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Evaluation of Phytochemical Analysis and *In vitro* Antioxidant activities of Stem and Leaf extract of *Andrographis echioides* (L.) Nees

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ABSTRACT: Plants are essential to human life on Earth and serve as its foundation. Their therapeutic uses are another important aspect of plants. Medicinal plants provide an excellent source of lead compounds for the finding new drugs with few adverse reactions, which is a very respectable source. The *Andrographis echioides* is a highly medicinal valuable plant widely spread in south India. The plant general name is called as "false water willow". The objective of the current study is to assess the in vitro antioxidant activity, quantitatively estimating, and qualitative phytochemical evaluation of different extraction of the *Andrographis echioides* both leaves and stems. The dried leaf while stem powders of *Andrographis echioides* was phytochemically analyzed, and it was found to include carbohydrates, proteins, amino acids, alkaloids, saponins, phenols, flavonoids, glycosides, cardiac glycosides, fixed fats and oils, gums, and Musilages. The DPPH, FRAP, Reducing Power, and Phosphoromolybdenum tests methods were utilized to investigate phytochemicals and in vitro antioxidant capabilities. The traditional usage of *Andrographis echioides* as a complementary medicine for treating specific medical disorders is supported by the presence of phytochemical components. Additional research should be done to extract particular phytochemical chemical ingredients, and these compounds should then be used in various studies to examine their biological impacts. A medicinal plant called *Andrographis echioides* is used to cure a variety of diseases. It may have antioxidant qualities. Finding a novel herbal remedy for oxidative stress from *Andrographis echioides* is the goal of the current research.

Keywords: DPPH, FRAP, Phytochemicals, *Andrographis echioides*, Reducing power assay, Secondary metabolites, Phosphomolybdenum.

INTRODUCTION

Medicines from Ayurveda, Siddha, and Unani constitute the majority of the medical industry. Siddha from south India (Tamil Nadu and Kerala), Ayurveda from north India, and Unani from Arabic medicine. In the past, people have promoted human health and treated a variety of diseases and afflictions using medicinal plants. India boasts 45,000 different plant species, making it the country with the highest biodiversity in the world. Recently, 20,000 potentially beneficial plants in India were identified. 500 traditional communities treat various illnesses (Chin *et al*., 2006). Medicinal plants are significant plant species that, in accordance with both modern scientific research and conventional medical practices, can be used to treat illnesses and improve human health. These plants are thought to be abundant sources of components for the synthesis and manufacture of pharmaceuticals (Oladeji *et al.,* 2019). Phytoconstituents are the numerous types of chemical components that make up plants (Mercy *et al.,* 2017). The secondary roles that phytoconstituents perform for plants include promoting plant development, protecting them by triggering defence mechanisms, and giving them colour, flavour, and aroma (Molyneux *et al.,* 2007). These plant-derived substances, such as flavonoids, quinine, terpenoids, etc., carry out specific biological processes that support therapeutic effects like anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant capabilities (Batiha and Beshbishy 2020). The presence of free radicals in the body causes damage to the DNA and other parts of the cell. It induces chain reaction which leads to the formation of many other free radicals thereby increasing the free radical oxidative stress (Mercy *et al.,* 2017; Batiha and Beshbishy 2020; Rajakumari *et al.,* 2020). The curiosity of man has led to the accumulation of information about medicines over thousands of years. Consequently, there are numerous efficient health care options available today. Since man was the only chemist for centuries, practically all medications utilized in the past were from plants. There currently exists a large amount of literature on the therapeutic benefits of many plants. Many plants now produce powdered medications, which are offered as plant products. Such circumstances need the investigation of the drug's cellular characteristics and the phytochemical testing of its ingredients (Iyengar 1974 and 1975; Iyenga and Yalc 1975). Therefore, the requirement for evolving standards for samples of unprocessed medicines has

become essential in pharmacognosy. In order to oversee the production of fake pharmaceuticals as well as the consistency of the products in a commercial management, proper scientific criteria must be devised (Israili and Issar 1975). Research has been done all over the world to discover hidden medications and to make use of herbs' therapeutic properties. The man's perception of the magical effects of plant products was altered by the scientific verification of the herbs. Drug production without scientific quality control would be detrimental to both the wellbeing of humans and conventional medical systems. The Acanthaceae family includes the current experimental plant, *Andrographis echioides* (L.) *Nees.* It is extensively dispersed in Sri Lanka and tropical India. Traditional uses for *Andrographis echioides* (L.) *Nees* include treating cuts and wounds with it for anti-inflammatory, antioxidant, febrifuge, cooling, and other benefits. To treat fever, the entire plant's extract is used (Nadkarni, 1976). The species of Indoneesiella is used in goitre, liver diseases (Nadkarni and Nadkarni 1976). fertility problems, bacterial (Qadrie *et al*., 2009). Diseases caused by fungi and malaria. This plant's leaf juice is used to treat fevers (Kirtikar and Basu 1975). About 40 different Andrographis species have been utilised to treat conditions like influenza, malaria, dyspepsia, and respiratory illnesses. The Andrographis species is also employed as an antidote for some insects' toxic stings (Kirtikar and Basu 1975; Chopra *et al*., 1980). To prevent hair loss and ageing, the leaf juice is combined with coconut oils and cooked (Pandi Kumar *et al*., 2007). Researchers have looked at the phytochemistry of *Andrographis echioides* and found that it contains a number of flavonoids (Harbone, 1994; Iinuma and Mizuno 1989). And labdane diterpenoids (Kleipool, 1952; Chan *et al.,* 1971; Balmain and Connolly 1973; Fujita *et al*., 1994; Matsuda *et al*., 1994; Reddy *et al.,* 2003). Only flavonoids have been identified as the primary constituents of Andrographis echioides (L) Nees extracts in earlier research (Govindachari *et al.,* 1956; Jayaprakasam *et al.,* 1999; Jayaprakasam *et al.,* 2001). Many constituents of plants have been linked to several kinds of diseases, including phenols as well coumarins, lignans, volatile oils, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, and alkaloids (Sharma *et al*., 2002). Research was done to identify the plant's preliminary phytochemical components and antioxidants capabilities.

