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PHYTOCHEMICAL STUDIES AND BIOLOGICAL ACTIVITY OF ALANGIUM SALVIFOLIUM

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ABSTRACT
In the present study the antibacterial activity of A.salvifolium leaves extract was carried out against various pathogenic microbes such as gram negative and gram positive bacteria of E. coli, K. Pneumoniae, and S. aureus by using disc diffusion method. The extract of Alangium salvifolium shows highest antibacterial activity was observed against Staphylococcus aureus followed by E. coli, and lowest inhibition show against Klebsiella pneumonia. Further the invitro antidiabetic effect of Alangium salvifolium by the α-amylase inhibitory activity also analyzed. The results indicated the IC_{50} value of *A. salvifolium* was 108.07 µg/ml for on α-amylase enzyme, compared with the standard drug Acarbose exhibited 50% inhibition on α-amylase enzyme at 56.84 µg/ml. The *A.salvifolium* revealed dose dependent inhibitory property for α-amylase enzyme.

KEYWORDS: Alangium salvifolium, invitro antidiabetic, antibacterial.

INTRODUCTION
Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Globally, about 60% of the healthcare products available in markets are known to be derived from plant origin. In India, medicinal plants are widely used by the people as: folk remedies, pharmaceutical preparations and also in different indigenous systems of medicine like Siddha, Unani and Ayurveda for the treatment of various diseases.[1] About 80% of the world population relies on plants and their products for primary health care. Awareness of plant based medications and therapeutics are continuously increasing worldwide and hence there are high acceptance and demand.[2] *Alangium salvifolium* Wang. belongs to the family *Alangiaceae* and is commonly known as sage leaved Alangium. It is a well-known traditionally used medicinal plant in India and it is also one of the most versatile medicinal plant having a wide spectrum of biological activities like antidiabetic, antiulcer, analgesic, anti-inflammatory, antimicrobial, antioxidant, anti-arthritic, diuretic, antifertility, anthelmintic, antiepileptic and antifungal.[3] *A. salvifolium* is a tall thorny tree and the genus contains 17 species of small trees, shrubs and lianas. *Alangium* name has been derived from the Malayalam word *Alangi*. Almost all the parts (root, bark, leaves, seeds and fruits) are known to have important therapeutic uses and are extensively used for different purposes in the indigenous herbal medicines. The synonyms of *A. salvifolium* are *A. decapetalum* Lam, *A. lamarckii* Thw, *A. latifolium* Miq.ex C.B. Clarke, *A. mohillae*Tul., *A. salvifolium* subsp. *Decapetalum* (Lam.) Wangerin, *A. sundanum* var. *Miqueliana* Kurz., *A.tomentosum* Lam., *Grewiasalviifolia* L.f, *Karangolummohillae* (Tul.) Kuntze and *Karangolum salvifolium* (L.f.) Kuntze.[4] The plant selected for study was based on its availability and its various therapeutic activities in various ailments mentioned in Ayurveda. In the present work, we have reported for the first time the results of the combined investigations on in-vitro antibacterial and antidiabetic activities of the extracts of *Alangium salvifolium*.

MATERIALS AND METHODS
GC–MS: The ethanolic extract was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS with with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization(CI) (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min. at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 min. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST)

having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. Identification of components was based on comparison of their mass spectra.

Plant material: The leaves of *Alangium salvifolium* were collected from Siluvinippatti village, Sivagangai District, Tamil Nadu. About 1.0 kg of shade-dried coarse powders of the plant material were extracted with 80%v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.

