European Journal of Medicinal Plants
4(10): 1240-1250, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Activity of Garlic (*Allium sativum*) on Some Pathogenic Fungi

Elham Abdelbasit Suleiman[1*] and Wafa Ballal Abdallah[2]

[1]Veterinary Research Institute, Mycology Department, Amarat, Khartoum, Sudan.
[2]Faculty of Science, University of Khartoum, Sudan.

Authors' contributions

Author EAS Designed the study, wrote the protocol, and the first draft of the manuscript.
Author WBA managed the literature search and performed the statistical analysis.
Both authors read and approved the final manuscript

Original Research Article

Received 16th March 2014
Accepted 25th June 2014
Published 15th July 2014

ABSTRACT

Aim: This study was conducted to investigate the *in vitro* antifungal activity of garlic (*Allium sativum*) on some pathogenic fungi.
Study Design: This is a comparative evaluation report on garlic as an antifungal agent.
Place and Duration: Department of mycology, Veterinary Research institute, between June-October 2013.
Methodology: Samples of garlic were obtained from a local market. It was thoroughly, cleaned, peeled and pulverized. Aqueous and organic extracts of garlic were obtained by maceration and Soxhlet extractor apparatus. The methanol and petroleum ether extracts were tested against *Candida albicans, Aspergillus, Curvularia* and some *Dermatophyte* species using cup diffusion and agar incorporated methods. Diameter of Inhibition zones of growth were measured in millimeter (mm) and expressed as Mean ±SD.
Results: The obtained results revealed that aqueous and petroleum ether extracts possess the stronger activity and a broader fungicidal spectrum against tested fungi compared to methanol extract. The study also showed that the dry coarsely- powdered garlic was found to be more potent to *Candida albicans* than the commercial Nystatin.
Conclusion: The study demonstrated the potent activity of garlic against tested fungi which encourages its use as a suitable alternative drug for controlling fungal infections because it has far less risk of side-effects than most known antifungal drugs and it can be used indefinitely in quite large amounts. Therefore, adding garlic to food (raw) or

**Corresponding author: Email: elham842001@yahoo.com;*

crushing and swallowing raw cloves which are cheaper is recommended as a powerful anti-fungal agent. Further purification and formulation of the garlic would give a true antifungal activity comparable to standard antibiotics.

Keywords: Garlic; antifungal; inhibition zone; Candida; dermatophytes; Aspergillus.

1. INTRODUCTION

Medicinal herbs are the use of natural herbs and plant's extracts for the treatment or prevention of diseases, disorders and promotion of good health [1,2]. It has a long tradition use outside of conventional medicine [3]. In the Sudan, the use of herbal treatment has been well known over the country since a long time ago. Due to the mutilating surgical and prolonged medical treatment by drugs, which are very expensive and have side effects, people started looking back on native treatment [4].

Garlic (*Allium sativum*) is one of the herbs that have been used worldwide. It is a species in the onion genus, *Allium* [5]. With the history of human use of over 7,000 years, garlic is native to central Asia and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe [6]. It is a member of the family *Amaryllidaceae* , Subfamily *Allioideae* which contains over 6,000 species including well-known edible plants such as onion, chives, leek, and shallot [5]. In Sudan, the northern regions are considered the most productive areas for garlic. The ancient Egyptians, Greeks, Chinese, Indians and Romans all advocated the therapeutic value of garlic in the treatment of ailments. It has been used for both culinary and medicinal purposes [6]. The biological activity of garlic was determined. The bactericidal effect of fresh garlic juice was tested by Louis Pasteur onto growing bacterial colonies. It was shown that fresh garlic juice inhibited the growth of most gram-positive and negative bacteria. The action was comparable *in vitro* with penicillin, streptomycin, chloramphenicol, tetracycline and erythromycin [6]. Previous studies further demonstrated that ethanol and aqueous extract exhibited growth inhibition of most gram positive and negative as well as multi- drug resistant bacteria [7,8].

