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Short Communication

Phytochemical and thin layer chromatographic studies on two medicinal plants of Dudhnoi area of Assam, India

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Abstract

Rabha peoples of Dudhnoi area of Assam use Vitex negundo for the treatment of diarrhea, asthma and cough. Another popular ethnomedicine Andrographis paniculata is used for the treatment of high blood pressure, ulcer, bronchitis, dysentery, malaria and different skin diseases. Phytochemical investigation on ethanolic leaf and stem extracts prove the presence of different important chemicals in these two medicinal plants. Thin layer chromatography also indicates the presence of multiple compounds in these two medicinal plants. Phytochemical and thin layer chromatographic investigation confirmed that chemical composition of leaf and stem extracts of the two samples are different.

Keywords: Vitex negundo, Andrographis paniculata, dysentery, diarrhea, malaria, asthma.

Introduction

Dudhnoi is an area of Assam having rich biodiversity of medicinal plants. This area of Assam offers an immense scope for ethnomedicinal studies as it is inhibited mainly by Rabh tribe having rich folklore[1-5]. Rabha peoples are dependent upon ethnomedicines for their day to day live hood practice. Vitex negundo and Andrographis paniculata are two important medicinal plants used by Rabha peoples of Dudhnoi area.

Vitex negundo, known as posotia, is used for the treatment of diarrhea, asthma and cough; whereas Andrographis paniculata, known as chirota tita, is used against high blood pressure, ulcer, bronchitis, dysentery, malaria and different skin diseases. Due to the importance of these two medicinal plants of Assam, present investigation was carried for possible discovery of new chemicals present in these plants. Here in this report only some results of phytochemical and thin layer chromatographic investigation on leaf and stem extracts of Vitex negundo and Andrographis paniculata in ethanol will be discussed.

Material and methods

Collection of plant sample: The samples of Vitex negundo and Andrographis paniculata was collected from different places of Dudhnoi area of Assam. After taxonomical identification, samples were washed and dried.

Preparation of extract: In a Soxhlet apparatus, 5gm of powdered material (leaf or stem) of each sample was extracted with 150ml of absolute ethanol for about 48 hours. After completion of extraction, the solvent was concentrated and filtered for further use[6,7].

Phytochemical assessment of the extracts: The ethanolic extracts were subjected to different chemical tests using standard procedure[8]. The presence of carbohydrates was tested with Fehling test, Benedict test and iodine test; for protein, ninhydrin test, biuret test, lead acetate test, and xanthoprotein test were carried out[9,10]. Phenols and tannins were detected by ferric chloride test, lead acetate test, bromine water test, acetic acid test and potassium permanganate test[9-14]. Similarly, presence of flavonoids was confirmed by pew test, alkaline reagent test, lead acetate test and ferric chloride test[9-14]. Dragendorff test, Wagner test and Mayer test were performed for confirmation of alkaloids[9-14]. Liebermann test, Salkowski test, Keller-kilani test and Legal test were used to test the presence of glcosides[9-14]. Steroid was identified using Liebermann's test[9-13]. Chloroform-sulfuric acid method was used to identify Terpenoid[9,10,12]. Stable foam formation method was used to detect saponins[9-14].

Thin layer chromatography: Glass plates coated with silica gel were used to study thin layer chromatographic behavior. The samples were spotted on this glass plates and then developed with different solvent systems. Then different visualizing reagents (Iodine, dilute sulfuric acid and ammonia) were used to detect spots on this glass plates[6,7].

Results and discussion

Table-1 and Table-2 show the different results obtained during phytochemical investigation on ethanolic extracts of Vitex negundo and Andrographis paniculata. From the Table-1, it is seen that leaf extract of Vitex negundo contains proteins, flavonoids, glycosides and steroids; on the other hand, leaf extract of Andrographis paniculata contains carbohydrates,

proteins, flavonoids, glycosides, alkaloids, steroids, phenols and tannins. Terpenoids and saponins were not detected in both of the leaf extracts. It is observed from the Table-2 that stem extract of Vitex negundo has proteins, flavonoids, glycosides and steroids as phytochemicals; on the other hand, alkaloids, flavonoids, steroids, terpenoids, phenols and tannins were present in the stem extract of Andrographis paniculata. In this time, carbohydrates and saponins were not detected in both of the stem extracts. Therefore, from Table-1 and Table-2, it is noticed that neither leaf extracts nor stem extract of Vitex negundo contain carbohydrates, alkaloid, terpenoids saponins, phenols and tannins. It is interesting that although leaf extracts of Andrographis paniculata show positive tests for carbohydrates, proteins and glycosides, negative results were obtained for stem extract. These observations explain that phytochemical composition of leaf and stem extracts of the two samples are different, although many compounds are common in both of the extracts.

