

Extraction and Various Pharmacological Activity of Allicin From Garlic

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Abstract: *Garlic has various pharmacological activities such as antibacterial, anticancer and antiinflammatory activities. In this work, fresh clove of garlic was used the raw material to prepare the garlic extracts. The aim of this work is to prepare the garlic extracts by using chemical and non-chemical extraction. For chemical extraction, different garlic/95% ethanol (w/v) ratios were 1:10, 1:15 and 1:20 using extraction methods including maceration, soxhlet, and microwave assisted extraction, respectively. For non-chemical extraction, the garlic extract was prepared by mixing the garlic and honey in the ratio of 1:1 (w/v). All garlic extracts were evaluated the bioactivities and allicin contents. The antioxidant activity and total phenolic contents of each extract were investigated by DPPH radical scavenging assay and Folin Ciocalteu method, respectively. The results revealed that the garlic extract obtained from soxhlet extraction using a mixture of garlic and ethanol in the ratio of 1:15 (w/v) displayed the highest antioxidant activity and total phenolic contents of 17.95 ± 1.05 μmol of trolox equivalents per gram of garlic and 26.88 ± 0.08 mg of gallic acid equivalents per gram of garlic, respectively. Each garlic extract was determined the content of allicin by HPLC-UV analysis. The results revealed that garlic extract obtained from the mixture of garlic and honey displayed the highest content of allicin at 8.12 $\mu\text{g/ml}$.*

Keywords: Soxhlet extraction, Microwave assisted extraction, Ultrasonic-assisted extraction, Supercritical-fluid extraction, Microwave Extraction, maceration. Pharmacological Activity

I. INTRODUCTION

Garlic (*Allium sativum*) is one of the traditional herbals and utilized as flavoring It is well known to acquire various phytochemical Several evidences highlight its pharmacological potential such as antibacterial, antifungal, anti inflammatory and antiviral activities In vitro studies verified its antiplatelets aggregation, anticancer and antioxidant activities Moreover, in vivo studies, both in human clinical trials and in animal modeling, have demonstrated beneficial aspects of garlic against various threats including hyperlipidaemia, hyperglycaemia, and atherosclerotic plaque formation, respectively Allicin and alliin is one of the main biologically active components of garlic . They have a potential to reduce various ailments Garlic cells encompass a (+)-S-allyl L-tysine sulfoxide commonly known as alliin an odourless compound along with alliinase enzyme which is present in the vacuoles produce diallyl thiosulfinate or allicin Alliin and the enzyme alliinase or alwhere Alliin is converted to allylsulfenic acid by alliinase. Allylsulfenic acid is further converted to allicin by the reduction of water. Allicin is an unstable compound and is liin lyase are located in separate regions in garlic and come in contact only when crushed or ground to produce allicin Alliin is converted to allicin enzymatically with the help of alliinase during cutting or grinding

II. METHODS

1. Soxhlet extraction.
2. Ultrasonic-assisted extraction
3. Pressurized Liquid extraction
4. Supercritical-fluid extraction
5. Microwave Extraction



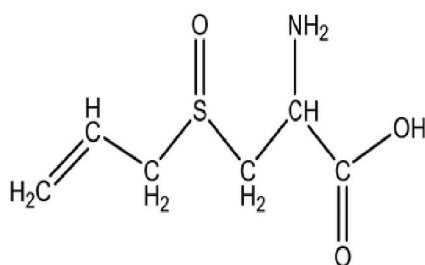
Garlic

Synonyms: Allium

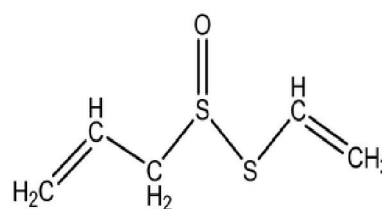
Biological: Garlic is the ripe bulb of *Allium sativum* Linn.

Family: Liliaceae.

Different Chemical Constituent



alliin



allicin

SOXHLET EXTRACTION

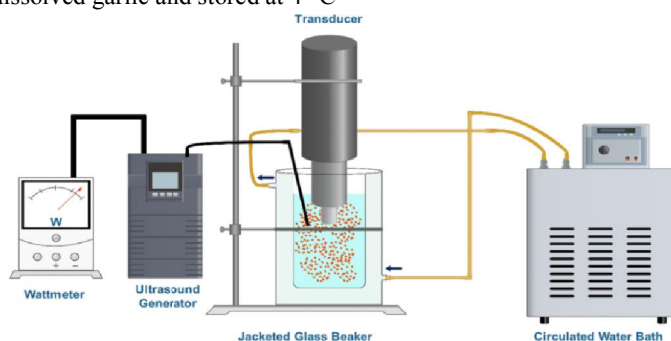
A mixture of garlic and ethanol in the ratio of 1:10, 1:15 and 1:20 (w/v) were carried out using 95% ethanol. 1 g of the sample was soaked in 200 ml of 95% ethanol for 48 hrs at room temperature. For soxhlet extraction, 1 g of fresh garlic was performed using a mixture of garlic and 95% ethanol in the same ratio of solvent to solid as maceration technique. The soxhlet condition was done for 1.5 hours at 70°C using soxhlet apparatus



