5. DISCUSSION

The beneficial medicinal effects of plant materials typically result from the secondary products present. It is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh et al., 2005a). The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study.

In the modern world, multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases (Agarwal et al., 1996).
In the present investigation, leaves and seeds of *Cassia alata*, *C. auriculata*, *C. fistula* and *C. tora* were tested against gram positive bacteria such as *Bacillus subtilis*, *B. pumilus*, *Micrococcus luteus*, *Staphylococcus aureus*, gram negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*, fungal strains such as *Aspergillus niger*, *A. flavus* and *A. fumigatus* and ten isolates of MRSA. Different crude extracts of four species of *Cassia* leaves and seeds exhibited varied levels of antimicrobial activity against tested pathogens. The mean zones of inhibition produced by all the extracts ranged from 6.3 ± 0.5 to 25.6 ± 0.2 mm against bacterial, fungal and MRSA strains. The differences in the antimicrobial activity of crude extracts may be due to degree of sensitivity of test microorganisms and nature and combination of phytochemicals present in crude extracts (Aquil and Ahmad, 2003) and may be due to the amount of antimicrobial agent present in the extract and their mode of action on different test microorganisms (Barbour *et al.*, 2004).

In the present study, among the crude extracts of *Cassia* species tested, the highest mean zone of inhibition (25.6 ± 0.2 mm at 1000 µg/disc) was recorded with ethyl acetate extract of leaves of *Cassia tora* against *Staphylococcus aureus*. Many of the *Cassia* species were well known to have biological properties and these plants are known to contain various active principles of therapeutic
value and possessed biological activity against number of diseases (Panichayupakaranat and Kaewsuwan, 2004). *Cassia fistula* leaf extracts showed antibacterial activity against a wide spectrum of bacteria such as *Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa* and *Proteus vulgaris* and the zones of inhibitions were 16, 16, 18 and 16 mm respectively at 5000 ppm (Perumalsamy et al., 1998). Perumalsamy and Iganicimuthu (2000) reported that the leaf extracts of *Cassia auriculata* exhibited significant broad spectrum activity against *Bacillus subtilis* and *Staphylococcus aureus*.

The methanol extracts of *Cassia siamea* showed a broad spectrum of antibacterial activity against *Bacillus subtilis, B. pumilus, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* with the mean zones of inhibition ranged from 7 to 24 mm (Chandrasekaran and Venkatesalu, 2004b). The same trend in *Cassia fistula* extract showed strong antibacterial and antifungal activities than *Mesua ferrea* (Ali et al., 2004a).

The methanol extract of leaves of *Cassia auriculata* exhibited the maximum antibacterial activity (18 mm at 75 µg/mL) against *Vibrio cholerae* and *Staphylococcus aureus* (Anushia et al., 2009). Singh and Karnwal (2006) pointed out the similar significant results in *Cassia fistula* leaf extract that possessed antifungal activity against *Candida albicans*. 
Roopashree et al. (2008) reported the similar results i.e., aqueous extracts of Cassia tora inhibited Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli at the concentration of 100, 200 and 250 µg/mL respectively but did not inhibit the growth of Bacillus subtilis. Ethanol extract of Cassia tora inhibited only B. subtilis and methanol extract of this plant showed effective inhibition against S. aureus and E. coli at the concentration of 64 µg/mL.

Our results are coinciding with the result of Chavan et al. (2011) i.e., the aqueous extracts of leaves of Cassia tora showed antibacterial activity against Pseudomonas aeruginosa, Lactobacillus sp., Proteus vulgaris, Staphylococcus aureus with the zones of inhibitions viz., 11, 13, 12 and 15 mm respectively and Staphylococcus aureus recorded the highest activity. The methanol extract of bark of Cassia fistula was found to possess good antifungal activity and they produced zones of inhibition against Candida albicans (12.5 mm), Aspergillus niger, (14.5 mm), Trichophyton mentagrophytes (9.5 mm) and Epidermophyton floccosum (16 mm) (Priya et al., 2010).

In this study, the antibacterial and antifungal activities of hexane, chloroform, ethyl acetate and methanol extracts of four species of Cassia against the selected seven bacterial strains, three mould fungal strains and ten MRSA were evaluated and the activities of the ethyl acetate extracts were found to be superior when compared to the other extracts. The activities of different extracts can be stated as ethyl acetate > methanol > chloroform > hexane. The standard drugs,
Ciprofloxacin and Ketoconazole inhibited effectively at a much lower concentration than the crude extracts. Variation of antimicrobial activity was recorded in the present study. This may be attributed to the nature of the plant material or its origin (Ncube et al., 2008). Moreover, the activity of the plant could be influenced by the climatic conditions in which it is growing, the plant part, or the solvent used for extraction, because plants have different constituents depending on those factors.

