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A Study on Phytochemical analysis, Antioxidant Activity & Detection of Bioactive Compounds Present from the Tuber of *Stephania glabra*

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Abstract

Stephania glabra is a perennial shrub whose tubers are used by folk medicine of Bangladesh and tribal of North- East India. It has been used for treating many ailments like asthma, scabies, stomach ache, inflammatory, tuberculosis, sleep disturbance etc. During the Present study, the species was examined for its Antioxidant activity and for the content of alkaloids, phenolic, tannins and carbohydrates. Column chromatography was done for the separation of the phytochemicals and by using TLC the pure active components were evaluated by calculating R_f value. Further studies were carried out to detect the presence of bioactive compounds by GC-MS and LC-MS.

Keywords: Stephania Glabra, Antioxidant, Phytochemicals, Column Chromatography.

Introduction

Medicinal plants have played a significant role in various ancient traditional system of medication. Medicinal plants also have played a vital role in the socio- cultural, spiritual and health care requirements of rural and tribal people of the emerging and developing countries. The herbal products today symbolise safety in comparison to that of synthetic or chemical products. For few centuries the synthetic products of modern age had suppressed their importance. However, the blind dependence on synthetic is over and the people are returning to natural products with hope of safety and security. India has a very rich heritage of plants based health care system,which was practice by the Ayurveda, Unani and Siddha. Due to high quality health care provided by them it has a very high acceptance in the society. Ayurveda, Siddha, Unani and folk (tribal) medicine are the major system of indigenous medicine. Indian medicinal system has Ayurvedic treatment essence which has miraculous effect. The Ayurvedic medicine doesn't have side effect and are safe for use. The forest of India is the principle repository of large number of medicinal and aromatic plants, which are largely collected as raw material for the manufacturing of drugs and perfumery products.

Stephania is a genus of flowering plant of the family *Menispermaceae.* It is mostly found in the Eastern and southern Asia and Australasia. The family have 68 genera with around 440 species which are distributed throughout low- lying tropical areas. Some of them are also present in temperate and arid regions. There are about 45 species in the genus of *Stephania.* The members of *Menispermaceae* family are medium sized climbing plant found in the tropics. They are perennial or deciduous plant growing to around four meter tall. The leaves are usually arranged spirally on the stem and are peltate, with the petiole attached near the centre of the leaf. The leaves are usually spiral and simple[1]. The flowers are zygomorphic cycle and the seeds are slightly curve or spiral with endosperm present or absent. Most of the tubers of Menispermaceae family are used. They have identified for alkaloids, antibacterial, antifungal, antiplasmodial and cytotoxic activities. Phenolic alkaloids from Menispermundauricum have shown effect against mycocardial cerebral ischemia in rabbit. It is used by inhabitants of Bangladesh as medicine [2].

Stephania glabra is a climbing herbaceous shrub with petal and membranous leaves. The roots of this species are tubers with fibrous roots below, which are round or oval with irregular shapes. The tubers may weigh around 5-20 kg. Stem are striate, glabrous, branchlets green, sparsely puberscent when the branch are young. Later the stems are glabrous. The leaves are simple,orbiculars, narrowly peltale, cordate or broadly ovate and usually dark green in colour .The leaves measures bare 4-16×3.13 cm across. It flowers on the axillary leaf opposite to old leafless stem, the flowers are umbellate cymes and are unisexual. It is greenish yellow in colour. Male flower have 6 sepals in series with 3 petals and with single filament. Female flowers are orange which has short style, stigma contains 4-5 lobe. Fruits are usually drupes, oval and globose.It has been used for treating dysentery, tuberculoses, hyperglycemias, cancer, fever, etc.The chemical components are mostly alkaloids, they also contains tannins, flavonoids, steroids, etc. They have anti- cancer activity, anti-inflammatory, analgesic activity and anti-hyperglycaemicactivity[3].

Therapeutic Uses

The plant tubers has been used for the treatment of asthma , tuberculosis, dysentery, hyperglycaemic, cancer, fever, intestinal complaints, sleep disturbance and inflammation in many Asian countries. It shows remarkable anti-psychotic, anti-diabetic, antipyretic, analgesic, antimicrobial and anti-hypertensive activities.

