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Isolation and Characterization of Secondary Metabolites of *Asplenium indicum* by Using Thin Layer Chromatography

Damayanti Jadhav*[1] and Manda Ghatage*[2]

[1*]Department of Botany, B V's M.B. S. K., Kanya Mahavdihyalaya, Kadegoan, Sangali, India. jadhavdamayanti@gmail.com

[2]Department of Botany, B V's M.B. S. K., Kanya Mahavdihyalaya, Kadegoan, Sangali, India. mandaghatge75@gmail.com

ABSTRACT: **TLC is a simple inexpensive technique used to support the identify the bioactive components. The bioactive components are generally found in most of the plant parts and used as traditional medicine. The present investigation was carried out to isolate and characterized bioactive components from epiphytic fern *Asplenium indicum* .This fern was collected from Mahabaleshwar from Satara district of Maharashtra. The extract is separated on silica TLC plates and bands are visualized under ultraviolet light and chromatogram of *Asplenium indicum* revealed 6 bands. Then chromatogram spray para anisaldehyde reagent and they exposed to iodine chamber chromatogram revealed 6 bands respectively. In the present work of TLC analysis of *Asplenium indicum* exhibited the presence of colour spots indicates the presence of bioactive constituents. These are flavonoids, phenols, steroids and terpenoids. TLC-IR spectrum of *Asplenium indicum* showed 7 functional groups. In present investigation of TLC-IR analysis of epiphytic ferns under study exhibited the presence of functional groups like primary amines, hydroxy, aromatics, alcohol, carboxylic acid, ester, ether alkanes etc. Therefore the epiphytic ferns may play important role in antibacterial, antifungal and antitumor biological activity. So this fern may be used in the pharmaceutical industries, for further use.**

***Index Terms*: *Asplenium indicum*, Bioactive components, Chromatogram, flavonoids, phenols, steroids, terpenoids.**

I. INTRODUCTION

Medicinal plants have significant role in human life as they are used as raw material for the extraction of active constitution for many synthetic drugs and production of many herbal and indigenous medicine (Ferdousi 2014).The use of traditional medicinal plant in rural areas is due to lack of both private and public hospitals, poverty, high unemployment rate, low-cost medicines and other health care organizations. The world health organization recommended evaluating the effective plants to be used as safe, modern drugs. Therefore in recent years new research started for drugs and dietary supplements derived from plants (Kumar *et al*., 2013). The leaves, roots, stems, fruits, barks and flowers are the essential parts of plants used for medicinal purposes (Sravanthi-Kota 2011).To discover new bioactive compounds from plants, which can be used as new modern techniques such as Thin-layer chromatography (TLC). This rapidly provides information about natural products.

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It is a chromatographic technique that is widely used within the pharmaceutical industry. It is used throughout the drug development process, mainly in purity tests for drug substances, reference standards, stability samples, and key intermediates.

Asplenium indicum Sledge, an epiphytic and evergreen fern of family Aspleniaceae grows up to 25cm in height. The epiphytic fern under study was collected from Mahabaleshwar forest in the rainy season. The tall and well graded forests supply an appropriate habitat for the growth of epiphytic ferns and fern- allies. Fern were collected on lateral branches of angiospermic plant species such as, *Ficus, Syzygium* and *Terminalia* at the average height ranging between 1 to 8 meter from soil level. Fern rhizomes were covered by leaf debris and mosses on the stem bark. This fern species where growing on the host plants and nearby rocks. Ferns are naturally abundant of phytochemicals and many types of bioactive compounds have been extracted from various fern species. The main bioactive compounds are flavonoids, phenols, steroids.

II. MATERIALS AND METHODS

In present study only leaf part of *Asplenium indicum* was included. The epiphytic fern species was collected on lateral branches of angiospermic plants from Mahabaleshwar area and identified by using Herbaria of Botany department, Shivaji University, Kolhapur and exhaustive literature as (Beddome 1883, Manickam and Irudayaraj 1992, Smith *et al.*, 2006 a b, Fraser Jenkins, 2008).The leaf material was washed carefully with water and put for drying for 15 days on blotting paper under laboratory conditions, with proper care. The dried plant material was powdered by using mortar pestle and grinder. 50gm powder was used for extraction of compounds using methanol as solvent. The powdered samples dissolved in methanol and carried out maceration at room temperature for overnight and filter through Whatman filter paper. These filtrate used for two different ways separation of bioactive compounds by using TLC and to identify functional groups using TLC- IR.

