ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.38132-38136, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANTIBIOTIC EFFECT OF ETHANOLIC EXTRACT OF SEEDS OF C*OFFEA CANEPHORA* BY DISC DIFFUSION AND DETERMINATION OF ITS MINIMUM INHIBITORY CONCENTRATION IN SELECTED STRAINS OF BACTERIA

Dr. Shamila Shetty, *Dr. Mohitha Shetty, Dr. Nandini Manjunath, Dr. Roshan Athikari and Dr. Mohammed Basil

Department of Periodontics, AJ Institute of Dental Sciences Mangalore

ARTICLE INFO

Article History:
Received 23rd June, 2016
Received in revised form
19th July, 2016
Accepted15th August, 2016
Published online 20th September, 2016

Key words:
Antibiotic effect,
Coffea canephora,
Disc diffusion method,
Minimum inhibitory concentration,
CGA (chlorogenic acid).

ABSTRACT

Aim: The aim of this study was to compare and evaluate the antibacterial efficacy of alcoholic extract of coffe a canephora on periodontal pathogenic bacteria Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn) and Aggregatibacter actinomycetemcomitans (Aa) with 0.2 % chlorhexidine gluconate and distilled water.

Materials and methods: The alcoholic extract of coffea canephora was obtained using percolation technique with ethanol as a solvent. Minimum inhibitory concentrations (MICs) and disk diffusion test were used to assess the antibacterial activity of alcoholic extract of coffea canephora on periodontal pathogenic bacteria with 0.2% chlorhexidine gluconate as positive control and distilled water as negative control.

Results: The disk diffusion test and the MIC showed that the *coffea canephora* extract showed antibacterial activity against the four periodontal pathogens which is comparable to chlorhexidine.

Conclusion: Alcoholic coffea canephora extract possess good antibacterial activity against Aa, Pg, Pi and Fn and thus would be beneficial for the prevention and treatment of periodontal disease.

Citation: Dr. Shamila Shetty, *Dr. Mohitha Shetty, Dr. Nandini Manjunath, Dr. Roshan Athikari and Dr. Mohammed Basil, 2016. "Antibiotic effect of ethanolic extract of seeds of c*offea canephora* by disc diffusion and determination of its minimum inhibitory concentration in selected strains of Bacteria", *International Journal of Current Research*, 8, (09), 38132-38136.

INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth and if not promptly recognised and correctly managed can ultimately lead to tooth loss. The prevalence and progression of this disease are related to a considerable increase in gram negative and anaerobic rods. Admist them, *porphyromonas gingivalis (Pg), prevotella intermedia (Pi), fusobacterium nucleatum (Fn) and aggregatibacter actinomycetemcomitans (Aa*) are actively implicated in the etiology of this disease. Periodontitis is characterised by a dysregulated host inflammatory / immune response to the above plaque bacteria in susceptible individuals (Wolff *et al*., 1994). Routine practice of antimicrobial adjunct to conventional periodontal therapy was well established owing to tissue penetrable nature of pathogenic bacteria (Academic report, 1996). The foremost chemical agents currently available are chlorhexidine, triclosan, cetyl pyridinium chloride and natural products.

Corresponding author: Dr. Mohitha Shetty,
Department of Periodontics, AJ Institute of Dental Sciences Mangalore

However, many of the synthetic agents including chlorhexidine and cetyl pyridinium chloride, have been provoking antimicrobial resistance of oral microorganisms. Thus, the use of natural antimicrobial agents as an alternative therapy for oral pathogens has been widely investigated (Walker *et al*., 1996). Chlorhexidine (CHX), a cationic bibisguanide is a gold standard among all antibacterial agents used in periodontal therapy, particularly because of its substantivity and broad spectrum anti-bacterial activity. However, CHX has been reported to have a number of side effects like brown discoloration of teeth, salt taste perturbation, oral mucosal erosions, and enhanced supragingival calculus formation, which limit its long term use (Eley *et al*., 1999) Medicinal plants have been investigated as a natural resource to treat microbial infections and also subsidise the development of new drugs with specific therapeutic properties (Newman *et al*., 2010). Among several plant species presenting antibacterial properties, coffee is the most popular in terms of consumption (Namboodiripad *et al*., 2008). Coffee, the genus coffea is a member of the family rubiaceae. There are several varieties of coffee cultivated worldwide, but those that are commercially grown include coffea canephora

