Antifungal studies in the plant extracts of Turmeric, Ginger, Onion and Garlic

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Abstract:-

Garlic, onion, ginger and turmeric are very common spices which are frequently used in preparation of food. These are herbal spices. The herb is a plan which has medicinal or aromatic qualities. Extracts taken from various parts like roots, fruits, or stems or essential oils of herbal plants perform important biological functions in our body. They can defend against attack from predators such as insects, fungi and herbivorous mammals i.e they have antioxidant and antimicrobial potential. This nature is due to different types of chemical substances present in the herbal plant which act upon the body.

In the present study , the inhibitory effects *in vitro* of these selected plant juice extracts taken singly & in combination, were studied against selected fungi. Selected four fungi are *A. niger, A. terreus, A.japinicus* and *P. expansum*. On the basis of observation we found that fungi are strongly inhibited by the garlic juice than turmeric, ginger, and other juice extracts and almost all fungi could not be inhibited be onion. In comparison to reference antifungal agent (Ketacanazole), garlic & ginger showed effective inhibition against selected fungi. Combination of these juice extracts also have shown antifungal activity.

Keyword: Herb, Allium cepa, allium sativum, Citrus aurentifolia, Extract.fungi.

INTRODUCTION:-.

Herbal plants are being used for curing many diseses since ancient time. 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. These phytochemicals have beneficial health effects when they are consumed by humans for longer time and they can be used to effectively treat human diseases(*Lai PK, Roy J; Roy (June 2004)*. More than 12,000 such compounds have been isolated so far. These phytochemicals are divided into two categories primary & secondary metabolites. Carbohydrates and fats which are found in all plants are under primary, while compounds which are found in a smaller range of plants, serving a more specific function are secondary metabolites like toxins,pigments and pheromones.Toxins are used to deter predation and pheromone are used to attract insects for pollination. It is the secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs .For examples quinine from the cinchona, morphine and codeine from the poppy are used in making drugs. (*Tapsell LC, Hemphill I, Cobiac L; et al. (August 2006).* Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal

medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects. *(Cai, Yun; Wang, Rui; Pei, Fei; Liang, Bei-Bei (2007)).*

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 known to people of ancient culture throughout the world and they were valued their preservative and medicinal powers.(Lawson L. D., Abrams G; 1998). 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 (Tessema, B; Mulu, A; Kassu, A; Yismaw, G (2006*).* 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. 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. Alcohol extracts show hypotensive analgesic and antileptazol properties. Onion extracts shows antibacterial properties. (Lawson L. D, Abrams G; 1998) .

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). **(Amagase H., Milner J.; 1993)**

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. **(Block E.; 1985) .Ra**w garlic is very strong, so eating too much could produce problems.. Symptoms of garlic allergy include skin rash, temperature and headaches. (**Lembo G., *et al;* 1991)** .

Onion (*Allium cepa)* *Allium cepa* L., also called the **garden onion**. 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.. **(Jandke J., *et al;* 1987)**

Lemon *(Citrus limon)*

Lemon Peel Oil consists mainly of terpenes, particularly limonene, also gamma terpinene and beta-phellandrene. There are small amounts of sesquiterpenes and aldehydes. **(Sies, H; 1995)**

Ginger- *Zingiber officinal* is a flowering plant in the family Zingiberaceae whose rhizome, **ginger root** or simply **ginger**, is widely used as a spice . The characteristic odor and flavor of ginger is caused by a mixture compounds of zingerone shogaols and gingerols (***Wood, C. (1988).***

Turmeric(*Curcuma longa)-* Turmeric is a rhizomatous herbaceous plant of the ginger family, The most important chemical components of turmeric are a group of compounds called curcuminoids which include curcumin (diferuloylmethane), demethoxycurcumin and bisdemethoxycurcumin..**(*Tayyem RF etal,2006)*** In addition, other important volatile oils include turmerone, atlantone, and zingiberene. Some general constituents are sugars, proteins, and resin.**(*Mishra S, 2008.)***

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. *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 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. **(Paniker C.K.J; 2005).**

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. **(Deshpande, S. D., and Koppikar; 1999)**

MATERIAL & METHODS-

Sampling -

About 500g of each plant samples were taken for this study. Collection of plant samples (garlic, onion *, Ginger, turmeric &* lemon) was done from the mandi market of Dehradun, Uttarakhand, India.

Preservation of samples- The samples were kept in refrigerator at 4º C in microbiology lab of Uttaranchal University.

Processing of Samples- First plant juice was extracted for antimicrobial activity.

At first, skins of the garlic, turmeric, ginger 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.while fruit parts of lemon were washed with sterilized 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, ginger, turmeric, 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 antifungal solutions

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

.Antifungal activity of plant sample juices

. Antifungal assay- In this study, the antifungal activity was studied against, *Aspergillus niger* (MTCC 2479), *Aspergillus terreus* (MTCC 279), *Aspergillus japinicus* (MTCC 1975)and *Penicillium expansum* (MTCC 2006).The cultures were obtained from the standard cultures maintained in the Microbiology lab of Uttaranchal University, 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 & DISCUSSION-

Antifungal activity-The antifungal agent taken as reference - Ketacanazole.

