An insecticidal compound, Sitosteryl β-D-glucopyranoside tetraacetate from *Peltophorum pterocarpum* DC. floral extract against *Sitophilus oryzae* L. (Coleoptera: Curculionidae).

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ABSTRACT

The insecticidal activity of yellow flamboyant, *Peltophorum pterocarpum* (DC.) Heyne. floral extract against the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) was evaluated by using contact and fumigant bioassays. The potential insecticidal compound isolated from the *P. pterocarpum* was Sitosterol- β- D-glucopyranoside tetraacetate. The LC_{50} values of the Sitosterol- β- D-glucopyranoside tetra acetate in contact and fumigation mode were observed as 27.16 and 66.54 respectively. Bergenin is reported earlier from this plant however, other compounds isolated Quercetin-3-O-rutinoside and Myricetin-3-O-galactoside along with insecticidal compound Sitosterol –β-D-glucopyranoside tetraacetate for the first time from the *Peltophorum pterocarpum.*

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INTRODUCTION

The yellow flamboyant, *Peltophorum pterocarpum* (DC.) Heyne (Ceasalpiniaceae) is a moderate sized, well-shaped, perennial, a dense crowned evergreen tree and is a native to South India. The antibacterial and anti-inflammatory activity of *P. pterocarpum* was well documented (Voravuthikunchai *et al.,* 2006; Sethuraman *et al.,* 1984) and the presence of plant sterols, sitosterols, lupeol in flowers of *P. pterocarpum* was reported by Varshney and Dubey (1969). However, the insecticidal activity of *P. pterocarpum* floral extract and possible utilization of this plant's role in insect pest management has not been explored so far. We have evaluated the efficacy of natural floral product from *P. pterocarpum* as an insecticidal compound against rice weevil, *Sitophilus oryzae* (L.) on stored maize. *Sitophilus oryzae* is the most important storage pest of raw cereals throughout the world. Both the larvae and adults feed on the grains creating huge loss and make it unfit for consumption. This further affects the quantity as well as quality of the grains (Gupta *et al.,* 1999).

The control of this insect pest relies heavily on the use of synthetic insecticides (Rice *et al.,* 1999). However, the negative environmental impacts increasing cost of application and erratic supply in developing countries due to foreign exchange constraints have necessitated research interest on the development of alternate chemicals such as phytochemicals (Bergy and Resh, 1994). Also *S. oryzae* has been reported to develop resistance to synthetic chemicals (Moina *et al.,* 1998). Plants may provide potential alternatives to the currently used insect-control agents because they constitute a rich source of bioactive chemicals. Naturally occurring toxicants from plants will not contaminate food products while acting as grain protectants (Arannilewa1 *et al.,* 2006). A number of plant species and their derivatives have been reported for their insecticidal activity against *S. oryzae* (Romzan *et al.,* 2007). Though there are reports for the utilization of different plant parts and plant materials in development of pesticides, only few floral extracts such as Chrysanthemums and Tagetes species have been exploited for their insecticidal activity towards the stored pests (Haouas *et al.,* 2008; Usha Rani *et al.,* 2007). The work entails the purification of the active isolates and the elucidation of the chemical structure of the active components of *P. pterocarpum* through the use of the full range

of modern spectroscopic and analytical techniques as well as by bioactivity guided fractionation.

MATERIALS AND METHODS

Insects

Parent stocks of *S. oryzae* were obtained from established laboratory culture reared on disinfested maize grains (*Zea Mays* L.) at CSIR- Indian Institute of Chemical Technology (IICT) Taranaka, Hyderabad, India. The beetles were reared and tested at the following conditions: temperature: 28±2°C and relative humidity 65±5%. Insects of age between 2 to 6d old were used for the experiments (Usha Rani *et al.,* 2011).

Plant material extraction

Fresh flowers of *P. pterocarpum* were collected from the trees growing in the premises of CSIR-Indian Institute of Chemical Technology, Hyderabad, India. Voucher specimens were deposited in the herbaria of IICT. The flower material was shade dried at room temperature (28±2ºC), pulverized and extracted with methanol in a soxhlet apparatus. The resulting extracts were concentrated under reduced pressure in a rotary evaporator and stored in the refrigerator till use. Insecticidal bioassays with *S. oryzae* were performed using all the solvent extracted crudes.

