International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **4(7),** 69-72, July **(2015)** *Int. Res. J. Biological Sci.*

Thin Layer Chromatographic investigation on leave of *Leucas Aspera* extracted in Ethanol and Dichloromethane

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Available online at: www.isca.in, www.isca.me
Received 6th May 2015, revised 30th May 2015, accepted 7th July 2015

Abstract

The state of Assam has an abundance of medicinal plants known to the native people. Luecas aspera is used by rural and tribal population in Assam in various problems like dysentery, diarrhea, sinusitis, headache, tonsil etc. For thin layer chromatographic study the leaves of Leucas aspera are extracted in ethanol and dichloromethane. For thin layer chromatography, glass plate and precoated silica gel sheets were used. Twenty nine different solvent systems were prepared to develop thin layer chromatographic plate. Four visualizing reagents, viz, Iodine, H_2SO_4, NH_3 solution, KOH solution in methanol were used to separate different spots in thin layer chromatographic plate. Dichloromethane was proved as better solvent to extract different compounds of Luecas aspera.

Keywords: Luecas aspera, dysentery, diarrhea, sinusitis, silica gel.

Introduction

Medicinal plants have always been the main source of medicine in India[1]. The forest in India is the principal repository of large number of medicinal plants. Medicinal properties of plants have been mentioned in Rigveda[2]. The medicinal plant wealth seems to be the first and foremost line of defense for the treatment of various diseases mostly in tribal and rural communities[1]. Large number of rural and tribal population in India depends on medicinal plant resources for primary health care still today[3]. As north-eastern region of India is one of the biodiversity hotspot so it contains so many flora which show different chemical properties which are directly or indirectly beneficial for society[4]. States of north east India are the store house of medicinal plants[5]. Assam is an evergreen state. The state of Assam bears a separate phytogeographic identity and diverse plant wealth within its much varied ecosystems[6]. This state has an abundance of medicinal plants known to the native people[7]. Leucas aspera is used by rural and tribal population in Assam in various problems like dysentery, diarrhea, sinusitis, headache, tonsil etc[8-14].

In Thin Layer Chromatography (TLC), a liquid (the mobile phase) is allowed to migrate across a solid (stationary phase)[15]. TLC is a good technique to analyze the compound of a medicinal plant. TLC is a simple, quick and inexpensive procedure to find out different components in a mixture[16]. Here in this report some of exciting results observed during the thin layer chromatographic investigation on leaf extract of Leucas aspera in ethanol and dichloromethane will be discussed.

Material and Methods

Collection of plant: The sample of Leucas aspera was procured from local market of Guwahati. The plant was taxonomically identified. The leaves were dried under shaded condition at room temperature. Leaves were crushed to powder and stored in air tight bottle.

Preparation of plant extract: 50 gm of the powdered leaves were extracted with 100 ml of solvent (absolute ethanol or dichloromethane) in a soxhlet apparatus for about 48 hours. The extracted solvent was concentrated on a water bath. Filtrate was stored in refrigerator for their future use.

Thin layer chromatography (TLC): For preparing thin layer chromatographic glass plates, silica gel G for TLC was used. Then the slurry of silica gel was prepared in water. Thin layer chromatographic glass plates were then prepared by coating the slurry on the glass plates. Then the glass plates were left overnight for air drying. The air dried plates were heated at 100-120 ^oC inside a hot air oven for 1-2hrs to remove the moisture and to activate the adsorbent on the plate. The samples were spotted on the activated plates. Commercial silica gel sheets (Keiselgel 60 F254, aluminum support; Merck) were also used. The samples were spotted on this precoated silica gel sheets. The plates were dried and developed in suitable solvents for rapid screening.

