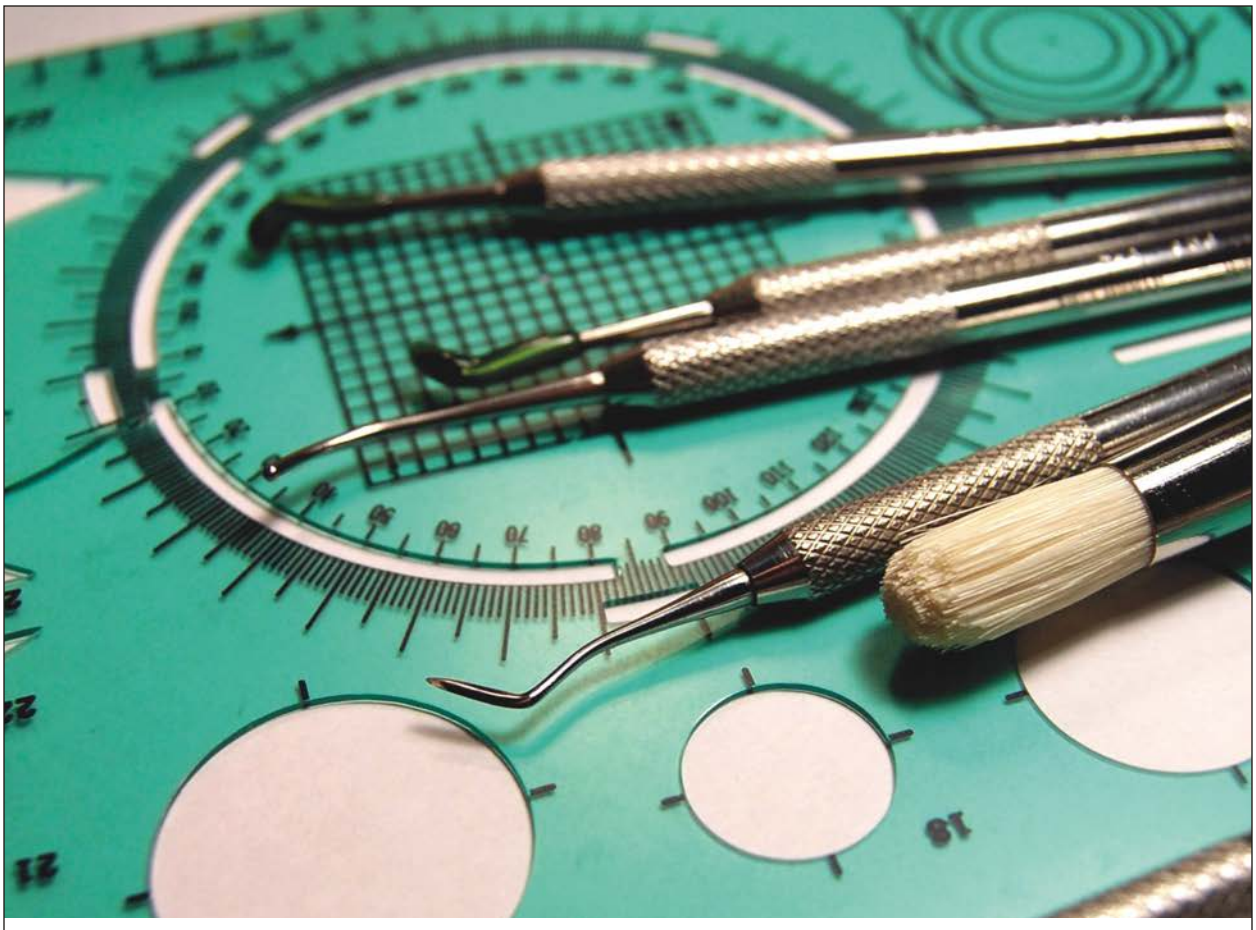

European Journal of Dentistry and Medicine

ISSN 1996-3378

Academic Journals Inc.

www.academicjournals.com

European Journal of Dentistry and Medicine 1 (1): 1-7, 2009
ISSN 1996-3378
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Anticaries Effects of *Baphia nittida* Extract in Albino Rats

