EVALUATION OF EFFICACY OF *CHICORIUM INTYBUS* EXTRACT ON PERIODONTAL PATHOGENS: AN *IN-VITRO STUDY*

Dissertation

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In the partial fulfillment of the requirement for the degree

Of

MASTER OF DENTAL SURGERY

In

PERIODONTOLOGY

By

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BATCH: 2018-2021

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All our dreams can come true, if we have the courage to pursue them.

Acknowledging the good you already have in your life, is the foundation for all abundance

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This one is for my love and life, my son ***Ivaan.***

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Knowledge is in the end based on acknowledgement

Dr Shikha Singh

CONTENT

S. No.	Particulars	Page No.
1.	List of Tables	**I**
2.	List of Graphs	**II**
3.	List of Illustrations	**III**
4.	List of Annexures	**IV**
5.	Abbreviations	**V-VI**
6.	Abstract	**1**
7.	Introduction	**2-3**
8.	Aim and Objectives	**4**
9.	Review of Literature	**5-12**
10.	Materials and Methods	**13-27**
11.	Results and Observations	**28-42**
12.	Discussion	**43-49**
13.	Conclusion	**50**

14.	Bibliography	**51-56**
15.	Appendices	**57-65**

LIST OF TABLES

S. NO.	TITLE	PAGE NO.
1.	Zone of inhibition (mm) w.r.t. *Porphyromonas gingivalis* among the study groups.	**28**
2.	Comparison of Zone of inhibition (mm) w.r.t. *Porphyromonas gingivalis* among the study groups.	**30**
3.	Zone of inhibition (mm) w.r.t. *Fusobacterium nucleatum* among the study groups.	**31**
4.	Comparison of Zone of inhibition (mm) w.r.t. *Fusobacterium nucleatum* among the study groups.	**33**
5.	Optical density value of extract against *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*	**34**
6.	Mean Optical density w.r.t. *P. gingivalis* among the study groups	**36**
7.	Comparison of OD w.r.t *P. gingivalis* among the study groups	**37**
8.	Optical Density w.r.t. *F. nucleatum* among the study groups	**38**
9.	Comparison of Optical Density w.r.t. *F. nucleatum* among the study groups	**38**
10.	MIC for *P. gingivalis* among the study groups	**39**
11.	MIC for *F.nucleatum* among the study groups	**41**

LIST OF GRAPHS

S. NO.	TITLE	PAGE NO.
1.	Zone of inhibition of *Porphyromonas gingivalis* given by different treatment group	**29**
2.	Zone of inhibition of *Fusobacterium nucleatum* given by different treatment group	**32**
3.	Optical density of *Chicorium Intybus* in both periodontal pathogens	**35**
4.	Minimum inhibitory concentration *of Chicorium Intybus* against *porphyromonas gingivalis*	**40**
5.	Minimum inhibitory concentration of *Chicorium Intybus* against *Fusobacterium nucleatum*	**42**

LIST OF ILLUSTRATIONS

S. NO.	TITLE	PAGE NO.
1.	*CHICORIUM INTYBUS* PLANT	**14**
2.	*CHICORIUM INTYBUS* COLLECTED LEAVES	**14**
3.	*CHICORIUM INTYBUS* SHADE DRIED LEAVES	**14**
4.	SAMPLES OF MEDICINAL PLANT FOR THE AUTHENTICATION FROM NISCAIR	**15**
5.	PLANT EXTRACT PREPARATION IN SOXHLET APPARATUS	**18**
6.	THE CLEAR ZONE FORMED AFTER COMPLETE EXTRACTION	**19**
7.	*CHICORIUM INTYBUS* EXTRACT OBTAINED FROM SOXHLET APPARATUS	**20**
8.	CONCENTRATED EXTRACT OF *CHICORIUM INTYBUS*	**20**
9.	ROTARY EVAPORATOR	**20**
10.	ATCC BACTERIAL STRAINS OF *PORPHYROMONAS GINGIVALIS* AND FUSOBACTERIUM NUCLEATUM	**22**
11.	ZONE OF INHIBITION OF PORPHYROMONAS GINGIVALIS	**23**
12.	ZONE OF INHIBITION OF FUSOBACTERIUM NUCLEATUM	**24**
13.	MINIMUM INHIBITORY CONCENTRATION PROCESS	**25**

LIST OF ANNEXURES

S. NO.	TITLE	PAGE NO.
ANNEXURE 1.	Data collection of Zone of inhibition, OD and MIC readings	**57-59**
ANNEXURE 2.	Statistical analysis	**60-61**
ANNEXURE 3.	IRC certificate	**62**
ANNEXURE 4.	IEC certificate	**63**
ANNEXURE 5.	Authentication letter from NISCAIR	**64**
ANNEXURE 6.	Work certificate from MRC Life Sciences, Lucknow	**65**
ANNEXURE 7.	Plagiarism report	**66**

ABBREVIATIONS

µl	microlitre
ATCC	American type culture collection
CHX	Chlorhexidine
CSIR	Council of scientific and industrial research
F.nucleatum	*Fusobacterium nucleatum*
mg	milligram
MIC	Minimum inhibitory concentration
ml	millilitre
mm	millimeter
MRS	De Man, Rogosa and Sharpe agar
NISCAIR	National institute of science and information resources
nm	nanometer
OD	Optical density
P. gingivalis	Porphyromonas gingivalis
PSI	Pound force per square inch
SD	Standard deviation
SPSS	Statistical package for Social Sciences
VSC	Volatile Sulphur compounds
w/v	Weight/volume

^{o}C	Degree celcius
et al	et alia

ABSTRACT

Chemical inhibitors of plaque play an important role in plaque control. Mouthwashes, dentifrices and Local drug delivery agents are commonly used methods to deliver antimicrobial agent and can be used by patient as an oral hygiene aid. The present study was undertaken to evaluate the efficacy of medicinal plant extract in periodontal pathogens. Two bacteria i.e.; *Porphyromonas gingivalis* and *Fusobacterium nucleatum* were taken to evaluate the zone of inhibition and minimum inhibitory concentration of the *Chicorium Intybus*. The bacterial strains were obtained from ATCC and were cultured in specific media under the laboratory environment. The extract was prepared and concentrated for the further use. Zone of inhibition was assessed along with its optical density value and Minimum inhibitory concentration taking chlorhexidine and amoxiclav as control groups. Herbal extract of *Chicorium Intybus* showed slightly higher zone of inhibition than Chlorhexidine but lesser than amoxiclav. It can thus be concluded that *Chicorium Intybus* can be effectively utilized as an adjunct to periodontal therapy in future in comparison to Chlorhexidine mouthwash for periodontitis patients taking into account that it needs more research on biosafety and further use on patients is needed in future.

INTRODUCTION

Research conducted over the past three decades has shown that several herbs contain a number of components enriched with antibacterial and antiplaque activity. In oral cavity, diseases like periodontitis have always been a matter of concern. Various antibiotics and oral rinses are given for such conditions. Periodontal diseases are polymicrobial oral infections by predominantly gram negative and anaerobic subgingival bacterial species such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*[1]

P. gingivalis has high proteolytic activity and is considered a keystone pathogen in the initiation and progression of periodontal disease. Apart from the mechanical debridement of periodontal pockets, clinical treatment involves the adjunctive use of antibiotics. Although due to increased and unrestricted consumption of antibiotics in humans, development of resistance against antibiotics has become a global threat. There is a need for change in prescription and use of antibiotics. Nowadays people prefer herbal medicines for lesser side effects. Therefore, introduction of alternatives which has antimicrobial effects with no or lesser side effects, could overcome the need of commercially available antibiotics and oral rinses like chlorhexidine.[2]

Very recently it has been shown that regular consumption of foods endowed with active molecules is associated with alterations of the oral microbial community in the direction of less periodontopathogenic microbiota. It therefore seems logical and senseful to encourage the consumption of such foods or to incorporate the active compounds for daily oral hygiene such as *Chicorium Intybus*.[3]

The ancient literature contains many references on the medicinal uses of *Chicorium Intybus* (Family- Asteraceae). It has been used as a local applicant in the treatment of acne, ophthalmia and throat infection lately. The plant has aromatic and healing properties. Aqueous and alcoholic extracts of Chicory were found to have anti-inflammatory action.[4]

Therefore, acid extract from this herb may decrease inflammation and bacterial infections. Many studies have been conducted to evaluate effect of consumption of

chicory extract systemically in non-surgical periodontal therapies on serum antioxidant and lipid status also.[5]

About 64 compounds have been detected from *Chicorium Intybus* such as Vitamin A, Potassium, Calcium, Phosphorus, phenolic acids, Anthocyanins, flavonoids and many more.[6]

Although few animal studies have been done on the toxic effects of *Chicorium Intybus* on rats.[7] But to best of our knowledge there is no relevant data on bio-safety of *Chicorium Intybus*. Moreover, till date antimicrobial effect of this on periodontopathogenic bacteria have also not been evaluated. Therefore, this study is being undertaken to evaluate the minimum inhibitory concentration of *Chicorium Intybus* and its antimicrobial effect on *Porphyromonas Gingivalis* and *Fusobacterium Nucleatum*. This will provide a clearance for the further use of *Chicorium Intybus* in the oral cavity.

AIM & OBJECTIVES OF THE STUDY

AIM: The aim of the study is to assess anti-microbial effects of *Chicorium Intybus.*

OBJECTIVES:

1. Authentication and preparation of *Chicorium Intybus* extract
2. To evaluate the anti-microbial effects of *Chicorium Intybus* on various oral bacteria.
3. To evaluate minimum inhibitory concentration of *Chicorium Intybus* extract on bacterial growth.

REVIEW OF LITERATURE

1. **Patel V.K et al (1983)**[11] conducted study to watch the effects of Chicory extract in gingival inflammation. 50 patients with gingivitis and bleeding gums with alcoholic extract of chicory roots were examined for three weeks. 42 of which turned up with the relief in inflammatory and bleeding conditions consistently. It was concluded that Chicory extract may be an effective medication for bleeding inflamed gums.

