ISOLATION AND PHYTOCHEMICAL SCREENING OF *ALANGIUM SALVIFOLIUM* LEAVE EXTRACT FOR ANTIDIABETIC ACTIVITY

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Abstract

The phytochemical analysis of leave Extracts of *Alangium salvifolium* showed the presence of various biologically active compounds. The biologically active compound of leaves extract of *Alangium salvifolium* is shows antidiabetic activity. Therefore, attempt was made to isolate the fractions containing active chemical compounds by column chromatography. The results indicated that during chromatographic separation the concentration of active compounds was increased

due to removal of unwanted substances, therefore the fractions showed good inhibitory effects at lower concentrations. The preliminary phytochemical analysis of leave extracts of *Alangium salvifolium* showed the presence of alkaloids, saponins and tannins. Therefore, from the R_f values it is concluded that the active fractions may contain these bioactive compounds responsible for antidiabetic activity.

Keywords: - *Alangium salvifolium,* phytochemical and isolation

1. INTRODUCTION

In modern era, Diabetes mellitus become the most serious problem world is facing. It may disturb the metabolisms. By this disturbance it may defect the secretion and action of insulin. In most countries diabetes mellitus shows the complications and may affect on people and in socioeconomic challenges. Around 25 to 30% of population may affected.[1,2] Diabetes may be developed by genetic and environment factor. At the early stage of diabetes, the metabolism of sugar is been stopped and the insulin is not formed. [3,4] This may result in lack of insulin and it may regulate the blood glucose level. This may break the fat, protein and glycogen and produces sugar. It led to the high sugar in blood and liver may release the ketones. Macromolecule's metabolism may be disturbed due to the distinguished in chronic hyperglycemia. [5,6] This result into impairment of secretion and action of insulin. This damage may occur for long term. This disease may also damage or failure of heart, eyes and nerves. It may lead to disability and death. Hyperglycemia may damage the organ system and may relate

the diseases how long it been present and how it may be controlled. Diabetes may also cause polyuria, weight loss and thirst.[7,8]

2. MATERIAL AND METHOD

2.1 Preparation of extracts

Alangium Salvifolium leaves were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the leave powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

2.2 Preliminary phytochemical studies

Extract phytochemical screening was carried out using normal methods. Extracts have been preliminarily phytochemically tested to classify different plant substances.

2.2.1 Qualitative chemical tests

2.2.1.1 Tests for carbohydrates

A. Molisch test: To 1ml of test + 2-3 drops of α- naphthol + Conc. sulphuric acid, appearance of purple ring.

B. Fehling's test: To 1 ml of test sample+ Fehling 's solution A and B + heat, brick red precipitate.

C. Benedict's test: To 1 ml of test sample, equal quantity of Benedict 's reagent was added and boiled. Red colour precipitate confirmed the presence of carbohydrates.

2.2.1.2 Test for alkaloids

Dil. HCL is pour into the extracts and then filter it.

A. Mayer's test: 1 ml of the filtrate + 1ml of Mayer`s reagent, cream precipitate

B. Hager's test: 1 ml of the filtrate + 1mL of Hager`s reagent, yellow precipitate

C. Wagner's test: 1ml of the filtrate + 1ml of Wagner 's reagent, reddish brown precipitate

2.2.1.3 Tests for terpenoids

A. Salkowski test: Chloroform solution of test sample was treated with equal amount of conc. sulphuric acid. Red colour shows the presence of steroid components.

B. Libermann - burchard test: To 2ml of test sample, chloroform was added before the addition of 2-3 drops of acetic anhydride and conc. sulphuric acid. Bluish colour show the presence of steroids.

2.2.1.4 Tests for flavonoids

A. Lead acetate test: Lead acetate solution was added to the extract. Flavonoid was confirmed on the basis of formation of yellow precipitate.

B. Alkaline reagent test: 1mL of test sample was dissolved in dilute sodium hydroxide solution that resulted in formation of yellow colour precipitate.

2.2.1.5 Tests for tannins and phenolic compounds

A. 5% $FeCl_3$ solution: In small amount of extract the 5% FeCl3 solution was added. A formation of deep blue-black colour complex.

