Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 1, 2013, pp. 65-69
© 2013 Vasile Goldis University Press (www.studiauniversitatis.ro)

TESTING OF THE ANTIFUNGAL EFFECT OF EXTRACTS OF BURDOCK, THYME AND ROUGH COCKLEBUR

Marian BUTU[1], Andreea DOBRE[1], Steliana RODINO[1,2,*], Alina BUTU[1], Dumitru LUPULEASA[3]
[1]National Institute of Research and Development for Biological Sciences, 060031, Splaiul Independentei 296, Bucharest, Romania, Tel/fax +40 212 200 880, steliana.rodino@yahoo.com
[2]University of Agronomic Sciences and Veterinary Medicine, 011464, Mărăşti Blvd. 59, Bucharest, Romania
[3]University of Medicine and Pharmacy "Carol Davila", 0010202, str. Traian Vuia 6, Bucharest, Romania

ABSTRACT. The present work studied the antifungal activity of the alcoholic extracts obtained from three native plant species: burdock (*Arctium lappa*), thyme (*Thymus vulgaris*) and rough cocklebur (*Xanthium strumarium*). The demonstration of the antifungal activity was realized using *Aspergillus ochraceus* and *Acremonium chrysogenum isolates*. The antifungal activity of the selected plant extracts was evaluated by radial growth measurement on potato dextrose agar amended with plant extracts of different concentrations. Fungi were completely inhibited in the variant using plant extracts of the following concentration: 0.3 g plant material / ml to 70o ethanol. When using concentration of 0.075 g plant material / ml to 70o ethanol, the development of fungi was significantly inhibited.

Keywords: plant extract, antifungal effect, *Arctium lappa, Thymus vulgaris, Thymus vulgaris*

INTRODUCTION

Pathogenic fungi are the main infectious agents attacking plants all over the world. They are the cause of alterations starting from the development stages of the plants until storage, causing various disorders related to appearance, nutritional value, organoleptic characteristics, etc. (Agriso, 2004).

Furthermore, phytopathogenic fungi development is also responsible for food contamination with mycotoxins, one of the main representatives being *Aspergillus*. This fungal specie is responsible of contamination of vegetal material with several aflatoxins and ochratoxin and patulin (Varga et al., 2004), generating teratogenic, carcinogenic, immunosuppressive and nephrotoxic effects (Creppy, 2002). Recent studies have shown that besides the pathogenic fungi from *Aspergillus spp* (K. Kamei and A. Watanabe, 2005), another saprophyte fungus, *Acremonium* joined the phytopathogenic group causing hyalohyphomycosis (S. Das et al., 2010).

The process of eliminating mycotoxins from foods can be realized by physical, chemical or biological methods. These methods must be efficient, cheap and must not alter the nutritional quality of foods and, in addition, the treatments should not leave any residues that might be capable of producing side effects. Using the methods of detoxification of the food contaminated with mycotoxins is currently restricted due to health issues that may arise and the possible reducing of the nutritional quality of food. Moreover, applying these methods results in high costs, fact that led to searching for new strategies and approaches such as the biological control of the crops.

Current control of phytopathogenic agents is done using synthetic fungicides. Along with increasing of the restrictions regarding their use due to adverse effects on human health and the environment (Harris et al., 2001), appeared the need to find alternatives to fungicides. Research is currently being carried for the development of biological methods that will work in the inhibition fungal growth, such as the use of antifungal active ingredients extracted from plants (Quiroga et al., 2001).

Burdock, thyme and rough cocklebur are three native plant species, spread all over the country, that produce secondary metabolites such as tannins, terpenoids, flavonoids and alkaloids whose activity has been demonstrated to be antifungal (Gujar et al., 2012).

Arctium lappa, commonly named Burdock, is known as medicinal herb with many therapeutic effects such as antioxidant, antidiabetic, anti-inflammatory, antitumor (Chan et al., 2011) and antimicrobial (Holetz et al., 2002). This is due to the different classes of bioactive secondary metabolites that this plant presents, such as flavonoids, lignans (Ferran et al, 2010) and fitoalexins. Recent studies demonstrated the antimicrobial activity of alcoholic extracts of *Arctium lappa* on the species of fungi *Staphylococcus aureus, Alternaria solani* and *Fusarium graminea* (Sun et al., 2008).

In vitro antimicrobial activity was determined for the extracts of *Thymus vulgaris*, commonly known as thyme, which was reported to inhibit the growth of bacteria such as *Bacillus subtilis*, *Enterococcus fecalis, Pseudomonas aeruginosa, Salmonella enteritidis*, and *Streptococcus pyogenes* (Güllüce et al., 2003), *Candida albicans* (Sahin et al., 2003).

