



## Antibacterial efficacy of commercially available activated carbon tooth powder: An *in vitro* study

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### ABSTRACT

There has been an indiscriminate use of commercial antimicrobials in the previous decades, leading to emergence of multidrug resistant bacteria. This has become a frequent occurrence, so natural antimicrobial agents have grabbed attention of researchers as potential alternatives. Of particular interest is cow dung which has been shown to have antibacterial and antiseptic properties. It is known to be used predominantly in rural areas. This paper aims to evaluate the antibacterial activity of *Goshala* Activated Carbon tooth powder against three strains of periodontopathogenic and cariogenic bacteria. Standardized strains of *Streptococcus mutans*, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were cultured in BHI (Brain Heart Infusion Broth) media and Minimum Inhibitory Concentration (MIC) of Activated Carbon tooth powder was assessed by serial dilution method. Likewise, Amoxicillin, Metronidazole and Chlorhexidine were tested against the same pathogens. *Porphyromonas gingivalis*, *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* were shown to be sensitive at an MIC of 50mg/ml, 25mg/ml and 0.8mg/ml for activated carbon tooth powder respectively. Given some limitations of this study, we can conclude that activated carbon powder presents a ray of hope in developing a targeted agent for aggressive periodontitis patients.



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### INTRODUCTION

Periodontal diseases and dental caries are two of the most prevalent globally prevalent diseases of the oral cavity affecting a large segment of the popu-

lation. Periodontitis and caries are infectious diseases of the oral cavity in which oral biofilms play a causative role. The presence of microorganisms in the oral cavity, their virulence and the host response decide the occurrence of a particular disease. Periodontitis is a chronic inflammatory disease of the tooth supporting structures resulting in alveolar bone and connective tissue loss and occurs due to a combination of various host, environmental and bacterial factors (Zambon, 1996). The phenomenon of initiation and progression of periodontal diseases are closely related to the presence of pathogenic bacteria in the subgingival biofilm.

The biofilm on tooth surfaces is also associated with dental caries. Dental caries is a multifactorial microbial infectious disease which is characterized by demineralization of the inorganic and destruc-

tion of the organic substance of the tooth. (Loesche, 1986) Host factors, substrates, microbes and time are the factors involved in the etiology of caries as represented by the Key's ring. There are more than 700 different bacterial species which colonize the oral cavity, but only a few of these are thought to be potential periodontal pathogens (Aas et al., 2005). *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* are known as the major pathogens of periodontal disease. Successful treatment of periodontal disease is associated with ensuring removal of and reducing these microorganisms (Holt and Ebersole, 2000). Likewise, *Lactobacillus*, *Streptococcus mutans* and *Actinomyces* are associated with dental caries.

For the past few decades, periodontitis has been treated by the removal of plaque biofilm and calculus from both the supragingival and subgingival surfaces by scaling and root planing. This modality aims at removing the whole of the biofilm rather than eliminating specific periopathogens (Drisko, 2014). Mechanical plaque control is also the primary modality in prevention of dental caries. Some patients cannot make effective use of mechanical plaque control and such patients could explore additional benefits from chemotherapeutic antiplaque agents (Haffajee et al., 1995). One group of chemical plaque agents, the antiplaque agents can decrease the rate of new plaque accumulation, decrease or remove existing plaque, suppress the growth of pathogenic microflora, or inhibit the production of virulence factors (Patil et al., 2016).

There has been an indiscriminate use of commercial antimicrobials in the previous decades, leading to emergence of multidrug resistant bacteria. This has become a frequent occurrence, so natural antimicrobial agents have grabbed attention of researchers as potential alternatives (Siddiqui, 1993). Now, herbal products have flooded the market even for oral health care. There are herbal toothpastes, activated charcoal toothpowders, essential oil infused oil-pulling melts and wooden toothbrushes with natural bristles. Folkloric medicine is a branch of medicine dealing with the traditional knowledge that developed over centuries which has been used for treatment of illnesses long before modern medicine. Traditional healers have used different parts of plants, natural stones, animal fat, oil and wastes in treatment of different diseases with great success. *Meswak* stick, *babool* stick, neem stick as toothbrushes and brick powder, ash and dried burnt cow dung powder as dentifrices have been in use since time immemorial (Ahmad et al., 2006).

