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Research Article

PHYTOCHEMICAL INVESTIGATION AND HPTLC OF *ALANGIUMSALVIFOLIUM*

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ABSTRACT
The phyto chemical studies on the leaves and stem bark of *Alangiumsalvifolium*resulted in isolation of proto emetine, cephaline, isotubulosine,alangimaridine are being reported from this plant. These compounds have been characterized on the basis of spectral and other data.

Keywords: Phytochemical, *Alagiumsalvifolium,* HPTLC.

INTRODUCTION

More than 400 plants are known to have been recommended and recent investigations have affirmed the potential value of some of these treatments. Present study aims to open avenues for the improvement of medicine use of indigenous plant *Alangiumsalvifolium* in the selected area for diabetes mellitus. Study aims the effect of aqueous and ethanolic extract of *Alangiumsalvifolium* on plasma insulin, blood glucose, glycogen content and total heamoglobin.

The plant *Alangiumsalvifolium*, Family: *Alangiaceae.* The root of the plant have been used in Skin diseases, Astringent, Anthelmintic, Purgative, Emetic, Diaphoretic, Antipyretic and Anti – tubercular properties[1-6]. Root bark is an antidote for several poisons. Root bark is very bitter and is reputed as a cure for skin diseases. The stem bark of the plant exerts a biphasic action and possesses marked hypotension in higher dose .The leaves of the plant was used as Anti-protozoal, Hypoglycemic, spasmolytic**.** The leaves are applied as poultice in rheumatism. Fruits are sweet, cooling, and purgative and used as a poultice for treating burning sensation and haemorrhage.

Modern methods describing the identification and quantification of active compounds in the plant material may be useful for proper standardization of herbals and their formulation. Also the world health organization assembly has the need of ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards[7, 8].

TLC plates do not give better resolution particularly when the medicine involves the use of several herbs and quantification of markers by conventional solvent extraction followed by colorimetry is laborious and expensive. HPTLC offers better resolution and estimation of active principles with reasonable accuracy in a shorter time [9].

EXPERIMENTAL

All the melting points were taken in veego-Vmp melting point apparatus and are uncorrected.IR

spectra were recorded on perkin –Elmer FTIR spectrometer. NMR spectra were recorded on Brukerspectrospier 200 MHz and the chemical shifts are referenced to TMS.

Plant material

The Plant *Alangiumsalvifolium* (L.f.) wang was collected from Alagarkovil Hills, Madurai during the month of May. Then was identified by Dr. Stephen Msc. Ph.D,Department of Botany, The American College, Madurai-2.

Preparation of the extract

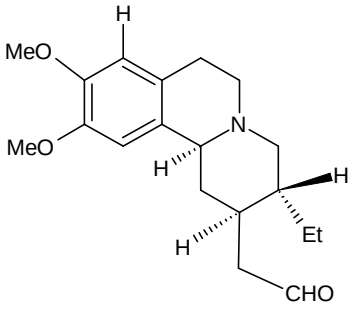
The leaves and stem bark were cut off and dried in shade for 45 days. Then about 3 kg of the shade dried leaves and stem bark was made in to coarse granules. The dried powder material of *Alangiumsalvifolium*were defatted with petroleum ether in a soxhlet apparatus. The defatted powder material was obtained and further extracted with ethanol and aqueous for 72 hrs in soxhlet apparatus. The resulting semisolid mass was dried and used for phyto chemical analysis and hyper glycemic results are tabulated 1.The chemical constituents of the extract were identified by

Preparation of column chromatography

Alcohol and aqueous extract obtained from the aerial parts of*Alangiumsalvifolium*was absorbed on silica gel (60-120 mesh) for column chromatography. The slurry was air dried to remove any absorbed moisture on surface and loaded on the top of the column of silica gel. The dis appearance or appearance of the existing spot was visualized on TLC. Various compounds isolated from the extract are listed below along with their spectral data.

Phytochemical investigation[10-12]

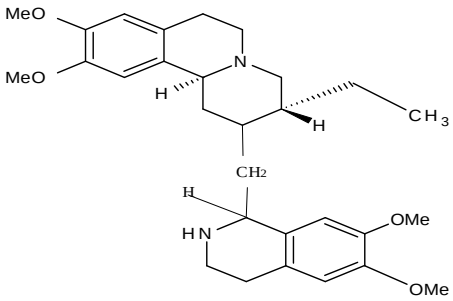
PROTO EMETINE



Compound A

This was obtained from benzene, chloroform fraction (8:2) of 110 mg. Rf.0.42.IR spectrum showed the band at 3392 cm^{-1} OH stretching, 2364 cm^{-1}CH stretching, 1741 cm^{-1} C=O stretching, 1498 cm^{-1} C-H bending, 1222 cm^{-1} C-O stretching. NMR signals ($CDCL_3$), 0.745-0.929 due to CH3 protons, 1.807-1.957 due to CH protons, 2.13 due to CH2 protons attached near to CH2-C=O. The UV absorption spectrum showed peaks at 245,260,280,290,300,320 and 330 nm. The UV max at 290 nm was observed in compound A.

