DISCUSSION

The discovery of penicillin stimulated the search for antibiotics of plant origin. Large scale screening of higher plants for antimicrobial substances were carried out throughout the world including India. These surveys have demonstrated that antibiotics are widely distributed in higher plants. Some of these investigations ended in the isolation, purification and characterisation of the active compounds. Although most of the purified substances with antimicrobial activity have been found to be toxic to animals and not therapeutically competitive with the products of microbial origin (Nickell, 1959), research continues with the hope that naturally occurring antimicrobial compounds may be found in some common easily growing green plants.

In a tropical country like India, hot and humid climate is highly conducive for infections caused by bacteria and fungi to human beings and animals. Apart from the systemic diseases, there are localised skin infections caused by yeasts (Candida sp.), bacteria (Staphylococcus aureus), and dermatophytic fungi (Trichophyton rubrum) etc. Spoilage of preserved food and diseases of economic plants are some of the other hazards created by bacteria and fungi. The present investigation was intended to find some remedial measures of plant origin to solve these problems.

For this purpose, a study has been made on the (i) screening of West Bengal plants for antimicrobial substance, (ii) selection of two plant species - Moringa oleifera Lamk. and Alpinia mutica Roxb. with high antimicrobial activity and finally (iii) isolation, chemical characterisation and microbiological studies of the active principles.

Screening of plants for antimicrobial activity was carried out on 38 West Bengal plants selected on a semi-random basis which were expected to contain specific type of chemical as evidenced by their folklore credentials. This method of selection of plants for screening
antimicrobial activity was chosen as the possibility of obtaining active plants was greater by this process (Roia and Smith, 1977).

Different morphological parts like root, stem, rhizome, leaf, flower bud, fruit and seeds of the selected plants were considered for the investigation as there are reports of antimicrobial substance to be concentrated only in some specific morphological parts of the plants (Osborn, 1943; Huddleson et al., 1944; Cavallito et al., 1945; Ghosh et al., 1980).

Collection of plants was carried out in different seasons throughout the year. Plants of different ages were also collected from the same locality as there are reports about accumulation of antimicrobial compounds influenced greatly by seasonal and age variation (Lucas and Lewis, 1944; Hayes, 1946; Ghosh et al., 1980).

The screening programme was conducted in two phases: (i) preliminary screening and (ii) final screening. Both fresh plant parts and air dried ones were used for preparing extracts as there are reports of presence of antimicrobial substance in some plants in fresh conditions only (Osborn, 1943; Freerksen and Bonicke, 1951) while others contain the active principle in air dried conditions (Atkinson, 1956). Water and ethanol (90%) were used as extraction-solvent for fresh materials and dried materials respectively during the preliminary screening. During the final screening various solvents viz. petroleum ether (60°-80°C), benzene, ethyl acetate, chloroform, ethanol (both dehydrated and 50% aqueous) and methanol were used for extracting the antimicrobial principles (if present). Water was used to extract the water soluble active substances and ethanol was used since it helps to extract bulk of the compounds present in the plants. Absolute ethanol was avoided during preliminary screening as it sometime destroys the antimicrobial substance, as in the case of antimicrobial substance of Moringa oleifera. The aqueous and 50% aqueous ethanol extracts of plants were sterilized by bacterial filters (pore size 0.1 μ) before testing their antimicrobial
activity. Sterilization of the extracts by bacterial filters was found necessary to avoid the chance of contamination and spoilage of the experimental plates.

Evaluation of the antimicrobial activity of the active principles present within the plant extracts was done by agar cup-plate diffusion method. This method was selected during the present investigation as it was found suitable for rapid assay and perfect sterilization of the test materials was not necessary. Moreover, it was suitable for both qualitative and quantitative assay represented by the area of the zone of inhibition in the result. The method was used by many investigators like Carlson et al. (1948), Florey et al. (1949), Gupta and Banerjee (1972), Ray and Majumdar (1976) etc.

The screening programme was carried out by testing the extracts against bacteria and fungi selected on the basis of (i) pathogenic to human being and animals, (ii) plant pathogenic, (iii) food spoiling and (iv) common soil inhabitants. The purpose was to screen the plants having compounds which may be used against (i), (ii) and (iii) types of organisms mentioned above.

During the preliminary screening aqueous and 90% aqueous ethanolic extracts of 126 morphological parts and latex (without extraction) from 38 species of plants were tested against 10 different bacteria and 8 different fungi.

