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Extraction Method and Isolation of Phytoconstituents from the Chloroform Extract of Plant Solanum Nigrum L. By Colunm Chromatography

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Abstract: ***Solanum Nigrum L. (Kaambal) (Kashmiri) has been traditionally used to treat pathological ailments like fever, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Creasy et al., 1981; Capizzi et al., 2003). Column chromatography in chemistry is a method used to purify individual chemical compound from mixtures of compounds. It is often used for preparative applications on scales from micrograms up to kilograms. The main advantage of column chromatography is the relatively low cost and disposability of the stationary phase used in the process. The classical preparative chromatography column is a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap and some kind of a filter (a glass frit or glass wool plug – to prevent the loss of the stationary phase) at the bottom.***

Keywords: Column, Chemical, Glass, Used, Chromatography.

INTRODUCTION

Nature is the world's best chemist: Many naturally occurring compounds have very complicated structures that present great challenges to chemists wishing to determine their structures or replicate them. The plant derived herbal compounds have a long history of clinical use, better patient tolerance and acceptance. Their high ligand binding affinity to the target introduce the prospect of their use in chemo preventive applications; in addition they are freely available natural compounds that can be safely used to prevent various ailments. Plants became the basis of traditional medicine system throughout the world for thousands of years and continue to provide mankind with new remedies. Natural products have coming from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, terrestrial vertebrates and invertebrates have importance as they provide an amazing source of new drugs as well as new drug leads and new chemical entities for further drug development (McCurdy & Scully, 2005; Chin *et al*., 2006). Morphine, vincristine, codeine, digitoxin, quinine, galantamine and taxol are just some of the typical examples of drugs that have been introduced from natural sources (Heinrich *et al*., 2004; Balunas & Kinghorn, 2005).

Natural products can be mainly divided into three groups such as primary metabolites, secondary metabolites and high molecular weight polymeric materials (Hanson, 2003). Primary metabolites including nucleic acids, amino acids, sugars; occur in all cells and play a central role in the metabolism and reproduction of the cells. High molecular weight polymeric materials such as cellulose, lignins and proteins take a part in the cellular structure. Secondary metabolites, small molecules which are not essential for the growth and development of the producing organism have importance because of their biological activities on other organisms.

Natural product term refers to any naturally occurring compounds but in most cases mean secondary metabolite (Hanson 2003; Sarker *et al*., 2005).

EXTRACTION METHOD

The plant *Solanum nigrum L*. was collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the plant was shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to (Rashmi *et al*., 2010). The dried plant was powdered (coarse) and subjected to Soxhlet appratus (Figure) using petroleum ether, ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed.

Fig. Showing Soxhlet apparatus

Identification of bioactive compound

Protocol for Column Chromatography

The chloroform extract was concentrated by distilling off the solvent and evaporated to dryness. The chloroform extract (5 gm) was suspended in n-hexane and ethanol and then resulting solutions were concentrated and were eluted with the solvent of increased polarity i.e. Non-polar - polar - highly polar.

Preparation of Column for isolation of phytoconstituets Adsorbent silica gel (60-120 mesh)

Activation	110 ^{0}C for 1 hour
Length of column	43 cm
Diameter	3.5 cm
Length of adsorbent	25 cm.
Rate of elution	5-10 drops/min.

Glass column was packed by wet filling. The slurry of adsorbent (silica gel; 230-400 mesh) was prepared by mixing the adsorbent in the n-hexane and used as stationary phase. It was then poured into glass column (43cm x 3.5 cm) (having sintered glass disc at its bottom) and allowed to settle. The air entrapped was removed by stirring with glass rod. A small amount of sand was kept at top the column to provide the latter a flat base. Excess of solvent was run off until the level of mobile phase fell to one cm just above the top of the sand layer.

