ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 1, pp. 035-040, January, 2010*

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

**RESEARCH ARTICLE**

# EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *Crinum asiaticum*

Ilavenil, S., B. Kaleeswaran, B. and Ravikumar, S.*

Department of Biotechnology, PRIST University Vallam, Thanjavur-603413,Tamilnadu, INDIA

**ARTICLE INFO**

***Article History:***
Received 10, January, 2010
Received in revised form
22, January, 2010
Accepted 24, January, 2010
Published online 29, January, 2010

***Key words:***
*Crinum asiaticum*
Antibacterial activity
Human pathogenic
MIC and MBC
Gas chromatography
Mass Spectrophotometry

**ABSTRACT**

Aqueous and ethanol extracts from the leaves of *crinum asiaticum* plants were investigated for their antibacterial activity against gram negative bacteria *Pseudomonas aeruginosa* (ATCC10031) *Klebsilla pneumoniae* (ATCC 10031) and *Escherichia coli* (ATCC 25922) and gram positive bacteria *Staphylococcus aureus* (ATCC 11632) and *Bacillus subtilis* (ATCC 23859) using the agar well diffusion, disc diffusion and broth dilution methods. These extract ranged between 0.5 -1.5 mg/ml. The patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. The different concentration of ethanol extract was significantly differed when compare to their aqueous extract. The maximum zone of inhibition and minimum inhibitory concentration (MIC) was found to be 1.5mg/ml. The minimum inhibitory concentrations (MIC) value of the ethanol extract of *Crinum asiaticum* were in the range from 0.187 mg/ml to 0.375 mg/ml. Phytochemical analysis, more than 22 compounds have been identified by Gas chromatography with mass spectrophotometry, n-Hexadecanoic acid (22.44%), 9, 12, 15-octadecatrienoic acid (15.42%), 9, 12-octadecadienoic acid (14.78%), 9, 10–Anthracenedione 2-amino (7.65%) and phytol (7.43%) are the major present components. We conclude that the leaf of *Crinum asiaticum* is a natural source of new antibacterial compounds.



## INTRODUCTION

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Davis. 1994). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions (Ahmad et al., 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Clark, 1996 and Cordell, 2000). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Chung. 2004, Nair, 2004 and De Boer *et al*., 2005).

* Corresponding author. drravinikesh@yahoo.co.in
Tel:+919443724611; Fax: +914362 265140

*Crinum* species produce tunicate bulbs that, at certain times of the year, are dormant. But few plants are more striking than this stately Amaryllidaecae (McFarland *et al*., 1948) when the heavy umbels of lily like flowers later appear. With this apparent disappearance and then the reappearance, it is not wondered that the plants entered in to folklore. The 130 species of *Crinum* (Verdoon, 1973; Brayan, 1989) have a pan tropical distribution with the center of diversity south of the Sahara (Fangan and Nordal, 1993). The ethanobotanical use of Crinum, as with many other medicinal plants, can be explained on the basis of chemical and physiological studies .In most cases these confirm the therapeutic value of the plants. However where plants have been studied chemically, a plant potential pharmaceutical value can be assumed, if it is used widely (Githens, 1949). The reason *Crinum* is used for medicinal purposes and in a number of countries for similar reason is possibly due to their alkaloids constituents, (Waller and Nowacki 1978).

*Crinum asiaticum* (Amaryllidaecae) is a bulbous herb found in many countries like India, Singapore, Malaysia and West Solomon Island. It has so many medicinal uses; in Southeast Asian countries *C. asiaticum*

has a medicinal reputation as potent folk medicine in the treatment of injury and inflamed joints and leaves as rheumatic remedy and to relief local pain .In Indonesia oiled and heated leaves are useful for stranger wounds by poisoned arrows, bites and sitting. In Malasia poultice of the leaves is applied to swelling, swollen, joints, and Lumbago pains and in case of headache and fever. The leaves are also emollient. It is also by traditional healers in North east Solomon Islands to treat inflammation (Wiart, 2000). Leaves are used for the treatment of skin disease and inflammation. It is also used to treat the ulcer and swelling (Goeltenboth *et al*., 1991). The seeds are considered as purgative (Duke and Ayensu, 1985). In the Trobriands, the stems fibers are used to treatment of Gonorrhoea. In Philippine, bulb are crushed and applied as an ointment (Wiart, 2000). This present study has been designed to determine the role of leaves extract of *Crinum asiaticum* for potential of antibacterial activity and it phytochemical analysis.

