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COMPARATIVE STUDY OF HYBRID AND NATIVE GARLIC (*ALLIUM SATIVUM*) BY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

Secondary metabolites found in the medicinal plants play important role in curing different diseases and used as important raw materials for the manufacturing of traditional and modern medicines. One of these medicinal plant garlic *(Allium sativum)* members of Liliaceae family reduces various risk factors associated with several diseases. Garlic has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotensin converting enzyme. It also reduces cholesterol and blood pressure Therefore; our aim was to compare the different secondary metabolites present in the hybrid and native aqueous and methanolic extracts of the garlic bulb. Phytochemicals screening revealed those alkaloids, reducing sugars, flavonoids, glycosides, tannin, saponins and amino acids. The antibacterial potency of aqueous and methanol extracts of hybrid-native garlic was determined invitro against three bacterial isolates (*Escherichia coli*, *Salmonella typhi* and *Enterococcus faecalis*) by agar-well diffusion method. These findings therefore justify the traditional medicinal use of garlic.

KEYWORDS: *Allium sativum, phytochemical, Escherichia coli, Salmonella typhi, Enterococcus faecalis.*

INTRODUCTION

Natural plants have been an essential part of the discovery of new therapeutic chemicals since ancient times. Almost every antibiotic is subject to the bacterial resistance problem. Therefore, newer antibacterial extracts from the plants and their semi-synthetic derivatives

have been examined to overcome resistance [Palaksha *et al., 2010*].

The use of plants as sources for useful medicines, foods, add-ons, flavors, dyes, binders and lubricants has been ongoing. Medicinal plants constitute sources of important drugs used alone or in combination with other plants in the treatment of diseases. Plant chemicals include alkaloids, glycosides, essential oil, tannins, steroids, resins, flavonoids, and protein so on. Substances found in plants include alkaloids. These substances are strong bioactive compounds that can be used for therapeutic purposes in medicinal plant parts [Length *et al., 2013*].

They provided strong support for the use and development of new medications and phytomedicine of these plants in herbal medicine. Several researches have shown that some of these bioactive plant origin principles have antimicrobial characteristics. The increased dependence on the use of medicinal plants in developed countries had resulted in several medication and chemotherapy products extracted and developed from these plants [Length *et al., 2013*].

Allium sativum L. is a wide-grown medicinal crop frequently referred to as garlic, which belongs to the Alliaceae family. It is used for thousands of years throughout the world. Records show that garlic was in use about 5000 years ago when the pyramids of Giza were built [Christian, *2017*].

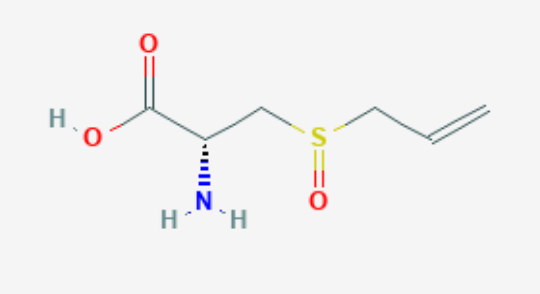
Garlic is a strong antibacterial agent against both gram-positive and gram- negative such as *E.coli*, *Salmonella spp*., *Streptococcus spp*., *Staphylococcus aureus*, also it's effective even against those strains that have become resistant to antibiotics. According to World Health Organization, new drugs can be obtained by plant sources. *A. sativum* has an active ingredient called Allicin which has an important role in many conditions, hence act as a potential phytochemical [Atheer, *2014*].

The use of garlic extract has a typical therapeutic activity, which relies on allicin, produced by alliin hydrolysis when the garlic samples are cut and crushed. Their use includes beneficial effects on the cardiovascular system, antibiotic, contraceptive, anti-inflammatory, hypoglycemic, anti-microbial, and hormone-like effects [Palaksha *et al., 2010*].