Duraipandiyan and Ignacimuthu (2007) had screened hexane, chloroform, ethyl acetate, methanol and water extracts of Cassia fistula flowers against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Enterococcus faecalis and one “Gram-negative” bacterium Pseudomonas aeruginosa. The results revealed that the highest zones of inhibition were recorded with ethyl acetate extract against S. epidermidis (23 mm), S. aureus (19 mm), B. subtilis (15 mm) and E. faecalis (13 mm).

Similarly, Phongpaichit et al. (2004) reported that crude methanol extracts from the leaves of Cassia alata, Cassia fistula and Cassia tora were investigated for their antifungal activities against three pathogenic fungi, Microsporum gypseum, Trichophyton rubrum and Penicillium marneffei. Among three species, the leaf extract of C. alata was the most effective against T. rubrum and M. gypseum with IC\textsubscript{50} values of 0.5 and 0.8 mg/mL, respectively. For P. marneffei, C. fistula was the most potent inhibitor with an IC\textsubscript{50} of 0.9 mg/mL.
Muthukumaran et al. (2011) screened the crude extract of *Cassia auriculata* for antimicrobial activity and recorded the differential zone of inhibition against *Escherichia coli* (17.9 mm), *Staphylococcus aureus* (18 mm), *Bacillus subtilis* (21 mm), *Pseudomonas aeruginosa* (18 mm) and fungi, *Candida albicans* (24 mm), *Candida tropicalis* (23 mm) and *Aspergillus niger* (22.3 mm) at 100 µg/mL concentration. Similarly, Doshi et al. (2011) recorded with 80% aqueous methanol extract of flowers of *Cassia auriculata* against *S. aureus* (16 mm), *S. typhi* (16 mm), *E. coli* (15 mm) and *B. subtilis* (12 mm).

The antibacterial and antifungal activities of individual plant may be due to the presence of phytochemicals. There are several evidences on the presence of antimicrobial metabolites like tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids, etc in the studied plant species (Olutiola et al., 1991; Une et al., 2001; Govindachari et al., 1969; Bhattacharya et al., 1999; Ganjewala et al., 2009). Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense (Parekh et al., 2005b). Tannins and saponins are plant metabolites well known for their antimicrobial properties (Tsechesche, 1971). Flavonoids have both antifungal and antibacterial activities. They possessed anti-inflammatory properties also (Ogundaini, 2005; Iwu, 1984). Saponins, flavonoids, terpenes and steroids are known to have antimicrobial and curative properties against several pathogens (Usman and Osuji, 2007; Hassan et al., 2004).
Flavonoids, another constituent of *Senn alata* stem bark extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al.*, 2002). Antifungal activities of anthraquinones and napthoquinones isolated from natural sources have been reported (Fuzellier *et al.*, 1982).

In the present investigation, ethyl acetate extract of leaves of *Cassia tora* possessed the highest mean zone of inhibition. According to Ayurveda, the leaves and seeds of *Cassia tora* are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardio tonic and expectorant (Ahmad *et al.*, 1998). The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders (Chan and Peria, 2001).

Studies on antimicrobial activity against human pathogens have been widely carried out for *Cassia tora*. Patel and Patel (1957) observed that the extract of seeds of *Cassia tora* inhibited the growth of *Micrococcus pyogenes* var. albus, *M. citreus*, *Corynebacterium diphtheria*, *Bacillus megaterium*, *Salmonella typhi*, *S. paratyphi*, *S. schottmuelleri* and *Escherichia coli*. The fungicidal activities of *Cassia tora* extracts were determined against *Botrytis cineria*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia grisea* and *Rhizoctonia solani* (Kim *et al.*, 2004).
Menghani and Soni (2012) reported that pet ether, benzene, chloroform, ethyl acetate, methanol and distilled water extracts of *Cassia tora* exhibited antibacterial and antifungal activities against, *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Shigella sonnei* and fungi, *Candida albicans, Aspergillus niger, A. flavus* and *Trichophyton rubrum*. *Cassia tora* leaves showed significant antimicrobial activity against fungi and Gram-positive bacteria.

