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Original Research

Ethnobotanical study, antifungal activity, phytochemical screening and total phenolic content of Algerian *Aristolochia longa*

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ABSTRACT

Aim: *Aristolochia longa* (from the family *Aristolochiaceae*) is widely used in Algerian traditional medicine. Here, we document ethnomedicinal uses by local population of Mascara province (West Algeria) and we evaluate the antifungal activity, the phytochemical composition and total phenolic content of aqueous extract (decoction) of the roots of *A. longa* from Algeria. **Materials and Methods:** The ethnobotanical investigation was carried out in Mascara Province (West Algeria). Antifungal activity was assessed against *Saccharomyces cerevisiae*. Total phenolic content was measured using the Folin–Ciocalteu's reagent. **Results:** Our results showed that *A. longa* is widely used to treat several ailments such as cancer (38%), skin infections (14%), and diabetes (11%). Crushed roots are commonly used (89%) mixed with honey, milk, water or other medicinal plants. *A. longa* aqueous extract induced growth inhibition of *S. cerevisiae* cells in a dose - and time - dependent manner. An effective suppression of *S. cerevisiae* (97.06% inhibition of proliferation) was obtained at the 500 μg/mL after 72 h. Results of the phytochemical screening revealed that *A. longa* aqueous extract contained various bioactive compounds, including polyphenols and flavonoids. Total phenolic content in *A. longa* aqueous extract was found to be 6.07 $\pm$ 0.12 mg (gallic acid equivalents)/g. **Conclusion:** *A. longa* may be considered as a promising source of new drugs for treating cancer and as a good antifungal agent.

KEY WORDS: Algeria, antifungal, *Aristolochia longa*, ethnobotany, Mascara, phenolic, phytochemistry

INTRODUCTION

Medicinal plants are one of major resources of therapeutic agents. They are used by 80% of the world population in health care [1]. Medicinal plant use knowledge developed by local populations in Africa is now disappearing. There is, therefore, an urgent need to document this traditional knowledge [2].

Aristolochia, Family *Aristolochiaceae*, is a large genus of herbs and twining plants, found in the tropical and temperate regions of the world [3]. *Aristolochia* species have well known beneficial effects. Some of them were found to possess anticancer activity in several bioscreening studies [4]. The *Aristolochia* species are cultivated as ornamentals and popularly used as sources of abortifacient, emmenagogue, sedative, analgesic, anti-inflammatory, anti-feedant, muscle relaxant, antihistaminic, and anti-allergic drugs [5]. We have recently published a review on biological activities of *Aristolochia* plants [6]. Phytochemical studies on plants of the *Aristolochia* genus have yielded various types of compounds with antitumor, antiplatelet aggregation, immunomodulating and antifertility activities [7]. *Aristolochia longa*, commonly known as "Berrostom" to the local population in Algeria, is widely used in traditional medicine. In our previous study, we demonstrated that the aqueous extract of A. *longa* induced cell growth inhibition of Burkitt's lymphoma (BL41) cells in a dose-dependent manner. The extract induced apoptosis, loss of mitochondrial membrane potential and the activation of caspases-9 and -3 followed by poly(adenosine diphosphate-ribose) polymerase cleavage [8].

As part of our continuing work to investigate and biologically evaluate Algerian medicinal plants used in cancer treatment, the present study aimed to document ethnomedicinal uses, and to evaluate phytochemical composition and antifungal activity of A. *longa*.

MATERIALS AND METHODS

Ethnobotanical Study

The ethnobotanical investigation was carried out in Mascara Province. Mascara (5941 km^2) is located in the north-west of Algeria, (at 360 km of Algiers) with Mediterranean climate and mean annual precipitations of about 450 mm. In 2010, the total population was 826,334, with male/female ratio of about 1.04. Ethnomedicinal uses of A. *longa* information were collected and

documented through casual conversations and semi-structured interview technique [9] with local herbal practitioners and knowledgeable residents of the study area. The main focus was to collect the oral information about the ethnomedicinal uses of A. *longa* by local population.

Preparation of *A. longa* Aqueous Extract

Roots of A. *longa* were collected in March 2009; in "Tissemssilet," an administrative region located in western Algeria. Botanic identification and authentication were made by Dr. Kada Righi (Department of Agriculture, Faculty of Nature and Life Sciences, Mascara University, Algeria). The roots were dried, pulverized and finely sieved. The aqueous extract of A. *longa* was prepared as follows: The dried roots were boiled for 20 min at 100°C, cooled to room temperature, and then filtered. The solution passing through the filter was collected, concentrated, lyophilized and stored in a desiccator at +4°C until use.