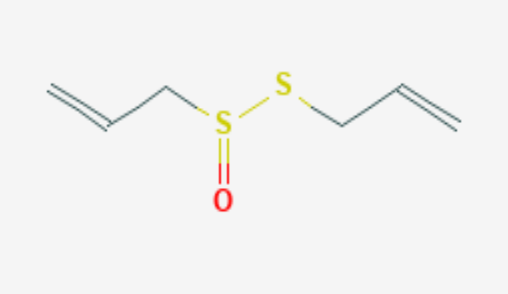
Due to biochemical reactions, there is a distinct flavor in the garlic. The predominant precursor to this flavor is alliin (Figure 1) or S-lyl-cysteine sulfoxide (ACSO). These sulfur

compounds are also responsible for the renowned medicinal properties of garlic. Other significant volatile compounds with potent bioactive properties are ajoenes, as well as several sulfur-containing compounds other than alliin, such as allicin, 1,2-vinyldithiin (Figure 1) and S-allyl-cysteine, and sulfides, such as diallyl-, methyl allyl-, and dipropyl mono-, di-, tri-and tetra-sulfides, formed after thiosulfinates has been decomposed [Martins *et al.*, *2016*]. Presence of all these compounds make it a potential agent in therapeutics. Hence it is necessary to explore the benefits and to be used as an important drug.

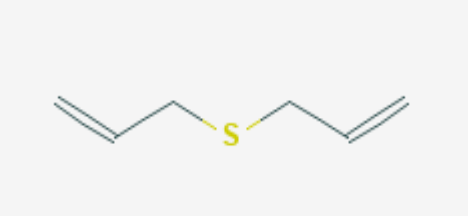
Chemical structures of *A. sativum L.* bioactive compounds



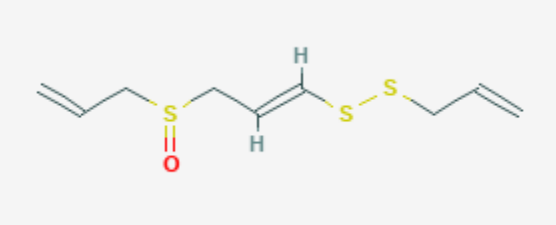
Alliin



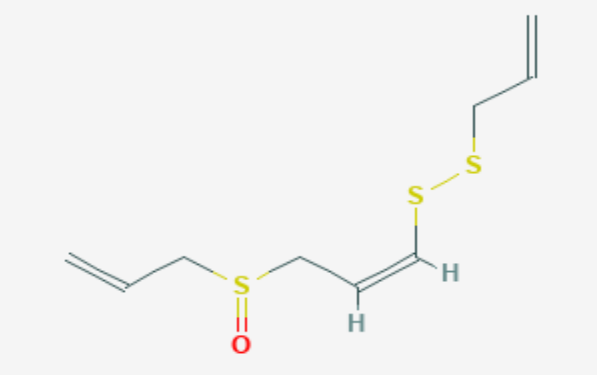
Allicin



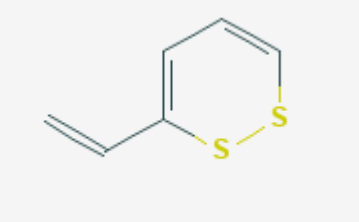
Allyl sulfide



(E)-ajoene



(Z)-ajoene



1, 2-vinyldithiin

Fig 1: Bioactive components of Garlic (*Allium sativum L.)* (NCBI).

MATERIALS AND METHODS

Collection of Garlic bulb

The *Allium sativum* plant was collected from the local market of Mysore during the month of March (Figure 2 A and B). First, the collected plant material was washed 3-4 times with tap water and then two times with de-ionized water. In order to avoid contamination, garlic bulb was kept in the dark after washing and kept at room temperature and under constant observation. With the help of a knife and chop board, bulb was chopped finely (Figure 2 C). For further use, the sample was stored.

A **B**

C

Figure 2: A- Native garlic bulb (*A. sativum L.*), B- Hybrid garlic bulb *(A. sativum L.*), C- Chopped garlic (*A. sativum L.*)

Preparation of garlic essential oil extract

Fresh garlic bulb was taken down and the essential oil was extracted with optimal conditions using a Soxhlet extractor. With 200ml of different solvents such as methanol and de-ionized

water, 50 g of fresh garlic was used. For this experiment, the operating temperature varied from 30°C to 40°C. The sample obtained was distilled to recover solvent at 10°C until all solvents were collected. The essential oil obtained from ginger was collected and stored for further use in room temperature. The extraction and yields of all the six solvents were noted (Table 2) [Singh *et al., 2017*].

