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Research Article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF RHIZOMES EXTRACT FROM *ARISTOLOCHIACLEMATITIS*

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ABSTRACT

Aristolochiaclematitis L is commonly used in Morocco in traditional medicine for the treatment of many disorders and ailments. The present study aims to assess the antibacterial activity of ethanolic extract of rhizomes of this plant using disc diffusion method. Seven bacteria strains (*Staphylococcucaureus*, *Bacillus cereus*, *Bacillus sp*, *Listeria ivanovii*, *Escherichia coli*, *Citrobacterfreundii*and *Salmonella sp*) and three fungi (*Candida albicans*, *Candida tropicalis* and *Aspergillusniger*).. All values are expressed as mean ± SD. The obtained results showed that ethanolic extract exhibited significant antimicrobial activity against all microorganisms used. The present study reveals that *A. clematitis* exerts beneficial effect by virtue of its antibacterial and antifungal effects. Therefore, this plant may be harnessed as drug formulation and as source for antibacterial and antifungal new compounds.

Keywords: Antibacterial, Antifungal,*Aristolochiaclematitis*, Rhizomes, Ethanol extract.

INTRODUCTION

Despite the advances achieved by modern pharmacology and medicine, 80% of the worldwide population benefits from the contributions of traditional medicine in terms of health care, especially in developing countries, in the absence of a modern medical system[1]. Therefore, traditional knowledge of medicinal plants and their use by population are not only useful for conservation of traditional knowledge and biodiversity but also for primary health care and drug development. Use of herbal medicines in Morocco represents a long history of human interactions with the environment[2]. Traditionally used medicinal plants produce a variety of compounds known for their therapeutic properties. For this reason, such plants should be investigated to better understand their properties, safety and efficiency. Otherwise, medicinal plants may be an important source of potentially useful new compounds for the development of drug agents.

Aristolochia is an important genus in the family of Aristolochiaceae. The genus Aristolochia consists of about 400 species of herbaceous perennials, under shrubs or shrubs. Its members are commonly known as birthwort, pipevine or Dutchman's pipe and are widely distributed in tropical, subtropical and temperate regions[3].Although acknowledged as toxic herbs due to the presence of aristolochic acides[4], Aristolochia species are still widely used in different traditional medicinal systemsworldwide[3,5,6]. In Indian traditional medicine, *Aristolochiabracteolata*, commonly known as Midmari, Kitamari, Kattachirubanaguda, Paniri, Aaduthinnapalai, Gadidagadapa and Gandhari,is used as gastric stimulant, purgative, aphrodisiac, antipyretic, anthelmintic, anti-inflammatory and it is also used in the treatmentof intermittent fever, malaria, parasitic infestations, various skin diseases, edema, intestinal disorders, cancer, lung inflammation, dysentery, snakebites, syphilis and gonorrhea. Otherwise, it facilitates deliverance by increasing uterine contractions[6,7].

Traditional Moroccan medicine still often uses Aristolochia species under the vernacular name of Bereztem for the treatment of numerous ailments, notably cancer, diabetes or digestive tract ailements[2,8].

The objective of the present study is to evaluate the antimicrobial potential of ethanolic extract obtained from rhizomes of *A. clematitis*.

MATERIAL AND METHODS

Plant material

A. clematitis was collected during spring 2015 in Taza city (Northeastern Morocco). Plant material was authenticated by a specialist. A voucher specimen (reference CA10/15) is kept on file in our laboratory.

Preparation of plant extract

The air-dried rhizomes of *A. clematitis*(500g) were powdered mechanically and sieved using a fine muslin cloth. The obtained powder was exhaustively extracted by soxhlet using ethanol 95% (V/V) at 35°C, giving 135g of the crude preparation. It was then filtered and concentrated using a rotary evaporator under reduced pressure at 35°C to prevent thermal decomposition of labile compounds. The crude extract (35g) was stored at +4°C until use.

Microorganisms

The antibacterial screening was conducted against four Gram positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Bacillus sp* (CIP 104717), *Bacillus cereus* (ATCC 33019) and *Listeria ivanovii* (ATCC 19119), and three Gram negative bacteria: *Escherichia coli* (CIP 54127), *Citrobacterfreundii* (ATCC 8090) and *Salmonella sp*.

For antifungal test, two yeasts (*Candida albicans*and *Candida tropicalis*) and one filamentous fungus (*Aspergillusniger*) were used.

All the microorganisms were procured from Pasteur Institute (Casablanca, Morocco). They were maintained by subculturing periodically and preserved at +4°C prior to use. For inocula preparations, bacteria and fungi were incubated for 24 h in Mueller Hinton Agar medium (MHA) and 3 days in Potato Dextrose Agar (PDA) respectively.

Antimicrobial test

Susceptibility of the microorganisms to plant extracts was determined by employing the disc-diffusion method (7). The bacterial and yeasts cultures in the exponential phase of growth or fungal spore solution were spread on MHA or PDA plates in order to give a population of approximately 10^8 CFU/plate.

