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Phytochemical Analysis and TLC Studies of *Eclipta alba* and *Scoparia dulcis* Plant Extract - Cold Extraction

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Abstract

Medicinal plants are the most extravagant bio-asset of medications of conventional frameworks of prescription, present day meds, nutraceuticals, nourishment supplements, society meds, pharmaceutical intermediates and substance elements for manufactured medications. Plants have extraordinary potential uses, particularly as customary prescription and pharmacological medications. A huge extent of the total populace relies upon traditional medication. The therapeutic plants are valuable for recuperating just as for relieving of human ailments in view of the nearness of phytochemical constituents. Phytochemical analysis was done on *Eclipta alba* and *Scoparia dulcis* to find their active compounds. In this experiment all of fifteen compounds- alkaloids, anthocyanin, balsams, carbohydrate, cardiac glycosides, fats and fixed Oil, flavonoids, steroids, phenol, phlobatanins, protein, resins, tannin, terpenoids and triterpene were seen in both plants. Thin layer chromatography strategy was utilized to screen the advancement of a response and recognize compounds present in every extract.

Keywords: Medicinal plants; Phytochemicals; TLC; Pharmacological medications

Introduction

Present day medication has developed from society solution and customary framework simply after intensive chemical and pharmaceutical screening. The utilization of synthetic compounds prompted to a decrease in the utilization of plants in current medication. In any case, engineered pharmaceutical can bring about reactions and therefore individuals are more incline to utilize natural compounds acquired from plants sources. Along these lines, plants remain a noteworthy source of medicinal compounds. In this study, two different plants used *Scoparia dulcis* and *Eclipta alba* which are known as medicinal plants and five different solvents (hexane, chloroform, ethyl acetate, acetone and methanol) were utilized for cold extraction. Phytochemical compounds screening was performed from plants *Scoparia dulcis* and *Eclipta alba* extracts and five dissolvable concentrates was kept running in five distinctive dissolvable frameworks in TLC to screen the advancement of a response, recognize mixes present in every blend.

Materials and Methods

Collection of plant materials

Whole plant of *Eclipta alba* and *Scoparia dulcis* (leaves, stem, roots, flowers and fruit) were collected from Kapasia, Bangladesh and the two plants examples were distinguished, ensured and the voucher example number (35897, 43794) was saved at the Bangladesh National Herbarium.

Preparation of the extracts

Both plants sample (1.5 kg) was washed under running tap water to expel soil flotsam and jetsam and then sun dried for 10 days until a constant weight was obtained. The dried plant were then cut into little pieces and crushed to powder shape utilizing a spotless electric blender. It was put away in impenetrable compartment until utilize.

Add up to 6 solvents were utilized for plant extraction. The powder of *Eclipta alba* (10 g) was consecutively macerated with 100 ml of every dissolvable solvent. It was left on shaker for 3 days at darkroom. At that point, the extracts were concentrated utilizing filter paper and put away at 4°C for the further examinations. The solvents were chosen from non-polar to polar solution.

Phytochemical analysis

Phytochemical analysis to screen the plants for the presence of alkaloids, anthocyanin, balsams, carbohydrate, cardiac glycosides, fats and fixed oil, flavonoids, steroids, phenol, phlobatanins, protein, resins, tannin, terpenoids and triterpene was performed.

Wagner's Reagent was used to test for alkaloids. One ml solution from each extract was fermented with hydrochloric acid and few drops of Wagner's reagent were added. A yellow or brown colour encourage was demonstrated the nearness of alkaloids [1]. About two ml of various concentrates was treated with 2 ml of NaOH and the nearness of blue green shading showed the presence of anthocyanin [2]. About three drops of alcoholic ferric chloride solution were added to 5 ml of 90% each of the extract. A dim green shading demonstrates the nearness of balsams [2]. 5 ml extract from each was taken to filter and filtrate is concentrated, then Benedict's reagent (2.5 ml) was added and bubbled for 5 minutes to bring the solution colour into brick red shading and this colour indicates the presence of carbohydrate Keller-Killani method was performed for cardiac glycosides test. In each concentrate/extract of (5 ml) test was treated with glacial acetic acid (2 ml) blended with one drop of ferric chloride solution and to concentrate this solution sulphuric acid (1 ml) was included. A brown ring was showed up at the interface demonstrated a deoxy sugar normal for cardenolides, trailed by the solution of a violet ring

underneath that brown ring. To 5 drops of each sample were included with 1 ml of 1% copper sulphate solution and a couple drops of 10% sodium hydroxide. The solution of a reasonable blue solution affirmed the test [3].

