RA Publications
Journal of Medical Science & Technology

Original Article Open Access

Anti bacterial activity and GC-MS analysis of ripened and un ripened cv.Amrutapani (Musa x paradisiaca L) Banana Extracts

N. Jyothirmayi [1], S.H.R.K.Prasad [2], N.Mallikarjuna Rao [3 *]

[1]Department of Biotechnology, K L University, Guntur, India
[2]Department of Biotechnology, Aksum University, Ethiopia
[3]Department of Biochemistry, Vishnu Dental College, Bhimavaram, India

Abstract

The present work assesses the antibacterial activity of solvent extracts (aqueous, ethanol, methanol and hexane) of ripe and unripe fruit pulps of Musa x paradisiaca L. cv. Amruthapani against Gram-positive (Bacillus cereus, Micrococcus flavus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa) pathogenic bacteria by Kirby-Bauer method along with, the minimum inhibitory concentrations (MICs). The phytochemical composition of non-polar solvent extract, hexane, was analyzed, qualitatively, by Gas Chromatography – Mass Spectrometry (GC-MS). The aqueous unripened and ripened pulp extracts of Amruthapani showed minimum inhibition at highest tested concentration against all the test organisms. Ethanolic ripened extracts exhibited good activity against all the test organisms in comparison with unripened pulp extracts and also the methanolic extracts. Hexane extracts showed very little activity against all test organisms even at high concentrations. A wide spectrum of bioactive compounds like phenols, ketones, aldehydes, alkanes, esters and terpenes were found to be present in the hexane extract and may be responsible for antibacterial activity exhibited.

Key words: Antibacterial, GC-MS, Gram-positive, Gram-negative, Musa x paradisiaca L cv. Amruthapani, Minimum Inhibitory Concentration (MIC), Zone of Inhibition.

*Corresponding Author: Dr Nanduri Mallikarjuna Rao, Professor and Head, Department of Biochemistry, Vishnu Dental College, Bhimavaram, Andhra Pradesh, India.

Received: June 1, 2014 Accepted: August 29, 2014. Published: September 20, 2014.

Introduction

Recent trends focus on the isolation, characterization and utilization of natural compounds as potential disease preventing agents. As the sources available for this kind of compounds are predominantly plants, especially the edible varieties, it would be worthwhile investigating the commonly consumed plants and/or their products.

Musa x paradisiaca L., commonly known as banana, is a widely consumed fruit all over the world. India is the largest producer and consumer of banana with an annual production of 11.7 million tonnes on 404,000 Ha, contributing to 27% of the world production and about 37% of the total fruit crop production in the country. A number of therapeutic uses of various cultivars of banana have been reported and documented, conventionally, worldwide. The term 'Banana' is a highly general term that encompasses the hundreds of cultivars available. Amruthapani is one of the cultivars of Musa x paradisiaca L. that is specific to the Indian sub-continent. Because of its white, firm and characteristically flavoured taste when ripe, this cultivar is liked by many.

Experiments carried out by [1-4] on various parts of banana plant (pulp, peel, petioles and leaves in ripe/unripe form using different solvents) are proof of the antimicrobial activity present in banana. Also, works of [5-7] have shown the presence of various phytochemicals that contribute to antimicrobial activity in the pulp and sap of different banana cultivars. However, no research has been carried out on Amruthapani (otherwise Silk Fig), a much-loved cultivar of Banana in the Indian sub-continent.

Hence, the present study was initiated to evaluate the antibacterial activity and phytochemical content (qualitative) of pulp extracts of Musa x paradisiaca L cv. Amruthapani in their ripe and unripe forms against pathogenic bacteria, in vitro.

Traditionally documented Medicinal uses of banana (Musa spp. in general) [8]: Flowers extracts used to treat bronchitis, dysentery and on ulcers; Cooked flowers syrup used against Diabetes; Astringent plant sap used as a medication to cure hysteria, epilepsy, leprosy, fevers, haemorrhages, acute dysentery and diarrhoea, and on haemorrhoids, insect and other stings and bites; whereas young leaves used as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and leaves works against dysentery, diarrhoea and malignant ulcers; Roots are an age-old application in digestive disorders, dysentery and other ailments and Seed mucilage cures ophthalmic cataracts and diarrhoea.