FIG. Soxhlet Extraction
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ULTRASONIC-ASSISTED EXTRACTION

Materials and method Plant Samples : Garlic cloves were purchased from local market which was originated from China. During the project, all the samples were maintained in lab at appropriate conditions (dark, 25 °C) **Reagents :** Methanol used was HPLC grade (Purity > 99.9 %) purchased from Sigma Aldrich (US) and deionised water used was purified by Q purification system (Millipore) (Massachusetts, USA) **Milli Pretreatments :** 10 g of garlic cloves were blended with 100 mL deionised water for 1 min in a commercial blender for blended samples. For sliced sample, 10 g of garlic cloves were sliced into small pieces with equal size distribution and mixed with 100 mL deionised water. Ultrasound-assisted extraction (UAE) was performed in an ultrasound cleaning bath (Sonorex DT1028/H, 500 mm × 300 mm × 200 mm interior dimensions) by the mode of the indirect sonication at the fixed-frequency of 35 kHz using as the working liquid. The 100 ml sample flask was charged with the pre-treated sample mixture and placed into the ultrasonic cavity for extraction process. Parameters optimised were extraction time (30 min, 60 min, 90 min, 120 min and 150 min), extraction temperature (25 °C, 30 °C, 35 °C) and particle size (Blended and sliced garlic bulb). After sonication the solution was then separated from impurities by centrifugation at 3,000 g for 2 min. Then the solution is filtered to remove the undissolved garlic and stored at 4 °C



SUPER CRITICAL EXTRACTION

This method is qualitatively accurate, environmentally-friendly and efficient for extraction of garlic. The super critical fluids are more efficient for the extraction than organic solvents for their transport properties like mass transfer, diffusivity and its penetration ability (Brunner, 1994). Carbon dioxide helps in the complete extraction and separation of the sample and has a quality advantage for its high selectivity. It also helps in the extraction of oxidation prone substances as it is not exposed to high temperatures or oxygen during treatment Maximum efficiency and yield of garlic are obtained at a temperature between 35-50°C and pressure of 300-400 bar with ethanol as the solvent . While in the paper by dynamic mode was used with 20g/min of CO₂ at a temperature of 50°C and a pressure of 100 psi. The garlic samples were cut, chopped and ground using a press for high yield of the enzyme They are added to de ionized water and vortexed for a few seconds and incubated at room temperature for maximum yield of allicin. Homogenates preparation was done by adding de ionized water and Alyl phenyl sulfonyl was added as standard and analyse

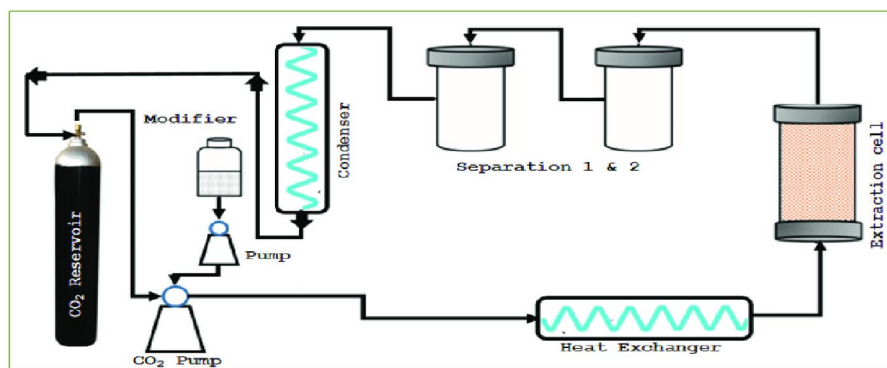


FIG. Supercritical Fluid Extraction

MICROWAVE EXTRACTION.

