

Comparative Effect of Some Herbal Extracts on Dental Caries Pathogens and Oral Health

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Abstract

The main objective of this study was to evaluate the comparative effect of some herbal extracts on dental caries pathogens and oral health. Different plant extracts were prepared from *Azadirachta indica* (Neem), *Pongamia pinnata* (Karanj), *Psidium guajava* (Guava) and *Punica granatum* (Pomegranate) in solvents like ethanol, acetone, methanol and water. These extracts were tested against some oral pathogens like *Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa* using disc diffusion method. *Azadirachta indica* as expected showed maximum inhibitory effect amongst all other plant extracts. But other plant extracts also showed positive results against the selected oral pathogens. After *Azadirachta indica*, the other plant extracts which had maximum effect was *Pongamia pinnata* (Karanj) which was followed by guava and pomegranate in its efficacy against dental caries and oral flora.

KEYWORDS: Dental caries, Disc diffusion, Oral flora, Oral pathogens.

Introduction

The significance of teeth cleanliness and oral health has been recognized from the earliest days of civilization to the 21st century (Almas K et al,1995). About 80% of the population in India living in rural areas still start their day with the chewing sticks obtained from different plants. The aim of this study was to make a comparative study on the effect of some herbal extracts on dental caries and oral health.

For this study, we have considered four plants like *Azadirachta indica*, *Pongamia pinnata*, *Psidium guajava* and *Punica granatum* (Johri.,2007) and prepared their extracts in different solvents like ethanol, acetone, methanol and water. The extracts prepared from them were tested against three common oral pathogens like *Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*. (Limsong, et al., 2004). The invitro study showed that all the four concerned plants have inhibitory effect on the oral flora.

MATERIALS & METHODS

Selection of Bacterial Strains

Bacterial strains of three different species (*Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) with enhancing activity in caries formation were selected from MTCC. Chandigarh.

Collection of Medicinal Plants

The medicinal plant samples were collected from the Ranchi region of Jharkhand. The different plants such as *Azadirachta indica* (Neem), *Pongamia pinnata* (Karanj), *Psidium guajava* (Guava) and *Punica granatum* (Pomegranate) were selected for testing its antibacterial studies and comparative analysis.

Preparation of Plant Extracts

The air dried plant parts were powdered properly and extracts were prepared in different solvents systems (acetone, ethanol, methanol and water) using a Soxhlet apparatus and rotaevaporatory system. All extracts were kept in -20 degree centigrade. Before starting the antimicrobial assay, extracts were soluted in distilled water in proportion of 1/20% w/v, this was the maximum concentration that could pass through the Millipore filter (30 mm in diameter), and then each solution was sterilized by the filter, gathered in sterilized tubes and kept in -20 degree centigrade.

Antibacterial Effect Checking of Medicinal Plant Extracts

Antibacterial effect of medicinal plant extracts were checked by Disc- diffusion method.

Disc Diffusion Method

The bacterial isolates were effectively swabbed on the prepared agar plates. After allowing the inoculums to dry at room temperature, four mm diameter sterile blank paper discs were aseptically put on agar surfaces and immediately impregnated with different dilutions of extracts. The first blank disc was impregnated with 100% w/v of the extracts and the other with 80, 40 and 20% w/v, respectively. Plates were incubated for 18 to 24 h, then the zones of inhibition measured and the average of diameters noted.

Antimicrobial effect of *Azadirachta indica* extracts

The antimicrobial activity of *Azadirachta indica* ethanol, acetone, methanol and water extracts against bacterial isolates (*Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) were tabulated using disc diffusion method. Every extract showed appreciable results against all the three selected oral pathogens.

Table 1: Zone of inhibition(average value) of different extracts of *Azadirachta indica* against oral bacteria

S.NO.	Bacteria	Acetone (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
1.	<i>Streptococcus mutans</i>	20	15.5	15	16
2.	<i>Lactobacillus fermentum</i>	18.5	15	13	15.5
3.	<i>Pseudomonas aeruginosa</i>	18	14	15	14.5

Antimicrobial effect of *Pongamia pinnata* extracts

The antimicrobial activity of *Pongamia pinnata* ethanol, acetone, methanol and water extracts against bacterial isolates (*Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) were tabulated using disc diffusion method. Every extract showed appreciable results against all the three selected oral pathogens.

Table 2: Zone of inhibition(average value) of different extracts of *Pongamia pinnata* against oral bacteria

S.NO.	Bacteria	Acetone (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
1.	<i>Streptococcus mutans</i>	18	13.5	12	14
2.	<i>Lactobacillus fermentum</i>	14.5	13	11	10.5
3.	<i>Pseudomonas aeruginosa</i>	14	13	12	9

Antimicrobial effect of *Psidium guajava* extracts

The antimicrobial activity of *Psidium guajava* ethanol, acetone, methanol and water extracts against bacterial isolates (*Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) were tabulated using disc diffusion method. Ethanol extract inhibit the growth of *Pseudomonas aeruginosa* (18mm) and the water extract revealed little activity against *Streptococcus mutans* .

Table 3: Zone of inhibition(average value) of different extracts of *Psidium guajava* against oral bacteria

S.NO.	Bacteria	Acetone (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
1.	<i>Streptococcus mutans</i>	15	5	4	7
2.	<i>Lactobacillus fermentum</i>	12	8	5.5	6.8
3.	<i>Pseudomonas aeruginosa</i>	18	18	5	—

Antimicrobial effect of *Punica granatum* extracts

The antimicrobial activity of *Punica granatum* ethanol, acetone, methanol and water extracts against bacterial isolates (*Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*) were tabulated using disc diffusion method. Every extract showed appreciable results against all the three selected oral pathogens.

