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Studies on the behavior of Ethanolic and Dichloromethanic leaf extract of *Cynodon dactylon* from Assam using Thin layer chromatography

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Abstract

Assam has an abundance of medicinal plants. Cynodon dactylon is used by the people of Assam in the treatment of dysentery, diarrhea, piles, fever, allergy, etc. For thin layer chromatographic study, ethanolic and dichloromethanic leaf extract of Cynodon dactylon was taken. Glass plates and precoated silica gel sheets were used for thin layer chromatographic investigation. Twenty different solvent systems and four visualizing reagents, viz, Iodine, H_2SO_4, NH_3 solution, KOH solution in methanol were used. Thin layer chromatography of ethanolic and dichloromethanic leaf extract of Cynodon dactylon are different both on glass plates and precoated silica gel sheets.

Keywords: Cynodon dactylon, Dysentery, Diarrhea, Piles, Fever, Allergy, Ethanol, Dichloromethane.

Introduction

Assam is a Northeast Indian state[1,2]. It has an abundance of medicinal plants[3]. A large number of people of Assam still depend on these medicinal plants for the treatment of various diseases[4]. So, they have the vast knowledge about various plants that are used as medicine. Cynodon dactylon is known as Dubari bon in Assam. Whole plant of Cynodon dactylon is used for the treatment of diarrhea and dysentery[5]. Paste of Cynodon dactylon is applied to cuts and wounds to stop bleeding[6]. Its juice is used in the treatment of piles. A solution of leaf juice with sugar and honey is used in the retention of urine[7]. Leaf juice is also used for the treatment of allergy and prickly heat[8]. A solution of leaf juice with honey and sugar candy is used for the treatment of fever[9].

Thin layer chromatography (TLC) is based on separating the compounds present in a complex mixture by putting it in contact with a mobile phase (liquid) and another static one (solid) which is fixed[10-12]. Thin layer chromatography (TLC) is used to identify the natural products of a medicinal plant. In this study an attempt has been made to compare the behavior of ethanolic and dichloromethanic leaf extract of *Cynodon dactylon* from Assam using thin layer chromatography.

Materials and Methods

Collection of plant sample: The sample of Cynodon dactylon was collected from nearby areas of Gopinath Nagar. The sample was then taxonomically identified. The leaves were washed with water, then dried and powdered.

Preparation of leaf extract: 25 gm of the powdered leaves were continuously extracted with 100 ml of solvent (absolute ethanol or dichloromethane) for about 48 hours using Soxhlet apparatus. Then the solvent was concentrated using a water bath.

Thin layer chromatography: Thin layer chromatographic glass plates were prepared by coating silica gel on the glass plates. The plates were first air dried and then dried in a hot air oven. Commercial silica gel sheets (Keiselgel 60 F254, aluminum support; Merck) were used as received. The plant samples were spotted on this glass plates or aluminum sheets. The plates or sheets were developed with suitable solvent mixtures. Then the TLC plates or sheets were visualized under different visualizing reagents.

Results and Discussion

For chromatographic study of leaf extract of Cynodon dactylon, twenty different solvent systems were selected as shown in Table-1. The leaves were extracted in ethanol and dichloromethane, as the former is a polar and the later is a non-polar solvent. Table-1 also shows the number of spots on TLC plates and R_f values of the brightest spots on TLC glass plates when the TLC plates were developed with different solvent systems under iodine as visualizing reagent. Commercial precoated silica gel sheets (Keiselgel 60 F254, aluminum support; Merck) were used with different solvent systems under different visualizing reagents as shown in Table-2.

