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Research Article

ANTICANDIDOSIC ACTIVITY OF LEAF, STEM AND ROOT EXTRACTS OF *Ajuga iva* L. FROM ALGERIA

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ABSTRACT. Superficial candidiasis is a real public health problem, especially in immunocompromised people and HIV-infected patients. Herbal medicines have been in use for many years and remain widespread in developing countries. *Ajuga iva* L. is important plant for its medicinal benefits among most communities in treating a wide range of ailments. The objective assigned to the present study is the evaluation of the anticandidosic activity of the methanolic extracts of the different organs of *Ajuga iva* L. The antifungal activity of the extracts is carried out by the diffusion method on agar medium against *Candida albicans*. Among different concentrations of extracts studied, 23.75 mg/ml of Leaf extracts showed maximum inhibition against the test organism under investigation.

Keywords: *Ajuga iva L., Guelma, methanolic extract, antifungal effect, Candida albicans*

INTRODUCTION

Candida yeast genus is the most common opportunistic pathogen among fungal infections causing high mortality and morbidity. It involves yeast pathogenic isolated from skin, mouth, intestinal tract and vagina. Over the past few decades, the incidence of candidiasis has increased especially with the growing number of immunocompromised patients [1]. Resistance of Candida yeast genus to current medications, including the azoles, was reported [2]. The search for new alternative strategies is therefore important in order to fight Candida infections. Phytotherapy represents a more effective and less toxic alternative than standard drugs as anti-candida agents [3, 4, 5]. Herbal medicines have been in use over the years in many parts of the world especially within developing countries [6]. More than 80% of the population in Africa use traditional medicine to serve their health needs, according to the World Health Organisation [5].

Ajuga iva is a small (5-10 cm) wooly, aromatic perennial herb with well documented anti-ulcerous, hypoglycemic and anti- inflammatory activities. Topically, it has been used for wound healing and breast hardness. It was mentioned as "*Chendgoura"* in traditional Algerian medicine.

Ethnopharmacological surveys have revealed that some 20 species of *Ajuga* plants are used in traditional medicine mostly in Africa, Asia and China. In North Africa, *Ajuga* plants are used to treat diabetes and hypertension [7,8]. Other reported activities of *Ajuga* plants include antibacterial, antifungal, anti-inflammatory, antimalarial/antiplasmodial, antimycobacterial, antioxidant, antipyretic, larvae and insect antifeedant and insect growth inhibitor activity [9].

The aim of this study was to evaluate anti-fungal effects of methanolic extract from leaf, stem and root of *Ajuga iva* L. against *Candida albicans*.

MATERIALS AND METHODS

Plant Material

The species *Ajuga iva* L. was harvested in the region of Guelma (north-*eastern* Algeria) in February 2019.

The identification of the plant was done with the key to determining the flora of Quezel and Santa [10]. Specimens were kept at the Laboratory of Cryptogamy and Medical Botany, Department of Pharmacy, Faculty of Medicine Annaba-Algeria.

Preparation of Methanolic Extracts

Dry parts (stem, leaf and root) of *Ajuga iva* L. have been ground and stored in glass bottles, hermetically sealed at low temperatures. 10 g of the vegetable powder was macerated in 100 ml of methanol with stirring for 24 hours at a temperature of 25 ± 2° C. The extract obtained was filtered and evaporated to dryness under reduced pressure at 50° C on a rotavapor. The dry residue is taken up in 3 ml of methanol and stored at -18 ° C until it is used [11].

Antifungal test

The test of the sensitivity of the fungi is carried out by the diffusion method in agar medium (the disk method). It is a method similar to that of the antibiogram which consists in determining the sensitivity of a fungi strain vis-à-vis one or more substances [11].

The antifungal activity of the methanolic extracts of the stem, leaf and root of *Ajuga iva* L. is evaluated vis-à-vis *Candida albicans*. This strain was kindly provided by the Microbiology Laboratory Manager at Annaba Medical School, Algeria.

Preparation of The Inoculum

For the preparation of the different concentrations of extracts, 2,5 mg of each freeze-dried extract (methanolic extract of leaf, stem and root), are introduced into a labeled tube, in which we added 1 ml of dimethylsulfoxide (DMSO), solvent without any antifungal effect. The tubes are vortexed until complete dissolution of the extract, and the dilutions are prepared to obtain X/4, X/8, X/16 and X/32 concentrations from the stock solution.

Seeding should be done within 15 minutes after the preparation of the inoculum. In 69 sterile Petri® dishes, 20 ml of agar are poured. After solidification of the medium, the latter is inoculated with 1 ml of fungi to be studied. Then, it is spread on the surface using a glass rake.

Sterile 5 mm diameter disks prepared in Whatman® n°1 papers are impregnated with a sterile metal forceps in each concentration and placed on the surface of the solidified medium (Sabouraud). The dishes were incubated for half an hour at room temperature, then for 24 to 48 hours in an oven at 37 ° C.

The reading is carried out by measuring the diameter of the inhibition zone (Ø), which translates into a translucent halo around each disc; the presence or absence of a halo would explain the sensitivity or the resistance of the germ vis-a-vis extracts tested; according to a symbolic notation scale from - to +++ [12].

