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A Study on Assessment of Biological Activities of Garlic (*Allium sativum*) Extract

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Abstract

Spices like alliums are indispensable for the preparation of our daily food and are reported to possess compounds, which have varied biological activites and also prevent the microbial spoilage of food. Hence, the present study was conducted with the main purpose of assessment of biological activites *viz.* antioxidant and antifungal activities of Garlic *(Allium sativum).* Antioxidant assay was carried out by *in-vitro* model using DPPH free radical scavenging activity. Antifungal activity of the ethanolic Garlic (*Allium sativum*) extract was determined by disk diffusion method. Results delineated that total polyphenols quantity was found to be highest (14.68 GAE) phytochemicals found in ethanolic Garlic (*Allium sativum*) extract when compared with total flavonoid quantities (4.12 GAE). The ethanolic Garlic (*Allium sativum*) extract showed a dose dependent DPPH scavenging activity. It showed the highest scavenging activity (45.43%) at 10 mg/mL and lowest (13.13%) at 2 mg/mL. Furthermore, the DPPH free radical scavenging activity of ethanolic Garlic (*Allium sativum*) extract was comparable with that of standard ascorbic acid. The tested ethanolic Garlic (*Allium sativum*) extract possesses potential antifungal activity against *Aspergillus fumigates, Candida tropicalis, Candida albicans,* and *Penicillium crysogenum.* In conclusion, our study results proved the biological activities *viz.* antioxidant and antifungal activities of ethanolic Garlic (*Allium sativum*) extract, and thus, it could be considered for the development of natural antioxidant and antifungal drugs.

Keywords: *Allium sativum*, Garlic, Antioxidant, Antifungal, Ethanolic extract

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Introduction

Free radicals form in our body as a result of biological oxidation. Oxidation is a natural process in organisms to produce energy to fuel biological cycles. Oxidation by-products of normal metabolism cause extensive damage to DNA, protein, and lipids, constituting a major contribution to ageing and to degenerative disease. Oxidative damage is associated with chronic degenerative diseases, including cancer, coronary artery disease, hypertension, and diabetes.[1] An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism.[2] Antioxidants occur naturally in many fruits and are able to neutralize free radicals by donating an electron and convert them into harmless molecules.[3]

Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from the synthetic products.[4] With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs.[5] This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics.[6,7] Pharmacological industries have shaped several new antibiotics and in the last three decades resistance to these drugs by microorganisms has increased. In general, bacteria are the microorganisms which have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[8] Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants.[9,10]

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Herbs and spices have been found to reduce inflammation, protect against infection, helps to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases.[11] Some common herbs such as cilantro, basil, thyme, onion, ginger, turmeric, garlic etc., offer great health benefits by virtue of their powerful phytochemical and antioxidant properties. Even though there is limited literature on the health effects of herbs and spices or extracts of these, the number of studies investigating the possible health effects of phytochemicals originating from herbs and spices is at large. Most of the products categorized as herbal and traditional plant medicines are also based on antioxidant-rich dietary plants or isolated phytochemicals. Spice oils have also been reported to prevent microbial spoilage of foods.[12,13] This preservative property of spices has been attributed to the presence of some antimicrobial principles contained in their oils.[14]

Garlic *(Allium sativum)*, belonging to family *Alliaceae* is a plant containing 1-2% essential oil on a dry basis with wide variation of chemical composition as a function of genetic diversity, habitat, and agronomic treatment of culture. Garlic has a long folklore history as a treatment for cold, cough, and asthma and is reported to strengthen immune system. It has many medicinal effects such as lowering of blood cholesterol level,[15] antiplatelet aggregation,[16] anti-inflammatory activity,[17] and inhibition of cholesterol synthesis.[18] Garlic has been long known to have antibacterial,[19] antifungal,[16] anticancer,[20,21] antioxidant, and antiviral activities. Therapeutic effects of garlic are due to the presence of allicin in the cloves. With this background the present study was conducted with the main objectives of evaluation of biological activites *viz.* antioxidant and antifungal activities of Garlic *(Allium sativum)*.