PHYTOCHEMICAL ANALYSIS

Using standard test procedures, phytochemical tests were performed for the methanol and aqueous garlic extracts. The test name, phytochemical and the reference is mentioned in the below table:

Table 1: Phytochemical analysis of garlic extracts.

SL. NO.	PHYTOCHEMICAL TESTS	TEST NAME	REFERENCE
1.	ALKALOIDS	MAYER'S TEST	Kumar *et al., 2011*
2.	CARBOHYDRATES	MOLISCH TEST	Usman *et al., 2009*
3.	STEROIDS	SALKOWSKI TEST	Singh *et al., 2017*
4.	TRITERPENOIDS	SALKOWSKI TEST	Singh *et al., 2017*
5.	FLAVONOIDS	ALKALINE REAGENT TEST	Singh *et al., 2017*
6.	REDUCING SUGAR	BENEDICT'S TEST	Singh *et al., 2017*
7.	AMINO ACIDS	NINHYDRIN TEST	Singh *et al., 2017*
8.	GLYCOSIDES	BORNTRAGER'S TEST	Singh *et al., 2017*
9.	PHENOLS AND TANNINS	$FeCl_3$ TEST	Singh *et al., 2017*
10.	PROTEIN	BIURET TEST	Singh *et al., 2017*
11.	SAPONINS	FROTH TEST	Kumar *et al., 2011*

Anti-microbial Activity of garlic

Test organisms

Standard bacterial cultures of *Escherichia coli, Salmonella typhi* and *Enterococcus faecalis* were collected from JSS Hospital, Mysore and from DFRL Mysore.

Cultures

The cultures were sub cultured in subsequent nutrient broth and definite media where they favored i.e., Mannitol Salt Agar (MSA) for *S. aureus* and MacConkey for *E. Coli* and Petri dishes were prepared on a slant [Length *et al., 2013*].

Antibacterial activity test

Agar diffusion method

The Agar diffusion method was used to test the antibacterial activity of the Garlic crude extract against standard isolates. Direct visual comparison of the growth of the test cultures

determined the antibacterial effect (Figures 6,7,8) All tests were conducted, and the results were reported as the average replication [Khusro *et al., 2013*].

Cork borer method

The antibacterial activity was evaluated using ruler to measure the diameter of the inhibition zone (the media was prepared in both methods according to the manufacturer's instructions) (Fig 3,4,5) [Lu *et al., 2011*].

RESULTS

Yield of extracts from successive extraction of Garlic bulb

The extraction of essential fresh and dry garlic oil by soxhlet extractor yield (ml) has been tabulated as follows:

Table 2: Yield of fresh hybrid and native garlic essential oil in different solvents.

SOLVENT USED	FRESH HYBRID GARLIC OIL YIELD IN ml	FRESH NATIVE GARLIC OIL YIELD IN ml
Methanol	20	19
Aqueous	25	22

Phytochemical analysis report

Standard methods are used for testing the presence or absence of various phytochemicals in the garlic extracts, that are given in the below table:

Table 3: Phytochemical analysis of bioactive compounds in garlic essential oil extract.

SL NO	BIOACTIVE COMPOUND	HYBRID GARLIC METHANOL EXTRACT	NATIVE GARLIC METHANOL EXTRACT	HYBRID GARLIC AQUEOUS EXTRACT	NATIVE GARLIC AQUEOUS EXTRACT
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Steroids	-	-	-	-
4	Triterpenoids	+	+	+	-
5	Flavonoids	+	+	+	+
6	Reducing sugar	+	+	+	+
7	Amino acids	+	+	+	+
8	Glycosides	-	+	+	-
9	Proteins	+	+	+	-
10	Phenols and tannins	-	+	+	-
11	Saponins	-	-	+	+