Table 1. *Sensitivity of fungal strains according to zones of inhibition*

Sensitivity	Inhibition zone
Not sensitive or resistant (-)	Diameter <10 mm
Sensitive (+)	Diameter between 10 to 16 mm
Very sensitive (++)	Diameter between 16 to 25 mm
Extremely sensitive (+++)	Diameter > 25 mm

RESULTS AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. In the present investigation *Candida albicans* was tested to determine antifungal activity of methanolic extract of *Ajuga iva* L. leaf, stem and root, the details of the results are shown in Table 2.

Table 2. *Inhibition Diameter (mm) of methanolic extracts of Ajuga iva* L.

Dilutions of leaf extract				Dilutions of stem extract				Dilutions of root extract			
x/4 (47.5 mg/ml)	x/8 (23.75 mg/ml)	x/16 (11.87 mg/ml)	x/32 (5.93 mg/ml)	x/4 (10 mg/ml)	x/8 (5 mg/ml)	x/16 (2.5 mg/ml)	x/32 (1.25 mg/ml)	x/4 (7.5 mg/ml)	x/8 (3.75 mg/ml)	x/16 (1.87 mg/ml)	x/32 (0.93 mg/ml)
6.3 mm	10.7 mm	8.3 mm	8.2 mm	8.4 mm	10.3 mm	7.4 mm	6.2 mm	6.2 mm	7.1 mm	6.2 mm	6.2 mm

mg: milligram, ml:milliliter, mm: millimeter

Among different concentrations (47,5mg/ml, 23,75mg/ml, 11,87mg/ml and 5,93mg/ml) of leaf extracts studied 23,75mg/ml showed the highest activity (10,7mm), followed by the concentration 5mg/ml of stem extract (10,3mm). Whereas methanol extract of root of *Ajuga iva* L. at concentrations of 7,5mg/ml, 3,75mg/ml, 1,87mg/ml and 0,93mg/ml could show no antifungal activity against *Candida albicans*. A low concentration gives more activity than a high concentration was noticed, a saturation of the molecular target by the active compounds could explain this effect [13].

In recent years, there has been an increasing amount of literature on the role of plant extracts in treating variety of diseases and their biological activities like antimicrobial, antibacterial, antiviral and antifungal activities [14, 15]. Antimicrobial or antifungal activity of medicinal plant is due to the presence of phenolic or saponins compounds [16, 17].

Ajuga iva contain a wide range of compound such as ajugapyrin A, bracteonin A and iridoids which had a wide range of biological and pharmacological activity. The plant contain also, a class of secondary metabolites which are produced by plants primarily as a defense against herbivores or against infection by microorganisms [18, 19], 20-hydroxyecdysone, cyasterone, ajugasterone, apigenin dihexoside, apigenin, carvacrol, ecdysterone, palmitic acid are also identified in different parts of *A. iva*. These compounds belong to different classes of chemical compounds such as steroids, flavonoids, fatty acids, and terpenoids [20]. Phenolic compounds in *A.iva* are known to be good antimicrobial agents [21, 22, 23].

The results provide the justification for the use of *Ajuga* in folk medicines to treat various infectious diseases and suggest that methanolic extract possesses some bioactive compounds with antifungal activities.

In the work conducted by Mouheb *et al*. (2018) *A.iva* aqueous extract showed a very weak or no activity against the used bacteria and fungi [24]. Makni *et al*. [25] from Tunisia obtained mixed results about the antibacterial and antifungal activity of *A.iva* methanolic and aqueous extract. The methanolic extract showed the highest inhibitory effect against *Escherichia coli, Staphylococcus aureus and Fusarium,* while the effect of the aqueous extract was weak with all tested strains.

In other study on *Ajuga remota*, the petroleum ether and methanol extracts showed antifungal activity against the dermatophytic fungi: *T. mentagrophytes* and *M. gypseum*. However, these extracts were not fungitoxic to the yeast *C. albicans* and the plant pathogen *C. cucumerinum*. These results may justify the traditional use of this species for the treatment of skin diseases that may be caused by fungal pathogens [26].

Many authors tested the anticandidotic activity of flavonoids [27, 28, 29]. Wabo et al. tested the anticandidotic activity of a new quinones as Pycnanthuquinone C isolated from *Pycnanthus angolensis* [30]. Alkaloids have also been reported to possess antifungal activity [29]. In addition to this, the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi and they may assume pharmacological importance [31]. Furthermore, saponins are a special class of glycosides that have soapy characteristics and it is consider as active antifungal agents [32]. In fact, it was reported in the literature, that the rate of diffusion of an antimicrobial through the agar is not always the same, it depends on diffusion and solubility properties, the concentration, and the molecular weight of the antimicrobial components; and also, on the agar depth, which can influence the antimicrobial diffusion and microorganisms growth [33].

There is also some evidence that minor components have a critical part in antifungal activity, possibly by producing a synergistic effect between other components.

CONCLUSION

The study of antifungal efficacy against *Candida albicans* revealed that the methanolic extracts of the leaf and the stem of the *Ajuga iva* L. from Guelma, Algeria have ability to kill or inhibit the growth of Fungi which experimentally support the use of this plant in traditional medicine by traditional healers. The results presented in this study are encouraging. A wider study is needed to identify the effective components, mode of action and possible toxic effect *in vivo* of these ingredients. Further investigations should be carried out in finding other activities of the extracts of leaf, stem and root. It is therefore important to investigate the potential of this plant as novel antifungal agents, targeting the multidrug resistant fungi of clinical importance.

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