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Phytochemical, Antidiabetic and Antioxidant Study of Aerial and Floral Methanolic Extract of Invasive Alien Species - *Antigonon leptopus* Hook. & Arn.

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Abstract: Invasive species are those that naturalize in the introduced area and causes biodiversity loss of the inhibiting area. *Antigonon leptopus* Hook. & Arn. is an invasive species representing the family Polygonaceae. The plant is a fast growing climber with heart shaped leaves and pink flowers. Present study pertains to estimate the phytochemicals present, anti-diabetic and antioxidant activity of the selected plant. The study was conducted in methanol extracts of aerial parts and flowers. Phytochemicals except carbohydrate were present in both extracts. The amount of phytochemicals, anti-diabetic and antioxidant activity were higher in floral extract than aerial extract. Further isolation of pure compounds and screening for bioactivity will provide more information about the pharmaceutical effects of the plant in treating various diseases.

Keywords: *Antigonon leptopus*, Invasive species, Anti-diabetic, Antioxidant, Phytochemical analysis

I. INTRODUCTION

The forest cover of India plays home to more than 18,000 of flowering plants endemic to India. Plants are treasure house of potential drugs. Over the years people have moved to Herbal remedies. Over 80% of people in developed countries depend on traditional medicine. Medicinal plants comprises these bioactive substances tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.*, 2015). The various climatic conditions favors for the growth of plants and their chemical constituents in different parts of the country. Each plant is unique in the part they play in our lives as food, medicine; aesthetics etc. there are many common plants that contribute to the poly herbal formulations.

Biological invasion of alien species is the second worst threat to the globe preceding habitat destruction according to Convention for Biological Diversity. Biological invasion is considered as a form of biological pollution and major cause for species extinction. Many invasive plant species cause economic and environmental damage, and referred to as alien pests or weeds. The number of naturalized alien/exotic species in India may be around 2000. A study conducted in 2007 reported a total of 173 invasive species under 44 families in India, Accounting to nearly 3% of total flora population of India.

Polygonaceae is one among the families with invasive species. *Antigonon leptopus* Hook. & Arn. is a smothering, habitat-transforming vine with showy pink flowers of the Polygonaceae (buckwheat) family. It is an invasive, fast growing, evergreen, perennial woody liana native to Mexico also found in the tropical islands of the world such as Asia and America (Raju *et al.*, 2001). Diabetes mellitus is a common and very prevalent disease affecting the people across the globe irrespective of their age. Diabetes mellitus is characterized by constant high levels of blood glucose caused by improper functioning of insulin. Even though there are large number of anti-diabetic drugs available search for new drugs are in demand due to the several limitations. Many ethnobotanical surveys have reported approximately 800 plants that may possess anti-diabetic potential.

Eventhough there are more than 2000 medicinal plants reported majority of them are left out by most of the pharmaceuticals and herbal practitioners. However, continuous use of these common plants may cause their gradual depletion in their sources. Humans are behind time taking the issue of biodiversity loss and its impact on the dwelling planet and their existence a serious matter concern. Excessive use of medicinal plants causes a decline in their sources. Drugs with anti-diabetic effects have side effects and even toxicity.

There arise the need to find novel, efficient techniques to overcome diabetes. To overcome this invasive species can be used. The present study aims the qualitative and quantitative phytochemical analysis, antidiabetic and antioxidant activity from the aerial and floral methanolic extract of *Antigonon leptopus*. And to make use of Invasive alien species- *Antigonon leptopus* for sustainable management of Floral diversity

II. MATERIALS AND METHODS

A. Study Area

Kozhikode, the central part of the former Malabar district, on the southwest coast second-largest urban region in the State of Kerala. The district covers an area of 28,79,131 km². The city lies between the coordinates 11° 15' 0" N latitude, and 75° 46' 12" E. Kozhikode features a tropical monsoon climate, the common annual temperature in Kozhikode is 26.2 °C. The highland region represents 26.80 per cent and the lowland region for 15.55 per cent of the total area of the district (Figure 1).

