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Studies on Antimicrobial and Phytochemical properties of Indigenous Indian Plants against Common Food Spoilage Microorganisms

Muhammad Abdullahi Sa'id and Er. Robinka Khajuria

Department of Microbiology and Bioprocess Engineering, School of Biosciences and Biotechnology, Lovely Professional University, Jalandhar-Delhi G.T. Road, National Highway 1, Phagwara, Punjab 144411, India.

ABSTRACT
The success of food quality control lies in the continuous search for new antimicrobial agents to counter the challenge posed by resistant microorganisms. Methanol, ethanol, and hot-water extracts of five medicinal spices [Allium sativum (garlic), Syzygium aromaticum (clove), Trachyspermum ammi (ajwain), Cuminum cyminum (cumin) & Trigonella foenum graecum (Methi)] traditionally used in India house-hold as flavor stimulants were investigated for in vitro antimicrobial activity against food borne pathogens namely Escherichia coli, Staphylococcus aureus, Bacillus subtilus, Klebsiella pneumoniae, Enterobacter spp., Saccharomyces cerevisiae, Rhodotorula spp., Aspergillus niger, Penicillium notatum, by disc diffusion method. Methanol-ethanol extracts of Syzygium aromaticum and Trachyspermum ammi showed the highest antimicrobial activity (25-48mm & 25-47mm respectively) against all the tested bacterial and fungal isolates, while Trigonella foenum graecum extracts revealed the least activity (0.0-8mm). Minimum inhibitory concentration (MIC) assay were determine for these four extracts against all the tested organisms. Methanol- clove revealed the highest antimicrobial activity at a minimum concentration of (10-20µg/ml) against all the tested microorganisms, followed by methanol-ajwain at concentration of (20µg/ml) against all the experimental organisms except Klebsiella pneumonia. The phytochemical analysis carried out revealed the presences of Tannins, Saponins, Flavornoids, Terponoids, Glycosides, Alkaloids, Phenolics which are responsible for the antimicrobial activity. The results of these experiments, provides justification for the use of spices in preservation of food stuffs.

Keywords: Antimicrobial activity, Phytochemical analysis, *E. coli, S. aureus, B. subtilus, K. pneumoniae, Enterobacter, S. cerevisiae, Rhodotorula, A. niger, P. notatum* .

INTRODUCTION

Food spoilage refers to undesirable changes occurring in food due to the influence of air, heat, light, moisture, which foster the growth of microorganisms. Many food products are perishable by nature and require protection from spoilage by bacteria, yeast, mold, enzymes and insects during their preparation, storage, and distribution to give them desired shelf life. Foods take different period of time to lose their natural form though spoilage, and on the context of preservation foods are classified as perishable (meat, fish, milk fruits and some vegetable), semi perishable (eggs, onions, potatoes, carrot, beans) and non-perishable (cereals, pulse nuts) (Nychas, 1995).

Most commonly followed practice for the prevention of food spoilage and maintaining food shelf life involves the use of chemical preservatives such calcium propionate, sodium nitrate, sodium nitrite, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite) and disodium EDTA (Sudheer*et al*., 2007).

Altough they tend to prevent food spoilage but these chemicals have been reported to exhibit residual toxicity, carcinogenic and teratogenic attributes (example sodium and potassium nitrate act as source of nitrite and thus promoting microbial growth). The past few decades have seen a mammoth rise in the manufacturing and consumption of preserved, ready to eat, consumables. Thus, increasing the average per individual consumption of these chemicals. This exponential growth in the food industry has again shifted the focus of research to the harmful effect of chemical preservatives added in food items and the exploration of naturally occurring antimicrobial substances which can replace chemical preservants. (Nychas, 1995).

Plant and plant products have been used for centuries as source of medicine against human diseases, as well a source for the preservation and extension of foods shelf life (Chattopadhyay and Bhattacharyya, 2007). The medicinal and antimicrobial value of the plants lies in some chemical substances that produce a definite physiological action on human body and targeted microbes. The most important of these bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga, 2005). Thus giving impetus to the concept of use of such medicinal plants as an alternate source of food preservants. (Narender, 2012).
The acceptance of traditional medicine as efficacious and safe alternative of health care by World Health Organization (WHO) in 1985 has gave impetus to the research related to antimicrobial activity of medicinal plants (Purshotam et al, 2011). Furthermore, many of the medicinal plants are rich source of antioxidants such as vitamin A, vitamin c, and vitamin E which prevent free radical damage, reducing risk of chronic diseases and also are already in used as flavoring to improve test in food (Jeanroy et al, 2013).

In this work, five Indian condiments, viz; *Allium sativum* (Garlic), *Syzygium aromaticum* (Clove), *Trachyspermum ammi* (Ajwain/Carom), *Cuminum cyminum* (Cumin) and *Trigonella foenum graecum* (Fenugreek/Methi) were investigated for their antimicrobial properties against *Escherichia coli, Staphylococcus aureus, Bacillus subtilus, Klebsiella pneumonia, Enterobacter, Aspergillus niger, Penicillium notatum, Saccharomyces cerevisiae and Rhodotorula*. Furthermore, the phytochemical profile, minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts ware examined.

MATERIALS AND METHOD

2.1 Sample collection and Identification

Bulbs of Syzygium aromaticum (clove) and bulbs of *Allium sativum (*garlic*)* were obtain from local sellers of herbal products in Phagwara city, while shade dried seeds of *Cuminum cyminum (*cumin*)*, *Trachyspermum ammi* (ajwain) and *Trigonella foenum graecum* (methi) were procured from local market of

Jalandhar city all within Punjab state of India. The plants were taken to the school of Biosciences-Department of Biotechnology Lovely Professional University, Punjab for identification.

2.2 Collection and Maintenance of test Organisms

The test organisms including *Escherichia coli, Staphylococcus aureus, Bacillus subtilus, Klebsiella pneumonia, Enterobacter, Aspergillus niger, Penicillium notatum, Saccharomyces cerevisiae and Rhodotorula* were obtained from School of Biotechnology and Biosciences Lovely Professional University, Punjab and the cultures were inoculated in Nutrient Broth (NB), Nutrient Agar slant, and incubated at 37^0C for 12hours. The slants and broth were stored at 4^0C and maintained in active stage by regular sub-culturing for further use.

2.3 Sample preparation

Clove and garlic bulbs were peeled, washed with sterile distilled water and then dried at room temperature for three weeks. The remaining moisture was removed by incubating in air-drier maintained at 40°C for 24hours. The dried seeds were pulverized into fine powder using an electric blender.

2.4 Preparation of Extracts

2.4.1 Aqueous (Hot water)

Extraction was carried out according to modified techniques of Keb-Llanes et al. (2002) and Victor et al. (2012). 20g air-dried powder of the five plants ware dissolved in 200ml distilled water respectively, boiled in water bath till one fourth of the extract was left after evaporation. The solution was filtered using muslin cloth and centrifuged at 5000 rpm for 15minutes, re-filtered using Whatman Filter No. 1 under aseptic conditions and then filtrate was collected in fresh sterilized bottles and stored at 4°C until further use.

2.4.2 Methanol Extraction

48g air-dried powders of the five plants were introduced into five hundred milliliter flasks separately and 200ml of 80% methanol was added to each. The mixtures were agitated overnight in a multifunctional oscillator at 120rpm after which the supernatants were separated from the residue by decanting and filtered through muslin cloth and then re-filtered by passing through Whatman filter NO.1. The samples were evaporated to remove traces of the extraction solvent with the aid of a rotary evaporator at 30°C and evaporated to dryness using an electric thermostatic drying oven at 40°C. The weight of the dry mass were determined and used to calculate the concentration of the extracts in each solution in mg/ml. while Stock solutions were prepared by dissolving 1g of the dried extracts in 1ml sterile distilled water to obtain a concentration of 10^3mg/ml and stored at 4°C in sterilized bottles until further use (Keb-Llanes et al., 2002).

2.4.3 Ethanol Extraction

48g air-dried powders of the five plants were introduced into five hundred milliliter flasks separately and 200ml of 80% ethanol was added to each. The mixtures were agitated overnight in a multifunctional oscillator at 120rpm after which the supernatants were separated from the residue by decanting and filtered

through muslin cloth and then re-filtered by passing through Whatman filter NO.1. The samples were then evaporated to remove traces of the extraction solvent with the aid of a rotary evaporator at 40°C and evaporated to dryness in a 100ml beaker using an electric thermostatic drying oven at 45°C. The weight of the dry mass were determined and used to calculate the concentration of the extracts in each solution in mg/ml, while Stock solutions were prepared by mixing well the appropriate amount of dried extracts with sterile distilled water to obtain different concentrations and stored at 4°C in sterilized bottles until further use (Victor et al., 2012).

