



DETECTION OF AMINO ACIDS FROM SELECTED WOUND HEALING PLANT SPECIES BY THIN LAYER CHROMATOGRAPHY

Ajay Shrirame and Dayanand Gogle

K.Z.S Sci. College, Bramhani, Kalmeshwar-441501
 PGTD Botany RTM, Nagpur, University, Nagpur-440033
Email-ajaysfri968@gmail.com

ABSTRACT:

The proteins are one of the most important nutrients in human and animal nutrition. It plays an important role in enhancing the repairing of wound. It is well accepted that sufficient protein is necessary for wound healing. This is due to the increase tissue regeneration and repair. Arginine and glutamic acids appears to be essential for efficient wound repair and immune function. In the present study five plant species viz. *Psuederantrhemum atropurpureum* (W. Bull) Radlk., *Cissus quadrangularis* L., *Clerodendrum serratum* L., *Clerodendrum infortunatum* L. and *Cymbopogon citrates* Stapf. were selected through their therapeutic ailments against wound healing. All the plant species were used for in different areas and localities for the treatments of wounds healing. The maximum TLC bands for amino acids were found in the *Cissus quadrangularis* and *Clerodendrum* species.

Keywords: *Psuederantrhemum atropurpureum* (W. Bull) Radlk., *Cissus quadrangularis* L., *Clerodendrum serratum* L., *Clerodendrum infortunatum* L. and *Cymbopogon citrates* Stapf., Amino acids standards, Thin layer chromatography kits.

INTRODUCTION:

In India wound is a very familiar problem in daily life people and animals. It is due to physical, chemical, thermal, microbial or immunological problem to the tissue.

The World Health Organization (WHO) has emphasized the importance of the traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first health remedies (Goleniowski et al., 2006). Plants have been utilized as medicine for thousands of year (Samuelsson, 2004 and Balick et al 1997). Globally, about 85% of all medications for primary health care are derived from plants (Farnsworth, 1988).

The plant based drugs can full filled the certain diet deficient like protein, vitamins, or minerals which is very useful in inflammation and cellular activity in the healing wound (Stadelmann et al., 1998). Arginine appears to be essential for efficient wound repair and immune function (Toriosian, 1994). Glutamine is used by inflammatory cells within the wound for proliferation and as a source of energy (Newsholme, 2001 and, Souba, 1992). Glutamine is

a necessary component of the process of tissue repair. Plants have active constituents, which are of potential wound healing and other valuable purpose. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population.

METHOD AND MATERIAL:

In the present investigation of five different plant species viz. *Psuederantrhemum atropurpureum* (W. Bull) Radlk., *Cissus quadrangularis* L., *Clerodendrum serratum* L., *Clerodendrum infortunatum* L. and *Cymbopogon citrates* Stapf were selected.

Preparation of samples: - It was done by the technique given by Singh and Tandan (1970).

Preparation of TLC plates: - The TLC plates were prepared by applying silica gel G slurry (1: 2 w/v) in distilled water by aluminum applicator on 10 x 20 cm thin glass plates. The plates were activated by heating at 50°C for overnight.

Solvent system: - n-Butanol: Acetic acid: Water (4: 1:1), (Harborne, J.B., 1998).

Detection: - The spot visualization was done by spraying Ninhydrin reagent (Harborne, 1998) and

heating the plates at 105°C for 10 min. The bands obtained were compared with Rf values of standard amino acids. The Rf values of each bands were calculated as follows;

$$\text{Rf value} = \frac{\text{Distance traveled by the solute (cm)}}{\text{Distance traveled by the solvent (cm)}}$$

RESULTS AND DISCUSSION

In the present study the results obtained were presented in table no.1 (plate no.1)

i. Free amino acids having 15 different Rf values were detected from five plants. All the obtained Rf values were compared with the 22 standards of amino acids. Out of these 22 amino acids, L-ArginineHCl, L-Cystein, L-LycineHCl, DL-Methionine, DL-Iso-leucine, DL-Threonine are detected in most of the species.

ii. Bound amino acids having 16 different Rf values were detected in five plants. All the obtained Rf values were compared with the 22 standards of amino acids. Out of these 22 amino acids, L-lycineHCl, DL-B-phenyl alanine present in all the sample were as L-tyrosine; L-cystein present in three sample.

CONCLUSION:

The maximum TLC bands of bioactive compounds were detected in *Cissus quadrangularis* and *Clerodendrum serratum* sp. On the basis of qualitative study it concluded that the plant species *Cissus quadrangularis* and *Clerodendrum serratum* sp. shows higher amount of amino acids. Thus, these plants could be more useful in wound healing.

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Table no.1.TLC analysis for detection of Free and bound Amino acids from selected plant sp.

