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Comparative studies on antimicrobial activities of garlic (*Allium sativu*), onion (*Allium cepa*) and lemon (*Citrus limon*): Individual & combined

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ABSTRACT

The inhibitory effects *in vitro* of the three plant juice extracts- garlic *(Allium sativum),* onion *(Allium cepa)* and lemon, taken singly & in combination, were studied against selected six bacteria (Three gram-negative; *E. coli, Kleibsella spp* and *Proteus spp*; & three gram-positive; *S. aureus, B. cereus* and *Micrococcus spp*) and four fungi (*A. niger, A. terreus, A.japinicus* and *P. expansum*) in which bacteria were found to be more susceptible to the plants juice extract than the fungi. The plants juice extracts showed the higher inhibitory effects against the gram positive bacteria than the gram-negative. Fungi are strongly inhibited by the garlic juice then the lemon juice and almost all fungi could not be inhibited be onion. In comparision to reference antibiotic (Choloroamphenicol) and antifungal agent (Ketacanazole), garlic and lemon showed effective inhibition against the bacteria and fungi respectively.

KEYWORDS

Allium cepa;
Allium sativum;
Citrus.

INTRODUCTION

Drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. The use of plants for medicinal purposes dates back thousands of years and even chimpanzees have been known to chew certain leaves, when suffering from gastrointestinal disturbances. Natural products once used to serve as the only source of medicine for the mankind in the ancient time. Many plants used today were know to people of ancient culture throughout the world and they were valued their preservative and medicinal powers[7].

The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently

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conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value[1].

Naturally occurring microbial inhibitors have been recovered from a wide variety of foods including onions, garlic, fruits, vegetables, cereals and spices. many of these antimicrobials contribute to the food stuffs natural resistance to deteriorations. The flavour components consist of such compounds as alcohols, aldehydes, esters, terpens, phenols, organic acids and others, some of which have not yet been identified. The widespread use of garlic and onion as a flavoring agent is well known. Garlic bulbs contain pectin, garlicin, volatile oils, allin and allistatins I and II. Alcoholic extract of garlic shows bactericidal, antibiotic, high hypoglycemic and fungicidal activities. The extract showed hypotensive, analgesic sedative and antileptazol properties. Onion and garlic also known to have medicinal properties. Onion bulbs contain tannin, pectin, quercetin and glycosides. Alcohol extracts show hypotensive analgesic and antileptazol properties. Onion extracts shows antibacterial properties[7]. Spice extractives, such as garlic shows bactericidal, antibiotic, high hypoglycemic and fungicidal activities. The extract showed hypotensive, analgesic sedative and antileptazol properties. Onion and garlic also known to have medicinal properties, oleoresin of rosemary, can provide inhibition of oxidative rancidity and retard the development of "warmed-over" flavor in some products.

Description about the plants

Garlic (*Allium sativum*)

Garlic has long been considered a herbal "wonder drug", with a reputation for preventing everything from the common cold and flu to the Plague. It has been used extensively in herbal medicine (phytotherapy, sometimes spelt phitotherapy). Raw garlic is used by some to treat the symptoms of acne and there is some evidence that it can assist in managing high cholesterol levels. It can even be effective as a natural mosquito repellent.

In general, a stronger tasting clove of garlic has more sulphur content and hence more medicinal value. Some people have suggested that organically grown garlic tends towards a higher sulphur level and hence greater benefit to health. Modern science has shown that garlic is a powerful antibiotic, albeit broad-spectrum rather than targeted. The body does not appear to build up resistance to the garlic, so its positive health benefits continue over time[4]. Raw garlic is very strong, so eating too much could produce problems.. Symptoms of garlic allergy include skin rash, temperature and headaches. Also, garlic could potentially disrupt anti-coagulants, so it's best avoided before surgery.

Constituents

Volatile oil, consisting of sulphur-containing compounds, including allicin (=S-allyl-2-propenthiosulphinate), allyl-methyltrisulphide, diallyldisulphide, diallyltrisulphide, diallyltetrasulphide, allylpropyldisulphide, ajoene, 2-vinyl-4H-l, 3 dithiin, and alliin, Properties-Anti-microbial, diaphoretic, cholagogue, hypotensive, anti-spasmodic.