B. 10% lead acetate solution: In few amount of extract, 10% lead acetate solution were added, white precipitate was formed.

C. Gelatin test: Extract was dissolved in water and add 2ml of gelatin solution it forms a white precipitate which show the presence of phenolic compounds.

2.2.1.6 Tests for saponins

A. Froth test*:* 1 ml of test sample + 20 ml of distilled water + shacked for 15min, Formation of persistent foam.

2.2.1.6 Test for proteins and amino acids

A. Ninhydrin test: 3 ml of the test solution + 3 drops of 5% ninhydrin + heat for 10 min + observe colour change

B. Biuret test: Test sample was treated with same volume of 1% copper sulphate and 4% sodium hydroxide solution and appearance of violet or pink colour was observed.

C. Million's test: extract is mixed with Million 's reagent a brick red colour show the presence of protein.

2.2.1.7 Tests for glycosides:

A. Borntrager's test: Dil. H_2SO_4 are add to the extract solution and boiled. The solution was filtered and cooled. Same amount of chloroform was added while shaking the mixture. Ammonia was added to the organic solvent layer. Change in color of ammonical layer to pinkish red colour confirmed the anthraquinone type glycosides.

B. Legal test: In a sample sodium nitroprusside and pyridine solution is added. It shows the colour changes.

C. Keller killani test: In a extract solution glacial acetic acid and ferric chloride are added. After that the solution are shift to the beaker which contain con. H_2SO_4.

2.2.1.8 Tests for fats and oils

A. Spot test: On a filter paper a small amount of extract was dropped and it will stand for some minutes to evaporate the solvent. After some minutes a small amount of oils are seen it indicate the presence of fixed oil.

2.3 *In vitro* antioxidant activity

2.3.1 ABTS radical cation decolorization assay–

ABTS solution was prepared by ABTS radical cation. It contains 2.45mM ammonium persulfate and it was stand for 12 to 16 hours at room temperature. It was use before the experiment. Different concentration (2 to 1000 μg/ml) of extract was added into 0.4ml. The absorbance was calculated at 745nm.

2.3.2 DPPH radical scavenging activity-

Take 1ml of extract and 1ml of DPPH solution and mix it. Fresh Methanol and DPPH is use as a control. Then it was put it into 20min dark place. After that absorbance is done at 517nm.

2.3.3 Scavenging of superoxide radical –

The superoxide radical is used for the measurement of scavenging activity for the inhibition of generation of O_2. DMSO method is use for the determination of this experiment. Potassium superoxide and DMSO is pour into the solution and filtered. The aqueous solution contains NBT, EDTA and potassium phosphate buffer was added into the filtrate.

2.3.4 Scavenging of nitric oxide radical

From sodium nitroprusside, nitric oxide is evaluated and it was measured by Griess reaction. Different concentration of extract was incubated in in sodium nitroprusside and in phosphate buffer. Tubes are incubated into 25^0C temperature for 5hr. In identical manner the test control was conducted in identical manner. After completion of 5h 0.5ml of incubation solution is removed and Griess solution is use for dilution. At diazotization of nitrite, the chromophore of absorbance with sulphanilamide with coupling of napthyl ethylene diamine at 546nm.

2.3.5 Iron chelating activity–

Reaction mixture contains O-phenathroline (0.05%), ferric chloride (2ml) and different concentration of test compound were incubated for 10to 15min. after that absorbance was taken at 510nm.

2.3.6 Total antioxidant capacity

Spectrophometric method is use for the determination of antioxidant property. In water 0.1ml of extract was dissolved into it and add reagent solution (1ml). In a thermal block these tubes are incubated and capped for 95min at 95^0C. at room temperature sample was cool and absorbance was taken 695nm.