10 ml of samples was evaporated and the paste of evaporated extract was used for TLC. The assimilation of Hexene: ethyl acetate 50:50 was used as solvent mixtures. The compounds in extracts were isolated by TLC using Merck silica pre-coated aluminium plates of 200µm thickness with solvent system of different polarities. The extract was spotted on the plate. The plates were dipped in TLC chamber and chromatogram was developed. The TLC plate removed in chamber and visualized in UV light (254 nm) and then spread reagent para anisaldehyde and some plates insert in prepared iodine chamber. Spots were marked. Then calculate Rf values by using following formula.

$$\mathrm{Rf} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

In filtrate methanol evaporated at room temperature. After completion of evaporation separate disk was prepared of all three epiphytotic ferns under study with 10 mg of potassium bromide by using mould it is placed under hydrous condition so as to get pellets for disc the plates were put in IR spectrophotometer for recording and measurement in the range of 400 to 4000 cm the transmission percentage was measured across the wave number the peak value of air was recorded and functional group where determined.

III. RESULT

The data of separation of bioactive compounds from Hexane: ethyl acetate (5:5) solvent system with iodine and Para anisaldehyde reagent of potential for *Asplenium indicum* by thin layer chromatography was showed in fig.1 and Table 1. Calculated Rf values by TLC pattern are useful to promote their identity and purity of the fern.

Fig 1(a). TLC in Hexane : Ethyl Acetate (50:50) (b). TLC in Para Anisaldehyade (c). TLC in iodine

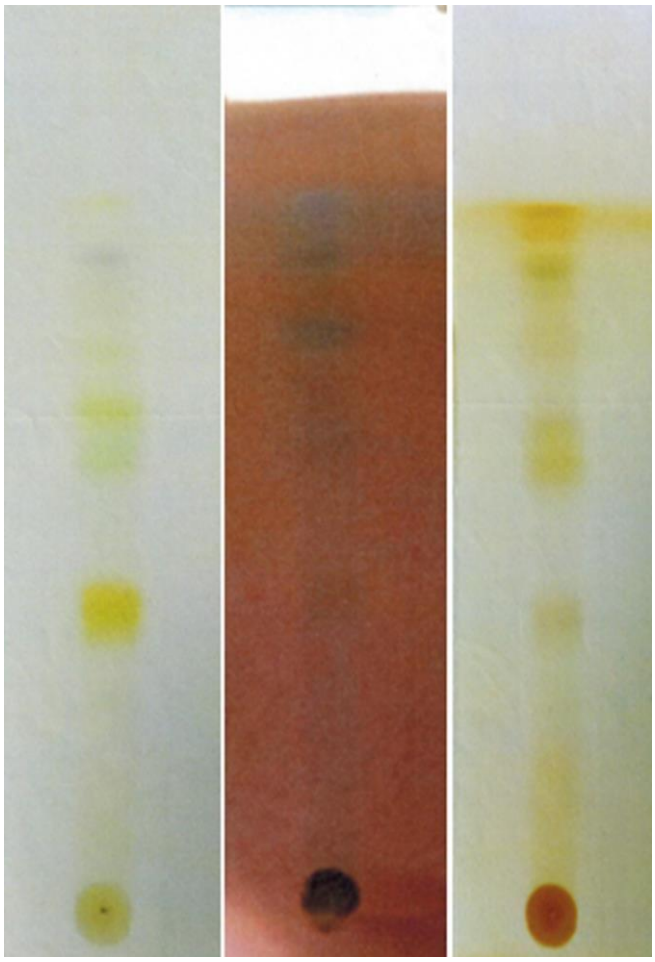

Fig 2: TLC-IR spectrum of *Asplenium indicum*

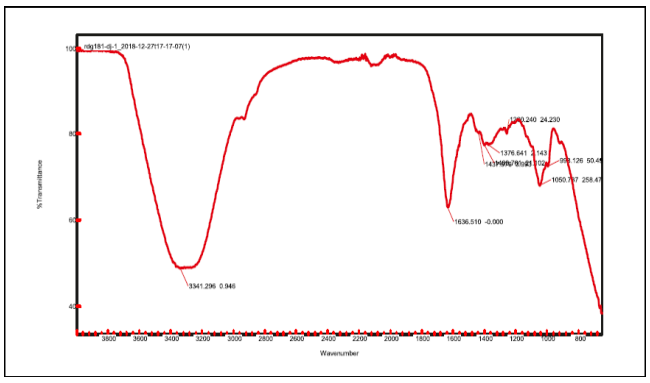

Table 1: Rf Value of *Asplenium indicum*

Plants Species	Mobile Phase	Solvent System	Rf Values					
Asplenium indicum	Hexane: ethyl acetate (5:5)	UV light	0.4	0.61	0.68	0.76	0.88	0.96
		Para A. Reagent	0.25	0.36	0.56	0.70	0.78	0.84
		Iodine Reagent	0.4	0.60	0.66	0.76	0.86	0.93

