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Holoptelea Integrifolia Bark And Leaves Phytochemicals, An Effective Ethno-Pharmacological Remedy For Treating Oral-Dental Infections

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Abstract: *Holoptelea integrifolia* (Ulmaceae) is a versatile medicinal plant encompassing a wide range of pharmacological properties. The present study is aimed at assessment of anti-bacterial activities of bark and leaf phytochemicals against the common oral bacterial pathogens namely *Staphylococcus aureus, Streptococcus oralis, Streptococcus mutans, Streptococcus gordonii, Lactobacillus brevis* and *Lactobacillus casei*. Bark and leaf dried powder samples were Soxhlet fractionated with solvents viz. petroleum-ether, chloroform, absolute-ethanol, 80%-methanol and distilled-water. Antibacterial potentials of the fractionated extracts against oral pathogens were evaluated with respect to zone of inhibition, MIC, MBC and IC_{50} values whereas antioxidant activities were assessed against $ABTS^{+}$ and DPPH free radicals. The results demonstrated significantly higher antibacterial activity in bark ethanolic extracts (HiBE) towards every tested oral bacterial pathogen as established by its greater zone of inhibition and lowest MIC, MBC and IC_{50} values. HiBE most potent antibacterial action against *L. casei* followed by *L. brevis*, *S. mutans*, *S. aureus, S. gordonii and S. oralis* HiBE showed remarkable $ABTS^{+}$ and DPPH free radicals scavenging activities with abundance of phenolics, flavanoids and tannins. Therefore, the present study ascertains *H. integrifolia* bark extracts as a commendable natural remedy for treating oral-dental infections.

Keywords: Antibacterial activity; antioxidant; *H. integrifolia* bark and leaves; oral bacterial pathogens; polyphenols; oral diseases

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1. INTRODUCTION

Oral diseases including dental caries and periodontal diseases are globally considered as major health problems. The relationship between oral diseases and natural microbiota in oral cavity is well recognized[1]. More than 750 bacterial species inhabit oral cavity and many of these have been recognized as a causative agent of oral diseases[2]. The worldwide requirement for the search of safe, effective and economical alternative methods to the chemotherapeutics to prevent and treat oral diseases is arising due to increased prevalence of antibiotic resistance in oral pathogens to currently employed antibiotics along with their undesirable side effects and economical considerations in developing countries[3-4]. Natural products derived from a variety of medicinal plants belong to families such as *Fabaceae, Ebenaceae, Bombaceae* and *Anonnaceae* have already been proven to be an excellent alternative treatment against bacterial pathogens in oral diseases[5-12]. *Holoptelea integrifolia* Planch (family *Ulmaceae*) commonly known as Indian elm is one of the most popular medicinal plant traditionally used in the treatment of several health maladies including leucoderma, eczema, leprosy, rickets, rheumatism, ringworm, malaria, intestinal cancer, and chronic wounds[13]. Pharmacological studies on crude extracts and purified compounds of leaves and barks of *H. integrifolia* demonstrated antibacterial, anti-inflammatory, anthelmintic, antidiarrhoeal, adaptogenic, antidiabetic, antitumor, antioxidant and wound healing properties[14]. Antibacterial investigations on the fractionated extracts of bark and leaves showed antibacterial activities against *Bacillus subtilis*, *Bacillus cerculences*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,Klebsiella pneumoniae , Citobacter freundii*, *Micrococcus luteus*, *P. fluorescens, Mycobacterium tuberculosis* H37RV[15-18]. Nonetheless, despite its remarkable antibacterial properties, the *H. integrifolia*, bark and leaves extracts have never been tested against oral pathogens. Therefore, the present study is focused on the 1) extraction of phytochemicals from the air-dried bark and leaves through soxhlet using five different solvents viz., petroleum ether, chloroform, absolute ethanol, 80% aqueous methanol and distilled water; 2) evaluation of fractionated extracts for antibacterial activities by determining their zone of inhibition, MIC, MBC and IC_{50} values against most common oral bacterial pathogens including *Streptococcus mutans, Streptococcus oralis, Streptococcus gordonii, Staphylococcus aureus, Lactobacillus brevis* and *Lactobacillus casei*; 3) assessment of extracts for free radical scavenging activities against $ABTS^{+\cdot}$ and DPPH free radicals.

