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Thin Layer Chromatographic Profile of *Ipomoea quamoclit* Linn Whole Plant.

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ABSTRACT

Present study is designed with an aim to establish thin layer chromatographic profile of an ethnomedicinally important plant, *Ipomoea quamoclit*, belongs to *Convolvulaceae* family. Plant was collected, shade dried and mechanically made into powder. This powder was subjected to cold maceration and preliminary phytochemical screening by standard methods. Thin layer chromatography study was conducted by using different mobile phases and detecting agents according to standard literature. In detection of alkaloids, three spots were identified whose R_f values were found to be 0.39, 0.46 and 0.73. In detection of carbohydrates, four spots were identified whose R_f values were found to be 0.41, 0.52, 0.79 and 0.87. In detection of saponins, four spots were identified whose R_f values were found to be 0.46, 0.59, 0.73 and 0.91. In detection of tannins, two spots were identified whose R_f values were found to be 0.41 and 0.79. In detection of flavonoids, four spots were identified whose R_f values were found to be 0.39, 0.45, 0.57 and 0.86. In detection of amino acids, five spots were identified whose R_f values were found to be 0.32, 0.52, 0.61, 0.68 and 0.81. In detection of phytosterols, four spots were identified whose R_f values were found to be 0.21, 0.42, 0.65 and 0.95.

Keywords: *Ipomoea quamoclit*, phytoconstituents, thin layer chromatography and R_f value.

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INTRODUCTION

Phytochemical evaluation of the crude drugs obtained from different natural sources is gaining much importance in current research due to the well known reason that the phytoconstituents that belong to different categories are responsible for the said pharmacological action of the crude drug under study. Structured preliminary phytochemical screening and thin layer chromatographic study is the most important aspect of today's pharmacognosy and phytochemistry research [1]. Keeping this in view the present work was designed to reveal the presence of different phytoconstituents present in whole plant of *Ipomoea quamoclit*. *Ipomoea quamoclit* also called as *Quamoclit pinnata* belongs to *Convolvulaceae* family is one of the most commonly seen plant in and around of the living area. In English it is called as Cypress Vine, Indian Pink and Cupid's Flower [2]. In Philippines, the leaves are used as poultices for bleeding haemorrhoids. The crushed leaves are used for carbuncles. The seeds are used as a laxative. In India, the powdered roots were given as sternutatory (substance that tends to cause sneezing) in Spain, the crushed leaves are used for ulcers and chest pain [3] where as in Siddha Medicine, decoction of leaves and stems is used to treat fever, is also used in diabetes [4] and in Thailand, is used for snake bites and as snuff, as a laxative and for haemorrhoids and in bloody cough [5]. Leaves and stems contain small amounts of alkaloids and cyanogenetic glycosides. Seeds have been reported to contain the resin glycosides, quamoclins I-IV and Jalapin. Pyrrolizidine alkaloids like mono and diesters of platynecine and minalobines like minalobine O and R, ipangulines like ipangualine B₂ and D₁₁ [6, 7] and ergoline alkaloids [8] and anthocyanins [9] were identified from the plant. Total alkaloid in seeds was found 0.012% [10]. After conduction of a thorough literature review, in the present study an attempt was made to reveal thin layer chromatographic profile of *Ipomoea quamoclit* Linn in a systematic way.

MATERIALS AND METHODS

Collection of plant material

For the present study, *Ipomoea quamoclit* whole plant was collected from the forest area near to the Madanapalli of Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V. University, Tirupati, A.P., India and a voucher specimen (RIPER/ASK/002) was preserved in Division of Pharmacognosy, RIPER, Anantapuramu for further reference.

Extraction

For the present study, 1000 gm of the powdered *Ipomoea quamoclit* was extracted by cold maceration method with ethanol: water (3:2) mixture as solvent. The maceration was continued for 72 hours after which, the contents were filtered and concentrated by rota evaporator. A resinous greenish extract was obtained which was calculated for the yield, designated with HAIQ and stored in desiccator till further study. [11]

Phytochemical Screening

Hydroalcoholic extract of *Ipomoea quamoclit* whole plant was subjected to preliminary phytochemical screening by using standard procedures to reveal the presence of different phytoconstituents in it. [11, 12]

Thin layer chromatography

TLC technique, now a day's became an important analytical tool for micro-analytical separation and determination of number of natural products. In the present study hydroalcoholic extract of *Ipomoea quamoclit* whole plant was subjected to TLC.

In this analysis pre coated aluminium plates (10 cm length) coated with silica gel were used and different solvent systems were employed, depending upon the nature of compound to be analyzed. For saturation of TLC chamber a sheet of filter paper was laid so as to cover three sides of the chamber from inside and which is soaked in the solvent system. The chamber was left undisturbed to ensure saturation.

A solution of hydroalcoholic extract of *Ipomoea quamoclit* whole plant was prepared in ethanol. The spots of identical volume were applied 2 cm away from the lower edge of plate with help of micro capillary tube. The solvent was allowed to evaporate after each application by air drying. The spotted plates were then placed vertically in the chamber with the bottom edge immersed in developing medium. The solvent system was allowed to run approximately up to 8 cm, then the plates were taken out and solvent front was marked.

The resolution of components of hydroalcoholic extract of *Ipomoea quamoclit* whole plant was studied by locating the spots on the chromatogram. The spots were identified first by visual observation and then using a suitable detecting agent.

Detection Method

The eluted spots, representing various fraction/compounds, were visualized by different detection methods as follows [13, 14].

Detection of alkaloids

Presence of alkaloids in HAIQ developed on TLC plates was done by using methanol: ammonium hydroxide (17:3 v/v) as mobile phase and spraying with Dragendorff's reagent. Orange brown colored spots were identified and calculated for the R_f values.

