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Research Article

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

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Abstract

Surge of severity and frequency of diseases made the search for bioactive compounds necessitated for medicinal plants to discover its resistance to maximum drugs as a therapeutic plant. In endemic countries where medicinal plants are often used to treat diseases. Phytochemical screening of *Scoparia dulcis* and *Eclipta alba* has revealed the presence of alkaloids, anthocyanin, balsams, carbohydrates, cardiac glycosides, fats and fixed Oil, flavonoids, steroids, phenol, phlobatanins, protein, resins, saponins, tannin, terpenoids and triterpene where anthocyanin was present in *Scoparia dulcis* but not in *Eclipta alba*. Thin layer chromatography technique was used to monitor the progress of a reaction, identify compounds present in each mixture.

Keywords: Ciprofloxacin; Floating tablets; *Sida acuta* gum; Matrix tablets; Sustained release property

Introduction

People from all continents have long used the indigenous plant infusions used for medicinal purpose for hundreds, if not thousands of years, dating back to prehistory. This medicinal plants catalog became the prototype for modern pharmacopoeias. Plants show therapeutic activity because of the presence of different phytochemicals. The use of and search for new drugs and the bioactive compounds derived from plants have accelerated in last few centuries. Researchers are trying to search new phytochemicals which could be lead to develop new treatment of infectious disease from natural products extracted from plants. In this study, two medicinal plants *Scoparia dulcis* and *Eclipta alba* were used and five solvents (hexane, chloroform, ethyl acetate, acetone and methanol) were used for hot extraction. Phytochemical compounds screening was performed from two medicinal plants *Scoparia dulcis* and *Eclipta alba* extracts. Five solvent extracts was run in five different solvent systems in TLC to monitor the progress of a reaction, identify compounds present in each mixture.

Materials and Methods

Collection of plant material

Whole plant of *Eclipta alba* and *Scoparia dulcis* (leaves, stem, roots, flowers and fruit) were collected from Kapasia, Bangladesh and the plant specimens were identified, certified and the voucher specimen number (35897, 43794) was deposited at the Bangladesh National Herbarium.

Preparation of the extracts

Both plants sample (1.5 kg) was washed under running tap water to remove dust and dirt and then sun dried for 10 days until a constant weight was obtained. The dried plant were then powdered using a conventional blender to get a very find powder and stored in an airtight glass container until further use. An amount of 15 grams of the powdered plants extracted sequentially using soxhlets apparatus with polarity increasing solvents started with n-hexane followed by ethyl acetate, chloroform, acetone and methanol each with a volume of 250 ml [1]. Then extracts were concentrated by removing excess solvent using soxhlet apparatus and stored at 4°C until further used.

Phytochemical analysis

Phytochemical analysis was performed to screen for the presence of active compounds were extracted from both the plants using 5 different solvents.

Wagner's method was used for the testing alkaloids. From each plant extracts (1 ml) sample solution was acidified with hydrochloric acid and few drops of Wagner's reagent were added. A yellow precipitate indicated the presence of alkaloids [2]. Each sample extracts of 2 ml were treated with 2 ml of NaOH and blue green color was formed what indicated the presence of anthocyanin [3]. Two drops of alcoholic ferric chloride solution were added to 5 ml of 90% each of the extract. A dark green color indicated the presence of balsams [3]. Benedict's reagent (2.5 ml) was added in 5 ml of concentrated solvent and boiled for 5 minutes, formation of the brick red color precipitate within 5 minutes and indicated the presence of carbohydrates. For cardiac glycosides test Keller-Killani method was performed. In this method, each extract of (5 ml) sample was treated with glacial acetic acid (2 ml) mixed with one drop of ferric chloride solution and 1 ml concentrated sulphuric acid which results in formation of a violet ring which indicates positive result [4]. To 5 drops of each sample 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide were added. The formation of a clear blue solution confirmed positive result [5]. One ml of each solvent extract was taken and 1 ml of NaOH and few drops of H_2SO_4 were added. Yellow bluish brown color formation confirmed the presence of flavonoids [4].

Acetic anhydride (2 ml) and 2 ml of H_2SO_4 was added in 2 ml of extract. The color changed from violet to green in some samples indicated the presence of steroids [6]. In 2 ml of plant extract 2 ml of ferric chloride solution ($FeCl_3$), was added, a deep bluish green solution is formed with presence of phenols [3]. One ml of plant extract was boiled with hydrochloric acid (1%) and formation of red precipitate indicated the phlobatannins presence [7]. Biuret test was done to see proteins presence in the plants. In 2 ml plant extract solution 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH were added and mixed carefully. Violet color formation is established existence of proteins [5].

