Thin Layer Chromatography (TLC)

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. It consists of three steps spotting, development, and visualization[12,13]. The R_f value is used to quantify the movement of the materials along the plate. R_f is equal to the distance travelled by the substance (stationary Phase) divided by the distance travelled by the solvent (Mobile Phase). The solvent system should be selected according to the nature of phytoconstituents e.g. carbohydrates, alkaloids, glycosides, flavonoids, steroids, fats, tannins and phenolic compounds[14].

Characterisation of phytoconstituents by FTIR

Infrared (IR) spectroscopy is a very useful tool for the identification of organic materials. However, with the development of Fourier transform infrared (FTIR) spectroscopy, it is very easy for the quantitative analysis of complex mixtures, as well as the investigation of surface and interfacial phenomena. FTIR works on the basis of functional groups and provide information in the form of peaks[15,16,17].

RESULTS AND DISCUSSION

The extraction was performed by successive hot continuous soxhlet extraction in order to the polarity of the solvents i.e. Petroleum Ether for defatting, hexane, n-butanol, butyl acetate, chloroform, ethyl acetate, acetone, methanol, ethanol, acetic acid, and aqueous and calculate the percentage w/w of extractive with reference to air-dried drug. The results of the Extractive values as a percentage yield are tabulated in Table 1. The Results of Phytochemical Investigation tabulated in Table 2.

Table 1: Percentage Yield (w/w) of leaves extracts of *Clitoria ternatea* Linn.

Extracts	Nature of Extracts	Percentage (%) Yield w/w
Petroleum Ether	Viscous	3.19
Hexane	Viscous	0.40
n-Butanol	Viscous	11.79
Butyl acetate	Powder	0.11
Chloroform	Powder	0.38
Ethyl acetate	Powder	0.21
Acetone	Powder	0.33
Methanol	Powder	3.05
Ethanol	Powder	0.24
Acetic acid	Powder	12.17
Aqueous	Powder	2.49

Table 2: Phytochemical Investigation of leaves extracts of *Clitoria ternatea* Linn.

Chemical Tests	Extracts										
	Pet. Ether	Hexane	n-Butanol	Butyl Acetate	Chloroform	Ethyl Acetate	Acetone	Methanol	Ethanol	Acetic Acid	Aqueous
Carbohydrates	+	+	-	-	-	-	+	+	-	+	+
Alkaloids	-	-	+	+	+	-	+	+	+	-	-
volatile oil	+	+	+	+	+	+	+	-	-	-	-
proteins	-	-	-	-	-	+	+	-	+	-	_
Amino acids	-	-	-	-	-	+	-	-	+	-	-
fats & Oils	+	+	-	-	-	-	-	-	-	-	-
Steroids	+	+	-	+	+	-	-	-	-	-	-
Cardiac Glycosides	-	+	-	+	-	-	-	-	-	-	-
Saponin Glycosides	-	+	-	-	+	+	-	-	-	-	+
Antraquinine Glycosides	-	-	+	-	-	-	+	-	-	-	-
Cynogenetic Glycosides	-	-	-	-	-	-	-	-	-	-	-
Coumarin Glycosides	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	+	-	-	-	+	+	-	-	-
Tannins & Phenolic Compounds	-	-	+	-	-	-	-	+	-	-	-

Leaves extracts of *Clitoria ternatea* Linn. were applied on prepared TLC silica plates and plates were developed in a chamber containing solvent, they dissolve and move with the solvent, each extract separated into bioactive compounds and move to different positions on the prepared silica plate. On expose to iodine vapours or spraying with different visualising reagents spots of various extracts became darker.

Table 3: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	Petroleum Ether	1 2 3.4	5	0.2 0.4 0.68
2.	Hexane	0.8 1.7 3.4	4.5	0.17 0.37 0.75
3.	Methanol	1.6 3.4	4.5	0.35 0.75
4.	Acetic Acid	3.4	5.0	0.68

For Solvent system: Acetonitrile: Chloroform: Methanol (5:9:2 or 4:9:1)

Plate 1 Plate 2 Plate 3 Plate 4

Figure 1: TLC plates for Petroleum Ether (Plate 1), Hexane (Plate 2), Methanol (Plate 3) & Acetic acid (Plate 4) of leaves extracts of *Clitoria ternatea* Linn.

