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# Insecticidal activity of ellagic acid, a major constituent of *Punica granatum* fruit peels against tomato leaf miner, *Tuta absoluta*

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**Abstract**
Ellagic acid was extracted and isolated from *Punica granatum* fruit Peels. It was structurally identified using different spectroscopic analyses (1H-NMR, 13C-NMR, IR spectrum and ESIMS). The toxicity of ellagic acid against both of *T. absoluta* eggs and second instar larvae was assessed. It showed high ovicidal and larvicidal activity. Also, the biochemical impacts of ellagic acid on the second instar larvae of *T. absoluta* were investigated. The target enzymes activities to be tested were the transaminases (GPT and GOT) in addition to alkaline phosphatase (AlP). Ellagic acid caused high declination of all tested enzymes. This declination explain the high biological dysfunction of the treated larvae.

**Keywords:** Ellagic acid, *Tuta absoluta*, *Punica granatum*, transaminases, alkaline phosphatase

## Introduction
Tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is a very serious pest inflicts severe quantity and quality damage of tomato crop. The larvae invade all parts of tomato plant leading to whole plant destruction [1]. Chemical insecticides was the fastest option for combating this pest but, by the time they lost their efficacy due to increasing the high application frequencies [2] and acquisition of resistance to the majority of them [3, 4], in addition to the high cost of their manufacturing and negative impact of them on human, natural enemies and environment. So, searching for new sustainable pesticides became very crucial and fateful matter.
Natural products of plant origin such as phenols, flavonoids, alkaloids, terpenoids, and others which are a part of plant defense system against microbes and herbivorous [5] can be used as green eco-friendly insecticides.
Recently, ellagic acid as a phenolic compound has gained momentum due to its various biological properties like anti-oxidant, anti-mutagenic, anti-carcinogenic, and anti-microbial activity in addition to support many biological function vital for human health [6]. The current study focused on ellagic acid extraction from pomegranate (*Punica granatum*) fruit peels as bio-renew utilization of agriculture wastes for producing compound of high value and assessment its insecticidal activity against both of *T. absoluta* eggs and larvae. Moreover, the impacts of this compound on transaminases and alkaline phosphatase enzymes activities of *T. absoluta* larvae were investigated.

## Materials and Methods
### Instruments
NMR spectra were obtained by JEOL 500 at Mansoura University. The transmission mode Infrared spectra between 4000 and 400 $cm^{-1}$ was obtained using FT-IR spectrometer (Perkin-Elmer Spectrum one model). ESI-Ms was performed at (Center of Applied Research and Advanced Studies (CARAS), Faculty of Pharmacy, Cairo University, Kasr Al-Aini Street. 11562.

### Plant material Processing and Extraction
Air dried peels of pomegranate fruits was processed as previously reported [7]. The ethyl acetate fraction was subjected to a silica gel column using methylene chloride ($MCl_2$) containing increasing amounts of methanol (MeOH). The sub-fraction containing major compound was separated from the column chromatography by a solvent mixture of 7 $MCL_2$: 3 MeOH, then, subjected to thin layer chromatography using Silica gel Merck Aluminum sheets 20x20 cm (TLC) using eluent systems of methylene chloride/ methanol (7: 3). Detection of the isolated compound was performed by using ultra violet (UV) light 254 and 365 nm in addition to p-anisaldhyde– sulfuric acid reagent [8].

### The insect pest
Laboratorial culture of *T. absoluta* larvae were maintained on leaves of tomato plants unsprayed before with any pesticides in net cages with an aluminium base (60 cm x 60 cm x 60 cm). Adults were supplied with 10% sugary solution for feeding.

### Bioassay
The isolated pure compound, ellagic acid was emulsified in water using 0.1% Tween-80. Serial dilutions were prepared and immediately applied by manual spraying. Water with 0.1% Tween-80 was sprayed as the control treatment. All treatments were performed under constant conditions of 25±2°C, 65±5% RH, and 14:10 hour light-darkness.

- **Ovicidal activity of ellagic acid**

Thirty freshly laid eggs were transferred to Petri dish (15 cm in diameter) containing clean tomato leaves and sprayed with the tested concentration of the pure compound with 0.1% Tween-80 to represent a replicate. Each concentration was represented by three replicates in addition to another three replicates of control. Eggs hatching was monitored daily for 7 days.

