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Oral and periodontal antibacterial activity of *Dodonaea angustifolia* plant extracts

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Abstract
This research study was done to evaluate the inhibitory and bactericidal effects of phytochemical extracts of the aerial part of the *Dodonaea angustifolia* plant found in Eritrea highlands where it is commonly known as *Tahses*. Phytochemical extraction of leaf, bark and stem part of the plant was done with ethanol, methanol and diethyl ether using Soxhlet extraction method. Agar well diffusion was used to evaluate the diameter of inhibition of different concentration of plant extracts against *Streptococcus mutans, Lactobacillus casei, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum* and *Capnocytophaga canimorsus*. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were also determined, in addition to compare the results of the plant extracts with the results of the susceptibility of these pathogenic microorganisms against conventional antibiotics. Completely randomized design (CRD) was used with three replications. The bioassay MIC test result showed that there was no inhibition of bacterial growth by the leaf, bark and stem extracts of diethyl ether, ethanol and methanol of concentration of 2.5mg/ml, 5mg/ml and 10mg/ml against all the bacterial species tested. However higher concentration of these plant extracts of 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml showed significant inhibition in all the tested bacterial species. Diethyl ether extracts of the bark and leaves demonstrated more inhibitory effect against the tested microorganisms as compared to ethanolic and methanolic extracts of leaves, bark and stem of the plant. Isolation and screening of *D. angustifolia* phytochemicals and their clinical trials against oral and periodontal pathogens would be ideal in determining their efficacy and pharmacological activities.

Keywords: *Dodonaea angustifolia*, phytochemicals, Eritrea, Tahses, bactericidal

1. Introduction
Medicinal plants constitute the major constituents of most indigenous medicines and a large number of allopathic medical preparations contain one or more component(s) of plant origin. The medicines that are in use today are definitely not the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries have continuously contributed to the type, quality, presentation and concept of medicinal preparation [1]. In the development of human knowledge for therapeutic use, scientists endeavored to isolate different chemical constituents from plants, subjected them to biological and pharmacological tests and then used them to prepare modern medicines [2]. There is increasing interest in the use of plant antioxidants for scientific research as well as for industrial (dietary, pharmaceutical and cosmetics) purposes. Significant activities of the other *Dodonaea* genus plants like *Dodonaea viscosa* crude extract have been reported against gram positive, gram-negative organisms as well as fungal *Candida albican* strain [2, 3].
Anticandidal activity in crude acetone-based extract of the leaves of *D. viscosa* has been reported when forty clinical isolates of *C. albican* including twenty samples each from HIV-positive and HIV-negative patients and a separate control strain, with MIC range of 6.25µg/ml to 25µg/ml [3]. *Dodonaea angustifolia* is found in many parts of Eritrea where it is commonly known as *Tahses*. Eritreans used it commonly to brush their teeth. It is therefore suspected to contain some oral antimicrobial activity. Studies that have been done on some *Dodonaea species* especially D. viscosa sowed that the plant contains diterpenoids, triterpenes, flavonoids, saponins and a complex mixture of other phenolic compounds [2, 3]. The therapeutic activity is associated with polyvalent pharmacological effects occurring due to a synergistic combination of several constituents instead of a single isolated one [2, 3].

The botanical classification of *D. angustifolia* is as follows:

Kingdom	:	*Plantae*
Division	:	*Spermatophyte*
Sub-Division	:	*Angiospermae*
Class	:	*Dicotyledonae*

Sub-class	:	*Magnoliales*
Order	:	*Sapindales*
Family	:	*Sapindaceae*
Genus	:	*Dodonaea*
Species	:	*Angustifolia*

2. Materials and Methods

2.1 Sample collection

The aerial part (stems, bark and leaves) of *Dodonaea angustifolia* of the family *Sapindaceae* was collected from hilly region near *Habrengaka* and *Balwa* villages in Anseba region, Eritrea. Identification was done by the special key given in Campbell flora [4]. The leaf, bark and stems of *D. angustifolia* were washed with sterile distilled water then shade dried and then powdered using pestle and mortar.