Onion (*Allium cepa*)

Onion in the general sense can be used for any plant in the Genus *Allium* but used without qualifiers usually means *Allium cepa* L., also called the garden onion. Onions (usually but not exclusively the bulbs) are edible with a distinctive strong flavour and pungent odour which is mellowed and sweetened by cooking. They generally have a papery outer skin over a fleshy, layered inner core. Used worldwide for culinary purposes, they come in a wide variety of forms and colors.

Constituents

The constituents of onion contain only traces (0.01%) of essential oil, which mostly consists of sulfur compounds. Onions contain two substances: sulfur and quercetin - both being strong antioxidants. They each have been shown to help neutralize the free radicals in the body, and protect the membranes of the body's cells from damage. It also contains the minerals Potassium, Phosphorus, Calcium, Magnesium, Sodium and Selenium. Also contains small amounts of iron, manganese, copper and zinc. Onion cells have two sections, one with enzymes called allinases, the other with sulfides. The enzymes break down the sulfides and generate sulfenic acids. Sulfenic acid is unstable and decomposes into a volatile gas called syn-ropanethial-S-oxide.

Lemon (*Citrus limon*)

Lemon juice-fresh, canned, concentrated and frozen, or dehydrated and powdered, is primarily used for

lemonade, in carbonated beverages, or other drinks. It is also used for making pies and tarts, as a flavoring for cakes, cookies, cake icings, puddings, sherbet, confectionery, preserves and pharmaceutical products. A few drops of lemon juice, added to cream before whipping, gives stability to the whipped cream.

Constituents

Lemon Peel Oil consists mainly of terpenes, particularly limonene, also gamma terpinene and beta-phellandrene. There are small amounts of sesquiterpenes and aldehydes. Among the aliphatic aldehydes are n-octyl aldehyde, n-nonyl aldehyde, and citral.

Pathogens and diseases

E. coli is responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis). *K. pneumoniae* causes pneumonia, a serious disease with high case of fatality. It occurs in middle aged or older persons who have medical problems such as alcoholism, chronic bronchopulmonary disease or diabetes mellitus. This disease is characterized by massive mucoid inflammatory exudates of lobar or lobular distribution, invoving one or more lobes in lungs. *K. pneumoniae* is a frequent cause of urinary tract infection. As most strains are resistant to antibiotics. *K. ozaenae* is a bacillus associated with ozena, a disease characterized by foul smelling nasal discharge. *K. rhinoscleromatis* causes rhinoscleroma, a chronic granulomatous hypertrophy of nose. *Proteus spp* are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial. These are frequently present in moist skin. Staphylococci produce two types of diseases- infections and intoxications. Coomon Staphylococci infections are- abcess, wound infection, osteomyelitis, arthritis, tonsillitis, sinusitis, UTI (Urinary tract infection) e.t.c. *Bacillus cereus* produces two patterns of food borne diseases. One is associated with a wide range of foods including cooked meat and vegetables. It is characterized by diarrhea and abdominal pain. Vomiting is rare, The second type is associated almost with the consumption of cooked rice, usually fried Chinese restaurant. The illness is characterized by acute nausea and vomiting after 1-5 hours after meal. Diarrhea is not common. Generally, Micrococci are parasitic on mammalian skin and are ordinarily nonpathogenic.

Aspergilosis is the group of diseases caused by *Aspergillus*. The symptoms include fever, cough, chest pain or breathlessness, which also occur in many other illnesses so diagnosis can be hard. Usually, only patients with already weakened immune system or who suffer other lung conditions are susceptible. *Penicillium* spp. are occasional causes of infection in humans and the resulting disease is known generically as penicilliosis. *Penicillium* has been isolated from patients with keratitis, endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis, and urinary tract infections. Most *Penicillium* infections are encountered in immunosuppressed hosts. Corneal infections are usually post-traumatic]. In addition to its infectious potential, *Penicillium verrucosum* produces a mycotoxin, ochratoxin A, which is nephrotoxic and carcinogenic.