3.23.2. Preparation of sample and loading:

The chloroform extract of *Solanum nigrum* L. were subjected to silica gel (230-400 mesh) column (43cm x 3.5 cm) chromatography for the isolation of phytoconstituents and elution was carried out from non polar to polar solvents n-hexane (100:0), n-hexane:chloroform (90:10), n-hexane: chloroform (50:50), n-hexane:chloroform (10:90), chloroform (100:0), chloroform:MeOH (90:10), chloroform:MeOH (50:50), chloroform:MeOH (10:90), MeOH (100:0).

Separation of bioactive constituents from chloroform (50%) extract chloroform extracts were dissolved in a minimum volume of n-hexane, adsorbed on silica gel (230-400 mesh), dried and applied on the column to separate possible phytoconstituets. n-hexane insoluble part was eluted gradiently with chloroform, chloroform, ethyl acetate and methanol mixtures. At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC) (silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm) using solvent system, Toluene:Ethy acetate:Acetic acid (36:12:5) for chloroformic extract. Presence of no. of spots was considered as criteria for selection of fraction for isolation of pure compound.

Showing Column Chromatography

RESULTS

Column chromatography:

The Chloroform crude extract was further purified by column chromatography and elution was carried out from non-polar to polar solvents by gradient elution method.

Isolation of phytoconstituents from Chloroform extract of *Solanum nigrum* Linn.

Different fractions like F1, F2, F3…….. F25 were eluted. Fraction F1, F2, F6 and F16 yield no residue after evaporation and rest of the fractions showed less quantity. Fraction F9 –F12 eluted with chloroform and methanol (90:10) showed single spot on TLC, afforded compound (F9-F12=1.59gm).The fractions F9-F12 which were eluted in considerable quantity and in pure form (after evaporating the respective mobile phase) and hence the fraction was subjected for further investigation that is for HPLC and spectral analysis (Table) .

Table: Different fractions of Column Chromatography and their Rf values:

S.No	Mobile phase	Ratio(%)	Total fractions obtained	No.of spots	R.F value
1	n-hexane	100:0	0	-	-
2	n-hexane:chloroform	90:10	0	-	-
3	n-hexane:chloroform	50:50	-	-	-
4	n-hexane:chloroform	10:90	F1	-	-
			F2	-	-
			F3	1	0.901
	Chloroform	100:0	F4	1	0.903
			F5	1	0.900
			F6	-	-
			F7	1	0.929
			F8	2	0.813,0.820
6	Chloroform:MeOH	90:10	F9	1	0.962
			F10	1	0.962
			F11	1	0.962
			F12	1	0.962
			F13	1	0.976
			F14	1	0.948

			F15	3	0.948,0.973,0.519
			F16	-	-
			F17	3	0.469,0.506,0.542
			F18	2	0.788,0.823
			F19	1	0.63
7	Chloroform:MeOH	50:50	F20	2	0.462,0.476
			F21	3	0.480,0.488,0.492
8	Chloroform:MeOH	10:90	F23	1	0.594
9	MeOH	100:0	F24	1	0.63
			F25	2	0.640,0.670

DISCUSSION

The Chloroform crude extract was further purified by column chromatography and elution was carried out from non-polar to polar solvents by gradient elution method. Different fractions like F1, F2, F3……..F25 were eluted. Fraction F1, F2, F6 and F16 yield no residue after evaporation and rest of the fractions showed less quantity. Fraction F9 –F12 eluted with chloroform and methanol (90:10) showed single spot on TLC and same peaks in UV, afforded compound (F9-F12=1.59gm). The fractions F9-F12 which were eluted in considerable quantity and in pure form (after evaporating the respective mobile phase) and hence the fraction was subjected for further investigation that is for HPLC spectral analysis.

CONCLUSION

Phytochemical screenings of the extracts were investigated according to the standard procedures. The crude extract of *Solanum nigrum* L. were investigated to preliminary phytochemical screening which showed the presence of various phyto-constituents i.e., alkaloids.Different chromatographic techniques such as paper chromatography (PC), thin layer chromatography (TLC), gas liquid chromatography (GLC), high performance liquid chromatography and column chromatography employ for separation and purification of natural products. Amon them column chromatography which is the oldest chromatographic technique, is still widely used especially for large scale isolation procedure.

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