- **Larvicidal activity of ellagic acid**

Clean tomato leaves were treated with different ellagic acid concentrations and let them dry before introducing to *T. absoluta* second instar larvae in ventilated plastic boxes. Each box contained ten larvae carefully transferred from their mines using a zero brush to represent a replicate. Mortality percentages were daily recorded along five days after treatments.

**Biochemical assay**

*T. absoluta* second instar larvae were treated with $LC_{50}$ of ellagic acid. Three live larvae and another three of control were collected in eppendorf tubes at 1st, 3rd and 5th day post- treatment and then frozen (-20 C°) until biochemical determination. Larvae were suspended in 3 ml bio-saline solution, 0.9% NaCl then centrifuged for 2 min. at 2000 rpm. The supernatants were immediately tested for glutamate pyruvate transaminase (GPT) and glutamate oxaloacetic transaminase (GOT) activities according to Bergmeyer and Herder (1980) [9]. Also, alkaline phosphatase (ALP) activity was estimated according to Tietz *et al*., (1983) [10].

**Statistical analysis**

Mortality percentages of eggs and the 2nd instar larvae were calculated and corrected by using Abbott's formula [11]. Estimation of $LC_{50}$, $LC_{90}$ and slope values was carried out according to Finney (1971) [12].

**Results and discussions**

**Characterization of ellagic acid**

Pure ellagic acid was isolated from ethyl acetate fraction as a yellow powder (R*f*: 0.41, 160 mg). It gives a pink color when treated with *p*-anisaldehyde reagent. It was insoluble in any solvent except DMSO.

**1H-NMR (DMSO-d6) ppm:** δ 7.441 (s, 2H, ArH), 10.3 (s, 4H, -OH), 13C-NMR (DMSO-d6) ppm: δ 159.282 (C7), 148.218 (C4), 139.795 (C3), 136.457 (C2), 112.449 (C1), 110.274 (C5), 107.59 (C6). The ESI-MS negative mode recorded ion peak at m/z at 301.1 [M-H]. Also, the IR spectrum exhibited broad band at the range 2856–3272 $cm^{-1}$ which is assigned to the -OH stretching. Also, it showed absorption band at 1693.16 cm-1 corresponding to C=O stretching, while, the bands recorded in the range 1622.93 – 1580.6 $cm^{-1}$ are attributed to aromatic ring vibrations. The absorption bands at 1194.47 and 1056.92 $cm^{-1}$ are due to ester linkage. Another band at 755.31 $cm^{-1}$ is attributed to bending vibration of the aromatic C-H.

All the previous spectral data supported that the compound of chemical formula of $C_{14}H_6O_8$ is ellagic acid, Fig.1.