Table 4: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	n-Butanol	3.9	4.9	0.79
2.	Chloroform	2.5	5.0	0.5
3.	Ethyl acetate	3.6	4.5	0.8
4.	Acetone	4.5	5.3	0.84
5.	Methanol	3.6 4.5	5.5	0.65 0.81
6.	Ethanol	2.5 4.2	4.7	0.53 0.89

For Solvent system: Butanol: Acetic acid: Water (4:5:1 or 8:12:2)

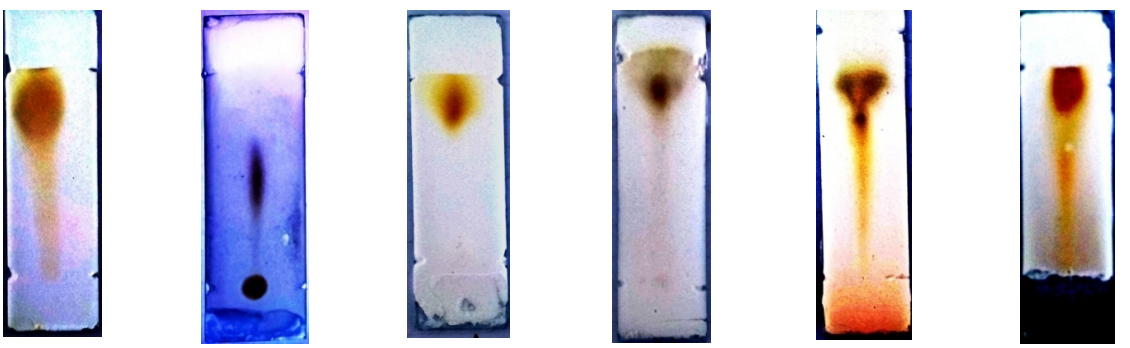

Plate 1 Plate 2 Plate 3 Plate 4 Plate 5 Plate 6

Figure 2: TLC plates for n-Butanol (Plate 1), Chloroform (Plate 2), Ethyl acetate (Plate 3), Acetone (Plate 4), Methanol (Plate5) & Ethanol (Plate 6) of leaves extracts of *Clitoria ternatea* Linn.

Table 5: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	n-Butanol	3		0.61
		4.4	4.9	0.89
2.	Acetone	2.5		0.48
		4.7	5.2	0.90
3.	Methanol	1		0.19
		2.8	5.2	0.53
		4.7		0.90

For Solvent system: Ethyl aectate:Methyl ethyl ketone: Acetic acid: Water (5:3:6:1 or 5:3:1:1)

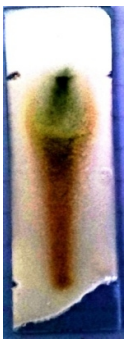

Plate 1

Plate 2

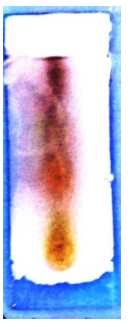

Plate 3

Figure 3: TLC plates for n-Butanol (Plate 1), Acetone (Plate 2), Methanol (Plate3) of leaves extracts of *Clitoria ternatea* Linn.

Table 6: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	Butyl acetate	2.5	5.0	0.50
		2.8		0.56

For Solvent system: Dichloromethane: Methanol: Formamide (8:2:2)

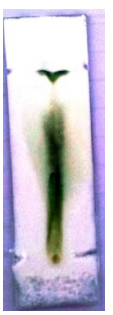

Figure 4: TLC plates for Butyl acetate of leaves extracts of *Clitoria ternatea* Linn.

