Research Paper

Volume : 3 | Issue : 9 | September 2014 • ISSN No 2277 - 8179

Phytochemical Screening and TLC Profiling of Seeds of Crotalaria Verrucosa Linn

Botany

KEYWORDS : Crotalaria verrucosa L., Hot continuous and successive extraction, Phytochemical screening, Thin layer chromatography.

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ABSTRACT

Seeds of Crotalaria verrucosa L. were screened for their phytochemical constitution following hot continuous and successive extraction by Soxhlet apparatus. Qualitative assay and thin layer chromatography was done using a range of solvents. Extraction process was carried using different solvents successively in the order of increasing polarity. Qualitative analysis of the extracts using standard procedures, revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids, phenols, steroids, coumarins, cardiac glycosides and phytosterols. TLC profiling of the extracts was performed using different solvent compositions, which yielded a wide array of compounds. Distinct qualitative manifestation of phytochemicals in screening test and chromatography of the seeds has demonstrated their potential for future drugs.

Introduction

Evaluation of higher plants for natural products has constantly been the basis of modern medicine. The fact, that the phytochemical constitution of the plant varies from organ to organ, with the developmental stages and growing conditions, makes it a rich source of secondary metabolites. Though, these phytochemicals represent smaller quantity in higher plants, including alkaloids, terpenoids, flavonoids, tannins, resins, etc. their taxonomical and chemical diversity is endowed with ambiguous function but with precision. They are widely used in human medicine, veterinary, agriculture, scientific research, and many more areas (Trease, GE., Evans, WC., 1978). Plants with ethnomedicinal importance need further studies for establishing their phytochemical constitution and potential for future medicine. In this perspective, seeds of *Crotalaria verrucosa* L. were screened for phytochemical constitution and chromatographic behavior.

Crotalaria verrucosa L. (Fabaceae, subfamily; Faboideae; common name, blue rattlesnake, called sana-pushpi in Sanskrit). It is a shrub, distributed all over India, in the tropical region of India from Himalayas to Ceylon. (Chopra, R.N., and Nayar, S.N.,1956). It is seen in almost all districts, weed ofn roadsides, waste places, gardens and fields. It is herbaceous, usually annul, plant with blue, sometimes white, flowers (Gamble, J.S., 2008).and is described in the Nighantas as bitter and an expellant of bile and phlegm.

A much branched herb 2-3 ft high; branches acutely angled, at first puberulous; afterwards glabrescent. Leaves 2-6 by 1 ¼-3 in., ovate- rhomboid or ovate –deltoid, obtuse or occasionally acute at the apex, tapering to the base, sub glabrous above, more or less downy and paler beneath; nerves prominent: flowers in terminal or lateral 12-20- flowered racemes 6-8in.long; pedicels3/16in. long: bracts at the base of the pedicels linear-lanceolate, 1/8in. long, those on the pedicels much smaller, subulate. Calyx membranous, 1/3in.lon, faintly pubescent: tube short, campanulate; teeth sub equal, triangular, acute. Corolla ¾ in. Long, exserted, bluish- purple and white, rarely entire white. Pods densely villous when young, softly pubescent when ripe, 1-1 ½ in. long, oblong- cylindrical, stalked. Seeds 10-15, yellow, 1/5 in. long, polished. (Theodore Cooke, C.I.E., 2006).

The juice of leaves is supposed to be efficacious in diminishing salivations and its leaves, tender stalks were prescribed by the doctors both internally and externally in case of scabies and impetigo (Watt, G., 1972). Juice of leaves diminishes salivation, juice used for scabies and impetigo, dyspepsia, blood impurities, diarrhoea, dysentery and leprosy (Nair, CKN., Mohan an, N., 1998,Khare, CP., 2007,Singh, J., 2008,Samant, SS., Dhar U and Palni LMS., 1998). It contains anacrotine crotaverrine and O 12-acetyl-crotaverine (Sharma, RK., Kasture, AV., and Kapoor KK.,1965,Suri, OP., Sawhney RS., and Bhatia MS.,1976).Used in scabies and impetigo (Nadakarni,K.M.,1976). The aqueous extract showed remarkable wound healing activity in animal model and it may be suggested for treating various types of wounds in animal and human beings (Meena Kumari., Eesha BR., and Mohanbabu Amberkar.,2010).Ainslie, speaking of the same plant, says "The slightly bitter, but not unpleasant tasted juice of the leaves and tender stalks is prescribed by the Tamil doctors, both internally and externally, in cases of scabies and impetigo". The crotalarias appear to be used medicinally on account of their mucilaginous and emollient properties; the leaves might be used as a poultice like *Althaea* leaves (William Dymock,C.J.H., Warden, and David Hooper.,1890).