For the of *Stephania glabra* the ethanol extraction was used for the study of Antimicrobial activity in which antibacterial and antifungal was evaluated using 5 bacteria species and 6 fungi species . The chemical constituent was mostly Alkaloids named Gindarudine. Hypotension activity, analgesic and antipyretic antithyperglycemiceffect, antihistamine activity and antihelmintic activity was studied. Pharmacological activity, anti- inflammatory activity, anti-arthritic activity, antioxidant activity, immunodulatory activity, anti-parasitic activity and chemical constituent of *Cissenpelas pareira* was studied[4].The tubers of *Stephania glabra* and leaves of *Woodfordia fructicisea*were dried and grinded to get a fine powder. The hydrodistillation of *Stephania glabra*tubers provided 0.33% v/w of volatile oil from 30gm of powdered material. It was so less that TLC was carried out and in *Woodfordia fructicisea* 0.33% of volatile oil was found. The volatile oil of both sampleswas lighter than water [5].

Phytochemical screening of *S. elegans* screened alkaloids, carbohydrates, glycosides, tannins etc. Quantitative determination of total phenolics, in-vitro antioxidant assay, cell culturing and maintenance, subculturing, statistical analysis. The total phenolic content was 23 mg GAE/g of dry sample FRAPassay reveals that methanolic extractwas increased by 20-80µg/ml. in –vitro antioxidant assay with increased concentration it contains high phenolic content which absorbs and neutralize free radicals scavenging activity which means it can use for treating cancer[6].In *Agertatum conyzoides* the phenolic contents was determined in n-hexane was 34mg and in acetone 25.70mg and the content of flavonoids found to reduce blood –lipid and glucose and to enhance human immunity. Tannins are an important source of protein. The phenolic and flavonoid content of n- hexane extract content is more potent in scavenging the free radical as compared to that of other extract[7].Medicinally important plants in high content of phenolic compound indicate that these compounds contribute to the antioxidant activity [8]. Quantitative and qualitative phytochemical analysis of leaves and fruits of *Passiflora foetida* gives idea about flavonoid. Saponin, alkaloids contain and phenolic content of leaves in which presence of carbohydrates, alkaloid, flavonoid, tannins, enzyme (catalase) organic acid (oxalic acid)vitamins A were found. Leaves showed more contains of alkaloids and phenolic whereas fruits showed higher flavonoids and saponins content[9].

With reference to the literature obtained,the main objective of this work is to study the phytochemicalanalysis, Antioxidant activity and Detection of Bioactive compounds present in its tuber for preparation of pharmaceutical medicines for the people who are in continuous use of natural plant products as their folk medicines.

Materials and Methods

Plant material and Preparation of extract:

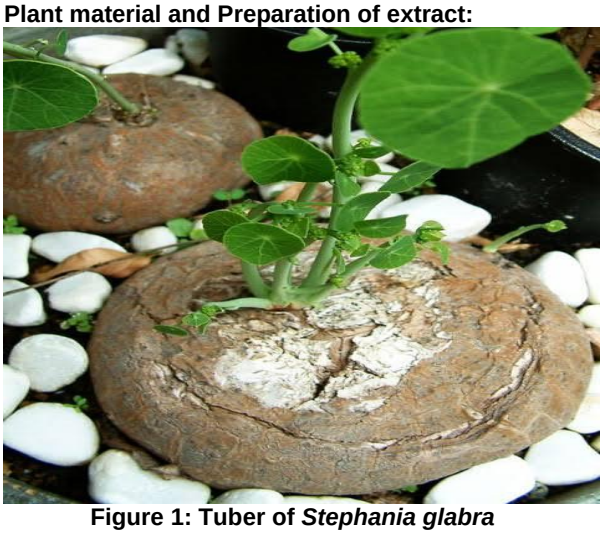

Figure 1: Tuber of *Stephania glabra*

The tuber of the plants of *Stephania glabra* was collected from forest of Yupia, Arunachal Pradesh on the September. It was identified by the Drug Testing laboratory, Jalukbari, Guahati-27 Assam. The tubers were cleaned by washing it with water and then it sliced into small pieces with help of the knife and was dried. After the plant dried it was grounded in powder with the grinder machine. The tuber powder of *Stephania glabra* was stored in the dry container. The dried powder of tuber 30 gm was extracted with 200 ml of methanol in the Soxhlet extraction with 60^0 C till the solvent in siphon tube of the extractor became colourless. After that the extraction was dried by condenser and the dried extract was kept in the refrigerator with 5^0 c.

Qualitative Determination of phytochemical

The extract was tested for the presence of phytoconstituents by using followingstandard methods-

Test for Alkaloids

Mayer's Test

To 2-3 ml of plant extract a few drops of Mayer's reagents was added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids.

