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Research Article

Antimicrobial Activity Of *Morus Alba* Leaf Extract Against Potent Oral Pathogens Isolated From Different Salivary Samples Of Individuals

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ABSTRACT

Over 700 species of microorganisms have been identified to exist in the oral cavity of healthy humans, and imbalance of host–microbial homeostasis can cause various oral diseases. Gram-negative anaerobic bacteria have been associated with periodontal diseases. In this study we investigated the possible antimicrobial activity of *Morus alba* leaf extract against oral bacterial and fungal microbiota isolated from saliva samples of 30 different individuals of different age categories with different physical health conditions- diabetes, COVID-19 and healthy. Herein, we compared the after clearance of COVID-19 recovered patients to those of healthy individuals with Type 2 Diabetes Mellitus patients. We exploit potential associations between some microorganisms and variables like teeth brushing and smoking habits. The phytochemical analysis was performed on qualitative basis indicated the presence of alkaloids, glycosides, flavonoids, steroids, tannins, saponins and Anthraquinone. Morus alba leaf extracts can form an excellent candidate for the management of dental caries can limits the transfer of multidrug resistance among the commensal oral microbes. We found common oral pathogens *Streptococcus mutans, Lactobacillus acidophilus, Candida albicans, Enterococcus faecalis, Escherichia coli, Salmonella Typhi, Shigella dysenteriae, Staphylococcus aureus, Bacillus subtilis, Pasturella multocida and Vibrio cholerae*. In testing the potent antibacterial activity against *Escherichia coli, Salmonella Typhi, Shigella dysenteriae, Staphylococcus aureus and Vibrio cholerae*, the mulberry extracts proved to be quite efficient, especially following water extraction. It thus appears that mulberry leaves can potentially be consumed as a good source containing antimicrobial

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properties against some pathogenic bacteria which cause dental, respiratory, and gastrointestinal tract infections.

INTRODUCTION

The human verbal depth is occupied by over 700 distinctive bacterial species, a few of which are disease-causing pathogens that can initiate gastrointestinal brokenness and tooth misfortune [1]. White spot injuries that create due to Streptococcus mutans (S. mutans) maintenance in plaque can amass around orthodontic brackets and lead to finish demineralization, based on thinks about utilizing mimicked cariogenic situations [2]. When streptococci gather in the mouth (by means of connection to the at first obtained pellicle), plaques (comprising the bacterial cells, glycoproteins, and polysaccharides) can shape. This at that point produces acids, which frame a strong cluster and cause demineralization [3,4]. Moreover, verbal pathogens too actuate dental mash malady and parasitic contaminations [5], and can cause both verbal and systemic maladies [6]. In this way, substances that can specifically repress dental microbes are essential to avoid infections caused by verbal microorganisms. To anticipate and/or smother the movement of bacterial verbal illnesses, the causal pathogen must be killed; this is ordinarily accomplished with mouth washes or antibiotics. Chlorhexidine gluconate (CHX) is a broadly utilized operator in mouthwashes that is compelling in avoiding plaque arrangement and verbal microbial development [7]. Octenidine (OCT) altogether repressed plaque arrangement, gingivitis, and verbal microbial development, and was more compelling than placebos and other common chemical operators utilized for plaque control [7]. Be that as it may, as of now utilized antimicrobial specialists such as CHX and OCT can apply destructive impacts on the body after long-term utilize, as well as having different side impacts, counting microbial medicate resistance [8,9]. To overcome these confinements, normal home grown extricates with tall pharmacological adequacy and moo harmfulness have as of late been connected to hinder the development of verbal pathogens and anticipate verbal illnesses [10,11]. For occasion, flavanone and flavanol (confined from normal home grown extricates) can hinder the glucosyltransferases that cause dental caries [12], whereas normal mulberry [13], green tea [14], and cardinal [15] extricates can repress verbal pathogens. Streptococcus mutans are Gram-positive microbes show in the verbal depression which is considered as the initiator organism for dental caries [16]. Streptococcus mutans actuates corrosive generation, the beginning occasion of caries arrangement, initiates other organisms like Lactobacillus acidophilus, Candida albicans and Enterococcus faecalis to shape a complex microbial community. The intuitive and colonization of other organisms with Streptococcus mutans are interceded through biofilm arrangement [17]. The glucans and the acids created by the Streptococcus mutans are past the buffering capacity of the salivary liquids. The related drop in pH of the target location clears way for the end of non-cariogenic commensal organisms. Being acidogenic and aciduric, Streptococcus mutans encourage the coaggregation and multiplication of the succeeding cariogens and leads to biofilm arrangement, demineralization and at last the cavitation of the tooth [18]. The end of spearheading biofilm initiators like Streptococcus mutans from the tooth surface can be a promising methodology for the anticipation of dental plaque amassing driving to cavitation. Endodontic irrigants such as chlorhexidine hypochlorite and other antimicrobial specialists against the cariogenic organisms moreover force genuine dangers like multidrug resistance which put forward the require of an effective antimicrobial specialist [19]. The non-specificity and wastefulness of current anti-microbials and the

plasmid interceded medicate resistance are the major rising challenges in dentistry. Subsequently particular antimicrobials to kill essential colonizers and prevention of multidrug resistance are well acknowledged. Phytochemicals are picking up consideration in dentistry and pharmaceutical owing to their flexible restorative values and negligible side effects. Morus alba (commonly known as mulberry or Tut) has a place to the Moraceae family is being developed all through the world for silk industry [20]. Ethano-medical properties of Morus alba is flexible which is apparent from its broad utilize in conventional therapeutic framework [21,22]. The plenitude of such restorative properties coined the title 'Kalpavriksha' to M. alba in India. The plant is a collection for copious dynamic phytoconstituents like; tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, and alkaloids. Prenylated flavonoids like moralbanone, kuwanon S, kuwanon G, kuwanon H, mulberroside C, cyclomorusin, eudraflavone B hydroperoxide, oxydihydromorusin, leachianone G and α- acetyl-amyrin from the root of Morus alba are moreover noteworthy [23]. Morus alba has moreover been hailed for its strong antimicrobial properties. Customarily Morus alba is chewed in toothache in arrange to maintain a strategic distance from advance cavitation of the carious tooth [24]. De Oliveira et al., detailed that the ethanolic extricate of Morus alba have both antibacterial and antifungal movement [25]. A few phenolic compounds like flavonoids, stilbenes and 2-arylbenzofurans contained in M. alba are great antimicrobials. Antimicrobial action of Morus alba is too credited to the phyto constituents like kuwanon C, mulberrofuran G, and albanol B [23]. Morus alba has been detailed to have therapeutic impacts against dental caries and hinder different verbal pathogens. The mulberry tree (Morus alba) appeared in Figure 1, an financially and environmentally noteworthy plant species with a worldwide nearness, serves different parts [26]. Strikingly, it is a imperative asset for the silk industry as the essential nourishment source for silkworms [26]. Past this conventional application, its takes off are moreover utilized as bolster supplements for animals and poultry due to their wealthy protein substance [26,27]. Besides, mulberry's tall dietary esteem and bioactive components make it a sought-after fixing in human nourishments and in different details of conventional Chinese pharmaceutical, hence underlining its multifaceted significance over divisions. Be that as it may, the generation of mulberry is debilitated by various illnesses, with bacterial shrink caused by Rp being one of the most damaging soil-borne maladies that limits solid generation [28,29]. The beginning episode of mulberry bacterial shrink was reported in 1969, beginning from Shunde City inside Guangdong territory, China. Since that essential revelation, the infection has continuously spread to include the larger part of mulberry development districts over Guangdong and past, influencing different parts of the nation [30].

Scientific classification

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots Clade: Rosids
Order: Rosales
Family: Moraceae
Genus: Morus
Species: M. alba
Binomial name: Morus alba

Figure 1: Mulberry Tree.

Mulberry Leaf Profile

The leaves are alternate, simple, broadly oval in shape, sharp pointed, 3 to 5 inches long with toothed margins. They are variable in shape, unlobed, 2-lobed, 3-lobed or multiple lobed. Usually, the different leaf shapes will be present on the same tree; occasionally, only a single leaf shape will occur on a tree.

Figure 2: Mulberry Leaf.

Table 1: Micromorphology of in mulberry leaf.

Number of stomata (per mm²)	Number of trichomes (per mm²)	Number of idioblasts (per mm²)
1063.80 ± 15.41	55.00 ± 1.00	41.20 ± 1.16

Table 2: Microscopy of in mulberry leaf.

Leaf thickness (µm)	Thickness of palisade parenchyma (µm)	Thickness of spongy parenchyma (µm)	Percentage of palisade parenchyma in mesophyll (µm)
263.77 ± 5.17	143.66 ± 2.42	71.83 ± 1.24	67.97 ± 1.48

