QUALITATIVE ANALYSIS OF SECONDARY METABOLITES FROM *CANAVALIA CATHARTICA* THOUARS AND *RANDIA SPINOSA* POIR.

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ABSTRACT

Phytochemical screening of the seed extract of wild bean, *Canavalia cathartica* Thouars and fruit extract of emetic nut, *Randia spinosa* Poir confirmed the presence of many major secondary metabolites like alkaloid, tannin, terpenoid, coumarin, glycoside, phenol, quinone and flavonoid. The results revealed absence of saponins. The presence of these secondary metabolites with a spectrum of anti-insect properties highlighted the scope of development of a new phyto-insecticide.

Keywords: *Canavalia cathartica*, *Randia spinosa*, secondary metabolites, qualitative analysis.

INTRODUCTION

Insects known to have diverse feeding habits compete for food and phytophagous insects among them have a direct impact on crop production [1]. 20 per cent of the total crop produced is destroyed annually by these

herbivorous insects. Managing these pests is an ever challenging act that necessitates continued updating of pest management strategies. Because of the problems posed by the synthetic insecticides, renewed interest on plant secondary metabolites demonstrating diverse responses against pests progressed. These compounds that are not directly involved in plant's growth and development were evolutionary arsenals in plant's fight against herbivory [2]. Their range in the plant kingdom is wide and some of them are alkaloids, tannins, terpenoids, coumarins, glycosides, phenols, quinones, flavonoids etc., [3]. For successful utilization of any botanical as a phyto insecticide, their qualitative analysis exhibiting range of secondary metabolites present need to be carried out, first. Hence, in the present study qualitative analysis of secondary metabolite constitution of two insecticidal plants *viz.*, wild bean, *Canavalia cathartica* Thouars (Fam.: Fabaceae) and emetic nut, *Randia spinosa* Poir. (Fam. Rubiaceae) was taken up.

MATERIALS AND METHODS

Collection and preparation of Plant materials:

Collection of *R.spinosa* berries and *C. cathartica* seeds:

The berries of *R. spinosa* and the seeds of *C. cathartica* were collected from the wild plants growing in Kodukkanpalayam village (Lat 11.713752º, Long 79.656074º), Cuddalore district, Tamil Nadu, India. The berries and seeds were shade dried and powered in an electric blender. They were then stored separately in air tight ziplock covers (7 inch x 5 inch). 50 g of *R. spinosa* berry and *C. cathartica* seed powders were immersed in 250 ml of three analytical grade solvents at room temperature sequentially *viz.*, petroleum ether (55ºC), chloroform (61.1ºC) and methanol (64.7ºC) for *R. spinosa* and n-hexane (64ºC), ethyl acetate (77.1ºC), and methanol (64.7ºC) for *C. cathartica* (From non-polar to polar) at room temperature for three days. The respective extracts were filtered. The filtrate was then evaporated to dryness in a rotary flash vaccum evaporator (Lab-Sil instruments®). The semisolid extractive thus obtained was stored in small glass vials, closed with aluminium foil to prevent the entry of light, and kept in -20 ˚C deep freezer (Blue star®) [4].

Phytochemical analysis of the plant extractives for the presence of secondary metabolites:

The collected extractives were subjected to phytochemical screening for the presence of alkaloid, flavonoid, saponin, tannin, terpenoid, coumarin, glycoside, phenol, and quinone by following standard procedures as detailed below.

Test for flavonoid:

One millilitre of the extractive was added to one ml of concentrated sulphuric acid and formation of. orange colour confirmed the presence of flavonoid [5] [6].

Test for phenol:

One ml of the extractive was treated with 3 % ferric chloride and the appearance of deep blue or green colour showed the presence of phenol [5] [6].

Test for quinone:

One millilitre of the extractive was treated with 5 ml of Hydro chloric acid. Formation of yellow precipitate indicated the presence of quinone [5] [6].

Test for terpenoid:

Two millilitres of the extractive were treated with two ml of acetic acid and three ml of concentrated sulphuric acid. Deep red colour development showed the presence of terpenoid [7].

Test for coumarin:

Two millilitres of the extractive was taken and 3 ml of 10% sodium hydroxide was added. Formation of yellow coloration indicated the presence of coumarin [7].