Tests for Amino Acids

Ninhydrin Test

2-3 drops of Ninhydrin solution was added to the 2-3 ml plant extract and was kept in water bath for 5 mins. Appearance of purple colour indicates the presence of amino acid.

Test for Carbohydrates

Molish's Test

To 2ml of plant sample extract was taken and few drops ofα- naphtholwas added and the mixture was shaken well. After which concentrated sulphuric acid was added slowly along the side of the tube. A violet ring is indicates the presence of carbohydrates.

Test for Phenol

Ferric chloride Test

The tuber extract (500mg) was dissolved in 5ml of distilled water. After that few drops of neutral 5% ferric chloride solution wereadded. A dark colour indicates the presence of phenolic compounds.

QuantitativeAnalysis

Total Phenol Assay

The total phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot (1 ml) of extracts or standard solution of Gallic acid (100, 200, 300, 400, and 500µg/ml) was added to 25 ml of volumetric flask, containing 9 ml of distilled water. Reagent blank using distilled water was prepared. 1ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken properly. Then 10 ml of 7% Na_2CO_3 solution was added to the mixture after 5 minutes. The volume was then made up to the mark.After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE) [10, 11].

Total Tannin Assay

The tannins were determined by Folin-Ciocalteu method. At first about 0.1 ml of the sample was added to a volumetric flask (10ml) containing 7.5 ml of distilled water and 0.5 ml of folin-Ciocalteuphenol reagent, 1ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled H_2O.Then the mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solution of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/ visible spectrophotometer. The tannin content was expressed in the terms of mg of GAE/g of extract.

Total Alkaloid Assay

The plant extract 1mg was dissolve in Dimethyl sulphoxide (DMSO), added 1ml of 2 N HCL and filtered. Then this solution was transferred to a separating funnel and 5 ml of bromocresol green solution, 5 ml of phosphate buffer were added .The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solution of atropine (20, 40, 60, 80 and 100µg/ml) were prepared in the same reagent blank at 470nm with an UV/ visible spectrophotometer. The total alkaloid content was expressed as mg of GAE/g of extract.

Total Carbohydrate Assay

Weigh 100 mg of the sample into a boiling tube. Hydrolyse by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCl and cool to room temperature. After that the sample is neutralising with solid sodium carbonate until the effervescence ceases. Then, the volume was Make up to 100 ml and centrifuge. Collect the supernatant and take 0.5 and 1 ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' serves as blank .Make up the volume to 1 mL in all the tubes including the sample tubes by adding distilled water. Then 4 ml of anthrone reagent was added and kept in a boiling water bath for eight minutes for heating. Cool rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube[11].

Antioxidant Assay

Antioxidant assay was determined by the DPPH assay according to the sharififar et.al, (2007). The antioxidant assay uses DPPH radicals as a reagent. When DPPH reacts with antioxidant compound, it isreduced. The change in colour (from deep violet to light yellow) is then measured. 950µl of 0.002% methanol solution of DPPH was added to 5 different concentration of (25µl, 50µl, 75µl, 100µl and 150µl) of the extract from *Stephania glabra* sp. tubers was taken. The absorbance was read at 517nm in spectrophotometer (Themofischer), after 30 mins of incubation in dark at room temperature. The inhibition of the DPPH free radical in percentage (I) was calculated using the following formula,

I% = (A Blank – A sample/A blank)* 100Where, 'A blank' is the absorbance of the control containing all the reagents except the extract.'A sample ' is the absorbance of the extract that is , the test compound. Ascorbic acid was taken as the standard. The concentration of the extract that provides 50% inhibition was calculated from the graph plotting inhibition concentration against the extract concentration.

Purification by using Chromatography

Purification by using Column Chromatography

The mixture is dissolved in fluid called the mobile phase, which carries it through a structure holding material called the stationary phase. The purpose of chromatography is to separate the components of the mixture for more advance used (and is thus a form of purification).Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids when carry out small-scale experiment. The stationary phase, a solid adsorbent is usually place in

a vertical glass column and the mobile phase, is added from the top and let flow down through the column by either gravity or external pressure.

Evaluation the volatile compound BY Thin layer Chromatography

TLC is the technique used to separate the pure components present in a mixture. This separation is possible due to the adhesion force of the molecules that are present in the mixture to a translate in to more or less movement of individual components, which allows its separation and identification.