2.2 Preparation of plant extracts

The plant material samples of *D. angustifolia* were surface-rinsed with tap water then with distilled water to remove surface dust and other solid contaminants. They were then dried in the shade and milled to a fine powder. Extracts were prepared using a method described by Eloff, (1999) [5] with slight modifications. Three solvents were used for extraction: Diethyl ether, methanol (Merck Chemicals Pty. Ltd, SA), and ethanol (Sigma-Aldrich, SA). Ten gram of powder was mixed with 100 ml of the solvent, vortexed for 30 minutes using Genie 2 vortexer (Lasec, SA) and centrifuged at 10000 rpm for 20 minutes using a micro centrifuge 5424 (Merck Chemicals Pty. Ltd, SA). The supernatant was collected in a pre-weighed 500 ml beaker. The above procedure was repeated three times using the same powder. All three supernatants were pooled together in the same beaker and the solvent was allowed to evaporate under a cold air stream. The beaker was weighed again with the dried plant extract. A yield of dried extract was calculated by subtracting the weight of the empty beaker from the weight of the beaker with the plant extract. The crude extracts were then stored at 4˚C for further analysis.

2.3 Preparation of concentrations

10%w/v stock solutions were prepared by mixing 5g from the dried leaf, bark and stem extract with 50 ml of 60%v/v ethanol solution. The stock solutions were then sterilized with 0.22µm Millipore membrane filter. Different concentrations of 2.5mg/ml, 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml was then prepared from the stock solutions using the formula $C_1V_1 = C_2V_2$. Negative control of 60%v/v ethanol solution was also prepared.

2.4 Test Microorganisms

The microorganisms used were Streptococcus mutan, Lactobacillus casei, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum and Capnocytophaga canimorsus. S. mutan and L. casei were obtained from Microbiology laboratory of Hamelmalo Agricultural College, while P. gingivalis, P. intermedia, F. nucleatum and C. canimorsus were obtained from Microbiology laboratory of Azel pharmaceutical plc.

2.5 Preparation of antibiotic solutions

A- Phosphate Buffer Solution: This solution was prepared according to [6].

B- Sodium hydroxide Solution (1M): This solution was prepared by dissolving 4 g of Sodium hydroxide in 100 ml of distilled, deionized water in volumetric flask.

2.6 Determination of antimicrobial activity

2.6.1 The Agar Well diffusion method

The agar well diffusion method was used for the determination of zone of inhibition of bacterial growth as a measure of antibacterial activity of the plant extracts [7, 8]. Every 100ml of cultured media were inoculated with 0.1ml of bacterial inoculum (containing 1.5×10^8 cell/ml after standardization with McFarland standard solution. The optical density was measured spectrophotometrically at 696nm. After proper homogenization it was poured into Petri dishes. Thereafter, 10mm wells were made by using sterilized cork borer. 100µL of plant extract solutions were then introduced into the wells. The plates were then incubated at 37˚C for 48 hours. The experiment was performed three times and the activity of plant extracts was determined by measuring the diameter of inhibition zone around each well in millimeter (mm).

10%v/v ethanol was used as negative control while 10mg/ml of erythromycin and 10mg/ml of chlorhexidine HCl was used as positive control.

2.6.2 Test for Minimum Inhibition Concentration (MIC)

10ml of 2.5mg/ml, 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml plant extracts concentrations were prepared from stock solutions by using nutrient broth and sabouraud dextrose broth and Dimethyl sulfoxide (DMSO) as solvent vehicle. Aliquot of 0.1ml of bacterial inoculum (containing1.5×10^8 cell/ml) was added to all test tubes, mixed well and incubated under anaerobic conditions for 48 hours at 37 ºC (for *S. mutan* and *L. casei)*, for 96 hours at 37 ˚C (for *C. canimorsus*) and for 7 days at 37˚C for *P. gingivalis, P. intermedia* and *F. nucleatum*. Results were recorded according to the turbidity appearance in the test tubes and compared with the control tubes. Two control tubes were used to determine bacterial growth. The first tube was contained broth and bacterial inoculum while the second tube was contained broth and plant extracts. The MIC was defined as the lowest concentration that prevents visible turbidity appeared clearly to the naked eye in the cultured broth [9].

