VI. Discussion
Discussion

Charak Samhita, an ancient classic, is the oldest text available on the treatment of diseases which specifies the use of hundreds of herbs in the complete treatment of bacterial diseases like leprosy, tuberculosis. Ayurveda has given due importance to the use of medicinal plants in the treatment of various infectious diseases. Literature available reveals the positive effects of the medicinal plants against a number of Gram-positive and Gram-negative bacteria (Pelczar et al, 1986). Nearly 6000 medicinal plants having curative properties are used for their medicinal values and personal hygiene. Indian systems of medicines, such as Ayurveda, Unani and Sidha's are regaining popularity and are of great demand in the country (Farooqi et al, 2003). Botanicals have been a source of medicinal agents since time immortal. They are promising candidates for development as chemotherapeutic agents and protect against malignancy (Redkar and Jolly, 2003). Modern man is searching the folkloric flora in the hopes of discovery of new therapeutic agents to treat human diseases (Svoboda, 1975). The antimicrobial effect of medicinal plants is well documented (Valero and Salmeron, 2003). Results of different studies provide evidence that some medicinal plants might be potential sources of new antibacterial agents even against some antibiotic resistant strains (Kone et al, 2004).

The present study was undertaken, considering the above advantages of medicinal plants. Keeping this in mind, commonly found fourteen plants were tried out for their anti-microbial activities using bioassay method, against seventeen common pathogens (Study Group A). Antimicrobial activity of the six effective plants was studied against the pathogens isolated from swab samples of burns wounds (Study Group B). Standard ATCC cultures were made available from Himedia and anti-microbial activity was studied against these four cultures (Study Group C). Results obtained were compared with anti-microbial activity of standard antibiotics, in all the three groups. By applying statistical analytical tests, the significance of the results obtained with respect to the various groups was derived. Phytochemical analysis of the plant extracts was carried out to detect the components.
Detailed analysis of various components of antimicrobially effective plant extracts was carried out by HPTLC at 200 nm, 254 nm and 366 nm. The antimicrobially most effective aqueous seed extract of Pongamia pinnata was subjected to TLC, its components separated and checked for antimicrobial activity against burns swab isolates. The aqueous Pongamia pinnata seed extract and its components were analysed for identification of their active chemical groups by IR spectroscopy and structures of components by GCMS.

1) **Pongamia pinnata**

*Pongamia pinnata* is a huge tree, each part of which finds varied uses in the life of mankind like seeds, flowers, fruits and leaves. This plant is found to be the anti-microbially most efficient, of all the samples studied during the course of investigation. Seeds of *P. pinnata* were extracted in distilled water, acetone and 0.1 ml of each extract was tested against each isolate for antimicrobial activity.

**Study Group A :**

Aqueous extract (0.31 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *S. aureus*, *Ent. fecalis*, *M. luteus*, Gram negative *E. coli*, *Kl. pneumoniae*, *Sal. typhi*, *Sh. Flexneri*, *C. albicans* and *Asp. niger* (Table 6). The acetone extract (0.0716 X 10^2 gm %) was found effective in inhibiting the growth of *S. aureus* and *Asp. niger* while Gram negative isolates were found to be resistant (Table 7). *M. luteus* was found to be most sensitive to the aqueous extract (19 mm) while *Asp. niger* to the acetone extract (21.5 mm). Amongst the test isolates, *S. aureus* was least sensitive to the aqueous (8.33 mm) and acetone (19 mm) extracts (Table 20). The aqueous extract of *P. pinnata* showed 71% efficiency while acetone extract showed 14% efficiency. Thus, the aqueous extract was found more potent than acetone extract against *S. aureus* and *Asp. niger* (Table 25, Bar diagram 1).

Response of Group A isolates to antibiotics showed that Lomefloxacin (LO) inhibits ten, Gentamicin (G), Cephotaxime (CX), Netillin (NT), Ofloxacin (OF), Pefloxacin (PF) inhibit nine, Norfloxacin (NX) inhibits eight, Nalidixic acid (NA) inhibits seven, Ceftazidime (CT), Chloramphenicol (CHLO),
Nitrofurantoin (NF) inhibits six, Ciprofloxacin (CP), Doxycycline (DO) inhibits five, Amikacin (AM) inhibits three, Augmentin (AU) inhibits two while Cefuroxime (CO) and Cefoperazone (CF) inhibit one of the isolates. On comparison, aqueous extract of *P. pinnata* can be said to be equipotent to *Lomefloxacin* in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group B:**
In primary screening, response of organisms from swab samples of burns wounds showed that, out of ten, six were sensitive to the aqueous extracts of *P. pinnata* (Table 28). Of the seven Group B isolates, five were susceptible to the aqueous extract (Table 30). Response to antibiotics showed that, Cephtoxime was most effective against seven isolates, Nalidixic acid, Nitrofurantoin, Norfloxacin, Netillin and Ofloxacin against six isolates, Ceftazidime against five and Ciprofloxacin against one. Hence the antimicrobial activity of *P. pinnata* was found equipotent to that of *Ceftazidime* (Table 31, 32, Bar diagram 3).

**Study Group C:**
The aqueous and acetone extracts were found to be effective against *S. aureus* ATCC 29213 while *Ent. fecalis* ATCC 29212, *E. coli* ATCC 25922 and *Ps. aeruginosa* ATCC 27853 were found to be resistant (Table 33, 34, 35, 37, Bar diagram 4).

MIC of aqueous extract of *P. pinnata* (0.31 X 10^2 gm%) for *M. luteus* was found to be 0.0256 gm of the plant material (Table 40, 41).

Preliminary qualitative chemical investigation of the aqueous extract showed the presence of alkaloids, flavonoids, glucose and proteins (Table 42). Analysis by HPTLC at 200 nm indicated the probable presence of anthroquinones (6.69%) and alkaloids (18.91%), at 254 nm, caffeic acid (4.13%, 19.3%, 5.88%), at 366 nm, Echinacosides (0.96 %), Cynarin (2.1%) and Caffeic acid derivatives (5.98%, 4.8%, 84.61%), at 254 nm flavonoids, Cascarosides A, B, C, D, sennosides (22.22%, 14.10%), Glucofrangulins and aloanosides (12.51%) and A-monoglycosides and frangulins A, B (20.11%) and at 366 nm, sily-christin (3.04%), taxifolin (4.15%) and iso-silybin (10.92%) (Table 43, 44, 45, 46, 47, Plates 15-17, Graphs 1-5).

Akki et al (2004) similarly reported the effectiveness of aqueous extract of *P. pinnata* against wound pathogens, species of *Bacillus, Pseudomonas,*
Enterococcus, Actinomycetes and one unidentified species. Wagh et al. (2005) observed a high degree of anti-fungal and anti-bacterial activity of *P. pinnata* against *Asp. niger*, *Asp. fumigatus*, *S. aureus* and *Ps. aeruginosa*. This can be attributed to the presence of 9-octadecenoic acid, methyl ester in maximum concentration. Kumar et al (2007) reported the antimicrobial effects of *Pongamia pinnata* seeds against acne-inducing bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* and MIC and MBC of 2.5, 5.0, 2.5, 5.0 mg/ml respectively.

2) *Curcuma longa*

*Curcuma longa* is used everyday in Indian kitchens to impart colour, flavour and a typical taste to foods and vegetable preparations. It is known to be an antiseptic, anti-inflammatory agent, used in case of emergencies as a dressing and as a gargle in case of throat infections.

In the present study, 0.1 ml each of the aqueous and acetone extracts of rhizomes of *C. longa* were used against each test isolate for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.17 X 10² gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *S. aureus*, *Ent. fecalis* and Gram negative *E. coli*, *Sal. typhi*, *Sh. flexneri*, *Ps. aeruginosa*, *P. vulgaris* as well as *C. albicans* (Table 6). *Asp. niger* was found to be resistant. The acetone extract (0.44 X 10² gm%) was found effective in inhibiting the growth of Gram positive *Ent. fecalis*, *M. luteus*, Gram negative *Ser. marsecesens* (Table 7).

*Ent. fecalis* was found to be most sensitive to the aqueous extract (19.66 mm) while *M. luteus* to acetone extract (19 mm). *Ps. aeruginosa* was least sensitive to the aqueous extract (15.33 mm) and *Ent. fecalis* to acetone extract (15.66 mm) (Table 16). Comparative efficiencies of aqueous and acetone extracts inhibiting *Ent. fecalis* were found to be statistically significant, aqueouq extract showed 64% efficiency while acetone extract showed 21% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *C. longa* inhibited nine of the Group A isolates and hence was equipotent to Gentamicin (G), Cephotaxime (AX), Netilill (NT),
Ofloxacin (OF) and Pefloxacin (PF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

Study Group B:
Primary screening of response of organisms in swab samples of burns wounds showed that, three out of ten and three out of the seven isolates of Group B were found susceptible to the aqueous extract of C. longa (Table 28-30). Cephotoxime is found to be most effective against all isolates, Nalidixic acid, Nitrofurantoin, Norfloxacin, Netillin and Ofloxacin against six, Ceftazidime against five and Ciprofloxacin against one. Hence the antimicrobial activity of C. longa was found more than that of antibiotic Ciprofloxacin (Table 31, 32, Bar diagram 3).

Study Group C:
The aqueous extract was found effective against the standard cultures of S. aureus ATCC 29213 and E. coli ATCC 25922 while acetone extract inhibited the growth of S. aureus ATCC 29213 (Table 33, 34, 35, 37, Bar diagram 4).

MIC of aqueous extract of C. longa (0.17 X 10^2 gm%) against Ent. fecalis is found to be 0.0472 gm of plant material (Table 40, 41).

Preliminary chemical investigation of the aqueous plant extract showed the presence of alkaloids, fats, glucose and triterpenoids. HPTLC analysis at 200 nm showed peaks probably corresponding to coumarins (1.25%) and triterpenoids (3.6%, 0.64%), at 366 nm, Echinacosides (2.69%), Eriodictyol (14.81%), Cichoric acid (4.3%) and Caffeic acid derivatives (10.86%, 63.06 %, 3.56%, 0.7%) (Table 43, 44, 45, 46, 47, Plates 15-17, Graphs 1-5).

