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SECLUSION OF BIOACTIVE COMPONENTS FROM FLOWER OF *SAMANEA SAMAN*

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Abstract

Phytochemical exploration is a prime stage for the discovery of active organic component. The *S. saman* flower extracts with different solvents were screened by standard phytochemical methods. The purpose of the existing investigation was to explore the existence of phytochemicals. Successive organic solvent extraction and chromatographic screening like TLC and CC were done. This analysis disclosed the occurrence of various secondary metabolites e.g., flavonoids, tannins, saponins, carbohydrates, terpenoids, phenols and glycosides in the aerial part (flower) of the plant. The existence of different compounds supports the use of S. saman for many disorders by customary experts. However, differentiation of organic constituents may proceed to detect a new medicine.

Key words: *Samanea saman*, solvent extraction, phytochemical screening, chromatographic techniques.

Introduction

Plants are the leading origin of crude ingredients for ancestral and modern medicine. Medicinal plants have been used for centuries before the advent of orthodox medicine. Phytochemicals are naturally existing organic substances that have inherent disorder preventing abilities (Yusaf *et al*., 2014; Afolabi *et al*., 2007). They are basically plant metabolites andare spontaneously produced in all portions of the plant. The amount and grade of photochemicals exist in plant portions may alter from one part to another (Lahlou, 2004). The most significant bioactive components of plant are steroids, flavonoids, alkaloids, tannins, terpenoids, glycosides, etc. Antibiotics or antibacterial ingredients like saponins, glycosides, flavonoids and alkaloids etc, are incurred to be dispersed in plants (Sardhara and Gopal, 2013; Tiwari *et al*., 2011; Edeoga *et al*., 2005).

Samanea saman (Rain tree), family fabaceae is rises upto thirty meter high. It is massive uplift disarmed, deciduous plant distributed throughout India. It has doubly pinnate leaves; bark irregular surface, gray and globose head of odorous reddish pink flower, four cm. extended bicolored stamens which is white in lower half and magenta upper half; devoloped pods are black-brown, 10-30 cm. extended and seeds 15-20 per pod, dark glossy brown (Anonymous, 2016; Staples and Elevitch, 2006). The rain tree is a ancestral medicine for colds, diarrhea, headache, intestinal diseases, acute bacillary dysentery, enteritis, diarrhea, sore throat and stomach pain (Thippeswamy *et al*., 2011; Ragasa *et al*., 2013; Parekh and Chanda, 2008). In the present investigation, numerous solvent extracts of *Samanea Saman* flower were qualitatively analyzed for the investigation of phytoconstituents using popular assessments. Successive organic solvent extraction and chromatographic screening like TLC and CC were done.

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Materials and Methods

Collection of plant materials

Flowers of *Samanea saman* were collected from Purba Medinipur area, W.B. in the month of April, 2016 and the materials were recognized taxonomically by the scientist of BSI Shibpur, Howrah.

Preparing of Plant Materials

The plant Materials (petals of flower) was shorted; shade dried and pounded into coarse powdered. The powdered examples were put away in a spotless glass compartment until required with appropriate marking for examination.

Preparation of plant extracts

Crude extract of flower was prepared by Soxhlet extraction technique. Around 20 gm of powdered plant material was consistently pressed into a thimble and

extricated with 250 ml of various solvents independently. Solvents utilized were pet. ether, chloroform, ethyl acetate, acetone, methanol, ethanol and water according to extremity. The procedure of extraction proceeds for 24 hours; after that the extracts was put into in a rotary evaporator for the concentration of extract and solvent recovery. Dried concentrate was kept in cold condition at 4ºC for their future use in phytochemical investigation.

Qualitative phytochemical analysis

• **Phytochemical Screening:**

The crude extracts were examined for the occurrence of bioactive compounds by utilizing standard methods. Preliminary screening for the secondary metabolites were carried out as per standard methods *e.g.*, alkaloids (Wagner's Test**)**, carbohydrate (Molisch test), cardiac glycoside (Keller Kelliani's test), flavonoids (Shinoda test), phenols (Ferric chloride test), phlobatannins (Precipitate test), amino acids and proteins (Ninhydrin test), saponins (Foam test), sterols (Libermann-Burchard test), coumarins (Sodium hydroxide test), tannins (Braymrer test), terpenoids (Swalkowski test), quinines (Hydrochloric acid test), oxalate (Acetic acid test) etc. (Trease and Evans, 1989; Kokate, 1994; Misra *et al*., 2011; Harborne, 1973; Sofowra, 1973).