MATERIAL AND METHODS

Sampling

About 500g of each three plant samples were taken for this study. The sampling site for the collection of plant samples (garlic - *Allium sativum*, onion - *Allium cepa and* lemon - *Citrus limon*) was the market Dehradun, Uttaranchal, India.

Preservation of samples

The samples were kept in refrigerator at 4º C.

Processing of samples

Preparation of plant juice extracts for antimicrobial activity

Garlic and onion

At first, skins of the garlic and the onion bulbs were peeled out and washed with sterilized water and air dried for 1 hour and cut in small pieces. Then the garlic and onion pieces were grinded in electric blender separately. Using the clean and dry muslin cloths, the crude juices was squeezed out, then it was further filtered through the whatmann filter paper No.1 under vacuum pressure. The filtrate was taken as for the experimental juice extracts sample.

Lemon

The fruit parts of lemon were washed with steril-

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ized water and air dried for 1 hour and cut in two pieces. Then the pieces were squeezed with the help of squeezer then it was filtered through whatmann filter paper No.1 under vacuum pressure. The filtrate was taken as for the experimental juice extracts sample.

Combination of juice extracts

The extracted juices of garlic, onion and lemon are mixed either of two samples (garlic and onion, garlic and lemon, onion and lemon) or of all three (garlic and onion and lemon) in the 1:1 and 1:1:1 ratio respectively.

Preparation of antibiotics and antifungal solutions

The stock solution of 10mg/ml was made first and further diluted to make concentration 1mg/ml using sterile distilled water.

Procedure

Antibacterial activity of plant sample juices

Antibacterial assay

Under modern system of medicine bacterial infections are generally treated by antibiotic. However results are not satisfactory and the organisms very often develop resistance to the antibiotic drugs. Prolonged courses of antibiotic also cause serious side effects. The bulb extracts of garlic and onion and fruit extract of lemon were screened for its antimicrobial activity against certain selected bacterial strains.

The following bacterial cultures were used for antibacterial activity:

- *Escherichia coli* (EC 536)
- *Kleibsella spp* (Isolate)
- *Staphylococcus aureus* (Isolate)
- *Proteus spp* (Isolate)
- *Bacillus cereus* (Isolate)
- *Micrococcus spp* (Isolate)

The cultures were obtained from the standard cultures maintained in the Microbiology department of DIBNS, Dehradun. These cultures were maintained on nutrient agar slants at first being incubated at 37º C for about 18 – 24 hours and then stored at 4º C as stock cultures for further antibacterial activity. Fresh cultures were obtained by transferring a loopful of culture into nutrient broth and then incubated at 37º C overnight. To test antibacterial acivity, the Cup well diffusion method was used.

Culture media preparation

The microbiological media were prepared as standard instruction provided by the HI-MEDIA Laboratories PVT. Limited Mumbai. The medium used for antibacterial activity were MHA, NA and NB were prepared and sterilized at 121º C at 15 lbs for 15 – 30 minutes in autoclave.

Plate preparation

25ml of pre autoclaved Muller Hinton agar (MHA) was poured into 90mm diameter pre sterilized petriplates and was allowed to solidify at room temperature.

Cup or hole well diffusion plate method

After the plates solidified the freshly prepared 24hrs microbial broth culture suspensions about 0.1ml was spreaded over the Muller Hinton agar (MHA) media using L-shaped sterilized glass spreader separately under aseptic condition using Laminar air flow (LAF).

Then four wells were made in each plate with the help of borer of 8mm diameter. In these well, about 0.1ml of each plant sample juice extracts were loaded and the antibiotics solutions 1mg/ml (0.1ml) were also loaded in the wells as reference. All tests were made in triplicate set.

This method depends upon the diffusion of the antibiotics or tested material (juices) from a vertical hole through, the solidified agar layer of a Petri dish to such an extent that growth of the added microorganism is prevented entirely in a circular area or zone around the hole containing a solution of tested material or antibiotics

The *agar well diffusion method* was used, 0.1ml of diluted inoculums (10^5 CFU/ml) of test organism was mixed in Muller Hinton agar media, shaken and poured in sterilized Petri plates. Wells of 8mm diameter were punched into the agar medium and filled with 50µl (200mg/ml) of the plants extracts (juices), solvent for blank or negative control.

