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Preliminary Phytochemical Evaluation and HPTLC Fingerprint Profile of *Jasminum azoricum* L

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ABSTRACT

The present study was aimed to develop the high performance thin layer chromatography (HPTLC) fingerprint profile of methanol extracts of leaves of *Jasminum azoricum.* This study was planned to develop a HPTLC fingerprint profile of extracts in the best solvent system in Toluene: Ethyl acetate: Formic acid: Methanol:: 7:5:1:0.5. The HPTLC method for the separation of the active constituents in extract has been developed and TLC of these extracts on silica gel pre-coated aluminium plates of MERCK by automatic TLC applicator and using solvent system HPTLC profiling of the extract confirm about the presence of various phytochemicals. HPTLC fingerprint scanned at 254 nm wavelength for methanol (leaf) and revealed the existence of six phytoconstituents whose R_f values ranged from 0.44 to 0.81 and in 366 nm it revealed the existence of eight constituents whose R_f values ranged from 0.44 to 0.87. Further research is necessary to extract the compounds from *J. azoricum* and it will give programmed for advanced phytochemical and pharmaceutical applications.

Keywords : *Jasminum azoricum* , HPTLC, phytoconstituents, derivatization

I. INTRODUCTION

Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as aminoacids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance[(8)].

Jasmine is an essential oil bearing plant belongs to the family Oleaceae. The fragrant world of jasmine comprises different varieties of bela, chameli and juhi. The distribution of the genus is wide but majority of the species centred around India, China and Malaya. It comprises about 200 species. The critical analysis of the species position revealed the true species to be 89 [(1)] .The genus *Jasminum azoricum* L. belonging to the family Oleaceae is a strong growing woody wine which will climb to 20 or more feet in height and produce a dense cover. The species is assessed as critically Endangered (CR) for the IUCN European Red List [(2)]. The traditional Indians use the fresh juice of the ground flowers in treating sores, itching and wounds. The medicated root paste of Jasmine plant is used in treating labour pain for centuries in the rural parts of India. The dried jasmine flowers are used in making herbal tea in China. The young leaves of *J.azoricum* plant is used in making herbal infusion for curing gallstones. It has also been used for culinary purposes especially in making candies, teas, puddings, desserts and dairy products. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some need to be explored[(12)]. The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities[(5)].

High performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environmental pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters [7][6][10].

II. METHODS AND MATERIAL

2.1 Collection of plant materials

The plant materials for the proposed study were collected from Arpookara (9°38'N:76°30' E) of Kottayam district, Kerala, India and was provisionally identified with the help of Flora of the Presidency of Madras.[3]

2.2 Preparation of plant extract

The plant material was collected from the mother plant. The mature leaves were detached and dried in shade at ambient temperature for a period of three weeks. The well dried samples were powdered separately by using an electric blender. The samples were prepared in 100 ml of 70% methanol separately, in conical flask and kept overnight in an orbital shaker for solvent extraction and the extract were centrifuged at 10,000 rpm for 10 minutes. The supernatant thus obtained was kept in a water bath to evaporate to dryness for removing the methanol. After evaporation the residue was subjected to phytochemical analysis for detecting the presence of total alkaloids, total phenols, total saponins, total steroids, total tannins, and total terpenoids.

2.3 Qualitative phytochemical analysis

The extracts were tested for the presence of bioactive compounds by using standard methods[4][9][11].

a) Total Alkaloids

Mayer's test: Two ml of extract was mixed with 0.2 ml of 1% HCl. Then 1 ml of Mayer's reagent was added. The precipitate or turbidity indicated the presence of alkaloids

b) Total Tannins Ferric chloride test: About two ml of the sample was mixed with three ml of 1% ferric chloride solution. Appearance of a blue, green or brownish colour indicated the presence of tannins.

c) Total Terpenoids

Salkowski test: About one ml of sample was mixed in three ml of chloroform, and three ml of concentrated H_2SO_4 was carefully added to form a layer. The development of yellow colour in the lower layer indicated the presence of terpenoids.

d) Total Steroids

Liebermann-Burchard test: To one of sample was added one ml of chloroform. Then five drops of acetic anhydride and one ml of concentrated sulfuric acid were added along the side of the test tube, the appearance of blue or green colour indicated the presence of steroids.

e) Total Phenols

Test for phenols: A small portion of the extract was mixed with two ml of ferric chloride solution. The appearance of green or blue colour indicates the presence of phenols.

f) Total Saponins

Frothing test: One ml of the extract was diluted with twenty ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins

g) Total Reducing sugar

Fehling's test: Few drops of Fehling's solution A & B in equal volume were added in dilute extracts and heated for thirty minutes and observed for the formation of brick red coloured precipitate.

2.4 High Pressure Thin Layer Chromatography (HPTLC) Profile.

2.4.1 Preparation of extract:

Take one gram of sample and boil for 5-10 minutes on a water bath. Concentrate the filtrate and make up to ten ml in a volumetric flask.