2. MATERIAL AND METHODS

2.1 *Chemicals*

The chemicals along with solvents used in the present study were of analytical grade (more than 99% pure) and procured from Merck Pvt. Ltd. India. Chemicals such as atropine, antibiotic discs amoxicillin (10 mcg) and ampicillin (10 mcg), bromocresol green, culture media such as nutrient agar, nutrient broth in addition to *Lactobacillus* MRS, brain heart infusion broth and agar were procured from Himedia Pvt Ltd. India. Catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and linalool were purchased from Sigma-Aldrich (St Louis, MO, USA). 2, 2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{+}$) was obtained from Calbiochem, Merck Pvt. Ltd. (Darmstadt, Germany).

2.2 *Plant Material*

H. integrifolia bark and leaves samples were harvested from Gumaniwala, Rishikesh (latitude 30.0777° N, longitude78.2462° E), Uttarakhand, India during the period of June-July and got authenticated by Botanical Survey of India (BSI), Dehradun, Uttarakhand, India, where it was deposited under the voucher number 116021.

2.3 *Preparation of Extracts*

The 50 g fine powder of shed-air-dried bark and leaves were processed individually for Soxhlet extraction (Borosil Pvt Ltd, India) using an eluotropic series of solvents including petroleum ether, chloroform, absolute ethanol, 80% aqueous methanol and distilled water with plant dry powder : solvent ratio (1:10) at temperature lower than their respective boiling points. Samples were saturated at reflux for 24 h before proceeding to fractionation for 8-10 h with each solvent. Extracts were filtered (Whatman filter paper No 1), dried at 45°C under reduced pressure by rotary evaporator (Perfitt Pvt. Ltd. India) and stored at -20°C till further analysis.

2.4 *Phytochemical Analysis*

To analyze phytochemicals, the dried extracts fractionated by organic solvent and distilled water both were reconstituted in absolute ethanol and distilled water respectively at a concentration of 10 mg/mL and further analyzed for total phenolic contents (TPC), total flavonoid contents (TFC), alkaloids, tannins and terpenoid contents. TPC in the individual extracts was evaluated by Folin-Ciocalteu colorimetric method using gallic acid as standard[19] while TFC in the extracts was determined as catechin equivalents using the protocol mentioned by Saini et al[19]. Alkaloid contents were estimated according to the method described by Patel et al.[20] using atropine as standard. Tannins contents were determined according to Folin-Denis method[21] using gallic acid (25-150 mg/mL) as standards while terpenoid contents were measured by the protocol described by Ghorai et al.[22] using linalool as standard (100 mg/mL).

2.5 *Antimicrobial Assays*

2.5.1 *Bacterial strains*

*Lactobacillus brevis (*MTCC1423), *Lactobacillus casei* (MTCC1750), *Staphylococcus aureus* sub-spp. *aureus* (MTCC1144), *Streptococcus gordonii* (MTCC2695), *Streptococcus mutans* (MTCC890), and *Streptococcus oralis* (MTCC2696) which are common oral pathogens were procured in lyophilized form from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India . *S. aureus, S. oralis* and *S. gordonii* and *L. brevis* were grown and maintained on broth and agar media of enriched brain heart infusion. *St. mutans* and *L. casei* cultures were grown and maintained on nutrient media while *L. casei* was maintained on MRS media.

