Ethnobotanical Study, Anatomical Study and Phytochemical Screening of Aristolochia longa L.

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ABSTRACT

Aristolochia longa L. (Aristolochiaceae) is used in Algerian traditional medicine. The ethnobotanical study conducted in the region of Setif (East Algeria) has as an objective of evaluating the potential of the region of Aristolochia longa L. used in the treatment of different diseases. A questionnaire was used which consisted of the diseases treated by this plant, the part of the plant used in the treatment and how to use this plant. The survey targeted 100 people from the local population. We also evaluated the phytochemical composition of the aerial parts (stems and leaves), fruits and tubers. Our results showed that A. longa is widely used to treat several ailments such as cancer (43%), diabetes (17%), and treatment of wounds in cattle (12%), and intestinal and stomach diseases (9 and 7%), the most used part is tubers by 70%. Crushed tubers are commonly mixed with honey (44%), milk (24%), water (8%). Results of the phytochemical screening revealed that A. longa contained various bioactive compounds, including polyphenols, flavonoids, saponins, terpenoids, and Alkaloids. In addition, there is very little information concerning the anatomical and morphological structure of this species of Setif region (Algeria), a preliminary study on anatomy of this plant is therefore reported in this paper. These preliminary results could be used to justify the traditional use of this plant and their bioactive substances could be exploited for therapeutic purposes such as antioxidant, antimicrobial and anti-inflammatory, and may be considered as a promising source of new drugs for treating cancer.

Keywords: Aristolochia longa L., ethnobotany Setif (Algeria), anatomical study of plant, phytochemical screening.

INTRODUCTION

The use of medicinal plants in treatment and prevention of diseases have increased dramatically over the last years ⁰¹. Up to 80% of the African population uses traditional medicine for primary health care ⁰². Plants remain to be the source for majority of people in developing countries to treat various health problems. They are a rich source of many natural products most of which have been extensively used for human welfare, and treatment of various diseases. A high diversity of secondary metabolites with interesting biological activities can be produced from plant extracts ⁰³.

Ethnobotany is the study of the relationships between plants and people with a particular emphasis on traditional cultures. The traditional use of plants to fulfil daily needs dates back to the beginning of human civilization and continues to date ⁰⁴. Ethnobotanical studies today are recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents ⁰⁵.

An ethnobotanical identification is the first stage in the quality assurance of traditional medicine and further in discovering new drug leads from the medicinal plants ⁰⁶. Documentation of the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. Therefore, establishment of the local names and indigenous uses of plants has significant potential societal benefits ⁰⁷, ⁰⁸.

The present work was carried out to explore Aristolochia longa L. used by the rural people of Setif, to ascertain the detailed information of this plant used by Setif people and their usage based on ethnobotanical knowledge.
MATERIALS AND METHODS

Ethnobotanical Study

The ethnobotanical investigation was carried out in Setif, a city located in the east of Algeria, at 300 km of Algiers. The survey targeted 100 people from the local population (elderly people and traditional healers). Parts of plants used, ailments treated, preparations and mode of uses were recorded.

Anatomical Study

Preparation of Sections for Anatomical Study

The utilized methods are those currently used for vegetal anatomy investigations. Cross-sections through the Young vegetative organs using a sharp blade have been executed. These sections have later been tinted using iodine-green and Carmen-red, using double coloration method according to Wagner method. The sections were later analysed and photographed using a photonic microscope.

Phytochemical Screening

Aristolochia longa L. was collected from 80 km North of Setif (Algeria). The aerial parts (stem and leaves), fruits and tubers were shadow-dried and pulverized to dry powder. And were screened in order to know the presence of phytochemical constituents (secondary and Primary metabolites), such as alkaloids, terpenoids, quinones, anthraquinones, flavonoids, tannins and saponins as secondary metabolites, carbohydrates and proteins as Primary metabolites, with the standard qualitative phytochemical procedures mentioned below:

Tests for Phenolic compounds

FeCl₃ Test: 0.5 g of the powdered sample of selected plant parts is boiled in 20 ml distilled water and then filtered using a filter paper. 5% (w/v) FeCl₃ is added to the filtered samples and observed for the presence of brownish green or blue black color.

