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Separation of antibacterial constituents from five Solanum species using Thin Layer Chromatography

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Abstract : Plants are the natural medicinal resources. The presence of bioactive compounds and secondary metabolites determined the medicinal values of the plants. The medicinal property of the plant denoted the presence of active compounds in it. The presence of maximum numbers of active compounds exhibit high medicinal values. Solanum species are medicinal herbs used for the treatment of diverse ailments (diabetes, cholera, bronchitis, high blood pressure) and as laxatives. *S. nigrum, S. torvum, S. trilobatum, S. surattense*and *S. melongena* are important medicinal plants. Many other*Solanum* species are also used for medicinal purposes. The medicinal uses and antibacterial activity of the Solanum species are mainly due to the presence of bioactive constituents in the plants. Hence, the present study was focused to screen and separate the bioactive antibacterial constituents from the methanol extracts of leaves of five Solanum species, *S. nigrum, S. torvum, S. trilobatum, S. surattense* and *S. melongena* using Thin Layer Chromatography (TLC). The chromatogram was observed under visible light, UV (365), UV (265) and Iodine chamber. Maximum numbers of bands were observed in leaves extracts of*S. surattense.*

Key words : Bioactive compounds, Secondary metabolites, Thin Layer Chromatography

1. Introduction

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal values of many plants still remain unexplored for its enumerable activity of compounds responsible for later. Yet, plant materials remain important resources to combat serious diseases of the world [1]. Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries [2, 3].

Solanaceae is a large plant family containing two thousand and three hundred species, nearly half of which belong to a single genus, *Solanum*. There are herbs, shrubs or small trees under this genus. This family comprises a number of plants widely known for the presence of variety of natural products of medicinal significance. Crude plant extract is beneficial in bronchial asthma and non-specific cough, influenza, difficult urination, bladder stones, rheumatism, etc. Solanum species are medicinal herbs used for the treatment of diverse ailments (diabetes, cholera, bronchitis, high blood pressure) and as laxatives. *S. nigrum, S. torvum, S. trilobatum, S. surattense* and *S. melongena* are important medicinal plants. The medicinal uses and antibacterial activity of the Solanum species are mainly due to the presence of bioactive constituents in the plants. Thin layer chromatographic technique is a useful analytical tool for the isolation of organic compounds. The antibacterial studies of the above medicinal plants were already investigated against some common human pathogenic bacteria and also against some plant and animal pathogens. Hence, the present study was focused to screen and separate the bioactive antibacterial constituents from the methanol extracts of leaves of five Solanum species, *S. nigrum, S. torvum, S. trilobatum, S. surattense* and *S. melongena* using Thin Layer Chromatography (TLC).

2. Materials and methods

Collection of plant materials

Fresh plant and plant parts were collected randomly from the region of Tirunelveli, India. Fresh plant material was washed; shade dried and then powdered using the blender and stored in bottles.

Methanol extraction

10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4 °C [4].

Thin Layer Chromatography

Thin Layer Chromatographic (TLC) analyses of the methanol extracts of leaves, fruits and flowers were done using pre-coated silica gel (Merck, GF254, 20 X 20 cm, 0.2 mm thickness). Increasing polar mobile phases were made with the mixtures of hexane, ethyl acetate and methanol (6:2:2). Spots were visualized with UV lamp fluorescent at 365 nm, 265nm, visible light and iodine spray.

3. Results and discussion

The data of quantitative separation of secondary metabolites from leaves of five Solanum species, *S. nigrum, S. torvum, S. trilobatum, S. surattense* and *S. melongena* by Thin Layer Chromatography is tabulated. Rf values obtained by TLC patterns are useful to establish their identity and purity of the herbs. In the present investigation methanol extracts of five different Solanum species were studied. The plates were first exposed to visible light then viewed through UV (365&265 nm) and in Iodine chamber to observe the variously coloured bands.

TLC chromatogram of five Solanum species under visible light revealed 7 bands in *S. nigrum*, 9 bands in *S. torvum*, 10 bands in *S. trilobatum*, 8 bands in *S. melongena* and 11 bands in *S. surattense* which is given in table (1). In all the species the highest Rf value was 0.97 with orange coloured bands. The least Rf value was 0.12 with light grey coloured bands in all the species. A dark blue coloured band was found in *S. surattense* with Rf value 0.18.