(robusta) and coffea Arabica. Bioactive profile of coffee contains many of the most important constituents known to exist within functional foods, namely, flavanoids (Catechins, anthcyanins) ferrulic acids and Caffeic acid and biologically active components such as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallic acid and Caffeine (Brown *et al*., 2007). A wide data is available regarding the role of antioxidants and other anti-inflammatory factors in periodontal pathogenesis. Coffee has been recognised to be the largest source of dietary antioxidants (Shiva Manjunath, 2011). Components of coffee that have been found to have such effect include Caffeic acid and chlorogenic acid (CGA) (Alexander Yashin, 2013). Caffeine has shown immunomodulatory effects, it modulates both innate and adaptive immune responses. It has been reported that Caffeine can suppress human neutrophil and monocyte chemotaxis,and also suppress production of the pro-infalammatory cytokine tumor necrosis factor (TNF) alpha. It was also noted that many of Caffeine's immunomodulatory effects occur at concentrations that are relevant to normal human consumption (Louise, 2006). CGA, is a polyphenotic natural compound. Structurally, it is an ester of Caffeic acid with the –hydroxylgroup of a quinic acid. It has been reported to possess many health benefits including anti bacterial, anti fungal, antiviral, antiphlogistic, antioxidant, chemopreventive and other biological activities Very few studies have been conducted on the antibacterial efficacy of ethanolic extract of roasted coffee on periodontal pathogens. Hence the aim of our present study was to evaluate the antibacterial activity of the ethanolic extracts of coffea Canephora against specific periodontal pathogens like Pg, Pi, Fn and Aa.

MATERIALS AND METHODS

Seeds of *Coffea Canephora* were shade dried, roasted, mechanically grinded and coarsely powdered. About 1000 gm of roasted seed powder was extracted with 99.9% ethanol in a Soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50ºC) using rotary evaporator. The extracted material was weighed and percentage yield followed by dilution to different concentration in percentages and microgram per ml (µg/ml).the disk diffusiontest and MIC microdilution test was carried out on the ethanloic coffea canephora extract, 0.2% chlorhexidine and distilled water and was repeated three times.

Disc Diffusion Test of Ethanolic extract of seeds of *Coffea Canephora*

Brain Heart Infusion agar media was used. The agar plates were brought to room temperature before use. Using a loop or swab, the colonies were trasfered to the plates. The turbidity was visually adjusted with broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. Alternatively, the suspension was standardized with a photometric device. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, a sterile cotton swabwas dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. Entire surface of agar plate was swabed three times, rotating plates approximately 60º between streaking to ensure even distribution. Inoculated plate was then allowed to stand for atleast 3 minutes but no longer than 15 min before making wells. Hollow tube of 5mm diameter was heated. It was then pressed on above inoculated Agar plate and removed immediately by making a well in the plate. Likewise, five wells were made on each plate. 75µl, 50 µl, 25 µl, 10 µl and 5 µl of compound were added into the respective wells on each plate. The plates were incubated within 15 min of compound application. It was incubated for 18-24 hrs at 37 ºC in incubator.

The diameter of inhibition zone to nearest whole millimeter was measured by holding the measuring device (Henry *et al*., 1992).

