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Research Article

Chemistry for New drug discovery

Qualitative and Quantitative Phytochemical Analysis of *Andrographis Echioides* Leaves.

S. Gurupriya* **and Dr.L. Cathrine[1]**

*Research Scholar, P.G. and Research Department of Chemistry, Holy Cross College (Autonomous) Affiliated to Bharathidasan University, Tiruchirappalli 620002, TamilNadu, India.

[1]Research Supervisor, P.G. and Research Department of Chemistry, Holy Cross College (Autonomous) Affiliated to Bharathidasan University, Tiruchirappalli 620002, TamilNadu, India.

Abstract: Plants are the crucial resources and backbone of human life on earth and another main significance of plants is their medicinal properties. Medicinal plants provide an estimable source of lead compounds for the discovery of noble new drugs with few undesirable side effects. In this study, the Qualitative phytochemical analysis, quantitative estimation, and proximate analysis of phytochemical compounds with the importance pharmacological significance in various organic solvent extracts of *Andrographis echioides* leaves. Preliminary phytochemical screening analyses were determined using standard protocol methods. Qualitative phytochemical analysis of leaves extracts from *Andrographis echioides* was analyzed for the primary and secondary metabolites of phytochemical constituents such as sugar, protein, amino acids, Carbohydrates, alkaloid, Flavonoids, Terpenoids, Glycosides, Tannins, Gum, Mucilage, phenolic compounds, Phytosterols, Coumarin, Emodin, Saponin, Oils, and Fats were screened in different solvent extracts. Quantitative estimation of secondary metabolites from *Andrographis echioides* leaves are determined by using Standard methods and proximate analysis of fibre, protein, fat, total ash, and carbohydrate were carried out by using standard. Among the nine different extracts of *Andrographis echioides* ,methanolic extract of the leaves showed the maximum amount of phytochemical was screened in *Andrographis echioides*. Quantitative estimation of leaves from *Andrographis echioides* contained maximum content of total phenols, flavonoids, Steroids, and Terpenoids with the minimum amount of saponins, alkaloids, and Tannin. The proximate analysis in percentage showed that *Andrographis echioides* leaves had the highest amount of Carbohydrates content 33.78% and Protein content 27.21%. and the lowest amount of Fibre content 10.56% Fat content 1.13% and ash content 7.32%. The presence of phytochemical compounds supports the traditional use of *Andrographis echioides* as an alternative treatment for curing certain health conditions. Further studies should be carried out to isolate specific phytochemical chemical constituents and should be used in different studies to explore their Biological effects.

Keywords: Phytochemical constituents, *Andrographis echioides,* leaves, primary metabolites, secondary metabolites, proximate analysis

***Corresponding Author**

S. Gurupriya , *Research Scholar, P.G. and Research Department of Chemistry, Holy Cross College (Autonomous) Affiliated to Bharathidasan University, Tiruchirappalli 620002, TamilNadu, India.