In the present research, the significant MIC (62.5 µg/mL) and MBC (125 µg/mL) values were recorded with crude ethyl acetate extract of *Cassia tora* against *Staphylococcus aureus* than the other tested *Cassia* species. The same trend and significant MIC values were observed with aqueous extract of *Cassia tora* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at 100 µg/mL, 200 µg/mL and 250 µg/mL respectively (Anushia et al., 2009).

Janaky *et al.* (2011) observed that ethyl acetate extract of *Cassia occidentalis* exhibited antibacterial effect against *Aeromonas* sp. (MIC 250 µg/mL, MBC 1000 µg/mL), *Bacillus subtilis* (MIC 125 µg/mL, MBC 250 µg/mL), MRSA (MIC 62.5 µg/mL, MBC 500 µg/mL) and *Vibrio fischeri* (MIC 250 µg/mL, MBC 1000 µg/mL).

Similarly, methanol extract of *Cassia laevigata* leaf exhibited MIC at 5 % (w/v) of the extract against *Fusarium oxysporum* and 7.5 % (w/v) against *Aspergillus niger*, whereas, the same extract of
C. fistula leaf showed MIC at 7.5% (w/v) against F. oxysporum and at 10% (w/v) against A. niger (Panigrahi et al., 2012). The leaves of Cassia nigricans showed the MIC value of $2 \times 10^3 \mu g/mL$ against Staphylococcus aureus and Corynebacterium pyogenes, while for Streptococcus pyogenes, Bacillus subtilis, Salmonella typhi and Escherichia coli, the value was $3 \times 10^3 \mu g/mL$. For Pseudomonas aeruginosa, Candida albicans and Neisseria gonorrhoea, the MIC value was $4 \times 10^3 \mu g/mL$ and for Klebsiella pneumonia the values was $5 \times 10^3 \mu g/mL$ (Ayo et al., 2007).

The present results differed with that of Lee et al. (2014) who reported that the strong activity was shown by methanol extracts of Cassia aurantifolia fruits against Haemophilus somnus with the MIC of 495 \mu g/mL. Its leaves showed much lower MIC (198 \mu g/mL) by chloroform extract than methanol (281 \mu g/mL) and n-hexane (810 \mu g/mL) extracts against Burkholdeira sp. Butanol extract of Sesbania grandiflora showed antibacterial specificity to Burkholdeira sp. with MIC of 135 \mu g/mL. Methanol extracts of Piper sarmentosum had MICs of 502 \mu g/mL, 1,005 \mu g/mL and 251 \mu g/mL against Escherichia coli, Burkholdeira sp. and Haemophilus parasuis respectively. The ethyl acetate and water extracts from Curcuma domestica roots have high specificities against Clostridium perfringens with MIC values of 306 \mu g/mL and 183 \mu g/mL, respectively.
Doughari et al. (2008) reported that the water, acetone, dichloromethane, hexane and methanol extracts of leaves of *Senna obtusifolia* recorded the MIC and MBC values from 200 to 2000 µg/mL, with the acetone extracts demonstrating the lowest MIC of 200 µg/mL: MBC of 300 µg/mL each against *Escherichia coli* and *Salmonella typhi*, followed by the dichloromethane extracts against *Salmonella typhi* (MIC 600 µg/mL, MBC 800 µg/mL) and *Staphylococcus aureus* (MIC 300 µg/mL, MBC 400 µg/mL). The ethanol extract of *Cassia siberiana* was active against *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Shigella dysenteriae* and *Salmonella typhi* with the MIC of $4 \times 102$ µg/mL against *Shigella dysentery, E. coli* and *Salmonella typhi*, $2 \times 102$ µg/mL against *Staphylococcus aureus and Streptococcus pyogens* (Olutayo et al., 2012).

In the present study, the active compound, hexadecanoic acid ethyl ester (a fatty acid) was isolated and identified from the ethyl acetate extract of leaves of *Cassia tora*. Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism – major metabolic fuel (storage and transport of energy), as essential components of all membranes, and as gene regulators. In addition, dietary lipids provide polyunsaturated fatty acids (PUFAs) that are precursors of powerful locally acting metabolites, *i.e.* the eicosanoids. Moreover, free fatty acids and their salts may function as detergents
and soaps owing to their amphipathic properties and the formation of
micelles. Fatty acids are carbon chains with a methyl group at one
end of the molecule (designated omega, ω) and a carboxyl group at the
other end. The carbon atom next to the carboxyl group is called the α
carbon, and the subsequent one, the β-carbon. The letter \( n \) is also
often used instead of the Greek \( ω \) to indicate the position of the double
bond closest to the methyl end. The systematic nomenclature for fatty
acids may also indicate the location of double bonds with reference to
the carboxyl group (\( Δ \)) (Rustan and Drevon, 2005).