Antifungal Activity Evaluation

Antifungal activity was evaluated using *Saccharomyces cerevisiae* from our laboratory collection (Département de Biologie, Faculté SNV, Université de Mascara). Sabouraud's dextrose broth was used for the preparation of fungal cultures and for the antifungal activity evaluation. A volume of 50 μl of overnight liquid cultures of yeast (density of 2×10^8 cells/mL) was added to each well of 96-well plates containing 50 μl media and various concentrations (0-500 μg/mL) of the extract in triplicate. The plates were incubated at 37°C. Absorbance was measured at 620 nm using a spectrophotometer (Shimadzu, Japan).

Phytochemical Screening

A. *longa* roots were screened for the presence of phytochemical constituents, such as alkaloids, terpenoids, anthraquinones, flavonoids, tannins, saponins, steroids and glycosides, with the standard qualitative phytochemical procedures described [10].

Determination of Total Phenolic Content

Total phenolic content was measured using the Folin–Ciocalteu's reagent as described [11]. Absorbance was measured at 760 nm using an ultraviolet spectrophotometer (Shimadzu, Japan). Gallic acid was used as a standard. A dose response linear regression was generated using the gallic acid standard absorbance, and the total phenolic content was expressed as mg gallic acid equivalents (GAE)/g dry weight of extract. Values were determined in triplicate.

Statistics Analysis

Mean data values are presented, with their standard deviations (mean ± SD). All statistical comparisons were made by Student's *t*-test, and statistical significance was defined as $P < 0.05$.

RESULTS

Ethnobotanical Study

In the present study, we interviewed 100 informants (female: 92; male: 8) in different locations of Mascara province (West Algeria). Almost all of the respondents knew and/or used A. *longa* for medicinal purposes. According to our results [Figure 1], A. *longa* is recognized to treat several ailments and health disorders. Cancer is the first ailment treated with the plant (39%), followed by skin infections (14%), diabetes (11%) and gastro-intestinal ailments (9%). Moreover, infertility and gynecological troubles are also treated with A. *longa*.

As shown in Figure 2, roots are the most frequently used part by local populations of Mascara (89%). Leaves (9%) and the entire plant (2%) are also used. The most usually method of preparation used is the mixture crushed roots - honey (63%), taken orally. Mixture with milk (13%) or other medicinal plants (12%) and decoction in water (11%) are also used to treat internal ailments. The paste (plant grinded with olive oil or water) is applied externally for skin infections and rheumatism [Figure 3].

Antifungal Activity of *A. longa* Aqueous Extract

To investigate the antifungal activity of the A. *longa* aqueous extract, *S. cerevisiae* cells were incubated with increasing

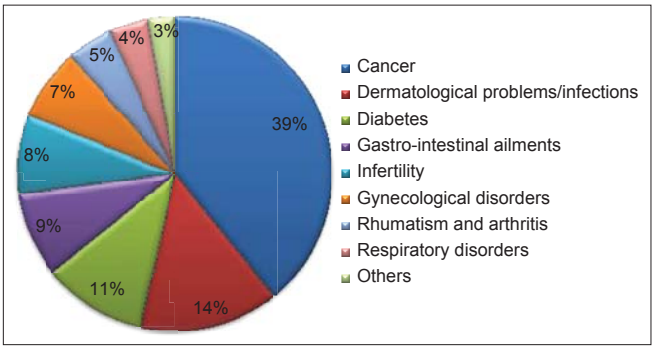

Figure 1: Ailments treatd with *Aristolochia longa*

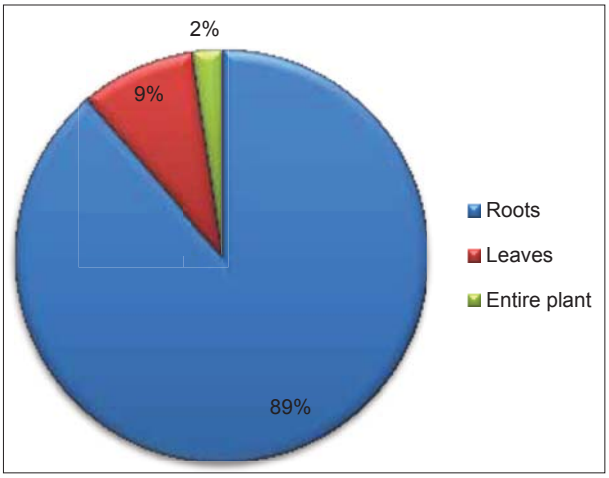

Figure 2: Plant parts used

concentrations (0-500 μg/mL). After 2, 48 and 72 h, cell viability was determined. Percent survival was determined when compared to untreated cells.