2.5 Preparation of inoculums

Active cultures for experiments were prepared from the stock cultures maintained at 4°C on slopes of nutrient agar and potato dextrose agar by transferring a loopful of cells to 50ml prepared Nutrient broth (NB) for bacteria and Potato dextrose broth (PDB) for fungi. The cultures were incubated without agitation for 24hours at 37°C and 27°C respectively and allowed to reach a turbidity equal to that of the standard 0.5 McFarland solution at 600nm which is equivalent to 106–108 CFU/ml (Sham, 2010).

2.6 Antimicrobial Sensitivity Assay

2.6.1 Disc diffusion method (Primary screening)

A modified protocol of Kirby-Bauer as described by Sham (2010) was adapted for the disc diffusion assay for primary screening of i*n vitro* antimicrobial activity of the crude extracts using Nutrient Agar (NA). The plates were prepared by pouring 15ml of molten media into sterile petriplates, allowed to solidify for 5minutes. 0.1ml of inoculum suspension was swabbed uniformly on the surface and left to dry for 5minutes. The same procedure was followed for the fungi using Potato dextrose agar (PDA). 0.01ml of (10^3µg/ml) concentrations of the extracts were loaded on 5mm sterile individual discs formed. The loaded discs were then placed on the surface of medium and the compound was allowed to diffuse for 5minutes before incubating at 37°C for 24hours and 27°C for 72hours for Bacteria and Fungi respectively. Negative control was prepared using respective solvent (Methanol & Ethanol), while Penicillin, Methicillin, Norfloxacin discs ware used as positive control for bacteria and fluconazole for Fungi. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeter. All assays were performed in duplicates.

2.6.2 Minimum Inhibitory Concentration (MIC) Assay

The Minimum Inhibitory Concentration (MIC) Assay was applied on extracts that proved their high efficacy against test organisms by the disk diffusion method; are methanol and ethanol extracts of both *Syzygium aromaticum* (clove) and *Trachyspermum ammi* (ajwain). The MIC was determined by monitoring growth of test organisms in a 96 well microtiter plate and ELISA reader. Seven different concentrations of the selected plant extracts were prepared (40, 30, 20, 10, 5.0, 2.5 and 1.25µg/ml) using sterile Mueller Hinton Broth (MHB) medium as a diluent. 100µl of an individual test organism and 100µl of different concentrated solutions of plant extracts-MHB were loaded into the 96-well microtiter plate separately, two

wells ware maintained as positive and negative control in which antibiotic/antifungal and extraction solvents (methanol/ethanol) were added respectively.

Blank solution containing broth medium only without extract, no test organisms was prepared in another well, incubated at 37^0C for 24hours and amount of growth was measured by reading optical density at 620 nm in a microtiter plate reader. 24 hours old culture of the test organisms were used throughout and highest dilution of the plant extract that retained its inhibitory effect resulting in absence of turbidity caused by microorganism was recorded as the MIC value of the extract.

2.6.3 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Assay

The minimum bactericidal/fungicidal concentration (MBC/MFC) is the lowest concentration of antimicrobial agent that reduces the viability of the initial microbial inoculums by ≥99.9%; it is an important characteristic of an antimicrobial compound showing if it is bactericidal or bacteristatic. It was determined by sub-culturing 100µl of the broth dilution with minimum inhibitory activity from Minimum Inhibitory Concentration (MIC) test on fresh extract free Nutrient Agar and Potato Dextrose Agar plates, incubated at 37^oc (for bacteria) and 27^oc (for fungi) for 24hours and 72hours respectively to enumerate the colony-forming unit recovered as a measure of microbial viability (Maryam et al, 2011).

2.7 Phytochemical Screening

Phytochemical screening for the presence of essential bioactive chemical constituents the five medicinal plants under study were carried using a combine modified standard procedures as described by Harborne (1973), Trease and Evans (1989), Sofowara (1993) and Sanjeet (2013).

2.7.1 Tannins

0.15ml of the extracts was dissolved in 10ml of distilled water and heated for 10 minutes, the mixture was allowed to cool and then few drops of 1% ferric chloride were added. Change of colour from yellow to green and dark green precipitate indicates a positive result.

2.7.2 Saponins

0.5ml of extract dissolved in 0.1ml of Ethyl acetate, heated for 2minutes to remove Ethyl acetate and distilled water was added to the mixture and shaken vigorously. Formation of persistent foam which lasted for at least 15 minutes indicates a positive result.

2.7.3 Flavonoids

0.2ml of extract was dissolved in10% NaOH, shaken gently and then few drops of HCl were added. Turning of yellow colour to colourless indicates a positive result.

2.7.4 Terpenoids

0.5ml of extract was mixed with 200µl chloroform, and then few drops of sulphuric acid were added to the mixture. Appearance of a reddish brown interface indicates a positive result.

2.7.5 Glycosides

0.1ml of extract was dissolved in 0.4ml acetic anhydride, cooled in ice for 3minutes and then few drops of sulphuric acid ware added. Change in colour from violet to blue to green indicates a positive result.

2.7.6 Alkaloids

0.5ml of extract was dissolved in 5ml of 1% aqueous HCl, heated for 2minutes in water bath and then 1 ml was taken from the mixture into a separate test tube. To the tube, few drops of Dragendorff's reagent were added and occurrence of orange-red precipitate indicates a positive result.

2.7.8 Phenolic Compounds

0.5ml of crude extract was dissolved in 95% ethanol and then few drops of neutral ferric chloride solution were added. The occurrence of black colour indicates a positive result.

RESULTS AND DISCUSSION

3.1 **Antimicrobial Assay (Primary Screening)**

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne and spoilage microorganisms. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavour (Nevas *et al.,* 2004, Souza *et al.,* 2005).

In other to ascertain the antimicrobial activity of all the fifteen extracts viz Methanol-Garlic, Methanol-Clove, Methanol-Cumin, Methanol-Ajwain, Methanol-Methi, Ethanol-Garlic, Ethanol-Clove, Ethanol-Cumin, Ethanol-Ajwain, Ethanol-Methi, Water-Garlic, Water-Clove, Water-Cumin, Water-Ajwain, and Water-Methi, the antimicrobial properties were tested against *Escherichia coli; Staphylococcus aureus, Bacillus subtilus, Klebsiella pneumonia, Enterobacter spp., Saccharomyces cerevisiae, Rhodotorula spp., Aspergillus niger, Penicillium notatum,* by disc diffusion method of antimicrobial assay. The results obtained are presented below.

3.1.1 Disc Diffusion (*Escherichia coli*)

It was observed that all the extracts except *Trigonella foenum graecum* (Fenugreek/Methi) have inhibitory effect against *Escherichia coli.* Ethanol-Ajwain revealed the highest zone of inhibition (21mm), followed by Ethanol-Clove (16mm). The results substantiates findings of Sulieman *et al*. (2007) who demonstrated the antibacterial activity of clove ethanolic extract against *E. coli, S. aureus* and *B. subtilis* and correlates the activity with high tannin content, eugenol (2 methoxy-4 allyl-phenol) and other bioactive components presence in the plant. Eugenol increases the permeability of cell wall there by allowing free movement of substances in and out of the cells, cause extensive lesion of cell membrane and reduction in the quantity of ergosterol, a specific fungal cell membrane component. The inhibitory actions of different extracts against *E. coli* have been presented in Table1 and Fig1 (a/b/c).

Table1. Primary Screening for *E.coli*

Crude Extract (10^3µg/ml)	Millimeter (mm) Sensitivity Zone	+ve Control (10 units)	-ve Control (80%)
Methanol-Garlic	10±0.50	8±0.00	6±0.00
Methanol-Clove	13±0.43	11±1.20	NS
Methanol-Cumin	6.5±1.0	8±0.00	NS
Methanol-Ajwain	20 ±0.50	9.5±1.0	NS
Methanol-Methi	NS	NS	NS
Ethanol-Garlic	13±0.55	10±1.0	6.5±0.30
Ethanol-Clove	16±0.50	9.51.0	7.5±0.50
Ethanol-Cumin	NS	15±1.0	8±0.00
Ethanol-Ajwain	21±0.00	12±0.50	6±0.00
Ethanol-Methi	NS	NS	NS
Water-Garlic	7±0.33	9±0.50	NS
Water-Clove	12±0.50	9±1.0	NS
Water-Cumin	6±0.50	6.5±0.50	NS
Water-Ajwain	8±0.88	10±1.0	NS
Water-Methi	NS	NS	NS

	F-Ratio	CD (5%)
Zone of Inhibition	702.74	0.424773
Extracts	2397.97	0.189964
Zone of Inhibition X Extracts	245.97	0.735728

