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Preliminary phytochemical investigation and TLC profiling of ethnomedicinally important plant, *Scoparia dulcis* Linnaeus

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Abstract

Ethnomedicinal plants have played a significant role in the field of natural product research and drug discovery since ancient times. *Scoparia dulcis* Linnaeus (Scrophulariaceae) is an ethnomedicinal plant mostly used as herbal remedy in indigenous communities for the treatment of diabetes mellitus and hypertension. The present study was undertaken to evaluate the phytochemical investigation and TLC profiling of four different solvent extracts of *Scoparia dulcis*. The result showed the presence of alkaloid, saponin, tannin and flavonoid while TLC profiling in different solvent system at different polarity index revealed the presence of diverse group of phytochemicals.

Key words: Ethnomedicinal plant, *Scoparia dulcis*, Phytochemical investigation, TLC profiling, phytochemicals

INTRODUCTION

Ethnomedicinal plants are of immense importance in modern drug research. These are natural resources yielding valuable herbal products which are often used in the treatment of various ailments (Dulger & Gonuz 2004). Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy (Rashid *et al* 1997; Cowan 1999). For this purpose the use of plant extracts in traditional medicine has been going on from ancient time (Foye 1995). *Scoparia dulcis* Linnaeus (Scrophulariaceae) is an important ethnomedicinal plant, commonly known as sweet broom weed, belongs to the family scrophulariaceae. It is a perennial herb widely distributed in tropical and subtropical regions (Zulfiker *et al* 2010a). In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach troubles, hypertension (Sadhu *et al* 2003), diabetes, bronchitis (Gonzales Torres 1986) and as analgesic and antipyretic agents (Farias Freie *et al* 1993). In view of its high reputation and wide acceptance in ethnomedicine, this plant has attracted not only wide publicity but also intensified research efforts by researchers (Branch & da Silva 1983; Denis 1988). More recently, a number of the speculated medicinal values of *S. dulcis* have been validated by scientific research. These include hypoglycaemic activity (Zulfiker *et al* 2010b; Jain 1985; Pari & Latha

2005), antitumor promoting activity (Nishino *et al* 1993), antiviral activity (Hayashi *et al* 1990), hyperlipidaemia activity (Orhue & Nwanze 2006), antioxidant and analgesic activity (Zulfiker *et al* 2010a,b). A significant analgesic activity was also demonstrated along with the antihyperalgesic activity for *S. dulcis* decoction (Ratnasooriya *et al* 2003). Later it was investigated the antibacterial and antifungal activity of *S. dulcis* (Latha *et al* 2006) by another author. The present study was undertaken to evaluate the phytochemical screening and TLC profiling (Yisa 2009; Okhale *et al* 2010; Muthumani *et al* 2010; Ali *et al* 2011) of different solvent extracts of this ethnomedicinal plant to ascertain the presence of diverse form of secondary metabolites which are responsible for these bioactivities.

MATERIALS AND METHODS

Collection, drying and grinding of plant materials

The fresh plant material (whole plant) was collected from Assam University Campus, Silchar, Assam during the flowering and fruiting periods. The identification was confirmed at Assam University Herbarium. The plant material was washed with water to remove soil debris, cut into small pieces and air dried at room temperature (25° to 30° C). The dried samples were then finely ground and were kept in an airtight clean container at room temperature until further uses.

Preparation of plant extracts

The plant extracts were prepared by successive extraction with solvents of increasing polarity, from a nonpolar such as hexane or petroleum ether to a more polar solvent such as methanol to ensure that a wide polarity range of compound could be extracted. The powdered samples (500 grams) were first placed in a stoppered container with the solvent petroleum ether (bp 40° - 60°) and allowed to stand at room temperature for a period of at least 3 days (72 hours) with frequent agitation until the soluble matter is dissolved. The extract was then filtered with Whatman No. 1 filter paper. The marc left after extraction was air dried and again extracted with solvent ethyl acetate for another 72 hours. This was followed by extraction with solvent acetone and finally methanol. All the four extracts were transferred into sterile bottles and kept in refrigerator until further uses.

Protocol for preliminary phytochemical screening

The preliminary phytochemical screening of the crude extracts for the presence of alkaloid, reducing sugar, steroid, flavonoid, saponin and tannin were done by following standard protocol.

Test for Alkaloids

Alkaloids solution produces white yellowish precipitate when few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (Siddiqui & Ali 1997).

Test for reducing sugar

To 0.5 ml of extracts solution, 1ml of water and 5 – 8 drops of Fehling's solution was added and observed for brick red precipitate.

Test for Steroid

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly. Green bluish colour observed for the presence of steroids (Siddiqui & Ali 1997).

Test for Flavonoid
Four millilitres of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 – 6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones (Siddiqui & Ali 1997).

Test for Saponins
About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing shows the presence of saponins (Iyenger 1995).

Test for Tannins
To 0.5 ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins (Iyenger 1995).