Test for flavonoids

For the confirmation of flavonoid in the selected parts of the plant, 0.5 g of powdered sample were added in a test tube and 10 ml of distilled water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each part of plant extract followed by addition of 1 ml concentrated H₂SO₄. Indication of yellow color shows the presence of flavonoid in each extract.

Test for tannins

A fraction of 0.5 g of the powdered sample was dissolved in 5 ml of water followed by a few drops of 10% ferric chloride. A blue-black green, or blue-green precipitate would indicate the presence of tannins.

Test for terpenoids

An amount of 0.8 g of the powdered sample was taken in a test tube, then poured 10 ml of methanol in it, shaken well and filtered to take 5 ml extract of plant sample. Ten 2 ml of chloroform were mixed in extract of selected part of plant sample and 3 ml of sulphuric acid were added in selected sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

Test for alkaloids

We have used two methods in this test:

- 0.2 g of the powdered sample were added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered.

Then took 5 ml of 2% HQ and poured in a test tube having the mixture of plant extract and hexane. Heated the test tube having the mixture, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

- About 5 g of powdered sample placed in the test tube and 20 ml methanol added to the tube, the mixture was heated in water bath and allowed to boil for two minutes. It was cooled and filtered. 5 ml of the filtrate was tested with two drops Wagner’s reagent (solution of iodine and potassium iodide). The presence of precipitate indicates alkaloid.

Saponins

200 mg plant material in 10 ml methanol, filtered, frothing test: 0.5 ml filtrate + 5 ml distilled water. Frothing persistence mean Saponin present.

Detection of Quinone

0.5 g of the powdered sample of selected plant parts is boiled in 20 ml distilled water and then filtered using a filter paper. Concentrated sulphuric acid (1ml) was added to 1ml of each of the plant parts extracts. Formation of red color indicated the presence of Quinones.

Detection of Anthraquinones

Take 1 ml of filtrate prepared previously (detection of Quinone), and few drops of 2% HCl were added. Appearance of red color precipitate indicated presence of anthraquinones.

Test for reducing Sugar

An amount of 0.50 g of the powdered sample of selected plant parts was added in 5 ml of distilled water. Then 1 ml of ethanol mixed in parts plant extracts. After that we took 1 ml of Fehling solution A and 1 ml of Fehling solution B in a test tube, heated it to boiling and then poured it in the aqueous ethanol extract. When color reaction was observed, it shows a positive result.

Test for Protein

Xanthoproteic reaction test: 5 ml volume of the filtrate obtained from boiling few grams of powdered parts of plant is heated with few drops of concentrated nitric acid; yellow color that changes to orange on addition of alkali indicates the presence of protein.

Statistical analysis

Questionnaires data were then transferred to Microsoft Excel 2007 and processed.

RESULTS AND DISCUSSION

Ethnobotanical Study

In the present study, we interviewed 100 informants (female:18; male:82) in different locations of Setif (East Algeria). Who used plants for medicinal purposes. Based on the information obtained, we found that only 50% of this group identified this plant and knew how to use and prepare it, while the other 50% did not recognize it. According to our results [Fig.01]. A. longa is known for treating several ailments and health disorders. Cancer is the first ailment treated with the plant (43%), followed by diabetes (17%), treatment of especially cattle’s wounds (12%), Intestinal and stomach diseases respectively, by 9% and 7%. Moreover, hemorrhoids (4%), mouth ulcers (3%), respiratory diseases (2%) and rheumatism (2%).
It has been reported that the tubers of this plant were used such as antitumor in Algeria\textsuperscript{16, 17}. In Morocco, the most widely uses of \textit{A. longa} are in cancer treatment\textsuperscript{18, 19} demonstrated that an aqueous extract of \textit{A. longa} induced apoptosis of BL41 cell line in a dose-dependent manner, by triggering the mitochondrial pathway.