Results and Discussion

Twenty nine different solvent systems were prepared, as shown in Table-1, to study the behavior of different component of leaf extract. Table-2 shows the number of spots on TLC plates and R_f values of the brightest spot on TLC plates when the TLC plates were developed with twenty one different solvent systems with ethanolic leaf extract under iodine as visualizing reagent. Similarly, table-3 shows the number of spots on TLC plates and

R_f values of the brightest spot on TLC plates when the TLC plates were developed with selected thirteen different solvent systems with dichloromethane leaf extract under iodine as visualizing reagent. The two tables are useful to compare the extraction ability of the two solvent, *viz*, ethanol and dichloromethane. Commercial precoated silica gel sheets (Keiselgel 60 F254, aluminum support; Merck) were used with different solvent system under different visualizing reagent as shown in table-4 and table-5.

Table-1
Solvent system and its composition

Solvent System	Composition	Ratio
SS-1	Ethyl acetate: Methanol	1:1
SS-2	Benzene: Chloroform	1:1
SS-3	Ethyl acetate: Benzene	1:2
SS-4	Chloroform: Methanol	2:3
SS-5	Butanol: Acetic acid	4:1
SS-6	Butanol: Ammonia	1:1
SS-7	Xylene: Ethyl acetate	2:1
SS-8	Ethyl acetate: Petroleum ether	9:1
SS-9	Chloroform: Xylene	1:1
SS-10	Methanol: Ammonia	19:1
SS-11	Ethyl acetate: Acetic acid: Water	9:5:1
SS-12	Ethyl acetate: Formic acid: Water	7:1:1
SS-13	Ethyl acetate: Methanol: Water	10:2:1
SS-14	Petroleum ether: Ethyl acetate: Methanol	17:2:1
SS-15	Dichloromethane: Methanol	19:1
SS-16	Benzene: Ethyl acetate	2:1
SS-17	Xylene: Ethyl acetate	2:1
SS-18	Xylene: Chloroform	1:1
SS-19	Ethyl acetate: Diethyl ether	9:1
SS-20	Methanol: Hydrochloric acid	9:1
SS-21	Hexane: Dichloromethane	1:1
SS-22	Hexane: Diethyl ether	9:1
SS-23	Acetone: Hexane	1:3
SS-24	Hexane: Ethyl acetate	1:1
SS-25	Hexane: Acetic acid	9:1
SS-26	Ethyl acetate: Formic acid: Acetic acid	20:1:2
SS-27	Benzene: Acetic acid: Water	6:7:3
SS-28	Chloroform: Methanol: Acetic acid	8:2:1
SS-29	Ethyl acetate: Formic acid: Acetic acid: Water	10:1:1 :2

In table-2, it is seen that with solvent system 13, highest number of separated spots is observed, next to it with solvent system 9. It is interesting that with most of the solvent systems, the R_f values of the brightest spot is around 0.8. Similarly, in table-3, highest number of separated spot is observed with solvent system 2, 14 and 18. Here in this Table also, with most of the solvent systems, the R_f value of the brightest spot is around 0.8. Table-4 and table-5 show that numbers of separated spots are same under most of the visualizing reagents. In Table-4 with solvent systems 1, 21 and 27 and in table-5 with solvent systems 2, 14, 17 and 25 numbers of separated spots are different under different of visualizing reagents. In table-4 and table-5, it is observed that iodine is a good visualizing reagent in most of the cases; but with solvent system 21 and 27 and with solvent system 2, 14, 17 and 25 iodine was not effective enough to visualize some compounds. From table-5, it can be inferred that ammonia solution is a powerful visualizing reagent with solvent system 2, 14, 17 and 25. It is also seen from the table-5 that dichloromethane can extract more compounds compared to ethanol which is more polar than dichloromethane as with solvent system 14 total six spots were obtained, but in table-4 maximum five spots are observed with solvent system 16.