Key: Penicillin 10mg/ml (+ve control), Methanol & Ethanol (-ve control), NS (Not significant)

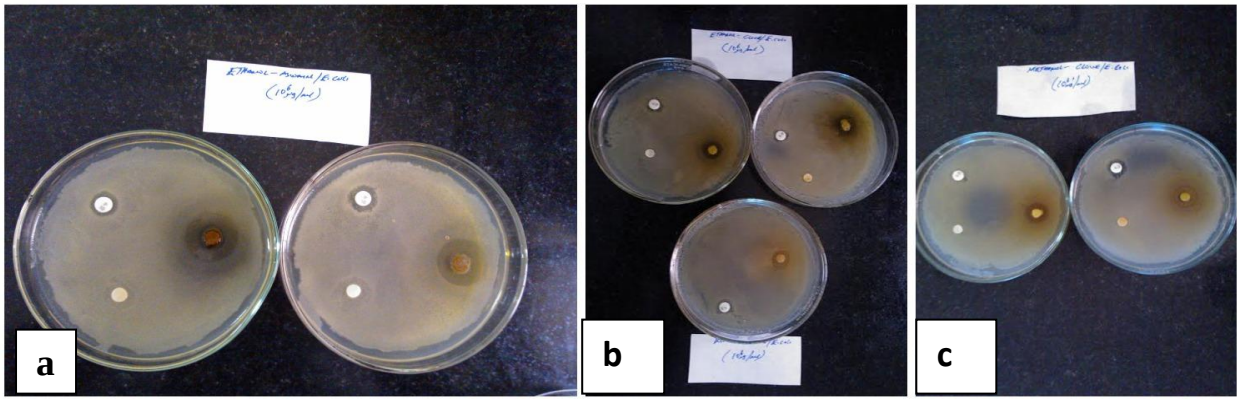

Fig.1 Antimicrobial activity of a) Ethanol-Ajwain, b) Methanol-Clove and c) Ethanol-Garlic extracts aginst E. coli.

3.1.2 Disc Diffusion (*Staphylococcus aureus*)

It was observed that all the extracts except *Trigonella foenum graecum* (Fenugreek/Methi) and *Cuminum cyminum* (cumin) have inhibitory effect against *Staphylococcus aureus.* Similarly Ethanol-Ajwain revealed the highest zone of inhibition (25mm), followed by Methanol-Clove (20mm). Zeinab et al (2014) report the antimicrobial activity of *Trachyspermum ammi (*ajwain*)* and attributed it with the presence of high phenolic compounds such as thymol, carvacrol/p-cymene and the mechanism of action involved damage in

membrane integrity with change in ph hemostasis. Similarly the antimicrobial activity of thyme and carvacrol have been studied by various researchers and reported to be a germicide, antispasmodic, and antifungal agent (Masih et al, 2012). Results are presented in Table2 and Fig2 (a/b/c)

Table2. Primary Screening for *Staphylococcus aureus*

Crude Extract (10^3µg/ml)	Millimeter (mm)		
	Sensitivity Zone	+ve Control (5 units)	-ve Control (80%)
Methanol-Garlic	9.5±0.55	NS	6.5±0.00
Methanol-Clove	19.5±1.20	NS	6.5±0.00
Methanol-Cumin	NS	NS	7±0.00
Methanol-Ajwain	18±1.0	NS	7±0.00
Methanol-Methi	NS	NS	6.5±0.00
Ethanol-Garlic	18.5±0.30	NS	7±0.50
Ethanol-Clove	20±0.00	NS	7±1.0
Ethanol-Cumin	8±0.00	NS	7.5±0.00
Ethanol-Ajwain	25±0.00	NS	7.5±0.00
Ethanol-Methi	NS	NS	NS
Water-Garlic	NS	NS	NS
Water-Clove	8±0.50	NS	NS
Water-Cumin	6.5±0.50	NS	7±0.00
Water-Ajwain	7±1.0	NS	NS
Water-Methi	NS	NS	NS

	F-Ratio	CD (5%)
Zone of Inhibition	586.87	0.418994
Extracts	4430.58	0 .187380
Zone of Inhibition X Extracts	391.48	0.725718

Key: Methicillin 5mg/ml (+ve control), Methanol & Ethanol (-ve control), NS (Not significant)

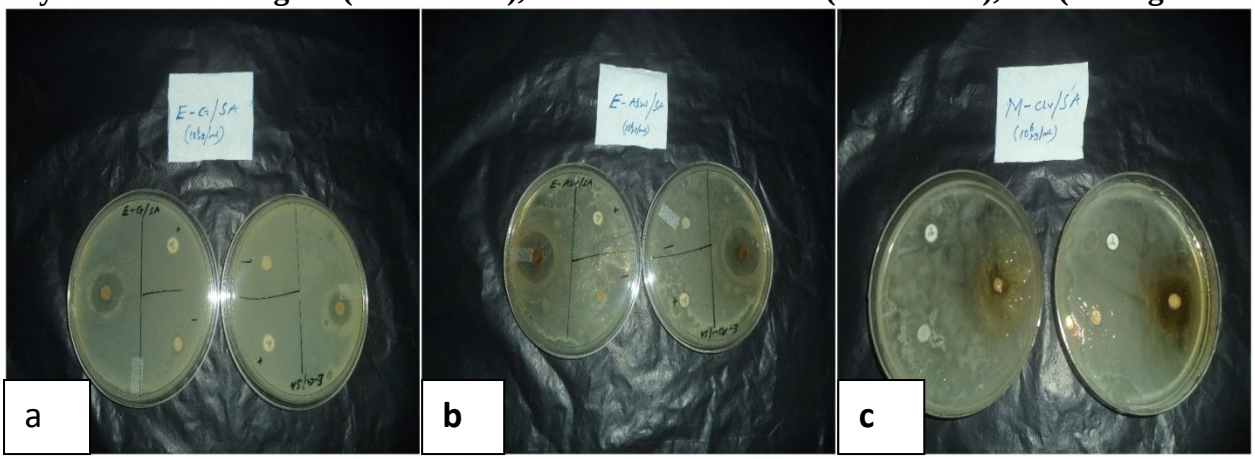

Fig.2 Antimicrobial activity of a) Methanol-Garlic, b) Ethanol-Ajwain and c) Methanol-Clove extracts aginst *S. aureus.*

3.1.3 Disc Diffusion (*Bacillus subtilus*)

It was observed that all the extracts have inhibitory effect against *Bacillus subtilus.* Methanol-Ajwain revealed the highest zone of inhibition (22mm), while *Trigonella foenum graecum* revealed the least activity (7mm). Researchers in different parts of the world have studied the antimicrobial activities of indigenous herbs and spices for over a century. Ibrahim et al (2006) has reviewed the antimicrobial effectiveness of

spices and herbs. Recent results of one Indian study (Krishna, 1999) indicated that cinnamon have potent antimicrobial activities against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas sp.* Results are presented in Table3 and Fig3 (a/b/c).

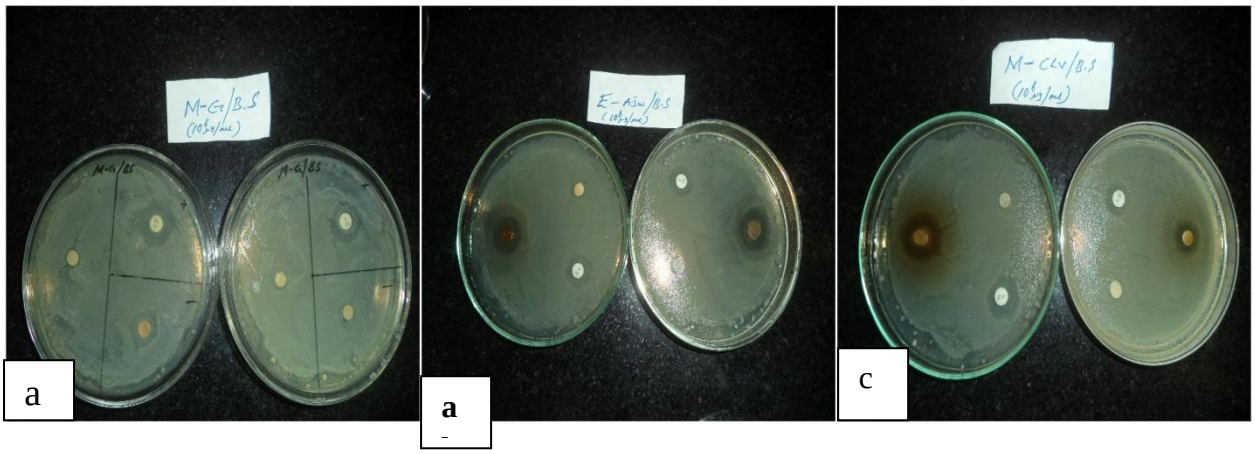

Fig.3 Antimicrobial activity of a) Ethanol-Garlic, b) Ethanol-Ajwain and c) Methanol-Clove extracts aginst *B. subtilus.*