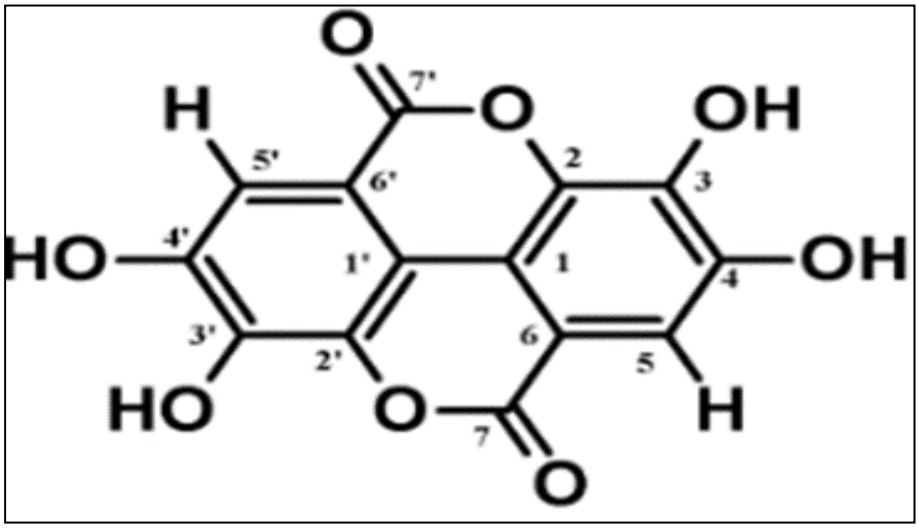


**Fig 1:** Ellagic acid structure

**Bioassay**

- **Ovicidal activity of ellagic acid**

It's obvious from the data in Table 1 that the pure isolated compound, ellagic acid showed a high ovicidal activity against *T. absoluta* eggs with $LC_{50}$ and $LC_{90}$ values: 18.339 ppm, 603.766 ppm, respectively. Upon comparing its toxicity with that of the parent extract previously tested against *T. absoluta* eggs showing LC50: 491.035, LC90: 47981.051 ppm [7], it was found that ellagic acid is more potent. Also, microscopic examination of ellagic acid-treated eggs detected structural malformation and shrinkage of the eggs as a result of eggs dehydration.

- **Larvicidal activity of ellagic acid**

Data in Table 1 showed that ellagic acid exhibited high insecticidal activity against *T. absoluta* 2nd instar larvae with $LC_{50}$ value: 15.16 ppm and $LC_{90}$ value: 332.49 ppm. In the same vein, when comparing the toxicity of ellagic acid with that of the parent extract previously showed $LC_{50}$: 83.508 ppm, $LC_{90}$: 5572.01 ppm against *T. absoluta* 2nd instar larvae [7], ellagic acid was the more toxic.

It is very clear that the second instar larvae were more susceptible to ellagic acid than eggs. Also, it showed cessation of food intake, weight loss, growth retardation and get paralyzed then die. The current data agreed with that reported the high larvicidal effect of ellagic acid against *Spodoptera litura* by hindering their growth and development [13]. The antifeedant manner of ellagic acid is attributed to its falling under the phenols class which prevent feeding of herbivores or by exert toxic effects on them [14]. They promote the Reactive Oxygen Species (ROS) in the insect mid-gut, where pH is alkaline. This results in direct oxidative damage of mid-gut proteins and lipids producing high levels of lipid per-oxidations, oxidized protein, and free ions in insect mid-gut leading to insect death [15].

Also, it was proved that ellagic acid exert high activity against acetylcholinesterase (AChE) in *Agrotis ipsilon* larval tissue because of its high binding affinity with AChE [16].

The high insecticidal efficiency of ellagic acid was previously proved against many insect pest like the red flour beetle *Tribolium castaneum* [17], *Corcyra cephalonica* [18] causing high mortality within a short time.

**Table 1:** Toxicity of ellagic acid against eggs and the 2nd instar larvae of *T. absoluta*

| | Ellagic acid toxicity | | | | | |
|---|---|---|---|---|---|---|
| | LC50 (ppm) and confidence limits at 95% | | LC90 (ppm) and confidence limits at 95% | | Slope ± SE | *X2* |
| Eggs | 18.34 | | 603.77 | | 0.8445± 0.079 | 5.881 |
| | 11.91 | 27.79 | 319.67 | 1431.22 | | |
| 2nd instar larvae | 15.16 | | 332.49 | | 0.9557± 0.147 | 0.1041 |
| | 7.39 | 29.55 | 137.84 | 1412.45 | | |

**Biochemical assay**

- **Transaminases (GPT & GOT) activities**

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) are key enzymes of non-essential amino acids formation, gluconeogenesis, anabolism and catabolism of protein [19]. Also, they act as catalytic agents in carbohydrates metabolism [20] and are considered the bridge between carbohydrates and protein metabolism [21]. It was found that insect- enzymes catalyzing amino acids metabolism were obviously impacted by insecticides or pathogens [22]. So, the present study aimed to evaluate the impact of ellagic acid on GPT and GOT of *T. absoluta* 2nd instar larvae to conceptualize its mechanism of insecticidal toxicity.

Data in Table 2 showed that the median -lethal concentration ($LC_{50}$) of ellagic acid caused a dramatic decrease in GPT and GOT activities comparing with the untreated control. GPT activity dropped significantly day after treatment and continued great decrease at the 3rd and 5th day post treatment recording inhibition % of: -43.14, -63.66 and -38.24% along days post treatment, respectively. Also, GOT activity declined gradually showing peak of declination at the third day of the treatment recording -58.54% inhibition of the enzyme activity. The current results agreed with that recorded a harsh suppression of GPT activity in adult *Schistocerca gregaria* fat body when treated with *P. granatum* butanol extract.