Assay of Antibacterial Activity: The leaves of *Alangium salvifolium* was shade dried at room temperature. The dried material was then homogenized to obtain coarse powder. 10gm powdered plant material was soaked in 20ml of ethanol overnight and then filtered through a Whatman No. 1 filter paper. The filtrate is used for the determination of antimicrobial activity. The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then discs were mixed with chemical compounds separately and control discs were prepared. Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*,(1966). [5] Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 ºC. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The ethanol extract soaked discs were placed on the each petri plates and also placed control and standard (Nitrofurantoin) discs. The plates were incubated at 37 ºC for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Enzyme inhibitory activities by α-amylase*:* The assay mixture containing 200 µl of 0.02 M sodium phosphate buffer, 20 µl of enzyme and *A. Salvifolium* extract and Acarbose at different concentration (20-100 µg/ml), separately was incubated for 10 minutes at room temperature followed by addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any test sample. The % inhibition was calculated according to the formula.[6]

$$\% \text{ Inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Statistical analysis

The values of results were expressed as mean value ± S.E.M. the variation in a set of data has been estimated by performing was analysis of variance (ANOVA). Individual comparison of group means values were done by using Graph pad prism 7.01.

RESULTS AND DISCUSSION

Chemical composition by GC-MS analysis: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. In the GC-MS analysis, 10 bio active phytochemical compounds were identified in the ethanol extract of *leaves* (Fig. 1 and Table 1)

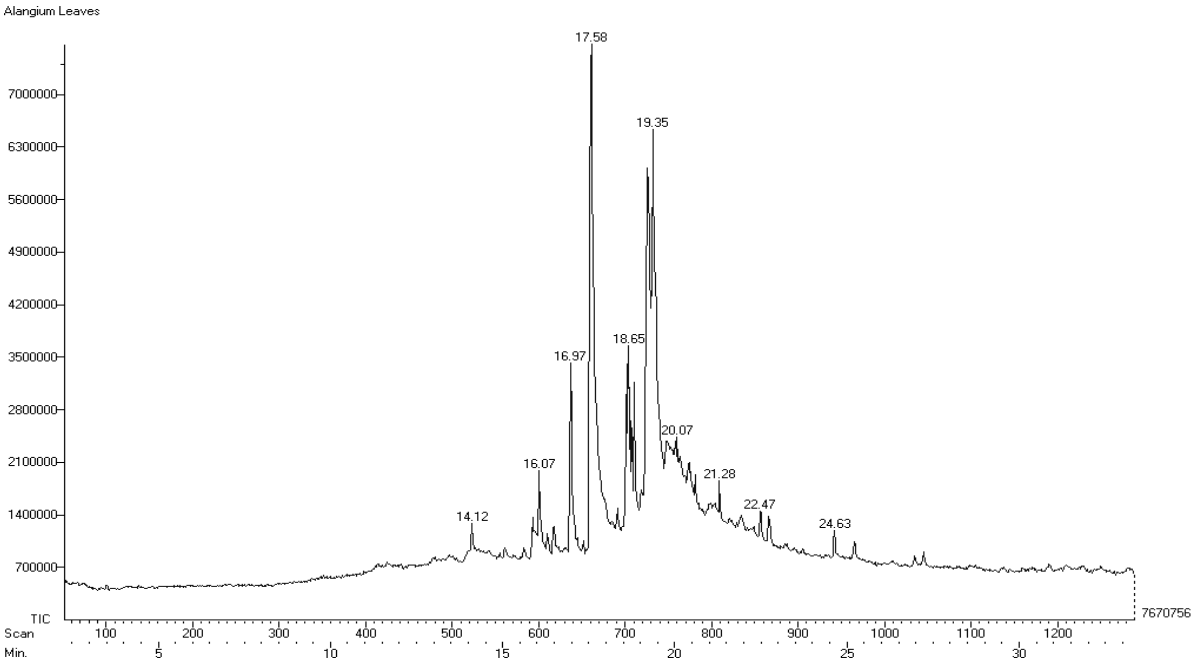
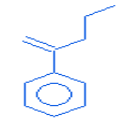
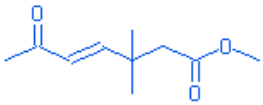
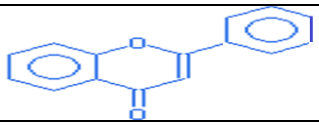
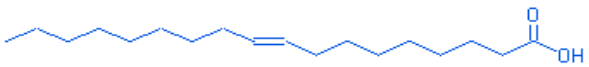
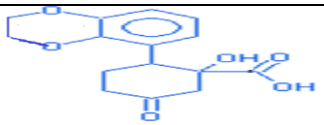


Fig.1. GCMS Chromatogra of *Alangium salvifolium* leaves.

