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Evaluation of Insecticidal and Anti-oxidant activity of Selected Medicinal Plants

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ABSTRACT
Methanolic extracts of some selected medicinal plants, *Arnica montana*, *Apis mellifica*, *Uva ursi*, *Urtica urens*, *Digitalis purpurea*, *Cicuta virosa*, *Sambucus nigra* and *Thuja occidentalis* were evaluated for their insecticidal (on the stored grain pest, *Tribolium castaneum*) and antioxidant activity. Permethrin was used as a reference standard for insecticidal activity and percentage mortality was found 100% at 100 mg/2ml. All crude extracts exhibited concentration and time dependent insecticidal activity. Among all extracts *Digitalis purpurea* exhibited the highest percentage mortality (60%). Anti-oxidant activity of theses extracts were determined by DPPH radical scavenging activity and phosphomolybdate methods while using ascorbic acid as a reference standard. Significant anti-oxidant activities were revealed by *U. ursi* at 100 mg/ml concentration (96% DPPH scavenging activity and 91.85% total anti-oxidant activity) and *D. purpurea* 1 mg/ml (94.25% DPPH scavenging activity and 92.28% total anti-oxidant activity); followed by, *T. occidentalis*, *A. mellifica*, *U. urens* *C. virosa*, *S. nigra* and *A. montana* in the descending series.

Keywords: Insecticidal activity, Medicinal plants, Antioxidant activity**,** DPPH radical scavenging activity.

1. Introduction
The term antioxidant refers to a broad range of substances, which have the ability to neutralize free radicals by donating one of their own electrons. Antioxidants act as scavengers preventing cellular and membrane damage. This process is called anti-oxidation. Antioxidants act in different ways by preventing free radical formation (metal chelation), by scavenging free radicals, by preventing the propagation of the oxidative chain reaction, by being part of the redox antioxidant network, or by regulating gene expression [1-4]. Anti-oxidants have been found to be beneficial for the treatment of lipid metabolism, atherosclerosis and cardiovascular disease in hemodialysis patients [5-6]. The major natural antioxidants include vitamin E, vitamin C (ascorbic acid), polyphenols, bioflavonoids and carotenoids.
Pesticides are essential to control pest infestation of food products. Immense toxic effects produced by synthetic pesticides and resistance development of plant pathogens to synthetic pesticides have turned the researchers' interest towards developing insecticides of natural origin [7-9]. Various plant products have been investigated for insecticidal, insect repellent and insect antifeedant activity [10-11]. Plants have been used as traditional protectant since ages [12].
Tribolium castaneum (Herbst) is considered as a major pest of stored grains. It is a pest of stored maize and a variety of stored products. Both adults and larvae feed on internally on maize grains. Adult as well as larval stages of this insect feed on milled products but can also attack broken grains if present in bulk storage. Flour beetles are secondary pests of all grains and primary pests of flour and other milled products. In grains, embryo or germ portion is preferred [13].

2. Material & Method
2.1. Collection and preparation of drug material
The medicinal plants; *Uva ursi*, *Urtica urens*, *Arnica montana*, *Apis mellifica*, *Cicuta virosa*, *Digitalis purpurea*, *Sambucus nigra* and *Thuja occidentalis* were collected from different places; they were identified and the voucher specimen (FSMP-08-09) was deposited in the herbarium of Research Institute of Pharmaceutical Sciences, University of Karachi.
Alcoholic extracts was prepared by percolation and extracts were concentrated by rota-evaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40 °C. The extracts obtained were stored in cool, dry place for further studies

2.2. Chemicals and animals
DPPH (1, 1-diphenyl, 2-picrylhydrazyl), Sulfuric acid, sodium phosphate, ammonium molybdate, methanol, Permethrin and ascorbic acid were obtained from Merck.
Tribolium castaneum adult and its larvae were collected from the stored grains in local market.