Table-1: Chemical constituents present in leaf extracts [(+) = Present; (-) = absent].

Phytochemicals	Test name/ Reagent	Extract of Vitex negundo	Extract of Andrographis paniculata
Carbohydrates	Fehling test	-	-
	Benedict test	-	-
	Iodine test	-	+
Proteins	Ninhydrin test	-	-
	Biuret test	-	-
	Lead acetate test	+	+
	Xanthoprotein test	-	-
Phenols and tannins	Ferric chloride test	-	+
	Lead acetate test	-	+
	Bromine water test	-	+
	Acetic acid test	-	-
	Potassium permanganate test	-	-
Flavonoids	Pew test	+	-
	Alkaline reagent test	-	-
	Lead acetate test	-	-
	Ferric chloride test	+	+
Alkaloids	Dragendorff test	-	+
	Wagner test	-	+
	Mayer test	-	+
Glycosides	Liebermann test	-	+
	Salkowski test	+	+
	Keller-kilani	+	-
	Legal test	-	-
Steroids	Liebermann test	+	+
Terpenoids	Chloroform-sulfuric acid test	-	-
Saponins	Shaking test for foaminess	-	-

Table-2: Chemical constituents present in stem extracts [(+) = Present; (-) = absent].

Phytochemicals	Test name/ Reagent	Extract of Vitex negundo	Extract of Andrographis paniculata
Carbohydrates	Fehling test	-	-
	Benedict test	-	-
	Iodine test	-	-
Proteins	Ninhydrin test	+	-
	Biuret test	-	-
	Lead acetate test	-	-
	Xanthoprotein test	+	-
Phenol and tannins	Ferric chloride test	-	+
	Lead acetate test	-	+
	Bromine water test	-	+
	Acetic acid test	-	-
	Potassium permanganate test	-	+
Flavonoids	Pew test	+	-
	Alkaline reagent test	+	+
	Lead acetate test	-	+
	Ferric chloride test	+	+
Alkaloids	Dragendorff test	-	+
	Wagner test	-	-
	Mayer test	-	+
Glycosides	Liebermann test	-	-
	Salkowski test	+	-
	Keller-kilani	+	-
	Legal test	-	-
Steroids	Liebermann test	+	+
Terpenoids	Chloroform-sulfuric acid test	-	+
Saponins	Shaking test for foaminess	-	-

Table-3 and Table-4 show that nineteen different solvent systems were used to study thin layer chromatography of Vitex negundo and Andrographis paniculata. In Table-3, it is observed that with leaf extract of Vitex negundo three spots were obtained with solvent system (Sl. No. 3, 4, 5, 6, 7, 10, 11, 12, 15, 16 and 19) and with Andrographis paniculata three spots were obtained with solvent system (Sl. No. 2, 3, 4, 6, 9, 10, 11, 15 and 16). The presence of multiple compounds in these two medicinal plants is indicated by the appearance of number of spots in thin layer chromatographic plates. In Table-4, it is interesting to observed that with stem extracts of Vitex negundo and Andrographis paniculata, maximum two spots were obtained in almost all solvent systems (Except with Sl. No. 9, 10 and 14) .

These results explain that chemical composition of leaf and stem extracts of the two samples are different.

Table-3: TLC of leaf extract.

Sl. No.	Solvent System		Extract of Vitex negundo		Extract of Andrographis paniculata	
	Composition	Ratio	Number of spots	R_f values	Number of spots	R_f values
1	Acetone: Ethyl acetate	1:2	2	0.56, 0.85	2	0.67, 0.95
2	Acetone: Ethyl acetate	2:1	2	0.34, 0.65	3	0.47, 0.56, 0.64
3	Hexane: Ethyl acetate	1:1	3	0.65, 0.75, 0.89	3	0.36, 0.46, 0.56
4	Hexane: Ethyl acetate	1:2	3	0.80, 0.85, 0.96	3	0.24, 0.45, 0.55
5	Hexane: Ethyl acetate	2:1	3	0.25, 0.36, 0.48	2	0.68, 0.96
6	Hexane: Ethyl acetate	3:1	3	0.25, 0.56, 0.85	3	0.45, 0.67, 0.95
7	Ethyl acetate: Petroleum ether	1:9	3	0.45, 0.70, 0.85	2	0.34, 0.78
8	Hexane: Acetic acid	9:1	2	0.25, 0.75	2	0.57, 0.65
9	Hexane: Ethyl acetate :Acetic acid	10:4:1	2	0.21, 0.30	3	0.51, 0.65, 0.89
10	Methanol: Hydrochloric acid	9:1	3	0.47, 0.74, 0.86	3	0.45, 0.56, 0.87
11	Hydrochloric acid: Acetic acid: Water	3:30:10	3	0.23, 0.56, 0.76	3	0.56, 0.67, 0.87
12	Ethyl acetate: Methanol: Water	5:1:1	3	0.50, 0.63, 0.85	2	0.34, 0.65
13	Chloroform: Methanol	5:1	2	0.30, 0.64	2	0.45, 0.56,
14	Toluene: Ethyl acetate	95:5	2	0.27, 0.47	2	0.45, 0.53
15	Toluene: Ethyl acetate	9:1	3	0.78, 0.83, 0.96	3	0.36, 0.45, 0.56
16	Hexane: Ethyl acetate: Acetic acid	5:4:1	3	0.36, 0.60, 0.78	3	0.35, 0.45, 0.57
17	Chloroform: Methanol: : Acetic acid	18:1:1	2	0.25, 0.67	2	0.64, 0.86,
18	Ethyl acetate :Formic acid: Water	8:1:1	2	0.66, 0.76	2	0.45, 0.78
19	Dichloromethane: Methanol	19:1	3	0.59, 0.76, 0.86	2	0.56, 0.87