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Abstract: The possible curative and preventive effect of *Baphia nittida* extract on insipient caries and caries-free rats were investigated. About 200 mg mL^{-1} of *Baphia nittida* extract were intra-orally administered to the teeth surfaces of different rat groups. Evidence of development of caries was determined by direct observation, probing, microbial count and radiography. The result of the study showed that there was no evidence of dental caries among the non-insipient caries rat groups fed simultaneously with 70% sucrose-in-diet and extract of *Baphia nittida*. Similarly, the rat groups having insipient caries did not exhibit signs of dental caries after the administration of *Baphia nittida* extract. There was a significant difference ($p<0.05$) between the pre and post microbial count of the insipient caries rat groups given *Baphia nittida* extract. Also, there was a significant difference ($p<0.05$) between the pre and post microbial count of the caries-free rat groups given *Baphia nittida* extract twice daily. The result generally suggested that *Baphia nittida* extract has *in vivo* preventive and curative potential on dental caries in rats simultaneously fed with 70% sucrose-in-diet.

Key words: Insipient caries, sucrose-in-diet, extract, plaque, rats, microbial count, teeth

INTRODUCTION

Dental caries is an infectious and communicable diseases which results in the gradual destruction of enamel, dentine and cementum by acid forming bacteria found in dental plaque, in the presence of sugar (Johnson and Beighton, 1992; Ingraham *et al*., 2001). It is one of the most common oral diseases showing marked geographical variation and severity of distribution all over the world (Ettinger, 1999; Talaro and Talaro, 2002). The prevalence is reported to be on the increase as a result of excessive sugar consumption especially in the developing countries (Adegbembo *et al*., 1995; El-Nadeef *et al*., 1998; Patro *et al*., 2008; Declerk *et al*., 2008; Umesi-Koleoso *et al*., 2007). Evidence on the association between amount and frequency of free sugar intake and dental caries has been previously highlighted (Moynihan and Peterson, 2004; Stecksen-Blicks *et al*., 2007; Kolker *et al*., 2007). Sugar free chewing gums has been reported to significantly slow the progression of caries (Morgan *et al*., 2008).

Dental caries is often associated with severe pain and discomfort especially when the pulp chamber is affected. It considerably impacts on self-esteem and quality of life and are expensive to treat. Dental caries ultimately results to loss of tooth minerals, which begins with the outer surface of the tooth and can progress through the dentine, to the pulp, thus culminating to tooth-loss (Johnson and Beighton, 1992; Moynihan and Peterson, 2004).

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Synthetic drugs and antimicrobial chemicals has been reported to be useful in the reduction of dental caries. They probably inhibits the development and maturation of biofilms as well as affect bacterial metabolism (Baehni and Takeuchi, 2003). Most of these drugs, especially the very effective ones, are either not available or are expensive, beyond the reach of the common people in the developing nations. Furthermore, as with the case with most drugs in recent times, these agents and chemicals are becoming increasingly prone to microbial resistance.

The usefulness of traditional medicinal plants for prophylaxis and treatment of ailments and their important role in the developmental progress of the pharmaceutical industry has long been recognized. In Nigeria, many a plant material has been used since ancient days in the treatment of diseases including oral problems. Although the antimicrobial potentials of some of these local plants has been enumerated (Prashant *et al*., 2007; Amadi *et al*., 2007), most are yet to be scientifically studied in the laboratory. The present study investigates the *in vivo* effect of *Baphia nittida*, a local plant used widely in southeastern Nigeria as chewing stick, on dental caries in albino rats.

MATERIALS AND METHODS

Collection of Plant Materials

The leaves and tender stems of *Baphia nittida* (Abosi) were collected from Abakpa Nike, Enugu, Enugu State, Nigeria. The plant was authenticated by a taxonomist of the Department of Applied Biology, Ebonyi State University, Ebonyi State, Nigeria. This study was conducted in 2003.

Extraction

The washed leaves and tender stems of *Baphia nittida* were dried at room temperature. They were then macerated using mechanical grinder (Fondicion, Corona). Forty gram of the ground plant material were introduced into 95% ethanol and allowed to stand for 24 h with constant agitation. They were subsequently filtered using Whatman's No.1 filter paper and evaporated to dryness in a water bath at 40°C (Ibrahim *et al*., 1997).

Experimental Rats

Twenty seven male albino rats were selected from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Only rats showing no sigus of dental caries and whose anterior teeth plaque samples contain *Streptococcus rattus* were included in the selection. The plaque samples were cultured in crystal violet blood agar and identified using standard methods (Chessbrough, 2002; Collins *et al*., 1995).