Table-2
TLC of Ethanolic leaf extract with different solvent systems

Solvent System	Number of Spots	R_f value of the brightest spot
SS-1	3	0.88
SS-2	3	0.92
SS-3	1	0.82
SS-4	3	0.88
SS-7	2	0.72
SS-8	3	0.85
SS-9	4	0.92
SS-10	3	0.48
SS-11	3	0.82
SS-12	2	0.89
SS-13	5	0.82
SS-14	2	0.39
SS-16	2	0.12
SS-18	3	0.90
SS-19	2	0.80
SS-20	2	0.82
SS-21	3	0.80
SS-23	3	0.72
SS-25	2	0.76
SS-27	3	0.45
SS-29	3	0.83

Table-3
TLC of dichloromethane leaf extract with different solvent systems

Solvent System	Number of Spots	R_f value of the brightest spot
SS-1	3	0.90
SS-2	5	0.86
SS-3	3	0.75
SS-12	2	0.89
SS-14	5	0.82
SS-15	1	0.85
SS-17	3	0.80
SS-18	5	0.80
SS-19	3	0.83
SS-21	2	0.82
SS-23	4	0.90
SS-25	4	0.85
SS-27	3	0.86

Table-4
Number of spots in TLC with ethanolic leaf extract under different visualizing reagents

Solvent System	Number of spots under visualizing reagent			
	Iodine	H_2SO_4	NH_3 Solution	KOH solution in Methanol
SS-1	4	3	-	-
SS-2	4	4	-	-
SS-3	3	3	-	-
SS-4	3	3	-	-
SS-7	4	4	-	-
SS-8	2	2	-	-
SS-9	3	3	3	3
SS-10	4	4	-	-
SS-11	2	2	-	-
SS-12	3	3	-	-
SS-13	3	3	-	-
SS-14	2	2	-	-
SS-16	5	5	5	5
SS-18	3	3	3	3
SS-19	2	2	2	2
SS-20	3	3	3	3
SS-21	2	3	3	3
SS-23	4	4	4	4
SS-25	3	3	3	3
SS-27	4	4	5	5
SS-29	2	2	2	2

Table-5
Number of spots in TLC with dichloromethane leaf extract under different visualizing reagents

Solvent System	Number of spots under visualizing reagent			
	Iodine	H_2SO_4	NH_3 solution	KOH solution in methanol
SS-1	2	2	2	2
SS-2	4	4	5	4
SS-3	3	3	3	3
SS-14	5	5	6	5
SS-17	4	4	5	4
SS-18	4	4	4	4
SS-19	2	2	-	-
SS-23	5	5	5	5
SS-25	4	4	5	4
SS-27	2	2	-	-

Conclusion

Leucas aspera is used by rural and tribal population in Assam in various problems like dysentery, diarrhea, sinusitis, headache, tonsil etc. The dried leaves were extracted in ethanol and dichloromethane to study phytoconstitutes present in them using thin layer chromatography. For thin layer chromatography, glass plates and precoated silica gel sheets were used. When visualized under iodine, maximum five spots were observed in thin layer chromatographic glass plate coated with silica gel G for TLC with the leaf extracted in ethanol and dichloromethane although the developing solvent systems are different. Ethyl acetate: Methanol: Water (10:2:1) was used as developing solvent of thin layer chromatography on glass plate coated with silica gel G for TLC with the leaf extracted in ethanol when five spots were observed. Similarly, Benzene: Chloroform (1:1), Petroleum ether: Ethyl acetate: Methanol (17:2:1) and Xylene: Chloroform (1:1) were the developing solvents of thin layer chromatography on glass plate coated with silica gel G for TLC with the leaf extracted in dichloromethane when five spots were observed. With these solvent systems maximum number of compounds can be isolated using thin layer chromatography. For thin layer chromatography of leaf extracted in dichloromethane with precoated silica gel sheets under ammonia solution as visualizing reagent maximum six spots were observed with solvent systems Petroleum ether: Ethyl acetate: Methanol (17:2:1) . Therefore, it can be inferred that dichloromethane is preferred over ethanol to extract more compounds from dried leaves of Leucas aspera.

Acknowledgement

The author is thankful to Arya Vidyapeeth College, Guwahati for providing necessary support to carry out the work.

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