Separation of Chemical constituents by chromatographic analysis

• **Column Chromatography:**

60-120 mesh size silica gel (Merck, Germany) was dissolved in the low polarity solvent hexane and tightly packed in 50 × 150 mm glass column up to 100 mm height without air bubbles. Then the flower extracts were loaded in separate glass columns and fractionated with solvents hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol at different extent of dissolvable blend which are shown in table 2 (Morhing *et al*., 2010; Koilpitchai *et al*., 2015).

Thin Layer Chromatography (TLC)

The various fraction collected from CC were subjected to TLC analysis. TLC plate were prepared by silica gel GF_{254} (Merck, Germany) of 60 mesh. Different fractions are loaded on the activated TLC plate using capillary tube and eluted by preparing various solvent systems at definite ratio as per polarity order (trial and error basis). The spot on the TLC plate was visualized by UV light (254 nm) and spraying Goldin's reagent (vanillin-sulphuric acid reagent) and R_f value was determined by measuring solvent and solute front which are shown in table 3 (Braithwaite and Smith, 1999; Shahzad *et al*., 2013).

Results and Discussion

In the preliminary phytochemical screenings of flower extracts with various solvents (pet. ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water) it is revealed to be present of flavonoids, saponins, tannins, carbohydrate, terpenoids, glycosides and phenolic compounds in flower extracts of *S. saman* (Table 1). This shows the plant constituents which have elevated level of restorative qualities. This could be liable for the adaptable therapeutic properties of plant (Oloyed, 2005; John *et al*., 2011).

The presence of saponins and phenols could give antibiotic property on the plant. This is supported by the findings of (Jacob and Burri, 1996; Eka, 1998). These compounds serve as natural antibiotics, which help the

Table 1: Result of phytochemical assessment of flower of *S. saman.*

S. No.	Phytochemicals/ Solvent Extracts	Pet. Ether	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water
1	**Alkaloids**	-	-	-	-	-	-	-
2	**Cardiac Glycosides**	-	-	-	+	+	+	-
3	**Carbohydrates**	+	+	+	+	+	+	+
4	**Flavonoids**	-	-	+	+	+	+	-
5	**phenols**	-	-	-	+	+	+	-
6	**Phlobatannins**	-	-	-	-	-	-	-
7	**Proteins**	-	-	-	-	-	-	-
8	**Saponins**	+	-	-	-	-	-	-
9	**Sterols**	-	-	-	-	-	-	-
10	**Tannins**	-	-	-	-	-	+	+
11	**Terpenoids**	-	+	+	+	+	+	-
12	**Quinones**	-	-	-	-	-	-	-
13	**Oxalates**	-	-	-	-	-	-	-

+ = present; - = absent.

Table 2: Solvent system used for the Column chromatographic isolation.

Fractions	Solvents	Ratio of solvents	Volume (ml)	Code of fractions
1	Pet.ether: Chloroform	8:2	20	C_1
2	Ethyl acetate: Mehanol	6:4	20	C_2
3	Acetone: Chloroform	9:1	20	C_3
4	Methanol: Chloroform	7:3	20	C_4

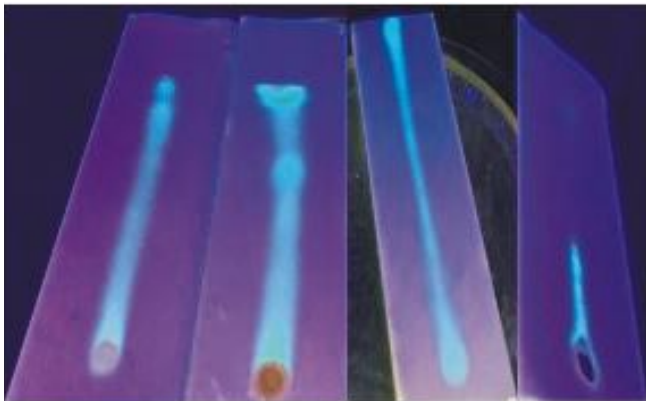

Fig. 1: TLC of the fractions.

