WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 8, 363-385. Conference Article ISSN 2277 – 7105

EVALUATION OF ANTIMICROBIAL EFFECT OF *ALLIUM SATIVUM* EXTRACT AND GOMUTRA

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Article Received on
05 March 2018,
Revised on 25 March 2018,
Accepted on 15 April 2018
DOI: 10.20959/wjpr20188-11023

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ABSTRACT

This study was designed to test the antimicrobial activity of various solvent extracts of *A. sativum* and gomutra. The antimicrobial activity for the extracts was observed against the common wound infection causing bacterial strains *Staphylococcus aureus* and *Pseudomonas sp* and fungal strains *Aspergillus niger* and *Penicillium sp.* The antimicrobial activity was tested by agar well diffusion method. The *A. sativum* extract was prepared with and without skin using various solvents like ethanol, methanol and distilled water. Based on the different solvents used for extraction the extracts were labeled as GJA, GJM, GJE, GJWSA, GJWSM and GJWSE. Almost all the solvent extracts exhibited antibacterial and antifungal activity against the tested organism, when compared to other solvents aqueous extract is more effective. Also those extracts with skin showed increased zone of inhibition. Preliminary phytochemical analysis of the plants constituents were assessed by using qualitative methods. Test was conducted for the following active components: alkaloids, flavanoids, steroids, phlobatannin, cardiac glycosides, phenols, saponins, volatile oil and glycosides. FTIR analysis was done to find out different functional groups present in the extracts. HPLC and GCMS were also done. Also MIC value of the various solvent extracts of *A. sativum* was determined. The MIC value for GJA, GJWSA, GJE and GJWSE against *Pseudomonas sp* is 1, 10, 10 and 1 mg/ml respectively. The MIC for GJA, GJWSA, GJE and GJWSE against *S. aureus* is 100, 100, 100 and 1 mg/ml respectively.

KEYWORDS: Garlic, phytochemical, antimicrobial activity, GCMS, FTIR, HPLC.

1.0 INTRODUCTION

In developing countries, the frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients.[1] The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to search for new infection-fighting strategies.[2,3]

Plants have been a vital source of natural products for human health and different parts of plants exhibit antimicrobial effect.[4] Plant products are the cheapest and safer alternative sources of antimicrobials as there are no side effects.[5,6,7] For a long time, plants have been an important source of natural products for human health. Worldwide, the antimicrobial properties of plants have been investigated by a number of studies and in many of the studies; it has been used as therapeutic alternatives because of their antimicrobial properties.[8] Plants have many antimicrobial properties especially in secondary metabolites such as alkaloids, phenolic compounds, etc.

Some plants are known to possess medicinal properties because they contain active substances that cause certain reactions, from relenting to the cure of diseases, on humans. [9] Knowledge on medicinal plants sometimes means the only therapeutic resource of some communities and ethnic groups.[10]

1.1 *Allium sativum*

A. sativum is commonly known as garlic. It is a species in the onion genus "*Allium*". Its close relatives include the onion, leek, chive, shallot and rakkyo.[11] It is a member of the Liliaceae family and it is the most popular herbs used worldwide to reduce various risk factors associated with several diseases.[12] Also, it is a bulbous perennial food plant of the family Alliaceae which give it a botanical name known as *Allium sativum* (which comes from old English genlac meaning "spear lack"). When crushed, *A. sativum* yield allicin, an antibiotic and antifungal compound (phytoncide). It has been claimed that due to its antibiotic properties, it can be used as home remedy to help speed recovery from streptococcal throat infection or other minor ailments.

1.2 Indian cow and gomutra

Cow is the most valuable animal in all Veda and it is called mother of all. The essential products we get from cow are urine, dung, milk, ghee and curd. These five ingredients are called as panchagawya. Gomutra has found therapeutic applications since days of yore. In almost the whole Indian continent, gomutra is consumed by the majority of the rural population as a traditional remedy.[13] Gomutra based preparations are able to counter viral, microbial, and fungal ailments. These potions promote powerful antimicrobial, antiviral, anti allergic, and antioxidant activity.[14] There are many research mainly centered on the exploration of the antimicrobial powers of gomutra and also its phyto chemical properties.[15]

The present study was designed to test the antimicrobial activity of various solvent extracts of *A. sativum* and gomutra. The antimicrobial activity for the extracts was observed against the common wound infection causing bacterial strains *Staphylococcus aureus* and *Pseudomonas sp* and fungal strains *Aspergillus niger* and *Penicillium sp.*

2.0 MATERIALS AND METHODS

2.1 Collection of *A. sativum*

The garlic (*A. sativum*) used for the study was purchased from the local market of Vellore District, Tamil Nadu, India.

2.2 Preparation of *A. sativum* extract

2.2.1 Aqueous extract

20g of raw *A. sativum* without skin was ground to paste and the volume was made up to 100ml with sterile distilled water and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

20g of raw *A. sativum* with skin was ground to paste and the volume was made up to 100ml with sterile distilled water and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

2.2.2 Ethanol extracts

20g of raw *A. sativum* without skin was ground to paste and the volume was made up to 100ml with ethanol and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

20g of raw *A. sativum* with skin was ground to paste and the volume was made up to 100ml with ethanol and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

2.2.3 Methanol extracts

20g of raw *A. sativum* without skin was ground to paste and the volume was made up to 100ml with methanol and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

20g of raw *A. sativum* with skin was ground to paste and the volume was made up to 100ml with methanol and allowed to stand for 72 hrs. Then the residue was filtered by Whatman No 1. filter paper. Then the extract was stored at 4°C till it was used.

2.3 Procurement of gomutra and analysis

Fresh gomutra was collected in sterile screw cap bottles and brought to the laboratory for testing. It was filtered through Whatman filter paper before being subjected to further testing. The gomutra was sent to a laboratory at Kanchipuram for analysis to confirm the absence of bacteria and fungi.

2.4 Labeling of the samples

The various solvent *A. sativum* juice extract and gomutra is labeled as shown in Table 1 throughout the study.

Table 1: Labeling of the samples.

Extract/ Sample name	Labeling
Aqueous extract of *A. sativum* without skin	GJA
Methanolic extract of *A. sativum* without skin	GJM
Ethanolic extract of *A. sativum* without skin	GJE
Aqueous extract of *A. sativum* with skin	GJWSA
Methanolic extract of *A. sativum* with skin	GJWSM
Ethanolic extract of *A. sativum* with skin	GJWSE

2.5 Phytochemical screening

To confirm the presence or absence of the following plant secondary metabolites phytochemical screening were carried out: phenols, alkaloids, steroids, cardiac glycosides, flavonoids, saponins, phlobatannins, volatile oil and glycosides.

2.5.1 Test for Phenols

Equal volumes of each extract and ferric chloride solution (which is prepared by dissolving 1352g of $FeCl_3$.6H2O in distilled water containing 20 ml of concentrated HCl dilute to 1 liter) are added together. A deep bluish green precipitate indicates the presence of phenol.

2.5.2 Test for Alkaloids

To each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with (2g of Iodine in 6g of Potassium iodide in 100 ml of distilled water). Formation brown or reddish brown precipitate indicates presence of alkaloids.

2.5.3 Test for Steroids

Into each extract was added to 2ml acetic anhydride and 2ml H_2SO_4. Color change from violet to blue or green indicates the presence of steroids.

2.5.4 Saponins

1g each extract was boiled with 5ml distilled water and filtered. 3ml distilled water was added to the filtrate and shaken vigorously for 5 minutes. Persistent frothing on warming indicates the presence of Saponins.

