Research Article

PHYTOCHEMICAL SCREENING OF SOLVENT EXTRACTS OF SWEET FLAG (SF) ON STORED PRODUCT INSECTS

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ABSTRACT

Investigations were carried out to test the preliminary phytochemical screening of sweet flag (SF) rhizome (*Acorus calamus* L.) extracts at the Department of Agricultural Entomology, Agricultural College and Research Institute, Coimbatore. Sweet flag (SF) rhizome extracts were obtained in different extraction methods (soxhlet and mechanical shaker) using various solvents *viz*., hexane, ethyl acetate and ethanol. In soxhlet apparatus, 75.34, 8.56 and 29.56 g of SF oil extracts comprising of various compounds were obtained from 1200g of sweet flag rhizome powder, when extracted sequentially with hexane, ethyl acetate and ethanol, respectively. A quantity of 47.84, 27.92 and 18.00 g of SF oil extracts were consisting of different compounds obtained from 1200g of sweet flag rhizome powder, when extracted sequentially with hexane, ethyl acetate and ethanol, respectively using mechanical shaker. In soxhlet apparatus extraction, various solvent extract of SF showed the presence of tannins, flavonoids, steroid, terpenoid, glycosides, carbohydrates and protein. In mechanical shaker extraction, various solvent extract of SF showed the presence of alkaloids, phenolic compounds tannins, flavonoids, steroids, terpenoids, glycosides, carbohydrates and protein. Among the two extraction methods used, the soxhlet extraction method resulted in higher oil yield in compare to the extraction by mechanical shaker. Interestingly, the insecticidal activity was recorded higher in mechanical shaker extraction method. This could be attributed to the higher solubility of extractable bioactive components such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides, carbohydrates and proteins that have insecticidal action. Phytochemicals have a synergistic effect that enhances the insecticidal action of solvent extracts of sweet flag.

INTRODUCTION

Sweet flag (*Acorus calamus* L.) is an herbaceous perennial with erect aromatic leaves ascending from a branched rhizome. It is cultivated in wetland where water stagnation is possible. Rice can also be cultivated with sweet flag as an intercrop (Nelson *et al*., 2007). Sweet flag has been used in many ways since biblical times. Saint Moses received instructions from God to use must frankincense, cinnamon, calamus, cassia, galbanum and sweet spices to make of the holy oil.

In India, sweet flag rhizome has been used in Siddha, Ayurveda and Unani traditional medicines. It is being used in many countries to cure various diseases, including cancer in China and Tibet (Motley, 1994). In Tamil Nadu, the extracts of sweet flag rhizome are given to babies while the bits of sweet flag rhizome are tied to emit fragrance and repel insects. It is being proved to be anti-cancer in humans (Gaidhani *et al*., 2009 and Linna *et al*., 2013).

Phytochemical is a broad term meaning plant (phyto) chemical referring to a wide variety of compounds that occur naturally in plants. Arunkumar and Muthuselvam (2009) Medicinal plants contain some natural products which perform definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids Edoga *et al*. (2005). These compounds are synthesized by primary or rather secondary metabolism of plants. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Treare and Evans, 1978). This paper focuses on the

extraction efficiency and phytochemical investigation in *A. calamus* (L.)**.**

MATERIALS AND METHODS

Extraction methods

Soxhlet extraction

The powdered sweet flag rhizome of 100 g was sequentially extracted with 700 ml of hexane (non polar), ethyl acetate (medium polar) and ethanol (high polar) solvent on soxhlet's extraction apparatus for 6, 10 and 10 h, respectively. The solvents were evaporated in a rotary vacuum evaporator at 40 °C. The obtained extracts were pale yellow to pale brown in colour, viscous liquid, having a pleasant woody and spicy odour.