2.4.2 Application of extract:

The extracts were applied with the linomat syringe (Linomat applicator-V) on the HPTLC PLATES (10x10 cm). Syringe was washed with the test solution and filled with extract prepared for the qualitative analysis.

2.4.3 Developing solvent system

A number of solvent systems were tried, for each extract for better resolution and maximum number of spots, but the satisfactory resolution was obtained in the solvent Toluene: Ethyl acetate: Formic acid: Methanol:: 7:5:1:0.

2.4.4 Development of the chromatogram

Two mobile and two stationary phase were used. Silica gel on the precoated plates act as the stationary phase. The solvent system was the used for the analysis. The plate was developed using the CAMAG Twin Trough Chamber. The sample went through the stationary phase and the components according to the binding capabilities of components with stationary phase. The plates were developed up to a distance of 80 mm after the run was completed, they were taken out of the chamber and dried in air.

2.4.5 Photo documentation

CAMAG HPTLC (Scanner 3) was used as a scanner in the absorbance mode at both the 254 and the 366 nm, the scanned data was subjected to integration through the software winCATS Planar Chromatography Manager. The fingerprint so developed was used for the detection of phytocomponents present in the samples and the chromatograms and the Rf values were noted. Bands were resolved and their colours were noted. Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when the TLC plates were sprayed with a detection reagent (sulfuric acid) and plate was heated to 110^0C for 1 minute.

III. RESULTS AND DISCUSSION

3.1 Phytochemical screening

The phytochemical tests on methanol extracts of J.azoricum leaves showed the presence of various phytoconstituents like terpenoids, steroids (Table 1).

Table 1 : Preliminary phytochemical screening of methanol extract of *Jasminum azoricum* leaves

Phytoconstituents	Methanol
Alkaloids	-
Tannins	-
Terpenoids	+
Steroids	+
Phenols	+
Saponins	-
Reducing sugar	-

"+" present, "-" absent

3.2 HPTLC Profile

HPTLC profile the best results were shown using Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system. TLC plate of *J.azoricum* methanol (leaf) extract scanned at 254 nm wavelength signified the existence of six phytoconstituents whose R_f values ranged from 0.44 to 0.81. Peak one showing with an R_f value of 0.44 with area 7.14%. Peak two with an R_f value of 0.49 area of 3.14%. Peak three with an R_f value of 0.54 and area 15.52%. Peak four showing R_f value of 0.68 with area 25.48%. Peak five showing an R_f value of 0.77 with an area 28.07%. Peak six showing R_f value of 0.81 with 20.65 area. The total peaks present in HPTLC profile of *J. azoricum,* is six with an area of 9282.8 (AU).(Table 2, Figure 1, Plate 1)

Table 2 : Area and peak of *Jasminum azoricum* L. at 254 nm

PEAK NO	Rf VALUE	AREA (AU)	% AREA (AU)
1	0.44	662.7	7.14
2	0.49	291.7	3.14
3	0.54	1440.7	15.52
4	0.68	2365.2	25.48
5	0.77	2605.6	28.07
6	0.81	1916.9	20.65

The methanol (leaf) extract scanned at 366 nm wavelength signified the existence of eight phytoconstituents whose R_f values ranged from 0.44 to 0.87. Peak one showing with an R_f value of 0.44 with area 10.87%. Peak two with an R_f value of 0.49 area of 4.26%. Peak three with an R_f value of 0.54 and area 17.15%.

Peak four showing R_f value of 0.60 with area 1.28%. Peak five showing an R_f value of 0.68 with an area 23.92%. Peak six showing R_f value of 0.77 with 21.46% area. Peak seven showing an R_f value of 0.81 with an area 19.74%. Peak eight showing an R_f value of 0.87 with an area 1.32 %. The total peaks present in HPTLC profile of *J.azoricum* , is eight with an area of 22845.3(AU). (Table 3, Figure 2, Plate 1). This is the first study to account the HPTLC fingerprint of methanol extracts of *J. azoricum* leaves showing topmost number of components using Toluene: Ethyl acetate: Formic acid: Methanol:: 7:5:1:0.5 as solvent system at a wavelength of 254 nm and 366 nm. The present study gives enough information regarding various phytochemicals present in the methanol extract of *J. azoricum.*

Table 3: Area and peak of *Jasminum azoricum* L . at 366 nm

PEAK NO	Rf VALUE	AREA(AU)	% AREA(AU)
1	0.44	2482.6	10.87
2	0.49	973.9	4.26
3	0.54	3918.9	17.15
4	0.60	293.1	1.28
5	0.68	5463.8	23.92
6	0.77	4903.4	21.46
7	0.81	4509.1	19.74
8	0.87	300.5	1.32