Curative Effect of *Baphia nittida* on Albino Rats

A total of (12) 8-10 week old male albino rats weighing 62-120 g harboring *Streptococcus rattus* on their teeth surface, without signs of dental caries were used. They were divided into four groups (A-D). To establish base-line data, the upper and lower anterior teeth of the rats were visnally observed and their texture determined using dental probe. Plaque samples from the upper and lower anterior teeth surfaces were also obtained using sterile swab sticks and probe and subsequently cultured in crystal violet blood agar to establish pre-microbial count.

Seventy percent sucrose-in-diet were fed to rat group A and B while group C and D rats were fed with normal diet without sucrose. Rat groups A and B were observed daily for development of white spot lesion. On determination of early signs of dental caries among the two rat groups, administration of *Baphia nittida* extract started. Two hundred milligram per milliliter of *Baphia nittida* extract was intra-orally administered on the anterior teeth of rat group A. However, while rat group A were given normal drinking water (containing 0.1 mg L^{-1} of fluoride), group B were given water containing

0.7 mg L^{-1} of fluoride . At this juncture, rat group D started receiving 70% sucrose-in-diet along with rat groups A and B, while group C rat continued with normal diet without sucrose. Rat group D was observed for the development of early caries signs. On confirmation of early caries on rat group D, the teeth surface of all the rat groups were observed visually and with dental probe. Also, plaque samples were collected from all the rats in all the groups for post microbial count. The radiograph of the lower interior teeth of all the rats were also done.

Preventive Effect of *Baphia nittida* Extract on Albino Rats

A total of (15) 8-10 week old male albino rat weighing 62-120 g harboring *Streptococcus rattus* on their teeth surface and without signs of dental caries were used. They were divided into groups A-E. To obtain baseline data, the upper and lower anterior teeth of the rats were observed visually and their texture determined using dental probe. Plaque samples from the upper and lower anterior teeth surface were also obtained to establish pre-microbial count.

Rat groups A, B, D and E were all fed with 70% sucrose-in-diet while group C were fed with normal diet. While group A rats were given 200 mg mL^{-1} *Baphia nittida* extract intra-orally once daily, group B were given same quantity of extract twice daily. However, whereas all the rat groups were given normal drinking water (containing 0.1 mg L^{-1} of fluoride), group D were given water containing 0.7 mg L^{-1} of fluoride . Group E rats served as control without plant extract or fluoride. The rat groups were fed and observed on daily basis until white spot lesions were observed in the anterior teeth surface of the group E rats.

Upon observation of the early caries sign on the teeth surface of the group E rats, the anterior teeth of all the rats in all the groups were observed visually and with dental probe. Plaque samples were also collected from all the rats to determine the post treatment microbial count. The radiograph of the lower anterior teeth of all the rats were also taken.

Determination of Insipient Caries

The early signal of insipient dental caries were determined by direct observation and probing. The plate count technique was used to determine the microbial count in the dental plaque sample (Ingraham *et al*., 2001). The radiographic examination of the lower anterior teeth of the rats were determined using an x-ray machine (ST-300) with a capacity of 0-150 kV and 50-300 mA at the Radiography Department of Ebonyi State University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria.

Statistical Analysis

The t-distribution was used to analyze the microbial counts obtained in this study.

RESULTS

Curative Effects of *Baphia nittida* Extract

There was no observable difference between the appearance of the anterior teeth surface of the rat groups showing early signs of dental caries and simultaneously given *Baphia nittida* extract and 70% sucrose-in-diet and the control group given normal diet, on visual observation. The result thus indicated that insipient caries signs became visually imperceptible with the administration of *Baphia nittida* extract.

Also, the texture and translucency of the upper and lower anterior teeth surface of the rats were similar to those of the control group given normal diet. However, the anterior teeth surface of the rat group given 0.7 mg L^{-1} of fluoride appeared grayish white and were non-sticky on probing.

There was a significant difference ($p<0.05$) between the pre and post microbial count of the insipient caries rat group simultaneously given 70% sucrose-in-diet and *Baphia nittida* extract. Thus, the microbial count of the rat showing early signs of dental caries drastically reduced upon simultaneous administration of *Baphia nittida* extract and 70% sucrose-in-diet. However, there was no significant difference ($p<0.05$) between the pre and post microbial count in the control groups given normal diet (Table 1).

