

ORIGINAL RESEARCH ARTICLE

Phyto Chemical Investigation and Determination of Crude Alkaloidal Content (Solasodine) in *Solanum Leave Dunal*(Dry and Fresh Berries)

P.Muthumani^{*1}, R. Meera¹, Sweetlin¹, P.Devi²

¹Department of Pharmaceutical Chemistry, ²Department of Pharmacognosy, K.M. College of Pharmacy, Uthangudi, Madurai, TamilNadu..

Received 18 April 2010; Revised 3 May 2010; Accepted 5 May 2010

ABSTRACT

The phytochemical studies on the plant of *Solanum laeve dunal* resulted in isolation of tomatilidine (16 un substituted 22,26 epiminocholestanes), Jervine being reported for the first time from this plant. These compounds have been characterized on the basis of spectral and other data. The alkaloid solasodine content present in the dry and fresh berries were determined in the isolated compounds (SA-I, SA-II, SA-III) of the extracts. The total alkaloidal content is more in SA-I compound than other compounds

Key words: Phytochemical investigation, IR, NMR, Solasodine.

Introduction

Alkaloids based on the steroid nucleus are not very widely distributed being restricted to plants of the Holarrhena, Solanum and Veratrum species. Among the bases there is relatively little variation in structural type, the following sub-group may be distinguished^[1].

Solasodine is the aglycone of solasonine (solancarpine, purapurine) of soladamine, and of solamargine and is prepared from these by hydrolysis with 3% hydrochloric acid at 100°C. The cooled reaction mixture deposits the sparingly soluble hydrochloride from which the free base may be regenerated. It is dimorphic but both forms melt at 198°-200°C^[2]

Solasodine is a weaker base with molecular formula C₁₂H₄₃O₂N and pK_b 6.30^[3]. One of the oxygen atom present as a secondary hydroxyl in position 3 as shown by the formation of a sparingly soluble digitonide. The functional nature of the second oxygen was more difficult to determine. More recent investigations have shown that the second oxygen is present as cyclic ether and the second active hydrogen is present on secondary nitrogen.

¹³C-NMR spectra of solasodine and their assignments have been reported. Mass

spectroscopic fragmentation pattern of solasodine has also been reported^[4]. The steroid bases of solasodine group occur naturally as the glycosides usually containing three sugars. On hydrolysis the glycosides yield the steroid in the aglycone form. The alkaloid content is usually determined by extraction apparatus, removal of the solvent and precipitation of bases with ammonia and weighing of the crude base. From the literature review of analytical methods for the determination of alkaloid, a number of methods have been reported, like potentiometric titration using HCl Hamid and Bakeshi^[5] colorimetric method using bromothymol blue, antimony trichloride^[6]. Among these the methods of Gupta and Basu^[7] acetic resorcinol-alkaloid complex, methyl orange-alkaloid phase-transfer complex method of Birner^[8] were mostly developed suitable HPLC methods are also developed^[9]. The HPLC methods were most developed for glycoalkaloids and are to sensitive to assay crude sample.

Anti tumor activity of β Solamarine, and Solapalmiotine isolated from *S. tripartitum* against cells derived from the human carcinoma of nasopharynx (Kupchan) against Sarcoma 180 in mice(cham) were reported Biological activities of α tomatine a glycol alkaloid like antifungal

activity, anti microbial and also anti diuretic was reported by Roddick and concluded that activities were exhibited by partially purified methanolic extracts and not purified alkaloid. Roddick has also reviewed some enzymes inhibiting activity of *Solanum* alkaloids and reported anti cholinesterase activity [10, 11, 12, 13, 14, 15]

MATERIALS AND METHODS

Plant material

The plant has been identified in the Kodaikanal area and has been authenticated by Dr.V.Balasubramanian M.Sc.Ph.D., Taxonomist, Saraswathi narayana College, Madurai.

The plant was collected during months of August-September which is the season for bearing the berries. Since almost all the reports suggest berries to be the main source of alkaloid. We have collected the plant during three stages 1.Berries at

green colour stage.2. Berries at greenish yellow 3. Berries at red colour (ripe)

Experimental

SPECTROPHOTOMETRIC METHOD:

Among the methods available in the literature two method of Jan Birner and Gupta and Basu have been tried. The method of Birner was more convenient when estimating the alkaloid in fresh berries. There fore the methods described here is essentially that of Jan Biner.