Minimum Inhibitory Concentration (MIC) of Ethanolic extract of seeds of *Coffea Canephora*

Each sample was done about nine dilution with Thioglycollate broth. In the initial tube 20microliter of the sample was added into the 380microliter of Thioglycollate broth. For dilutions 200microliter of Thioglycollate broth was added into the next 9 tubes separately. Then from the initial tube 200 microliter was transferred to the first tube containing 200 microliter of Thioglycollate broth. This was considered as 10-1 dilution From 10-1 diluted tube 200microliter was transferred to second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each sample. From the maintained stock cultures of required organisms, 5 microliter was taken and added into 2ml of Thioglycollate broth. In each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity (Schwalve *et al*., 2007).

Statistical Analysis

One way ANOVA and Pearson Chi-Square test. $p<0.05$ was considered as statistically significant (Table 1, 2 & 3).

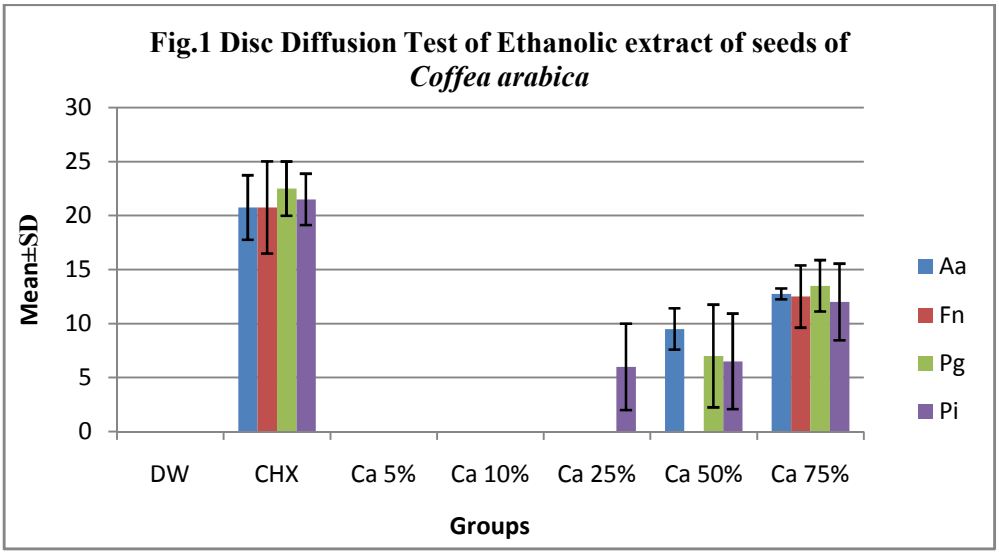
RESULTS

An inhibition zone of less than 8 mm in diameter (including the disc) is considered as the separation point between resistant and susceptible strains as follows: "Resistant" indicated by "R" <8 mm, "Susceptible" indicated by "S" >8 mm, DW-Distilled water, CHX-Chlorhexidine, Cc- *Coffea Canephora*, Aa-*Aggrebacter actinomycetemcomitans*, Fn-*Fusobacterium nucleatum*, Pg-*Porphyromonas gingivalis*, Pi-*Prevotella intermedia* (Fig. 1).

Table 1. Disc Diffusion Test of Ethanolic extract of seeds of *Coffea Canephora*

	Aa	Fn	Pg	Pi
DW	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CHX	20.75±2.98**	20.75±4.27**	22.50±2.51**	21.50±2.51**
5%	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
10%	0.00±0.00*	0.00±0.00*	0.00±0.00*	0.00±0.00*
25%	0.00±0.00*	0.00±0.00*	0.00±0.00*	6.00±4.00*
50%	9.50±1.91**	0.00±0.00*	7.00±4.76**	6.50±4.43**
75%	12.75±0.50**	12.50±2.88**	13.50±2.38**	12.00±7.95**

Observations are Mean±SD, One way ANOVA and Pearson Chi-Square test, *p>0.05-Not SignifiCcnt, **p<0.01-Highly SignifiCcnt. DW-Distilled water, CHX-Chlorhexidine, Aa-*Aggrebacter actinomycetemcomitans*, Fn-*Fusobacterium nucleatum*, Pg-*Porphyromonas gingivalis*, Pi-*Prevotella intermedia*.