2.5.2 *Antibacterial activity*

Antibacterial activities of fractionated extracts of *H.integrifolia* bark and leaves were evaluated using zone of inhibition assays as per Kirby-Bauer cup well method (Agar diffusion

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assay)[23] in which 100 µL each of the oral pathogen culture broth which was grown for 24-48 h (approx.10^6 CFU/mL) were spread plated on their respective culture media agar plate. 50 µL of each fractionated extracts of *H.integrifolia* bark and leaves (1.0 mg/mL) was poured in the well (6 mm diameter) made with the help of a sterile cork borer on inoculated bacterial agar plate. Antibiotic solutions including ampicillin (50 µg/mL) and amoxicillin (50 µg/mL) were considered as positive control, whereas absolute ethanol was used as negative control. Plates were refrigerated at 2–8°C for 2 h to promote effective diffusion of extracts as well as standards followed by incubation at 37°C for 24 to 48 h. Measurement of the diameters of zones of inhibition developed around the was done using Hi-media zone scale (Himedia Pvt Ltd. India),

2.5.3 *Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)*

For MIC evaluation, inoculums were prepared by growing each oral bacterial pathogen for 2-6 h in their respective culture broth with constant shaking (100 rpm). Turbidity of the growing culture was adjusted equivalent to that of 0.5 McFarland standards (approx. $5x10^5$ CFU/ml)[24] with sterile broth. Afterwards, 1.0 mL diluted extracts (0.005-10 mg/mL) in respective culture media) was added to 1.0 mL of each bacterial culture broth followed by incubation at 37°C for 24 h in case of *S. aureus, S. oralis, S. gordonii, S mutans* and *L. brevis* whereas with *L. casei*,the incubation period was kept to be 48 h at 35°C. Negative and solvent control with broth and ethanol devoid of extracts were also setup. Ampicillin (50 µg/mL) and amoxicillin (50 µg/mL) were used as the positive controls. The minimum extracts concentration that exhibited absence of any visible bacterial growth was considered as MIC. The % inhibition of growth was determined by measuring absorbance at 600 nm and then plotting it against extracts concentration to establish the extracts concentration which displays 50% inhibition (IC_{50}) against each oral bacterial pathogen. For determining MBC, 50µL of bacterial suspension treated with extracts in MIC experiment were spread plated on respective culture media agar plates and incubated for 24-48 h. The lowest extracts concentration exhibiting absolute inhibition of bacterial growth was considered as MBC.

2.5.4 *Antioxidant Activity*

Antioxidant activities of *H. integrifolia* bark and leaves fractionated extracts were determined in terms of free radical scavenging activities against $ABTS^{+\cdot}$ cationic (ABTSRSA) and DPPH free radicals scavenging activity (DPPHRSA) as per the methods described by Saini et al.[19] .

3. STATISTICAL ANALYSIS

In order to avoid the possibility of any inconsistency,all the experiments were performed in triplicates in case of all the fractionated extracts of *H. integrifolia* bark and leaves. The results were expressed with respect to mean of three independent experiments (n = 3) along with standard error (SE). Statistical analysis including standard error and level of significance test using one way –ANOVA of the data was performed using MS Excel and Prism 3 pad software (Microsoft, Redmond, WA, USA) respectively.

4. RESULTS

4.1 Extraction

Characteristics and yield (% w/w) of the fractionated extracts are presented in the Table 1. Among leaves and bark extracts, HiLM showed significantly higher yield ($p<0.001$) than the rest of the extracts. Leaves and barks both showed better extract yields in alcoholic and distilled water extracts as compared to that in non-polar solvents.

Table 1. Characteristics and yield of H. integrifolia leaves and bark extracts.

Part used	Solvent	Characteristics	Total % yield (g) (w/w)
Leaves	Pet. Ether (HiLPE)	Light green	1.66±0.04
	Chloroform (HiLCH)	Blackish green	1.15±0.11
	Abs.Ethanol (HiLE)	Brown green, sticky	2.10±0.06
	80 % Methanol (HiLM)	Brown green, sticky	8.27±0.20
	Distilled Water (HiLDW)	Pale brown sticky	7.39±0.14
Bark	Pet. Ether (HiBPE)	Reddish yellow, waxy	3.01±0.0
	Chloroform (HiBCH)	Reddish waxy	0.40±0.037
	Abs. Ethanol (HiBE)	Dark Brown, sticky, waxy	4.4±0.23
	80%Methanol (HiBM)	Dark brown, sticky, waxy	4.14±0.86
	Distilled Water (HiBDW)	Brown sticky	3.44±0.33