Shrinivas and Prabhakaran (1987) reported similar findings, in the clinical bacteriological study of C. longa in case of conjunctivitis. Tang and Eisenbrand (1992) observed that aqueous extract of C. longa is effective against pathogens isolated from wounds, Actinomycetal species and two unidentified species. Rhizomes of C. longa are considered to have natural antibacterial, anti-inflammatory, antineoplastic and analgesic activities because they contain a number of monoterpenoids, sesquiterpenoids, and curcuminoids. Soni et al (1992) reported that extracts of C. longa, at concentrations of 5-10 mg/ml, greatly reduced aflatoxin production by more than 90% in case of Aspergillus parasiticus. Chandi et al (1999) observed
antibacterial activity of *Curcuma longa* stem and root extract and reported that they were effective against *S. aureus*, *E. coli* and a wide variety of pathogenic bacteria. Antimicrobial properties of essential oils of *Curcuma longa* studied against *Sal. typhi*, *Kl. pneumoniae*, *E. coli*, *S. aureus* and *B. subtilis*, in different concentrations showed antimicrobial activity against all but maximum activity against *E. coli* (Dubey et al, 2005). Joshi and Shete (2006) studied haladi for anti-candidal activity and reported inhibition of antibiotic resistant clinical strains of *Salmonella*, *Shigella*, *Vibrio*.

3) **Cassia auriculata**

*Cassia auriculata* is a plant, leaves of which are commonly crushed in rural areas and used as a dressing pack for healing wounds and to reduce edema, which may be attributed to alkaloids and flavonoids. It is available commonly in ample and is known to be medicinal. Its topical and external use is advisable, but oral consumption requires further scientific research. 0.1 ml of each of the aqueous and acetone leaf extracts was used against each test isolate, for antimicrobial activity.

**Study Group A**:

The aqueous extract (0.0014 X 10^2 gm%) was found effective in inhibiting the growth of eight organisms in nutrient agar medium, three Gram positive and five Gram negative bacteria. The susceptible Gram positive strains were *B. subtilis*, *S. aureus*, *M. luteus*. Of the Gram negative isolates, *E. coli*, *Kl. pneumoniae*, *Sh. flexneri*, *Ps. aeruginosa* and *P. vulgaris* were found to be susceptible (Table 6). The acetone extract (0.015 X 10^2 gm%) was found effective in inhibiting the growth of one Gram negative organism, *P. vulgaris* in nutrient agar medium. No Gram positive bacteria were found susceptible to the extract (Table 7).

*Ps. aeruginosa* was found to be most sensitive to the aqueous extract (24.5 mm) while *P. vulgaris* to acetone extract (21.16 mm). *Sh. flexneri* was least sensitive to the aqueous extract (16.5 mm) as concluded from the zone sizes (Table 13).

Comparative efficiencies of aqueous and acetone extracts inhibiting *C. auriculata* were statistically very highly significant, aqueous extract of
showed 57% efficiency while acetone extract showed 7% efficiency (Table 25, Bar diagram 1).

On comparison of the antimicrobial effect of the plant extract with that of antibiotics, it was observed that aqueous extract of *C. auriculata* inhibited eight of the Group A isolates and hence found equipotent to Norfloxacin (NX) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group B:**
Primary screening of swab samples of burns wounds showed that, out of ten, two were sensitive to the aqueous extracts of *C. auriculata* (Table 28). Of the seven isolates (B), four were found to susceptible to the aqueous extract (Table 30). Cephotoxime was found to be most effective against seven isolates, Nalidixic acid, Nitrofarantoin, Norfloxacin, Netillin and Ofloxacin against six isolates, Ceftazidime against five and Ciprofloxacin against one. Hence the antimicrobial activity of *C. auriculata* was found more than that of Ciprofloxacin (Table 31, 32, Bar diagram 3).

**Study Group C:**
The aqueous extract was found to be effective against the standard culture of *S. aureus* ATCC 29213 and *Ps. aeruginosa* ATCC 27853 and the acetone extract inhibited the growth of *S. aureus* ATCC 29213 (Table 33, 34, 35, 37, Bar diagram 4).

Chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, fats and glycosides (Table 42).
Its aqueous extract on HPTLC analysis at 254 nm probably indicated the presence of Cascarosides A, B, C, D, Sennosides (15.09% and 9.75%), Glucofrangulins, Aloinosides (4.38%), Aloin and Rhein (2.81%), Deoxyaloin (2.71%) and A-monoglycosides, frangulins A, B (1.51%), at 366 nm, flavonoids xantho-eriodictyol (8.86%), sily-christin (6.62%) and taxifolin (17.78%) (Table 43, 44, 45, 46, 47, Plates 15-17, Graphs 1-5).

In similar studies, aqueous extract of *C. auriculata* is found effective against pathogens isolated from wounds, *Bacillus* species, *Actinomycetal* species and two unidentified species (Tang and Eisenbrand, 1992). Murugan et al (2006) studied the efficiency of aqueous and acetone extracts of *C. auriculata* on the candidial and other superficial mycoses. It was observed that growth decreased with increase in extract concentration and both the extracts
exhibited significant antifungal activity, comparable with antibiotics. The antimicrobial potency of ethanol extract of Cassia species was checked against *E. coli*, *S. aureus*, *Ps. aeruginosa*, *C. albicans*, *Asp. niger*, *Asp. flavus*, by disc diffusion method and was found to be significant (Selvamani and Latha, 2004). *C. auriculata* was one of the active plants, amongst eighteen, which exhibited antimicrobial activity against *B. subtilis*, *S. aureus*, *S. epidermidis*, *Ent. fecalis*, *E. coli*, *K. pneumonia*, *Ps. aeruginosa*, *Erwinia sp*, *P. vulgaris* at three different concentrations of 1.25, 2.5 and 5 mg/disc. (Samy and Ignacimuthu, 2000; Duraipandiyan et al., 2006).

4) *Phyllanthus niruri*

Commonly known as Bhui-awala, *Phyllanthus niruri* is used as an antiseptic in emergencies. 0.1 ml of each aqueous and acetone leaves extract was used against each test isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.06 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *S. aureus*, *E. fecalis*, Gram negative *Sal. typhi*, *Sal. paratyphi B*, *P. vulgaris*, *Ser. marsescens* and *C. albicans* (Table 6). The acetone extract (0.095 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *Ent. fecalis*, Gram negative, *Sal. typhi*, *Sal. paratyphi B* and *P. vulgaris*. *C. albicans* and *Asp. niger* were found to be resistant (Table 7).

*S. aureus* was found to be most sensitive to the aqueous extract (19.83 mm) while *P. vulgaris* to acetone extract (15 mm). *P. vulgaris* was least sensitive to the aqueous extract (14.5 mm) and *Sal. paratyphi B* to acetone extract (13.83 mm), as concluded from the zone sizes (Table 18).

Comparative efficiencies of aqueous and acetone extracts inhibiting *Sal. typhi* were statistically highly significant, *Ent. fecalis* very highly significant, while those of *Sal. paratyphi B* and *P. vulgaris* found comparable. The aqueous extract of *P. niruri* showed 57% efficiency while acetone extract showed 29% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *P. niruri* inhibited eight of the Group A isolates and hence was equipotent to *Norfloxacin* (NX) in its antimicrobial action (Table 26, 27, Bar diagram 2).
**Study Group B:**

Primary screening of organisms from swab samples of burns wounds showed that, out of ten, one was sensitive to the aqueous extract of *P. niruri* (Table 28). Of the seven isolates (B), two were found susceptible to the aqueous extract (Table 30). Cephotoxime is found to be most effective against seven isolates, Nalidixic acid, Nitrofurantoin, Norflaxacin, Netilin and Ofloxacin against six isolates, Ceftazidime against five and Ciprofloxacin against one. Hence the antimicrobial activity of *P. niruri* was found more than that of antibiotic Ciprofloxacin (Table 31, 32, Bar diagram 3).

**Study Group C:**

The aqueous extract was found to be effective against the standard culture (study Group C) of *S. aureus* ATCC 29213, *Ent. fecalis* ATCC 29212 and *E. coli* ATCC 25922 and the acetone extract inhibited the growth of *S. aureus* ATCC 29213, *Ent. fecalis* ATCC 29212 (Table 33, 34, 35, 37, Bar diagram 4).

MIC of aqueous extract of *P. niruri* (0.06 X 10^2 gm%) for *S. aureus* was found to be 0.1826 gm of plant material (Table 40, 41).

Qualitative chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, glycosides, reducing sugars and triterpenoids (Table 42).

Its aqueous extract on HPTLC analysis at 200 nm probably showed peaks corresponding to alkaloids (6.42%), at 366 nm, Eriodictyl (3.62%), Cichoric acid (15.48%), Cynarin (2.29%), Eriodictyl (3.62%), Chlorogenic acid / Eriocitrin (5.5%) and Caffeic acid derivatives (2.85%, 5.07%, 3.27 %, 4.77%, 34.39%, 14.54%, 6.2%, 2.02%), Flavonoid analysis at 254 nm, Cascarosides A, B, C, D, senosides (7.68%), Aloin, Rhein (20.79%), deoxyaloin (19.46%) while at 366 nm xantho-eriodictyl (8.52%) and silychristin (10.74%) (Table 43, 44, 45, 46, 47, Plates 15-17, Graphs 1-5).

In similar studies, Akki et al (2004) observed the wound healing activity of *P. niruri* aqueous leaf extract and reported that aqueous extract is found effective against pathogens isolated from wounds, *Pseudomonas species*, *Enterococcus species*, *Actinomycetal* species and two unidentified species.
5) *Azadirachta indica*

*Azadirachta indica* is known to have extraordinary medicinal properties and is used in bathing water regularly. In winters, it is crushed and eaten raw and is known to cure most of the common diseases. 0.1 ml of each aqueous and acetone extracts of leaves of *A. indica* was used against each test isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.0925 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, S. aureus, Ent. fecalis*, Gram negative *E. coli, Sal. typhi, Sh. flexneri* and *P. vulgaris* (Table 6). The acetone extract (0.035 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, S. aureus, Ent. fecalis*, Gram negative *E. coli, C. albicans* and *Asp. niger* (Table 7). *Sh. flexneri* was found to be most sensitive to the aqueous extract (16 mm) while *Asp. niger* to acetone extract (19.16 mm). *Sal. typhi* was least sensitive to the aqueous (7.5 mm) and *B. subtilis* to acetone extract (7.83 mm), as concluded from the zone sizes (Table 11).

Comparative efficiencies of aqueous and acetone extracts inhibiting *S. aureus, Ent. fecalis, E. coli* were statistically very highly significant while those for *B. subtilis* comparable. The aqueous extract of *A. indica* showed 50% efficiency while acetone extract showed 43% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *P. niruri* inhibited seven of the Group A isolates and hence was equipotent to Nalidixic acid (NA) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group B:**

Primary screening of isolates from swab samples of burns wounds showed that out of ten, five were sensitive to the aqueous extracts of *A. indica* (Table 28). Of the seven isolates (B), three were found to be susceptible to the aqueous extract (Table 30). Cephotoxime is found to be most effective against seven isolates, Nalidixic acid, Nitrofurantoin, Norfloxacin, Netillin and Ofloxacin against six isolates, Cefazidime against five and Ciprofloxacin against one. Hence the antimicrobial activity of *A. indica* was
found more than that of antibiotic Ciprofloxacin (Table 31, 32, Bar diagram 3).