## MATERIALS AND METHODS

### Plant Material

The leaves of *Crinum asiaticum* has been collected from Thanjavur District, Tamilnadu, India and identified using standard procedures. Voucher specimens have been deposited in PRIST University, Thanjavur, Tamilnadu, India.

### Preparation of extract

Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles. The dried plants pulverized by an electrical blender and passed through the 20 mesh sieve. A powdered plant was extracted successfully with ethanol by using as soxhlet apparatus and water extracted by cooled maceration. The extraction was carried out for 24 hrs at room temperature with mild shaking (Chopra *et al*., 1992). The extract were filtered and concentrated at $45^0$C using rotary vacuum evaporator. The extract obtained was vacuum dried and used for further investigation.

### Bacterial samples

The bacterial strains studied are *Staphylococcus aureus (*ATCC 11632), *Escherichia coli* (ATCC 25922) *Pseudomonas aeruginosa (*ATCC 10145), *Klebsiella pneumonia (*ATCC 10031) and *Bacillus subtilis* (ATCC 23859) are obtained from Hi media Bangalore were used and determined the antibacterial activity of leaves of *Crinum asiaticum.*

### Antibacterial Assay

Disc diffusion method was used to determine the zone of inhibition against chosen bacteria by the *crinum asiaticum* plant extract (Rabe and Van Staden, 1997). Antibacterial activity of plant extracts was tested by a modified well in agar method (Sinclair and Dhingra, 1995). Quantitative evaluation of antibacterial effect of ethanol and aqueous extract of *Crinum asiaticum* was determined by the broth dilution method (Abutbul *et al*., 2004).Determination of MIC and MBC values of the extracts was determined using the two fold serial micro dilution method. Final concentration ranging from 1.5 mg/ml to 0.00589 mg/ml. The tested extracts were added to sterile nutrient broth into micro titer plates before the diluted bacterial suspension (final inoculums of 105 bacteria/ml) were added. Each extract was assayed in triplicate. The antibiotic (penicillin & streptomycin) were used as positive control ranging from 1mg to 0.00781mg/ml. The MIC values were taken as the lowest concentration of the extracts in the wells of the micro titer plate that showed no turbidity after 24 hours of incubation at 37°C. The turbidity of the wells in the micro titer plate was interpreted as visible growth of the microorganisms. The MBC was determined by subculture of the well showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value.

### GC Mass analysis

All analysis was conducted with a GC equipped with mass spectrometry (GC-MS-QP2010- Shimadzu). The chromatographic conditions were as follows: Column: DB-% ms (length 30.0 m, Diameter 0.25 mm, Film thickness 0.25 µm). The 10µl DG ethanolic extract was injected into the GC-MS in split less mode at $200^o$C. The column oven temperature was held at $45^o$C for 1 minute, then programmed at 10 different rates to $280^o$C and held for 15 minutes. Helium carrier gas was maintained at a flow rate of 1.4 ml/min.