Rough cocklebur, *Xanthium strumarium*, contain in their structure a series of compounds such as glycosides and phytosterols, which have antibacterial, antifungal, antirheumatic, cytotoxic, effect. Antifungal activity is also given by the presence of terpenes, such as limonenels and carveol (Kamboj et al., 2010).

The present study evaluated in vitro growth inhibition of *Aspergillus ochraceus* and *Acremonium*

***Correspondence:** Steliana Rodino, National Institute of Research and Development for Biological Sciences, 060031, Splaiul Independentei 296, Bucharest, Romania, Tel/fax +40 212 200 880, steliana.rodino@yahoo.com*
Article received: November 2012; published: February 2013

chrysogenum species using alcoholic extracts obtained from these three selected plants of the local flora.

MATERIALS AND METHODS

Preparation of fungi

The fungi used for all the tests belong to the collection of the Biotechnologies Department laboratory of NIRDBS. The fungi are kept in PDA agar petri dishes under 25 ºC. Cultures of 1 week old were used for all the testing.

Collection of Plant Materials

Arctium lappa and *Xanthium strumarium* belong to wild flora and were collected locally from their natural habitat, in the month October, from agricultural fields located in Baragan Plain region in Southern Romania. The fresh plants were well cleaned with tap water in order to remove the dust and then dried artificially at 40oC. The dried samples were cut in small pieces and stored in proper conditions for further use in the experimentation scheme.

Thymus vulgaris vegetal material was purchased from SC Plafar SRL, a Romanian supplier of medicinal plants.

Extraction Procedure

Plant extracts were obtained by cold percolation method, using as solvent 70% ethanol to 15g of dried and ground plant material. For *Arctium lappa* was used the root, the leaves for *Thymus vulgaris*, and whole plant for *Xanthium strumarium*.

Plant extracts were tested in different concentrations. In order to determine the minimum concentration of extract that inhibits the growth of fungi, were used the following concentrations 0.3 g/mL, 0.15 g/mL, and 0.075 g/mL.

Determination of antifungal properties of the extracts

The antifungal effect of the preparations obtained from *Arctium lappa, Thymus vulgaris* and *Xanthium strumarium*, on mycelial growth of the tested fungi was assessed with the radial growth method as described by Banso et al. (1999). Freshly autoclaved Potato dextrose agar (PDA) agar was amended with the plant extract in a ratio of 1:10, when cooled down to approximately 40 oC and poured into plastic Petri dishes (9 cm). Control Petri dishes were used containing nonamended PDA or PDA with added alcohol (10:1). Consecutively, the Petri plates were inoculated with 6 mm mycelial discs of the tested fungi which were placed upside down in the center of each plate. The petri plates were incubated for 7 days at 25oC. Radial growth of each fungal colony was recorded at exactly 24 h, 120 h and 168 h intervals.

The experiments were run in 3 replicates.

RESULTS AND DISCUSSIONS

Aspergillus ochraceus

At 24 hours of incubation, both the control and the Petri dishes treated with the plant extracts did not show any change in the development of fungi.

On the 5th day, at 120 hours of incubation, the level of development of the fungi varies depending on the treatment incorporated in the PDA. Maximum concentration of plant extract, no matter what plant material was used, had total inhibitory effect on *Aspergillus inoculum*. The Petri plates amended with 0.15 g/mL extract presented a small increase of the fungal colonies, their diameter being of approx. 1.8 cm, 2 cm and 3 cm. Plant extracts of 0.075 g / mL showed a lower level of inhibition, fungal growth being visible since the 3rd day, and on the 5th day of the experiment the diameter of fungal colonies being about 3 cm. It is to be noted the existence of secondary colonies and also different level of development of the fungi depending on the extract incorporated in the PDA medium. The fungal colonies from the Petri plate treated with *Xanthium strumarium* (Figure 1) extract was in an earlier development stage (vegetative hyphae formation) compared with *Aspergillus* cultured on medium treated with extracts of *Arctium lappa* (Figure 2) and *Thymus vulgaris* presenting reproductive hyphae formation (the formation of spores) (Figure 3). Control plates are also in vegetative development stage (Figure 4).