MATERIALS AND METHODS

A. Collection and identification of plant material

Fresh *Andrographis echioides* leaves and stem were procured in May, Palamalai Hills Salem district, Tamil Nadu, India. The Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India, confirmed the taxonomic identification of the plant sample. The plant name and vouchers a specimen code (BSI/SRC/5/23/2023-24/Tech-355) were both verified from the plant list. Both the stems and leaves were washed under the running water to eliminate any surface materials, followed by grinding into a fine powder and left to air dried before being kept until use.

B. Chemicals

Potassium persulfate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH%), Trolox, Butylated Hydroxy Toluene (BHT), Rutin, Gallic acid, Tannic acid, ferrous chloride, hydrogen peroxide, ferrous ammonium sulphate, ferric acid, sodium nitroprusside, etc. are examples of compounds with 6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxilic acid. The chemicals were bought from Sigma Aldrich in the USA, Himedia in Mumbai, and SRL in Mumbai. Analytical-grade chemicals and solvents were employed throughout.

C. Preparation of the Plant extract

70g of the plant sample's powder (leaf and stem) were packaged individually in little thimbles for the shoxlet equipment. 400 mL of several organic solvents, including petroleum ether, chloroform, ethyl acetate, and ethanol, were used to extract the material. The polarity nature of the solvents is used. The thimble was air dried each time before extraction. The material was macerated in hot water with continuous stirring for 24 hours before the aqueous extract was filtered using Whatman No. 1 filter paper. The various solvent extracts from the (Model: Evator EV11) evaporator and air drier were concentrated using a rotary vacuum.

D. Extract recovery percentage

The yield percentage was calculated by measuring the quantity of the crude extract collected before each process of extraction by applying the following formula:

$$\%\ \text{of extract recovery} = \frac{\text{Amount of recoverable extract (g)}}{\text{Amount Plant sample (g)}} \times 100$$

E. Qualitative Phytochemical Analysis

According to established procedures, the major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oil & fat gums, and mucilages were all analyzed in the Andrographis echioides leaf and stem powder (Raaman, 2006).

(i) Detection of Carbohydrates

Molish's test (Ramakrishnan et al., 1994). The plant powder was filtered after dissolving in 5 mL of water and about 100 mg. To 2 mL of the filtrates, two drops of an alcoholic solution of -naphthol were added, then 1 mL of concentrated sulphuric acid was gently added along the test tube's sides and lets to stand. Carbohydrates were denoted by a violet ring.

(ii) Detection of Proteins

***Biuret test* (Gahan, 1984).** After being diluted in 10 mL of distilled water, the plant powder (100 mg) was filtered using Whatman No. 1 filter paper. One drop of 2% copper sulphate solution was added to a 2 mL sample of the filtrate for treatment. This was followed by the addition of extra potassium hydroxide pellets and 1 mL of 95% ethanol. The presence of proteins was shown by the ethanolic layer's pink tint.

(iii) Detection of Amino acids.

Ninhydrin test (Yasuma and Ichikawa 1953). To 2 mL of aqueous filtrate, two drops of a solution of ninhydrin (10 mg of ninhydrin in 200 mL of acetone) were added. The emergence of a distinctive purple colour acted as an indication that amino acids were present.

(iv) Detection of Alkaloids

Hager's test (Wagner et al., 1996). 5 mL of diluted hydrochloric acid was mixed with 50 mg of plant powder free of solvents before filtering the mixture. 2 mL of Hager's reagent (picric acid saturated aqueous solution) was added to the filtrate. The test resulted in a noticeable yellow precipitate.

(v) Detection of Saponins

Frothing test (Kokate, 1999). The plant powder (50 mg) can be made up to 20 mL by diluting it with distilled water. For 15 minutes, the suspension was shaken in a graded cylinder. The two-centimetre layer of foam that formed indicates the presence of saponins.

(vi) Detection of Phenolic compounds

Ferric chloride test. 50 mg of plant powder added into 5ml of distilled water, one or two drops of 5% of neutral ferric chloride solution was added to this mixture. The dark green colour was appeared to indicate the phenolic compound

(vii) Flavonoids

Alkaline reagent test (Raaman, 2006). The extract was processed with a 10% ammonium hydroxide solution in an aqueous solution. The presence of flavonoids was shown by a large white precipitate.

(viii) Detection of Glycosides

Borntrager's test (Evans, 1997). 50 mg of Plant powder was dissolved in concentrated hydrochloric acid for 2 hrs on water bath and after filtrate solution. Then 2ml of filtrate solution mixed into 3ml of chloroform and shaken well, the chloroform layer was separated then added into 10% of ammonia solution. The presence of glycosides to indicated pink in colour.