2. **Okamoto A.C et al (2000)**[8] examine the influence of subinhibitory concentrations of chlorhexidine, triclosan, penicillin G and metronidazole on hemolytic activity and bacteriocin-like substance production of oral F. nucleatum. A high resistance to penicillin G was observed and 63% of the isolates were beta-lactamase positive. All the tested isolates were susceptible to metronidazole. F. nucleatum isolates grown with or without antimicrobials were alpha-hemolytics. Bacteriocin-like substance production was increased in isolates grown with penicillin G. Impaired production of hemolytic or antagonic substances can suggest a role in the regulation of oral microbiota.

3. **Roldan S et al (2003)**[9] evaluated the microbial effects of a newly formulated mouthwash (Halita®) on oral halitosis patients, containing chlorhexidine, cetyl pyridinium chloride and zinc lactate. It was a double blind, placebo controlled parallel study. At baseline and at 2 weeks post treatment, full mouth organoleptic odor scores, level of volatile sulphur compounds (VSC) and the Winkel Tongue Coating Index were recorded. High prevalences were observed for *Fusobacterium nucleatum*, *Prevotella intermedia* and *Porphyromonas gingivalis* in tongue coating, saliva and subgingival plaque samples. The test mouthwash demonstrated efficacy in reducing the microbiological parameters in three oral niches in moderate to severe halitosis patients without periodontitis.

4. **Petrovic J et al (2005)**[10] evaluated the antibacterial activity of *Chicorium Intybus* extract in water, ethanol ethyl acetate base. All extracts showed antibacterial activity, ethyl acetate being the most active. Water ectract inhibits agrobacterium radiobacter sp. Tumefaciens, pseudomonas fluorescence and P. aeruginosa.

5. **Asad M et al (2006)**[11] evaluated RNA interference as a tool to engineer high nutritional value in *Chicorium Intybus* authors generated a transgenic chicory plants with suppressed inulin degradation. The hairpin constructs were made and chicory was transformed by agrobacterium tumifaciense, strain (C58C1). They explained that transgenics should be selected and check by means of molecular techniques.

6. **Robert C et al (2006)**[12] used the Plackette-Burman experimental design to examine the impact of extraction parameters on yields and compositions of pectins extracted from chicory roots *(Chicorium Intybus).* In result, the acid extraction of chicory roots resulted in average yield of 11% containing 86% of sugars. It was found that extraction temperature, time, protease pretreatment, water purity, and water washing of pulps significantly affected yield and pectin composition with an increase of yield and purity of pectin in harsher extraction conditions.

7. **Sena N.T et al (2006)**[13] investigated the antimicrobial activity of 2.5% and 5.25% sodium hypochlorite and 2.0% chlorhexidine gel and liquid as endodontic-irrigating substances against selected single-species biofilms. Single-species biofilms of Enterococcus faecalis, Staphylococcus aureus, Candida albicans, Prevotella intermedia, Porphyromonas gingivalis, Porphyromonas endodontalis and Fusobacterium nucleatum were generated on a cellulose nitrate membrane placed on agar medium. The biofilms were then immersed in the endodontic-irrigating substances for 30 s and also for 5, 10, 15, 30 and 60 min, with and without mechanical agitation. Mechanical agitation improved the antimicrobial properties of the chemical substances tested using a biofilm model, favouring the agents in liquid presentation, especially 5.25% NaOCl and 2% chlorhexidine.

8. **Schmidt M et al (2007)**[7] conducted study to evaluate the toxicological effects of chicory roots. It was a 28 day sub chronic toxicity study in male and female Sprague-dawley rats. They concluded that there were no treatment related toxic effects from *Chicorium Intybus* administered orally at 70, 350, or 1000 mg/kg/day. There was no mutagenic activity in the Ames test, supporting its use as a therapeutic agent.

9. **Ardila C.M et al (2010)**[14] tested the antimicrobial sensitivity of two periodontal pathogens (Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans) to a panel of five orally administrable antibiotics (clindamycin, metronidazole, amoxicillin, moxifloxacin and amoxicillin/clavulanic acid) in periodontal disease. Susceptibility testing revealed a sensitivity of 100% of A. actinomycetemcomitans and P. gingivalis to moxifloxacin and amoxicillin/clavulanic acid but moderate susceptibilities were found for the rest of antibiotics tested.

10. **Rassouli M. B et al (2010)**[15] investigated the effect of aqueous extract of *Chicorium Intybus* leaves on offspring sex ratio in rat. All rats in 1 group were injected with either 1.0 or 0.7 /kg body weight of an aqueous extract of chicory leaves for 30 days at 72 hours intervals. Group 2 was injected with distilled water in same way. Blood PH, Na, K, Ca and Mg were measured in all groups. After mating results showed that there was significant increase in Na and K levels as well as the sex ratio of male to female offspring (10.23%) in experimental group.

11. **Japoni A et al (2011)**[15] evaluated the antimicrobial sensitivity of Porphyromonas gingivalis to a panel of eight orally administrable antibiotics in chronic periodontal diseases and to evaluate factors associated with periodontitis in adult patients. Selected colonies of P. gingivalis were used to evaluate the antibacterial activities of penicillin, metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin, doxy-cycline, ciprofloxacin and azithromycin. Susceptibility testing revealed a sensitivity of 100% of P. gingivalis to azithromycin, doxycycline and amoxicillin/clavulanic acid but lower susceptibilities were found for the rest of antibiotic agents evaluated.

12. **Signoretto C et al (2011)**[3] evaluated the mode of antimicrobial action of chicory and mushroom on *P.intermedia* **(**a periodontopathogenic bacterium**).** Concentration used, resulted in bacteriostatic effect in mushroom extract and slightly bacteriocidal effect in *Chicorium Intybus* extract. Changes in RNA and DNA were noticed. The discovery of antibiotic like mode of action suggests that these extracts can be employed for daily oral hygiene.

13. **Bratt D.A et al (2012)**[17] investigated the oral health benefits of a number of foods. Both antigingivitis and anticaries effects were investigated by assays examining the prevention of biofilm formation and coaggregation, disruption of pre-existing biofilms, and the food's antibacterial effects. Assays investigating interactions with gingival epithelial cells and cytokine production were carried out. Anti-caries properties such as interactions with hydroxyapatite, disruption of signal transduction, and the inhibition of acid production were investigated. The mushroom and Chicory homogenates shows promise as anti-caries and anti-gingivitis agents.

14. **Rams T.E et al (2013)**[18] evaluated the occurrence of b-lactamase-positive subgingival bacteria in chronic periodontitis subjects of USA origin, and assessed their in vitro resistance to metronidazole at a breakpoint concentration of 4 lg/mL. Dilutions were also plated onto culture media plates supplemented with 2 lg/mL of amoxicillin, a combination of 2 lg/mL of amoxicillin plus 2 lg/mL of the b-lactamase inhibitor clavulanic acid, or 4 lg/mL of metronidazole. 52.1% study subjects yielded b-lactamase-producing subgingival bacterial test species, with Prevotella intermedia / nigrescens, Fusobacterium nucleatum and other Prevotella species most frequently identified as b-lactamase-producing organisms. Of the b-lactamase producing bacterial test species strains recovered, 98.9% were susceptible in vitro to metronidazole at 4 µg / ml.

15. **Street R. A et al (2013)**[19] reviewed the data on *Chicorium Intybus* traditional uses, scientific validation and phytochemical composition in detail. This review focus on the economic and culturally important medicinal uses of C. intybus. Although toxicological data on *C. Intybus* is currently limited. Authors concluded that this herb remains an extremely versatile plant, amenable to genetic manipulation and knowledge relating to the various medicinal uses of C. Intybus has been supported by phytochemical isolation and investigations into biological activity.

16. **Frauenhoffer M.A. et al (2014)**[20] investigate antibiotic susceptibility of an in vitro biofilm by isothermal microcalorimetry (IMC). Titanium disks containing a 72 h three-species biofilm (Streptococcus sanguinis, Fusobacterium nucleatum,

and Porphyromonas gingivalis) were placed with nutrient agar supplemented with increasing concentrations of amoxicillin, metronidazole or their combination and incubated anaerobically. S. sanguinis and P. gingivalis were incubated anaerobically in media supplemented with antibiotics at 37°C for 24 h, and their viability was determined by live/dead staining, conventional culturing, and IMC. In all biofilm samples incubated with antibiotics a prolonged lag phase was observed compared to controls. Maximum growth rate was significantly lower for samples either treated with amoxicillin or metronidazole in comparison to controls.

17. **Sharma R et al (2014)**[21] evaluated the microbial activity of different combinations of chicory-coffee solution and their anti-adherence effect on streptococcus mutans to glass surface. Pure chicory had shown less bacterial count compared to all other groups. Authors concluded that chicory exerted antibacterial effect against S. mutans while coffee reduced significantly the adherence of S. mutans to the glass surface.

18. **Karpinski T.M et al (2015)**[22] evaluated pharmaco-biological activity and application through collecting online databases from pubmed/medline. For this total 75 papers were enrolled. Authors concluded that CHX has strong biocidal activity against Gram-positive bacteria and weaker activity against Gram-negative bacteria. It is also active against yeasts, some dermatophytes and some lipophilic viruses. Also, CHX exhibits cytotoxic activity on human cells, can cause colorization of teeth and fillings, and its activity depends on the pH of the environment and the presence of organic substances.

19. **Krylova S et al (2015)**[23] conducted study on animals like rats and dogs to evaluate Gastroprotective effect of *Chicorium Intybus* extract. The effect is attributed to the antisecretory activity of the plant and stimulation of defence barrier function of the gastric mucosa. Results showed the aggressive factors (hypersecretion and hyperacidity) predominated, while the defense factors were reduced, which was manifested by peptic digestion of the gastric mucosa. The statistical differences were considered significant.

20. **Saad E.M et al (2015)**[24] studied and experimented on molecularly imprinted polymer synthesized and applied for the extraction of chicoric acid from chicory herb. A computational study was developed to find a suitable template to functional monomer molar ratio for Molecularly imprinted polymer (MIP) preparations. The MIP's were synthesized in a non-covalent approach via thermal free radical polymerization, using two methods i.e; bulk and suspension. Results showed best binding ability towards chicoric acid was with MIP prepared using bulk polymerization.