Table 7: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	Petroleum Ether	1		0.20
		1.4		0.28
		3	4.9	0.61
		3.7		0.75
		4		0.81
2.	Hexane	0.6		0.10
		1.3		0.23
		1.7		0.30
		3	5.5	0.54
		3.2		0.58
		4.4		0.80

For Solvent system: Hexane: Acetone (9:1)

Plate 1

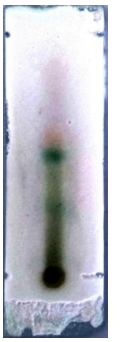

Plate 2

Figure 5: TLC plates for Petroleum ether (Plate 1) and hexane (Plate 2) of leaves extracts of *Clitoria ternatea* Linn.

Table 8: R_f values for different leaves extracts of *Clitoria ternatea* Linn.

S.No.	Extracts	Distance travelled by Mobile Phase (Spots) (Cm)	Distance travelled by Stationary Phase (Cm)	R_f Values
1.	n-Butanol	3.7	5.2	0.71
		4.1		0.78

For Solvent system: Benzene: Acetic acid: Water (4:8:2)

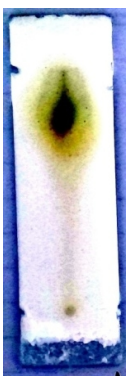

Figure 6: TLC plates for n-Butanol of leaves extracts of *Clitoria ternatea* Linn.

FTIR Analysis for Extracts

Figure 7 and Table 9 are the IR spectrum and peak values of eleven extracts of *Clitoria ternatea* Linn. leaves. FTIR technique determining the assignments of different functional groups present in the compounds on the basis of their wavenumber (cm^{-1}). The FTIR spectrums were used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The extracts of Aparajita (*C.ternatea* Linn) were passed into the FTIR and the functional groups of the components were separated based on its peak ratio.

The dry extract of petroleum ether results prove the presence of alcohols, phenols, alkanes, aldehydes, nitro compounds and sulfonic acids at major peaks 3402, 2913, 1740, 1372 and 1075 respectively.

Hexane extract results suggests that alkanes, aldehydes, nitro compounds and benzenes at peaks 2924, 1728, 1373 and 759 are present respectively.

n-Butanol has presence of oximes, alkanes, nitro compounds and sulfonic acids at peaks 3321, 2934, 1372, 1077 respectively.

Butyl acetate extract has presence of alkanes, aldehydes, nitro compounds, amines and cyclic compounds at major peaks 2965, 1740, 1372, 1229, 1025.

Chloroform extract has presence of primary amines, alkanes, alkyl sulfoxides and vinylidenes at major peaks 3342, 2976, 1045 and 882 respectively.

Ethyl acetate extract has presence of primary amides, alkanes, alcohols at peaks 3342, 2945 and 1015 respectively.

Acetone extract has presence of alkanes, alkenes, isopropyl groups, alcohols and phenols at peaks 2924, 1647, 1362, 1066 and 675.

Methanol extract has presence of alcohols and phenols, alkanes and primary amides at peaks 3331, 2945, 1637 respectively.

Ethanol extract has the presence of primary amides, alkanes, alkyl sulfoxides and benzenes at peaks 3342, 2976, 1045 and 882 respectively.

Acetic acid extract has presence of carboxylic acids, primary amides, hydrocarbons, alcohols and sulfones at major peaks 1710, 1403, 1240, 1015 and 607 respectively.