According to Ayurveda, The leaves are hot, sharp, bitter; expectorant; emetic; cure "kapha", biliousness, dyspepsia, fever, blood impurities, and throat and mouth diseases, heart complaints. The juice of its leaves is used in medicine; it is supposed to be efficacious in diminishing salivation. It is prescribed by the Tamil doctors, both internally and externally, in cases of scabies and impetigo (Kiritikar, K.R., and Basu, B.D., 1999). The leaf decoction is given orally to cure jaundice (Senthil kumar, M., and Gurumoorthi, P., 2006). The aqueos ethanolic extract of aerial parts of *Crotalaria verrucosa* has shown very significant hepatoprotection against paracetamol induced hepatotoxicity study models in wistar rats (Lekharani, C., and Yanadaiah, J.P., 2013).

Recent studies on the aqueous and ethanolic extracts of aerial parts of *C. verrucosa* were found to be affective against fertility and estrogenic implantation in *Albino* rats (SubodhKumar Singal and Pradeepa M.S.,2011).

Methodology

Crotalaria verrucosa seeds were collected from Osmania University campus, in wild condition from mature pods in the month of November, 2013. The plant was authenticated by Prof. P. Ramachandhra reddy, Department of Botany, Osmania University (O.U) and the specimen was submitted to the Herbarium, Hyderabadens, Department of Botany, O.U, Hyderabad (Voucher No.0146).

The seeds were dried in hot air oven at 45 oC for 15 days to attain constant weight (20.Raman, N., 2006).were obtained by electronic balance machine (Type BL-22OH, NO.D427600501). Dried seeds were ground and meshed through 0.3mm mesh. (Jayanth scientific IND. Mumbai.) And stored in airtight sterile container

Successive extraction using Soxhlet apparatus

Successive extract of was carried out using Soxhlet apparatus. 20 gr *Crotalaria verrucosa* L. seed powder was taken in Wattmans No.1 filter paper, placed in Soxhlet thimble and 200mlof solvent was taken in the round bottom flask (still pot). The seed powder was extracted successively with n-hexane at 70 oC, petroleum ether at 60 oC, ethyl acetate at 77 oC and chloroform at 61 oC, acetone at 56 oC, ethanol at 78 oC, Methanol at 65 oC, and water at 80 oC. Extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of cycling of fresh solvent. Six hours of duration was allocated to each solvent for hot continuous and successive extraction. The extracts were cooled, filtered through Whatman No.1 filter paper and the extraction was done in the order of increasing polarity of the solvents i.e., from hexane to water and proceeded for phytochemical screening.

Aqueous extraction

20 g of the seed powder was taken in a flask and heated with 200ml of distilled water for five hours at 80 oC by agitating gently at regular intervals. The contents were then filtered through Whatman's No.1filter paper (W and R balson Ltd, England) and the filtrate was used for preliminary phytochemical screening.

Screening of Phytochemicals

Detection of alkaloids.

Extracts were dissolved in dilute Hydrochloric acid and filtered

a) Mayer's Test (Evans, W.C., Trease, 1997).

To a 2ml ml filtrate two to three drops of Mayers reagent were added by the side of the test tube a white or creamy precipitate indicated the test as positive

b) Wagner's Test (Wagner, H., 1993).