For flavonoids test 1 ml of every dissolvable concentrate/extract and 1 ml of NaOH was taken and few drops of sulphuric acid were added. The yellowish-brown shade demonstrated the presence of flavonoids. 2 ml of concentrate was added to 2 ml of ferric chloride solution ($FeCl_3$), a profound pale blue green solution is framed with nearness of phenols [2]. One ml extract was boil with hydrochloric acid (1%) brought about the development of red precipitate which demonstrated the nearness of phlobatannins [4]. To 2 ml of the test solution included 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH. Blend completely. Development of purple or violet shading affirmed proteins [3]. One ml test was broken down in acetone (1 ml) and the solution was have poured in distilled water (2 ml). Turbidity demonstrated the confirmation of resin [1]. 10 ml of distilled water was added to 0.5 cm^3 of each concentrate. Shake the substance enthusiastically with the test tube for 2 minutes. The nearness of foaming or percolating shows the nearness of saponins [5]. About 2 ml of acetic anhydride was added to concentrate 2 ml of H_2SO_4. The shading changed from violet to blue or green in a few specimens showed the presence of steroids. To each extract of (1–2 ml) test, few drops of $FeCl_3$ (5%) solution was added brought about the development of green shading was demonstrate the nearness gallotannins, while brown shading was showing the nearness of pseudo tannins [6]. To 1 ml of every dissolvable concentrate of the both plants extract, 2 ml of chloroform was included, trailed by 3 ml of conc. H_2SO_4 to shape a layer. A reddish-brown coloration of the border demonstrated the nearness of terpenoids [6]. For the confirmation of triterpene presence, Lieberman Burchardt test was done. Chloroform solution of the extract with few drops of acetic acid and 1 ml concentrated sulphuric acid gives dark red at the intersection of 2 layers pointing the presence of triterpene.

Thin layer chromatographic studies

Every dissolvable concentrate was exposed to thin layer chromatography (TLC) according to customery one dimensional climbing strategy utilizing silica gel plate 60 F254, 7 × 6 cm (Merck). Glass vessels were utilized to recognize the example on the TLC plates. Each plate was connected with 4 µl of tests at separation of 1 cm at 5 tracks. In the twin trough chamber with various dissolvable frameworks Hexane: Acetic acid (9:1) solvent system I. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1). While, in solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2) and solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1). The solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1) were utilized. After pre-immersion with mobile phase for 20 min for improvement. After the run plates are dried, the plates were perused under UV and development of the solvents was set apart on the TLC plates. The development of the dynamic compound was communicated by its retention factor (Rf), which esteems were determined for various examples utilizing the accompanying formula [7].

Rf = (Distance travelled by the solute)/(Distance travelled by the solvent front on TLC plates)

The Chloroform extract of *Eclipta alba* in solvent system I, 4 spots were visible and Rf values are 0.14, 0.28, 0.38 and 0.39. In solvent system II, also 4 spots were detected with Rf values 0.54, 0.60, 0.66 and 0.71. All other used solvent system (III, IV and V) gave same results. Single spots were found and their Rf values are 0.61, 0.64 and 0.72. On the other plant name *Scoparia dulcis*, Chloroform extract in solvent system I, 7 spots were detected with Rf values 0.11, 0.14, 0.19, 0.22, 0.25, 0.30 and 0.36. No spots were visible for rest of the solvent system (Table 1).