Table3. Primary Screening for *Bacillus subtilus*

Crude Extract (10^3µg/ml)	**Millimeter (mm)**		
	Sensitivity Zone	**+ve Control (10 units)**	**-ve Control (80%)**
Methanol-Garlic	9±0.30	15±0.00	8±0.50
Methanol-Clove	19±0.88	15±0.00	7.5±0.00
Methanol-Cumin	6.5±0.00	12±1.20	6.5±0.00
Methanol-Ajwain	22±0.30	15±0.00	9±1.20
Methanol-Methi	7±0.30	13±0.50	7±0.30
Ethanol-Garlic	9±0.50	14±0.50	8.5±0.50
Ethanol-Clove	20±1.0	13±0.30	8.5±0.50
Ethanol-Cumin	10±0.50	12±0.50	7±0.30
Ethanol-Ajwain	20±0.00	15±0.00	8±0.30
Ethanol-Methi	7±0.00	14±1.0	0±0.00
Water-Garlic	7±0.00	14±0.00	7±0.00
Water-Clove	9±0.00	14±0.00	7±0.00
Water-Cumin	7±0.00	12±0.00	7±0.00
Water-Ajwain	7±0.00	15±0.00	7±0.00
Water-Methi	7±0.00	13±0.00	7±0.00

	F-Ratio	CD (5%)
Zone of Inhibition	92.49	0.732407
Extracts	847.83	0.327542
Zone of Inhibition X Extracts	51.06	1.26857

Key: Penicillin 10mg/ml (+ve control), Methanol & Ethanol (-ve control), NS (Not significant)

3.1.4 Disc Diffusion (*Klebsiella pneumonia*)

It was observed that all the extracts have inhibitory effect against *Klebsiella pneumonia.* Similarly methanol-clove revealed the highest zone of inhibition (22mm), while *Trigonella foenum graecum* revealed

the least activity (7mm). The results obtained as presented under chapter-4, show that ethanol-cumin is active against (*B. subtilus, K. pneumonia, A. niger & P. notatum,)* while methanol-cumin and water-cumin shows a less significant activity. This result is similar to previous researcher's results, Afolayan Meyer and Kuhnt (1995) reported the inactivity of cumin extracts against (*E.coli, Enterobacter, K. neumoniae & Rhodotorula spp.*). The activity is attributed to the presence of bioactive compounds (alkaloids, flavonoids, tannin, aldehyde group-compouds etc) and action mechanism involve binding of this compounds to membrane proteins and partition in the lipid bilayer of sensitive microorganisms (Mamta & Alka, 2012). Results are presented in Table4 and Fig4 (a/b/c).

Table4. Primary Screening for *Klebsiella pneumoniae*

Crude Extract (10^3µg/ml)	**Millimeter (mm)**		
	Sensitivity Zone	**+ve Control (10 units)**	**-ve Control (80%)**
Methanol-Garlic	NS	26±0.00	7±0.00
Methanol-Clove	22±0.00	26±0.00	7.5±0.30
Methanol-Cumin	9±0.00	26±0.00	7±0.50
Methanol-Ajwain	18±0.30	26±0.00	8±0.50
Methanol-Methi	9±0.50	26±0.00	7±0.50
Ethanol-Garlic	10±0.00	25±0.50	7±0.30
Ethanol-Clove	13±0.33	26±0.00	8±0.50
Ethanol-Cumin	NS	26±0.00	7±0.50
Ethanol-Ajwain	13±0.50	25±0.50	6.5±0.00
Ethanol-Methi	10±1.0	25±0.50	7±0.50
Water-Garlic	7±0.00	25.5±0.30	NS
Water-Clove	11±0.00	26±0.00	7±0.00
Water-Cumin	7±0.00	26±0.00	NS
Water-Ajwain	7±0.00	26±0.00	NS
Water-Methi	7±0.00	25.5±0.30	NS

	F-Ratio	CD (5%)
Zone of Inhibition	92.33	0.732407
Extracts	8826.87	0.327542
Zone of Inhibition X Extracts	61.69	1.26857

Key: Norfloxacin 10mg/ml (+ve cont.), Methanol & Ethanol (-ve cont.), NS (Not significant)

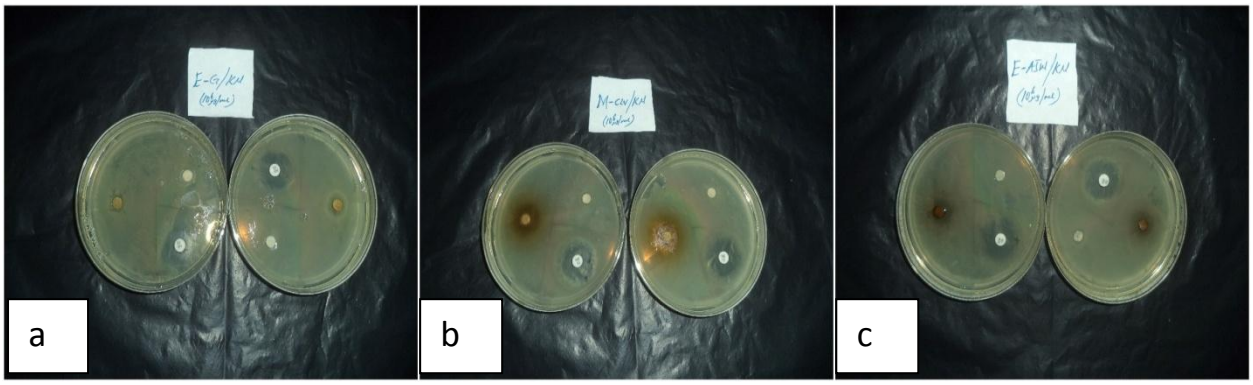

Fig.4 Antimicrobial activity of a) Ethanol-Garlic, b)Methanol-Clove and c)Ethanol-Ajwain extracts aginst *K. pneumoniae.*

3.1.5 Disc Diffusion (*Enterobacter*)

It was observed that all the extracts have inhibitory effect against *Enterobacter spp*. It was observed that all the extracts except *Trigonella foenum graecum* (Fenugreek/Methi), *Cuminum cyminum* (cumin) and *Allium sativum* (garlic) have inhibitory effect against *Enterobacter*. Ethanol-clove revealed the highest zone of inhibition (34mm), followed by Mthanol-ajwain (20mm). Results are presented in Table5 and Fig5 (a/b/c).

Table5. Primary Screening for *Enterobacter*

Crude Extract (10^3µg/ml)	**Millimeter (mm)**		
	Sensitivity Zone	**+ve Control (10 units)**	**-ve Control (80%)**
Methanol-Garlic	NS	26±0.00	7±0.30
Methanol-Clove	27±0.33	26±0.00	6.5±0.00
Methanol-Cumin	NS	26±0.00	NS
Methanol-Ajwain	20±0.50	26±0.00	NS
Methanol-Methi	NS	26±0.00	NS
Ethanol-Garlic	10±0.50	26±0.00	8±080
Ethanol-Clove	34±1.20	26±0.00	NS
Ethanol-Cumin	NS	26±0.00	NS
Ethanol-Ajwain	18±0.50	26±0.00	6.5±0.00
Ethanol-Methi	NS	26±0.00	NS
Water-Garlic	NS	26±0.00	NS
Water-Clove	9.5±0.00	26±0.00	NS
Water-Cumin	NS	26±0.00	NS
Water-Ajwain	8±0.50	26±0.00	NS
Water-Methi	NS	26±0.00	NS

	F-Ratio	CD (5%)
Zone of Inhibition	329.38	0.651352
Extracts	14483.72	0.291293
Zone of Inhibition X Extracts	267.90	1.12817

Key: Norfloxacin10mg/ml (+ve cont), Methanol & Ethanol (-ve cont), NS (Not significant)

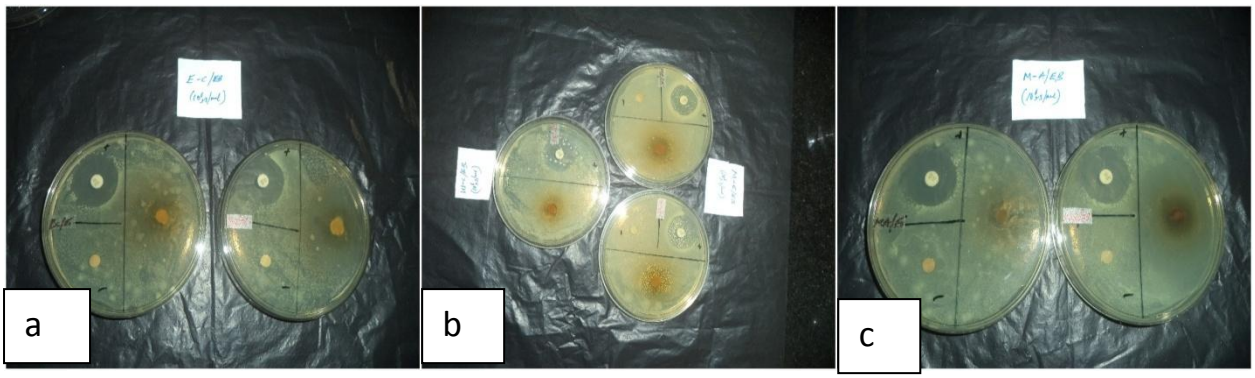

Fig.5 Antimicrobial activity of a) Ethanol-Garlic, b) Methanol-Clove and c) Methanol-Ajwain extracts aginst Enterobacter.