Fractionation by chromatography

The materials extracted from *P. pterocarpum* flowers with methanol exhibited insecticidal activity to *S. oryzae* and was subjected to column chromatography over silica gel (200g-acme's 100-200 mesh) and eluted with chloroform/ ethyl acetate combination of increasing polarity. Bioactivity was detected in the fraction obtained by eluting with chloroform/ ethyl acetate 99:1 and the fraction was further subjected to Thin Layer chromatography yielded four sub-fractions.

Analytical techniques

^{1}H NMR spectra were measured on a Bruker 300 MHz spectrometer. Column chromatography was carried out using 100-200 mesh silica gel (Merck). Thin layer chromatography (TLC) was performed using precoated silica gel 60 F_{254} TLC plates, visualized under a UV lamp.

Contact toxicity of the tested plant extracts

The dermal and oral toxicity of the test samples was determined by food grain treatment method (Usha Rani *et al.,* 2011). Fresh, uninfected 30 g of maize seeds were treated with the methanol diluted samples of the crude flower extracts, fractions and pure active compounds at different concentrations uniformly with a micro applicator. Controls received solvent only. After evaporating the solvent for about 15 min (at room temperature) 20 adult insects (5-10 d old, mixed sexes) of *S. oryzae* were released separately into each jar containing the treated diet. Mortality counts were made visually after every 24 hrs, by probing the insect body with a slender paintbrush to make sure it is dead. The experiments were continued till the end of 4 days. Each set of experiments consisted of 30 replicates and all the experiments were performed in similar conditions and were repeated 3 times.

Vapor toxicity of the tested plant extracts

The vapor toxicity of the plant extracts was evaluated in fumigation experiments (Usha Rani *et al.,* 2011). In brief, small plastic airtight containers (5cm height × 5.8cm diameter) (100 cc capacity) were used as fumigation chambers and filled with 30 g of the test insect-diet. The floral crude extracts, column fractions and the isolated pure compounds all in 5 different doses were applied individually to a small ball of absorbent cotton (weighing 300 mg) which was attached underneath the aluminum screw cap of each container. An additional cotton ball treated only with acetone served as control. Solvent from the treated cotton balls was allowed to evaporate at room temperature for about 10 min and then 20 unsexed adults of *S. oryzae* were placed into the center of every container and, the container is sealed. Each set of experiments consisted of 30 replicates and all the experiments were performed in similar conditions and were repeated 3 times. All tests were carried out at 28±2ºC temperature, and 65±5% relative humidity. Experimental conditions, mortality counts, number of treatments and statistical analysis were the same as those described in the former methods.

Statistical analysis

Toxicity data of different concentrations of each compound were subjected to probit analysis using AnalystSoft, Biostat analysis program (Biostat, 2008) to determine ED_{50} representing the concentrations that caused 50% feeding deterrence with 95% fiducial limits.

RESULTS AND DISCUSSION

Subfractions-1 yielded colorless solid, mp 146° C when subjected to TLC on silica gel 60 F 254 TLC plate using chloroform: ethyl acetate: methanol (2:2:1) mixture under UV-light of wavelength 254 nm observed as black spot at r.f-0.48 identified as Sitosterol- β- D-glucopyranoside tetra acetate (1) from the analysis of its various spectral data. Subfractions-2 yielded colorless solid, mp 146° C when subjected to TLC on silica gel 60 F 254 TLC plate using ethyl acetate : acetic acid : formic acid : water (100:11:11:25) mixture under UV-light of wavelength 254 nm observed as black spot at r.f 0.58 identified as bergenin (2) from the analysis of its various spectral data. Subfraction-3 yielded a pale color solid mass when subjected to TLC as described above and is observed as orange-brown spot under UV light 365 nm at r.f 0.38 after spraying TLC plate with NP/PEG 4000 and has been identified as Quercetin-3-O-rutinocide (3, rutin) from a consideration of various spectral data of its hydrolysis product (7% H_2SO_4, 100° C, 2 hrs) quercetin (3). Subfraction- 4 yielded a pale brown solid mass, when subjected to TLC as described above observed as orange brown spot under UV light 365 nm at r.f 0.45 after spraying TLC plate with NP/PEG 4000 and has been identified as myricetin-3-O-galactocide (5) from examination of various spectral data of its hydrolysis product (7% H_2SO_4, 100° C, 2 hrs) myricetin (4)