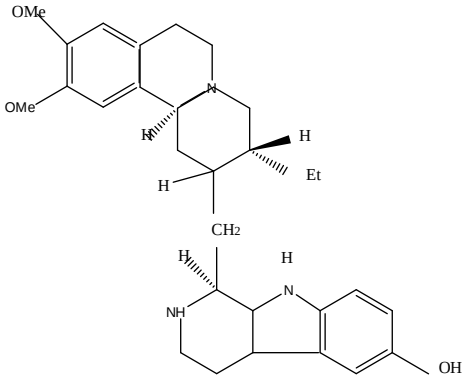
CEPHALINE



Compound B

This was obtained from chloroform and ethyl acetate fraction (7:3) of 120 mg. The Rf value is 0.71.IR spectrum showed the band at 3392 cm^{-1} OH stretching, 2362 cm^{-1} CH stretching, 1622 cm^{-1} C=O stretching, 1544 cm^{-1} NH bending, 1375 cm^{-1} CH bending, 1222 cm^{-1} C-O stretching, 1163 cm^{-1} C-O stretching. NMR signals ($CDCL_3$) 0.979 due to CH_3 protons,1.2 due to CH_3 protons, 2.181 due to CH_2 proton attached to CH2-C=O.

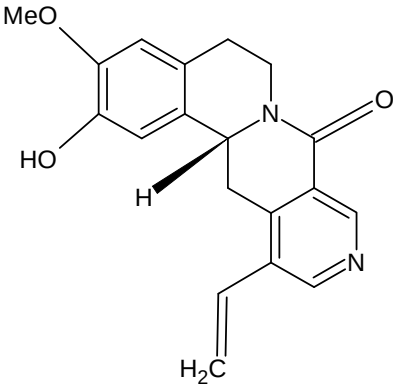
Isotubulosin



Compound C

This was obtained from chloroform and ethyl acetate fraction (5:5) of 125 mg. The Rf value is 0.73. IR spectrum showed the band at 3398 cm^{-1} OH stretching, 2923 cm^{-1} CH stretching, 2853 cm^{-1} H-C=O stretching, 1655 cm^{-1} –C=C- stretching, 1561 cm^{-1} C-C stretching, 1459 cm^{-1} C-C stretching, 1020 cm^{-1} C-N stretching. The UV absorption spectrum peaks observed at 282, 328, 341,365,416, 661 nm.

ALANGIMARIDINE



Compound D

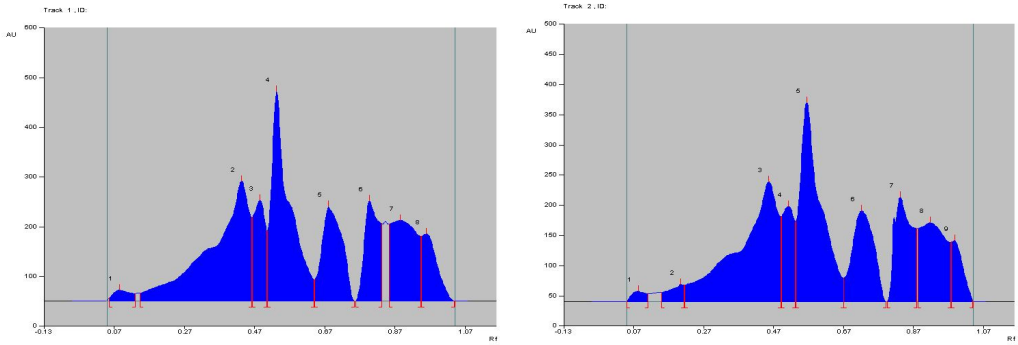
This was obtained from ethyl acetate and methanol fraction 7:3 of 110 mg. The Rf value is 0.41. The IR spectrum showed as 2852 cm^{-1}, 2362 cm^{-1}CH stretching , 1739.67 cm^{-1} C=Ostretching, 1652.88 cm^{-1} C=Ostretching, 1461.94 cm^{-1} C-H stretching, 1074.28 cm^{-1} C-O stretching. NMR signals ($CDCL_3$) 0.688-1.000 CH_3 protons, 1.418CH protons, 1.887CH protons, 2.093CH_2 proton attached near to CH_2-C=O. TheUV absorption spectrum showed peaks at 415, 447, 510, 537, 606, 662nm.