The radiographic study indicated no signs of radiolucency among the rat groups given *Baphia nittida* extract as well as those of the control groups given normal diet. There was however some evidence of radiolucency among the rat group given 70% sucrose-in-diet without plant extract.

Preventive Effects of *Baphia nittida* Extract

The visual appearance of the upper and lower anterior teeth surface of caries-free rats given simultaneously 70% sucrose-in-diet and *Baphia nittida* extracts were similar to those of the control group given normal diet without sucrose. Similarly, there was no difference in the texture and translucency of the same rat groups given 70% sucrose-in-diet and *Baphia nittida* extract and those of the control group given normal diet. However, the anterior teeth surface of the rats given only 70% sucrose-in-diet had milky-white spots and were sticky on probing after a period of about six weeks. Those of the rat group given 0.7 mg L^{-1} fluoride were however grayish white in color and non sticky on probing.

There was a significant difference between the pre and post microbial count ($p<0.05$) of the caries-free rats simultaneously given 70% sucrose-in-diet and *Baphia nittida* extract twice daily. No significant difference ($p<0.05$) however were observed for the rat groups given the same plant extract once daily as well as the control groups given normal diet (Table 2).

No signs of radiolucency was observed among the rat groups simultaneously given 70% sucrose-in-diet and *Baphia nittida* extract once and twice daily. The same was the case for the control groups given normal diet. There was however, evidence of radiolucency among the rat group given 70% sucrose-in-diet without plant extract or fluoride.

Table 1: Effect of *Baphia nittida* extract and fluoride on the microbial count of albino rats showing early signs of dental caries

	Mean microbial counts ($\times 10^4$ cells mL^{-1})	
Treatment	Before treatment	After treatment
Baphia nittida	90.70±19.00^a	2.05±1.76^b
Fluoride	2930.00±4570^a	11.70±15.80^a
Control A (normal feed)	7.57±6.45^a	4.03±0.55^a
Control B (70% sucrose)	7.07±1.61^a	4.66±1527.53^b

^{ab}Different alphabetical superscript in a row indicate that the means are significantly different at the probability level $p<0.05$, but similar superscripts in a row indicate a lack of significant difference between the means ($p>0.05$)

Table 2: Effect of *Baphia nittida* extract and fluoride on the microbial count of caries free albino rats

	Mean microbial counts ($\times 10^4$ cells mL^{-1})	
Treatment	Before treatment	After treatment
Baphia nittida (once daily)	6.40±3.14^a	15.43±25.61^a
Baphia nittida (twice daily)	3.93±1.01^a	22.67±13.65^b
Fluoride	7.57±6.45^a	4.03±0.55^a
Control A (normal feed)	28.10±36.30^a	1.01±0.96^a
Control B (70% sucrose)	4.83±3.01^a	1966.67±2631.22^b

^{ab}Different alphabetical superscript in a row indicate that the means are significantly different at the probability level $p<0.05$, but similar superscripts in a row indicate a lack of significant difference between the means ($p>0.05$)

DISCUSSION

The result of this study revealed that there was no evidence of early signs of dental caries among the insipient caries rat groups fed simultaneously with 70% sucrose-in-diet and *Baphia nittida* extract. This is suggestive of probable curative potential of the plant extract. This was indicated by the lack of white spot lesion, rough texture and bright light reflection of the anterior teeth surfaces of the rat groups given the plant extract. Further corroboration was obtained by the significant reduction ($p<0.05$) in the microbial count of the rat groups given the plant extract. Reduction in the number of cariogenic bacteria on the teeth surface has been reported to result to decline in the incidence and prevalence of dental caries in a community (Adetunji *et al*., 1996). Previous studies has shown that *Streptococcus mutans* usually increase at the sites that later becomes carious especially in the presence of sucrose diet (Linossier *et al*., 2003).

However, that the microbial count in the rat group given fluoride water (0.7 mg L^{-1}) did not reduce significantly is somewhat surprising, since the anti-caries properties of fluoride has long been established (Marinho *et al*., 2003; Reelick *et al*., 2003; Addai *et al*., 2002; Moynihan and Peterson, 2004). This was quite obvious among the insipient caries rat groups were the mean microbial counts before and after fluoride treatment were drastically reduced and yet statistically insignificant. It is most probable, that the simultaneous administration of 70% sucrose-in-diet and fluoride may have affected the anti-caries activity of the fluoride. The caries inhibitory activity of fluoride containing dentifrices in rats has been previously highlighted (Guggenheim *et al*., 1997).

