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A Study On Assessment Of *In-Vitro* Anti-Inflammatory Activity Of Garlic(*Allium sativum*) Extract Using Albumin Denaturation Method

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Abstract
The most widely used medications in the world today to treat inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs). However, long-term use of NSAIDs leads to side effects like gastrointestinal irritation. Therefore, there is a renewed interest in finding new anti-inflammatory drugs and medicines from natural sources. Thus, present study was conducted with the main purpose of assessment of *in-vitro* anti-inflammatory activity of Garlic (*Allium sativum*) extract using albumin denaturation method. Results revealed that there was dose dependent inhibition (%) was observed in standard as well as ethanolic Garlic (*Allium sativum*)extract. Furthermore, the inhibition (%) *of* ethanolic Garlic (*Allium sativum*) extract at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of ethanolic Garlic (*Allium sativum*) extract was better than that of standard drug i.e., Aspirin. The major phytochemicals present in ethanolic Garlic (*Allium sativum*) extract were found to be alkaloids, flavonoids, glycosides, saponins, steroids, phenolic compounds, tannins and terpenoids. In conclusion, results of our study clearly demonstrated that ethanolic Garlic (*Allium sativum*) extract possess anti- inflammatory activity. Thus, it could be recommended that ethanolic Garlic (*Allium sativum*) extract could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs.

Keywords: *Allium sativum*, Garlic, Ethanolic extract, Anti-inflammatory, Albumin denaturation

Introduction

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 thatcan lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases.[1] Some common herbs such as cilantro, basil, thyme, onion, ginger, turmeric, garlic etc., offer great health benefits by virtue of their powerful phytochemicals. 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 isat large.[2,3]
Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[4] The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protectiveattempt by the organism to remove the injurious stimuli and to initiate the healing process. Proinflammatory cytokines (e.g., tumor necrosis factor - a [TNF-a], interleukin [IL-6], and IL-1b) are produced in large quantities by activated macrophages/monocytes that

stimulate cellular responses via increasing prostaglandins (PGs) and reactive oxygen species (ROS). In addition, lipid peroxidation (malondialdehyde [MDA]) is produced by free radicals attacking the cell membranes. Thus, inflammatory effect results in the accumulation of MDA.[5] Many studies have indicated that flavonoids in herbs possess anti-inflammatory activities via scavenging ROS and reducing proinflammatory cytokines (e.g., NF-aB, TNF-a, IL-1b, and IL-6), such as ursolic acid,[6-8] and lupeol.[9]

Garlic *(Allium sativum)* as shown in Figure 1, 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,[10] antiplatelet aggregation,[11] anti-inflammatory activity,[12] and inhibition of cholesterol synthesis.[13] Garlic has been long known to have antibacterial,[14] antifungal,[11] anticancer,[15,16] antioxidant, and antiviral activities. Therapeutic effects of garlic are due to the presence of allicin in the cloves.

Figure 1: Showing Garlic (*Allium sativum*) plant and cloves

With this background the present study was conducted with the main objective of evaluation of *in-vitro* anti-inflammatory activity of Garlic (*Allium sativum*) extract using albumin denaturation method.

Materials and Methods Collection of Garlic Cloves

The cloves of Garlic (*Allium sativum*) were procured from the local markets ofChikkaballapura, 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.[17]

Phytochemical Screening

Phytochemical screening was carried out on the ethanolic Garlic (*Allium sativum*) extract by using standard procedure to detect constituents as described by Sofora (1993),[18] Trease and Evans (1989),[19] and Herborne (1973).[20]

Test for Alkaloids

Approximately 0.2g of ethanolic Garlic (*Allium sativum*) extract was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The ethanolic Garlic (*Allium sativum*) extract in **s**mall quantity was mixed with water and heated on water

bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green coloration indicates the presence of tannins and phenolic compounds.