2.4 Isolation of active component of extract by Column Chromatography

2.4.1 Column chromatography

Column chromatography is use for the separation of natural plant materials. Silica gel around 65gm is linked with adsorption cotton and packed the columns from the base by petroleum-ether slurry. Take care of the column and avoid air bubbles. In methanol, around 3g of extract (*Alangium Salvifolium)* is dissolved. By isocratic elution, the substances are elucidated by using chloroform: ethyl acetate is use for mobile phase and the collected the elute. The collected elution is monitor by elusion by different components. This is use for the determination of existence and homogeneity of compounds in fractions. The Rf spot is spotted the fraction and evaporating it at 45^0C.

2.4.2 Characterization of Alangium Salvifolium

2.4.2.1 UV-Visible spectroscopy

UV spectra of the compound were taken on Perkin-Elmer instrument dissolved in Methanol. Spectra 's was recorded.

2.4.2.2 IR Spectroscopy

Infrared spectroscopy is one of the successful analytical methods that can detect chemicals. The strategy is based on the basic fact that the chemical material is selectively absorbed in infra-red areas. The molecules vibrate as IR radiation is absorbed and induces spectrum absorption. It is an ideal instrument for observational analysis since the compound distribution is similar with optical isomers. It is most helpful in defining pureness and gross structural data. This method is useful in the field of natural ingredients, forensic chemistry and comparative examination of agricultural products.

2.4.2.3 NMR Spectroscopy

NMR spectroscopy is a research technique that profits from those nuclei's magnetic characteristics. It describes the physical and chemical characteristics of atoms or their molecules. This relies on the nuclear magnetic resonance effect that can provide an exhaustive information about the composition, dynamics and reaction state of the molecules. The magnetic field intramolecular around an atom in a molecule changes the frequency of resonance and thereby facilitates the knowledge of the electronic structure of a molecule.

2.4.2.4 Mass spectroscopy

Charged molecules or molecular fragments produced in a high vacuum setting are regulated using electrical or magnetic fields to assess molecular weight. In matrix 3-nitrobenzyl alcohol, the compound is embedded in positive ion form. Scan wavelength is 50-1000. Mass spectroscopy is an important physiochemical instrument used to elucidate structural compounds from natural materials, including plants. The central principle of mass spectroscopy is the use of separate physical means for sample ionization and ion separation depending on the mass (m) and load (z) ratios of m to z. The ionization technique available includes electrospray ionization.

3. RESULT AND DISCUSSION

3.1 Phytochemical Screening

Test	Pet ether	Ethyl acetate	Methanol
Carbohydrate			
Molish test	-ve	+ve	+ ve
Fehlings test	- ve	+ve	+ ve
Benedit 's test	-ve	- ve	+ ve
Alkaloids			
Mayers test	+ ve	+ ve	+ ve

Hagers test	+ ve	+ ve	+ ve
Wagners test	- ve	+ ve	+ ve
Terpenoids & Steroids			
Salwoski	-ve	+ ve	+ ve
Libberman Burchard	- ve	-ve	-ve
Flavonoids			
Lead acetate	+ve	+ ve	+ ve
Alkaline reagent test	- ve	+ ve	+ ve
Tannin & Phenolics			
Ferric chloride	- ve	+ ve	+ ve
Lead acetate	- ve	+ ve	+ ve
Gelatin	- ve	+ ve	+ve
Saponins			
Froth	- ve	- ve	-ve
Protein & amino acids			
Biurets	- ve	- ve	-ve
Ninhydrin	- ve	- ve	- ve
Glycosides			
Borntrager	- ve	+ ve	+ ve
Legal test	- ve	+ ve	+ ve
Killer killani	- ve	+ ve	+ ve
Fats			
Spot test	+ ve	- ve	- ve