Chlorhexidine gluconate (5%w/v *in vivo* dose) and erythromycin (500mg/ml) were used as positive control. The tests were done in triplicates. 40µL of 0.2mg/ml of Iodonitrotetrazolium violet (INT) added to each well, incubated further 24hrs Bacterial growth was indicated by formation of red formazin colour.

2.6.3 Test for Minimum Bactericidal Concentration (MBC)

The MBC was determined as a concentration where 99.9% or more of the initial inoculums is killed by taking 0.1ml from prepared tubes and spread on the culture media plates by sterilized cotton swap stick then the plates were incubated for 24 hours at 37 ºC. The results were recorded by existing or not existing of bacterial growth [10, 11].

Data Analysis

Laboratory analytical results expressed as mean (n = 3) were subjected to analysis of variance (ANOVA) using SAS software, version 9.2, 2nd edition of 2010. Separation of the means was performed using Duncan significance test ($p < 0.05$).

3. Results and Discussions

3.1. Diameter of Zones of inhibition

The agar well diffusion method was used for the determination of antibacterial effect of the crude extracts of

the leaves, bark and stem of *D. angustifolia* plant. All the leaf, bark and stem extracts demonstrated a degree of inhibition at all tested microorganisms as shown in tables 1, 2 and 3. This may due to the fact that the crude extracts of *D. angustifolia* plant contains terpenes, phenols, flavonoids and saponins which have a great effect as antimicrobial agents and therefore have potential antimicrobial effect [12].

Table 1: Diameter of inhibition zones of leaf extracts of *D. angustifolia* against oral and periodontal pathogens