The chemistry of the *Allium* species has been dominated by many sulfur containing compounds that give them a characteristic flavour and exhibit potent anti-fungal properties [9]. Among the most studied are allicin, alliinase and allin (S-allyl cysteine sulfoxide), the precursor of allicin which upon crushing of garlic bulb, it is hydrolyzed by the enzyme allinase to its active form allicin [10]. It is responsible for most of the biological property of garlic. One gram of allicin (S-methyl-l-cysteine sulfoxide has been equated to 15 IU of penicillin in its antibiotic activity. However, a variety of non sulfur compounds, work synergistically to provide various health benefits [11]. Freshly pressed juice of garlic has a strong antifungal effect on the major pathogenic moulds, yeast and dermatophytes [12]. The action of garlic on yeast and fungi is perhaps even more dramatic. Some studies revealed garlic as effective as fluconazole [8] at inhibiting *Candida albicans.* Odorless capsules, liquid extract and tablets were available for treating intestinal yeast infection. Fresh garlic was significantly more potent against *Candida albicans.* The essential oil, water, and ethanol extracts, and the juice also, inhibited the *in vitro* growth of *Candida and Aspergillus species* [12]. Moreover, Ajoene is also known to have effective broad antifungal properties helpful in preventing yeast infection (*Candida albicans*) and treating athlete's foot (*Tinea pedis*) [13]. Garlic changes its characteristics because of the complexity of its intrinsic chemistry, and processing procedures [11]. Thus, standardization marker compounds are important for ensuring consistent effects.

Garlic oil and recently available stabilized allicin, revealed a considerable activity against various phytopathogenic fungi. In a field study with peanut, garlic oil was found to be protective against *Gibberella zeae*, (a fungal parasite of maize). It was inhibited by garlic oil (8,000-10,000ppm) more effectively than by pesticides. Moreover, larvae of *Trogoderma granarium Everts*, another parasite on maize kernels, was controlled by 1-2% garlic oil. The present study aims to evaluate antifungal activity of garlic against pathogenic fungi.

2. MATERIALS AND METHODS

2.1 Test Microorganisms

Eight fungal strains were used. *Aspergillus niger* (ATCC9763), *Candida albicans* (ATCC7596) were obtained from National Council for Research, Khartoum, Sudan. Clinical isolates: *Aspergillus flavus, Microsborum canis, Curvularia lunata, Microsporum audouinii, Tricophyton mentagrophytes, Tricophyton soudenense* were obtained from the stock of the Mycology Department, Veterinary Research Institute, Khartoum, Soba, Sudan. They were maintained on Sabouraud's Dextrose Agar (SDA) media.

2.2 Sample Preparation

Fresh bulbs of garlic were purchased from a local market in Khartoum North, Sudan. The cloves were separated, peeled and washed to obtain the edible portion. 1000 grams were crushed using mortar and pestle. They were kept under shade for two days to dry. They were pulverized with a blender to a fine powder and kept for further analysis.

2.3 Extraction of Garlic Powder

Extraction was carried out according to the method described by Harborne [14]. One kg of dried plant sample was successively extracted with (2.5L) of petroleum ether and 80% methanol using Soxhlet extractor. The extraction was carried out for about 4hr and 8hr, respectively. Solvents were evaporated under reduced pressure and temperature below their melting point using Rotary evaporator. Finally, the extracts were allowed to dry in petri dishes until complete dryness. The obtained extracts were weighed and kept in a refrigerator for further study. The percentage yield was calculated using the formula below:

$$\% \text{ Yield} = \text{Weight of extract} / \text{Weight of plant sample} * 100$$

2.4 Preparation of the Aqueous Extract

Hundred grams of the garlic sample were soaked in 500 ml of hot distilled water, and left till cooled down with continuous stirring at room temperature. The extract was filtered using whatman n filter paper no.1 to give crude aqueous extract of 200mg/ml. This was collected in a sterile vial and kept in deep-freeze. The Freeze extract was then dried using freeze-drier till powdered extract obtained and kept at 4ºC until used [14].

2.5 Preparation of Samples for Antimicrobial Analysis

Four samples were used for this study: crude methanol extract, petroleum ether, aqueous and dry fraction. One gram of each sample was weighed and dissolved in 10ml of the solvent used for the extraction to give 100mg ml^{-1}. This was serially diluted until a

concentration of 6.25 mg ml^{-1} of the content was obtained. Different concentrations of the dry-powdered garlic (50mg, 100mg, and 200 mg) were prepared and incorporated into the Sabouraud's Dextrose Agar (SDA) media after sterilization. Control groups were run simultaneously in order to check their growth inhibitory effects.