Table 2. TLC–IR functional group of *Asplenium indicum*

Peak Values	Functional Group	*A. indicum*
998.126	C-H Alkene	+
1050.787	C-O Alkyl Halide, esters, ethers	+
1260.240	C-O alkyl halide ethers, esters.	+
1376.641	C-H Alkane	+
1437.976	C-H Alkane	+
1636.510	C=C Alkene	+
3341.296	O-H alcohol, hydroxy, phenol, carboxylic acid	+

The plates were first exposed through UV (254 NM) and observed colour band. Chromatogram of .*Asplenium indicum* revealed 6 bands. The highest Rf value was 0.96.When chromatogram spread with Para anisaldehyde reagent *Asplenium indicum* revealed 6 bands. The least Rf value was 0.25 in *Asplenium indicum*. When the chromatogram exposed to iodine chamber *Asplenium indicum* revealed 6 bands. The highest Rf value was 0.93. Thin-layer chromatography is an important technique for separating chemical substances. The results of TLC-IR studies on *Asplenium indicum* showed IR spectra.TLC-IR spectrum of *Asplenium indicum* showed seven different spectroscopic peak values. Functional groups have frequency range from 998.126 cm^{-1} to 3341.296 cm^{-1}. The result showed different peak values viz. 998.126, 1050.787, 1260.240, 1376.641, 1437.976, 1636.510 and 3341.296 with the functional groups C-H alkene, C-O alkyl halide ethers, esters, C-H alkane, C=C alkene, O-H alcohol hydroxy, phenol, carboxylic acid respectively.

IV. DISCUSSION AND CONCLUSION

The range of 0.44 to 0.7 and greenish yellow colour on TLC indicates that presence of flavonoids (Kaya *et al*., 2012, Yamuna Devi *et al*., 2012). In present study reported that TLC plate of showed Rf value range from 0.44 to 0.70 having yellowish green colour indicates that the presence of flavonoids. Grey colour spot indicates presence of steroids and violet colour spot indicates presence of terpenoids. (Gerlach *et al*., 2018). In our findings TLC of para anisaldehyde terpenoids and steroids showed very similar pattern visualized as grey, violet spot. The range of 0.40 to 0.95 indicated the presence of phenol (Prabhu et al., 2011, Kristanti and Tanjung 2015). In our study TLC of iodine showed Rf value range from 0.40 to 0.95 indicates that present of phenol. TLC is a simple and beneficial analytical technique that has been used to separate bioactive components present in the sample extracts from natural resources like plants. Polyphenolic contains several –OH groups so they are significantly used as chemo preventive agents against cardio vascular and degenerative diseases. So consider as cardio protective and also acts against cancer, antibacterial and antiviral activities (Halpern 1998, Okuda 2005, Esposito 2002). Flavonoids are important group of active compounds and their action is by the inhibition of DNA, RNA and protein synthesis of bacteria (Dzoyem 2013). The TLC analysis revealed that the presence of Polyphenols and Flavonoids due to (O-H) are stretching, terpens due to (C-H) group. (Maobe 2013). Flavonoids are important group of active compounds and their action is by the inhibition of DNA, RNA and protein synthesis of bacteria (Dzoyem 2013). (Santhi *et al*., 2011) reported that steroids play significant role in immune suppressor. They are mainly used in the pharmaceutical industry as it is associated with the sex hormones. Fang (2010) worked on TLC separation of functional groups in plants, like primary amines, amines aromatics these are antimicrobial and antifungal agents. Mendoza (2018) reported that alcohol, carboxylic acid, ester, ether showed antioxidant and antitumor activity. In present investigation of TLC analysis of epiphytic ferns under study exhibited the presence of colour spots indicates presence of phytoconstituents. The yellow colour bands on TLC showed presence of flavonoids, phenols. Grey spot showed presence of steroids and violet colour on TLC plates indicates terpenoids. These bioactive components play significant role in biological activity. TLC-IR showed different functional groups O-H, C=C Stretching. The presence of characteristic functional groups like primary amines, hydroxy, aromatics, alcohol, carboxylic acid, ester, ether alkanes etc. Therefore the epiphytic fern under study may play important role in antibacterial, antifungal and antitumor like biological activity. TLC profile of epiphytic ferns may be used in the pharmaceutical industries, for further use.

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