- **Alkaline phosphatase (AlP) activity**

Data in Table 2 indicated that ellagic acid exhibited gradual inhibition of ALP activity along the experimental period recording -16.4, -29.09 and -53.26% inhibition of the enzyme activity at 1st, 3rd and 5th day after treatment, respectively. The present results were agreed with that reported significant inhibition in AlP activity in *S. litturalis* when treated with $LC_{25}$ of *Euphorbia pulcherrrima* petroleum ether extract [23]. Also, it was agreed with previous studies [24, 25] proved that AlP activity was negatively impacted with different stress, disease and toxic chemicals.

ALP is an essential enzyme for catalysis the ransphosphorylation reaction [26]. Also, it is closely related to insect development and moulting. So, any inhibition of this enzyme obstruct the insect development.

**Table 2**: Enzyme activities (U/L) in *T. absoluta* 2nd instar larvae treated with $LC_{50}$ of ellagic acid

| Enzyme | activity at different times post treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1st day | | 3rd day | | 5th day | |
| | mean ± SD (U/L) | Change % | mean ± SD (U/L) | Change % | mean ± SD (U/L) | Change % |
| GPT | 81.5± 1.8 | -43.14 | 24.47± 2.08 | -63.66 | 8.4± 1.65 | -38.24 |
| Control | 143.33± 2.05 | ----- | 67.33± 2.87 | ----- | 13.6± 2.04 | ----- |
| GOT | 259.67± 2.55 | -10.25 | 81.87± 5.53 | -58.54 | 73.67± 2.84 | -53.69 |
| Control | 289.33± 4.78 | ----- | 197.47± 3.27 | ----- | 159.07± 10.46 | ----- |
| AlP | 27.67± 0.87 | -16.4 | 14.7± 0.37 | -29.09 | 8.6± 0.54 | -53.26 |
| Control | 33.1± 1.07 | ----- | 20.73± 0.49 | | 18.4± 0.41 | ----- |

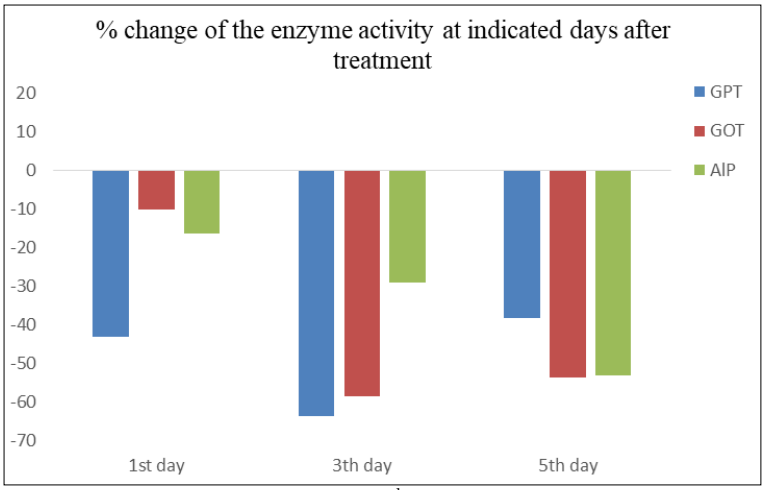


**Fig 2:** Enzyme activities (U/L) in *T. absoluta* 2nd instar larvae treated with $LC_{50}$ of ellagic acid

**Conclusion**

Bio-renew utilization of agriculture wastes like pomegranate Fruits peels for producing a high value compound like ellagic acid is a distinctive step for sustainable insecticide in parallel with environmental cleanup of agricultural wastes. Ellagic acid showed high ovicial and larvicidal activity against *T. absoluta* eggs and larvae. This high toxicity was clearly confirmed by the high declination of all tested

enzymes. This support ellagic acid as a promising green insecticide can be Integrated in Pest Management Program for controlling *T. absoluta.*

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