One of the most predominant maladies affecting the teeth and the supporting tissues, such as the bone, periodontal tendons (PDL), and cementum, is periodontitis [31]. In grown-up populaces, the predominance of periodontal malady, especially in its mellow to direct shapes, is critical [32]. In differentiate, the rate of its extreme shape increments with age, especially between the third and fourth decades of life [33]. A few components contribute to periodontal illness [34]. The bacterial biofilm that creates on dental surfaces and its byproducts have been recognized as the essential cause of periodontitis [35-37]. By decimating the connections encompassing the tooth, the poisons discharged by periodontal pathogens have a pivotal affect on the onset of periodontal infection [38]. Periodontal pathogens classified as the “red complex” are most habitually related with the graduation and improvement of perio- (odoús implies “a tooth”) + -itis. When cleared out untreated, periodontitis spreads from the gingival incendiary reaction to the more profound tissues, changing the bone’s homeostasis and pulverizing the connective tissue connection, coming about in

the misfortune of alveolar bone, ultimately leading to tooth misfortune [39]. In grown-up populaces, the predominance of periodontal infection, especially in its gentle to direct shapes, is critical [38]. In differentiate, the frequency of its extreme shape increments with age, especially between the third and fourth decades of life [37]. A few components contribute to periodontal illness [39]. The bacterial biofilm that creates on dental surfaces and its byproducts have been recognized as the essential cause of periodontitis [37-39]. By pulverizing the connections encompassing the tooth, the poisons discharged by periodontal pathogens have a pivotal affect on the onset of periodontal infection [10]. Periodontal pathogens classified as the "red complex" are most habitually related with the graduation and improvement of periodontal infections among all the bacterial complexes found in biofilm. T. denticola, P. gingivalis, and T. forsythia are the three bacterial species that make up the ruddy complex. The coexistence and lifted levels of all the pathogens in the ruddy complex have been recognized in organize III and IV periodontitis [40,41]. On the other hand, organize IV periodontitis of the molar incisor and a few organize III and IV periodontitis are commonly related with Aggregatibacter actinomycetemcomitans (A. a) [41,42] (Figure 3). Along with neighborhood components, counting plaque and calculus, the patient's systemic wellbeing, financial status, way of life choices, age, sex, ethnicity, hereditary qualities, natural variables, and have reaction moreover influence how the illness advances [44]. Metabolic disorder, smoking, diabetes, and corpulence are extra critical supporters (Figure 4) [45,46]. Periodontopathogens have inconvenient impacts on patients' generally wellbeing, in expansion to their harming impacts on the periodontium [17]. Clearing out periodontal illness untreated may incline the persistent to different systemic maladies. Cardiovascular illness, diabetes, affront resistance, verbal and colon cancer, stomach related disarranges, antagonistic pregnancy results, respiratory tract contaminations, pneumonia, and Alzheimer's illness are among these conditions [18]. Along with nearby variables, counting plaque and calculus, the patient's systemic wellbeing, financial status, way of life choices, age, sex, ethnicity, hereditary qualities, natural variables, and have reaction too influence how the illness advances [44]. Metabolic disorder, smoking, diabetes, and corpulence are extra noteworthy donors (Figure 4) [45,46]. Periodontopathogens have inconvenient impacts on patients' generally wellbeing, in expansion to their harming impacts on the periodontium [47]. Taking off periodontal infection untreated may incline the quiet to different systemic maladies. Cardiovascular malady, diabetes, affront resistance, verbal and colon cancer, stomach related clutters, unfavorable pregnancy results, respiratory tract diseases, pneumonia, and Alzheimer's illness are among these conditions [48]. Pathogenic enteric microbes are the primary causative specialists of human gastrointestinal tract diseases and stay a unmistakable open wellbeing concern around the world [51]. They can cause genuine irresistible maladies such as cholera with Vibrio cholerae, diarrhea with Shigella sp. or salmonellosis with Salmonella sp. [52]. Whereas rehydration treatment coupled with anti-microbial treatment can be endorsed amid the sickness, particularly in the case of serious the runs, imprudent anti-microbial treatment can lead to the improvement of multi-drug safe microscopic organisms and/or constant carriers [53]. The dissemination of antimicrobial resistance is of worldwide concern, and common compounds with tall pharmacological action and moo cytotoxicity can be an elective either in supplement or for substitution of anti-microbials [54]. Phytochemical compounds in plant extricates have pulled in expanding consideration for the

treatment of bacterial diseases. In Asia, Morus alba L. (Moraceae or mulberry tree) is one of the traditional medicinal plants that has been utilized in phytomedicine and drug store for the avoidance of diabetes, hypertension and cerebral pains, and has been utilized as a diuretic operator [55].

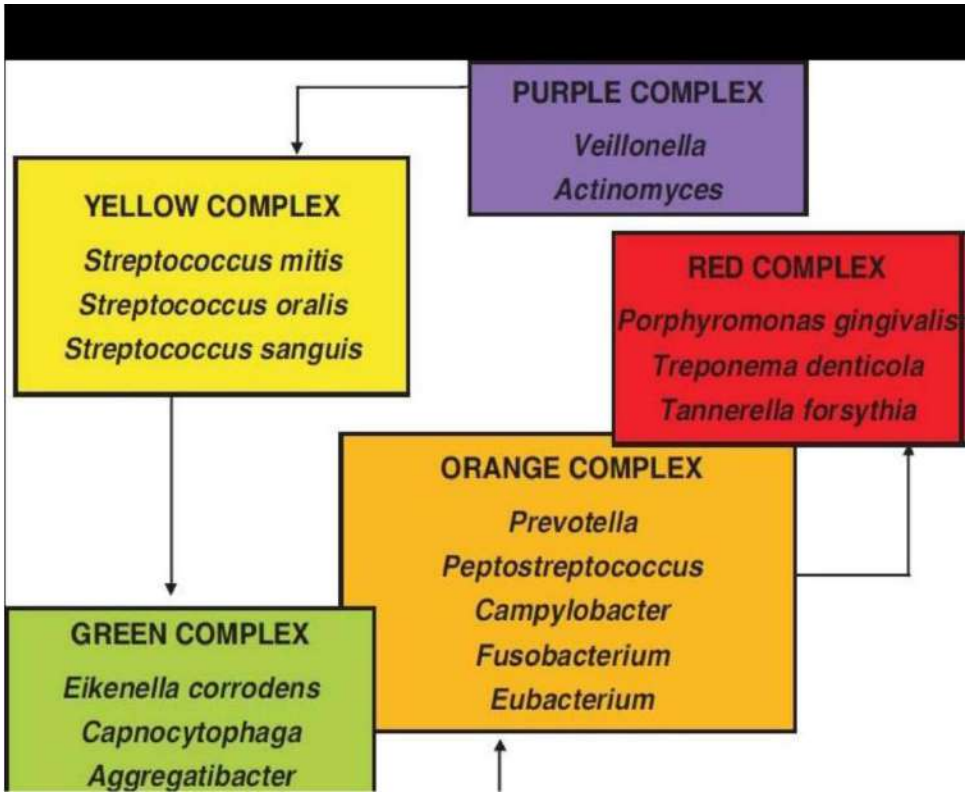


Figure 3: Microbial complexes involved in the progression and development of periodontal diseases [43].

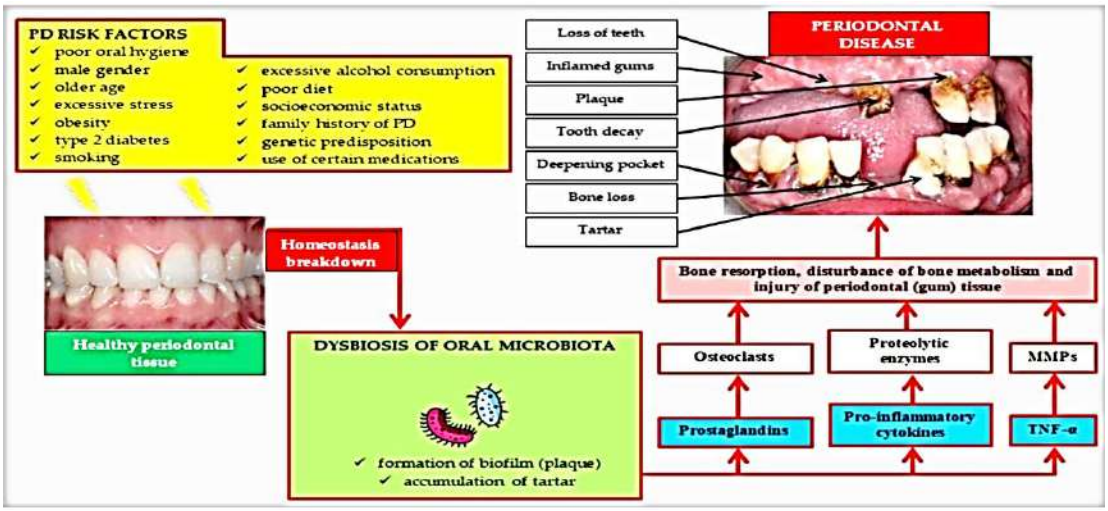


Figure 4: Personal, social, systemic, and local risk factors associated with oral dysbiosis lead to periodontal disease development and progression through activating pathogenic pathways [49].

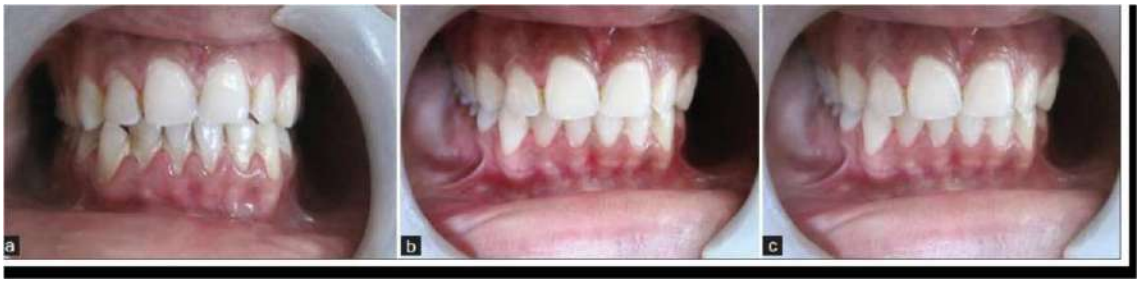


Figure 5: Results of a study on beneficial anti-inflammatory effects of Mulberry leaf + scaling treatment [(a) baseline; (b) one-month post-op; and (c) three-month post-op] to reduce plaque-induced gingivitis [50].