2.5.5 Cardiac glycosides

The extract was treated with 2ml glacial acetic acid with a drop of Ferric Chloride solution and underplayed with 1ml H_2SO_4. A browning at the interface indicates the presence of cardiac glycosides.

2.5.6 Phlobatannins

A few drops of 1% HCl were added to 1ml of test extract and was boiled. A reddish precipitates indicate the presence of phlobatannins.

2.5.7 Flavonoids

5ml Ammonium solution was added to the aqueous filtrate of extract and then a few drops of concentrated H_2SO_4. Yellow coloration indicates the presence of Flavonoids.

2.5.8 Volatile oil

A small quantity of the test extract was shaken with dilute NaOH and 0.1ml HCl. The formation of a white precipitate indicates a positive result.

2.5.9 Glycosides

A small amount of the alcoholic extract was taken in 1mlof water in a test tube and a few drops of aqueous NaOH were added. A yellow coloration indicates the presence of glycoside.

2.6 Maintenance of pure culture

2.6.1 Test organism

Test organisms were collected from MTCC and an antibacterial activity of various extracts of *A. sativum* and gomutra was tested against *Pseudomonas sp* and *S. aureus*.

2.6.2 Standardization of broth culture

To the test tube containing 10ml of nutrient broth, 0.1ml of the bacterial inoculum was added and mixed for homogeneity and was incubated for 3 hrs. Then the broth cultures were estimated for the presence of $l0^8$ cfu/ml of bacterial inoculums. Standardization of broth culture was done by comparing the turbidity to that of 0.5 McFarland turbidity standards.

2.7 Agar well diffusion method

The antibacterial activity of the aqueous, ethanol and methanol extracts of *A. sativum* and the synergistic effect of aqueous, ethanol and methanol extracts of *A. sativum* with gomutra was evaluated by agar well diffusion method. The Muller-Hinton agar was prepared and poured into sterile petri dishes and allowed to solidify. The test organism was inoculated onto the sterile agar plates. Four wells of 6mm in diameter each were aseptically bored using a sterile cork borer on each agar plates. 100µl of the different extracts of *A. sativum* and gomutra were added to each well. Also 100 µl of *A. sativum* with gomutra (50 µl + 50 µl*)* was added to a well. The effect of solvent on the bacteria was also checked by adding the respective solvent on one well. The plates were then incubated at 37°C for 18-24 hrs and room temperature for 3-4 days for bacteria and fungi respectively. Effects of the extracts were assessed by measuring the diameters of zones of inhibition.

2.8 FTIR spectral analysis

The FTIR spectra were performed and recorded with a Fourier transform infrared spectroscopy. The infrared radiation is propagated through the sample to obtain the corresponding spectrum, which was averaged from several data acquisitions. FTIR spectra were acquired in the wave number range of 500-4000 cm-1.

2.9 HPLC analysis

The HPLC analysis was done with aqueous and ethanolic extracts of *A. sativum* both with and without skin. The extracts were sent to VIT University, Vellore for HPLC analysis with HPLC model 1525. The flow rate was 0.5 ml/min. The injected volume was 20µl and the UV detector was set at 254 nm.

2.10 GCMS analysis

The bioactive compound was identified in ethanolic extracts of *A. sativum*. The extracts were sent to VIT University, Vellore for GC-MS analysis and results with identity of compound were collected.

2.11 Calculation of minimal inhibitory concentration (MIC) by broth dilution method

Serial dilutions of aqueous and ethanolic extract of *A. sativum* with and without skin (GJA, GJWSA, GJE and GJWSE) were prepared in Mueller-Hinton broth with 1ml of standard inoculums of the microorganisms. To the first tube 1mg/ml of *A. sativum* juice was added and serially diluted. A tube of the growth medium without *A. sativum* juice, served as a growth control. An uninoculated tube of the medium was incubated to serve as a negative growth control. After 24 hrs of incubation, the tubes were examined for turbidity, indicating growth of the microorganisms. The lowest concentration of the garlic extracts that inhibits growth of the organism was designated the minimum inhibitory concentration (MIC).[16]

3.0 RESULTS

3.1 Extraction of *A. sativum* clove juice

The *A. sativum* clove juice was prepared using different solvents (aqueous, methanol and ethanol) and filtered using Whatman filter paper (Figure 1a, 1b, 1c and 1d).

3.2 Phytochemical analysis

The phytochemical analysis of the current study revealed the presence or absence of phenol, alkaloids, steroids, cardiac glycosides, flavonoids, saponins, phlobatannins, volatile oil and glycosides (Table 2; Figure 2 – 10).

3.3 Gomutra analysis to confirm the absence of microorganisms

The gomutra culture showed no presence of any bacteria or fungi. Also the urine analysis showed the absence of bile salts and pigments (Figure 11).

3.4 Agar well diffusion assay

3.4.1 Antifungal assay

The results of the antifungal assay reveal that the extracts possessed antifungal properties in various degrees on test organisms (Graph 1 and 2).

3.4.1.1 *A. sativum* extract without skin

Zone of inhibition was observed in all solvent extracts but the aqueous *A. sativum* extract showed the maximum zone of inhibition against both *A. niger* (17mm) and *Penicillium sp* (14mm) (Table 3a and 3b). The aqueous, methanolic and ethanolic *A. sativum* extract without skin when mixed with FCU showed 15, 15 and 16 mm zone of inhibition against *A. niger,* respectively (Figure 9a). The aqueous, methanolic and ethanolic *A. sativum* extract without skin when mixed with FCU showed 16, 1 and 1 mm zone of inhibition against *Penicillium sp* respectively (Figure 10a and 11a). There was no zone of inhibition with FCU against both the tested fungi.

3.4.1.1 *A. sativum* extract with skin

Zone of inhibition was observed in all solvent extracts but the aqueous *A. sativum* extract showed the maximum zone of inhibition against both *A. niger* (18mm) and *Penicillium sp* (17mm) (Table 3a and 3b). The aqueous, methanolic and ethanolic *A. sativum* extract with skin when mixed with FCU showed 16, 14 and 1 mm zone of inhibition against *A. niger* respectively (Figure 9b). The aqueous, methanolic and ethanolic *A. sativum* extract with skin when mixed with FCU showed 16, 1 and 1 mm zone of inhibition against *Penicillium sp* respectively (Figure 10b and 11b). There was no zone of inhibition with FCU against both the tested fungi.

3.4.2 Antibacterial assay

The results of the antibacterial assay reveal that the extracts possessed antibacterial properties in various degrees on test organisms (Graph 3 and 4).

3.4.2.1 *A. sativum* extract without skin

Zone of inhibition was observed in all solvent extracts but the aqueous extract without skin showed the maximum zone of inhibition against both *Pseudomonas sp* (17mm) and *S. aureus* (14mm) (Table 4a and 4b). When the aqueous, methanolic and ethanolic *A. sativum* extract without skin was mixed with FCU, it showed 18, 1 and 4 mm zone of inhibition against *Pseudomonas sp* respectively (Figure 15a). Only the aqueous *A. sativum* extract without skin

mixed with FCU showed 13 mm zone of inhibition against *S. aureus* (Figure 16a). There was no zone of inhibition with FCU against both the bacteria.

