

Comparison of Antibacterial Activities of Selected Species of Zingiberaceae Family and Some Synthetic Compounds

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Abstract: This study describes the antibacterial activity of ginger (G), mangoginger (M) and turmeric (T) and mixtures thereof i.e. ginger and mangoginger (GM), ginger and turmeric (GT), turmeric and mangoginger (TM), and a mixture of peels (P) and 4 synthetic compounds (HC-1, HC-2, HC-3 and HC-4). Extracts of these compounds were studied on *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. Both aqueous (heated and unheated extracts) and organic solvents, 1, 4-Dioxan, N,N-dimethylformamide (DMF), were used for antibacterial assay. Growth inhibition was evaluated by the disc diffusion and agar ditch methods. The antibacterial activity of heated extracts was greater than that of the unheated extracts of all the 3 spices alone or mixtures thereof against all the 3 bacterial strains. When the organic acid extracts were studied, the antibacterial activity of 1, 4-Dioxan extracts of T, GT and TM showed the highest activity against *E. coli*. The 1, 4-Dioxan and DMF extracts of M showed the highest activity against *B. subtilis*. The antibacterial activity of G in 1, 4-Dioxan showed the highest activity against *S. aureus*. Extracts in DMF did not show any activity. The synthetic compounds in 1, 4-Dioxan showed better antimicrobial activity than did DMF extracts. In conclusion, amongst the studied members of Zingiberaceae mangoginger and mixtures containing mangoginger showed maximum antibacterial activity and the synthetic compound HC-3 showed antibacterial activity equivalent to that of mangoginger. Both natural and synthetic compounds extracted in 1, 4-Dioxan gave better results. Therefore, these 2 compounds can be used as lead molecules in drug designing.

Key Words: Zingiberaceae, Antibacterial activity, Natural and synthetic compounds.

Introduction

Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative (1) and medicinal value, which forms one of the oldest sciences (2). Yet it is only in recent years that modern science has started paying attention to the properties of spices.

Medicinal and spice plants are renewable raw materials. Their production is an alternative to the over-production of traditional crops in agriculture. They also have an increasing economic importance. Spices can be

defined as "any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms, that contribute flavour, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages" (3).

Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances being derived from a plant does not mean it is always harmless. One drug used for one ailment could actually be detrimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, mutagens and abortifacient.

Considering the above we selected *Zingiber officinale* Rosc., *Curcuma amada* Roxb. and *Curcuma longa* L. of the family Zingiberaceae to evaluate their antibacterial

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activity against the Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacterium *Escherichia coli*.

Zingiber officinale Rosc. (Ginger), belonging to the family, Zingiberaceae is widely used around the world as a spice or food additive and medicine. This plant is a perennial herb consisting of an underground stem or rhizome, bearing erect leafy shoots. The pungent element of ginger is the oleoresin-gingerols, shogaols and zingerone, which are credited with anti-nausea or antiemetic, abortifacient, antimicrobial, antiinflammatory (4) antioxidant (5) anticoagulant, antihypercholesterolemic, antihypertensive, antihyperglycaemic, anti-spasmodic, aperient (6) alexeteric, circulatory stimulant, counter irritant, sialagogue and vasodilator effects.

Curcuma longa L. (turmeric) and *Curcuma amada* Roxb. (mangoginger) belong to the family Zingiberaceae. When added to various food preparations turmeric preserves their freshness and nutritive values and gives flavour and colour. *Curcuma amada* Roxb., or mangoginger contains rhizomes that smell of fresh mango and are used as a flavouring spice in pickles and other Indian dishes. These plants are perennial herbs consisting of short and thick underground rhizomes and reduced stems, bearing tufts of large, broad, bright green leaves. The active constituents of turmeric, which provide biological activity, are the phenolic compounds known as curcuminoids.

The significance of turmeric in medicine has changed considerably since the discovery of the antioxidant (7) and antimicrobial properties of its naturally occurring phenolic compounds. They also have antiinflammatory (8), anticancer, hepatoprotective, antiallergic, wound healing, anti-tumour, antispasmodic and anti-HIV (9) properties.

The future of the natural habitat of medicinal plants is being threatened by ever increasing anthropogenic activities. Increased commercialisation has resulted in over-harvesting of medicinally useful plants, which has diminished their number so they are now in danger of extinction. To overcome this alarming problem, the discovery of novel active compounds is the need of the day. Previously, all drugs and medicinal agents were derived from natural substances, especially from higher plants. Nowadays new chemotherapeutic agents are synthetically derived, based on rational drug design.

The antibacterial activity of spice extracts of the family Zingiberaceae and few synthetic compounds were evaluated against a few clinically important bacterial strains.

Materials and Methods

Plant materials

Fresh rhizomes of ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), and mangoginger (*Curcuma amada*) belonging to the family Zingiberaceae were collected locally.

Test-microorganisms

Gram-negative *Escherichia coli* (Enterobacteriaceae), Gram positive *Bacillus subtilis* (Bacillaceae), and Gram positive *Staphylococcus aureus* (Micrococcaceae) were used for in vitro antibacterial activity. These bacterial strains were obtained from a private clinical microbiological laboratory (Micro Care Laboratory, Rajkot).

Preparation of unheated extracts

Ginger (G), mangoginger (M), turmeric (T), mixtures of ginger and mangoginger (GM), ginger and turmeric (GT), and turmeric and mangoginger (TM) and a mixture of peels (P) were prepared by taking 25 g of fresh rhizomes, and for mixture extracts spices were mixed in a ratio of 1:1, washed under tap water, air dried and crushed in a homogeniser in 100 ml of distilled water. The slurry was placed in a 250 ml flask and put on a rotary shaker at 190-220 rpm for 3-4 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was reduced to 25 ml and then autoclaved at 121 °C and 15 lb pressure for 20 min. These extracts were cooled and immediately processed for antibacterial activity.

Preparation of heated extracts (decoction)

Fresh rhizomes (25 g) were washed under tap water, air dried and crushed in a homogeniser. The slurry was placed in a conical flask and 200 ml of distilled water was added to the flask and boiled over a low flame for of 5-6 h until the volume reduced to about 25 ml. The slurry was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min at room temperature. The supernatant was collected and autoclaved at 121 °C and 15 lb pressure for 20 min.

These extracts were cooled and immediately processed for antibacterial activity.

Preparation of crude solvent extracts

Crude solvent extracts of selected spices were prepared by crushing 25 g of plant materials in 100 ml of 1, 4-Dioxan (non polar) and DMF (polar) in 250 ml flasks. These flasks were then put on a rotary shaker at 180–190 rpm for 24 h. After 24 h, these mixtures were centrifuged at 5000 rpm for 5-7 min at room temperature. The supernatants were collected and the solvent was evaporated until the final volume was reduced to about 25 ml. These extracts were stored at 4 °C in airtight bottles.

Preparation of synthetic compounds

A total of 10.8 ml of p-cresol, 10.4 g of malonic acid, 40-50 ml of POCl₃ and 30 g of anhydrous ZnCl₂ was placed in a round bottom flask and refluxed at 70 °C in a water bath for 18–20 h. The reaction mixture was cooled to room temperature and ice cold water was added. The product was filtered and dissolved in 10% Na₂CO₃ solution. The solution was left overnight and then acidified by adding dilute HCl. The product was filtered and washed several times with cold water. This is denoted as compounds I (HC-1).

Five grams of compound I, 25 ml of glacial acetic acid, and 20 ml of POCl₃ were placed in a round bottom flask and the mixture was heated in a water bath for 2–4 h. The resulting solution was cooled to room temperature and ice was added. The product was filtered and washed several times with cold water. This is denoted as compound II (HC-2).