Other than being antinociceptive, a few mulberries were detailed to display antibacterial movement against a few Gram-positive and Gram-negative microscopic organisms such as Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Cutibacterium acnes, Pseudomonas aeruginosa, Staphylococcus epidermidis and Streptococcus pyogenes [56,57]. Hence, bioactive compounds of mulberry natural products can be investigated in nourishment, wellbeing care and restorative businesses [58]. COVID-19, which is caused by the novel coronavirus extreme intense respiratory disorder coronavirus 2 (SARS-CoV-2), has spread quickly around the world and postures a genuine danger to open wellbeing universally [59]. Past considers have illustrated that microbiomes in the body are pivotal to advancement and support of resistant homeostasis. The verbal depression has the moment biggest microbiome after the intestine in the human body [60]. Past ponders have affirmed that verbal microbiota play critical parts in the pathogenesis of numerous irresistible infections [61]. Past ponders have moreover detailed that various viral pneumonia cases counting COVID-19, appeared bacterial–fungal coinfection, which may impact malady movement and result [15–17]. Li et al. [62] detailed the changes in the bacterial community composition of patients with COVID-19. According to the HOMD, human verbal microbiota comprises 13 phyla (Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, SR1, Synergistes, Tenericutes, and TM7). An overpowering number of species-level phylotypes (96%) drop into six phyla (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, and Fusobacteria). Three phyla (TM7, SR1, and Chloroflexi) are still not spoken to by a single developed verbal species [63]. A developing number of ponders have been announcing a near affiliation between diabetes and vulnerability for a few verbal ailments, such as periodontitis [64], inferred from the deregulation of the verbal microbiota balance that increases the foundation of pathogenic life forms, causing the deregulation of the verbal microbiota harmony, and vice-versa. In the show think about we pointed to test the major phytochemicals in the ethanolic extricate and fluid extricate of Morus alba leaf and to investigate its antimicrobial properties against the verbal pathogens separated from 30 diverse categories of volunteers with diverse age bunches and diverse wellbeing conditions.

MATERIALS & METHODS

Collection of Plant Materials and Authentication.

Leaves of Morus alba were collected from Rampurhat, Birbhum India. Moris alba herbarium specimen was authenticated by Dr. Vijay Kumar Mastakar, Scientist in- charge, Acharya Jagdish Chandra Bose Indian Botanic Garden Botanical Survey of India, P.O. Botanic Garden, Howrah: 711103.

Preparation of Morus alba crude Ethanolic extract.

Fresh leaves of M. Alba were collected and were washed thoroughly with distilled water and then weighed. These leaves were dried by evaporation in oven.100 g Morus alba leaf powder was soaked in 70% ethanol for 72 h in a shaker at room temperature. After soaking the extract was filtered using a muslin cloth in a petri dish and dried using an air flow system. The dried extract was removed, weighed, dissolved in sterile distilled water and kept aseptically in air tight containers (figure 6 and 7).

Preparation of Morus alba crude Aqueous extract.

Fresh leaves of M. Alba were collected and were washed thoroughly with distilled water and then weighed. These leaves were dried by evaporation in oven.100 g Morus alba leaf powder was soaked in water for 72 h in a shaker at room temperature. After soaking the extract was filtered using a muslin cloth in a petri dish and dried using an air flow system. The dried extract was removed, weighed, dissolved in sterile distilled water and kept aseptically in air tight containers (figure 6 and 7).

Phytochemical Screening.

The powdered leaves were subjected to phytochemical screening for the presence of the alkaloids, tannins, saponins, steroids using standard photochemical protocol used (figure 8).

Quantitative Estimation of Phytochemicals [65].

i. Extraction of Alkaloids

The Paste of plant material (25g) was mixed with 5% Na2CO3 solution and transferred to a 500 ml flask, by adding 50 ml of chloroform. The solution was refluxed for 20 minutes, cooled, filtered and transferred to the agitator for 5 minutes. The upper layer was removed and made volume up to 5 ml. Further 1% H2SO4 (25 ml) was added and extracted using 20 ml of CHCl3. The aqueous phase was separated, and ammonium hydroxide was added to alkaline it and then extracted with 10 ml portions of CHCl3 successively. Then chloroform layers were washed with water (5 ml) followed by reducing volume (5 ml) by distillation. The absolute alcohol (2 ml) was added to the residues and evaporated at 100° C to dryness and solid residue obtained were crude alkaloids. The percentage yield of alkaloids was determined.

% yield of alkaloids= Weight of alkaloids obtained × 100/ Total weight of Sample

ii. **Extraction of Flavonoids**

The paste plant material (25 g) was mixed with 100 ml ether in flask and refluxed for 1 hour at temperature 60°C, filtered and dried at 20° C. The filtrate was mixed with methanol of concentration 100 ml, refluxed (2 hour), filtered and evaporated. The crude flavonoids were determined by the following formula (Vongsak, 2013).

% yield of flavonoids = Weight of flavonoids obtained × 100 / Total weight of Sample

iii. Extraction of Tannic Acid

The paste (25 g) of plant material was mixed with acetone and water (70% and 30%) and employed for extraction. Purification of this material was carried out in order to remove the pigments and phenolics by following Hagerman (1996).

% yield of Tannic acid = Weight of tannic acid obtained × 100 / Total weight of Sample

iv. Saponins Extraction

The plant material (25 g) was placed in soxhlet extractor with solvent (200 ml) i-e ether for six hours at temperature 60°C to be defatted. The residue was kept in open air for overnight to evaporate the solvent. This plant material was then placed in the thimble of soxhlet extractor again alongwith methanol solvent (200 ml) till the colorless extraction. The solvent was evaporated and calculated percent yield of saponins by following Sharma et al., (1982).

% yield of saponins = Weight of saponins obtained × 100 / Total weight of Sample

v. Extraction of Steroids

The powdered plant material (10 g) and Gemmo-therapeutically treated M. alba was then weighed and transferred to a round bottom flask with ethyl acetate (100 ml). The solution was refluxed for 20 minutes at 40°C and then filtered. The filtrate was mixed with 5% KOH (2-50 ml). Two layers were separated: ethyl acetate layer and steroids layer (The ethyl acetate layer was extracted with of 5% HCl (2-50 ml) by evaporation) while steroid layer persist.

% yield of steroids = Weight of steroids obtained × 100 / Total weight of Sample

vi. Extraction of Glycosides

The grounded plant material (25 gram) was boiled with 90 ml ethyl alcohol and filtered. The filtrate was mixed with lead sub acetate solution (30 ml) to remove chlorophyll and other pigments. The filtrate was treated with distilled water (45 ml saturated with H2S) to remove lead sub acetate. The pure filtrate was then dried on an electric water bath and the percentage (%) yield of crude glycosides was calculated.

% Yield of Glycosides = Weight of glycosides obtained × 100 / Total weight of Sample.

Phytoconstituent analysis [65]

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols (figure 8).

i. Tests for alkaloids

a **Dragendorff 's test.**

By adding 1 mL of Dragendorff"s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

b **Mayer's test.**

Few drops of Mayer's reagent were added to 1 mL of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids.

c **Hager's test.**

Two milliliters of extract were treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids.

ii. Tests for flavonoids

a Alkaline reagent test. Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

b Shinod's test. Ten drops of dilute HCL and a piece of magnesium were added to 1 mL of extract, the resulting deep pink colour indicating the presence of flavonoids.

iii.Test for phenolic compounds and tannins

a Ferric chloride test. Two milliliters of 5% neutral ferric chloride solution were added to 1 mL of extract, the dark blue colouring indicating the presence of phenolic compounds and tannins.

b Lead tetra acetic acid test. One milliliter of lead tetra acetate solution was treated with 0.5 mL of extract, precipitate formation indicating the presence of phenolic compounds and tannins.

iv. Tests for proteins

a Biuret test. Two drops of 3% copper sulphate and few drops of 10% sodium hydroxide were added to 1 mL of extract, violet or red colour formation indicating that proteins are present.

b Ninhydrin test. Two drops of 0.2% freshly prepared ninhydrin solution added to 1 mL of extract. Production of purple colour shows the presence of proteins.

v. **Test for Tanic Acid, Steroids and Anthraquinone**

Plant material was extracted with 20 ml of distilled water for 5 minutes. Then, 5 drops of FeCl3 were added. Blue black precipitates indicated the presence of tannins. Violet coloration appears which indicated the presence of steroids. On extraction of plant material with absolute ethanol, then addition of n, n dimethyl aniline solution, no red coloration appeared confirms Anthraquinone in M. Alba leaves.

Figure 6: Preparation of Morus alba crude leaf Aqueous/ethanolic extract.

Standard Microbial Culture and Maintenance

The oral bacterial strains (ATCC, CDL, Kolkata) used were E.coli (ATCC 8739), S. aureus (ATCC 6538), B.Subtitis (ATCC 6633) and Candida albicans (ATCC 10231). The strains were revived by suspending them in BHI (Brain Heart Infusion) broth as lyophilized culture followed by subculturing them on specific selective media (figure 9 and 10).

Dispersion of Medium

15 ml of medium was poured in Petri plates (9 cm) for gel formation (2-3 mm).

Figure 7: Preparation of Morus alba crude leaf ethanolic extract.

Figure 8: Identification Tests performed for Phytoconstituent analysis of Morus alba Leaf extract.

Sample Collection and Isolation of Oral microorganisms from Individuals

A total of 32 volunteers aged 18–60 years provided dental biofilm samples (in which 5 of them are previously reported dental caries, 5 of them are recovered from COViD19 infection, 5 of them are Diabetic, 5 of them are healthy, 5 of them are having cleaned their mouth with mouth wash, 5 of them are haven't cleaned their mouth with mouth wash) the subjects were trained to sample and completed the questionnaire. All participants were informed about the aim of this study and signed informed consent before entering the study, the dental condition and diet habits were recorded for each participant. The modified protocol of a previous study was used to collect oral microorganisms [66]. Briefly, supragingival biofilm samples were collected using a sterile cotton swab in the morning before tooth brushing and breakfast. On the other hand, microorganisms also isolated from freshly extracted human maxillary incisors extracted for caries reasons. Infected teeth and the swabs containing biofilm samples were separately placed into 2 mL of 0.9% normal saline water and stored at 4°C to use for not more than 24 h before the experiment. Samples were then sonicated for 30 s, vortexed to disperse, and the suspension dilutions were plated on blood Agar with the addition of 5% defibrinated sheep blood. Single pure colonies were in a given sector of a plate, well-isolated colonies were selected and subcultured for isolation on a solid medium to ensure purity (as shown in figure 11, 12 and 13).