3.4.2.2 *A. sativum* extract with skin

The aqueous *A. sativum* extract with skin showed the maximum zone of inhibition against both *Pseudomonas sp* (16mm) and *S. aureus* (16mm) (Table 4a and 4b). When the aqueous and methanolic *A. sativum* extract with skin was mixed with FCU it showed 12 and 2 mm zone of inhibition against *Pseudomonas sp* respectively (Figure 15b). Whereas the ethanolic and methanolic *A. sativum* extract when mixed with FCU didn't show any zone of inhibition against *Pseudomonas sp* and *S. aureus* respectively. Whereas the aqueous and ethanolic *A. sativum* extract when mixed with FCU showed 14 and 2 mm zone of inhibition against *S. aureus* respectively (Figure 16b). There was no zone of inhibition with FCU against both the bacteria.

3.5 FTIR

Graph (5, 6, 7 and 8) shows the typical FTIR spectrum of *A. sativum* extracts and Graph 9 shows the FTIR analysis of gomutra. The FTIR analysis of *A. sativum* aqueous extract without skin (GJA) is shown in Table 5a and Graph 5. The FTIR analysis of *A. sativum* aqueous extract with skin (GJWSA) is shown in Table 5b and Graph 6. The FTIR analysis of *A. sativum* ethanolic extract without skin (GJE) is shown in Table 5c and Graph 7. The FTIR analysis of *A. sativum* ethanolic extract with skin (GJWSE) is shown in Table 5d and Graph 8. The FTIR analysis of FCU is shown in Table 5e and Graph 9.

3.6 HPLC Analysis

The HPLC analysis of various solvent extracts of *A. sativum* for allicin were analyzed using UV detector and shown in Graph 11 – 14. The retention time for allicin is between 2.5 to 4 minutes. The HPLC analysis of the FCU was also recorded as shown in Graph 10. The chromatogram obtained by analyzing a solution of standard allicin is also shown in Graph 11.

3.7 GC/MS analysis

For identification of bioactive compounds in ethanolic solvent extract of *A. sativum* without skin, GC-MS analysis was performed (Graph 15). The ethanol extract showed presence of N-hexadecanoic acid as main compound with other 8 compounds in trace quantity (Table 6). Other minor compounds included alpha-D-glucopyranoside - O-alpha D-Glucopyranosyl, and

Oleic acid. From the results it can be interpreted that the identified compound may be responsible for the antimicrobial activity of garlic.

3.8 Calculation of MIC of various solvent extracts of *A. sativum*

MIC value of the various solvent extracts of *A. sativum* was determined. The MIC value for GJA, GJE, GJWSA and GJWSE against *Pseudomonas sp* is 1, 10, 10 and 1 mg/ml respectively (Graph 16). The MIC for GJA, GJE, GJWSA and GJWSE against *S. aureus* is 100, 100, 100 and 1 mg/ml respectively (Graph 17).

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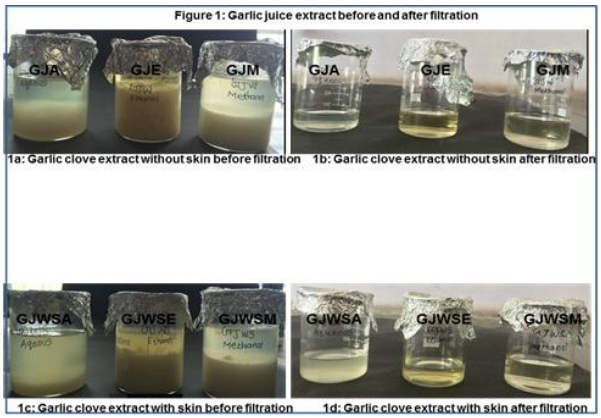

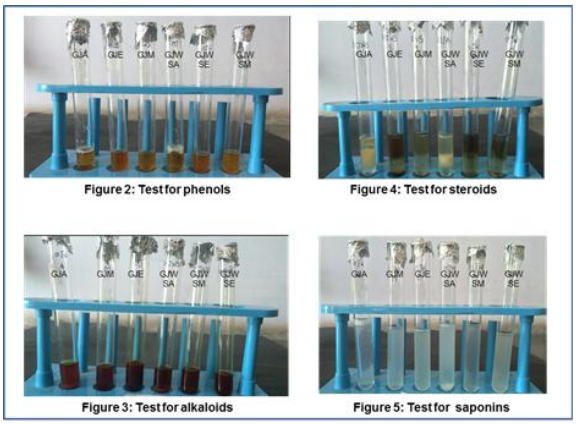

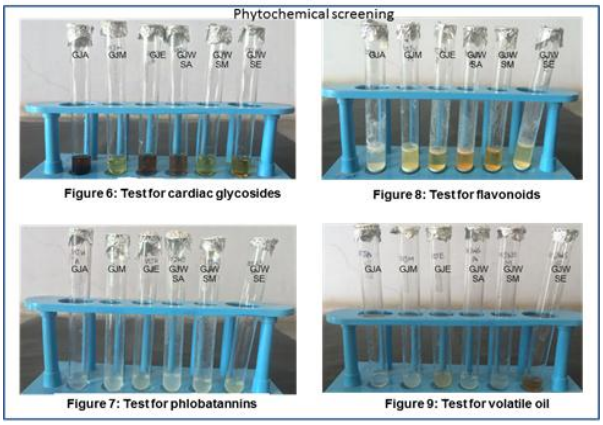

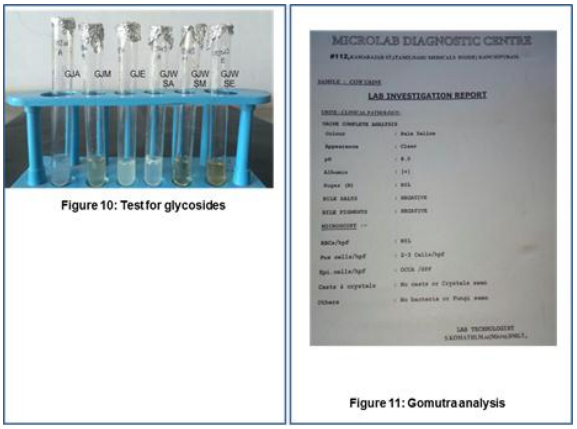

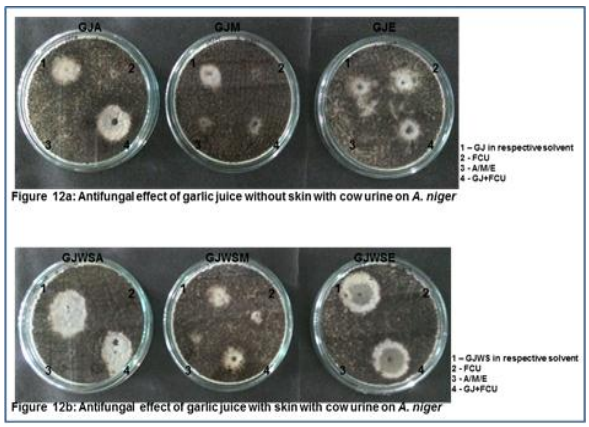

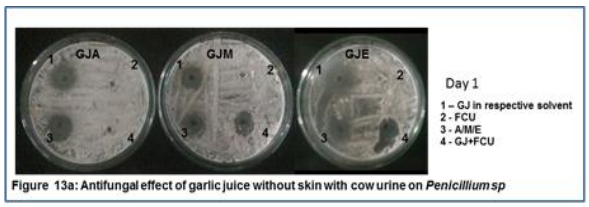

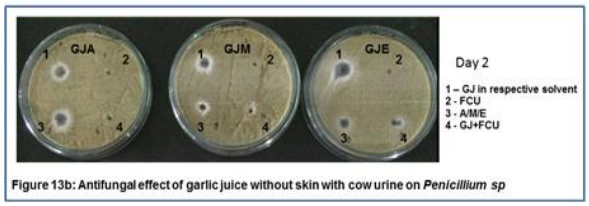

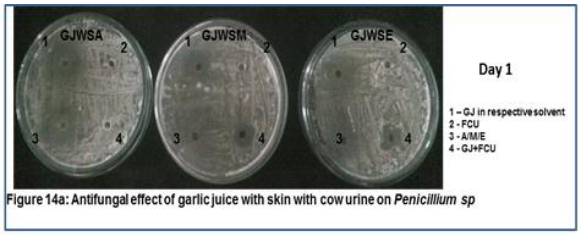

Figure 14a: Antifungal effect of garlic juice with skin with cow urine on *Penicillium sp*