2.6 Agar Dilution Method (MIC)

2.6.1 MIC=Minimal inhibition concentration

One gram of ether extract was added to 10ml of the solvent. Serial dilutions of extract were made by transferring 5ml from tube1 to tube2 containing 5ml of solvent. The two-fold dilution was continued up to tube3. Each dilution was tested on *A. flavus*, *C. albicans* and some dermatophytes. The concentration of garlic at which no growth of the organism was observed was considered as the minimum inhibitory concentration (MIC) of garlic for that organism. The results were expressed in terms of growth of organisms (+) or inhibition of growth (-) after addition of the extract [15].

2.7 Preparation of Fungal Inoculums

Inoculum for *Candida albicans*, was prepared from overnight growth (18 h) to 48 h old cultures on SDA. 2ml amounts of sterile distilled water were inoculated with a loop full of the isolate. For filamentous fungi sterile distilled water with 0.05% Tween 80 was added to the surface growth and the spores and hyphae were scraped off with a sterile wire loop. The concentration of the suspension was adjusted to about $1x10^6$ cells/ml. Suspensions were stored in the refrigerator until used.

2.8 *In vitro* Testing of Antimicrobial Activity of the Extracts

2.8.1 The cup-plate agar diffusion method

The cup-plate agar diffusion method Perez [16] was adopted with some minor modifications to assess the antifungal activity of the prepared extracts. One gram of each extract was weighed and dissolved in 5ml of the solvent used for the extraction to give 200mg /ml 0.1 ml of 100 mg/ml the standardized fungal stock suspension was thoroughly mixed with 20 ml molten sterile Sabouraud dextrose agar and maintained at 25ºC. The agar plates were left to set and in each of these plates 4 cups (10mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Cups were filled with 0.2ml of each extract using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 2-3 days. Activity of each extract was tested in triplicate. The diameters of zones of inhibition were measured in millimeter using a transparent well calibrated ruler, averaged and the mean values were tabulated.

2.8.2 Agar incorporation technique

The anti-fungal susceptibility of the extracts and the standard drugs ketoconazole and Nystatin were determined using agar incorporation technique in Sabouraud's Agar Medium supplemented with 0.05mg/ml chloramphenicol and 0.5mg/ml cycloheximide. Each plate was inoculated with the test organism. The control group was inoculated with the same organism. All plates were incubated at 25^0C for three to seven days except for

dermatophytes which were incubated for up to three weeks at the same temperature [17]. Different concentrations of 50, 100 and 200mg/ml dry coarsely powdered garlic were also, tested using incorporated technique. Parallel experiments were run in the same way using solvent vehicle (petroleum ether and 80% methanol).

2.9 Statistical Analysis

The mean values were expressed as the Mean± Standard Deviation (SD) and were analyzed using one-way ANOVA using the program SPSS version19.0 for Windows Differences were considered significant at P<0. 05.

3. RESULTS

The percentage yield of garlic extract obtained is shown in Table (1).

Table 1. Percentage yield of garlic obtained after extraction.

Solvent	Weight (gram)	Yield %
Methanol (80%)	40.62	4.00
Petroleum ether	1.08	0.10

In the present study the antifungal activity was investigated. Previous studies demonstrated that garlic has a broad range of antifungal activity. Our result further supported this finding. Both aqueous and petroleum ether extracts displayed antifungal activity using cup diffusion test. Clear zones of inhibition were observed. *Curvularia lunata* was found to be more sensitive (45mm) than other tested fungi (Fig. 1). Zones of inhibition on petroleum ether extract against tested fungi are shown in (Table (2). While the methanol extract showed no effect.

Table 2. Antimicrobial activity of garlic extracts against *C. albicans* and molds at a concentration of 10mg/ mL.

Organism	Methanol	Petroleum ether	Aqueous extract
	Diameter of inhibition zone (mm)		
Candida albicans	0.0	23±0.06	16±1.41
Aspergillus niger	0.0	16±1.09	14±1.63
Aspergillus flavus	0.0	38±1.26	23±0.82
Curvularia lunata	0.0	45±1.34	45±1.15

Inhibition zone diameters are expressed as Mean ± (SD).