Leaf Extracts	Conc. (mg/ml)	*C. canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
-ve cont.	10%Eth	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Di Methyl ether Extract	2.5	8.6 ± 0.3^{c}	12.7 ± 0.4^{b}	11.6 ± 0.3^{c}	9.2 ± 0.4^{b}	14.3 ± 0.2^{a}	9.4 ± 0.3^{b}
	5	10.2 ± 0.2^{c}	14.6 ± 0.2^{b}	13.2 ± 0.2^{c}	11.7 ± 0.4^{c}	16.0 ± 0.3^{a}	10.5 ± 0.3^{c}
	10	12.9 ± 0.3^{c}	15.5 ± 0.2^{b}	17.6 ± 0.2^{c}	13.7 ± 0.1^{c}	17.6 ± 0.2^{a}	12.8 ± 0.1^{c}
	20	16.4 ± 0.3^{b}	17.7 ± 0.3^{b}	21.8 ± 0.1^{a}	15.4 ± 0.3^{c}	18.6 ± 0.2^{a}	14.7 ± 0.1^{c}
	30	17.8 ± 0.2^{c}	20.8 ± 0.1^{b}	22.5 ± 0.3^{a}	16.9 ± 0.2^{c}	20.5 ± 0.3^{a}	17.4 ± 0.1^{c}
	40	18.8 ± 0.2^{c}	21.8 ± 0.1^{b}	24.6 ± 0.2^{a}	19.4 ± 0.2^{c}	23.0 ± 0.3^{a}	18.6 ± 0.2^{c}
	50	21.6 ± 0.2^{b}	24.1 ± 0.3^{ab}	25.0 ± 0.3^{c}	21.4 ± 0.3^{c}	24.6 ± 0.3^{b}	20.7 ± 0.2^{c}
Ethanol Extract	2.5	18.3 ± 0.4^{a}	12.7 ± 0.4^{b}	11.6 ± 0.3^{c}	11.9 ± 0.2^{a}	13.4 ± 0.3^{b}	13.9 ± 0.3^{a}
	5	19.6 ± 0.5^{a}	14.6 ± 0.2^{b}	13.2 ± 0.2^{c}	13.3 ± 0.4^{b}	14.6 ± 0.1^{b}	15.4 ± 0.3^{a}
	10	21.8 ± 0.2^{a}	15.5 ± 0.2^{b}	15.6 ± 0.2^{c}	15.9 ± 0.2^{b}	16.5 ± 0.2^{b}	17.2 ± 0.2^{a}
	20	23.6 ± 0.1^{a}	17.7 ± 0.3^{b}	16.8 ± 0.1^{c}	17.6 ± 0.1^{b}	19.0 ± 0.3^{a}	23.0 ± 1.4^{a}
	30	24.4 ± 0.2^{a}	20.8 ± 0.1^{b}	18.0 ± 0.3^{c}	18.8 ± 0.2^{b}	20.1 ± 0.3^{a}	22.3 ± 0.2^{a}
	40	25.1 ± 0.2^{a}	21.8 ± 0.1^{b}	20.6 ± 0.2^{b}	21.9 ± 0.2^{b}	22.5 ± 0.2^{a}	24.8 ± 0.2^{a}
	50	25.2 ± 0.4^{a}	24.1 ± 0.3^{ab}	23.0 ± 0.3^{a}	24.7 ± 0.3^{b}	25.7 ± 0.1^{a}	25.8 ± 0.2^{a}
Methanol Extracts	2.5	11.1 ± 0.3^{b}	13.6 ± 0.3^{ab}	11.4 ± 0.1^{a}	11.9 ± 0.2^{a}	11.6 ± 0.1^{c}	9.4 ± 0.2^{b}
	5	13.5 ± 0.1^{b}	14.6 ± 0.1^{b}	13.6 ± 0.1^{a}	13.3 ± 0.4^{b}	12.5 ± 0.1^{c}	13.4 ± 0.2^{b}
	10	15.3 ± 0.3^{b}	15.6 ± 0.1^{b}	16.6 ± 0.1^{a}	15.9 ± 0.2^{b}	13.6 ± 0.1^{c}	15.5 ± 0.2^{b}
	20	16.4 ± 0.1^{b}	16.7 ± 0.3^{c}	18.9 ± 0.3^{a}	17.6 ± 0.1^{b}	15.4 ± 0.2^{b}	17.6 ± 0.2^{b}
	30	18.9 ± 0.3^{b}	18.8 ± 0.3^{c}	19.4 ± 0.3^{a}	18.8 ± 0.2^{b}	16.5 ± 0.1^{b}	19.0 ± 0.2^{b}
	40	23.6 ± 0.1^{b}	20.7 ± 0.2^{c}	21.1 ± 0.2^{a}	21.9 ± 0.2^{b}	17.4 ± 0.1^{b}	21.6 ± 0.1^{b}
	50	25.9 ± 0.3^{a}	23.1 ± 0.4^{b}	23.1 ± 0.3^{a}	24.7 ± 0.3^{b}	18.4 ± 0.1^{c}	22.6 ± 0.1^{b}
Erythro	10	27.5 ± 0.3^{a}	10.5 ± 0.5^{b}	11.4 ± 0.9^{a}	9.3 ± 0.3^{b}	12.5 ± 0.2^{c}	9.8 ± 0.1^{b}
CHX	10	12.4 ± 0.4^{b}	11.6 ± 0.2^{b}	#NI	#NI	8.5 ± 0.5^{b}	#NI

Identical small letters refer to not significant differences between bacteria at probability of 5% ($P\leq0.05$) by Duncan's multiple range test (Duncan, 1955). Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate
#NI = No Inhibition observed; Resistant; Intermediate; Susceptible

Table 2: Diameter of inhibition zones of bark extracts of *D. angustifolia* against oral and periodontal pathogens