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Materials and Methods

Collection of Garlic Cloves

The cloves of Garlic (*Allium sativum*) were procured from the local markets of Chikkaballapura, Karnataka, India.

Extraction

300 gms of fresh Garlic (*Allium sativum*) cloves were finely chopped into pieces in 400 mL ethanol in ice bath, and was kept in an air tight bottle at 0°C in a refrigerator for 24 hrs. They were crushed with a motor pestle and were filtered using a Whatman filter paper no 1 in cold room. The residue was resuspended in 400 mL of ethanol and kept at 0°C in a refrigerator for 24 hr and the procedure was repeated. The filtrate was concentrated under vacuum in Rota evaporator at <50°C. The final powder extract was stored in a container and kept in the refrigerator.[22]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the ethanolic Garlic (*Allium sativum*) extract was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[23] The phenolic content of the ethanolic Garlic (*Allium sativum*) extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in ethanolic Garlic (*Allium sativum*) extract.[24] The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled

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water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay.[25,26] Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in ethanol and mixed with sample solution (3mL, containing 20-100ug) in ethanol. The control was also run which contains only methanol. The hydrogen atom or electron donation abilities of ethanolic Garlic (*Allium sativum*) extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.,* Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Fungal Strains

The pathogenic fungal strains *viz. Aspergillus niger, Aspergillus fumigates, Candida tropicalis,* and *Penicillium crysogenum* were isolated from clinical samples of local hospital in and around Chikkaballapura and confirmed by various microscopic evaluation like Gram's staining.[27] Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne.[28] All the fungal pathogens were further confirmed by suitable biochemical tests,[29] and used for antifungal activity studies.

Stock cultures were maintained at 4ºC on the slant of nutrient agar. Active Cultures for experiments were prepared by transferring a loopful of cell organisms from the stock cultures to test tubes of nutrient broth for fungi. Streaking was done on Yeast Extract Peptone Dextrose

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(YEPD) agar plates and incubated for 24-48 hours at 37ºC in which the assay was performed by disc diffusion method.

Determination of Antifungal Activities

The antifungal activity of the ethanolic Garlic (*Allium sativum*) extract was determined by disk diffusion method on Muller Hinton Agar (MHA) medium. Cultured colonies were picked from Yeast Extract Peptone Dextrose (YEPD) agar plates and were added in peptone water and was incubated for around 30 minutes. The MHA medium was poured in the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moisture with the fungal suspension. The anti-fungal discs were placed on MHA plate with the help of sterile forceps and different concentration of ethanolic Garlic (*Allium sativum*) extract were loaded on discs. Fluconazole was used as positive control and double distilled water was used as negative control. Then all the plates were incubated in upright position at 37ºC for 24-48 hours. The inhibition zones were measured on the underside of the plates.[30]

Results

Quantitative estimation of phytochemicals in ethanolic Garlic (*Allium sativum*) extract was represented in Table 1. Results revealed that total polyphenols quantity was found to be highest (14.68 GAE) phytochemicals found in ethanolic Garlic (*Allium sativum*) extract when compared with total flavonoid quantities (4.12 GAE).

Table 1: Quantitative analysis of phytochemicals present in ethanolic Garlic (*Allium sativum*) extract

Chemical Components	Ethanolic Garlic (*Allium sativum*) extract
Total phenolics	14.68 GAE
Total flavonoids	4.12 GAE

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The ethanolic Garlic (*Allium sativum*) extract showed scavenging activity against the free radicals. The ethanolic Garlic (*Allium sativum*) extract showed a dose dependent DPPH scavenging activity. It showed the highest scavenging activity (45.43%) at 10 mg/mL and lowest (13.13%) at 2 mg/mL. Furthermore, the DPPH free radical scavenging activity of ethanolic Garlic (*Allium sativum*) extract were comparable with that of standard ascorbic acid as shown in Table 2.