Table 1: Phytochemicals identified in the leaves of *Alangium salvifolium.*

Sl.No.	RT	Name of the compound	Structure
1	14.12	Benzene, 1-(methylenebutyl)-	
2	16.07	4-Heptenoic acid,3,3-dimethyl-6-oxo-methyl ester	
3	16.97	Flavone	
4	17.58	n-Hexadecanoic acid	
5	18.65	Oleic acid	
6	19.35	Octadec-9-enoic acid	
7	20.07	cycleohexan-1-ol-3-one-1-carboxylic acid,6-(2,3-dimethoxyphenyl)	
8	21.28	Isopropyl stearate	
9	22.47	Tricosane-2,4-dione	
10	24.63	1-Docosanol,acetate	

Inhibitory activity of *A. Salvifolium* on α-amylase enzyme

The finding of inhibitory activity of *A. salvifolium* is represented in the table 2. The IC_{50} value of *A. salvifolium* was 108.07 µg/ml for on α-amylase enzyme (Fig.2). The standard drug Acarbose exhibited 50% inhibition on α-amylase enzyme at 56.84 µg/ml (Fig.3). The *A.salvifolium* revealed dose dependent inhibitory property for α-amylase enzyme.

Table 2: Percentage inhibition of alpha-amylase by *A.salvifolium* and Acarbose.

Concentration (µg/ml)	% inhibition by *A.salvifolium*	IC_{50} (µg/ml) *A.salvifolium*	% inhibition by Acarbose	IC_{50} (µg/ml) Acarbose
20	34.23±0.2		44.35±0.6	
40	45.35±0.8		65.23±0.5	
60	67.08±0.3	108.07	96.98±0.5	56.84
80	85.28±0.7		119.4±0.3	
100	106.65±06		130.33±0.1	

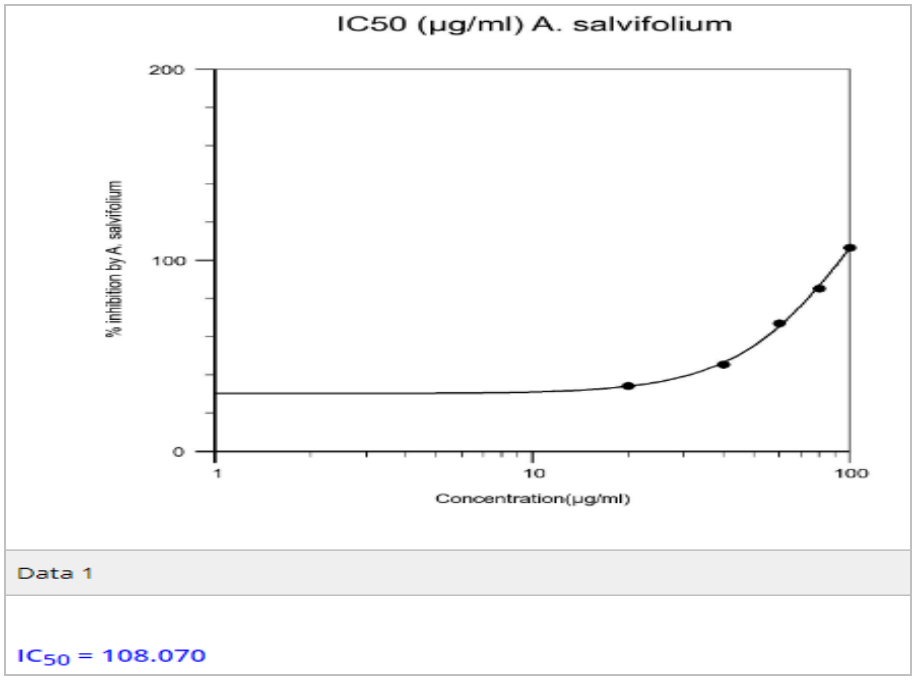


Fig. 2: IC_{50}(µg/ml) of *A. salvifolium*.