As shown in Fig.02, tubers are the most frequently used part (70%), fruits (16%) and the leaves (14%) are also used. The most commonly used method is tuber powder with honey taken orally (44%), Mixture with milk (24%) or water (8%) and decoction in water (10%) are also used to treat internal ailments. The paste (plant grinded with olive oil) is (10%) applied externally for skin infections and rheumatism [Fig.03]. The lowest percentage was used for maceration of leaf and fruit (4%).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Ailments treated with \textit{Aristolochia longa} L.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Plant parts used}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Preparation methods of \textit{Aristolochia longa} L.}
\end{figure}
Our results have been confirmed by the results of the study conducted by 20 in the Mascara area (West Algeria), regarding of diseases treated by *Aristolochia longa* L. Noting that cancer is the most treated by this plant (39%). Also the most used vegetative part was tubers by 89%, and the most commonly used method is tubers powder with honey (63%). According to 21 the roots were mixed with honey and taken orally to treat cancer.

**Morphological Study**

After the morphological study of the plant *Aristolochia longa* L., We concluded that it was a perennial herb about 30 cm long, and grows in moist streams, the subterranean vegetative organs present tubers reaches 6-12 centimeters (cm) in length, aerial stems start out at the base of the tubers, The unbranched stem has alternate, large, smooth-edged, heart-shaped and leaves with small petiole that clasp the stem with enlarged, about 4.5 cm long and 4 cm wide, the simple leaves are alternate and cordate. The solitary flowers have an unpleasant odor and they are tubular with purple-brown color, about 6 centimeters long [Fig.04]. Flowering period is between April and June. Fruits are oblong or ellipsoid capsules open with six valves and seeds compressed with cordate base. It has bitter taste with characteristic odour. This description is identical to the description of 22.

The *A. longa* group, whose most common member is *A. paucinervis*, is mainly morphologically characterized by the elongated rootstock and the short size of petiole and peduncle 23. The chromosome numbers are 2n = 12; 24; 36, denoting the presence of polyploidy, even if a proper polyploid series has not yet been demonstrated24.

![Morphology of Aristolochia longa L.](image-url)

**Figure 4: Morphology of Aristolochia longa L.**
Anatomical study

The structure of the vegetative apparatus:

The structure of the subterranean vegetative organs

- Root

When a seed germinates, the first structure to appear is the root or the radical (Fig. 05a). This becomes the primary root. The different tissues in the root have a distribution which is common to all dicotyledonous plants and is shown in Fig. 05.

The anatomical study which was performed on young fresh root showed the presence of pith, and vascular tissues with its number was two, cortex and epidermis (Fig. 05b)

The epidermis is a single layer of round cells on the outside that protects the inner tissues, cuticle and stoma are absent, The cortex Present under the epidermis consisting of several layers of thin-walled parenchyma cells have leucoplasts to store starch, chlorenchima is usually absent in the cortex of roots, Intercellular spaces between parenchyma cells facilitate the movement of water from the root hair cells on the outside of the plant to the xylem on the inside of the plant. Endodermis cells, are barrel-shaped forming the innermost layer of the cortex. They are densely arranged single cell layer. The radial and transverse cell walls are thickened with a water-impermeable, waxy suberin layer, known as the Casparian strip. This layer helps to regulate the flow of water from the cortex into the stele, rather than allowing the water to spread to all the root cells. To aid in directing water, there are also thin-walled passage cells in the endodermis, directly opposite the xylem, allowing water to move into the xylem rapidly.