Test for alkaloid:

One ml of the extractive was added with six drops of Mayer's reagent. The formation of yellowish cream precipitate indicated the presence of alkaloid [8] [9].

Test for glycoside:

0.5gm of extractive was taken and dissolved in two ml glacial acetic acid. One drop of ferric chloride solution and one ml of concentrated sulphuric acid were added. Brown ring at the interface indicated the presence of glycoside.

Test for saponin:

One ml of extractive was mixed with five ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponin [8] [9].

Test for tannin:

One ml of extractive was treated with three drops of 1% lead acetate solution. Formation of yellow or red precipitate indicated presence of tannin.

RESULT AND DISCUSSION

In the present study, qualitative analysis of methanol, ethyl acetate and n-hexane seed extract of *C. cathartica* revealed the presence of phytochemical constituents such as alkaloid, tannin, terpenoid, coumarin, glycoside, phenol, quinone and flavonoid (Table 1). *R. spinosa* fruit methanol,

chlorofom and petroleum ether extract revealed the presence of alkaloid, tannin, terpenoid, coumarin, glycoside, phenol, Quinone, and flavonoid (Table 2).

Alkaloids are present in *Canavalia* seed ethyl acetate extract and all the extracts of *R. spinosa*. Alkaloids are not favoured by many herbivorous insects. They are important growth inhibitors and feeding deterrents [10].

The results revealed the absence of saponins in both the plants. However, tannins were found in all the extracts tested. They have a strong deleterious effect to phytophagous insects. They bind to the proteins and reduce their absorption efficiency and cause midgut lesions [11]. They are also effective feeding deterrents to many insect [12].Terpenoid was found to occur in all the extracts of both the plants except *Canavalia* seed hexane extract. These are most metabolically diverse class of plant bioactive natural products acting as antifeedants or toxins or as modifiers of insect development [13].

Glycoside was present in all the extracts of both the plants except *Canavalia* seed ethyl acetate extract.

Phenols were detected in all the extracts except *Randia* fruit methanol extract. As a corrosive substance, phenol denatures proteins and generally acts as a protoplasmic poison. Phenol may also cause peripheral nerve damage (*i.e.,* demyelination of axons) [14]. Similarly, quinone was absent in the *Canavalia* seed ethyl acetate extract. Quinones also exhibit direct toxicity to insects [15].

Flavonoid was present in all the tested extracts. They are polyphenolic compounds [16], and are reported to have an important role in plant defense against insects [17] [18].

Qualitative analysis of *C. cathartica* and *R. spinosa* had exhibited the presence of major secondary metabolites. Proper isolation and characterization of the active ingredient might lead to the development of a new phyto-insecticide.

CONCLUSION

The present study revealed that the qualitative analysis of *C. cathartica* and *R. spinosa* had major secondary metabolites such as alkaloid, tannin, terpenoid, coumarin, glycoside, phenol, quinone and flavonoid. These secondary metabolites have a insecticidal activities so, it leads to the development of a new phyto-insecticide.

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Table1: Preliminary phytochemical screening of different solvent extracts of *C.cathartica*

Phytochemicals	**Canavalia cathartica seed extracts**		
	Methanol	**Ethyl acetate**	**n-hexane**
Alkaloids	-	+	-
Saponin	-	-	-
Tannins	+	+	+
Terpenoids	+	+	-
Coumarins	+	+	-
Glycoside	+	-	+
Phenols	+	+	+
Quinons	+	-	+
Flavonoids	+	+	+

+: Presence of phytochemicals; -: Absence of phytochemicals

Table2: Preliminary phytochemical screening of different solvent extracts of *R. spinosa*.

Phytochemicals	***Randia spinosa* seed extracts**		
	Methanol	**Chloroform**	**Pet-ether**
Alkaloids	+	+	+
Saponin	-	-	-
Tannins	+	+	+
Terpenoids	+	+	+
Coumarins	+	+	+
Glycoside	+	+	+
Phenols	-	+	+
Quinons	+	+	+
Flavonoids	+	+	+

+: Presence of phytochemicals; -: Absence of phytochemicals