Observations are Mean±SD, DW-Distilled water, CHX-Chlorhexidine, Cc- *Coffea Canephora*, Aa-*Aggrebacter actinomycetemcomitans*, Fn-*Fusobacterium nucleatum*, Pg-*Porphyromonas gingivalis*, Pi-*Prevotella intermedia*.

Table 2. Assessment of susceptibility and resistance by Disc diffusion method of Ethanolic extract of seeds of *Coffea Canephora*

S.No.	Sample	DW	CHX	Cc 5%	Cc 10%	Cc 25%	Cc 50%	Cc 75%
1	Aa 1	R	18mm	R	R	R	08 mm	12 mm
2	Aa 2	R	20 mm	R	R	R	10 mm	13 mm
3	Aa 3	R	20 mm	R	R	R	08 mm	13 mm
4	Fn 1	R	18 mm	R	R	R	R	10 mm
5	Fn 2	R	10 mm	R	R	R	R	15 mm
6	Fn 3	R	18 mm	R	R	R	R	15 mm
7	Pi 1	R	20 mm	R	R	08	10 mm	15 mm
8	Pi 2	R	21 mm	R	R	08	08 mm	15 mm
9	Pi 3	R	20 mm	R	R	08	08 mm	10 mm
10	Pg 1	R	22 mm	R	R	R	10 mm	14 mm
11	Pg 2	R	20 mm	R	R	R	08 mm	15 mm
12	Pg 3	R	22 mm	R	R	R	10 mm	15 mm

An inhibition zone of less than 8 mm in diameter (including the disc) is considered as the separation point between resistant and susceptible strains as follows: "Resistant" indiCcted by "R" <8 mm, "Susceptible" indiCcted by "S" >8 mm, DW-Distilled water, CHX-Chlorhexidine, Cc- *Coffea Canephora*, Aa-*Aggrebacter actinomycetemcomitans*, Fn-*Fusobacterium nucleatum*, Pg-*Porphyromonas gingivalis*, Pi-*Prevotella intermedia*.

Table 3. Minimum Inhibitory Concentration (MIC) of Ethanolic extract of seeds of *Coffea Canephora in* microgram per millilitre (µg/ml)

Sample	DW	CHX	Cc 100 µg/ml	Cc 50 µg/ml	Cc 25 µg/ml	Cc 12.5 µg/ml	Cc 6.25 µg/ml	Cc 3.12 µg/ml	Cc 1.6 µg/ml	Cc 0.8 µg/ml	Cc 0.4 µg/ml	Cc 0.2 µg/ml
Aa1	R	S	S	S	R	R	R	R	R	R	R	R
Aa 2	R	S	S	S	R	R	R	R	R	R	R	R
Aa 3	R	S	S	S	R	R	R	R	R	R	R	R
Fn 1	R	S	S	S	R	R	R	R	R	R	R	R
Fn 2	R	S	S	S	R	R	R	R	R	R	R	R
Fn 3	R	S	S	S	R	R	R	R	R	R	R	R
Pi 1	R	S	S	S	R	R	R	R	R	R	R	R
Pi 2	R	S	S	S	R	R	R	R	R	R	R	R
Pi 3	R	S	S	R	R	R	R	R	R	R	R	R
Pg 1	R	S	S	R	R	R	R	R	R	R	R	R
Pg 2	R	S	S	R	R	R	R	R	R	R	R	R
Pg 3	R	S	S	R	R	R	R	R	R	R	R	R

DW-Distilled water, CHX-Chlorhexidine, Cc- *Coffea Canephora*, Aa-*Aggrebacter actinomycetemcomitans*, Fn-*Fusobacterium nucleatum*, Pg-*Porphyromonas gingivalis*, Pi-*Prevotella intermedia*. S-Susceptibility, R-Resistance, µg/ml-microgram per millilitre