(ix) Detection of Flavonol of glycosides

Magnesium and Hydrochloric acid reduction (Harborne, 1998). 50 mg of plant powder was added into 5 ml of alcohol and then added into few pieces of magnesium ribbons. Drop by drop, concentrated hydrochloric acid was poured into the test tube. Flavonol glycosides were present when a pink or scarlet colour appeared.

(x)Detection of Cardiac glycosides

Keller Killiani test (Ngbede et al., 2020). One mL of glacial acetic acid with one drop of ferric chloride solution was used to dissolve 100 mg of plant powder. A layer of concentrated sulfuric acid (1 mL) was then placed underneath this. The presence of cardiac glycosides was suggested by a brown ring that was obtained at the interface.

(xi) Detection of Phytosterols

Libermann and Burchard's test (Finar, 1986). 50 mg of plant powder were dissolved into 2 ml of acetic anhydride solution. Then few drops of concentrated sulphuric acid were added gently along the test tube's sides. The presence of phytosterols was indicated by a variety of colour changes.

(xii) Fixed oils and fats

Saponification test (Kokate, 1999). A small amount of extract was mixed with a few drops of 0.5 N alcoholic potassium hydroxide solutions and a drop of phenolphthalein. The mixture was then heated up for two hours on a boiling water bath. The production of soap or the partial neutralisation of alkali are indicators that fixed oils and fats are present.

(xiii) Detection of Gums and mucilage's.

Absolute alcohol test (Whistler and Be Miller 1993). 100 mg of the plant powder was dissolved in 10 mL of distilled water, and 25 mL of pure alcohol was then added while stirring continuously. Gums and mucilage were indicated by white or hazy precipitation.

F. Quantification of Secondary Metabolites

(i) Quantification of total phenolics. The method described by (Siddhuraju and Becker 2003) was used for analysing the total phenolic content. The test tubes were filled by fifty microliter triplicates of the extracts (1 mg/1 mL) and distilled water was added to make up the amount of 1 mL. Following that, 2.5 mL of sodium carbonate solution (20%) and 0.5 mL of the Folin-Ciocalteu phenol reagent (1:1 with water) were subsequently added to each tube. The reaction mixture was quickly swirled around, the test tubes were left in the dark for 40 minutes, and the absorbance was measured at 725 nm in comparison to the reagent blank. Results were represented as equivalent amounts of gallic acid after the analysis was carried out in triplicate.

(ii) Quantification of Total Tannins. After treatment with Polyvinyl Polypyrrolidine (PVPP), the tannins were calculated using the same extract (Siddhuraju and Manian 2007). A 100×12 mm Eppendorf tube was filled with 100 mg of PVPP, 1 mL of distilled water, and finally 1 mL of the sample extracts. The mixture underwent a vortex before spending 15 minutes at 4°C in the freezer. The material was then centrifuged at room temperature for 10 minutes at 4000 rpm to separate the supernatant. Besides the tannins, which would have precipitated with the PVPP, this supernatant solely contains simple phenolics. By using the aforementioned process, the supernatant's phenolic content was determined and expressed as the amount of non-tannin phenolics.

Tannins = Total phenolics – Non tannin phenolics.

(iii) Quantification of total flavonoids. The (Zhishen *et al*. (1999), technique was used to measure the flavonoid content of the plant extracts. Two millilitres of distilled water were added to each of the test tubes containing around 100 µL of the plant extracts. As a control, 2.5 mL of distilled water were placed in a test tube. All of the test tubes were then filled with 150 µL of 5% $NaNO_2$, and they were let to sit at room temperature for 6 minutes. After the test tubes had been incubated, 150 µL of 10% $AlCl_3$ was added to each one, including the blank. At room temperature, each test tube was incubated for 6 minutes. All test tubes were then filled to made with distilled water to a total of 5 mL before 2 mL of 4% NaOH was added. All test tube contents were thoroughly vortexed before being let to stand at room temperature for 15 minutes. At 510

nm, the flavonoids' pink colour that emerged was measured spectrophotometrically. Based on the calibration curve: y = 0. 0255x, R2 = 0.9812, where x was the absorbance and y were the quercetin equivalent (mg/g), the total flavonoid content was determined as quercetin equivalents (mg/g).

G. Invitro Antioxidant Activity

(i) DPPH scavenging activity. According to the approach of (Gursoy *et al*., 2009), the antioxidant activity of the extracts was assessed in terms of hydrogen donating or radical scavenging capacity using the stable radical DPPH. Using methanol, sample extracts were collected in volumes ranging from 20 to 100 µL at varied concentrations. The aliquots of samples and standards (BHT and rutin) were combined with approximately 3 mL of a 0.004% methanolic solution of DPPH and vigorously shaken. A negative control was created by mixing 3 mL of methanolic DPPH solution with 100 µL of methanol. The tubes were let to stand at 27°C for 30 minutes. At 517 nm, the absorbance of the samples and the control was calculated in comparison to a methanol blank. The IC50 value, or the concentration of the sample needed to inhibit 50% of DPPH concentration, was used to express the samples' radical scavenging ability.