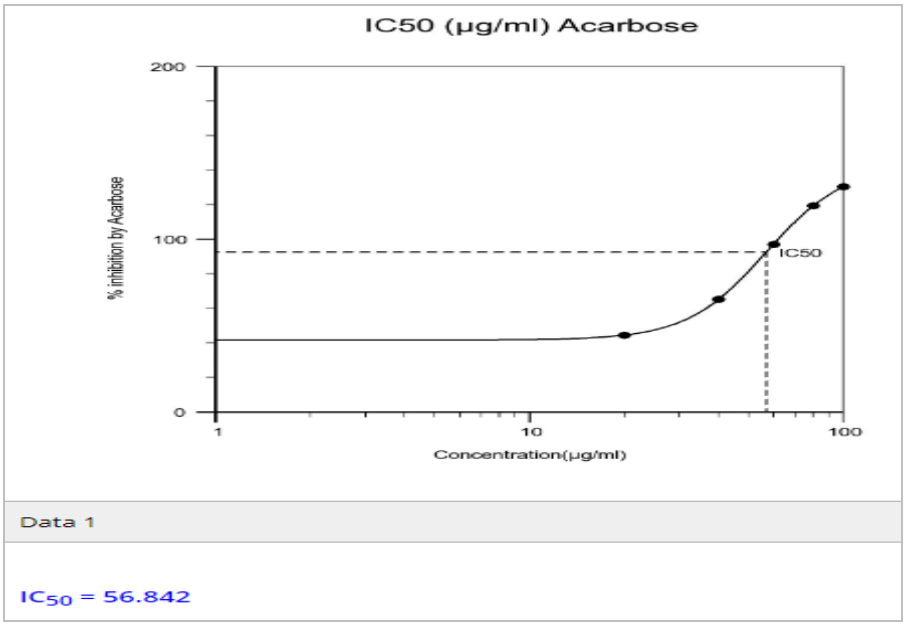


Fig. 3: IC_{50}(µg/ml) of Standarad Acarbose.

Diabetes patients are identified by abnormal postprandial increase of blood glucose level. The α-amylase and α-glucosidase enzyme play chief role to enhance the glucose level in blood by catalyzing the release of α-glucose from the non-reducing end of the substrate. The increment of blood sugar following carbohydrates meal can be reduced by inhibiting the α-amylase and α-glucosidase enzyme.[7] These enzymes are present in epithelium of the small intestine, and enzyme enable the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides.[8] Consequently, on inhibiting the α-amylase and α-glucosidase enzyme in the small intestine, it decrease conversion rate of hydrolytic cleavage of oligosaccharide and the process of carbohydrate digestion spreads to the lower part of small intestine. This digestion process of carbohydrate delays the total absorption rate of glucose and declines the postprandial blood glucose peak in diabetic patients.[9-11]

The findings of present study, *A.salvifolium* demonstrated inhibition against α- enzyme. The results implies that the extract of *A.salvifolium* were potent inhibitors of α-amylase enzyme. Moreover, the IC_{50} values of *A.salvifolium* are nearer to IC_{50} values of Acarbose and therefore can be potentially useful as an effective therapy for postprandial hyperglycemia with minimal side effects.

Antibacterial Activity

The present study investigated the antimicrobial activity of leaves extract tested againt human pathogenic bacteria like *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* compared with the standarad, the diameters of inhibition zones increased for all the test pathogens (Table 3 & Fig.4). The leaves extract could inhibit different typical pathogenic bacteria. Thus, *A.salvifolium* could be considered as excellent broad-spectrum antibacterial agents.

Table 3 : Assay of Antibacterial Activity.