Of particular interest is cow dung which has been shown to have antibacterial and antiseptic properties (Waziri and Suleiman, 2012). Cow dung as a slurry is spread all over the earthy floor inside and outside the house. This is known to keep pests away, lower the ambient temperature and provide insulation as well. It is also used as fuel in wooden fires. Dried burnt cow dung is used as a toothpowder. Herbal and natural products, therefore, need to be tested for their effectiveness and safety and researched upon further.

Thus, this *in-vitro* study was carried out to determine the efficacy of a commercially available activated carbon toothpowder against known oral pathogens as aim.

## MATERIALS AND METHODS

The study employed an *in vitro* experimental design. Ethical clearance to conduct the study was obtained from the Institutional Ethics Committee of SRM Kattankulathur Dental College and Hospital, Potheri (Ref No:1507/IEC/2018). Three standardized strains of well-known pathogenic bacteria were obtained from Department of Microbiology, SRM Kattankulathur Medical College and Hospital, Potheri. *Streptococcus mutans*, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were used in this study. Based on Socransky's groundbreaking research, one bacterium each from the yellow, red and green complex were selected which were positively implicated as etiologic agents of various oral pathologic conditions (Socransky and Haffajee, 1992). Namely, *Streptococcus mutans* in dental caries, *Porphyromonas gingivalis* in chronic periodontitis and *Aggregatibacter actinomycetemcomitans* in localized aggressive periodontitis.

The main material of interest is *Goshala*<sup>TM</sup> Active carbon toothpowder (Mfd by Gaunaturals, Machilipatnam, Andhra Pradesh) which is commercially available (Figure 1). Each 15g of this toothpowder is primarily composed of *Gomaya Bhasma* (12g). Other additives like camphor 1.4g (*karpooram*), sea salt 1.2g (*saindhava lavana*) and carom seeds 1.2g (*ajwain*) are present. Amoxicillin, Metronidazole and Chlorhexidine were used as positive controls.

Serial dilution was done for each drug from 100mg/ml up to 0.2mg/ml. Nine dilutions of the drug were done with Brain Heart Infusion broth for Minimum inhibitory concentration. In the initial tube 20  $\mu$ l of drug was added to 380  $\mu$ l of BHI broth. 20  $\mu$ l of BHI broth was added into next nine tubes. From the initial tube 200  $\mu$ l was transferred to the next one giving us 2-1 dilution. This serial

**Table 1: Effect of Various Antibacterial agents against *Porphyromonas Gingivalis***

Sample concentration in mg/ml	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Amoxicillin	S	S	S	S	S	S	S	S	R	R
Metronidazole	S	S	S	S	S	S	S	S	S	R
Chlorhexidine	S	S	S	S	S	S	S	S	S	R
Goshala	S	S	R	R	R	R	R	R	R	R

*Porphyromonas gingivalis* is susceptible to Amoxicillin at 0.8mg/ml, Metronidazole at 0.4mg/ml, Chlorhexidine at 0.4mg/ml and Goshala™ active carbon toothpowder at 50mg/ml. S = Sensitive and R = Resistant

**Table 2: Effect of various Antibacterial agents against *Streptococcus Mutans***

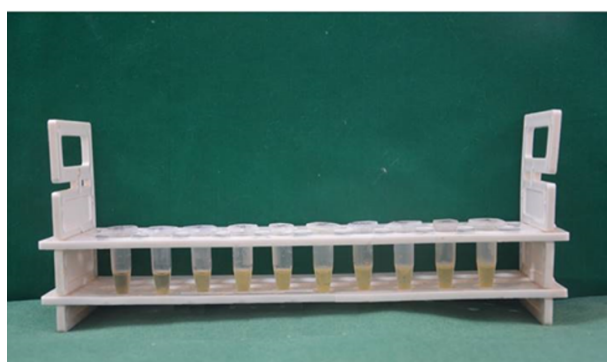
Sample concentration in mg/ml	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Amoxicillin	S	S	S	S	S	S	S	S	S	S
Metronidazole	S	S	S	S	S	R	R	R	R	R
Chlorhexidine	S	S	S	S	S	S	S	R	R	R
Goshala	S	S	S	R	R	R	R	R	R	R