**Study Group C**:

The aqueous and acetone extracts were found to be effective against study Group C culture of *Ent. fecalis* ATCC 29212 (Table 33, 34, 35, 37, Bar diagram 4).

MIC of aqueous extract of *A. indica* (0.0925 X 10^2 gm%) for *Sh. flexneri* was found to be 0.0864 gm of plant material (Table 40, 41).

Qualitative chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, glycosides, proteins and reducing sugars (Table 42). Analysis by HPTLC at 200 nm probably showed peaks, corresponding to terpenes (10.22%, 0.27%), saponins (8.65%) while at 366 nm, Echinacoside (1.19%), Rutin / Sinensetin (1.7%), Chlorogenic acid / Eriocitrin (4.53%), Eriodictyol (12.88%), Cichoric acid (1.74%), at 254 nm flavonoids - Cascarosides A, B, C, D, Sennosides (7.59%), Glucofrangulins, Aloinosides (51.19%), and at 366 nm xantho-eriodictyol (7.66%), sily-christin (12.6%), taxifolin (26.76%) and iso-silybin (40.97%) (Tables 43-47, Plates 15-17, Graphs 1-5).


Kumar and Khanum (2004) in similar studies observed that ethanolic extract of *A. indica* leaves showed anti-acne activity. It is inhibitory against *Propionibacterium acne*. Akki et al (2004) studied the wound healing
activity of *A. indica* leaf extract and reported that aqueous extract is found effective against pathogens isolated from wounds, *Pseudomonas* species, *Enterococcus* species, *Actinomycetal* species and two unidentified species. Muley and Pawar (2005) reported that fungus affected groundnuts, gram and green gram seeds when treated with crude aqueous extracts of *A. indica* for various time intervals like 5, 10, 15 minutes showed that within 30 min, growth of dominant fungi like *Asp. flavus*, *Asp. niger*, *Fusarium moniliforme* were inhibited, which may be due to the bioactive compounds present in the leaves. Bipte and Musaddiq (2005) reported that biotoxicity of aqueous extracts of green-leaves is more at all concentrations while neem tender twigs, seeds, dry leaves were found inhibitory at 75 - 100% concentration over the control. Mohite et al (2005) reported that, neem extract showed significant parasitocidal activity on chloroquine resistant *Plasmodium falciparum* isolates. Seed kernels of neem were tested against two fungal species, *Aspergillus flavus* and *Curvularia lunata* and compared with two commercial antifungal products, neemark and phytolan. Plant extracts were superior over commercial antifungal products in inhibition of spore germination. Both aqueous and acetone extracts of *A. indica* were found to show 38% and 32% efficiency (Karade et al, 2001). Aqueous, alcohol, xylene, hexane, chloroform, ether leaf extracts of *A. indica* showed antimicrobial activity against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Ent. fecalis*, *Sal. typhi*, *V. cholerae*, *P. mirabilis*, *Ps. aeruginosa* ATCC 27853, *St. pneumoniae* ATCC 49619 and *Yersinia enterocolitica* (Tambekar and Kharate, 2005). Joshi and Shete (2006) studied neem for anti-candidal activity and reported inhibition of antibiotic resistant clinical strains of *Salmonella*, *Shigella* and *Vibrio*. Antibacterial activity of aqueous neem extract showed highest inhibition of *P. vulgaris*, *Ps. aeruginosa*, *S. aureus*, *B. anthracis*, *P. mirabilis*, *Sal. paratyphi B*, *Sal. paratyphi A* and *Kl. pneumoniae* sequentially and acetone extracts against *Sal. paratyphi A*, *S. aureus*, *P. vulgaris*, *Kl. pneumoniae*, *B. anthracis*, *P. mirabilis* and *Sal. paratyphi B* respectively (Gharge and Gune, 2007). Thus, the results obtained in the present study were found to be comparable with the above literature.
6) *Eucalyptus globulus*

*Eucalyptus globulus* is commonly called as Nilgiri and used in case of common colds to give relief from the running nose. 0.1 ml of each aqueous and acetone extracts of leaves of *E. globulus* were used against each test isolate for antimicrobial activity.

**Study Group A:**
The aqueous extract (0.025 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *M. luteus*, Gram negative isolates, *Kl. pneumoniae*, *Sh. flexneri*, *Ps. aeruginosa* and *P. vulgaris* (Table 6). The acetone extract (0.054 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, *M. luteus*, *Ent. fecalis*, *S. aureus* while Gram negative *Kl. pneumoniae*, *Sal. typhi*, *Sal. paratyphi B*, *Sh. flexneri*, *Ps. aeruginosa* and *P. vulgaris* and *C. albicans* (Table 7).

*B. subtilis* was found to be most sensitive to the aqueous extract (21.83 mm) while *M. luteus* to acetone extract (29 mm). *Kl. pneumoniae* (16 mm) was least sensitive to the aqueous extract and *B. subtilis* (14.33 mm) to acetone extract, as concluded from the zone sizes (Table 17).

Comparative efficiencies of aqueous and acetone extracts inhibiting *Shigella flexneri* were statistically significant, those for *B. subtilis*, *M. luteus*, *P. vulgaris* very highly significant while for *Kl. pneumoniae*, *Ps. aeruginosa* comparable. The aqueous extract of *E. globulus* showed 43% efficiency while acetone extract showed 79% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *E. globulus* inhibited six of the Group A isolates and hence was equipotent to *Ceftazidime* (CT), *Chloramphenicol* (CHLO), *Nitrofurantoin* (NF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group B:**
Primary screening of response of organisms from swab samples of burns wounds showed that out of ten, three were sensitive to the aqueous extracts of *E. globulus*. (Table 28). Of the seven isolates (B), four were found susceptible to the aqueous extract (Table 30). Cephotoxime was found to be most effective against seven isolates, Nalidixic acid, *Nitrofurantoin*, Norfloxacin, Netillin and Ofloxacin against six isolates, *Ceftazidime* against
five and Ciprofloxacin against one. Hence the antimicrobial activity of *E. globulus* was found more than that of antibiotic Ciprofloxacin (Table 31, 32, Bar diagram 3).

**Study Group C:**

The aqueous extract was found to be effective against the standard cultures (study Group C) of *S. aureus* ATCC 29213, *Ent. fecalis* ATCC 29212 and the acetone extract inhibited the growth of *Ent. fecalis* ATCC 29212 (Table 33, 34, 35, 37, Bar diagram 4). MIC of aqueous extract of *E. globulus* (0.025 X 10^2 gm%) for *B. subtilis* was found to be 0.2800 gm of plant material (Tables 40, 41).

Chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, glycosides and reducing sugars (Table 42). Its aqueous extract on HPTLC analysis at 200 nm probably showed peaks corresponding to anthroquinones (16.76%), at 366 nm, Echinacosides (2.19 %, 2.24%), Eriodictyl (17.02%), Cichoric acid (16.84%), Cynarin (7.41%) and Caffeic acid derivatives (12.1%, 8.39%, 4.61%, 3.62%, 8.35%), at 254 nm flavonoids - Cascarosides A, B, C, D, sennosides (3.56%), Glucofrangulins and aloinosides (5.99%) and at 366 nm flavonoid - taxifolin (16.24%).

In similar studies, Akki et al (2004), reported the wound healing activity of *E. globulus* leaf extract and reported that aqueous extract is found effective against pathogens isolated from wounds, *Pseudomonas* species, *Enterococcus* species, *Actinomycetal* species and two unidentified species. Timande and Nafde (2004) undertook a study to check the activity of essential oil of *Eucalyptus* against *E. coli*, *Salmonella* species, *Shigella* species, *P. vulgaris*, *Ps. aeruginosa*, *S. aureus*, *B. cereus*, *B. subtilis*, *Corynebacterium* species NCIM 2640 and fungi *Asp. niger*, *A. terrus*, *P. chrysogenum* and the results were found to be very encouraging. Leaves of nilgiri were tested against two fungal species, *Aspergillus flavus* and *Curvularia lunata* and compared with two commercial antifungal products, neemark and phytolan. Plant extracts were found to be superior over commercial antifungal products in inhibition of spore germination. Acetone extract was found to be effective showing 30% efficiency (Karade et al, 2001).
7) *Allium sativum*

Leaves and bulbs of *Allium sativum* are commonly used as a spice in Indian kitchens. It has a very strong aromatic flavour which imparts a typical taste to food. It is medicinal in its properties and used since olden times, in the form of water, acetone, oil extract and bulblets. 0.1 ml of aqueous and acetone extracts of bulblets were used against each test isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.034 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, S. aureus* and *Ent. fecalis*, Gram negative *E. coli, Kl. pneumoniae, Sal. typhi, Sh. flexneri* and *Ps. aeruginosa* (Table 6) The acetone extract (0.0425 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *S. aureus, Ent. fecalis*, Gram negative *E. coli, Kl. pneumoniae, Sal. typhi, Sh. flexneri*, while *C. albicans* and *Asp. niger* were found to be resistant (Table 7).

*Sh. flexneri* was found to be most sensitive to the aqueous extract (22 mm) while *Ent. fecalis* to acetone extract (19 mm). *Ps. aeruginosa* was least sensitive to the aqueous extract (8.83 mm) and *Sal. typhi* to acetone extract (10.5 mm), as concluded from the zone sizes (Table 8).

Efficiencies of aqueous and acetone extracts inhibiting *Ent. fecalis* and *Kl. pneumoniae* were statistically comparable, *S. aureus* significant, *E. coli* highly significant and *Sal. typhi, Sh. flexneri* very highly significant. The aqueous extract of *A. sativum* showed 57% efficiency while acetone extract showed 43% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *A. sativum* inhibited eight of the Group A isolates and hence was equipotent to Norfloxacin (NX) in its antimicrobial action (Tables 26, 27, Bar diagram 2).

**Study Group C:**

The aqueous extract was found to be effective against the members of study Group C viz. *S. aureus* ATCC 29213, *Ent. fecalis* ATCC 29212 and *E. coli* ATCC 25922, and the acetone extract inhibited the growth of *Ent. fecalis* ATCC 29212 and *E. coli* ATCC 25922 (Table 33, 34, 35, 37, Bar diagram 4).
MIC of aqueous extract of *A. sativum* (0.034 X 10^2 gm %) for *Sh. flexneri* was found to be 0.2646 gm of plant material (Tables 40, 41).

Preliminary qualitative chemical investigation of the plant extract showed the presence of glucose, alkaloids, fats, proteins, reducing sugars and triterpenoids (Table 42).

