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In vitro Antimitotic Activity of Gall Extract of *Pistacia terebinthus*

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Abstract

Pistacia terebinthus L. - turpentine tree - is a perennial flowering plant in the Mediterranean region, some aphids species induce the formation of galls in *Pistacia terebinthus* formerly called Carobs of Judea whose tannin content equals 60%, used in traditional medicine as a stimulant, diuretic, astringent for the treatment of asthma and other respiratory and urological affections. In the present study, the antimitotic activity of *Pistacia terebinthus* galls was evaluated using meristematic cells of *Allium cepa* roots assay our results reveal that the methanolic extract decreased the root length and dividing cell number significantly after 96 h and compared to control ($p < 0.05$) the mitotic index of extract at the concentration 4 mg/ml was 31% and has significant activity near to the standard methotrexate. Overall, the methanol extract of *P. terebinthus* galls revealed the presence of the phytochemical's compounds such as gallic acid, caffeoylquinic acid, which affect plant mitosis and can be used as an antimitotic drug.

Key Words: *Allium cepa*, Antimitotic activity, Galls, Caffeoylquinic acid, *Pistacia terebinthus*

1. Introduction

Pistacia terebinthus L. (the Terebinth) is a deciduous tree or shrub from North Africa, referring to the Anacardiaceae, a fairly large family that includes 600 species and 70 genera (Bozorgi, 2013). The genus *Pistacia* has ten species, characterized by having alternate, pinnate leaves (Lin., 1984) and secretory cavities that contain resinous compounds. The plant owes its name to the oleoresin known as CHIO turpentine, it is the first turpentine known by Discords and this name was later extended to the oleoresin of conifers (Denoël., 1958). This tree is one of the components of the Mediterranean shrub, particularly in Algeria, where there are large stands, especially in the Tessala park, known

as Betoum el Kiffan in Arabic and Hejji Kabyle (Lapie,1914).

Pistacia terebinthus produces a rich mixture of substances, including resin, essential oils, proteins, organic acids, sugars, flavonoids, and tannins. The leaves are often attacked by aphids, which stimulate the plant to form galls in the leaves. The gall contains a mixture of 60% resin, 36% tannins and 4% essential oil (Pulaj. 2019). Several studies have been engaged in different locations where the species grows in the wild to evaluate and compare its composition and biological proprieties, including as a bioherbicide and antifungal. anti-inflammatory (Giner, 2002; Kordali, 2003; Kıvçak, 2004; Remila, 2015). While the antimitotic properties of this species have never been evaluated.

Some plant-derived compounds such as combretastatin, paclitaxel, colchicine, and vincristine are important antimitotic agents of cytotoxic drugs (Mukhtar, 2014). A large number of new drugs isolated from plants have been used for treating cancer. Most of these drugs are the secondary plant metabolites including phenolics and alkaloid (Calzada, 2020) These phytoconstituents are usually active against various types of cancers (Conforti, 2008).

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals which modify the normal course of mitosis, In *Allium cepa* L. root tip model root system of plant cells, it is commonly used as a test for investigating environmental pollution factors, the toxicity of chemical compounds and evaluating potential anticancer properties. It is easy to make preparations of onion roots; they contain rather homogenous meristematic (Kuras, 2016). This study aimed to evaluate the composition of the methanol extracts of galls from *Pistacia trebinthus* and to test them for potential antimitotic activity on meristematic cells of Allium cepa roots.

2. Material and Methods

Pistacia terebinthus galls induced by aphids were collected by Dr. Bellifa Nazim in September and November in Tessala (35°16'22.9"N 0°47'08.8"W) state of Sidi Bel Abbes Western Algeria, the plant material was authenticated by Dr. Ferkous Houssem botanist of the pharmacy department Sidi bel abbes and Pr Alvarez R university Leon Spain, A specimen has kept in the herbarium of the department (Fig. 1).

Galls were dried and reduced into powder (100 g) separated into 3; Soxhlet extraction technique was adopted to get crude extracts using methanol as solvent. The extract obtained was concentrated in a rotary evaporator at low pressure and temperature to give crude methanol extracts, tannins were precipitated by ammoniac to give 48 g than the residue was extracted by chloroform according to the Stas Otto Method (Cortes, 2019). TLC was carried (DCM- MeOH-H2O, 77:13:10) to give gallic acid Rutin and caffeoylquinic acid, these compounds were determined using UV visible and Infrared spectroscopy (Jahangirian, 2011) using the spectral database (Bio-Rad) as well as a direct comparison of TLC with authentic samples (Sigma) available in our laboratory.