Evaluation of the bioactive compounds by Gas Chromatography – Mass Spectrometry:

The Gas chromatography is a physical methods of separation in which the components to be separated are distributed between two phases, one being stationary bed of large surface area, and the other a gas that percolates through the stationary bed. Gas Chromatography played a fundamental role in determining howmany components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule[12].

Evaluation of the bioactive compounds by Liquid Chromatography- Mass Spectrometry:

LC-MS is a hyphenated technique which combines the separating power of High Performance Liquid Chromatography (HPLC) with the detection power of mass spectrometry. Mass spectrometry is atechnique, which involves the production and subsequently separation and identification of the charged species. The associated acronym, LC-MS covers a broad range of application areas.

RESULTS

Qualitative analysis of Phytochemical of plants extracts:

Alkaloid Test

When few drops of Mayer's reagent were added a white precipitate was formed which indicates the present of alkaloids (Table 1).

Amino Acid Test

After the addition of few drops ninhydrin solution to 2-3 ml of plant extract and keeping it for 5 mins in water it did not turn into purple which indicates the absent of the amino acid (Table 1).

Carbohydrates Test

There was formation of violet ring after the addition of sulphuric acid to the mixture of α-napthol and 2-3 ml of plant extract, which indicates the presents of carbohydrates (Table 1).

Phenol Test

Dark colour was formed when 5%of ferric chloride was added to the plant extract which indicates the presence of phenol (Table 1).

Table 1: Qualitative analysis of Phytochemical of Tuber sample

Sl .No	Phytochemical	Test Results
1	Alkaloids	Present
2	Amino Acids	Absent
3	Carbohydrates	Present
4	Phenolic compounds	Present

Quantitative analysis for Phytochemical

Total Phenolic contain

The total phenolic content was reported as Gallic acid equivalents (fig. 1) with reference to a standard curve (standard curve equation: y = 2.578x+0.072, R^2= 3.008). The total phenolic content measured in methanolic extract was 29.80±0.072 µg GAE/g of dry sample. (Table 2).

Total Tannin Contain

The total tannin content was reported as Gallic acid equivalents (fig. 1) with reference to a standard curve (standard curve equation: y =0.519x+1.104, R^2= 2.630). The total phenolic content measured in methanolic extract was 5.028±1.104 µg GAE/g of dry sample. (Table 2).

Total Alkaloids and Carbohydrates Content

The total estimation of alkaloids with reference to standard curve (standard Curve equation y=0.506 +0.229,R^2=0.9). The total alkaloids content is 4.47±0.229 µg/g.(**Table 2).**

Total Carbohydrates Content

The total content of carbohydrates in the sampleis measured by using anthrone method, with reference with the standard curve (Standard curve equation: y=0.480+0.381, R^2=1.467) the total carbohydrates contain in methanolic extract was 6.73±0.381µg /g of dry sample. (**Table 2).**

Table 2: Quantitative Analysis for Phytochemicals of Stephania *glabra*

Sl.No	Concentration (µl)	Phenol	Tannin	Alkaloid	Carbohydrate
		Absorbance (550)nm	Absorbance at (725 nm)	Absorbance at (470 nm)	Absorbance at(630 nm)
1	Blank	0	0	0	0
2	0.2	1.05	1.17	0.205	0.45
3	0.4	1.67	1.35	0.309	0.605
4	0.6	2.18	1.39	0.46	0.694
5	0.8	2.59	1.55	0.54	0.727
6	1	2.32	1.59	0.62	0.871
7	Sample	3.008	2.63	0.9	1.467

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Evaluation of antioxidant Activity

The Antioxidant study, carried out using the DPPH radical scavenging assay demonstrated that the extract has a high amount of antioxidant activity. The methanol extract obtained fromthe tuber of *Stephaniaglabra* species has about 88.99% radical scavenging activityas compared to the positive control Ascorbic acid which exhibits 95.25%. Therefore the extract may also be suitable as an Anti- inflammatory substances provided the molecules present in the extract known to possess such anti- oxidant properties are identified (**Table-3, Figure-2).**

Table 3: Evaluation of antioxidant activity by DPPH method and comparison with standard solvent Ascorbic acid:

				Tuber sample		Ascorbic acid	
Sl. No.	**Conc. of plant extract(µl)**	**Conc. of methanol (µl)**	**Conc. of DPPH sol(0.1mM)**	**Absorbance at 517 nm**	**Scavenging activity (%)**	**Absorbance at 517 nm**	**Scavenging activity (%)**
1	25	145	950	0.058	88.99	0.025	95.25
2	50	120	950	0.075	85.76	0.027	94.87
3	75	95	950	0.078	85.19	0.030	94.30
4	100	70	950	0.082	84.44	0.030	94.30
5	150	20	950	0.09	82.92	0.097	81.59

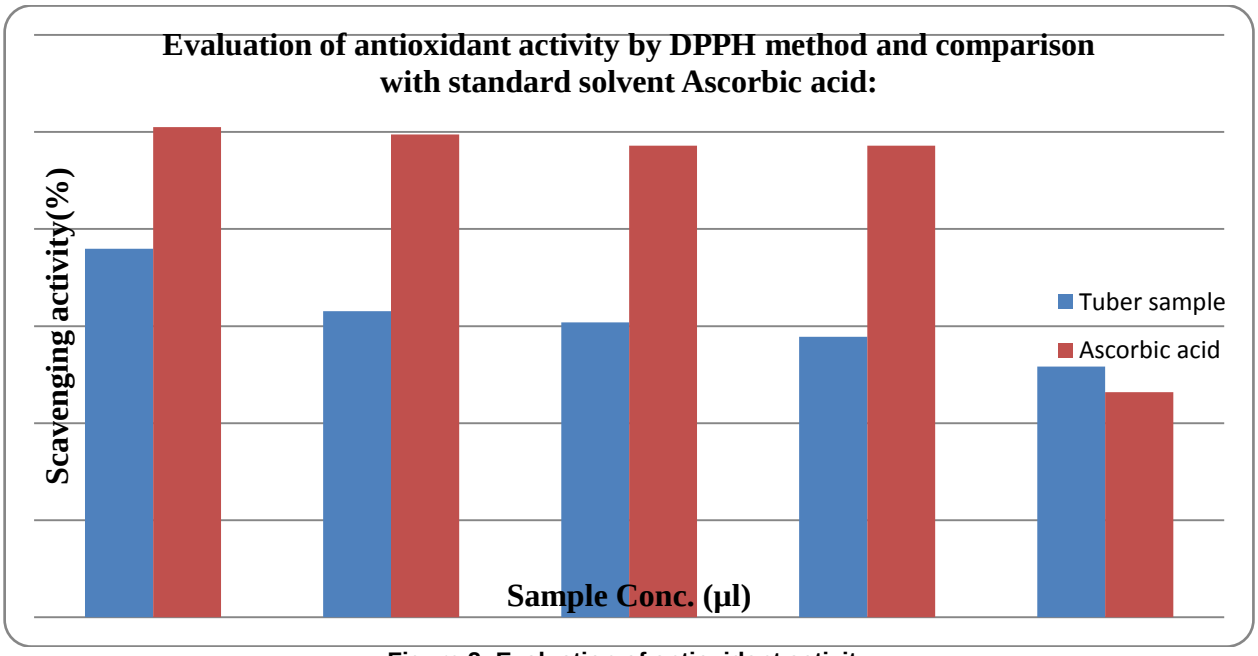

Figure 2: Evaluation of antioxidant activity

Chromatography:

Thin Layer Chromatography (TLC)

During the development and viewing of TLC Plate, the starting point and solvent front are marked and all the spot are observed. The location of the each spot on the plate is then represented numerically by calculating a retention factor (R_f). This is accomplished by marking the following measurement and calculation as shown.