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1. INTRODUCTION

Nature has been a resource for therapeutic agents for thousands of years. Various medicinal plants have been used for years in everyday life for ailments all over the world[1]. W.H.O survey reported 80% of the population in the world uses traditional medicines for primary healthcare in both early and developed countries.[2,3]Natural remedies derived from herbs[4] food or raw materials are used by pharmaceutical and food industries. It has been approximating that 14%-28% of privileged plant species are used medicinally and 74% of pharmacologically active plant-derived components were discovered by following ethano medicine use of the plants.[5] Medicinal Plant products have been parts of phytomedicines since days of yore. This can be from barks, leaves, flowers, roots, fruits, seeds[6]. Information about the phytochemical constituents of plants is attractive because such information will be valuable for the synthesis of complex chemical substances.[7-8] Phytochemical studies are based on exploring plants for their use of the creation of Novel recuperative drugs. Medicinal plants provide an estimable source of a lead compound in discovering noble and new drugs with huge less side or undesirable effects.[9]Everyday researchers are entering for new-fangled drugs with better or improved therapeutic actions. Traditional medicines are exceedingly very important since the early primeval periods because of their truthfulness in the use of different ailments and human sufferings. These are the reservoirs of potentially helpful phytochemical compounds that could provide newer leads and clues for recent drug design.[10] Phytochemicals are bioactive chemicals of plant origin and it served as raw resources for new drug discovery.[11] Phytochemicals are classified as primary and secondary metabolites, dependent on their part in plant metabolism. Primary constituents consist of common sugars, amino acids, proteins, purines, and pyrimidines of nucleic acids, chlorophylls, etc. Secondary constituents are the enduring plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, saponins, phenolics, flavonoids, and glucosides.[12] The significance of these phytochemical compounds of plant kingdom include alkaloids, tannins, steroids, Terpenoids, Flavonoids, glycosides, phenolic compounds, volatile oils, etc. be synthesized from recurrent types of healing plants that have prospective healing and pharmacological activities and are used in different disorders.[13] *Andrographis echioides* (L) *Nees* is an imperative herb widely disseminated in south India. This is commonly known as False Water willow. *Andrographis echioides* belong to the family *Acanthaceae*, utilized for different restorative purposes in South Asia especially India. However, information on the chemical composition and bioactivity of this species is currently not available.[14] Based on the literature, this plant has pharmacological properties such as antimicrobial activity, anti-inflammatory, diuretic, anthelmintic, Pain-relieving, antipyretic, hepato-defensive, and antioxidant effect.[15] The leaves juice of Andrographis *echioides* is utilized to cure fevers.[16] *Andrographis echioides* overflowed with coconut oil is utilized to decrease the falling and turning gray of hair.[17,18] Thus, the main aim of this research work is to carry out Qualitative phytochemical analysis, Quantitative determination, and Proximate analysis for the phytochemical compounds in *Andrographis echioides*.

2. MATERIALS AND METHODS

1.1 Collection and Authentication of plant

The Andrographis echioides were collected together in May month from Mullipatti, Pudukkottai, Tamil Nadu, India. They were duly processed and mounted in standard Herbarium sheets. The plant (*Andrographis echioides*) was recognized, authenticated, and established by Dr. S. John Britto, Director, Rapinat Herbarium, St. Joseph College, Tiruchirappalli, and Tamil Nadu for identifying the plants. The voucher specimen number is SGP001 (7.06.2017).

1.2 Extraction of plant material

Andrographis echioides leaves were collected washed exhaustively in tap water to evacuate undesirable material, rinsed with distilled water. After the leaves were dried at room temperature for 30 days. The dried leaves were ground well into coarse powder and stored in airtight containers and 25g coarse leaves powder was then exposed to Progressive extraction in 250ml of methanol solvent by using Soxhlet apparatus. The accumulated methanolic leaves extract was stored and then used for further investigation. Similar procedures were followed for different solvents like Ethanol, Chloroform, Dichloromethane, Aqueous, Ethyl acetate, Petroleum ether, Hexane, and Acetone.

1.3 Qualitative phytochemical analysis of Primary and secondary metabolites from Andrographis echioides leaves extract.

Qualitative phytochemical speculation was carried out by utilizing standard procedures Sofowara (1993), Trease and Evans (1989), and Harborne (1973, 1984).[19-22]

1.4 *Quantitative determination phytochemical analysis of secondary metabolites from Andrographis echioides leaves*

1.4.1 Determination Total Phenolic content

The measure of Total phenolic contents of *Andrographis echioides* leaves was resolute through the Folin-Ciocalteu reagent technique as Portrayed by Kim *et al*(2003)[23] Briefly, A diluted methanolic leaves extract (5 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask containing 9ml deionized water. 1 ml Folin-Ciocalteu's phenol reagent was added to the reaction mixture and shaken. After 5 min, 10 ml 7% Na_2CO_3 solution was treated to the test solution was diluted to 25 ml deionized water and uniform altogether. The reaction mixture was kept in the dark for 90 min at 23°C, after which the absorbance was Scrutinized at 750 nm. Above the tests were performed in triplicates.

1.4.2 Determination Total Flavonoids content

The total flavonoid contents of the *Andrographis echioides* leaves were resolved to utilize the aluminum chloride colorimetric technique as depicted by Katasani *et al*(2011).[24] 2g leaves powder was taken in a test tube and 10 ml methanol was poured into it. The blend was shaken well and filtered to take 5ml methanolic leaves extract of the plant sample. About 5 ml methanolic leaves extract or Quercetin standard Flavonoid compound was blended in with 1.5 ml of methanol, 0.2 ml 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 2.8 ml distilled water and it stayed at room temperature for 30 minutes. The absorbance of the

reaction blend was estimated at 510 nm utilizing a UV-Visible spectrophotometer. Above the tests were performed three times simultaneously.