Bark extract	Conc. (mg/ml)	*C. Canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
-ve cont.	10%Eth	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Diethyl ether extract	2.5	12.3 ± 0.1^{a}	12.0 ± 0.3^{c}	9.9 ± 0.2^{c}	10.8 ± 0.4^{b}	11.2 ± 0.6^{b}	9.8 ± 0.2^{c}
	5	14.0 ± 0.3^{a}	13.6 ± 0.1^{c}	15.7 ± 0.4^{b}	13.7 ± 0.6^{b}	12.8 ± 0.1^{b}	11.2 ± 0.8^{b}
	10	15.6 ± 0.1^{a}	15.1 ± 0.3^{b}	23.7 ± 0.2^{a}	16.5 ± 0.5^{ab}	15.0 ± 0.7^{ab}	13.8 ± 0.2^{b}
	20	18.1 ± 0.3^{a}	18.1 ± 0.3^{b}	24.2 ± 0.2^{a}	19.3 ± 0.5^{a}	17.1 ± 0.3^{a}	16.3 ± 0.4^{b}
	30	19.1 ± 0.3^{a}	19.6 ± 0.3^{b}	26.5 ± 0.2^{a}	21.3 ± 0.5^{a}	19.9 ± 0.6^{a}	20.5 ± 1.0^{a}
	40	20.8 ± 0.8^{b}	21.9 ± 0.1^{a}	28.6 ± 0.1^{a}	23.3 ± 0.5^{a}	22.3 ± 0.3^{a}	23.1 ± 0.3^{a}
	50	23.1 ± 0.3^{b}	23.0 ± 0.2^{b}	29.1 ± 0.3^{a}	25.5 ± 0.2^{a}	24.3 ± 0.3^{a}	24.3 ± 0.3^{a}
Ethanol extract	2.5	11.1 ± 0.2^{a}	15.9 ± 0.1^{a}	12.6 ± 0.1^{b}	13.5 ± 0.7^{a}	13.3 ± 0.4^{a}	12.3 ± 0.2^{a}
	5	12.1 ± 0.1^{b}	16.8 ± 0.0^{a}	14.0 ± 0.3^{b}	15.7 ± 0.1^{a}	14.2 ± 0.3^{a}	14.7 ± 0.1^{a}
	10	13.2 ± 0.2^{b}	18.2 ± 0.0^{a}	14.7 ± 0.1^{b}	17.7 ± 0.6^{a}	16.1 ± 0.3^{a}	16.3 ± 0.3^{a}
	20	14.5 ± 0.2^{b}	19.7 ± 0.3^{a}	17.2 ± 0.2^{b}	18.7 ± 0.4^{ab}	17.9 ± 0.4^{a}	17.8 ± 0.3^{a}
	30	15.0 ± 0.3^{b}	20.7 ± 0.1^{a}	18.2 ± 0.3^{b}	20.5 ± 0.2^{a}	19.4 ± 0.1^{a}	18.5 ± 0.1^{a}
	40	16.2 ± 0.2^{c}	22.8 ± 0.4^{a}	22.7 ± 0.3^{b}	21.7 ± 0.0^{b}	21.7 ± 0.0^{a}	19.4 ± 0.1^{c}
	50	18.1 ± 0.2^{c}	24.6 ± 0.1^{a}	24.5 ± 0.5^{b}	23.7 ± 0.5^{b}	23.3 ± 0.6^{a}	21.7 ± 0.6^{b}
Methanol Extract	2.5	11.1 ± 0.6^{a}	13.6 ± 0.5^{b}	8.4 ± 0.1^{c}	11.9 ± 0.2^{ab}	11.6 ± 0.1^{b}	9.8 ± 0.2^{b}
	5	12.9 ± 0.3^{b}	14.7 ± 0.2^{b}	9.6 ± 0.1^{c}	13.3 ± 0.4^{b}	12.6 ± 0.1^{b}	13.1 ± 0.3^{a}
	10	15.5 ± 0.4^{a}	15.6 ± 0.1^{b}	13.2 ± 0.3^{a}	15.6 ± 0.5^{b}	13.7 ± 0.1^{b}	15.3 ± 0.3^{a}
	20	17.2 ± 0.5^{a}	17.4 ± 0.2^{b}	16.7 ± 0.1^{b}	17.6 ± 0.1^{b}	14.1 ± 0.3^{b}	17.6 ± 0.2^{a}
	30	19.2 ± 0.6^{a}	18.8 ± 0.3^{b}	18.8 ± 0.6^{b}	18.7 ± 0.1^{b}	16.5 ± 0.1^{b}	18.6 ± 0.1^{a}
	40	24.1 ± 0.3^{a}	22.0 ± 0.4^{a}	19.7 ± 0.5^{b}	21.9 ± 0.2^{b}	17.9 ± 0.4^{b}	21.6 ± 0.1^{b}
	50	26.0 ± 0.2^{a}	24.2 ± 0.4^{a}	22.8 ± 0.2^{a}	24.7 ± 0.3^{ab}	21.5 ± 0.2^{b}	22.4 ± 0.2^{b}
Erythro	10mg/ml	24.5 ± 0.3^{a}	10.5 ± 0.5^{b}	11.4 ± 0.9^{a}	9.3 ± 0.3^{b}	12.5 ± 0.2^{c}	9.8 ± 0.1^{b}
CHX	10mg/ml	9.4 ± 0.4^{b}	12.6 ± 0.2^{b}	11.76^{a}	#NI	#NI	#NI