3.1.6 Disc Diffusion (*Saccharomyces cerevisiae*)

It was observed that aqueous extract of all the five plants does not have inhibitory effect against *Saccharomyces cerevisiae*. However, methanol and ethanol extracts are effective with ethanol-clove showing the highest zone of inhibition (36mm). The in activity may be due to less quantity of the bioactive components, denaturation or inability of the water to dissolve them [Table6 and Fig6 (a/b/c)].

Table6. Primary Screening for *Saccharomyces cerevisiae*

Crude Extract (10^3µg/ml)	Millimeter (mm)		
	Sensitivity Zone	+ve Control (25 units)	-ve Control (80%)
Methanol-Garlic	NS	33±0.00	NS
Methanol-Clove	28±0.55	33±0.00	NS
Methanol-Cumin	NS	33±0.00	NS
Methanol-Ajwain	18±1.0	32.5±0.33	NS
Methanol-Methi	NS	33±0.00	NS
Ethanol-Garlic	14±1.0	33±0.00	NS
Ethanol-Clove	36±0.50	33±0.00	NS
Ethanol-Cumin	NS	33±0.00	NS
Ethanol-Ajwain	25±0.50	33±0.00	NS
Ethanol-Methi	NS	33±0.00	NS
Water-Garlic	NS	33±0.00	NS
Water-Clove	NS	33±0.00	NS
Water-Cumin	7±0.00	33±0.00	NS
Water-Ajwain	NS	33±0.00	NS
Water-Methi	NS	33±0.00	NS

	F-Ratio	CD (5%)
Zone of Inhibition	623.35	0.468449
Extracts	52813.07	0.209497
Zone of Inhibition X Extracts	623.35	0.811377

Key: Fluconazole 25mg/ml (+ve cont.), Methanol & Ethanol (-ve cont.), NS (Not significant)

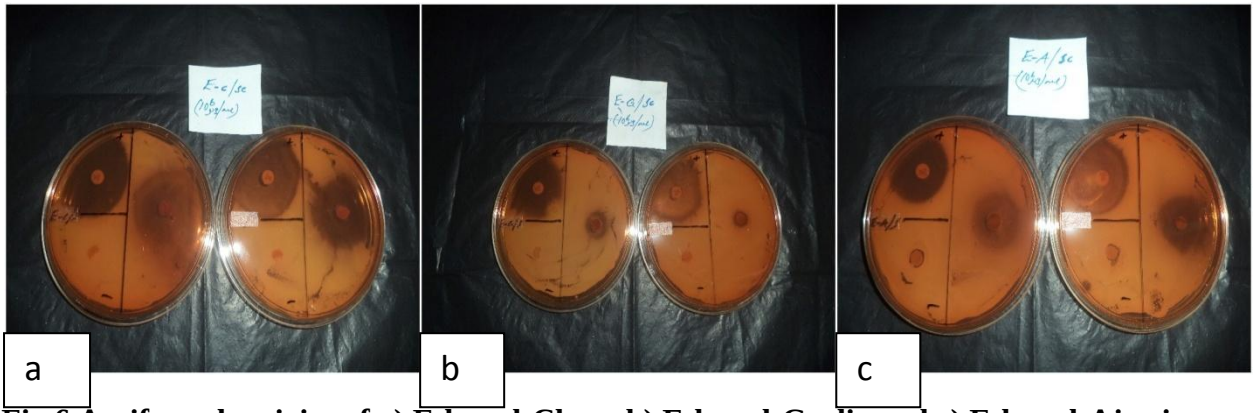

Fig.6 Antifungal activity of a) Ethanol-Clove, b) Ethanol-Garlic and c) Ethanol-Ajwain extracts aginst *S. cerevisiae.*

3.1.7 Disc Diffusion (*Rhodotorula*)

Similarly it was observed that aqueous extract of all the five plants does not have inhibitory effect against *Rhodotorula*. However, methanol and ethanol extracts are effective with methanol-clove showing the

highest zone of inhibition (48mm), followed by methanol-ajwain (46mm). Results are presented in Table7 and Fig7 (a/b/c).

Table7. Primary Screening for *Rhodotorula*

Crude Extract (10^3µg/ml)	Millimeter (mm)		
	Sensitivity Zone	+ve Control (25 units)	-ve Control (80%)
Methanol-Garlic	NS	NS	NS
Methanol-Clove	48±1.20	NS	NS
Methanol-Cumin	NS	NS	NS
Methanol-Ajwain	46±0.50	NS	NS
Methanol-Methi	NS	NS	NS
Ethanol-Garlic	16.5±0.50	NS	NS
Ethanol-Clove	46±0.50	NS	NS
Ethanol-Cumin	11±0.00	NS	NS
Ethanol-Ajwain	47±0.30	NS	NS
Ethanol-Methi	NS	NS	NS
Water-Garlic	NS	NS	NS
Water-Clove	NS	NS	NS
Water-Cumin	NS	NS	NS
Water-Ajwain	NS	NS	NS
Water-Methi	NS	NS	NS

	F-Ratio	CD (5%)
Zone of Inhibition	2001.40	0.436102
Extracts	14157.00	0.195031
Zone of Inhibition X Extracts	2001.40	0.755351

Key: Fluconazole 25mg/ml (+ve cont.), Methanol & Ethanol (-ve cont.), NS (Not significant)

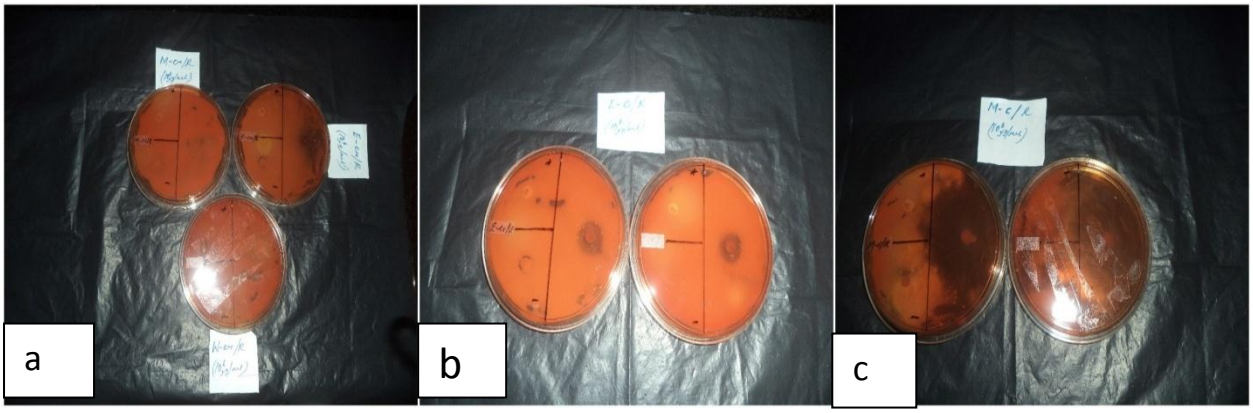

Fig.7 Antifungal activity of a) Methanol-Ajwain, b) Ethanol-Garlic and c) Methanol-Clove extracts aginst *Rhodotorula.*

3.1.8 Disc Diffusion (*Aspergillus niger*)

Similarly it was observed that aqueous extract of all the five plants does not have inhibitory effect against *Rhodotorula.* However, methanol and ethanol extracts are effective with methanol-clove showing the highest zone of inhibition (42mm), followed by methanol-ajwain (41mm). Ethanol-garlic also revealed a good zone of inhibition (21mm), these results are similar to those reported by previous researchers on garlic antimicrobial properties, which was related to garlic's chemical complexity, various solvent extract,

different bioactive materials (Harunobu, 2006). The main mechanism of action involved the inhibition of certain thiol-containing enzymes in the microorganisms by the rapid reaction of thiosulfinates with thiol groups. These enzymes are involved in maintaining cell wall rigidity of the organisms (Serge, 1999). The results revealed that ethanol have more potential in dissolving the bioactive compounds in garlic compared to methanol and water. Results are presented in Table8 and Fig8 (a/b/c).