Materials and Methods

Plant: Musa cultivar – Amruthapani

Tested material: Unripened and ripened pulp extracts of Musa x paradisiaca L. cv. Amruthapani prepared using polar and non-polar solvents – distilled water, ethanol, methanol and hexane. The extracts were obtained using a mortar and pestle, filtered, concentrated to dryness under vacuum and then collected. The left over powder was considered 100%. Different concentrations of the extracts such as 100, 250, 500, 750 and 1000µg/ml were prepared by re dissolving the extract powder in the same solvent and tested[9].

Studied activities: The minimum inhibitory concentration was determined by Vander-Berghe Da and Vlietinck (Agar Dilution) method [10-13]. Antibacterial activity was studied by Bauer AW *et al.* (Diffusion method) method [14-17]. Triplicates of the assay were run for standardizing the result. Simultaneously the activity of standard antibiotic, Chloramphenicol (30µg/ml) and solvents (served as controls) was also tested against the microorganisms under study in similar conditions.

Preliminary Phytochemical analysis was carried out for the non-polar solvent (Hexane) extract by Gas Chromatography-Mass Spectrometry GC-MS unit of Agilent Technologies 6890, mass detector model 5973 run in spilt mode was used for assay with MS source at 230^{0}C, MS Quadrupole at 150^{0}C and Helium as carrier gas at 80 bar pressure.

Identification of components

The results of the GC-MS analysis were interpreted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of each of the unknown compound was compared with the spectrum of the known compounds stored in the NIST library. The name, molecular weight, structure, nature of compound and activity of the compounds were determined.

Used bacterial strains

Escherichia coli (NCIM 2931), Pseudomonas aeruginosa (NCIM 5029), Bacillus cereus (NCIM 2106) and Micrococcus flavus (NCIM 2376) obtained from National Chemical Laboratory, Pune, India. The characteristics of the test micro-organisms are given in (**Table 1**).

Table 1: Bacterial strains used in the present study

S.No	Microorganism	Characteristics	Diseases caused
1	Escherichia coli (NCIM 2931)	Gram negative rod-shaped bacterium, facultative anaerobe.	Urinary tract infections, gastroenteritis, neonatal meningitis, pneumonia
2	Pseudomonas aeruginosa (NCIM 5029)	Gram-negative, aerobic, rod-shaped bacterium with unipolar motility	Urinary tract infections, respiratory infections, dermatitis, soft tissue infections, opportunistic human pathogen.
3	Micrococcus flavus (NCIM 2376)	Gram - positive cocci, non - motile, aerobic	Urinary tract infections, respiratory infections
4	Bacillus cereus (NCIM 2106)	Gram-positive, rod-shaped, swarming motility, facultative anaerobe	Food-borne illnesses, nausea, vomiting and diarrhoea

Results

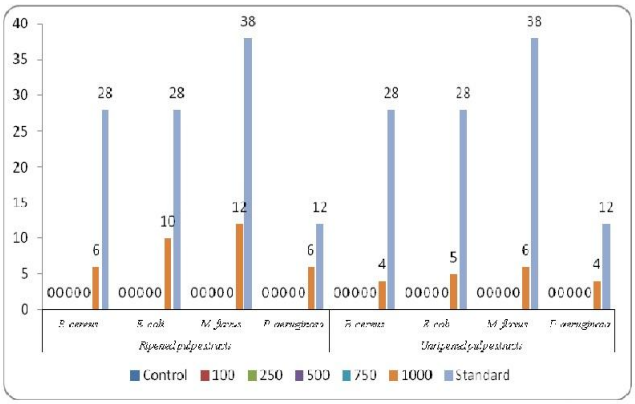
Determination of zone of inhibition of Musa x paradisiaca L. cv. Amruthapani

The zone of inhibition acquired by testing the solvent extracts – aqueous, ethanol, methanol and hexane, of unripened and ripened pulp of Musa x paradisiaca L, cv Amruthapani, at given concentrations against the test organisms can be seen in Graphs 1, 2, 3 and 4, respectively.