Fatty acids (FA) and their corresponding esters are one
group of chemicals found in nature considered to have little or no
toxicity, with proven antimicrobial activity and it showed that fatty
acids esterified with monohydrad acids were inactive against
microorganisms, those esterified with certain polyhydric alcohols yielded
antimicrobial derivatives (Kabara et al., 1972; Conley and Kabara, 1973).
Fatty acids are widely occurring in natural fats and dietary oils and
they play an important role as nutritious substances and metabolites
in living organisms (Cakir, 2004). Many fatty acids are known to have
antibacterial and antifungal properties (Russel, 1991). Recently, a
number of novel fatty acid derivatives of carbohydrates have been
synthesized and their antimicrobial activity has been assessed
(Devulapalle et al., 2004; Ferrer et al., 2005). Generally, long-chain
fatty acids have activity against Gram-positive bacteria, while short-
chain fatty acids are more active against Gram-negative bacteria. Lauric acid (a medium-chain fatty acid) is regarded as the most active, with reported activity against both Gram-positive and Gram-negative bacteria (Kabara, 1983). Lauric acid and gentamycin combined have been reported to show activity against MRSA. Vegetable oils are also known to have antibacterial and antifungal properties attributed to fatty acids (Erdenoglu and Kusmenoglu, 2003).

In the present investigation, like crude extracts of Cassia species, the isolated compound, hexadeconoic acid ethyl ester also possessed differential effects against tested pathogens. The mean zones of inhibition ranged between 10.0 and 27.1 mm against all the tested pathogens. The highest mean zone of inhibition (27.1 ± 0.28) was recorded against Staphylococcus aureus. Hexadecanoic acid ethyl ester was reported in some Cassia species and from other medicinal plants, which possessed biological properties. Sermakkani and Thangapandian (2012) reported hexadecanoic acid ethyl ester from the leaf methanol extract of Cassia italica and leaves of Hugonia mystax (Rajeswari et al., 2012). Gnanavel and Saral (2013) identified the hexadecanoic acid ethyl ester from the leaves of Abrus precatorius, which showed biological activities. Hexadecanoic acid ethyl ester was identified in the seed oil of Brachystegia eurycoma and screened against Escherichia coli, Salmonella typhi and Staphylococcus aureus (Igwe and Okwu, 2013). The flower extract of Cassia javanica showed hexadecanoic acid ethyl ester and it exhibited antimicrobial activities against Bacillus subtilis, Streptococcus pyogenes,
Candida albicans, Fusarium solani and Trichophyton rubrum (Bhuvaneswari and Gopalakrishnan, 2014). The leaves of Cassia roxburghii contained hexadecanoic acid and exhibited biological properties (El-Souda and Farrag, 2014). Hexadecanoic acid was isolated from Allium roseum var. grandiflorum also (Rouis-Soussi et al., 2014).

In the present study, hexadecanoic acid ethyl ester produced the lowest MIC (7.81 µg/mL) against Staphylococcus aureus. Similar results were reported with fatty acids of some plants against bacteria and fungi. A new fatty acid (Z)-17-methyl-13-octadecenoic acid, from the sponge Polymastia penicillus and that compound displayed antiprotozoal activity against Leishmania donovani (Carballeira et al., 2009). Seanego and Ndip (2012) identified high levels of linoleic acid, 1,2-benzenedicarboxylic acid and 2,3-dihydro-3,5-dihydroxy-6-methyl ester and these fatty acids showed antibacterial activity against Streptococcus pyogenes, Staphylococcus aureus, Plesiomonas shigelloides and Salmonella typhimurium.