Equimolar weights of compound II and amine were dissolved in 25–30 ml methanol. A few drops of glacial acetic acid were added and the mixture was refluxed in a water bath for 10–12 h. It was cooled to room temperature and poured into ice cold water. The product obtained when p-NO₂ aniline was added is denoted as compound III (HC-3) and the product obtained when m-NO₂ aniline was added is denoted as compound IV (HC-4).

Antimicrobial assay of aqueous extracts

The antibacterial activity was determined by agar disc diffusion (10): 28 ml of molten Mueller Hinton Agar No. 2 along with 0.2 ml of inoculum (10⁸ cells/ml) was poured into a Petri plate (Hi-media). The complete experiment was performed under strict aseptic conditions. The test compound was placed on the disc (7 mm) and then allowed to dry; then the disc was placed on the upper layer of the seeded agar plate. The plates were incubated at 37 °C for 24 h. Microbial growth was determined by measuring the diameter of the zone of inhibition around each disc. The disc was impregnated with an equivalent volume of sterile distilled water to serve as a control. The control values have been subtracted from the test values and the results are plotted in the graphs.

Antibacterial assay of crude solvent extracts

The antibacterial activity was determined by agar well diffusion (11): 28 ml of molten Mueller Hinton Agar No. 2 along with 0.2 ml of inoculum was poured into a Petri plate (Hi-media). The plates were allowed to solidify, after which a well was made in the plates with the help of

The following table shows some details of the synthetic compounds used:

No. Code	IUPAC Name	Molecular formula	Molecular weight	Rf value	Solvent system benzene:acetone
1 HC-1	4-hydroxy-6-methyl-chromen-2-one	C ₁₀ H ₈ O ₃	176.169	0.58	7:3
2 HC-2	3-acetyl-4-hydroxy-6-methyl-chromen-2-one	C ₁₂ H ₁₀ O ₄	218.205	0.55	7:3
3 HC-3	4-hydroxy-6-methyl-3-[1-(4-nitro-phenylimino)-ethyl]-chromen-2-one	C ₁₈ H ₁₄ N ₂ O ₅	338.314	0.61	8:2
4 HC-4	4-hydroxy-6-methyl-3-[1-(3 nitro-phenylimino)-ethyl]-chromen-2-one	C ₁₈ H ₁₄ N ₂ O ₅	338.314	0.57	8:2

a cup-borer (85 mm); and this was filled with the plant extract. The wells loaded with pure solvents (DMF or 1,4-Dioxan) served as controls. The plates were incubated at 37 °C for 24 h. Microbial growth was determined by measuring the diameter of the zone of inhibition.

Antibacterial assay of synthetic compounds

The antibacterial activity was determined by using agar well diffusion: 20 mg of each synthetic compound was dissolved in 1 ml of 1, 4-Dioxan/DMF. The assay was performed exactly as described above. All assays were done in 4 replicates and the mean values are presented.

Results

All the spices studied possessed some antibacterial activity against *E. coli*, *B. subtilis*, and *S. aureus*. The antibacterial activities of heated and unheated extracts of G, M and T and their mixtures GM, GT, TM and P against *E. coli* are shown in Figures 1a and 1b, respectively. The antibacterial activities of G, M and T extracts (heated) were almost identical; however, the extract of M had maximum activity, followed by G and T in that order. The unheated spice extracts of G and T showed similar effects, and the unheated extract of M did not show any activity. The antibacterial activity of mixtures of spices and peels showed good antibacterial activity against *E. coli*. The activity of heated extracts was greater than that of unheated extracts. The maximum activity was that of peels, followed by the 3 spices mixtures i.e. GT, GM and TM. All the unheated extracts showed less activity and maximum difference was observed in the peel extract.

The antibacterial activity of G, M, and T against *B. subtilis* is shown in Figure 2a and the antibacterial activity of the mixtures, i.e. GM, GT, TM and P, are shown in Figure 2b. The antibacterial activities of heated and unheated extracts of G, M and T were almost identical and the heated extracts also showed slightly higher activity compared to unheated extracts. Mixtures of heated extracts of GM and GT were slightly more active against *B. subtilis* compared to those of TM and P.

The antibacterial activities of heated extracts of G, M and T against *S. aureus* are shown in Figure 3a and the antibacterial activities of their mixtures (GM, GT, TM and P) are shown in Figure 3b. Heated and unheated extracts of G were more active against *S. aureus* compared to the heated and unheated extracts of M; and heated and

unheated extracts of T did not show any activity against *S. aureus*. The mixture of heated extracts of GT displayed the highest activity, followed by the mixture of GM and the mixture of heated P extracts. When the mixtures of unheated extracts were compared, GM showed the highest activity, followed by the mixture of unheated peels extracts and the mixture of GT. When the extract of TM was considered both heated and unheated extracts failed to show any activity against *S. aureus*.

The antibacterial activities of 1, 4-Dioxan and DMF extracts of G, M and T against *E. coli* are shown in Figure 4a while the antibacterial activities of 1, 4-Dioxan and DMF extracts of mixtures of GM, GT, TM and P against *E. coli* are shown in Figure 4b. The 1, 4-Dioxan extract of T showed the highest activity compared to the 1, 4-Dioxan extracts of G and M. DMF extract of G and M showed little, but almost identical activity against *E. coli*, while the DMF extract of T failed to show any activity. The mixture of 1, 4-Dioxan extracts of GT and TM showed the same activity while GM failed to show any activity against *E. coli*. On the other hand, only the mixture of DMF extracts of GM showed antibacterial activity, while the other 2 extracts failed to show any activity.

The antibacterial activities of 1, 4-Dioxan and DMF extracts of G, M and T against *B. subtilis* are shown in Figure 5a while the activity of their mixtures are shown in Figure 5b. The 1, 4-Dioxan extract of M was highly active against *B. subtilis*, followed by T and G. The DMF extract of M also showed the highest activity, followed by T and G. The mixture of 1, 4-Dioxan extracts of TM showed the highest activity, followed by GM and GT. Similar activity was found with the mixture of DMF extracts.

The antibacterial activities of 1, 4-Dioxan and DMF extracts of G, M and T against *S. aureus* are shown in Figure 6a while the activity of their mixtures are shown in Figure 6b. The 1, 4-Dioxan extract of M showed the highest activity, followed by T and G. However, the DMF extract of all the 3 spices did not show any activity. The mixture of 1, 4-Dioxan extracts of GM showed the highest activity, followed by TM and GT. Here too the mixture of DMF extracts did not show any activity.

The antibacterial activities of 4 synthetic compounds, i.e. HC-1, HC-2, HC-3 and HC-4, in 1, 4-Dioxan as well as in DMF against *E. coli* are shown in Figure 7a. In 1, 4-