1.4.3 Determination of Total Tannin content

The total Tannins assay was conducted according to Bajaj and Devsharma method (1977).[25] 2g leaves powder was taken in a test tube and 10 ml methanol was poured into it. The blend was shaken well and filtered to take 5ml methanolic leaves extract of the plant sample.5ml of *Andrographis echioides* methanolic leaves extract or Tannic acid standard Tannin compound containing 7.5 ml of water. Add 0.5 ml of Folin-Denis reagent and 1.0 ml of Na_2Co_3 Solution and dilute to the mark with water. Mix well and determine the absorbance at 760 nm after 30 min utilizing UV-Visible spectrophotometer. Above the tests were performed three times simultaneously.

1.4.4 Determination of Steroids

The steroids were determined by the method *Attarde Daksha et al*(2010).[26] *Andrographis echioides* methanolic leaves extract 5ml dissolved in chloroform to 10 ml, and diluted solution(3ml) blended with 2.0 ml of Liberman-Burchard reagent and 2.0 ml of chloroform. The tubes were covered with black carbon paper and kept in an ice bucket in a dark place for 15 min. secured Libermann-Burchard reagents react with the sterol to produced characteristic green color, their absorbance was determined on a spectrophotometer at 640 nm. Cholesterol is used as the standard for Steroids. Above the tests were performed three times simultaneously.

1.4.5 Determination of Terpenoids

Terpenoids were determined by the method Indumathi *et al* (2014).[27] 2g leaves powder was taken in a test tube and 10 ml methanol was poured into it. The blend was shaken well and filtered to take 5ml methanolic leaves extract of the plant sample. Then 2 ml chloroform was blended in the extract of the plant sample and 3 ml sulphuric acid was added in plant extract and read the absorbance at 538 nm. The absorbance of the standard was determined on a spectrophotometer and the standard Linalool (20 to 80 µg/ml) graph was plotted. Terpenoids content was expressed as milligrams of Linalool equivalents per gram of dried sample.

1.4.6 Determination of Alkaloid

The alkaloid was assessed by the method of Fazel Shamsa *et al* (2008) and Mallikarjuna Rao *et al* (2012).[28,29] 2g leaves powder was taken in a test tube and 10 ml methanol was poured into it. The blend was shaken well and filtered to take 5ml methanolic leaves extract was dissolved in dimethyl sulphoxide (DMSO) and 1ml of 2 N HCl. Above 1ml of the test, solutions were transferred to separating funnels, and added 5 ml of phosphate buffer pH 4.7 and 5 ml bromocresol green solution were added. The mixture was shaken and the complex formed was extracted with 5 ml of chloroform by overwhelming shaking and gathered in a 10-ml volumetric flask and diluted to the volume with chloroform and read the absorbance at 470nm utilizing UV/Visible spectrophotometer. Atropine was used as standard (1 mg/mL). The above tests were performed three times simultaneously.

1.4.7 Determination of Saponin

The Saponin was estimated by the method Madland *et al* (2013).[30] 2g leaves powder was taken in a test tube and 10 ml methanol was poured into it. The blend was shaken well and filtered to take 5ml methanolic leaves extract of the plant sample. About 5 ml of methanolic leaves extract of *Andrographis echioides* were treated with vanillin-acetic acid reagent (400 µl) and 1.6 ml of perchloric acid. This reaction mixture was kept on a water bath at 70-75° for several min. It was then cooled on an ice bath for 2 min and 2.5 ml of glacial acetic acid was poured into it. Sapogenin (1 mg/mL) was used as a standard and read the absorbance at 550 nm utilizing UV/Visible spectrophotometer. Above the tests were performed three times simultaneously.