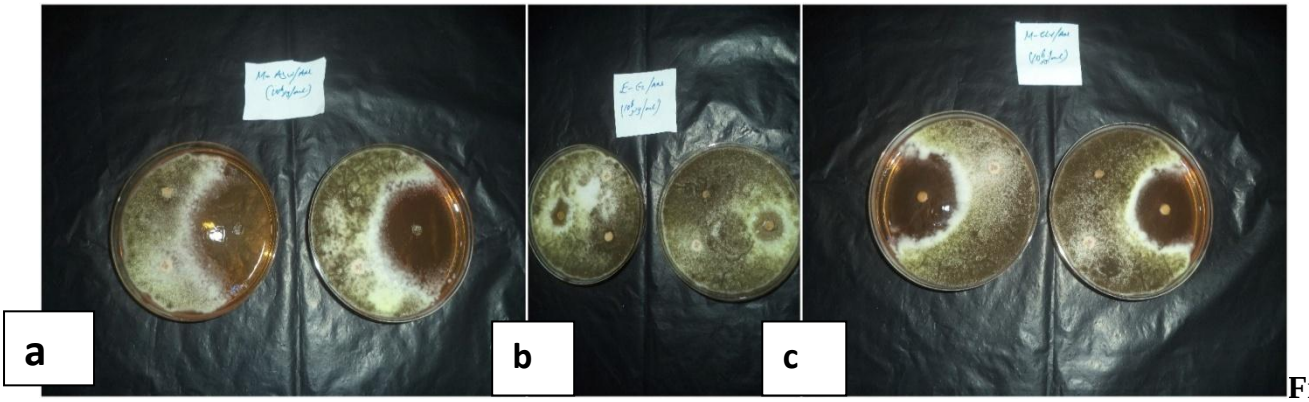

Fig.8 Antifungal activity of a) Methanol-Ajwain, b) Ethanol-Garlic and c) Methanol-Clove extracts aginst *A. niger.*

Table8. Primary Screening for *Aspergillus niger*

Crude Extract (10^3µg/ml)	Millimeter (mm)		
	Sensitivity Zone	+ve Control (25 units)	-ve Control (80%)
Methanol-Garlic	NS	NS	NS
Methanol-Clove	42±0.50	NS	NS
Methanol-Cumin	NS	NS	NS
Methanol-Ajwain	41±1.0	NS	NS
Methanol-Methi	NS	NS	NS
Ethanol-Garlic	21±0.00	NS	NS
Ethanol-Clove	34±1.0	NS	NS
Ethanol-Cumin	7±0.00	NS	NS
Ethanol-Ajwain	32±1.0	NS	NS
Ethanol-Methi	NS	NS	NS
Water-Garlic	NS	NS	NS
Water-Clove	NS	NS	NS
Water-Cumin	NS	NS	NS
Water-Ajwain	NS	NS	NS
Water-Methi	NS	NS	NS

Key: Fluconazole 25mg/ml (+ve cont.), Methanol & Ethanol (-ve cont.), NS (Not Significant)

6.1.9 Disc Diffusion (*Penicillium notatum*)

Similarly it was observed that aqueous extract of all the five plants does not have inhibitory effect against *Rhodotorula.* However, methanol and ethanol extracts are effective with methanol-clove showing the highest zone of inhibition (45mm), followed by ethanol-ajwain (44mm) and ethanol-garlic (22mm). Results are presented in Table9 and Fig9 (a/b/c).

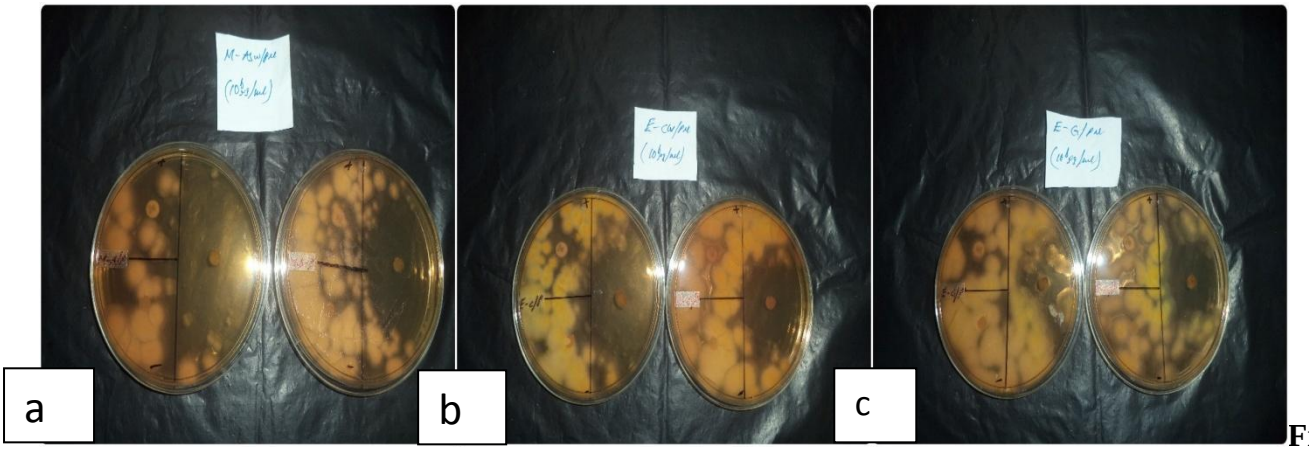

Fig.9 Antifungal activity of a) Methanol-Ajwain, b)Ethanol-Clove and c)Ethanol-Garlic extracts aginst *P. notatum*

Table9. Primary Screening for *Penicillium notatum*

Crude Extract (10^3µg/ml)	Millimeter (mm)		
	Sensitivity Zone	+ve Control (25 units)	-ve Control (80%)
Methanol-Garlic	NS	NS	NS
Methanol-Clove	45±0.00	NS	NS
Methanol-Cumin	NS	NS	NS
Methanol-Ajwain	43±0.50	NS	NS
Methanol-Methi	NS	NS	NS
Ethanol-Garlic	22±0.50	NS	NS
Ethanol-Clove	44±0.50	NS	NS
Ethanol-Cumin	25±1.0	NS	NS
Ethanol-Ajwain	44±1.0	NS	NS
Ethanol-Methi	NS	NS	NS
Water-Garlic	NS	NS	NS
Water-Clove	NS	NS	NS
Water-Cumin	NS	NS	NS
Water-Ajwain	NS	NS	NS
Water-Methi	NS	NS	NS

	F-Ratio	CD (5%)
Zone of Inhibition	4189.71	0.287926
Extracts	35102.82	0.128764
Zone of Inhibition X Extracts	4189.71	0.498702

Key: Fluconazole 25mg/ml (+ve cont.), Methanol & Ethanol (-ve cont.), NS (Not significant.)

3.2.1 Minimum Inhibitory Concentration (MIC) for Methanol-Clove Extract

Methanol-clove exhibited the highest antimicrobial efficacy at concentration of (20µg/ml) against *Escherichia coli, Staphylococcus aureus, Bacillus subtilus, Enterobacter spp., Saccharomyces cerevisiae* and 30µg/ml against *Klebsiella pneumonia.* Results are presented in Table10 and Fig10.

Table10. Minimum Inhibitory Concentration (MIC) for Methanol-Clove Extract (595nm)

	Extract-MHB Solution (µg/ml)								
Test Organisms	**40**	**30**	**20**	**10**	**5.0**	**2.5**	**1.25**	**+ve**	**-ve**

E. *coli*	NS	NS	NS	1.300	1.378	1.399	1.931	0.019	0.108
S. aureus	NS	NS	NS	1.265	1.371	1.482	1.993	0.263	0.879
B. subtilus	NS	NS	NS	1.610	1.660	1.670	1.842	0.315	0.543
K. pneumoniae	NS	NS	1.302	1.411	1.618	1.667	1.960	0.373	1.002
Enterobacter	NS	NS	NS	1.592	1.722	1.800	1.831	0.267	0.965
S. cerevisiae	NS	NS	NS	0.900	1.317	1.554	1.584	0.168	2.009
Rhodotorula	0.051	0.460	0.753	0.900	1.139	1.686	1.800	0.007	2.965
A. niger	NS	NS	0.789	0.957	1.407	1.409	1.516	0.051	3.002
P. notatum	NS	NS	0.080	0.859	1.288	1.527	1.560	0.070	1.012

Key: +ve control (Streptomycin 7.3µg/ml & Fluconazole), -ve control (80% methanol), *NS (Not significance)*

3.2.2 Minimum Inhibitory Concentration (MIC) for Ethanol-Clove Extract

Ethanol-clove also exhibited a relatively high antimicrobial efficacy against some of the tested microbes but at comparably more concentration (30-40µg/ml) compared to methanol extract. Results are presented in Table11.