Ultraviolet-Visible spectroscopy: The chloroform fraction (7 g) was resolved by column chromatography (silica gel, 180 g) using a step gradient of a DCM-MeOH solvent system to give carboxylic phenol acid. The evaluation of the anti-mitotic activity of the MeOH fraction was made as described by Sehgal et al. (2006) with modifications (Grant, 1982; Fiskesjo, 1988; Melappa, 2017). Using *Allium cepa* root meristematic cells which have been used extensively in the screening of drugs with a natural antitumoral origin (Fig 2).

Fig 1 : Galls of *Pistacia terebinthus*

Fig 2 Antimitotic Activity after treatment of *A. cepa* root with different MeoH extracts of Galls

A. cepa bulbs (70 ± 10 g) were purchased from the local market and grown in beakers at room temperature for 48 h the bulbs with roots measuring 2–3 cm were transferred to beakers containing extracts at different dilutions (0.5, 1, 2 and 4 mg/mL) in tap water.

A blank with water was used as control. Methotrexate was used as a standard control. All the groups were incubated at 25±2 °C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h. After, the root tips were fixed with fixing the solution of acetic acid and alcohol. Squash preparations were made by staining the treated roots with acetocarmine stain and Giemsa and May Grünwald stain. For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 3-5 fields of view using high resolution (100x) light microscopy (Leica). The mitotic index was calculated as

Mitotic Index = Number of dividing cells/Total number of cells x 100

3. Results and Discussion

In the present work, we displayed the strong anti-mitotic effect of methanol extract of the galls from Pistacia terebinthus towards *Allium cepa* root development; this model has been used for evaluating anti-mitotic activity (Raheel, 2017).

As already mentioned, aphids induce the host plant to secrete a range of phytochemical compounds such as polyphenols and terpenes, which under normal conditions, the plant would not generate this explains the difference in content between the leaf of *P. terebeinthus* and the extract of the galls, this can be explained by the interference of the metabolism of the plant and the aphid for example the pathways of development of auxin and cytokinin in plants. The high phenolic content helps explain the use of this plant in traditional medicine. Indeed, these compounds are widely known for their antiviral, antispasmodic, anti-tumor, hypocholesterolemic, anti-inflammatory, anti-hypertensive and antimicrobial activities. The extract was purified by TLC or CC and pure compounds were obtained: Hydroxamic acid and evaluated for antimitotic activity showed significant activity near to the standard. The MeOH extract of galls from *Pistacia terebinthus* presented strong and dose-dependent anti-mitotic activity in terms of decrease in mitotic index, mean root length and the number of dividing cells, the results were correlated with water used as a control in which 78% mitotic index was observed with actively dividing cells at various stages of mitosis (Fig. 3).

The results were analyzed based on mitotic index and are presented in Table 1 reflect the effect of various extracts on mitotic index. The methanolic extract of *Pistacia terebinthus* galls decrease the root length and dividing cell number significantly after 96h and compared to control ($p< 0.05$) as shown in the Table 1.

The mitotic index of *P. terebinthus* extract at the concentration 4 mg/ml was 31% and has significant activity proximate to the standard methotrexate 30% (0.1 mg/ml) Table1 shows the antimitotic activity of different extracts and methotrexate. Our results on these meristematic cells model corroborate previous works in which the mitotic index of extract decrease dividing cells number as root length was described Raheel, R 2017. And had an excellent anti-mitotic activity that was comparable to the activity of methotrexate. A maximum number of non-dividing cells were observed.