In order to establish the alkaloid content the spectrophotometric was used for determination of total alkaloid content with authentic solasodine sample kindly supplied by Prof.Jaggie and Kapoor (**Table 1**).

The method has been critically examined by several workers note Bradly^[16] for possible interferences.

Table-1:Solanium Glyco Alkaloids

Glycoalkaloid	Alkamine	Sugar
α -Solamine	Solanidine	L-Rhaamnose D-Glucose,D-galactose
β -Solanine	Solanidine	D-Glucose,D-galactose
γ -Solanine	Solanidine	D-Galactose
α -Chaconine	Solanidine	2-L-Rhamnose,D-glucose
β -Chaconine	Solanidine	L-Rhaamnose D-glucose
γ -Chaconine	Solanidine	D-glucose
Solacauline	Solanidine	2D-Xylose D-glucose
Tetroside from <i>S.acaulia</i>	Solanidine	D-Xylose,2D-glucose,D-galactose
Demissine	Demissidine(Solanidan 3β -ol)	D-Xylose,2D-glucose,D-galactose
Solasonine	Solanidine	L-Rhaamnose, D-glucose,D-galactose
Solamargine	Solasodine	2L-Rhaamnose, D-glucose
Solasodamine	Solasodan	2L-Rhaamnose, D-glucose,D-galactose
Tomatine	Tomatidine	D-Xylose,2D-glucose,D-galactose
Trioside from <i>S.polyadenium</i>	Tomatidine	D-Xylose,2D-glucose.
A, β and γ -Soladulcine	Soladulcidine(Solasodan 3β -ol)	D-Xylose,L-Rhamnose,D-galactose and D-glucose

Preparation of standard curve

Reagents:

standard solution of solasodine: weigh out exactly 10mg of pure solasodine and dissolve in 25ml of 20% aceticacid A.R., dilute as aliquot a further 10 times with 20% aceticacid. This solution contains 40mcg/ml.

Acetate Buffer pH 4.7 dissolve 5.44g sodium acetate A.R. in water and 2.40ml of aceticacid and adjust volume to 100ml with water. Methyl orange 0.05% dilution in water. Chloroform A.R

Procedure A.

Preparation of standard curve Into four suitable separators are pipetted 0,1,2 and 3 of 40mcg/ml standard solution and the volume of each is made up to 5ml with 20% aceticacid, to each separator 5 ml of acetate buffr and 1ml of methyl orange are added. After shaking for 10 sec. 5 ml of chloroform is added. The separators are stopped and shaken for 3 min. after standing for a few minutes chloroform layers are withdrawn into dry test tubes, dried with small amount of anhydrous Na₂SO₄ and absorbances read on a

spectrophotometer at 420nm using 10mm cells. From the reading standard curves is constructed.

Estimation of crude alkaloid in fresh berries

One hundred grams of fresh berries is homogenized with 100ml 2% acetic acid in a suitable blender to produce a fine pulp which is further diluted with 400ml of 2% acetic acid, transferred into two 200ml conical flasks and shaken for 3 hrs. the volume is measured volume is transferred into a 150ml beaker, heated till boiling and the alkaloid precipitated by addition of 1:2 ammonia in water until the pH reaches 9.5-10.0. the content is transferred into 100ml conical centrifuge tube and spun for 15min at 2000 rpm. The supernatant is removed by suction or decanting and the precipitate dissolved in 1NHCl. The solution is transferred to a 100ml volumetric flask and adjusted to the mark with 1NHCl. It is then filtered through filter paper into a dry vessel and 5 ml pipette into a small flask for hydrolysis on a 100°C water bath by refluxing for 2 hrs.

To the flask is then added 5ml of 1N NaOH and 20 ml of concentrated acetic acid the contents transferred in to a 100ml volumetric flask and adjusted to the mark with water. Each ml of this solution is equivalent to 5mgs of fresh berries.

1 to 3ml of this solution is transferred into a separator and the procedure followed as for standard curve.

Estimation of crude alkaloid in dry leaves

One hundred mgs of finely powdered material and 40ml of 95% ethanol are refluxed in a 100ml flask for 30min. the extract is then filtered; the residue is washed twice with 2ml of ethanol. The washings are added to the original filtrate and transferred into a 50ml standard flask, the volume being adjusted to the mark with 95% ethanol. 5ml of this solution is pipette into a test tube and ethanol completely removed by evaporation on a water bath. The residue is treated with 3ml of 1N HCl and refluxed for two hours for hydrolysis.

