Isolation of Active Compounds from Root Extracts of *Alangium salvifolium* by Chromatography

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Abstract:

The phytochemical analysis of Root Extracts of *Alangium salvifolium* showed the presence of various biologically active compounds. The antimicrobial activity of the root extract of *Alangium salvifolium* is due to the presence of biologically active compounds. 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 root bark 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 antimicrobial activity.

Keywords:
Alangium salvifolium, phytochemicals, tannins, saponins.

Introduction:

Alangium salvifolium (L.f.) Wang (Alangiaceae) is a small shrub or small tree is used in native medicine (Witt,1908). Numbers of bioactive compounds are separated from stem bark and leaves (Rastogi and Mehrotra, 1990). The root bark of *A. salvifolium*is used in leprosy and skin diseases. *A. salvifolium* contains active biochemical constituents like alkaloids, flavonoids, steroids, glycosides, tannins, oil and saponins (Prathyusha P, Subramanian MS. 2010), quantification of gallic acid has also been reported (Natvarbhai M. P., et al., 2010).Evaluation and quantification of primary metabolites has also been carried out (Babeetsingh Tanwer, Rekha Vijayvergia 2010). In our study we used the root bark for isolation of the active compounds.

The phytochemical analysis showed the presence of various biologically active compounds. The antimicrobial activity of the root extract may be due to the presence of sterols, alkaloids, saponins and tannins, because these compounds are biologically active and known to possess antimicrobial activity (Tanira et al.,1994).

Material and Methods:

Root extracts of *Alangium salvifolium* were prepared in acetone and methanol by Soxhlet extraction method. The root bark powder was extracted

successively with ether, chloroform, acetone and methanol solvents. Preliminary phytochemical analysis of extracts showed the presence of important biologically active compounds. Therefore, attempt was made to isolate the fractions containing chemical compounds by column chromatography.

A) Chromatography of Acetone and Methanolextracts

a) Preparation of column:

A neutral glass column of about 3cmx60 cm fitted with a sintered glass disc at the bottom to facilitate filtration was used. The column was cleaned thoroughly using chromic acid and then washed with distilled water till it was free from acid. It was then washed with acetone and dried. The column was fixed vertically on a stand and filled with chloroform. About 250 gm of silica get G (Column chromatographic grade) was mixed with chloroform to make slurry. This slurry was slowly poured in the column from the top and allowed to settle. After all the silica gel had settled, a plug of cotton wool was placed over it. The stop cock was opened and liquid was allowed to run from the bottom of the column till about 1 mm remained above the column.

b) Introduction of the sample:

The dried residue of acetone/methanol extract (5gm) obtained from root bark of *Alangium salvifolium* was dissolved in 5ml of acetone/methanol and the solution was poured carefully with the help of a pipette on the top of the column. The stopcock was opened carefully to allow the solution to get adsorbed on the column.

c) Elution of the Column:

The column was then eluted successively with different solvent and the mixture of solvents in the following order as shown below:

Acetone extract

1) Chloroform
2) Chloroform : ethyl acetate (9:1)
3) Chloroform : ethyl acetate (8:2)
4) Chloroform : ethyl acetate (1:1)
5) Ethyl acetate
6) Ethyl acetate : acetone (9:1)
7) Ethyl acetate : acetone (8:2)
8) Ethyl acetate : acetone (1:1)
9) Acetone

Methanol extract

1) Ethyl acetone
2) Ethyl acetate: acetone (9:1)
3) Ethyl acetate: acetone (8:2)
4) Ethyl acetate: acetone (1:1)
5) Acetone

6) Acetone: methanol (9:1)
7) Acetone: methanol (8:2)
8) Acetone: methanol (1:1)
9) Methanol

For each solvent 6 fractions of 30 ml each were collected. Total fractions amounted to 54.

B) Thin layer chromatography of root bark extract of *Alangium salvifolium:*

The thin layer chromatography was carried out to find out the number of compounds present in the extract. The detail of the procedure followed was as given below:

a) Preparation of the plates:

About 25 gm of silica gel G was taken in a glass mortar and about 35 ml of distilled water was added. The mixture was stirred with glass rod until it become homogenous. Then an additional 15ml of distilled water was added with stirring. The stirring was continued for about 2 minutes and immediately the silica gel G suspension was spread with a spreader on the TLC plates. The prepared plates were air dried and activated in an oven at 110°C for 1 hour. The activated plates were kept in desiccators till required.

b) Application of sample:

For application of the test samples on plates, glass capillaries were used. The distance between two spots was kept minimum of 1 cm. The spots of the samples were marked on the top of the plate to know their identity.

c) Chromatographic chamber:

Chromatographic rectangular glass chamber (16.5 cm x 29.5 cm) was used in the experiments. To avoid insufficient chamber saturation and the undesirable edge effect a smooth filter paper approximately 15x40 cm was placed in the Chromatographic chamber (in a U shape) and soaked in the solvent. The moistened paper was pressed to the chamber wall so that it adhered to the walls.