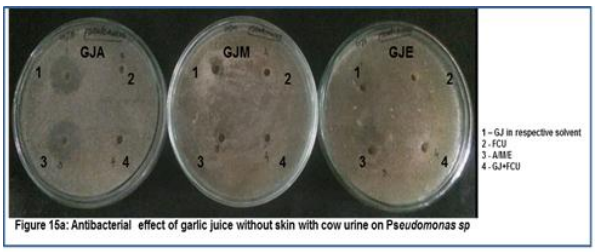

Figure 15a: Antibacterial effect of garlic juice without skin with cow urine on P*seudomonas sp*

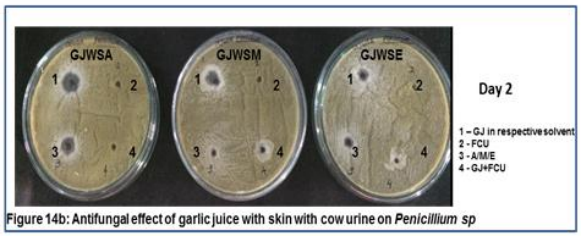

Figure 14b: Antifungal effect of garlic juice with skin with cow urine on *Penicillium sp*

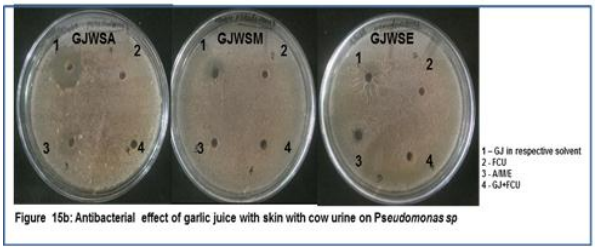

Figure 15b: Antibacterial effect of garlic juice with skin with cow urine on P*seudomonas sp*

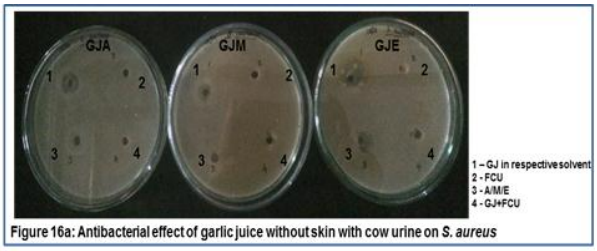

Figure 16a: Antibacterial effect of garlic juice without skin with cow urine on *S. aureus*

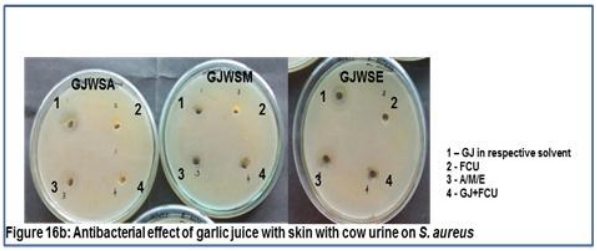

Figure 16b: Antibacterial effect of garlic juice with skin with cow urine on *S. aureus*

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Table 2: Phytochemical analysis of *A. sativum* clove juice extract with different solvents.

S. No.	Chemical constituents	GJA	GJM	GJE	GJWSA	GJWSM	GJWSE
1	Phenols	-	-	-	-	-	-
2	Alkaloids	+	+	+	+	+	+
3	Steroids	-	+	+	-	+	+
4	Saponin	+	-	+	+	-	-
5	Cardiac glycosides	+	-	+	-	-	+
6	Phlobatannin	-	-	-	-	-	-
7	Flavanoids	-	+	+	+	+	+
8	Volatile oil	+	+	-	+	+	-
9	Glycosides	-	+	-	-	+	+

Note: + = Present; - = Absent

Table 3a: Anti fungal activity of *A. sativum* juice extract without skin.

Organism	Zone of Inhibition in mm								
	Aqueous extract			Methanolic extract			Ethanolic extract		
	GJA	FCU	GJA+FCU	GJM	FCU	GJM+FCU	GJE	FCU	GJE+FCU
A.niger	17	-	15	15	-	15	16	-	16
Penicillum spp	14	-	16	5	-	1	3	-	1

Table 3b: Antifungal activity of *A. sativum* juice extract with skin.

Organism	Zone of Inhibition in mm								
	Aqueous extract			Methanolic extract			Ethanolic extract		
	GJWSA	FCU	GJWSA +FCU	GJWSM	FCU	GJWSM +FCU	GJWSE	FCU	GJWSE +FCU
A.niger	18	-	16	16	-	14	3	-	1
Penicillum spp	17	-	16	3	-	1	4	-	1

Table 4a: Antibacterial activity of *A. sativum* juice extract without skin.

Organism	Zone of Inhibition in mm								
	Aqueous extract			Methanolic extract			Ethanolic extract		
	GJA	FCU	GJA+FCU	GJM	FCU	GJM+FCU	GJE	FCU	GJE+FCU
Pseudomonas sp	17	-	18	2	-	1	6	-	4
S. aureus	14	-	13	-	-	-	2	-	-

Table 4b: Antibacterial activity of *A. sativum* juice extract with skin.

Organism	Zone of Inhibition in mm								
	Aqueous extract			Methanolic extract			Ethanolic extract		
	GJWSA	FCU	GJWSA +FCU	GJWSM	FCU	GJWSM +FCU	GJWSE	FCU	GJWSE +FCU
Pseudomonas sp	16	-	12	4	-	2	-	-	-
S. aureus	16	-	14	-	-	-	3	-	2

Table 5a: FTIR analysis of aqueous garlic extract without skin (GJA)

Wave Number (cm-1)	Functional group
671.23	Aliphatic bromo compounds, C-Br stretch
933.55	Silicate ion
1045.42	Silicate ion
1128.36	Tertiary amine C-N stretch
1269.16	Aromatic ethers, aryl-O stretch
1419.61	Carbonate ion
1639.49	Alkenyl C=C stretch
2357.01	Carboxylic acid O-H stretch
2931.80	Carboxylic acid O-H stretch
3439.08	Amide N-H stretch

Table 5b: FTIR analysis of aqueous garlic extract with skin (GJWSA)

Wave Number (cm-1)	Functional group
632.65	Alcohol, OH out of plane bend
869.90	Carbonate ion
933.55	Silicate ion
1026.13	Silicate ion
1056.99	Silicate ion
1128.36	Tertiary amine C-N stretch
1280.73	Aromatic ethers, aryl-O stretch
1419.61	Carbonate ion
1456.26	Carbonate ion
1641.42	Alkenyl C=C stretch
1739.79	Alkyl carbonate
2135.20	Transition metal carbonyls/Alkynyl C+C stretch
2370.51	Carboxylic acid O-H stretch
2927.94	Carboxylic acid O-H stretch
3437.19	Amide N-H stretch