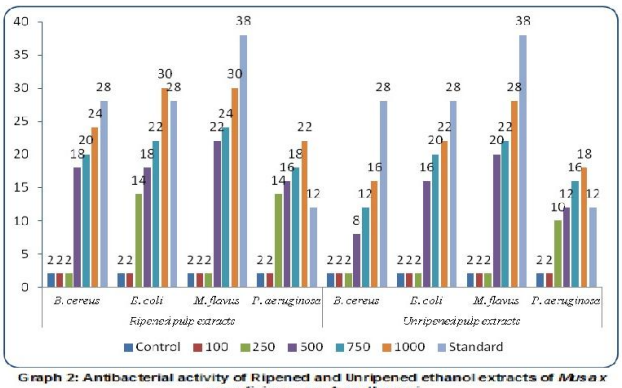
Unripened pulp solvent extracts - The aqueous extract showed little activity against all test

organisms at highest tested concentration of 1000 µg/ml. The zone of inhibition acquired by testing the ethanolic extracts of Musa x paradisiaca L. cv Amruthapani was such that initial activity was exhibited by the extract at 250µg/ml against P. aeruginosa and at 500µg/ml against all others. The methanolic extract exhibited activity beginning at the lowest concentration of 100µg/ml against B. cereus. Least zone of inhibition was observed to be at 250µg/ml against E. coli and highest activity was observed at 1000 µg/ml against M. flavus. The hexane solvent extract exhibited by the extract on all test organisms beginning at 750µg/ml.

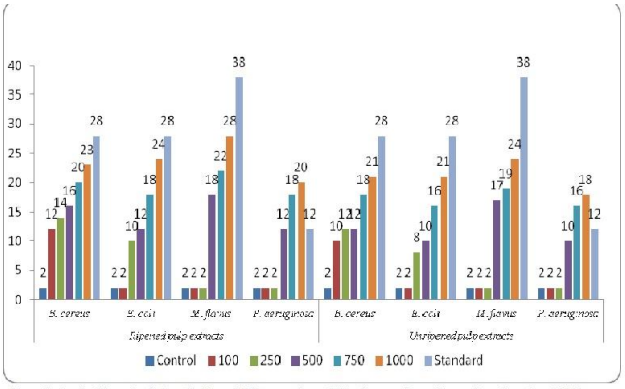
Ripened pulp solvent extracts - Similar to unripened extracts, the ripened aqueous extracts also showed minimum activity against all test organisms at the highest concentration of 1000µg/ml. The zone of inhibition acquired by testing the ethanol extract was as follows - minimum activity was exhibited by the extract at 250µg/ml against E. coli and P. aeruginosa and maximum zone of inhibition was seen against E. coli and M. flavus at 1000µg/ml. The activity exhibited by this extract at 1000µg/ml against E. coli was greater than that of the reference antibiotic, Chloramphenicol. The methanolic extracts of the same cultivar showed mediocre zones of inhibition at the least concentration of 100µg/ml against B. cereus, followed by E. coli at 250µg/ml, ending with the highest zone of inhibition at 1000µg/ml against M. flavus. **Figure 1** shows the Zone of inhibition of methanolic extract of ripened Musa x paradisiaca L. cv Amruthapani on Escherichia coli. The activity exhibited by hexane solvent extract was very little compared to other extracts but, better than that of the unripe pulp extract. Here also, activity against all test organisms was seen at 750µg/ml concentration, the best being on M. flavus.