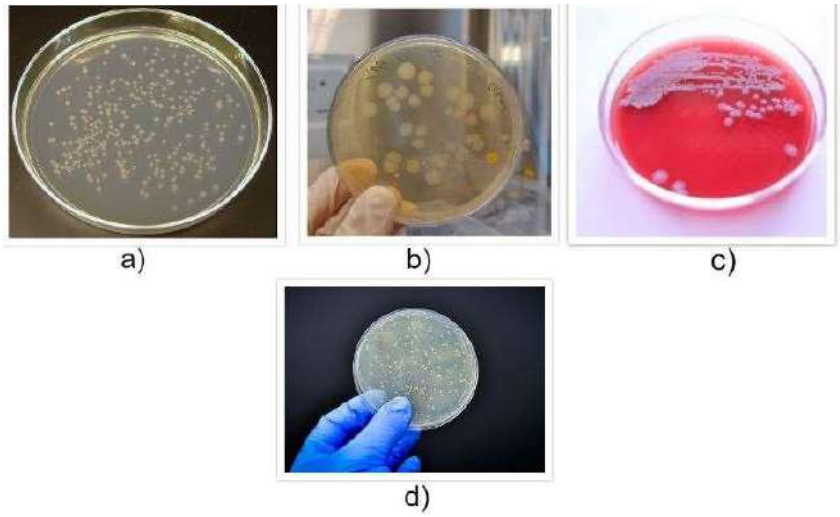


Figure 9: Standard Microbial Culture and Maintenance of standard oral bacterial strains (ATCC, CDL, Kolkata)- a) E.coli (ATCC 8739), b) S. aureus (ATCC 6538), c) B.Subtitis (ATCC 6633) and d)bCandida albicans (ATCC 10231).

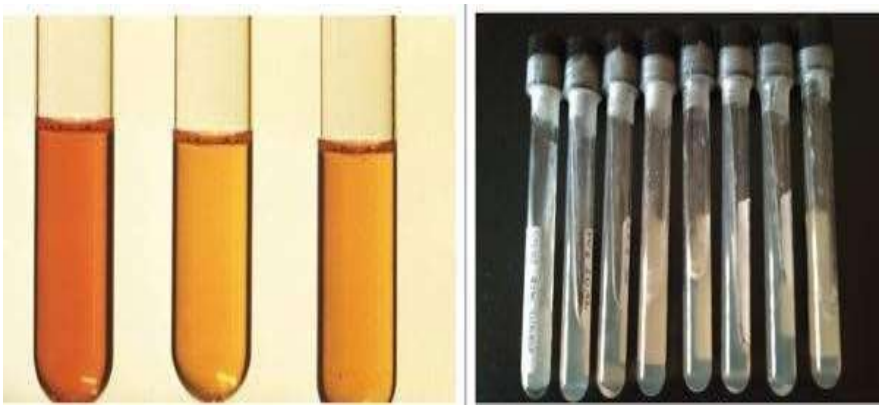


Figure 10: Suspending lyophilized culture of standard oral bacterial strains (ATCC, CDL, Kolkata) a) E.coli (ATCC 8739), b) S. aureus (ATCC 6538), c) B.Subtitis (ATCC 6633) and d. Candida albicans (ATCC 10231) in BHI (Brain Heart Infusion) broth.

Screening of Strains Based on Cariogenicity

The isolated microorganisms were screened based on their acidogenicity and aciduricity using a method described previously with some modification [67]. Briefly, all isolated microorganisms were inoculated onto slant of the agar to determine the ability of organisms to ferment glucose, lactose, and sucrose, leave the cap on loosely and incubate the tube at 37 °C in ambient air for 18 to 24 h. Changing the color of the slant agar to yellow suggesting fermentation of sugar from which strains were selected. Isolated strains overnight culture was inoculated into "acid medium" and conventional medium, respectively, the survival rate of selected microorganisms was measured, immediately after resuspension (Time 0) and after 60 min (Time 60); the isolates with a survival rate more than 90% were selected. The acidogenicity of strains was classified according to previously published descriptions, low ($pH \geq 5.5$), moderate ($3.5 \leq pH \leq 5.5$), and high ($pH \leq 3.5$); high and moderate acid-producing microorganisms were selected (data not shown). Finally, four strains were selected with high acidogenicity and high acid tolerance (figure 14).

Figure 11: The process of isolation of potentially microorganisms in human oral biofilms.

Figure 12: Visible growth of potential microorganisms of human oral biofilms isolated from individuals.

Figure 13: Incubation of potential oral microorganisms isolated from individuals volunteers.

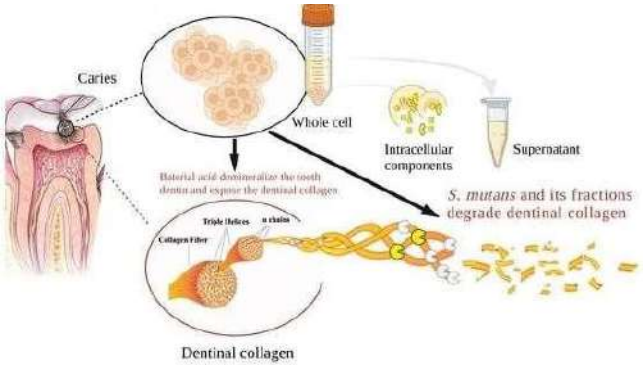


Figure 14: Screening of Strains Based on Cariogenicity.

Enumeration of Viable CelI Count

The overnight broth culture was serially diluted with autoclaved distilled water upto 10° dilution and 100 ul of each dilution was spread on to Nutrient agar plates and incubated overnight at 37°C. After 12 -18 hours incubation the number of viable colonies were counted using total viable plate count method.

C.F.U/ nL original sample = Number of colonies /plate x (1/mL aliquot plated) x dilution factor

Isolation and screening of biofilm forming oral bacteria

10 colonies with visually distinguishable morphologies were randomly selected and isolated by directly streaking on Nutrient agar plates and incubated for another 12-18 hours. The isolated colonies were then restreaked after incubation onto nutrient agar plates to obtain pure cultures. The viability of the isolated cultures was checked.

Bacterial Identification—Morphological

After selecting potential cariogenic oral microorganisms, single colonies were inoculated on the nutrient agar and incubated at 37 °C in ambient air for 18 to 24 h. Gram staining reactions were performed for each selected strains, and the microbe morphology was observed under the microscope [67].

Physical characterization Oral microorganisms

a. **Colony morphology:**

Shape. size. colour. elevation and margin of colony and appearance are observed in overnight plate culture on Nutrient agar media noted down.

b. **Cell morphology:**

The gram-stained cells were viewed under light microscope under 100x oil immersion to determine the morphological characterization of the cells.

c. **Motility test:**

It is used to check the ability of bacteria to migrate away from the line of inoculation.

Preparation of Disc and Media

Wicks paper disc of 10 mm was used. Agar media was prepared. The solution of peptone and agar-agar were prepared by distilled water and yeast extract and NaCl were added. The solution was autoclaved.

Essay of antibacterial activity

The bacteria preserved in NaCl at 0.9 % were inoculated in the liquid Mueller-Hinton medium and were incubated at 37 ºC in agitation (100 rpm) during 18 h. Afterwards a suspension was prepared for each of the bacterial strains at 0.5 ml. The microorganisms were inoculated in the agar medium, previously melted down and maintained at 45 ºC. The growth in lawn occurred at a final concentration of approximately 108 cells/mL. After the medium solidification, 6 perforations of 0.8 cm diameter were made, where 100 µL of the mulberry extract, as well as of the negative and positive controls, were put. Ten repetitions were made per treatment for each microorganism. The 10 Petri dishes per microbial group were incubated

at 37 ºC during 24 h. Then, the diameter of the microorganism growth inhibition halo was measured and the percentage of the relative inhibitory effect was calculated with regards to the positive control, as expressed in the following equation:

$$\%\ \text{inhibitory effect} = \frac{\text{mean of the diameter of the inhib.halo of the extract}}{\text{mean of the diameter of the inhib.halo positive control}} \times 100$$

Testing Antibacterial Activity (Standard)

The bacteria (S. aureus, B.Subtitis, Candida albicans and E. coli) incubated at (37± 0.2° C) on nutrient agar. Petri plates (9 cm) were incubated with 0.1ml – 10 ml of cultured media (110 -118 bacteria per mL). Agar media was distributed into each inoculated Petri dishes. Discs of ethanolic extracts and aqueous extract (0.1 µg, 0.2 µg, 0.3 µg,0.4 µg,0.5 µg,0.6 µg,0.7 µg,0.8 µg, 0.9 µg and 10 µg) per ml of Morus alba leaf were placed on agar medium. Medium were placed at 37o C for 24 - 48 hours. Commercially available antibiotics Cephalosporin were used as standard reference.

Testing Antibacterial Activity (Test)

The isolated microorganisms were incubated at (37± 0.2° C) on nutrient agar. Petri plates (9 cm) were incubated with 0.1ml – 10 ml of cultured media (110 -118 bacteria per mL). Agar media was distributed into each inoculated Petri dishes. Discs of ethanolic extracts and aqueous extract (0.1 µg, 0.2 µg, 0.3 µg,0.4 µg,0.5 µg,0.6 µg,0.7 µg,0.8 µg, 0.9 µg and 10 µg) per ml of Morus alba leaf were placed on agar medium. Medium were placed at 37o C for 24 - 48 hours.

RESULTS

Qualitative and Quantitative analysis of phytoconstituents

Table 3: Qualitative and Quantitative analysis of phytoconstituents in Morus alba leaf extract

Constituent	Result	% age yield
Alkaloids	Positive	40%
Glycosides	Positive	20.05%
Flavonoids	Positive	14%
Saponins	Positive	11.5%
Tannic Acid	Positive	11.9%
Steroids	Positive	3.5%
Anthraquinone	Negative	0.5%

Antibacterial activity of leaf extract of Morus alba on different strains of standard microorganisms

Table 4: Antibacterial activity of plant leaves ethanolic extract of Morus Alba showing inhibition zones for different strains of standard microorganisms.

