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Research Article

ANTIMICROBIAL ACTIVITY OF SOME SPICES AGAINST SELECTED MICROBES

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ABSTRACT

The aim of the study is to assess the antimicrobial activity and to determine the Minimum Inhibitory Concentration (MIC) of various spice extract on some bacterial and fungal strains. The antimicrobial activity of alcoholic and aqueous extracts of asafoetida, ginger, cinnamon and cardamom extract was tested against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicians* & *P. chrysogenum* by agar well diffusion method. The crude extract showed a broad spectrum of antimicrobial activity by inhibiting both the groups of bacteria and fungus. Agar well diffusion assay for antimicrobial activity yielded the inhibitory zone of 16 to 34 mm diameter for asafoetida, 12 to 18mm diameter for cinnamon, 15 to 35mm diameter for ginger and 13 to 21 mm for cardamom extracts. The MIC value ranged between 12.5 mg/ml to 3.125 mg/ml with an exception of cinnamon alcohol extract against *E. coli* for which the calculated MIC was 25mg/ml.

Keywords: Antimicrobial, Spices, Agar well diffusion, Minimal inhibitory concentration

INTRODUCTION

Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents with antimicrobial properties. Herbal medicines are increasingly used as dietary supplements for treatment against different human disorders. Spices can be defined as "any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms, that contribute flavor, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages"[1].

Herbs and spices have been used for thousands of years to enhance the flavor, colour and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative[2] and medicinal value, which forms one of the oldest sciences[3] A large number of plants are used to combat different diseases[4,5] and possess antimicrobial activity[6-14]. Several spices particularly garlic, black pepper, clove, ginger, cumin, cardamom, cinnamon and caraway are used extensively in the Indian diet and in Indian medicine. Garlic with its antimicrobial properties is widely used for a number of infectious diseases. Eugenol, an active principle of clove is used as an antiseptic and possesses local anesthetic activity; it is therefore used for toothache[15]. Use of spices for medical benefits can be justified as these are easily absorbed by our body and generally do not have any adverse effects.

MATERIALS AND METHODS

Spice samples and extract preparation

Asafoetida, ginger, cinnamon and cardamom were bought from the local market of Kanpur.

Extracts were prepared by the method of Clarkson and Bibby, 1969[16]. Both water and alcohol extracts of spices were used. Water extracts were made by extracting 5 gm of ground spice in 100 ml distilled water in a Soxhlet extraction apparatus for four hours at 100° C. To prepare alcohol extracts 5 gm of ground spice was added to 100 ml of absolute alcohol and agitated at room temperature for eight hours in a wrist-action shaker. Thereafter, the mixture was allowed to stand for 12 hours; the alcohol evaporated without heat, and the residue was mixed with 100 ml of distilled water at 80° C.

Test-microorganisms

The following microorganisms*: B. subtilis* (MTCC 121), *S. aureus* (MTCC 96), *E. coli* (MTCC 739), *P. aeruginosa* (MTCC 2453), *C. albicians* (MTCC 3017) & *P. chrysogenum* (MTCC 160) were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24h at 37°C on Nutrient Agar and Potato Dextrose Agar medium (Hi Media, Mumbai, India) respectively following refrigeration storage at 4°C .

Preparation of inoculum

A loopful of inoculum was taken from a pure culture of the respective bacteria/fungus inoculated into 10 ml of Muller Hinton broth (Hi Media, Mumbai, India), respectively. The broth suspension was then incubated at 37°C for 3 h. The growth so obtained was used as inoculum for the sensitivity assay.

Antimicrobial activity test

Agar well diffusion method[17] and microbroth dilution[18] methods. From the primary isolation medium 2-3 colonies of investigated micro-organism were taken by flamed loop, suspended in Mueller-Hinton broth, and they were incubated at 37°C. The suspension for inoculation was prepared from the broth cultures. The number of cells in 1 ml of suspension for inoculation measured by the McFarland nephelometer was 1 X 10^7 cfu/ml. A volume of 1 ml of this suspension was homogenized with 9 ml of melted (45°C) Mueller-Hinton & Nutrient agar and poured into Petri dishes. For screening, wells of 8mm diameter were punched out of the solid media and 100 µl of extract at concentration of 50 mg/ml, 37.5mg/ml, 25mg/ml and 12.5mg/ml were used in the wells. After incubation for 24-48 hours, Zone of inhibitions were measured and expressed in mm.

The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the extracts capable of inhibiting the growth of the bacterium tested. The MIC was determined by the broth microdilution test using an inoculum of 1x10^7 cfu/ml in a 96 well microtiter plate. Final concentrations of the investigated extracts were 50 mg/ml, 37.5mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. Both tests were done in triplicates. Gentamycin (10 mcg), amoxicillin (10 mcg) and fluconazole (10 mcg) were used as positive controls.

RESULTS AND DISCUSSION

The antimicrobial activities of alcoholic and aqueous extracts were determined. Antimicrobial activity detected at various concentrations was found to be a linear function of concentration.

Figure 1-4 shows the antimicrobial activity of alcoholic and aqueous extract of asafoetida against selected microbes on nutrient agar and Muller Hinton agar medium. Alcoholic extract of asafoetida shows better activity against *P. chrysogenum, S. aureus* and *E. coli* whereas the aqueous extract shows maximum inhibitory effect against the fungal strain of *C. albicans.* Alcoholic extract (24-34 mm inhibitory zone) of asafoetida yielded better results than aqueous extract (16-25 mm inhibitory zone). The results are in accordance with Thyagaraja et al., 1996[19] who had established the inhibitory effect of asafoetida against food spoilage fungi.