Table 4: Zone of inhibition(average value) of different extracts of *Punica granatum* against oral bacteria

S.NO.	Bacteria	Acetone (mm)	Ethanol (mm)	Methanol (mm)	Water (mm)
1.	<i>Streptococcus mutans</i>	10	9.5	4	7
2.	<i>Lactobacillus fermentum</i>	8	9	5	5.4
3.	<i>Pseudomonas aeruginosa</i>	13	12	5	–

Conclusion

From the results mentioned above, it is clear that extracts obtained from *Azadirachta indica* had maximum inhibitory effect against the considered oral pathogens specially against *S. mutans*. Also, the extracts prepared in the solvents other than distilled water had more efficacy. The maximum effect was shown in higher concentrations of plant extracts like 100% and 80% w/v. However distilled water extract had shown better inhibitory effect over the ethanolic extract in case of *Azadirachta indica*. But other plant extracts also showed positive results against the selected oral pathogens. After Neem, the other plant extracts which had maximum effect was Karanj which was followed by guava and pomegranate in its efficacy against dental caries and oral flora. Also, acetone extracts had more antibacterial effect than the methanol extracts. This may be because acetone has better extracting capacity over methanol which may be attributed to the ability to extract the natural antimicrobial compounds such as alkaloids, flavanoids, terpinoids and phenolic compounds from the plant.

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REFERENCES

1. Arima H, Danno G. Isolation of antimicrobial compounds from guava (*Psidium guajava* L.) and their structural elucidation. Biosci Biotechnol Biochem. 2002; 66:1727-1730.
2. Johri. M. Neem: Rightly Called the "Village Pharmacy", IST 2007 15:42:27.
3. Almas K, Ali-Lafi TR. The natural toothbrush. World Health Forum. 1995; 16(2):206-10
4. Parekh J, Karathia N, Chanda S. Screening of some traditionally used medicinal plants for potential antibacterial activity. Indian J Pharm Sci; 2006; 68:832-4
5. Bhavnani SM, Ballow CH. Curr. Opin. Micro- iol., 2000; 3: 528-34
6. Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH. Triterpenoids from the leaves of *Psidium guajava*. Phytochemistry. 2002; 61:399-403.

7. Belemtougri RG, Constantin B, Cognard C, Raymond G, Sawadogo L. Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. *J Zhejiang Univ Sci B*. 2006; 7:56-63.
8. Qadan F, Thewaini AJ, Ali DA, Afifi R, Elkhawad A, Matalaka KZ. The antimicrobial activities of *Psidium guajava* and *Juglans regia* leaf extracts to acne-developing organisms. *Am J Chin Med*. 2005; 33:197-204.
9. A. Abubakar, M. N. Salka, F. B. Hassan, *Asian J. Plant Sci. Res.*, **2011**, 1, 1, 95.
10. A. Palombo, *Adv Access*, **2009**, 10.
11. R.Subapriya, S. Nagini, *Curr Med Chem. Anticancer Agents*, **2005**, 5(2), 149.
12. L. E. Wolinsky, S.Mani, S.Nachnani, S.Lin, *J. Dent.Res*, **1996**, 75, 816.
- 13.K.Almas, *Indian J Dent Res*, **1999**, 10(1), 23.
- 14.G.M. Prashant, G.N.Chandu, K.S. Murulikrishna, M.D.Shafiulla, *Indian J Dent Res*, **2007**, 18(4), 148.
15. A.Vank, S.Tandon, S.R.Rao, N.Udupa, *Ind J Dent Res*, **2001**, 12, 133.
16. S.Chetan, Sonawane, Deepali M. Jagdale, Sushama D. Patil, Leena J. Patil and Vilasrao J. Kadam. *Der Pharmacia Sinica*, **2011**, 2 (5):267-272.
17. John G Holt, Noel R Krieg, Peter H A Sneath, James T Staley, Stanly T Williams, *Streptococcus* and *Fusobacterium*, in *Bergeys manual of determinative bacteriology* edited by William R Henayl, (Lippincott Williams & Wilkins, New York **2000**), 296 & 532.
18. A.Vanka, S.Tandon, S.R. Rao, N. Udupa, P.Ramkumar, *Indian J Dent Res*, **2001**, 12(4), 193.
- 19.Chatterjee A, and Pakrashi S.C. The treaties of Indian Medicinal Plants. New Delhi, India: CSIR, 1991, p190-192.
- 20.Duraipandiyar, V., Ayyanar, M. and S. Ignacimuthu. (2006) Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine*, 6:35 doi:10.1186/1472- 6882-6-35
- 21.Erdogrul, O.T. Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology*. 2002, 40:269-273.
- 22.Hussain, A., Virmani, O.P. and Popli, S.P. Dictionary of Indian Medicinal Plants. Lucknow, India: CIMAP, 1992:384
- 24.Selwitz RH, et al. Dental caries. *The Lancet*. 2007; 369:51.
- 23.Gonzalves W. Oral health. In: South-Paul JE, et al. *Current Diagnosis & Treatment in Family Medicine*. 2nd ed. New York, N.Y.: The McGraw-Hill Companies; 2008.