The chamber was allowed to saturate for 24 hours before use. The experiments were carried out at room temperature in diffused day light.

Solvent system:

Number of solvent system was tried but the satisfactory resolution was obtained in the Benzene: Ethyl acetate solvent system.

Spraying equipment:

Compressed air sprayer with fine nozzle was used to detect constituents on TLC plates. The sprayer was filled with about 50 ml of the detecting reagent and it was sprayed on the TLC plates.

Thin layer chromatography of each fraction was carried out to find out the homogeneity. Benzene: ethyl acetate (8:2) solvent system was used for resolution because satisfactory resolution was obtained with this solvent

system. The detecting reagents used were 50% sulphuric acid and ferric chloride solution. The chromatographic pattern of each fraction was studied thoroughly and the fraction (belonging to the same eluting solvent) which gave identical pattern in respect of R_f and colour were mixed. The fractions which did not showed any component were discarded. The remaining fractions were concentrated to a small volume by keeping them in room temperature for some time. By doing this fraction 3 and 7(named as A and B) of acetone extract and 2, 4 and 7(named as C, D and E) of methanol extract yield sufficient powdered material. The TLC results are given in the table No.1 and 2.

C) Antimicrobial activity of different chromatographic fractions of root bark of *A. salvifolium:*

The Antimicrobial activity of different chromatographic fractions obtained from methanol and acetone extracts of root bark of A. salvifolium was determined by agar disc diffusion technique. The concentration of compound was taken as 100µg/ disc. The results of antimicrobial activity of various fractions obtained are shown in the table No.3.

Table 1: TLC results of Acetone extracts of *A. salvifolium*

Eluting solvent	Fraction number	Solvent system	H_2SO_4 spraying reagent		$FeCl_3$ spraying reagent	
			No. of spots obtained	Rf value	No.of spots	Rf value
Chloroform	1	Benzene : Ethyl acetate	-	-	-	-
Chloroform:Ethyl acetate(9:1)	2		2	0.8,0.75	-	-
Chloroform:Ethyl acetate(8:2)	3		1	0.75	-	-
Chloroform:Ethyl acetate(1:1)	4		1	0.75	-	-
Ethyl acetate	5		2	0.70, 0.65	1	0.65
Ethyl acetate:Acetone(9:1)	6		1	0.60	-	-
Ethyl acetate:Acetone(8:2)	7		1	0.55	-	-
Ethyl acetate:Acetone(1:1)	8		2	0.50,0.40	1	0.50
Acetone	9		-	-	-	-

Table 2: TLC results of Methanol extracts of *A. salvifolium*

Eluting solvent	Fraction number	Solvent system	H_2SO_4 spraying reagent		$FeCl_3$ spraying reagent	
			No. of spots obtained	Rf value	No.of spots	Rf value
Ethyl acetate	1	Benzene	-	-	-	-
Ethyl acetate:Acetone(9:1)	2		2	0.9,0.85	-	-
Ethyl acetate:Acetone(8:2)	3		1	0.85	-	-

Ethyl acetate:Acetone(1:1)	4		2	0.85,0.84	1	0.84
Acetone	5		2	0.82,0.80	-	-
Acetone:methanol(9:1)	6		1	0.80	-	-
Acetone:methanol(8:2)	7		2	0.60,0.50	1	0.50
Acetone:methanol(1:1)	8		1	0.50	-	-
Methanol	9		-	-	-	-

Table 3: Results of antimicrobial activity of extracts of *A. salvifolium*

Test organism	Zone of inhibition(mm)				
	Acetone fraction		Methanol fraction		
	A	B	C	D	E
1) S.aureus	10	8	9	8	8
2) B.subtilis	9	8	8	-	-
3) E.coli	-	-	-	-	-
4) V.cholerae	14	10	12	10	9
5) Sh.dysenteriae	13	11	11	9	8
6) P.vulgaris	-	-	-	-	-
7) S.typhi	12	11	11	10	-
8) Ps.aeruginosa	14	12	14	9	10

Result and discussion:

The results of antimicrobial activity of various fractions obtained are shown in the table No.3. The results indicated that the chromatographic fractions obtained were active against the test microorganisms. The activity of the acetone fractions was found to be more than methanol extracts. Of the two fraction of acetone extracts the fraction A was more active than B fraction.

The methanol extracts yields three major fractions, of which the fraction C was found to be more active than the D and E fractions. The preliminary phytochemical analysis of root bark extracts of *A. salvifolium* showed the presence of alkaloids, saponins and tannins. Therefore, from the R_f values it is concluded that the active fractions contain these bioactive compounds responsible for antimicrobial activity. 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.

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