Table 2: Antioxidant activity of ethanolic Garlic (*Allium sativum*) extract

Concentration of extract (mg/mL)	**DPPH Scavenging Activity (%)**	**Concentration of Ascorbic Acid (mg/mL)**	**DPPH Scavenging Activity (%)**
2	13.13	2	26.27
4	24.18	4	47.19
6	29.21	6	54.38
8	37.81	8	69.58
10	45.43	10	81.31

The highest zone of inhibition (15.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (11.5 mm) was observed against *A. niger* at 100 mg/mL. At 50 mg/mL the highest zone of inhibition (13.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (9.5 mm) was observed against *C. tropicalis*. Whereas the highest zone of inhibition (10.5 mm) was seen against *A. fumigates* and lowest zone of inhibition (6.0 mm) against *C. tropicalis* at 25 mg/mL. At 12.5 mg/mL the highest zone of inhibition (8.5 mm) was observed against *A. fumigates* and lowest zone of inhibition (3.5 mm) was observed against *C. tropicalis.* The reference standard fluconazole showed highest zone of inhibition (23.5 mm) against *P. crysogenum* and the lowest zone of inhibition (16.5 mm) against at *A. niger.* These findings revealed that the tested ethanolic Garlic (*Allium sativum*) extract possesses potential antifungal

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activity against *A. niger*, *A. fumigates*, *C. tropicalis* and *P. crysogenum* as shown in Table 3 and Figure 1.

Table 3: Antifungal activities of ethanolic Garlic (*Allium sativum*) extract

Fungal strains	Zone of inhibition(mm)					
	Negative Control	Positive Control	Ethanolic Garlic (*Allium sativum*) extract			
			12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
A. niger	-	16.5	6.5	8.5	10.5	11.5
A. fumigates	-	18.0	8.5	10.5	13.5	15.5
C. tropicalis	-	17.0	3.5	6.0	9.5	13.0
P. crysogenum	-	23.5	6.5	8.0	12.5	14.5