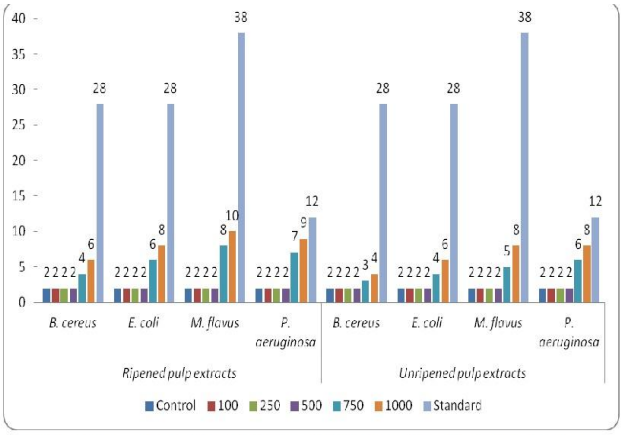
Graph 1: Antibacterial activity of Ripened and Unripened aqueous extracts of *Musa x paradisiaca* L. cv Amruthapani.



Graph 2: Antibacterial activity of Ripened and Unripened ethanol extracts of *Musa x paradisiaca* – cv. Amruthapani



Graph 3: Antibacterial activity of Ripened and Unripened methanol extracts of *Musa x paradisiaca* – cv. Amruthapani



Graph 4: Antibacterial activity of Ripened and Unripened hexane extracts of *Musa x paradisiaca* L. cv. Amruthapani

Minimum Inhibitory Concentration (MIC)

MIC of unripened and ripened pulps of *Musa x paradisiaca* L. cv Amruthapani is summarized in Table 2.

Phytochemical analysis

The primary phytochemical screening of non-polar solvent extract ie. hexane extract, of the selected ripe *Musa x paradisiaca* L cv. Amruthapani by Gas Chromatography – Mass Spectrometry (GC-MS) method indicated the

presence of secondary plant metabolites such as esters, ketones, phenols, aldehydes and other hydrocarbons. The chromatogram obtained from this analysis can be seen in **Fig 3**. The chromatogram has a total of ten peaks at various retention times. A list of the various compounds identified at the different retention times, their molecular mass, nature and activity are given in **Table-3.**

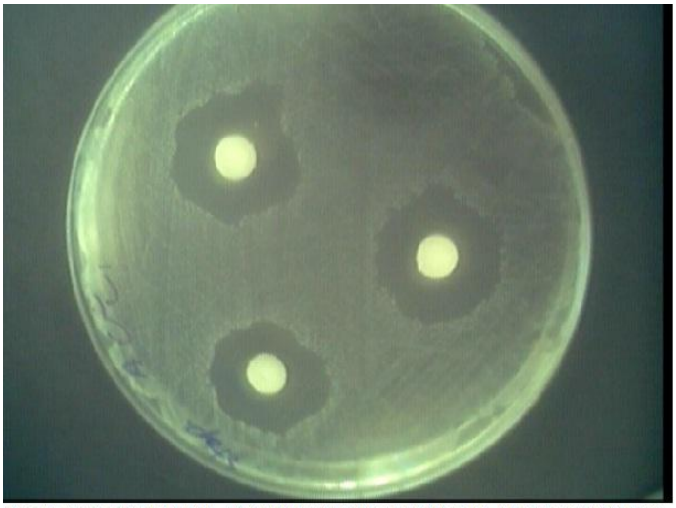


Fig 1: Zone of inhibition of methanolic extract of ripened *Musa x paradisiaca* L. cv Amruthapani on *Escherichia coli*.

