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Antimicrobial and pesticidal activities of soya saponin

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Abstract:
Soya saponin is a triterpenoid compound of 30 carbon atom. It's recorded that they have an *in-vitro* antimicrobial activity against Gram- positive bacteria (*Staphylococcus aureus* and *S. epidermiditis*), Gram- negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Agrobacterium tumefaciens, Erwinia amylovora* and *E. carotovora*), *Candida albicans* and plant pathogenic fungi (*Fusarium oxysporium* and *Botrytis cinerea*). And also have an insecticidal activity against stored product insects. Toxicity of soya saponin against *Tribolium castaneum* was more than *Rhyzopertha dominica* and *Sitophilus oryzae* and its LC_{50} against *T. castaneum* were 44.63, 18.16, 6.83, 4.03 and 2.741 (µg / cm^2) after 24, 48, 72, 96 and 120 hours respectively. In addition, nematicidal activity of soya saponin against root-knot nematode *Meloidogyne incognita* was determined.

Key words: soya saponin, antimicrobial activity, nematicidal activity, insecticidal activity.

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1. Introduction

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals found their way into the arsenal of antimicrobial drugs prescribed by physician [1]. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [2]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Among substances involved in a plant, a defense is saponins which are heterosides synthesised by several plants. They were reported to have a defensive role which was highlighted for the first time by Appelbaum *et al.* (1969) [3]. Saponins or saponosides set up a large and frequent group of heterosides in plants. Characterised by their surface active properties, saponin dissolves in water forming a foaming solution due to their tension-activity [4]. Saponin is naturally occurring surface active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria [5, 6]. Saponin consists of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methyl pentose glycosidically linked to a hydrophobic aglycone (sapogenin) in nature. Experiments demonstrating the physiological, immunological and pharmacological properties of saponin have provoked considerable clinical interest in these substances.

Saponin compound showed different biological activities against bacteria, fungi and insects. It was recorded that saponin had a significant inhibiting effect on *P. aeruginosa, E. coli, Proteus mirabilis, Salmonella typhi, Klebsiella pneumonia* and *Bacillus subtilis* bacteria [7]. The insecticidal activity of saponin is due to their interaction with cholesterol, causing a disturbance of the synthesis of ecdysteroids. This substance is also protease inhibitors or cytotoxic to certain insects [8].

The present study aimed to study antimicrobial activities of soya saponin. And its usage as natural pesticide through detection of its effects on plant pathogen fungi, bacteria, stored product insects and root-knot nematode.

2. Material and methods

2.1 Antibacterial activity of soya saponin extract

The antibacterial activity was tested by agar well diffusion method [9]. The test microorganisms were aseptically swabbed on sterile Luria bertani (LB) agar plates for bacteria and potato dextrose agar (PDA) plates for fungi and yeast. Wells of 6 mm diameter were made aseptically in the inoculated plates and then adding 50μl of extracted soya saponin (5mg/ml).

Standard used were tetracycline disc 30 μg for bacteria, Bioarc 2.5 mg/ml (commercial name of active ingredient *Bacillus megaterium* ATCC 14581) for fungi and fluconazole 20 mg/ml for *C. albicans*. The plates were incubated at 37°C for 24 hours for bacteria and 20°C for 5 days for fungi in an upright position. The experiment was carried in triplicates and the zone of inhibition was recorded using a ruler.

2.2 Bioassay of soya saponin extract against the stored product insects' adults

A direct contact application assay [10, 11] was used to evaluate the insecticidal activity of soya saponin extract against the adults of three stored product insects. A series of concentrations were prepared in acetone. One milliliter of each concentration was applied on the bottom of a glass Petri dish (9 cm diameter) to give a series of concentrations. The solvent was allowed to evaporate for 5 min prior to the introduction of insects. Twenty adults of each insect were placed into each Petri dish, and the control dishes were treated with acetone alone. All the treatments were replicated four times. Mortality was recorded after 1-, 2-, 3-, 4- and 5-days of treatment, Mortality percentages were corrected according to Abbott equation [12], subjected to probit analysis [13] and LC_{50} values were calculated.