Cells were treated with increasing concentrations (from 0.00 to 500 μg/mL) of A. *longa* aqueous extract for 72 h and cell growth was measured as described in M and M. Percent survival was determined when compared to untreated cells. The difference in cell growth between untreated and A. *longa* aqueous extract-treated cells was found to be significant ($P < 0.001$). Data are represented as the mean ± standard deviation of three experiments per treatment group.

According to our results [Figure 4], A. *longa* aqueous extract induced growth inhibition of *S. cerevisiae* cells in a dose- and time- dependent manner. At 250 μg/mL, A. *longa* aqueous extract resulted in 30% and 67.65% inhibition of cell viability of *S. cerevisiae* cells after 48 and 72 h, respectively. At 500 μg/mL, the maximal inhibitory dose on the cell growth, the extract suppressed effectively the growth of *S. cerevisiae* cells after 72 h of treatment (97.06% inhibition of proliferation). We demonstrate a strong antifungal effect of A. *longa* aqueous extract as assessed against *S. cerevisiae* cells.

Phytochemical Screening of *A. longa*

Phytochemical screening of A. *longa* aqueous extract showed the presence polyphenols, flavonoids, tannins, C-heterosides, carbohydrates, and saponins. However, alkaloids, coumarins and O-heterosides were not detected [Table 1].

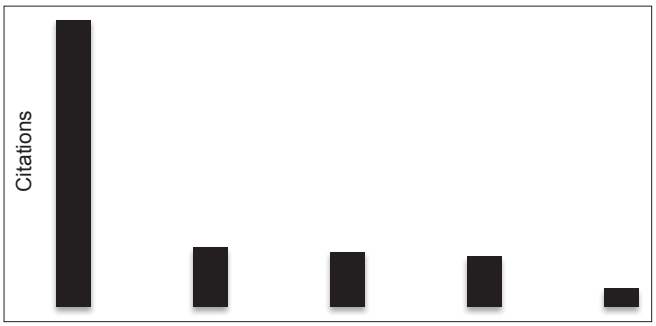

Figure 3: Preparation methods

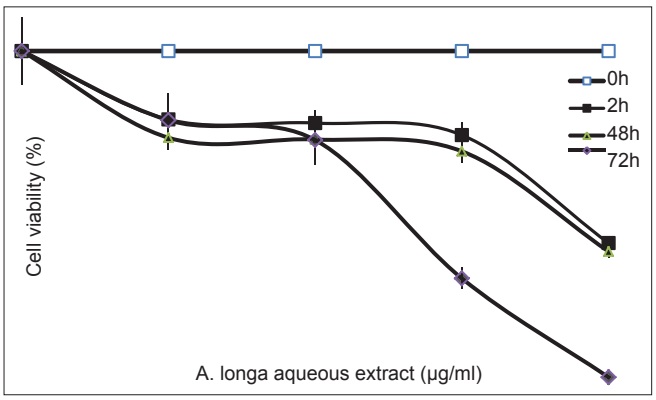

Figure 4: Effect of *Aristolochia longa* aqueous extract on growth *Saccharomyces cerevisiae* cells

Total Phenolic Content

Total phenolic content was estimated by using Folin–Ciocalteu reagent. Folin–Ciocalteu is a widely used and reported method for a simple and rapid estimation of total phenolics [12]. A standard curve [Figure 5] was obtained using gallic acid as a standard.

Total phenolic content in A. *longa* roots was found to be 6.07 ± 0.12 mg (GAE)/g.

DISCUSSION

Plants have played a vital role in the prevention and treatment of disease since prehistoric times [13]. Although local populations of Mascara have a long history of traditional use of medicinal plants, no studies have documented the traditional knowledge, which it is disappearing. The present study aimed to record the ethnomedicinal uses and to investigate the antifungal and the phytochemical profile of A. *longa*. The latter is widely used in Algeria.