Plant extract sample and acetone was mixed in at 1:1 ratio and distilled water (2 ml) was poured in mixture. Turbidity was seen which specified the resins presence [2]. Distilled water (10 ml) and 0.5 ml of

each extract was mixed. The solution was shaken dynamically for 2 minutes. Frothing or bubbling attendance showed the occurrence of saponins [8]. In each extract of (2 ml) sample, few drops of $FeCl_3$ (5%) solution was added and green color formed means tannins are present [4]. Salkowski's method was used for testing terpenoids presence. One ml of each solvent extract, 2 ml of chloroform and 3 ml of conc. H_2SO_4 was mixed. A reddish brown coloration layer formation confirmed the terpenoids presence [4]. To see triterpene presence in both the plant extracts Lieberman Burchardt method was used. Wherein chloroform solution of the extract, few drops of acetic acid and 1 ml concentrated sulphuric acid were varied, which made deep red junction of 2 layers, indicating the existence of triterpene.

Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel plate 60F254, 7 × 6 cm (Merck). Glass capillaries were used to spot the sample on the TLC plates. Each plate was applied with 4 µl of samples at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system 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 used. After pre-saturation with mobile phase for 20 min for development. After the run plates are dried, the plates were read under UV and movement of the solvents were marked on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), which values were calculated for different samples using the following formulae [9].

$$R_f = \frac{Distance\ traveled\ by\ the\ solute}{Distance\ traveled\ by\ the\ solvent\ front\ on\ TLC\ plates}$$

Results

The phytochemical active compounds for both the plants were qualitatively analyzed for whole plants and the results are listed in Table 1. Phytochemical screening shows presence of alkaloids, balsams, carbohydrates, cardiac glycosides, fats and fixed Oil, flavonoids, steroids, phenol, phlobatanins, protein, resins, saponins, tannin, terpenoids, triterpene were present in both plants extracts whereas only anthocyanin was present in *Scoparia dulcis* but not in *Eclipta alba*.

In Hexane extract of *Eclipta alba* alkaloids, fats and fixed oil, flavonoids, protein, terpenoids and triterpene while ethyl acetate gave positive result for alkaloids, fats and fixed oils, phenol, protein and tannin. In chloroform extracts fats and fixed oil, steroids, protein and triterpene was found in most of the compounds are present in acetone extract (balsams, carbohydrates, cardiac glycosides, fats and fixed oil, flavonoids, phlobactannins, tannin and terpenoids). Minimum result was shown by methanol extract. Only resins and saponins are present.

In *Scoparia dulcis*, hexane extract gave the results for fats and fixed oil, flavonoid, protein, terpenoids and triterpene. Few important compounds like alkaloids, anthocyanin, balsams, fats and fixed oil, phenol, protein and tannin are present in ethyl acetate extract. In chloroform extract anthocyanin, balsams, fats and fixed oil, steroids, phenol, protein, tannin and terpenoids. Acetone extracts also showed good results for presence of balsams, carbohydrates, cardiac glycosides, flavonoids, phlobactanins, tannin and terpenoid are present. Lowest number of compounds (resins, saponins and tannin) are present in methanol extract.

All five extract of TLC solvents given some spots, which shows the presence of number of phytochemicals. Five different solvent systems were used to obtain the active compounds in TLC. The ratio of hexane: ethyl acetate: acetic acid solvents was used.

The hexane extract of *Scoparia dulcis* plant in solvent system I showed 4 visible spots Rf values 0.15, 0.22, 0.26 and 0.32 while hexane extract of *Eclipta alba* in solvent system II gave 2 spots detected Rf value 0.52 and 0.64. Other solvent system did not show any result.

The Ethyl acetate extract of *Scoparia dulcis* in solvent system I, 2 spots were visible with Rf values 0.19 and 0.26 and in solvent system III, 1 spot detected Rf value 0.69. On the other side, *Eclipta alba* ethyl acetate extract, in solvent system II, 2 spots were detected and Rf values 0.49 and 0.53. In solvent system III, lV and V, for each of them 1 spots was detected Rf values 0.97, 0.51 and 0.51.

The Chloroform extract of *Scoparia dulcis* in solvent system I, 2 spots were visible Rf values 0.20 and 0.26. In solvent system II and III, 1 spot was detected Rf values 0.56 and 0.69. Chloroform extract of *Eclipta alba* in solvent system II, 2 spots were detected Rf values 0.49 and 0.53.

	Eclipta alba					*Scoparia dulcis*				
Test	**Hexane**	**Ethyl Acetate**	**Chloroform**	**Acetone**	**Methanol**	**Hexane**	**Ethyl Acetate**	**Chloroform**	**Acetone**	**Methanol**
Alkaloids	+	+	-	-	-	-	+	-	-	-
Anthocyanin	-	-	-	-	-	-	+	+	-	-
Balsams	-	-	-	+	-	-	+	+	+	-
Carbohydrates	-	-	-	+	-	-	-	-	+	-
Cardiac glycoside	-	-	-	+	-	-	-	-	+	-
Fats and Fixed Oil	+	+	+	+	-	+	+	+	+	-
Flavonoids	+	-	-	+	-	+	-	-	+	-
Steroids	-	-	+	-	-	-	-	+	-	-
Phenol	-	+	-	-	-	-	+	+	-	-
Phlobatanins	-	-	-	+	-	-	-	-	+	-
Protein	+	+	+	-	-	+	+	+	-	-
Resins	-	-	-	-	+	-	-	-	-	+
Saponins	-	-	-	-	+	-	-	-	-	+
Tannin	-	+	-	+	-	-	+	+	+	+
Terpenoids	+	-	-	+	-	+	-	-	+	-
Triterpene	+	-	+	-	-	+	-	+	-	-

Table 1: Phytochemical test results of *Eclipta alba* and *Scoparia dulcis*- Hot Extraction.