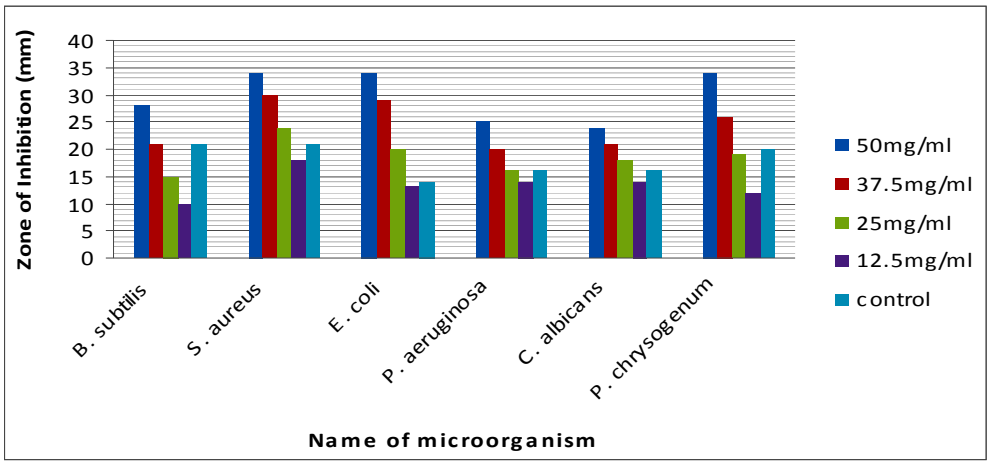

Fig. 1: Antimicrobial activity of Asafoetida Alcohol extracts (N.A.).

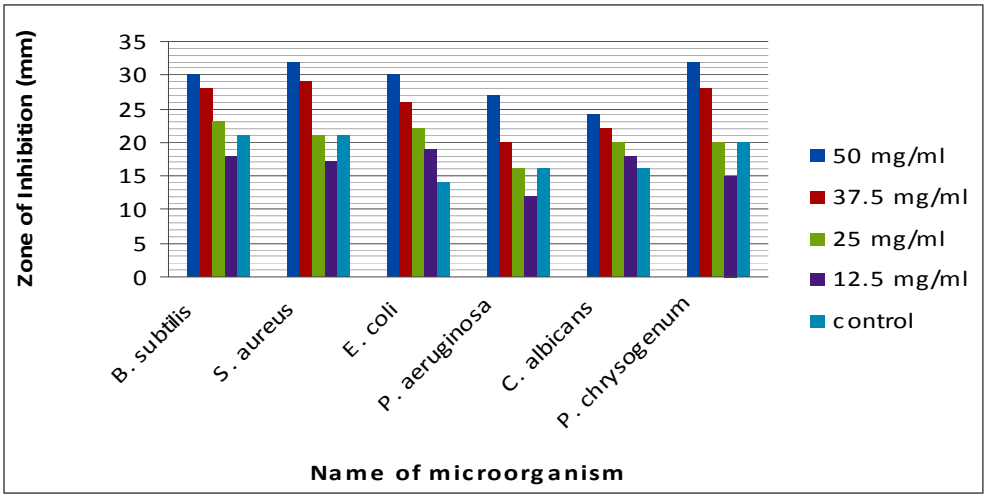

Fig. 2: Antimicrobial activity of Asafoetida alcohol extract (M.H.A.)

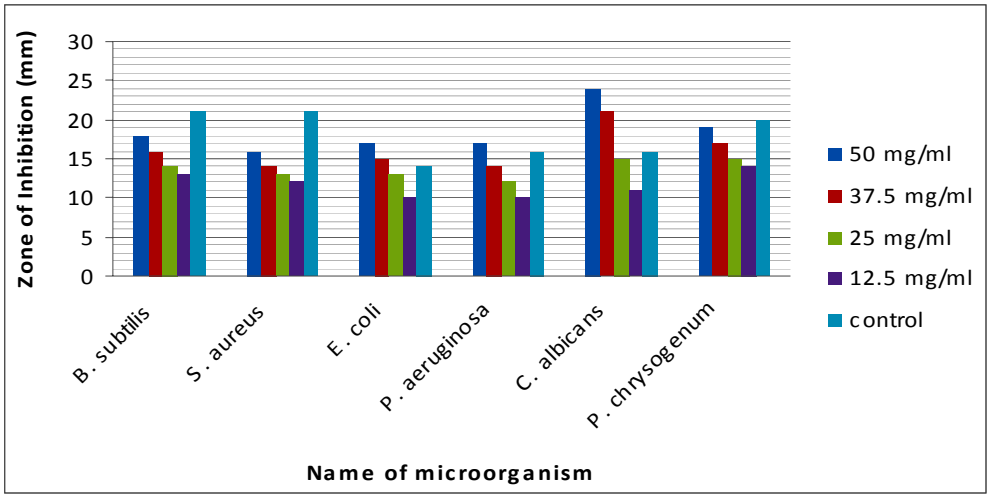

Fig. 3: Antimicrobial activity of Asafoetida aqueous extract at (N.A.).

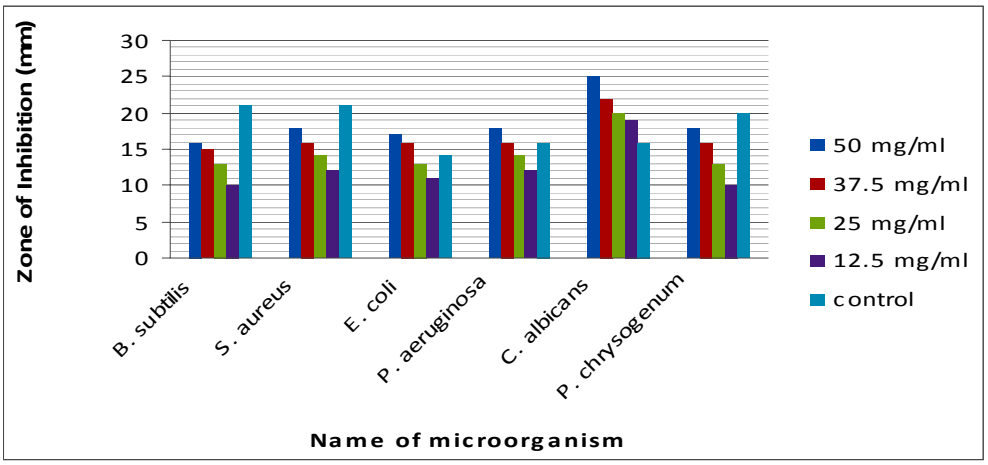

Fig. 4: Antimicrobial activity of Asafoetida aqueous extract at (M.H.A.).