Table-4: TLC of stem extract.

Sl. No.	Solvent System		Extract of Vitex negundo		Extract of Andrographis paniculata	
	Composition	Ratio	Number of spots	R_f values	Number of spots	R_f values
1	Acetone: Ethyl acetate	1:2	2	0.67, 0.78	2	0.66, 0.87
2	Acetone: Ethyl acetate	2:1	2	0.89, 0.97	2	0.55, 0.98
3	Hexane: Ethyl acetate	1:1	2	0.55, 0.98	2	0.56, 0.97
4	Hexane: Ethyl acetate	1:2	2	0.56, 0.76	2	0.50, 0.76
5	Hexane: Ethyl acetate	2:1	2	0.77, 0.97	2	0.70, 0.95
6	Hexane: Ethyl acetate	3:1	2	0.53, 0.76	2	0.54, 0.75
7	Ethyl acetate: Petroleum ether	1:9	2	0.61 0.87	2	0.56, 0.62
8	Hexane: Acetic acid	9:1	2	0.67, 0.96	2	0.80, 0.95
9	Hexane: Ethyl acetate :Acetic acid	10:4:1	1	0.74	2	0.60, 0.75
10	Methanol: Hydrochloric acid	9:1	1	0.94	2	0.65, 0.98
11	Hydrochloric acid: Acetic acid: Water	3:30:10	2	0.72, 0.98	2	0.45, 0.79
12	Ethyl acetate: Methanol: Water	5:1:1	2	0.56, 0.74	2	0.50, 0.75
13	Chloroform: Methanol	5:1	2	0.55, 0.89,	2	0.45, 0.88
14	Toluene: Ethyl acetate	95:5	2	0.61, 0.93	1	0.81
15	Toluene: Ethyl acetate	9:1	2	0.56, 0.82	2	0.80, 0.87
16	Hexane: Ethyl acetate: Acetic acid	5:4:1	2	0.55, 0.94	2	0.85, 0.94
17	Chloroform: Methanol: : Acetic acid	18:1:1	2	0.67, 0.87	2	0.40, 0.54
18	Ethyl acetate :Formic acid: Water	8:1:1	2	0.45, 0.56	2	0.51, 0.90
19	Dichloromethane: Methanol	19:1	2	0.50, 0.76	2	0.58, 0.77

Conclusion

Dudhnoi area of Assam offers an immense scope for ethnomedicinal studies as it is inhibited mainly by Rabh tribe. Rabha peoples of this area use Vitex negundo for the treatment of diarrhea, asthma and cough. Similarly, Andrographis paniculata is used by them in the treatment of high blood pressure, ulcer, bronchitis, dysentery, malaria and different skin diseases. Phytochemical investigation on ethanolic leaf extracts prove the presence of proteins, flavonoids, glycosides and steroids in Vitex negundo; and carbohydrates, proteins, flavonoids, glycosides, alkaloids, steroids, phenols and tannins in Andrographis paniculata. Similarly, it is proved that stem extract of Vitex negundo has proteins, flavonoids, glycosides and steroids as phytochemicals; on the other hand, alkaloids, flavonoids, steroids, terpenoids, phenols and tannins were present in the stem extract of Andrographis paniculata. From this study it is inferred that phytochemical composition of leaf and stem extracts of the two samples are different, although many compounds are common in both of the extracts. In thin layer chromatography also, it is seen that chemical composition of leaf and stem extracts of the two samples are different. As the thin layer chromatographic plates gave multiple spots with different solvent systems, further study on leaf and stem extracts of the two samples may lead to possible discovery of new chemicals present in leaf and stem of these plants.

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