Sr. No.	Tested Microorganism	Inhibition zone (mm)	
		Standard (Cephalosporin)	Test (Ethanolic extract)
1.	S. Aureus	19 ± 4.5	8 ± 3.1
2.	B.Subtitis	25.5 ± 8.6	9 ± 2.5
3.	Candida albicans	25.5 ± 8.8	9 ± 2.7
4.	E. Coli	25 ± 9.3	7 ± 3.3

Table 5: Antibacterial activity of plant leaves aqueous extract of Morus alba showing inhibition zones for different strains of standard microorganisms.

Sr. No.	Tested Microorganism	Inhibition zone (mm)	
		Standard (Cephalosporin)	Test (Aqueous extract)
1.	S. aureus	19 ± 4.5	7 ± 3.1
2.	B.Subtitis	25.5 ± 8.6	8 ± 2.5
3.	Candida albicans	25.5 ± 8.8	8 ± 2.7
4.	E. coli	25 ± 9.3	6 ± 3.3

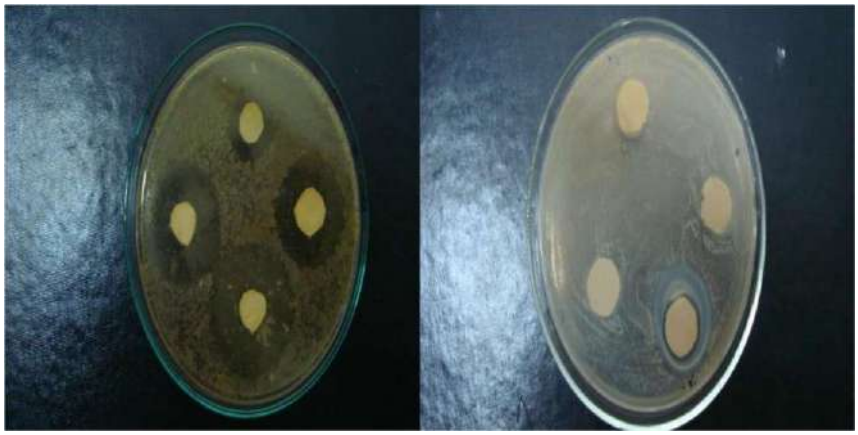


Figure 15: Inhibition zone produced by Aqueous extract of Morus alba against E.Coli, Staphylococcus aureus, Bacillus subtilis and Candida albicans.

Table 6: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) suffering from Type-2-Diabetes.

Sr. No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#2	S. mutans, E. faecalis, C. albicans, Lactobacillus acidophilus	Gram-positive
2.	#7	P. gingivalis, Prevotella intermedia, Salmonella Typhi	Gram-negative
3.	#12	Campylobacter rectus, P. gingivalis, Prevotella intermedia	Gram-negative
4.	#5	Porphyromonas endodontalis, Campylobacter rectus, P. gingivalis, Salmonella Typhi	Gram-negative
5.	#10	Treponema socranskii, Porphyromonas endodontalis, Campylobacter rectus, Shigella dysenteriae	Gram-negative

Table 7: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) recovered from COViD-19.

Sr .No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#1	S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum	Gram-positive
2.	#3	P. gingivalis, Prevotella intermedia, Alloprevotella rava	Gram-negative
3.	#16	P. gingivalis, Prevotella intermedia, Alloprevotella rava	Gram-negative
4.	#20	Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Aggregatibacter actinomycetemcomitans	Gram-negative
5.	#25	Veillonella, Treponema socranskii, Campylobacter rectus, Fusobacterium, Salmonella Typhi, Shigella dysenteriae	Gram-negative

Table 8: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) in good health.

Sr. No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#13	S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus subtilis	Gram-positive
2.	#23	P. gingivalis, Prevotella intermedia, Alloprevotella rava	Gram-negative
3.	#26	P. gingivalis, Prevotella intermedia, Alloprevotella rava	Gram-negative
4.	#28	Veillonella, Porphyromonas endodontalis, Campylobacter rectus	Gram-negative
5.	#30	Veillonella, Treponema socranskii, Campylobacter rectus, Fusobacterium	Gram-negative

Table 9: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) who are Smokers.

Sr. No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#4	S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Staphylococcus aureus	Gram-positive
2.	#8	P. gingivalis, Prevotella intermedia, Alloprevotella rava, Actinomyces	Gram-negative
3.	#11	P. gingivalis, Prevotella intermedia, Alloprevotella rava, Klebsiella pneumoniae, Veillonella, Actinomyces	Gram-negative
4.	#18	Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Klebsiella pneumoniae, Veillonella, Actinomyces	Gram-negative
5.	#24	Veillonella, Treponema socranskii, Campylobacter rectus, Fusobacterium, Klebsiella pneumoniae, Veillonella, Actinomyces, Pasteurella multocida	Gram-negative

Table 10: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) who had brushed/washed their mouth.

Sr No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#6	Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum	Gram-positive
2.	#9	Alloprevotella rava	Gram-negative
3.	#14	NIL	Gram-negative
4.	#15	Campylobacter rectus	Gram-negative
5.	#29	Campylobacter rectus	Gram-negative

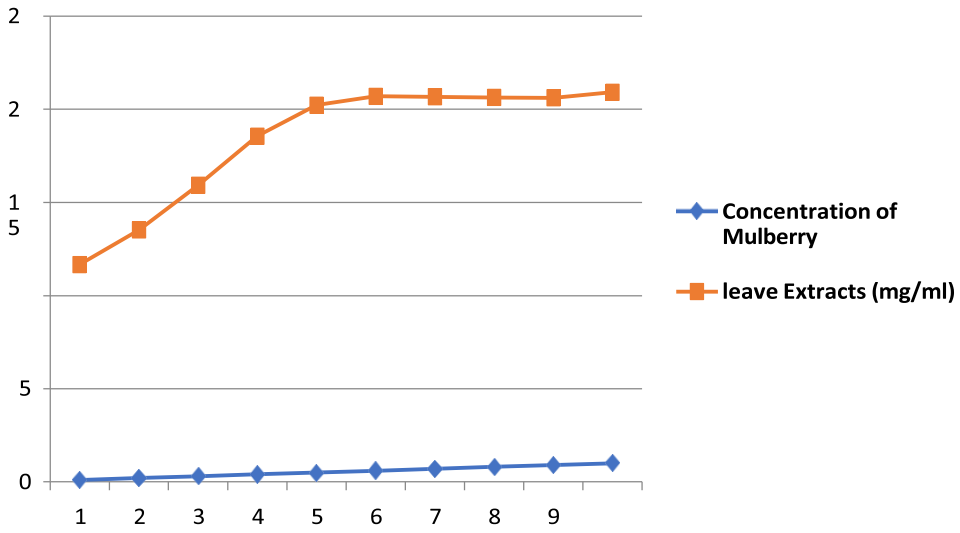
Table 11: Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) who are non-smokers.

Sr No.	Sample No.:	Isolated Oral Microorganism	Straining Property
1.	#17	S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus subtilis	Gram-positive
2.	#19	P. gingivalis, Prevotella intermedia, Alloprevotella rava, Actinomyces	Gram-negative

3.	#21	P. gingivalis, Prevotella intermedia, Alloprevotella rava, Klebsiella pneumoniae, Veillonella, Actinomyces, Shigella dysenteriae, Pasteurella multocida	Gram-negative
4.	#22	Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Klebsiella pneumoniae, Veillonella, Actinomyces	Gram-negative
5.	#27	Veillonella, Treponema socranskii, Campylobacter rectus, Fusobacterium, Klebsiella pneumoniae, Veillonella, Actinomyces, Shigella dysenteriae, Pasteurella multocida	Gram-negative

Table 12: Antimicrobial Effectiveness test of Morus alba leaf extract (Ethanolic) at different concentrations on isolated oral microorganism.

Sr. No.	Concentration of Mulberry leave Extracts (µg/ml)	Zone of Inhibition (in mm)
1.	0.1	9.6
2.	0.2	11.2
3.	0.3	11.8
4.	0.4	12.2
5.	0.5	12.6
6.	0.6	14.2
7.	0.7	14.4
8.	0.8	14.8
9.	0.9	15.4
10.	1	17.2

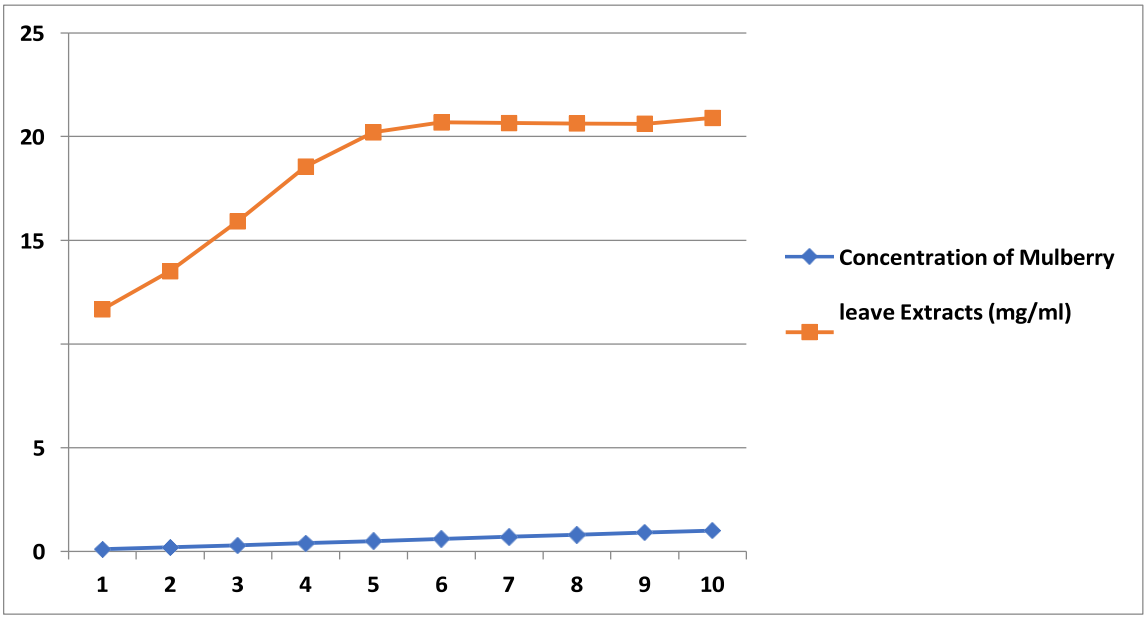


Graph 1: Antimicrobial Effective concentration on isolated oral microorganism with Morus alba leaf extract (Ethanolic).