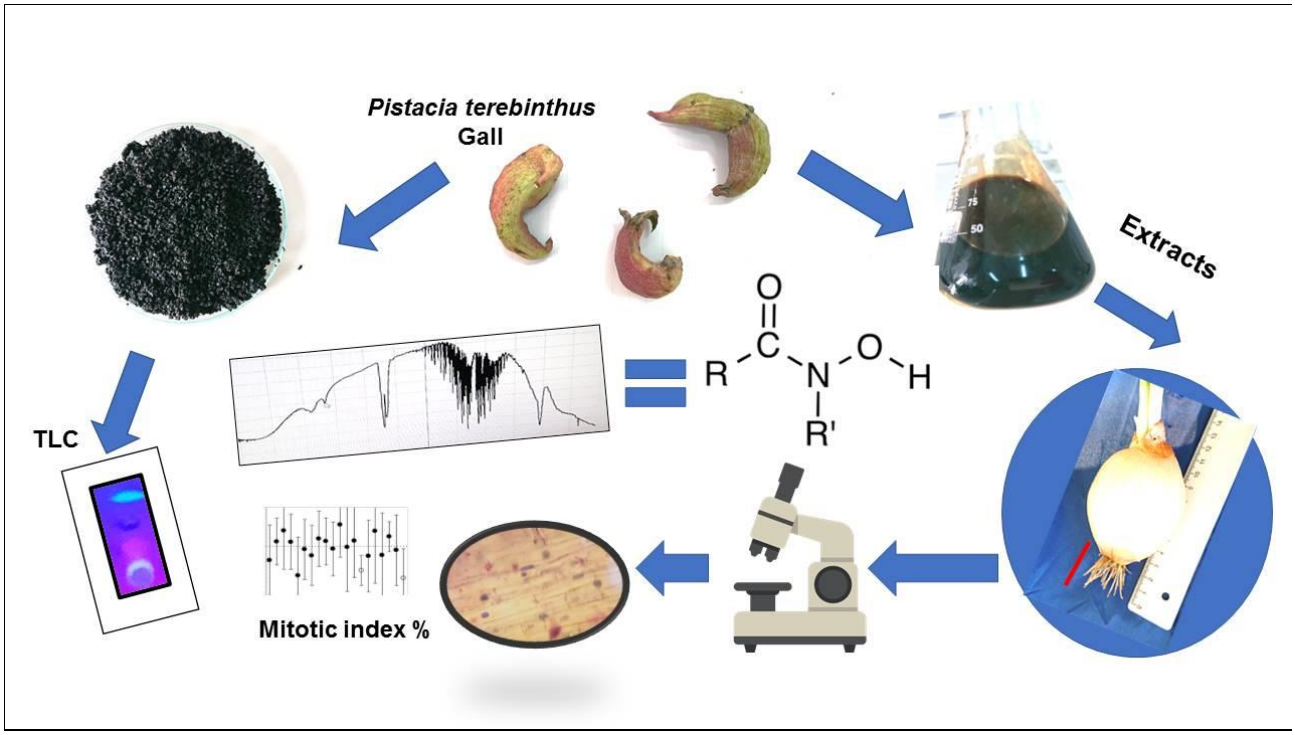

Figure 3. Dividing cells at various stages of mitosis

Table 1: The effect of different extracts on mitotic index

Extract concentration (mg/mL)	Mean root Length (mm)	Number of dividing cells	Average number of total cells	Mitotic Index (%)
Water	45 ±1.5	47	60	78
0.5	40 ±2	41	60	68
1	40 ±1.2	37	60	61
2	30 ±2.3	23	60	38
4	20 ±1.6	19	60	31
Methotrexate (0.1 mg/mL)	15 ± 1.5	18	60	30

±Values are expressed as Mean± SD for triplicates

Cytotoxicity tests using in vivo plant systems, such as Allium cepa, are validated by several researchers, who have jointly conducted in vitro animal tests and the results obtained are similar. The *Allium cepa* test is one of the few direct methods of measuring cellular damage and analysis of cytotoxicity and genotoxicity because the roots are in direct contact with the extract often associated with microtubular disturbances.

Phytochemical characterization of the MeOH extract of Gall from Pistacia revealed the presence of the phytochemicals- Alkaloid, polyphenols, flavonoids, terpen, saponin glycosides and reducing sugars. In our study, it may be suggested that the extract may be acting through the pathway inhibiting tubulin that is required for DNA synthesis that arrest cell division. Methotrexate is known as an anticancer drug which competes with folic acid for the enzyme reductase Also Adamaskis et al had reviewed the antimitotic activity of Bisphenol and Taxol by immunofluorescence microscopy in meristematic root cells their study reported an elevation of tubulin

acetylation on *A. cepa* root. caffeoylquinic acid is a potent moiety not only in the field of cancer therapy but also as a mutagenic agent. Among the various derivatives of carboxylic phenol acid, is considered as a potent anticancer agent. Scientists from different corners synthesized different phenolic acid moieties groups and have been evaluated as antimitotic agents. (Zhu, 2011).

Caffeoylquinic acids have attracted considerable interest recently because of their capacity to inhibit a variety of enzymes such as metalloproteases, some carboxylic phenol acid, such as caffeic acid have been used clinically for the treatment of cancer or iron-overload diseases. Much of the activities of these carboxylic phenol acids were thought to be due to their metal chelating properties (Witte, 2000; Jahangirian, 2011).

4. Conclusion

In conclusion, our results indicate the following: caffeoylquinic acid affects plant mitosis it maybe explains by disrupting microtubule organization. this result can explain the resistance of plants against aphids and open the possibility of exploiting *P. terebinthus* galls as a source of therapeutic agents.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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