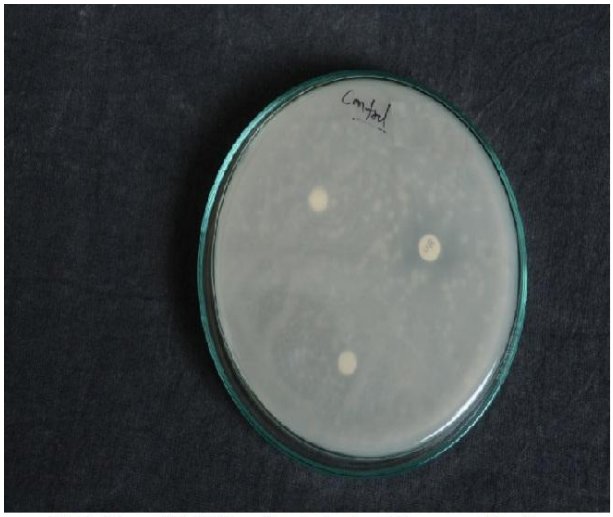


Fig 2: Zone of inhibition of control solvent Hexane on *E.coli*

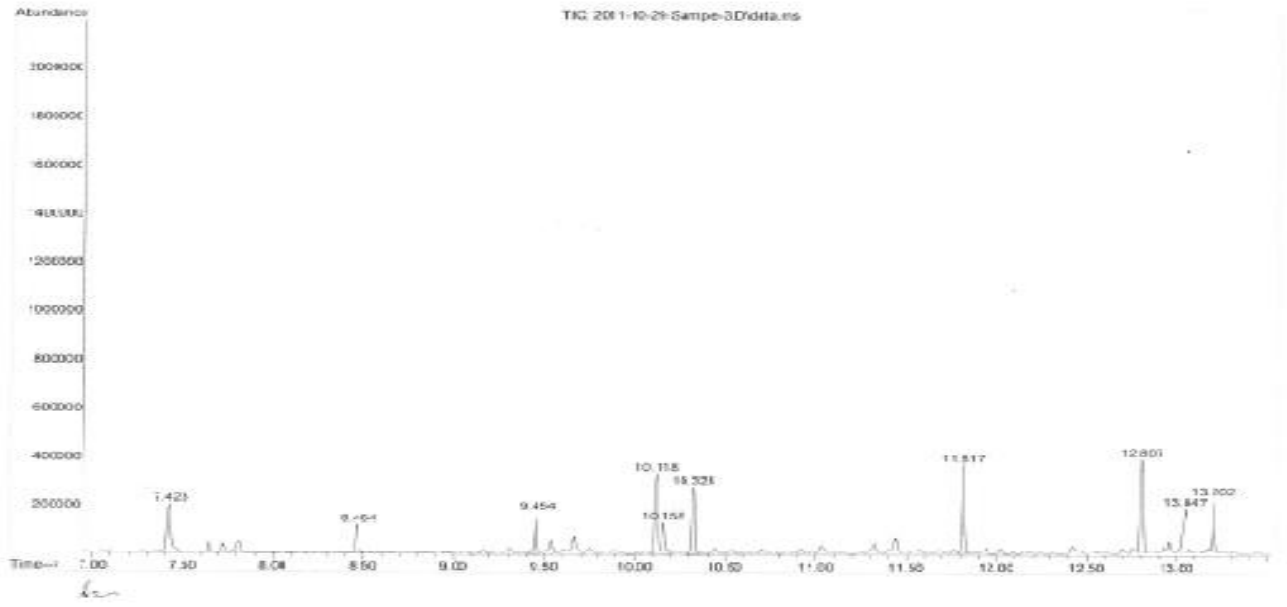


Fig 3: GC-MS chromatogram of hexane extract of Ripe Amruthapani

Discussion

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. In the present study, aqueous, ethanolic, methanolic and hexane extracts of *Musa x paradisiaca* L. cv. Amruthapani, were tested against selected Gram positive and Gram negative bacteria species. The results obtained indicate that the different extracts of the cultivar under study exhibit antibacterial activity and among the various extracts, ethanolic extracts have shown better activity as compared to other extracts with respect to similar bacteria. Also, from the values of zones of inhibition obtained, it was observed that the ethanol fraction was more potent in the case of both ripened and

ISSN: 1694-1217 JMST. An open access journal

unripened *Musa x paradisiaca* L. cv Amruthapani extracts. This may partly indicate that the pulp extracts of *Musa x paradisiaca* L. cv. *Amruthapani* have broad inhibitory activities to pathogenic micro-organisms and are promising potential antibacterial agents from natural plant sources [18].