Agar incorporated method displayed antifungal activity of the extracts demonstrated by –ve growth on SDA treated plates. Petroleum ether and aqueous extracts of garlic were found to inhibit the growth of the standard organism of *Candida albicans* and *Aspergillus niger* and the clinical isolates of *A. flavus*, *Curvularia lunata, Microsporum audouinii, Trichophyton soudanense* and *Trichophyton mentagrophytes* at a concentation of 10mg/ml (Table 3; Figs 2&3). The dry coarsely- powdered crude garlic was found to show the strongest antifungal activity than the organic and aqueous extracts against tested fungi. Different concentrations of the dry crude garlic, 2.5mg, 5mg, and 10mg/ml were found to affect the growth of *Candida albicans, Microsporum audouinii, Microsporum canis* and *Tricophyton soudanense.* The

minimum concentration inhibited the growth was found to be 2.5mg/ml (Table 4). The vehicle (petroleum ether) revealed resistance to tested fungi (Fig. 4).

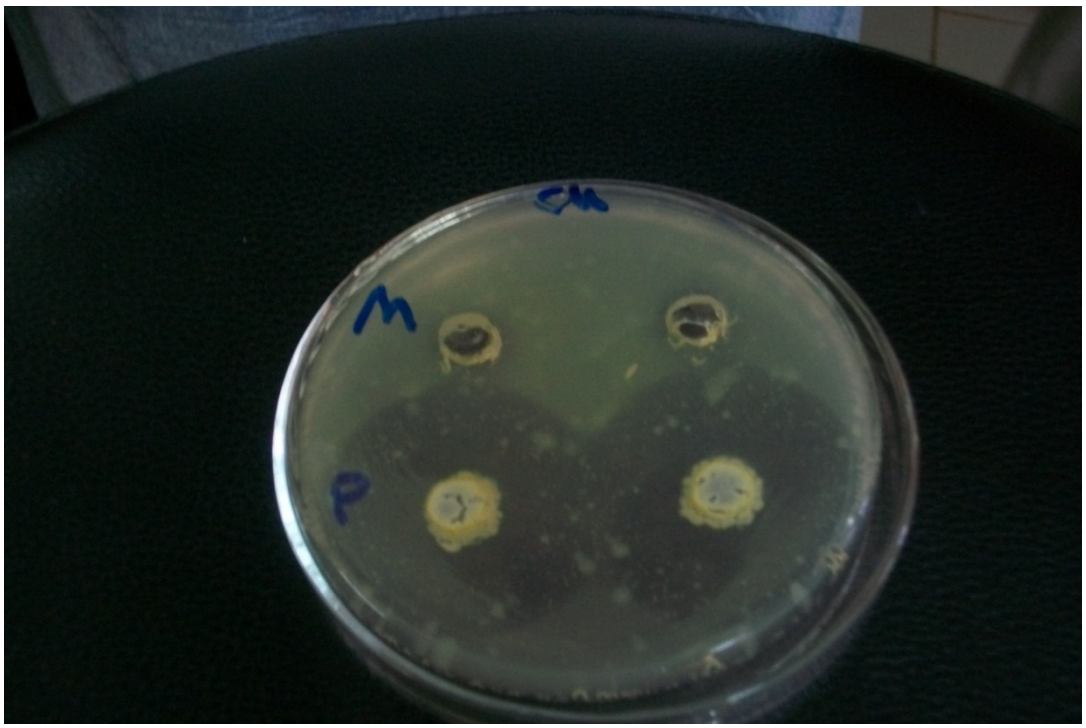

Fig. 1. Zone of inhibition (mm) of *Curvularia lunata* on petroleum ether extract.
Ca: Candida albicans, M: methanol extract of garlic, P: petroleum ether extract of garlic

Table 3. Antimicrobial activity of garlic extracts by agar incorporated method at a concentration of 10 mg/ml of the extract.