Table 1. Phytochemical evaluation of *Alangium Salvifolium* leaves

3.2 *In vitro* antioxidant activity

3.2.1 DPPH scavenging activity

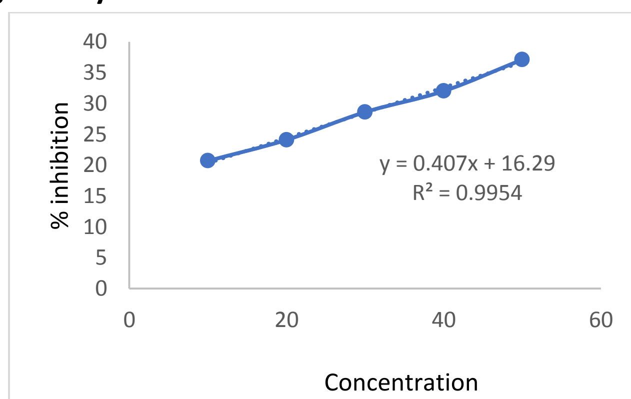

Figure 1. DPPH assay activity of aqueous extracts of *Alangium Salvifolium*

3.2.2 Hydrogen peroxide radical scavenging activity

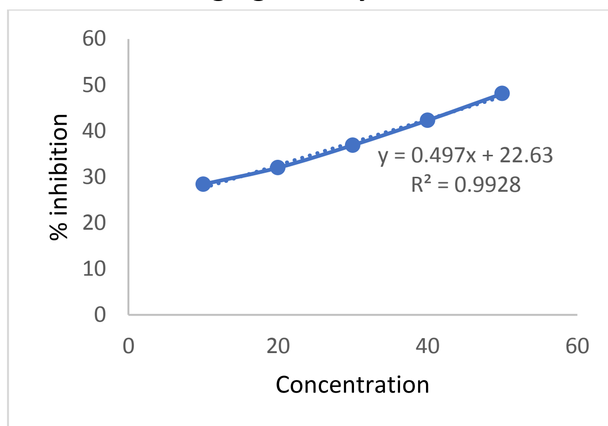

Figure 2. Different concentration of H_2O_2 radical of aqueous extracts of *Alangium Salvifolium*

3.2.3 Nitric Oxide Scavenging

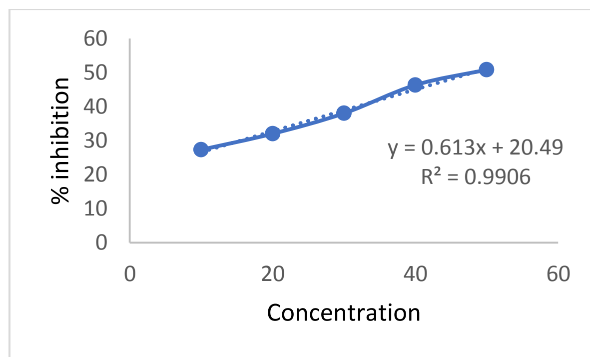

Figure 3. Scavenging effect of aqueous extracts of *Alangium Salvifolium* on nitric oxide assay