Fernandes et al. (2013) isolated triterpene esters from edible fruits of Manilkara subsericea, which exhibited antimicrobial activity against Staphylococcus aureus. The major fraction of fatty acid methyl esters (FAMEs) isolated from Linum usitatissimum seeds oil and inhibited effectively the growth of Aspergillus flavus than Aspergillus ochraceus. Antifungal potency may be due to the abundance of linoleic and α-linolenic acids in linseed oil (Abdelillah et al., 2013).
Five compounds of fatty acids esters such as cyclopentane-tridecanoic acid, methyl ester, tartronic acid (p-ethoxyphenyl), diethyl ester, 7,10-octadecadienoic acid, methyl ester, heptadecanoic acid, 16-methyl methyl ester, and 9-Octadecenoic acid [Z]-, 2-hydroxyl-1-[hydroxymethyl], ethyl ester were isolated from petroleum ether extract of dried fruiting bodies of *Pleurotus eous* against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* (Suseem and Saral, 2013).

In the present study, Gram-positive bacteria were more susceptible than the Gram-negative bacteria for the tested compound and extracts of *Cassia* species. The greater resistance of Gram-negative bacteria to plant extracts has been documented previously for *Plectranthus* sp. (Rabe and van Staden, 1994), seeds of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004a) and bark of *Cassia siamea* (Chandrasekaran and Venkatesalu, 2004b). The reason for different sensitivity between gram positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms (Avato et al., 1997). The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). The resistance of Gram-negative bacteria towards antibacterial substances is related to lipopolysaccharides in their outer membrane (Sawer et al., 1997;
Gao et al., 1999). Similar results were reported recently in many medicinal plants against bacterial strains (Daboor and Haroon, 2012; Gopalakrishnan et al., 2013).

Our results are in accordance with the findings of Nobmann et al. (2009) who reported that the CFA derivatives, lauric ether of methyl α-D-glucopyranoside and lauric ester of methyl α-D-mannopyranoside had showed more effective activity against Gram-positive than Gram-negative bacteria. The same trend was also observed for the fatty acid and monoglyceride controls, in accordance with previous studies (Conley and Kabara, 1973; Ruzicka et al., 2003). Abdelilah et al. (2013) reported that free fatty acids of Linum usitatisimum seeds showed the highest antifungal activity against Aspergillus flavus and A. ochraceus and may be due the abundance of linoleic and α-linolenic acids. Chandrasekaran et al. (2011) studied FAME extracts from halophytic plant, Sesuvium portulacastrum, which exhibited strong antimicrobial activities against Gram-positive and Gram-negative bacteria and some fungal strains such as Candida albicans, C. krusei, C. tropicalis, C. parapsilosis, Aspergillus flavus, A. fumigatus and A. niger. Additionally, FAME extracts of Excoecaria agallocha leaves showed antibacterial and antifungal principle for clinical application (Agoramoorthy et al., 2007).

In this study, the MBC values of both crude extracts and pure compound were found to be two fold higher than corresponding MIC values. In some other studies also similar trend i.e., the MBC values of methanol
extract of aerial parts of *Lythrum salicaria* (Becker *et al.*, 2005), ethanolic extract of root of *Synclisa scabrida* (Okoli and Iroegbu, 2005) and aerial parts of *Zuccagania puncatata* (Zampini *et al.*, 2005), seeds of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004a), leaves of *Cleistanthus collinus*, and *Strychnos nux-vomica* (Kalaivanan *et al.*, 2014) were identical or two fold higher than the corresponding MIC values.

With regard to MRSA strains tested, all MRSA showed variable susceptibilities to the *Cassia* species extracts and pure compound. Among the MRSA strains, the highest susceptibility (mean zone of inhibition of 22.6 mm at 1000 µg/disc) was showed by ethyl acetate extract of seeds of *Cassia tora* and hexadecanoic acid ethyl ester (mean zone of inhibition of 26.0 mm at 200 µg/disc) against MRSA 1. Methicillin-resistant *Staphylococcus aureus* is a bacterium that is resistant to many antibiotics. In the community, most MRSA infections are skin infections. The MRSA causes life-threatening bloodstream infections, pneumonia and surgical site infections. Methicillin–resistant *Staphylococcus aureus* strains have acquired a gene that makes them resistant to all beta-lactim antibiotics. Hospital associated strains of this organism are serious nosocomial pathogens that have become resistant to most common antibiotics, and treatment can be challenging. In the present study, the MRSA strains showed more susceptibility to compound, hexadecanoic acid ethyl ester. The compound was isolated
from the leaves of *Cassia tora*, which may be controlled safely than the other antibiotics. Development of drug resistance in bacteria is a common clinical problem. Use of plant derived bioactive molecules have been reported in many cases (Ahmad and Beg, 2001) such as methicillin resistant *Staphylococcus aureus* (Linuma *et al.*, 1996; Aquil *et al.*, 2005), multi-drug resistant tuberculosis (Jimenez-Arellanes *et al.*, 2003; Camacho-Corona *et al.*, 2008) and multidrug-resistant enteric bacteria (Ahmad and Aqil, 2007).