Isolation and identification of pure compound

Sitosterol- β- D-glucopyranoside tetra acetate(1): FABMS: M^+ m/e 744; ^1H NMR: ($CDCl_3$), δ 0.70, 0.80, 0.81, 0.85, 0.99, 1.03 (CH_3-18, 27, 29, 26,19, 21) 2.1-2.3 singlets 4 x $OCOCH_3$; 3.49 m H-3, 3.69m H-5', 4.13 dd 6'A, 4.26 dd H-6'B; 4.59 d H-1'; 4.96 m H-2'; 5.08 m H-4' 5.21 m H-3'; 5.37 m H-6 has been identified as Sitosteryl β-D-glucopyranoside tetraacetate agrees to elemental composition $C_{43}H_{68}O_{10}$. The total extraction route is given in Figure 1.

Bergenin (2): UV λmax 275 nm (MeOH); ESIMS: M^+ m/e 328 agrees to $C_{14}H_{16}O_9$; IR (KBR) cm^{-1} 3400 (phenolic OH), 1705 and 1620 (carbonyl); ^1H NMR δ DMSO D6; 9.6 & 8.4 (br, 8 & 10-OH); 7.12 (s, H-7); 5.2-5.5 (br, OH at 3,4 & CH_2OH); 4.9 (d, H-11) and 3.8 (s,OCH_3) confirms its identity.

Quercetin (3): UV λmax 272 and 358 nm (MeOH); ESIMS: M^+ m/e 302 agrees to $C_{15}H_{10}O_7$; ^1H NMR δ DMSO D6: 12.60 (br, 5-OH); 10.90 (br 7-OH); 9.44 (br 3-OH); 7.77 (s, br 2^1-H); 7.68 (s, br 6^1- H); 7.01 (δ, br 5^1-H); 650 (s,br 8-H); 6.30 (s, br 6-H) confirms its identity.

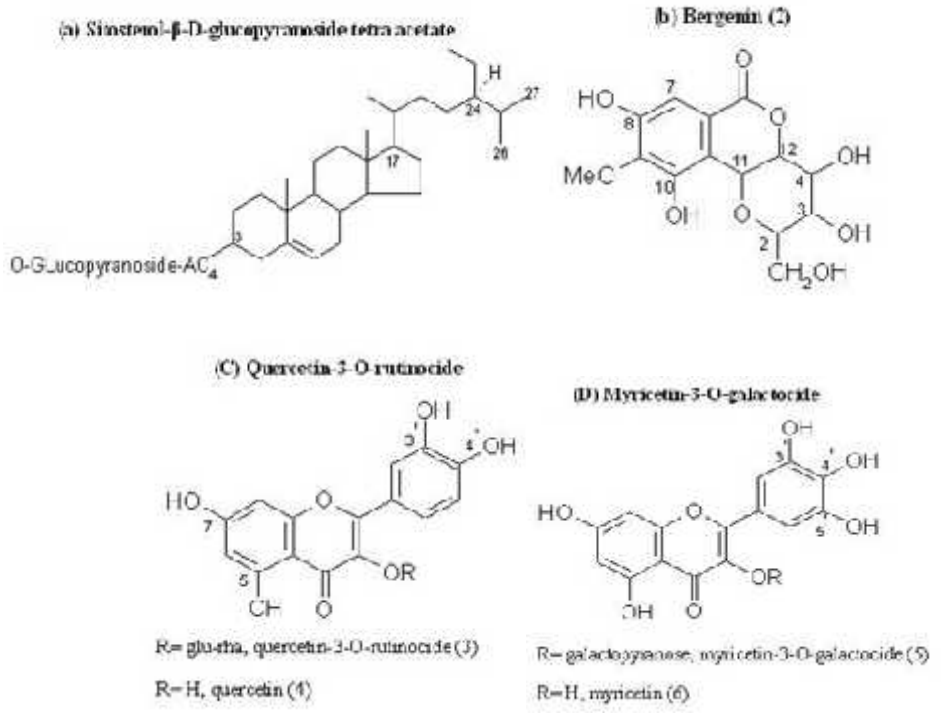
Myricetin (4): UV λmax 272 & 358 nm (MeOH); ESIMS: M^+ m/e 318 agrees to $C_{15}H_{10}O_8$; ^1H NMR δ DMSO D6: 12.57 (br, 5-OH); 10.85 (br, 7-OH); 9.86 (br, 4^1-OH), 9.40 (br, 3 & 3^1 OH), 7.33 (s, br 2^1 & 6^1 H); 6.44 (s, br 8-H); 6.30 (s, br 6-H) confirms its identity.