To a 2ml of filtrate two drops of Wagner's reagent (Iodine in Potassium Iodide) is Added by the side of the test tube, formation of brown/reddish precipitate indicated the

Presence of alkaloids

c) Dragendroff's Test (Wagner. X.S., Bladt, Z., Gain and E.M., Suie1996).

2ml of Filtrate is taken and 1 to 2 ml of Dragendroff's reagent is added. Appearance of a prominent yellow precipitate denoted the presence of alkaloids.

d) Hager's Test (Waldi, D., 1965).

To 2ml of Filtrates 1 of Hager's reagent (saturated aqueous picric acid solution) was added. A prominent yellow precipitate indicated the presence of alkaloids

Detection of carbohydrates (Ramkrishnan, S, K.G., Prasannan., and.Rajan, R., 1994**).**

The extract was dissolved in 5ml of water and filtered; the filtrate was subjected to the following tests.

a) Molisch's Test: To 2ml of filtrate, two drops of alcoholic solution of α- napthol was added, the mixture was shaken well and 1ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand, appearance of a violet ring indicated the presence of carbohydrates.

b) Fehling's Test:

One ml of filtrate was boiled on water bath with 1 ml each of Fehling solution 'A' and 'B'. A red precipitate indicated the presence of sugar.

C) Barfoeds test:

To1ml of filtrate, 1ml of Barfoeds reagent was added and heated on a boiling water bath for 2min. Appearance of red precipitate indicated the presence of sugars

Detection of glycosides (Mariappansenthilkumar,2013).

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Borntrager's Test (Modified):

5ml of extract was taken,5ml of 5% $feCl_3$ and 5ml dil. HCl were added. Contents were heated for 5min in boiling water bath followed by cooling. To this mixture, 5 ml of benzene was added and shaken well. Organic layer was separated and equal volume of dilute ammonia solution was added. Appearance of pinkish red color in the ammonical layer indicated the presence of glycosides

Detection of saponins (Kokate, C.K., 1999. Sindhu,S., and Uma,G.,2013).

a) Froth Test: Extracts is diluted with distilled water and made up to 20ml and suspension was shaken in a graduated cylinder for 15 min. Formation of foam layer of about two centimeters indicated the presence of saponins.

b) Foam Test: In a test tube, about 5ml of the extract was taken and a drop of sodium bi carbonate was added .the mixture was shaken vigorously and kept for 3 min. Formation of honey comb like froth showed the presence of saponins

Detection of proteins (Fisher,D.D., 1968, Ruthmann, A.C.,1970).

The extract was dissolved in 10ml of distilled water and filtered through Whatman no. 1 filter paper and the filtrate is subjected to tests for proteins.

a) Millon's test (Rasch, E.,ands wift. H., 1960).

To 2ml of filtrate 0.5ml of millon's reagent was added. Formation of a white precipitate indicated the presence of proteins.

b) Biuret test (Gahan, P.B., 1984).

To 2 ml of filtrate 0.5ml of biuret reagent was added. Appearance of pink color indicated the presence of proteins

Detection of amino acids

Ninhydrin test (Yasuma, A., and Ichikawa1953). Two drops of ninhydrin solution (10mg of ninhydrin in 200 ml of acetone) were added to two ml of aqueous filtrate. Formation of a characteristic purple color indicated the presence of amino acids in the extracts.

Detection of cardiac glycosides (Sasidharan, S., and Chen,Y., 2011).

Kellar - Kiliani test: 2ml filtrate was taken, to this 1ml of glacial acetic acid, 1ml ferric chloride and 1ml concentrated sulphuric acid were added. Green-blue coloration of solution appeared indicating the presence of cardiac glycosides.

Detection of flavonoids (Sindhu, S., and Uma, G.,2013).

In a test tube containing 0.5 ml of the fruit extract, 5-10 drops of dilute HCL and small piece of Zn were added and the solution was boiled for few minutes. Presence of flavonoids resulted in reddish pink or dirty brown.

Detection of resins (Anjali soni, and Sheetal Sosa, 2013).

To 2 ml of extract, 5-10 drops of acetic anhydrate was added, dissolved by gently heating and then 0.5 of Sulphuric acid was added.Bright purple color was produced indicating the presence

Volume : 3 | Issue : 9 | September 2014 • ISSN No 2277 - 8179

of resins.