TLC profiling of the fractions
The crude extracts of *Scoparia dulcis* Linnaeus *viz*., Petroleum ether (PE), Ethyl acetate (EA), Acetone (A) and Methanol (M) were subjected to TLC in solvents of different polarity at different ratios. Each of the four extracts was run in five different solvent systems *viz*., PE, PE: EA=9:1, PE: EA=4:1, PE: M=9.5:0.5 and PE: EA: M= 8:1:1 on analytical plates over Silica gel (TLC grade, Merck India).

In each case, if the components of the sample are coloured, they can be observed directly. If not, they can sometimes be visualized by shining ultraviolet light (365 nm) on the plate or by allowing the plate to stand for a few minutes in a closed container in which the atmosphere is saturated with iodine vapour. Sometimes the spots can be visualized by spraying the plate with a reagent such as $K_2Cr_2O_7$ that will react with one or more of the components of the sample. Finally, the *Rf* values of each were calculated out.

RESULTS

The preliminary phytochemical screening showed the presence of alkaloid, saponin, tannin and flavonoid in four crude extracts (Table 1). The TLC profiling of four different crude extracts in different solvent systems confirms the presence of diverse group of phytochemicals (Table 2).

Table 1. Preliminary phytochemical screening of *Scoparia dulcis* Linnaeus

Extracts	Alkaloid	Reducing sugar	Steroid	Saponin	Tannin	Flavonoid
Petroleum Ether	+	-	-	+	+	+
Ethyl Acetate	-	-	-	+	+	+
Acetone	+	-	-	+	-	-
Methanol	-	-	-	+	+	+

*Here '+' indicates the presence and '-' indicates the absence of specific secondary metabolite

DISCUSSION

Phytochemicals are the dependable sources for the treatment of different ailments. Their screening provides useful information regarding the presence of different secondary metabolites of clinical significance. Till date many researches have already been carried out on *Scoparia dulcis* Linnaeus in different parts of the world, but very little work has been

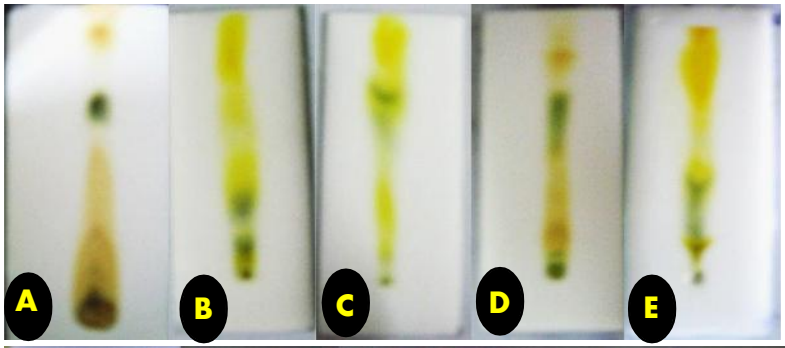

Fig. 1: TLC profiling of Petroleum ether extract with A. Pure Petroleum ether, B. PE:EA=9:1, C. PE:EA=4:1, D. PE:M=9.5:0.5 and E. PE:EA:M=8:1:1

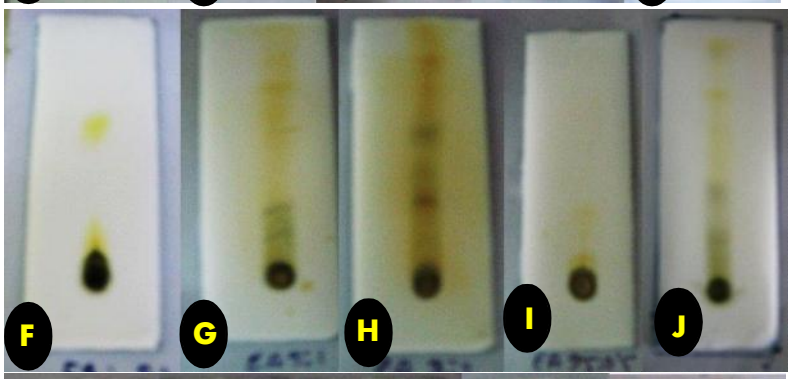

Fig. 2: TLC profiling of Ethyl acetate extract with F. Pure Petroleum ether, G. PE:EA=9:1, H. PE:EA=4:1, I. PE:M=9.5:0.5 and J. PE:EA:M=8:1:1