21. **Javid A.Z et al (2016)**[5] conducted study to evaluate the effect of consumption of Chicory leaf extract in adjunct with nonsurgical periodontal therapies on serum antioxidant and lipid status. They concluded that consumption of Chicory leaves in adjunct to nonsurgical treatment has beneficial effect against periodontal disease.

22. **Eslami H et al (2017)**[25] evaluated antifungal effects of chicory extracts on candida glabrata and candida krusei in a Laboratory environment. They used minimum inhibitory concentration and agar well diffusion method and compared and also compared to nystatin in taste effects. Author concluded that *Chicorium Intybus* could be considered an effective antifungal drug against infections caused by C. krusei and C. glabrata.

23. **Balejo R D P et al (2017)**[26] conducted study to evaluate effects of chlorhexidine preprocedural rinses on bacteremia in periodontal patients. Periodontal probing depth, clinical attachment level, plaque, and gingival indices were measured and subgingival samples were collected. Blood samples were collected before dental scaling, 2 and 6 minutes after scaling. Total bacterial load and levels of P. gingivalis were determined in oral and blood samples by real-time polymerase chain reaction, while aerobic and anaerobic counts were determined by culture in blood samples. He concluded that in all polymerase chain reaction revealed higher blood bacterial levels than culture. while gingivitis patients presented lower bacterial levels in blood than periodontitis patients. Individuals who experienced bacteremia showed worse mean clinical attachment level and more subgingival bacteria. The pre-procedural rinse did not reduce induced bacteremia.

24. **James P et al (2017)**[27] included 51 studies that analysed a total of 5345 participants. To assess the effectiveness of chlorhexidine mouthrinse used as an adjunct to mechanical oral hygiene procedures for the control of gingivitis and plaque compared to mechanical oral hygiene procedures alone or mechanical oral hygiene procedures plus placebo/control mouthrinse. There is high-quality evidence of a large reduction in dental plaque with chlorhexidine mouthrinse used as an adjunct to mechanical oral hygiene procedures for 4 to 6 weeks and 6 months. There is no evidence that one concentration of chlorhexidine rinse is more effective than another.

25. **Haydari M et al (2017)**[28] compared the plaque and gingivitis inhibiting effect of commercial products containing 0.2%, 0.12% and 0.06% chlorhexidine in a modified experimental gingivitis model. In three groups of healthy volunteers, experimental gingivitis was induced and monitored over 21 days and simultaneously treated with the commercial solutions containing 0.2%, 0.12% and 0.06% chlorhexidine. The maxillary right quadrant of each individual received mouthwash only, whereas the maxillary left quadrant was subject to both rinsing and mechanical oral hygiene. As a result the commercial mouthwash containing 0.2% chlorhexidine resulted in statistically significantly lower plaque scores than the 0.12 and 0.06% mouthwashes after 21 days use.

26. **Babaei H et al (2018)**[6] evaluated the effects of Chicory extract on serum oxidative stress markers, lipid profile and periodontal status in patients with chronic periodontitis. In this study 40 patients were taken for trial. Intervention group received methanolic extract capsules for 8 weeks. In control group participants received a placebo capsule for 8 weeks. All participants had nonsurgical periodontal therapy. The results showed Mean serum Total Antioxidant Capacity, Uric acid, and HDL-C increased. LDL-C and total cholesterol decreased significantly. A significant difference was seen in mean pocket depth.

27. **Khoobani M et al (2019)**[29] designed an experiment to determine the effect of different levels of *Chicorium Intybus* powder and a probiotic blend (PrimaLac®) on productive performance,blood biochemical parameters, and ileal microbiota in broiler chickens. Results showed that the body weight gain of broilers fed the

probiotic blend or 0.10% *Chicorium Intybus* was significantly higher than those fed on the other treatments given.

28. **Cova C. M et al (2019)**[30] investigated industrial leftover chicory for the ultrasound assisted extraction, microwave assisted extraction and their simultaneous combination, using either ethanol/ water or water alone with the aim of designing a green and efficient extraction process. Results have shown that in ethanol solutions polyphenol recovery values of up tp ~3g of gallic acid equivalents per kg of fresh material in only 15min, while conventional extraction required 240 minutes to obtain the same results.

MATERIALS AND METHOD

Place of the study where it is conducted: -

1. The study was conducted in The Department of Periodontology of BBDCODS, BBDU Lucknow.
2. SCHOOL OF PHARMACY, BBDU, Lucknow.
3. MRD Life Sciences, Lucknow.

Study Sample and size

Two bacterial strains with 2 kwik stik each.

a. *Porphyromonas gingivalis*
b. *Fusobacterium nucleatum*

Ethanolic extract of *Chicorium Intybus* 65 ml

MATERIALS AND EQUIPMENT USED IN THE STUDY WITH SPECIFICATIONS AND COMPANY

MEDICINAL PLANT:

The present study was undertaken to evaluate the efficacy of medicinal plant extracts in chronic periodontitis of the following plant:

Chicorium Intybus (C. Intybus)

***Chicorium* Intybus – (kasni)**

Family name: Asteraceae

Common name: chicory, succory, blue daisy, kasni

Part used: Leaves

Chemical constituents: Vitamin A, Potassium, Calcium, Phosphorus, phenolic acids, Anthocyanins, flavonoids and many more.

Safety profile: The plant has been reported to have no side effects and toxicity. It was used as a coffee substitute and is used along with pure coffee powder now. It has been used in ayurvedic medicines for hepatic diseases and for body intoxication systemically.

It may cause adverse effects if taken at high doses regularly.

***Fig.1: CHICORIUM INTYBUS* PLANT**

***FIG.2: CHICORIUM INTYBUS* COLLECTED LEAVES**

***Fig.3: CHICORIUM INTYBUS* SHADE DRIED LEAVES**

Plate - 1

Fig.4: **SAMPLES OF MEDICINAL PLANT FOR THE AUTHENTICATION FROM NISCAIR**

Plate - 2

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL:

The leaves of *C. Intybus* were collected from a farm of Kasganj and Atrauli and it was authenticated by the National institute of science and information resources (NISCAIR), council of scientific and industrial research (CSIR), Delhi **[Authentication No. NISCAIR/RHMD/ Consult/2019/3429-30]**

METHODOLOGY: IN DETAIL

1. METHOD OF PREPARATION OF *CHICORIUM INTYBUS* EXTRACT

Ethanol based Extract of *Chicorium Intybus* was prepared in School of Pharmacy, BBDU, Lucknow. The collected leaves were shade dried and grinded to make a course powder. The dried course powder of leaves (80.20gm) was packed well and was subjected with ethanol by continuous hot extraction for 72 hours. The preparation was filtered through a sterilized Whatman filter paper and concentrated on evaporator. Obtained extract was weighed and percentage yield was found out to be 10.83 %. The extract was stored in airtight bottle at 4ºC for further use.

TEST MICROORGANISM:

The strains of two oral pathogenic anaerobic bacteria namely, ***Porphyromonas gingivalis*** **(P. gingivalis) (ATCC 33277)** and ***Fusobacterium nucleatum*** **(F. nucleatum) (ATCC 25586)** [American type culture collection] were obtained from **Gyan Scientific Traders (India) Pvt. Ltd; Lucknow**. The strains were grown in meat broth media and after growth they were transferred and cultured on the specific media recommended for each organism i.e.; **MRS** agar **(De Man, Rogosa and Sharpe agar)** and incubated in an anaerobic environment at 37ºC using gas packs. Further identification of the strains was done by vitek 2 compact and gram staining. All strains were maintained by subculturing.

SCREENING OF ANTIMICROBIAL ACTIVITY

The screening for antimicrobial activity of the two bacterial strains i.e., *P. gingivalis* and *F. nucleatum* was done. Sensitivity of these bacterial strains towards the extract of the medicinal plant was assessed by using well diffusion method. A minimum of 3-4 colonies from the sub cultured, pure isolate of the test micro-organism was touched with the sterile loop under aseptic conditions. 4 media was transferred to plates and left for solidification. 20 µl of each sample were loaded and placed in wells of 6mm

diameter to determine the inhibition zone. A zone of inhibition was determined. Chlorhexidine gluconate 0.2% w/v and Amoxicillin- clavulanic acid combination was taken as positive control groups. Sterile saline was taken as negative control. The tests were carried out thrice for meticulous analysis. The diameter of zone of inhibition was measured after incubating for 2 days at 37ºC. This process was done in MRD life Sciences in Lucknow.