Results obtained from the preliminary screening indicated that 7 plants out of 38 plants tested, contained antimicrobial compounds. By comparing this result with those of some previous investigators like Dhar et al., 1968 (one antibacterial plant obtained from 285 plants tested); Bhakuni et al., 1969 (one antibacterial and one antifungal from 300 plants tested); Gupta and Banerjee, 1972 (four antifungal from 174 plants tested) the result obtained seems to be quite encouraging. Among the antimicrobially active plants studied in the present programme, all
morphological parts of Moringa oleifera have exhibited antimicrobial property. It has activity against common soil inhabitant bacteria (B. subtilis), human pathogenic bacteria (S. aureus), enteropathogenic bacteria (E. coli, Proteus retgeri etc.), human pathogenic dermatophyte (T. mentagrophytes), and food spoiling fungi (A. niger, S. cerevisiae etc.) and plant pathogenic fungi (Fusarium solani).

Croton bonplandianum, Cephalandra indica and Holarrhena antidysenterica were all active against Gram-positive bacteria - B. subtilis and S. aureus. Among these, latex of C. bonplandianum has shown greater activity in comparison to the others. It was also active against Gram-negative enteropathogenic bacteria - P. retgeri.

Rhizome of Alpinia mutica, Curcuma amada and Zingiber spectabile, all belonging to Zingiberaceae were active against Gram-positive bacteria, yeast and fungi including dermatophytes.

Negative results obtained with the plant parts during the preliminary screening programme, may not necessarily be due to absence of any antimicrobial principle. The possible reasons of obtaining negative results may be:

(i) The active principle might have been destroyed by enzymatic activities in the aqueous extracts (Skinner, 1955).

(ii) In addition to the antibiotic, there might be some growth stimulating agents for the test organism in the extract (Boas, 1934).

(iii) In addition to the antibiotic, the presence of antibiotic-antagonistic substance might be there which has led to a false negative result.

Considering the degree of antimicrobial activity as indicated by the diameter of zone of inhibition, the plants selected for final
screening were (i) *Moringa oleifera*, (ii) *Croton bonplandianum*, (iii) *Alpinia mutica*, (iv) *Curcuma amada* and (v) *Zingiber spectabile*.

Final screening of the above mentioned plants was carried out in two steps. In the first step various solvent extracts of the selected plants were tested against 4 different microorganisms, thereby selecting the most suitable solvent for extraction of antimicrobial principle from each plant part. In the second step the range of antimicrobial activity of each plant part was tested against a wide range of bacteria and fungi with the help of selected solvent extracts.

The results obtained from the final screening have indicated that 50% aqueous ethanol, acetone, dehydrated ethanol, ethyl acetate and dehydrated ethanol were the most suitable solvents for extracting the antimicrobial principles from *Moringa oleifera*, *Croton bonplandianum*, *Alpinia mutica*, *Curcuma amada* and *Zingiber spectabile* respectively. Except latex extract of *C. bonplandianum*, others have shown antibacterial and antifungal activity against most of Gram-positive bacteria and the dermatophytic fungi.

In several instances, the findings of other workers have been corroborated (e.g. *M. oleifera* - Bhatnagar et al., 1961; *C. amada* - Ghosh et al., 1980), while in other cases different results have been obtained (e.g. *Nerium indicum* - Ahmed et al., 1993; *Acacia nilotica* ssp. *indica* - Almagboul et al., 1988). This, however, is not unexpected since phytocomponents are known to vary depending on factors such as time of collection, age of the plant, climate and habitat (Hossein et al., 1955).

Selection of plants for antimicrobial compounds and their purification and characterization was based on certain parameters viz., pH tolerance, thermostability, seasonal variation, availability & range of antimicrobial activity of their antimicrobial principles. The pH stability was tested in citrate-phosphate buffers and phosphate buffers
ranging from pH 3 to pH 8. After the treatment of the extracts with buffers at different pH, the pH of each sample was adjusted between pH 5.0-8.0, as most microorganisms are found to grow within this range of pH (Bushnell et al., 1950). The extracts were filtered through sterile bacterial filters to avoid contamination of the assay plates.