(ii) Phosphomolybdenum assay. According to the approach of (Prieto *et al.*, 1999), the green phosphomolybdenum complex production was used to assess the antioxidant activity of the samples. The blank was a test tube filled with around 300 µL of methanol. 100 µL of sample (1 mg/mL of the appropriate organic solvents) and standards (BHT and Rutin) were divided into three identical test tubes, and 300 µL of methanol was added to each test tube. 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to each test tube, and the contents were thoroughly mixed by vortexing. The test tubes' mouths were foil-wrapped, and they were incubated in a water bath at 95°C for 90 minutes. After the samples had reached room temperature, the mixture's absorbance at 695 nm was calculated in comparison to the reagent blank. The results were expressed as milligrams of ascorbic acid equivalents (AAE)/g extract using ascorbic acid as the reference standard.

(iii) Ferric reducing antioxidant power (FRAP) assay. According to the method described by (Pulido *et al.*, 2000), the antioxidant capabilities of various extracts from samples were calculated. 900 mL of freshly made FRAP reagent were combined with 90 mL of distilled water, 30 mL of test sample, and methanol (blank) before being incubated at 37 °C. The standards used utilised are BHT and Rutin. In a water bath, all the test tubes were incubated for 30 minutes at 37°C. The test sample was finally diluted by a factor of 1/34 in the reaction mixture. 2.5 mL of 20 mM TPTZ in 40 mM HCl and 2.5 mL of 20 mM FeCl3 were combined to create the FRAP reagent. 25 mL of 0.3 M acetate buffer (pH-3.6) and $6H_2O$. The measurement of the absorbance of the blue colour formed was read instantly at 593 nm against the reagent blank at the time of incubation. The calibration curve was created using methanolic solutions with known $FeSO_4.7H_2O$ concentrations ranging from 500 to 4000 M. The expression for the parameter Equivalent Concentration was the amount of antioxidant with a ferric TPTZ-reducing capacity equal to 1 mM $FeSO_4.7H_2O$.

(iv) Reducing power assay. The method described by (Hseu *et al.*, 2008) was used for evaluating the reduction power of the plant extracts. An increasing number of test tubes were filled with various aliquots of extracts (ranging from 50 to 250 mg) and standards (BHT and Rutin), which were then diluted with methanol to a volume of 1 ml. The blank was a test tube containing one millilitre of methanol. Then, phosphate buffer (2.5 ml, 0.2 M, pH-6.6) and potassium ferricyanide (2.5 ml, 1%) were added to each test tube, excluding the blank. After mixing, the solutions were incubated at 50 °C for 20 min. During incubation, each reaction mixture received 2.5 mL (10%) of trichloroacetic acid, which was then centrifuged for 10 minutes at 3000 rpm. Ferric chloride (0.5 mL, 0.1%) and distilled water (2.5 mL) were combined with the upper layer of the solution and let to stand for 10 minutes. At 700 nm, the absorbance was measured.

RESULT AND DISCUSSION

A. Percentage of recovered extract

Table 1 displays the leaf and stem extract of the sample from various solvent extracts with percentage yields. Aqueous leaf extract produced the highest yield (9.56%), whereas leaf ethyl acetate extract produced the second-highest yield (5.53%). The lowest yield percentages were discovered for extracts of petroleum ether, chloroform, and ethanol. Among all the extract, aqueous extract exhibited maximum yield in all parts of the selected samples.

Table 1: Percentage yield of *Andrographis echioides* plant extracts.

Sr. No.	Solvents	Leaf	Stem
1.	Petroleum ether	1.01	0.49
2.	Chloroform	1.89	1.55
3.	Ethyl acetate	5.53	1.01
4.	Ethanol	1	4.85
5.	Water	9.56	4.28

B. Qualitative Phytochemical Screening

A. echioides stem and leaves were qualitatively screened for significant primary and secondary phytochemicals, and the results are reported in (Table 2). The findings showed that the major metabolites, including carbohydrates, proteins, and amino acids, were present in every extract from each part of present analysis. All areas of the study contained secondary metabolites including an alkaloid, saponins, phenolic compounds, tannins, glycosides, flavonol glycosides, cardiac glycosides, phytosterol, cardiac glycosides, gums, and mucilage. The high intensity of the colour developed, shown by the +++sign, indicates a high concentration of a certain secondary metabolite, while the -sign denotes the lack of a chemical component. However, gums and mucilage's as well as fixed oils and fats were not present in either sample.

Table 2: Qualitative screening of phytochemicals in *Andrographis echioides* Leaf and stem.

SAMPLE	LEAF					STEM				
Extract Phyto Chemical	P.E	C.F	E.A	E	H.W	P.E	C.F	E.A	E	H.W
Carbohydrate	+	+	++	++	+	+	+	++	++	+
Protein	+	-	+	++	+	+	-	+	+	+
Amino acid	+	+	+	++	+	+	+	+	+	+
Alkaloids	+	+	++	+	+	+	+	++	+	+
Saponins	-	-	+	+	++	-	-	+	+	++
Phenols	++	+	++	+	+	+	+	++	+	+
Flavonoid	+	+	++	++	+	+	+	+	++	+
Glycoside	+	+	+	+	+	+	+	+	+	+
Flavonoid glycoside	+	-	++	+	+	+	-	+	+	+
Cardiac glycoside	++	+	+	+	+	++	+	+	+	+
Phytosterol	+	++	++	++	+	+	++	+	+	+
Fixed oils & Fats	+	+	-	-	-	++	-	-	-	-
Gums & Musilages	+	-	-	-	+	-	+	-	-	-

(+): Presence of chemical compound, (-): Absence of chemical compound, (+) < (++): Based on the intensity of characteristic colour, P.E- Petroleum ether, C.F- Chloroform, E.A- Ethyl acetate, E- Ethanol, H.W- Hot Water.