Fig.5: **PLANT EXTRACT PREPARATION IN SOXHLET APPARATUS**

Plate - 3

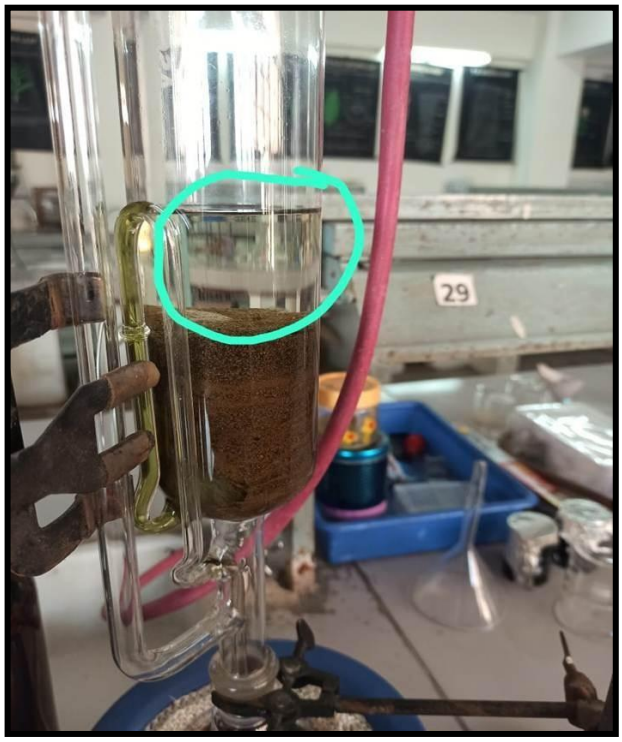


Fig.6: **THE CLEAR ZONE FORMED AFTER COMPLETE EXTRACTION**

Plate - 4

Fig.7: CHICORIUM INTYBUS **EXTRACT OBTAINED FROM SOXHLET APPARATUS**

Fig.8: **CONCENTRATED EXTRACT OF *CHICORIUM INTYBUS***

Fig.9: **ROTARY EVAPORATOR**

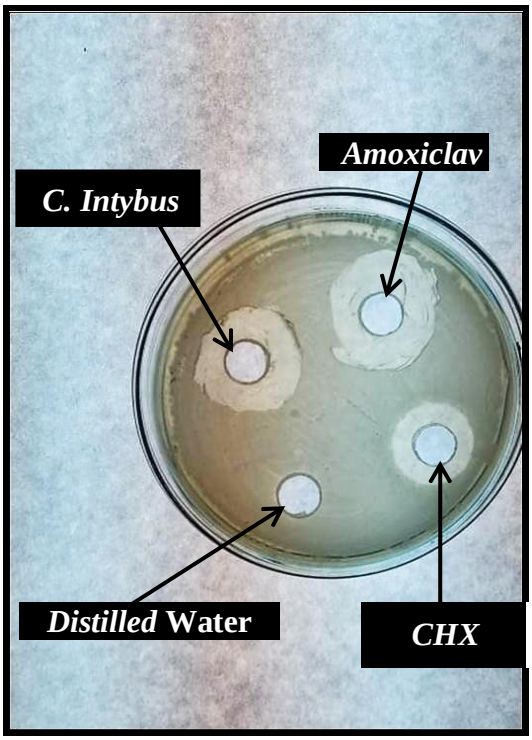
Plate - 5

MINIMUM INHIBITORY CONCENTRATION TEST:

3ml of MRS broth media was prepared in each test tubes and sterilized by autoclaving at 121ºC for 15 min at 15psi. It was cool down at room temperature. Extract was then serially diluted by taking 0.3ml volume. 20 µl bacterial cultures were inoculated and incubated at 37ºC for anaerobic conditions. Optical density (OD) was taken at 620nm. Minimum inhibitory concentration was evaluated and calculated thrice to remove standard error.

Fig.10: **ATCC BACTERIAL STRAINS OF *PORPHYROMONAS GINGIVALIS* AND FUSOBACTERIUM NUCLEATUM**

Plate - 6



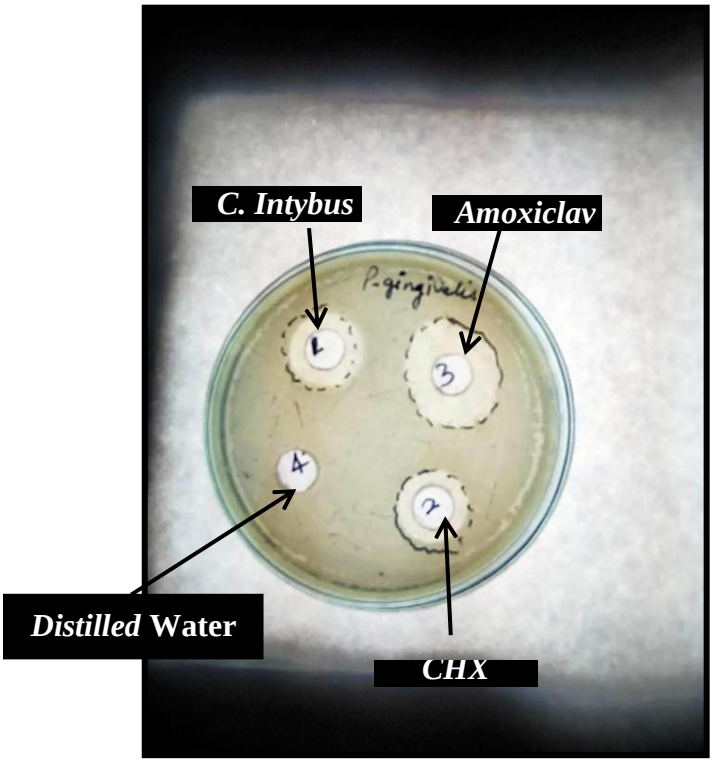


Fig.11: **ZONE OF INHIBITION OF PORPHYROMONAS GINGIVALIS**

Plate - 7

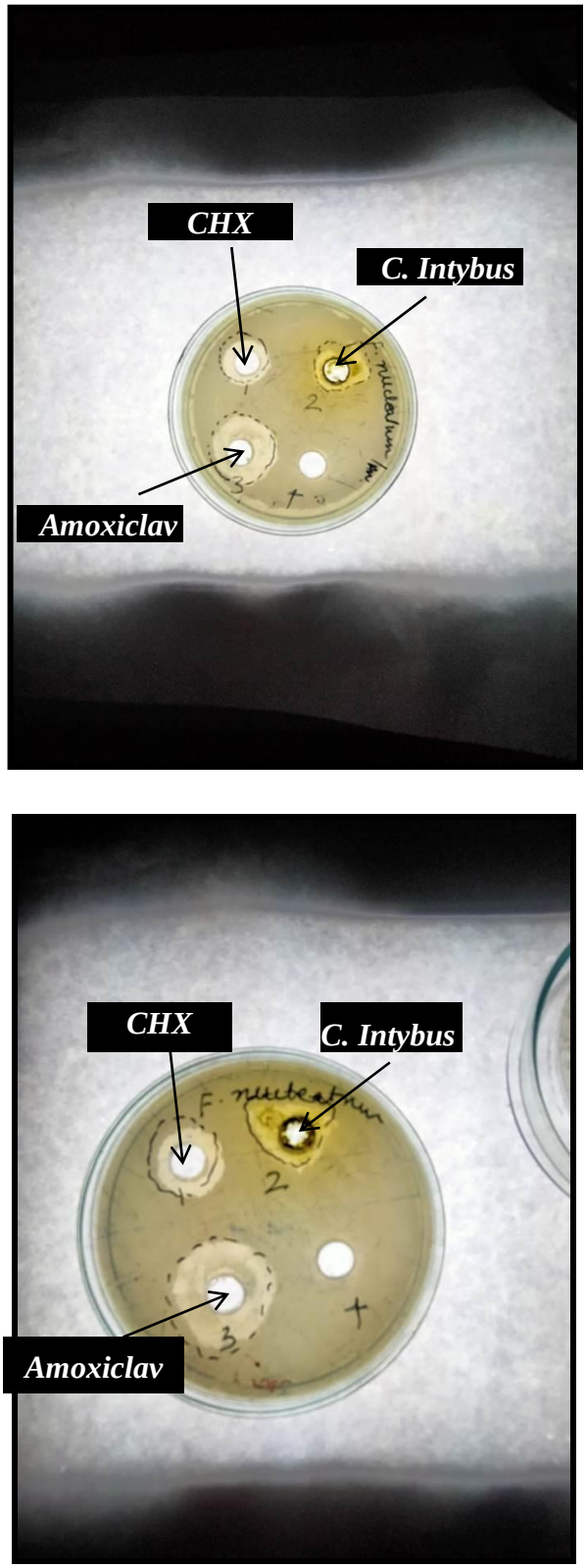


Fig.12: **ZONE OF INHIBITION OF FUSOBACTERIUM NUCLEATUM**

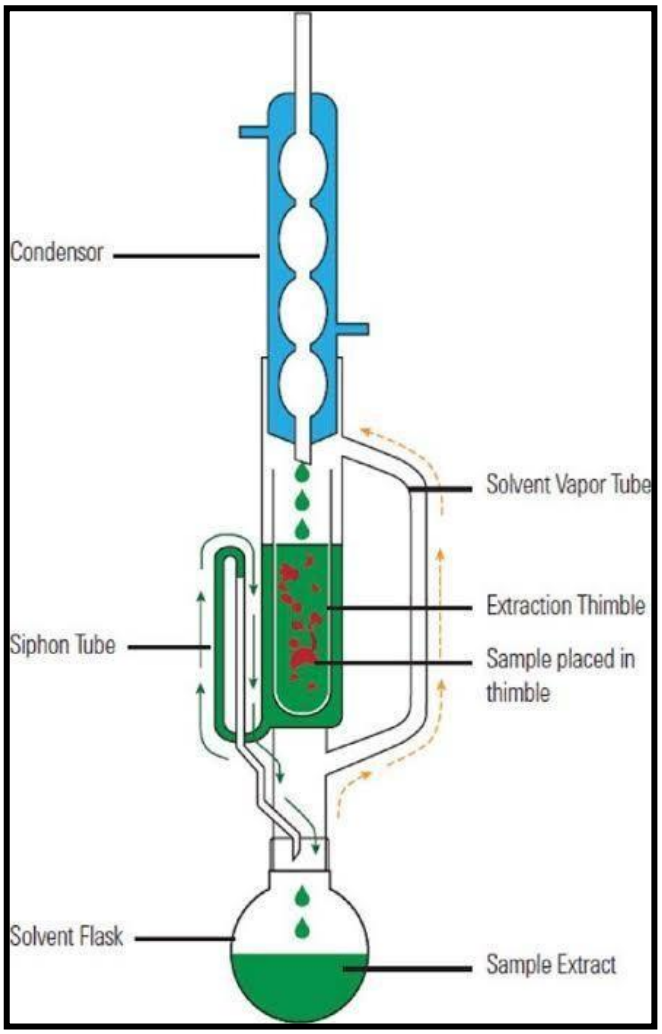
Plate - 8

Fig.13: **MINIMUM INHIBITORY CONCENTRATION PROCESS**

Plate - 9

Theory:

Hot extraction of the medicinal plant was done in Soxhlet apparatus.



In the laboratory, a fat extractor (**Soxhlet extractor**) is used for extraction. The Soxhlet apparatus uses the solvent reflux and siphon principle to continuously extract the solid matter by pure solvent, which saves the solvent extraction efficiency and high efficiency. The solid material is finely ground prior to extraction to increase the area of solid-liquid contact. The solid material is then placed in a filter paper holder and placed in an extractor. The bottom end of the extractor is connected to a round bottom flask containing a solvent, and is connected to a reflux condenser. The bottom flask is heated to boil the solvent, the vapor rises through the branch pipe of the extractor, is condensed and drops into the extractor, and the solvent is contacted with the solid for extraction. When the solvent surface exceeds the highest point of the siphon, the solvent containing the extract is siphoned back. The flask, thus extracting

a portion of the material, is repeated such that the solid material is continuously taken as a pure solvent and the extracted material is concentrated in the flask.