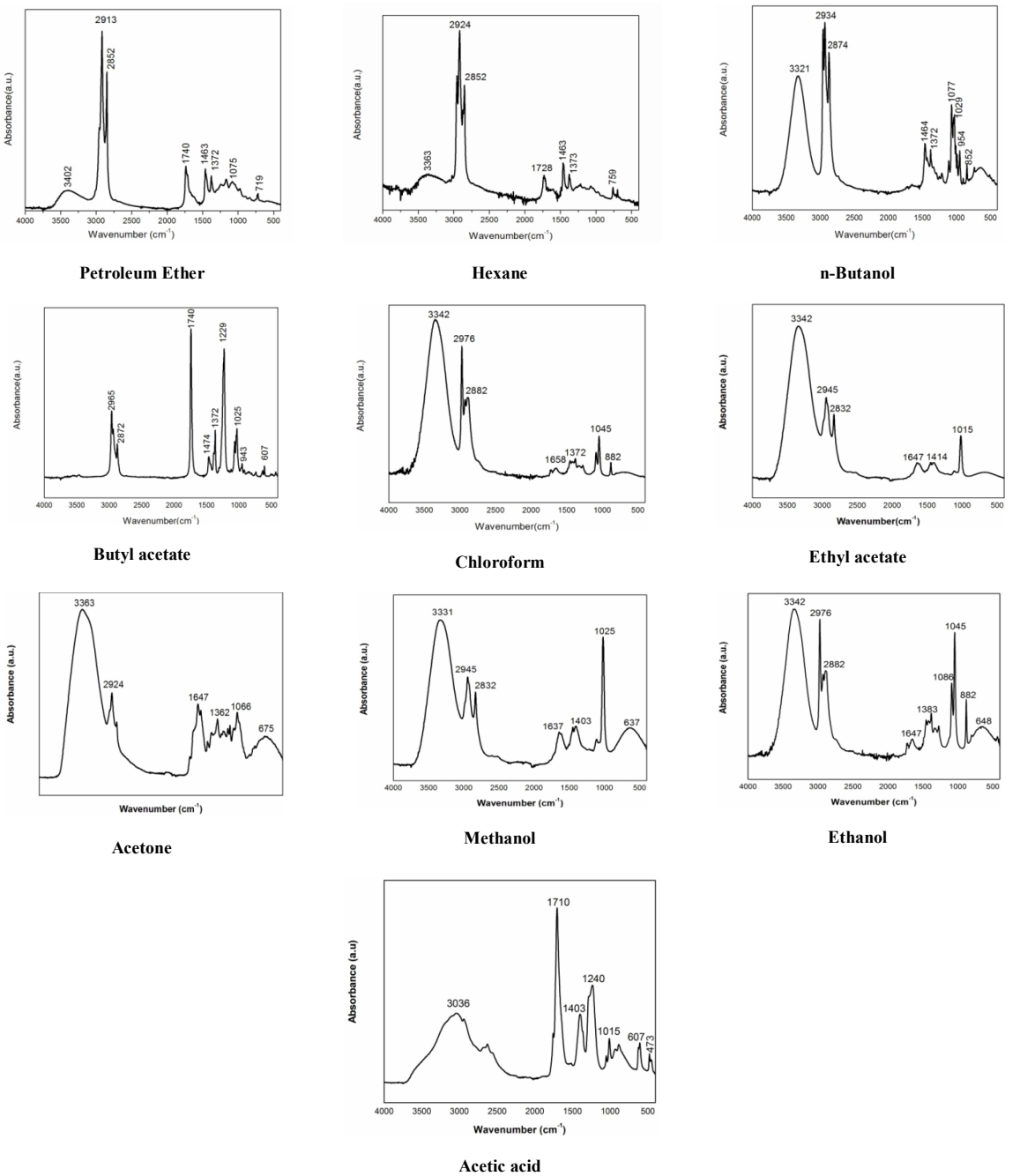

Figure 7: Infrared spectra of the different extracts of *Clitoria ternatea* Linn.

Table 9: FTIR data for different extracts leaves of *Clitoria ternatea* Linn.