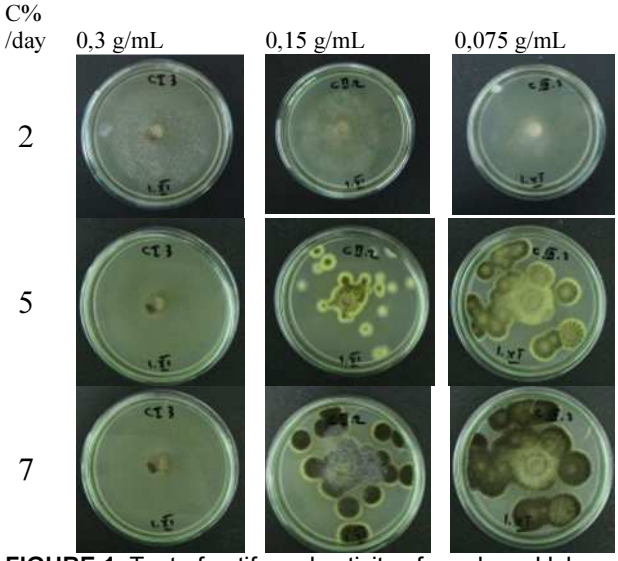

FIGURE 1. Test of antifungal activity of rough cocklebur extract

In the 7th day, at 168 hours after inoculation, the fungi cultured on medium treated with extracts of 0.3 g/mL did not show any sign of development. In the case of the Petri plates treated with extracts of 0.15 g/mL the primary cultures of fungi reached diameters of about 4 to 5 cm. It is to be noted that the thyme extract inhibited the development stage of the fungi (vegetative hyphae) compared with the state of

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 1, 2013, pp.65-69
© 2013 Vasile Goldis University Press (www.studiauniversitatis.ro)

development of fungi treated with extracts of burdock, respectively cocklebur (reproductive hyphae). The fungi that were treated with 0.075 g/mL grown considerably in the case of *Thymus vulgaris* extract treated plates, the colonies merged and reached the Petri plate edges. In the case of the plates containing burdock extract, the main colony merged with the secondary ones by increasing size up to 4 cm. The mycelium of the inoculum developed in plates treated with cocklebur extract, is still in a vegetative state, the development being low compared to secondary mycelium which formed reproductive hyphae. The control with no alcohol also reached the edges of the Petri dish.