2.3. DPPH scavenging activity
3.96 mg of DPPH was dissolved in 20 ml of methanol to get stock solution. With 0.5 ml of sample solution was added to 1 ml of DPPH solution separately. These solution mixtures were kept at room temperature in dark for 30 minutes (incubation period) [14]. Its absorbance was measured at 517 nm. Low absorbance of the reaction mixture indicated higher free radical scavenging activity using the equation.

$$\% \text{ scavenging DPPH free radical} = 100 \times (1\text{-AE/AD})$$

AE = It is absorbance of the solution, when extract has been added at a particular level
AD = It is the absorbance of the DPPH solution with nothing added (blank without extract)

2.4. Total anti-oxidant activity (Phospho molybdate method)
Total antioxidant capacity (TAC) was measured by phospho molybdate using ascorbic acid as the standard. An aliquot of 0.1 ml of the extract (20 mg - 100 mg /ml) was combined with 1 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated on a boiling water bath at 95 °C for 90 min. After the sample had cooled to room temperature, the absorbance was measured at 695 nm [15]. The total antioxidant was expressed as mg equivalents of ascorbic acid by using the standard ascorbic acid graph.

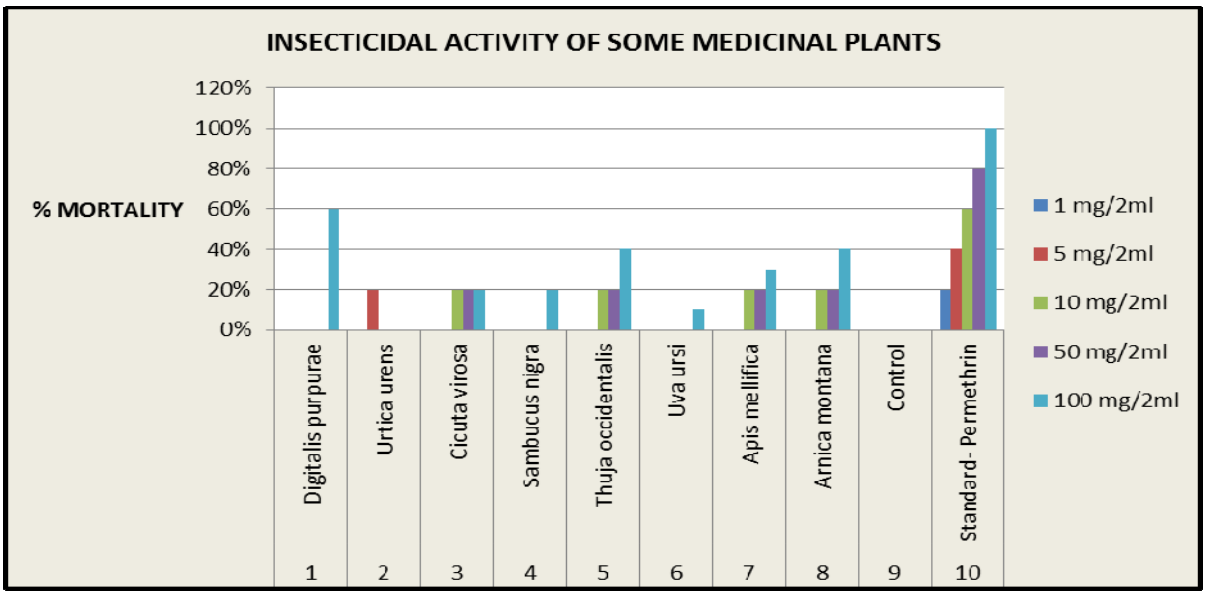
2.5. Insecticidal Activity
The dilution of the each drug extracts is prepared in following concentrations: 1 mg/2 ml, 5 mg/2 ml, 10 mg/2 ml, 50 mg/2ml and 100 mg/2 ml. One piece of filter paper was kept in the petri dish and 2 ml of the drug was poured over it; then dried over 24 hrs. This was done separately for each of the drug in five different concentrations. Twenty adults each of *T. castaneum* were placed in each of the petri dish and their motility, behavior (that is, whether the insects are attracted or repelled by the drug) and mortality is monitored. Permethrin was used as standard and methanol as a control. All these were kept without food for 24 hours. The insects were observed at intervals for 24 hrs. and number of fatalities were noted in each drugs' respective concentration [16].

2.6. Statistical Analysis
Results of the study were presented as a mean plus or minus standard error of mean (Mean ± SEM). Differences between control and treatment groups were analyzed by student t- test [17].