Results expressed as Mean ± SE of triplicates

4.2 Phytochemical Analysis

Phytochemical analysis of all the fractionated extracts of *H. integrifolia* leaves and bark was done with respect to TPC, TFC, alkaloids, tannin and terpenes (Table 2). TPC values using gallic acid as standard (R^2=0.982) was observed to be highest ($p<0.001$) in HiLE followed by HiLM, HiLDW, HiBE, HiBDW and HiBM (Table 2). TFC, determined as catechin equivalent (R^2=0.999) was even though found to be highest in HiLM however the value was no significantly different ($p>0.05$) to that of HiLE and HiLDW. Tannins contents measured as tannic acid equivalent (R^2= 0.996) was highest in HiBE followed by HiBM, HiBDW, HiLE, HiLM and HiLDW. It is also very much noteworthy to mention that leaves possessed significantly higher ($p<0.001$) levels of both TPC and TFC than bark extracts while bark possessed higher ($p<0.001$) tannin contents than leave extracts (Table 2). Alkaloid content determined as atropine equivalent (R^2=0.999) was observed to be significantly higher ($p<0.001$) in HiBCH followed by HiBPE. Terpenes content evaluated linalool as standard (R^2=0.944) was maximum in HiBPE followed by HiBCH, HiBLPE and HiLCH (Table 2).

Table 2. Phytochemical analysis of H. integrifolia leaves and Bark extracts

Extracts	TPC (mgGAE/gDW)	TFC (mg CE/g DW)	Tannin (mg TE/g DW)	Alkaloids (mg AE/ g DW)	Terpenes (mg LE/g DW)
HiLPE	Nd	Nd	Nd	Nd	15.75 ±0.30
HiLCH	Nd	Nd	Nd	Nd	12.28±0.15
HiLE	129.87±3.9	229.50±25.57	28.36±1.0	Nd	Nd
HiLM	89.19±1.3	244.40±3.43	14.11±0.15	Nd	Nd
HiLDW	63.64±1.6	193.40±6.65	9.88±0.46	Nd	Nd
HiBPE	Nd	Nd	Nd	1.539±0.022	53.46±1.35
HiBCH	Nd	Nd	Nd	4.576±0.033	42.97±0.24
HiBE	25.41±0.10	59.58±2.70	48.99±1.48	Nd	Nd
HiBM	17.48±0.39	59.66±1.60	43.69±3.17	Nd	Nd
HiBDW	17.84±4.0	25.31±0.94	19.16±1.39	Nd	Nd

1. *GAE, gallic acid equivalents; CE, catechin equivalents; AE, atropine equivalents; TE, tannic acid equivalents; LE, linalool equivalents; DW, dry weight' Nd, not detected*
2. *Each value is expressed as mean±SE (n=3).*

4.3 Zone of inhibition

The fractionated extracts of *H. integrifolia* leaves and barks were screened for zone of inhibition (ZOI) against common oral bacterial pathogens namely *S. aureus, S. oralis, S. mutans*, *S. gordonii, L. casei* and *L. brevis* (Table 3). Among the fractionated extracts of both leaves and bark, HiBE showed noteworthy ZOI against all the tested pathogens followed by HiBM, HiBCH, HiLE and HiLM extracts. Leaves extracts HiLE showed highest ZOI against *S. aureus, S. oralis, S. mutans*, and *S. gordonii,* however it remained ineffective against *L casei* and *L. brevis.* Similar pattern of inhibition was also showed by HiLM. While, HiBCH extracts strongly inhibited most of the bacterial pathogens, it stayed futile against *S. gordonii*. Surprisingly, in contrary to HiBCH, HiLCH showed potent inhibition only against *S. gordonii* (Table 3).

Table 3. Zone of inhibition of H. integrifolia extract against tested bacterial species associated with dental problems.