In similar studies, Jain (1993) observed antimicrobial activity of garlic against five drug resistant strains, *St. pneumoniae, St. pyogenes, Citrobacter freundii, Edwardsiella tarda, Kl. aerogenes* and *Ps. aeruginosa*. Tumane et al (2000), studied 64 plant extracts against standard cultures of *S. aureus* (NCTC-3750), *E. coli* (ATCC-1948), *P. mirabilis* (NCIM-2087), *B. subtilis* (NCIM-2063), *B. stereothermophilus* (NCIM –2328), clinical isolates of *Kl. aerogenes, Kl. pneumoniae, Vibrio, Sal. typhimurium, Sal. paratyphi* and *Sh. flexneri* of which, only twenty seven plant extracts exhibited antimicrobial activity. Extract of *A. sativum* revealed comparatively higher potential of anti-bacterial activity. Direct crude aqueous extracts exhibited highest degree of antibacterial activity which confirms traditional therapeutical claims for aqueous dose forms of these plant extracts. High diffusion rate is associated with soluble agents in aqueous agar media. Tumane et al (2002) observed that the aqueous extract of *A. sativum* exhibited highest degree of antimicrobial activity by inhibiting the growth of eight isolates out of fourteen. It was reported that almost all clinical isolates studied were found to be resistant to one or more antibiotics while bulb extract of *A. sativum* was found to be effective against almost all, twenty five strains of *S. aureus*, twenty strains of *E. coli*, ten strains of *Kl. aeruginosa*, ten strains of *Kl. pneumoniae*, ten strains of *P. mirabilis*, ten strains of *P. vulgaris*, five strains of *Sal. typhimurium*, five of *Sal. paratyphi*, five of *Sh. flexneri*, ten of NAG *Vibrio*. Vijaykumar et al (2004) reported that low concentration of aqueous garlic extract is inhibitory and lethal to *C. albicans*. Kothari et al (2005) observed that crude extract of *A. sativum* was most anti-microbially effective against *E. coli, B. subtilis, S. aureus, Ps. aeruginosa, C. albicans, Asp. niger*. Ebenezer et al (2005) and Kirubhakaran et al (1999) studied antibacterial effect of garlic extract against various bacterial milk contaminants namely *S. aureus, Ent. fecalis, Sal. enteritidis, Micrococcus luteus, B. subtilis, B. cereus* and *E. coli*, in different concentrations, 1%, 2%, 3%, 4%,
and neat. It was found to be effective at all concentrations, neat showing maximum inhibition against *S. aureus*. Jha et al (2005) reported that garlic was found to inhibit the growth of *Staphylococcus*, *Bacillus*, *Brucella*, *Vibrio* species, *E. coli*, *Salmonella*, *Enteritidis*, *Klebsiella*, *Mycobacteria*, *C. albicans*, *Aspergillus* and *Saccharomyces cerevisiae*. Aqueous bulb extract of garlic showed anti-dandruff activity under conditions (Samuel et al, 2005).

Patil and Mali (2006) reported that aqueous extract of garlic shows more antibacterial activity than alcoholic extract. It inhibits *B. cereus*, *S. aureus*, *Sal. typhi*, *E. coli*, *Kl. pneumoniae*, *Ps. aeruginosa* and *P. vulgaris* but is more effective against *B. cereus*, *S. aureus*, *Sal. typhi* and *P. vulgaris*. Joshi and Shete (2006) studied garlic, neem, haladi, Korfad for anti-candidal activity. Garlic showed the highest activity while a combination of garlic and onion followed. They reported inhibition of antibiotic resistant clinical strains of *Salmonella*, *Shigella*, *Vibrio*. Zaika et al (1988) reported that, aqueous and alcoholic extracts of *A. sativum* were found to have broad antimicrobial spectrum against Gram positive and Gram negative pathogens, more against Gram negative ones. Waghmare (2007) reported that aqueous extract was found to be more effective and inhibited *B. cereus*, *S. aureus*, *Sal. typhi*, *P. vulgaris*, *E. coli*, *Kl. pneumoniae*, *Ps. aeruginosa*, in that order.

8) *Piper betel*

*Piper betel* is a very popular plant, leaves of which are consumed by millions of Indians, after food, as a digestive. It has also recently found a very significant place in medicine. 0.1 ml of each aqueous and acetone extracts leaves of *P. betel* was used against each test isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.05 X 10² gm%) was found effective in inhibiting the growth of Gram positive *S. aureus*, Gram negative *Sal. paratyphi B*, *Sh. flexneri*, *P. vulgaris*, *Ser. marsescens* and *C. albicans* (Table 6). The acetone extract (0.03 X 10² gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis*, Gram negative *Sal. paratyphi B*, *Sh. flexneri*, *P. vulgaris* while *C. albicans*, *Asp. niger* were found to be resistant (Table 7).
Sal. paratyphi B was found to be most sensitive to the aqueous extract (19 mm) while P. vulgaris to acetone extract (15.16 mm). C. albicans was least sensitive to the aqueous extract (10 mm) and Sal. paratyphi B to acetone extract (11.16 mm), as concluded from the zone sizes (Table 19).

Comparative efficiencies of aqueous and acetone extracts inhibiting Sal. paratyphi B, Sh. flexneri were statistically very highly significant while those for P. vulgaris comparable. The aqueous extract of P. betel showed 43% efficiency while acetone extract showed 29% efficiency (Table 25, Bar diagram 1).

Aqueous extract of P. betel inhibited six of the Group A isolates and hence was equipotent to Ceftazidime (CT), Chloramphenicol (CHLO), Nitrofurantoin (NF) in its antimicrobial action (Tables 26, 27, Bar diagram 2).

**Study Group C :**

The aqueous extract was found to be effective against the members of study Group C, S. aureus ATCC 29213, Ent. fecalis ATCC 29212 and E. coli ATCC 25922 and the acetone extract inhibited the growth of E. coli ATCC 25922 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the plant extract showed the presence of alkaloids, fats, flavonoids, reducing sugars and tannins (Table 42).

George et al (1947) studied the antibacterial activity of P. betel leaf extract and reported that antibacterial activity was exhibited against S. aureus and E. coli. Santhanum and Snagarajan (1990) studied the wound healing activity of P. betel. Garg and Jain (1994) observed that, alkaloids and flavonoids seem to be the ingredients responsible for various features of P. betel. Its essential oil also exhibits a biological activity.

Similarly, Banginwar and Tambekar (2003) and Burade et al (2005) studied the anti-bacterial effect of various plant extracts on the growth of Vibrio cholerae. 21 different plant extracts were screened of which betel leaf showed highest activities. Burade et al (2005) reported that alcoholic extracts of the leaf were reported to show high degree of activity against pathogens. Wiart et al (2004) reported that various Piper species are known to have broad spectrum anti-bacterial activity. Mullaicharam et al (2005) reported
that, activity of *P. betel* ethanolic extract cream showed significant antifungal activity against *C. albicans* and *C. krusei* as compared to Griseofulvin. Burade et al (2005) reported the antimicrobial activity of *P. betel* leaves extract. Alcoholic extracts of the leaf showed high degree of activity against all test microbes and the antimicrobial activity may be due to the presence of tannins. Leaf extract of *Piper betel* on phytochemical screening showed the presence of carotenoids, flavonoids, fixed oils, steroids, alkaloids, phenolic compounds and saponins. They also showed high degree of activity against all microbial pathogens under investigation (*E. coli*, *B. subtilis*, *Kl. pneumoniae*, *S. aureus*, *Asp. niger* and *Rhizopus* species) at all concentrations. (Uma and Sasikumar, 2005). Essential oil of *P. betel* shows biological activity and antimicrobial activity against *E. coli*, *B. subtilis*, *S. aureus*, *V. cholerae* and *Asp. niger* (Gangwar and Kumar, 2006). Leaf extracts of *Piper betel* showed activity against *S. aureus* and essential oil against *E. coli*, *Sal. typhi*, *Ps. aeruginosa* (Ahmed et al, 1998). Antibacterial activity of *Piper betel* leaf extract was found against *S. aureus*, *E. coli* and a wide variety of pathogenic bacteria (Chandi et al, 1999). Aqueous, alcohol, xylene, hexane, chloroform, ether leaf extracts of *P. betel* showed antimicrobial activity against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Ent. fecalis*, *Sal. typhi*, *V. cholerae*, *P. mirabilis*, *Ps. aeruginosa* ATCC 27853, *St. pneumoniae* ATCC 49619 and *Yersinia enterocolitica* (Tambekar and Kharate, 2005).

9) *Catharanthus roseus*

*Catharanthus roseus* is very commonly found in the surroundings. Normally considered to be just a harmless weed, it has now gained tremendous importance. It is known to contain anti-cancer agents like Vinchristine, Vinblastine and is used as a chemotherapeutic agent for various malignancies. 0.1 ml of both aqueous and acetone extracts of leaves of *C. roseus* were used against the test isolates, for antimicrobial activity.

**Study Group A:**
The aqueous extract (0.0386 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive strain *Ent. fecalis*, Gram negative *E. coli*, *Kl. pneumoniae*, along with yeast *C. albicans* (Table 6). The acetone extract
(0.005 X 10^2 gm%) was not found effective in inhibiting the growth of any of the test isolates (Table 7).

*K. pneumoniae* was found to be most sensitive to the aqueous extract (21.66 mm) while *Ent. fecalis* and *C. albicans* was least sensitive to the aqueous extract (14.83 mm), as concluded from the zone sizes (Table 14).

The aqueous extract of *C. roseus* showed 29% efficiency while acetone extract showed 0% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *C. roseus* inhibited four of the Group A isolates and hence had more efficiency than Amikacin (AM), Augmentin (AU), Cefuroxime (CO) and Cefoperazone (CF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C:**

The aqueous extract was found to be effective against study Group C culture of *Ent. fecalis* ATCC 29212 and *E. coli* ATCC 25928 and the acetone extract inhibited the growth of *E. coli* ATCC 25928 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, reducing sugars, triterpenoids and tannins (Table 42).

10) **Calotropis gigantea**

Leaves of *Calotropis gigantea* plant are used for worshipping Vayu devata, Hanuman. In rural areas, its white milky secretions are used as wound-packs and oedema-reducing packs, the effect may be contributed to the presence of flavonoids and triterpenoids. 0.1 ml each of aqueous and acetone extracts of leaves of *C. giganteae* was tested against each test isolate for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.08 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *M. luteus*, Gram negative *Sal. typhi*, *Sal. paratyphi* B and yeast *C. albicans* (Table 6). The acetone extract (0.004 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *S. aureus*, Gram negative *K. pneumoniae*, *Ps. aeruginosa* and *P. vulgaris* (Table 7).
Sal. paratyphi B was found to be most sensitive to the aqueous extract (15.33 mm) while P. vulgaris to acetone extract (13.16 mm). C. albicans was least sensitive to the aqueous extract (11.33 mm) and S. aureus to acetone extract (11.66 mm), as concluded from the zone sizes (Table 12).