Table11. Minimum Inhibitory Concentration (MIC) for Ethanol-Clove Extract (595nm)

	Extract-MHB Solution (µg/ml)								
Test Organisms	**40**	**30**	**20**	**10**	**5.0**	**2.5**	**1.25**	**+ve**	**-ve**
E. *coli*	NS	NS	NS	0.017	0.632	0.837	0.995	0.049	1.654
S. aureus	NS	NS	0.017	0.040	0.826	1.170	1.963	0.366	0.956
B. subtilus	NS	0.062	0.255	0.367	0.812	0.996	1.046	0.365	1.033
K. pneumoniae	NS	0.059	0.138	0.465	0.761	0.884	1.022	0.407	2.206
Enterobacter	NS	0.605	1.085	1.168	1.634	1.789	1.883	0.444	1.432
S. cerevisiae	0.009	0.036	0.331	0.799	0.896	0.948	1.081	0.314	2.543
Rhodotorula	NS	0.000	0.205	0.656	0.764	1.105	1.114	0.039	1.023
A. niger	0.003	0.473	0.759	0.792	1.220	1.284	1.444	0.034	2.001
P. notatum	0.702	0.996	1.121	1.586	1.945	2.001	2.032	0.013	1.009

Key: +ve control (Streptomycin 7.3µg/ml & Fluconazole), -ve control (80% Ethanol), NS (No significance)

3.2.3 Minimum Inhibitory Concentration (MIC) for Methanol-Ajwain Extract

Methanol-Ajwain extracts exhibited the second high antimicrobial efficacy for MIC assay, in which the microbial growth was prevented completely at concentration 0f (10-30µg/ml). Results are presented in Table12.

Table12. Minimum Inhibitory Concentration (MIC) for Methanol-Ajwain Extract (595nm)

	Extract-MHB Solution (µg/ml)								
Test Organisms	**40**	**30**	**20**	**10**	**5.0**	**2.5**	**1.25**	**+ve**	**-ve**
E. *coli*	NS	NS	NS	0.009	0.118	0.278	0.406	0.371	1.324
S. aureus	NS	NS	0.016	0.176	0.304	0.935	1.080	0.850	1.124
B. subtilus	NS	NS	NS	NS	0.196	0.554	1.321	0.661	0.928
K. neumoniae	0.090	0.495	0.922	1.076	1.610	1.690	1.896	0.842	1.112
Enterobacter	0.036	0.089	0.554	0.651	0.898	0.917	1.206	0.883	1.002
S. cerevisiae	NS	NS	NS	0.015	0.065	0.104	0.406	0.150	1.342
Rhodotorula	0.022	0.078	0.156	0.388	0.522	0.775	0.978	0.666	1.672

A. niger	NS	NS	0.008	0.193	0.498	0.686	1.047	0.271	1.333
P. notatum	NS	0.004	0.102	0.117	0.238	0.296	0.414	0.435	1.657

Key: +ve control (Streptomycin 7.3µg/ml & Fluconazole), -ve control (80% methanol), *NS (No significance)*

3.2.4 Minimum Inhibitory Concentration (MIC) for ethanol-Ajwain Extract

Ethanol-Ajwain extracts also exhibited good antimicrobial efficacy for MIC assay, in which the microbial growth was prevented completely at concentration 0f (20-40µg/ml). Results are presented in Table13.

Table13. Minimum Inhibitory Concentration (MIC) for Ethanol-Ajwain Extract (595nm)

	Extract-MHB Solution (µg/ml)								
Test Organisms	**40**	**30**	**20**	**10**	**5.0**	**2.5**	**1.25**	**+ve**	**-ve**
E. *coli*	NS	0.061	0.398	0.609	0.890	0.899	1.071	0.361	1.342
S. aureus	NS	NS	0.009	0.265	0.489	0.757	0.887	0.711	1.400
B. subtilus	0.004	0.184	0.514	0.686	0.965	1.156	1.232	0.856	1.089
K. neumoniae	NS	0.009	0.390	0.605	0.612	0.769	0.801	0.934	1.241
Enterobacter	0.035	0.119	0.240	0.635	0.748	0.994	1.008	0.909	1.119
S. cerevisiae	NS	NS	NS	0.018	0.191	0.214	0.570	0.363	0.689
Rhodotorula	NS	0.091	0.214	0.303	0.347	0.473	0.668	0.660	0.912
A. niger	NS	NS	0.012	0.079	0.143	0.373	0.609	0.260	1.023
P. notatum	0.027	0.128	0.420	0.654	0.883	1.302	1.479	0.580	1.117

Key: +ve control (Streptomycin 7.3µg/ml & Fluconazole), -ve control (80% Ethanol), *NS (No significance)*

Streptomycin and Fluconozole were used as positive and negative controls at concentration of (7.3µg/ml & 15µg/ml) for bacteria and fungi respectively. Similar results have been reported previously but at relatively higher concentrations. European Medicines agency (13th September, 2011) reported the antimicrobial efficacy of clove and ajwain extracts but at a high concentration range of 50µg/ml to 120µg/ml. Similarly International Research Journal of Biological Sciences published an article in August 2012 in which the antimicrobial efficacy of clove and ajwain extracts were reported but again at a high concentration range of 35µg/ml to 100µg/ml.

3.3 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The two best extracts (Clove and Ajwain) were tested for bactericidal/ fungicidal or bacteristatic/fungistatic action and he results obtained revealed that some of the extracts have a bactericidal action and some are bacteristatic depending on the test organisms and concentration used. Antimicrobial agents are usually regarded as bactericidal/fungicidal if the MBC/MFC is no more than four times the MIC. Results are presented in Table14 and Table15.

Table14. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) for Clove Extract

Test Organisms	Methanol		Ethanol	
	MIC (µg/ml)	MBC (colonies)	MIC (µg/ml)	MBC (colonies)
E. *coli*	20	>400	10	120
S. aureus	20	128	10	>400
B. subtilus	20	116	30	CG
K. pneumoniae	30	168	30	CG
Enterobacter	20	CG	40	76
S. cerevisiae	20	01	30	CG
Rhodotorula	40	100	30	180
A. niger	30	100	40	CG
P. notatum	30	110	40	>400

Key:*MIC (*Minimum Inhibitory Concentration*), *MBC (Minimum Bactericidal Concentration)

CG (Confluent growth), & > (Greater than).

Table15. Minimum Bactericidal/Fungicidal Conc. (MBC/MFC) for Ajwain Extract

Test Organisms	Methanol		Ethanol	
	MIC (µg/ml)	MBC (colonies)	MIC (µg/ml)	MBC (colonies)
E. *coli*	10	16	30	>400
S. aureus	20	>400	20	>400
B. subtilus	10	120	40	76
K. neumoniae	40	>400	30	>400
Enterobacter	30	CG	40	>400
S. cerevisiae	10	01	10	56
Rhodotorula	30	01	30	48
A. niger	20	40	10	01
P. notatum	30	CG	40	CG

Key:*MIC (*Minimum Inhibitory Concentration*), *MBC (Minimum Bactericidal Concentration)

CG (Confluent growth), & > (Greater than).

In general, antimicrobial activity of the spices is known to be due to procession of various bioactive compounds and volatile aromatic secretions. Considering the large number of different groups of chemical compounds present in the essential components, it is most likely that antibacterial potential is not attributable to one specific mechanism but rather are several targets in the cell (Carson et al, 2002). However, hypotheses have been proposed by different workers which involve:

- Hydrophobic and hydrogen bonding of active compounds to membrane proteins
- Partition in the lipid bilayer
- Perturbation of membrane permeability consequent to its expansion and increased fluidity Causing the leakage of ions and other cell contents

- Inhibition of membrane embedded enzymes
- Destruction of electrons transport systems,
- Disruption of proton motive force (PMF) and
- Coagulation of cell contents (Mamta & Alka, 2012).

Not all of these mechanisms are separate targets; some are even affected as a consequence of another mechanism being targeted.

Results suggest that the tested gram +ve bacteria are more susceptible as compared to the gram –ve strains used. The greater susceptibility may be due to the absence of an outer membrane which makes them more sensitive to external environmental changes such as Temperature, pH, natural extracts, essential oils and other antimicrobial substances (Nevas *et al.*, 2004, Souza *et al.*, 2005). On the other hand, the lipopolysacharides in the cell membrane of *gram –ve strains* could provide a barrier to many antimicrobial agents, rendering them more resistant to certain agents than g+ve strains. Similarly yeasts were more susceptible as compared to molds possibly due to their less complexity and thus prone to environmental changes.