1.5 Determination of Proximate analysis

The parameters determined for proximate analyses include ash, crude protein, fat, fibre, and carbohydrate. *Andrographis echioides* leaves were carried out using the methods described by AOAC (2010).[31]

3. RESULTS

1.6 Qualitative Phytochemicals analysis of primary and secondary metabolites from Andrographis echioides leaves extract.

Therapeutic plants are utilized for finding and screening the phytochemical constituents which are exceptionally useful for the assembling of new drugs. Qualitative phytochemical screening of *Andrographis echioides* leaves extracts was done with methanol, ethanol, Petroleum ether, Hexane, Dichloromethane, Ethyl acetate, acetone, chloroform, aqueous for nine extracts are summarized in theTable-1.

Table1: Qualitative Phytochemicals analysis of primary and secondary metabolites from *Andrographis echioides* leaves

S. No	Phytochemicals	Extracts								
		Aq	ME	ET	E.AC	ACE	DCM	CH	PE	HE
1.	Carbohydrates i)Molish's Test	++	+++	++	++	-	+	+++	+++	-
2.	Protein	-	+	+	++	+	+	-	-	-
3.	Amino acid	-	+	-	-	-	-	-		
4.	Sugar i) Fehling's Test ii) Barfoed's Test iii) Benedict's Test	+	+	-	-	-	-	+	+	-
5.	Tannin	+	+++	++	+++	+++	-	-	-	-

6.	Saponin	++	++	++	-	+	+++	-	-	-
7.	Flavonoids i)Alkaline Reagent Test	+	+++	++	+++	+	-	-	+	-
8.	Steroids i)Liberman-Burchard	+	+++	++	+	-	-	++	+	++
9.	Terpenoids	+	+++	++	++	+	-	++	+	-
10.	Alkaloids i)Mayer's Test ii)Wagner's Test	-	+++	+	++	++	+	-	-	-
11.	Phenolic compounds i)Ferric Chloride Test ii) Lead Acetate Test iii) Gelatin Test	++	+	+	++	-	-	++	+	+
12.	Glycosides i)Keller-Killani test ii) Legal's Test	+	++	++	+	-	-	+	++	+
13.	Coumarins	++	+++	++	+++	-	+	-	-	-
14.	Emodins	-	+	-	-	-	-	-	-	-
15.	Oil & fats i) Spot Test ii) Saponification Test	-	+++	++	-	-	-	-	-	-
16.	Gum & Mucilage	-	-	-	-	-	-	-	-	-

+++: highly present, ++: moderately present, +: Low, -: absent.

The present investigation of Qualitative Phytochemical analysis of leaves extracts separates from *Andrographis echioides* were analyzed for the presence of alkaloids, carbohydrates, saponin, protein, amino acids, Terpenoids, Steroid, Phenolic compounds, Coumarins, Emodins, Flavonoids, glycosides, Oil, fats, and Gum and Mucilage were screened in Nine different solvent extracts. The maximum amount of the Phytochemical constituents exists in various polar solvents like Methanol, Aqueous, and ethanolic extract. The hexane extract was able to have a very less amount of phytochemical compounds like Steroid, Phenolic compounds, and Glycosides. The above study concluded that the methanolic extract of leaves of *Andrographis echioides* has the potential to act as a source of useful drugs because of the presence of various phytochemical constituents. The previous study showed that the phytochemical screening of whole plant methanolic extract of *Andrographis echioides* revealed the presence of alkaloids, coumarins, flavonoids, phenols, and tannins as compared with ethyl acetate, aqueous, petroleum ether, and chloroform extracts (Ranjith Singh et al., 2017).[32] The aqueous, chloroform, and ethanol extracts of *Andrographis echioides* leaves showed the presence of major active compounds such as phenol, alkaloids, flavonoids, steroids, saponin which are important secondary metabolites which are used in traditional medicine to cure various ailments as well as in the modern medicine (Jeevanantham and Zahir Hussain, 2018).[33] The whole plant extracts of *Andrographis echioides* in acetone, petroleum ether, and methanol extracts showed the active compounds present in high concentrations such as flavonoids, phenolic compounds, glycosides, and terpenoids. Saponin, alkaloids, carbohydrates, and amino acids are in low concentration (Debasish Singha Roy et al., 2020)[34]. [35]Kanchana et al., 2004 reported that the petroleum ether, chloroform, ethyl acetate, and hydro-alcoholic extracts of the whole plant of *Andrographis echioides* contain flavonoids, saponins, tannins, phenols, terpenoids, and steroids. *Andrographis echioides* are utilized for finding and screening for the phytochemical constituents which are exceptionally useful for the assembling of new medications for the treatment of different illnesses.