DISCUSSION

Antimicrobial activity has been extensively studied in recent decades due to the persistence of antimicrobial resistance in bacterial population. Thus, there has been made many efforts to search for new antibacterial agents from natural products, mainly in response to the staggering concern of consumers over the safety of synthetic products. Coffee, one of the most popular drink, and highly consumed in the world, has properties that can combat many diseases (Meckelberg *et al*.,

2014). Some of them include reducing the risk of type 2 diabetes, gallstones, liver cancer, Parkinsons disease and alzheimers disease (Farah *et al*., 2008). Coffee is classified in the rubiaceae family and coffea genus. Coffea canephora and coffea Arabica, known as "robust coffee" and "Arabic coffee", respectively are commonly used (Namboodiripad *et al*., 2008 and Brown *et al*., 2007).

Main components of coffee are caffeine, volatile and non volatile organic acids, phenols and aromatic compounds which have shown antimicrobial activity. The non-volatile organic acids present in coffee are chlorogenic and caffeic acids, which inhibit the growth of some gram- positive and gram negative bacteria. During roasting of coffee, 30 to 50 percent of CGA decomposes. However, volatile compounds, ketones and aldehydes are shown to have antimicrobial activites. Some phenolic compounds inhibit the growth of certain gram-positive and gram-negative bacteria (Fardiaz *et al*., 1995). It has been shown that, coffee consumption maybe protective against periodontal bone loss in adult males (Nathanng *et al*., 2003). Green coffee been extract was shown to be effective at a very low concentration against four pathogenic bacteria-Pg, Pi, Fn amd Aa (Bharath *et al*., 2015). Antonio *et al* 2011, has shown that the coffea canephora extract has an antibacterial effect against streptococcus mutans and also shows a preventive effect against demineralization of tooth enamel (Antonio *et al*., 2012). A study by FM da silva *et al* stated, that coffee consumption may not be effective on periodontal diseases, since the evaluated extract showed only inhibitory properties against tested cariogenic bacteria and not the bacteria involved in periodontal diseases (da Silva *et al*., 2014). However, important microorganism such as Aa was not investigated in that study. Most of the studies mentioned in the literature, determines the in vitro antibacterial activity of aqueous coffee extract. But previous studies have confirmed that alcoholic solvents like ethanol and methanol are more suitable than other solvents such as water in extracting compounds of medicinal plants (Ahmad *et al*., 1998). After a thorough study of literature and to the best of our knowledge, there are no invitro studies on the ethanolic extract of *coffea canephora*, to confirm its antibacterial activity on periodontal pathogens. In this study, we have compared and analysed the antibacterial efficacy of ethanolic extract of coffea canephora using the MIC microdilution method and the disk diffusion method, with 0.2% chlorhexidine as positive control and distilled water as negative control. The disk diffusion test for ethanolic coffea canephora extract revealed, the zone of inhibition (ZOI) for Aa was 8 to 10mm at 50% concentration and 12-13mm at 75% concentration. For Fn, the ZOI was 10 to 15mm at 75% concentration. Pi showed ZOI of 8mm at 25% concentration, 8 to10 mm 8 to 10 mm at 50% concentration and 10 to 15% at 75% concentration. For Pg, ZOI was 8 to 10mm at 50% concentration and 14 to 15mm at 75% concentration. zone of inhibition, in case of 0.2% chlorhexidine was in the range of 18 to 22mm for all the four organisms and they were all resistant for distilled water. (Table 1, 2) The MIC values for coffea canephora extract obtained for the periodontal pathogens showed that Aa, Fn and Pi were sensitive at 50 µg/ml and Pg was sensitive at 100 µg/ml. All the pathogens were sensitive to 0.2% chlorhexidine and resistant to distilled water. (Table 3) Within the limitations of our study we can state that the ethanolic extract of coffea canephora has proven effective on the four periodontal pathogens Aa, Fn, Pi and Pg and is comparable to chlorhexidine.