Mortality

The crude floral extract of *P. pterocarpum* showed insecticidal activity to the major stored product pest, *S. oryzae*. Among the fractions collected with different solvents, ethyl acetate proved to be effective in producing the toxic symptoms in *S. oryzae*. Further purification of the ethyl acetate fraction with chloroform and ethyl acetate gradients by column chromatography followed by bioassays of each sub fractions lead to the isolation of four bioactive compounds Sitosterol-β-D-glucopyranoside tetra acetate (a), Berganin (b), Quercetin-3-O-rutinoside (c) and Myricetin-3-O-galactoside (d) (Figure. 2). Out of the compounds obtained, Sitosterol-β-D-glucopyranoside was tested against *S. oryzae* adults and was found to be very potent in producing toxicity to the treated insects. The effectiveness of the tested extracts, separated fractions and the pure compounds depended on the type of individual sample and also between the modes of exposure.

The crude acetone extract of the *P. pterocarpum* flowers, its column eluted fractions, and Sitosterol-β-D-glucopyranoside tetra acetate, had high contact toxicity to *S. oryzae*. The duration of exposure, and the test chemical concentration played an important

Fig 2. Isolation and identification of compounds from the flower extract of *Peltophorum pterocarpum* (DC.) Heyne.



role in generating the toxicity. At a concentration of 125 mg/30g diet the ethyl acetate floral extract produced 100% toxicity to *S. oryzae* with LC_{50} value of 8.93 mg/30g (Table 1). No mortality was observed in controls. Among the chromatographic fractions, ethyl acetate eluted column fractions was lethal to the test insects producing 100% mortality

Table 1. Insecticidal activity of crude fraction and isolates from flower extracts of *P. pterocarpum* against *S. oryzae* after 24 hrs of treatment in contact and fumigation mode.

Compound	**Activity (95% FL)**	
	Contact Toxicity LC_{50} (mg/30g)(LCL-UPL)	**Fumigant toxicity** LC_{50} (mg/30g) (LCL-UPL)
Crude	33.6 (30.4- 36.8)	90.3 (86.9-93.8)
EA fraction	8.93 (8.39- 9.15)	62.8 (60.7- 64.9)
Sitosteryl β-D-gluco-pyranoside tetraacetate	0.047-(0.021-0.069)	0.127(0.073- 0.154)
Bergenin	0. 141 (0.073-0.246)	--
Quercetin-3-O-rutinocide	--	---
Myricetin-3-O-galactocide	0. 179 (0.079-0.256)	---

Fl: Fiducial limits, UCL: Upper confidence limit. LCL: Lower confidence limit.
LC_{50}: concentrations that cause 50% mortality.