Incubation

The Petri plates were incubated for 24 hours at 37º C in the incubator.

Measurement of zone of inhibition

After the incubation, the diameter of clear zone of inhibition produced around the well (or hole) were measured in mm and the diameter of inhibition by the juices extracts were compared with the reference antibiotics.

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Determination of minimum inhibitory concentration (MIC) of the juice extracts and the antibiotics

Minimum inhibitory concentration (MIC) level of any antimicrobial substance is the lowest concentration of the drug inhibiting the bacterial growth. The MIC value of those microorganisms against a particular fraction is considered which exhibit a maximum activity in preliminary screening process either by disc diffusion or by serial dilution.

The MIC of the juices and extracts were estimated by serial dilution method and compared with the MIC of the antibiotic taken as reference. The confirmatory test to find out the MIC, the two border lines dilution of visible and non-visible growth were sub-cultured in Nutrient agar media to observe the growth after incubation.

Antifungal activity of plant sample juices

Antifungal assay

Fungal infections are the most common among the human population and a number of therapeutic agents are also available in the market but most of them are effective as topical applications. Rare drugs are available for deep mucosal infection.

In this study, the antifungal activity was studied against following cultures.

- *Aspergillus niger* (MTCC 2479)
- *Aspergillus terreus* (MTCC 279)
- *Aspergillus japinicus* (MTCC 1975)
- *Penicillium expansum* (MTCC 2006)

The cultures were obtained from the standard cultures maintained in the Microbiology department of DIBNS, Dehradun. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25º C for about 72 – 96 hours and then stored at 4º C as stock cultures for further antibacterial activity. Fresh cultures were obtained by transferring a loopful of culture into Sabouraud Dextrose broth (SDB) and then incubated at 25º C for 72 hours. To test antifungal acivity, the Cup well diffusion method was used.

Culture media preparation

The microbiological media were prepared as standard instruction provided by the HI-MEDIA Laboratories PVT. Limited, Mumbai. The medium used for antifungal activity were SDA, SDB were prepared and sterilized at 121º C at 15 lbs for 15 – 30 minutes in autoclave.

Plate preparation

About 25ml to 30ml of pre autoclaved SDA was poured into 90mm diameter pre sterilized petriplates and was allowed to solidify at room temperature.

Cup or hole well diffusion plate method

After the plates solidified the freshly prepared 72hrs fungal broth culture suspensions about 0.1ml was spreaded over the SDA media using L-shaped sterilized glass spreader separately under aseptic condition using Laminar air folw.

Then four wells were made in each plate with the help of borer of 8mm diameter. In these well, about 0.1ml of each plant sample juice extracts were loaded and the antifungal drug solutions 1mg/ml (0.1ml) were also loaded in the wells as reference

Incubation

The Petri plates were incubated for 72 hours at 25º C in the incubator.

Measurement of zone of inhibition

After the incubation, the diameter of clear zone of inhibition produced around the well (or hole) were measured in mm and the diameter of inhibition by the juices extracts were compared with the reference antifungal agent (Ketacanazole).

RESULT AND DISCUSSION

Antibacterial activity

Juice extracts obtained from the *Allium sativum* and *Allium cepa,* and *Citrus limon* were assessed as singly and in combination for the inhibitory effects against selected bacterial pathogens namely *E. coli, Kleibsella spp, Staphylococcus aureus, Proteus spp, S. aureus* and *Bacillus cereus*. The antibiotic taken as references was; Chloroamphenicol.