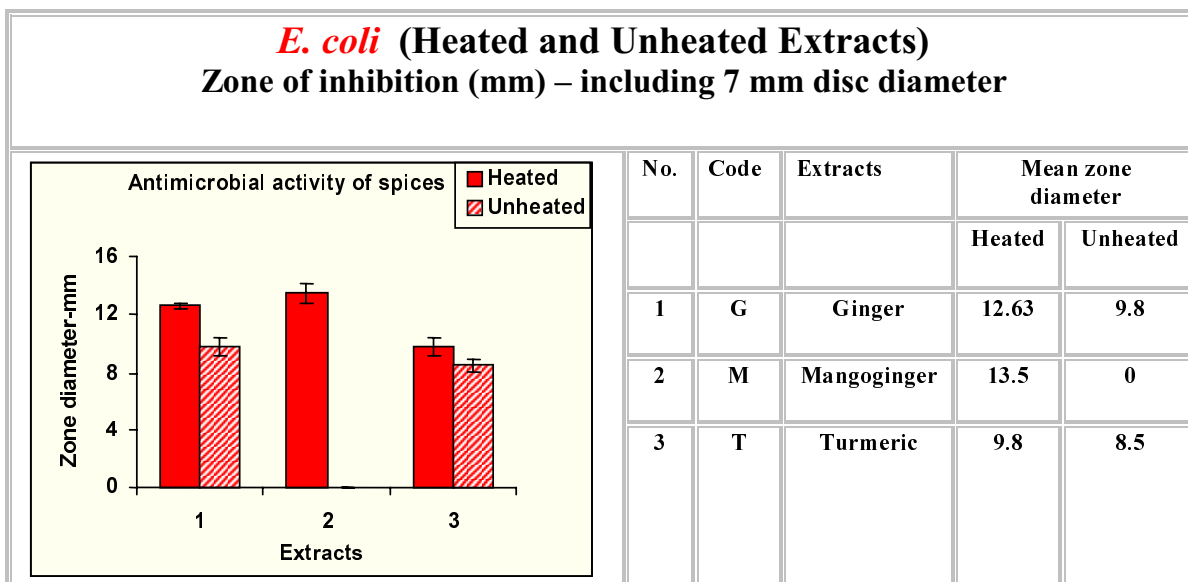


Figure 1a.

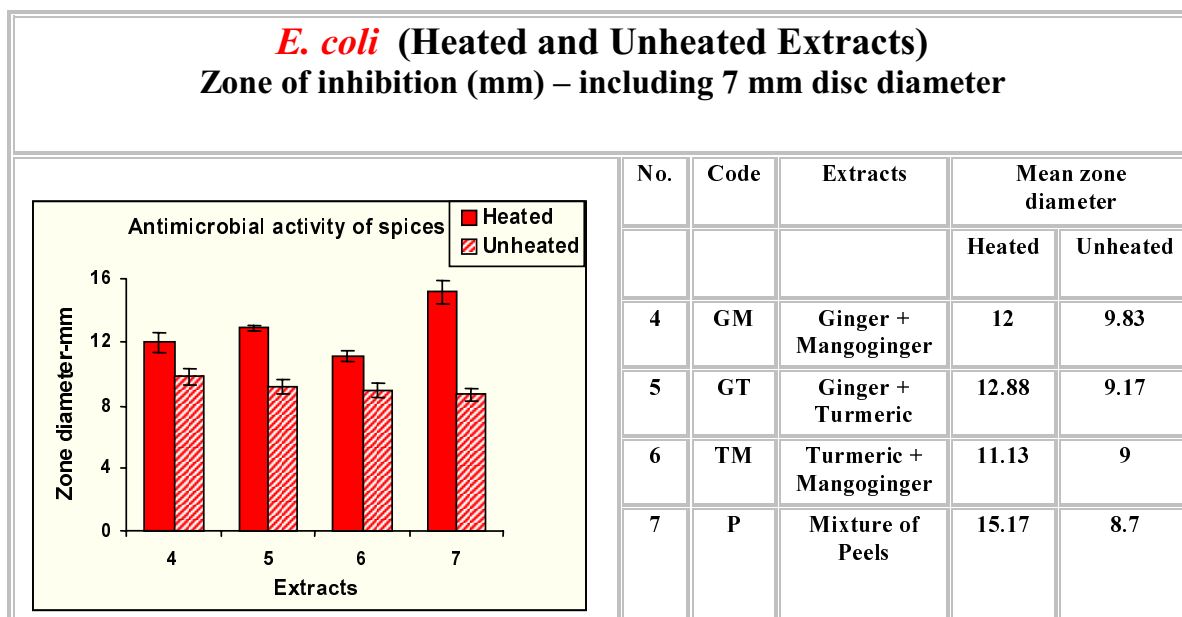


Figure 1b.

Dioxan, HC-2 and HC-3 showed the highest activity followed by HC-1 while HC-4 had the lowest activity. The synthetic compound HC-1 in DMF showed very little activity against *E. coli* while the other compounds did not show any activity. The antibacterial activities of the 4 synthetic compounds in 1, 4-Dioxan and in DMF against

B. subtilis are shown in Figure 7b. HC-3 in 1, 4-Dioxan showed the highest activity, followed by HC-2 and HC-1; HC-4 did not show any activity. HC-1, HC-2 and HC-3 in DMF showed almost identical moderate activity while here too HC-4 showed negligible activity. The antibacterial activities of synthetic compounds against *S.*

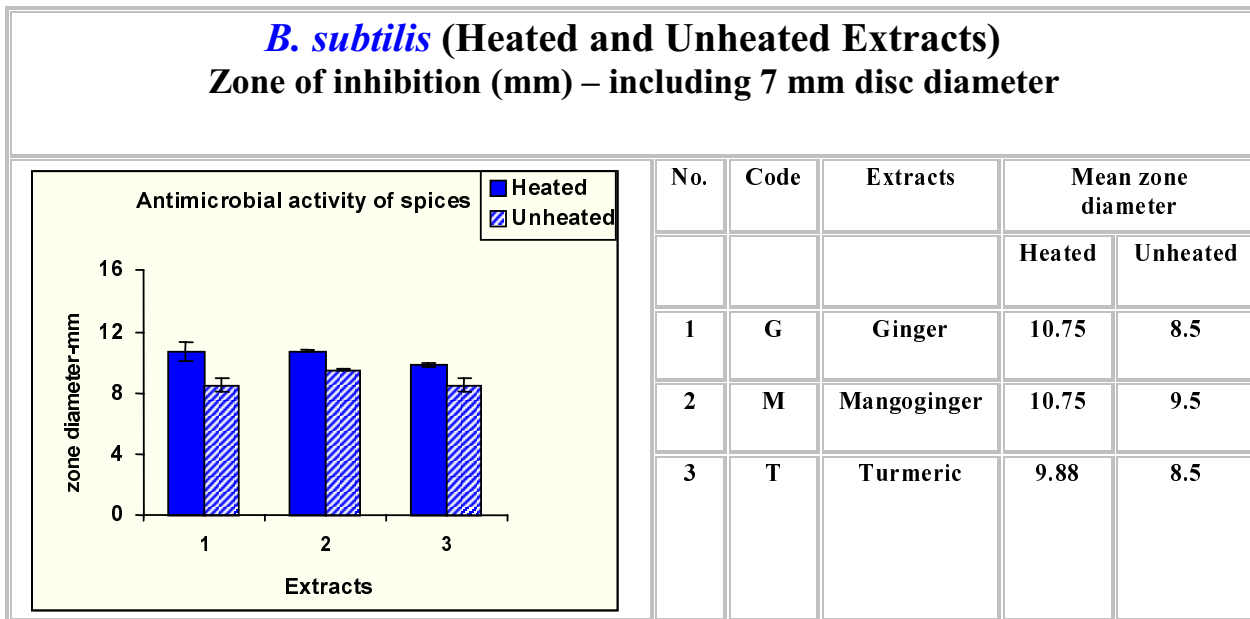


Figure 2a.