C. Quantification of secondary metabolites

(i) Quantification of Total phenolics content in *Andrographis echioides.* Various leaf and stem extracts of *A. echioides* were examined for their total phenolic content, and the results are displayed in Fig. 1. When compared to other plant extracts, the stem ethyl acetate extract (855.55 mg GAE/g extract) was shown to contain more total phenolics. When compared to the leaf, *A. echioides* stem extracts had higher phenolic levels.

(ii) Quantification of Total Tannin content in *Andrographis echioides.* The total amount of tannins in various *A. echioides* leaf and stem extracts were depicted in Fig. 2. The ethyl acetate stem extract (832.76 mg GAE/g extract) was found to contain more total tannins than other extracts. When compared to leaf, ethyl acetate produced better results when extracting tannins from stem.

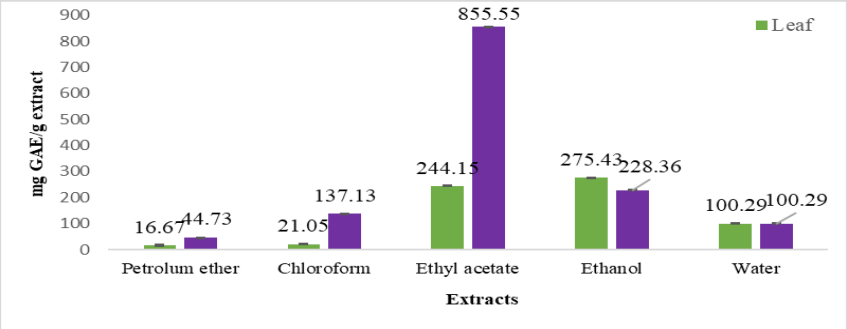

Fig. 1. Total phenolic content of the *Andrographis echioides* Leaf and Stem Extracts.

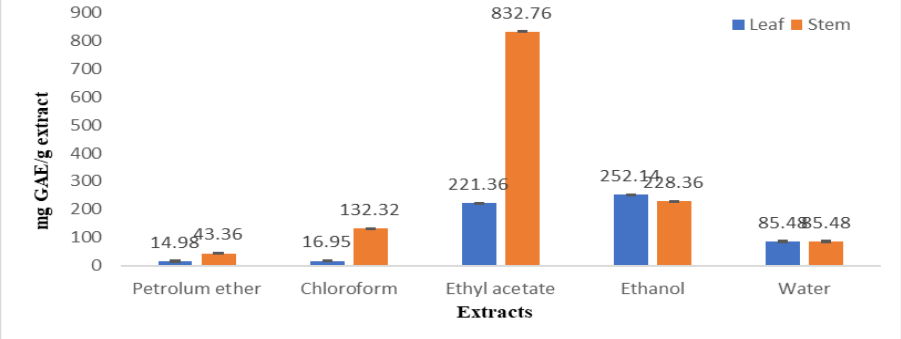

Fig. 2. Total Tannin content of *Andrographis echioides* Leaf and Stem Extract.

(iii) Determination of flavonoid content in *Andrographis echioides.* *A. echioides* stem and leaf flavonoid contents were examined, and the results are displayed in Fig. 3. In comparison to the other solvent extracts, the ethyl acetate stem extract (120.19 mg RE/ g extract) and ethyl acetate leaf extract (110.05 mg RE/ g extract) were shown to have higher flavonoid concentrations.

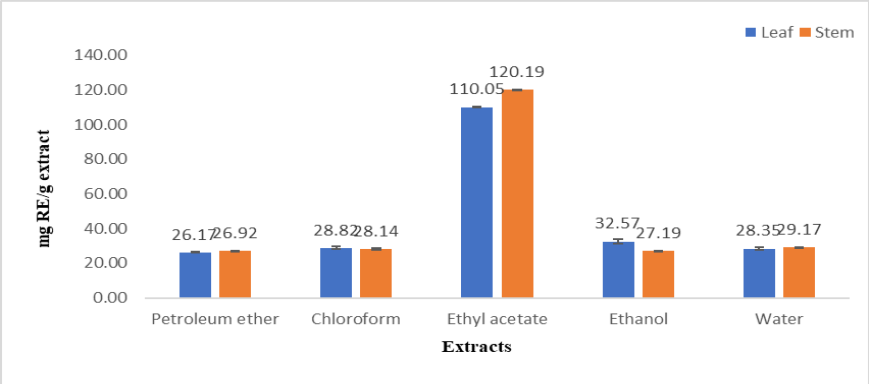

Fig. 3. Total Flavonoid content of *Andrographis echioides* Leaf and Stem Extract.

D. In vitro Antioxidant activity

(i) DPPH radical scavenging activity. *A. echioides* leaf and stem extract was tested for its ability to scavenge DPPH radicals, and the findings are shown in Fig. 4. Results were presented as IC50 values, where a lower IC50 value denotes a higher level of antioxidant activity. Stem ethyl acetate extracts with IC50 values of 26.43 g/mL and leaf 28.13 g/mL had the highest DPPH radical scavenging activity. Rutin has (IC50: 7.93 µg/ML) a naturally occurring antioxidant, was utilized as a reference compound along with Butylated Hydroxyanisole (BHA), a synthetic antioxidant, which has an (IC50 of 6.35 µg/mL). The nature of phenolics may be connected to the extracts capacity to scavenge free radicals, which could promote their electron transfer/hydrogen donating mechanism. Comparing stem ethyl acetate extract to other solvent extracts, these findings showed that it significantly reduces the number of free radicals present.