Observations and discussion

Antibacterial activity of plant juice extracts

Applied singly (TABLE 1, & Figure 1)

Garlic juice extracts singly produced the highest zone of inhibition of *B. cereus* (51.33 ±1.24 mm) followed by *Micrococcus spp* (42.33 ±0.47mm), *S. aureus* (37.66 ±0.47 mm), *E. coli* (34± 0.81mm), *Kleibsella spp* (22.33 ±0.47 mm) and *Proteus spp*

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(18.66 ±0.47 mm). The order of bacteria which were inhibited more strongly by the garlic juice extracts:
B. cereus> *Micrococcus spp*> *S. aureus*> *E. coli*> *Kleibsella spp*> *Proteus spp*

From the observation, *B. cereus* was found to be the most sensitive bacteria to garlic juice and the garlic juice showed greater potency than the Chloroamphenicol which was used as references

TABLE 1 : Mean diameter (mm) and SD of zone of inhibition produced by the plant juice extracts (applied singly) against the bacteria

Name of bacteria	G	O	L	C	Most potent plant juice
E. coli	34 ± 0.81	ND	23.4 ± 1.35	21 ± 0.81	G (34 ± 0.81mm)
Kleibsella spp	22.33 ± 0.47	ND	12 ± 0.81	26 ± 0.81	G (22.33 ± 0.47mm)
Proteus spp	18.66 ± 0.47	ND	ND	21.33 ± 0.47	G (18.66 ± 0.47mm)
S. aureus	37.66 ± 0.47	ND	37.66 ± 0.0	12.33 ± 0.47	L (37.66 ± 0.0mm)
B. cereus	51.33 ± 1.24	20.33 ± 2.03	21.66 ± 0.47	32.66 ± 0.81	G (51.33 ± 1.24mm)
Micrococcus spp	42.33 ± 0.47	ND	29.33 ± 0.47	52 ± 0.81	G (42.33 ± 0.47mm)
Most Sensitive bacteria	*B* (51.33± 1.24 mm)	*B* (20.33± 2.03 mm)	*S* (37.66± 0.94 mm)	*M* (52± 0.8 mm)	The most sensitive bacteria *B. cereus* and potent plant juice G

G = Garlic, O = Onion, L = Lemon, C = Chloroamphenicol (reference), ND = Not detected, SD = Standard deviation, B = B. cereus, S = S. aureus, M = Micrococcus sp, E = E. coli

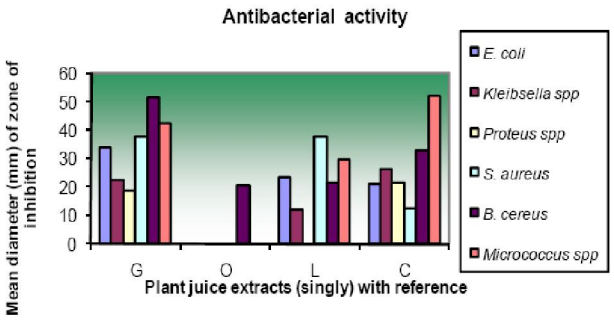

Figure 1 : Antibacterial activity of plant juice extracts (applied singly) and the antibiotics against the selected bacteria.

(32.66± 0.81mm) against the bacteria

The author Cavallito C.J. & Bailey J.H.,[9] described that garlic contains the antimicrobial agent; allicin (sulphur containing compound), inhibits the growth of both gram negative and gram positive bacteria.

Onion juice extracts could only inhibit *B. cereus* (20.33 ±2.03mm) and other all bacteria did not show the zone of inhibition denoted by ND (not detected). Onion produced less zone of inhibition than the chloroamohenicol.

Lemon juice extracts showed the highest zone of inhibition of *S. aureus* (37.66 ±0.94 mm) followed by *Micrococcus spp* (29.33 ±0.47 mm). The order of bacteria which were inhibited more strongly by the lemon juice extracts:
S. aureus> *Micrococcus spp*> *E. coli*> *B. cereus*> *Kleibsella spp*

The result agrees with the claim made by Elestin K.L; 1997, according to the author, lemon juice and oil are effective in killing germs and it is taken as prevention of stomach infection.

