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Comparative phytochemical and TLC investigation on two antidiabetic plants of Goalpara district of Assam, India

Pankaj Kalita
Chemistry Department, Arya Vidyapeeth (Autonomous) College, Assam, India
pkmscphd@outlook.com

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Abstract

Stevia rebaudiana and Kalanchoe pinnata are two antidiabetic plants used by peoples of Assam. Phytochemical and TLC studies indicate that properties of ethanolic leaf extracts and ethanol stem extracts of the two antidiabetic plants are not the same.

Keywords: Phytochemical, TLC, Investigation, Antidiabetic.

Introduction

Plants, having medicinal properties, are widely used against various health problems in India[1]. Indian people in rural areas still depend on medicines derived from plants for health care[2]. North-eastern India has medicinal plant resources showing acceptable chemical properties[3]. Northeast Indian states, including Assam, are the repository of large number of medicinal plants which are available in the forests[4,5]. Stevia rebaudiana and Kalanchoe pinnata are two antidiabetic plants used by peoples of Assam. Here, a report on comparative phytochemical and TLC investigation on these plants is discussed.

Material and Methods

Samples Collection: The samples of Stevia rebaudiana and Kalanchoe pinnata were collected from Goalpara district of Assam; and were washed with distill water. Then the samples were dried and stored for further experiments.

Preparation of extract: 5 gm of the grinded leaf or stem sample was continuously refluxed with 150 ml of dry ethanol for about 2 days using Soxhlet apparatus. After filtration, the filtrate was concentrated on a rotary evaporator before stored in a refrigerator[6,7].

Thin layer chromatography: Micro slides coated with silica gel were activated inside a hot air. Then these slides were developed for different plant samples using well known procedures[6,7].

Phytochemical assessment of the extracts: phytochemical studies on the ethanolic extracts were carried out using different standard procedures[8]. Different phytochemicals such as carbohydrate (using Fehling test, Benedict test and iodine test), protein (using different tests as given in Table-3 and Table-4), phenols and tannins (using different tests as given in Table-3 and Table-4), flavonoids (using different tests as given in Table -3 and Table-4), alkaloids (using different tests as given in Table-3 and Table-4) and steroid (using Liebermann's test) were tested[9-14]. Liebermann, Salkowski and other tests (given in Table-3 and Table-4) were carried out for identification of glycosides[9-14]. Terpenoids were identified using chloroform-sulfuric acid method[9,10,12]. Saponins were detected by stable foam formation method[9-14].

Results and Discussion

Nineteen mixtures of solvents were used (given in the Table-1 and Table-2) for chromatographic study. In chromatographic study, with different solvent mixtures, leaf extract of Stevia rebaudiana gives maximum three spots whereas with Andrographis paniculata four (Table-1).

The appearance of different number of spots in TLC plates indicates that chemical behavior of leaf extracts of Stevia rebaudiana and Kalanchoe pinnata are not the same. In the Table-2, it is observed that stem extracts of Stevia rebaudiana and Kalanchoe pinnata give only two spots in all the solvent systems.

Table-3 shows that leaf extract of Stevia rebaudiana composed of proteins, flavonoids, glycosides, alkaloids, saponins, phenols and tannins; on the other hand, leaf extract of Kalanchoe pinnata contains carbohydrates, flavonoids, glycosides, saponins, phenols and tannins.

Again, from Table-4, it is seen that stem extract of Stevia rebaudiana contains proteins, flavonoids, glycosides, saponins, phenols and tannins and with stem extract of Kalanchoe pinnata, carbohydrates, flavonoids, glycosides, saponins, phenols and tannins were detected.

Table-1: TLC of leaf extracts.