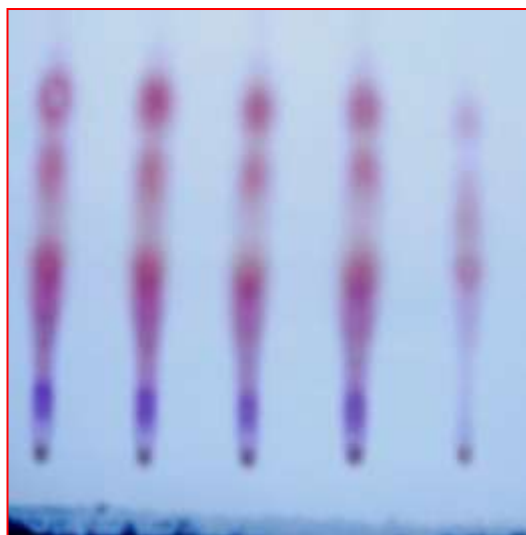
Standard Amino acids.	Color	Standard (Rf)	Plant Sp. for free amino acids					plant Sp. for bound amino acids				
			1	2	3	4	5	1	2	3	4	5
DL-Alanine	pink++	0.41	-	-	++	-	++	-	++	++	-	-
DL-2-Amino-n-Butyric Acid	pink+++	0.45	-	+++	-	+++	-	-	-	-	-	+++
L-ArgenineHCl	blue+++	0.10	++ +	+++	-	+++	+++	+++	+++	-	-	+++
DL-Aspartic Acid	blue++	0.31	-	-	-	-	-	-	-	-	++	-
L-CysteinHCl	blue++	0.27	-	++	++	-	-	-	++	++	-	-
L-Cystein	blue+	0.24	++	++	++	++	-	+	+	-	+	-
DL-Dopa	pink++	0.54	-	-	-	-	-	-	-	-	-	-
L-Glutamic Acid	pink+++	0.40	-	--	-	-	-	-	-	-	-	-
Glycine	pink++	0.32	++	++	-	-	++	-	++	++	-	-
L-HistidineHCl	Blackish (purple)++ +	0.10	-	-	-	-	-	-	-	-	-	-
L-Hydroxyproline	yellow++	0.30	-	-	-	-	-	-	-	-	-	-
DL-Iso-leucine	pink+++	0.59	++ +	+++	+++	+	-	-	+++	-	-	-
DL-nor-leucine	pink+++	0.61	-	-	-	-	-	+++	-	-	-	-
L-leucine	pink++	0.62	-	-	-	-	-	-	-	-	-	-
L-LycineHCl	brownish +++	0.08	++ ++	+	++	++	++	++	+++	++	++	++
DL-Methionine	pink+++	0.52	++ +	++	-	++	++	-	-	-	-	-
L-Ornithine HCl	brownish blue++	0.07	-	++	++	++	-	-	-	-	-	-
DL-B-Phenyl alanine	Black (purple)++ +	0.64	++	+++	++	++	++	++	++	++	++	+++
L-Proline	yellow++	0.27	-	++	++	++	-	-	++	+++	++	-
DL-Serine	pink+++	0.34	++	-	+++	-	+++	+++	-	-	-	-
DL-Threonine	pink++	0.37	++	++	-	++	-	-	-	-	++	++
DL-Tryptophan	pink+++	0.65	-	-	-	-	++	-	-	-	-	-
L-Tyrosine	pink+++	0.65	-	-	+	+	+	-	+	++	+	++
DL-Valine	pink+++	0.51	-	-	-	-	-	+++	-	+++	+++	+++
Total band			9	11	9	11	9	7	10	8	8	7

Plant species: 1.*Psuederantherum atropurpureum*(W. Bull) Radlk., 2.*Cissus quadrangularis*L., 3. *Clerodendrum serratum*L., 4. *Clerodendrum in fortunatum* L. and 5. *Cymbopogon citrates* Stapf.,

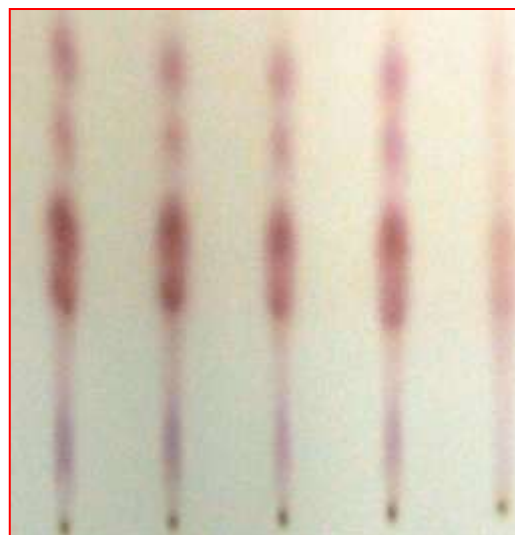
Plate no. 1 . TLC plate of standard amino acids :



TLC detection for freeamino acids of plant sp. TLC detection for bound amino acids of plant sp.



1 2 3 4 5



1 2 3 4 5