1.7 Quantitative Determination of secondary metabolites from Andrographis echioides leaves

Since the methanolic leaves extract confined a maximum amount of phytochemical compounds (as clear from Phytochemical screening) simply these solvents alone were chosen for the quantification of secondary metabolites from *Andrographis echioides*. The phytochemicals with the highest quantity were phenol, followed by flavonoids, steroids, and terpenoids individually, as appeared in Table 2. Quantitative determination of secondary metabolites such as Alkaloids, Saponin, Terpenoids, Tannin, total phenols, and Flavonoids, steroids were analyzed by using Standard chemicals of Calibration curve are plotted as shown in the below Figure: 1-7. The most elevated convergence of phenol (192.96 ± 13.44), flavonoids (120.88 ± 8.42), alkaloids (41.740 ± 2.87), steroids (106.25 ± 7.42), tannin (52.858 ± 3.64), terpenoids (71.008 ± 4.97), and saponin (22.024 ±1.23) were found in the methanolic leaves extract of *Andrographis echioides*. Previous investigations have portrayed that the entire plant of methanolic extract of *Andrographis echioides* revealed the maximum extent of flavonoids (6.21 mg/g), anthraquinones (11.93 mg/g), terpenoids (12.56 mg/g), steroids (4.96 mg/g) and alkaloids (10.84 mg/g) of crude extract as equated to the chloroform extract indicated the maximum extent of flavonoids (2.92 mg/g), anthraquinones (4.64 mg/g) and alkaloids (4.93 mg/g) of crude extract (Ranjith Singh et al., 2017).[32]

Table 2: Quantitative Estimation of Secondary metabolites from *Andrographis echioides* leaves

S.No	Name of the Phytochemical compounds	Methanolic

	(Secondary metabolites)	Leaves extract (mg/g)
1.	Total phenol (Milligrams of Gallic acid (GAE) equivalents per gram)	192.968±13.44
2.	Flavonoids (Milligrams of Quercetin equivalents per gram)	120.882± 8.42
3.	Alkaloids (Milligrams of atropine equivalents per gram)	41.740± 2.87
4.	Steroids (Milligrams of Cholesterol equivalents per gram)	106.455± 7.42
5.	Tannin (Milligrams of tannic acid equivalents per gram)	52.8588± 3.64
6.	Terpenoids (Milligrams of Linalool equivalents per gram)	71.008 ± 4.97
7.	Saponin (Milligrams of Sapogenin equivalents per gram)	22.024 ± 1.23

Values were expressed as Mean ± SD for Triplicates

At the 0.05 level, the population means are significantly different.

FIGURE-1-7: Quantitative determination of Secondary Metabolite from the Leaves of *Andrographis echioides*