Table 1: TLC fingerprint profile of five Solanum species under visible light

NB	Colour of bands	S1	S2	S3	S4	S5
1	Orange	0.97	0.97	0.97	0.97	0.97
2	Light green	-	0.82	0.82	0.82	-
3	Brownish green	0.78	0.78	0.78	-	0.78
4	Light yellow	0.69	-	0.69	0.69	0.69
5	Dark green	-	0.63	0.63	-	0.63
6	Bluish green	-	0.59	0.59	0.59	0.59
7	Grayish green	.52	-	0.52	0.52	0.52
8	Ash green	-	0.48	-	0.48	0.48
9	Blackish green	0.31	0.31	0.31	-	0.31
10	Greenish yellow	0.20	0.20	0.20	0.20	0.20
11	Dark blue	-	-	-	-	0.18
12	Light grey	0.12	0.12	0.12	0.12	0.12

Under UV light (365 nm) chromatogram revealed 5 bands in *S. nigrum*, 7 bands in *S. torvum*, 7 bands in *S. trilobatum*, 6 bands in *S. melongena* and 8 bands in *S. surattense* which is given in Table 2. The highest Rf value was 0.97 with dark brown coloured band and the least Rf value was 0.11 with dark grey band in all the species. A reddish brown coloured band was observed in *S. surattense* with Rf value 0.85.

Table 2 : TLC fingerprint profile of five Solanum species under UV light (365 nm)

NB	Colour of bands	S1	S2	S3	S4	S5
1	Dark brown	0.97	0.97	0.97	0.97	0.97
2	Reddish brown	-	-	-	-	0.85
3	Brown	0.82	0.84	-	0.83	0.83
4	Blackish grey	-	0.79	0.78	-	0.78
5	Black	0.72	-	0.72	0.75	0.74
6	Dark orange	-	0.69	0.65	0.69	0.68
7	Brownish grey	0.34	0.54	0.52	-	0.56
8	Grey	-	0.25	0.23	0.25	-
9	Dark grey	0.11	0.11	0.11	0.11	0.11

Under UV light (265 nm) the chromatogram revealed 6 bands in *S. nigrum*, 5 bands in *S. torvum*, 6 bands in *S. trilobatum*, 5 bands in *S. melongena* and 7 bands in *S. surattense* which is given in Table 3. In all the species the highest Rf value was 0.87 with bluish grey coloured bands. The least Rf value was 0.11 with pink coloured bands. A purple colour band was found in *S. surattense* with Rf value 0.70.

Table 3: TLC fingerprint profile of five Solanum species under UV light (265 nm)

NB	Colour of bands	S1	S2	S3	S4	S5
1	Bluish grey	0.87	0.87	0.87	0.87	0.87
2	Light green	0.75	-	0.75	-	0.75
3	Purple	-	-	-	-	0.70
4	Green	0.65	-	0.63	-	0.65
5	Light yellow	-	0.55	0.60	0.61	-
6	Light brown	0.43	0.43	-	0.45	0.46
7	Dark brown	0.36	0.32	0.34	0.35	0.38
8	Pink	0.11	0.11	0.11	0.11	0.11

When the chromatogram was exposed to iodine vapour, it revealed 6 bands in *S. nigrum*, 5 bands in *S. torvum*, 6 bands in *S. trilobatum*, 5 bands in *S. melongena* and 7 bands in *S.*

surattense which is given in Table (4). The highest Rf value was 0.91with brown coloured bands and least Rf value was 0.15 with purple coloured bands in all the species. 2 brownish orange coloured bands were observed in*S. surattense* and *S. nigrum* with Rf values 0.68.

Table 4 : TLC fingerprint profile of five Solanum species under Iodine vapour

NB	Colour of bands	S1	S2	S3	S4	S5
1	Brown	0.91	0.91	0.91	0.91	0.91
2	Green	0.85	-	0.85	-	0.82
3	Light green	0.78	0.76	-	0.77	0.79
4	Brown	-	0.69	0.65	-	-
5	Brownish orange	0.68	-	-	-	0.68
6	Orange	-	0.54	0.52	0.53	0.51
7	Light grey	0.37	-	0.33	0.32	0.34
8	Purple	0.15	0.15	0.15	0.15	0.15

A simple, robust and reproducible TLC method for the separation of phytochemicals was reported in Vitex trifolia by Alfi khatib*et al*. (2010) [5] in Radix polygoni by Gao*et al*. (2007) [6] and in Mucuna pruriens by Misra and Wagner (2007)[7]. Gabriela (2009)[8] suggested that the colours of the separated spots in TLC and their position relative to standard substances are important characteristics for the plant extract identification. The phytochemical analysis and antibacterial activity of the selected *Solanum* species was already tested against *Xanthomonas campestris* by John de *et al*. (2011) [9] and got best results. Hence, in this study the antibacterial constituents of the*Solanum* species were separated successfully through TLC techniques. The active compounds will be identified through further advanced chromatographic techniques in future.

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