Extracts	Peak Value Wave number (Cm^{-1})	Functional groups & class	Assignment & Remarks
Petroleum ether	3402	OH in alcohols and phenols	O-H stretch
	2913	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	2852	CH_3 attached to O or N	CH stretching mode
	1740	C=O in aldehydes	C=O stretch
	1463	CH_3 in aliphatic comounds	CH_3 anti deformation
	1372	NO_2 in aliphatic nitro compounds	NO_2 sym stretch
	1075	SO_3H in sulfonic acid	SO_3 sym Stretch
Hexane	2924	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	2852	CH_3 attached to O or N	CH stretching modes
	1728	C=O in aldehydes	C=O stretch
	1463	CH_3 in aliphatic comounds	CH_2 scissors vibration
	1373	NO_2 in aliphatic nitro compounds	NO_2 sym Stretch
	759	O-disubst benzenes	CH out of plane deformation
n-Butanol	3321	OH in oximes	O-H stretch
	2934	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	1464	CH_3 in aliphatic compounds	CH_3 antisym stretching
	1372	NO_2 in aliphatic nitro compounds	NO_2 sym stretch
	1077	SO_3H in sulfonic acid	SO_3 sym Stretch
Butyl acetate	2965	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	2872	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	1740	C=O in aldehydes	C=O stretch
	1474	CH_2 in aliphatic comounds	CH_2 scissors vibration
	1372	NO_2 in aliphatic nitro compounds	NO_2 sym Stretch
	1229	C-C-N in amines	C-C-N bending
	1025	Carbon ring in cyclic compounds	Ring breathing mode.
Chloroform	3342	NH_2 in primary amides	NH_2 antisym stretch
	2976	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	1045	S=O in alkyl sulfoxides	S=O stretch
	882	CH_2=C< in vinylidenes	CH_2 out of plane way
Ethyl acetate	3342	NH_2 in primary amides	NH_2 antisym stretch
	2945	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	2832	CH_3 attached to O or N	CH stetching mode
	1647	N-H in primary amides	NH deformation
	1414	C-N in primary amides	C-N stretch
	1015	CH-O-H in cyclic alcohols	C-O stretch
Acetone	2924	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	1647	C=C in alkenes	C=C stretch
	1362	Isopropyl group	CH_3 deformation
	1066	CH-O-H in cyclic alcohols	C-O stretch
	675	Ar-OH in Phenols	OH- out of Plane deformation
Methanol	3331	OH in alcohols & Phenols	O-H stretch
	2945	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	2832	CH_3 attached to O or N	CH stretching mode
	1637	N-H in primary amides	NH deformation
	1403	C-N in primary amides	CH_3 deformation
	1025	CH-O-H in primary alcohols	C-O stretch
Ethanol	3342	NH_2 in primary amides	NH_2 antisym stretch
	2976	CH_3 & CH_2 in aliphatic compounds	CH antisym & sym stretching
	1647	N-H in primary amides	NH deformation
	1383	CH_3 in aliphatic compounds	CH_3 deformation
	1045	S=O in alkyl sulfoxides	S=O stretch
	882	1,2,4-trisubst benzenes	CH out-of-plane deformation
Acetic acid	1710	C=O in carboxylic acids	C=O stretch
	1403	C-N in primary amides	C-N stretch
	1240	t-butyl in hydrocarbons	Skeletal vibrations
	1015	CH-O-H in cyclic alcohols	C-O stretch
	607	SO_2 in sulfones	SO_2 scissoring

CONCLUSION

In the present study, the leaves of *Clitoria ternatea* Linn.(Aparajita) belongs to the family Fabaceae were collected and authenticated. The leaves was subjected to size reduction to get coarse powder and extraction with various solvents such as petroleum ether (60^0-80^0C), hexane, n-butanol, butyl acetate, chloroform, ethyl acetate, acetone, methanol, ethanol, acetic acid and aqueous by successive hot continuous soxhlet method. Each extract was concentrated by distillation the solvent and then evaporated to dryness on water bath. The concentrated extracts stored carefully for phytochemical, separation and characterisation of bioactive compounds.

As per the phytochemical investigation Carbohydrates, glycosides, flavonoids, tannins, saponins, steroids, volatile oils, fats and oils were found to be present in the various extracts of the leaves of *Clitoria ternatea* Linn. After the phytochemical investigation, the extracts were subjected for thin layer chromatography for separation of bioactive compounds. TLC

profiling of all eleven extracts shows an impressive result that directing towards the presence of number of phytoconstituents. This variation in Rf values of the phytoconstituents provides a very important information in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds. The leaves extracts of Aparajita FTIR analysis results proved the presence of oximes, alcohols, phenols, alkanes, alkenes, carboxylic acids, aldehydes, nitro compounds, amides, amines and alkyl halides compounds.

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