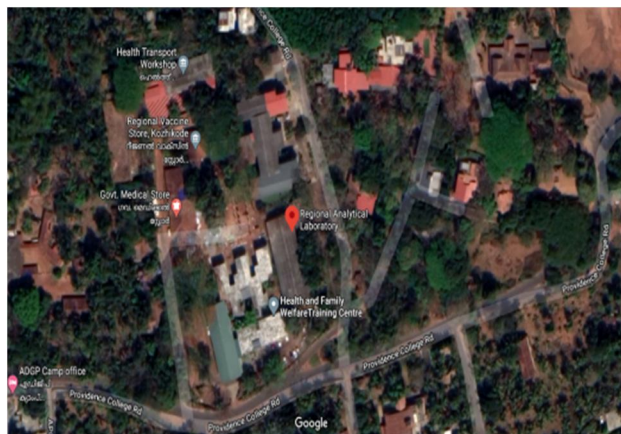


Figure 1: Study area

B. Sample Collection

Fresh material of *Antigonon leptopus* is collected from New Bazar, a rural locality at an altitude of 11m from the sea level of Kozhikode, Kerala (Figure 2,3).

The plant materials (aerial parts and flowers) were washed and cleaned properly and allowed to shade dry, until all plants became well dried for grinding. After drying, the plant materials are ground into fine powder. The plant material is then stored in air tight containers for further use.



Figure 2: Leaf of *Antigonon leptopus*



Figure 3: Flower of *Antigonon leptopus*

C. Extraction

The extracts from *A.leptopus* flower and aerial parts are prepared using Soaking method.15 gm of dried and finely ground samples are soaked in methanol, hydro alcoholic polar solvent for 24 hrs to get the plant extract. The extracts were filtered using Whatmann no. 41 filter paper. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator to obtain the crude plant extract. Further phytochemical analysis is conducted using this plant extract

III. QUALITATIVE ANALYSIS

The plant extract were tested for phytochemicals using the following tests- Test for alkaloids, carbohydrates, flavonoids, glycoside, phenol, protein, saponins, steroids, tannins, and terpenoids. (Thilagavathi *et al.*, 2015)

- 1) *Test for Alkaloids:* 2ml of extract is acidified with 1 ml of dilute hydrochloric acid. Then 1ml of Dragendorff's reagent was added. The orange to red precipitate represents the presence of alkaloids.
- 2) *Test for Tannins:* To 2ml of each extract a few drops of 10% lead acetate were added. The presence of tannins is indicated by white precipitate.
- 3) *Test for Saponins:* To 1ml of extract 9ml of distilled water was added, shaken vigorously for 15 seconds and extract were allowed to settle for 10min. Formation of stable foam indicates the presence of saponins.
- 4) *Test for Steroids:* 2 ml of plant sample is treated with 10 ml Chloroform. 1ml of acetic anhydride was added 2ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The blue green colour indicates the presence of steroids.
- 5) *Test for Triterpenoids:* 2 ml of plant sample is treated with 10 ml Chloroform. 1ml of acetic anhydride was added 2ml of concentrated sulphuric acid was added along the sides of the test tube, the appearance of red, pink colour or violet colour at the junction indicates the presence of Triterpenoids.
- 6) *Test for Glycosides:* To 1ml of each extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The presence of blue-green colour indicates glycosides.
- 7) *Test for Flavonoid:* 1.5ml of methanol solution is added to 4 ml of sample extract. The solution was heated and metal magnesium was added to this solution 5-6 drops of Con. HCl acid were added and colour was observed for flavonoids and orange colour for flavones.
- 8) *Test for Carbohydrates:* Fehling A and Fehling B reagents in equal volume were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate indicate presence of reducing sugars
- 9) *Test for Proteins:* Crude extract is mixed with 2ml of Millon's reagent, white precipitate which turned red upon gentle heating confirmed the presence of protein.
- 10) *Test for Phenol:* To 2 ml of each extract a few drops of aqueous ferric chloride is added. The formation of blue, violet, purple, green, red colour upon addition indicates the presence of phenol.

IV. QUANTITATIVE ANALYSIS

A. Determination of Alkaloid

Alkaloids are estimated using Harborne (1973) method. 5 gm. of sample is treated with 30ml 10% glacial acetic acid and incubated for 5 hr. The sample is then filtered, the filtrate is reduced to $\frac{1}{4}$ th of its original volume by evaporation. 10 ml conc. Ammonium hydroxide is added drop wise with continuous stirring and allowed to settle. The solution is filtered into a pre-weighed filter paper. The residue is washed with 10 ml dil. Ammonium hydroxide. The filter paper is dried and weighed to estimate the total alkaloid content. The total alkaloid content is calculated using the formula,

$$\% \text{ of Alkaloid} = \frac{\text{final weight} - \text{initial weight}}{\text{weight of sample}} \times 100$$