	Eclipta alba					**Scoparia dulcis**				
Test	**Hexane**	**Ethyl acetate**	**Chloro form**	**Acetone**	**Methanol**	**Hexane**	**Ethyl acetate**	**Chloro form**	**Acetone**	**Methanol**
Alkaloids	+	+	-	-	-	+	+	-	-	-
Anthocyanin	-	+	+	+	-	-	+	+	+	+
Balsams	-	+	+	+	+	-	+	+	+	+
Carbohydrate	-	-	+	+	-	-	-	-	-	-
Cardiac glycoside	+	-	-	-	-	+	-	-	-	-
Fats and Fixed oil	+	+	+	+	-	-	+	-	-	-
Flavonoids	+	-	-	+	+	+	-	-	-	-
Steroids	-	+	+	+	+	+	-	-	-	-
Phenol	-	-	-	+	+	-	+	+	+	+
Phlobatanins	-	-	-	+	+	-	+	-	-	-
Protein	-	+	+	+	+	-	-	+	-	-
Resins	-	-	+	+	+	-	-	+	-	-

Tannin	-	+	+	+	-	-	+	+	+	+
Triterpenoids	+	+	-	+	-	+	+	_	_	_
Triterpene	+	+	+	+	-	+	-	-	-	-

Table 1: Phytochemical test results of *Eclipta alba* and *Scoparia dulcis* – Cold extraction.

Acetone extract in *Eclipta alba* plant, 2 spots were visible and Rf values are 0.22 and 0.27 in solvent system I and in solvent system II, 4 spots were detected with Rf value 0.52, 0.58, 0.64 and 0.71. In *Scoparia dulcis* plant, acetone extract gave 7 spots in solvent system I and their retention of factor were 0.08, 0.12, 0.17, 0.22, 0.25, 0.31 and 0.35. Among all solvent system three of them gave (solvent system III, IV and V) only 1 spots for TLC experiments and their Rf values are 0.59, 0.64 and 0.71 for *Eclipta alba* while four solvent system gave one spots for all and their values are 0.75, 0.14, 0.15 and 0.27 respectively for *Scoparia dulcis* plant (Table 2).

No	Extract name		Solvent System I		Solvent system II		Solvent System III		Solvent system IV		Solvent system V	
			EA	SD	EA	SD	EA	SD	EA	SD	EA	SD
1	Hexane	No of spots	3	6	2	-	1	-	1	-	-	-
		Rf Value	0.15	0.14	0.65							
			0.22	0.19	0.68							
			0.29	0.23		-	0.65	-	0.61	-	-	-
				0.27								
				0.3								
				0.34								
2	Ethyl acetate	No of Spots	2	6	4	-	1	-	1	-	1	-
		Rf Value	0.16	0.12	0.54							
			0.27	0.15	0.6							
				0.2	0.66	-	0.59	-	0.64	-	0.71	-
				0.23	0.72							
				0.28								
				0.23								
3	Chloroform	No of Spots	4	7	4	-	1	-	1	-	1	-
		Rf Value	0.14	0.11	0.54							
			0.28	0.14	0.6							
			0.88	0.19	0.66	-	0.61	-	0.64	-	0.72	-
			0.39	0.22	0.71							
				0.25								
				0.3								
				0.36								
4	Acetone	No of Spots	2	7	4	1	1	1	1	1	1	1
		Rf Value	0.22	0.08	0.52							
			0.27	0.12	0.58							
				0.17	0.64	0.75	0.59	0.14	0.64	0.2	0.71	0.3

				0.22	0.71							
				0.25								
				0.31								
				0.35								
5	Methanol	No of spots	3	7	4	1	1	1	1	1	1	1
		Rf Value	0.17	0.11	0.51							
			0.21	0.14	0.57							
			0.27	0.17	0.64	0.77	0.59	0.11	0.15	0.2	0.71	0.3
				0.22	0.7							
				0.26								
				0.31								
				0.35								

Table 2: Rf values of TLC solvent system for different extracts of *Eclipta alba* and *Scoparia dulcis.*

In *Eclipta alba* plant, methanol extract gave 3 spots with 0.17, 0.21, 0.27 values in system I and 4 spots were visible and their Rf values are 0.51, 0.57, 0.64, 0.70 in solvent system II. While 7 spots were detected for methanol extract of solvent system I in *Scoparia dulcis* plant and 0.11, 0.14, 0.17, 0.22, 0.26, 0.31 and 0.35 are their Rf value. Three of the left solvent system gave same result. Only 1 spots detected and their values are 0.59, 0.15, and 0.71 while methanol extract of *Scoparia dulcis* plant also gave same result for solvent system I, II, III and IV. Their Rf values are 0.77, 0.11, 0.15 and 0.26.