The aqueous and acetone extracts of C. gigantea showed 29% efficiency each (Table 25, Bar diagram 1).

Aqueous extract of C. gigantea inhibited four of the Group A isolates and hence was observed to have more efficiency than Amikacin (AM), Augmentin (AU), Cefuroxime(CO) and Cefoperazone(CF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C:**

The aqueous extract was found to be effective against the standard culture of S. aureus ATCC 29213 while acetone extract was ineffective against all (Table 33, 34, 35, 37, Bar diagram 4).

Qualitative chemical investigation of the plant extract showed the presence of flavonoids, reducing sugars and triterpenoids (Table 42).

Similarly, Mascolo et al (1988) reported that C. gigantea shows anti-inflammatory activity. Sen et al (1992) observed that, the major constituents present in C. gigantea are cardenolides, lignans and flavanol glycosides which fulfill structural criteria for being a good antioxidant. It inhibits Salmonella species and is used more for topical and local application. Mueen et al (2003) reported that testing of ethanolic extracts of leaf and latex of C. gigantea showed that latex extracts exhibited greater capacity of free radical scavenging activity while leaf extract showed moderate free radical scavenging activity. Argal and Pathak (2005-a) reported that alcoholic extract of C. gigantea flowers was found to reduce the number, frequency, and wetness of faeces in diarrhoea. Ethanol extracts of flowers of C. gigantea showed significant bacterial activity against Bacillus species, Pseudomonas species and S. aureus. On phytochemical screening they showed the presence of flavonoids, tannins, steroids, saponins and sterols.

Leaf extract on phytochemical screening showed the presence of carotenoids, flavonoids, fixed oils, steroids, alkaloids, phenolic compounds and saponins. They also showed high degree of activity against all microbial pathogens under investigation (E. coli, B. subtilis, Kl. pneumoniae, S. aureus, Asp.
niger- and Rhizopus species), at all concentrations (Uma and Sasikumar, 2005)

11) *Terminalia arjuna*

According to the vaidus and ayurvedic experts, the bark of *Terminalia arjuna* is known to strengthen heart muscles and is anti-microbial to a limited extent. Its activity may be due to factors like alkaloids, sterols and tannins. 0.1 ml of each aqueous and acetone extracts of barks of *T. arjuna* was used against each test isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.074 X 10² gm%) was found effective in inhibiting the growth of Gram positive *S. aureus, M. luteus* and Gram negative *Ps. aeruginosa* (Table 6). The acetone extract (0.05 X 10² gm %) was found effective in inhibiting the growth of Gram positive *S. aureus, M. luteus, B. subtilis, Ent. fecalis*, Gram negative, *E. coli, Kl. pneumonia, Sal. typhi, Sal. paratyphi B* and *Sh. flexneri* (Table 7).

*Ps. aeruginosa* was found to be most sensitive to the aqueous extract (19.16 mm) while *Kl. pneumonia* to acetone (21.33 mm) extract. *S. aureus* was least sensitive to the aqueous (14.83 mm) extract and *B. subtilis* to acetone (11.66 mm) extract, as concluded from the zone sizes (Table 21). Comparative efficiencies of aqueous and acetone extracts inhibiting *M. luteus* were statistically very highly significant while those for *S. aureus* were comparable. The aqueous extract of *T. arjuna* showed 21% efficiency while acetone extract showed 64% efficiency. (Table 25, Bar diagram 1).

Aqueous extract of *P. betel* inhibited three of the Group A isolates and hence was equipotent to Amikacin (AM) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C:**

The aqueous extract was found to be effective against the standard culture of *E. coli* ATCC 25928 and the acetone extract inhibited the growth of *Ent. fecalis* ATCC 29212 and *E. coli* ATCC 25922 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the plant extract showed the presence of alkaloids, fats, sterols and tannins (Table 42).

12) *Andropogum citratum*

*Andropogum citratum* is very commonly found in Indian households and its leaves are a common ingredient of the morning tea. It is an aromatic plant with its leaves, imparting a very distinct flavour. It is known as a good digestive, which may due to alkaloids, flavonoids and triterpenoids. Literature shows that it has medicinal properties. It is found to be a permanent member of “Ajibai’s Batwa”, which is a collection of common household items, used to control common upper respiratory tract infections. 0.1 ml of each aqueous and acetone extracts of leaves of *A. citratum* was used against each study Group A isolate, for antimicrobial activity.

**Study Group A:**

The aqueous extract (0.06 X 10² gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, M. luteus* and Gram negative *Sal. typhi* (Table 6). The acetone extract (0.052 X 10² gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis, Sal. typhi* while others were resistant (Table 7). *Sal. typhi* was found to be most sensitive to the aqueous (20.33 mm) and acetone (14.16 mm) extracts. *M. luteus* (15 mm) was least sensitive to the aqueous and *B. subtilis* (12.33 mm) to acetone extracts, as concluded from the zone sizes (Table 10). Comparative efficiencies of aqueous and acetone extracts inhibiting *B. subtilis* were statistically significant while those for *Sal. typhi* comparable. The aqueous extract of *A. citratum* showed 21% efficiency while acetone extract showed 14% efficiency (Table 25, Bar diagram 1).
Aqueous extract of *A. citratum* inhibited three of the Group A isolates and hence was equipotent to Amikacin (AM) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C:**
The aqueous and acetone extracts were found to be effective against the study Group C culture of *E. coli* ATCC 25922 (Table 33, 34, 35, 37, Bar diagram 4).

Chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, reducing sugars and triterpenoids (Table 42).

In similar studies, leaves of lemon grass have been tested against two fungal species, *Asp. flavus* and *Curvularia lunata*. When compared with two commercial antifungal products, neemark and phytolan, plant extracts were found superior to commercial antifungal products in inhibition of spore germination. Both aqueous and acetone extracts of *A. citratum* were found to show some efficiency (Karade et al, 2001). Mohite et al (2005) studied the effect of herbal extracts on chloroquine resistant *Plasmodium falciparum* isolates and observed that lemon-grass extracts, along with neem and tulsi showed significant parasitocidal activity.

13) *Aloe vera*:
An essential member of “Ajibai’s batwa”, *Aloe vera* is a plant known to work wonders with skin, hair and acidity. It is now a common ingredient of anti-aging, anti-wrinkle creams. Its active principle seems to be alkaloids. 0.1 ml of each aqueous and acetone extracts of the juicy extract found between the two skin layers of plant leaf was used against each test isolate for antimicrobial activity.

**Study Group A:**
The aqueous extract (0.015 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis* and *M. luteus*. Of the Gram negative, all the eight isolates were found to be resistant. (Table 6). The acetone extract (0.0128 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *B. subtilis* and *M. luteus*. Of the Gram negative isolates, all were found to be resistant. (Table 7).
B. subtilis was found to be most sensitive to the aqueous (13.5 mm) extract while M. luteus to acetone (15 mm) extract. M. luteus was least sensitive to the aqueous (15 mm) extract and B. subtilis to acetone (12.33 mm) extract, as concluded from the zone sizes (Table 9).

Comparative efficiencies of aqueous and acetone extracts inhibiting M. luteus were statistically significant while those for B. subtilis were comparable. The aqueous and acetone extracts of A. vera showed 14% efficiency each (Table 25, Bar diagram 1).

Aqueous extract of A. vera inhibited two of the Group A isolates and hence was equipotent to Augmentin (AU) in its antimicrobial action (Table 26, 27, Bar diagram 2).

Study Group C:
The aqueous and acetone extracts were found to be effective against only one member of study Group C, Ent. fecalis ATCC 29212 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the plant extract showed the presence of alkaloids, fats and proteins (Table 42).

Similar study on anti-bacterial activities of A. vera, as a means to develop effective and safe antimicrobial drug was carried out (Pal-Datta et al, 2002). Aqueous, alcohol, xylene, hexane, chloroform and ether leaf extracts of A. vera showed antimicrobial activity against S. aureus ATCC 25923, Ps. aeruginosa ATCC 27853, St. pneumoniae ATCC 49619 and Yersinia enterocolitica (Tambekar and Saratkar, 2005). Aloe vera was studied for anti-candidal activity and reported inhibition of antibiotic resistant clinical strains of Salmonella, Shigella and Vibrio to some extent (Joshi and Shete, 2006).

14) Cleome viscosa

Cleome viscosa shrub with milky white secretion is known to enhance lactation, during and post-pregnancy. However, its anti-microbial effects do not seem to be very prominent. 0.1 ml of each of the aqueous and acetone extracts of leaves of Cl. viscosa were used against each test isolate, for antimicrobial activity.
Study Group A:

The aqueous extract (0.06 X 10^2 gm%) was found effective in inhibiting the growth of *C. albicans*. All Gram positive and Gram negative bacteria were found to be resistant. (Table 6). The acetone extract (0.02 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive *M. luteus* and Gram negative *Ps. aeruginosa* (Table 7).

*C. albicans* was found to be most sensitive to the aqueous (16.83 mm) extract while *Ps. aeruginosa* to acetone (14.5 mm) extract as concluded from the zone sizes. *Sar.luteus* was found to be least sensitive to the acetone (12.83 mm) extract (Table 15).

The aqueous extract of *Cl. viscosa* showed 7% efficiency while acetone extract showed 14% efficiency (Table 25, Bar diagram 1).

Aqueous extract of *Cl. viscosa* inhibited one of the Group A isolates and hence was equipotent to Cefuroxime(CO) and Cefoperazone (CF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

Study Group C:

The aqueous and acetone extracts were found to be effective against the standard culture (study Group C) of *Ps. aeruginosa* ATCC 27853 (Table 33, 34, 35, 37, Bar diagram 4).

Qualitative chemical investigation of the plant extract showed the presence of alkaloids, flavonoids, fats and glycosides (Table 42).

Similar studies on methanol extract of *P. betel* showed antimicrobial activity against *E. coli*, *Shigella* species, effective in the treatment of diarrhea and dysentery. It showed anti-fungal activities against *Penicillium notatum*, *Penicillium chrysogenum*, *Asp.niger*, *C. albicans*, as compared to griseofulvin (Parimala devi et al, 2006).

15) Triphala

It is a popular ayurvedic drug, prescribed as a digestive and has been used in this study as a standard. 0.1 ml of each of the aqueous and acetone extracts of Triphala mixture was tested against each isolate, for antimicrobial activity.

Study Group A:

The aqueous extract (0.0675 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive strains *S. aureus, M. luteus, B. subtilis, Ent.*
fecalis and Gram negative isolates E. coli, K. pneumoniae, Sal. typhi, Sh. flexneri and Ser. marcescens (Table 6). The acetone extract (0.0125 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive Ent. fecalis, M. luteus and Gram negative E. coli, Sal. typhi, Sh. flexneri (Table 7).