B. Determination of Flavonoid

Total flavonoid is estimated using Bohm and Kocipai- Abyazan (1994) method, Cameron *et. al.*, 1993. 5 gm. of sample is treated with 30 ml 80 % methanol and incubated for 2 hours. The solution is filtered through Whatmann filter paper No 42 (125 mm) into a pre weighed crucible. The filtrate is then evaporated to dryness. The crucible is further weighed and total flavonoid content is calculated using the following equation.

$$\% \text{ of Flavonoid} = \frac{\text{final weight} - \text{initial weight}}{\text{weight of sample}} \times 100$$

C. Determination of Tannin

Estimation of tannin is conducted following Folin- Ciocalteu method proposed by Polshettiwar and Ganjiwale (2007). 20 μ l of sample is treated with 980 μ l distilled water and 4.5 ml Na₂CO₃ and incubated for 10 minutes. 0.5 ml Folin's reagent is then added and incubated for 30 minutes.

For control test 1 ml distilled water is added with 4.5 ml Na₂CO₃, incubated for 10 minutes. Then 0.5 ml Folin's reagent is added and incubated for 30 minutes. The optical density at 725 nm is measured using spectrophotometer. Prepare a stock standard of 100 mg /100 ml Tannic acid. Pipette out 10 ml stock and made up to 100 ml with distilled water and used as working standard. Tannic acid dilutions (0 to 1 ml) were used as working standard solutions for standard graph preparation. The results of tannins are denoted in terms of tannic acid in mg/ml of extract. The total Tannin content is calculated using the formula

$$\% \text{ of Tannin} = \frac{\text{OD of test sample}}{\text{OD of standard}} \times \frac{\text{concentration of standard}}{\text{volume of test sample}}$$

D. Determination of Polyphenol.

The amount of total phenols in the plant extracts were estimated by the method proposed by Mallick and Singh (1980). 20µl sample is treated with 6.980 ml distilled water and 2 ml Na₂CO₃ and 0.8 ml Folin's Reagent. To the control test tube 7 ml distilled water with 2 ml Na₂CO₃ and 0.8 ml Folin's reagent is added. They are incubated for 2 hours. The optical density at 765 nm is measured. Prepare a stock standard of 100 mg /100 ml Gallic acid. Pipet out 10 ml stock, made up to 100 ml with distilled water, and used as working standard. For standard graph preparation Gallic acid dilutions (0 to 1 ml) were used. The results of phenols are defined in terms of Gallic acid in mg/ml of extract.

$$\% \text{ of Polyphenol} = \frac{\text{OD of test sample}}{\text{OD of standard}} \times \frac{\text{concentration of standard}}{\text{volume of test sample}}$$

E. Determination of Glycoside

Glycosides were quantitatively determined according to Solich *et al.*, 1992 by some modifications. For determination of glycosides 1 ml of each extract were treated with 1 ml freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). To control test 2 ml Baljet's reagent was added. After 1 hour, the mixture was diluted with 10 ml distilled water and the absorbance was measured at 495 nm

$$\% \text{ of Glycoside} = \frac{\text{OD of test sample}}{17} \times 100$$

V. IN-VITRO EVALUATION OF ANTIOXIDANT ACTIVITY

A. Antioxidant Activity by DPPH Method

The free radical scavenging potential of plant extracts was measured in- vitro by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method by Blois, 1958

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. 1 ml of freshly prepared DPPH solution (0.004% w/v) was treated with 1ml of sample extract. The control test contains 1 ml DPPH only. The test tubes are incubated at dark for 30 minutes. Methanol is used as blank. Absorbance was read at 523nm using spectrophotometer. The scavenging DPPH radical was calculated using the following formula

$$\% \text{ of Antioxidant activity} = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

VI. IN-VITRO EVALUATION OF ANTI-DIABETIC ACTIVITY

A. Anti-diabetic Activity by INHIBITION of alpha Amylase Enzyme

A total of 500 µl of test samples were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing 500 µl of α-amylase (0.5mg/ml) solution and were incubated at 25 C for 10 min. After these, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer was added to each tube. The reaction mixtures were then incubated at 25° C for 10 min. The reaction was stopped with 1.0 ml of 3,5 dinitrosalicylic acid colour reagent. The test tubes were incubated in a boiling water bath for 5-7 minutes, then cooled to room temperature. The reaction mixture was diluted by adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with standard anti-diabetic drug Glimiperide 100 mg (Nair *et al.*, 2011)

$$\% \text{ of Anti-diabetic inhibition} = \frac{\text{Abs Control} - \text{Abs Extract}}{\text{Abs Control}} \times 100$$