This method improves the stability and the quantity of allicin and evaluated post extraction. Microwave extraction has several advantages viz., short extraction time, less solvent consumption, environment friendly, low cost and high quality. Domestic microwave is used for extraction and involves two methods, one with solvent and the other is without solvent. The solvent assisted microwave extraction is more efficient as it helps in cell breakage. Microwaves have a higher transforming capacity of allicin as garlic absorbs the microwave energy and converts it to heat energy. In , garlic samples were added to a round bottom flask with a condenser. The flask is further kept in a microwave extractor where the temperature, reflux time and percentage power are set for the extraction of allicin. The solvent used for this method is usually ethanol along with pre fixed extraction temperature, time and microwave power. Excess exposure to microwave radiation leads to the decomposition of allicin.

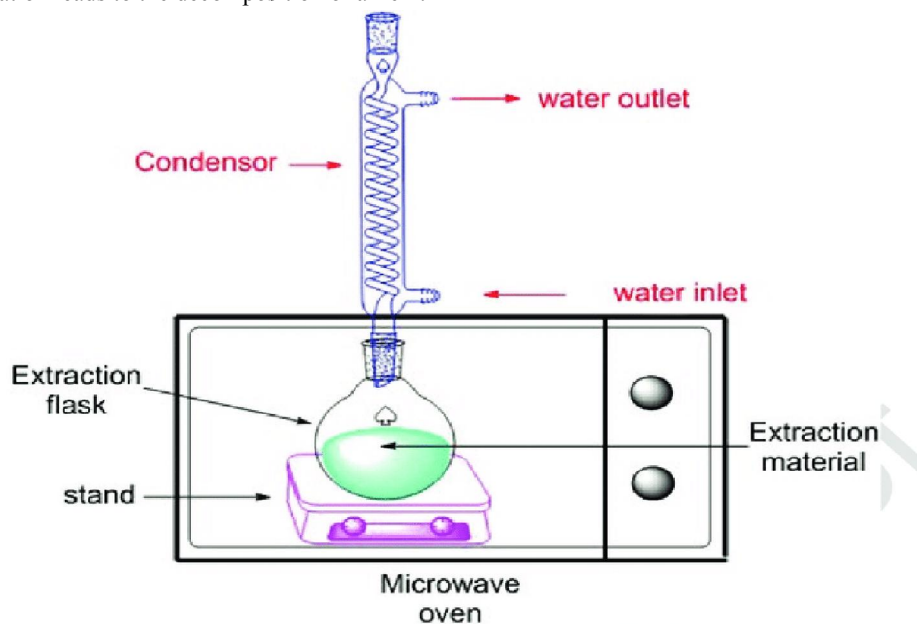


Fig: Microwave Extraction

PRESSURIZED LIQUID EXTRACTION

Materials and Methods-

Plant Material : Fresh garlic was obtained at local supermarkets (Campinas, SP, Brazil). The outer skin of the garlic cloves was peeled off. The garlic cloves were cut into small cubes (approximately 0.3 cm on all side) using a kitchen knife. The garlic samples were prepared immediately before PLE extraction was performed in order to avoid the degradation of allicin.

Methods:

It is a method of extraction of compounds using liquids at high pressure. It has a simple operation, works faster and consumes lesser solvent than other techniques. Its working principle is that of static extraction with the assistance of superheated liquids and is a substitute for other extraction methods. Extraction is carried out at high temperatures and pressure that enhances the extraction performance as the solvent possesses high solubility and mass transfer properties. The sample is often pre-treated which includes cutting into pieces or ground for easier diffusion due to smaller particle size before extraction. Ethanol is usually adopted as the solvent for the extraction of allicin. It contains a HPLC pump, an extraction vessel consists of a blocking valve and back pressure, manometer, solvent reservoir and a sampling bottle. The garlic was peeled and cut into pieces to keep the allicin intact before the extraction. The pressure, temperature and time were pre-determined before the extraction. The extraction of allicin was carried out at a fixed temperature of 313K and pressure of 6MPa. Once the sample is subjected to high pressure, ethanol was removed using vapour evaporation.

and alliin is extracted from the sample. Care was ensured while extracting and storage area must be clean, cool and dry.