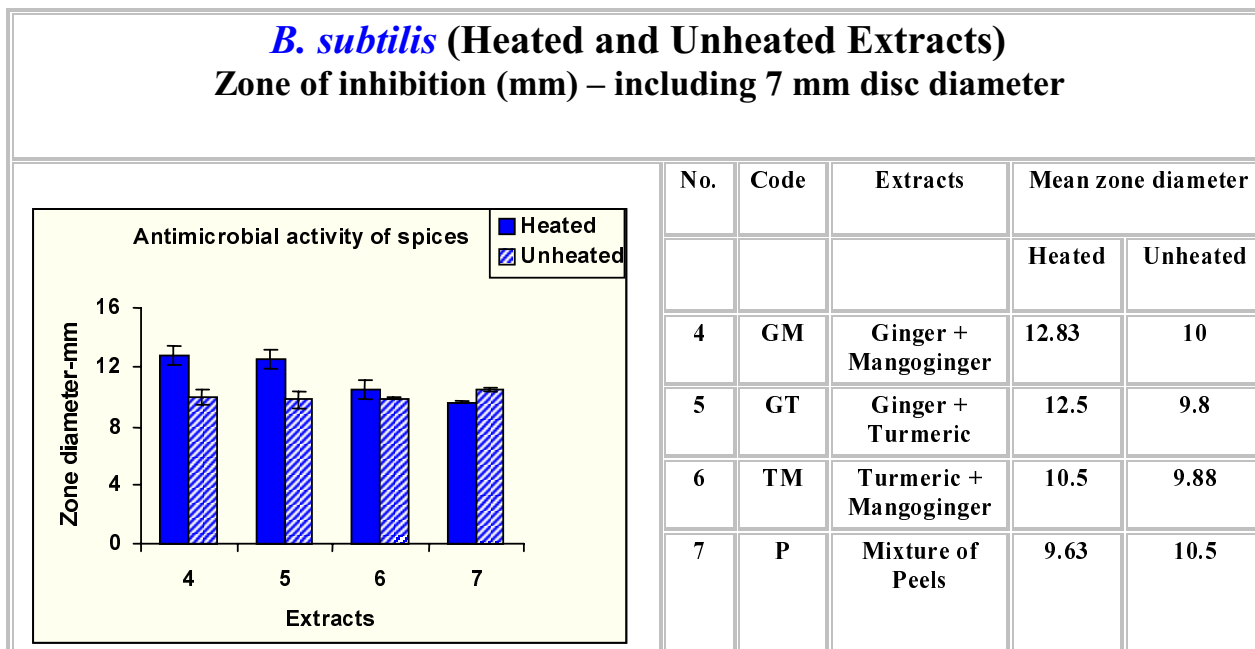


Figure 2b.

aureus in 1,4-Dioxan and in DMF are shown in Figure 7c. In 1, 4-Dioxan, HC-2 showed the highest activity followed by HC-3, HC-1 and HC-4 in that order. The synthetic compounds in DMF failed to show any activity against *S. aureus*.

A comparison of the antibacterial activities of natural and synthetic compounds in 1, 4-Dioxan as well as in DMF against *E. coli* is shown in Figure 8a. T, mixtures of GT and MT and the synthetic compounds HC-2 and HC-3 in 1, 4-Dioxan showed almost identical activity followed by

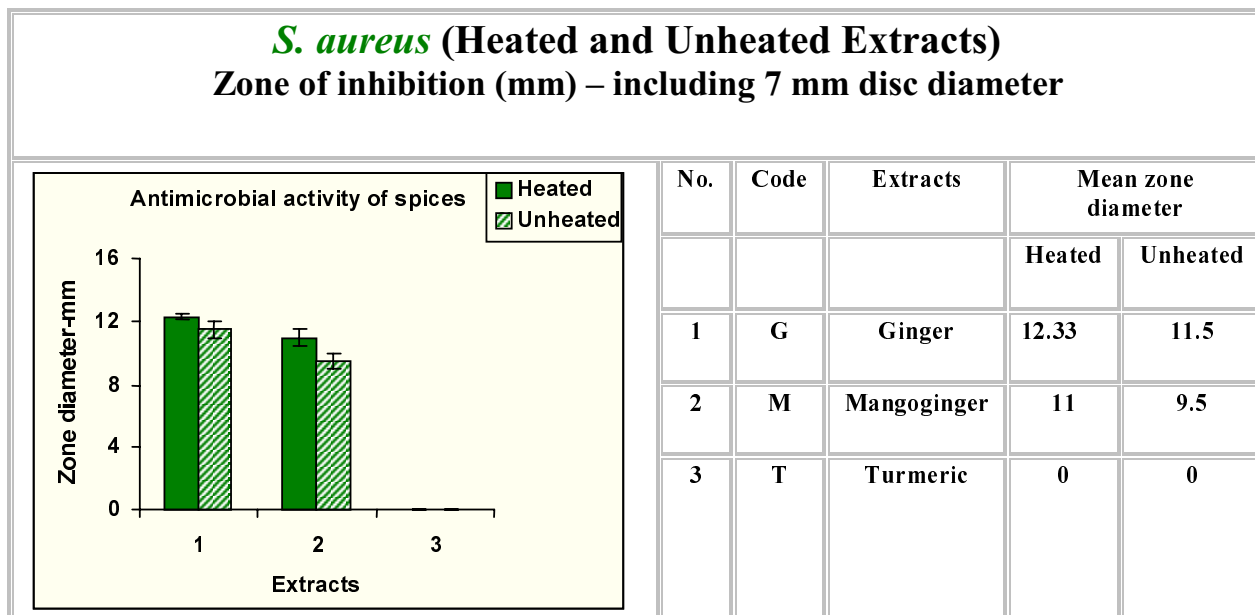


Figure 3a.

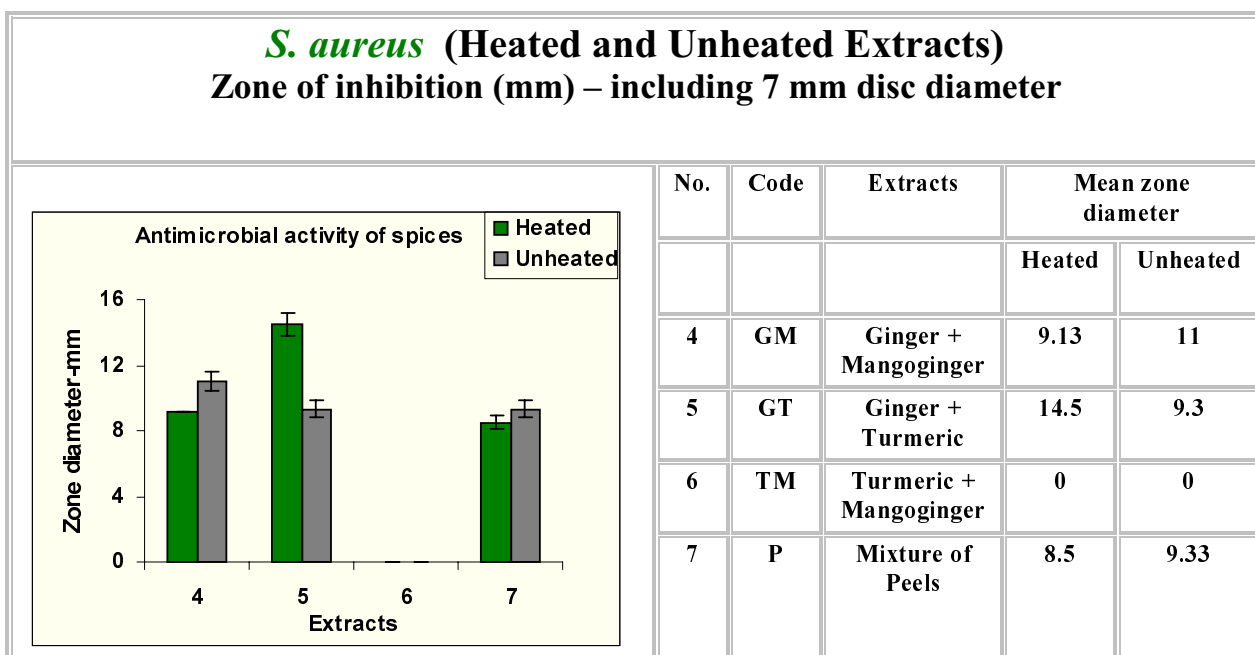


Figure 3b.