E. coli was found to be most sensitive to the aqueous (22.16 mm) extract while E. coli and Sal. typhi to acetone (19.5 mm) extract. Sh. flexneri was least sensitive to the aqueous (16.16 mm) extract and Ent. fecalis to acetone (15.33 mm) extract, as concluded from the zone sizes (Table 22).

Aqueous extract of Triphala inhibited nine isolates of study Group A and hence its antimicrobial activity is comparable with that of Curcuma longa (Table 6).

Comparative efficiencies of aqueous and acetone extracts inhibiting Ent. fecalis were statistically highly significant while those for M. luteus, E. coli, Sal. typhi, Sh. flexneri were comparable. The aqueous extract of Triphala showed 64% efficiency while acetone extract showed 36% efficiency (Table 25, Bar diagram 1).

Aqueous extract of Triphala inhibited nine of the Group A isolates and hence is equipotent to Gentamicin (G), Cephotaxime (CX), Netillin (NT), Ofloxacin (OF) and Pefloxacin (PF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

Study Group C

The aqueous extract was found to be effective against the standard culture of Ent. fecalis ATCC 29212 and E. coli ATCC 25922 and the acetone extract inhibited the growth of Ent. fecalis ATCC 29212, E. coli ATCC 25928 and P. aeruginosa ATCC 27853 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the powder showed the presence of alkaloids, fats, glycosides, sterols and tannins (Table 42).

Emblica officinalis or Phyllanthus emblica, is the richest natural source of Vitamin C and is known for its antioxidant and immunostimulant properties since time immemorial and is extensively used in Indian Ayurvedic medicines (Reddy and Lokes, 1992). Mehta et al (1993) reported antimicrobial efficacy of Triphala. They studied the antibacterial activity of E. officinalis fruit extract against S. aureus,
Kl. aerogenes, E. coli, Sal. typhi, Sh. dysenteriae, V. cholerae. Paranjpe (1997) reported the topical application of E. officinalis in treatment of Acne vulgaris. Leaves of amla were tested against two fungal species, Asp. flavus and Curvularia lunata. They were compared with two commercial antifungal products, neemark and phytolan and plant extracts were superior over commercial antifungal products in inhibition of spore germination. Both aqueous and acetone extracts of E. officinalis were found to show 95% and 100% efficiency (Karade et al, 2001). Fruit and leaf extract of E. officinalis showed antimicrobial activity against E. coli, S. aureus, B. subtilis, Sal. typhi and Ps. aeruginosa (Chavan et al, 2005). Leaves of E. officinalis exhibited antimicrobial activity against B. cereus NCIM 2322, S. aureus ATCC 6538, P. vulgaris MTCC 742, E. coli NCIM 2931 and Ps. aeruginosa NCIM 2200 (Deshpande et al, 2005). Studies of aqueous extract of dried fruits of T. chebula for anti-fungal activity against four dermatophytes revealed that two anthropophilic dermatophytes – Trichophyton tonsurance and T. rubrum reported Minimum fungicidal timing (MFT) of 24 and 48 hrs. and the two geophilic species – Microsporum fulvum shows no effect upto 48 hrs. and M. gypseum reported the MFT as minutes 1 hr. (Dutta et al, 2004). Aqueous, alcohol, xylene, hexane, chloroform, ether leaf extracts of T. chebula showed antimicrobial activity against E. coli ATCC 25922, S. aureus ATCC 25923, Ent. fecalis, Sal. typhi, V. cholerae, P. mirabilis, Ps. aeruginosa ATCC 27853, St. pneumoniae ATCC 49619 and Yersinia enterocolitica (Tambekar and Saratkar, 2005).

16) Kofit (herbal cough syrup)
Ayurveda bio-enhanced “Kofit” herbal cough formula is prescribed by the doctors for treating common cough and sore throat. In this study, it has been used as a sample with known composition but unknown antimicrobial activity. 0.1 ml of the Kofit syrup was directly tested against each isolate for antimicrobial activity.

Study Group A:
Kofit was found effective in inhibiting the growth of Gram positive B. subtilis, S. aureus and Gram negative Sal. typhi, Sal. paratyphi B, Ps. aeruginosa and Ser. marsecens (Table 6).
Ser. marsescens was found to be most sensitive (20.33 mm) and B. subtilis least sensitive (13.5 mm) to the syrup, as concluded from the zone sizes (Table 24).

Kofit inhibited six isolates of study Group A and hence its antimicrobial activity was comparable with that of Eucalyptus globulus and Piper betel (Table 6). It showed 43% efficiency (Table 25, Bar diagram 1).

Since Kofit inhibited six of the Group A isolates, it was found equipotent to Ceftazidime (CT), Chloramphenicol (CHLO), Nitrofurantoin (NF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C:**
The syrup was found effective against the study Group C culture of S. aureus ATCC 29213 and E. coli ATCC 25922 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation showed the presence of alkaloids, fats, proteins, reducing sugars and triterpenoids (Table 42).

**17) Face pack**
It is an anti-acne powder, containing natural medicinal plants and herbs, claiming to demolish pimples and black heads and bringing fairness. It is routinely used in few beauty parlours. It is found to be effective against acne. In this study, it has been used as an unknown ayurvedic product with known medicinal activity. 0.1 ml of each of the aqueous and acetone extracts of the face pack powder has been used against each test isolate, for antimicrobial activity.

**Study Group A:**
The aqueous extract (0.055 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive S. aureus and Gram negative isolates, Sal. typhi, Sal. paratyphi B, Ps. aeruginosa (Table 6). The acetone extract (0.048 X 10^2 gm%) was found effective in inhibiting the growth of Gram positive S. aureus and the Gram negative Ps. aeruginosa (Table 7).

S. aureus was found to be most sensitive to the aqueous (19.5 mm) and acetone (19 mm) extracts. Sal. paratyphi B was least sensitive to the aqueous (14.33 mm) extract and Ps. aeruginosa to acetone (18 mm) extract, as concluded from the zone sizes (Table 23).
On comparison, the difference between antimicrobial activity of plant extracts and antibiotics with respect to the isolates of Group A was found to be statistically insignificant. Hence their antimicrobial activities are comparable (Table 27, Bar diagram 2).

Aqueous extract of the face pack inhibited four isolates of study Group A and hence its antimicrobial activity was comparable with that of *Catharanthus roseus* and *Calotropis gigantea* (Table 6). The aqueous extract of face pack showed 29% efficiency while acetone extract showed 14% efficiency (Table 25, Bar diagram 1).

Aqueous extract of the face pack inhibited four of the Group A isolates and hence was found to have more efficiency than Amikacin (AM), Augmentin (AU), Cefuroxime (CO) and Cefoperazone (CF) in its antimicrobial action (Table 26, 27, Bar diagram 2).

**Study Group C**

The aqueous extract was found to be effective against the standard culture of *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853. The acetone extract inhibited the growth of *S. aureus* ATCC 29213 (Table 33, 34, 35, 37, Bar diagram 4).

Preliminary qualitative chemical investigation of the face pack powder sample showed the presence of fats, alkaloids and triterpenoids (Table 42).

Thus it was observed that:-

- Aqueous extracts of *P. pinnata* showed maximum antimicrobial effect against ten isolates, *C. longa* and *Triphala* showed antimicrobial effect against nine isolates, *A. sativum*, *C. auriculata* and *P. niruri* against eight isolates, *A. indica* against seven isolates, *E. globulus*, *P. betel* and Kofit against six isolates, *C. roseus*, *C. gigantea*, Face pack against four, *T. arjuna* and *A. citratum* against three isolates, *A. vera* against two and *Cl. viscosa* against one (Table 8).

In general, aqueous extracts were found to be better than acetone extracts and can be used as broad spectrum antibiotics to restrict the growth of common pathogens.
• Of all the test isolates, *B. subtilis* and *S. aureus* were found to be most sensitive to eleven of the aqueous plant extracts under study, *Sal. typhi* to ten of the samples, *M. luteus*, *Sh. flexneri* to eight of the samples, *Ent. fecalis*, *E. coli*, *Ps. aeruginosa*, *C. albicans* to seven of the samples, *Kl. pneumoniae*, *P. vulgaris* to six of the samples, *Sal. paratyphi B* to five of the samples, *Ser. marcescens* to four of the samples and *Asp. niger* to one of the samples (Table 6).

**Study of sensitivity of the isolates of Group A:**

Sensitivity of the isolates was checked against various antibiotics and plant extracts;

1) *B. subtilis* was found sensitive to eight antibiotics, Augmentin, Cephotaxime, Ceftazidime, Netillin, Ofloxacin, Norfloxacain, Nalidixic acid and Nitrofurantoin (Table 26). It was sensitive to eleven of the aqueous samples under study; *A. sativum*, *A. vera*, *A. citratum*, *A. indica*, *C. auriculata*, *C. longa*, *E. globulus*, *P. niruri*, *P. pinnata*, Triphala, Kofit and six of the acetone extracts (Tables 6, 7, Plate 9).

2) *S. aureus* was found sensitive to twelve antibiotics, Cephotaxime, Ceftazidime, Gentamicin, Lomefloxacin, Netillin, Ofloxacin, Pefloxacain, Norfloxacain, Nalidixic acid, Nitrofurantoin, Doxicyclin and Chloramphenicol (Table 26). It was sensitive to eleven of the aqueous samples under study- extracts of *A. sativum*, *A. indica*, *C. auriculata*, *C. longa*, *P. niruri*, *P. betel*, *P. pinnata*, *T. arjuna*, Triphala, Face pack, Kofit and seven of the acetone extracts (Tables 6, 7).

3) *Ent. fecalis* was found sensitive to nine antibiotics, Cephotaxime, Ceftazidime, Gentamicin, Lomefloxacin, Netillin, Ofloxacin, Pefloxacain, Nalidixic acid and Nitrofurantoin. (Table 26). It was sensitive to seven of the aqueous samples under study- extracts of *A. sativum*, *A. indica*, *C. auriculata*, *C. roseus*, *C. longa*, *P. niruri*, *P. pinnata*, Triphala and seven of the acetone extracts (Tables 6, 7, Plates 8, 11).

4) *M. luteus* was found sensitive to nine antibiotics, Cephotaxime, Gentamicin, Lomefloxacin, Netillin, Ofloxacin, Pefloxacain, Nalidixic acid, Nitrofurantoin and Norfloxacain (Table 26). It was sensitive to eight of the aqueous samples under study- extracts of *A. vera*, *A. citratum*, *C. gigantea*,...
C. auriculata, E. globulus, P. pinnata, T. arjuna, Triphala and six of the acetone extracts (Tables 6, 7).