The results of pH stability determination have shown that antimicrobial principles of fruit pulp extracts of Moringa oleifera and rhizome extract of Alpinia mutica have a wider range of pH tolerance in comparison to that present in latex of Croton bonplandianum and rhizome extract of Curcuma amada and Zingiber spectabile.

Effect of heat on the antimicrobial principles of the plant extracts was carried out to determine the thermostability of the antimicrobial principles of plant extracts since their use as chemotherapeutic agent, food preservative or as plant protectant are dependent on this criterion. Results of treatment of plant extracts at different temperatures have shown that antimicrobial principles present in fruit pulps of Moringa oleifera and rhizomes of Alpinia mutica retained their antimicrobial activity up to 90°C and only a partial loss of activity was observed at 100°C. A very interesting observation was, the antimicrobial activity of Alpinia mutica rhizome extract increased up to 80°C over that of the extract kept at 30°C (control).

The results also indicate that latex extract of Croton bonplandianum, and rhizome extracts of Curcuma amada and Zingiber spectabile retained their antimicrobial activity only up to 60°C. There was a sharp decline in activity beyond this temperature.

Latex of C. bonplandianum and fruit pulps of M. oleifera have not shown any seasonal variation of antimicrobial activity. On the other hand, rhizomes of A. mutica, C. amada and Z. spectabile have shown marked seasonal variation in antimicrobial activity. The seasonal variation is
perhaps related to the germination and formation of new rhizomes. The activity decreased when the rhizomes started germinating utilizing the stored food and metabolites while the activity increased with aging of the newly formed rhizomes (Ghosh et al., 1980).

On the basis of sparse availability of the active plant parts, Croton bonplandianum, Zingiber spectabile and Curcuma amada were not studied further. Although rhizomes of C. amada are available in the local markets but we have observed that the activity was not uniform (even sometimes absent) in some collections (Ghosh et al., 1980). This may be due to variation in the age of the rhizome or locality of growth of the rhizome. Moringa oleifera fruit pulps and Alpinia mutica rhizomes were, however, found to possess uniform activity irrespective of their place of collection. They were also available in sufficient quantity locally.

The results of final screening of plants for antimicrobial activity have shown that Moringa oleifera fruit pulps and Alpinia mutica rhizomes contained antimicrobial principles active against a wide range of bacteria and fungi. On the other hand, Croton bonplandianum, latex has shown activity only against Gram-positive bacteria. Rhizomes of Curcuma amada and Zingiber spectabile, however, have shown both antibacterial and antifungal activity.

Considering all these points the fruit pulps of Moringa oleifera Lamk. and rhizomes of Alpinia mutica Roxb. were selected for further investigations.

From the review of the phytochemicals and pharmacological activities of Moringa oleifera Lamk. and Alpinia mutica Roxb. it was evident that the plant M. oleifera is rich in phytochemicals having physiologically and antimicrobially active substances (Chopra et al., 1958; Wealth of India, 1962). The antibiotic present in the root of M. oleifera has been identified and studied by Kurup and Rao (1954) and
and Kurup et al. (1954), but there is hardly any report on the detailed studies of the phytochemicals present in other morphological parts of this plant. Further, regarding *A. mutica*, no detailed study on the phytochemicals derived from it has been reported, although there are some reports regarding the presence of some physiologically and antimicrobially active principles in the allied species *A. galanga* (Janssen and Scheffer, 1985; Wealth of India, 1985).

Isolation and purification of the antimicrobial compounds from *M. oleifera* fruit pulp and *A. mutica* rhizome was carried out by extracting the compound with ethanol (50% aqueous or dehydrated) and separation of the compounds by column chromatography.

The antimicrobial principle of freeze dried fruit pulp powder of *Moringa oleifera* could be effectively extracted with 50% aqueous ethanol at room temperature. Extraction with dehydrated ethanol or with solvents was not possible, since the compound lost its antimicrobial activity in them. Purification of the active principle could be achieved by column chromatography on neutral alumina column and eluting successively with different solvents with increasing polarity. The compound 'MA' was obtained from the petroleum ether (60°-80°C) fraction of column eluates. The compound was purified to the chemical state of purity by removing the petroleum ether (60°-80°C) soluble impurity from it. The compound appeared homogeneous on thin layer chromatograms developed with five different solvent systems. The compound 'MA' showed strong growth inhibitory action against the test organisms *Bacillus subtilis* and *Saccharomyces cerevisiae*.