2.3 Nematicidal activity of soya saponin extract against *Meloidogyne incognita*

The eggs of the root-knot nematode, *M. incognita*, were isolated from infested roots of egg plant (*Solanum melongena* L.). Sodium hypochlorite (NaOCl) was used for the isolation of nematode eggs from root galls according to Hussey and Barker (1973) [14] and then they were passed through 200 and 400 mesh sieves to obtain free eggs. The mean numbers of used eggs was 100 eggs per 1 ml. The eggs hatchability was recorded after 6 days.

For juveniles' mortality, the obtained eggs were put in the incubator at 27°C for 72 hours. The suspension of three-days-old second-stage larvae (juveniles) of *M. incognita* was passed through a 325 mesh sieve and then the retained larvae were collected on the sieve by backwashing into a 250-ml beaker, using tap water. The larvae per 1 ml were counted under a microscope which showed 80 larvae/ ml. The treatments were carried out in glass vials (15 ml). The tested soya saponin concentrations were 5000, 10000 and 20000 mg L^{-1} in addition to the untreated control. The total volume of the suspension in both eggs hatchability and larvae mortality tests were 5 ml and every treatment was replicated three times. The reduction in eggs hatchability and the larval mortality were determined.

2.4 Statistical analysis:

All the data are expressed as mean ± SE. Differences between groups were assessed by *Student t*-test. Statistical analysis was performed using the computer software Microsoft Excel 2003 version 11.5612.5606. Significance was set at $p < 0.05$.

3. Results

3.1 Bioactivity of soya saponin extract against human and plant pathogenic microorganisms:

It is observed that soya saponin extract had a bioactivity against human and plant pathogenic microorganisms. Agar well diffusion method was used to study bioactivity of soya saponin compared with standards in presence of control. The result showed that the inhibition zone of standards was more than soya saponin extract inhibition zone but without significant difference at $p < 0.05$ according to *Student t*- test with an exception in the case of *F. oxysporium*. Concentration of soya saponin is 5 mg/ml. The effect of soya saponin ranged from 30 mm inhibition zone (*S. epidermiditis*) to 13.3 mm inhibition zone (*E. coli*). These results were recorded in the table (1).

Table (1): Bioactivity of 50 μl soya saponin extract (5mg\ml) against different pathogenic microorganisms.

Microorganism	Inhibition zone (mm) ± SE		
	Control	Standard antibiotic	Soya saponin extract
E.coli	0	21.1 ± 2.9	13.3 ± 2.9
S. aureus	0	34.7 ±4.4	26.7 ±4.4
S. epidermiditis	0	45.1 ± 2.9	30.0 ± 2.9
P. aeruginosa	0	29.5 ± 7.6	20 ± 7.6
A. tumefaciens	0	27.7 ± 1.4	20 ± 2.9
E. amylovora	0	32 ±1.7	29.3 ±2.9
E. carotovora	0	33 ± 1.6	28 ± 2.1
F. oxysporium	0	33.3 ± 0.18	22.3 ± 1.4
B. cinerea	0	33.7 ± 0.18	26.0 ± 0.6
C. albicans	0	28.5 ± 2.1	19± 2.1

3.2Toxicity of soya saponin against three stored-product insects:

Toxicity of soya saponin extract by residual film technique against three stored-product insects the rice weevil, *S. oryzae*, the lesser grain borer, *R. dominica*, and the red flour beetle, *T. castaneum*, was carried out and results are shown in Tables (2, 3 and 4). According to the concentration which required killing 50% of treated insects (LC_{50}), the toxicity of soya saponin extract against *S. oryzae* increased with the increasing the time after exposure. The LC_{50} was decreased from µg / cm^2 after 1 day of exposure to µg / cm^2 after 5 days of exposure (Table 2).