Detection of carbohydrates

Presence of carbohydrates in HAIQ developed on TLC plates was done by using ethyl acetate: pyridine: water (40:20:20 v/v upper phase) as mobile phase. After development, plates were dipped in 10% sulphuric acid and plates were heated in oven at 100^o C. Dark brown colored spots were identified and calculated for the R_f values.

Detection of saponins

Presence of saponins in HAIQ developed on TLC plates was done by using chloroform: glacial acetic acid: methanol: water (64:32:12:8 v/v) as mobile phase. After development, plates were detected by using anisaldehyde in sulphuric acid as spraying reagent. Blue violet colored spots were identified and calculated for the R_f values.

Detection of tannins

Presence of tannins in HAIQ developed on TLC plates was done by using toluene: ethyl acetate (93:7 v/v) as mobile phase. After development, plates were detected by using vanillin sulphuric acid as spraying reagent. Blue violet colored spots were visualized after ten minutes of heating the plate at 100^o C and were identified, calculated for the R_f values.

Detection of flavonoids

Presence of flavonoids in HAIQ developed on TLC plates was done by using ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26 v/v) as mobile phase. After development, plates were detected by using natural product-poly ethylene glycol reagent (NP-PEG) as spraying reagent. Orange yellow colored spots were visualized after observing under UV at 365 nm, they were identified, calculated for the R_f values.

Detection of amino acids

Presence of amino acids in HAIQ developed on TLC plates was done by using n-butanol: glacial acetic acid: water (4:1:1 v/v) as mobile phase. After development, plates were detected by using ninhydrin reagent (0.1% solution of Ninhydrin in n-butanol) as spraying reagent. Red violet colored spots were visualized after spraying with reagent; they were identified, calculated for the R_f values.

Detection of phytosterols

Presence of phytosterols in HAIQ developed on TLC plates was done by using chloroform: ethyl acetate: formic acid (5:4:1 v/v) as mobile phase. After development, plates were detected by dipping in solution prepared by adding 15gm vanillin, 250 ml ethanol and 2.5 ml of concentrated sulphuric acid for 5 seconds and then first dried at room temperature followed by hot air oven at 100⁰ C. blue colored spots were visualized after spraying with reagent; they were identified, calculated for the R_f values.

RESULTS AND DISCUSSIONS

Extraction

A thick green viscous matter about 36.4 gm was obtained from 1000 gm of *Ipomoea quamoclit* whole plant by maceration and the percentage was found to be 3.64% w/w.

Preliminary phytochemical analysis

Preliminary phytochemical analysis of whole plant of *Ipomoea quamoclit* was carried out and it showed the presence of alkaloids, carbohydrates, saponins, phytosterols, phenolic compounds, tannins, flavonoids, proteins, amino acids, terpenoids, gums and mucilages.

Thin layer chromatography of *I.quamoclit* whole plant

After conducting thin layer chromatographic studies, different phytoconstituents were characterized as follows. In detection of alkaloids, three spots were identified whose R_f values were found to be 0.39, 0.46 and 0.73. In detection of carbohydrates, four spots were identified whose R_f values were found to be 0.41, 0.52, 0.79 and 0.87. In detection of saponins, four spots were identified whose R_f values were found to be 0.46, 0.59, 0.73 and 0.91. In detection of tannins, two spots were identified whose R_f values were found to be 0.41 and 0.79. In detection of flavonoids, four spots were identified whose R_f values were found to be 0.39, 0.45, 0.57 and 0.86. In detection of amino acids, five spots were identified whose R_f values were found to be 0.32, 0.52, 0.61, 0.68 and 0.81. In detection of phytosterols, four spots were identified whose R_f values were found to be 0.21, 0.42, 0.65 and 0.95. The results were tabulated in table 1.

Table 1: Thin layer chromatography of *I.quamoclit* whole plant

S. No.	Phytoconstituent	Mobile phase	Detecting agent	R _f values
01.	Alkaloids	methanol: ammonium hydroxide (17:3 v/v)	dragondroff's reagent- orange brown spots	0.39, 0.46 and 0.73
02.	carbohydrates	ethyl acetate: pyridine: water (40:20:20 v/v upper phase)	dipped in 10% sulphuric acid and plates were heated in oven at 100 ⁰ C- dark brown spots	0.41, 0.52, 0.79 and 0.87
03.	saponins	chloroform: glacial acetic acid: methanol: water (64:32:12:8 v/v)	anisaldehyde in sulphuric acid- blue violet spots	0.46, 0.59, 0.73 and 0.91
04.	Tannins	toluene: ethyl acetate (93:7 v/v)	Vanillin sulphuric acid- blue violet spots	0.41 and 0.79
05.	Flavonoids	ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26 v/v)	natural product-poly ethylene glycol reagent (NP-PEG) followed by UV 365- orange yellow spots	0.39, 0.45, 0.57 and 0.86
06.	Amino acids	n-butanol: glacial acetic acid: water (4:1:1 v/v)	ninhydrin reagent (0.1% solution of Ninhydrin in n-butanol)- red violet spots	0.32, 0.52, 0.61 and 0.81
07.	Phytosterols	chloroform: ethyl acetate: formic acid (5:4:1 v/v)	dipping in solution prepared by adding 15gm vanillin, 250 ml ethanol and 2.5 ml of concentrated sulphuric acid for 5 seconds and then first dried at room temperature followed by hot air oven at 100 ⁰ C- blue colour	0.21, 0.42, 0.65 and 0.95

CONCLUSION

Present study concludes the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, amino acids and phytosterols in the hydroalcoholic extract of *Ipomoea quamoclit* whole plant by preliminary phytochemical careening and thin layer chromatographic methods.

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