5) *E. coli* was found sensitive to ten antibiotics, Amikacin, Cephotaxime, Ceftazidime, Gentamicin, Lomefloxacin, Ofloxacin, Pefloxacin, Nalidixic acid, Nitrofurantoin and Doxicyclin (Table 26). It was sensitive to seven of the aqueous extracts of *A. sativum, A. indica, C. auriculata, C. roseus, C. longa, P. pinnata*, Triphala and four of the acetone extracts (Tables 6, 7).

6) *Kl. pneumoniae* was found sensitive to eight antibiotics, Amikacin, Ciprofloxacin, Gentamicin, Lomefloxacin, Ofloxacin, Norfloxacin, Doxicyclin and Chloramphenicol (Table 26), six of the aqueous samples under study - extracts of *A. sativum, C. auriculata, C. roseus, E. globulus, P. pinnata*, Triphala and four of the acetone extracts (Tables 6, 7).

7) *Sal. typhi* was found sensitive to ten antibiotics, Ciprofloxacin, Ceftazidime, Gentamicin, Lomefloxacin, Netilin, Ofloxacin, Pefloxacin, Norfloxacin, Doxicyclin and Chloramphenicol (Table 26). It was sensitive to ten of the aqueous samples under study - extracts of *A. sativum, A. citratum, A. indica, C. gigantea, C. longa, P. niruri, P. pinnata*, Triphala, Face pack, Kofit and six of the acetone extracts (Tables 6, 7, Plate 9).

8) *Sal. paratyphi B* was found sensitive to ten antibiotics, Cephotaxime, Gentamicin, Lomefloxacin, Netilin, Ofloxacin, Pefloxacin, Norfloxacin, Nalidixic acid, Doxicyclin and Chloramphenicol (Table 26). It was sensitive to five of the aqueous samples under study - extracts of *C. gigantea, P. niruri, P. betel*, Face pack, Kofit (Tables 6, 7, Plate 10) and four of the acetone extracts.

9) *Sh. flexneri* was found sensitive to nine antibiotics, Cephotaxime, Ceftazidime, Lomefloxacin, Netilin, Ofloxacin, Pefloxacin, Norfloxacin, Nalidixic acid, Nitrofurantoin and Chloramphenicol (Table 26). It was sensitive to eight of the aqueous samples under study - extracts of *A. sativum, A. indica, C. auriculata, C. longa, E. globulus, P. betel, P. pinnata*, Triphala (Tables 6, 7, Plate 10) and five of the acetone extracts.

10) *Ps. aeruginosa* was found sensitive to five antibiotics, Augmentin, Cephotaxime, Ciprofloxacin, Norfloxacin, Chloramphenicol (Table 26). It was also sensitive to seven of the aqueous samples under study – *A. sativum,*
C. auriculata, C. longa, E. globulus, T. arjuna, Face pack, Kofit (Tables 6, 7) and four of the acetone extracts.

11) *P. vulgaris* was found to be sensitive to ten antibiotics, Amikacin, Cephotaxime, Ciprofloxacin, Cefuroxime, Cefoperazone, Gentamicin, Lomefloxacin, Netillin, Pefloxacin, Chloramphenicol (Table 26). It was sensitive to six of the aqueous samples under study - extracts of *A. indica*, *C. auriculata*, *C. longa*, *E. globulus*, *P. niruri*, *P. betel* and five of the acetone extracts (Tables 6, 7, Plate 8).

12) *Ser. marsescens* was found sensitive to seven antibiotics, Ciprofloxacin, Gentamicin, Lomefloxacin, Netillin, Ofloxacin, Pefloxacin, Norfloxacin (Table 26), to four of the aqueous extracts of *P. niruri*, *P. betel*, Triphala, Kofit and one of the acetone extracts (Tables 6, 7).

13) *C. albicans* was found resistant to all the antibiotics (Table 26), but sensitive to seven of the aqueous extracts of *C. gigantea*, *C. roseus*, *C. longa*, *C. viscosa*, *P. niruri*, *P. betel*, *P. pinnata* and acetone extracts of *A. indica*, *E. globulus* (Tables 6, 7, Plate 12).

14) *Asp. niger* was found resistant to all antibiotics (Table 26) although it was found sensitive to aqueous extract of *P. pinnata* and acetone extracts of *A. indica* and *P. pinnata* (Tables 6, 7).

*Asp. niger* was found sensitive to the aqueous extract of *P. pinnata* while it was found resistant to all other plant extracts as well as to all the antibiotics (Table 6, Plate 11 (b)).

*C. albicans* was found sensitive to aqueous extracts of *Calotropis gigantea* (11.3 mm), *Catharanthus roseus* (14.8 mm), *Cleome viscosa* (16.8 mm), *Curcuma longa* (16 mm), *Phyllanthus niruri* (15.2 mm), *Piper betel* (10.3 mm) and *Pongamia pinnata* (10.8 mm). On the contrary, it was found resistant to all the antibiotics (Table 26, Plate 12). Hence these observations support in addition, anti-fungal and anti-yeast activity of aqueous plant extracts under study.

- The Master chart (Table 39) depicts that, Aqueous extracts of *C. longa*, *P. niruri* and *P. pinnata* were the most effective of all extracts, killing eleven of the pathogenic and standard cultures, followed by *A. sativum* and *C. auriculata* inhibiting ten, *A. indica*
inhibiting eight, *E. globulus* and *P. betel* inhibiting seven, *C. roseus* and *T. arjuna* inhibiting five, *A. citratum* and *C. gigantea* inhibiting four, *A. vera* inhibiting three and *C. viscosa* inhibiting two of the isolates and standard cultures.

Lomefloxacin and Gentamycin were the most effective of all antibiotics used, as they inhibited thirteen of the pathogenic and standard cultures, followed by Ofloxacin, Pefloxacin, Norfloxacin inhibiting twelve, Netillin inhibiting eleven, Cefotaxime, Nalidixic acid inhibiting ten, Norfloxacin inhibiting nine, Ceftazidime, Chloramphenicol inhibiting seven, Ciprofloxacin, Doxicyclin inhibiting six, Amikacin, Cefoperazone inhibiting four, Augmentin inhibiting three and Cefuroxime inhibiting one.

*S. aureus* was found to be the most sensitive of all the cultures tested, as it was inhibited by twenty of the plant extracts and standard antibiotics used. It showed maximum zone of inhibition for Chloramphenicol (42.3 mm) amongst the antibiotics and maximum zone of inhibition for aqueous extract of *Cassia auriculata* (23.16 mm) amongst the extracts under study.

- **Primary screening results of swab samples from burns wounds and response of Study Group B to aqueous plant extracts:**

In an additional study, conducted to support the antimicrobial efficacy of the plant extracts against the samples from the burns patients, it was observed that, aqueous extract of *P. pinnata* was found to be most effective against six of the burns swab samples, *A. indica* to five, *C. longa* and *E. globulus* to three, *C. auriculata* to two and *P. niruri* to one of the samples. Sensitivity of the samples to antibiotics revealed that Ofloxacin inhibited all ten samples and was the most efficient, Ciprofloxacin and Cephotoxime inhibited nine, Ceftazidime and Norfloxacin inhibited eight, Netillin inhibited three, Nitrofurantoin two and Nalidixic acid one, being the least efficient. This suggests that the antimicrobial activity of *P. pinnata* and *A. indica* is more than that of antibiotics Netillin, Nitrofurantoin and Nalidixic acid while that of *C. longa*, *E. globulus*, *C. auriculata* and *P. niruri* is equipotent.

Of the seven burns isolates, *St. pyogenes* was susceptible to all the plant extracts, *M. luteus* and *B. subtilis* to four extracts, *Ps. aeruginosa* to three
extracts, *Ent. fecalis* and *B. cereus* to two of the extracts and *S. aureus* isolate was found resistant to all the plant extracts.

Aqueous extract of *P. pinnata* was found to be most effective against five burns isolates, *C. auriculata, E. globulus* were found to be effective against four, *A. indica* and *C. longa* were found to be effective against three and *P. niruri* was found to be effective against two of the isolates. The response of the same burns swab samples was checked towards antibiotics. Cephotoxime is found to be most effective against seven isolates, Nalidixic acid, Nitrofurantoin, Norfloxacin, Netillin and Ofloxacin against six isolates, Ceftazidime against five and Ciprofloxacin against one. It clearly indicates that the antimicrobial activity of *P. pinnata* is equipotent to that of Ceftazidime and that of *C. auriculata, E. globulus, A. indica, C. longa* and *P. niruri* more than that of Ciprofloxacin.

On comparison of % efficiencies of number of plant extracts and antibiotics inhibiting Group B isolates, it was noted that the difference for *Ps. aeruginosa, M. luteus* and *B. subtilis* was statistically comparable and that for *B. cereus* and *Ent. fecalis* statistically significant (Table 32, Bar diagram 3).

- Comparison of response of common isolates found in study Groups A, B and C to aqueous plant extracts and antibiotics was done by statistical analysis. Efficiencies of plant extracts and antibiotics in inhibiting *S. aureus* of study Groups A and C, *Ent. fecalis* of Groups A and C while *Ps. aeruginosa* of Groups A and B were observed to be statistically comparable (Table 38).

It has been reported that natives of India use *A. indica* leaves as poultices, ointments and liniments for burns and its seed oil is widely used in asian medicine and has been therapeutically confirmed as an anti-inflammatory and antibacterial agent (Martindale, 1989). Nagoba et al (1998) hinted at the use of plants in the treatment of a severe electric burn complicated by multiple antibiotic resistant *Ps. aeruginosa* case. Bonjar et al (2003) reported the use of plants in treatment of burns, infections, dermatophytic diseases, as antiseptics and as anti-inflammatory agents. *Ps. aeruginosa* is the most prevalent pathogen in burns wounds, capable of causing life-threatening
illnesses, clinical infections of wounds, giving rise to blue-green pus, meningitis, fatal sepsis, septicemia and pneumonia in cystic fibrosis. Chah et al (2006) conducted a study to evaluate methanolic extracts of *Ageratum conyzoides*, *Anthocleista djalonensis*, *Napoleona imperialis*, *Ocimum gratissimum* and *Psidium guajava* for antibacterial and wound healing properties, using the excision wound model, against eleven wound isolates, *S. aureus* (four strains), *E. coli* (two strains), *Ps. aeruginosa* (one strain), *Proteus* species (three strains), and *Shigella* species (one strain). Extract of *Napoleona imperialis* inhibited growth of all the test bacterial strains. More than 90% wound healing was recorded in the extract. Babu et al (2003) reported the positive influence of extracts of *Tridax procumbens* on healing of burn wounds in rats. These reports correlate with the findings in the present study suggesting the use of the plant extracts of the study Group B in the treatment of burn wounds.

