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Anticariogenic and anti-inflammatory activity of coffee bean powder ethanolic extract

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Abstract:
Introduction: Coffee bean powder has a rich source of natural antioxidant and anti-inflammatory properties due to the presence of chlorogenic and phenolic acid components. The chlorogenic and phenolic acids along with other aromatic compounds are presented to have antibacterial effects against pathogenic microorganisms thereby preventing the invasion of dental caries over the tooth surface. The anticariogenic agents in coffee bean powder were found to be effective against s.mutans and e.faecalis. Inflammation is well understood as a critical biological response protecting the host's body from pathogens to maintain tissue homeostasis. Hence, the current study aims to evaluate the anticariogenic and anti-inflammatory activity of coffee bean powder ethanolic extract.
Materials and methods: 40 mg of coffee bean powder was diluted in 25ml of ethanolic solution and was boiled for 20 minutes to produce 20ml of coffee bean powder ethanolic extract. Anticariogenic activity was carried against the strains of s.mutans, e.faecalis, s.aureus and c.albicans by disc diffusion method in MHA agar and the zone of inhibition was determined. Anti-inflammatory activity was achieved by using Muzushima and Kabayashi method of test with specific alteration and the reagent was bovine serum albumin with diclofenac sodium as standard.
Results and discussion: The zone of inhibition of s.mutans in 100µL of coffee bean powder ethanolic extract was 14mm. The zone of inhibition of c.albicans in 100µLwas 13mm which is higher than the standard antibody. The effectiveness inhibition of bacteria is found to increase with increase in the concentration of the extract. Similarly, anti-inflammatory activity observed a value of 84.8% in 50µL concentration which is higher than the standard diclofenac sodium compound.
Conclusion: Hence the current study concludes that coffee bean powder ethanolic extract can be used as a potent anti-cariogenic and anti-inflammatory agent against dental caries and tissue injuries.

Keywords: Innovative synthesis, green chemical, nanoparticles, Anticariogenic activity

Introduction:

The coffee tree is a dicotyledon seeded tree that belongs to the family *Rubiaceae* which has over 100 species of coffee, with the three main common types namely Arabica, Robusta, and Liberica. The coffee fruit appears yellow and red when matured, and the green bean (1.0~1.5 cm in length and 0.6~0.7 cm in width) usually consists of two hemicycles that face each other (Thiagamani *et al.*, 2017). Coffee bean powder has a rich source of novel phenolic antioxidants such as chlorogenic acids. This chlorogenic acid in coffee bean powder benefits cardiovascular health, diabetes, neuroprotection, hypertension and metabolic syndromes(Bagchi, Moriyama and Swaroop, 2016). The coffee bean powder has an stimulating effect on the cardiovascular, endocrine and central nervous system (Durazzo and Lucarini, 2019). Plant derived extract substances are found to have bacterial resistant antimicrobial activity. Coffee bean extract has got special attention due to its antimicrobial activity against gram positive and gram negative bacteria. The chlorogenic acid, phenol and other aromatic compounds are represented to have antibacterial effects against pathogenic microorganisms (Bakkir, 2017)(Pane *et al.*, 2012). Dental caries is one of the most prevalent diseases of people that is present in the tooth region. Caries are formed due to consumption of sugar and fermentable carbohydrates. Caries are also caused due to less production of saliva and tooth position (Selwitz, Ismail and Pitts, 2007)(Pitts *et al.*, 2017). The pathological and protective factors play an important role in the initiation and progression of caries (Marthaler, 2004). The anticariogenic agent in coffee bean powder was found to be effective against the bacteria *streptococcus mutans*, caries producing microorganisms (Ferrazzano *et al.*, 2011). Inflammation is referred to as a critical biological response of protecting the host's body from pathogens that helps to maintain tissue homeostasis. In the process of inflammation, macrophage plays a central role in through its ability to produce pro-inflammatory mediators, such as nitric oxide (NO), cytokines, and chemokines. Coffee is a multifaceted mixture of various bioactive compounds comprising anti-inflammatory properties. However, the mechanisms by which coffee wields anti-inflammatory effects remains indistinct and the active ingredients has not yet been identified (Lee *et al.*, 2020). Pyrocatechol, a degradation product, which is a derivative of chlorogenic

acid during roasting, as the active ingredient that presents the anti-inflammatory activity in coffee (Funakoshi-Tago *et al.*, 2020) .

