Medicinal importance of *Atropa belladonna* plant (tropane alkaloids) (Review)

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Abstract

The name Atropa is thought to have been derived from the Greek goddess Atropos while, meaning a beautiful lady. Belladonna has also been used over the past centuries to relax muscles, especially of the stomach and intestines, to help relieve intestinal colic, and to treat peptic ulcers. It also relaxes the urinary tract, removing spasms. The ancients used the plant to treat Parkinson's disease, as it works to reduce tremors and stiffness. Modern medicine proved that belladonna alkaloids inhibit the parasympathetic central nervous system that controls the various involuntary activities of the body. These alkaloids increase heartbeats. It has been shown that belladonna is a sedative used to remove the pain of diseases accompanied by bouts of muscle contractions.

Key words: secondary prodect ,Atropine, secondary metabolism
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Introduction

1-Botanical description

The name Atropa is thought to have been derived from the Greek goddess Atropos while Belladonna is driven from the Italian language, meaning a beautiful lady. The plant has other names, including deadly nightshade, dwale, banewort, devils cherries, and devils herb. [1]. The genus Atropa involves a group of four species of

erect perennial herbs, having leaves alternately arranged and bell-shaped flowers with five petals. The most famous of them is A. belladonna L which grows as a shrub of 1.5 m in height. The root is conical in shape with several branches. The leaves are large oval pointed at the base with yellowish-green apex color. The flowers are small, 3.5-2.5 cm long, borne on an axis curved downward curved downward. The petals are bell-shaped with a purple color. The fruits are small in semi-spherical shape, soft and juicy, green in color, and turn purple at maturity of 3-10 mm in diameter [2]. The seeds are renal in shape, brown with a yellowish tint, and difficult to germinate due to the hardness of the seed coat [3]. Germination occurs within several weeks of planting; however, it can be accelerated by using gibberellin acid [4]. As for the seedlings, they need to use sterilized soil to prevent the Damping off disease.

Medicinal importance of belladonna

The name belladonna refers to the plant use by Italian women for to widen the pupils of their eyes, which makes them more attractive [2]. Belladonna has also been used over the past centuries to relax muscles, especially of the stomach and intestines, to help relieve intestinal colic, and to treat peptic ulcers. It also relaxes the urinary tract, removing spasms. The ancients used the plant to treat Parkinson's disease, as it works to reduce tremors and stiffness [5]. Modern medicine proved that belladonna alkaloids inhibit the parasympathetic central nervous system that controls the various involuntary activities of the body. They reduce fluids such as saliva, secretions of the stomach, intestines, and trachea, as well as the activity of the urinary tract and bladder [6]. These alkaloids increase heartbeats. It has been shown that belladonna is a sedative used to remove the pain of diseases accompanied by bouts of muscle contractions, especially cases of whooping cough, asthma, epilepsy, and acute bronchitis. Belladonna extract helps to reduce heart pain, as well as treat liver and gallbladder problems. Externally, belladonna is used for the treatment of gout and ulcers [7].

however, extracts are prepared from it, and alkaloids are separated from it in a pure crystalline form, which is involved in the composition of many medicines. Researchers found that the liquid extract of the belladonna plant contains 0.3% of alkaloids, while the dry extracts contain 1%. The liquid extract is used in the formulation of liquid medicines such as syrups and drops, and the dry extract is used in the form of tablets and medical powders [8].

2-Secondary metabolism compounds

Secondary metabolites, in general, are the direct relationship between the plant producing them and the surrounding environmental conditions, such as resistance against insects and diseases and attraction to pollinators, It is a response to stress [9]. Plants produce a huge number of organic compounds that do not have a direct function in the growth or development processes. These compounds are known as secondary metabolites, or secondary products, produced from the primary metabolism compounds (carbohydrates, proteins, and fats) of great importance for the processes of plant growth and development [10] [11]. Metabolites, from the economic point of view, have great importance as some of them, in particular, are used as natural chemical compounds such as medicines, flavors, aromas, insecticides, and pigments. About 75% of all drugs are of natural plant origin as pure compounds or derived from natural substances, examples of these compounds are Atropine, Scopolamine, Morphine, and Codeine [12].

3-Alkaloids

Plant alkaloids are synthesized within some cells of some medicinal plant tissues

and accumulate in the same cells or transfer to other locations, saved as solid alkaline compounds or oily liquid compounds. These products are present with organic or mineral acids as salts, characterized by a bitter taste and mostly colorless [13].