The toxicity of soya saponin extract against *R. dominica* was increased with the increasing of the exposure time, where the LC_{50} was decreased from 318.3 µg / cm^2 after 1 day of exposure to become 26.3 µg / cm^2 after 5 days of exposure Table (3). Regarding the toxicity of soya saponin extract against *T. castaneum*, LC_{50} value 44.6 µg / cm^2 after one day and 2.7 µg / cm^2 after 5 days Table (4). According to the LC_{50} values, soya saponin extract was about 5 times more toxic against *T. castaneum* than *S. oryzae* after 5 days of exposure. Also, soya saponin extract was about 9.6 times more toxic against *T. castaneum* than *R. dominica* after 5 days of exposure.

Table (2): LC_{50} of soya saponin extract against *S. oryzae*.

Time after exposure (hr)	LC_{50} (µg / cm^2)	Confidence limits		Slope ± SE
		Lower limit	Upper limit	
24	161.903	131.688	204.84	1.344 ± 0.136
48	88.117	72.625	107.835	1.323 ± 0.108
72	36.384	30.222	43.988	1.386 ± 0.11
96	23.083	19.223	27.807	1.524 ± 0.139
120	13.41	11.072	16.13	1.655 ± 0.191

Table (3): LC_{50} of soya saponin extract against *R. domenica* adults.

Time after exposure (hr)	LC_{50} (µg / cm^2)	Confidence limits		Slope ± SE
		Lower limit	Upper limit	
24	318.266	248.623	425.806	1.051± 0.103
48	113.382	92.262	138.08	1.293± 0.107
72	58.994	48.33	71.679	1.314± 0.107
96	31.015	25.276	37.35	1.503± 0.14
120	26.333	21.271	31.762	1.529± 0.143

Table (4): LC_{50} of soya saponin extract against *T. castaneum*.

Time after exposure (hr)	LC_{50} (µg / cm^2)	Confidence limits		Slope ± SE
		Lower limit	Upper limit	
24	44.63	35.626	58.776	1.283 +/- 0.137
48	18.16	14.766	22.52	1.219 +/- 0.104
72	6.83	5.588	8.349	1.28 +/- 0.106
96	4.03	3.361	4.804	1.592 +/- 0.141
120	2.741	2.234	3.323	1.576 +/- 0.182

3.4 Nematicidal activity of soya saponin extract

Nematicidal activity of soya saponin extract against the egg and juveniles of root-knot nematode, *M. incognita*, was carried out and results are presented in Tables (5 and 6). Soya saponin extract achieved 22.3, 29.1 and 54.7% reduction in eggs hatching at concentrations of 50, 100 and 200 mg L^{-1} respectively (Table 5). Regarding the results on juveniles of root-knot nematode, soya saponin at concentrations of 50, 100 and 200 mg L^{-1} caused 23.4, 46.5 and 61.8% reduction of treated juveniles respectively (Table 6).

Table (5): Effect of soya saponin extract on eggs hatching of root-knot nematode, *M. incognita*.

Conc. (mgl^{-1})	Control		Soya saponin extract	
	Egg hatching ± SE	Reduction percentage	Egg hatching ± SE	Reduction percentage
50	98.67 ± 0	0%	76.67 ± 3.18	22.3%
100	98.67 ± 0	0%	70 ± 4.04	29.06%
200	98.67 ± 0	0%	44.67 ± 4.91	54.73%

Table (6): Effect of soya saponin extract on Juveniles of root-knot nematode, *M. incognita*.

Conc. (mgl^{-1})	Control		Soya saponin extract	
	Mortality ± SE	Reduction percentage	Mortality ± SE	Reduction percentage
50	0 ± 0	0%	20 ± 2.87	23.35%
100	0 ± 0	0%	26.33 ± 2.33	46.47%
200	0 ± 0	0%	25.33 ± 2.90	61.78%

4. Discussion

In this study, about ten human and plant pathogenic microorganisms were used to evaluate the antimicrobial activity of soya saponin. The results showed a significant antimicrobial activity of soya saponin against human and plant microorganisms with inhibition zone ranged from 30 mm in (*S. epidermiditis*) to 13.3 mm (*E. coli.*). The results obtained by Abid *et al*. (2012) [15], Jeffrey *et al*. (2010) [16] and Karimi *et al*. (2011) [17] are in agreement with the present finding.