Extracts	Zone of inhibition (mm) against Bacterial species					
	S. aureus	*S. oralis*	*S. mutans*	*S.gordonii*	*L casei*	*L. brevis*
HiLPE	-	-	-	-	-	St
HiLCH	-	-	-	20.33±0.88	-	-
HiLE	19.66±0.33	23.02±0.89	18.66±0.66	19±0.57	-	-
HiLM	15.33±0.88	16.33±0.88	14±0.57	14±0.57	-	-
HiLDW	-	-	-	-	-	-
HiBPE	-	-	St	-	-	-
HiBCH	14.0±0.12	16.02±2.3	13.0±1.3	-	10.66±0.33	12.33±0.80
HiBE	19±1.0	21.33±0.882	17.33±0.882	17.05±0.40	16.0±06	17.23±2.3
HiBM	11.8±0.3	14.8±0.71	11.33±0.882	15.33±0.88	10.06±0.88	9±0.32
HiBDW	-	-		-	10±1.08	-
Positive control						
Amox	37	40	30	35	18	41
Amp	39	65	25	39	18	48
Negative control						
Absolute ethanol	-	-	-	-	-	-

Results expressed as Mean ± SE of triplicates; Amox and Amp: antibiotics amoxicillin and ampicillin coated on the disc (10 mg/mL). "-" indicates absence of zone of inhibition against bacterial species."St" indicates static growth

4.4 MIC and MBC

Fractionated extracts showing the notable ZOI were further evaluated for MIC and MBC against *S. aureus, S. oralis, S. mutans*, *S. gordonii, L. casei* and *L. brevis*. As evident in the Table 4, HiBE exhibited lowest MIC and MBC against all the tested oral bacterial pathogens excluding *S. oralis* which was much more sensitive to HiBM. HiBM was the second most effective extract against all the oral pathogen as explained by its lower MIC and MBC values than rest of the active extracts. Among leaves extracts, HiLE and HILM showed comparable MIC and MBC values. Moreover, the IC_{50} of all the fractionated extracts were also evaluated and compared against all the tested oral bacterial pathogens as shown in Table 5. The IC_{50} values of the extracts were in accordance to their MIC and MBC values (Table 4 and Table 5). The order of sensitivity of HiBE based on its IC50 values against pathogens was found to be *L. casei* ≥*L. brevis*>*S. mutans*>*S. aureus* >*S. gordonii*≥*S. oralis* (Table 5) while the sensitivity of HiBM in descending order was observed to be *S. oralis*> *L. casei* ≥*L. brevis*> *S. mutans*≥*S. aureus*>*St gordonii.*

Table 4. Antibacterial activity of H. integrifolia leaves and bark extracts

Extracts	Antibacterial activity (mg/mL)											
	S. aureus		*S. oralis*		*S. mutans*		*S. gordonii*		*L casei*		*L. brevis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
HiLPE	-	-	-	-	-	-	-	-	-	-	-	-
HiLCH	-	-	-	-	-	-	0.25	2.0	-	-	-	-
HiLE	0.25	1.0	0.1	0.5	0.05	0.5	0.05	0.25	-	-	-	-
HiLM	0.1	0.5	0.1	0.5	0.05	0.5	0.1	0.5	-	-	-	-
HiLDW	-	-	-	-	-	-	-	-	-	-	-	-
HiBPE	-	-	-	-		-	-	-	-	-	-	-
HiBCH	0.1	1.0	0.1	0.5	0.05	0.5			0.25	2.0	0.1	1.0
HiBE	0.05	0.5	0.1	0.5	0.05	0.25	0.05	0.25	0.01	0.05	0.01	0.05
HiBM	0.05	0.5	0.05	0.25	0.05	0.25	0.05	0.5	0.05	0.25	0.05	0.25
HiBDW	-	-	-	-	-	-	-	-	0.25	NA	-	-

Results expressed as Mean of triplicates; MIC, Minimal Inhibitory Concentration; MBC, Minimal Bactericidal Concentration; MIC and MBC values are expressed in mg/ml. – indicates absence of antibacterial activity