Wagner's test for the test of alkaloids showed the presence of reddish brown precipitate, Molisch test for the test of carbohydrates showed the presence of violet ring at the junction, Alkaline reagent test for the test of flavonoids showed the presence of colorless solution, Benedict's test for the test of reducing sugar showed the presence of green/yellow/red color, Ninhydrin test for the test of amino acids showed the presence of blue color solution and Oil test for the test of volatile oils showed the presence of upper layer green colour solution in both methanol and aqueous extracts of hybrid and native garlic (*A. sativum L.).*

Salkowski test for the test of steroids did not show the presence of red colour in the lower layer in all the extracts. Borntrager's test for the test of glycosides showed the presence of red color solution, and Ferric chloride test for the test of phenols and tannins showed the presence of violet/green/blue colored solution in methanol and aqueous extract of native and hybrid garlic respectively. Salkowski test for the test of triterpenoids showed the presence of golden yellow colour at the bottom, Biuret test for the test of proteins showed the presence of violet/pink colored solution in methanol extract of hybrid and native garlic and in aqueous extract of hybrid garlic.

Froth test for the test of saponins resulted in foam formation in aqueous extract of native and hybrid garlic.

Anti-microbial activity

The antimicrobial activity of the garlic extracts against *E. coli*, *E. faecalis* and *S.typhi* by measuring the diameter of zone of inhibition are shown in the Tables 4, 5, 6 respectively.

Zone of Inhibition (mm)

Table 4: Zone of inhibition of Garlic extract against *Escherichia coli.*

Dilution of Garlic extract(µl/ml)			
Garlic extract	**Concentration of extract**		
	30	40	50
Hybrid methanol	5 ± 2	5 ± 2	5± 4
Native methanol	7 ± 2	7 ± 4	7 ± 5
Hybrid aqueous	-	-	9±3
Native aqueous	8±2	8±5	8 ± 6
Control	-	-	-

Zone of Inhibition (mm)

Table 5: Zone of inhibition of Garlic extract against *Enterococcus faecalis.*

Dilution of Garlic extract(µl/ml)			
Garlic extract	**Concentration of extract**		
	30	40	50
Hybrid methanol	8± 4	8± 5	8± 6
Native methanol	10± 5	10 ± 8	10 ± 10
Hybrid aqueous	-	-	9±2
Native aqueous	6 ± 3	6 ± 3	6 ± 4
Control	-	-	-

Zone of Inhibition (mm)

Table 6: Zone of inhibition of Garlic extract against *Salmonella typhi.*

Dilution of Garlic extract(µl/ml)			
Garlic extract	**Concentration of extract**		
	30	40	50
Hybrid methanol	15 ± 9	15 ± 10	15 ± 13
Native methanol	14±5	14±7	14± 8
Hybrid aqueous	-	-	14±3
Native aqueous	8 ± 3	8 ± 4	8 ± 6
Control	-	-	-