The acid is neutralized by adding 3ml of 1N NaOH, two mo of concentrated acetic acid is then added and the contents transferred to a 10ml standard flask, the volume being adjusted to the mark with water. One ml of this solution is equivalent to 1mg of dry material. One to 3 ml is then pipetted into separator and the procedure followed as for A.

Based on the quantitative estimation and T.L.C. berries which are ripe have been chosen for purification. The quantitative estimation results

were described along with description of isolated compound.

ISOLATION OF SOLASODINE (alkaloid content) FROM FRESH BERRIES:

In some tropical Solanum species, the glycol alkaloids are concentrated in fruits only and attempts to raise these plants as industrial crops is being undertaken in South America. There are two approaches to recover glycol alkaloids from Solanum fruits.

1. Extraction of dried plant material successively with petroleum ether, methanol and crystallizing
2. Extraction of fresh materials (berries) directly with 2-5% acetic acid. Extraction of fresh berries Telk^[17].

1kg of fresh berries were homogenized in a warring blender with 1.5 lit of 3% aqueous acetic acid, the resulting thick slurry was filtered through a filter cloth. The filtrate and washings are mixed with w volumes of ethanol stirred fro 2 hours, centrifuged and filtrate discarded. The brown coloured residue was re-suspended in ethanol (500ml) and extracted for 2 hours. The alcoholic concentrate was made alkaline with ammonia (pH 9.5) and kept in refrigeration after 2 days a precipitate was formed, this was centrifuged. The precipitates from the combined extracts are extracted with hot 100ml ethanol three times, which recovers most of the glycol alkaloid from the precipitate. The ethanol extracts were refluxed with 2-5 gm of activated charcoal filtered through Buckner funnel, to the filtrate concentrated HCl was added to make it 3N and refluxed for 1 hour and the alcohol was removed by distillation on cooling the alkaloid crystallized as a light brown solid. It was designated as compound- B (SA-II).**Table 2**

Table-2 Determination of alkaloid as solasodine content

S.No	Sample	Total alkaloid	% of total alkaloid based on dry weight
1	SA-I	282	2.82
2	SA-II	226	2.26
3	SA-III	156	1.56

ISOLATION OF SOLASODINE FROM DRY LEAVES:

500 gms of dried leaves were taken and one hundred mgs of finely powdered material and 40ml of 95% ethanol are refluxed in a 100ml flask for 30min. the extract is then filtered; the residue is washed twice with 2ml of ethanol. The washings are added to the original filtrate and transferred into a 50ml standard flask, the volume being adjusted to the mark with 95% ethanol. 5ml of this solution is pipette into a test tube and ethanol completely removed by evaporation on a water bath. The residue is treated with 3ml of 1N HCl and refluxed for two hours for hydrolysis.

The acid is neutralized by adding 3ml of 1N NaOH, two mo of concentrated acetic acid is then added and the contents transferred to a 10ml standard flask, the volume being adjusted to the mark with water. One ml of this solution is equivalent to 1mg of dry material.

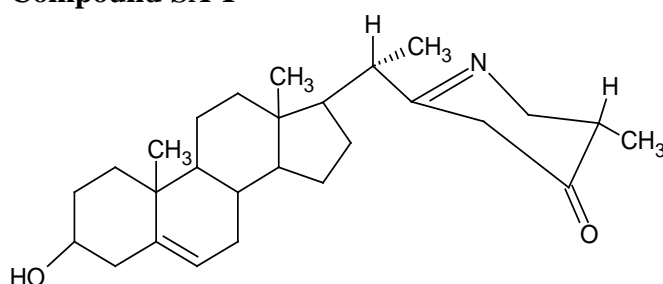
Experimental

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Perkin –Elmer FTIR using KBr discs. PMR on Bruker spectrospeir 200MHz NMR instrument using CDCl₃ as solvent and TMS as internal reference (Chemical shifts in δ , ppm) Elemental analysis of all the synthesized compounds were performed on a Perkin Elmer 2400. Series – II

Phytochemical investigation ^[18, 19, 20]