In *Eclipta alba* plant, few compounds like alkaloids, cardiac glycosides, fats and fixed oil, flavonoids, terpenoids and triterpene are present in hexane extract. Previously alkaloids presence in hexane extract was found in few of the journal [8-10]. Ethyl acetate extracts revealed that alkaloids, anthocyanin, balsams, fats and fixed oil, steroids, protein, tannin, terpenoids and triterpene has been found in this study. Chloroform extract confirmed existence for anthocyanin, balsams, carbohydrate, fats and fixed oil, steroids, protein, resins, tannin and triterpene. Few of the research and studied has been done by the researcher on this compound [11,12].

The hexane extract explored the presence of alkaloids, cardiac glycosides, flavonoids, steroids, terpenoids and triterpene in *Scoparia dulcis* plant. The ethyl acetate showed that alkaloids, anthocyanin, balsams, fats and fixed oil, phenol, phlobatanins, tannin and triterpenoids are available in this plant. Few of the compounds are present in ethyl acetate extract has been recorded previously [13]. The chloroform extract showed positive results for anthocyanin, balsams, phenol, protein, resins and tannin. But minimum and same result for compounds presence recorded in acetone and methanol extract (anthocyanin, balsams, phenol and tannin) in our research.

The phytochemical screening of *Eclipta alba* and *Scoparia dulcis* revealed that both plants are a huge reservoir of different secondary metabolites like alkaloids, anthocyanin, balsams, cardiac glycoside, fats and fixed oils, flavonoids, steroids, phenols, phlobatanins, proteins, resins, tannin, terenoid and triterpene. The whole plants were used to do extraction by n-hexane, ethyl acetate, chloroform, acetone and methanol. Almost same result was found in some papers written by Sharma et al. [10], Dalal et al. [9], Mithun et al. [14], Lunavath et al. [8], Gani et al. [12]. In paper written by Gani et al. [12] methanolic extract of the weed showed the presence of alkaloids and steroids. In paper detailed by Sharma et al. [12] the phenolic gathering indicated bring about ethyl acetic acid derivation separate, which was comparative the outcome acquired in the current explore. As announced by Mithun et al. [14] the entire plant some portion of *Eclipta alba* is accounted for to have demonstrated the nearness of bioactive compound glycosides, saponins and triterpenes, while the methanol concentrate brought about the certification of eight bioactive mixes of steroidal alkaloids.

Diverse Rf esteems was recorded for all extraordinary dissolvable framework. This variety in Rf estimations of the phytochemicals determines an exceptionally critical intimation in comprehension of their extremity and aides in choice of suitable dissolvable framework for division of unadulterated mixes chromatography strategy. The determination of appropriate dissolvable framework for a particular plant concentrates must be accomplished by breaking down the R_f estimations of mixes in various dissolvable frameworks. Diverse R_f estimations of the bioactive compound additionally uncover a thought regarding their extremity. For division of unadulterated compound from plant extricate, blend of solvents with variable extremity in various proportion can be utilized. This information will help in choice of reasonable dissolvable framework for further partition of compound from these plant separates [15].

In this examination, phytochemical investigation of *Eclipta alba* and *Scoparia dulcis* were performed. From this outcome, it can be presumed that different phytochemicals including alkaloids, anthocyanin, balsams, carbohydrates, cardiac glycoside, fats and fixed Oil, flavonoids, steroids, phenol, phlobatanins, protein, resins, tannin, terpenoids and triterpene are available in the plants. These plants are rich in optional metabolites and have various uses in customary pharmaceutical to treat a few sicknesses, ethnic restoratively trustworthy as antibacterial, antimicrobial, antidiabetics and anticancer. It has the potential for improvement into a phytomedicine.

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