Organism	Methanol	Petroleum ether	Aqueous
Candida albicans	+	–	–
Aspergillus niger	+	–	–
Aspergillus flavus	+	–	–
Curvularia lunata	+	–	–
Microsporum audouinii	+	–	–
Tricophyton mentagrophytes	+	–	–

+ indicates growth of fungi (-ve response) ,– indicates no growth (+ve response)

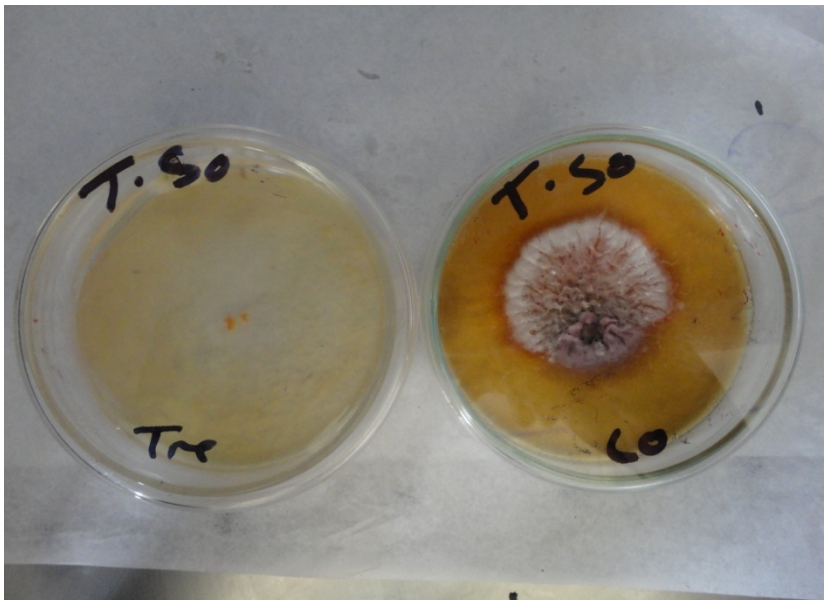

Fig. 2. Growth of *Trichophyton soudanense* on SDA control plate (right) and inhibition of growth on petroleum ether extract (left) plate at a concentration of 10mg/ml.
Tre= treated plate, Co= control plate

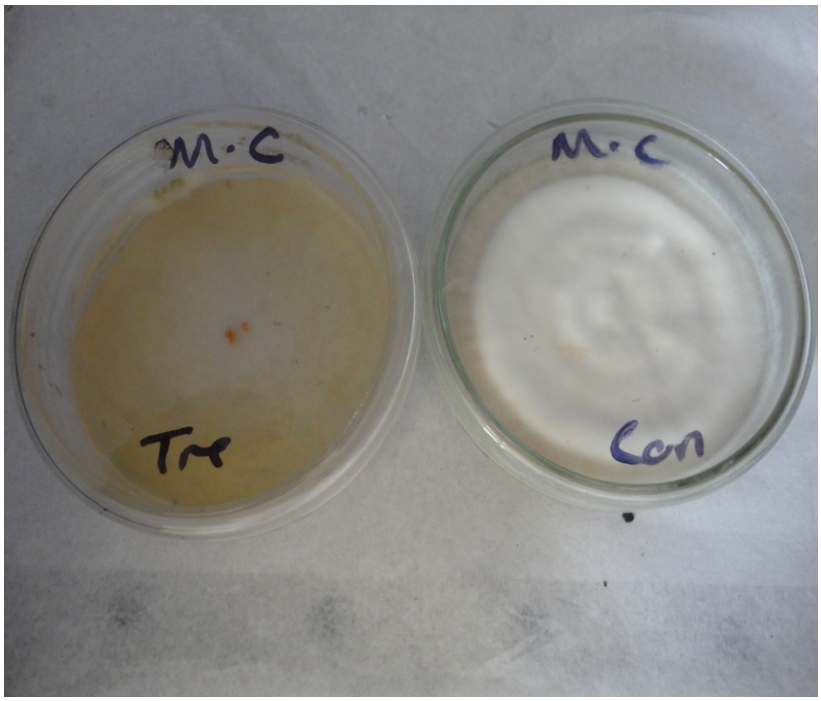

Fig. 3. Growth of *Microsporum canis* on SDA control plate(right) and inhibition of growth on petroleum ether extract (left) plate at a concentration of 10mg/ml

Table 4. Antifungal activity of dry crude powdered garlic on some pathogenic fungi

Organism	2.5mg/ml	5mg/ml	10mg/ml
C. albicans	+	+	+
M. audouinii	+	+	+
M. canis	+	+	+
T. soudanense	+	+	+

+ indicates sensitivity of garlic.