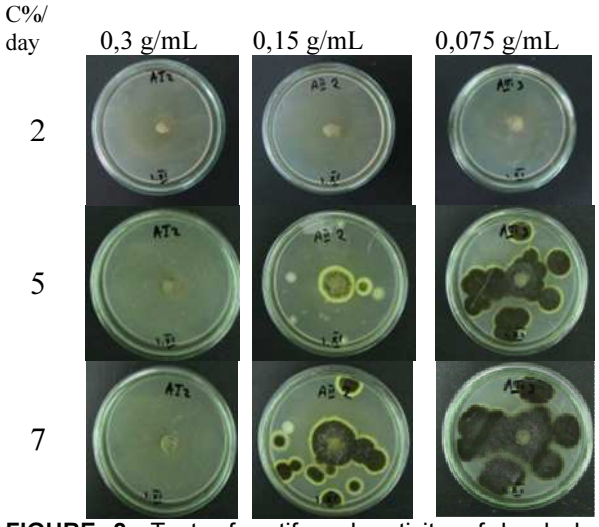

FIGURE 2. Test of antifungal activity of burdock extract

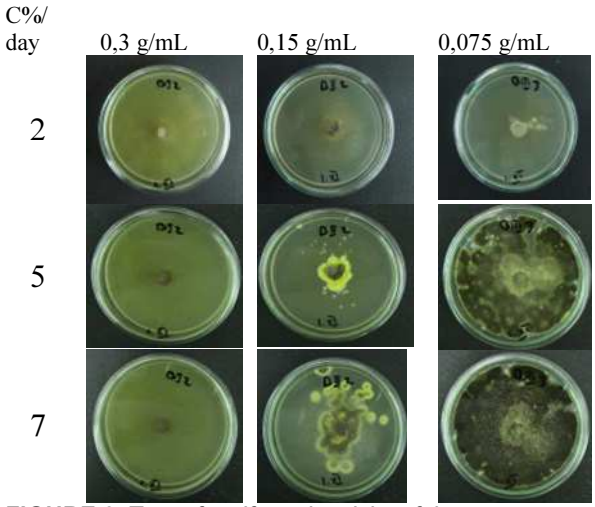

FIGURE 3. Test of antifungal activity of thyme extract

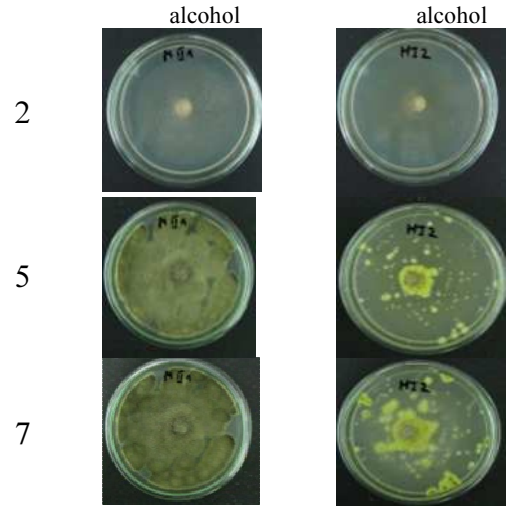

FIGURE 4. Control samples for test of antifungal activity on Aspergillus ochraceus

Acremonium chrysogenum

At 24 h after inoculation, both fungi on plates treated with extracts, as well as the control, did not show any signs of development.

After 120 hours of incubation, the plates treated with extracts of 0.3 g /mL did not show any sign of development (Figures 5,6,7). At concentrations of 0.15 g/mL, fungi grow a few millimeters. On the plates treated with thyme extract (Figure 7), the development of secondary infection was inhibited by this fungus with antibacterial properties. It is to be noted that that the plates treated with extract of 0.15 g/mL cocklebur (Figure 5) were the most permissive environment for the development of fungi. Results similar to those observed in the above case were observed on plates treated with plant extract at minimum concentration. The control presented, a high degree of development situated above the average growth of fungi when treated with plant extracts (Figure 8).

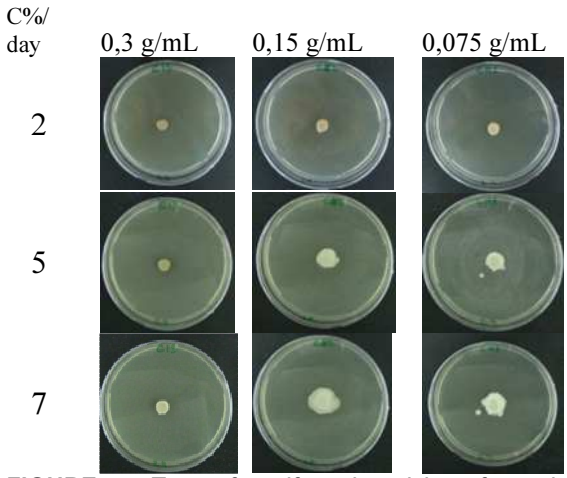

FIGURE 5. Test of antifungal activity of rough cocklebur extract

day Control without Control with

© 2013 Vasile Goldis University Press (www.studiauniversitatis.ro)

On the 7th day of the experiment, the effectiveness of the treatment with the selected plant extracts of maximum concentration of the vegetal material taken into study was fully demonstrated. The fungal growth on Petri plates treated with extract of 0.15 g/mL was minor, the colonies having dimensions around 1 cm. It is remarkable the highlight of a halo of inhibition of bacteria by the 0.15 g/mL concentration extract. Also, it must be mentioned in the variant where the fungi were treated with extract of minimum concentration the fungal colonies size did not reach the dimensions of the control.

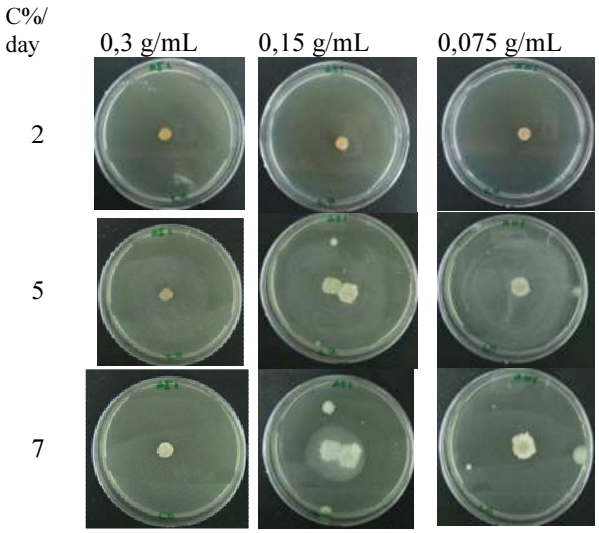

FIGURE 6. Test of antifungal activity of burdock extract

Acremonium chrysogenum, and respectively *Aspergillus ochraceus* development was similarly in the presence of alcoholic plant extracts of concentration 0.15 g/mL. This phenomenon can be explained by the relatively small size (max 3 cm) at which mature *Acremonium* fungal colony reaches, which determines the directly proportional increase of the inoculs according to the maximum diameter that the fungal colony can reach at maturity.