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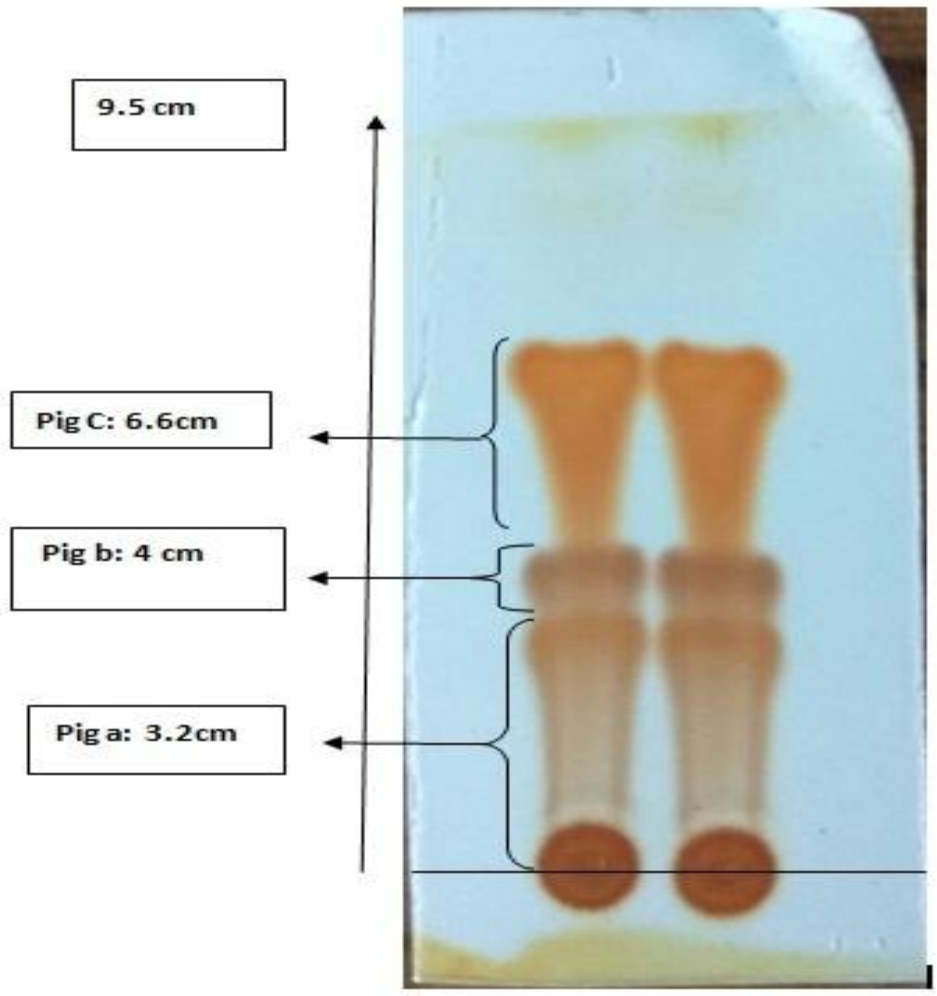

Figure 3: TLC plate showing R_f value.

Table 4: showing the different R_f value of different pigments.

S.No.	Pigments	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	R_f value
1	Pig a	3.2cm	9.5 cm	0.34
2	Pig b	4 cm	"	0.42
3	Pig c	6.6 cm	"	0.69

Gas Chromatography – Mass spectrometry (GC-MS)

GC-MS was done out to identify some Bioactive component present in the sample.

To know whatare bioactive compound are presents, 9 highest peaks was taken n was measured from the GC-MS library which was already present. The common bioactive compounds are PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL, PHENOL, 3,5-BIS(1,1-DIMETHYLETHYL, 5-EICOSENE, 1-HEXADECANOL, TRIFLUOROACETIC ACID,N-TRIDECYL ESTER,BEHENIC ALCOHOL, 6H-DIBENZO[A,G]QUINOLIZINE, 5,8,13,13A-TETRAHYDRO-2,3,9,10-TETRAMETHOXY, BENZENE, 1-METHOXY-4-METHYL-2-(1-METHYL ETHYL, BENZENE, 1-METHOXY-4-METHYL-2-(1-METHYLETHYL), TRIFLUOROACETATE. (Figure-4).

Liquid chromatography-Mass spectrometry (LC-MS)

The LC-MS was done to known the bioactive Compound structure present in the sample. Through LC-MS we can know the definite structure of the component present in the sample and the highest number of bioactive compound structure present .Crystal was formed after the purified. (Figure-5)