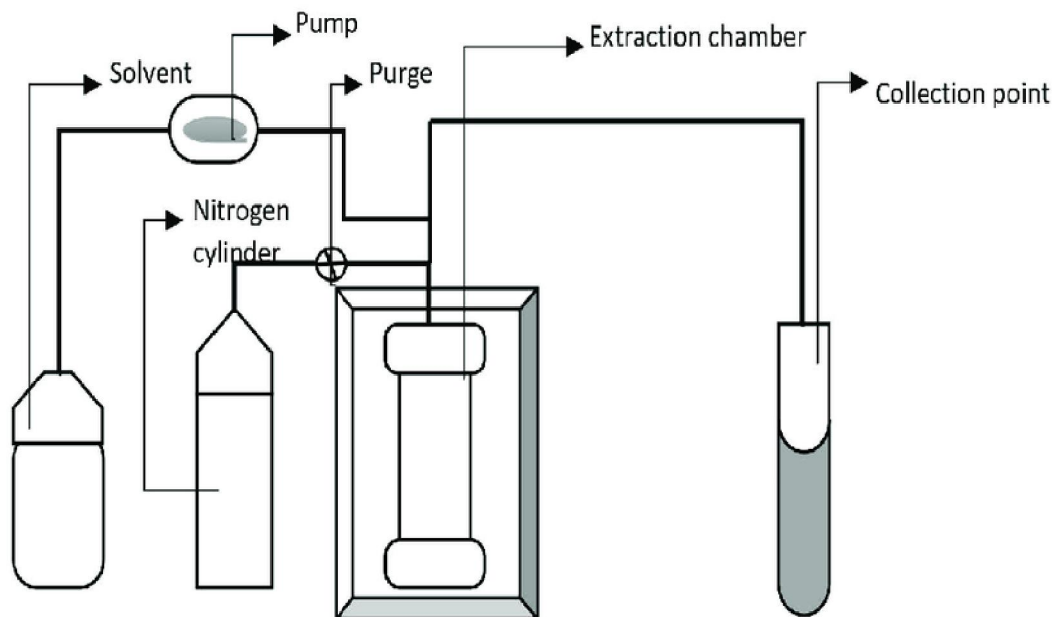


FIG: PRESSURIZED LIQUID EXTRACTION

PHARMACOLOGICAL ACTIVITY OF ALLICIN FROM GARLIC

Antibacterial

Alliin and other sulfur compounds are thought to be the major compounds responsible for the antimicrobial effect of garlic. Garlic is effective against a number of gram-negative, gram-positive, and acid-fast bacteria, including *Staphylococcus*, *Salmonella*, *Vibrio*, *Mycobacteria*, and *Proteus* species (7). Aqueous, ethanol and chloroform extracts of garlic inhibited the growth of the pathogenic bacteria, though with varying degrees of susceptibility. The gram positive *Staphylococcus aureus* was more susceptible to the toxic effects of garlic than its gram negative counterparts. It has been shown that the aqueous extract of garlic can be used alongside conventional antibiotics to fight agents of nosocomial infections that are so prevalent in hospitals (5). An *in vitro* study on the effects of aqueous and ethanolic extracts of garlic against specific bacteria such as *Escherichia coli* and *sal. typhi* showed that the aqueous extract had little or no inhibition while the ethanolic extract had a higher inhibitory effect. Alliin in its pure form was found to exhibit antibacterial activity against multidrug-resistant enterotoxigenic strains of *E. coli*.

Antifungal

Alliin (diallyl-dithiosulfinate), which is produced by the garlic enzyme alliinase from the alliin, has been shown to have wide-range antifungal specificity. An *in vivo* study showed that antibody-alliinase conjugates and alliin are effective against murine pulmonary aspergillosis (20). One study showed that alliin from garlic has antifungal activity particularly against *Candida albicans* (10). Another *in vitro* study showed both intrinsic antifungal activity of alliin and its synergy with the azoles, in the treatment of candidiasis (21). Studies on the effect of Amphotericin B (AmB) against *C. albicans* showed that alliin enhances significantly the effect of AmB against *Candida albicans*, *Saccharomyces cerevisiae* and against *Aspergillus fumigatus* *in vitro* and *in vivo* (22, 23). It was found in another study that polymyxin B (PMB), is effective against various yeasts and filamentous fungi when used in combination with alliin.

Anti-parasitic

An ultrastructural study showed that alliin is able to produce morphological changes in the male *Schistosoma mansoni* (37). Another study indicated that Alliin has antiparasitic activity against *Plasmodium*

falciparum and *Trypanosoma brucei brucei* (38). It is also effective against some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia* (10). Diallyl trisulfide is a chemically stable final transformation product of allicin. The activity of diallyl trisulfide was investigated against several important protozoan parasites *in vitro*. The results indicated that the compound has the potential to be used in treatment of several human and animal parasitic diseases such as *Trypanosoma sp*, *Ent. histolytica* and *Giar. lamblia* (39). Ajoene isolated from *A. sativum* is an inhibitor of human glutathione reductase and *Trypa. cruzi* trypanothione reductase. The antiparasitic and cytostatic actions of ajoene may at least in part be due to the multiple effects on key enzymes of the antioxidant thiol metabolism (40).

III. CONCLUSION

In this paper, different methods of extraction of Garlic was discussed and analysed. The extraction process is very important for maintaining the stability as well as quantity of the garlic. The extraction process must be chosen according to the particle size of garlic and the end product required

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