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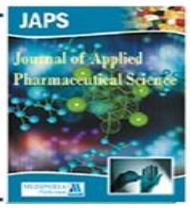

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Antimicrobial screening of Garlic (*Allium sativum)* extracts and their effect on Glucoamylase activity *in-vitro*

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ABSTRACT

Amongst the various extracts, Petrol-ether and Chloroform extracts of garlic were found to be good antimicrobial agents against *Escherechia coli, Pseudomonas Aeruginosa, Bacilli Subtilis* and *Staphylococcus Aureus,* and moderate inhibitors of Glucoamylase, an enzyme prominent in carbohydrate metabolism. Whereas, the Ethyl acetate and Methanolic extracts did not exhibit antimicrobial activity and found moderate to good activators of Glucoamylase.

Keywords: *Allium Sativum,* Glucoamylase, antimicrobial, Glucoamylase inhibitor, Hypoglycemic.

INTRODUCTION

Nature has provided a complete storehouse of remedies to cure ailments of mankind. About 80% of the world's population depends wholly or partially on traditional medicines for its primary health care needs (Kunwar *et al.,* Adhikari *et al.,* 2005). Herbal medicines as the major remedy in traditional medical system have been used in medical practice for thousands of years and have made a great contribution to maintain human health (Rahman *et al.,* Khan *et al.,* Jamal *et al.,* 2011). Herbal treatments are becoming increasing by popular as the herbal preparations have no or less side effects (Rajasekaran *et al.,* Sivagananam *et al.,* Narayanan *et al.,* 2001). Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of order to validate their use in folk medicine. Systematic screening of them may result into the discovery of novel active compounds (Tomoko *et al.,* Hiromu *et al.,* Takashi *et al.,* Yuka *et al.,* 2002). In modern medicine, the beneficial effects on glycemic levels are well documented. The preventive effect of activity of these drugs against progressive nature of diabetes and its micro and macro vascular complications was modest and not always effective (Kasiviswanath *et al.,* Ramesh *et al.,* Kumar *et al.,* 2005). Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies (Raghavan *et al.,* Krishnakumari *et al.,* 2006). Garlic (*Allium Sativum Linn.*) is a common spicy flavoring agent used since ancient times. Garlic has been cultivated for its characteristic flavor and medicinal properties (Zargari *et al.,* 1997). In the 1970's, Jain and coworkers investigated the effect of extracts of garlic with water or several different organic solvents on oral glucose tolerance in normal and alloxan induced diabetic rabbits (Jain *et al.,* Vyas *et al.,* Mahatma *et al.,* 1973). However, Swanstton-Flatt et al. (1990) failed to find a hypoglycemic effect of garlic powder in animals with streptozotocin (STZ) induced diabetes (Swanstton *et al.,* Day *et al.,* Bailey *et al.,* Flatt *et al.,* 1990).

Brahmachari and Augusti assayed the hypoglycemic potency of the ether extract of garlic in normal as well as alloxan diabetic rabbits (Brahmachari *et al.*, Augusti *et al.*, 1962). Oral hypoglycemic agents are a boon to diabetic patients on non-insulin dependent diabetes mellitus. Besides all the agents are associated with a number of side effects (Carry *et al.*, Garlof *et al.*, Griffin *et al*., Edward *et al*., Wagner *et al*., 2001). In the present research work, attempt is made for screening of various extracts of garlic for antimicrobial activity and to evaluate the effects of crude extracts on glycogen debranching enzyme, i.e., glucoamylase, *in vitro.*

MATERIALS AND METHODS

Plant Material

Garlic bulbs (*Allium Sativum Linn.*) were collected from local market of Santacruz (E), Mumbai. The garlic bulbs were cleaned properly and de-skinned and weighed.

Preparation of garlic extracts

300g of garlic bulbs were chopped and subjected to successive Soxhlet extraction with different solvents such as Petrol-ether, Chloroform, Ethyl Acetate and Methanol. Prior to extraction, the plant material was dried in the air oven below 50°C. The extracts obtained were then concentrated and weighed.

Antimicrobial Screening

Bioassay study was done of all extracts against four micro-organisms, *Staphylococcus Aureus* (NCTC 3750), *Pseudomonas Aeruginosa* (Fisch's Immuno Type 4), *Bacilli Subtilis* (ATCC 9373) and *Escherechia Coli* (ATCC 10148) by agar cup diffusion method (Barn *et al.*, Victor *et al.*, Editor *et al.*, 1998). All bio-assays were carried out in triplicate and average values are recorded.

Glucoamylase Activity

1mL of the reaction mixture containing 0.5 mL of starch solution (5mg/mL prepared in 100mM acetate buffer pH 4.5), suitable amount of glucoamylase enzyme (0.1mL) and 0.4mL of buffer (100mM) was incubated at 37°C. for 30 minutes. The reaction was terminated by keeping the test-tubes in boiling water bath for 1-2 minutes, cooled under running tap water and liberated glucose was estimated by DNS method. A unit activity (U) is defined as the mg of glucose liberated per mg of protein per minute.

RESULTS AND DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant. Although, it is usually not attributed to a single compound but a combination of metabolites. Plant extracts have been used for thousands of years, in pharmaceuticals, alternative medicines and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. The percentage yields of the extracts obtained from Soxhlet extraction are recorded **(Table1)**.

Table. 1: % Extraction with different solvents.

Serial no.	Solvent	% Extraction
1	Petrol-ether	0.14
2	Chloroform	0.97
3	Ethyl-Acetate	6.88
4	Methanol	7.55

Amongst the solvents used, ethyl acetate and methanol rendered excellent yields, whereas, pet-ether and chloroform extracts resulted in reasonably low yields. All the four extracts were tested for antimicrobial activity and was observed that pet-ether and chloroform extracts with maximum antibacterial activity whereas ethyl acetate and methanol did not inhibit the growth of micro-organisms **(Table 2)**. Further, the effect of all four extracts on glucoamylase was studied, *in vitro.* It is observed that, the ethyl acetate and methanol extracts increase the activity of glucoamylase whereas the chloroform extract was found to be good inhibitor and the petrol ether extract showed moderate inhibition **(Table 3)**. Thus we report, the petrol ether and chloroform extracts as antimicrobial and inhibit the effect of glucoamylase, *in vitro.* In contrast, the ethyl acetate and methanolic extracts were found to be initiators of glucoamylase, *in vitro*.

Table. 2: Bioassay of garlic extracts.

Extract	*Bacillus Subtilis*	*Escherichia Coli*	*Pseudomonas Aeruginosa*	*Staphylococcus Aureus*
Petrol ether	12	18	18	16
Chloroform	15	17	17	19
Ethyl Acetate	----	----	----	----
Methanol	----	----	----	----

Inhibition zone excluding diameter of borer (8mm).

Table. 3 : Effect of garlic extracts on glucoamylase activity, *in vitro.*

Fraction	Activity	% Increase/Inhibition
Control	10.75	----
Pet Ether	7.89	-26.60
Chloroform	6.81	-36.65
Ethyl acetate	15.07	+40.18
Methanol	16.15	+50.23

CONCLUSION

In the present study, we have carried out antimicrobial screening of the garlic extracts and their effects on glucoamylase, *in vitro.* Petrol ether and Chloroform extracts are found to be antibacterial and inhibitors of glucoamylase whereas ethyl acetate and methanol extracts are activators of glucoamylase. Hence, we report Petrol-ether and chloroform extracts of *A.Sativum* as hypoglycemic agents in Diabetes Type II and ethyl acetate and methanol extracts may be used in Glycogen Storage Disease Type III.

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