Table.1.a. 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	T	L	Gi	K	Most potent plant juice extracts
A. niger	20.66±0.47	ND	21.16±0.11	ND	ND	16.5±0.5	G (20.66±0.47mm)
A. terreus	45.33±0.47	ND	43.1±0.27	16.1±0.2	18.2±0.47	13.33±0.47	G (45.33±0.47mm)
A.japinicus	16±0.0	ND	14±1.0	ND	ND	14.33±0.47	G (16±0.0mm)
P. expansum	22±0.81	ND	23±0.1	15.9±0.1	16.66±0.2	22.66±0.94	G (22±0.81mm)
Most Sensitive Fungi	*A.t*(45.33±0.47 mm)	ND	*A.t*(43.1±0.27 mm)	*A.t*(16.1±0.2 mm)	*A.t* (18.2±0.47mm)	*P.e*(22.66±0.94mm)	Most sensitive fungi *A. terreus* and potent plant juice **G**

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

Table 2.a 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	17.5±0.2	ND
A. terreus	ND	ND	ND	ND	14.31±0.21	ND
A. japinicus	16.66 ±0.47	ND	ND	ND	14.83±0.11	GO(16.66±0.47)
P. expansum	ND	18.66 ±0.94	ND	ND	23.11 ±0.24	GL (18.66± 0.94 mm)
Most Sensitive Fungi	*Aj* (16.66 ±0.47mm)	*Pe* (18.66 ±0.94mm)	ND	ND	*Pe* (23.11 ±0.24mm)	Most sensitive fungi *P. expansum* and potent combination **GL**

Aj= A. japinicus, Pe= P. expansum

Discussion: Antifungal activities the plant juice extracts - applied singly (Table 1.a, Figure.1.a)

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 follows as:

A. terreus> P. expansum> A. niger> A. japinicus

Turmeric and Ginger extracts also produced the highest zone of inhibition against *A. terreus.*

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 mentagrophytes*, and *T.rubrum* are further species responding to garlic. **(Hughes & Lawson; 1991).**

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 & discussion: Antifungal activities the plant juice extracts - applied in combination (Table 2a, Figure 2b)

The combination of **garlic & onion** juice **(GO)** produced 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).

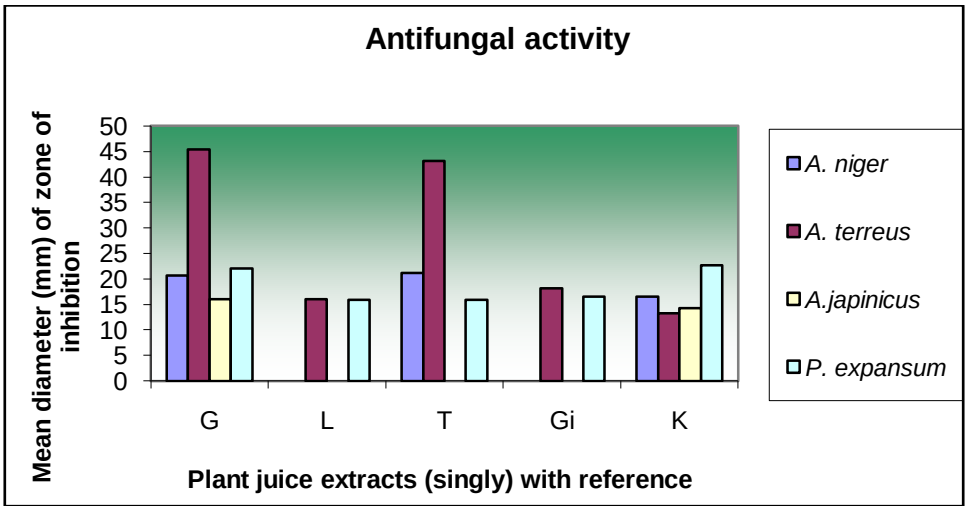

Figure 1.b: plant juice extracts (applied singly) against the selected fungi.

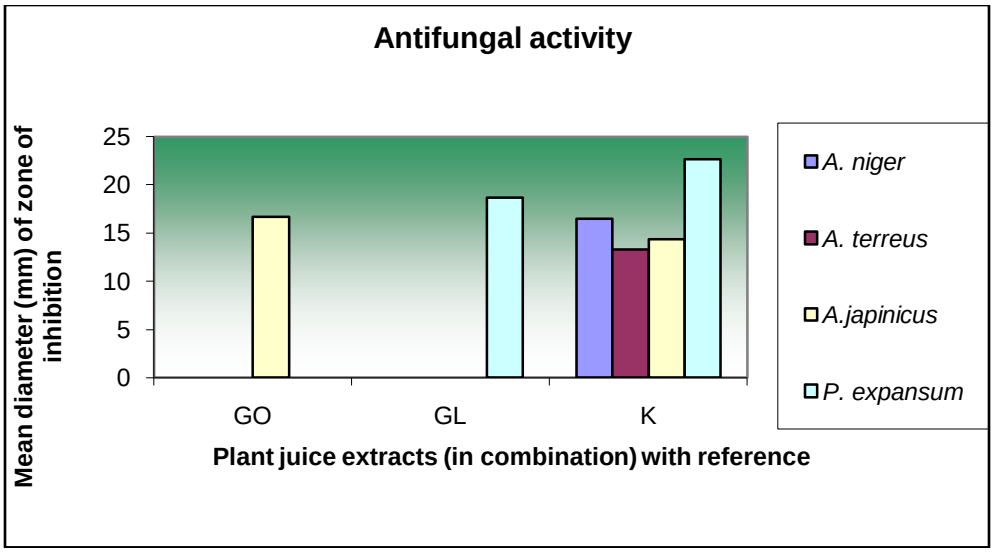

Figure 2b: Antifungal activity (applied in combination)

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