Cinnamon has been traditionally reported to be of high medical importance. It has been reported to increases the hydroxyproline content in tissues, which is reduced in degenerative diseases like osteoarthritis[20]. Some of the constituents of cinnamon have proven value against bacteria and fungi, including the molds that produce the carcinogenic aflatoxins[21-23]. Figure 5-8 shows antimicrobial activity of the alcoholic and aqueous extracts on nutrient agar and Muller Hinton agar medium against selected microbes. Both alcoholic and aqueous extracts of cinnamon were effective and showed inhibitory zone in the range of 12-18mm. Aqueous extract (14-18 mm inhibitory zone) of cinnamon yielded better results than alcoholic extract(12-16 mm inhibitory zone).

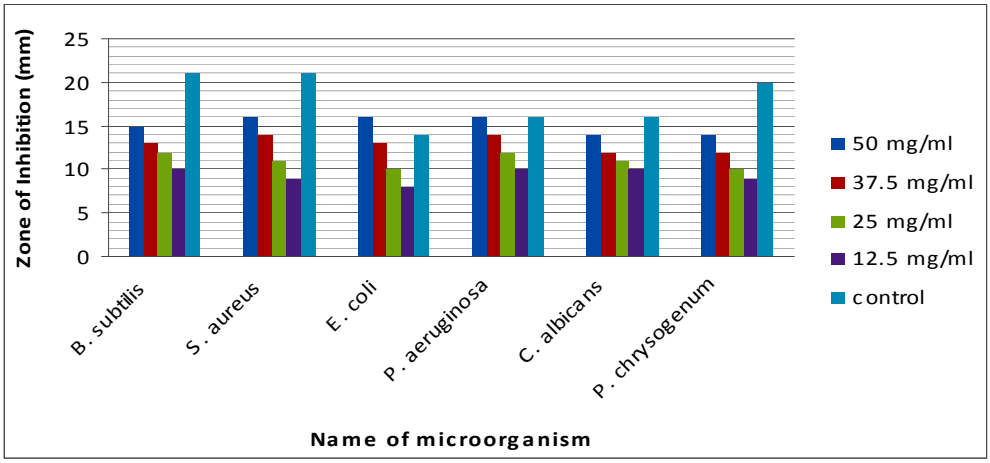

Fig 5: Antimicrobial activity of Cinnamon Alcohol extract at (N.A.).

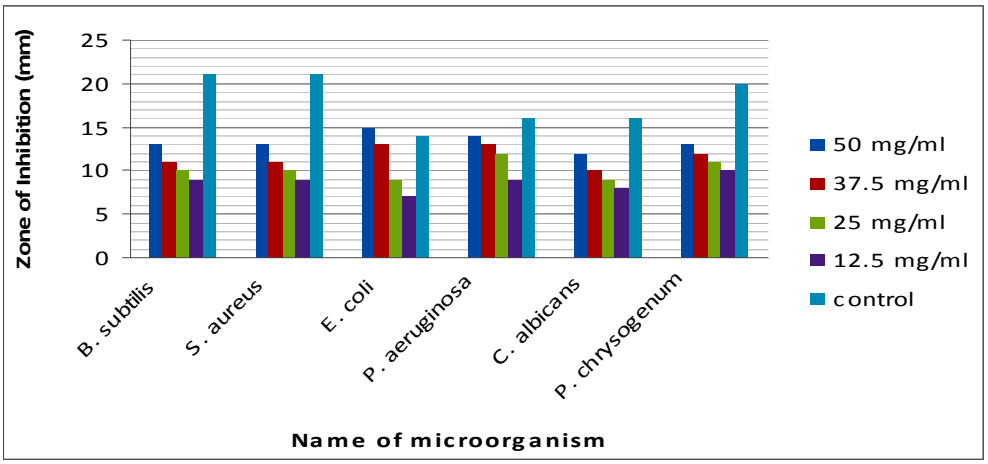

Fig. 6: Antimicrobial activity of Cinnamon Alcohol extract at (M.H.A.).

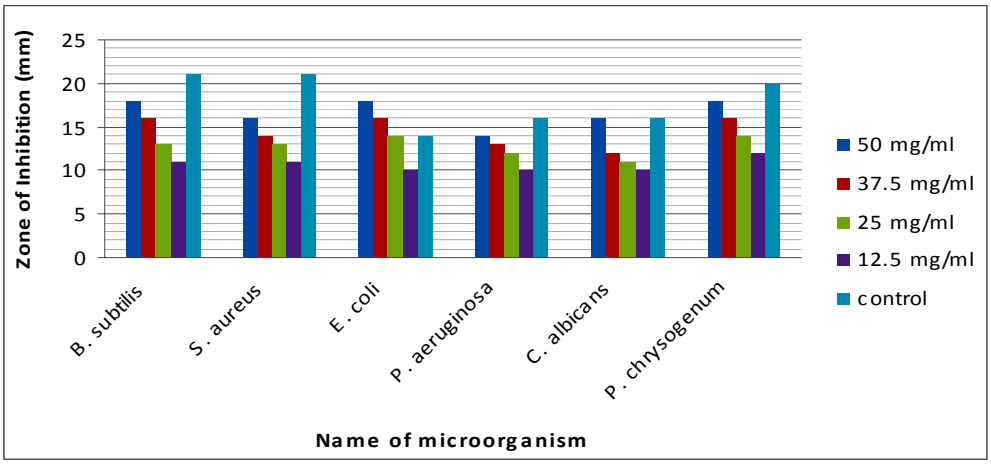

Fig. 7: Antimicrobial activity of Cinnamon aqueous extract at (N.A.).