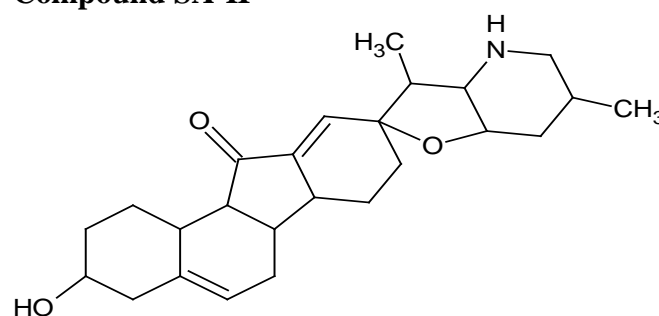
These plant parts were dried, crushed into a coarse form and extracted. Melting points were recorded on a Veego-Vmp-I apparatus. Infra-red-Spectra were recorded on a shimadzu & FT-IR Perkin Elmer spectrometer by using potassium bromide pellet and nujol mul for solid and semisolid compounds respectively. NMR spectra were recorded on a H¹ NMR dueteriated chloroform (CDCl₃) 500 MHz Tetra methylsilane as internal standard.

Compound SA-I



From dried berries after rechromatography, yield 58mgs, appearance white amorphous, soluble in ether and chloroform, soluble in acetic acid on heating, M.P. 203°C. T.L.C. chloroform: Ethylacetate (2:1) Rf = 0.24, spraying reagent Dragon dorff reagent, Vanillin sulphuric acid and also gave red spot with iodine. U.V spectrum: 1ml of chloroform solution (40 μ g) made upto 5ml with absolute methanol and measured showed absorbance maximum at 250nm to 260nm. Fig no 1. IR spectra (KBr pellet) about 10mgs on perkin Elmer spectrum 3371cm⁻¹)O-H stretching, 2945 and 2842 cm⁻¹ C-H stretching, 1672 cm⁻¹ C=C stretching, 1020 cm⁻¹ and 1136 cm⁻¹ C-O stretching, 1246 cm⁻¹ C-N symmetric stretching. NMR spectra 0.8-1.8 δ ppm due to CH₃ and CH₂ proton in rings, 2.2 δ ppm CH₂ adjacent to C=C, 4.2 δ ppm O-H proton, 5, 2 δ ppm may be C=CH proton.

Compound SA-II



The compound was isolated from fresh berries (crude), yield 125mgs, appearance brownish crystals, yield 125mgs, soluble in ether, chloroform and absolute methanol, M.P. 160°C. T.L.C. Chloroform:Ethyl acetate (2:1) Rf value 0.26., spraying reagent Dragon dorff reagent, Vanillin sulphuric acid and also gave red spot with iodine.

U.V spectrum: 1ml of chloroform solution (40 μ g) made upto 5ml with absolute methanol and measured showed absorbance maximum at 270nm to 280nm. Fig 2. IR spectra (KBr pellet) 3670-3708cm⁻¹)O-H stretching, 2936 cm⁻¹ C-H stretching, 1708-1741 cm⁻¹ C=O stretching, 1381-1465 cm⁻¹ CH₃ bending, 1044 cm⁻¹ C-O stretching, 1230 cm⁻¹ C-N stretching. NMR spectra 0.7-1.3 δ ppm characteristic of steroids, 3.8 δ ppm 3 α -H, 5.2 δ ppm C=C-H proton.

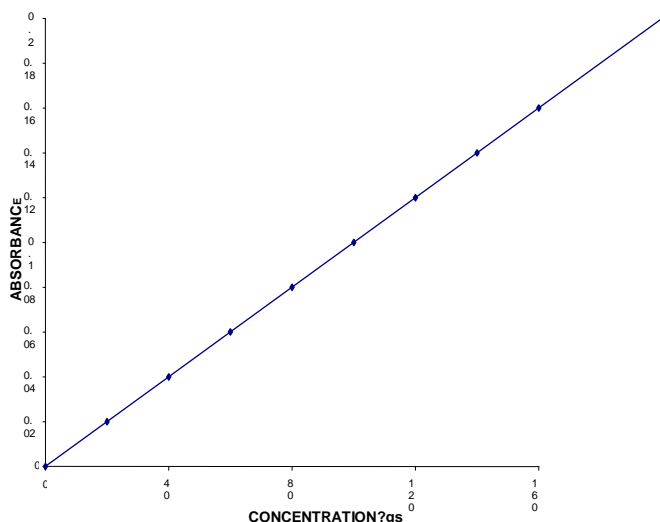
Compound –C-SA-III

The compound was isolated from dried leaves (crude), yield 56mgs, appearance greenish powder, soluble in ether, chloroform and absolute methanol, M.P. 160°C. T.L.C. Chloroform: Ethyl