The anti-cariogenic activity mainly focuses on the causative agents namely *streptococcus mutans, candida albicans, enterococcus faecalis* and *staphylococcus aureus (Ferrazzano et al., 2009)*. *Streptococcus mutans* are known as the primary causative factor of dental caries in humans since they are isolated from the dental plaque and are referred to as cariogenic oral bacteria (Brandão *et al.*, 2007). Firm adhesion of *streptococcus mutans* occurs when the oral bacteria that is present on the tooth surface start synthesizing sucrose dependent glucosyltransferase activity (Yadav *et al.*, 2017). This activity helps in forming an insoluble glucan layer that provides adhesion capability to the bacteria (Chung *et al.*, 2006). The known antibiotics that are effective against *Streptococcus spp* are penicillin, amphicillin, erythromycin, vancomycin and chlorhexidine which helps in preventing occurrence of dental caries (Hwang, Shim and Chung, 2004). Conversely, immense use of chemical compounds may affect the intestinal and oral flora that can cause side effects like tooth staining, diarrhoea and vomiting (Choi *et al.*, 2007). Correspondingly, *Enterococcus faecalis* is attained to be occasionally isolated from primary endodontic infection which are chiefly produced due to treatment failure . It is non-sporing, facultatively anaerobic gram-positive coccus that is ovoid in shape with a diameter of 0.5-1mm (Rôças, Siqueira and Santos, 2004). This bacterium is acquired from diverse environments, likely the gastrointestinal tract of humans, and in other mammals like reptiles, birds, insects and so on. In association with oral cavity, it is predominantly isolated from marginal periodontitis, periradicular abscesses and infected root canals (Siqueira *et al.*, 2002).

Akin to these bacteria's, fungal organisms like *Candida albicans* are also present within the vicinity of the oral cavity that can cause fatal systemic infections (Akdeniz *et al.*, 2002). This fungus is recurrently isolated from the human mouth, however, only few carriers acquire clinical signs of candidiasis(Devi, Subathra Devi and Gnanavel, 2014)(Gupta, Ariga and Deogade, 2018)(Saravanan *et al.*, 2018)(Needhidasan, Samuel and Chidambaram, 2014). This reflects the capacity of the yeast to colonize various oral surfaces that predispose to other subsequent conditions of the host (Cannon *et al.*, 1995). *Candida* adheres to complement receptors, specific sugar deposits over surfaces, extracellular matrix proteins within the oral cavity. Thus, oral candidiasis marks the growth and penetration of the fungi in the oral cavity of undermined physical and immunological defences of the host's system (Salvatori *et al.*, 2016). The incidence of *Staphylococcus aureus* in the oral cavity is observed for the contribution of periodontal infections in recent studies. The *s.aureus* are found to be associated with conditions like angular cheilitis, suppurative parotitis, dentoalveolar infections and denture stomatitis (Smith *et al.*, 2003). It is also noticed in immunocompromising systemic conditions like rheumatoid arthritis (Koukos *et al.*, 2015). Similar to anticariogenic activity, anti-inflammatory activity is also known to comprise various productive factors since inflammation includes a complex series of protective and reparative mechanisms in response to any tissue injury, autoimmune condition or infections (Schinella *et al.*, 2002). Hence, the current study evaluates the anticariogenic and anti-inflammatory activity of coffee bean powder ethanolic extract.