Extraction using mechanical shaker

Thirty grams of sweet flag rhizome powder were sequentially soaked in 150 ml of hexane, ethyl acetate and ethanol solvent for overnight (16 hr) separately. Shaking was done in a mechanical shaker at 250 rpm for 1hr on next day and the solvents were filtered through using whatman No 1 filter paper. The filtered solvents were evaporated in a rotary vacuum evaporator at 40 °C. The obtained extracts were pale yellow to dark brown in colour, viscous liquid, having a pleasant woody and spicy odour

Phytochemical screening for sweet flag rhizome extracts

i) Test for alkaloids

Dragendorff's test: To a few ml of filtrate, one or two drops of dragendorff's reagent were added. Formation of reddish brown precipitate indicates the presence of alkaloids.

ii) Test for phenolic compounds

a) Ferric chloride test: The extract was dissolved in 5 ml of distilled water and to this 5 per cent of ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds.

b) Lead acetate test: 5 mg extract was dissolved in 5 ml of distilled water and to this 3ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

iii) Test for tannins: Extracts were treated with few drops of lead acetate solution. The formation of white precipitate indicated the presence of tannins.

iv) Test for flavonoids

Alkaline reagent test: An aqueous solution of extract was treated with 10 per cent of ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

v) Test for saponins: The extracts (5 ml) was taken in a test tube and a pinch of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3min. A honeycomb like froth indicated the presence of saponins.

vi) Test for steroids

a. Salkowski's Test: Dissolved a little sample in 2 ml of chloroform in a dry test tube and 2 ml of conc. H_2SO_4 was added and shaken gently. The upper layer turns red and the layer shows a yellow colour with green fluorescence.

b. Liebermann-Burchard reaction: Dissolved a little sample in 2 ml of chloroform in a dry test tube and added 10 drops of acetic anhydride and 2 drops of sulphuric acid and shaken gently. A red colour was formed which quickly changes through blue to green.

vii) Test for terpenoids: The extract (2 ml) was added to 2 ml of acetic anhydride and concentrated H_2SO_4. Formation of blue, green rings indicated the presence of terpenoids.

viii) Test for glycosides

a) Liebermann's test: Crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was then cooled in ice and few drops of concentrated sulphuric acid was added. A colour changes from violet to green indicated the presence of glycosides.

b) Salkowski's test: Crude extract mixed with 2 ml of chloroform and then 2 ml of concentrated sulphuric acid was added carefully and shaken gently. A reddish brown colour indicated the presence of glycosides.

c) Keller-Killani test: Crude extract was mixed with 2 ml of glacial acetic acid containing two drops of 25% ferric chloride solution. The mixture was poured into another test tube containing 2 ml of concentrated sulphuric acid. A brown ring at the interphase indicates presence of glycosides

ix) Test for carbohydrates

a) Fehling's test: The filterate (1 ml) was taken in a test tube and 1 ml of Fehling solution A and B was added and heated. Red precipitate indicated presence of carbohydrates.

b) Barfoed's test: The filterate (1 ml) was taken in a test tube and 1 ml of Barfoed's reagent was added and heated on boiling water bath for 2 min. Red precipitate indicated presence of carbohydrates

c) Benedict's test: The filterate (0.5 ml) was taken in a test tube and 0.5 ml of suggested Benedict's reagent was added. The mixture was then heated on a boiling water bath for 2min. A characteristic coloured precipitate indicated presence of carbohydrates.

Table 1. Quantity of extract obtained in different solvent and extraction methods

Solvents	Quantity of extract obtained (g) / 1200 gm of sweet flag rhizome powder	
	Soxhlet apparatus	Mechanical shaker
Hexane (Non polar)	75.34	47.84
Ethyl acetate (Medium polar)	8.56	27.92
Ethanol (High polar)	29.56	18.00

x) Test for proteins

a. Millon's test: The filterate (0.5 ml) was taken in a test tube and few drops of millon's reagent was added. Reddish brown colour was observed which indicates the presence of proteins.