Table 5. IC_{50} values of H. integrifolia leaves and bark extracts

Extracts	IC50 Values (mg/mL)					
	S. aureus	*S. oralis*	*S. mutans*	*S. gordonii*	*L. casei*	*L. brevis*
HiLCH	--	--	--	0.896±0.091	--	--
HiLE	0.455±0.023	0.195±0.008	0.133±0.01	0.109±0.01	--	--
HiLM	0.328±0.023	0.244±0.012	0.072±0.002	0.235±0.09	--	--
HiBCH	0.419±0.013	0.224±0.023	0.151±0.051	--	0.786±0.071	0.262±0.023
HiBE	0.120±0.021	0.192±0.029	0.101±0.015	0.187±0.016	0.011±0.0	0.0126±0.002
HiBM	0.121±0.012	0.069±0.004	0.116±0.003	0.216±0.002	0.084±0.001	0.0914±0.0032
HiBDW	--	--	--	--	0.562±0.028	--

4.5 Determination of Antioxidant activity

Antioxidant activities of the fractionated extracts were determined with respect to free radical scavenging activities namely DPPH radical scavenging activity (DPPH-RSA) and ABTS+ radical scavenging activity (ABTS-RSA). The HiLE and HiBE showed highest ABTS-RSA with lowest IC_{50} values with no significant difference ($p>0.05$) followed by HiBM, HiLM, and HiBDW with significant difference ($p<0.001$) (Table 6). DPPH-RSA was highest in HiLE followed by HiLM, HiBM, HiBE, and HiBDW with significance difference ($p<0.001$) as evident by their increasing IC 50 values from lowest to highest..

Table 6. Free radical scavenging activities of H. integrifolia leaves and bark extracts

Extracts	IC50 Value (µg/mL)	
	$ABTS^{+}$-RSA	DPPH-RSA
HiLCH	-	-
HiLE	2.09±0.027	0.313±0.009
HiLM	4.190±0.140	1.065±0.033
HiBCH	-	-
HiBE	2.205±0.112	1.471±0.021
HiBM	3.281±0.060	1.299±0.028
HiBDW	18.874±0.108	2.190±0.040

5. DISCUSSION

Dental caries and dental plaques are the most common biofilm-infectious diseases caused by the uncontrolled growth of acid-producing and acid-tolerant bacterial sp. which adhere to the slimy layer on the tooth surface containing salivary polymers and food debris that can lower the plaque pH resulting in tooth demineralization and caries[25-26]. Some of the acidogenic and acid tolerant bacterial sp. which actively participate in the biofilm formation include *S. mutans, S. oralis, S. gordonii, S. aureus, L. brevis* and *L. casei*[27-29]. Present study is the first report demonstrating anti-cariogenic potentials of *H. integrifolia* leaves and bark extracts against the above mentioned most common cariogenic bacterial pathogens. To confirm the appropriate separation of antibacterial compounds, dried powders of leaves and stem-bark of *H. integrifolia* were individually subjected to soxhlet-fractionation using the solvents in the order viz. petroleum ether, chloroform, absolute ethanol (100%), 80% aqueous methanol and distilled water[30]. The observations showed remarkably higher antibacterial activity in HiBE against all the tested oral bacterial pathogens as evident by its higher zone of inhibition and lowest MIC, MBC and IC_{50} values (Table 3, 4, 5) followed by HiBM. Leaves alcoholic extracts namely HiLE and HiLM were also very effective against majority of the tested oral pathogens except *L. brevis* and *L. casei.* While, antioxidant activities against ABTS cations were comparable in bark and leaves extracts however DPPH scavenging activities were significantly higher in leaves (HiLE and HiLM) than in bark (HiBE and HiBM). Among phytochemicals, TPC and TFC were significantly higher in HiLE and HiLM while tannins were higher in HiBE and HiBM. Terpenes were found to be