Detection of triterpenoids (Sindhu, S., and Uma, G.,2013).
The extract was dissolved in one ml of chloroform; 1ml of acetic anhydride was added followed by the addition of 2ml of concentrated H_2SO_4. Formation of reddish violet color indicated presence of triterpenoids

Detection of steroids (Yasuma, A., and Ichikawa1953).
Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulfuric acid were added. Appearance of dark green color showed the presence of steroids.

Detection of Phenolic compounds
Ferric chloride test (Anjali soni, and Sheetal Sosa, 2013)
Take to 2ml of extract, treated with 3-4 drops of Ferric chloride solution formation of bluish black color indicates presence of phenols

Detection of tannins (Prashanth Tiwari and Bimlesh. 2011).
1 ml of extract was taken and few drops of 1% lead acetate were added. Formation of yellowish precipitate indicated the presence of tannins.

Detection of phytosterols (Anjali soni, and Sheetal Sosa, 2013).
Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled followed by the addition of concentrated sulfuric acid. Formation of a brown ring at the junction indicated the presence of phytosterols.

Detection of Quinones (Poongothai, A., and Sreena, K.P., 2011).
Dilute NaOH was added to 1ml of crude extract blue green or red coloration indicated the presence of quinones.

Detection of coumarins (Sasi kumar, R., and. Balasubramanian, P.,2014).
3ml of 10% NaOH was added to 2ml of aqueous extract. Yellow coloration of the contents indicated the presence of Coumarins.

Detection of leucoanthocyanins (Jhansi Rani, D., and Rahini Devi, R., 2013).
1ml of aqueous extract was added to 1ml of isoamyl alcohol. Upper layer turned red in color indicating the presence of leucoanthocyanins.

Detection of anthraquinone (Ayoola, GA., Coker, HAB., 2008).
To 1gr of the powdered plant material, chloroform was added and shaken for 5mints. Contents were filtered and to the filtrate, 5ml of was ammonia solution was added and agitated gently. A bright pink color in the upper aqueous layer indicated the presence of anthraquinone.

Detection of fixed oils (Kokate, C.K.,1999).
A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Thin Layer Chromatography (TLC) of the crude extracts
TLC needs an extremely small quantity of the sample (less than a milligram) and an extremely short time (5 to 10 min) for effective qualitative separation of the compounds in the sample. It helps to determine the number of compounds in a sample also to identify unknown compounds by a comparison with suspected reference or standards (David Krupadanam, GL., Vijayaprasad, D.,Varaprasadarao,K., and Reddy, KLN.,2001).

Crude extracts of the seeds of *C. verrucosa* obtained by using eight different solvents composition were subjected to TLC profiling under varied proportions of two different solvent systems (mobile phases), hexane + ethyl acetate and chloroform + methanol.

Chromatography (TLC) was carried out on Precoated Silical gel plates (TLC-grade; Merck India) at room temperature. For each extract, fourteen different mobile phases were used. These are, Hexane + ethyl acetate (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 0:10) and Chloroform + methanol (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 0:10).

TLC plates were marked with a pencil, 1cm from the base and labeled. In each case 1cm was measured from the base of the TLC plate, marked with a pencil and labeled. Capillary tube was used to spot the plates with the crude extract. Eight spots were made on each plate.

Derivatisation of TLC plates was done by spraying the reagent, charic solution.

Spray reagent: Mix110ml ethano,l 2.8 ml ansaldyhyde , 1ml acetic acid and 3.2 ml H2SO4 (Stahl, E., 1969. Fisher, W.,Wimmer, H., and Wiley,NY.,1994).

Different bands were observed and corresponding Rf values are determined. Rf value of each spot was calculated using the formula,

$$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

Results and discussions
Qualitative assay of the whole seeds of *Crotalaria verrucosa Linn*. Revealed the presence of diverse group of phytochemicals. The results are presented in table.1 to enable their comparative study with respect to the solvents used for extraction.