Accurate determination of bacterial susceptibility to antibiotics is essential to the successful management of bacterial infections and to the comparative analysis of antimicrobial agents. This can be done by a number of techniques, which include the disc diffusion method, the broth dilution assay and the E tests. The effectiveness of antibiotics can be assessed by their ability to suppress bacterial growth, described by the MIC, or by their ability to kill bacteria, characterized by the minimal lethal concentration (MLC). MIC is usually derived by means of tests in solid media, whereas both MIC and MLC can be determined in broth dilution assays. A number of reports have been dedicated to comparing the effectiveness of these methods.

The agar diffusion technique is commonly used for determination of MIC in solid media. It involves the application of antibiotic solutions of different concentrations to cups, wells or paper discs, placed on the surface of or punched into agar plates seeded with the test bacterial strain. Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear 'zones' without bacterial lawn. The diameter of these zones increases with antibiotic concentration. The value of MIC is determined as the zero intercept of a linear regression of the squared size of these inhibition zones, plotted against the natural logarithm of the antibiotic concentration.

A number of factors affect the accuracy and reproducibility of the agar diffusion method, including thickness and uniformity of the gel, the choice of cut-off size for the inhibition zones and breakpoints, temperature etc. When these factors are controlled or taken into consideration, analysis of data from the agar diffusion assays relies on theoretical models, which incorporate a number of important additional assumptions. It is important to understand these assumptions, which justify the use of these theoretical models and, at the same time, introduce some limitations in the validity of each model. Theoretical analysis of antibiotic diffusion data by the disc method is built on the assumption that antibiotics diffuse freely and the diffusion-limiting factor is hydrodynamic viscous drag.

RESULTS AND OBSERVATIONS

The current study was conducted in two parts. In the in-vitro analysis the antimicrobial activity of *Chicorium Intybus* was evaluated against two pathogens; *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Also, the minimum inhibitory concentration was tested for both the microorganisms.

Chlorhexidine mouth wash and amoxiclav were taken as the other control groups.

The following treatment groups were evaluated:

Group 1: *C.intybus* extract

Group 2: Chlorhexidine mouthwash

Group 3: Amoxicillin and clavulanic acid (amoxiclav) combination

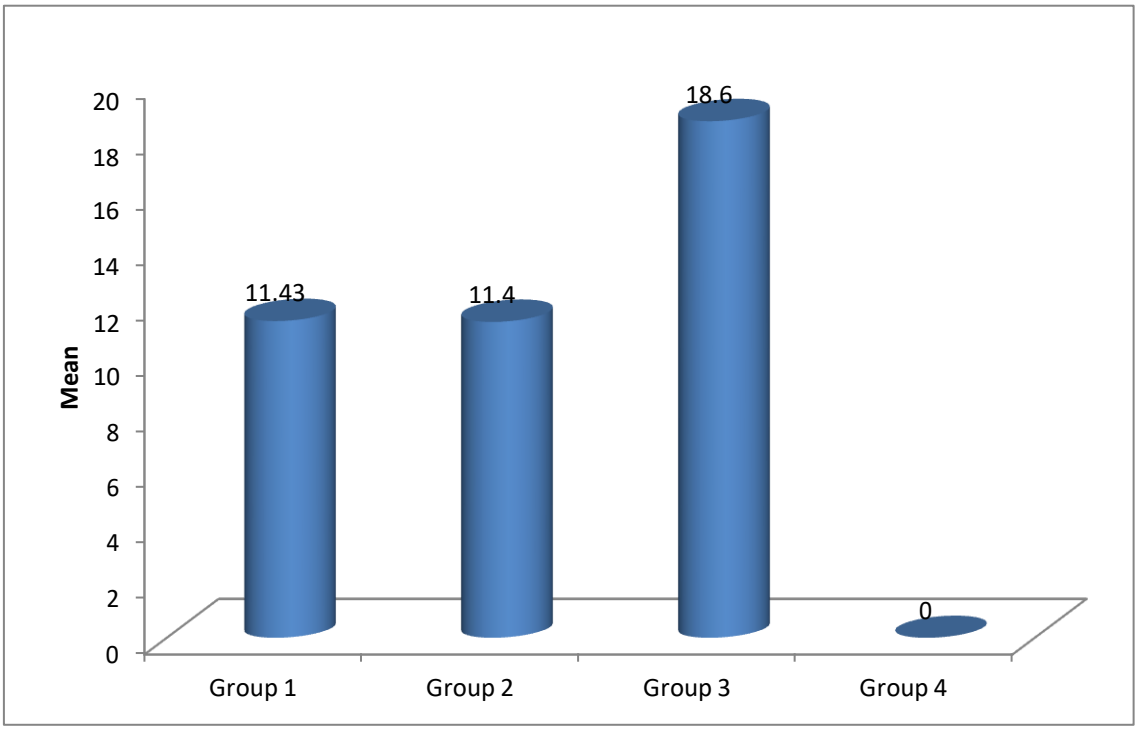
Group 4: distilled water

Data was summarized as Mean ± SD.

Table 1: Zone of inhibition (mm) w.r.t. *P. gingivalis* among the study groups

Group	Mean	SD
Group 1	11.43	0.29
Group 2	11.40	0.17
Group 3	18.60	0.20
Group 4	0	0

Mean ± SD zone of inhibition (mm) w.r.t. *P. gingivalis* was 11.43±0.29, 1I1.40±0.17, 18.60±0.20 and 0 respectively as shown in table 5, graph 3. Hence zone of inhibition was maximum in group 3.



Graph 1: Zone of inhibition (mm) w.r.t. *P. gingivalis* among the study groups

Table 2: Comparison of Zone of inhibition (mm) w.r.t. *P. gingivalis* among the study groups

Groups	t test	p value
Group 1 vs 2	.500	.667
Group 1 vs 3	35.346	.001*
Group 1 vs 4	68.600	<.01*
Group 2 vs 3	47.135	<.01*
Group 2 vs 4	114.000	<.01*
Group 3 vs 4	161.081	<.01*

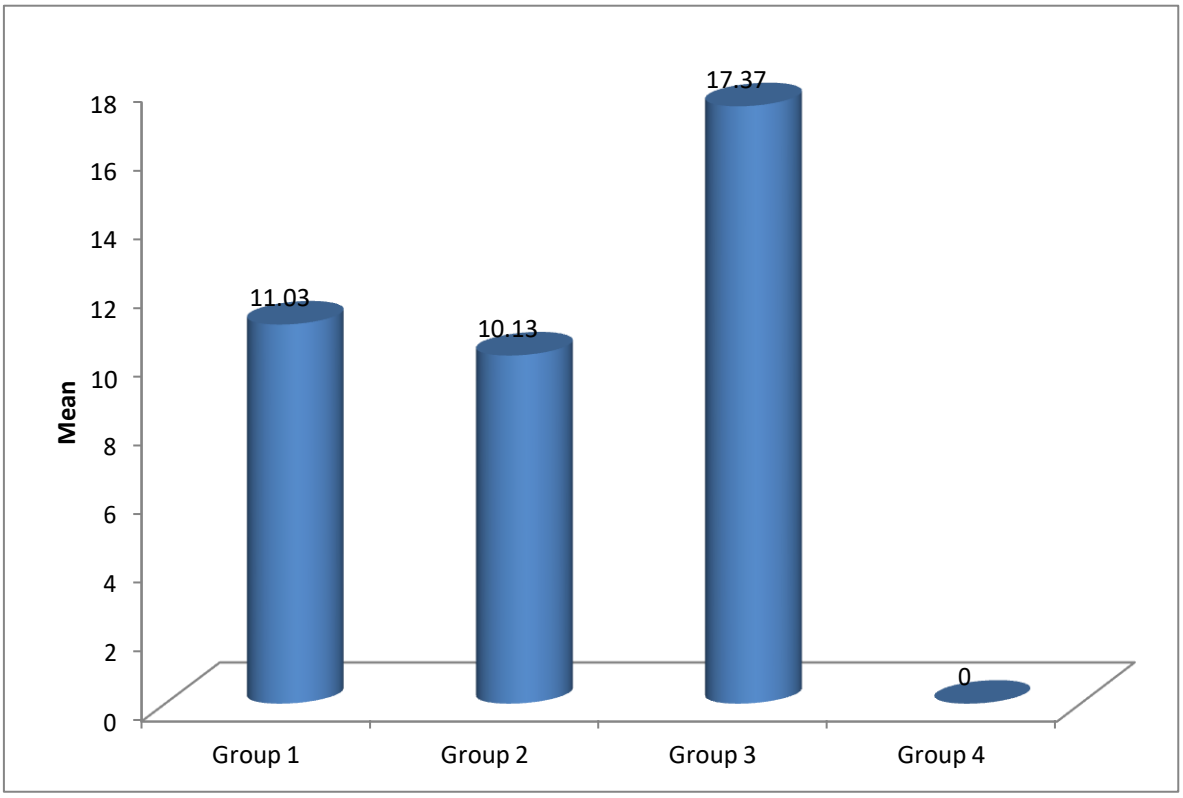
*: statistically significant

Table 2 shows the comparison of zone of inhibition (mm) w.r.t. *P. gingivalis* among the study groups. Statistically significant difference was found when control group was compared with all other groups. No difference was found between group 1 and 2 w.r.t zone of inhibition (mm). When zone of inhibition (mm) w.r.t. *P. gingivalis* between group 3 and group 1 as well as group 2, it was found to be statistically significant as $p<0.05$.

Table 3: Zone of inhibition (mm) w.r.t. *F. nucleatum* among the study groups

Group	Mean	SD
Group 1	11.03	0.70
Group 2	10.13	0.55
Group 3	17.37	0.51
Group 4	0	0

Mean±SD zone of inhibition (mm) w.r.t. *F. nucleatum* was 11.03±0.70, 10.13±0.55, 17.37±0.51 and 0 respectively as shown in table 3, graph 2. Hence zone of inhibition was maximum in group 3.