3. Results
3.1. Insecticidal activity
No mortality took place at the exposure of 1-50 mg/2 ml conc. of *D. purpurea* but with the increased in conc. paralyzing effect was observed in insects. Percentage mortality of *D. purpurea* was 60%, at 100 mg/2 ml. Gradual decreased in motility was observed with *U. urens* as the conc. was increased. Similarly 20% mortality was observed on exposure to 5 mg/2 ml conc. of *U. urens*. *C. virosa*, *T. occidentalis* and *A. mellifica*. They caused concentration and time dependent increased in mean paralysis and mortality time. *C. virosa* at the concentrations of 10, 50 and 100 mg 2 ml revealed 20% mortality respectively. Whereas, on exposure of 100 mg/2 ml of *T. occidentalis*, 40% mortality was observed. Mean mortality time was found 30% on exposure to 100 mg/2 ml of *A. mellifica*. No mortality was seen with *S. nigra* at concentrations 1-50 mg/2ml; but at the concentration 100 mg/2 ml, 20 % mortality was observed. Gradual and very pronounced paralyzing effect was observed with the increased in the concentration of *U. ursi*. 10% mortality was only observed on exposure to 100 mg/2 ml of *U. ursi*. *A. montana* produced concentration and time dependent increased in percentage mortality from the concentrations 1-100 mg/2 ml. The percentage mortality was observed in the following descending series: *D. purpurea* > *C. virosa* > *T. occidentalis* > *A. mellifica* > *S. nigra* > *A. montana*. *D. purpurea* showed the highest percentage mortality in comparison to the other (Table 1, Graph 1).

Table 1: Percentage mortality of insects in different concentrations of some medicinal plants

S. No.	Name of Medicinal plants	1 mg/2 ml	5 mg/2 ml	10 mg/2 ml	50 mg/2 ml	100 mg/2 ml
1	*Digitalis purpurea*	0%	0%	0%	0%	60%
2	*Urtica urens*	0%	20%	0%	0%	0%
3	*Cicuta virosa*	0%	0%	20%	20%	20%
4	*Sambucus nigra*	0%	0%	0%	0%	20%
5	*Thuja occidentalis*	0%	0%	20%	20%	40%
6	*Uva ursi*	0%	0%	0%	0%	10%
7	*Apis mellifica*	0%	0%	20%	20%	30%
8	*Arnica montana*	0%	0%	20%	20%	40%
9	Control	0%	0%	0%	0%	0%
10	Standard- Permethrin	20%	40%	60%	80%	100%