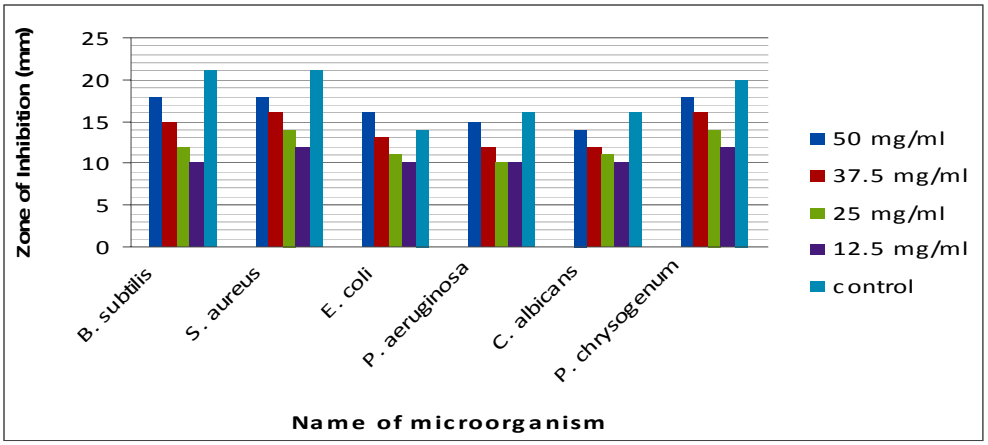

Fig. 8: Antimicrobial activity of Cinnamon aqueous extract at (M.H.A.).

Figure 9-12 shows antimicrobial activity of the alcoholic and aqueous extract of ginger against selected microbes on nutrient agar and Muller Hinton agar medium. Ginger showed optimum activity against the microbial spices of *E. coli*, *P. aeruginosa* and *C. albicians.* Alcoholic extract (23-35 mm inhibitory zone) of ginger yielded better results than aqueous extract (15-21 mm inhibitory zone). The activity shown here may be argued to the presence of [6]-gingerol, an active ingredient of ginger (Zingiber officinale*) Masuda* et al., 2004[24] have determined the structures of more than 50 antioxidants isolated from the rhizomes of ginger and have reported gingerol to possess substantial antioxidant and anti-inflammatory activity. It has potent anti-angiogenic activity in vitro and in vivo[25]. Our results compare well with previous observations[26,27].

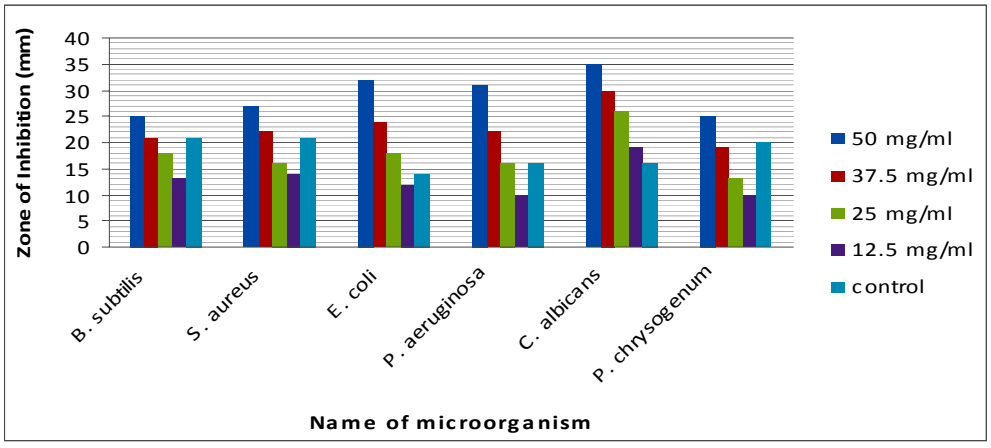

Fig. 9: Antimicrobial activity of Ginger alcohol extract at (N.A.).

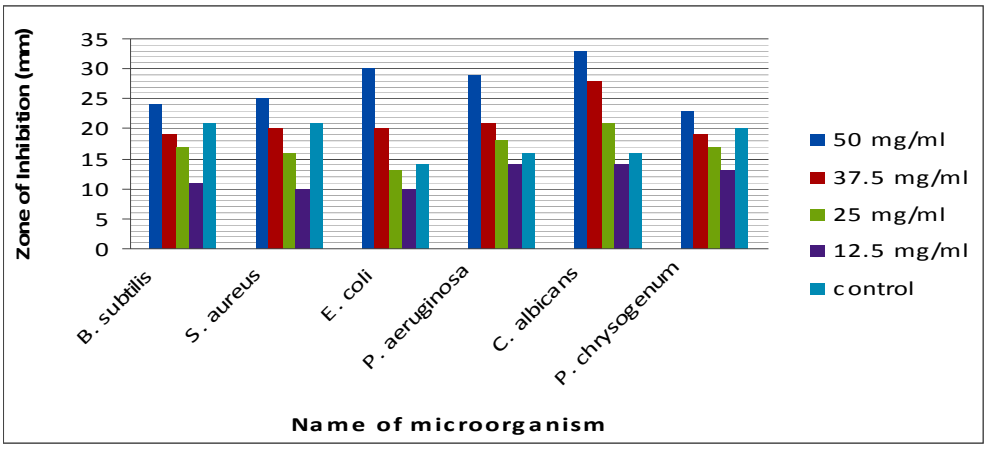

Fig. 10: Antimicrobial activity of Ginger alcohol extract at (M.H.A.).

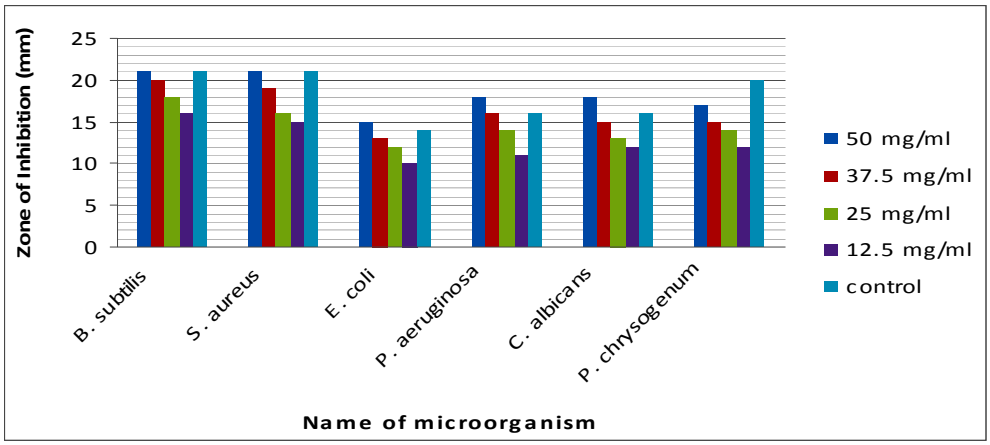

Fig. 11: Antimicrobial activity of Ginger aqueous extract at (N.A.).