The insipient-caries curative property of *Baphia nittida* extract was further highlighted by the fact that the radiograph of the rat group simultaneously given 70% sucrose-in-diet and *Baphia nittida* extract were not radiolucent compared with those of the group given only 70% sucrose-in-diet that had evidence of radiolucency.

In the same vein, that no early sign of dental caries were observed among the caries-free rat groups simultaneously given 70% sucrose-in-diet and *Baphia nittida* extract once and twice daily tends to suggest that the plant extract probably posses preventive activity. Unlike the rat group given only 70% sucrose-in-diet where white spot lesion, rough texture and dull light reflection were recorded, the anterior teeth surface of the rats given plant extract were the same with those of the control group given normal diet.

The result of the study further showed that significant difference ($p<0.05$) between the pre and post microbial count was only obtained for caries-free rat group given *Baphia nittida* extract twice daily. Although one may not absolutely assert that twice daily administration of plant extract is essential for the preventive activity of the plant extract, since other criteria including visual observation, probing and radiography did not indicate same, it is not unlikely however, that twice daily administration may have some degree of advantage.

Previous study had indicated the anticaries property of polyphenol compounds from *Camellia sinensis* in *Streptococcus mutans* infected rats (Saito, 1990). Linke and LeGeros (2003) also reported the *in vivo* anticaries properties of black tea extract in hamsters. Further, the inhibitory activity of mango and neem extracts against *Streptococcus mutans*, *S. mitis*, *S. salivarius* and *S. sanguis* has also been highlighted (Prashant *et al*., 2007). It is important to note, that the *in vitro* inhibitory activity of the plant material used in this study against *S. mutans* has been reported (Amadi *et al*., 2007). Further studies to identify and characterize the active ingredients in the medicinal plant used in this study is recommended.

CONCLUSION

It is therefore most probable that the extract of *Baphia nittida* posses some degree of preventive and curative potential against dental caries in albino rat. Further investigation of this plant material is hereby suggested.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Okeke Clement, of the Department of Botany, Nnamdi Azikiwe University, Awka and The Association for Scientific, Identification, Conservation and Utilization of Medicinal Plants of Nigeria (ASICUMPON) Enugu, Nigeria, for their inestimable assistance in the accomplishment of this study.

REFERENCES

Addai, F.K., I.K. Nuamah and G.E. Parkins, 2002. Brief chewing of *Garcinia manii* after a glucose rinse. Med. Sci. Monit., 8: 746-750.

Adegbembo, A.O., M.A. El-Nadeef and A. Adeyinka, 1995. National survey of dental caries status and treatment needs in Nigeria. Int. Dent. J., 45: 35-44.

Adetunji, O.F., B.O. Akinshipe, E.O. Ogunbodede and C.O. Ijaware, 1996. Bacteriological studies of dental caries in Ile-Ife, Nigeria. Cent. Afr. J. Med., 42: 249-252.

Amadi, E.S., A. Oyeka, R.A. Onyeagba, I. Okoli and O.C. Ugbogu, 2007. Studies on the antimicrobial effects of *Spondias mombin* and *Baphia nittida* on Dental caries organism. Pak. J. Biol. Sci., 10: 393-397.

Baehni, P.C. and Y. Takeuchi, 2003. Antiplaque agent in the prevention of biofilm-associated oral disease. Oral. Dis., 9: 23-29.

Chessbrough, M., 2002. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, Cambridge, UK., ISBN 0-521-66546-9, pp: 157-165.

Collins, C.H., P.M. Lyne and J.M. Grange, 1995. Microbiological Methods. 7th Edn. Butterworth-Heinemann Ltd., Oxford, UK., ISBN:0-7506-0653-3 pp: 360-366.

Declerk, D., R. Leroy, L. martens, E. Lesaffre, M.J. Garcia-Zattera, S. vanden Brouk, M. Debyser and K. Hopenbrouwer, 2008. Factors associated with prevalence and severity of caries experience in pre school children. Community Dent. Oral Epidemiol., 36: 168-178.