Observations and discussion

Antibacterial activity of plant juice extracts Applied in combination (TABLE 2, Figure 2)

The garlic & onion juice combination (GO) produced the highest zone of inhibition of *Micrococcus spp* (40.33±0.47mm) followed by *B. cereus* (36±0.0mm), The reference- chloroamphenicol showed the higher inhibitory effect than this combination against the *B. cereus*. The order of bacteria which were inhibited more strongly by the GO combination:
Micrococcus spp> *B. cereus*> *S. aureus*> *E. coli*> *Kleibsella spp*> *Proteus spp*

From the observation, GO combination inhibits *Micrococcus spp* more strongly than the *B. cereus* although *B. cereus* was inhibited strongly by garlic when applied singly.

The garlic & lemon juice combination (GL) showed the highest zone of inhibition of *Micrococcus spp* (33.33±0.47mm) followed by *B. cereus* (30.66±0.47mm). The order of bacteria which were inhibited more strongly by the GL combination:
Micrococcus spp>*B. cereus*> *S. aureus*> *E. coli*> *Proteus spp*> *Kleibsella spp*

In GL combination, all bacteria, except *Proteus spp*, are less sensitive than the GO combination. The

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author Onyeagba *et al*. 2000 expained that the zone of inhibition produced by the aqueous extracts of garlic (*Allium sativum* Linn) and lime (*Citrus aurantifolia* Linn) combination against *Bacillus spp, Staphylococcus aureus* and *E. coli* were 17mm, 17mm and 9mm respectively and the ethanolic extracts of the same combination gainst the respective bacteria were 13mm, 19mm and 11mm.

The onion & lemon juice combination (OL) produced the highest zone of inhibition of *E. coli* (22.33 ± 0.47mm) followed by *S. aureus* (20.66 ± 0.47mm),

Combination of all three juice extracts- garlic, onion & lemon (GOL) showed the highest zone of inhibition of *Micrococcus spp* (38±0.0mm) followed by *B. cereus* (32.66±0.47mm), The order of bacteria which were inhibited more strongly by the GOL combination:

Micrococcus spp>*B. cereus*> *S. aureus*> *E. coli*> *Proteus spp*> *Kleibsella spp*

In the case of combination of garlic with other plant extracts Onyeagba *et al*. 2000 reported that the three aqueous extracts combination of garlic (*Allium sativum* Linn), lime(*Citrus aurantifolia*, Linn) and ginger (*Zingiber officinale* Roscoe) produced the zone of inhibition 13mm, 17mm and 9mm against *Bacillus spp, Staphylococcus aureus* and *E. coli*. And the ethanolic extracts combination of garlic (*Allium sativum* Linn), lime(*Citrus aurantifolia*, Linn) and ginger (*Zingiber officinale* Roscoe) produced the zone of inhibition 13mm and 13mm against *Bacillus spp, Staphylococcus aureus* and this combination could not inhibit *E. coli*.

TABLE 2 : Mean diameter (mm) and SD of zone of inhibition produced by the plant juice extracts (applied in combination) against the bacteria

Name of bacteria	GO	GL	OL	GOL	C	Most potent plant juice combination
E. coli	22.66 ± 0.47	27.66 ± 0.47	22.33 ± 0.47	23.33 ± 0.47	21 ± 0.81	GL (27.66 ± 0.47 mm)
Kleibsella spp	18.33 ± 1.24	16.33 ± 0.47	ND	15 ± 0.0	26 ± 0.81	GO (18.33 ± 1.24 mm)
Proteus spp	17 ± 0.81	19.33 ± 0.47	ND	17.66 ± 1.24	21.33 ± 0.47	GL (19.33 ± 0.47 mm)
S. aureus	27.66 ± 0.47	30 ± 0.0	20.66 ± 0.47	23.66 ± 0.47	12.33 ± 0.47	GL (30 ± 0.0mm)
B. cereus	36 ± 0.0	30.66 ± 0.47	16 ± 0.81	32.66 ± 0.47	32.66 ± 0.81	GO (36 ± 0.0mm)
Micrococcus spp	40.33 ± 0.47	33.33 ± 0.47	18.6 ± 0.47	38 ± 0.0	52 ± 0.81	GO (40.33 ± 0.47 mm)
Most Sensitive bacteria & its zone of inhibition	*M*(40.33 ± 0.47 mm)	*M*(33.33 ± 0.47 mm)	*E* (22.33 ± 0.47 mm)	*M* (38 ± 0.0 mm)	*M* (52 ± 0.81 mm)	Most sensitive bacteria *Micrococcus spp* & potent combination GO