The antimicrobial activities of saponin could be explained by having surface active properties, might insert into the lipid bi-layer bind to cholesterol forming domains enriched with cholesterol-saponin complexes and finally lyse cells [18]. On the other hand, the absence of cholesterol in the membrane structure does not inhibit pore formation by saponin and the interaction of saponin with aquaporins, leading to the cellular rupture [19, 20]. It's suggested that saponin might disturb the permeability of the bacterial outer membrane. About 90% of the surface of naturally cholesterol-free gram-negative bacteria cell wall outer membranes are covered by lipopolysaccharide (LPS). It's concluded that saponin might interact with the lipid A part of Proteus LPSs and thereby increase the permeability of bacterial cell wall [21]. It was recorded that fungicidal properties of saponin were screened in vitro against *F. oxysporum* and showed significant antifungal activity. These results are in agreement with Maria *et al*. (2015) [22]. The major mechanism suggested for the antifungal activity of saponins is their interaction with membrane sterols. It was recorded that anti-yeast properties of saponin were screened in vitro against *C. albicans*. These results are in agreement with Miyakoshi *et al*. (2000) [23].

On the other hand, the toxicity of soya saponin was evaluated by residual film technique against three stored-product insects and achieved a variable toxicity. According to the LC_{50} values, soya saponin was about 5 times more toxic against *T. castaneum* than *S. oryzae* after 5 days of exposure. Also, soya saponins were about 9.6 times more toxic against *T. castaneum* than *R. dominica* after 5 days of exposure. These results are in agreement with Shah and Sadiq (2014) where they mentioned that, the extracted crude saponin from *Teucrium stocksianum* showed overwhelming results against *T. castaneum*as compared to *R. dominica* [24]. Furthermore, Da Silva *et al.*, (2012) recorded a strong toxic activity of saponin isolated from *Midicago truncatula* seed flour towards the adults of the rice weevil *S. oryzae* [25].

Root-knot nematodes are obligate sedentary endoparasites of many plant species. Their wide host range encompasses more than 3000 plant species. It has been reported to cause yield loss up to 20 to 33% [26]. Among different nematode management strategies chemical control has proved generally effective but a majority of these chemicals are highly expensive and often hazardous. So, searching for new alternatives becomes very important. In the present study, soya saponin reduced eggs hatching and caused a considerable mortality of treated juveniles. These results are compatible with the findings of Ibrahim and srour, (2013) where they reported that, saponin treatments led to significant reduction in the numbers of a larva of root knot-nematodes in tomato roots and in the soil [27]. Omar *et al.*, (1994) also reported that saponins solutions reduced the total population [28], number of eggs masses and viable juveniles of the root knot-nematode *Meloidogyne javanica*. Nematocidal activity of saponin extract from *Medicago spp* was reported by Argentieri *etal.*, (2008) [29]. They found that saponin from *Medicago arborea, Medicago arabica,* and *Medicago sativa* to different concentrations all possess nematocidal activity against the plant parasitic nematode *Xiphinema index*. The exposure of juveniles of *M. incognita* to eight different steroids and triterpenoid saponins led to significant reduction in the motility of the juveniles [30].

Different modes of actions of saponin have been investigated [31]. For example, saponins can slow down the passage of food through the gut of insects [32] and also lower the absorption of steroids required for the synthesis of many compounds which are necessary for moulting process of insects. So it might have an inhibitory action on the synthesis of ecdysteroids and hydroxyecdysone from steroids which are among the most important requirements for moulting process in insects [33]. The inhibitory action of saponin on the digestive proteases of insects has also been recorded [34]. Further studies will be needed to understand more and more about the mode of action of plant saponin on the insect pests. Finally, toxicity tests of plant extracts are crucial to the process of searching for new, active substances that may be synthesised and used commercially in the future. To achieve this, numerous steps are needed to lead to the discovery of new insecticides.

5. Conclusion

From the previous results and discussion, it is obvious that saponins especially which extracted from soya beans have a highly antimicrobial activity and an insecticidal activity so it is recommended to have a cooperation with industrial companies to formulate it as antimicrobial and insecticide especially it is safe for human and environment.

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