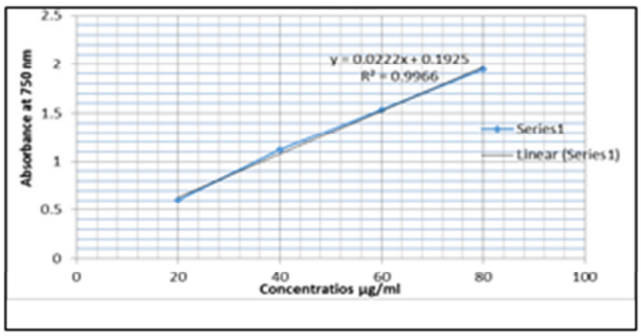

Fig: 1 Standard Curve for Phenol using Gallic acid

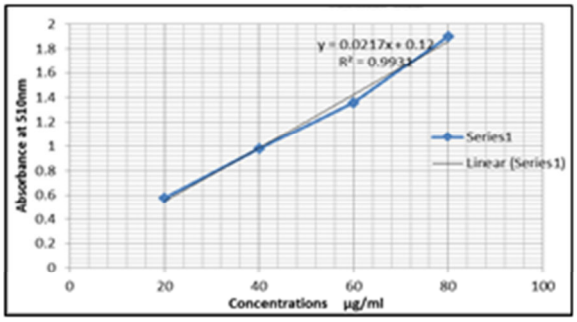

Fig: 2 Standard Curve for Flavonoids using Quercetin

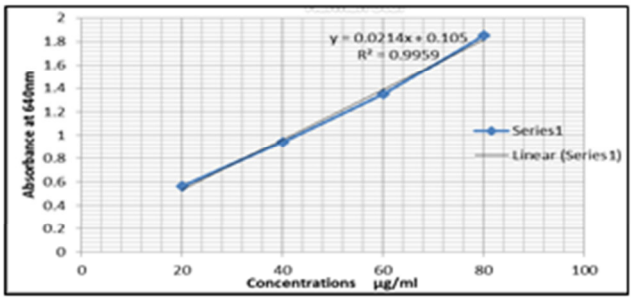

Fig: 3 Standard Curve for steroids using cholesterol

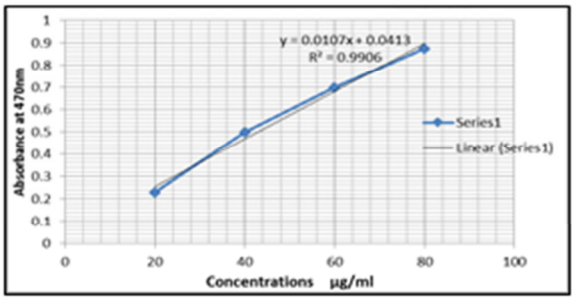

Fig: 4 Standard Curve for alkaloids using atropine

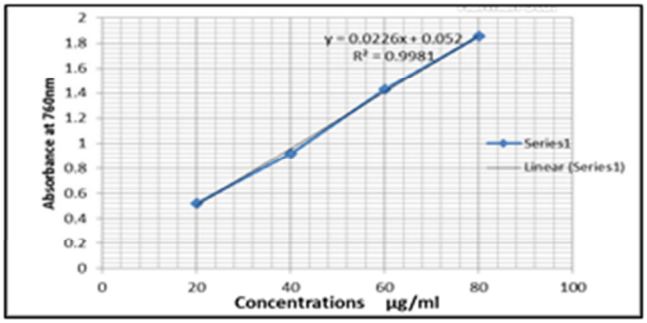

Fig: 5 Standard Curve for tannin using Tannic acid

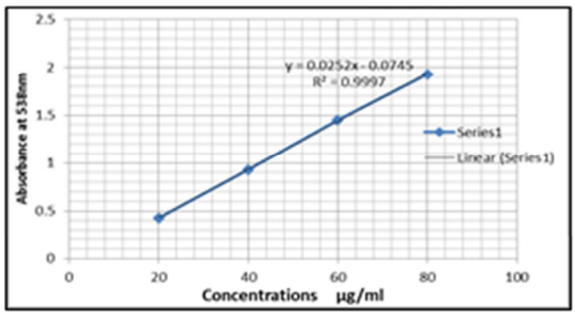

Fig: 6 Standard Curve for terpenoids using Linalool

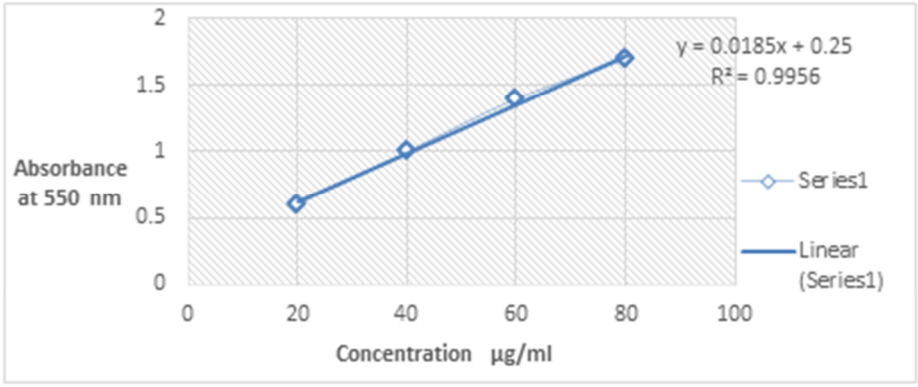

Fig: 7 Standard Curve for Saponin using Sapogenin

3.3 Proximate analysis of Andrographis echioides leaves

The parameters determined for proximate analysis in **(%)** include ash, crude protein, fat, fibre, and carbohydrate of *Andrographis echioides* leaves are shown in Table-3.