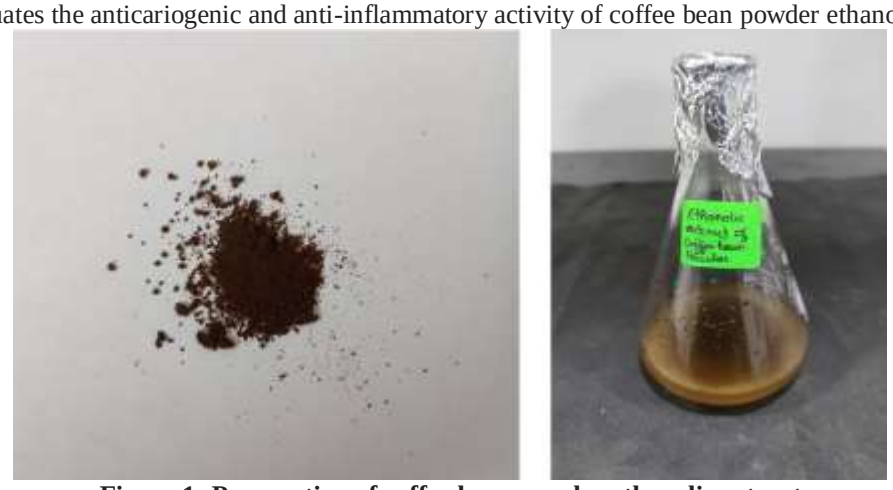

Figure 1: Preparation of coffee bean powder ethanolic extract

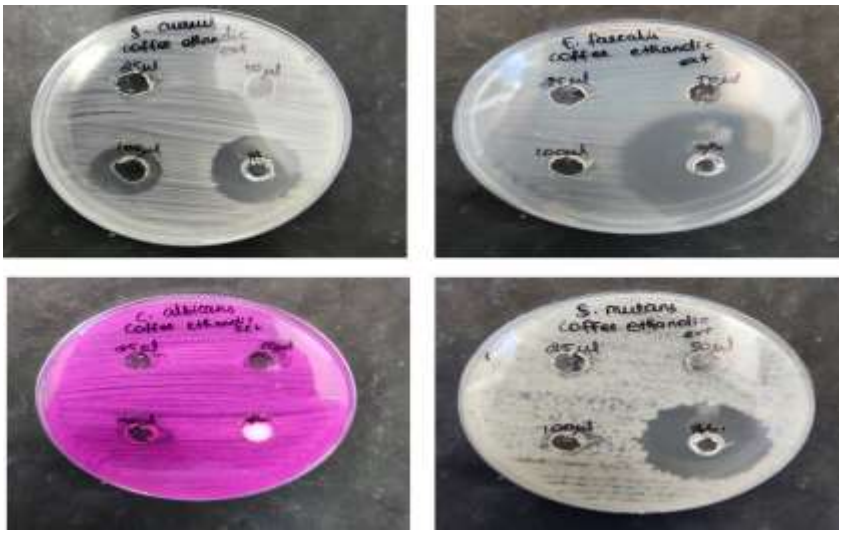

Figure 2: Anticariogenic activity of coffee bean powder ethanolic extract against strains of s.aureus, e.faecalis, c.albicans and s.mutans

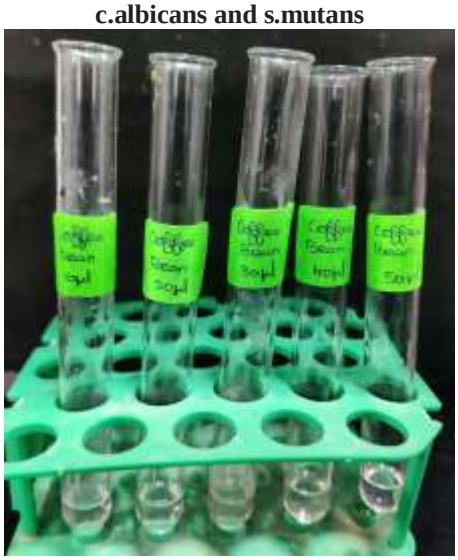

Figure 3: Anti-inflammatory activity of coffee bean powder ethanolic extract after incubation for 20 minutes at 55º C

Materials and methods:

Coffee bean powder was purchased commercially. 40mg of coffee bean powder was diluted in 25ml of ethanolic solution. Filtration was done and coffee bean powder ethanolic solution was obtained. The solution was boiled for 20 minutes and 20ml of coffee bean powder ethanolic extract was produced.