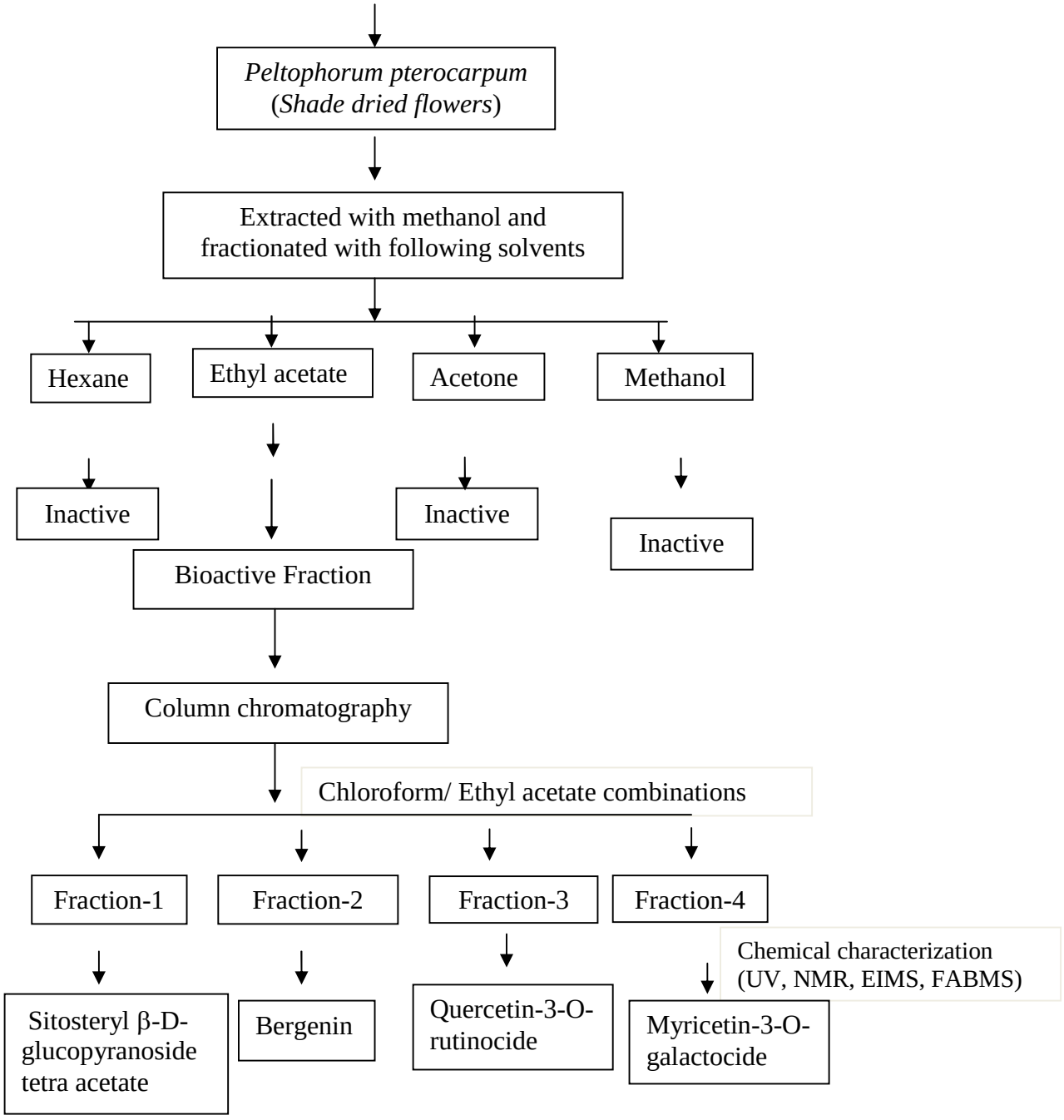
at a concentration of 20 mg/30g diet. Other fractions isolated failed to show any toxicity even at 50mg/ 30g diet tested, thus it is evident that the compounds responsible for the bioactivity is concentrated in ethyl acetate fraction.

The isolated chemical, Sitosteryl β-D-glucopyranoside tetra acetate was highly toxic to *S. oryzae* and a dosage of 0.080 mg/30 g diet was sufficient to kill 100% test insects within 24 hrs of treatment (Table 1). The mortality with this compound was concentration dependent and increased with the increase of dosage. In all the control experiments, insects moved and lived normally, with no mortality. Among all the test samples evaluated, Sitosteryl β-D-glucopyranoside tetra acetate was found to be the most effective compound inducing mortality at considerably low dose as well as in shorter duration of exposure time in contact application method. With the other isolated compounds, a higher amount of the chemicals are required to achieve the 100% mortality.