*Streptococcus mutans* is susceptible to Amoxicillin at 0.2mg/ml, Metronidazole at 6.25mg/ml, Chlorhexidine at 1.6mg/ml and Goshala™ at 25mg/ml. S = Sensitive and R = Resistant

**Table 3: Effect of various antibacterial agents against *Aggregatibacter Actinomycetemcomitans***

Sample concentration in mg/ml	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Amoxicillin	S	S	S	S	S	S	S	S	R	R
Metronidazole	S	S	S	S	S	S	S	S	R	R
Chlorhexidine	S	S	S	S	S	S	S	R	R	R
Goshala	S	S	S	S	S	S	S	S	R	R

*Aggregatibacter actinomycetemcomitans* is susceptible to Amoxicillin at 0.8mg/ml, Metronidazole at 0.8mg/ml, Chlorhexidine at 1.6mg/ml and Goshala™ active carbon toothpowder at 0.8mg/ml. S = Sensitive and R = Resistant

**Figure 1: Goshala™ active carbon tooth powder****Figure 2: Serial dilution of the antibacterial agent with bacterial culture Concentrations from 0.2mg/ml to 100mg/ml are cultured with bacterial isolate and observed for turbidity**

dilution was repeated for each microorganism. From the maintained stock cultures of the required organisms, five  $\mu\text{l}$  was taken and added into two ml of BHI broth. In each tube of serially diluted drug, 200  $\mu\text{l}$  of culture suspension was added (Figure 2).

Facultative anaerobes were incubated at 37°C for 48-72 hours in CO<sub>2</sub> jar. Obligate anaerobes were incubated in anaerobic jars for 48-72 hours. Tubes were incubated for 24 hours and observed for turbidity (Wheat, 2001; King, 2002). The culture was adjusted with broth to give a turbidity equivalent to the McFarland 0.5 standard. This was done by photometrical analysis by determining OD<sub>450</sub> (Escmid, 2000; Schwalbe et al., 2007).

## RESULTS AND DISCUSSION

Bacterial inhibition displayed by *Goshala*<sup>TM</sup> toothpowder (at different concentrations) and controls against *P. gingivalis*, *S. mutans* and *A. actinomycetemcomitans*, are depicted in Tables 1, 2 and 3.

*Porphyromonas gingivalis* is susceptible to Amoxicillin at 0.8mg/ml, Metronidazole at 0.4mg/ml, Chlorhexidine at 0.4mg/ml and *Goshala*<sup>TM</sup> active carbon toothpowder at 50mg/ml (Table 1). *Streptococcus mutans* is susceptible to Amoxicillin at 0.2mg/ml, Metronidazole at 6.25mg/ml, Chlorhexidine at 1.6mg/ml and *Goshala*<sup>TM</sup> at 25mg/ml (Table 2). *Aggregatibacter actinomycetemcomitans* is susceptible to Amoxicillin at 0.8mg/ml, Metronidazole at 0.8mg/ml, Chlorhexidine at 1.6mg/ml and *Goshala*<sup>TM</sup> active carbon toothpowder at 0.8mg/ml (Table 3).

With due consideration to available evidence pertaining to side effects and emergence of uncommon infections with the usage of synthetic antimicrobial agents, including tetracyclines, this study was conducted in the quest of identifying a possible alternative or an “adjunct” in the treatment of aggressive periodontitis. Several plant products such as *tulsi*, neem, lemon, and others have been tested for their antimicrobial properties in the past with considerable success. Resistance to currently used chemotherapeutics is the major factor that necessitates the search for alternative safe, efficacious and cost-effective treatment options, particularly in developing countries (Hussain et al., 2009).