M = Micrococcus spp, E = E. coli

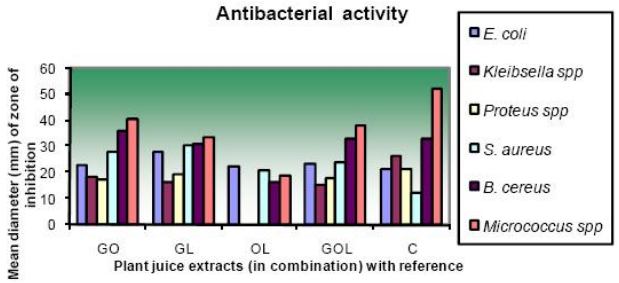

Figure 2 : Plant juice extracts (applied in combination)

Observations and discussion (TABLE 3, Figure 3)

The minimum inhibitory concentration (MIC) of the garlic juice extracts was found to be 35 µl/ml against *E. coli* while for onion juice 180 µl/ml and MIC of chloroamphenicol was found to be 155 µg/ml.. The order of all three juice extract having low MIC value against *E. coli*

Garlic> Lemon.

The order of all three juice extract having low MIC value against *B. cereus*

Garlic> Lemon> Onion.

TABLE 3 : Minimum inhibitory concentration (MIC) of the plant juice extracts against the selected bacteria with reference chloramphenicol

Name of bacteria	G	O	L	C
E. coli	35 µl/ml	ND*	50 µl/ml	155 µg/ml
B. cereus	25 µl/ml	180 µl/ml	55 µl/ml	80 µg/ml

ND*= Due to high MIC value, it could not be detected.

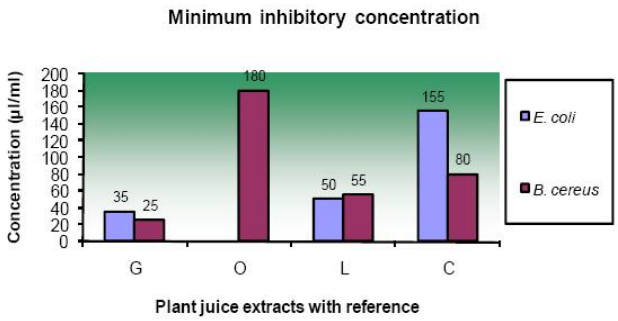

Figure 3 : (MIC) of the plant juice extracts with reference

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Antifungal activity

The antifungal agent taken as reference - Ketacanazole.

Discussion

Antifungal activities the plant juice extracts

Applied singly (TABLE 4, Figure 4)

Garlic juice extracts produced the highest zone of inhibition of *A. terreus* (45.33±0.47 mm) followed by *P. expansum* (22±0.81 mm), The order of fungi which were inhibited more strongly by the garlic juice extracts: *terreus> P. expansum> A. niger> A. japinicus*

The first published evidence by Schmidt and Marquardt, 1936 demonstrated the extraordinary fungistatic and fungicidal action of freshly pressed garlic juice and dried garlic with epidermophyte cultures . In 1960, several workers carried out some model experiments with various yeast strains (*Saccharomyces cerevisiae, S.ellipsoideus, S.carlsbergensis*) and enzymatically-produced allicin. Effectiveness against *Aspergillus parasiticus, Aspergillus ochraceus, Penicillum patulum, P.roqueforti*, and *P.citrinum* has also been reported. *Saccharomyces cerevisiae, Candida albicans, Microsporum canis, Trichophyton*

TABLE 4 : Mean diameter (mm) and SD of zone of inhibition produced by the plant juice extracts (applied singly) against the fungi

