minutes and by asking the subject to spit in funnel, saliva(3ml) was collected in vial. By following the above mention method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1) in a sterile vile containing 1ml of normal saline and were used to inoculate on the agar plates. All samples were refrigerated within 30 minutes and frozen within [4] hours. (If collection is being carried out in the field, it may not be practical to freeze the samples until the end of the day, but samples should be kept cold until they are returned to the lab).

Antimicrobial assay

The microbial inhibition assay was prepared using the agar well diffusion method. Sterile 8.0 mm diameter of well were impregnated with the extract of different concentrations ranging from 62.5µg to 4000µg per well. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid Muller Hinton Agar medium in plates. After the media was solidified; a well was made in the plates with the help of a cup-borer (8.0mm). The well was filled with different concentrations of the extract (62.5µg to 4000µg/ well) and plates were incubated at 37 ± 0.1°c for 24 hours. After incubation, the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. Chlorhexidine was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded.
This is an procedure for the extract as discussed with Hon. Deshpande Madam.

Plant materials and preparation of extracts

*Morinda Pubescence*was collected from Western Pune, Maharashtra, India. The taxonomic identification was carried out with the help of Flora of Botany Presidency and Flora of Maharashtra and herbaria were prepared by following standard methods. The specimens were also compared with the authentic herbaria of BSI, Western circle Pune, Maharashtra,India for confirming the identification, its number is BSI/WC/Tech /2009/95.Air shade dried and pulverized plant material was used. Extracts were prepared using exact weighed sample powder in the measured volume of solvents like, acetone, ethanol. Vacuum dried extracs are used for the experiment.Solvents used after distillation

RESULTS AND DISCUSSION

This study compares antimicrobial activity of "*Morinda Pubescence*"with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg. The zone of inhibition are measured by excluding the diameter of well. The mean value of average zone of inhibition of "*Morinda Pubescence*" with 0.2% chlorhexidine and S-flo in ten salivary samples has taken for comparison. These zones of inhibition are directly proportional to the concentration.

Table 1. represents the mean value of average zone of inhibition of the "*Morinda Pubescence*" at five different concentrations. Table 2.represents the mean value of average zone of inhibition of chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg at five different concentrations. Results were obtained after 24 hours of incubation. Number 1,3 and 4 in Fig 2 represents zone of inhibition of chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg respectively. Fig 1 represents average zones of inhibition (mm) of "*Morinda Pubescence*".

The demand for plant based medicines, healthproducts, pharmaceuticals, food supplement, cosmetics etc are increasing in both developing and developed countries, due to the growing recognition that thenatural products are non-toxic, have less side effects and easily available at affordable prices.[4]The global market for herbal medicines currently stands at over $60 billion annually. The sale of herbal medicines is expected to get higher at 6.4% an average annual growth rate.[5]

Tooth decay disease is caused by specific types of bacteria that produce acid in the presence of fermentable carbohydrates such as sucrose,fructose, and glucose.[6,7]Dental caries (tooth decay) is the single most common chronic disease of childhood.[8]

Dental caries is an infectious disease of bacterial origin. Therefore, it seems rational to use an antimicrobial approach to prevent and control dental caries. Chlorhexidine is an antimicrobial agent that has been studied extensively over the last 30 years for its ability to suppress the levels of mutans streptococci in the mouth and for its potential to prevent and control dental caries.

This study proves that the antimicrobial activity of "*Morinda Pubescence*" at higher concentration is comparable with 0.2% chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg.Statistically, Kruskal-Wallis test followed by post-hoc test proved that all results are comparable as the p value is 0.0001 which is significant (p< 0.5).

TABLES

Table 1: Mean value of Zones of inhibition of "Morinda Pubescence"

Concentration (µg)	Mean value of average zone of inhibition (mm)
50	4
100	5.2
200	7.2
400	9.2
800	14

Table 2: Mean value of Zones of inhibition of standard antimicrobial agent

Antimicrobial agent	Mean value of average zone of inhibition
0.2% chlorhexidine	20,0000
Amoxicillin 125mg	404.000
Amoxicillin 250mg	48.4000

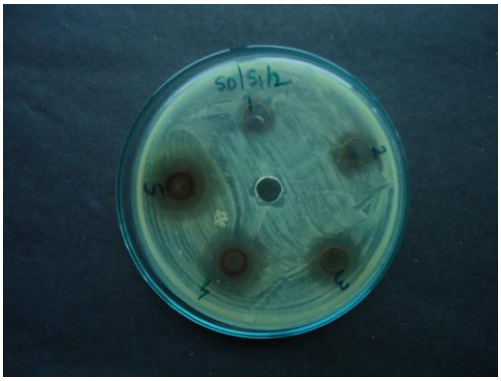

Fig 1: average zones of inhibition (mm) of "*Morinda Pubescence*"

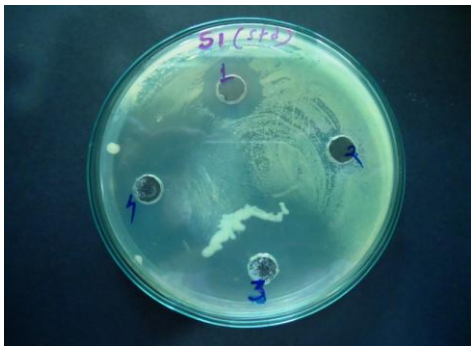

Fig 2: Here '1', '2' and '3' represents zone of inhibition of standard antimicrobial agent 0.2% Chlorhexidine, amoxicillin 125mg and amoxicillin 250mg respectively.

CONCLUSION

The antimicrobial activity of "*Morinda Pubescence*" at higher concentration is comparable with 0.2% chlorhexidine. This study need to increase concentration to confirm the antimicrobial activity of active compound of"*Morinda Pubescence*" againsed Amoxicillin. This study has confirmed the antimicrobial potentials of the plant, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

ACKNOWLEDGEMENTS

Agharkar Research Institute, Pune, India.

Deshpande's Oral Health Clinic, Pune.

REFERENCES

1. Tomoko N., Takashi A., Hiromu Y., et al: Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin resistant Staphylococcus aureus. J. Health Sci; 2000: 48: 273–276.
2. Sieradsxi K., Roberts R. B., Haber S. W., Tomosaz A. The development of vancomycin resistance in a patient with methicillin-resistant Staphylococcus aureus infection. N. Engl. J. Med; 1999: 340: 517–523.
3. B. P. Katz and E. Huntington ; J DENT RES ; 2004, 83, (Spec Iss C), C109 - C112.
4. Krishnan, R. Indian Drug ManufacturedAssociation Bulletin, 1998, 13: 318-320.
5. Inamdar, N., S. Edalat, V.B. Kotwal and S. Pawar, 2008. Herbal Drugs in Milieu of Modern Drugs. Int. J. Green Pharm., 2 (1): 2-8.
6. HardieJM . "The microbiology of dental caries". Dent Update 1982, **9** (4): 199–200, 202–4, 206–8.
7. Holloway PJ; Moore, W.J. The role of sugar in the etiology of dental caries". J Dent 1983, **11** (3): 189–213.
8. U.S. Department of Health and Human Services. Oral Health in America: A Report of the Surgeon General. Rockville, MD: U.S. Department of Health and Human Services, National Institute of Dental and Craniofacial Research, National Institutes for Health, 2000.