Table 13: Antimicrobial Effectiveness test of Morus alba leaf extract (Aqueous) at different concentrations on isolated oral microorganism.

Sr. No.	Concentration of Mulberry leave Extracts (mg/ml)	Zone of Inhibition (in mm)
1.	0.1	11.66
2.	0.2	13.51
3.	0.3	15.92
4.	0.4	18.55

5.	0.5	20.21
6.	0.6	20.69
7.	0.7	20.66
8.	0.8	20.63
9.	0.9	20.61
10.	1	20.91



Graph 2: Antimicrobial Effective concentration on isolated oral microorganism with Morus alba leaf extract (Aqueous).

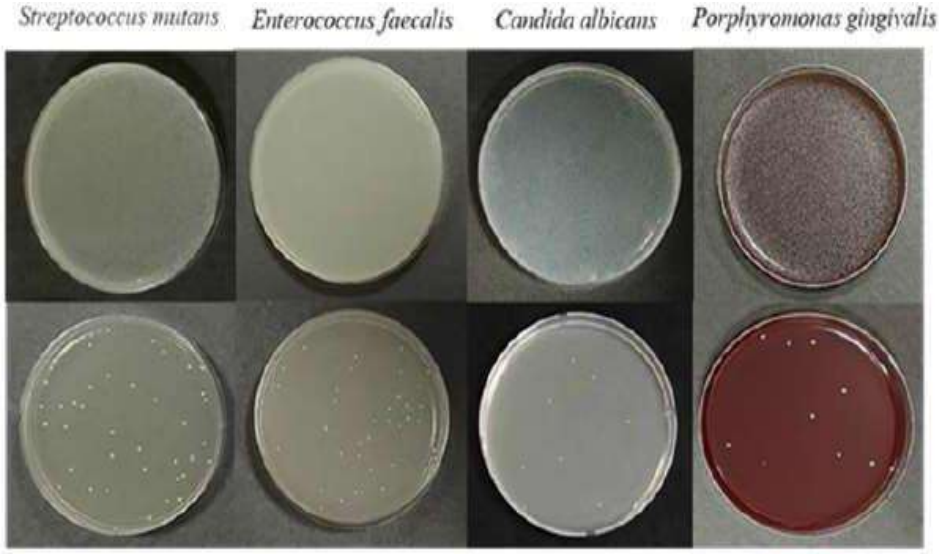


Figure 16: Colony-forming units (CFU) of oral pathogens following treatment with aqueous extract of Morus alba. Each white dot on the plates indicates an oral pathogen colony- Top (Control) and Bottom (Test).

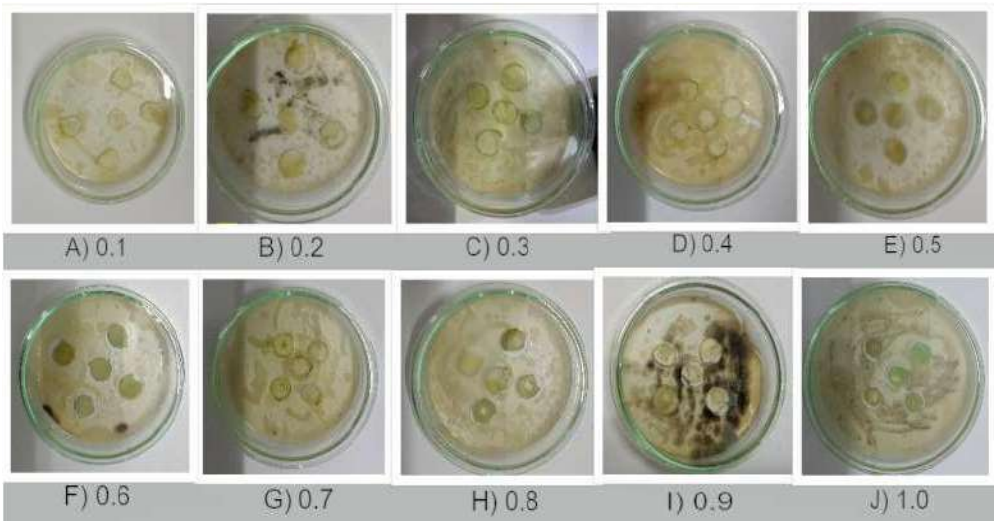


Figure 17: Antimicrobial Effectiveness test of Morus alba leaf extract (Aqueous) at different concentrations on isolated oral microorganism.

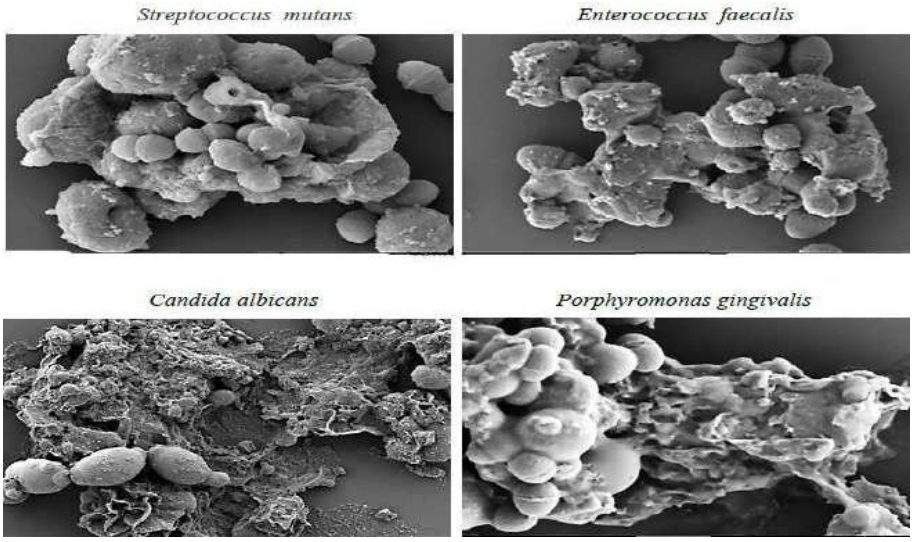


Figure 18: Microscopic images of isolated oral microorganism after treated with Morus alba leaf extract (Aqueous) at effective concentration on isolated oral microorganism.

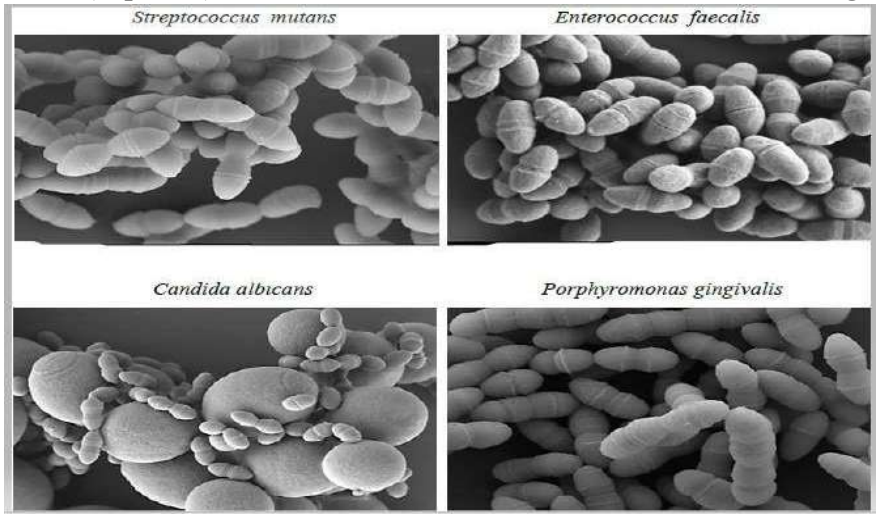


Figure 19: Microscopic images of isolated oral microorganism after (untreated).

DISCUSSION

Various microbial species inhabit the oral cavity, and the growth of pathogenic bacteria can cause oral diseases. Frequent consumption of refined carbohydrates accelerates the demineralization of tooth enamel due to dental caries, causing imbalances in remineralization and demineralization. S. mutans refers to a group of seven closely related species that are collectively known as mutans Streptococci. S. mutans are commonly found in the mouth, pharynx, and intestines. Oral diseases such as caries, endodontic infections, periodontitis, and periimplantitis have also been linked to E. faecalis, the predominant human Enterococcus. E. faecalis has been observed to comprise 3.7–35% of the oral microbiota in periodontitis patients [35]. Candida albicans is a commensal fungal species that commonly colonizes human mucosal surfaces. C. albicans has been observed in carious dentin/dentine tubules [36]. P. gingivalis is the major causative agent of periodontitis, a chronic inflammatory disease that causes tooth loss and degeneration of the gingiva, alveolar bones, and periodontal ligaments [37]. Oral diseases such as dental caries, periodontal disease, periapical lesions, and oral candidiasis are the main causes of tooth loss and oral malodor, for which effective management methods are urgently needed. Mouthwashes containing chemical agents are commonly used to eliminate bacteria and/or inhibit microbial activity. Qualitative and Quantitative analysis of phytoconstituents in Morus alba leaf ethanolic and aqueous extract yields about 40% alkaloids and 14% Flavonoids as the antimicrobial activity of Morus alba leaves is due to the presence of flavonoids and active phenols (Table 3). The antibacterial activity of leaf ethanolic extract of Morus alba on standard strains shows that Candida albicans is the highest with 9 mm ± 2.7 mm inhibition zone as compared to Standard antibiotic: Cephalosporin with 25.5 mm ± 8.8 mm (Table 4). The antibacterial activity of leaf aqueous extract of Morus alba on standard strains shows that Candida albicans is the highest with 8 mm ± 2.7 mm inhibition zone as compared to Standard antibiotic: Cephalosporin with 25.5 mm ± 8.8 mm (Table 5). Inhibition zone produced by Aqueous extract of Morus alba against E.Coli, Staphylococcus aureus, Bacillus subtilis and Candida albicans are illustrated in figure 15. Oral Microorganisms isolated from Individual volunteers with different age groups (18-60) suffering from Type-2-Diabetes, recovered from COViD-19, individuals with good health, Smokers, individuals who had brushed/washed their mouth and non-smokers are illuminated in table 6 to 11 respectively. Individual volunteers with different age groups (18-60) suffering from Type-2-Diabetes has microbial isolates of gram-positive bacteria: S. mutans, E. faecalis, C. albicans, Lactobacillus acidophilus and gram-negative bacteria: P. gingivalis, Prevotella intermedia, Salmonella Typhi, Campylobacter rectus, Porphyromonas endodontalis, Treponema socranskii, Shigella dysenteriae (Table 6). Individual volunteers with different age groups (18-60) recovered from COViD-19 has microbial isolates of gram-positive bacteria: S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum and gram-negative bacteria: P. gingivalis, Prevotella intermedia, Alloprevotella rava, Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Aggregatibacter actinomycetemcomitans, Treponema socranskii, Fusobacterium, Salmonella Typhi, Shigella dysenteriae (Table 7). Individual volunteers with different age groups (18-60) who are in good health has microbial isolates of gram-positive bacteria: S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus

subtilis and gram-negative bacteria: P. gingivalis, Prevotella intermedia, Alloprevotella rava, Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Treponema socranskii, Fusobacterium (Table 8).

Individual volunteers with different age groups (18-60) who are in good health has microbial isolates of gram-positive bacteria: S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Staphylococcus aureus and gram-negative bacteria: P. gingivalis, Prevotella intermedia, Alloprevotella rava, Actinomyces, Klebsiella pneumoniae, Veillonella, Porphyromonas endodontalis, Campylobacter rectus, Treponema socranskii, Pasteurella multocida (Table 9). Individual volunteers with different age groups (18-60) who had brushed/washed their mouth has microbial isolates of gram-positive bacteria: Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum and gram- negative bacteria: Alloprevotella rava, Campylobacter rectus (Table 10).

Individual volunteers with different age groups (18-60) who are non-smokers has microbial isolates of gram-positive bacteria: S. mutans, E. faecalis, Stomatobaculum longum, Lachnoanaerobaculum, Oribacterium asaccharolyticum, Aggregatibacter actinomycetemcomitans, Lactobacillus acidophilus, Staphylococcus aureus, Bacillus subtilis and gram-negative bacteria: P. gingivalis, Prevotella intermedia, Alloprevotella rava, Actinomyces, Klebsiella pneumoniae, Veillonella, Shigella dysenteriae, Pasteurella multocida, Porphyromonas endodontalis, Campylobacter rectus, Treponema socranskii, Fusobacterium (Table 11). S. mutans, which causes dental caries, and E. faecalis, which is a causative agent of dental pulp disease, were reduced by less than 3 log CFU/mL under treatment with the aqueous extract of Morus alba leaf. Additionally, the numbers of C. albicans, a fungal strain that infects the oral mucosa, and P. gingivalis, which causes periodontal disease, were reduced by less than 4 log CFU/mL (figure 16). Antimicrobial Effectiveness test of Morus alba leaf extract (Aqueous) at different concentrations on isolated oral microorganism has been shown in (figure 17). Comparative microscopical examination for morphology of each microbial strain isolates with untreated group showed that the oral microorganisms exhibited disorganized damage after treatment with the Morus alba leaf aqueous extract; particularly, C. albicans showed an overall damaged morphology (figure 18 and 19).

CONCLUSION

There is an abundance of evidence that pure phytochemicals, essential oils, and plant extracts have the potential to be converted into medications that can be used to treat or prevent periodontitis. More studies on the safety and efficacy of these products are required to ascertain whether they have medicinal value, either alone or in combination with conventional treatment options, which can help reduce the overall burden of oral diseases globally, although the numerous clinical trials for these products are encouraging. This study developed a method of testing a bacterium for its cariogenicity. Thus, aqueous extract of Morus alba leaf has significant efficacy in inhibiting oral pathogens. These results indicate that the mixed herbal extracts had stronger effects on Gram-positive fungi and Gram-negative bacteria than on Gram-positive bacteria. Morus alba leaf aqueous extract is mostly effective against C. albicans. Washing/brushing of mouth can significantly reduce oral microbial growth. This disagrees with the findings of a previous study in literature reviews which found that the ethanolic extract of Morus alba leaf was more effective against Gram-positive bacteria than

Gram-negative bacteria. As examined in this study the antimicrobial effects of aqueous extracts of Morus alba on oral pathogens isolated from different individuals and found that the abundances of the four evaluated strains of oral pathogens were reduced after treatment.

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REFERENCES

1. Bumm, C.V.; Folwaczny, M.; Stokes, L.N.; Olsen, I.; Dewhirst, F.E. Infective endocarditis and oral health—A Narrative Review. Cardivasc. Diagn. Ther. 2021, 11, 1403–1415.
2. Toz Ertop, M.; Cicek, O.; Erener, H.; Ozkalayci, N.; Demir Cicek, B.; Comert, F. Evaluation of the Demineralization Development around Different Types of Orthodontic Brackets. Materials 2023, 16, 984.
3. Huang, R.; Li, M.; Gregory, R.L. Bacterial interactions in dental biofilm. Virulence 2011, 2, 435–444.
4. Chen, X.; Daliri, E.B.; Tyagi, A.; Oh, D.H. Cariogenic Biofilm: Pathology-Related Phenotypes and Targeted Therapy. Microorganisms 2021, 9, 1311.
5. Rajendra Santosh, A.B.; Muddana, K.; Bakki, S.R. Fungal Infections of Oral Cavity: Diagnosis, Management, and Association with COVID-19. SN Compr. Clin. Med. 2021, 3, 1373–1384.
6. Bui, F.Q.; Almeida-da-Silva, C.L.C.; Huynh, B.; Trinh, A.; Liu, J.; Woodward, J.; Asadi, H.; Ojcius, D.M. Association between periodontal pathogens and systemic disease. Biomed.
7. J. 2019, 42, 27–35.
8. Grover, V.; Mahendra, J.; Gopalakrishnan, D.; Jain, A. Effect of octenidine mouthwash on plaque, gingivitis, and oral microbial growth: A systematic review. Clin. Exp. Dent. Res. 2021, 7, 450–464.
9. Katsura, H.; Tsukiyama, R.I.; Suzuki, A.; Kobayashi, M. In vitro antimicrobial activities of bakuchiol against oral microorganism. Antimicrob. Agents Chemother. 2001, 45, 3009–3013.
10. Inhartová, L.; Michalíková, K.; Šrédlová, K.; Cajthaml, T. Biodegradability of Dental Care Antimicrobial Agents Chlorhexidine and Octenidine by Ligninolytic Fungi. Molecules 2020, 25, 400.
11. Pytko-Polo ´ nczyk, J.; Stawarz-Janeczek, M.; Kryczyk-Poprawa, A.; Muszy´ nska, B. Antioxidant-Rich Natural Raw Materials in the Prevention and Treatment of Selected Oral Cavity and Periodontal Diseases. Antioxidants 2021, 10, 1848.
12. Schönknecht, K.; Surdacka, A.; Rudenko, L.; Scherer,W. Effectiveness of composed herbal extract in the treatment of gingivitis and oral and pharyngeal mucosa—Review of studies. Wiad. Lek. 2021, 74, 1737–1749.
13. Kováˇc, J.; Slobodníková, L.; Trajˇcíková, E.; Rendeková, K.; Muˇcaji, P.; Sychrová, A.; Bittner Fialová, S. Therapeutic Potential of Flavonoids and Tannins in Management of Oral Infectious Diseases–A Review. Molecules 2022, 28, 158.
14. Lin, Z.; Gan, T.; Huang, Y.; Bao, L.; Liu, S.; Cui, X.; Wang, H.; Jiao, F.; Zhang, M.; Su, C.; et al. Anti-Inflammatory Activity of Mulberry