Name of fungi	G	O	L	K	Most potent plant juice extracts
A. niger	20.66 ± 0.47	ND	ND	16.5 ± 0.5	G (20.66 ± 0.47mm)
A. terreus	45.33 ± 0.47	ND	16.33 ± 0.47	13.33 ± 0.47	G (45.33 ± 0.47mm)
A.japinicus	16 ± 0.0	ND	ND	14.33 ± 0.47	G (16 ± 0.0mm)
P. expansum	22 ± 0.81	ND	15.66 ± 0.94	22.66 ± 0.94	G (22 ± 0.81mm)
Most Sensitive Fungi	(45.33 ± 0.47 mm)	ND	(16.33 ± 0.47mm)	(22.66 ± 0.94mm)	Most sensitivefung *A. terreus* and potent plant juice G

G = Garlic, O = Onion, L = Lemon, K = Ketacanazole (reference), ND = Not detected, At = A. terreus, Pe = P. expansum

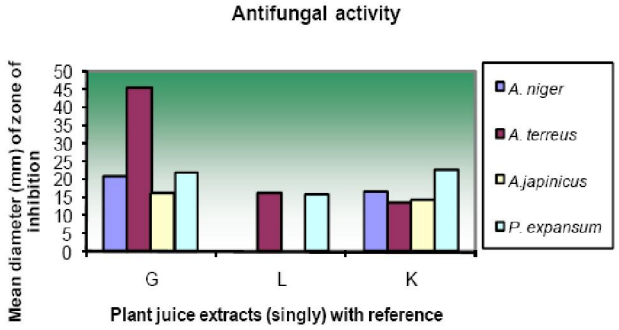

Figure 4 : Plant juice extracts (applied singly) against the selected fungi.

mentagrophytes, and *T.rubrum* are further species responding to garlic[6].

Onion juice extracts could not produce the significant zone of inhibition against any fungal strains.

Shelef, L.A., 1983, suggested that onion has inhibitory effect against *Aspergillus flavis* and *Aspergillus parasiticus* in higher concentration. However all selected fungi were found to be resistant to the onion, it might be due to less concentration of juice applied. In contrast to garlic, fungi are less sensitive to lemon although the lemon had higher inhibitory effects against *A. terreus* than the effect shown by the ketacanazole.

Observations and discussion

Antifungal activities the plant juice extracts

Applied in combination (TABLE 5, Figure 5)

The combination of garlic & onion juice (GO) pro-

TABLE 5 : Mean diameter (mm) and SD of zone of inhibition produced by plant juice extracts (in combination) against the fungi

Name of fungi	GO	GL	OL	GOL	K	Most potent plant juice combination
A. niger	ND	ND	ND	ND	16.5 ± 0.5	ND
A. terreus	ND	ND	ND	ND	13.33 ± 0.47	ND
A. japinicus	16.66 ± 0.47	ND	ND	ND	14.33 ± 0.47	GO(16.66 ± 0.47)
P. expansum	ND	18.66 ± 0.94	ND	ND	22.66 ± 0.94	GL (18.66 ± 0.94 mm)
Most Sensitive Fungi	*Aj* (16.66 ± 0.47mm)	*Pe* (18.66 ± 0.94mm)	ND	ND	*Pe* (22.66 ± 0.94mm)	Most sensitive fungi *P. expansum* and potent combination GL

Aj = A. japinicus, Pe = P. expansum

Full Paper

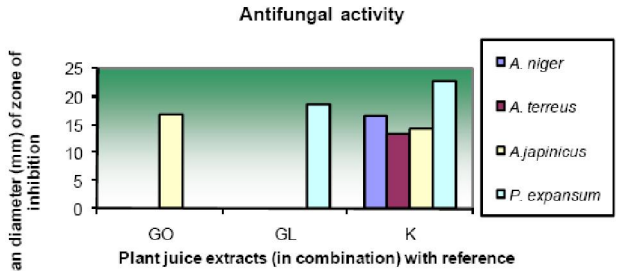

Figure 5 : (Applied in combination) against the selected fungi.

duced zone of inhibition only against *A. japinicus* (16.66±0.47 mm). This combination was found to be more potent against *A. japinicus* than the ketacanazole. Other fungi were not inhibited by this combination. The combination of garlic & lemon juice (GL) showed inhibitory effect only against *P. expansum* (18.66±0.94 mm).

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