## RESULTS

### Antimicrobial activity

Our study indicates that the ethanolic extract possessed the better zone of inhibition when compared with aqueous extract. The antimicrobial potential of ethanol and aqueous extract of *Crinum asiaticum* has been investigated against some human pathogenic bacteria like *Klebsilla pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.* The crude extract ranging from 0.5 – 1.5mg /ml were used , more inhibitory activity was found to be in 1.5 mg/ml of ethanol extract against both gram negative and gram positive bacteria. However, the other two concentration also having least inhibitory activity against all the tested bacteria. The maximum zone of inhibition were observed in ethanolic extract range from 13±0.45 mm for *Bacillus subtilis* and to 18±0.21 mm for K. *pneumonia* in well in agar diffusion method. But in case of disc diffusion method the same extract was more active against only *P.aeruginosa* (13± 0.31mm) and *K. pneumonia* (13± 0.02mm) and less active against *E.coli,* followed by *S. aureus and Bacillus subtilis*. An increase in the concentration to 1.5mg/ml suppressed the growth of almost all the tested bacteria. From the above results it was found that *E.coli* have been suppressed at all the 3 concentrations of the ethanolic extract in both well and disc diffusion method where as *Klebsilla pneumoniae, Escherichia coli* and *Staphylococcus aureus* activity were effectively reduced in well in agar diffusion method only at all the concentration of ethanolic extract, but at 0.5 mg/ml concentration there was no zone inhibition were observed against *P.aeruginosa and B.subtilis* in both well and disc method (Table 1&2).

The effect of *C. asiaticum* ethanolic extract on the growth of K. *pneumoniae* S. *aureus, Bacillus subtilis*, *and*

**Table 1. Antimicrobial activity of leaves of *Crinum asiaticum* (Zone of inhibition in mm)**

| Microorganism | 0.5mg/ml | 1.0mg/ml | 1.5mg/ml | Streptomycin 10µg/ml | Penicillin 10µg/ml |
|---|---|---|---|---|---|
| *S. aureus* | 8± 0.32 | 8± 0.16 | 13±0.45 | 18± 0.03 | 16± 0.04 |
| *P.aeruginosa* | - | 7± 0.20 | 16± 0.31 | 23± 0.021 | 20± 0.07 |
| *B.subtilis* | - | 8± 0.13 | 13± 0.23 | 28± 0.03 | 21± 0.22 |
| *E. coli* | - | 12± 0.12 | 15± 0.12 | 18± 0.12 | 20± 0.62 |
| *K.pneumoniae* | 8±0.72 | 10± 0.43 | 18±0.21 | 16± 0.20 | 21± 0.10 |

***Zone of inhibition were expressed as STD ± SEM (P < 0.05 level in mm)***

*P.aeruginosa* and *E. coli* in broth dilution technique has been studied. It has significant effect on all the tested bacteria for after 24 hrs incubation and it results are presented in figure 1 and 2. The inhibitory activity ranged between 0.5 mg and 1.5mg/ml of ethanolic extract, the inhibitory effect increased with increasing concentration of plant extract , highest inhibitory activity was found to be in 1.5mg /ml for *P.aeruginosa* but in case of aqueous extract does not show any inhibitory activity. It may be due to insoluble properties of compounds in aqueous medium. The MIC and MBC values of ethanolic extract of *Crinum asiaticum* against five human pathogenic bacteria were shown in Table 3.

The same MIC values of ethanolic extract were observed against *P.aeruginosa, B.subtilis* and *E. Coli* but in case of *K.pneumoniae* and *S. aureus* low MIC values has been identified (0.375mg/ml)when the compared with other three bacteria, but we didn't observed any minimum bactericidal activities in ethanolic extract of this plant. It indicates that plant extract possessed only bacteriostatic effect. Suppose MBC values may be observed when the concentration of this plant extract will be increased against the tested bacteria.

***Table: 2 Antimicrobial activity of ethanolic extract of Crinum asiaticum (Disc diffusion method)***

| Microorganism | 0.5mg/ml | 1.0mg/ml | 1.5mg/ml | streptomycin 10µg/disc | Penicillin 10µg/disc |
|---|---|---|---|---|---|
| ***S. aureus*** | - | 7± 0.42 | 11± 0.12 | 16± 0.61 | 18± 0.192 |
| ***P.aeruginosa*** | - | 7± 0.32 | 13± 0.31 | 23± 0.24 | 14± 0.60 |
| ***B.subtilis*** | - | 7± 0.22 | 10± 0.82 | 30± 0.04 | 28± 0.53 |
| ***E. coli*** | 8± 0.02 | 9± 0.02 | 9± 0.22 | 17± 0.31 | 21± 0.07 |
| ***K.pneumoniae*** | - | 8± 0.62 | 13± 0.42 | 14± 0.09 | 17± 0.08 |