Table 5c: FTIR analysis of ethanolic garlic extract without skin (GJE)

Wave Number (cm-1)	Functional group
514.99	Aliphatic iodo compound, C-I stretch
592.15	Alcohol, OH out of plane bend
677.01	Aryl thioethers,Φ-S (C-S stretch)
871.82	Carbonate ion
929.69	Silicate ion
1053.13	Silicate ion
1257.59	Aromatic esters, aryl-O stretch/ Aromatic primary amine CN stretch
1409.96	Carbonate ion
1462.04	Carbonate ion
1527.62	Aromatic C=C bending
2079.26	Cyanide ion, thio cyanate and other related ion
2358.94	CO2
2933.73	Carboxylic acid O-H stretch
3441.01	Amine N-H stretch

Table 5d: FTIR analysis of ethanolic garlic extract with skin (GJWSE)

Wave Number (cm-1)	Functional group
522.71	Aliphatic iodo compound, C-I stretch
594.08	Alcohol, OH out of plane
873.75	Carbonate ion
931.62	Silicate ion
1053.13	Silicate ion
1124.50	Sulphate ion
1257.59	Aromatic esters, aryl-O stretch/ Aromatic primary amine CN stretch
1406.11	Carbonate ion
1458.18	Carbonate ion
1517.98	Aromatic C=C bending
1641.42	Aromatic C=C bending
1739.79	Aldehyde/alkyl carbonate
1836.23	Five membered ring anhydride
2362.80	CO2
2929.87	Carboxylic acid O-H stretch
3427.51	Amine N-H stretch

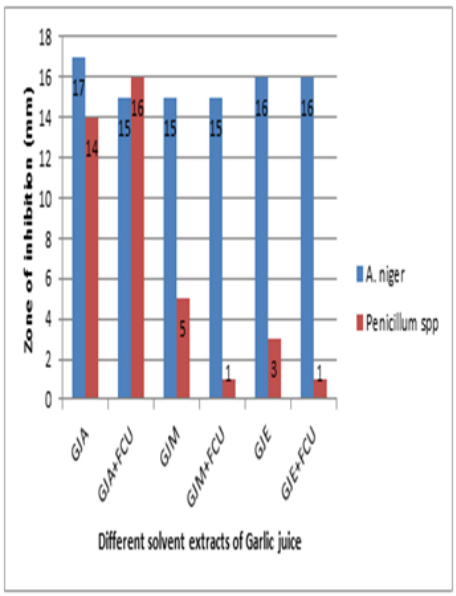
Table 5e: FTIR analysis of fresh cow urine (FCU)

Wave Number (cm-1)	Functional group
505.35	Aliphatic iodo compound, C-I stretch
580.57	Disulphide C=S stretch
1008.77	Phosphate ion
1359.82	Nitrate ion
1408.04	Ammonium ion
1463.97	Carbonate ion
1641.42	Aromatic C=C bending/Primary amine NH bend
2077.33	Cyanide ion, thio cyanate and other related ion/Transition metal carbonyls
2357.01	CO2
3520.09	Dimeric OH stretch

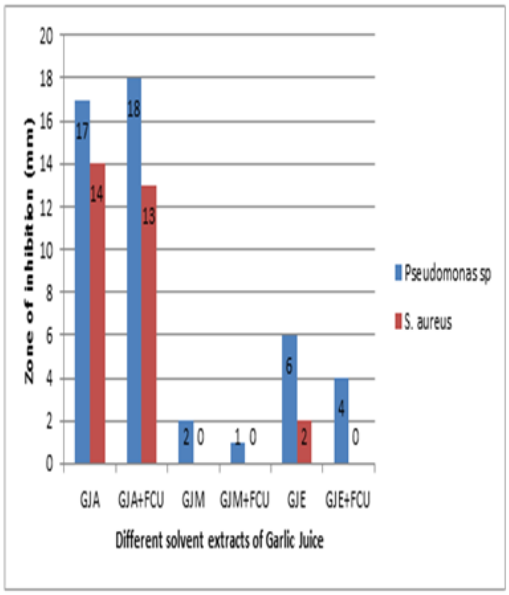
Table 6: Compound Identification by GCMS.

S.No	Retention time	Compound
1	5.19	1,2-ETHANEDIOL, MONOACETATE
2	8.29	ACETIC ACID, (ACETYLOXY)-
3	12.68	2-AMINO-OCTADEC-7-ENE-1,3-DIOL BUTANEBORONATE
4	13.33	PENTANOIC ACID, 2-(AMINOOXY)-
5	15.56	ALPHA.-D-GLUCOPYRANOSIDE, O-.ALPHA.-D-GLUCOPYRANOSYL-(1.FWDARW.
6	17.62	EICOSANOIC ACID
7	19.02	N-HEXADECANOIC ACID
8	20.68	OLEIC ACID
9	27.54	TETRACOSANOIC ACID, TRIMETHYLSILYL ESTER

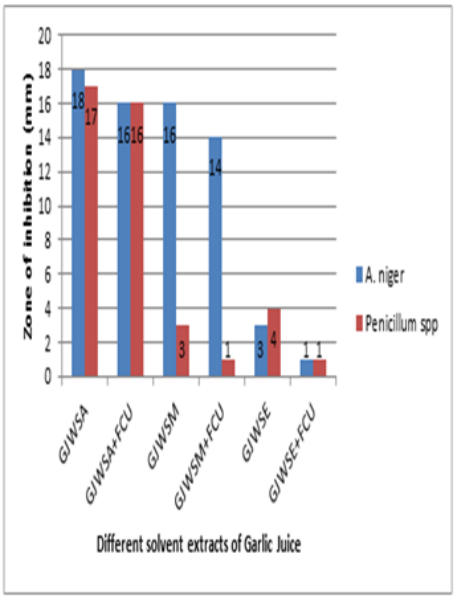
GRAPHS



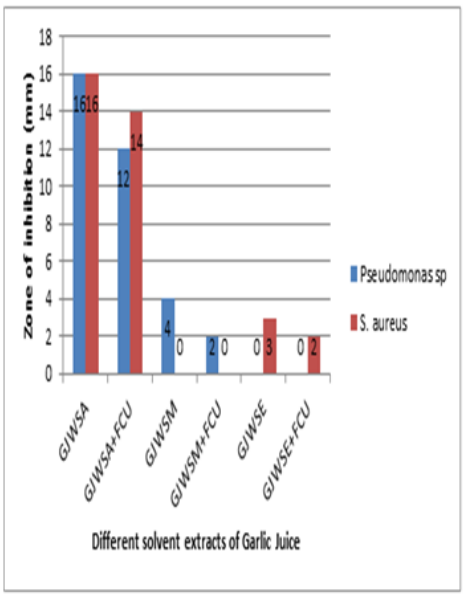
Graph 1: Antifungal activity of Garlic Juice without skin