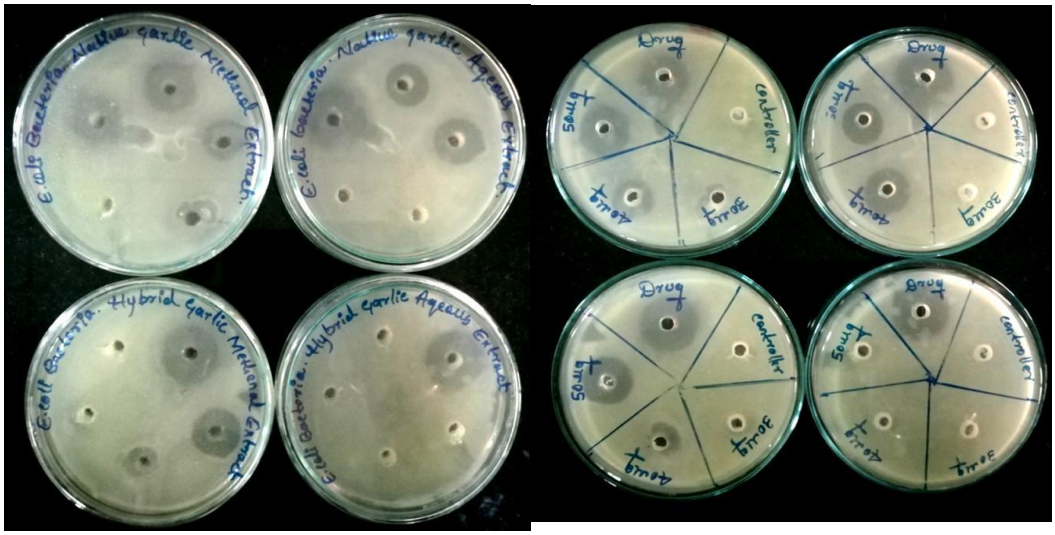


Figure 3: Zone of inhibition of Garlic extract against *Escherichia coli.*

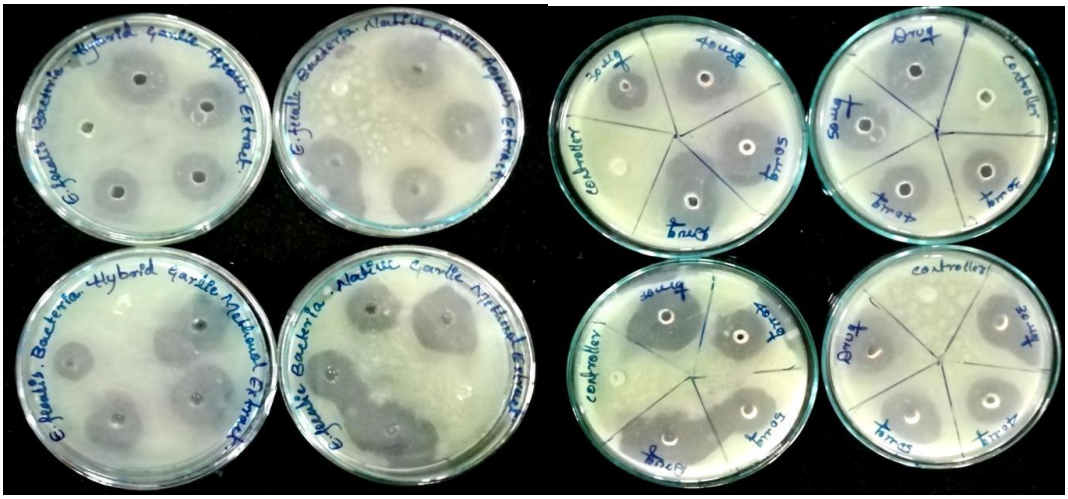


Figure 4: Zone of inhibition of Garlic extract against *Enterococcus faecalis.*

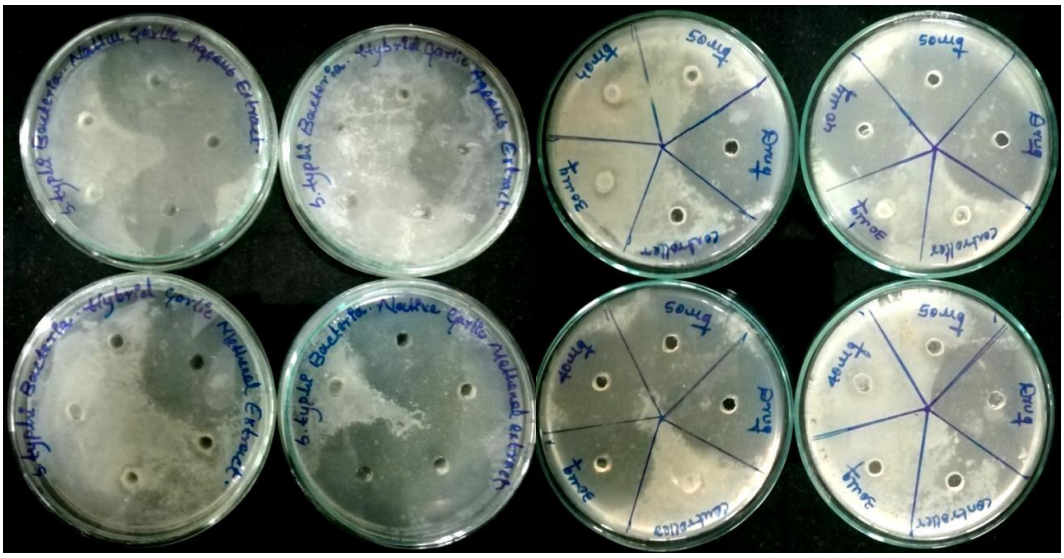


Figure 5: Zone of inhibition of Garlic extract against *Salmonella typhi.*

DISCUSSION

Antibacterial evaluation of garlic aqueous and methanol extracts showed significant antibacterial potency against the test organisms. The garlic phytoconstituents have been known for a long time and have been commonly reported with antibacterial properties. The antimicrobial activity of garlic extracts has been associated with several bioactive compounds for many years [Garba *et al.*, *2014*]. The presence of alkaloids, glycosides, saponins, steroids, proteins, carbohydrates, oils, and acidic compounds have been revealed by phytochemical screening with the hybrid and native garlic bulb extract in this study.

The different dimensions of the inhibition areas acquired in this study show the potential of indigenous and garlic extractor bioactive values. The various MICs in the garlic extract from

the sample specimens are linked with the document stating that the susceptibility of micro-organisms to garlic foods is different. Consequently, the current research demonstrates *A.sativum* native bulb extract which has helpful antimicrobial characteristics. Further research is required in the testing of the antimicrobial function of the relevant crop parts to isolate effective values out of the plant.

In this study, native garlic extract showed higher activity against the test organisms compare to hybrid garlic extract. Gram negative *E. coli* was most susceptible to the active principle present in native garlic with a maximum zone of growth inhibition for methanol and aqueous extract. Although, gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agent. Impressive activity against this gram- negative bacterium was observed. *E. coli* are incriminated in gastrointestinal and urinary tract infections: the sensitivity of *E. coli* to the extracts is an indication of the therapeutic potentials of these extracts against such infections. The garlic aqueous and methanol extracts have an appreciable potency against *E. faecalis* with inhibition zones.