The pharmacologically active compounds were isolated from *Cassia* and other plants, which may support to the present research. *Cassia tora* seeds also contain Rubrofusarin and its triglucoside, Quercetin, 6-O-β-D glucoside and 6-O-β-D-gentiobioside (Chatterjee and Pakrashi, 1992). A new naphthopyrone glycoside was isolated and characterized as 10-[[β-Dglucopyranosyl-(1→6)-O-β-D-glucopyranosyl]oxy]-5-hydroxy-ethoxy-2-methyl-4Hnaphtho[1,2-b]pyran-4one (isorubrofusarin gentiobioside) from the roasted seeds of *Cassia tora*. Along with isorubrofusarin gentiobioside, alaternin and adenosin were isolated and identified (Lee *et al.*, 2006). Three naphthopyrone glucosides, cassiaside, rubrofusarin- 6-O-β-D-gentiobioside and toralactone-9-O-β-D-gentiobioside were isolated from the seeds of *Cassia tora* using an *in vitro* bioassay based on the inhibition of advanced glycation end products (AGEs) formation (Lee *et al.*, 1998).
Ali et al. (2004b) reported that three lectins, i.e. CSL-1, CSL-2 and CSL-3, purified from the Cassia fistula seeds were tested for their antibacterial activities against different pathogenic bacteria, i.e. Bacillus subtilis, B. megaterium, Streptococcus haemolyticus, Streptococcus aureus, Sarcina lutea, Shigella sonnei, Escherichia coli, Klebsiella sp., Shigella shiga, S. boydii, S. flexneri, Shigella dysenteriae, Salmonella typhi and Pseudomonas aeruginosa.

The phenolic glycosides and their glycones and several other compounds of Cassia fistula were examined on Escherichia coli, Pseudomonas aeruginosa and some strains of Staphylococcus aureus. Among them torachrysone, toralactone, aloe-emodin, rhein and emodin showed noticeable antibacterial effects on four strains of methicillin-resistant Staphylococcus aureus (Hatano et al., 1999).

Two antimicrobial compounds namely Methylgallate and Protocatechuic acid isolated from Sebastiania brasiliensis which possessed antimicrobial activity against Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Mucor sp. and Aspergillus niger (Penna et al., 2001).

Two antibacterial compounds were detected from the seeds of Mimusops elengi, 2,3-dihyro-3,3’4’5,7-pentahydroxyflavone and 3,3’,4’,5,7-pentahydroxyflavone. The compounds showed strong inhibitory activity against Gram-positive and Gram-negative bacteria (Hazra et al., 2007).
Two compounds *viz.*, benzo *(g)* pteridine-8-hydroxy-7,10-dimethyl-2,4-dione and 1,2-benzenedicarboxylic dinonyl ester isolated from *Ephedra alata* showed antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus fumigatus*, *Penicillium italicum*, *Syncephalastrum racemosum* and *Candida albicans* (Ghanem and El-Magly, 2008).

Three phenolic compounds, 4-hydroxyphenylethylvanillate, a new natural product, acteoside and quercetin isolated from leaf extract of *Buddleja salviifolia* exhibited a broad spectrum of antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (Pendota et al., 2013). The bioactive compound, Plumbagin, isolated from *Aristea ecklonii* possessed antimicrobial activity against *Trichophyton mentagrophytes* and *Microsporum canis* (Mabona et al., 2013).

The two compounds namely methyl gallate and a phenyl propanoid glucoside, 1-6-di-0-coumaroyl glycol pyranoside were obtained from *Terminalia phanerophelebia*, which possessed antimicrobial activity against *Mycobacterium tuberculosis*, *M. aurum*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Hexadecanoic acid ethyl ester has been identified to have antibacterial and antifungal activity against range of species studied. Since natural substances have proved to have less side effects and less unwanted reaction with environment, using natural materials for biological prevention of microbes is more desirable (Mehrabian et al., 2000). Hence, the compound Hexadecanoic acid ethyl ester can be used as a natural product against bacterial and fungal pathogens studied. However, a detailed pharmacological investigation of this compound in *in vivo* condition is necessary.