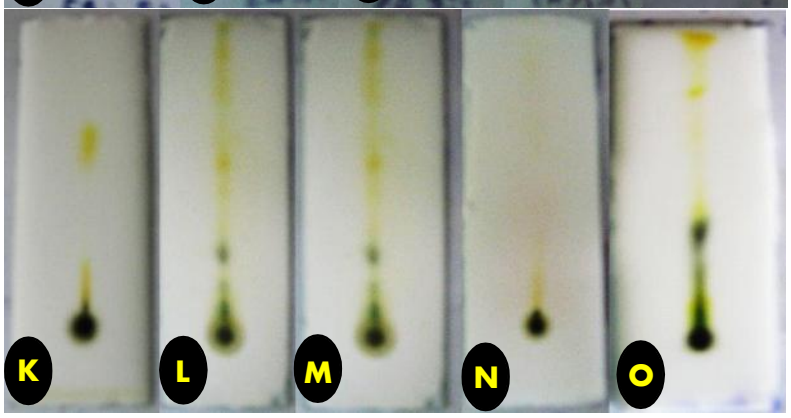

Fig. 3: TLC profiling of Acetone extract with K. Pure Petroleum ether, L. PE:EA=9:1, M. PE:EA=4:1, N. PE:M=9.5:0.5 and O. PE:EA:M=8:1:1

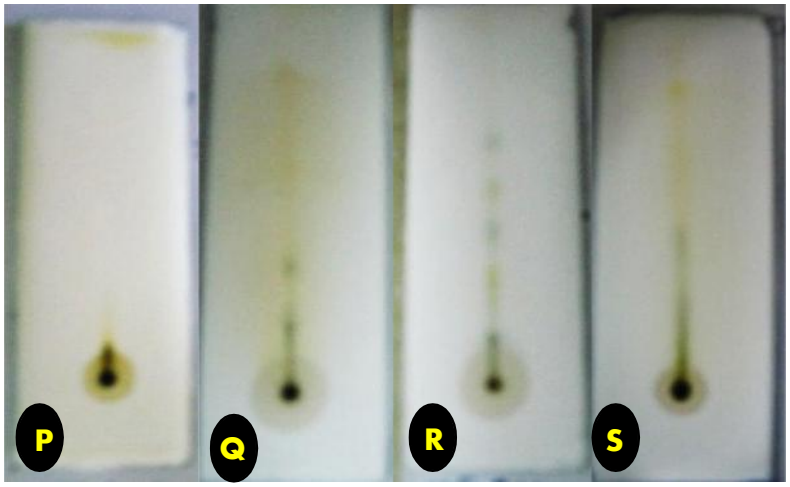

Fig. 4: TLC profiling of Methanol extract with P. Pure Petroleum ether, Q. PE:EA=9:1, R. PE:EA=4:1 and S. PE:EA:M=8:1:1

Table 2. The Retention factors of each of the four extracts in five different solvent systems

Extracts	Developing Solvent Systems				
	PE	**PE:EA=9:1**	**PE:EA=4:1**	**PE:M=9.5:0.5**	**PE:EA:M=8:1:1**
Petroleum ether	0.08,0.17,0.69	0.11,0.24,0.4, 0.61,0.82	0.07,0.23,0.5, 0.68,0.87	0.06,0.12,0.18, 0.40,0.65,0.81, 0.89	0.10,0.32,0.52, 0.66
Ethyl Acetate	0.1,0.25,0.55, 0.79	0.08,0.15,0.19, 0.25,0.6,0.75, 0.91	0.15,0.3,0.33, 0.56,0.83,0.9	0.06,0.11,0.27, 0.63	0.11,0.2,0.35, 0.73,0.90
Acetone	0.13,0.31,0.55, 0.78	0.08,0.11,0.17, 0.27,0.91,0.95	0.06,0.12,0.22, 0.32,0.43,0.54, 0.77,0.93	0.06,0.14,0.56, 0.81	0.08,0.32,0.64, 0.75,0.95
Methanol	0.1,0.2	0.16,0.21,0.3, 0.56,0.78	0.15,0.27,0.47, 0.5,0.61,0.85	0.11,0.16,0.28	0.19,0.39,0.8,0.9

undertaken for the North-East India, especially in Assam. In the present study, preliminary phytochemical screening of the extracts of this species from Southern Assam revealed the presence of alkaloid, saponin, tannin and flavonoid. Also the TLC profiling at different solvent ratios showed the presence of diverse group of phytochemicals which reflects an idea about the polarity of these components as well as provides useful information regarding the selection of appropriate solvent system for their separation. These current findings are in accordance with previously documented works from Nigeria (Yisa 2009; Okhale *et al* 2010), Kerala (Muthumani *et al* 2010), Ebiraland (Ali *et al* 2011). Thus, current study also justifies the presence of diverse group of secondary metabolites in this plant from Southern Assam. These screened secondary metabolites are validated to have various medicinal properties *viz.* Alkaloids had been reported to have anticancer, antidiabetic, anti-aging and antiviral activities (Evans & Trease 2002). Saponin has been reported to be cardio-tonics (Evans & Trease 2002). Tannins are responsible for curing diabetes, diarrhoea, sore throat, skin ulcer and dysentery. Flavonoids are used to cure cancer, inflammations and allergies (Evans & Trease 2002; Cushine & Lamb 2005). Therefore, further investigation is necessary to obtain more information and also to isolate some bioactive compounds from this ethnomedicinal plant.

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