Cultivar	Extract		MIC value
	Ripe/ Unripe	Solvent	
Amruthapani	Unripe	Aqueous	1000µg/ml
		Ethanol	500µg/ml (all), 250µg/ml (*P. aeruginosa*)
		Methanol	100µg/ml (*B. cereus*), 250µg/ml (*E. coli*), 500µg/ml (*M. flavus* and *P. aeruginosa*).
		Hexane	750µg/ml for all test organisms
	Ripe	Aqueous	1000µg/ml
		Ethanol	250 µg/ml (*E. coli* and *P. aeruginosa);* 500µg/ml (others)
		Methanol	100µg/ml (*B. cereus*), 250µg/ml (*E. coli*), 500µg/ml (*M. flavus* and *P. aeruginosa*).
		Hexane	750µg/ml for all test organisms

Table 2: Minimum Inhibitory Concentration (MIC) values of unripened and ripened pulps' extracts of *Musa x paradisiaca* L. cv Amruthapani.

S No	RT	Phyto-component	Mol formula	MW	Compound nature	Activity
1	6.616	Parachlorophenol	C_6H_5ClO	128.55	Phenol	Disinfectant
2	7.423	Benzaldehyde, 4-propyl-	$C_{10}H_{12}O$	148.22	Aldehyde	Antimicrobial
3	10.158	Diethyl phthalate	$C_{12}H_{14}O_4$	222.23	phthalate ester	nutritional supplements, bind cosmetics and fragrances
4	13.047	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	phthalate ester (Volatile)	antiparasitic, plasticizer, insect repellent
5	11.811	Octadecane	$C_{18}H_{38}$	254.4943	Alkane	anti-corrosion agents
6	8.470	Tetradecane	$C_{14}H_{30}$	198.3880	Alkane	Paraffin, perfuming agent, standard material in chromatographic analysis
7	9.454	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.3239	Phenol	Antioxidant No. 33
8	10.118	Hexadecane	$C_{16}H_{34}$	226.4412	Hydrocarbons – Alkanes	Aroma compound
9	10.329	Dodecanoic acid, 1-methylethyl ester	$C_{15}H_{30}O_2$	242.3975	Ester	Lubricant
10	12.807	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.3707	Ketone	Antioxidant
11	13.202	Eicosane	$C_{20}H_{42}$	282.5475	Terpene	Paraffin

Table 3: GC-MS analysis of Ripe Hexane pulp extracts of *Musa x paradisiaca* L cv Amruthapani

Preliminary phyto-chemical screening of plants is very useful for determination of the active constituents in different solvents and their yields. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds[19]. The responsibility of antibacterial activity exhibited by the extracts can be attributed to these chemicals. Further phyto-chemical studies are required to determine the purified fractions/bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents [18].

Conclusion

The ever-growing resistance of microbes to existing drugs and also the inefficiency of the drugs have given rise to an era of research wherein plants are the primary source of new and potent antimicrobial compounds [20]. *Musa x paradisiaca* L. cv Amruthapani, a commonly consumed and commercially important plant of India, is a rich source of bioactive compounds with diverse chemical structure. As of now, little work has been done on the biological activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigation is needed to exploit the bioactive principles of local varieties of *Musa x paradisiaca* L. cv Amruthapani for therapeutic utility. In the present study antibacterial activity of *Musa x paradisiaca* L. cv Amruthapani pulp extracts towards significant pathogenic microbes has been investigated. Further detailed investigations may lead to development of new antibiotics of high potency. The present study suggests that the ethanol extract of *Musa x paradisiaca* L. cv Amruthapani plants is a potential source of natural antibacterial agents. After this screening experiment, further work should be performed to describe the antibacterial activities in more detail as *in vivo*. Also phyto-chemical studies will be necessary to isolate the active constituents and evaluate the antibacterial activities against a wide range of bacterial population [21].

Acknowledgements

The authors are grateful to Dr V Himabindu, HOD, Department of Environmental Chemistry, JNTU-H, Hyderabad, for the constant support and encouragement extended. The authors are also grateful to the Management of K L University, Guntur, for providing the basic facilities required for completing the work.