Alkaloids, and plants containing them, occupy the first rank of therapeutic importance for their physiological effect on humans and animals. They usually exist in plants as a free form or as salts of some organic acids [14]. Also, alkaloids are among the most vital metabolic components known as organic waste, existing in plants where they are synthesized and stored and regarded as a reserve source of nitrogen [15]. Alkaloids are characterized by severe toxicity within the cell sap, letting them play their role in protecting the plants producing these compounds. Biologically, they act within plants as compensatory or replacement substances as organic compounds during growing under environmentally abnormal conditions or inappropriate climates Or act as a plant defense material. Alkaloids can be classified into the following groups: [13].

1. Non-hetro cyclic alkaloids, involving the phenyl-alkylamine group
2. Hetro cyclic alkaloids, divided according to the number of heterocyclic rings and nitrogen atoms into:

- pyrrolidine alkaloids
- pyrrolizidine alkaloids
- Pyridine and piperidine alkaloids
- Polyacetyl alkaloids
- Isoquinoline alkaloids
- Indol alkaloids
- Steroidal alkaloids

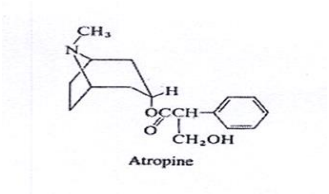
Prrolidine alkaloids include a small group classified in its turn into two divisions:

1. Simple pyrrolidine involving hygrine, hygroline, and cuscohygrine.
2. Tropane alkaloids involving Atropine, hyoscyamine, Hyoscine, and cocaine. [16].

4-Tropane alkaloids

Solanaceae is one of the medicinal, prominent plant families, for their members' content of several alkaloids of various chemical structures and molecular weights having therapy effectiveness and physiological activities, the most important of them are:

1. **Atropine,** exists majority in belladonna *A. belladonna* L. by 0.8 -1.2%. It is characterized by: its prismatic crystalline texture, melts at 118 °C, is not photoactive, easily soluble in alcohol and chloroform, slightly soluble in ether, and very difficult to dissolve in water. The chemical formula for this alkaloid is $C_{17}H_{23}O_3N$ [13].

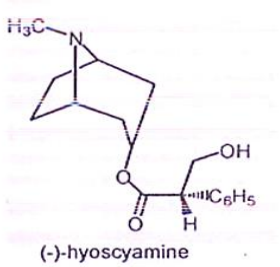


Atropine structure (Leemr, 2007)

Atropine is an ancient medication used as an analgesic and antispasmodic. It acts on the motor system affecting harmonic movement and has applications in ophthalmology, cardiology, and the digestive system [17] [18]. Atropine also has an inhibitory effect on the autonomic nervous system. Moreover, this alkaloid, at high doses, causes rapid heartbeats, increasing the rate by 30 beats/minute, so it is used for treating cases of a drop in the heart rate to less than 60 beats/minute accompanied by a drop in blood pressure, in the case of myocardial infarction without affecting peripheral blood vessels, systolic and diastolic blood pressure, and breathing [19]. Atropine is used as an antispasmodic and convulsing, as it reduces gastrointestinal tension and secretions in the presence of peptic ulcers and prolongs the stay of antacids in the stomach. It has also been noted that it can inhibit saliva secretion by 11-12 times in high doses [20]. In the ophthalmology

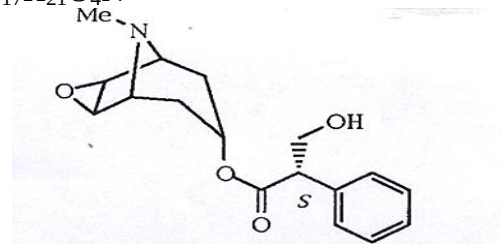
domain, atropine works to dilate the pupil, as it helps to eye funduscopic examination [12] [6].

2. **Hyoscyamine,** it exists in high amounts in Egyptian henbane *H. muticus* L. and in low amounts in *Datura spp*. and belladonna *A. belladonna*. It is solid in texture of white crystals, needle-like in shape, similar to natural silk threads, melts at 109 °C, is difficult to dissolve in water, and quickly dissolves in ether, alcohol, and chloroform. It has the same chemical formula and the same therapeutic importance as the alkaloid, atropine [13].