Solvent System		Extract of Stevia rebaudiana		Extract of Kalanchoe pinnata	
Composition	Ratio	Number of spots	R_f values	Number of spots	R_f values
Acetone: Ethyl acetate	1:2	3	0.74, 0.62, 0.53	2	0.43, 0.56
Acetone: Ethyl acetate	2:1	2	0.27, 0.66	2	0.67, 0.82
Hexane: Ethyl acetate	1:1	3	0.42, 0.93, 0.85	4	0.22, 0.37, 0.42, 0.54
Hexane: Ethyl acetate	1:2	2	0.85, 0.92	2	0.65, 0.82
Hexane: Ethyl acetate	2:1	3	0.45, 0.54, 0.84	3	0.19, 0.45, 0.63
Hexane: Ethyl acetate	3:1	2	0.32, 0.63	2	0.44, 0.54
Ethyl acetate: Petroleum ether	1:9	2	0.68, 0.79	2	0.65, 0.82
Hexane: Acetic acid	9:1	2	0.4, 0.7	3	0.67, 0.75, 0.92
Hexane: Ethyl acetate :Acetic acid	10:4:1	3	0.23, 0.33, 0.49	3	0.54, 0.78, 0.85
Methanol: Hydrochloric acid	9:1	2	0.19, 0.40	3	0.72, 0.86, 0.91
Hydrochloric acid: Acetic acid: Water	3:30:10	3	0.38, 0.62, 0.77	3	0.28, 0.63, 0.86
Ethyl acetate: Methanol: Water	5:1:1	2	0.23, 0.39	2	0.68, 0.79
Chloroform: Methanol	5:1	2	0.44, 0.74	3	0.46, 0.56, 0.81
Toluene: Ethyl acetate	95:5	3	0.27, 0.41, 0.73	3	0.28, 0.45, 0.83
Toluene: Ethyl acetate	9:1	3	0.35, 0.46, 0.56	2	0.46, 0.64
Hexane: Ethyl acetate: Acetic acid	5:4:1	2	0.34, 0.48	2	0.51, 0.69
Chloroform: Methanol: : Acetic acid	18:1:1	2	0.46, 0.59	2	0.24, 0.42
Formic acid: Water: Ethyl acetate	1:1:8	1	0.57	2	0.34, 0.45
Dichloromethane: Methanol	19:1	1	0.67	2	0.46, 0.67

Table-2: TLC of stem extracts

Solvent System		Extract of Stevia rebaudiana		Extract of Kalanchoe pinnata	
Composition	Ratio	Number of spots	R_f values	Number of spots	R_f values
Acetone: Ethyl acetate	1:2	2	0.46, 0.67	2	0.36, 0.67
Acetone: Ethyl acetate	2:1	2	0.45, 0.57	2	0.51, 0.65
Hexane: Ethyl acetate	1:1	2	0.45, 0.59	2	0.52, 0.67
Hexane: Ethyl acetate	1:2	2	0.68, 0.81	2	0.71, 0.87

Hexane: Ethyl acetate	2:1	2	0.45. 0.59	2	0.67, 0.79
Hexane: Ethyl acetate	3:1	2	0.57, 0.73	2	0.46, 0.59
Ethyl acetate: Petroleum ether	1:9	2	0.47, 0.8	2	0.56, 0.76
Hexane: Acetic acid	9:1	2	0.69, 0.89	2	0.67, 0.84
Hexane: Ethyl acetate :Acetic acid	10:4:1	2	0.68, 0.82	2	0.67, 0.79
Methanol: Hydrochloric acid	9:1	2	0.79, 0.89	2	0.69, 0.87
Hydrochloric acid: Acetic acid: Water	3:30:10	2	0.57, 0.77	2	0.71, 0.83
Ethyl acetate: Methanol: Water	5:1:1	2	0.58, 0.71	2	0.62, 0.76
Chloroform: Methanol	5:1	2	0.68, 0.79	2	0.68, 0.84
Toluene: Ethyl acetate	95:5	2	0.68, 0.86	2	0.79, 0.89
Toluene: Ethyl acetate	9:1	2	0.66, 0.78	2	0.68, 0.79
Hexane: Ethyl acetate: Acetic acid	5:4:1	2	0.69, 0.88	2	0.64, 0.79
Chloroform: Methanol: : Acetic acid	18:1:1	2	0.79, 0.89	2	0.78, 0.86
Ethyl acetate :Formic acid: Water	8:1:1	2	0.68, 0.81	2	0.57, 0.71
Dichloromethane: Methanol	19:1	2	0.67, 0.79	2	0.72, 0.89

Table-3: Chemical constituents present in leaf extracts [(+) = Present; (-) = absent].