The results on the toxic effects of *P. pterocarpum* flowers extracted with various solvents in fumigation experiments were presented in Table 1. The *P. pterocarpum* crude extract showed 50% fumigant toxicity at a concentration of 90 mg/100cc. Among the fractions, ethyl acetate fraction showed toxicity with LC_{50} value of 62.8 mg/100cc and rest of the fractions were inactive even at higher concentrations tested. The isolated chemical, Sitosteryl β-D-glucopyranoside tetra acetate was less toxic to exposed insects in vapor form than the contact application mode and showed minimal activity within higher LC_{50} value. Other isolated compounds did not exhibit fumigation toxicity. The present work deals with the effects of isolates from flowers of *P. pterocarpum* extracts and its toxicity against *S. oryzae.* The plant under examination possesses varied medicinal properties and contains numerous biologically active compounds. Among the four isolated and identified compounds, Sitosteryl β-D-glucopyranoside tetra acetate reported for the first time is found to contain insecticidal activity. A bioflavonoid Bergenin was reported also been reported earlier in from this plant which is also the main compound isolated from the root extracts of *P. africanum* and was reported earlier for its antibacterial and anti-oxidant properties (Bizimenyera *et al.,* 2007). Botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules (Isman, 2006; Zettler and Authur, 2000). Secondary metabolites isolated from many plants have lethal effects on stored commodity pests (Rajasekharreddy et al., 2010) and the use of plant extracts in the control of stored products insects is an ancient practice (Qi *et al.,* 1981). The crude and the chromatographic fractions of the shade dried flowers of *P. pterocarpum* were moderately toxic against *S. oryzae*. However, the isolated pure compound, Sitosteryl β-D-glucopyranoside tetra acetate was highly lethal in contact mode of application. The toxicity of Sitosteryl β-D-glucopyranoside tetra acetate may be due to its easy penetration into the insect cuticles, as found with (2n-octyl*cyclo*prop-1-enyl)-octanoic acid (I) from *Sterculia foetida* L. (Usha Rani *et al.,* 2010).

In contact method, the dose and exposure time played an important role in producing the lethal effects. Insect mortality and the duration of the exposure were directly proportional in all the treatments with pure compound. Mortality of 100% was reached 1 DAT at 125mg/30g diet after treatment with the crude extract. In case of column fractions, ethyl acetate eluted fraction concentrated the toxic chemicals and caused highest mortality to *S. oryzae*, whereas the compounds eluted with the other solvents failed to produce significant mortality against *S. oryzae.* The concentrations employed also played a major role in determining the toxic efficacy of Sitosteryl β-D-glucopyranoside tetra acetate. Though, the compound at the lower dose failed to exhibit greater toxic symptoms, the percentage mortality has gradually increased with the increased concentrations of the active compound. When weevils were not in contact with the sample (fumigation), mortality rates varied depending on the solvents used (Table 1). Among the isolated compounds Sitosteryl β-D-glucopyranoside tetra acetate exhibited toxicity to *S. oryzae* and other compounds did not have any toxic effect, which may be due to the release of insufficient amounts of

Fig 1. Extraction and isolation of identification process of Sitosteryl ß-D- glucopyranoside tetra acetate



volatiles from the test compounds. Thus contact application appears to be superior over the other method employed as a lesser quantity of the test material was required to achieve 100% mortality. Also it appears that the chemical penetration in to insect body through oral or cuticular entry is higher in this method of application.

Previous study suggests that the ethanol and ethyl acetate extracts of *P. pterocarpum* leaf extracts were effective against *E. coli* and *S. aureus* (Jagessar *et al.*, 2007). Quercetin-3-O-rutinoside and Myricetin-3-O-galcatoside was reported earlier for their antimicrobial activity (Kotkar *et al.*, 2001). Few plant flowers are known for their insecticidal activity against stored product pests. The floral crude extracts and some of their purified fractions of *Tagetes erecta* L. were reported for highest toxicity against both the larvae and adults against *Tribolium castaneum* (Herbst) (Farjana *et al.*, 2009; Usha Rani *et al.*, 2007). Ethanolic extracts from the flowers of

Verbascum cheiranthifolium Boiss. was demonstrated for its activity against the adults of *Sitophilus oryzae* L. The most important insecticidal compounds from floral extracts are the pyrethrins derived from the extracts of chrysanthemum flowers (Klaasen *et al.,* 1996).

Although few reports are available on antibacterial and antifungal properties of *P. pterocarpum* floral extracts, this is the first report on the insecticidal activity against stored product insect pests. Therefore, a product based on *P. pterocarpum* floral extract or its active compound, Sitosteryl β-D-glucopyranoside tetra acetate may have potential to control to this destructive insect. Unlike synthetic insecticides, botanical insecticides contain mixtures of biologically active compounds, whose biological effectiveness can be additionally increased by a suitable synergic effect (Pavela, 2008). It is unlikely that the insect would acquire resistance to the entire biologically active complex (Koul *et al.,* 2007). It has been recognized that some plant extracted insecticidal compounds could be developed into products suitable for insect control because they are selective to pests, and have no or little harmful effect against non-target organisms or the environment (Schmutterer, 1992; Isman, 2000). The present study may improve the effectiveness of botanical derivatives from floral extracts as insecticides will benefit agricultural sectors of developing countries as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazard.

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