Hyoscyamine structure [7].

3. **Hyoscine, or Scopolamine**, is one of the tropane alkaloids and represents the major component of the plant genus Datura and Scopolia, present in a viscous form or the form of colorless solid crystals. It melts at 59 °C and is rapidly soluble in organic solvents. Its chemical formula is $C_{17}H_{21}O_4N$



Scopolamine structure [7].

The most prominent therapeutic use of the alkaloid is the inhibitory effect on the autonomic nervous system. It is similar to atropine in the anticonvulsant effect, yet it is distinguished, by the strong influence on certain secretory glands such as; sweat glands, salivary glands, and tracheal secretions. It is used to treat acute pain arising from function disorders of the digestive system and the urinary and genital systems and to treat acute pain accompanied by spasms during childbirth. Hyoscine leads to drowsiness symptoms, so it is used to treat cases of dizziness that affect travelers by sea and air and to treat dizziness that affects patients after surgeries [7].

4-Plant tissue culture technique

The cell and plant tissue culture expression refers to cultivating all plant parts outside the living body, in vitro, whether it is a single cell, tissue, or organ in sterile conditions to achieve a goal such as solving a scientific or economic problem. All over the world, there is a great interest in this technique in recent years because it represents the ideal way to reproduce some difficult to plants to propagate [21].

This technique acquired great importance, as some developed countries have used it to increase the production of secondary metabolite compounds, especially in medicinal plants, which are considered primary sources for producing many drugs obtained from plant extracts after increasing their quantity or concentration; however, getting compounds from plants has many difficulties [22], involving that the amount of the active compounds in the plant extract is considerably scarce and it is hard to purify. Also, the purification process requires large quantities of plants, which may grow in inaccessible areas; furthermore, the required concentration of the compound depends on a specific stage of plant growth and under certain environmental conditions. For this reason, the trend was to use the technique of in vitro culture, whose benefits include the possibility of producing secondary metabolite compounds under controlled laboratory growth conditions through which, cell growth can be regulated by controlling the nutritional medium ingredient [23].

Thus, the importance of plant tissue culture technology emerged as a source for drug and pharmaceutical production, not to mention that some of these compounds cannot be prepared in the laboratory [24] [25]. To increase the production of secondary metabolites, plants characterized by their abundant production of such compounds must be used [24][26]. And the possibility of producing these vehicles throughout the year.

The researchers proved the possibility of using the plant tissue culture technique for producing tropane alkaloids [16]. Many studies were able to develop appropriate practical and economic approaches for producing tropane alkaloids relying upon this technique, especially after confirming the presence of tropane in the callus of the A. belladonna plant since this technique provided ideal control for the cultivation conditions of heat, light, and humidity, in addition to that the cultivated explants are free from microbial and insect infestations and their effects. [15].

5-Producing alkaloids *in vitro*

Research studies confirmed that the basic structure of alkaloids, especially the tropane group, is derived from some amino acids and other biological molecules [13]. So to increase the production of the secondary metabolite compounds, the culture media must contain the intermediate compounds that produce them, namely amino acids. It was clear that adding them to the cell suspension increased the production of tropane alkaloids and Indol alkaloids [27] [28] [29].

[30] confirmed that tryptophan is the most effective amino acid in producing Scopolamine and Hyoscyamine alkaloids in *Scopolia spp*. plants. [31] studied the amino acid's active role in synthesizing tropane alkaloids in the cell suspension of *A. belladonna*.

[32] reached the possibility of stimulating tropane alkaloids in the cell suspension culture of *Atropa*, *Datura*, and *Hyosyamus* plants by regulating the osmotic potential. [33] reported that using the growth regulators 2, 4-D at a concentration of 1 mg/L and kinetin at a concentration of 0.1 Mg. l^{-1} raises the alkaloids percentage in the callus induced from the *D. metel* leaves culture, reaching 5.1 mg/L. gm dry weight, while using NAA at a concentration of 4 mg/L, the alkaloids percentage in the callus decreased to 0.1 mg/gm dry weight.