The stele, or vascular cylinder (responsible for transporting water and minerals) (Fig. 05c), consists of the pericycle, phloem, cambium and xylem. The pericycle is the outermost layer of the stele under the endodermis, and consists of one or more rows of thin-walled meristematic parenchyma cells. It is in close contact with the xylem and phloem tissues of the root. It functions in the formation of lateral roots. Two (2) Vascular bundles show radial arrangement in ring, xylem (is responsible for transporting water and dissolved mineral salts) and phloem (is responsible for transporting food) bundles are arranged alternatively in different radii. Bundles of xylem are exarch wherein the metaxylem is present towards the center and the protoxylem at the outer side (Fig. 05d). The metaxylem elements are polygonal angled in outline in cross section. The cambium separates the xylem and phloem tissues from each other. This is the area where secondary growths of xylem and phloem tissues occur. Finally, we find the Pith are significantly absent or occur rarely. In conclusion the specimen studied is a section of the dicotyledonous root according to 25.

Figure 5: Cross sections of radicle of *A. longa* L. a) Where to take samples to make cross-sections (Black circle), b) general view, C) vascular cylinder, d) Vascular bundles(phloem and xylem(the metaxylem is present towards the center and the protoxylem at the outer side).
- Tubers

The rootstock rich in storage parenchyma is to be considered a more evolute organ probably derived from the subterranean stem (Fig. 06a, yellow circle). Its shape (elongated or oblong to fusiform). The rootstock, whatever shape it may have, can be simple or lobed to branched (Fig.06a).

Section of elongated tuberous rootstock type shows secondary tissues added to the primary tissue previously observed.

The roots of gymnosperms and most dicotyledonous undergo secondary growth. Most of the dicotyledonous roots show secondary growth in thickness, similar to that of dicotyledonous stems. The secondary vascular tissues originate as a result of the cambial activity. The phellogen gives rise to the periderm (Fig.06b).

On the initiation of secondary growth, a few parenchyma cells beneath each group of phloem become meristematic and thus as many cambial strips are formed as the number of phloem groups. The cambial cells divide tangentially again and again and produce secondary tissues. Thereafter some of the cells of single layered pericycle become meristematic lying against the protoxylem groups, which divide and form a few layers of cells. The first formed cambium now extends towards both of its edges and reaches the inner most derivatives of the pericycle, thus giving rise to a complete ring of cambium. It produces the secondary xylem towards the inner side and secondary phloem towards the outer side (Fig.06b).

The cambial cells that originate from the pericycle lying against the groups of protoxylem function as ray initials and produce broad vascular rays. These rays are traversed in the xylem and phloem through cambium; this is characteristic feature of the roots. Normally, such rays are called medullary rays.

Simultaneously the periderm develops in the outer region of the root. The single layered pericycle becomes meristematic and divides, giving rise to cork cambium or phellogen. It produces a few brownish layers of cork cells or phellem towards outside, and the phellem on the inside. The pressure caused by secondary tissues ruptures the cortex with endodermis, which is ultimately sloughed off. Lenticels may also be formed.

Figure 6: Secondary growth in root, a) Where to take samples to make cross-sections for tubers (Black circle), b) Cross sections of tuber of A. longa L. (general view)

The structure of the aerial vegetative apparatus

- Stem

A species have erect square stems, Observations by light microscope showed the presence of pith, vascular bundles, cortex and epidermis(Fig.07a) whereas epidermis is single layered Covered with a thin cuticular layer, there are no glandular and eglandular hairs on the epidermis, angular collenchyma tissue (supporting tissue) is located under the epidermis in the cortex in four corners of the stem (Fig.07b); The cortex is characterized by lacunous parenchyma tissue. Cambium is distinguishable and 4-5 layered. There are two large vascular bundles on the two corners, in contrast, the other two small vascular bundles exist in the other two corners. These vascular bundles are surrounded by a sclerenchyma (supporting tissue) which appears bluish green (Fig.07b), and this vascular bundle is made up with xylem and phloem and between theme there is the cambium. Xylem and phloem elements are clear. Cambium is distinguishable (Fig.07c). The pith consists of large parenchymatic cells. Our results were similar to the results obtained by the in his study about several stems cross-sections of different Aristolochia types: Aristolochia gigantea, A. leuconeura, A. grandiflora, A. ovalifolia, A. triactina, A. leuconeura and A. serpentaria. We observed similarities in the existing tissues and their arrangement.
- **Leaf**