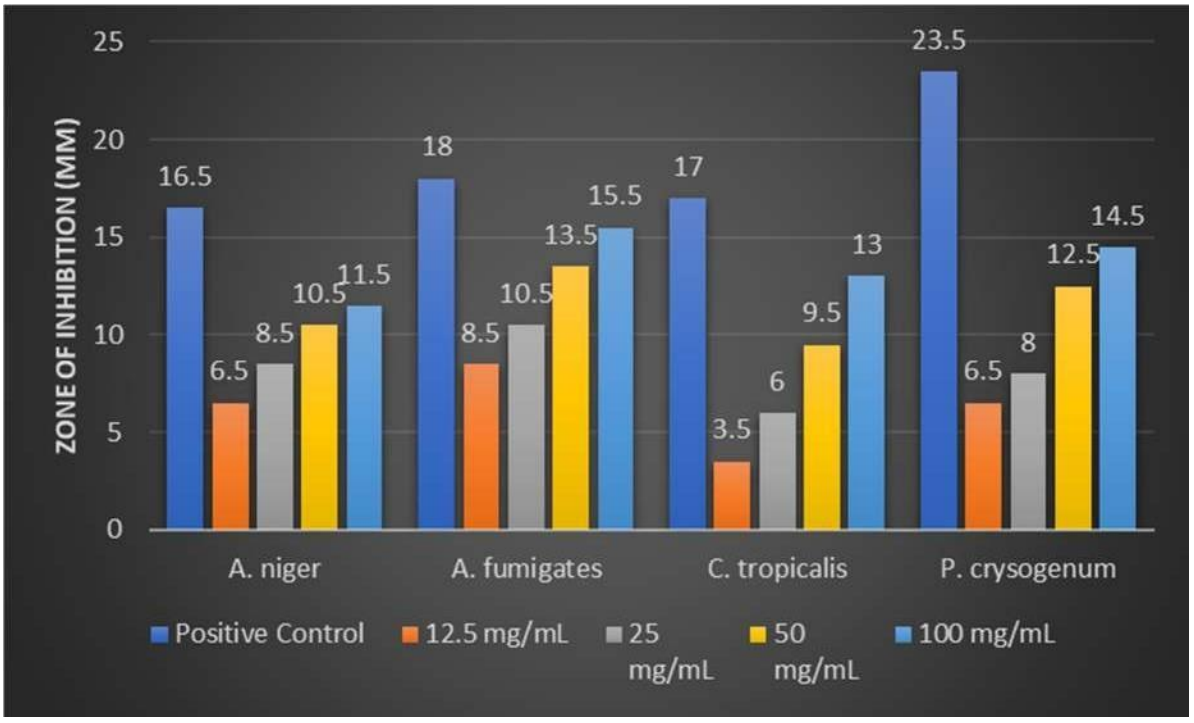

Figure 1: Antifungal activities of ethanolic Garlic (*Allium sativum*) extract

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Discussion

DPPH is often used to determine free radical scavenging activity of natural compounds due to its stability as a radical.[31] The presence of unpaired electron imparts a strong absorbance at 517 nm, giving the radical a purple color. With the exposure to antioxidants, it undergoes reduction, decreasing absorbance due to the formation of yellow colored anti-radical diphenyl picryl hydrazine (DPPH). The degree of colour change from purple to yellow is a measure of scavenging potential of the antioxidants in the extracts in terms of hydrogen donating ability.[32] The frequency of life-threatening infections caused by pathogenic microorganisms is becoming an alarming factor of morbidity and mortality in immuno-compromised patients in developing countries. Many pathogenic microorganisms are constantly developing resistance to these agents. Since many of the existing antifungal drugs have undesirable side effects or are very toxic, show drug-drug interactions or develop resistance affecting treatment planning. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these shortcomings. The herbal extracts are easily available and cheaper having added advantage of widespread availability, minimal side effects, cost effective and efficiency in long term usage. Considering the side effects and disadvantages of fluconazole, these herbal extracts mainly can be considered as a better alternative antifungal agent.[33] With this background, in the current we aimed for evaluation of antioxidant and antifungal activities of Garlic *(Allium sativum)* extract.

Our study results depicted that the ethanolic Garlic (*Allium sativum*) extract showed a dose dependent DPPH scavenging activity. It showed the highest scavenging activity (45.43%) at 10 mg/mL and lowest (13.13%) at 2 mg/mL. Furthermore, the DPPH free radical scavenging activity of ethanolic Garlic (*Allium sativum*) extract were comparable with that of standard ascorbic acid. In accordance with our study findings, Dilis and Trichopoulon (2010) revealed that the extracts of many spices and herbs have become popular in recent years for their

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antimicrobial and antioxidant properties and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications.[34] Furthermore, Hossain et al., (2008) reported that spices are rich sources of polyphenolic compounds having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been implicated in the prevention of many chronic diseases such as cardiovascular diseases, cancer and inflammation etc.[35]

Extensive research has proved that antimicrobial activity of spices can prevent growth of pathogens in foods. Many studies reported the inhibitory activities of spices and herbs against food borne pathogens. Concurrently, our study findings delineated that the ethanolic Garlic (*Allium sativum*) extract possess potential antifungal activity against *A. niger*, *A. fumigates*, *C. tropicalis* and *P. crysogenum.* Literature reports evidenced that the antimicrobial activities of commonly used herbal spices form the basis for many applications including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies.[36] Iram et al., (2012) revealed that the organo Sulphur compounds present in garlic is thought to scavenge free radicals and also inhibit microbial growth via interaction with Sulphur containing enzymes.[37]

Conclusion

Our study results proved the biological activities *viz.* antioxidant and antifungal activities of ethanolic Garlic (*Allium sativum*) extract, and thus, it could be considered for the development of natural antioxidant and antifungal drugs. However, further studies are recommended to elucidate the exact mechanism of action of particular phytochemical responsible for biological activities of Garlic (*Allium sativum*) extract.

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