The phytochemical screening in the present study following hot continuous method of extraction using Soxhlet apparatus, revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids, phenols, steroids, coumarins, cardiac glycosides, and phytosterols in *C. verrucosa* fruit. Screening tests for carbohydrates, proteins, quinons, anthra quinons, leucoanthocyanins, amino acids, fixed oils, and resins were found to be negative in this method of extraction using different solvents.

Table. 1: Qualitative Analysis of Crude extracts of crotalaria verrucosa .L

Solvent / Phytochemicals	hexane extract	Petroleum ether extract	Ethyl acetate extract	Chloroform extract	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract
ALKALIODS								
Mayer's Test	-	-	+	-	+	+	+	+
Wagner Test	-	-	+	-	+	+	+	+
Dragendroffs Test	-	-	+	-	+	+	+	+
Hager's Test	-	-	+	-	+	+	+	+
FLAVANOIDS	-	-	+	-	+	+	+	+
TANNINS	-	-	-	-	-	+	+	+
SAPONINS								
Froth Test	-	-	-	-	-	-	+	+
Foam test	-	-	-	-	-	-	+	+
CARBOHYDRATES								
Molish test	-	-	-	-	-	-	-	-
Fehling test	-	-	-	-	-	-	-	-
Barfoed test	-	-	-	-	-	-	-	-
Benedict test	-	-	-	-	-	-	-	-

GLYCOSIDES Modified Borntrager's Test	+	+	+	+	+	+	+	+
PROTIENS Millons test Biuret test	- -	- -	- -	- -	- -	- -	- -	- -
TRITERPINOIDS	+	+	+	-	+	-	-	-
PHENOLS	-	-	+	-	-	-	+	-
STERIODS	+	-	+	-	-	-	-	-
COUMARINS	-	-	-	-	-	-	+	+
CARDIAC GLYCOSIDES	+	-	-	-	+	-	+	+
QUINONS	-	-	-	-	-	-	-	-
LEUCOANTHOCYANINS	-	-	-	-	-	-	-	-
ANTHRA QUINONS	-	-	-	-	-	-	-	-
PHYTOSTEROLS	-	+	+	+	+	+	+	+
AMINOACIDS	-	-	-	-	-	-	-	-
FIXED OILS	-	-	-	-	-	-	-	-
RESINS	-	-	-	-	-	-	-	-

Alkaloids were identified in ethyl acetate, ethanol, methanol, and aqueous extracts; flavonoids were found in ethyl acetate, acetone, ethanol, methanol, and aqueous extracts; qualitative test for tannins was positive in ethanol, methanol, and aqueous extracts; positive results were obtained for the presence of saponins in methanol and aqueous extracts; glycosides were detected by modified Borntrager's test in all the extracts; triterpenoids were noticed in hexane, petroleum ether, ethyl acetate and acetone; phenols were found in ethyl acetate and methanol extracts; steroids were present in hexane and ethyl acetate extracts; tests for coumarins were positive in methanol and aqueous extracts; cardiac glycosides were identified in hexane, acetone, methanol and aqueous extracts, phytosterols were found in all the extracts except hexane. Extraction process using the solvent, methanol could yield most of the phytochemicals, followed by aqueous extract, ethyl acetate and acetone. The results differed depending upon the method of extraction. Quite a number of biologically active phytochemicals were identified in the extraction by the method followed for extracts and screening tests done.

Table. 2: Rf Values of compounds separated by varied solvent Composition.