In this study, we attempted to obtain information on the antimicrobial efficacy of activated tooth powder, particularly against three oral pathogens namely *A. actinomycetemcomitans*, *Streptococcus mutans* and *P. gingivalis*; as these microbes are implicated in initiation and progression of various oral diseases, especially aggressive periodontitis and dental caries. Results in this *in vitro* experiment showed that *Goshala*<sup>TM</sup> activated carbon toothpowder at a concentration of 0.8mg/ml can effectively inhibit the growth of *A. actinomycetemcomitans*, comparable to that of Amoxicillin and Metronidazole, and

better than Chlorhexidine which had an MIC of 1.6 mg/ml. We can infer from this that the active carbon toothpowder is effective at half the concentration of Chlorhexidine, which is the gold standard of chemical plaque control.

The exact mechanism of action of activated carbon/charcoal needs to be better understood which is said to be a combination of physical and chemical processes (Yamamoto et al., 2002; Karnib et al., 2013). Physical process which could include separating the bacteria from the microenvironment and damaging the cell wall and membranes and chemical process being generation of Reactive Oxygen Species (ROS)-dependent and ROS-independent oxidative stress. Other possible explanations are inhibition of adsorption by altering the surface free energy of the system and changes in pore size distribution. This inhibits biofilm growth (Akasaka and Watari, 2009; Shi et al., 2007).

These results could shed some light on new approaches to manage aggressive forms of periodontitis especially as an antibacterial agent in dentifrices. Everyone receiving treatment for periodontal disease might not respond to mechanical debridement alone. Some have postulated the reason to be the fact that some periopathogens including *P.gingivalis* and *A. actinomycetemcomitans* invade the host gingival tissues and thus evade elimination by mechanical debridement. Recurrence of disease is caused by these hidden pathogens which act as the source for recolonization of the periodontal pocket. The adjuvant therapy by use of local drug delivery has been suggested for such microorganisms (Etienne, 2003).

Mechanical plaque control is achieved by the use of cleaning aids such as manual and electric toothbrushes, interdental brushes, dental floss, oral irrigators etc. Chemical plaque control agents are available in various delivery formats like toothpastes, gels, mouth rinses, varnishes, lozenges and sprays to name a few. The use of dentifrices plays a major role in plaque control as it is the most commonly employed chemical aid (Stuart, 1997). Nearly 50% of the population in semirural and rural areas are known to use toothpastes and many of them do not use toothbrushes (Punitha and Sivaprakasam, 2011).

Various local drug delivery agents have been tested and found to be successful adjuncts to scaling and root planning. They play a supportive rather than primary role in management of periodontal disease. In this therapy, the antimicrobial agent is placed within the periodontal pocket by means of a carrier medium and sustained release in a local area

over a period of time is seen. Various antimicrobial agents have been used successfully for this purpose. Some include chlorhexidine, tetracycline and metronidazole to name a few (Matesanz-Pérez *et al.*, 2013; Divya and Nandakumar, 2006).

With research nowadays trending towards natural substances, herbal extracts of neem, turmeric, aloe vera and *tulsi*, among others, have been adapted into toothpaste, gel as well sustained release chip form. The current study focuses on a natural material obtained quite easily and with known antibacterial action. So it follows logically that activated carbon be used as a local drug delivery agent or chemical plaque control agent. This could potentially be a veritable gold mine of a material. The challenge lies in translating this knowledge into clinical application. Before trying to use this substance in interventional therapy, several factors need to be taken into account. The first being biocompatibility. It is necessary to conduct studies that determine what kind of, if any, reaction is elicited by the cells and tissues of a human. Other properties of the agent in question, such as abrasiveness, pH, solubility, pharmacokinetics and pharmacodynamics including biocompatibility, as well as identification of the active compound(s) have to be ascertained.

## CONCLUSIONS

This study is the first step towards developing an adjunctive antibacterial agent which is effective, natural, easily available, local, environmentally sustainable and cheap among other qualities. It is safe to say that while additional studies are required before a randomised human clinical trial can be performed, activated carbon powder presents a ray of hope in developing a targeted agent for aggressive periodontitis patients.

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## Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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