Experiment	Test results for extract of Stevia rebaudiana	Test results for extract of Kalanchoe pinnata
Detection of Carbohydrates	Fehling test -ve	Fehling test +ve
	Benedict test - ve	Benedict test – ve
	Iodine test - ve	Iodine test – ve
Detection of Proteins	Ninhydrin test - ve	Ninhydrin test – ve
	Biuret test - ve	Biuret test – ve
	Lead acetate test + ve	Lead acetate test – ve
	Xanthoprotein test - ve	Xanthoprotein test – ve
Detection of Phenols and tannins	Ferric chloride test + ve	Ferric chloride test – ve
	Lead acetate test + ve	Lead acetate test + ve
	Bromine water test - ve	Bromine water test + ve
	Acetic acid test - ve	Acetic acid test – ve
	Potassium permanganate test - ve	Potassium permanganate test + ve

Detection of Flavonoids	Pew test - ve	Pew test – ve
	Alkaline reagent test - ve	Alkaline reagent test + ve
	Lead acetate test - ve	Lead acetate test - ve
	Ferric chloride test + ve	Ferric chloride test + ve
Detection of Alkaloids	Dragendorff test - ve	Dragendorff test – ve
	Wagner test + ve	Wagner test – ve
	Mayer test + ve	Mayer test – ve
Detection of Glycosides	Liebermann test + ve	Liebermann test + ve
	Salkowski test - ve	Salkowski test + ve
	Keller-kilani test + ve	Keller-kilani test – ve
	Legal test - ve	Legal test – ve
Detection of Steroids	Liebermann test - ve	Liebermann test – ve
Detection of Terpenoids	Chloroform-sulfuric acid test - ve	Chloroform-sulfuric acid test – ve
Detection of Saponins	Shaking test for foaminess + ve	Shaking test for foaminess + ve

Table -4: Chemical constituents present in stem extracts [(+) = Present; (-) = absent].

Experiment	Test results for extract of Stevia rebaudiana	Test results for extract of Kalanchoe pinnata
Detection of Carbohydrates	Fehling test - ve	Fehling test + ve
	Benedict test - ve	Benedict test – ve
	Iodine test - ve	Iodine test -ve
Detection of Proteins	Ninhydrin test + ve	Ninhydrin test – ve
	Biuret test - ve	Biuret test – ve
	Lead acetate test + ve	Lead acetate test – ve
	Xanthoprotein test + ve	Xanthoprotein test – ve
Detection of Phenol and tannins	Ferric chloride test + ve	Ferric chloride test + ve
	Lead acetate test + ve	Lead acetate test + ve
	Bromine water test - ve	Bromine water test – ve
	Acetic acid test + ve	Acetic acid test + ve
	Potassium permanganate test + ve	Potassium permanganate test – ve
Detection of Flavonoids	Pew test + ve	Pew test – ve

	Alkaline reagent test - ve	Alkaline reagent test – ve
	Lead acetate test + ve	Lead acetate test – ve
	Ferric chloride test + ve	Ferric chloride test – ve
Detection of Alkaloids	Dragendorff test - ve	Dragendorff test – ve
	Wagner test - ve	Wagner test – ve
	Mayer test - ve	Mayer test – ve
Detection of Glycosides	Liebermann test + ve	Liebermann test – ve
	Salkowski test + ve	Salkowski test + ve
	Keller-kilani test - ve	Keller-kilani test – ve
	Legal test - ve	Legal test – ve
Detection of Steroids	Liebermann test - ve	Liebermann test – ve
Detection of Terpenoids	Chloroform-sulfuric acid test - ve	Chloroform-sulfuric acid test – ve
Detection of Saponins	Shaking test for foaminess + ve	Shaking test for foaminess + ve

Conclusion

Phytochemical studies on leaf extract of Stevia rebaudiana proves the presence of proteins, flavonoids, glycosides, alkaloids, saponins, phenols and tannins, whereas Kalanchoe pinnata proves the presence of flavonoids, saponins, carbohydrates, glycosides, phenols and tannins.

Similarly, it is proved that stem extracts of Stevia rebaudiana contains proteins, flavonoids, glycosides, saponins, phenols and tannins; and Kalanchoe pinnata contains carbohydrates, flavonoids, glycosides, saponins, phenols and tannins.

Although Stevia rebaudiana and Kalanchoe pinnata are used as antidiabetic ethnomedicinal plants from thin layer chromatographic and phytochemical studies it is seen that behavior of ethanolic leaf extract and ethanolic stem extract of the two samples are not the same. Further study on these two samples may lead to possible discovery of new antidiabetic compounds.

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