The antimicrobial principles of heat dried (80°C) rhizome powder of *Alpinia mutica* could be almost quantitatively extracted with dehydrated ethanol. Purification of the active principles was achieved by
column chromatography on neutral alumina column and eluting with different solvents, successively with increasing polarity. At least 3 different compounds designated as AA, AB and AC were present in the eluate fractions of which AA and AB were further purified by passing through neutral alumina columns while the compound AC was purified by recrystallization. The compounds thus obtained were found to be homogeneous on thin layer chromatograms developed with six different solvent systems. However, homogeneity of AC was later proved to be eluding, as it was proved to be a mixture of two closely related compounds. Strong antimicrobial activity was exhibited by the compounds AA and AB against the test organisms Bacillus subtilis and Saccharomyces cerevisiae. Although the pooled fraction containing the compound AC exhibited strong antimicrobial activity against B. subtilis and S. cerevisiae, yet the purified white crystals of AC retained very little of it. However, the brown gummy residue of the fraction still contained marked antimicrobial property. Unsuccessful attempts were made to purify the antimicrobial compound from the gummy residue by column chromatography and thin layer chromatography.

The melting point of MA (from M. oleifera) was found to be 15°±1°C. It was found highly soluble in Polar solvents like ethanol, methanol, 1,4-Dioxan and dimethylsulfoxide. The compound was found stable up to 80°C between pH 3-5. By raising the pH the antimicrobial property was lost.

The compound was found unstable and lost its antimicrobial activity when dissolved in water (100%) or ethanol (100%) and kept for 48 h. Similar observations were reported by Dayrit et al. (1990) with antimicrobial principle from M. oleifera seeds.

When treated with Hydroiodic acid, the active compound MA liberated iodine.

From elemental analysis of the compound MA the following result
was obtained: C, 65.2%, H, 4.5%, N, 6.84%, S, 15.69%, O, 7.79%. From the elemental analysis, the possible empirical formula constructed is \( C_{22}H_{18}O_{2}N_{2}S_{2} \).

U.V. light absorption curves of compound MA and its inactive product were prepared. Maximum absorption in case of active compound MA was at 217.6 nm and that of the inactive product of MA was 228.9 nm.

The compound MA did not react to Dragendorff's reagent, Aniline phthalate and Ninhydrin indicating absence of alkaloid, reducing sugar and amino acid in MA.

The physical and chemical properties of MA and the U.V. absorption spectra of MA and its inactive product indicated that the compound MA was the same antimicrobial compound obtained by Kurup and Rao (1954) isolated from \( M. \) oleifera root. The structure of the compound proposed by them was

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\text{Dayrit et al. (1990) identified the active antimicrobial compound from } M. \text{ oleifera seeds as:}
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\[ 4 - \{ \overset{\infty}{\text{Rhamnosyloxy}} \} \text{ benzyliothiocyanate} \]

Earlier workers tried to purify the antibiotic 'Pterygospermin' present in the root of \( M. \) oleifera. Rao et al. (1946), Rao and George (1949), Kurup and Rao (1952), Rao and Kurup (1953) were among them.
However, it was not possible by them to isolate the antibiotic compound to the chemical state of purity.

The compounds AA, AB and AC purified from _A. mutica_ rhizome were tested for physical and chemical properties.

Compound AA is a transparent liquid with a characteristic odour. Compound AB is brown in colour and semisolid at room temperature. Compound AC is white crystalline solid.

Melting point of compound AB and AC were recorded in sulphuric acid baths. For AB the melting point was recorded 46°±1°C and for AC it was recorded 136°±1°C. The compound AA was found highly soluble in petroleum ether (60°-80°C) and less soluble in other solvents like methanol, ethanol, benzene, acetone, chloroform etc. and insoluble in water.

The compound AB was found highly soluble in benzene, ethanol and methanol. It was found less soluble in acetone and chloroform. It was found insoluble in petroleum ether (60°-80°C) and water.

The compound AC was found highly soluble in methanol and water. It was found less soluble in ethanol and chloroform and insoluble in petroleum ether (60°-80°C), acetone and benzene.

The antimicrobial activity of compounds AA and AB was found stable up to 80°C between pH 3-8. Thermostability of the compound AC was not tested since very little antimicrobial activity was originally present in it.