(ii) Phosphomolybdenum assay. The phosphomolybdneum assay was depends on the depletion of the green phosphate/Mo (V) complex with the maximum absorbance at 695 nm and the reduction of Mo (VI) to Mo (V) by the antioxidant agent. Various solvent extracts of the leaf and stem of *A. echioides* were examined for their overall antioxidant capacity, and the results are depicted in Fig. 5. In comparison to leaf extracts, the stem demonstrated greater activity in majority of the solvents among the several parts examined. The leaf and stem extracts' antioxidant properties can therefore be correlated with their free radical scavenging activity, which is comparable to that of the naturally occurring antioxidant ascorbic acid. The best antioxidant capacity was demonstrated by the ethyl acetate extract of the stem (247.48 mg AAE/g extract), followed by the ethanol extract of the stem (238.26 mg AAE/g extract), and then the leaf (147.27 mg AAE/g extract).

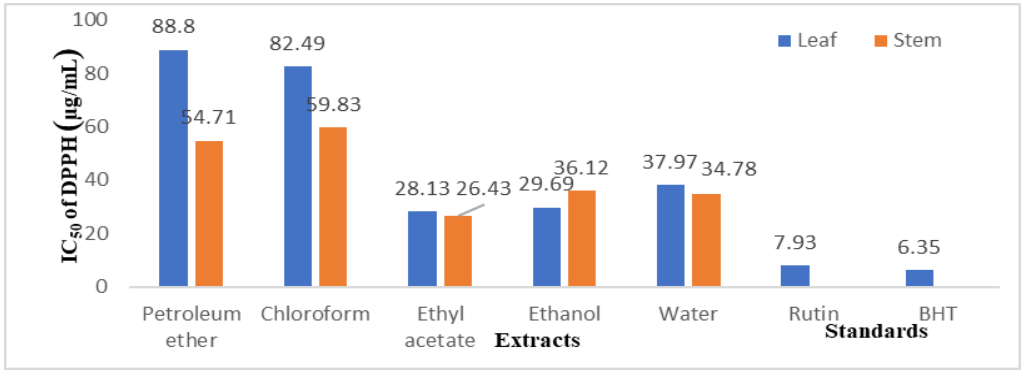

Fig. 4. DPPH scavenging activity of *Andrographis echioides* Leaf and Stem Extracts.

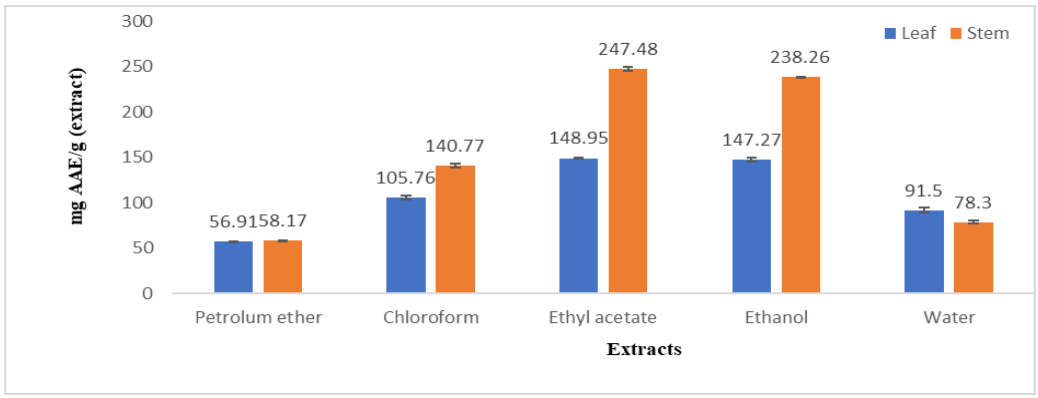

Fig. 5. Phosphomolybdenum assay of *Andrographis echioides* Leaf and Stem Extracts.

(iv) Ferric Reducing Antioxidant Power (FRAP) assay. The reducing potential of an antioxidant was used in the FRAP experiment to react with a ferric 2, 4, 6-tripyridyl-S-triazine (Fe (III)-TPTZ) complex to create a coloured ferrous 2, 4, 6-tripyridyl-S-triazine (Fe (II))-TPTZ complex by a reductant at low pH. When comparing the two plant samples, *A. echioides* indicated 593 nm, ferric reducing capacity. Ethyl acetate leaf extracts (136.04 mM Fe (II)/mg extract) and stem extracts (130.12 mM Fe (II)/mg extract) had the highest reducing antioxidant potential. This reducing ability can be compared to that for Rutin (268.89 mM Fe (II)/mg) and conventional BHT (275.55 mM Fe (II)/mg extract). In order to function as primary and secondary antioxidants, compounds with reducing power shown that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes. Therefore, based on the findings, it was determined that ethyl acetate leaf and stem extract had better reduction power than the other plant samples that have been selected (Fig. 6).