S. No.	Mobile phase and solvent extract	R_f values
I	Hexane + Ethyl Acetate (9:1)	
	Hexane	i) 0.25, ii) 0.37, iii) 0.5, iv) 0.75, v) 0.97.
	Petroleum ether	-
	Ethyl acetate	i) 0. 5, ii) 0.70, iii) 1.0.
	Chloroform	-
	Acetone	-
	Ethanol	-
	Methanol	-
	Aqueous	-
II	Hexane + Ethyl Acetate (8:2)	
	Hexane	i) 0.51, ii) 0.54, iii) 0.85.
	Petroleum ether	i) 0.37, ii) 0.85, iii) 0.94.
	Ethyl acetate	i) 0.34, ii) 0.62, iii) 0.88, iv) 0.97.
	Chloroform	i) 0.28.
	Acetone	i) 0.22.
	Ethanol	-
	Methanol	-
	Aqueous	-
III	Hexane + Ethyl Acetate (7:3)	
	Hexane	i) 0.52, ii) 0.56, iii) 0.67, iv) 0.84.
	Petroleum ether	i) 0.43, ii) 0.54, iii) 0.67, iv) 0.86.
	Ethyl acetate	i) 0.43, ii) 0.54, iii) 0.67, iv) 0.86. v) 0.89.
	Chloroform	-
	Acetone	i) 0.41.
	Ethanol	i) 0.17.
	Methanol	i) 0.43, ii) 0.82.
	Aqueous	-
IV	Hexane + Ethyl Acetate (6:4)	
	Hexane	i) 0.12, ii) 0.27, iii) 0.75, iv) 0.90.
	Petroleum ether	i) 0.67, ii) 0.75, iii) 0.95.
	Ethyl acetate	i) 0.50, ii) 0.75, iii) 0.95.
	Chloroform	-
	Acetone	i) 0.35.
	Ethanol	-
	Methanol	i) 0.12, ii) 0.30, iii) 0.35.
	Aqueous	-
V	Hexane + Ethyl Acetate (5:5)	
	Hexane	i) 0.16, ii) 0.34, iii) 0.53, iv) 0.74, v) 0.88, vi) 0.95.
	Petroleum ether	i) 0.16, ii) 0.34, iii) 0.60, iv) 0.69, v) 0.79, vi) 0.88, vii) 0.97.
	Ethyl acetate	i) 0.16, ii) 0.72, iii) 0.60, iv) 0.88, v) 0.95.
	Chloroform	i) 0.95.
	Acetone	i) 0.16, ii) 0.34, iii) 0.51.
	Ethanol	i) 0.34.
	Methanol	i) 0.16, ii) 0.69, iii) 0.93.
	Aqueous	-
VI	Ethyl Acetate	
	Hexane	i) 0.13, ii) 0.45, iii) 0.65, iv) 0.78, v) 0.93.
	Petroleum ether	i) 0.95.
	Ethyl acetate	-
	Chloroform	-
	Acetone	i) 0.71.
	Ethanol	-
	Methanol	-
	Aqueous	-
VII	Chloroform	
	Hexane	i) 0.15, ii) 0.18, iii) 0.27, iv) 0.57, v) 0.84.
	Petroleum ether	i) 0.12, ii) 0.18, iii) 0.33, iv) 0.60, v) 0.63.
	Ethyl acetate	i) 0.09, ii) 0.60, iii) 0.93.
	Chloroform	i) 0.93.
	Acetone	i) 0.15.
	Ethanol	-
	Methanol	-
	Aqueous	-
VIII	Chloroform + Methanol(9:1)	
	Hexane	i) 0.18, ii) 0.30, iii) 0.44, iv) 0.55, v) 0.60, vi) 0.69
	Petroleum ether	i) 0.30 ii) 0.53, iii) 0.67, iv) 0.76.
	Ethyl acetate	i) 0.13, ii) 0.25, iii) 0.30, iv) 0.53, v) 0.67, vi) 0.81.
	Chloroform	-
	Acetone	i) 0.65.
	Ethanol	i) 0.16.
	Methanol	-
	Aqueous	-
IX	Chloroform + Methanol(8:2)	
	Hexane	i) 0.31, ii) 0.71.
	Petroleum ether	i) 0.62.
	Ethyl acetate	i) 0.56, ii) 0.93.
	Chloroform	-
	Acetone	-
	Ethanol	-
	Methanol	i) 0.25.
	Aqueous	i) 0.31.
X	Chloroform + Methanol(7:3)	
	Hexane	i) 0.86.
	Petroleum ether	i) 0.91.
	Ethyl acetate	i) 0.63, ii) 0.94.
	Chloroform	-
	Acetone	-
	Ethanol	i) 0.69.
	Methanol	i) 0.27 ii) 0.33, iii) 0.66.
	Aqueous	i) 0.27.
XI	Chloroform + Methanol(6:4)	