3.2.4 Reducing power assay

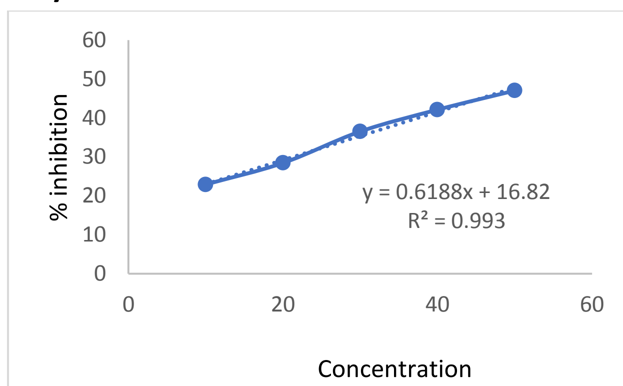

Figure 4. Scavenging effect of *Alangium Salvifolium* on reducing power assay

3.2.5 Estimation of tannins

Plant extract	Tannins
Alangium salvifolium	15-20%

Table 2. Tannins of the selected plants

3.2.6 Estimation of total phenolic content

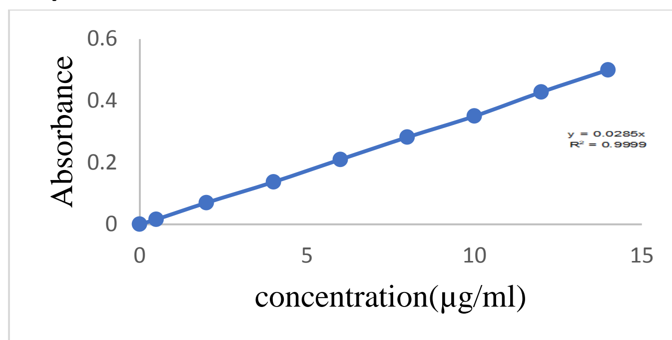

Figure 5. Calibration curve of Gallic acid

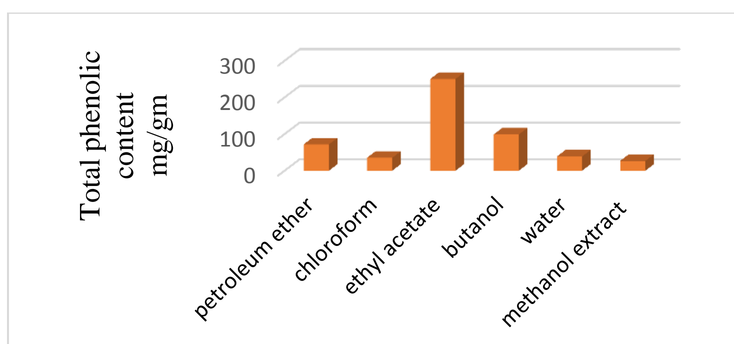

Figure 6. Total phenolic content of different fractions of selected plants

3.2.7 Estimation of total flavonoid

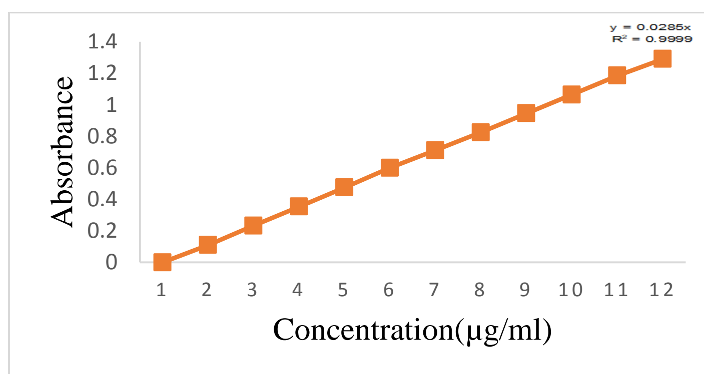

Figure 7. Calibration curve of Rutin

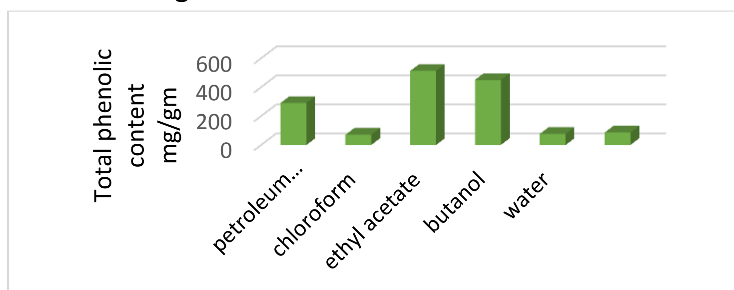

Figure 8. Total phenolic content of different fractions of selected plants

3.3 Isolation of active component of extract by Column Chromatography

Fraction solvent	Eluting solvent	No. of spots obtained	R_f value
1	Chloroform	0	0
2	Chloroform: ethyl acetate (9:1)	2	0.8,0.76
3	Chloroform: ethyl acetate (8:2)	1	0.76
4	Chloroform: ethyl acetate (1:1)	1	0.76
5	Ethyl acetate	2	0.71,0.66
6	Ethyl acetate: Acetone (9:1)	1	0.61
7	Ethyl acetate: Acetone (8:2)	1	0.56
8	Ethyl acetate: Acetone (1:1)	2	0.51,0.41
9	Acetone	0	0