HPTLC – DETERMINATION OF *ALANGIUM SALVIFOLIUM* (L.F.) WANG[13-17]

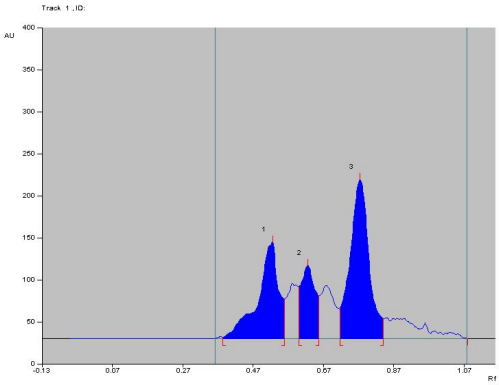
A simple, precise, sensitive, rapid, and reproducible, HPTLC method for the simultaneous estimation of alkaloids in alcoholic extract of *Alangiumsalvifolium*wang.The detection was done densitometrically using UV detector in absorbance mode. The R_f value of the sample proves the good resolution between them. The method is so sensitive to detect the constituents in the nanogram level.

MATERIALS AND METHODS

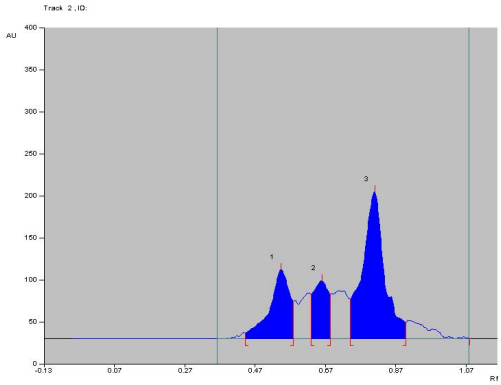
The Instrument Used is CAMAG.The Software WINCATS 1.4.3. The Sample Applicator is Linomat V. The Scanning instrument isDensitometry TLC Scanner III. The HPTLC Plates Silica gel 60 F 254. The Solvent System used is n-Butanol : Glacial Acetic Acid : Water(4:1:1). The derivatization reagent is Dragendroff reagent. The Sample solution is EEAS. The samples were applied using linomat V applicator in the form of bands having 6mm band width on precoated silica gel 60, f254 aluminium plates of 20*10 cm size and 0.25 mc thicknesses. The plates were pre washed with methanol and activated at 60 °c for 5 minutes before use. Application speed was 0.1 mcl/s and space between bands as 5mm. Linear ascending development was carried out in a twin trough glass chamber saturated with mobile phase. The mobile phase or the solvent system used for the detection of alkaloid is [n-Butanol : Glacial AceticAcid : Water] (4:1:1).The optimized saturation time with 30 min at room temperature. The plates were developed till 75 mm. After drying the plate, it was scanned at 273nm, using win CATS software. Dragendroff reagent was sprayed and R_f value was scanned.



Dragendorff Reagent



TRACK 1



TRACK 2

UV 254nm

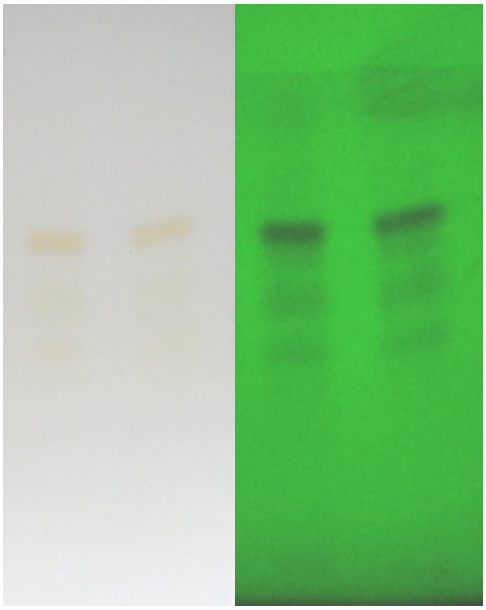
Track	Peak	Rf value	Area
1	1	0.44 Rf	1263.0 AU
1	2	0.50 Rf	6727.1 AU
1	3	0.61 Rf	10404.6 AU
1	4	0.78 Rf	23937.8 AU
1	5	0.87 Rf	1120.2 AU
1	6	1.04 Rf	937.8 AU
2	1	0.53 Rf	6661.1 AU
2	2	0.65 Rf	9406.7 AU
2	3	0.81 Rf	21017.0 AU
2	4	0.94 Rf	149.2 AU