Graph 2: Zone of inhibition (mm) w.r.t. *F. nucleatum* among the study groups

Table 4: Comparison of Zone of inhibition (mm) w.r.t. *F. nucleatum* among the study groups

Groups	t test	p value
Group 1 vs 2	4.323	.050
Group 1 vs 3	-23.212	.002*
Group 1 vs 4	27.208	.001*
Group 2 vs 3	-108.500	<.01*
Group 2 vs 4	31.868	.001*
Group 3 vs 4	58.617	<.01*

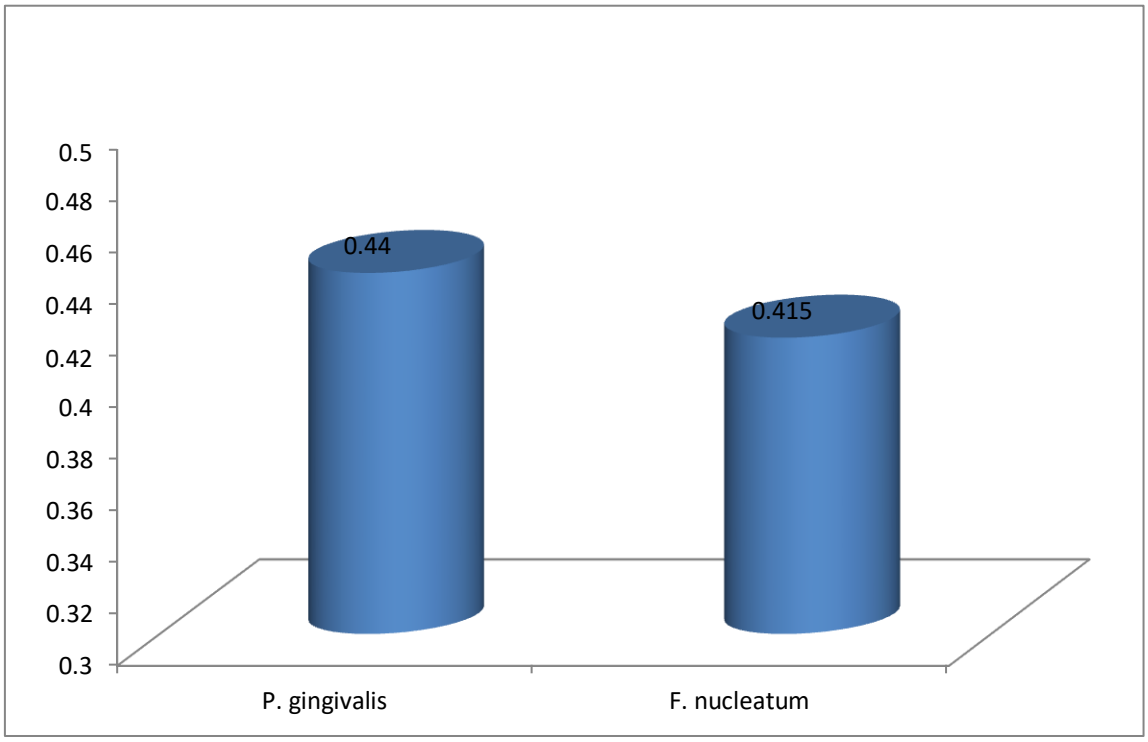
*: statistically significant

Table 4 shows the comparison of zone of inhibition (mm) w.r.t. *F. nucleatum* among the study groups. Statistically significant difference was found when control group was compared with all other groups. No difference was found between group 1 and 2 w.r.t zone of inhibition (mm). When zone of inhibition (mm) w.r.t. *F. nucleatum* between group 3 and group 1 as well as group 2, it was found to be statistically significant as $p<0.05$.

Table 5: OD Value of *Chicorium Intybus* in both anaerobes

Organism	Mean	Std. Error	SD
P. gingivalis	.4400	.06071	.25759
F. nucleatum	.4150	.05326	.22597

Table 5, graph 3 shows the OD of anaerobic pathogens. Mean OD w.r.t. P. gingivalis and F. nucleatum was 0.44 and 0.42 respectively.



Graph 3: OD of anaerobic pathogens

OD values for all the 3 groups:

Table 6: Optical density w.r.t. *P. gingivalis* among the study groups

Group	Mean	SD
Chicorium Intybus extract	0.44	0.26
0.2% Chlorhexidine	0.39	0.22
Amoxiclav	0.47	0.20

Mean±SD Optical density (OD) w.r.t. *P. gingivalis* was 0.44±0.26, 0.39±0.22 and 0.47±0.20 in control, 0.2% Chlorhexidine and Amoxiclav group respectively as shown in table 6 Hence OD was maximum in Amoxiclav group.

Table 7: Comparison of OD w.r.t *P. gingivalis* among the study groups

Groups	t test	p value
Chicorium Intybus extract vs 0.2% Chlorhexidine	2.96	0.009*
Chicorium Intybus extract vs Amoxiclav	1.17	0.26
0.2% Chlorhexidine vs Amoxiclav	4.26	0.001*

*: statistically significant

Table 7 shows the comparison of OD w.r.t *P. gingivalis* among the study groups. Statistically significant difference was found when control group was compared with 0.2% Chlorhexidine group. No difference was found between control and Amoxiclav group. When OD w.r.t.

P. gingivalis was compared between 0.2% Chlorhexidine and Amoxiclav group, it was found to be statistically significant as $p<0.05$.

Table 8: Optical Density w.r.t. *F. nucleatum* among the study groups

Group	Mean	SD
Chicorium Intybus extract	0.415	0.23
0.2% Chlorhexidine	0.52	0.26
Amoxiclav	0.55	0.21

Mean±SD OD w.r.t. *F. nucleatum* was 0.415±0.23, 0.52±0.26 and 0.55±0.21 in control, 0.2% Chlorhexidine and Amoxiclav group respectively as shown in table 8, Hence OD was maximum in Amoxiclav group.

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Table 9: **Comparison of Optical Density w.r.t. *F. nucleatum* among the study groups**

Groups	t test	p value
Control vs 0.2% Chlorhexidine	3.86	0.001*
Control vs Amoxiclav	5.88	<0.01*
0.2% Chlorhexidine vs Amoxiclav	1.36	0.19

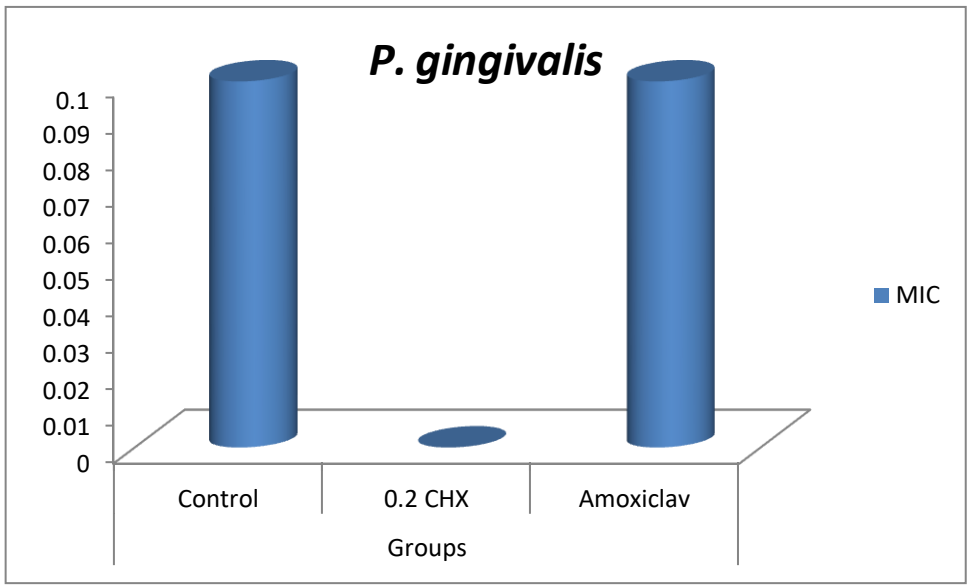
*: statistically significant

Table 9 shows the comparison of OD w.r.t *F. nucleatum* among the study groups. Statistically significant difference was found when control group was compared with 0.2% Chlorhexidine group and Amoxiclav group. No difference was found between 0.2% Chlorhexidine and Amoxiclav group.

MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES:

TABLE 10. MIC for *P. gingivalis*

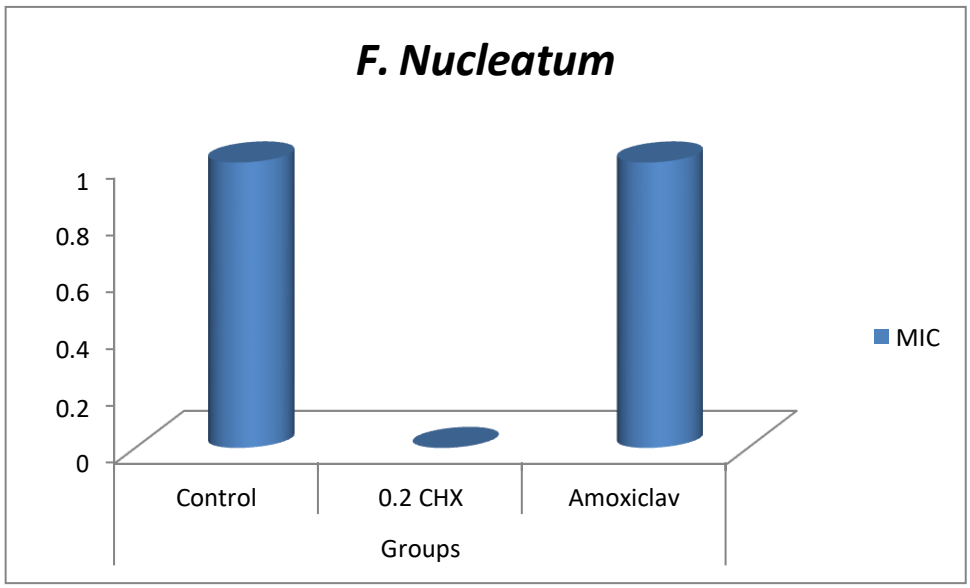
Group	Mean MIC
Chicorium Intybus extract	0.1 mg/ml
0.2 CHX	0.0002 %
Amoxiclav	0.1 mg/ml



Graph 4: Indicates that MIC for *P. gingivalis* among all the groups. *Chicorium* (0.1 mg/ml) *Intybus* showed the equal MIC value to that of Amoxiclav (0.1mg/ml), Chlorhexidine (0.0002%) being lowest in MIC.