Table 3. TLC results of Acetone extracts of *A. salvifolium*

Fraction solvent	Eluting solvent	No. of spots obtained	Rf value
1	Ethyl acetate	0	0
2	Ethyl acetate: Acetone (9:1)	2	0.9,0.86
3	Ethyl acetate: Acetone (8:2)	1	0.86
4	Ethyl acetate: Acetone (1:1)	2	0.86,0.85
5	Acetone	2	0.83,0.81
6	Acetone: methanol (9:1)	1	0.81
7	Acetone: methanol (8:2)	2	0.61,0.51
8	Acetone: methanol (1:1)	1	0.51
9	Methanol	0	0

Table 4. TLC results of Methanol extracts of *A. salvifolium*

3.3.1 Characterization of *Alangium Salvifolium*

3.3.1.1 IR-Spectroscopy

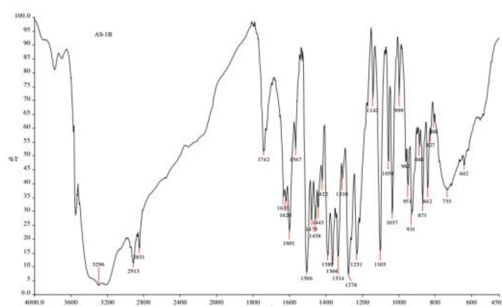

Figure 9. Characterization Of *A. Salviifolium* Compound by Infra-Red Spectroscopy