El-Nadeef, M.A., A.O. Adegbembo and E. Houkala, 1998. The association of urbanization with the prevalence of dental caries among school children in Nigeria New Capital Territory. Int. Dent. J., 48: 44-49.

Ettinger, R.L., 1999. Epidemiology of dental caries: A broad review. Dent. Clin. North. Am., 43: 679-694.

Guggenheim, B., F. Lutz and R. Schmid, 1997. Caries and plaque inhibition in rats by five topically applied dentifrices. Eur. J. Oral Sci., 105: 258-263.

Ibrahim, M.B., M.O. Owonubi and J.A. Onaolapo, 1997. Antibacterial effects of extracts of leaf, stem and root bark of *Anogiessus leiocarpus* on *Staphylococcus aureus* NCTC 6571, *Streptococcus pyogenes* NCTC 8198, *Escherichia coli* NCTC 10418 and *Proteus vulgaris* NCTC 4636. J. Pharm. Res. Dev., 2: 20-26.

Ingraham, T.L., C.A. Ingraham and H. Prentiss, 2001. Introduction to Microbiology. Wadsworths Publishing, USA., ISBN: 0-534-16728-4, pp: 558-559.

Johnson, N.W. and D. Beighton, 1992. Dental Caries. In: Oral Diseases in the Tropics. Prabhu, S.R., D.F. Wilson, D.K. Daftary and N.W. Johnson (Eds.). Oxford University Press, Oxford, London, ISBN: 0192620088, pp: 553-581.

Kolker, J.L., Y. Yuan, B.A. Burt, A.M. Sandretto, W. Sohn, S.W. Lang and A.L. Ismail, 2007. Dental caries and dietary patterns in low income African American children. Pediatr. Dent., 29: 457-464.

Linke, H.A. and R.Z. LeGeros, 2003. Black tea extract and dental caries formation in hamsters. Int. J. Food Sci. Nutr., 54: 89-95.

Linossier, A., A. Vargas, G. Zillmann, M. Arriagada, R. Rojas and R. Villegas, 2003. *Streptococcus mutans*- a semi-qualitative method to assess the risk to oral infection in preschool Chilean children. Rev. Med. Chil., 131: 412-418.

Marinho, V.C., J.P. Higgins, S. Logan and A. Sheiham, 2003. Fluoride mouth-rinse for preventing dental caries in children and adolescents. Cochran Database Syst. Rev.,

Morgan, M.V., G.G. Adams, D.L. Bailey, C.E. Tsao, S.L. Fischman and E.C. Reynolds, 2008. The anticaries effect of sugar- free gum containing CPP-ACP nanocomplexes on approximal caries determined using digital bitewing radiograph. Caries Res., 42: 171-184.

Moynihan, P. and P.E. Peterson, 2004. Diet, Nutrition and the prevalence of dental disease. Pubicl. Health Nutr., 7: 201-206.

Patro, B.K., B. Ravi Kumar, A. Goswami, V.P. Mathur and B. Nongkynrih, 2008. Prevalence of dental caries among adult and elderly in an urban resettlement colony of New Delhi. Indian J. Dent. Res., 19: 95-98.

Prashant, G.M., G.N. Chandu, K.S. Murulikrishna and M.D. Shafiulla, 2007. The effect of mango and neem extract on four organisms causing dental caries: *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis* and *Streptococcus sanguis*: An *in vitro* study. Indian J. Dent. Res., 18: 148-151.

Reelick, N.F., M. Guldenmundt and J. Bleeker, 2003. The effectiveness of a school-based fluoride rinsing program for dental risk groups. Ned. Tijdschr. Tandheelkd., 110: 276-280.

Saito, N., 1990. Anticaries effects of polyphenol compounds from *Camallia sinensis*. Nichidai. Koko Kagaku., 16: 154-163.

Stecksen-Blicks, C., P.L. Holgerson and S. Twetman, 2007. Caries risk orofile in two-year old children from Northern Sweden. Oral Health Prev. Dent., 5: 215-221.

Talaro, K.P. and A. Talaro, 2002. Foundations in Microbiology. 4th Edn. McGraw Hill Comp. Inc., New York, pp: 654-658. ISBN: 0-07-112275-3.

Umesi-Koleoso, D.C., P.O. Ayanbadejo and O.A. Oremosu, 2007. Dental caries trend among adolescents in Lagos South West Nigeria. West Afr. J. Med., 26: 201-205.