TABLE 11. MIC for *F. nucleatum*

Group	Mean MIC
Chicorium Intybus extract	1 mg/ml
0.2 CHX	0.00002 %
Amoxiclav	1 mg/ml



Graph 5: shows that MIC of Chicorium *Intybus* (1mg/ml) is equivalent to Amoxiclav (1mg/ml) and higher than Chlorhexidine (0.00002%) in case of *F. nucleatum*.

DISCUSSION

Periodontitis is a multifactorial disease which starts with inflammatory lesions in gingiva, which, if left without treatment, may progress and eventually involve and compromise the entire periodontal apparatus of the affected teeth. The progression of periodontitis can be controlled by conventional periodontal therapy as mechanical debridement by scaling and root planning and further by use of chemical plaque control agents as adjunctive.[31]

Broad spectrum antimicrobial mouth rinses have gained popularity as adjuncts to conventional periodontal therapy. **Loe and schiott in 1970** established the potential for microbial mouth rinses in clinical practice using an experimental gingivitis study model and found that 0.2% chlorhexidine mouth wash can effectively prevent plaque and gingivitis in the absence of other chemical and mechanical oral hygiene methods.[32]

We used chlorhexidine in our study as positive control as it is considered as the gold standard against which the efficacy of alternative anti-plaque agents can be measured. It exhibits both anti-bacterial and anti-plaque properties and acts by altering integrity of cell membrane of bacteria. Substantivity is an important property in chlorhexidine, which refers to its oral retentiveness.

However, its extended use causes local side effects like brown discoloration of teeth and tongue, altered taste, oral mucosal ulcerations which actually reduces its acceptability in patients.

Due to these limitations a continuous search for alternative is being carried out. Nature has been a source of medicinal plants for years. A number of studies have been done to explore the medicinal properties of plants in a quest to formulate new, economical, widely available antibacterial agent with fewer side effects.

Hence effectiveness of one medicinal plant; *Chicorium Intybus* was assessed against periodontal pathogens – *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*

C.Intybus commonly known as 'kasni' and 'chicory'. About 64 compounds have been extracted from chicory. Chicory leaves contains anthocyanins, potassium, calcium, phosphorus, phenolic acid and flavonoids.[3,23]

M Saeed et al in his research in 2017 suggested that Chicoric acid extracted from chicory may reduce inflammation and bacterial infections. Chicorium intybus is a perennial herbaceous plant which is less frequently studied than the majority of plants. Its leaves have a range of uses, including as salad, as fodder plant, feed additives and as a medicine for the preparation of infusions and decoction i.e., a medicinal concentration prepared by heating.[33]

Its roots contain maximum of inulin and other carbohydrates so it is used in the preparation of substances like chewing gums. Chicory is considered as versatile medicinal plant as it possesses antidiabetic, hepatoprotective, sedative, immunological, reproductive, cardiovascular, analgesic, antimicrobial, antitoxic, antiulcerogenic, anticarcinogenic and anti-inflammatory properties. These properties have been known for years, even the ancient Romans and Greeks used *Chicorium Intybus* as medicine.[34,38]

According to **N M Abd El-Mageed (2011) and A Mushtaq et al (2013)**[36], the health benefits of chicory are from its peculiar phytochemical composition, as it has high content of flavonoids, anthocyanins, cinnamic and quinic acids. This plant is gaining attention because of its low cost and high polyphenol content. *Chicorium Intybus* is used as prebiotics against some species of pathogenic bacteria for both *in vitro* and *in vivo*. **Mushtaq et al**. found the hepatoprotective activity of aqueous-ethanolic extract of fresh dried leaves of chicory with different concentrations. The significant effects were seen in biochemical parameters regarding the liver enzymes such as alanine phosphatase, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and total bilirubin.

Various studies on antibiotics and herbal medicines susceptibility patterns of *Porphyromonas gingivalis, Fusobacterium nucleatum* and other periodontopathogenic bacterial strains have been studied from decades in which Minimum inhibitory concentration and resistance tests are performed.

To best of our knowledge, there is no relevant history of any study performing these tests on oral bacteria using *Chicorium Intybus* to initiate further use in our oral cavity. So, an *in vitro* study was carried out to evaluate the antibacterial activity of medicinal plant extract against two periodontal anaerobic pathogens namely *P. gingivalis* (ATCC 33277) and *F. nucleatum* (ATCC 25586). Also, the minimum inhibitory concentration of herbal extract was evaluated for both the bacterial growth.

For the determination and comparison of the antimicrobial activity of the chlorhexidine, amoxiclav and *Chicorium Intybus* extracts, the zone of inhibition was observed against the two bacterial strains by well diffusion method. The extent of the antimicrobial activity was based on the diameter of the zone of inhibition. The antimicrobial activity of the extract dissolved in distilled water was carried out in triplets for detailed analysis. CHX and Amoxiclav was taken as positive controls and distilled water as negative control. Clear zone for distilled water was observed indicating that it does not have pharmacological activity of their own.

The data of the In-vitro study was statistically analysed and it was observed that the mean zone of inhibition for *P. gingivalis* was highest in group 3 (18.60±0.20), followed by group 1 (11.43±0.29), group 2 (11.40±0.17) and group 4 (0 as negative control).

For *F. nucleatum*, the mean zone of inhibition was highest in group 3 (17.37±0.51), followed by group 1(11.03±0.70), group 2(10.13±0.55) and group 4 (0).

On the basis of this present study, comparing individual group with each other, it was observed that the comparison of zone of inhibition (mm) w.r.t *P. gingivalis*, no difference was found between group 1 and 2 and it is considered as non-significant as p value > 0.05. Inter-comparison between group 3 with group 1 and group 2 was found to be statistically significant as $p<0.05$. Similar result was observed for *F. nucleatum* in inter group comparison.

In current study, test was done to evaluate Minimum inhibitory concentration. MIC are considered 'Gold Standard' for evaluating the susceptibility of microbes towards antimicrobials and are used to evaluate the performance of other methods of

susceptibility tests. MICs are used in to confirm unusual resistance, to give a fixed value when borderline result is obtained by other method of testing or when disc diffusion method is not required.

MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of a microbe or organism after overnight incubation, although some anaerobes require extended time for this.

3ml of MRS broth media was prepared in 6 test tubes and after autoclaving at 21˚C for 15 minutes at 15 psi, it was set to cool at room temperature. Serial wise distribution of extract was done by taking 0.3 ml volume in which 20 µl bacterial culture was inoculated and it was incubated at 37˚C for anaerobic conditions.

Optical density was taken at 620nm. Minimum inhibitory concentration of Chicorium Intybus extract was evaluated and calculated thrice to remove standard error. Initially an optical density (OD) value is seen for each test tube and then the MIC is measured according to the McFaland standards. The mean OD for *Porphyromonas gingivalis* was found to be 0.44±0.25 and OD for *Fusobacterium nucletum* was 0.41±0.22.[40,46] Statistically significant difference was found when control group was compared with 0.2% Chlorhexidine group. No difference was found between control and Amoxiclav group. When OD w.r.t. *P. gingivalis* was compared between 0.2% Chlorhexidine and Amoxiclav group, it was found to be statistically significant as $p<0.05$.

Statistically significant difference was found when control group was compared with 0.2% Chlorhexidine group and Amoxiclav group. No difference was found between 0.2% Chlorhexidine and Amoxiclav group.

The mean MIC of *Chicorium Intybus* for *Porphyromonas gingivalis* was 0.1 mg/ml. and for *Fusobacterium nucleatum* was 1 mg/ml.

The mean MIC of 0.2% CHX for *Porphyromonas gingivalis* was 0.0002 % mg/ml. and for *Fusobacterium nucleatum* was 0.00002 % mg/ml

And the mean MIC of Amoxiclav for *Porphyromonas gingivalis* was 0.1 mg/ml and for *Fusobacterium Nucleatum was* 1 mg/ml.

This indicates that MIC of Chicorium Intybus and Amoxiclav is equal in both the periodontal pathogens.

BR Chandrashekhar et al in 2018, assess the antimicrobial efficacy of various herbs and their combinations on *F. nucleatum* and *P. gingivalis*, along with 0.2% CHX as positive control group. The zone of inhibition of CHX in *P. gingivalis* was 19.50(0.55) mm and in *F. nucleatum* it was 18.08 (1.02) mm.

Md. Jalaluddin et al in 2019 evaluated the antimicrobial activity of *Curcuma Longa* extract in comparison to CHX being positive control group. Zone of inhibition of CHX in *P. gingivalis* was 24.92±1.22.[40]

Taking into account the result in our study, the difference in values is because of the different culture media used, different techniques, conditions and laboratories experimenting protocols.

Different studies have been done **[I. Milazzo et al (2003) and Eva M. Kulik et al (2019)]**[41,2] to evaluate MIC of Amoxiclav on *P. gingivalis* and *F. nucleatum* since years which ranges from 0.03-0.6 mg/L and 0.03-0.5 mg/L respectively in comparison to 0.1 mg/ml and 1mg/ml in our study. In-Vitro studies over the years potent antimicrobial activities are shown by Amoxiclav against periodontal pathogens in comparison to other antimicrobial drugs.

Yuuki Sakaue et al (2016) studied antibiofilm bactericidal effects of few herbs on periodontal pathogen grown in BHI growth media. MIC at 620nm of 0.2% CHX which was found to be 0.25 µg/ml. **In 2017, Roza Haghgoo [43]** conducted an In Vitro study comparing antibacterial effect of CHX and different concentration of Cyperus rotundus extract. Pathogens were grown on muiller hinton blood agar media. MIC of CHX was 0.5 mg/ml[42].