Leaf Flavonoids In Vitro and In Vivo. Int.J. Mol. Sci. 2022, 23, 7694.
15. Vyas, T.; Nagi, R.; Bhatia, A.; Bains, S.K. Therapeutic effects of green tea as an antioxidant on oral health—A review. J. Fam. Med. Prim. Care 2021, 10, 3998–4001.
16. Takasago, K. Addition of cariogenic pathogens to complex oral microflora drives significant changes in biofilm compositions and functionalities. Microbiome 2023, 11, 123.
17. Van Houte J. Role of micro-organisms in caries etiology. J Dent Res. 1994;73(3):672–81.
18. Gross EL, Leys EJ, Gasparovich SR, Firestone ND, Schwartzbaum JA, Janies DA, et al. Bacterial 16S Sequence Analysis of Severe Caries in Young Permanent Teeth. J Clin Microbiol. 2010;48(11):4121–8.
19. Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA. Streptococcus mutans, Candida albicans, and the Human Mouth: A Sticky Situation. Heitman J, editor. PLoS Pathog. 2013; 17;9(10):e1003616.
20. Smitha C, Usha R. Hydroxy apatite – alginate dental composite loaded with Myristica fragrans and Syzygium aromaticum extracts for the management of dental caries. Int J Pharm Bio Sci. 2016; 17(1):438–44.
21. Bandna Devi NS. Morus alba Linn: A phytopharmacological review. Int J Pharm Pharm Sci. 2013; 5(2):14–8.
22. Ramandeep Singh AB. Traditional uses, phytochemistry and pharmacology of Morus alba Linn.: A review. J Med Plant Res. 2013;7(9):461–469.
23. Oh H, Ko E-K, Jun J-Y, Oh M-H, Park S-U, Kang K-H, et al. Hepatoprotective and free radical scavenging activities of prenyl flavonoids, coumarin, and stilbene from Morus alba. Planta Med. 2002; 68(10):932–934.
24. Du J, He Z-D, Jiang R-W, Ye W-C, Xu H-X, But PP-H. Antiviral flavonoids from the root bark of Morus alba L. Phytochemistry. 2003; 62(8):1235–1238.
25. Islam B, Khan SN, Haque I, Alam M, Mushfiq M, Khan AU. Novel anti-adherence activity of mulberry leaves: inhibition of Streptococcus mutans biofilm by 1- deoxynojirimycin isolated from Morus alba. J Antimicrob Chemother. 2008;62(4):751–7.
26. de Oliveira AM, Mesquita M da S, da Silva GC, de Oliveira Lima E, de Medeiros PL, Paiva PMG, et al. Evaluation of Toxicity and Antimicrobial Activity of an Ethanolic Extract from Leaves of Morus alba L. (Moraceae). Evid Based Complement Alternat Med. 2015;1–7.
27. Lu, Q.-Y.; Ma, Y.; Smith, W.K.; Yu, J.; Cheng, Y.-Y.; Zhang, P.; Han, T.-T. The Identification of Tautoneura mori as the Vector of Mulberry Crinkle Leaf Virus and the Infectivity of Infectious Clones in Mulberry. Phytopathology 2022, 112, 435–440.
28. Jiao, F.; Luo, R.; Dai, X.; Liu, H.; Yu, G.; Han, S.; Lu, X.; Su, C.; Chen, Q.; Song, Q.; etal. Chromosome-Level Reference Genome and Population Genomic Analysis Provide Insights into the Evolution and Improvement of Domesticated Mulberry (Morus alba). Mol. Plant 2020, 13, 1001–1012.
29. Yuan, T.; Qazi, I.H.; Li, J.; Yang, P.; Yang, H.; Zhang, X.; Liu, W.; Liu, J. Analysis of changes in bacterial diversity in healthy and bacterial wilt mulberry samples using metagenomic sequencing and culture-dependent approaches. Front. Plant Sci. 2023, 14, 1206691.
30. Li, P.; Yang, Z.; Tang, B.; Zhang, Q.; Chen, Z.; Zhang, J.;Wei, J.; Sun, L.; Yan, J. Identification of Xanthones from the Mangosteen Pericarp that Inhibit the Growth

of Ralstonia solanacearum. ACS Omega 2019, 5, 334–343.
31. Dai, F.; Wang, Z.; Luo, G.; Tang, C. Transcriptional analysis of different mulberry cultivars in response to Ralstonia solanacearum. Can. J. For. Res. 2016, 46, 152–162.
32. Petersen, P.E.; Baehni, P.C. Periodontal health and global public health. Periodontology 2000 2012, 60, 7–14.
33. Eke, P.I.; Dye, B.A.; Wei, L.; Thornton-Evans, G.O.; Genco, R.J. Prevalence of periodontitis in adults in the United States: 2009 and 2010. J. Dent. Res. 2012, 91, 914–920.
34. Kassebaum, N.J.; Bernabé, E.; Dahiya, M.; Bhandari, B.; Murray, C.J.; Marcenes, W. Global burden of severe periodontitis in 1990–2010: A systematic review and meta-regression. J. Dent. Res. 2014, 93, 1045–1053.
35. Bartold, P.M.; Van Dyke, T.E. Van Dyke, Periodontitis: A host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000 2013, 62, 203–217.
36. Socransky, S.S.; Haffajee, A.D.; Cugini, M.A.; Smith, C.; Kent, R.L., Jr. Microbial complexes in subgingival plaque. J. Clin. Periodontol. 1998, 25, 134–144.
37. Darveau, R.P. Periodontitis: A polymicrobial disruption of host homeostasis. Nat. Rev. Microbiol. 2010, 8, 481–490.
38. Boyd, R.L.; Leggott, P.; Quinn, R.; Buchanan, S.; Eakle,W.; Chambers, D. Effect of self-administered daily irrigation with 0.02% SnF2 on periodontal disease activity. J. Clin. Periodontol. 1985, 12, 420–431.
39. Listgarten, M.A.; Lindhe, J.; Hellden, L. Effect of tetracycline and/or scaling on human periodontal disease. Clinical, microbiological, and histological observations. J. Clin. Periodontol. 1978, 5, 246–271.
40. Slots, J. Subgingival microflora and periodontal disease. J. Clin. Periodontol. 1979, 6, 351–382.
41. Dzink, J.L.; Socransky, S.S.; Haffajee, A.D. The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. J. Clin. Periodontol. 1988, 15, 316–323.
42. Wara-aswapati, N.; Pitiphat, W.; Chanchaimongkon, L.; Taweechaisupapong, S.; Boch, J.A.; Ishikawa, I. Red bacterial complex is associated with the severity of chronic periodontitis in a Thai population. Oral Dis. 2009, 15, 354–359.
43. Newman, M.G.; Takei, H.; Klokkevold, P.R.; Carranza, F.A. Carranza's Clinical Periodontology; Elsevier Health Sciences: London, UK, 2011.
44. Chandki, R.; Banthia, P.; Banthia, R. Biofilms: A microbial home. J. Indian Soc. Periodontol. 2011, 15, 111–114.
45. Bartold, P.M.; Van Dyke, T.E. Van Dyke, Periodontitis: A host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000 2013, 62, 203–217.
46. Genco, R.J.; Borgnakke,W.S. Risk factors for periodontal disease. Periodontology 2000 2013, 62, 59–94.
47. Lalla, E.; Papapanou, P.N. Diabetes mellitus and periodontitis: A tale of two common interrelated diseases. Nat. Rev. Endocrinol. 2011, 7, 738–748.
48. Kwon, T.; Lamster, I.B.; Levin, L. Current Concepts in the Management of Periodontitis. Int. Dent. J. 2021, 71, 462–476.
49. Bui, F.Q.; Almeida-da-Silva, C.L.C.; Huynh, B.; Trinh, A.; Liu, J.; Woodward, J.; Asadi, H.; Ojcius, D.M. Association between

periodontal pathogens and systemic disease. Biomed. J. 2019, 42, 27–35.
50. Czerniuk, M.R.; Surma, S.; Roma´ nczyk, M.; Nowak, J.M.;Wojtowicz, A.; Filipiak, K.J. Unexpected Relationships: Periodontal Diseases: Atherosclerosis–Plaque Destabilization? From the Teeth to a Coronary Event. Biology 2022, 11, 272.
51. Ajmera, N.; Chatterjee, A.; Goyal, V. Aloe vera: It's effect on gingivitis. J. Indian Soc. Periodontol. 2013, 17, 435–438.
52. Getie, M.; Abebe, W.; Tessema, B. Prevalence of enteric bacteria and their antimicrobial susceptibility patterns among food handlers in Gondar town, Northwest Ethiopia. Antimicrob. Resist. Infect. Control 2019, 8, 111.
53. Santamaria, J.; Toranzos, G.A. Enteric pathogens and soil: A short review. Int. Microbiol. 2003, 6, 5–9.
54. Riddle, M.S. Travel, diarrhea, antibiotics, antimicrobial resistance and practice guidelines—A holistic approach to a health conundrum. Curr. Infect. Dis. Rep. 2020, 22, 1– 10.
55. Kartal, M. Intellectual property protection in the natural product drug discovery, traditional herbal medicine and herbal medicinal products. Phytother. Res. 2007, 21, 113– 119.
56. Choi, S.W.; Jang, Y.J.; Lee, Y.J.; Leem, H.H.; Kim, E.O. Analysis of functional constituents in mulberry (Morus alba L.) twigs by different cultivars, producing areas, and heat processings. Prev. Nutr. Food Sci. 2013, 18, 256–262.
57. Miljkovic, V.; Nikolic, G.; Mihajlov-Krstev, T.M.; Arsic, B. Antibacterial activities of fruits extracts of three mulberry species (Morus alba L., Morus rubra L. and Morus nigra L.) and bilberry (Vaccinium myrtillus L.). Acta Med. Median. 2018, 57, 5–12.
58. Chen, H.; Yu,W.; Chen, G.; Meng, S.; Xiang, Z.; He, N. Antinociceptive and antibacterial properties of anthocyanins and flavonols from fruits of black and non-black mulberries. Molecules 2017, 23, 4.
59. Jan, B.; Parveen, R.; Zahiruddin, S.; Khan, M.U.; Mohapatra, S.; Ahmad, S. Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review. Saudi J. Biol. Sci. 2021, 28, 3909–3921.
60. Zhu, N., et al., A novel coronavirus from patients with Pneumonia in China, 2019. N Engl J Med, 2020. 382(8):727–733.
61. Dewhirst, F.E., et al., The human oral microbiome. J Bacteriol, 2010. 192(19):5002–17. https://doi.org/10.1128/JB.00542-10.
62. Deo, P.N. and R. Deshmukh, Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol, 2019. 23(1):122–12.
63. Ren, Z., et al., Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. Gut, 2021. 70(7):1253–1265.
64. Sizova, M. V., et al. "New approaches for isolation of previously uncultivated oral bacteria." Applied and environmental microbiology 78.1 (2012): 194-203.
65. Mealey, B. L., Oates, T. W., and American Academy of, P. (2006). Diabetes mellitus and periodontal diseases. J. Periodontol. 77, 1289–1303.
66. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica (Bucur). 2019 Dec;14(4):350-356.
67. Chen X, Daliri EB-M, Chelliah R, Oh D-H. Isolation and Identification of Potentially Pathogenic Microorganisms Associated with

Dental Caries in Human Teeth Biofilms. Microorganisms. 2020; 8(10):1596.

68. Krzyściak, W.; Papież, M.; Jurczak, A.; Kościelniak, D.; Vyhouskaya, P.; Zagórska-Świeży, K.; Skalniak, A. Relationship between pyruvate kinase activity and cariogenic biofilm formation in Streptococcus mutans biotypes in caries patients. Front. Microbiol. 2017, 8, 856.

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