HC-1. The natural compounds G and M and the synthetic compound HC-4 in 1, 4-Dioxan showed very little activity. The mixture of GM did not show any activity. Natural as well as synthetic compounds in DMF did not show any significant activity against *E. coli*. A comparison of the

antibacterial activities of natural and synthetic compounds in 1, 4-Dioxan as well as in DMF against *B. subtilis* are shown in Figure 8b. Almost all natural and 3 synthetic compounds showed significant antibacterial activity. Maximum activity was shown by the natural compounds

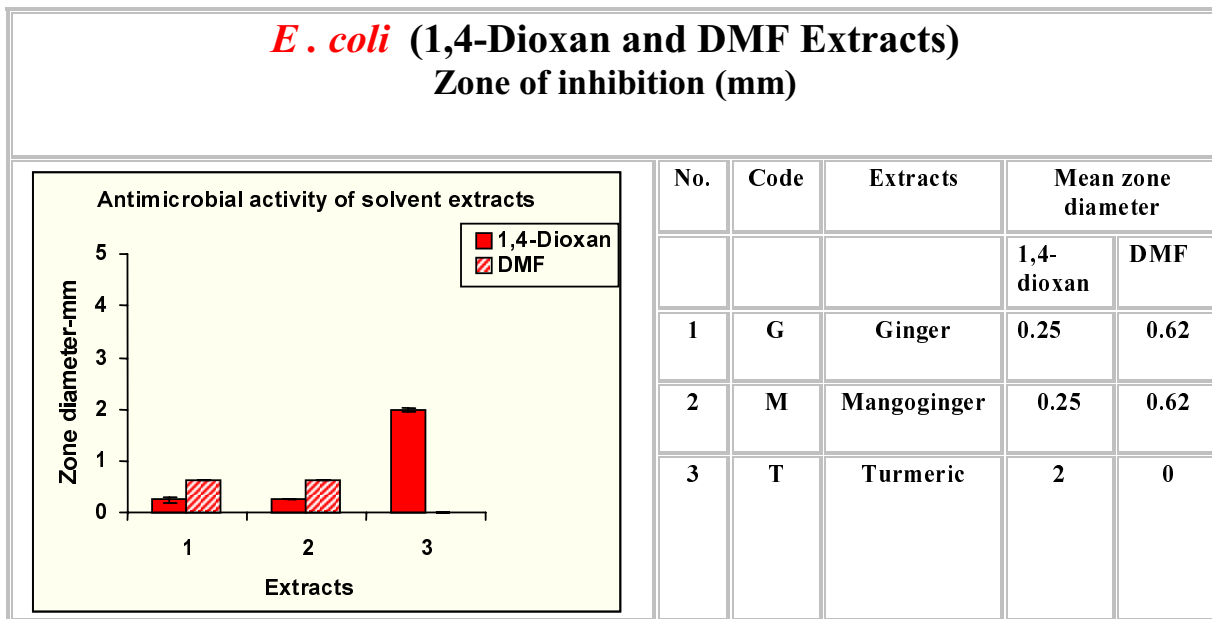


Figure 4a.

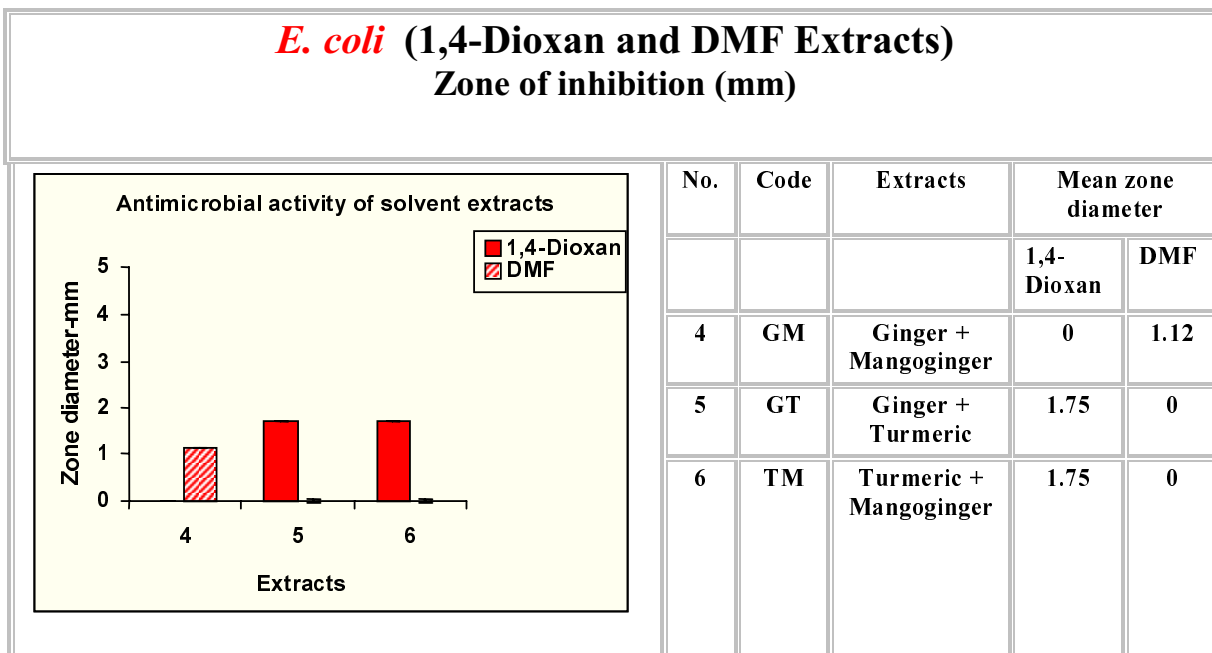


Figure 4b.

M and TM, and this was almost equal to that of 3 synthetic compounds, i.e. HC-1, HC-2 and HC-3; followed by GM, T and GT in that order. HC-4 did not show any activity. The antibacterial activity in DMF also

showed similar results. However, the antibacterial activity of synthetic compounds was almost half of that shown in 1, 4-Dioxan. A comparison of the antibacterial activities of natural and synthetic compounds against *S. aureus* is

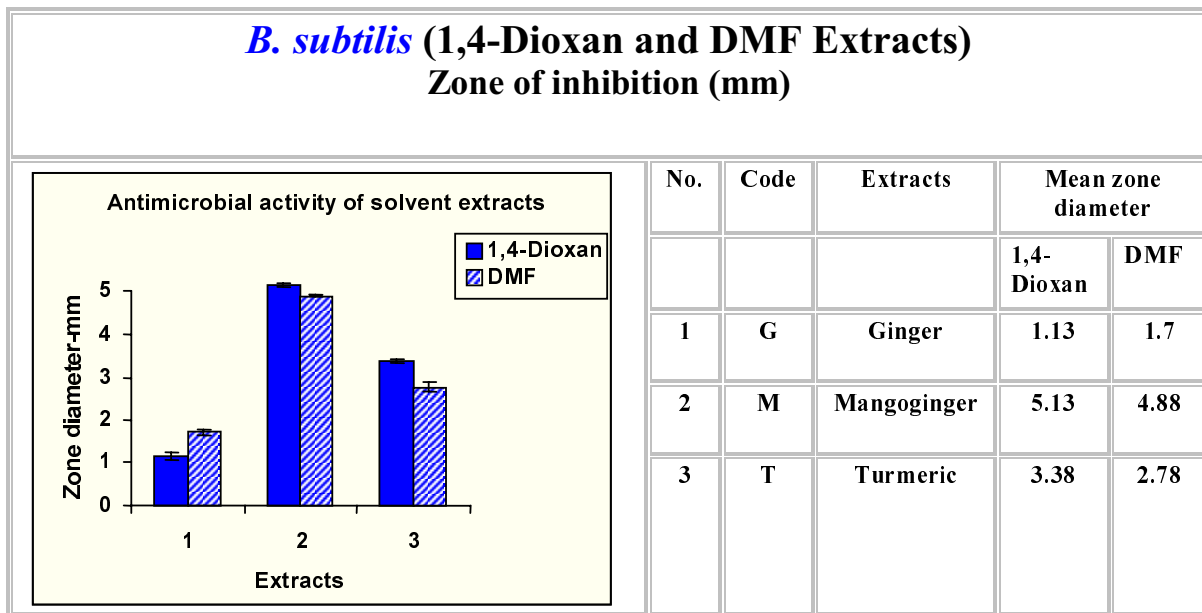


Figure 5a.