Table 1: Preliminary phytochemical screening of *A. longa* roots

Constituents	Result
Phenolic compounds	+++
Flavonoids	+++
Gallic tannins	-
Catechic tannins	+++
Sterols and triterpenes	-
Alkaloids	-
Quinones	-
Carotenoids	-
Anthocyanins	-
Coumarins	-
Anthraquinones	-
Carbohydrates	+
C-heterosides	+
O-heterosides	-
Saponins	++

+: Presence, -: Absence, *A. longa: Aristolochia longa*

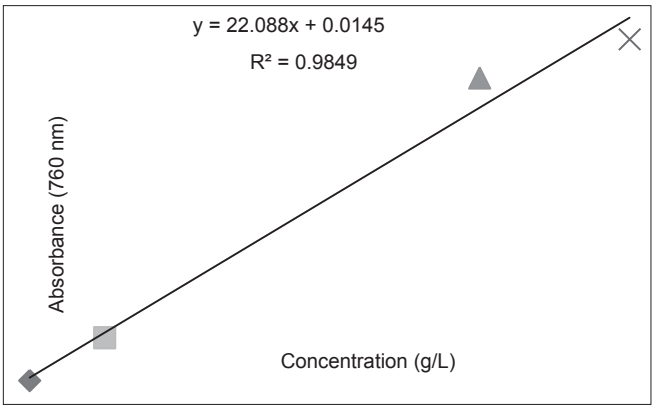

Figure 5: Calibration curve of gallic acid

Our results showed that the first ailment treated by local population using A. *longa* is cancer. Our findings are in consistence with previous studies. It has been reported that the most widely uses of A. *longa* in Algeria are in cancer treatment [14-16]. The use of this plant as an anti-cancer has been also reported in Morocco [17]. A*rthroleptis brevipes*, A*rachis monticola* and A*grimonia pilosa* are used as anticancer plants in Mexico [18]. We have recently demonstrated that an aqueous extract of A. *longa* induced apoptosis of BL41 cell line in a dose-dependent manner, by triggering the mitochondrial pathway [8]. Furthermore, our results revealed that skin infections are the second ailment group treated with A. *longa* in Mascara. Similar findings have been reported for A*ristolochia bracteolate* and A*cacia albida* used in Nigeria [19]. Current antifungal drugs are insufficient due to the limited selection of medications, their adverse side-effects and the emergence of resistance to them by fungal pathogens [20]. According to our results, A. *longa* aqueous extract induced growth inhibition of *S. cerevisiae* cells in a dose- and time- dependent manner. At 500 μg/mL, the maximal inhibitory dose on the cell growth, the extract almost abolished the proliferation of *S. cerevisiae* cells after 72 h of treatment (97.06% inhibition of proliferation). A. *longa* is considered as good antimicrobial drug [21]. Aristolochic acids, isolated from this plant inhibited *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [22]. Several plant extracts from the genus A*ristolochia* have been shown to possess strong *in vitro* antimicrobial activity [23-25].

Secondary metabolites produced by plants possess several interesting biological activities, and are a source of pharmacologically active principles against pathogenic microorganisms [26]. Since biological activities of medicinal plants are closely related to their chemical compounds [27], thus strong antifungal activity of the A. *longa* aqueous extract may be due to the detected compounds. Phytochemical screening of A. *longa* aqueous extract revealed the presence of polyphenols, flavonoids, tannins, c-heterosides, carbohydrates, and saponins. Phenolic compounds, flavonoids and saponins may be responsible for the antifungal activity. The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins. On the other hand, saponins appear to act by disrupting the membrane integrity of fungal cells [28]. In the current study, total phenolic content of the A. *longa* aqueous extract was measured and found to be 6.07 ± 0.12 mg (GAE)/g. In A. *longa* total phenolics and flavonoids were estimated as 1.47 mg/g and 0.8 mg/g, respectively [29].

CONCLUSION

We present evidence that A. *longa* is widely used by local populations of Mascara province (West Algeria) to treat several ailments such as cancer. Crushed roots are commonly used mixed with honey, milk, water or other medicinal plants. Moreover, a strong antifungal effect (dose- and time- dependent manner) of A. *longa* aqueous extract as assessed against *S. cerevisiae* cells was demonstrated. Phytochemical screening revealed the presence of bioactive compounds that may contribute to the antifungal activity of the A. *longa* aqueous extract, such as flavonoids or saponins. Thus, A. *longa* may be considered as a promising source of new drugs for treating cancer and as a good antifungal agent.

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