From their negative response to colouring reagents viz. Dragendorff's reagent, Aniline phthalate and Ninhydrin (Chapter V, page 131 of this thesis) it was evident that the compounds AA, AB and AC are devoid of alkaloid, reducing sugar and amino acid.
The compound AA of *A. mutica* showed no peak in 200-400 nm region in the U.V. spectrum.

The I.R. data of compound AA (Table 25) suggested the presence of a straight chain aliphatic structure.

The $^1$H NMR spectrum showed the presence of a broadened triplet (δ 0.88 ppm, J = 6Hz), a singlet (δ 1.24). The triplet indicated the compound might be a straight chain hydrocarbon with methyl groups at the two ends. The singlet at δ 1.24 indicated the presence of a methylene group. The relative intensities of two peaks were 3:8.

From these data it seems the compound AA is likely to be a saturated straight chain hydrocarbon.

The U.V. spectrum of compound AB of *A. mutica* showed a peak at 277 nm (Table 26) indicating the presence of unsaturation.

The I.R. data of compound AB (Table 27) showed the presence of conjugated ester, hydroxyl group, vinyl (CH$_2$-CH-) and aromatic moiety.

The $^1$H NMR data (Table 28) indicated the presence of ethyl cinnamate moiety, a hydrocarbon unit and a vinyl group (CH$_2$=CH-). A tertiary hydroxyl group, three methyl singlets for three -CH$_3$ groups and expected triplet for the methyl protons of the carbethoxy group was centered at δ 1.34. The aliphatic region is not clear due to signal overlap. Total number of proton in the aliphatic region is approximately 27. A sesquiterpene like structure is indicated from the $^1$H NMR data.

The EI-mass spectrum data (Table 29) indicated an ethyl cinnamate moiety (peaks at m/z 176, 131, 103 and 77). The molecular ion could not be located due to feeble peaks in higher mass regions. The compound possibly undergoes thermal fragmentation to eliminate the aliphatic part. The ethyl cinnamate part carries the ion current.
From these data the compound AB seems to be a sesquiterpene alcohol attached to an ethyl cinnamate moiety. This structural assignment needs further confirmation.

The U.V. spectral data (Table 30) of the compound AC of A. mutica indicated the presence of unsaturation in the molecule.

The I.R. spectrum indicated the presence of hydroxy and trans substituted double bonds.

The $^1$H NMR spectral data (Table 31) showed the presence of two types of olefinic bonds and also of a hydroxy group in the molecule. The general pattern of the spectrum was reminiscent of $\Delta^3\alpha - 3\alpha$ -hydroxy steroids with the 10- and 13-methyl resonances assignable to singlets at $\delta$ 0.7 and 1.05 ppm respectively. The 3-carbonyl proton accounted for the multiplet observed at around $\delta$ 3.5. The broad doublet centered at 5.35 with $J = 6$ Hz may have originated from the 6-H of the $\Delta^2$ steroid. The other olefinic proton signals in conjugation with I.R. band at $960$ cm$^{-1}$ suggested the presence of a trans disubstituted double bond, but the relative intensity observed indicated a mixture.

The compound showed two distinct molecular ions at $m/z$ 414 and 412 with intensity ratio of approximately 1:1 in mass spectrum, thus proving the compound to be a 1:1 mixture of two steroids. The spectrum showed strong peaks at $m/z$ 273 and 255 and another pair at $m/z$ 231 and 213 which from known fragmentation behaviour of steroids can be explained as M-side chain, M-side chain-18, M-side chain 42 (part of and M-side chain-42-18 peaks). Since primary fragmentation revealed the presence of only one hydroxy group (distinct M-18 peak at $m/z$ 396 and 394) the molecular formulas of the two compounds should be $C_{29}H_{50}O$ and $C_{29}H_{48}O$. The location of M-side chain fragment at $m/z$ 273, further proved that the side chain consists of a ten-carbon unit. In conjugation with other (particularly $^1$H NMR) spectral evidences structure of two compounds could thus be tentatively identified as $\beta$-sitosterol (stigmasta-5-en-3$\beta$-ol)
and stigmasterol (stigmasta-5, 22-dien-3-ol) which occur together in many plants. The mass fragment at m/z 271 strongly supported the presence of stigmasterol. The triplet like olefinic proton signal centered at δ 5.1 could then be assigned to the side chain protons of stigmasterol. Although the above spectral data do not strictly rule out the presence of other homologues, it may be said that components of compound AC have high similarity in the structures of sitosterol and stigmasterol.