Graph 1: Insecticidal activity of some medicinal plants

3.2. Anti-oxidant activities

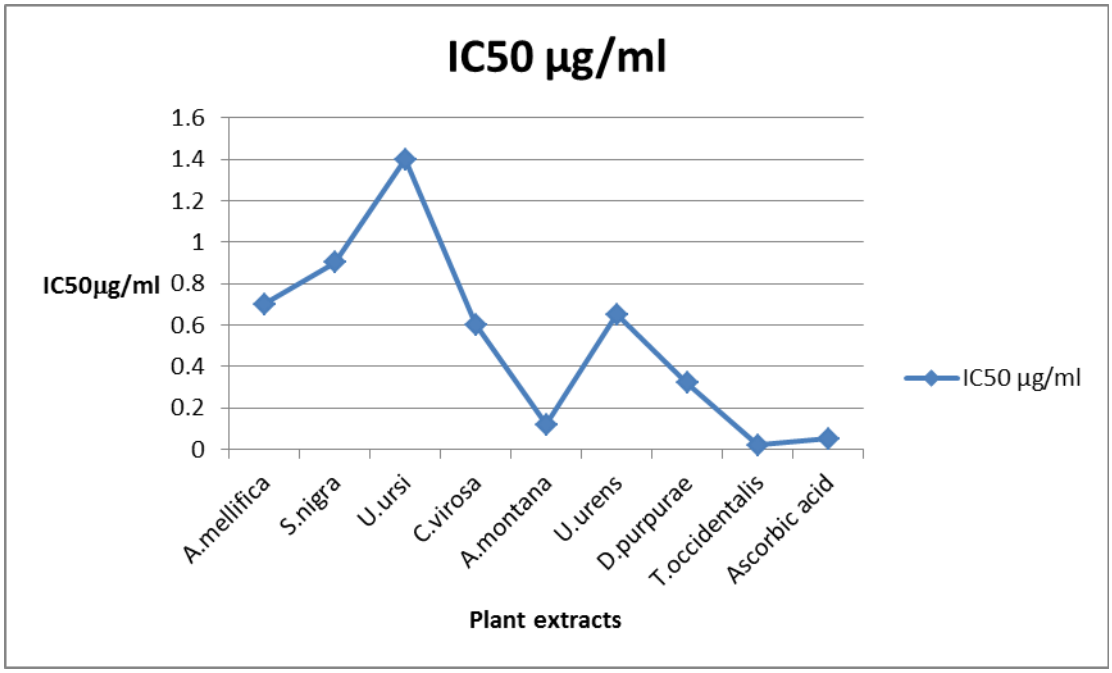
3.2.1. DPPH Scavenging activity

A. mellifica at the dose of 1mg exhibited 88.93% DPPH scavenging activity. *S. nigra*, at 100 mg showed highest DPPH scavenging activity (76.24%). *U. ursi* (100 mg) had potent DPPH scavenging activity (96%). 1 mg/ml of *C. virosa* revealed strongest DPPH scavenging activity (80.01%). Whereas, *A. montana* at 5 mg/ml exhibited 71.52% DPPH scavenging activity. *U. urens* (50 mg/ml) showed 82.16% DPPH scavenging activity. 94.25% DPPH scavenging activity was exhibited by 1 mg/ml of *D. purpurea*. *T. occidentalis* at 1 mg/ml dose revealed 92.45% DPPH scavenging activity. The reference standard ascorbic acid (50 mg/ml) had 98.62% anti-oxidant activity (Table 2 and Graphs 2).

Table 2: Percentage DPPH scavenging, TAC and IC_{50} Value of different plant extracts

Plant extract	Conc.mg/ml	% DPPH Scavenging	Total Anti-oxidant activity (TAC)	IC_{50} µg/ml
A. mellifica	1	88.93%	82.98%	0.7
	5	72.89%	69.96%	
	10	85.33%	79.08%	
	50	81.64%	76.33%	
	100	48.19%	38.87%	
S. nigra	1	66.89%	50%	0.9
	5	62.60%	45.72%	
	10	49.74%	41.44%	
	50	41.16%	39.61%	
	100	76.24%	68.91%	
U. ursi	1	34.81%	32.51%	1.4
	5	92.71%	88.07%	
	10	64.23%	85.74%	
	50	77.27%	74.19%	
	100	96%	91.85%	
C. virosa	1	80.01%	74.27%	0.6
	5	61.57%	56.21%	
	10	27.01%	22.51%	
	50	63.37%	54.75%	

	100	42.53%	37.11%	
A. montana	1	53%	50.28%	0.12
	5	71.52%	63.68%	
	10	17.49%	15.92%	
	50	43.99%	37.91%	
	100	52.48%	48.69%	
U. urens	1	66.46%	64.63%	0.65
	5	55.66%	52.65%	
	10	17.83%	14.87%	
	50	82.16%	77.95%	
	100	42.02%	39.57%	
D. purpurea	1	94.25%	92.28%	0.32
	5	71.61%	68.38%	
	10	49.74%	46.44%	
	50	62.43%	59.14%	
	100	1.45%	0.56%	
T. occidentalis	1	92.45%	89.97%	0.02
	5	73.41%	70.82%	
	10	46.99%	43.23%	
	50	62.43%	59.62%	
	100	61.57%	57.88%	
Ascorbic acid	1	94%	92.24%	0.05
	50	98.62%	96.23%	
	100	95.02%	92.48%	



Graph 2: IC_{50} of different plant extracts

3.2.3. Total anti-oxidant activity

The results of TAC also showed significant antioxidant activity. *A. mellifica* at 1 mg/ml exhibited 82.98% total anti-oxidant activity by phosphomolybdate method. *S. nigra*, 100 mg showed highest total antioxidant activity at 68.91%. *U. ursi* had potent total antioxidant activity (91.85%) at 100mg/ml. *C. virosa* revealed total antioxidant activity (74.27%) at 1 mg/ml. *A. montana* exhibited 63.68% total antioxidant activity at 5 mg/ml. *U. urens* (50 mg) had 77.95% total anti-oxidant activity whereas *D. purpurea* and *T. occidentalis* showed 92.28% and 89.97% total anti-oxidant activity at 1 mg/ml respectively. The results of total antioxidant activity were compared with ascorbic acid (Table 2 and Graph 2).