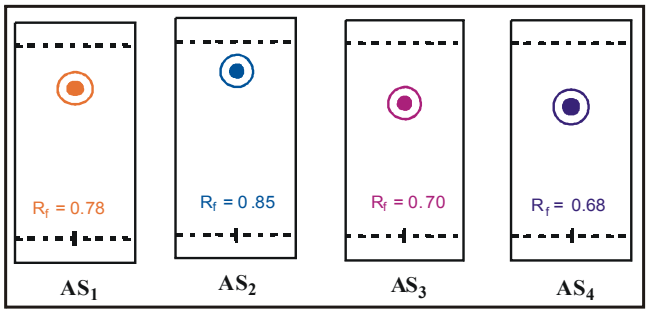

Fig. 2: TLC of the isolated compounds.

body to fight infections and microbial invasion combat microbes and viruses and knock out some tumor cells, particularly lung and blood cancers (Poornima and Ravishankar, 2009; Amin *et al*., 2013). Tannins are known to hinder pathogenic fungi. Flavonoids for the most part present in areal parts like blossoms assume some metabolic job and control improvement in living framework. They are likewise engaged with defensive capacity in creatures and are utilized as medication particularly the flavonol glycosides. The flavonoids and phenolics in plant have been accounted for to apply various organic impacts including antioxidant, free radical scavenging capacities, anti inflammatory, anti carcinogenic and so on (Das *et al*., 2010; Sodipo *et al*., 2000).

The different crude extract of pet. ether, chloroform, ethyl acetate, acetone, ethyl alcohol, methanol and water were collected by successive solvent extraction techniques, the concentrated crude extracts were subjected to TLC and column chromatographic techniques for separation of phytoconstituents. The ethanol extract was chromatographed on silica gel column by eluting solvents at definite proportion. From the pet.ether: chloroform (8:2, v/v) fraction (C-1), a pale yellow or cream colour compounds marked as AS_1 was obtained. The ethyl acetate fraction was charged on silica gel column and eluted, the ethyl acetate: methanol (6:4, v/v) eluent fraction (C-2), a pale yellow colour compound marked as AS_2 was obtained. The acetone soluble fraction (C-3) was found to contain a pale yellow compound chromatographically by eluting acetone: chloroform (9:1, v/v) with clear distinct on TLC plate, which was further purified on TLC plate, marked as AS_3. The methanol soluble fraction (C-4) gave a brown compound which was separated by elution of acetone: chloroform (9:1, v/v), yielded a compound labeled as AS_4.

TLC has been a good analytic technique for isolation and identification of various compounds. Number of UV-VIS components from the ethanol extract of areal parts (flowers) of *Samanea saman* has been identified through their respective R_f values. TLC of the four fractions *i.e*., C-1, C-2, C-3 and C-4 showed a distinct spots of compound AS_1, AS_2, AS_3 and AS_4 having R_f values 0.78, 0.85, 0.70 and 0.68 respectively.

Conclusion

The secondary metabolites are known to be biologically active and play significant roles in bioactivity of medicinal plants. Phytochemicals found in flower of *Samanea saman* indicates their prospective as a source of herbal medicines. The presence of these phytochemicals in this plant enhances their pharmaceutical and therapeutic potentials in the traditional treatment against various diseases affecting humans and animals. The phytochemical examination of the extracts, the seclusion of accountable bioactive compounds and their organic action are vital for future investigations. Further examinations are hence recommended to find out their distinctive pharmacological exercises. Solvent extraction, preliminary

Table 3: Summary of TLC after Column Chromatographic separation.

Fractions	Colour	Solvent System		R_f Value
		Solvents	Ratio	
C_1	Light Yellow	Pet. Ether: Chloroform	8:2	0.78
C_2	Pale Brown	Ethyl acetate: Methanol	6:4	0.85
C_3	Pale Yellow	Acetone: Chloroform	9:1	0.7
C_4	Brown	Methanol: Chloroform	7:3	0.68

phytochemical screening and chromatographic study (TLC and CC) helps in isolation, purification, authentification from adulteration for the quality control of herbal raw drugs and such analysis exhibited a significant role in medicinal chemistry for the formulation of life saving drugs.

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