It is interesting to be noted that the emergence of reproductive hyphae, generating spores, visible in the case of *Aspergillus ochraceus* only occurs in the 7-day of incubation. The significance of this observation is the delayed sporulation phase of the life cycle of fungus due to the activity of the alcoholic extracts obtained from *Arctium lappa*, and *Xanthium strumarium*.

Another important thing to mention is that not even at the final stage of the experiment, in the 7-day of incubation, the fungi treated with thyme extract did not produce spores. This observation reveals that from the three alcoholic extracts the one of 0.15 g/mL concentration obtained from *Thymus vulgaris* presented the most lasting effect.

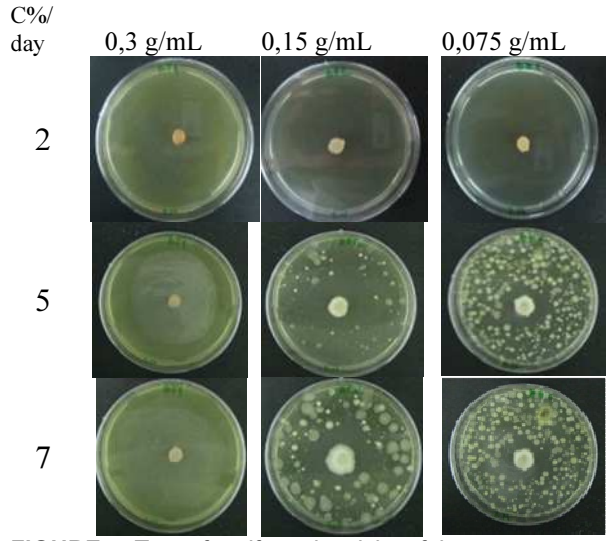

FIGURE 7. Test of antifungal activity of thyme extract

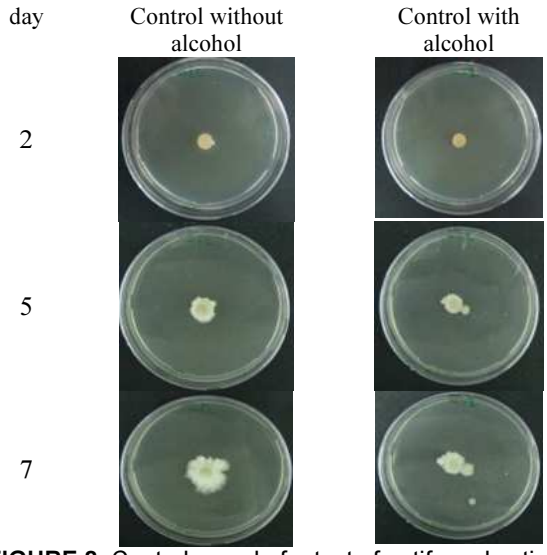

FIGURE 8. Control sample for test of antifungal activity on *Acremonium chrysogenum*

It is observed the antibacterial activity of *Acremonium chrysogenum* isolate rendered by the presence of an inhibition halo on the plates presenting a secondary infection with bacterial strains.

The effect of the plant extracts with minimum concentration used on growth of *Aspergillus ochraceus* strain is low. However, it can be concluded that extracts of burdock, respectively cocklebur at this concentration had a better effect than the extract of thyme.

The fungal colonies of *Acremonium chrysogenum* had a very slow development when undergoing treatment with plant extracts obtained with minimum concentration 0.075 g/mL.

The use of plant extracts in treating infections was applied from ancient times, long before the discovery of synthetic products.

Plant composition showed particular interest to scientists because plant extracts have a very wide range of application, from flavor drinks, preservation of

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 23, issue 1, 2013, pp.65-69
© 2013 Vasile Goldis University Press (www.studiauniversitatis.ro)

stored food crops to the perfume industry. Furthermore, the antifungal activity of extracts obtained from plants has a wide application in raw and processed food preservation, pharmaceuticals, alternative medicines, and natural therapies (Hammer et al. 1999).

The experiment presented in this study reveals important preliminary data regarding to the utilization of plant extracts in fungal attack control. It sets the foundation rock for future experiments on different fungal strains using diverse vegetal material and extraction methods.