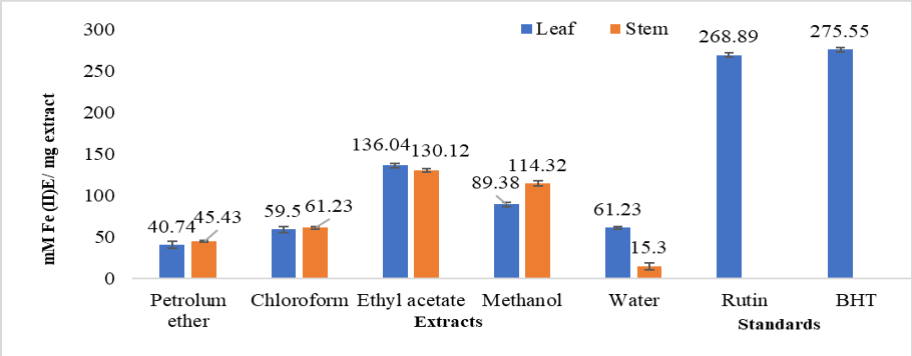

Fig. 6. Ferric Reducing Antioxidant Power (FRAP) Assay of *Andrographis echioides* Leaf and Stem Extracts.

(v) Reducing Power Assay. A significant indication of a compound's potentially antioxidant action is its capacity for lowering. In this test, the Fe3+ ferricyanide complex would be reduced to the ferrous form if reducing agents (antioxidants) were present in the evaluated samples. In Fig. 7 and 8, the results of an investigation into the reductive ability of the *A. echioides* leaf and stem extracts to change Fe^{3+} to Fe^{2+} are shown. All of the extracts' reducing power indicated dependent on concentration activity between 100 and 500 µg/mL of samples. The reaction mixture's increase in absorbance revealed that the samples had reducing power. The reductants in the extract work by stopping the production of peroxide or by interacting with free radicals to stop a chain reaction involving free radicals. The stem ethyl acetate extracts' (0.933 Absorbance at 700 nm) considerable contribution to the current study's conclusion is possible.

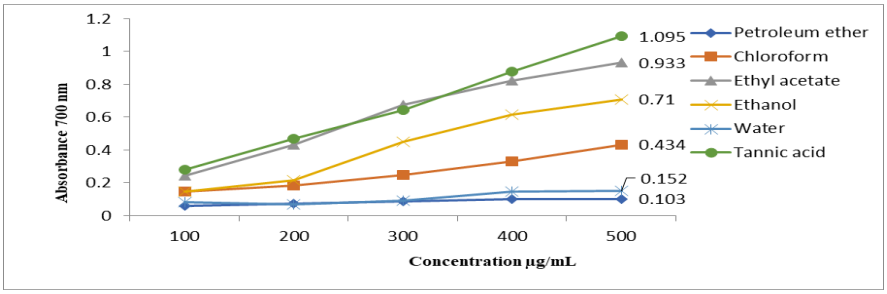

Fig. 7. Reducing power assay of *Andrographis echioides* Leaf.

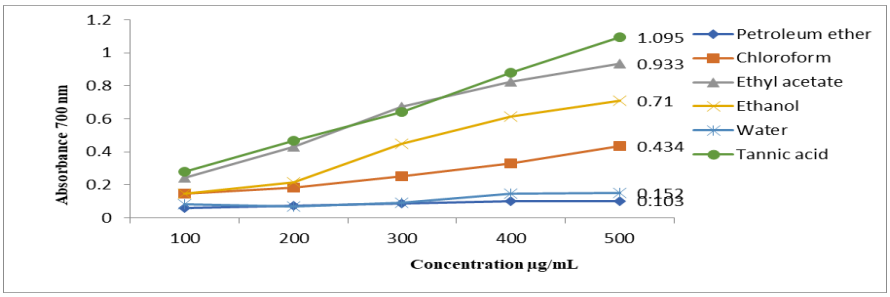

Fig. 8. Reducing power assay of *Andrographis echioides* Stem.

Secondary metabolites are mostly found in plants and are used for a variety of biological processes in both humans and animals (Ovuakporie-Uvo O *et al.*, 2019). In order to construct barriers against predators, plants are produced as secondary metabolites that are formed as a consequence of necessary metabolism. Plants are produced as secondary metabolites, or compounds that result from essential metabolism in order to build barriers against predators. Secondary metabolites are important in the pharmaceutical sector even though they are frequently unimportant for the development and reproduction of organisms. The early screening of