The leaves are composed of the following tissues: adaxial epidermis (upper surface), palisade mesophyll, spongy mesophyll and abaxial epidermis (lower surface) (Fig. 8a). Anatomical investigation showed that the leaves of *A. longa* were characterized by a single layer of thick cutinized epidermal cells on both sides of the leaf (Fig. 8a). Epidermal cells are rectangular in shape and are compactly arranged. Collenchymatic cells are located under the upper and lower epidermis. Mesophyll consists of palisade parenchyma cells (elongated cell) and therefore appeared to be the main photosynthetic tissue as the chloroplast occurred in great abundance in this tissue, and of spongy parenchyma cells which was characterized by intercellular spaces which were assumed to occur as a result of cell lysis. The vascular bundles are collateral in arrangement with phloem is placed over the xylem. These bundles are surrounded by lacunous parenchyma tissue, in the median region of the leaf; there is a large vascular bundle. It appeared that small vascular bundle was contained in the intercellular spaces of spongy parenchyma cells. There are many glandular hairs (trichomes) only on the lower epidermis (Fig. 8b). These glandular hairs are multicellular characterized by a hook shaped terminal cell (Fig. 8c). They are similar in appearance to the existing hairs on the outer surface of the *Aristolochia bracteolata* leaf as described by 27.

Stomata cells are more common on the lower epidermis, there type is Ranunculaceus (Fig. 9a, b and c), 27 have found the same type of stomata on the surface of *Aristolochia bracteolata* leaves. We also note the presence of internal secretory glands in the form of channels (Fig. 9a-c).
Anatomy of the flower of Aristolochia longa L.

The flowers grow in the leaf axils (the single flower). They are inflated and globose at the base, continuing as a long perianth tube, ending in a tongue-shaped, brightly colored lobe. The perianth is constituted of three joined sepals (gamosepalous) of a petaloid aspect (Fig.10 a) and is morphologically divided into three regions. The longitudinal section of the plant flower(Fig.10b) is shown that is zygomorphic and hermaphrodite flower(bisexual), where there are six stamens. They are united with the style that appeared short, forming a gynostemium (Fig11.a; Fig12a,b). The Longitudinal section showing also pistil (=ovary+style+ stigma), the sepals(perianth) are above the ovary; such a flower is said to have an inferior ovary, or the flower is said to be epigynous (Fig.10b). This ovary has six carpels fused (6locules)(Fig.11b). This agreed with studies of 22, 28.

The anthers of stamens consists of two lobes (Fig12a and b) containing pollen, which has a spheroidal shape, without
aperture and with thicker exine (Fig. 12c), this observation corresponds to 29 study, but 28 was mentioned that the pollen of the Aristolochiaceae family has only one aperture.

The fruit is dehiscent capsule (Fig. 13a) with 6 valves (Fig. 13 b,c) and contain many endospermic seeds, which are triangle-shaped (heart-shaped) flattened or ± convex and smooth (Fig. 13d), it is similar to the description of 23.

These flowers have a specialized pollination mechanism. The plants are aromatic and their strong scent attracts insects. The inner part of the perianth tube is covered with hairs, acting as a fly-trap. These hairs then wither to release the fly, covered with pollen.

From the study that we have conducted, the floral formula of Aristolochia longa L. has been concluded and can be written as following:

This result is in accordance with the studies of 28 and 30.

Figure 10: Longitudinal section of A. longa L. flower showing pistil (=ovary+style+stigma). (The ovary is inferior)

Figure 11: The female reproductive part of the A. longa L. flower (carpel). a) 6 stigmas(sti), b) Cross-section through the ovary of A. longa L. showing 6 connate carpels (a compound pistil).
Stages of germination and the formation of tubers of *Aristolochia longa* L.