Table 2. Phytochemical screening of the solvent extracts of sweet flag rhizome

Sl. No	Phytochemicals	Qualitative tests	S - H	S - EA	S - E	M - H	M - EA	M - E
1	**Alkaloids**	Dragendroffs reagent	-	-	+	+	+	+
2	**Phenolic compounds**	a) Ferric chloride	-	-	+	-	-	+
		b) Lead acetate	-	-	+	+	+	-
3	**Tannins**	Lead acetate test	+	+	-	+	+	+
4	**Flavonoids**	Alkaline reagent test	+	-	-	+	+	+
5	**Saponins**	Foam test	-	-	-	-	-	-
6	**Steroids**	a) Salkowski's	+	+	-	+	-	-
		b) Libermann-Burchard	+	+	-	+	+	-
7	**Terpenoids**	Acetic anhydride test	+	-	-	+	-	-
8	**Glycosides**	a) Libermann's	-	-	-	-	-	-
		b**)** Keller-Killiani	+	-	-	+	-	-
		c) Salkowski's	+	+	+	+	+	+
9	**Carbohydrates**	a**)** Fehling's reagent	-	-	+	-	-	+
		b**)**Barfoed reagent	-	-	-	-	-	-
		c) Benedict's reagent	-	+	+	-	+	+
10	**Protein**	Millon's	+	+	+	+	+	+

H- Hexane EA- Ethyl acetate E- Ethanol S- Soxhlet apparatus extraction M- Mechanical shaker extraction. Qualitative analysis shows visually observable product and depicted as presence (+) or absence (-) of phytochemicals in the extracts.

RESULTS AND DISCUSSION

Quantity of extract obtained in different solvents and extraction methods

In soxhlet apparatus, 75.34 (6.67%), 8.56 (0.06%) and 29.56 (2.46%) g of extracts comprising of various compounds were obtained from 1200g of sweet flag rhizome powder, when extracted sequentially with hexane, ethyl acetate and ethanol respectively. A quantity of 47.84 (3.98%), 27.92 (2.32%) and 18.00 (1.50%) g of extracts were consisting of different compounds obtained from 1200g of sweet flag rhizome powder, when extracted sequentially with hexane, ethyl acetate and ethanol respectively using mechanical shaker (Table1).

Phytochemical screening of the solvent extracts of sweet flag rhizome

In soxhlet apparatus extraction, hexane extract of sweet flag rhizome showed the presence of tannins, flavonoids, steroid, terpenoid, glycosides and protein. Ethyl acetate extract of sweet flag rhizome showed the presence of tannins, steroid, glycosides, carbohydrates and proteins. Ethanolic extract of sweet flag rhizome showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycoside carbohydrates and proteins. In mechanical shaker extraction, hexane extract of sweet flag rhizome showed the presence of alkaloids, phenolic compounds tannins, flavonoids, steroids, terpenoids, glycosides and protein. Ethyl acetate extract of sweet flag rhizome showed the presence of alkaloids, phenolic compounds, tannins, flavonoids, steroid, glycosides, carbohydrates and proteins. Ethanolic extract of sweet flag rhizome showed the presence of alkaloids, phenolic compounds, flavonoids, tannins, glycoside carbohydrates and proteins (Table 2).

Among the two extraction methods used, the soxhlet extraction method resulted in higher oil yield in compare to the extraction by mechanical shaker. Interestingly, mechanical shaker extraction was showed higher insecticidal activity on stored product insects than soxhlet extraction. This could be attributed to the higher solubility of extractable bioactive components such as alkaloids, flavonoids, phenolic compounds, tannins, glycosides, carbohydrates and proteins that have insecticidal action. Phytochemicals have a synergistic effect that enhances the insecticidal action of solvent extracts of sweet flag. The variation in the extraction yield could also be due to the difference in polarity of the solvents used, which plays a key role in increasing the solubility of phytochemical compounds. Neha *et al*. (2012) also reported that ethanolic extract of sweet flag showed the presence of alkaloids, carbohydrates, glycosides and tannins. The present findings could be corroborated with the findings of Asha and Kumar (2015) who reported that methanolic extract of sweet flag

rhizome showed the presence of flavonoids, tanins, saponins, steroids, carbohydrates and phenolic compounds.

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