***(Zone of inhibition were expressed as STD ± SEM (P < 0.05 level in mm)***

**Table 3.** ***Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of ethanolic extract of Crinum asiaticum***

| Concentration (mg/ml) | *P.aeruginosa* | | *K.Pneumoniae* | | *E. coli* | | *S. aureus* | | *B.subtilis* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Ethanolic extract | 0.187 | - | 0.375 | - | 0.187 | - | 0.375 | - | 0.187 | - |
| Penicillin G | 0.007 | >0.015 | 0.015 | >0.031 | 0.007 | 0.015 | 0.007 | 0.015 | 0.015 | >0.031 |
| Streptomycin | 0.003 | >0.015 | 0.007 | 0.015 | 0.003 | 0.007 | 0.015 | 0.031 | 0.007 | 0.015 |

**GC-MS analysis**

Based on GC-MS chromatogram and library search data, the ethanolic extract exhibited 22 components as presented in Table 4 and figure 3. The major components were n- Hexodecanoic acid appeared at retention time (rt) 17.78 min, 9,12,15-octadecatrienoic acid (rt) 20.77, 9,12-Octadecadienoic acid (rt) 20.88, 9,10-Anthracenedione, 2 amino (rt) 23.10, 1-Monolinoylglycerol trimethysilyl ether (rt) 28.72 and phytol (rt) 20.23.

Table 4. GC-MS analysis of ethanolic extract of *C.asiaticum*

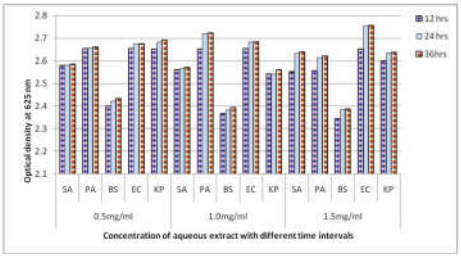
| S.No | RT | Name of the compound | Molecular formula | Molecular weight | Peak Area |
|---|---|---|---|---|---|
| 1 | 3.46 | Azocine, octahydro- | $C_7H_{15}N$ | 113 | 0.52 |
| 2 | 3.56 | Butane, 1, 1-diethoxy-3-methyl- | $C_9H_{20}O_2$ | 160 | 0.84 |
| 3 | 3.72 | Glycerin | $C_3H_8O_3$ | 92 | 5.47 |
| 4 | 4.65 | 1-Butanamine, 2-methyl-N-- | $C_{10}H_{21}N$ | 155 | 0.85 |
| 5 | 5.27 | 1H-Azonine, octahydr1nitroso | $C_8H_{16}N_2O$ | 156 | 0.71 |
| 6 | 6.16 | 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl- | $C_6H_8O_4$ | 144 | 1.00 |
| 7 | 7.07 | 2-Furancarboxaldehyde, | $C_6H_6O_3$ | 126 | 6.44 |
| 8 | 7.58 | 3-Octyn-1ol | $C_8H_{14}O$ | 126 | 1.01 |
| 9 | 8.57 | Benzene, (2,2-diethoxyethyl)- | $C_{12}H_{18}O_2$ | 194 | 0.64 |
| 10 | 10.00 | 2-Piperidinecabooxylic acid, (+)- | $C_6H_{11}O_2$ | 129 | 1.39 |
| 11 | 10.74 | β-D-Glucopyranose | $C_6H_{12}O_6$ | 180 | 0.76 |
| 12 | 11.70 | Dodecanoic acid | $C_{12}H_{24}O_2$ | 200 | 1.18 |
| 13 | 15.76 | 3, 7, 11, 15-Tetramethyl-2-hexadecen-1ol | $C_{20}H_{40}O$ | 296 | 1.76 |
| 14 | 17.78 | n-Hexadecacanoic acid | $C_9H_{32}O_2$ | 256 | 22.44 |
| 15 | 18.07 | Hexadecacanoic acid. Ethyl ester | $C_{18}H_{36}O_6$ | 284 | 2.44 |
| 16 | 18.23 | β-D-Mannofuranoside, farnesyl- | $C_{21}H_{36}O_6$ | 384 | 0.54 |
| 17 | 20.23 | Phytol | $C_{20}H_{40}O$ | 296 | 7.43 |
| 18 | 20.66 | 9, 12, 15-Octadecatrienoic acid(Z, Z)- | $C_{18}H_{32}O_2$ | 280 | 14.78 |
| 19 | 20.77 | 9, 12, 15-Octadecatrienoic acid(Z, Z, Z)- | $C_{18}H_{30}O_2$ | 278 | 15.42 |
| 20 | 20.88 | 9, 12, - Octadecatrienoic acid Ethyl ester | $C_{20}H_{36}O_2$ | 308 | 4.14 |
| 21 | 23.10 | 9, 10-Anthracenedione, 2- amino- | $C_{14}H_9NO_2$ | 223 | 7.65 |
| 22 | 28.72 | 1-Monolinoleoylglycerol trimethylsilyl ether. | $C_{27}H_{54}O_4Si_2$ | 498 | 2.59 |