No	Extract Name		Solvent system 1		Solvent system 2		Solvent system 3		Solvent system 4		Solvent system 5	
			SD	EA	SD	EA	SD	EA	SD	EA	SD	EA
1.	Hexane	No. of spots	4	-	-	2	-	1	-	-	-	-
		Rf value	0.150.22 0.26 0.32	-	-	0.52 0.62	-	0.584	-	-	-	-
2.	Ethyl Acetate	No. of drops	2	-	-	2	1	1	-	1	-	1
		Rf value	0.19 0.26	-	-	0.49 0.53	0.694	0.972	-	0.507	-	0.507
3.	Chloroform	No. of drops	2	-	1	2	1	1	-	1	-	1
		Rf value	0.20 0.26	-	0.557	0.49 0.53	0.694	0.972	-	0.507	-	0.507
4.	Acetone	No. of drops	-	-	1	-	1	-	-	-	-	-
		Rf value	-	-	0.555	-	0.959	-	-	-	-	-
5.	Methanol	No. of drops	-	-	-	-	-	-	-	-	-	-
		Rf value	-	-	-	-	-	-	-	-	-	-

*SD - *Scoparia dulcis* , EA - *Eclipta alba*

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

In each solvent system III, IV and V, 1 spot was detected Rf values 0.97, 0.51 and 0.51.

Acetone extract of *Scoparia dulcis* in solvent system II and III, 1 spots detected Rf value 0.55 and 0.96 while acetone extract of *Eclipta alba* didn't show any result.

TLC studies of the methanol extract of both the plants (*Scoparia dulcis* and *Eclipta alba*) did not show any result in all 5 solvents (Solvent I, II, III, IV and V) (Table 2).

Discussion

Hexane extract of *Eclipta alba* showed the presence of alkaloids, fats and fixed oil, flavonoids, protein, terpenoids, triterpene. In previous studies alkaloids was detected in the hexane extract of *Eclipta alba* [10,11]. Chloroform extract of plants revealed the presence of fats and fixed oil, steroids, protein and triterpene. This all compounds have already been studied by some of the researchers [10]. Ethyl acetate extract gives the presence of alkaloids, fats and fixed oil, phenol, protein, tanins in this study.

The hexane extract showed that fats and fixed oil, flavonoids, protein, terpenoids and triterpene are present in *Scoparia dulcis* also phytochemical screening of the ethyl acetate extract revealed the presence of alkaloids, anthocyanin, balsams, fats and fixed oil, phenol, protein, tannins [12].

The chloroform extract of *Scoparia dulcis* showed that anthocyanin, balsams, fats and fixed oil, steroids, phenol, protein, tannin and triterpene.

The phytochemicals detected in the acetone extracts of both plants (balsams, carbohydrate, cardiac glycoside, fats and fixed oil, flavonoids, phlobatannins, tannins and terpenoids) have been associated with antimicrobial activities in our study.

The phytochemical screening of *Eclipta alba* showed the presence of different groups of secondary metabolites (alkaloids, anthocyanin, balsams, cardiac glycoside, fats and fixed oils, flavonoids, steroids, phenols, phlobatanins, proteins, resins, saponin, tannin, terenoid and triterpene) when the entire plant was extracted in n- hexane, ethyl acetate, chloroform, acetone and methanol. Similar result was also reported by Sharma and Sharma et al. [10,11,13]. In paper reported by Sharma and Sharma et al., the phenolic group showed result in ethyl acetate extract, which was similar the result obtained in the current research. As reported by Mithun et al. and Maged et al., the whole plant part of *Eclipta alba* is reported to have shown the presence of bioactive compound viz., glycosides, saponins and triterpenes, while the methanol extract resulted in the identification of eight bioactive compounds of steroidal alkaloids [13,14].

Different Rf values was recorded for all different solvent system. This variation in Rf values of the phytochemicals specifies a very significant clue in understanding of their polarity and helps in selection of appropriate solvent system for separation of pure compounds chromatography technique. The selection of suitable solvent system for a specific plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system. Different Rf values of the bioactive compound also reveal an idea about their polarity. For separation of pure compound from plant extract, mixture of solvents with variable polarity in different ratio can be used. This data will help in selection of suitable solvent system for further separation of compound from these plant extracts [9,10].

Conclusion

Phytochemical analysis of *Scoparia dulcis* and *Eclipta alba* were performed and found various phytochemicals, including alkaloids, anthocyanin, balsams, carbohydrates, cardiac glycoside, fats and fixed oil, flavonoids, steroids, phenol, phlobatanins, protein, resin, saponin, tannin, terpenoid, triterpene are present in both plants. *Soparia dulcis* and *Eclipta alba* plants are rich in secondary metabolites and it has potential for development into a phytomedicine. This phytochemical reported here for its potential drug activity in plant could be useful for monograph development and for quality control purposes [12].

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