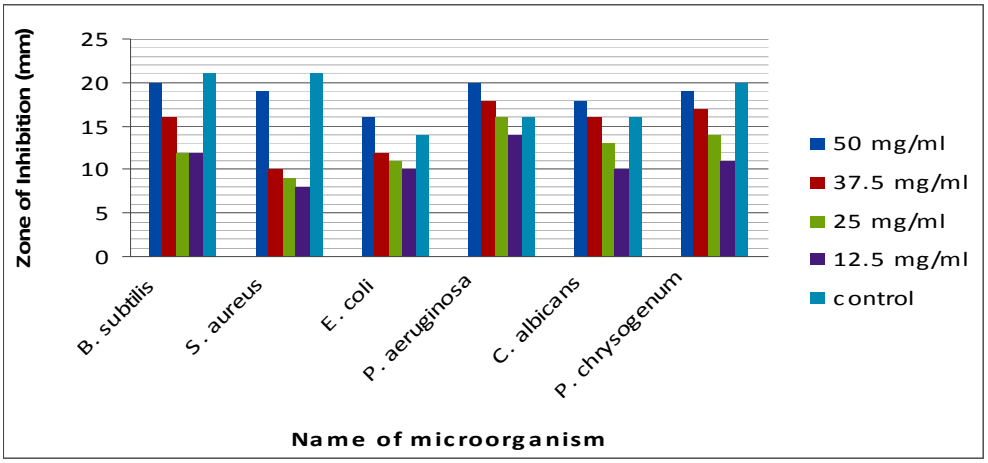

Fig. 12: Antimicrobial activity of Ginger aqueous extract at (M.H.A.).

Figure 13-16 shows the antimicrobial activity of the alcoholic and aqueous extract of cardamom against selected microbes on nutrient agar and Muller Hinton agar medium. Cardamom shows maximum inhibitory activity against *B. subtilis* and *S. aureus* (21 mm inhibitory zone) and least activity against *P. aeruginosa* and *C. albicans.* Both alcoholic (13-20 mm inhibitory zone) and aqueous extract (14-21 mm inhibitory zone) of ginger shows similar inhibitory activity against the selected microbes. The results compare well with the findings of Jazila El Malti et al., 2007[28] have demonstrated the antimicrobial activity of cardamom against both Gram-positive and Gram-negative bacterial species. Agaoglu et al., 2007[29] have also reported the antimicrobial effects of seed extract of cardamom on some microorganisms including pathogens such as *M. smegmatis, K. pneumonia, S. aureus, E. coli, E. faecalis, M. luteus and C. albicans.*

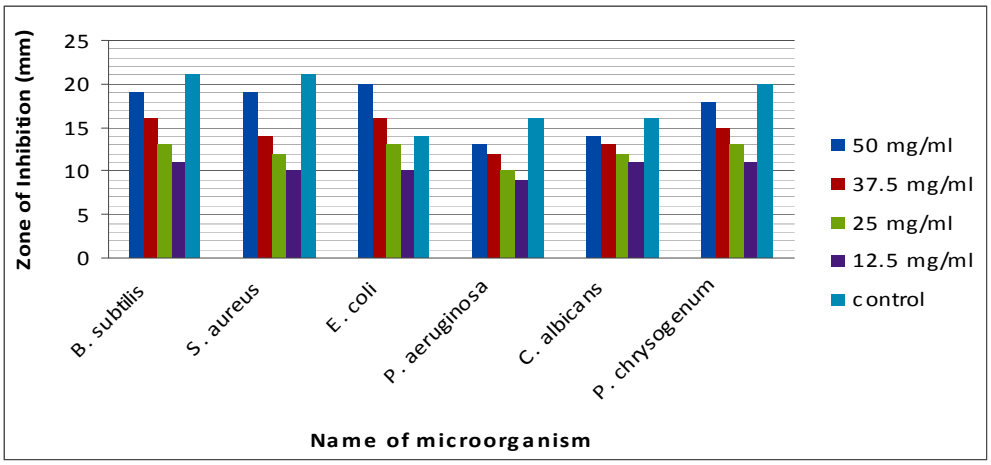

Fig. 13: Antimicrobial activity of Cardamom alcohol extract at (N.A.).

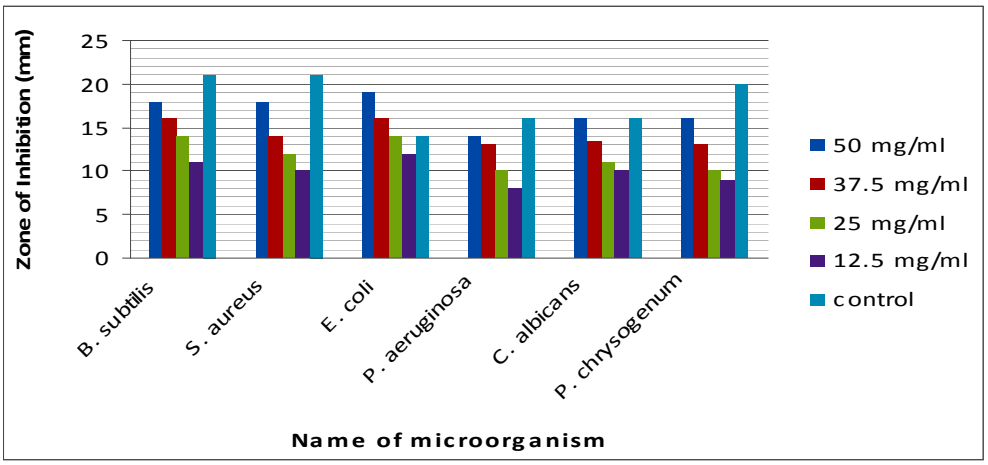

Fig. 14: Antimicrobial activity of Cardamom alcohol extract at (M.H.A.).