Table-1
TLC of ethanolic and dichloromethanic leaf extract on glass plates

Solvent System	Composition	Ratio	Ethanolic leaf extract		Dichloromethanic leaf extract	
			Number of spots	R_f value of the brightest spot	Number of spots	R_f value of the brightest spot
SS-1	Ethyl acetate: Methanol	1:1	2	0.92	2	0.91
SS-2	Benzene: Chloroform	1:1	3	0.88	4	0.86
SS-3	Chloroform: Methanol	2:3	2	0.92	-	-
SS-4	Xylene: Ethyl acetate	2:1	3	0.69	-	-
SS-5	Ethyl acetate: Petroleum ether	9:1	3	0.83	-	-
SS-6	Chloroform: Xylene	1:1	3	0.91	-	-
SS-7	Methanol: Ammonia	19:1	2	0.45	-	-
SS-8	Ethyl acetate: Acetic acid: Water	9:5:1	3	0.86	-	-
SS-9	Ethyl acetate: Formic acid: Water	7:1:1	3	0.89	1	0.89
SS-10	Ethyl acetate: Methanol: Water	10:2:1	2	0.82	-	-
SS-11	Petroleum ether: Ethyl acetate: Methanol	17:2:1	3	0.29	5	0.88
SS-12	Benzene: Ethyl acetate	2:1	4	0.04	-	-
SS-13	Xylene: Chloroform	1:1	2	0.9	4	0.84
SS-14	Ethyl acetate: Diethyl ether	9:1	2	0.78	2	0.89
SS-15	Methanol: Hydrochloric acid	9:1	3	0.88	-	-
SS-16	Hexane: Dichloromethane	1:1	2	0.85	2	0.11
SS-17	Acetone: Hexane	1:3	3	0.82	5	0.91
SS-18	Hexane: Acetic acid	9:1	2	0.86	4	0.9
SS-19	Benzene: Acetic acid: Water	6:7:3	3	0.41	2	0.82
SS-20	Ethyl acetate: Formic acid: Acetic acid: Water	10:1:1:2	2	0.83	-	-

Table-2
TLC of ethanolic (I) and dichloromethanic (II) leaf extract on precoated silica gel sheets under different visualizing reagents

Solvent system	Number of spots under visualizing reagent							
	Iodine		H_2SO_4		NH_3 Solution		KOH Solution in Methanol	
	I	II	I	II	I	II	I	II
SS-1	3	2	2	2	-	2	-	2
SS-2	4	4	4	4	-	5	-	4
SS-3	2	-	2	-	-	-	-	-
SS-4	3	-	3	-	-	-	-	-
SS-5	3	-	3	-	-	-	-	-
SS-6	3	-	3	-	3	-	3	-
SS-7	2	-	2	-	-	-	-	-
SS-8	2	-	2	-	-	-	-	-
SS-9	3	1	3	-	-	-	-	-
SS-10	2	-	2	-	-	-	-	-
SS-11	3	5	3	5	-	6	-	6
SS-12	4	-	4	-	5	-	4	-
SS-13	2	4	2	4	3	4	2	4
SS-14	2	2	2	2	2	2	2	2
SS-15	3	-	3	-	3	-	3	-
SS-16	2	2	2	-	2	-	2	-
SS-17	3	5	3	5	4	5	4	5
SS-18	3	4	3	4	3	5	3	4
SS-19	3	2	3	2	4	-	3	-
SS-20	2	-	2	-	2	-	2	-

In Table-1, it is seen that in dichloromethanic leaf extract, five spots were obtained with solvent system 11 and solvent system 17 (Sl. No. 11 and 17); but in ethanolic leaf extract, four spots is observed only with solvent system 12 (Sl. No. 12). It is seen that with most of the solvent systems, the R_f value of the brightest spot is 0.8 or 0.9 in both ethanolic and dichloromethanic leaf extract. Table-2 shows that numbers of separated spots are not same under different visualizing reagents (Sl. No. 1, 11 13, 17, 18 and 19). Dichloromethanic leaf extract gives more separated spots as seen in this Table-2 (Sl. No. 11, 13, 17 and 18). It is also observed that with ethanolic leaf extract, maximum five spots is seen with solvent system 12 (Sl. No. 12) under ammonia solution as visualizing reagent and with dichloromethanic leaf extract maximum six spots is seen with solvent system 11 (Sl. No. 11).

Conclusion

Cynodon dactylon is known as dubari bon in Assam. It is an important and widely used medicinal plant of Assam. It is used for the treatment of fever, diarrhea, dysentery, piles, allergy, etc. The dried leaves of Cynodon dactylon were extracted in ethanol and dichloromethane for thin layer chromatography. Both glass plates and precoated silica gel sheets were used in thin layer chromatography. When thin layer chromatography was performed on glass plates, under iodine, with ethanolic leaf extract, Benzene: Ethyl acetate (2:1) gave maximum number of separated spots; but with dichloromethanic leaf extract, Petroleum ether: Ethyl acetate: Methanol (17:2:1) and Acetone: Hexane (1:3) gave maximum number of separated spots. When thin layer chromatography was performed on precoated silica gel sheets, under ammonia solution as visualizing reagent, with dichloromethanic leaf extract maximum six spots is seen with solvent system Petroleum ether: Ethyl acetate: Methanol (17:2:1), but with ethanolic leaf extract, maximum five spots is seen with solvent system Benzene: Ethyl acetate (2:1). Finally, it can be inferred that thin layer chromatography of ethanolic and dichloromethanic leaf extract of Cynodon dactylon are different both on glass plates and precoated silica gel sheets.

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