Test for Glycosides

About 0.6g of ethanolic Garlic (*Allium sativum*) extract was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The ethanolic Garlic (*Allium sativum*) extract was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of ethanolic Garlic (*Allium sativum*) extract was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2 g of ethanolic Garlic (*Allium sativum*) extract was dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of ethanolic Garlic (*Allium sativum*) extract and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3 g of ethanolic Garlic (*Allium sativum*) extract was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

***In-vitro* Anti-inflammatory Activity Assay**

The *in-vitro* anti-inflammatory activity of ethanolic Garlic (*Allium sativum*) extract was determined using modified method of Saleem *et al.,* (2011). Control, Standard (Aspirin), and different concentrations of ethanolic Garlic (*Allium sativum*) extract (i.e., 100-1000 µg/mL) were prepared as follows;

Control: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and final volume was made up to 50 ml with double distilled water.

Standard (Aspirin): 2 ml of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations (100-1000 µg/mL) of standard drug (Asprin) were taken and final volume was made up to 50 ml.

Extract: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations of ethanolic Garlic (*Allium sativum*) extract (i.e., 100-1000 µg/mL) were taken and final volumewas made up to 50 ml.

The reaction mixtures of control, standard (Aspirin), and different concentrations of ethanolic Garlic (*Allium sativum*) extract (i.e., 100-1000 µg/mL) were incubated at 37ºC for 15 minutes and heated at 70ºC for 5 minutes. After cooling the turbidity was measured at 660 nm.Percentage inhibition of albumin denaturation was calculated using the following formula (Chandra *et al.,* 2012).

Inhibition (%) = $(1 - A2/A1) \times 100$

Where,

A1 = Absorption of the control sampleA2 = Absorption of the test sample

Results

The results of qualitative phytochemical analysis of ethanolic Garlic (*Allium sativum*) extract was represented in Table 1. Results revealed that the major phytochemicals present in ethanolic Garlic (*Allium sativum*) extract were found to be alkaloids, flavonoids, glycosides, saponins, steroids, phenolic compounds,

tannins and terpenoids. Whereas, reducing sugars were found to be absent in ethanolic Garlic (*Allium sativum*) extract (Table 1).

Table 1: Qualitative photochemical analysis of ethanolic Garlic (*Allium sativum*) extract

PhytochemicalComponents	Ethanolic Garlic (*Allium sativum*) extract
Alkaloids	+
Flavonoids	+
Glycosides	+
Reducing sugars	-
Saponins	+
Steroids	+
Phenolic compounds	+
Tannins	+
Terpenoids	+

'+': Present; '-': Absent

The results of *in-vitro* anti-inflammatory activities of standard and ethanolic Garlic (*Allium sativum*) extract was presented in Table 2 and Figure 2. Results revealed that the mean inhibition (%) exhibited by standard was found to be 100.60, 132.82, 186.25, and 308.20 at the concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL respectively. Similarly, the mean inhibition (%) exhibited by ethanolic Garlic (*Allium sativum*) extract at concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µg/mL was found to be 39.55, 98.56, 209.32, and 435.33 respectively. These findings depicted that there was a dose dependent inhibition (%) was observed in standard as well as ethanolic Garlic (*Allium sativum*) extract. Furthermore, the inhibition (%) *of* ethanolic Garlic (*Allium sativum*) extract at the concentration of 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of ethanolic Garlic (*Allium sativum*) extract was better than that ofstandard drug i.e., Aspirin.

Table 2: Effect of ethanolic Garlic (*Allium sativum*) extract on *in-vitro* anti-inflammatoryactivity

Concentration(µg/mL)	Inhibition (%)	
	Standard	Ethanolic Garlic (*Allium sativum*) extract
250	100.60	39.55
500	132.82	98.56
750	186.25	209.32
1000	308.20	435.33