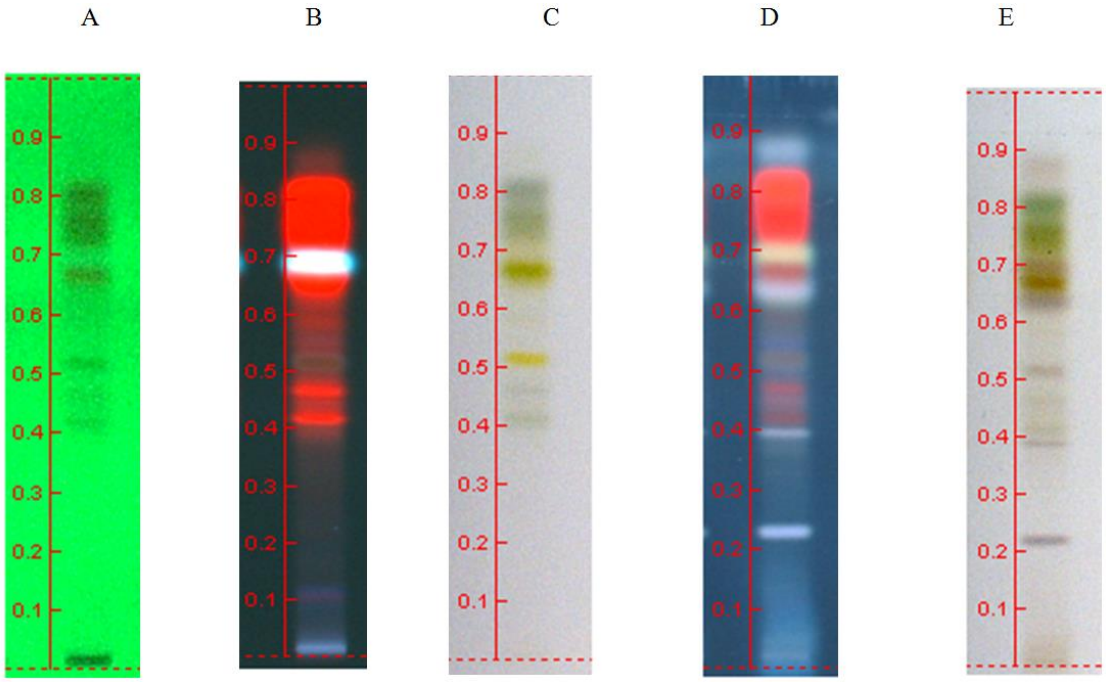

Plate 1: Phytochemical profile of *Jasminum azoricum* L. before (A, B & C) and after (D & E) derivatization

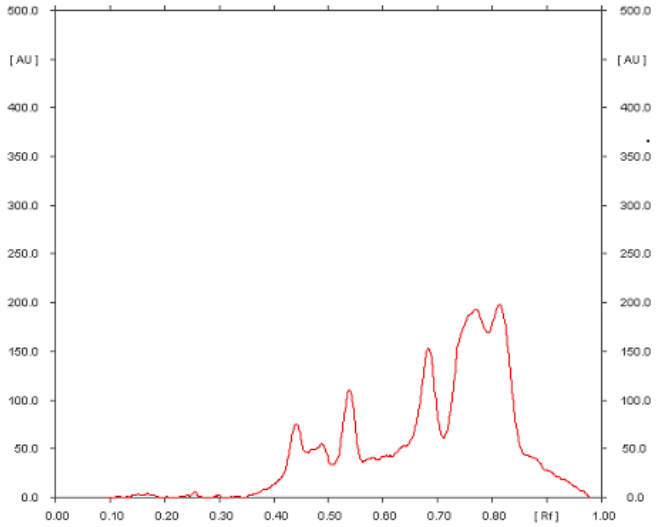

Figure 1 : An overview of *Jasminum azoricum* L. sample at 254 nm before derivatization.

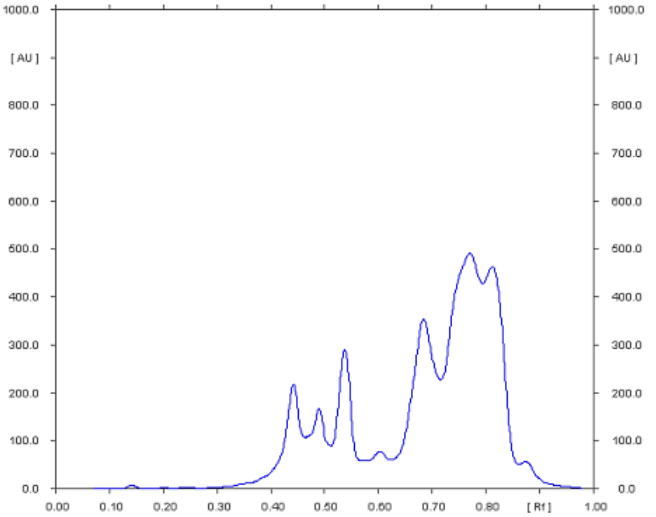

Figure 2 : An overview of *Jasminum azoricum* L. sample at 366 nm after derivatization.

IV. CONCLUSION

Phytochemical fingerprint developed by using HPTLC is a superior technique to check the variability present in plant species. It is extreme tensible for selection of stationary and mobile phase, developing techniques, detection and derivatization. Further research is necessary to extract the compounds from *Jasminum azoricum* and it will give a programme for advanced phytochemical and pharmaceutical applications.

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