## DISCUSSION

The antimicrobial potential of ethanol and aqueous extract of *Crinum asiaticum* has been investigated against some human pathogenic bacteria like *Klebsilla pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.* Similar results were reported by Parekh and Chanda, 2007. They are analyzed the thirty-four Indian Medicinal plants belonging to 28 different families were screened for potential antibacterial activity against *Staphylococcus* species. The activity of aqueous and alcoholic extracts was performed by agar disc diffusion and agar well diffusion method. The alcoholic extracts were more active than the aqueous extracts for all the plants studied. The alcoholic extract of *Woodfordia fruticosa* showed the best antibacterial activity. Ethanolic leaf extract of *Nyctanthes arbortristis of* were tested against 10 bacterial strains at different concentration of extracts as 50mg/ml, 100mg/ml, 200mg/ml and 300mg/ml solvent. The extract showed better inhibitory activity against all the tested pathogens. (Sathiya *et al.*, 2008).

Figure-1

Effect of Aqueous extract of C. *asiaticum* on human pathogenic bacteria in broth culture




Similarly our results indicate that ethanolic extract possessed more inhibitory activity than the aqueous extract. The above results are presented in Table 1&2 .The bacteria used in these study include such as *E.coli* known to cause urinary tract infection, *K. pneumonia* which are associated with causative agent for pneumonia (Pelczar *et al.,* 2006).

**Figure- 2**

Effect of ethanolic extract of C. *asiaticum* on human pathogenic bacteria in broth culture

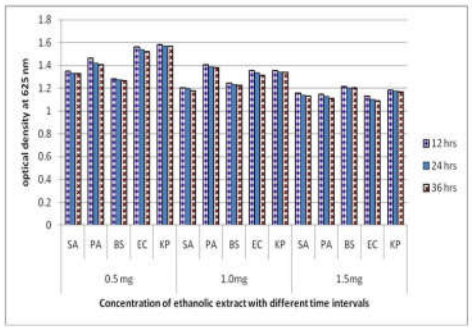
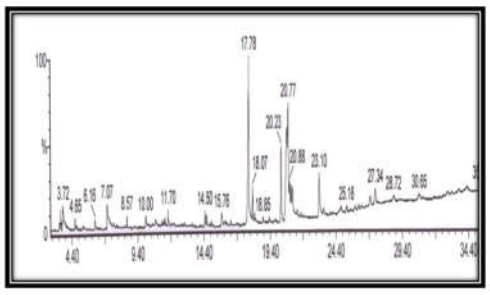



**Figure : 3**

GC-MS Chromatogram of *crinum asiaticum* ethanolic extract



Ethanolic extract exhibited the highly susceptibility on *P.aeruginosa* and *K. pneumonia.* This probably indicates that there are bioactive ingredients that are inhibitory to the growth of these common pathogens. (Etani *et al*., 1998; Okigbo and Kalu, 2005) reported that organic solvent extract is better than aqueous extract similarly our aqueous plant extract didn't possess any antibacterial activity.