VII. RESULTS AND DISCUSSION

A. Qualitative Analysis

Primary and secondary metabolites are abundant in plants. These metabolites makes each plant unique and useful to mankind. Various phytochemicals are present in *Antigonon leptopus*. The results for the qualitative phytochemical analysis of aerial extract and floral extract is represented in Table 1

Table 1: Preliminary phytochemical analysis of aerial and floral methanolic extract of *Antigonon leptopus*

Phytochemical constituents	Aerial parts	Flower
Alkaloid	+++	+++
Flavanoid	+++	+++
Tannin	++	++
Polyphenol	++	+
Glycoside	+	+
Saponin	+	+
Protein	+	-
Carbohydrate	-	-
Steroid	+	+
Triterpenoid	+	+

+++ indicates high concentration, ++ indicates moderate concentration, - indicates absence

From the tests conducted the aerial methanol extract showed the presence of phytochemicals like alkaloids, flavonoids, tannin, glycosides, polyphenols, saponin, protein, steroid and terpenoids but carbohydrates are absent. Among these phytochemicals Alkaloids and flavonoids are present in higher concentration than the others. Tannin, polyphenol and glycosides are found in moderate concentration whereas saponins, steroids and terpenoids show low concentration. In case of the floral extract, phytochemicals such as alkaloids, flavonoids, tannin, polyphenol, glycosides, saponins, steroids and terpenoids tested positive. However, protein and carbohydrates were absent. alkaloids and flavonoids are present in higher concentration than tannin, glycosides and polyphenols. Saponins, steroids and terpenoids show very low concentration. Carbohydrate was altogether absent in both extracts.

B. Quantitative Phytochemical Analysis

The alkaloid content is comparatively higher in the flower sample of the selected plant. The total alkaloid content in flower is 9.65 % whereas in aerial parts it is only 2.1% .The percentage of total flavonoid contents are 3.44 % and 9.3 % in aerial parts and flower respectively. It can be concluded that flavonoid is more in floral extract than in aerial parts.The polyphenol is vaguely higher in flower i.e. 0.114 mg/ml in contrast with the aerial parts which is 0.034 mg/ml. Moderate amount of tannin were found in aerial and floral extract of *A. leptopus* 0.0569 mg/ml and 0.884 mg/ml of tannin content were found in aerial and floral extract respectively. 1.05 mg/ml and 2.29 mg/ml of glycoside were present in aerial and floral extract respectively. The quantity is higher in flower compared to Aerial parts. (Figure 4; Table 2).

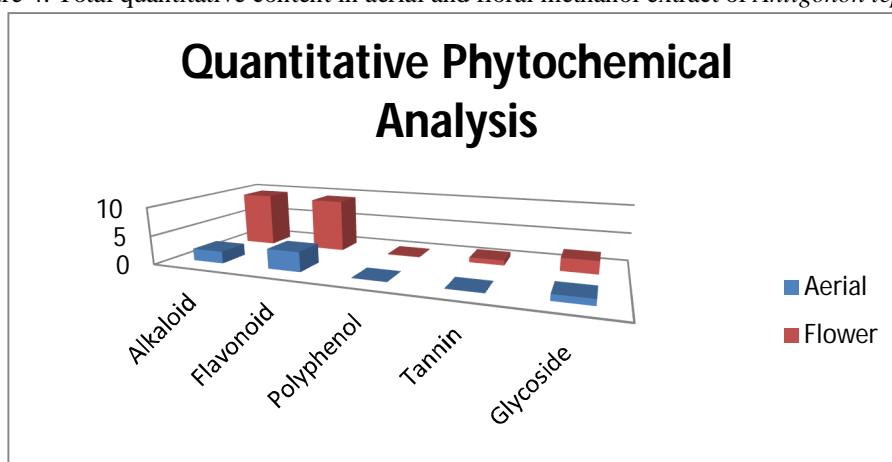
The quantity of phytochemicals were more abundant in flower in comparison with aerial parts. The percentage of alkaloids, flavonoids, tannins, polyphenols and glycosides were all higher in floral extract than the aerial extract. Surendar *et al.*, (2010) have found the presence of alkaloids, triterpenoids, steroids, flavonoids, phenols and coumarins in the *A. leptopus* and they have examined the anti-inflammatory property of phenolic compounds. Phenolic compounds have strong antioxidant property and prevent antioxidative damage to biomolecules like DNA, lipids and proteins (Liu and Mori,1993). Alkaloids have been associated with medicinal uses for a long time.