CONCLUSIONS

At the finalization of the experiments performed in order to evaluate the antifungal effect of the alcoholic plant extracts obtained from three selected plant species of the native flora, *Arctium lappa, Thymus vulgaris* and *Xanthium strumarium*, on *Acremonium chrysogenum*, and respectively *Aspergillus ochraceus* isolates, the maximal inhibition growth of the mycelium during the entire ongoing of the testing (7days), could be observed when using extracts of concentrations of 0.3 g / mL

ACKNOWLEDGMENTS

This work has been funded by the research contract PN-II-PT-PCCA 106/2012.

REFERENCES

Agrios, G., N., 2004. Losses caused by plant diseases, Plant Pathology. Elsevier, Oxford, UK; p.: 29-45;

Banso, A, Adeyemo, SO, Jeremiah, P. 1999. Antimicrobial properties of Vernonia amygdalina extract. J. Appl. Sci. Manage. 3: 9-11.

Chan, Y., S., Cheng, L., ,N., Wu, J., H., Chan, E., Kwan, Y., W., Lee, S., M., Y., Leung, G., P., H., Yu, P., H., Chan, S., W., 2011, A review of the pharmacological effects of Arctium lappa (burdock), Inflammopharmacology, 19 (5); p.: 245-254;

Creppy, E., E., 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicological Letters;127, p.:18–28;

Das, S., Saha, R., Dar, S., A., Ramachandran, V., G., 2012, Acremonium Species: A Review of the Etiological Agents of Emerging Hyalohyphomycosis, Springer Science;

Ferracane, R, Graziani, G., Gallo, M., Fogliano, V., Ritieni, A., 2010, Metabolic profile of the bioactive compounds of burdock (Arctium lappa) seeds, roots and leaves, J Pharm Biomed Anal, 51(2); p.:399-404;

Gujar, J., Talwankar, D., 2012, Antifungal potential of crude plant extract on some pathogenic fungi, World Journal of Science and Technology, 2(6); p.:58-62;

Güllüce, L., Sökmen, M., Daferera, D., Ağar, G., Özkan, H., Kartal, N., Polissiou, M., Sökmen, A., Şahin, F., 2003, In Vitro Antibacterial, Antifungal, and Antioxidant Activities of the Essential Oil and Methanol Extracts of Herbal Parts and Callus Cultures of Thymus vulgaris, J. Agric. Food Chem., 51 (14);p: 3958–3965;

Hammer KA, Carson CF, Riley TV. 1999. Antimicrobial activity of essential oils and other plant extracts. Appl Microbiol. Jun;86(6):985-90

Harris, C., A., Renfrew, M., J., Woolridge, M.,W., 2001, Assessing the risk of pesticide residues to consumers: recent and future developments, Food Additives and Contamination, 18; p.: 1124-1129;

Holetz, F., B., Pessini, G., L., Sanches, N., R., Cortez, D., A., G., Nakamura, C., S., Filho, P., B., D., 2002, Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases, Mem. Inst. Oswaldo Cruz, .97(7); p: 1027-1031;

Kamboj, A., Kumar, A., 2010, Phytopharmacological review of Xanthium strumarium L. (Cocklebur), International Journal of Green Pharmacy, 4 (3); p.:129-139;

Kamei, K., Watanabe, A., 2005, Aspergillus mycotoxins and their effect on the host, Medical Mycology, 43(1) p.: 95-99;

Quiroga, E., N., Sampietro, A., R., Vattuone, M., A., 2001, Screening antifungal activities of selected medicinal plants, Journal of Ethnopharmacology, 74 (1); p:. 89–96;

Sun, J., Wang, X, Zang, N., Shi, C., Li, Q., Chen, D., 2008, Antifungal Activity in vitro of Arctium Ethanol Extract, Journal of Henan Agricultural Science;

Şahina, F., Karamana, İ., Güllüceb, M., Öğütçüa, H., Şengülc, M., Adıgüzelb A., Öztürkb, S., Kotana, R., 2003, Evaluation of antimicrobial activities of Thymus vulgaris L., Journal of Ethnopharmacology, 87 (1); p.:61–65;

Varga, J., Juhász, A., Kevei, F., Kozakiewicz, Z., 2004, Molecular diversity of agriculturally important Aspergillus species, European Journal of Plant Pathology 110; p:627–640.

© 2013 Vasile Goldis University Press (www.studiauniversitatis.ro)