Table 3: Proximate analysis for the leaves of *Andrographis echioides*

S. No.	Proximate Tests	Leaves sample (%)
1.	Fiber	10.56
2.	Protein	27.21
3.	Fat	1.13
4.	Total Ash	7.32
5.	Carbohydrates	33.78

The results of the proximate analysis in percentage showed that *Andrographis echioides* leaves had the highest amount of Carbohydrates content of 33.78 % and Protein content of 27.21% *and the* lowest amount of Fibre content 10.56%, ash content 7.32% and Fat content 1.13%. Carbohydrate constituent a significant class of naturally exacerbates of organic compounds that are fundamental for the upkeep and sustenance of life in plants and animals and provide crude materials to several industries.[36]

4. DISCUSSION

Plants are the major source for secondary metabolites, they are meant for several biological activities in humans and animals. [37] Plants are secondary metabolites that are shaped as results of essential digestion and created for barriers against predators. Secondary metabolites are commonly not significant for the development and proliferation of organisms, but they play an essential role in the pharmaceutical field. Preliminary screening of phytochemicals is an important advance, in the revealing of the bioactive principle present in restorative plants and consequently, may prompt drug discovery and enlargement.[38] Quantitative Determination of *Andrographis echioides* leaves contained higher values of phenolic and flavonoid content make the plant useful for the formulation of the different drugs for human uses for treating various diseases. The obtained results explained the presence of possible phytoconstituents, potential usefulness, and justified the traditional uses. Secondary metabolites may be helpful in protection against various diseases. Instances of such metabolites are tannins, flavonoids, and alkaloids; they are known to be the mind behind the therapeutic potentials of plants. For Example, Alkaloids play a role in medicines, anti-spasmodic, and antibacterial natural compounds.[39]Saponin is used as a potential ointment in the recovery process of wound healing and hormone synthesis in the pharmaceutical industry. Saponins are known to have anti-inflammatory, hemolytic, and cholesterol binding properties.[40,41] Terpenoids are valuable in the prevention and therapy of several diseases, including cancer.[42] Tannin compounds play a major role in plants to protect them from predators and also in growth regulation.[43] Tannins have astonishing astringent properties.[44] They are known to accelerate the recuperating of wounds and excited mucous membranes. Phenolic compounds are widely found in the secondary products of medicinal plants, which are potent antioxidants free radical-scavengers, and anti-inflammatory agents.[45] Steroids were responsible for central nervous system activities.[46] Flavonoids occur naturally in plants, fruits, and food products. It effectively scavenges most free radicals including singlet oxygen.[47] It exhibits vast pharmacological and therapeutic properties like antiviral, anti-inflammatory, anticancer, anti-diabetic, and antiallergic activities.[48-50] Flavonoids are liable for powerful water-soluble anti-oxidant and free extreme forager, which forestall oxidative cell harm and also have strapping anti-cancer action.[51] It also helps in overseeing Diabetic actuated oxidative stress. Proximate analysis is an important criterion in the determination of contamination and the quality of the sample used for the experiment.[52] Plants contribute to satisfying human needs in terms of energy and nutrition. The nutrients present in plants are carbohydrates, proteins, and fats. This plant would be a promising source of carbohydrate, protein fat, and fibre and may be recommended as nourishment to people suffering from malnutrition. Carbohydrates are known to be important components in many foods, and digestible carbohydrates are considered an important source of energy.[53]

5. CONCLUSION

Andrographis echioides leaves are utilized for screening the phytochemical constituents which are extremely useful for the assembling of new drugs for the treatment of several diseases. From the above exploration, it may very well be concluded that this plant has enormous potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various Phytocompounds that are essential for good health, it can also be used to improve the health status of society. The research is in progress to discover innovative, dynamic, and novel drugs for curing various newly emerged dangerous diseases.

6. AUTHORS CONTRIBUTION STATEMENT

Professor Dr.L.Cathrine gives the guidelines for the research study and S. Gurupriya carried out the research work.

7. ACKNOWLEDGEMENT

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8. CONFLICTS OF INTERESTS

Conflict of interest declared none.

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