All the tested human pathogenic bacteria highly sensitive to the *Crinum asiaticum* ethanolic extract used in this investigation, so these reports supporting the medicinal application of *C. asiaticum* in folk remedies in the treatment of infection caused by these bacteria . The ethanolic extract showed to inhibit the growth of both gram negative and gram positive bacteria, so the above statement give the clear information about our ethanolic plant extract possessed a broad spectrum activity, Wiart *et al.* (2000 ) reported that the *C. asiaticum* which consist alkaloid such as crinamine and lycorine alkaloid responsible for control the growth *of Baccilus cerus* and *P.aeruginosa.*

The MIC and MBC values of ethanolic extract of *Crinum asiaticum* against five human pathogenic bacteria were shown in Table 3. The antibacterial activity of plant extracts was not likely to be due to any one main active constituent but to the combined action of additional other compounds (Essawi and Srour, 2000). Similar result was obtained from the antimicrobial activities of *Rauvolfia tertraphylla* and *Physalis minima* leaf and callus extracts (Shafriff *et al.,* 2006). On the other hands, GC-MS chromatogram and library search data, the ethanol extract exhibited 22 components as presented in Table 4 and figure 3. The 9, 12-Octadecadienoic and Hexodecanoic acid is suggested to be a essential fatty acid and it used in pharmaceutical and cosmetic production (Dictionary of natural products, 1982).

## CONCLUSION

Over all conclusions of our results support the valuable use of this plant in traditional medicine for treatment of above the tested bacterial causing infection. The ethanolic extract of the leaves of *Crinum asiaticum* showed greater activity antibacterial activity than the corresponding water extract. These observation may be attributed to two reasons: firstly the nature of biological active components whose activity can be enhanced in the presence of ethanol: secondly the stronger extraction capacity of ethanol could have been produced number of active constitute responsible for antibacterial activity because more than 22 components has been identified in ethanolic extract by GC-MS analysis and further, phytochemical separation and pharmacological studies of this plant is in under progress.

## ACKNOWLEDGEMENT

The authors thank to PRIST University, Thanjavur, Tamilnadu, India for their financial and excellent technical support

## REFERENCES

Abutbul, S., Golan-Goldhirsh .,A., Barazani. O. and Zilberg, D. 2004**.** Use of *Rosmarinus officinalis* as a treatment against Streptococcus iniae in tilpia (*Oreochromis sp*). Aquaculture, 238, 97-105.

Ahmad I, Mehmood Z. and Mohammad F. 1998. Screening of some Indianmedicinal plants for their antimicrobial properties. J. Ethnopharmacol., 62: 183-193.

Bhavnani SM, Ballow CH. 2000. New agents for Gram-positive bacteria.Curr Opin Microbiol., 3: 528-534.

Bolkan, H.A. and Reinert, W.R. 1994. Developing and implementing IPM strategies to assist farmers: an industry approach. *Plant Disease,* 78: 545–550.

Bryan, J.E., 1989. Bulbs, vol. II. A-H. Timber Press, Portland.

Chopra, R.N., Nair. S.L. and Chopra. J.C. 1981. Glossary of Indian medicinal plants*. CSIR* publication*,* New Delhi, 50.

Chung, PY, Chung, LY. and Ngeow, YF. 2004. Antimicrobial activities of Malaysian plant species. Pharm. Biol., 42: 292-300.

Clark, AM. 1996. Natural products as resource for new drugs. Pharm. Res., 13: 1133-1141.

Cordell, GA. 2000. Biodiversity and drug discovery a symbioticrelationship. Phytochemistry, 55: 463-480.