Anticariogenic activity:

Anticariogenic activity of coffee bean powder ethanolic extract against the strains of staphylococcus aureus, enterococcus faecalis, streptococcus mutans and candida albicans was evaluated. MHA agar was utilised for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. The media was poured into the sterilized plates and left stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The coffee bean powder ethanolic extract with different concentrations were loaded and the plates were incubated for 24 hours at 37° C. After the incubation time, the zone of inhibition was measured.

Anti-inflammatory activity:

The anti-inflammatory activity for coffee bean powder ethanolic extract was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations. 0.05ml of coffee bean powder ethanolic extract of various fixation (10µL,20µL,30µL,40µL and 50µL) was added to 0.45mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 minutes and then heated at 55° C in a water bath for 30 minutes. The samples were cooled and the absorbance was estimated spectrophotometrically at 660nm. Diclofenac sodium was used as the standard. DMSO is utilized as a control.

Results and Discussion:

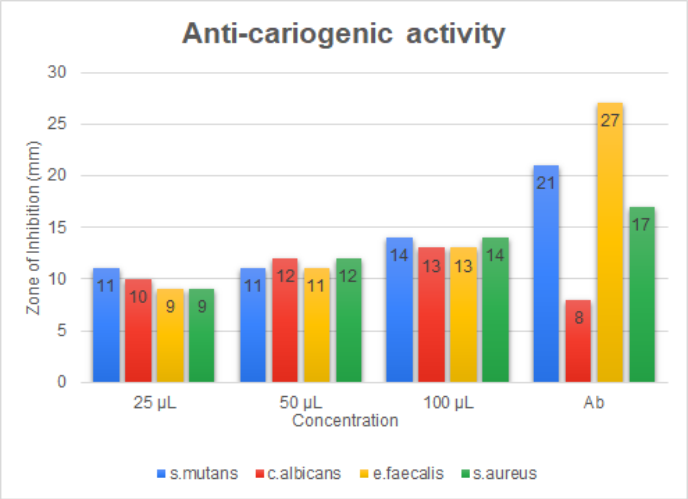

Figure 4: The graph represents the anticariogenic activity of coffee bean powder ethanolic extract. X-axis indicates the concentration of nanoparticles and Y axis is zone of inhibition

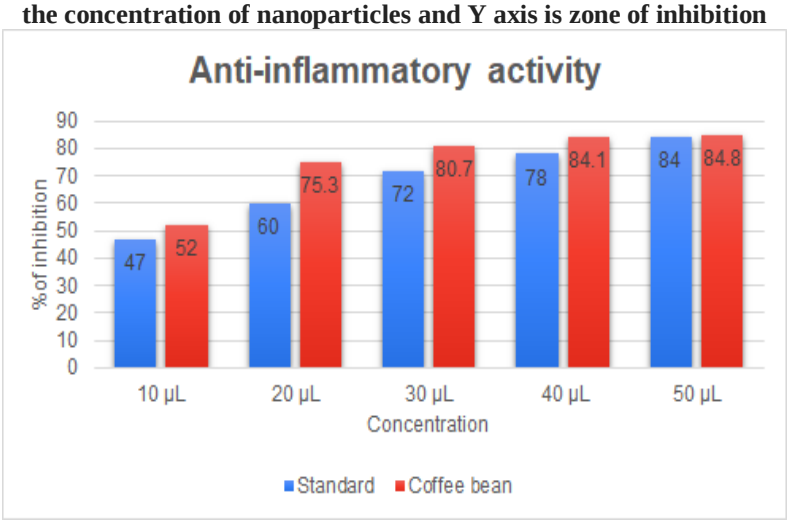

Figure 5: The graph represents the anti-inflammatory activity of coffee bean powder ethanolic extract.X-axis indicates the concentration of nanoparticles and Y axis is percentage of inhibition