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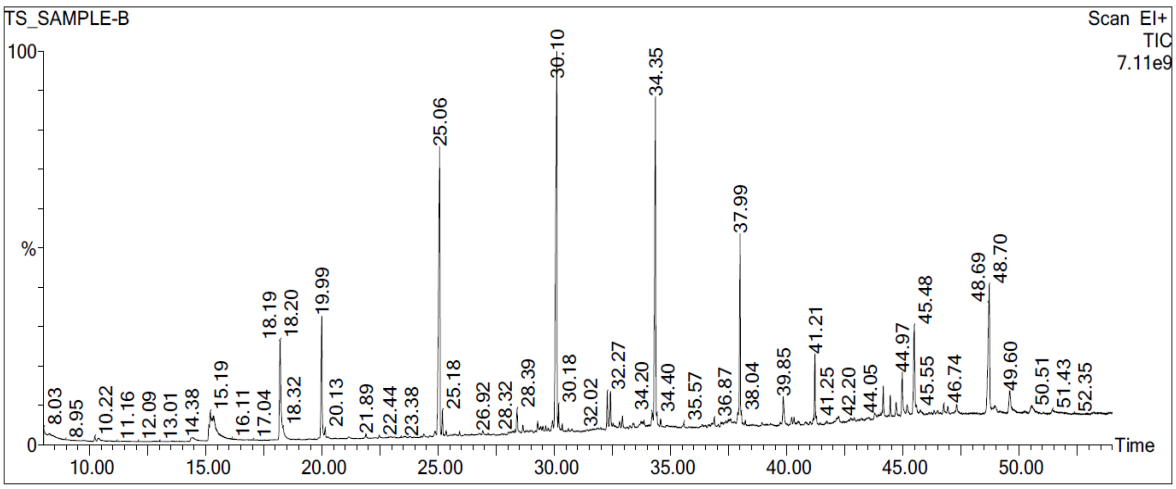

Figure 4: The results of GC-MS peak.

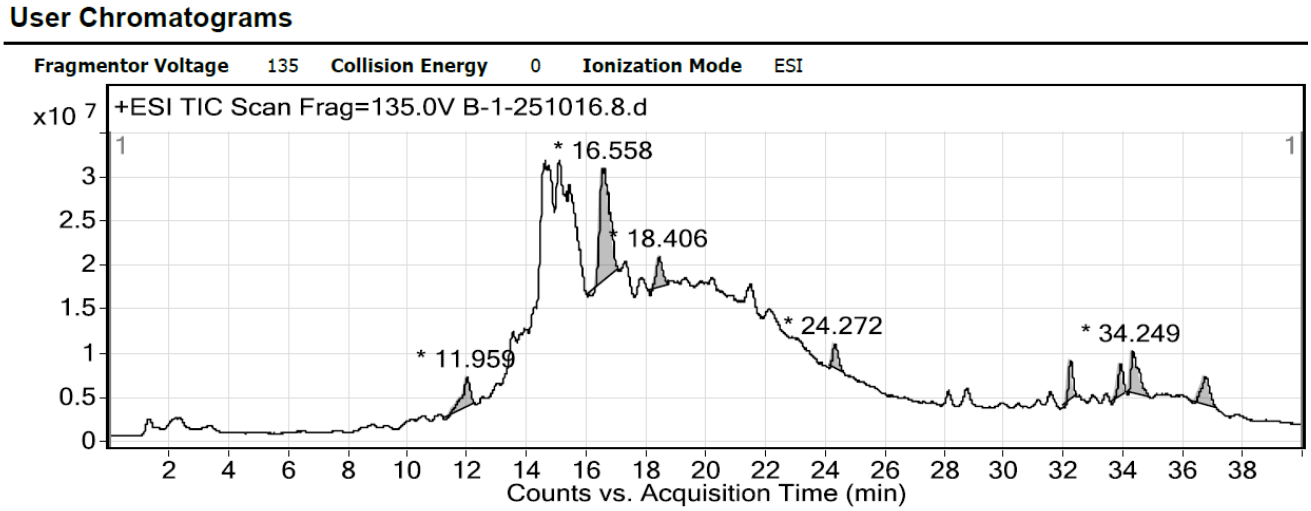

Figure 5: The peak showing the Results of LC-MS

Discussion

Stephania glabra is a climbing herbaceous shrubs which has tuber as roots in folklore to the decoction of Stephania glabra roxb. Menispermaceae is used in the treatment of fever,gastric problem, anathematic dysentery, as in anti – helminthic in rheumatic body ache, blood dysentery, leprosy and even as anti – cancer drugs.Quantitative analysis was carried out for determination of Phenol, Tannin, alkaloids and carbohydrates, which advocate this plant to be an important source for providing therapeutic agents specially for antioxidant and anticancer activity. Phenol content was 29.80 µg GAE/g, tannin 5.0 µg GAE/g and alkaloids 4.47 µg/g. so its seen that the phenolic compound is rich in *Stephania glabra* as compared to *Cukrasiatubularis*with phenolic content of 17.2mg GAE/g and *Baliospermummontanum*(2.72 mg GAE/g) [8]. Carbohydrates was carried out by anthrone method and found 6.73 µg/gm.*Stephania glabra* was elucidated for its antioxidant potential by DPPH which revealed excellent radical scavenging activity and reducing abilities with scavenging activity 88.99% as compared with methanolic extract of *S.elegans* as determined by (IC50 value was found to be 22µg/ml with reference to *S. elegans with 41.66by ABTS methods and 52.96 by FRAP* [6].Column chromatography was carried for purification and further studies.TLC was determined with 3 spots with R_f = 0.34, 0.4, 0.66 cm for pigment of a, b and c respectively.Bioactive compound was determine by LC- MS methods and GC- MS library with highest peak and compound identified are phenol, dimethyethyl, eosin , hexadecanol, trifluoroacetic acid, behenic acid, alcohol, dibenzo, quinolizine , benzene etc.