Fig. 4. Growth of *T. mentagrophytes* (left), growth of *M. audouinii* (right) on petroleum ether treated plates at a concentration of 10mg/ml

Significant antifungal effect was expressed as MIC of petroleum ether extract against tested fungi is shown in Table 5. MIC value of 2.5mg/ml was found to show the strongest activity of petroleum ether extract against test microorganisms. It inhibited the growth of Aspergillus and dermatophyte species tested except *C. albicans* where MIC value of 10mg/ml was found to inhibit its growth.

Table 5. Minimum inhibitory concentration (MIC) of petroleum ether extract of garlic

Organism	Petroleum ether extract mg/ml		
	10	5	2.5
Candida albicans	–	+	+
Aspergillus niger	–	–	–
Aspergillus flavus	–	–	–
Curvularia lunata	–	–	–
M. audouinii	–	–	–
T. mentagrophytes	–	–	–

- indicates +ve response (sensitivity) + indicates –ve response (resistance)

4. DISCUSSION

Garlic (*Allium sativum*) is a spice with global recognition. In the present study, it has been shown to inhibit the growth of fungi when *in vitro* tested. The antimicrobial activity of garlic is

believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase [18] and ajoene [19]. Previous studies reported that allicin is a pure, bioactive and the most powerful medicinal compound isolated from garlic [19]. It has strong antimicrobial and antifungal activities. Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi [19].

In the present study, the methanol extract of garlic has no effect on fungi tested. This might be due to insolubility of the phytoconstituents ingredients in methanol. Whereas, petroleum and aqueous extracts displayed greater antifungal activity against *C. albicans* and tested molds. Similar results were obtained by previous coworkers [8,18] where inhibition of *C. albicans* using fresh crushed homogenate garlic was shown. Our study demonstrated high potency of petroleum ether extract against *Curvularia lunata*. Singh and his colleagues disclosed similar result of inhibition of spore germination of *Curvularia lunata* due to ajeone fraction [20] as germination of hyphae was used as indicator in suspect testing of broth and agar method [14].

Inhibition of growth of dermatophytes observed in this study was similar to previous findings. Several studies by Dikasso et al. [12] and yoshda et al. [19] had previously demonstrated antifungal potency of garlic where inhibition of *Trichophyton* and *Microsporum* species using fresh garlic juice was shown due to stronger activity of ajoene. Mason and his colleagues successfully treated athelets foot using garlic relating that to ajoene fraction of garlic [9]. Moreover, all extracts of garlic in higher concentrations showed that the antifungal effects increased with increased concentrations [8]. However, the crude coarsely powdered garlic had greater antifungal activity compared to other extracts. This is due to the complex chemistry of Allium plants as variations in processing, yield quite different preparations thus, highly reactive thiosulfinates, such as allicin which disappear during processing and are quickly transformed to other types of organo-sulfur compounds. So, efficacy and safety are contingent upon processing methods [11]. Hence, some of the antimicrobial function might be lost. This finding supports the suspicion of Hassan and his collaborators [21] who believed that the whole herbs contain many ingredients, and they may work together to produce a beneficial effect. This fact supports our finding where dry-powdered garlic was found to have stronger antifungal effect. This might be due to damage caused by garlic to the outer surface of the fungal cells together with alterations in the fat content of the cell. It is also probable that garlic may reduce the adhesion of fungal cells to epithelial membrane. Moreover, the organo sulphur compound present in garlic (allicin) is thought to inhibit fungal growth via interaction with sulphur containing enzymes [19]. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity [18].

Comparing antifungal effect of crude dry-powdered garlic with Nystatin and Clorox, it was found that the dry-powdered garlic was most potent against *C. albicans.* It was shown that the antifungal activity of garlic exceeds that of nystatin and Clorox. Previous study disclosed same result [5]. This might be due to the assumption that the very best form of garlic is the raw and crushed, preferably certified the organic extract. When raw garlic is crushed it starts a chemical process which creates allicin which exhibits potent anti-fungal properties [9].

Moreover, comparative studies on the effects of garlic juice and the pharmaceutical preparations, nystatin, against *Candida albicans,* have shown that the antimycotic activity of

garlic exceeds that of all the drugs investigated [5]. This finding supports our result. Thus, use of fresh garlic or its extract is highly recommended as a potent anti-fungal agent.