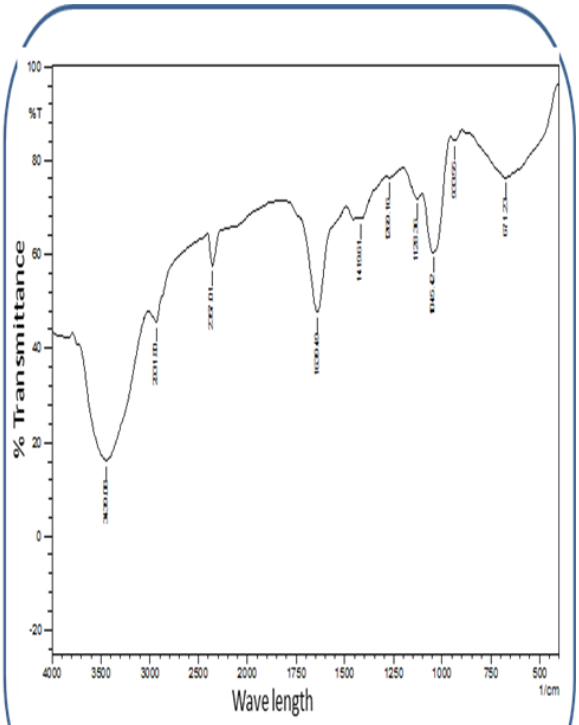
Graph 3: Antibacterial activity of Garlic Juice without skin



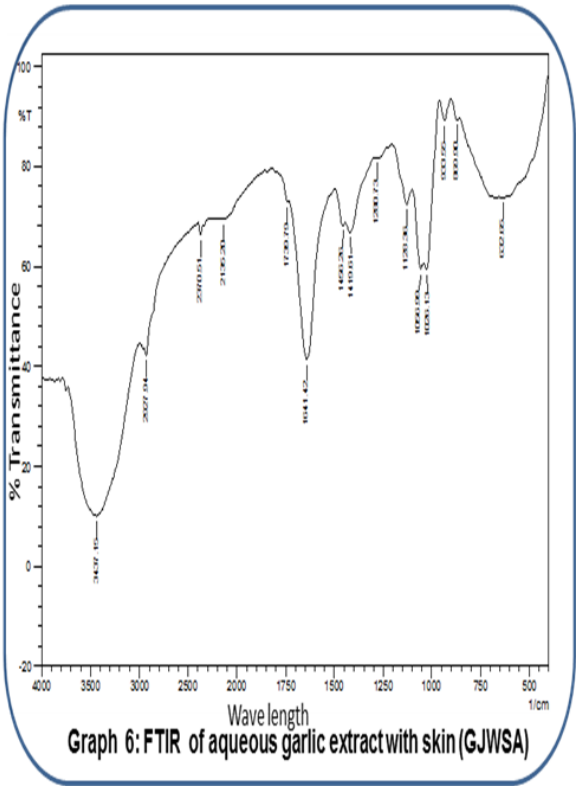
Graph 2: Antifungal activity of Garlic Juice with skin



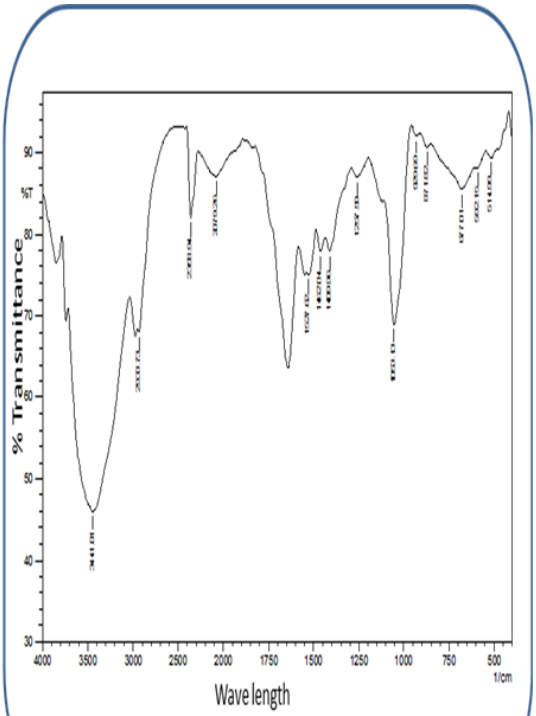
Graph 4: Antibacterial activity of Garlic Juice with skin



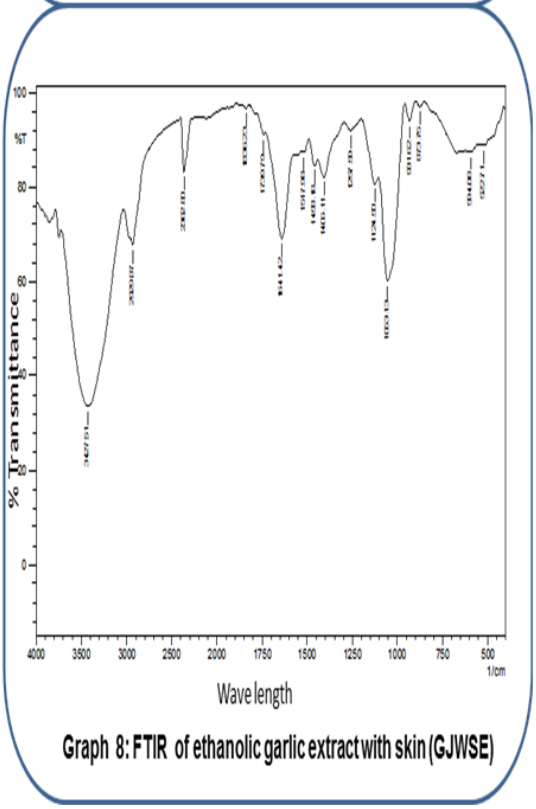
Graph 5: FTIR of aqueous garlic extract without skin (GJA)



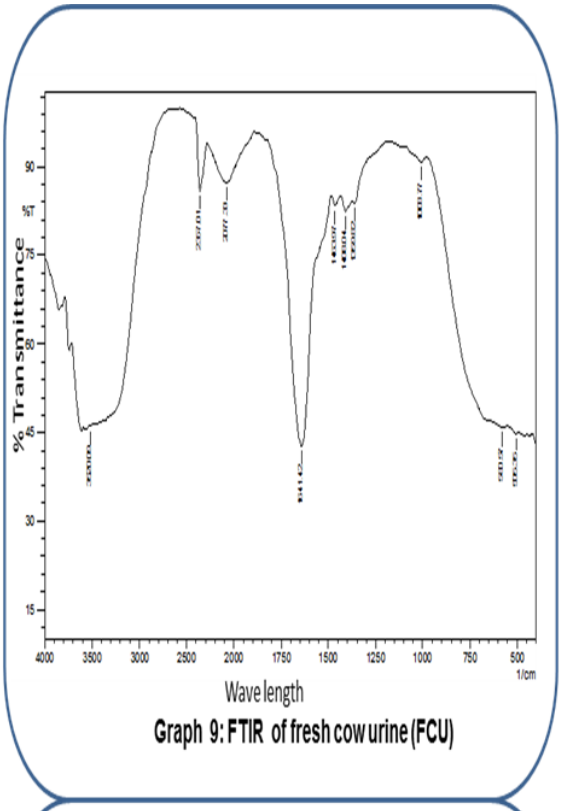
Graph 6: FTIR of aqueous garlic extract with skin (GJWSA)