phytochemicals, which could result in the creation of novel medications, is a crucial step towards finding the bioactive principles present in therapeutic plants (Tiwari and Rana 2015). Evaluation of *Andrographis echioides* in Quantitative Formulation The plant's leaves, which have higher phenolic and Tannin content values, can be used to create a variety of pharmaceuticals for treating a range of human ailments. The acquired results supported the traditional applications and provided an explanation for the existence of any probable phytoconstituents. Secondary metabolites may aid in the prevention of a number of diseases. These compounds, such as tannins, flavonoids, and alkaloids as illustrative examples, are regarded to be the brains behind the therapeutic potential of plants. One such example is alkaloids, which are organic substances with antibacterial and antispasmodic characteristics (Palanichamy *et al.,* 2018). In the pharmaceutical sector, saponin is being investigated as a potential medication for the treatment of hormone production and wound healing. It is established that saponins have anti-inflammatory, haemolytic, and cholesterol-binding properties (Rajalakshmi and Cathrine 2016; Vinha *et al.,* 2012). Plants depending mainly on tannin compounds for both growth regulation and predator resistance (Suresh *et al.,* 2011). Tannins have astringent qualities that are astounding (Katie and Thorington 2006). They have a reputation for prompting the recovery of wounds and agitated mucous membranes. Phenolic compounds are highly potential ability of antioxidants, Free radicle scavenging activity, and anti-inflammatory agents, are abundantly present in the secondary by products of medicinal plants (Prabavathy and Vallinachiyar 2013). The activities of the central nervous system were caused by steroids (Okuda and Ito 2011). Flavonoids are organic compounds found in plants, fruits, and food items. Most free radicals, including singlet oxygen, are successfully scavenged by it (Sultana *et al.,* 2018). It demonstrates a wide range of pharmacokinetics and healing qualities, including anti-viral, anti-inflammatory, anti-cancer, anti-diabetic, and anti-allergic effects (Bravo, 1998; Shukla *et al*., 2009; Di Carlo *et al.,* 1999). Flavonoids are believed to be powerful insoluble in water antioxidant compounds and free radical scavengers that prevent oxidative cell damage and also have substantial anti-cancer effect. (Montoro *et al*., 2005). It additionally helps in controlling oxidative stress brought on by diabetes. Proximate analysis is essential for identifying contaminant and the standard of the sample utilised for the experiment (Kumar and Pandey 2013). Plants help humans meet some of their dietary and energy needs. The nutrients that are present in plants are proteins, lipids, and carbohydrates. This plant may be recommended as food for undernourished people since it has the potential to be a healthy source of fibre, protein, fat, and carbs. Digestible carbohydrates are recognised as being an important source of energy and as a necessary component of many diets (Nasreen and Radha 2011). One or more unpaired electrons are chemical compounds known as free radicals. They are extremely unstable, and in an effort to become stable, they destroy other molecules by stealing their electrons (Thambiraj and Paulsamy 2012). According to (Chowdhury *et al*., 2017), the presence of some groups of phytochemicals, such as flavonoids, alkaloids, and tannins, had a cytotoxic effect. It was claimed that the flavonoids that give food its colour and aroma have anticancer qualities. In addition, the presence of saponin is thought to have cholesterol-lowering effects as well as cytotoxic, anti-bacterial, and anti-viral characteristics (Bailly and Vergoten 2020). Free radicals are molecules with an unpaired electron in their outermost orbital that are capable of independent existence. Because of the unpaired electrons, it is unstable and extremely reactive.

To achieve stability, they either strive to give or take an electron. The hydroxyl radical, superoxide radical, anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical are a few examples of free radicals that can occur in the body (Vivekanandhan *et al.,* 2021; Wahab *et al.,* 2018). In methanol solution, DPPH, a stable free radical with a nitrogen centre, turns violet. It was reduced out by the yellow substance diphenylpicryl hydrazine. The Ethyl acetate extracts of both leaf and stem of *Andrographis echioides* demonstrated concentration-dependent DPPH scavenging activity, indicating the antioxidant activity of these compounds. The ability of DPPH radicals to reduce was measured by a drop in their absorbance at 517 nm. Stable DPPH radical reacted with the Ethyl acetate extract of *Andrographis echioides* stem material. The phosphate-molybdenum (VI) to phosphate-molybdenum (V) reduction of the sample leads to the development of a phosphate/Mo complex that is bluish green in colour at an acidic pH. This complex is the basis for the phosphate-molybdenum (V) assay. The phosphomolybdenum method is used frequently in the laboratory to evaluate the total antioxidant activity of plant extracts (Prieto *et al.,* 1999). According to current study, when the concentration of the extract and regular vitamin C increases, so does the extract's overall antioxidant activity. Using the phosphomolybdenum method, identical results on the overall antioxidant activity of various plant extracts have already been documented (Yadav and Yogesh 2015; Phatak and Hendre 2014; Sangeeta and Venkatlakshmi 2017). The FRAP data showed a similar pattern to that of phenol, flavonoids, and DPPH, with ethanolic extracts coming in first, then hydroethanolic, and then aqueous extracts. The Fe3+/ferricyanide complex is reduction to the ferrous formation in various plant extracts because of the existance reducers. Therefore, Fe2+ can be measured and monitored using the increase in blue colour density in the reaction media at 700 nm (Bougandoura and Bendimerad 2012). In order to assess the antioxidant activity of plant polyphenols, reducing power is frequently used. Reductones, which operate as antioxidants by giving an atom of hydrogen to break down free radicals, are typically linked to the reducing power (Adesegun *et al.,* 2009).

CONCLUSIONS

The phytochemical components and in vitro antioxidant activity are screened in the leaves and stems of *Andrographis echioides*. Utilising various solvents and their polar nature, phytochemicals and in vitro anti-oxidant properties are estimated. The Ethyl acetate extract of the stem contained the highest concentration of antioxidants found in the plant and the greatest number of secondary metabolites. The examination is particularly beneficial for creating novel medications to treat a variety of diseases. According to the analysis mentioned earlier, the plant has a great deal of potential to be exploited in drugs and as a potential source of significant medicines. Because it contains several phytocompounds that are essential for good health, it can be used to improve society's overall health. Research is currently being performed to uncover innovative, dynamic, and unique remedies for a number of serious diseases that have lately been identified.

FUTURE SCOPE

More research is required to prove the antioxidant capacities of the plants in vivo. The active components and the mechanisms of action of these plants must be thoroughly validated by science before they can be employed as a source of Phyto antioxidants. Therefore, more study of the *Andrographis echioides* (L.) *Nees.* plant stem is required for the development of natural medicines.

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Conflict of Interest. None.

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