The different solvents used differ in their ability to dissolved different bioactive components of the planst. Methanol and ethanol extracts had similar amounts of phytochemicals; but the methanol extract contained high concentration of carbohydrate, saponin, tannins and moderate concentrations of flavonoids, resin, protein, alkaloids, and glycosides. While the ethanol extract contains moderate concentration of these phytochemicals, except tannin that was present in high concentration. The aqueous (hot-water) extract contains the least concentration of the phytochemicals. These concentrations influenced their overall activity on the inhibition of microbial growth (Anosike et al., 2012).

3.4 Phytochemical Screening

Phytochemical constituents such as tannins, alkaloids, flavonoids, phenols, saponins, glycosides, and other aromatic compounds are secondary metabolites of plant serving as defense mechanism against prediction by many microorganisms. The five medicinal plant seeds tested in the present study revealed the presence of most of the bioactive constituents as summarized in [Table-16 & Fig16 (a-g)] and most were found to be present in methanol and ethanol extracts, with aqueous (hot-water) extract showing negative for some.

Table16. Phytochemical Screening

Crude Extract	Tannins	Saponins	Flavornoids	Terponoids	Glycosides	Alkaloids	Phenolics
M-Garlic	-ve	+ve	+ve	+ve	-ve	-ve	-ve
M-Clove	+ve	+ve	-ve	+ve	+ve	+ve	+ve
M-Cumin	+ve	+ve	+ve	+ve	+ve	+ve	+ve
M-Ajwain	+ve	+ve	+ve	+ve	+ve	+ve	+ve
M-Methi	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E-Garlic	-ve	+ve	+ve	+ve	+ve	+ve	+ve

E-Clove	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E-Cumin	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E-Ajwain	+ve	+ve	+ve	+ve	+ve	+ve	+ve
E-Methi	+ve	-ve	-ve	+ve	+ve	+ve	+ve
W-Garlic	-ve	-ve	+ve	+ve	+ve	-ve	-ve
W-Clove	+ve	+ve	-ve	+ve	+ve	+ve	+ve
W-Cumin	+ve	+ve	+ve	+ve	+ve	+ve	+ve
W-Ajwain	+ve	+ve	+ve	+ve	+ve	+ve	+ve
W-Methi	-ve	- ve	+ve	-ve	+ve	-ve	+ve

Key: M-Methanol, E-Ethanol, W-Water, +ve: Positive, -ve: Negative.

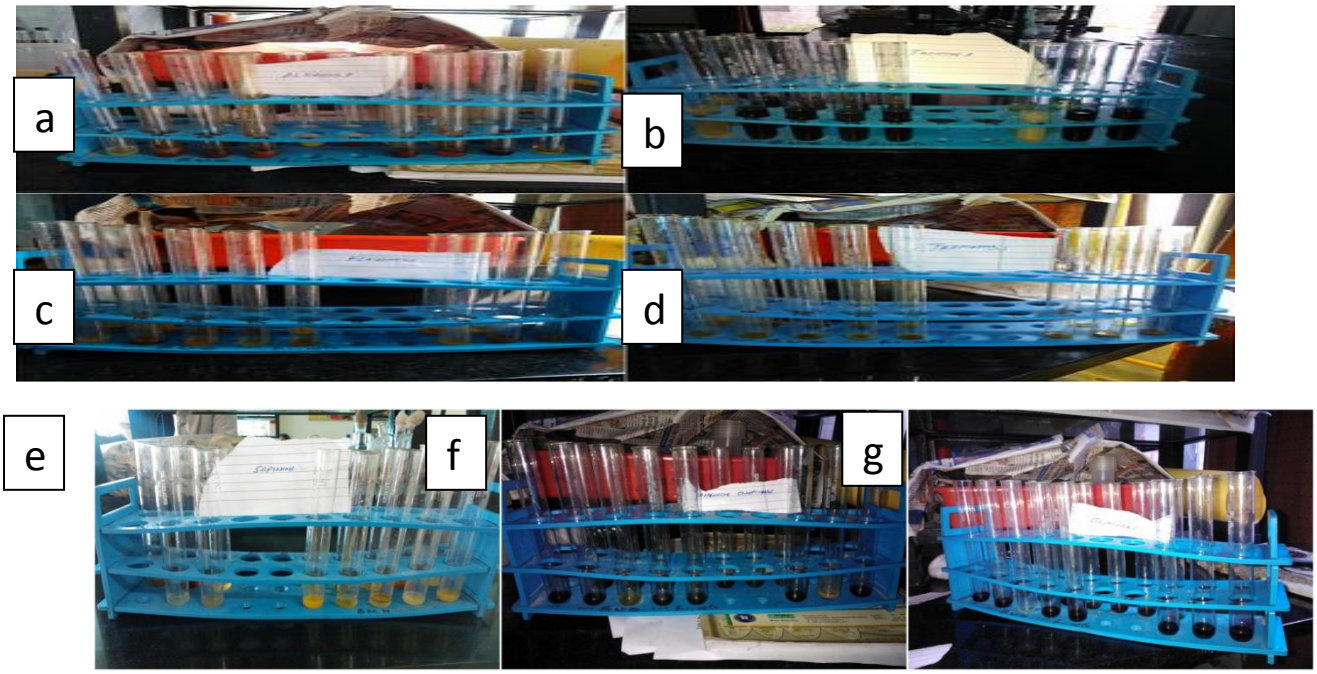

Fig.21 Phytochemical screening for the presence of bioactive components [a) Alkaloids, b)Tannins, c) Flavornoids, d)Terponoids, e)Saponins, f)Phenolic compounds & g) Glycosides]

CONCLUSION

The Methanol, ethanol, and hot-water extracts of five medicinal spices *[Allium sativum* (garlic), *Syzygium aromaticum* (clove), *Trachyspermum ammi* (ajwain), *Cuminum cyminum* (cumin) *& Trigonella foenum graecum* (Methi)] traditionally used in India house-hold as flavor stimulants were investigated for in vitro antimicrobial activity against food borne pathogens namely *Escherichia coli, Staphylococcus aureus, Bacillus subtilus, Klebsiella pneumoniae, Enterobacter spp., Saccharomyces cerevisiae, Rhodotorula spp., Aspergillus niger, Penicillium notatum,* by disc diffusion method. Methanol-ethanol extracts of *Syzygium aromaticum* and *Trachyspermum ammi* showed the highest antimicrobial activity (25-48mm & 25-47mm respectively) against all the tested bacterial and fungal isolates, while *Trigonella foenum graecum* extracts revealed the least activity (0.0-8mm). Minimum inhibitory concentration (MIC) assay were determine for these four extracts against all the tested organisms. Methanol- clove revealed the highest antimicrobial activity at a minimum concentration of (10-20µg/ml) against all the tested microorganisms, followed by methanol-ajwain at concentration of (20µg/ml) against all the experimental organisms except *Klebsiella*

pneumonia. The phytochemical analysis carried out revealed the presences of Tannins, Saponins, Flavornoids, Terponoids, Glycosides, Alkaloids, Phenolics which are responsible for the antimicrobial activity. The results of these experiments, justified the use of spices in preservation of food stuffs.

The output of this research work indicate that both methanol and ethanol extracts of (*Allium sativum*, *Syzygium aromaticum*, *Trachyspermum ammi*, *Cuminum cyminum & Trigonella foenum graecum*) effectively arrested food borne pathogens in culture media and may be further considered for their possible applications in food processing and preservation sectors. Further studies must be undertaken to elucidate the safety, stability and organoleptic aspects of the essential extracts in different food systems before these substances can be reliably used in commercial applications. Furthermore, the exact mechanism of modes of action of these natural extracts is still not so well understood, thus, it would be the next line of research.

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MUHAMMAD ABDULLAHI SA'ID

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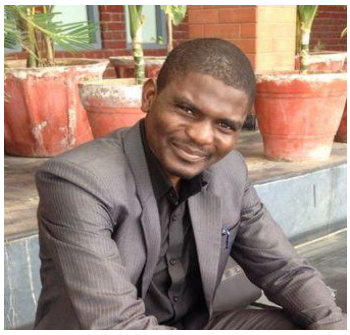
Authors Profile



Muhammad Abdullahi Sa'id
B.Sc. Applied Biology (BUK, Nigeria), M.Sc. Microbiology (LPU, India)
Email: yaalhaj99@yahoo.com

Er. Robinka Khajuria (Assistant Professor LPU, India)

Email: robinka.16766@lpu.co.in