Davis, J. 1994. Inactivation of the antibiotics and the dissemination of resistance genes. Science, 264: 375-382.

De Boer, HJ, Kool, A. and Broberg, A . 2005. Antifungal and antibacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol., 96: 461-469.

Dictionary of natural products. 1982. A Suggested validation lexicon. Pharmaceutical Technology Volume one A-C, p. 1266.

Duke, J.A. and Ayensu, E. S. 1985. Medicinal plants of China, Volume One. Reference Publications, Inc.

Essawi, T. and Srour, M. 2000. Screeing of some Palestian medicinal plants for antibacterial activity. *J. Ethnopharmcacol*

Etani, E., Agai, M., Tsujihata, S., suksmoto, T.T., and Ohta, M., 1998. Antibacterial action of vinegar against food-bone pathogenic bacteria including *Escherichiacoli.,* 0157. H7.J. Food Production, 6: 953-959.

Fangan, B.M. and Nordal, I. 1993. A Comparative analysis of morphology, chloroplast- DNA and distribution within the genus *Crinum* (Amaryllidaceae). Journal of biogeography, 20: 55-61.

Githens, T.S., 1949. Drug Plants of Africa. University of Pennsylvania Press, Philadelphia.

Goeltenboth, F., Holdworth, D. K., SAkulas, H., Thredgold, H., Woodley, E. and Yauieb, A. 1991. In: Woodly, E (eds.), Medical Plants of Papua New Guinea. Wau Ecology Institute, F. & T. Mullerbader, Germany.

McFarland, J.H., Hatton, R.M. and Foley, D.J. 1948. Garden Bulbs in Color. J. Horace Mc Farland, Harrisburg.

Monroe, S. and Polk R.2000. Antimicrobial use and bacterial resistance. Curr. Opin. Microbiol., 3: 496-501.

Nair, R. and Chanda, SV.2004. Antibacterial activity of some medicinal plants of Saurashtra region. J. Tissue Res., 4: 117-120.

Okigbo, R.N and Nmeka, I.A. 2005. Control of yamtuber rot caused by *Fusarium axysporium* Schlecht, *Aspergillus niger Vau Tiegh and Aspergillus flavus* Link with leaf extract of *Xylopia aethiopica* (Dunal) A Rich and Zingiber officinalc Roscoe. African J. Biotechnol., Kenya.

Pelczar, M.J., Chan, E.C. and Kruz, NR. 2006. Microbiology. 5th edition. TataMcGraw-Hill Publishing Company Ltd., New Delhi.

Parekh, J. and Chanda, V., 2007. *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal plants. *Turk. J. Biol.,* 31: 53-58.

Rajab, M. S., Cantrell. C. L., Franzblau. S. G. and Fischer, N. H. 1998. Antimycobacterial activity of (E)-phytol and derivatives: A preliminary structure-activity study. Planta medica., vol. 64, no 1, pp. 2-4.

Rabe, T. and Van Staden. J.1993. Isolation of an Antibacterial Sesquiterpenoid from Warburgia salutaris. *Journal of Ethanopharmacol.,* 73: 171-174.

Sathiya, M., Parimala, P. and Muthuchelian, K. 2008. Preliminary phytochemical screening and antibacterial studies on the ethanolic leaf extract of *Nyctanthes arbortristis* L. *Ethanobotanical leaflets,* 12: 337-342.

Shariff, N., Sudarshana, M.S., Umesha, S. and Hariprasad, P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *African Journal of Biotechnology*, 5: 946 – 950

Sinclair, J.B. and Dhingra, O.D. 1995. Basic Plant Pathology Methods**,** p. 287-305.

Verdoorn, I.C. 1973. The genus *Crinum* in southern Africa. Bothalia., 11, 27-52.

Waller, G.R., Nowacki, H. and Farnsworth, N.R. 1978. Alkaloid Biology and Metabolism in Plants. Plenum Press, New York.

Wiart, C. 2000, Medicinal Plants of Southeast Asia. Pelanduk Publications