Rushali Ramdas Khobragade et al in 2020, compared Indigenous mouthwash with 0.2% CHX in prevention of plaque and gingivitis. They conducted this research in vitro as well as in vivo. In an In-vitro study *P.gingivalis*, *B.forsythus*, *S.mutans* and *Lactobacilus* grown in BHI broth were tested for MIC and efficacy of CHX and indigenous mouthwash. They found CHX has highest zone of Inhibition and MIC for *P.gingivalis* i.e.; 25mm and12.5mg/ml, respectively[44].

So previous literature proves that CHX and Amoxiclav are effective against periodontal pathogens. But these findings reinforce the earlier findings that variation in the media can affect the MIC values of a compound and that MIC values are method dependent. It may be that the constituents of the agar media could have influenced some of the antimicrobial properties of the different test groups. The presence exogenous proteins in the media or the ability of media components to reduce the antimicrobial activity or the binding of the mouthwash components to protein in the media can influence the antimicrobial efficacy.

On the basis of our present in-vitro study it was observed that the effect of *Chicorium Intybus* was as good as chlorhexidine. *C.Intybus* has demonstrated almost similar effect in the test involved. Thus, it can prove to be beneficial and more cost-effective alternative to CHX.

However, it was the first attempt to assess the effects of *Chicorium Intybus* for the further use in periodontitis patients in future, and so the current study had certain limitations. The study was limited to analyse the antimicrobial effects on two periodontal pathogens only. More pathogens causing periodontal disease could be examined in future studies using *C. Intybus.*

The gross extract was used, prepared by leaves including all its contents. Technologies can be used to extract the individual different anti-inflammatory and anti-microbial components, without using non-essential elements present in herbal extract. This may increase its efficacy further.

Due to present situation of pandemic spread of corona virus, the study was not able to extend for the patients of chronic periodontitis. So, a crossover trial with a wash out period could have been done using a larger sample size.

CONCLUSION

Within limitations, the present study concluded that:

- *C.Intybus* inhibited the two periodontal pathogens; *P. gingivitis* and *F. nucleatum* as shown in this study.
- *C.Intybus* had similar anti-microbial effects as CHX.
- Amoxiclav proved to be more effective than *C.Intybus* and CHX

Hence, evaluating the results it can be concluded that *C. Intybus* has better or similar effects as compared to CHX, so it can be effectively use as an adjunct to mechanical periodontal therapy in future studies.

Also, further clinical studies may play an imperative role to investigate the properties of the medicinal plant used.

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ANNEXURE 1.DATA COLLECTION

SENSITIVITY TEST

Under Anaerobic conditions

Pathogens	Zone of inhibition (mm)			
	1	2	3	4
P. gingivalis	11.1	11.2	18.6	0
P. gingivalis	11.6	11.5	18.4	0
P. gingivalis	11.6	11.5	18.8	0
F. nucleatum	11.1	10.5	17.8	0
F. nucleatum	10.3	9.5	16.8	0
F. nucleatum	11.7	10.4	17.5	0

OPTICAL DENSITY VALUES OBTAINED

For Chicorium Intybus

Pathogens	OD 620 nm					
	P. gingivalis			*F. nucleatum*		
1	0.11	0.14	0.15	0.14	0.19	0.18
2	0.23	0.21	0.25	0.19	0.29	0.24
3	0.29	0.25	0.31	0.29	0.36	0.26
4	0.48	0.52	0.52	0.35	0.54	0.37
5	0.59	0.69	076	0.55	0.79	0.48
6	0.72	0.75	0.95	0.69	0.88	0.68

0.2 % Chlorhexidine:

Pathogens	OD 620 nm					
	P. gingivalis			*F. nucleatum*		
1	0.11	0.13	0.14	0.13	0.18	0.17
2	0.21	0.23	0.28	0.32	0.35	0.36
3	0.26	0.29	0.34	0.39	0.42	0.41
4	0.39	0.34	0.48	0.53	0.59	0.59
5	0.46	0.51	0.62	0.77	0.67	0.83
6	0.66	0.73	0.84	0.83	0.89	0.97

Amoxiclav:

Pathogens	OD 620 nm					
	P. gingivalis			*F. nucleatum*		
1	0.25	0.19	0.21	0.29	0.21	0.25
2	0.38	0.35	0.3	0.34	0.35	0.37
3	0.47	0.39	0.33	0.47	0.58	0.48
4	0.58	0.43	0.43	0.56	0.68	0.62
5	0.66	0.54	0.59	0.68	0.74	0.76
6	0.73	0.79	0.84	0.75	0.84	0.88

MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES

FOR *P. GINGIVALIS*

Group	**Mean MIC**
Chicorium Intybus extract	0.1 mg/ml
0.2 CHX	0.0002 %
Amoxiclav	0.1 mg/ml

MIC FOR *F. NUCLEATUM*

Group	Mean MIC
Chicorium Intybus extract	1 mg/ml
0.2 CHX	0.00002 %
Amoxiclav	1 mg/ml

ANNEXURE 2.

STATISTICAL ANALYSIS

Data were tabulated and examined using the Statistical Package for Social Sciences Version 22.0 (IBM SPSS Statistics for Mac, Armonk, NY: IBM Corp, USA). Descriptive statistical analysis had been carried out in the present study. Results on continuous measurements are presented as Mean±SD. Categorical data has been presented as frequency distribution. The statistical power calculation was based on the assumption that the data were normally distributed. P-value of <0.05 was considered as significant. Difference between two groups was determined student T test for continuous data.

The statistical analysis for the present study was done by applying the following formulae:

1. **Mean**: The mean (or average) is the most popular and well-known measure of central tendency. It can be used with both discrete and continuous data, although its use is most often with continuous data. The mean is equal to the sum of all the values in the data set divided by the number of values in the data set. So, if we have n values in a data set and they have values x_1, x_2, ..., x_n, the sample mean, usually denoted by $\bar{x}$ (pronounced x bar), is:

$$\bar{x} = \frac{(x_1 + x_2 + \cdots + x_n)}{n}$$

This formula is usually written in a slightly different manner using the Greek capitol i.e.:

Sample Mean	Population Mean
$\bar{x} = \frac{\Sigma x}{n}$	$\mu = \frac{\Sigma x}{N}$

where Σx is sum of all data values

N is number of data items in population

n is number of data items in sample

2. **Standard deviation**: the standard deviation (SD, also represented by the lower case Greek letter sigma σ or the Latin letter s) is a measure that is used

to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}}$$

σ = **lower case sigma**
$\sum$ = **capital sigma**
$\bar{x}$ = **x bar**

3. **t test**: A student *t*-test is any statistical hypothesis test in which the test statistic follows a Student *t*-distribution under the null hypothesis. It can be used to determine if two sets of data are significantly different from each other. It is most commonly applied when the test statistic would follow a normal distribution if the value of a scaling term in the test statistic were known. When the scaling term is unknown and is replaced by an estimate based on the data, the test statistics (under certain conditions) follow a student's *t* distribution.

ANNEXURE 3

IRC

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

(Revised)

The project titled **"Evaluation of Efficacy of Chicorium Intybus Extract on Periodontal Pathogens: An *In-Vitro* Study."** submitted by **Dr Shikha Singh** Post graduate student from the **Department of Periodontology** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **21th June, 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE 4

IEC

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 29 (Revised) **BBDCODS/07/2021**

Title of the Project: Evaluation of Efficacy of *Chicorium Intybus* Extract on Periodontal Pathogens: An *In-Vitro* Study.

Principal Investigator: Dr. Shikha Singh **Department:** Periodontology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Shikha Singh,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 2nd July, 2021.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 02/07/21

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

ANNEXURE 5

AUTHENTICATION LETTER

सीएसआईआर - राष्ट्रीय विज्ञान संचार एवं सूचना स्रोत संस्थान
CSIR - National Institute of Science Communication and Information Resources
वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद् Council of Scientific & Industrial Research
(विज्ञान एवं प्रौद्योगिकी मंत्रालय, भारत सरकार Ministry of Science & Technology, Govt. of India)
NISCAIR

RAW MATERIALS HERBARIUM AND MUSEUM, DELHI (RHMD)

Authentication No.-NISCAIR/RHMD/Consult/2019/3429-30 **22 /04/2019**

CERTIFICATE FOR CRUDE DRUG SAMPLE AUTHENTICATION

This is to certify that leaf sample of *Chicorium intybus*, Chicory, Kasni, received from Dr Shikha Singh vide letter No. Nil, Dated 18th April 2019 for authentication has been found **correct as leaf of *Cichorium intybus* L. which is commonly known as Chicory, Succory, Wild endive, Kasni.** The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic samples deposited in the Raw Material Herbarium and Museum, Delhi (RHMD).

Identification pertains to the quantity/quality of specimen/sample(s) received in RHMD. This certificate is not issued for any judicial purpose.

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ANNEXURE 6
WORK CERTIFICATE

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To Whom It May Concern

This is to certify that ***Dr. Shikha Singh****, final year MDS,* ***Babu Banarasi Das College of Dental Sciences,*** *has done her Antibiotic sensitivity test (Agar well diffusion method) & MIC test in Microbiology Department MRD LifeSciecnes Pvt. Ltd. Lab.*

Pallavi Sharma
Research Scientist
MRD LifeSciences Pvt. Ltd.
Lucknow

MRD LifeSciences Pvt. Ltd. Lucknow

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ANNEXURE 7

PLAGIARISM REPORT

Curiginal

Document Information

Analyzed document	urkund.docx (D110044893)
Submitted	7/2/2021 10:40:00 AM
Submitted by	Dr. Vandana Pant
Submitter email	drvandanapant@bbdu.ac.in
Similarity	2%
Analysis address	drvandanapant.bbduni@analysis.urkund.com

Sources included in the report

W	URL: https://healthdocbox.com/Dental_Care/119023934-J-lasers-med-sci-2016-winter-7-1-21-25.html Fetched: 12/10/2020 1:22:15 PM	1
W	URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5813340/ Fetched: 7/17/2020 7:19:53 AM	1