Conclusion

The plant *Stephania glabra* is a plant with many curative principle and economic value .The investigation concluded that the phytoconstituent phenolic compounds, tannins, alkaloids and carbohydrates are present and are responsible for many biological activities. It has scavenging activity of 88.99 % in antioxidant .Column chromatography was carried to purify the compound. TLC isolates the bioactive compound present in plant sample. GC-MS

and GC- MS detected the bioactive compound present. It can be further investigated for its antimicrobial activity. It can yield rich dividends in term of discovery of new drugs and new drugs target in medicinal plant research.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Reference

1. *Deepak Kumar Semwal and Ruchi Badoni Semwal.Efficacy and safety of Stephania glabra:an alkaloid-rich traditional medicinal Plant. Natural product research .2015 ,29(5):396-410 ·*
2. *Rownak Jahan, Mst, Afsana Khatun, Nusratan Nahar, Farhana Ishrat Jahan, Use of Menispermaceae family plants in folk medicine of Bangladesh.Advances in Natural and Applied Sciences.2010, 4(1): 01-09.*
3. *Semwal D. K, Badoni R, Semwal R , Kothiyal S. K, Singh, G. J , Rawat U. The genus Stephania (Menispermaceae): Chemical and pharmacological perspectives. Journal of Ethanopharmacology. 2010. 132(2):369-383.*
4. *Hemraj, Avneet Gupta, Ajay Thakur. Neeraj Upmanya., Pharmacological activities of Stephania glabra, Woodfordia frucicosa and Cissempelos Pareira. A review, International Journal of Pharmacy and Pharmaceutical science .2012. 4. (3) 16-23.*
5. *Hemraj, Avneet Gupta, Ajay Thakur. Neeraj Upmanya., Hydrodistillation of Stephania glabra tubers and Woodfordia fructicosa leaves., Asian Journal of Pharmaceutical and Clinical Research. 2012,5(3) 105-107.*
6. *Ravin Sharma , Gourav Chandan, Anterpreetchahal, Reena V Saini., Antioxidant and Anticancer activity of methanolic extract from Stephania elegans., Int J PharmPharm Sci.2016.9(2) ,245-249.*
7. *Mudasis Sultana, Pawan Kumar Verma, Rajinder Raina, Shahid Prawez , MA Das., Quantitative analysis of total phenolic, flavonoids and tannin content in acetone and n- hexane of Ageratum conyzoides., International Journel of Chemtech Research., 2012.4(3), 996-999*
8. *Biju John, Sulaiman CT, Satheesh George, URK Reddy., Total Phenolics and Flavonoids in selected Medicinal Plants from Kerala. International Journal of Pharmacy and Pharmaceutical Science.2014, 6(1):406-408.*
9. *Mathew Geoge., Qualitative and Quantitative Phytochemical Analysis on the leaves and fruits of Passiflora foetida., International Journal of Pharmaceutical Science Invention.2017, 6 (3),26-30.*
10. *Lin Y, Xu W, Huang M, Xu W, Li H, Ye M, Zhang X, Chu K. Qualitative and Quantitative Analysis of Phenolic Acids, Flavonoids and Iridoid Glycosides in YinhuaKanggan Tablet by UPLC-QqQ-MS/MS. Molecules, 2015, 20(7):12209-28*
11. *Neeru Agarwal. Divya K Minj. KHusboo Rani., Estimation of Total carbohydrates present in dry fruits ., IOSR-Journal of Environmental science , Toxicology and Food Technology., 2015.1(6), 24-27+*
12. *Syed Zameer., Hussain and Khushnuma Maqbool., GC-MS principle , Technique and its application in food science., INTJ-Curr sci. 2014 13(E) 116-126.*