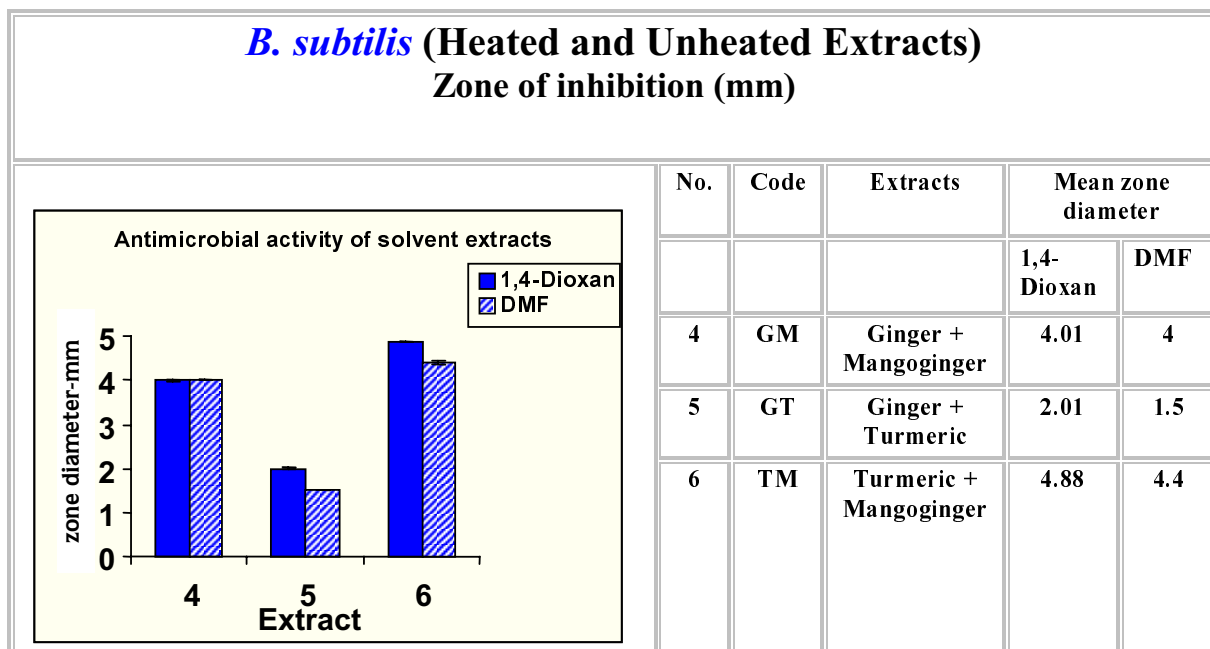


Figure 5b.

shown in Figure 8c. The antibacterial activity of synthetic compounds was considerably greater than that of natural compounds against *S. aureus*. Maximum activity was shown by HC-2, followed by HC-3, HC-1 and HC-4 in

that order. Among the natural compounds, maximum activity was shown by M, and all the others showed less, but very similar activity. The DMF extracts of both natural and synthetic compounds did not show any activity.

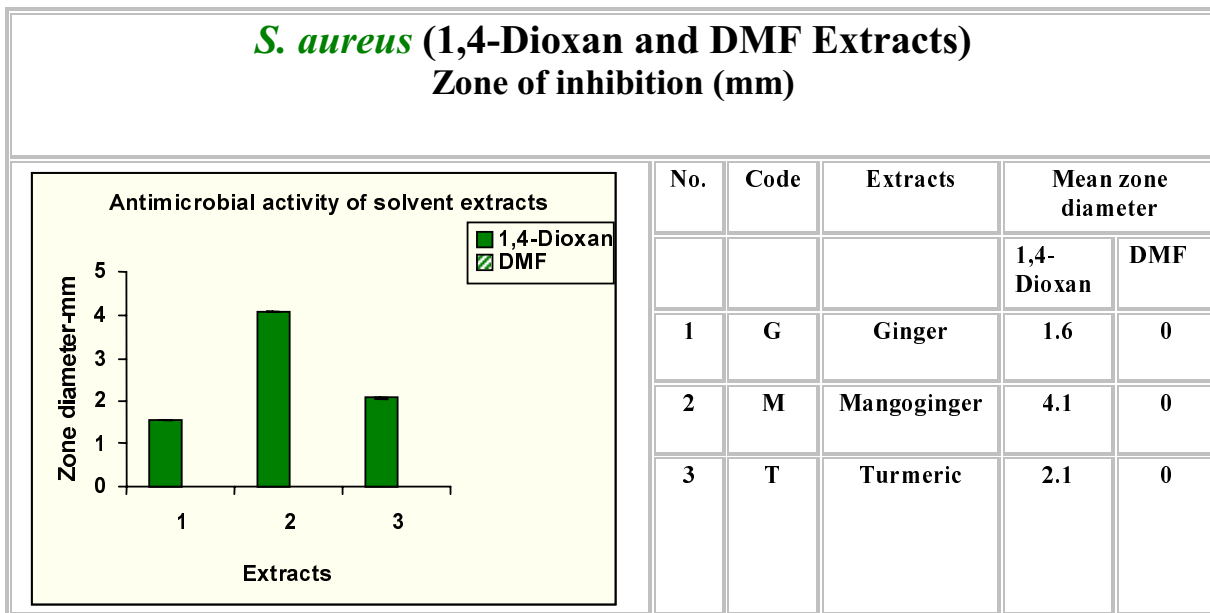


Figure 6a.