As stated above the antimicrobial activity of *Alpinia mutica* rhizome is due to a mixture of several compounds. the most abundant amongst these is a sesquiterpene alcohol attached to an ethyl cinnamate moiety (compound AB). Two other compounds (compound AA and AC) were purified, having hydrocarbon and sterol like structure respectively. Chromatographic purity in case of compound AC was found deceptive, which was proved from its spectral data as a mixture of two chemically similar compounds β-sitosterol and stigmasterol.

Terpenes, cinnamates, sterols and hydrocarbons have been reported from different species of *Alpinia*.

Tumann & Tkotz (1972) reported the rhizome of *A. officinarum* has been found to contain sterol glucosides like β-sitosterol (m.p. 140°C), stigmasterol (m.p. 170°C) and camposterol (m.p. 157°C).
l'-acetoxy-chavicolacetate, l'-acetoxy eugenol acetate are terpenes reported from A. officinarum and A. galanga (Ogiso and Kobayashi, 1974; Janssen and Scheffer, 1985).

d-camphene, m.p. 52°C, d-camphor, m.p. 179.75°C and d-borneol, m.p. 208°C are other terpenes present in the rhizome of some species of Alpinia (Ultac, 1936; Kafuka, 1917 and Nadkarni, 1989).
Methyl cinnamate is reported by a good number of workers to be present in the rhizome of *A. galanga*, *A. officinarum*, and other species of *Alpinia* (Goldfien, 1937; Nigam and Radhakrishnan, 1963; Nadkarni, 1989).

A hydrocarbon $C_{15}H_{30}$ was reported from *A. galanga* rhizome (Wealth of India, 1985).

From the study of antimicrobial spectrum the compound MA from *M. oleifera* fruit pulp was found to be active against a wide range of microorganisms including human and animal pathogenic bacteria e.g. *Staphylococcus aureus* and *Escherichia coli*, common soil bacteria e.g. *Bacillus subtilis*, human dermatophytes e.g. *Trichophyton rubrum* and *Epidermophyton floccosum*, systemic human pathogenic fungi e.g. *Aspergillus fumigatus*, plant pathogenic fungi e.g. *Fusarium solani* and food spoiling fungi e.g. *Saccharomyces cerevisiae*. The MIC of compound MA was determined 2 µg/ml (against *Staphylococcus aureus*) to 15 µg/ml (against *Curvularia lunata*).

The compounds AA and AB from *A. mutica* rhizome were found active against a wide range of microorganisms. The compounds have shown a similar antimicrobial spectrum. The organisms include human and animal pathogenic bacteria e.g. *Staphylococcus aureus*, common soil bacteria e.g. *Bacillus subtilis*, human dermatophytes e.g. *Trichophyton rubrum* and *Epidermophyton floccosum*, plant pathogenic fungi e.g. *Fusarium solani* and
Botrytis allii, and food spoiling fungi, e.g. Saccharomyces cerevisiae and Aspergillus niger.

However, some difference in activity between AA and AB was also noticed. While AA was active against human systemic pathogens Aspergillus fumigatus, AB was not.

MIC of AA was determined 150 µg/ml against T. mentagrophytes and T. tonsurans. MIC of AB was 120 µg/ml against T. rubrum and 130 µg/ml against T. mentagrophytes.

The antimicrobial spectrum of compound AC from A. mutica rhizome was not determined since the crystals exhibited very feeble antimicrobial activity against different organisms.

It may be concluded that the antimicrobial compound is perhaps present in all the morphological parts of M. oleifera. The compound has strong antimicrobial property against various bacteria and fungi but the active compound is unstable. The activity is retained in 50% aqueous ethanol solution but lost in both dehydrated ethanol and water. Further studies are required to find ways for increasing the stability of the compound.

Antimicrobial compounds of A. mutica rhizome has strong antimicrobial property against Gram-positive bacteria and may fungi including the dermatophytes. Possibility of therapeutic use of these compounds against dermatophytes cannot be ruled out, although pharmacological studies including animal toxicity, LD₅₀ dose, effect of prolonged administration of the antimicrobial compounds are necessary. The compounds may also be used as food preservative, if found non-toxic to human being. The compounds may also find their application in the control of some diseases of crop plants caused by fungi.