higher in HiBPE and HiBCH than HiLPE and HiLCH. Alkaloids were exclusively observed in the HiBPE and HiBCH extracts. The antibacterial activity of *H. integrifolia* leaves and bark extracts were in accordance with previous studies[15-17,31]. Reddy et al.[15] studied antibacterial and antioxidant activities of methanolic extracts of bark (MSBE) and leaves (MLE) of *H. integrifolia*. Bark extracts were highly effective against *B. subtilis*, *S. aureus* and *K. pneumoniae* while leave extracts demonstrated better antibacterial activity against *B. cerculences* and *P. aeruginosa.* However *E. coli* was equally sensitive to both bark and leaves extracts. Reddy et al.[15] further proposed that higher antibacterial activity of bark extracts might be due to its higher TPC and DPPH free radical scavenging activity. Similar pattern of differential sensitivity of tested oral pathogens was observed in the present study. *S. aureus, S. oralis, L. brevis* and *L. casei* were found to be highly sensitive to HiBE and HiBM while *S. mutans*, and *S. gordonii* were more sensitive to the HiLE and HiLM. In contrast to Reddy et al.[15], we observed significantly higher TPC/TFC and DPPH free radical scavenging activity in the HiLE and HiLM extracts than HiBE and HiBM extracts which contained significantly higher tannin content and comparable ABTS free radical scavenging activity. Higher antibacterial activity of HiBE and HiLE might be due to tannin and TPC/TFC respectively which is quite justifiable. Tannins have been shown to exert antibacterial activity against various pathogenic bacterial strains owing to their strong metal chelating properties in addition to inactivation of membrane-bound proteins and impairment of cell membrane[32]. TPC and TFC have been shown to disrupt membrane potential of bacterial cells, may also bind enzymes active site and alters microbial cell metabolism[33-34]. Durga and Paarakh[16] evaluated *H. integrifolia* bark extracts namely petroleum ether, benzene, chloroform, methanol and aqueous extracts for antibacterial activities against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* and found chloroform extracts highly inhibitory against all the tested bacterial spp. Comparable results were obtained in the present study with HiBCH extracts being inhibitory to *S-. mutans, S. oralis, S. aureus, L. brevis* and *L. casei* except *S. gordonii* whereas HiLCH showed potent inhibition only against *St. gordonii*. Antibacterial activity of HiBPE and HiBCH extracts might be due to abundance of terpenes and alkaloids. Terpenes have been shown to exert antibacterial action since they cause changes in membrane permeability and K^+ leakage[35]. Moreover, Vinod et al.[31] reported isolation of a terpenoid namely 1, 4- naphthalenedione in diethyl ether leaf extracts of *H.integrifolia* which showed potent antibacterial activityagainst lactam resistant strain of *S.aureus*. Low levels of alkaloids in HiBPE and HiBCH extracts might also have contributed in their antibacterial actions as theyare known to possess antibacterial activity owing to their capability of forming hydrogen bonds with proteins and enzymes due to the presence of proton-accepting nitrogen atom along with polycyclic moieties in addition to proton-donating amine hydrogen and phenolic hydroxyl groups[36]. Antibacterial effect of HiLCH which exclusively contained moderate level of terpenes similar to the findings of Ahmad et al.[17] which showed antibacterial potential of chloroform leaf extract of *H. integrifolia* against *Citrobacter freundii, Micrococcus luteus, Pseudomonas aeruginosa*, and *Pseudomonas fluorescens* using disk diffusion method and found that it was highly effective against *C. freundii* followed by *P. fluorescens P. aeruginosa*, and *M. luteus*.

6. CONCLUSION

The present study ascertains *H. integrifolia* bark and leaf extracts as an abundant source of polyphenols with potent antibacterial action against oral bacterial pathogens besides commendable free radical scavenging activities. Therefore, *H. integrifolia* bark and leaf extracts could serve as a great natural remedy for treating oral-dental infections.

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9. AUTHORS CONTRIBUTION STATEMENT

Mrs Savita Petwal has carried out the entire experimental work besides data analysis. Dr. Madhuri Kaushish Lily has made substantial contribution in the preparation of manuscript as well as experimental design in addition to data analysis and results interpretation. Dr. Koushalya Dangwal being the corresponding author of this article has made active participation in the manuscript preparation, experimental design, along with data analysis and results interpretation. The authors have made discussion regarding the methodology as well as results and contributed to the final manuscript.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

11. REFERENCES

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