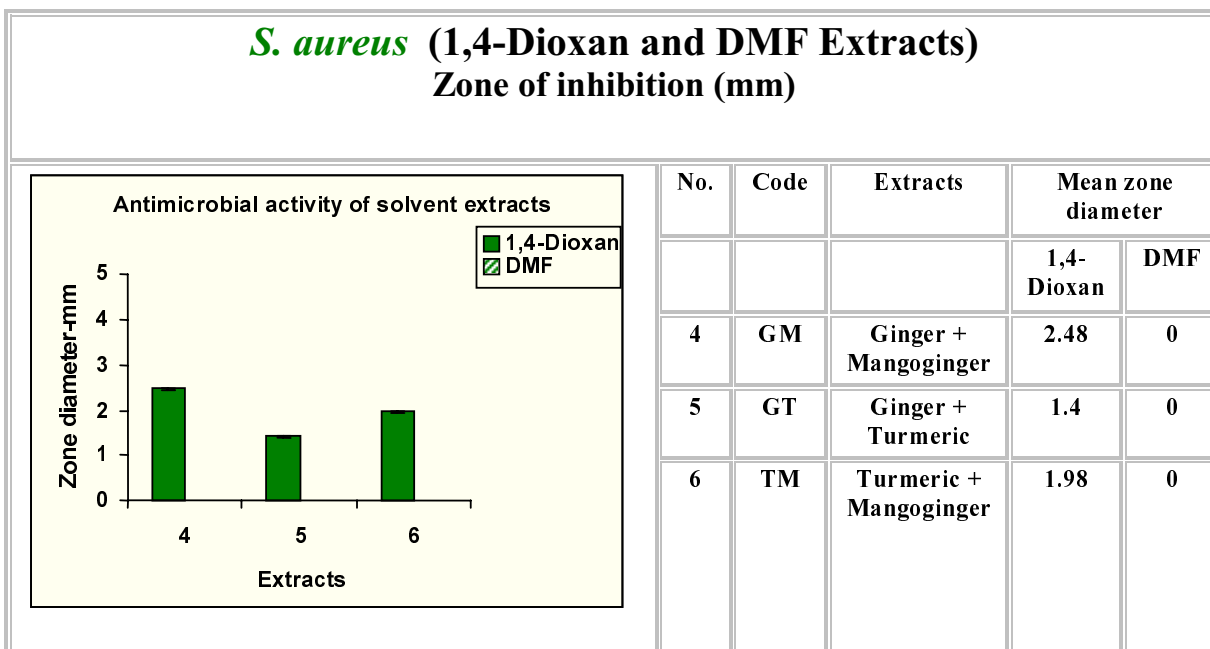


Figure 6b.

Discussion

The results of our experiments showed that different bacterial species exhibited different sensitivities towards these compounds. The sensitivities of bacterial species

against phenolic compounds of the family *Zingiberaceae* also varied in the 2 different species of the same genus.

Extracts of the 3 individual spices as well as mixtures there of showed more activity against Gram positive

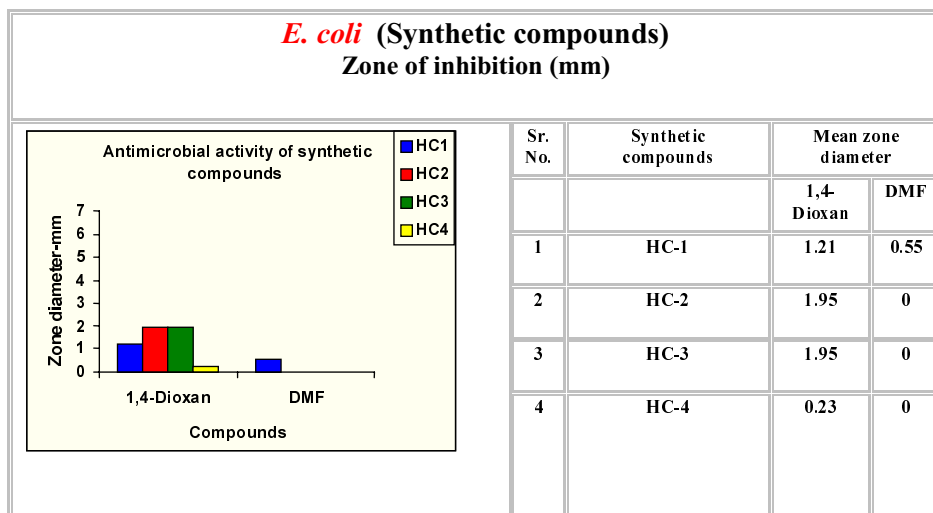


Figure 7a.

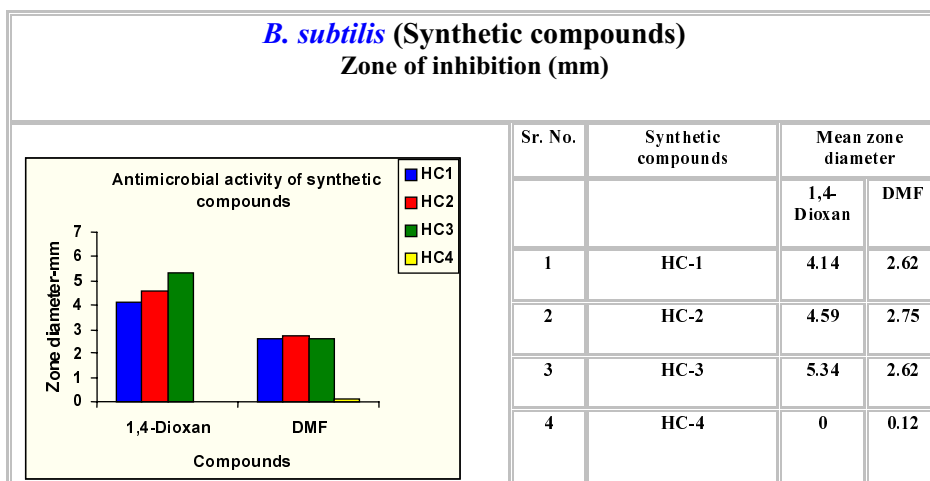


Figure 7b.

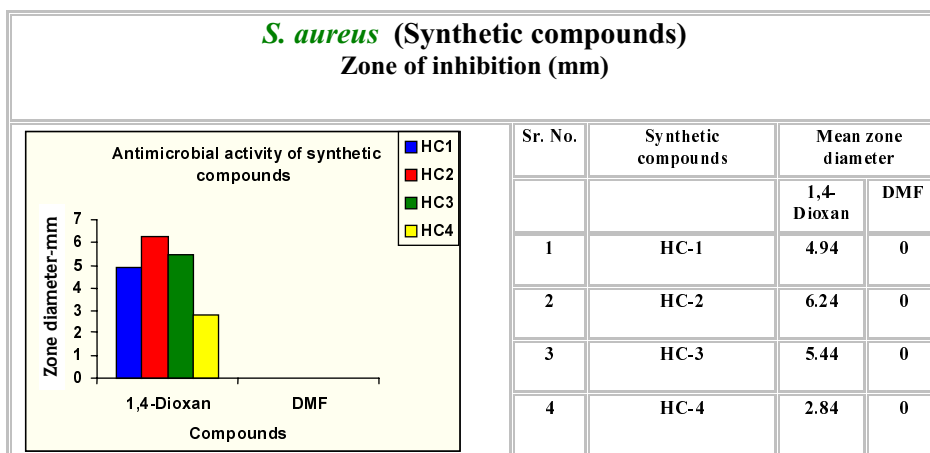


Figure 7c.

***E. coli* (Natural and Synthetic Compounds)**
Zone of inhibition - mm

Sr. No	Code	Extracts	Mean zone diameter	
			1,4-Dioxan	DMF
1	G	Ginger	0.25	0.62
2	M	Mangoginger	0.25	0.62
3	T	Turmeric	2	0
4	GM	Ginger & Mangoginger	0	1.12
5	GT	Ginger & Turmeric	1.75	0
6	TM	Mangoginger & Turmeric	1.75	0
		Synthetic Compounds		
1		HC-1	1.21	0.55
2		HC-2	1.95	0
3		HC-3	1.95	0
4		HC-4	0.23	0

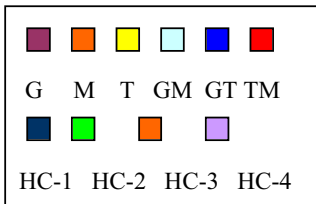
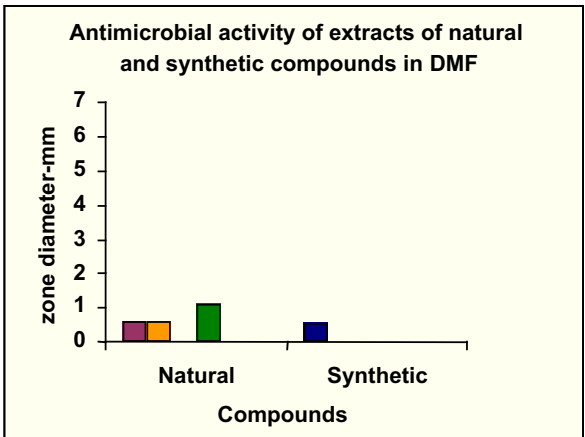
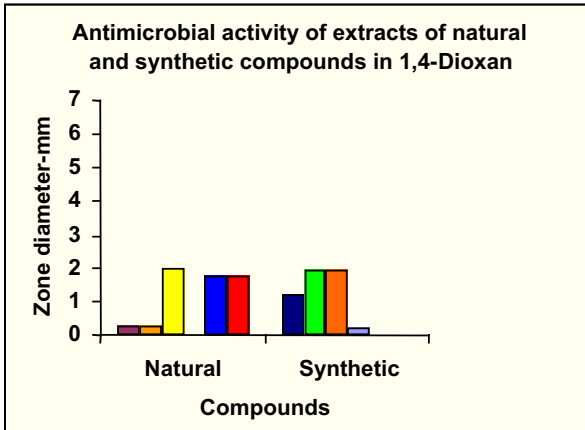


Figure 8a.