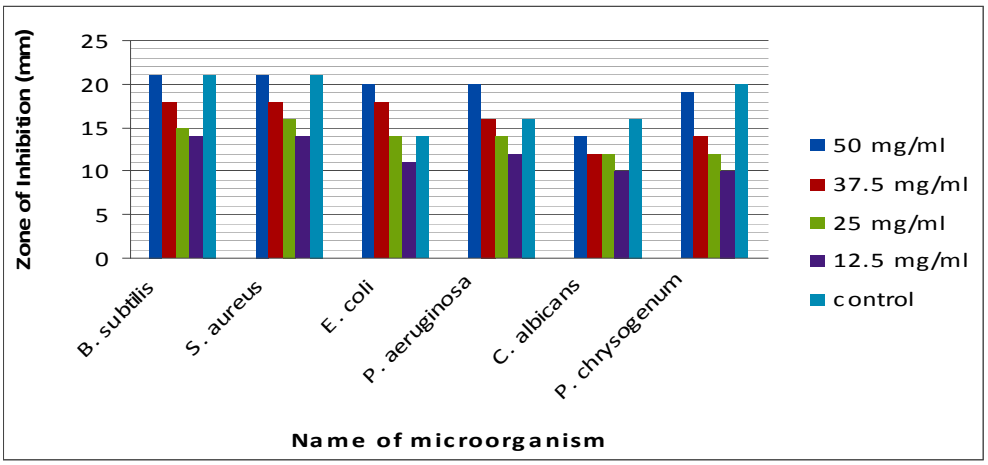

Fig. 15: Antimicrobial activity of Cardamom aqueous extract at (N.A.)

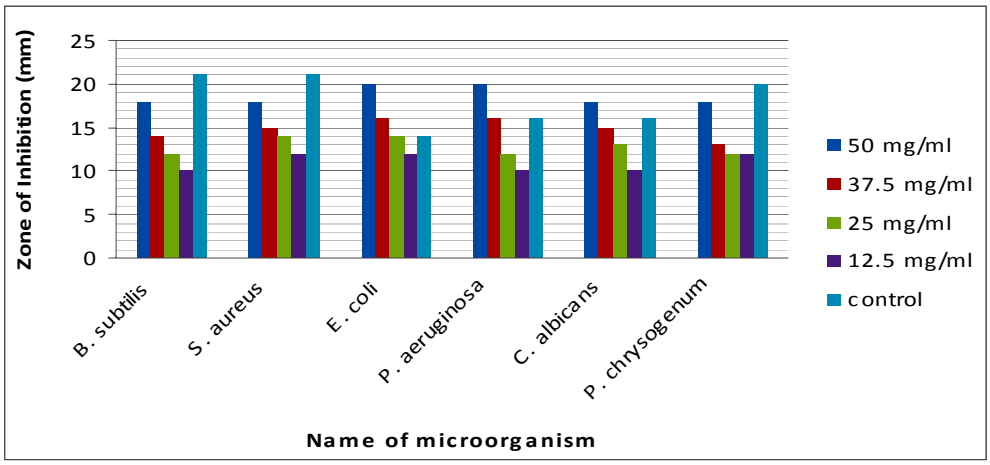

Fig.16: Antimicrobial activity of Cardamom aqueous extract at (M.H.A.).

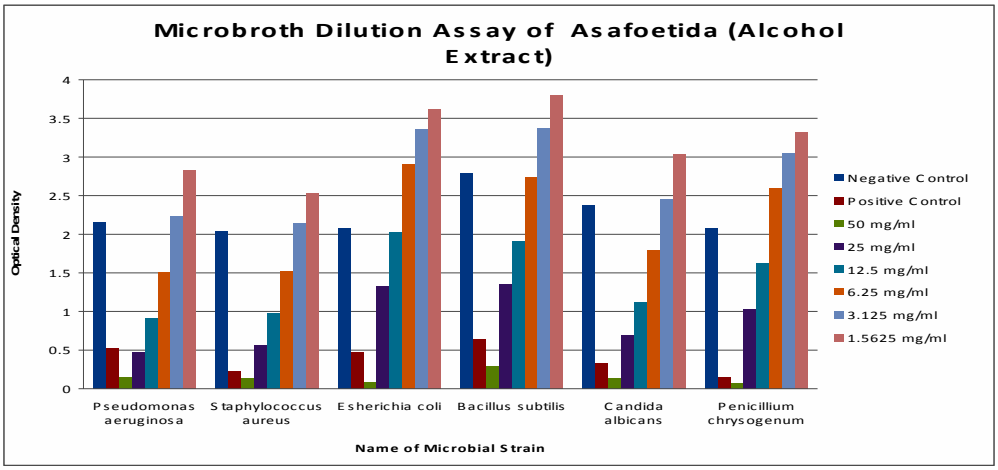

Fig. 17: Microbroth Dilution assay for alcoholic extract of Asafoetida

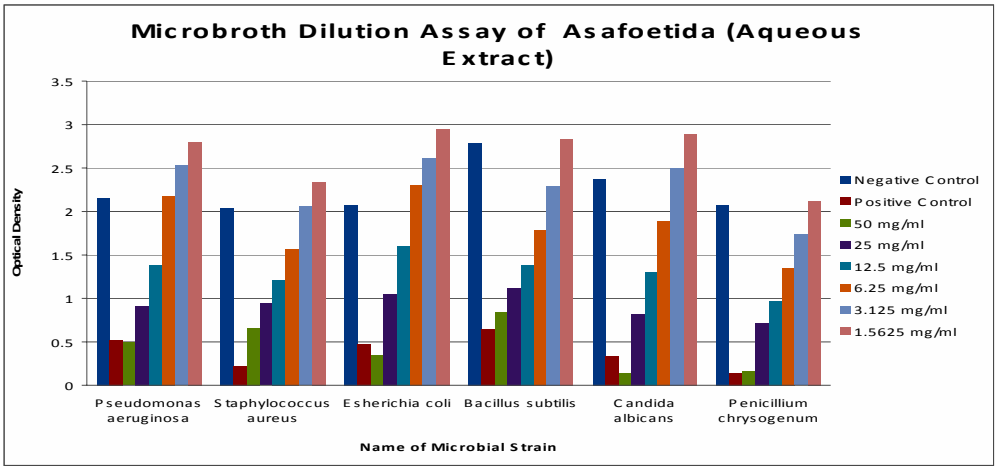

Fig. 18: Microbroth Dilution assay for aqueous extract of Asafoetida:

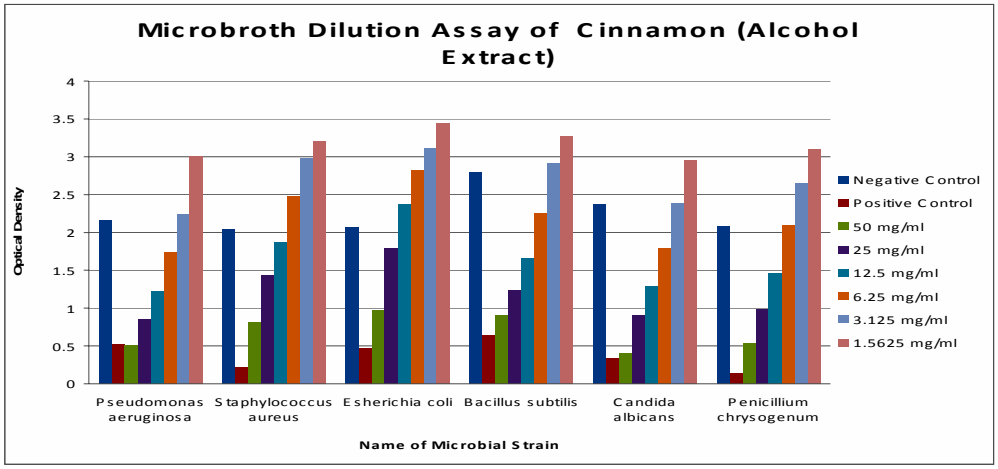

Fig. 19: Microbroth Dilution assay for alcoholic extract of Cinnamon

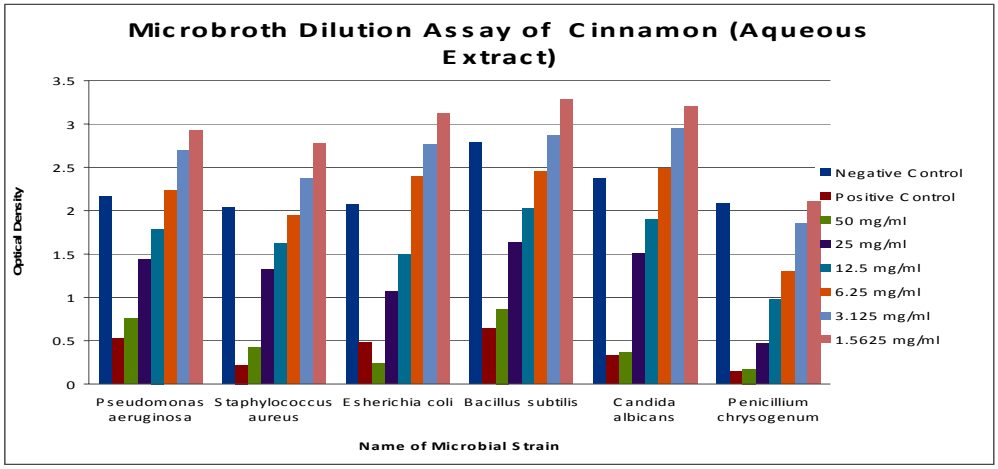

Fig. 20: Microbroth Dilution assay for aqueous extract of Cinnamon

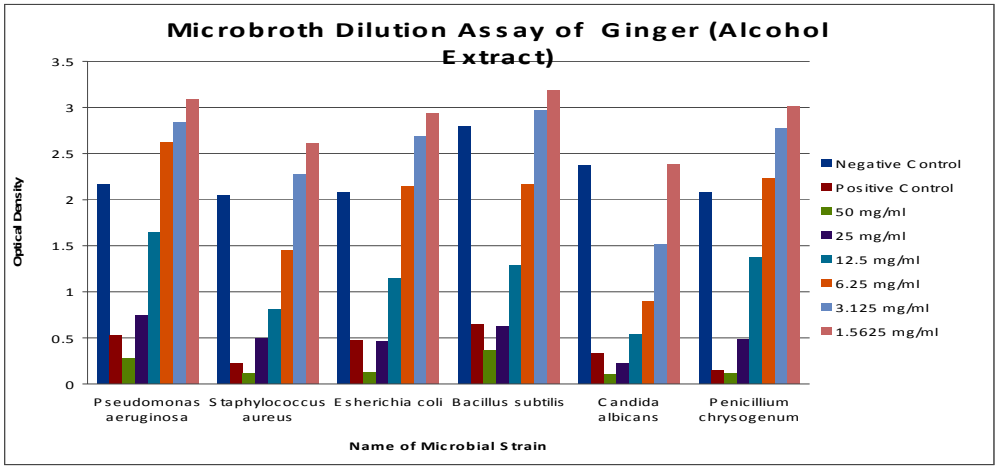

Fig. 21: Microbroth Dilution assay for alcoholic extract of Ginger:

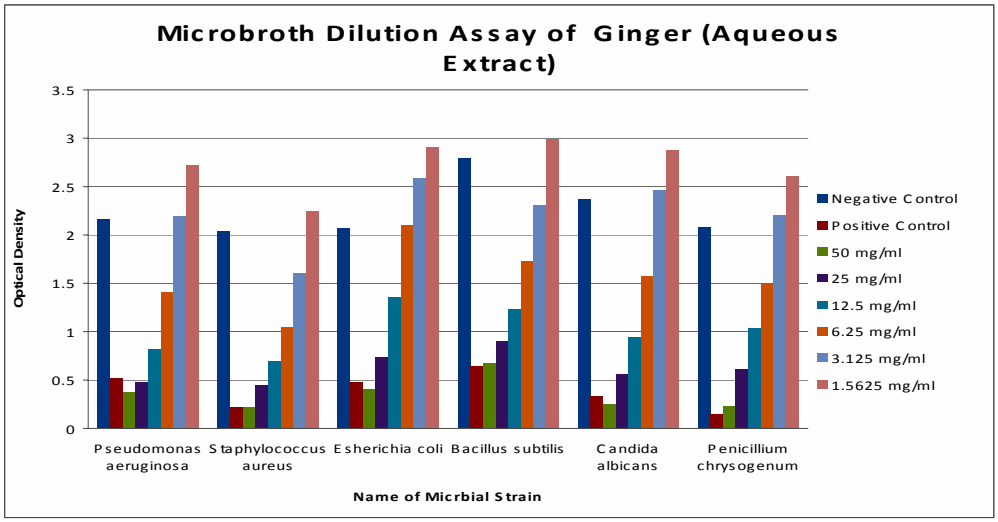

Fig. 22: Microbroth Dilution assay for aqueous extract of Ginger

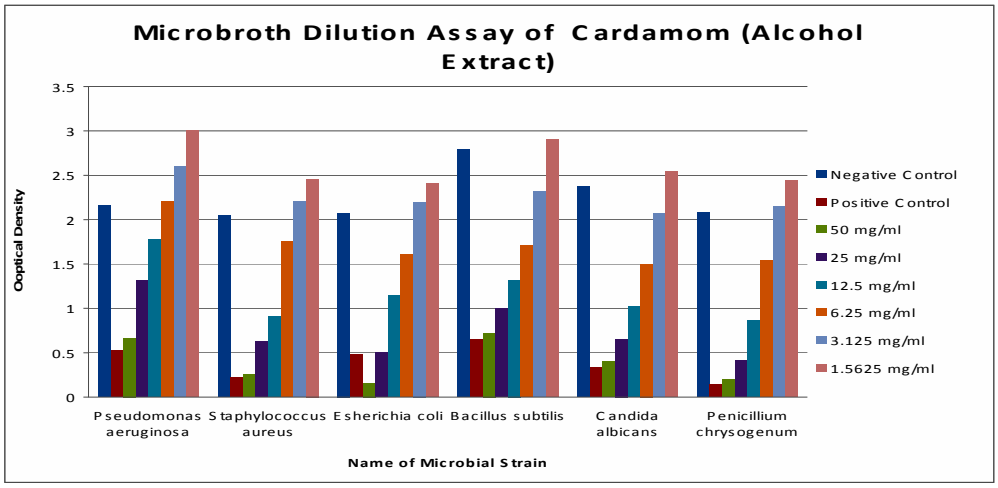

Fig. 23: Microbroth Dilution assay for alcoholic extract of Cardamom

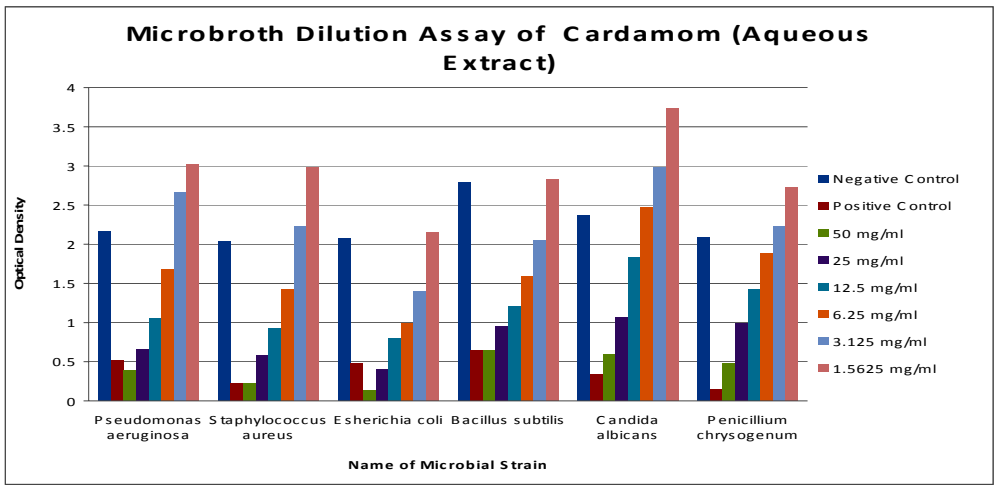

Fig. 24: Microbroth Dilution assay for aqueous extract of Cardamom

The Minimum Inhibitory Concentration (MIC) for each extract against selected microbes was carried out and the results are shown in table 1. The MIC value ranged between 12.5 mg/ml to 3.125 mg/ml with an exception of cinnamon alcohol extract against *E.coli* for which the calculated MIC was 25mg/ml.

Table 1: Minimum inhibitory concentration (MIC) values of different spice extracts

S. No.	Name of extract	Minimum inhibitory concentration (MIC) (mg/ml)					
		P. aeruginosa	*S. aureus*	*E. coli*	*B. subtilis*	*C. Albicans*	*P. chrysogenum*
1	asafoetida Alcohol	6.25	6.25	12.5	6.25	6.25	12.5
2	asafoetida Aqueous	12.5	6.25	12.5	3.125	6.25	3.125
3	cinnamon Alcohol	6.25	12.5	25	6.25	6.25	12.5
4	cinnamon Aqueous	12.5	6.25	12.5	6.25	12.5	3.125
5	ginger Alcohol	12.5	6.25	12.5	6.25	3.125	12.5
6	ginger Aqueous	6.25	3.125	12.5	3.125	6.25	6.25
7	cardamom Alcohol	12.5	6.25	6.25	3.125	3.125	6.25
8	cardamom Aqueous	6.25	6.25	3.125	3.125	12.5	6.25

CONCLUSION

The above results signify the fact that natural products like spices can be seen as alternatives to chemical preservatives used in various food industries so as to minimize their side effects and simultaneously improving the shelf life of the food products. The inhibitory factor responsible for the antimicrobial activity can further be identified and used as an alternative to currently used drugs against the pathogenic microbes under study. Nowadays microbes are increasingly developing resistance against the drugs in use. To combat against these drug resistant microbes, a large library of novel compounds is required. Natural products from plants may give us a solution to this alarming problem.

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