We planted the seeds of *Aristolochia longa* L. and we followed their germination and how the tubers were formed. After about two months, the seeds were spread and gave the results shown in the following figures. In Figure 14a we note the appearance of the vegetative part over the soil represented by the stem and leaves, and the radicle under the soil. After that, we notice a small hypertrophy under the seed (under the cotyledon); this indicates the beginning of the tuber’s formation (Fig. 14b,c,d). After 4 months we notice a good appearance of the tuber With the demise of the seed (Fig.14 e, f, g).

Figure 14h shows the young tuber clearly. As for the Figure 14i, it is an old tuber which seems woody tuber, this tuber is considered as perennating organs, therefore this plant is a perennial plant. Various forms of plant tuber are illustrated in Fig.14j. According to [31], tubers are formed from the enlargement of certain tissues in the plant, where the accumulation of savings is mainly carbohydrates, and thus we consider them as storage tissues. He has also concluded that the tubers can be produced from under the cotyledon (hypocotyle), such as radish (*Raphanus sativus*) and turnip (*Brassica rapa* L.), and the mechanism for forming tubers varies in terms of the organ responsible for creating this tuber, that is, according to the type of plant.
The results of the anatomical structure of the tuber plant which were previously explained have been consistent with \(\text{interpretation of the secondary structure of the}^{25}\text{"dicotyledon root. This makes us conclude that the tuber resulting from the enlargement of the hypocotyle part of a plant is similar to the anatomical structure of the root. With this experiment, we deduce that the tuber of this plant "Aristolochia longa L." is a root tuber.}

Fig.14. Stages of germination and the formation of Aristolochia longa L. tuber (a – j).
Phytochemical Screening of A. longa L.

This test is a preliminary estimate of the presence of active ingredients in plant material, The results obtained were listed in Table 01.

| Constituents      | aerial parts | fruits | tubers |
|-------------------|--------------|--------|--------|
| Phenolic compounds| +            | +      | +      |
| Flavonoids        | +            | +      | +      |
| Tannins           | +            | +      | +      |
| Terpenoids        | -            | +++    | +      |
| Alkaloids         | +            | +++    | ++     |
| Saponins          | +            | -      | -      |
| Quinones          | -            | -      | -      |
| Anthraquinones    | -            | -      | -      |
| Proteins          | +            | +      | +++    |
| Carbohydrates     | +            | +      | +++    |

*: Presence, -: Absence

Phytochemical screening of A. longa extract showed the presence of polyphenols, flavonoids, tannins (tannins catéchiques), terpenoids, alkaloids, saponins, carbohydrates and proteins. However, quinones and anthraquinones were not detected [Table 01]. Our results have been approved by benraba et Medlah (2014) results.

Species belonging to the genus Aristolochia have often been reported as important medicinal plants in ethno medicinal studies. According to 32 Aristolochia species contain secondary metabolites that are important natural toxins and traditional medicines, and as mentioned 33 the genus Aristolochia is an important source of physiologically active compounds that belong to different chemical classes, which is the subject of research in numerous pharmacological and chemical studies. Phytochemical investigations of these species revealed both the presence of aporphinic tetrahydroprotoberberinic, benzyltetrahydroisoquinolinic, and bisbenzyltetrahydroisoquinolinic alkaloids, Quinones, coumarins, flavonoids, and fatty acids are frequently isolated from plants of the genus. However, the most prominent compounds in Aristolochia are terpenoids, constituents of the essential oils isolated from the plant species. The majority of the identified terpenoids are kaurane, clerodane, and labdane diterpene derivatives 33.

CONCLUSION

- As a result of this field investigation of Aristolochia longa L. plant in Setif area (Algeria) we found that this plant has been used and spread only in the recent years, and we have concluded that the most used part is tubers for the purpose of special treatment of cancer by taking the powder of these tubers with honey in a small amount and for a specified period.
- The anatomical study of root, stem and leaf of Aristolochia longa L. showed that it has a typical structure of the dicotyledon plant, flowers have a tubular form, bisexual and zygomorphic. From the stages of tuber formation and the cross section of a young tuber it is proved that a plant tuber is a root tuber.
- Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, saponins, tannins. Thus, A. longa L. may be considered as a promising source of new drugs for treating cancer.

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