The aqueous and methanol extracts of native garlic show an appreciable potency against *S. typhi* with an inhibition zones respectively, a bacterium that has been reported to have possibly developed resistance to most antibiotics even before their discovery. Comparing the native and hybrid garlic, antibacterial property is high in native one. From both methanol and aqueous extract, they showed the inhibition zones in all three bacterial strains.

This study has demonstrated the effectiveness of native garlic against clinical isolates of *E. coli*, *E. faecalis* and *S. typhi* that are associated with various infectious diseases. This has provided justification that if well processed, native garlic can be used to develop bioactive substances that may have promising effect on the treatment of some diseases.

CONCLUSION

This study concludes the comparison of hybrid garlic extract and native garlic extract and the antimicrobial activity of *Allium sativum* extract against *E.coli*, *S.typhi*, *E.faecalis*. It is expected that the use of natural products as therapeutic agents in microorganisms will probably not produce resistance. Research should continue to isolate and purify the active ingredients of this natural herb and use it in experimental animals. Recent growth in the popularity of alternative medicinal products and natural products has renewed interest in garlic and its derivatives as potential natural remedies. This review may be useful for

enhancing our understanding of garlic therapeutic effects and improving our future research plans. Although it has been shown that garlic has significant clinical potential either by itself or as an adjuvant in various disorders.

It is concluded from this study that different bacterial strains have susceptibility to garlic hybrid and native methanol-aqueous extracts. Since the introduction of antibiotics, many bacterial pathogens have increased resistance, we need to focus on alternative drugs that have a long history to avoid such emerging diseases and can be easily accessible and affordable. As a substitute for antibiotics, garlic can, therefore, is a potential spice. Finally, studies should be identified in the mode of action and interaction with garlic hybrid and native extract antibiotics. A detailed study of the physiological role and antibacterial activity of hybrid and native garlic is required.

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