S. No.	Bacteria	Zone of Inhibition (mm in diameter)		
		Control	Standard *	Leaf (20 µg)
1	*Escherichia coli*	-	24	20
2	*Klebsiella pneumoniae*	-	23	18
3	*Staphylococcus aureus*	-	26	24

***Nitrofurantoin (100µg)**

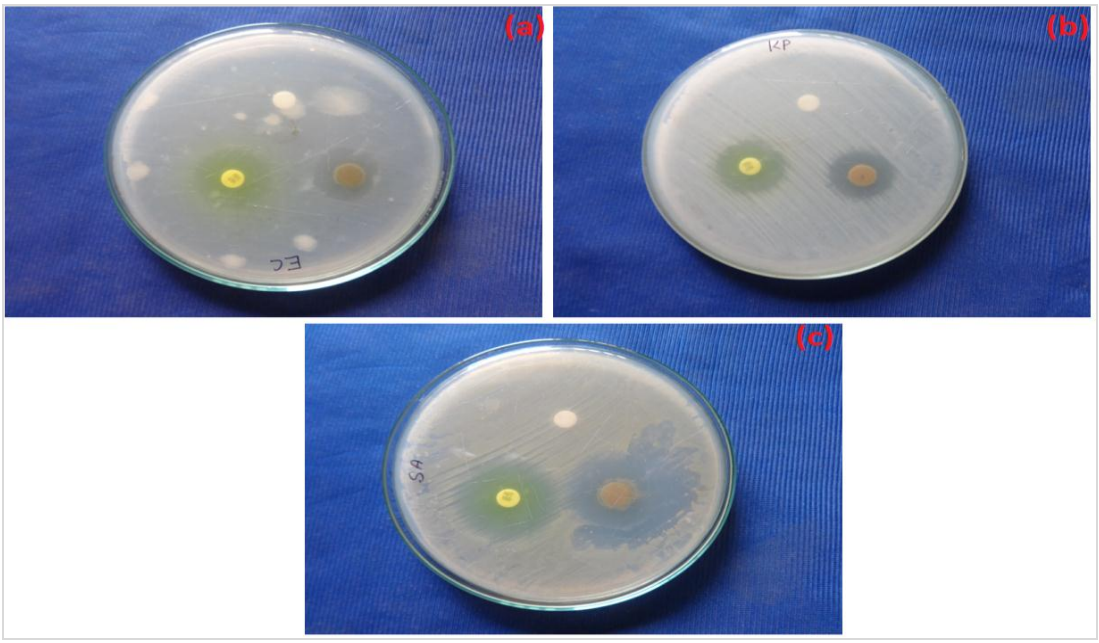


Fig.4: Antibacterial activity of *A.alvifolium* leaves on human pathogenic bacteria (a) *Escherichia coli* (b) *Klebsiella pneumonia* and *(c) Staphylococcus aureus*

In the present study the antibacterial activity of *A.salvifolium* leaves extract was carried out against various pathogenic microbes such as gram negative and gram positive bacteria of *E. coli, K. Pneumoniae, and* S. *aureus* by using disc diffusion method. The leaf extract of *A. salvifolium* showed broad spectrum of antibacterial activity. The extract of *Alangium salvifolium* shows highest antibacterial activity was observed against *Staphylococcus aureus* followed by *E. coli,* and lowest inhibition show against *Klebsiella pneumonia.*

CONCLUSION

In this study ethanol extract of the leaves of *A.salvifolium* was evaluated for their antibacterial and antidiabetic potency It is concluded that the leaves of *Alangium salvifolium* can be used as a potential

antimicrobial and antidiabetic agent. The *Alangium salvifolium* is the most inexpensive plant and used to cure and prevent a lot of disease such as epilepsy, scabies, gonorrhea, jaundice, hepatitis, diabetes, syphilis and asthma. The manifold benefits of *Alangium salvifolium* made it a miracle plant of nature. Numerous studies have been conducted on different parts of *Alangium salvifolium*, but this plant has not yet developed as a drug by pharmaceutical industries. More research work can be done on plant so that a drug with multifarious effects will be available in the future market. *Alangium salviifolium* bioactive components can be further developed into naturally based cosmetic, externally used products and herbal drugs for treatment of dermatomycotic infections. Further focus on developing contemporary formulations after extensive analysis of its bioactivity, pharmacokinetics and pharmacodynamics, safety, etc. using appropriate animal models followed by clinical trials will provide a novel entity for the treatment of various diseases from *A. salvifolium*.

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