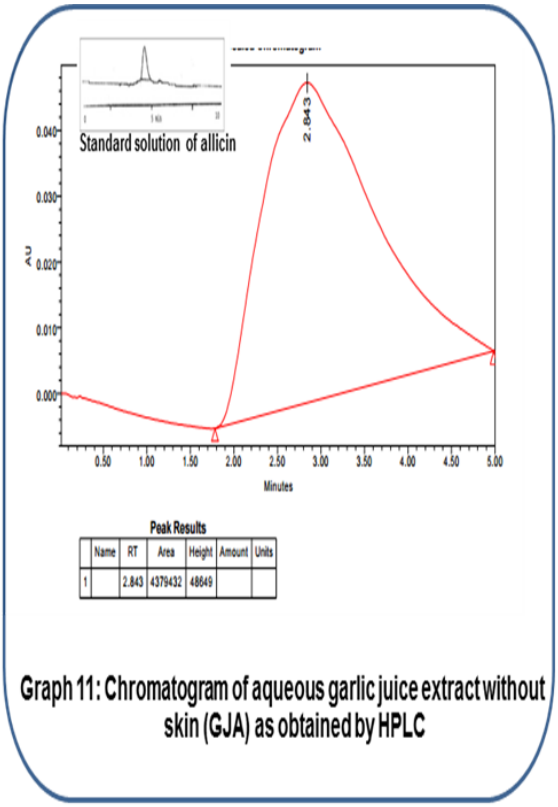
Graph 7: FTIR of ethanolic garlic extract without skin (GJE)



Graph 8: FTIR of ethanolic garlic extract with skin (GJWSE)

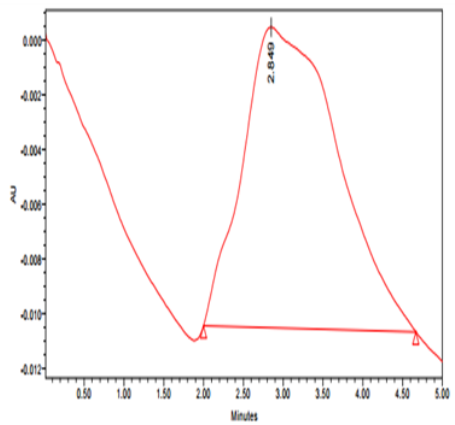


Graph 9: FTIR of fresh cow urine (FCU)



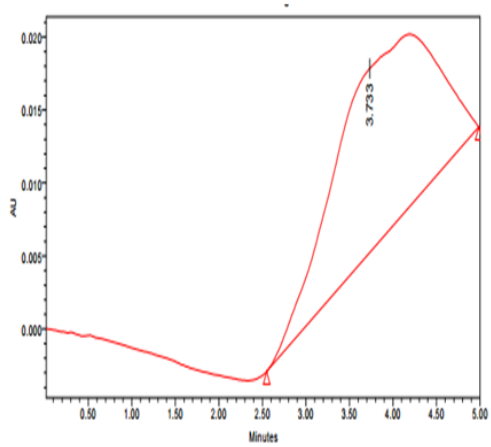
Peak Results						
	Name	RT	Area	Height	Amount	Units
1		2.843	4379432	48649		

Graph 11: Chromatogram of aqueous garlic juice extract without skin (GJA) as obtained by HPLC



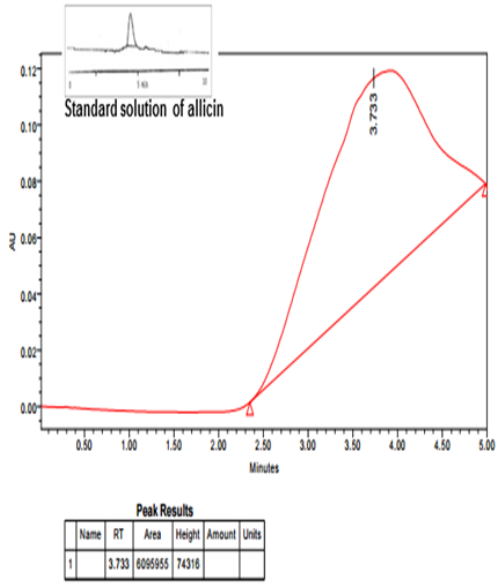
Peak Results						
	Name	RT	Area	Height	Amount	Units
1		2.849	916776	10987		

Graph 10: Chromatogram of Fresh Cow Urine (FCU) as obtained by HPLC



Peak Results						
	Name	RT	Area	Height	Amount	Units
1		3.733	1054904	12610		

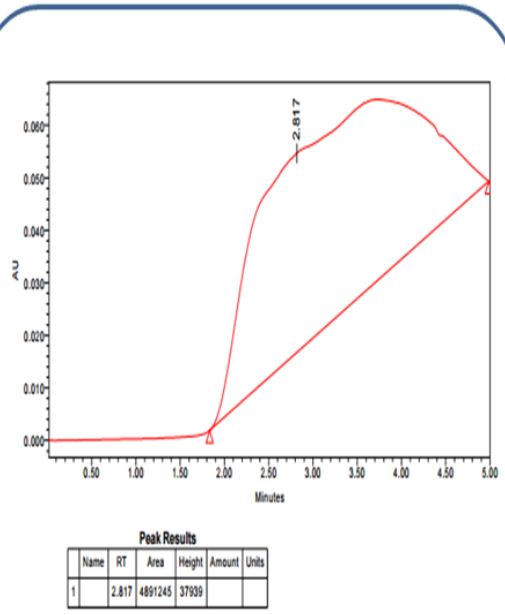
Graph 12: Chromatogram of aqueous garlic juice extract with skin (GJWSA) as obtained by HPLC



Peak Results

	Name	RT	Area	Height	Amount	Units
1		3.733	6095955	74316		

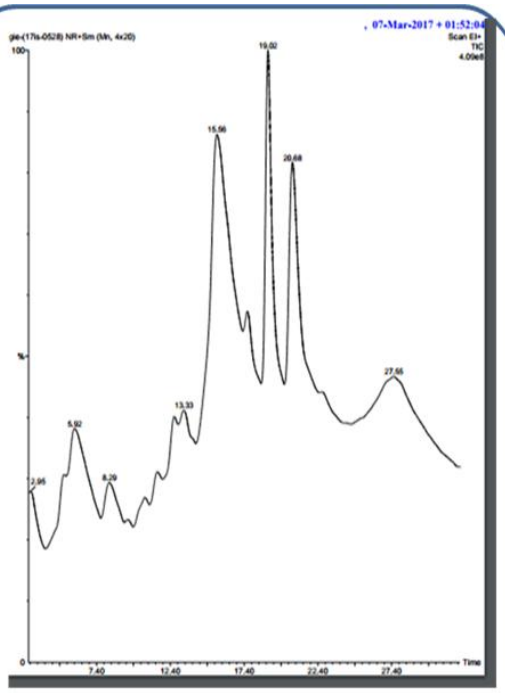
Graph 13: Chromatogram of ethanolic garlic juice extract without skin (GJE) as obtained by HPLC