Anticariogenic activity of coffee bean powder ethanolic extract:

Figure 4 presents the anticariogenic activity of coffee bean powder ethanolic extract. The zone of inhibition of streptococcus mutans for each value of the extract are 11mm in 25µL and 50µL, 14mm in 100µL. The standard value of the zone of inhibition of the antibody was 21mm which is significantly higher than the test sample values.This was supported by a previous study proclaiming that coffee bean extract was equally effective to chlorhexidine mouthwash against strains of s.mutans (Gowtham *et al.*, 2020). The zone of inhibition of Candida albicans showed 10mm and 12mm of inhibition in 25µL and 50µL. In 100µL, it presented an inhibition of 13mm. The zone of inhibition of the test sample is found to be greater than the standard since the standard measured a zone of inhibition of 8mm. This was aided by a study asserting that the ethanolic extract of coffea arabica produced an inhibitory effectiveness over the growth of candida albicans and the inhibitor zone was increased with an increase in the concentration of the extract (Rakatama, Pramono and Yulianti, 2018). The highest zone of inhibition of coffee bean ethanolic extract against strain of e.faecalis observed in 100µL concentration. When compared with the standard, the standard showed greater value of inhibition than the test sample. Similarly, the highest zone of inhibition observed against s.aureus was 14mm in 100µL concentration of the test sample which is lesser than the standard that has 17mm zone of inhibition.

Anti-inflammatory activity of coffee bean powder ethanolic extract:

The anti-inflammatory activity of coffee bean powder ethanolic extract was indicated in figure 5. The percentage of inhibition of the extract was found to be increasing gradually with respect to increase in the concentration of the extract. 10µL, 20µL, 30µL, 40µL and 50µL of concentration showed 52%, 75.3%, 80.7%, 84.1% and 84.8% respectively. When compared with the standard Diclofenac sodium, the percentage of inhibition of the extract was found to be higher than the standard value that was supported by preceding articles have stating that the methanolic extract of coffee bean powder possessed elevated capacity of free radical scavenging on DPHH (1,1-diphenyl-2-picrylhydrazyl) (Pergolizzi *et*

al., 2020). Our team has extensive knowledge and research experience that has translate into high quality publications (Rajeshkumar *et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019; M. Gomathi *et al.*, 2020; Rajasekaran *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Santhoshkumar *et al.*, 2019),(Raj R, D and S, 2020),(Saravanan *et al.*, 2018),(Gheena and Ezhilarasan, 2019),(Ezhilarasan, Sokal and Najimi, 2018),(Ezhilarasan, 2018),((Dua *et al.*, 2019; A. C. Gomathi *et al.*, 2020; Vairavel, Devaraj and Shanmugam, 2020),(Ramesh *et al.*, 2018; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021) .,(Gnanavel, Roopan and Rajeshkumar, 2019),(Markov *et al.*, 2021)

Conclusion:

The practise of using herbal medicines is growing owing to its potential and ability to treat various diseases. The significant action of coffee bean powder ethanolic extract is observed to have no side effects and is safe to consume. (Rajendran *et al.*, 2019)(Ashok, Ajith and Sivanesan, 2017)(Malli *et al.*, 2019)(Mohan and Jagannathan, 2014)(Menon *et al.*, 2018)(Samuel, Acharya and Rao, 2020)(Praveen *et al.*, 2001)(Neelakantan *et al.*, 2011)('Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases', 2019)(Kumar *et al.*, 2006).Based on the results of the current study, it is concluded that coffee bean powder ethanolic extract can be used as a potent anticariogenic and anti-inflammatory drug for the treatment of dental caries and tissue injuries.

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Conflict of interest:

The author declare that there is no conflict of interest

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