5. CONCLUSION

Petroleum ether, methanol and water extracts of garlic were investigated for their antimicrobial activity. Petroleum ether, and aqueous extracts were shown to have great antifungal activity against dermatophytes, *C. albicans* and other mould tested. The crude dry garlic was shown to have the strongest antifungal activity than aqueous and petroleum ether extracts. The antifungal potential of dry garlic against *C. albicans* exceeds that of the commercial Nystatin. The study indicated that garlic has strong antifungal activity. Further research on purification and formulation may be needed to understand the mechanisms through which this effect is exerted.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOLEDGEMENS

The authors are indebted to Sudan Academy of Science (SAS) for permission to carry this study. Technical assistance of Mycology Department Staff is highly appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Deepak A, Anshu S. Tribal Formulations and Traditional Herbal Practices. In: Indigenous Herbal Medicines. Aavishkar Publishers Distributor, Jaipur- India. 2008;440.
2. El- Astal ZY, Ashour AERA, Kerrit AAM. Antimicrobial activity of some medicinal plant extracts in Palestine. Pak J Med Sci. 2005;21:187-193.
3. Owolabi OJ, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella africana* (Bignoniaceae) stem bark. Afr J Biotechnol. 2007;6:1677-1680.
4. Elfadil AG, Gummaa SA, Musa HA, Khalid HE, Sirro AA. Herbal Treatment of Eumycetoma Caused by *Madurella mycetomatis*. In: The proceedings of The International Conference of Mycetoma, Khartoum, Sudan. 2002;33:34.
5. Block E. Garlic and other Alliums: The Lore and the Science. Royal Society of Chemistry, Cambridge, UK. 2010;152-166.
6. Ensminger AH. Foods & nutrition encyclopedia, Volume 1. CRC Press. 1994;750.
7. Virendra VP, Shalini T, Nirmala K, Chetan Ni, Kalpagam P. *In vitro* evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic. J. Pharmacogn. and Phytochem. 2013;2(3):143-148.

8. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. *In Vitro* Antimicrobial Properties of Aqueous Garlic Extract Against Multidrug-Resistant Bacteria and *Candida* Species from Nigeria. J Med Food. 2004;7(3):327- 333.
9. Lawson LD, Wood SG, Hughes BG. HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. Planta Med. 1991;57:263-270.
10. Reuter HD, Sendl A. *Allium sativum* and *Allium ursinum*: Chemistry, pharmacology, and medicinal applications. In: Wagner H, Farnsworth NR, eds. Economic and medicinal plants research, London. Academic Press. 1994;6:55–113.
11. Amagase H, Petesch B, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr. 2001;131:955-962.
12. Dikasso D, Lemma H, Urga K, Debella A, Addis G, Tadele A, Yirsaw K. Investigation on the antifungal properties of freshly pressed garlic juice on major pathogenic fungi. J Ethiop Med Pract. 2001;3(1):16-20.
13. Ledezma E, Apitz-Castro R. Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. Revista Iberoamericana de Micologia 2006;23(2):75–80.
14. Harborne J. B. Phytochemical Methods. 2nd edition. Chapman and Hall. 1984;(4):4-7.
15. Minakshi D, Amit KD, Banerjee A. Antimicrobial screening of some Indian spices. Phytother Res. 1999;13:616–618.
16. Perez C, Paul M, Bazerque P. An antibiotic assay by agar well diffusion method. Acta Biol Med Exp. 1990;15:113-115.
17. Warnock DW. Methods with antifungal drugs. In: Medical Mycology a practical approach. 1st Ed. E G V. Evans and M D Richardson. IRL Press. Oxford, New York, Tokyo. 1989;235-247:11.
18. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect 1999;1(2):125-9.
19. Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H, Nakagawa S. Antifungal activity of ajoene derived from garlic. Appl Environ Microbiol. 1987;53(3):615–617.
20. Singh UP, Pandey VN, Wagner KG, Singh KP. Antifungal activity of ajoene, a constituent of garlic (*Allium sativum*) Canad. J Bot. 1990;68(6):1354-1356.
21. Hasan SS, Ahmed SI, Bukhari NI, Loon WC. Use of complementary and alternative medicine among patients with chronic diseases at outpatient clinics. Complement Ther Clin Pract. 2009;15(3):152-157.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=575&id=13&aid=5330