Peak Results

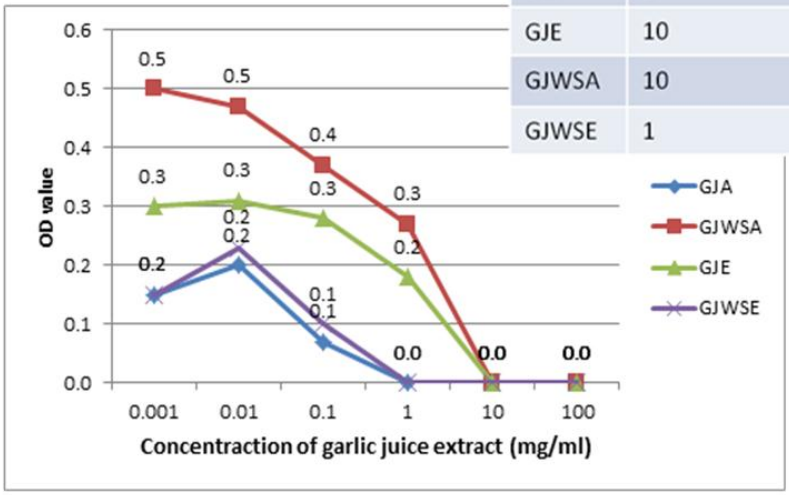
	Name	RT	Area	Height	Amount	Units
1		2.817	4891245	37939		

Graph 14: Chromatogram of ethanolic garlic juice extract with skin (GJWSE) as obtained by HPLC



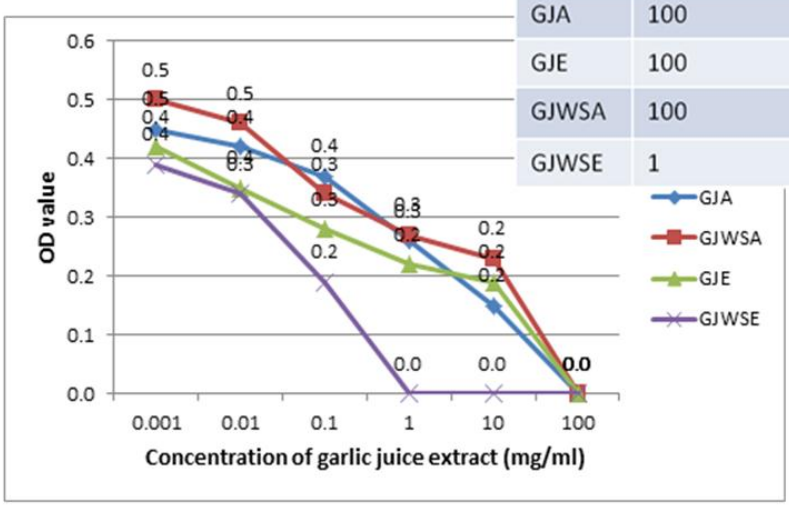
Graph 15: Chromatogram of GJE as obtained by GCMS

Sample	MIC (mg/ml)
GJA	1
GJE	10
GJWSA	10
GJWSE	1



Graph 16: MIC of different solvent extracts of garlic against *Pseudomonas sp*

Sample	MIC (mg/ml)
GJA	100
GJE	100
GJWSA	100
GJWSE	1



Graph 17: MIC of different solvent extracts of garlic against *S. aureus*

DISCUSSION

A medicinal plant is any plant which contains substances that can be used for therapeutic purpose of which are precursors for the synthesis of useful drugs. The Phytochemical screening of *A. sativum* extract in this study revealed the presence of phenols, alkaloids, flavonoids, glycosides, saponins, volatile oil, cardiac glycosides, phlobatannins, and steroids.

The presence of some of these bioactive components confirms with similar research.[17] The presence of alkaloids, flavonoids, saponin, and cardiac glycosides is in agreement with the work done by other researchers.[18] These classes of compounds especially alkaloids, saponins and flavonoids are known to have curative activity against several pathogens.[19] Saponins are produced by plants to fight against infections by parasites and in humans it helps the immune system and also protect against viruses and bacteria. It has been found out that saponins possess cholesterol lowering, cytotoxic permeabilization of the intestine and also exhibit structure dependent biological activities.[20]

Flavonoids are water soluble polyphenolic molecules which in together with carotenes are also responsible for the coloring of fruits, vegetables and herbs. Some flavonoid containing plants have antimicrobial properties.[21] Flavonoids detected in *A. sativum* bulbs could be used for the treatment of various disease conditions like edema, toothache, fever, common cold, diarrhea and dental caries. These could be possible as the root extracts contains some antibacterial activities. The flavonoids act on bacteria by inhibiting its protein synthesis. [22]

The morbidity and mortality due to microbial diseases have drastically decreased after the development of effective and safe drugs to deal with bacterial infections. Unfortunately, the emergence of drug-resistant organisms has become a serious threat.

The present study has demonstrated that *A. sativum* juice extract effectively inhibited the growth of both *Pseudomonas sp* and *S. aureus* though their sensitivity to the extract varied based on the presence or absence of skin and the different solvents used for extraction. The MIC values were found to be in the range of 1 to 10 mg/ml in case of *Pseudomonas sp* and 1 to 100 mg/ml in the case of *S. aureus* (Graph 16 and 17).

In previous studies, it has been reported that a component named allicin that is present in *A. sativum* exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action.[23] The structural differences of the bacterial strains may also play a role in the bacterial susceptibility to *A. sativum* constituents. The HPLC analysis of the various solvent extracts of *A. sativum* confirmed the presence of allicin.

The present study showed that *A. sativum* extracts also have antifungal activity which is in accordance with.[24] It is clear that *A. sativum* juice may be useful as an antimicrobial agent

against the *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*.[25] The present study suggests that *A. sativum* is active against organisms that cause wound infection. It has been previously reported that *A. sativum* is active against organisms that are found to be resistant to conventional antibiotics.[26, 27] Moreover, studies also indicate that combination of *A. sativum* extracts with conventional antimicrobials leads to partial or total synergism.[28,29]

The susceptibility of some bacterial strains to the extract *A. sativum* may be a pointer to its potential as a drug that can be used against these susceptible bacterial strains. Furthermore, the search for alternative drugs or natural antibacterial remedies is important due to antibacterial resistance, especially, among Gram-negative bacteria.[30]

The antibacterial activity of aqueous, ethanolic and methanolic extracts of *A. sativum* was determined using *Pseudomonas sp* and *S. aureus*. From the result of the zone of inhibition in the microbial study, it was seen that all the extracts demonstrated antibacterial activity. The aqueous extract demonstrated the higher activity followed by ethanolic and methanol extracts.

Findings from this work reveal that FCU doesn't show any antimicrobial activity. Also the combination of *A. sativum* juice with FCU didn't show any synergistic effect. This study also reveals that *A. sativum* had both antibacterial and antifungal activity. This indicates that the extracts could also be used in the treatment of some wound infections caused by gram negative and gram positive bacteria.

Further research should be conducted to impregnate the bandages with active compounds of *A. sativum* and analyze whether it is active against infections in a real wound setting.

CONCLUSION

The phytochemical screening and investigation into the antibacterial potential of the extract of *A. sativum* showed or highlighted the antibiotic spectra of the *A. sativum* extract under assay, suggesting a promising lead as an alternative antibiotic and it yielded scientific support to their use in traditional ayurvedic medicine. The *A. sativum* extract was found to exhibit slight synergistic activity with gomutra against some tested organism.

From the entire experiment, it can be concluded that *A. sativum* juice extract with skin have the potential natural antibacterial which cause wound infection and anti fungal compounds.

The activity was influenced by gomutra and it was more effective in aqueous extract. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will always need to be considered. Hence I conclude that further research should be carried out on the dosage, in vivo evaluation of the garlic extracts in bandages against infections in a real wound setting.

ACKNOWLEDGEMENT

The authors are grateful to all those who have helped in completion of this project on time and the authors / editors / publishers of all articles, journals and books from where the literature for this article has been reviewed and discussed.

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