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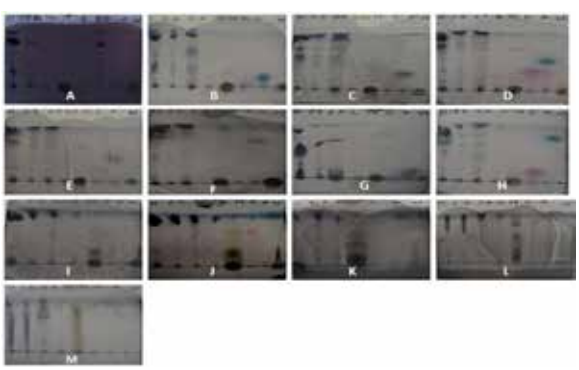
	Hexane	-
	Petroleum ether	-
	Ethyl acetate	-
	Chloroform	-
	Acetone	-
	Ethanol	-
	Methanol	i) 0.12, ii) 0.20, iii) 0.35, iv) 0.43, v) 0.58.
	Aqueous	i) 0.25.
XII	Chloroform + Methanol(5:5)	
	Hexane	i) 0.18, ii) 0.72, iii) 0.18.
	Petroleum ether	i) 0.18, ii) 0.72, iii) 0.87.
	Ethyl acetate	i) 0.18, ii) 0.81, iii) 0.90.
	Chloroform	-
	Acetone	-
	Ethanol	-
	Methanol	i) 0.15, ii) 0.30, iii) 0.39, iv) 0.45, v) 0.54, vi) 0.75, vii) 0.87, viii) 0.93.
	AQUEOUS	-
XIII	Methanol	
	Hexane	i) 0.70, ii) 0.89.
	Petroleum ether	i) 0.74, ii) 0.89.
	Ethyl acetate	i) 0.21, ii) 0.65, iii) 0.70, iv) 0.95.
	Chloroform	-
	Acetone	i) 0.87.
	Ethanol	i) 0.85.
	Methanol	i) 0.12, ii) 0.48, iii) 0.63, iv) 0.74, v) 0.85.
	Aqueous	-

TLC profiling of different crude extracts of *C. verrucosa* confirms the presence of a variety of Phytochemicals. The photographs of the chromatography are presented in Plate 1. and the results are shown in Table 2. Separation of the compounds was variously dependent upon the solvent used for extraction and also the mobile phase used in TLC which reflects the chemical nature and polarity of the compound. The data obtained provides useful information regarding the selection of appropriate solvent system for their extraction and separation. Distinct qualitative manifestation of Phytochemicals in chromatography of the seed extracts demonstrates their potential for future drugs.



Figer 1: Thin layer chromatography of the extracts in 13 different mobile phases

A. Hexane + Ethyl acetate 9:1; B. Hexane + Ethyl acetate 8:2; C. Hexane + Ethyl acetate 7:3;
D. Hexane + Ethyl acetate 6:4; E. Hexane + Ethyl acetate 5:5; F. Ethyl acetate; G. Chloroform;
H. Chloroform + Methanol 9:1; I. Chloroform + Methanol 8:2; J. Chloroform + Methanol 7:3;
K. Chloroform + Methanol 6:4; L. Chloroform + Methanol 5:5; M. Methanol.

Conclusion

The extraction of Phytochemicals from the seeds of *Crotalaria verrucosa* L. following hot continuous successive method using eight different solvents yielded variety of Phytochemicals. Appearance of a different compounds and disparity in Rf values with respect to the solvent used for extraction and chromatography reflects an idea of their polarity and solubility. This information will help in selecting the appropriate solvent for extraction and chromatographic separation. The chemical constituents of the seeds attests that it can be a potential source of future drugs and vital for good health. The outcome of the analyses will certainly facilitate their quantitative estimation and isolation of pharmacologically active chemical compounds.

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