[34] were able to extract hyoscyamine and hyoscine alkaloids from induced callus of *D. innoxia* leaves, stems and roots grown on nutrient media containing growth regulators NAA and BA. [35] concluded that the amino acid phenylalanine increased the production of hyoscyamine and scopolamine alkaloids when cultivating the transformed roots of the Datura plant, *D. metel*. [36] stated that adding the amino acid phenylalanine to the cell suspension of *Saliva officinalis* increased the production of rosmarinic acid, While [37] concluded that the amino acid, ornithine, is the main stimulator of one of the tropane alkaloids, which is Hyoscyamine alkaloid in the Datura plant. [38] Mentioned that adding tropic acid to the callus induced from the stem of different types of Datura increased the alkaloids content, especially when adding 0.25 mmol and 0.5 mmol of the acid. [39] referred to the superiority of the treatment of 10 mg/L of phenylene with 60 g/L of sucrose, giving the highest concentration of Atropine amounting to 528.73 µg/g dry weight, and the highest concentration of Hyoscyamine amounting to 70.29 µg/g dry weight, while the treatment of 20 mg/L of phenylene and 60 g/l of sucrose gave 174.55 µg/g dry weight of hyoscine.

6-Elicitors

Alkaloid compounds can be produced by regulating and controlling physical factors (light, heat, and type of culture containers) and nutrients (carbon source and nitrogen source - nitrate or ammonium-, starting compound molecules, and phosphates). A two-step culture system is used to increase the

production of secondary metabolite compounds. The first step is the ideal (growth and maintenance medium). The second step is culturing in a medium for the producing secondary compounds (the production medium), where development practically relies upon the production medium through controlling nutrients, raising the sucrose level to 10-4%, and decreasing phosphate, nitrogen, and 2, 4-D [10].

Studies reported the possibility of stimulating cultured plant parts to produce secondary compounds when subjected to stress, represented by adding some substances to the nutrient medium. It was observed that increasing the concentration of sucrose in the medium from 2% to 4% resulting in increasing the polyphenol compound concentration in the callus of the rose bush plant [40].

An experiment involving callus growth and development of the vegetative parts of C. rouses plant in the MS nutrient media supplemented with 80, 100, and 120 g/L sucrose and free from growth regulators showed that the amount of callus increased with the increase in the concentration of sucrose in the nutrient media, as each gram of the fresh cultured part produced amount of callus weighted 11.82, 18.86, and 27.25gm respectively [41].

[42] reported that the addition of different concentrations of sucrose (40, 60, 80, and 120g / L) in the callus culture media of the periwinkle stimulated the production of alkaloid, Vincristine, whose content in the weight of the produced callus was 129.6, 120.75, 39.1, and 7.68 ppm, respectively, referring that the high concentrations of sucrose stimulated the production of the compound and then its accumulation in the cells.

[43] showed that the highest production of alkaloids in *D. stramonium* was after five weeks of culturing in a nutrient medium supplemented with 20 g / L sucrose, while the highest fresh and dry weight of the cell mass was in the nutrient media supplemented with 40 g / L sucrose.

[44] referred that when adding sucrose at concentrations 30, 60, 90, or 120 g/L to the culture medium of the poppy plant *P. somniferum* L., the concentration of 30 g/L was superior sucrose in the average fresh and dry weight of callus. Investigating the effect of sugar and tyrosine on producing effective substances from callus the study also showed that adding sucrose at the concentrate of 90 g / L to the MS medium supplemented with 30 mg / L of tyrosine gave the highest amount of morphine and codeine alkaloids, reaching 2.8 and 2.9 mg / g fresh weight of the callus respectively.

[38] concluded that the medium that gave the best production of the highest alkaloids percentage in the Egyptian henbane plant, *H. muticus* L. was the medium supplemented with 43 mmol of sucrose + 0.5 mmol of Tropic acid + 2 g/L of yeast extract.

The addition of 60 g/L of mannitol was the most effective in the average fresh and dry weight of callus induced from the leaves of the sage plant, reaching 3.18 g and 0.148 g, respectively [45], and adding sucrose at the concentration of 20 g/L gave the highest amount of the Thujone compound of 134.1 µg/g dry weight of callus.

7-Precursors

Precursor compounds are defined as molecules that interfere with secondary metabolite compounds, with occurring structural changes in some cases. When precursor molecules are added to the cultures, they interfere with them towards enhancing the biosynthetic pathways of the secondary metabolite compounds. Available information about the acceleration of the biosynthesis of alkaloids as a result of adding amino acids into the medium enhances the fact that the amino acid acts not only as a precursor compound but also as a stimulator [10].