Conclusion

The present study has demonstrated that ethanolic extract of coffea canephora has significant antibacterial potential against periodontal pathogens-Pg, Pi, Aa and Fn which is comparable to chlorhexidine. The in vitro determination of their concentration in GCF and serum samples might help us to know the ideal dosage required for antimicrobial and regenerative activity of ethanolic *coffea canephora* extract to treat periodontitis.

REFERENCES

Academic report by AAP. Systemic antibiotics in periodontics. *J Periodontol* 1996;67:831-8

Ahmad, I., Mehmood, Z., Mohammed, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.,* 62: 183 193.

Alexander yashin, 2013. yakov yashin*et al*. antioxidant and antiradical activity of coffee. *Antioxidants,* 2,230-245.

Antonio, A.G. *et al*. 2012. Effect of Coffea canephoraAqueous Extract On Microbial Counts in Ex Vivo Oral Biofilms: *A Case Study Planta Med*, 78: 755–760

Bharath, N, Sowmya, N.K., Mehta, D.S. 2015. Determinationof antibacterial activity of green coffee bean extract on periodontogenicbacteria like *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans*: An *in vitro* study. *Contemp Clin Dent*., 6:166-9.

Brown, A.C. 2007. Understanding food: principles and preparation. 3rd ed. Belmonth, CA: Cengage Learning.

Chou, T. 1992. Wake up and smell the coffee -caffeine, coffee, and the medical consequences. *The Western Journal of Medicine*, 157:544-553.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbial. Rev.,* 12: 564 582.

da Silva, F.M., *et al*. 2014. Antibacterial Effect of Aqueous Extracts and Bioactive Chemical Compounds of *Coffea canephora* against Microorganisms Involved in Dental Caries and Periodontal Disease. *Advances in Microbiology*, 4, 978-985.

Eley, B. M. 1999. Antibacterial agents in the control of supragingival plaque — a review; *British Dental Journal,* 186: 286–296

Farah, A., Monteiro, M., Donangelo, C.M., Lafay, S. 2008. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *J Nutr*., 138:2309‑15.

Fardiaz, S. 1995. Antimicrobial activity of coffee *(Coffea robusta)* extract. *ASEA Food J,* 10:103‑6.

Henry, D. 1992. Isenberg.clinical microbiology procedures handbook. American society for Microbiology, vol 1.1992.

Karunanidhi, A., Thomas, R., van Belkum, A., Neela, V. 2013. *In vitro* antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia* including the trimethoprim /sulfamethoxazole resistant strain. *Biomed Res Int.,* 392058.

Louise A. 2006. Horrigan *et al*. immunomodulatory effects of caffeine: friend or foe? pharmacology and therapeutics., vol111(3),877-892.

Meckelberg, N. K.C Pinto *et al*. 2014. Antibacterial effect of coffee: calcium concentration in a culture containing teeth/biofilm exposed to coffea canephora aqueous extract.

Namboodiripad, P.A., Kori, S. 2008. Can coffee prevent caries? *Journal of Conservative Dentistry*, 12:17-21.

Nathan, N.G. 2013. Kaye et al. Coffee consumption and periodontaldisease in males, *J Periodontol*, 851042-1049.

Newman, D.J., Cragg, G.M., Wrigley, S.K., Thomas, R., Nicholson, N. and Bedford, C. 2010. In: *Functional Molecules from Natural Sources*, RSC Publications, Cambridge, 3-36.

Shiva Manjunath. RG .2011. Role of Antioxidants as an adjunct in periodontal therapy. *ISPCD*, Vol2; issue 2, May.

Schwalve, 2007. Moore and Goodwin. Antimicrobial susceptibility protocols.

Walker, C.B. 2000. The acquisition of antibiotic resistance in the periodontal microflora. *Periodontol*, 1996; 10:79-88

Wolff, L., Dahlén, G., Aeppli, D. 1994. Bacteria as risk markers for periodontitis. *J Periodontol*., 65 5 Suppl: 498‑510.

****