Commercial paper discs (6 mm in diameter), sterilized at 120 °C for 15 min, were first impregnated separately with 20 µl of three concentrations of extract (38, 75 and 150 mg of extract/ml), and were then deposed on the surface of the inoculated plate. The plates were kept at 4°C for 2 h and then incubated for 24h (bacteria and yeasts) or 3 days (filamentous fungi) at 37°C under aerobic conditions and the diameter of the inhibition zone around each disc was then measured and recorded. Negative control was set up with equivalent quantities of solvents used in the extraction. Ampicilline (30µg /disc) and Fluconazole (30 µg/disc) were used as positive control. All the experiments were performed in triplicate and the results (mm of zone of inhibition) were expressed as mean value ± standard deviation.

Antimicrobial efficiency of extracts was evaluated according to the following scale:

Ø ≤ 9mm : Non-significant antimicrobial activity

9mm< Ø ≤ 13mm : Moderate antimicrobial activity

13mm< Ø ≤ 17mm : Significant antimicrobial activity

Ø > 17mm : Very significant antimicrobial activity

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA). P Values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

It is well known that the widespread use of commercially antibiotics led to the consequence of emergence of antimicrobial resistance that ultimately led to the threat to global public health. The medicinal plants represent therefore an important source of potentially new compounds for the development of new antibiotics. The present study deals with the antibacterial and antifungal potentials of ethanol extract obtained from rhizomes of *A. clematitis*.

Antibacterial activity

The antibacterial activity of the plant extract, assessed by diameter of inhibition (mm), is reported in table 1. The results showed that ethanol extract exhibits broad-spectrum antibacterial activity against all Gram positive and Gram negative bacteria tested in a dose-dependent manner, albeit to varying extent. At 150mg of extract/ml, a very significant antibacterial effect (Ø > 17mm) is obtained against *E. coli*, whereas *B. cereus,L. ivanovii*and *C. freundii*aresignificantly inhibited (13mm< Ø ≤ 17mm). Otherwise, a moderate effect (9mm< Ø ≤ 13mm) was obtained against *B. sp, S. sp and S.*

aureus. In the same conditions, Ampicillin, used as antibiotic standard, showed a very significant effect (Ø > 17mm) against *B. sp, L. ivanovii, E. coli* and *C. freundii* whereas *S. aureus, B. cereus* and *S. sp* were significantly inhibited. It is to note, that negative control (ethanol) exhibited no significant effect (data not shown).

The antibacterial potential of ethanol extract of rhizomes from *A. clematitis,* against all bacteria strains tested, is clearly demonstrated. To our knowledge it is the first time that this activity is reported for *A. clematitis*. However, previous studies have reported the antibacterial potential extracts from different parts ofotherspecies of Aristolochia as *A. paucinervis Pomel*[9],*A. cymbifrea*[10,11], A mollissima[12], *A. indica*[13,14], *A. bracteata*[15]and *A. galeata*[16].

Antifungal activity

The antifungal activity of the ethanol extract of rhizomes from*A. clematitis* wasalso evaluated against three fungal strainsand recorded in table 2. The diameter of inhibition against fungal pathogens ranged between 15-9mm. At the high concentration (150mg of extract/ml) the three fungal strains were significantly inhibited (13< Ø ≤ 17mm) . In the same conditions, Fluconazol, used as antifungal standard at 30µg/disc, excreted a very significant antifungal activity against yeast strains (*C albicans and C. tropicalis*) and moderate effect against filamentous fungus (*A.niger*). It is to note, that negative control (ethanol) exhibited no significant effect (data not shown).

Our results are corroborated by those of Nacza-Farkaset al[17]who proved that moderate antifungal activity (MIC = 33mg/ml) was observed with ethanolicextract of aerial parts of *A. clematitis*against various species of Candida. This antifungal effect was also reported by various studies using other species of Aristolochia as *A. indica*[14]and*A. bracteata*[15].

CONCLUSION

In the present study, the antimicrobial effect of ethanol extractof rhizomes from *A. clematitis* was clearly established by the measurement of inhibition diameters. Our results were consistent with traditional uses of *A. clematitis,* which is prescribed against various infectious diseases as gastro-intestinal infection, skin diseases, syphilis and gonorrhea. Furthermore, the detection of antimicrobial activities indicates that this plant may be a source for antibacterial and antifungal new drugs. Bioassay-guided research in progress could reveal new, renewable and more potent compounds in this plant.