Flavonoids are hydroxylated phenolic substances synthesized by plants and have been found to be antimicrobial substances against wide array of microorganisms *in vitro* (Yadav and Agarwala 2011). Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy,1990).

Table 2: Total quantitative content in aerial and floral methanol extract of *Antigonon leptopus*

Parameters	Aerial	flower
Alkaloid (%)	2.1 %	9.55 %
Flavonoid (%)	3.44%	9.3 %
Polyphenol (mg/g DW)	0.034 %	0.114%
Tannin (mg/g DW)	0.0569 %	0.884 %
Glycoside (mg/g DW)	1.05 %	2.29 %

Figure 4: Total quantitative content in aerial and floral methanol extract of *Antigonon leptopus*



C. Antioxidant Property

The inhibition by the extracts were 54.03% and 64.59% respectively. The results clearly states that the floral extract shows higher % of inhibition than the aerial part extract (Figure 5;Table 3). In the study conducted by Udayaprakash *et al.*, 2014 screened free radical scavenging potential of methanolic leaf extract of *A. leptopus* by DPPH assay. In the experiment by Ngoitaku,2016 tea prepared by brewing dried flowers were evaluated for antioxidant activity by FRAPS method. Total phenolic content was higher in tea that was brewed for more time. The tea samples exhibited ferric reducing potential. The study carried out by Pradhan and Bhatnagar, 2016 revealed dose dependent antioxidant activity of various solvent extracts of *A. leptopus* leaf by DPPH radical scavenging activity and FRAP assay. Ethyl acetate and methanol extracts showed higher antioxidant potential compared to chloroform and hexane extracts.

In the present study, floral extract exhibits more phenol content than aerial extract and thus having higher antioxidant activity. It can be suggested that the phenolic compounds significantly contribute to the antioxidant potential of the selected plant species. Flavonoids are important antioxidants and help in removal of oxidative stress. The main drawback of synthetic antioxidants is the side effects when taken *in vivo* (Chen *et al.*,1992) .

D. Anti- Diabetic Property

The α -amylase inhibition shown by the aerial parts is 64.15% and of floral extract is 75.19 % (Figure 6;Table 3). In the study conducted by Sujatha *et al.*, 2012 the methanolic extract of flower of *A. leptopus* has shown to exhibit anti- hyperglycemic. The enzymatic inhibitory assay revealed that the inhibition activities of the *E. cymosa* methanolic fractions against α -amylase to be dose-dependent, strong inhibition were observed at the highest dose investigated (Ogundajo and Ashafa.,2017). A dose-dependent increase in percentage inhibitory activity against α -amylase enzyme therefore can be an important strategy in management of blood glucose (Narkhede *et al.*,2011).

Plant-derived antioxidants are potential α -amylase and α -glucosidase inhibitors, which indicate their great potentials in the management of diabetes (Kumavat *et al.*,2012). dotriacontanyl docosanoate, triacontanol and a mixture of oleanolic acid and ursolic acid were obtained by fractionation of *Phyllanthus amarus* extract. All compounds tested for alpha-amylase inhibition assay and results revealed that the oleanolic acid and ursolic acid mixture has potential alpha-amylase inhibiting activity (Ali *et al.*,2006).

Table 3: Antioxidant and Anti-diabetic activity of the aerial and the floral extract of *A. leptopus*

Parameters	Aerial part extract	Floral extract
Antioxidant activity	54.03%	64.09%
Anti-diabetic activity	64.15%	75.19%

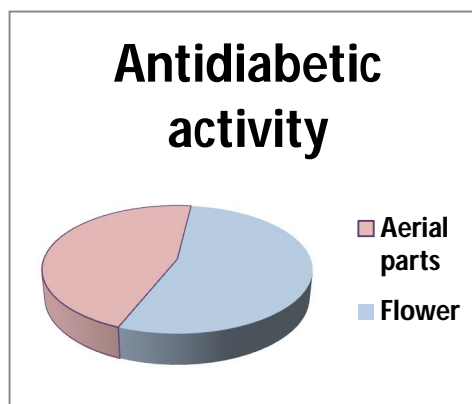
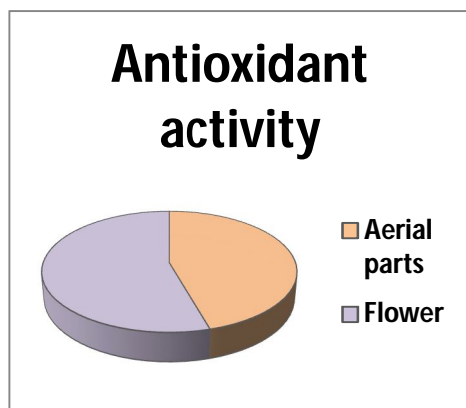