References

1. William E. Scott. *et al*. Antibiotic substances from the banana (*Musa sapientum*). J Clin Invest. 1949; 28(5 Pt 1): 899–902.
2. Matook Saif Mokbel, Fumio Hashinaga. Antibacterial and Antioxidant Activities of Banana (*Musa*, AAA cv. Cavendish) fruits peel. American Journal of Biochemistry and Biotechnology. 2005; 1 (3):125-131.
3. Fagbemi Josephine Ferdinand. *et al*. Evaluation of the antimicrobial properties of unripe banana (*Musa sapientum* L.), lemon grass (*Cymbopogon citratus* S.) and turmeric (*Curcuma longa* L.) African Journal of Biotechnology .2009; 8 (7):1176-1182.
4. Suhaila Mohamed, Zaharia Hassan, Norhashimah Abd Hamid, Antimicrobial Activity of some Tropical Fruit Wastes (Guava, Starfruit, Banana, Papaya, Passionfruit, Langsat, Duku, Rambutan and Rambai). PertanikaJ. Trap. Agric. Sci. 1994; 17(3):219-227.
5. Oliveira L. *et al,*. Lipophilic extracts from banana fruit residues: a source of valuable phytosterols. J Agric Food Chem. 2008; 56(20): 9520-9524.
6. Malik IO. *et al*. In vitro availability of minerals of some tropical and citrus fruits as influenced by antinutritional factors. Nahrung. 2004; 48(1):65-68.
7. Pothavorn P *et al*. Sap phytochemical compositions of some bananas in Thailand. J Agric Food Chem. 2010; 58(15):8782-8787.
8. Gurumaa A. Go Banana: Banana Guide benefit and Nutrition facts http://www.gurumaa.com/health-go-bananas .php ; 2008.
9. S H K R Prasad, L Swapna, N Madan Prasad, Efficacy of *Euphorbia tirucalli* (L.) towards microbicidal activity against human pathogens. International Journal of Pharma and Bio Sciences. 2011; 229-235.
10. Vander-Berghe Da; Vlietinck N; Screening methods for antibacterial and antiviral agents

from higher plants. In: Dey PM, Harborne JB (eds). Methods in Plant Biochemistry, London, ,Academic Press,1991
11. Panda B.R. *et al.* Antibacterial activity of the leaves of *Cocculus hirsutus*, Indian Drugs. 2007; 44(2):108-110.
12. Kumar V P *et al* .Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol. 2006; 67:241-245.
13. Mishra U S. *et al.* Antibacterial and analgesic effects of the leaves of *Dichrostachys cinerea*, International Journal of Pharmacy And Pharmaceutical Sciences. 2009; 1(2):230-234.
14. Bauer AW *et al.* Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966; 45: 493-496.
15. Barry A C. Standard diffusion methods for antibiotic susceptibility of common rapid growing bacterial pathogens. Baltimore, USA, Park Press, 1976.
16. Benson H J. Microbiological applications, 5th ed. USA: WMC. Brown Publ, 1990; 4.
17. Pelczar M J. Microbiology. 5th ed. New Delhi, Tata Mc Graw-Hill Publishing Company Limited, 2004.
18. Rajesh Kowti *et al.* Antimicrobial activity of ethanol extract of leaf and flower of *Spathodea campanulata P*. Beauv. Res J Pharm Biol Chem Sci 2010; 1(3):691.
19. Babeet Singh Tanwer, Rekha Vijayvergia. Phytochemical evaluation and quantification of primary metabolites of *Alangium salviifolium*. International Journal of Pharma and Bio Sciences; 2010; 1(3): 430-436.
20. Ramappa Raghavendra, Gurumurthy D, Mahadevan. *In vitro* antimicrobial activity of various plant latex against resistant human pathogens. Int J Pharm Pharm Sci. 2011; 3(4):70-72.
21. Abdollah Ghasemi Pirbalouti *et al.* Antibacterial Activity of Some Folklore Medicinal Plants Used by Bakhtiari Tribal in Southwest Iran. International Journal of Biology; 2010; 2(2):55-63.