- The comparative MICs of the six plant extracts, in the form of weight of plant material showed that, *P. pinnata* (0.31 X 10^2 gm%) was effective in inhibiting *M. luteus* in the minimum dose of 0.0256 gm. This was the minimum plant material required to inhibit the isolate. The dose of *C. longa* (0.170 X 10^2 gm%) for *Ent. fecalis* was 0.0472 gm while that for *A. indica* (0.0925 X 10^2 gm%) for *Sh. flexneri* was 0.0864 gm MIC of *P. niruri* (0.06 X 10^2 gm%) for *S. aureus* was 0.1826 gm, of *Allium sativum* (0.034 X 10^2 gm%) for *Sh. flexneri* was 0.2646 gm The effective dose observed for *E. globulus* (0.025 X 10^2 gm%) for *B. subtilis* was 0.2800 gm which was the maximum weight of the plant material required to inhibit the isolate. MIC indicates the therapeutic efficacy, lower the MIC value, greater is its therapeutic efficacy. In this context, *P. pinnata* and *C. longa* are more efficient as antimicrobial agents. 100 gm of *P. pinnata* seeds yielded 0.31 X 10^2 gm of powder in aqueous extract and its minimal effective dose against the most susceptible isolate *M. luteus* was found to be 0.0256 gm. In spite of maximum availability in the form of powder in aqueous extract, minimum effective dose was required to inhibit the growth of the isolate and thus *P. pinnata* is observed to be the most potent and effective medicinal plant as an anti-microbial agent (Tables 40, 41).
Analytical study of plant extracts revealed the presence of proteins, reducing sugars, glycosides, flavonoids, sterols, fats, triterpenoids, tannins and alkaloids. Moreover, when the antimicrobial activity and contents of the most effective extracts were co-related it was found that,

- **P. pinnata** inhibited *B. subtilis*, *S. aureus*, *Ent. fecalis*, *M. luteus*, *E. coli*, *K. pneumoniae*, *Sal. typhi*, *Sh. flexneri*, *C. albicans*, *Asp. niger* and showed the probable presence of Anthroquinones, Alkaloids and Flavonoids - Silychristin, Taxifolin, Iso-silybin, Echinacosides, Cynarin, Caffeic acid derivatives, Cascarosides A, B, C, D, Sennosides, Glucofrangulins, Aloinosides, A-monoglycosides, Frangulins A and B.

- **C. longa** was lethal for *B. subtilis*, *S. aureus*, *Ent. fecalis*, *Sal. typhi*, *Sh. flexneri*, *Ps. aeruginosa*, *P. vulgaris*, *C. albicans* and reported to probably contain Coumarins, Triterpenoids, Echinacosides, Eriodictyl, Cichoric acid and Caffeic acid derivatives.

- **C. auriculata** inhibited *B. subtilis*, *S. aureus*, *M. luteus*, *E. coli*, *K. pneumoniae*, *Sh. flexneri*, *Ps. aeruginosa*, *P. vulgaris* and was found to probably contain Flavonoids - Xantho-Eriodictyl, Sily-christin, Taxifolin, Cascarosides A, B, C, D, Sennosides, Glucofrangulins, Aloinosides, Aloin, Rhein, Deoxyaloin, A-monoglycosides and Frangulins A, B.

- **P. niruri** was antimicrobially effective for *B. subtilis*, *S. aureus*, *Ent. fecalis*, *Sal. typhi*, *Sal. paratyphi B*, *P. vulgaris*, *C. albicans*, *Ser. marseecens*, *C. albicans* lab isolates and tested to probably contain Alkaloids and Flavonoids - Xantho-eriodictyl, Sily-christin, Eriodictyl, Cichoric acid, Cynarin, Eriodictyl, Chlorogenic acid / Eriocitrin, Caffeic acid derivatives, Cascarosides A, B, C, D, Sennosides, Aloin, Rhein and Deoxyaloin.

- **A. indica** was lethal for *B. subtilis*, *S. aureus*, *Ent. fecalis*, *E. coli*, *Sal. typhi*, *Sh. flexneri*, *Ps. aeruginosa*, *P. vulgaris*, *C. albicans* and *Asp. niger* and found to probably contain Terpenes, Saponins, Caffeic acid and Flavonoids - Xantho-eriodictyl, Sily-christin, Taxifolin, Iso-silybin, Echinacoside, Rutin / Sinensetin, Chlorogenic acid / Eriocitrin, Eriodictyl, Cichoric acid, Cascarosides A, B, C, D, Sennosides, Glucofrangulins and Aloinosides.
• *E. globulus* was found inhibitory for *B. subtilis, M. luteus, K. pneumoniae, Sh. flexneri, Ps. aeruginosa, P. vulgaris, C. albicans* and showed to probably contain Anthrquinones and Flavonoid - Taxifolin, Anthrquinones, Flavonoid - Taxifolin, Echinacosides, Eriodictyl, Cichoric acid, Cynarin, Caffeic acid derivatives, Cascarosides A, B, C, D, Sennosides, Glucofrangulins and Aloinosides. (Table 43, 44, 45, 46, 47, Plates 15-17, Graphs 1-5).

Banginwar and Tambekar (2003), reported that active principles like tannin, aliphatic ketones, beta-carotenoids, fatty acids, essential oils and phenolic compounds in the extract influence the anti-bacterial activity. Many medicinal plants owe their physiological activities, molecular interactions between alkaloid molecules and chemically defined components of the affected organisms, to their content of alkaloids (Havsteen, 1983). Antioxidant principles from natural resources provide enormous scope in correcting the imbalance between pro-oxidant and antioxidant homeostasis responsible for diseases (Tiwari, 2001). Free radical scavenging activity has been ascribed to two classes of natural compounds as flavonoids and polyphenolics (Clark et al, 1985). Flavonoids are a class of natural products of high pharmacological potency and exert multiple biological effects such as anti-inflammatory, anti-oxidant, anti-allergic, anti-viral and anti-malignancy chemotherapy (Havsteen, 1983). Flavonoids, phenolic compounds are present to a great extent in many fruits and vegetables and have a major role in prevention of several forms of cancer, cardiovascular diseases, cytotoxicity of LDL and are hydrogen donating free radicals with essentially the presence of free hydroxyl Groups and catechol moiety (Shetgiri and D’Mello, 2003). Tannins and alkaloids, plant secondary metabolites are known to possess anti-microbial activity (Cowan, 1999). Flavonoids and alkaloids have been found to be responsible for antimicrobial activity. Quercitin, Rutin are flavonols (Jadhav and Kharya, 2005) showing very good free radical scavenging activity, flavon-3-ols like Catechin too reduce free radicals (Shetgiri and D’Mello, 2003).

The most effective aqueous seed extract of *Pongamia pinnata* which showed good antimicrobial activity against isolates was subjected to TLC and its
components separated. Each component was tested for antimicrobial activity against burns swab samples and their isolates. On the basis of size of zone of inhibition, effectivity of components PP\textsubscript{1} and PP\textsubscript{2} against \textit{S. aureus} may be considered equipotent to Cephotoxime and Ceftazidime respectively (Plates 18-20).

- The extract and its components were analysed for identification of their active groups by IR spectroscopy. The aqueous extract of PP showed the probable presence of hydroxy (O-H \textsubscript{str}), aliphatic (C-H \textsubscript{str}), carbonyl (C=O \textsubscript{str}), amide (C=O \textsubscript{str}), aromatic nitro / carboxylic acid groups. The first component - PP\textsubscript{1} indicated the presence of aliphatic (C-H \textsubscript{str}), amide (C=O \textsubscript{str}), primary and secondary amines (N-H \textsubscript{def}) and the second component - PP\textsubscript{2} showed N-H \textsubscript{str} / O-H \textsubscript{str}, C-H \textsubscript{str}, C = O - O and weak carbonyl, (C=O-NH), carbonyl amide groups (Tables 48-50, Graphs 6-8).

Novak et al (1969) reported the antimicrobial activity of some \textit{N}-substituted amides of long-chain fatty acids. Goodman and Gilman (2006) reported that Sulphanilamide group has all the structural pre-requisites for anti-bacterial action. All members of sulfonamide group differ in the nature of N substitution, which governs solubility, potency and pharmacokinetic property. A free amino group in the para position is required for antibacterial activity. Amide (NH\textsubscript{2}) group has variable effects on antibacterial activity of a molecule. Tripathi (2008) reported that aromatic Nitro group, Nitrobenzene group is responsible for antimicrobial activity.

- GCMS analysis of PP showed the presence of Benzopyran, Caryophyllene, Octadecenoic acid and PP\textsubscript{1} showed the presence of Pentanone. PP\textsubscript{2} showed no peaks at all, probably because its components possess a higher boiling range which could not be detected by the temperature conditions applied in the present GCMS analysis (Table 51, Graphs 9-11).

Benzopyran is an ingredient of pigments, coumarins and is an antimicrobial, antifungal agent (Satoh et al, 1996). Caryophyllene is a sesquiterpene, present in essential oils. It is an anti-fungal, anti-microbial agent, known to inhibit \textit{E. coli}, \textit{Salmonella typhi} and \textit{Shigella} species (Sabulal et al, 2006). Antimicrobial activities of Caryophyllene, hexadecenoic acid, Germacrene,
Pentanone have been reported (Formisano et al, 2006; Hegazi and Abd El Hady, 2001). Caffeic acid has been reported as showing a high inhibitory activity against *Staphylococcus aureus* and *Candida albicans*. Octadecenoic acid has been reported as a promising antifungal and antimicrobial agent (Ahmed et al, 2001).

Thus, it can be concluded that antimicrobial activity of the plants may be attributed to the presence of specific constituents in them.

- The interviews with general practitioners in ayurvedic medicine revealed that the average age group of the patients approaching for consultation was 5-50 years, mostly females, diverted from the use of antibiotics, stressing on natural remedies and ready to wait for long periods for results. Their complaints were commonly related to skin, hair, upper respiratory tract, digestive system, joints and menstrual cycle. The practitioners stressed on natural remedies, in the right amount, at the right time, but only after consultation with the experts. They also observed an increased awareness towards hazards of antibiotics and an increased affinity towards using natural remedies, as far as possible. Thus it can be concluded that a definite inclination towards the use of natural products, as drugs, has increased. Keeping this in mind, the antimicrobial activity of medicinal plants can be utilized for clinical purpose to control common infections.

Looking at the response of the pathogens to the plant extracts and considering the harmful side effects of the antibiotics (Gulhati, 2005) and the changing mental set-up of the patients, it can be said that a switch over to natural plants as remedies for commonly occurring infections may prove beneficial to man-kind. However, the dosage and duration of such treatments, needs more attention.