[46] stated that adding the amino acid phenylalanine at the concentration of 10 mg/L to *P. bracteatum* callus culture media increased the production of theb aine alkaloid compared to the media to

which this amino acid was not added. Adding the amino acid phenylalanine to the culture medium of suspension cells of S. officinalis stimulated the production of Rosmarinic acid by 100% [47]. [48] showed that adding ornithine and phenylalanine to *D. stramonium* callus medium increased the alkaloid content five times compared to the free medium. [49] concluded that methyl jasmonate was the most effective in increasing tropane alkaloids in *D. stramonium* plants. [50] Showed that treating the root hairs of *Brugmansia candida* with Salicylic acid increased the accumulation of Scopolamine and Hyoscyamine alkaloids. [51] concluded that adding the amino acid phenylalanine at a concentration of 40 mg/L to the medium of cell cultures of *S. fruticosa* stimulated the production of rosmarinic acid compared to untreated media. And adding phenylalanine to *S. officinalis* cell suspension increased the production of r osmarinic acid [36]. In a study on the effect of the interaction between the concentrations of sucrose and the amino acid tyrosine on the average fresh weight of poppy plant callus and on the production of active compounds, [44] noticed an increase in the average weight of the fresh callus in the MS nutrient media supplemented with 30 g/L sucrose and 30 mg/L. of the amino acid tyrosine, reaching 688.6 mg. in addition to a response in the production of alkaloids by increasing the concentrations of tyrosine added to the medium, as the highest value was obtained from the MS medium supplemented with 30 mg/liter of tyrosine, prducing1.5 and1.2 mg / g morphine and codeine, respectively. [45] concluded that the addition of 20 g / L of sucrose to the MS media gave the best results of increasing the amount of Thujone and Borneol amounting to 134.1 and 151.2 µg / g dry weight of callus, respectively.

8-Alkaloid extraction

Extracting and purifying alkaloids from plants includes isolating resins, fats, dyes, and other components from plant extracts. It is known that alkaloids are generally present in the form of salts dissolved in water, and their insolubility means that they exist in the form of a free base [52]. The first step following converting the plant parts into powder or granules of an appropriate size is the extraction process which includes the release of the free bases and precipitation of organic acids and tannins, as well as extraction of free bases with suitable organic solvents such as chloroform, ether, etc [53]. The last extract still contains fats, resins, and other materials. Treating it with dilute acid leads to converting the free base alkaloid into soluble salts and entering the aqueous phase separated from the organic phase. Having these two phases separated from each other, the alkaloid could be released, as a free base, by adding an alkaline solution to the aqueous phase, then reseparating it with an organic solvent. So we have the solution phase separated from the aqueous phase, which is isolated and evaporated to remove the organic solvent. It is preferable to accompany the extraction process with shaking and mixing practices; however, during the phase separation process, it is preferable that it be slow to give an opportunity to increase the extracted quantity, and the solvent must have characteristics suitable for the extraction process, involving is its ability to dissolve the substance, to be extracted, readily and does not interact chemically with it as well as it can be easily disposed of after the extraction process [54].

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الأهمية الطبية لنبات الأتروبا بلادونا (قلويدات تروبان)

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المستخلص

يُعتقد أن اسم أتروبا مشتق من الإلهة اليونانية أتروبوس، مما يعني سيدة جميلة. كما استخدم بيلادونا على مدار القرون الماضية لإرخاء العضلات، وخاصة في المعدة والأمعاء، للمساعدة في تخفيف المغص المعوي، وعلاج القرحة الهضمية. كما أنه يريح المسالك البولية ويزيل التشنجات. استخدم القدماء النبات لعلاج مرض باركنسون، حيث يعمل على تقليل الهزات والتصلب. أثبت الطب الحديث أن قلويدات البلادونا تثبط الجهاز العصبي السمبتاوي المركزي الذي يتحكم في الأنشطة اللاإرادية المختلفة للجسم. هذه القلويدات تزيد من دقات القلب. لقد ثبت أن البلادونا مسكن يستخدم لإزالة آلام الأمراض المصحوبة بنوبات من تقلصات العضلات.

الكلمات المفتاحية: منتج ثانوي، الأتروبين، التمثيل الغذائي الثانوي