3.3.1.2 NMR Spectrum

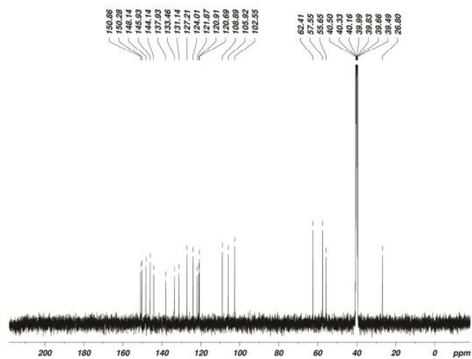

Figure 10. Characterization Of *A. Salviifolium* Compound By ^{1}H-NMR Spectrum

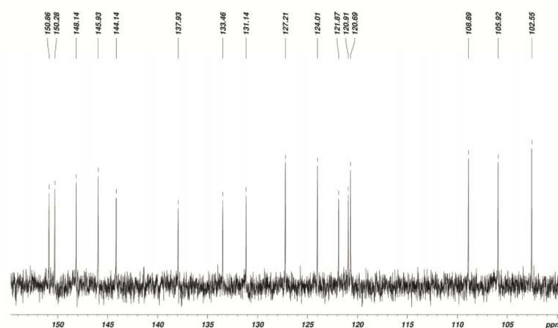

Figure 11. ^{1}H-NMR Spectrum of *A. Salviifolium*

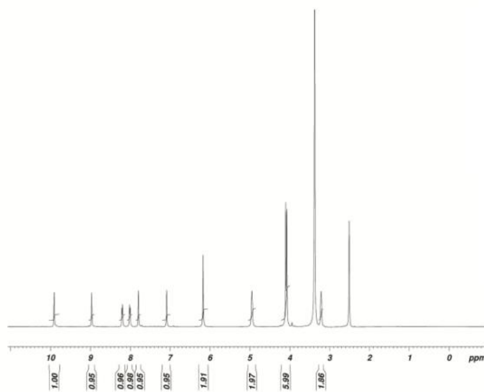

Figure 12. Characterization Of *A. Salviifolium* Compound By^{13}C-Nmr Spectrum

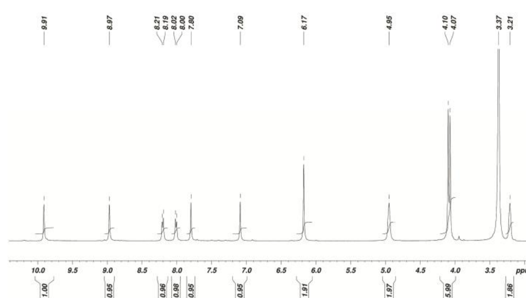

Figure 13. ^{13}C-Nmr Spectrum of *A. Salviifolium*

3.3.1.3 Mass spectroscopy

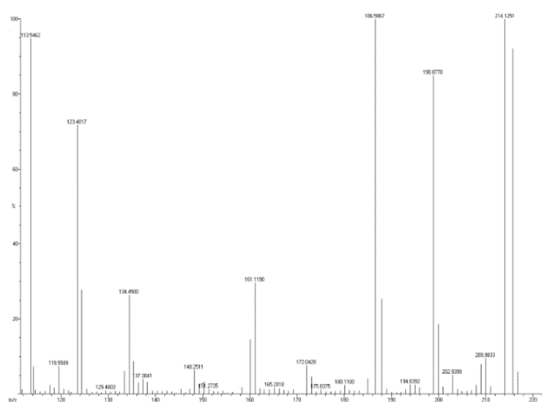

Figure 14. Characterization Of *A. Salviifolium* Compound by Mass Spectrum

3.3.1.4 UV-Visible spectroscopy

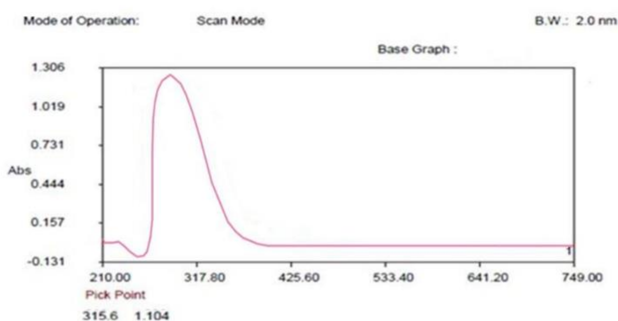

Figure 15. UV Spectra of *Alangium salvifolium*

4. SUMMARY AND CONCLUSION

Now a days Diabetes mellitus shows a major cause of death. It is the one of the six major systemic complications. For the treatment of this hormone therapy is use. Diabetes is reduced by the using of drugs. Now a day, traditional and indigenous medicine system is use for the treatment of diabetes. The extractive value indicate that aqueous extractive value shows the higher values as compared to ethanol solvents. In crude extract the phytoconstituents are present.

The crude drug extraction produces the better yields. It contains different phytoconstituents. It was presence due to the nature and the solvents used. During phytochemical investigation of ethanol and aqueous extracts of *Alangium salvifolium* leaves, it has been observed that the maximum number of phytoconstituents. From results it was concluded that the maximum amount of therapeutic compounds are release in ethanol and aqueous extracts of *Alangium salvifolium* leaves.

In ethanol and aquous extract flavonoids and polyphenol is present in *Alangium salvifolium* leaves. These are used for the management of free radical and use in the controlled of diabetes.

The stable DPPH is reduced by ethanol and aqueous extracts of *Alangium salvifolium* leaves. This changes the colour from purple to yellow. Due to the discolouration of degree the extract shows the scavenging potential.

In the present study, the aqueous extracts of *Alangium salvifolium* leaves demonstrated the highest capacity to neutralize DPPH radicals compared to ethanol extracts.

In hydrogen peroxide radical scavenging activity, the ethanol and aqueous extracts of *Alangium salvifolium* leaves show the presence of phenolic group. It donates the electrons for the neutralization. Aqueous extract shows the highest scavenging as compared to ethanol extract.

The ethanol and aqueous extracts reduce the formation of nitric oxide in the reaction system. In the reducing power assay, the ethanol and aqueous extracts strongly reduced the free radical. In both studies the aqueous extract showed higher reducing capacity of nitric oxide and free radicals compared to the ethanol extract.

The aqueous extracts of *Alangium salvifolium* leaves has the greater scavenging and has less capacity. Thus, polyphenol and flavonoid present in extract exhibits the therapeutic properties.

From the findings of *in vitro* antioxidant study, concluded that the aqueous extracts of *Alangium salvifolium* leaves shows the alleviating the oxidative stress which introduced diabetes due to the reduction cellular component. Hence the aqueous extract of *Alangium salvifolium* leaves were selected for the preparation of antidiabetic herbalformulation.

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