4. Discussion

Interest in the use of medicinal plants as insecticides has increased over a decade due to environmental concerns and development of resistance to the synthetic insecticides in insects. Naturally occurring insecticides are derived from plants source [18]. The insecticidal constituents of various medicinal plant extracts and essential oils present in them are mono-terpenoids. Due to their high volatile nature they have significant insecticidal activity that might be of utility for controlling stored-product insects [19-22].

Pyrethrum is an oleoresin extract of dried chrysanthemum flowers. The extract contains about 50% active insecticidal ingredients known as pyrethrin. Pyrethrins, cinerins, and jasmolins are the keto-alcoholic esters of chrysanthemic and pyrethroid acids. These potent lipophilic esters quickly penetrate many insects and paralyze their nervous systems. Both crude pyrethrum extract and purified pyrethrins are present in numerous commercially available products. These products are commonly used for indoor pest control. They are not stable enough to be used on crops [23].

Concentration and time dependent increased in insecticidal activity was observed in all the eight medicinal plants tested in comparison with permethrin, as a standard. *D. purpurea* (100 mg/2 ml) was found to have highest percentage mortality (60%), among them. *D. purpurea* contains triterpenoids, cardiac glycosides such as digitoxin, gitoxin, and digoxin which are accredited for the insecticidal activity of the plant. Although the insecticidal effect of *D. purpurea* was found to be slightly lower than the standard drug, permethrin but it may be used as an effective insecticide.

Free radicals play a significant role in a wide variety of pathological conditions. Antioxidants can fight against free radicals and protect us against numerous diseases. They produce their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Medicinal plants having vitamin C, E, carotenoids, flavonoids, polyphenols possess remarkable antioxidant activity that is responsible for inhibiting or preventing the harmful effects of oxidative stress [24].

The imbalance between antioxidant defense systems and free radicals may cause damage to Anti-oxidant activity of the eight extracts was measured by DPPH free radical scavenging method and their scavenging activity was compared with the standard antioxidant ascorbic acid. The DPPH method is a simple, rapid and convenient method for screening of many samples for radical scavenging activity [25]. The electron donation ability of natural products can be measured by 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [26-27].

Anti-oxidant activity was found by DPPH method in descending order as follows: *U. ursi* (100 mg) 96%, *D. purpurea* (1 mg) 94.25%, *T. occidentalis* (1 mg) 92.45%, *A. mellifica* (1 mg) 88.93%, *U. urens* (50 mg) 82.16%, *C. virosa* (1 mg) 80.01%, *S. nigra* (100 mg) 76.24% and *A. montana* (5 mg) 71.52%

The phosphomolybdate method is a quantitative method of analysis, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The antioxidant capacity of the fractions was measured using spectrophotometer by phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 765 nm. Researches revealed that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [28-31].

The results of total anti-oxidant activity revealed following results in descending series: *D. purpurea* (1 mg) 92.28%, *U. ursi* (100 mg) 91.85%, *T. occidentalis* (1 mg) 89.97%, *A. mellifica* (1 mg) 82.98%, *U. urens* (50 mg) 77.95%, *C. virosa* (1 mg) 74.27%, *S. nigra* (100 mg) 68.91%, and *A. montana* (5 mg) 63.68%. Potent anti-oxidant activity of *U. ursi* extract may be attributed to its flavonoids, phenolic constituents [32], hydroquinone, its derivatives [33] and arbutin [34]. *T. occidentalis* also possess significant anti-oxidant activity due to the presence of vitamin C, flavonoids, phenolic compounds and coumarin [35]. The pronounced anti-oxidant activity of *D. purpurea* may be due to the presence the flavonoid chrysoeriol in it [36-42]. Graph 2 represents IC_{50} value of all extracts.

The results of insecticidal and anti-oxidant activity reveals that medicinal plants can be effectively used due to their environmental friendly effects for the control of pest, storage of food and therapeutic effect against various pathologies at a lower cost and with lesser hazardous effects in comparison to the currently available synthetic drugs

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