***B. subtilis* (Natural and Synthetic Compounds)**
Zone of inhibition - mm

Sr. No.	Code	Extracts	Mean zone diameter	
			1,4-Dioxan	DMF
1	G	Ginger	1.13	1.7
2	M	Mangoginger	5.13	4.88
3	T	Turmeric	3.38	2.78
4	GM	Ginger & Mangoginger	4.01	4
5	GT	Ginger & Turmeric	2.01	1.5
6	TM	Mangoginger & Turmeric	4.88	4.4
		Synthetic Compounds		
1		HC-1	4.14	2.62
2		HC-2	4.59	2.75
3		HC-3	5.34	2.62
4		HC-4	0	0.12

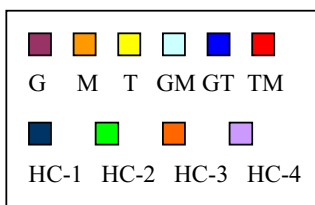
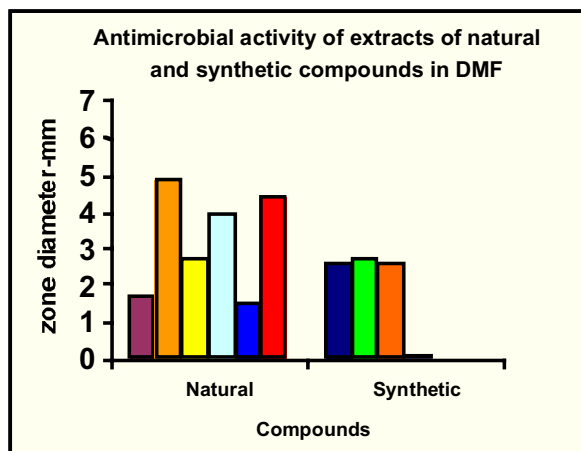
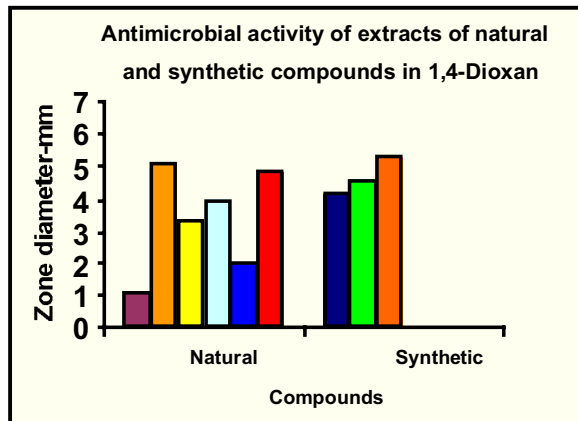


Figure 8b. (continued)

bacteria compared to Gram negative bacteria. The decreasing order of sensitivity of selected species of Gram positive and Gram negative bacteria against extracts of spices (under study) was *B. subtilis* > *S. aureus* > *E. coli*.

Gram negative bacteria were also more resistant than Gram positive bacteria, as also shown by Zaika (12). These variations in inhibition may be because of differences in the composition and structure of the cell

***S. aureus* (Natural and Synthetic Compounds)**
Zone of inhibition - mm

Sr. No.	Code	Extracts	Mean zone diameter	
			1,4-Dioxan	DMF
1	G	Ginger	1.6	0
2	M	Mangoginger	4.1	0
3	T	Turmeric	2.1	0
4	GM	Ginger & Mangoginger	2.48	0
5	GT	Ginger & Turmeric	1.4	0
6	TM	Mangoginger & Turmeric	1.98	0
		Synthetic compounds		
1		HC-1	4.94	0
2		HC-2	6.24	0
3		HC-3	5.44	0
4		HC-4	2.84	0

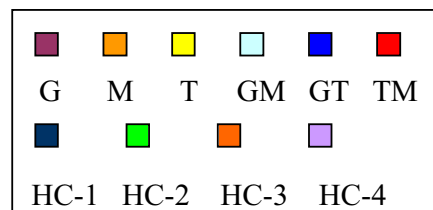
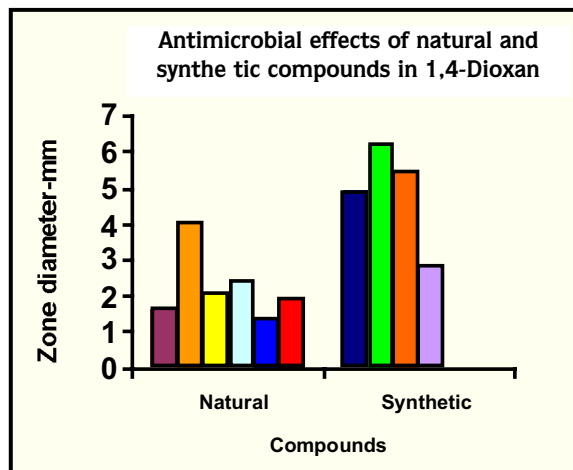


Figure 8c. (continued)

surface between Gram positive and Gram negative bacteria. In addition to the cell wall and cell membrane, Gram negative bacteria have an outer membrane composed of a phospholipid bilayer, which may be a protective barrier against these phenolic compounds. Moreover, the cell walls of Gram positive bacteria have a large amount of peptidoglycan and a small amount of lipid, while in the case of Gram negative bacteria, due to the presence of an outer membrane, a large amount of lipid and a small amount of peptidoglycan is found (13).

Most of the phenols are protein denaturing agents; they can change the cell permeability, which may lead to swelling and rupture of the bacterial cells. Most of them are metal chelators that attach to the active site of metabolic enzymes, reducing enzyme activities and therefore slowing bacterial metabolism and reproduction. However these are only possible only when phenols enter the living bacterial cell. As Gram negative bacteria have an additional outer membrane on their cell wall, the entry of phenols may be interrupted and its effects are less

serious. However, Gram positive bacteria lack the outer membrane and therefore they are more susceptible to, easily entering phenols.

Extracts of individual spices and mixtures there of were prepared by heating, and without heating as well as in organic solvents. From the results, it appears that heated extracts have the highest activity against Gram positive and Gram negative bacteria, because under the effect of high temperature biologically active compounds are easily extracted from the plant tissue. However, the curcumin present in turmeric is insoluble in water but soluble in organic solvents, and therefore the activity of turmeric was higher when its active compound was extracted by organic solvents like 1, 4-Dioxan and DMF. Individual extracts as well as mixtures of extracts of different spices did not show any remarkable differences in their activity, and they therefore do not have any effects on each other when they are present in mixture form. Synthetic compounds HC-3 with NO₂ at the para

position proved better than HC-4 which has NO₂ at the meta position.

Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

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