Values are expressed as Mean; n=3

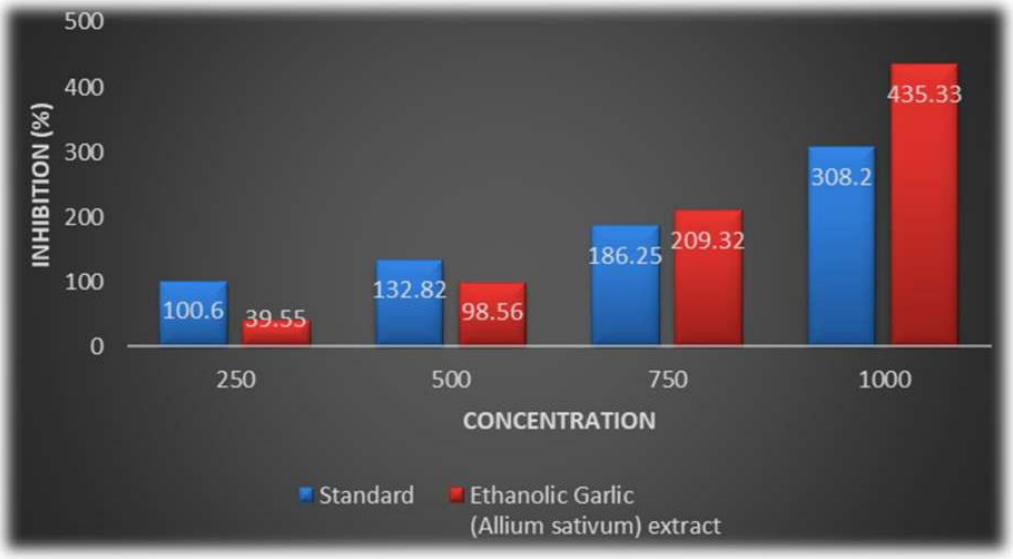

Figure 2: Effect of ethanolic Garlic (*Allium sativum*) extract on *in-vitro* anti-inflammatoryactivity

Discussion

A number of factors, such as bacterial infection, chemical injury, and environmental pollution, can cause inflammation, which is a complicated process that can cause cell damage or death. The most widely used drugs in the world today are NSAIDs.[21,22] The NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Market demand exists for orally active molecules that are moreeffective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms. Different methods such as inhibition of phosphatases, aminotransferases, cotton pellet granulation techniques, inhibition of heat-induced hemolysis, inhibition of albumin denaturation, membrane stabilizing, platelet aggregation, have been used to study the anti-inflammatory potentials of drugs or agents.[23] With these viewpoints, the present study was conducted with the main purpose of assessment of *in-vitro* anti-inflammatoryactivity of ethanolic Garlic (*Allium sativum*) extract using albumin denaturation method.

Denaturation of protein has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulfide bonding.[24] Denaturation of protein causes the production of autoantigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. Hence, by inhibition of protein denaturation, inflammatory activity can be inhibited.[25] Concurrently, in our study, there was dose dependent inhibition (%) was observed in standard as well as ethanolic Garlic (*Allium sativum*) extract. Furthermore, the inhibition (%) *of* ethanolic Garlic (*Allium sativum*) extract at the concentrationof 750 µg/mL was comparable with that of standard drug i.e., Aspirin. While, at the concentration of 1000 µg/mL inhibition (%) of ethanolic Garlic (*Allium sativum*) extract was better than that of standard drug i.e., Aspirin.

Furthermore, major phytochemicals present in ethanolic Garlic (*Allium sativum*) extractwere found to be alkaloids, flavonoids, glycosides, saponins, steroids, phenolic compounds, tannins and terpenoids. In accordance with our study findings Ali and Ibrahim (2019) revealed that the phytochemical screening of *Allium sativum* aqueous and ethanol extracts indicated the existence of alkaloid, steroid, terpenoids, flavonoids, phenol, anthraquinones, saponin, tannin and glycoside.[26] Phytochemicals provide plants with their colour, flavor, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites.[27]

Conclusion

The results of present preliminary study clearly demonstrated that ethanolic Garlic (*Allium sativum*) extract possess anti-inflammatory activity. Hence, it could be recommended that ethanolic Garlic (*Allium sativum*) extract could be employed for the management of inflammatory conditions and could be considered for development of natural anti-inflammatory drugs. However, further studies are recommended to elucidate the exact mechanism of action of particular phytochemical responsible for anti-inflammatory activity of Garlic (*Allium sativum*) extract.

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