Figure 5: Antioxidant activity of the aerial and floral extract of *A. leptopus*

Figure 6: Anti-diabetic activity of the aerial and floral extract of *A. leptopus*

E. Invasive Character Of *A. leptopus*

Thomas and Tinde van An del (2019) in the review concluded that *Antigonon leptopus* is the inexhaustible invasive species in the Dutch Caribbean island of St. Eustatius forming a thick carpet bed which makes them a threat to the biodiversity. The overwhelming presence is said to have significant negative consequences for plant and animal diversity. *A. leptopus* is classified as one of the most aggressive weed occurring in tropical and insular ecosystem. Wherever *A. leptopus* invades, it completely smothers native trees, out-competes understory plants Langeland *et al.*, 2008; PIER, 2012). *A. leptopus* has 2 distinct modes of reproduction, sexually by seeds and vegetatively by stems and underground tubers (USDA-NRCS, 2011). The distinct reproductive behaviour aids its survival as a successful weed *A. leptopus* influence native plant communities by displacing native species, changing community structures and altering ecological functions.(Figure 6, 7)

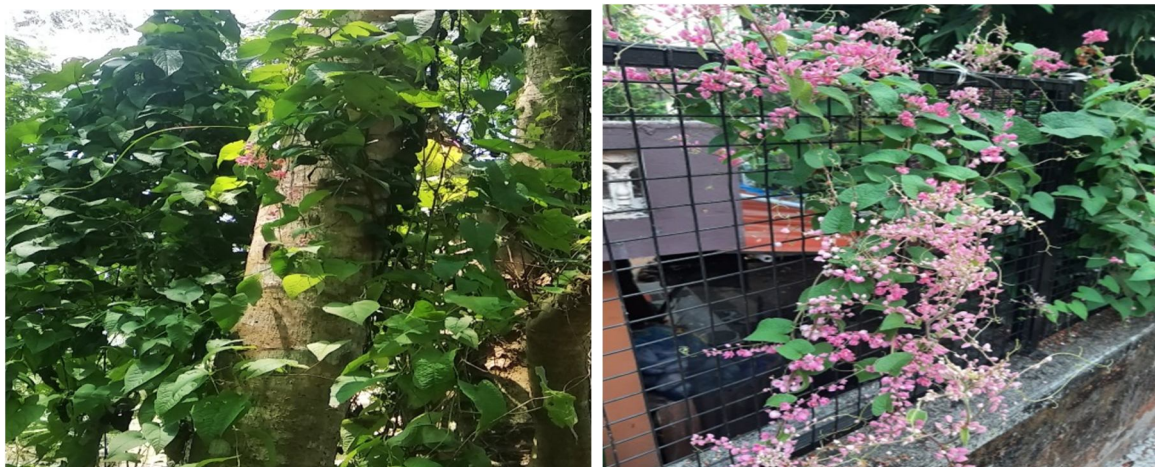


Figure 6,7: Invasive Character of *A. leptopus*

VIII. CONCLUSION

The study was conducted in methanol extract and the phytochemicals tested include alkaloids, flavonoids, tannin, polyphenol, glycoside, saponin, steroids, carbohydrates and protein. Both extracts showed the presence of almost all phytochemicals. The aerial extract showed the presence of alkaloids, flavonoids, tannin, polyphenol, glycoside, saponin, steroids, protein but not carbohydrates. The floral extract showed the presence of phytochemicals except for carbohydrates and steroids.

The quantitative analysis concluded that the total alkaloid and flavonoid content was much higher than other phytochemicals. On the whole, the quantity of phytochemicals, antioxidant activity, and anti-diabetic activity was higher in floral extract than in aerial extract.

As the present anti-diabetics drugs have various side effects and considering the rate of biodiversity loss and its impact on the future generation. We can be sustainable and use the particular invasive species for treating diabetes. From the present study, we can conclude that the particular invasive species *A. leptopus* can be used as a potential anti-diabetic drug.

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