Dragendorff Reagent

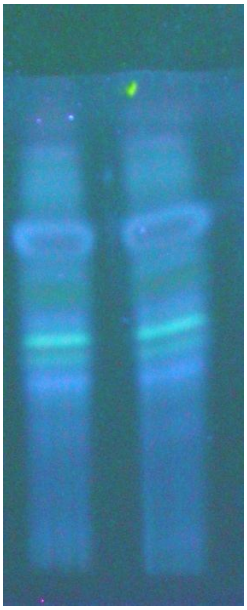
Track	Peak	Rf value	Area
1	1	0.09 Rf	797.4 AU
1	2	0.43 Rf	18612.8 AU
1	3	0.49 Rf	4857.9 AU
1	4	0.53 Rf	15515.7 AU
1	5	0.68 Rf	7892.4 AU
1	6	0.80 Rf	6009.7 AU
1	7	0.89 Rf	8668.9 AU
1	8	0.96 Rf	4196.0 AU
2	1	0.08 Rf	496.7 AU
2	2	0.20 Rf	872.4 AU
2	3	0.46 Rf	15711.6 AU
2	4	0.51 Rf	3857.9 AU
2	5	0.57 Rf	13917.4 AU
2	6	0.72 Rf	6740.5 AU
2	7	0.83 Rf	6307.8 AU
2	8	0.92 Rf	7037.4 AU
2	9	0.99 Rf	2293.1 AU

UV366nm

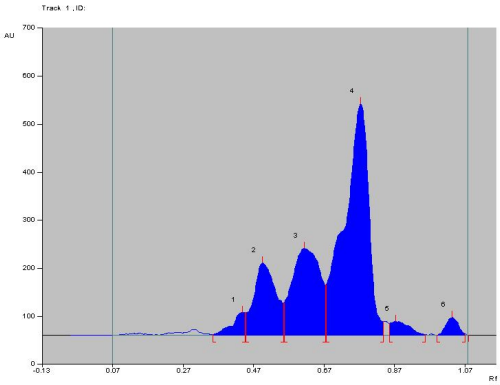
Track	Peak	Rf value	Area
1	1	0.53 Rf	4837.9 AU
1	2	0.63 Rf	2510.2 AU
1	3	0.78 Rf	7247.4 AU
2	1	0.55 Rf	3390.8 AU
2	2	0.66 Rf	2137.7 AU
2	3	0.81 Rf	7837.1 AU



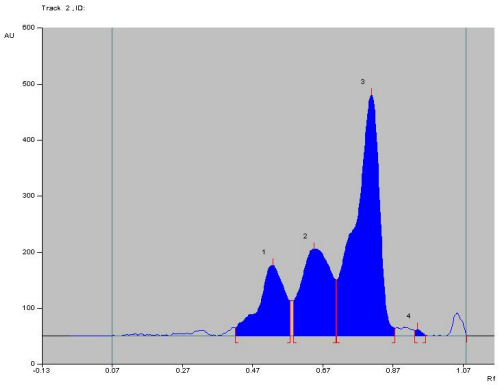
Vis. Light UV 254nm



Dragendorff Reagent



TRACK 1



TRACK 2

PEAK DISPLAY at UV 254nm

RESULTS AND DISCUSSION

The melting point of the isolated compounds were found out by open capillary tube method and the results are uncorrected. The purity of the compounds was checked by TLC using silica gel G as an adsorbent, ethyl acetate and chloroform (9:1) were used as mobile phase. The spot was visualized by iodine vapour or dinitrophenyl hydrazine solution. The structure of the isolated compounds was characterized by its IR and H NMRspectral analysis, where it complies with the normal values.

Using EEAS involves silicagel 60F 254, TLC plate as stationary phase and nButanol:Glacial acetic acid:water (4:1:1) as mobile phase gives good separation of Alkaloids (Rf=0.53,0.63,0.78) at track 1 and (Rf=0.55,0.66,0.81) at Track 2 using Dragondroff reagent as spraying agent present in Ethanolic Extract of stem bark and leaf extracts of*Alangiumsalvifolium* (L.f) wang. The TLC plate and HPTLC photographed chrome plate was visualized under UV light at 254 nm and 366nm.

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