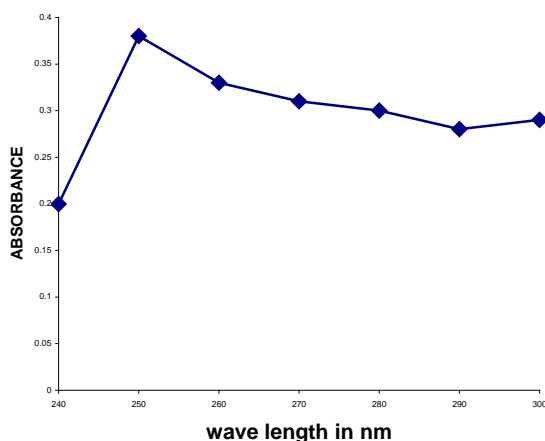
acetate (2:1) R_f value 0.78., spraying reagent Dragendroff's reagent, Vanillin sulphuric acid and

also gave red spot with iodine.

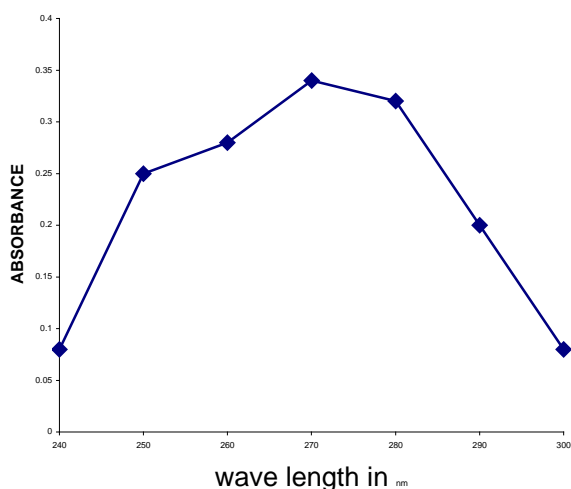
STANDARD CURVE



UV SPECTRUM OF COMPOUND A (SA - I)
Fig 1



UV SPECTRUM OF COMPOUND B (SA - II)
Fig 2



References

- Schreiber K : Alkaloids, Editor R.H.F. Manske, Vol I.
- Bell RC, Briggs LH , Caroll JJ: *J.Chem Soc*,1942,1-2.
- Schreiber K :Alkaloids,Editor R.H.F. Manske, Vol X ,1
- Esahak Ali, Ajit. K.Chakravarthy, Prkrashi SC: *Tetrahedron*, 1977;33(11):1371-1377.
- Bakshi VN, Hamied YK: *Ind.J.Pharm*, 1971;33:54-55.
- Balcar E, Zalecka M, Biul Inst Roslin Leenmickyych: *Chem Abstract*, 1962;8: 59,1963, 2593.
- Gupta U, Basu PK : *Ind J Exp Biol*, 1981; 19: 205-1206.
- Jan Birner: *J. Pharm. Sci*,1969; 53(2): 258-289.
- Bill E Chem and Linda Wilson : *Planta medica*, 1987;58: 59-62.
- Mitscher C ,Jvarker JV ,*Experientia* .1976;32(4):415
- Wilson RF: *Toxicol appl.pharmacol*.1961;3:39.
- Kupchan SM, Davies AP, Barboutis SJ: *Jr. Org Chem*. 1969;34(12):3888-3893.
- Kupchan S M: *Science*,1965;150:1827.
- Bill E: Cham., *Planta Med*.1987;58:34.
- Roddick J: *Phytochem*.1974;13:9.
- Bradly V, Collins DJ: *Aust .J.Bot*, 1979;26: 723-754.
- Telek J: *Planta medica* ,1997;41:92.
- John R.Dyer Applications of absorption spectroscopy of organic compounds, 1st edition, Prentice-Hall of India (P),New Delhi,1969, 33-38.
- Robert M. Silverstein, Francis X. Webster, Spectrometric identification of organic compounds, John Wiley and sons, Inc.1998.
- McLafferty FW, Interpretation of mass spectra, 2nd edition, W.A. Benjamin. Inc. Publishers, New delhi.

P.Muthumani et al. / A New Stability- Phyto Chemical Investigation and Determination of Crude Alkaloidal Content (Solasodine) in *Solanum Leave Dunal*(Dry and Fresh Berries)

IJPBA, Sep.-Oct. 2010, Vol. 1, Issue, 4