Table 1: Antibacterial effect of ethanol extract of rhizomes from *A. clematitis* (n=3, M± SD)

	Bacteria strain	Diameter of inhibition (mm)			
		Ethanol extract mg/ml			Ampicillin
		38	75	150	10µg/disc
Gram positive	*Bacillus. Sp*	7.00±2.94	8.75±2.75	9.75±3.80	20.00±0
	Bacillus cereus	8.25±1.71	13.25±0.96	17.00±2.80	16.50±2.10
	Listeria ivanovii	9.00±1.41	10.00±2.16	16.25±1.00	22.50±3.50
	Staphylococcus aureus	8.00±1.83	9.75±2.63	13.00±5.60	13.50±0.70
Gram negative	*Escherichia. coli*	8.25±1.30	14.25±0.96	20.50±3.10	31.50±2.10
	Citrobacterfreundi	7.50±0.58	9.50±0.58	16.00±5.90	25.50±3.50
	Salmonella sp	6.25±1.26	9.00±1.41	10.75±2.50	15.00±1.40

Table 2: Antifungal effect of ethanol extract of rhizomes from *A. clematitis*(n=3, M± SD)

Fungal strain	Diameter of inhibition (mm)			
	Ethanol extract mg/ml			Fluconazol
	38	75	150	10µg/ml
Candida tropicalis	9.0±0	10.0±0	15.0±0	17.5±3.5
Candida albicans	9.5±0.7	10.5±0,7	14.5±2,1	20.0±0
Aspergillusniger	9.0±0	10.0±0	15.0±0	11.5±2.1

REFERENCES

1. Northridge ME and Mack RJR. Integrating ethnomedicine into public health. Am J Public Health. 2002 ;92:1561.
2. Bellakhdar J. La pharmacopée traditionnellemarocaine (Moroccan Traditional pharmacopeia). Ibis Press, Paris (France), 1997.
3. Ping-Chung K, Chiun L and Shung W. Chemical constituents and pharmacology of the Aristolochia species. J Tradit Complement Med. 2012;2(4): 249-66.
4. Debroe ME. Chinese herbs nephropathy and Balkan endemic nephropathy: toward a single entity, aristolochic acid nephropathy.Kidney International. 2012; 81:513-15.
5. World Health Organisation. IARC monographs on the evaluation of carcinogenic risks to human. Some traditional herbal medicines. Some mycotoxins, naphthaleneand styrene. Vol 82, IARC Press. Lyon (France). 2002;69-118,
6. Chawla P, Chawla A, Shah G, Baghel US and Dhawan RK. Review on pharmacology and biological activities of Aristolochia. As J Res Biol Pharma Sc. 2013;1(2):97-106.
7. Heinrich M, Chan J, Wanke S, Neinhuis C and Simmonds M. Local uses of Aristolochia species and content of nephrotoxic aristolochic acid 1 and 2- A Global assessment based on Bibliographic sources. Journal of Ethnopharmacol. 2009;125: 108-44.
8. Yamani A, Bunel V, Antoine MH, Husson C, Stévigny C, Duez P, Elachouri M and Nortier J. Substitution between Aristolochia and Bryonia genus in North-Eastern Morocco: toxicological implications. Journal of Ethnopharmacol. 2015; 26:166:250-60.
9. Ghadi CA, Weber M, Mory F, Benharref A, Lion C, Jana M and Lozniewski A. Antibacterial activity of Aristolochiapaucinervis Pomel. Journal of Ethnopharmacol. 1999;67(1):87-92.
10. De Barros Machado T, Leal ICR, Kuster RM, Amaral ACF, Kokis V, de SilvaMG and dos Santos KRN. Brazilian phytopharmaceuticals – evaluation against hospital bacteria. Phytotherapy Research. 2005;19:519–25.
11. Alviano WS, Alviano DS, Diniz CG, Antoniolli AR, Alviano CS, Farias LM, Carvalho MAR, Souza MMG and Bolognese AM. In vitro antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Archives of Oral Biology. 2008; 53:545–52.
12. Yu JQ, Liao ZX, Cai XQ, Lei JC and Zou GL. Composition antimicrobial activity and cytotoxicity of essential oils from Aristolochiamollissima. Environm ental Toxicology and Pharmacology. 2007; 23:162–67.
13. MuruganM and Mohan VR. Efficacy of different solvent extracts of Vitextrifolia L. and Aristolochiaindica L. for potential antibacterial activity. Science Research Reporter. 2012; 2(1):110-14.
14. Surendra Kumar M, Rajeswari M and Astalakshmi N. Evaluation of antimicrobial activities of Aristolochiaindica L. Int J Pharm Pharm Sci. 2011;3(4):271-72.
15. Deepa T, Kamalakannan P, Elamathi R, Kavitha R and Sridhar S. Studies on antibacterial, antifungal activity and phytochemical analysis ofAristolochiabracteata Retz. J Chem Pharm Res. 2012;4(3):1449-53.
16. Aleixo AA, Camargos VN, dos Santos Pereira Andrade AC, Silva Herrera KM, de AzambujaRibeiro RIM and dos Santos KM. Antibacterial and cytotoxic antibacterial potential of ethanol extract and fractions from AristolochiagaleataMart. ex Zucc. J Med Plant Res. 2014;8(6):326-30.
17. Nacsa-Farkas E, Kerekes E, Kerekes EB, Krisch J, Roxana P, Vlad DC, Ivan P and Vágvölgyi C. Antifungal effect of selected European herbs against Candida albicansand emerging pathogenic non-albicansCandida species. Acta BiologicaSzegediensis. 2014;58(1):61-64.