Identical small letters refer to not significant differences between bacteria at probability of 5% ($P\leq0.05$) by Duncan's multiple range test (Duncan, 1955). Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate
#NI = No Inhibition observed; Resistant; Intermediate; Susceptible

C. canimorsus showed least susceptibility to inhibition from diethyl ether leaf extracts and methanolic stem extracts of concentration of 2.5mg/ml (8.6±0.3mm and 7.8±0.2mm respectively). However, there was increase in the length of inhibition with increase in the concentration of the extracts in all the extracts of the leaf, bark and stem against all the tested

microorganisms. Diethyl ether, ethanol and methanol of the leaf, bark and stem extract were bioactive against all the tested microorganisms and no significant differences at Dancun's test of P≤0.05 was shown.

Furthermore, all the *D. angustifolia* plant part extracts also showed longer zone of inhibition in all the microorganisms than the positive controls used in the experiments i.e. erythromycin antibiotic and chlorhexidine gluconate which is one of the most commonly used as chemical control method in oral and periodontal pathogenic infections. 10% v/v ethanol solution which was used as negative control however did not show any inhibition in any of the tested microorganism.

Table 3: Diameter of inhibition zones of stem extracts of *D. angustifolia* against oral and periodontal pathogens

Stem extract	Conc. (mg/ml)	*C. Canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
Diethyl ether extract	2.5	12.5±0.2ᵃ	11.7±0.2ᵇ	9.5±0.3ᵇ	10.6±0.2ᵇ	10.5±0.4ᶜ	8.8±0.2ᵇ
	5	13.8±0.1ᵃ	13.4±0.2ᵇ	11.5±0.3ᶜ	13.5±0.3ᵇ	12.6±0.2ᶜ	11.4±0.2ᵇ
	10	15.8±0.2ᵈ	14.6±0.2ᵇ	14.0±0.3ᵇ	15.7±0.4ᵇ	14.5±0.3ᵇ	13.7±0.2ᶜ
	20	18.0±0.3ᵃ	17.5±0.3ᵇ	15.2±0.1ᵇ	18.4±0.3ᵃ	17.2±0.1ᵇ	16.8±0.2ᵇ
	30	19.3±0.3ᵃ	19.6±0.2ᵇ	16.4±0.2ᵇ	20.6±0.2ᵃ	19.6±0.2ᵃ	19.6±0.2ᵃ
	40	20.3±0.7ᵃ	21.8±0.1ᵇ	18.2±0.6ᵇ	23.4±0.2ᵃ	21.8±0.2ᵃ	22.4±0.2ᵃ
	50	22.5±0.3ᵃ	23.8±0.1ᵃ	20.6±0.2ᵇ	25.1±0.2ᵃ	24.1±0.3ᵃ	24.3±0.2ᵃ
Ethanol extract	2.5	10.6±0.1ᵇ	15.7±0.1ᵃ	12.9±0.3ᵃ	13.8±0.2ᵃ	13.7±0.1ᵃ	12.5±0.1ᵃ
	5	11.8±0.1ᵇ	16.7±0.1ᵃ	14.4±0.3ᵃ	15.7±0.1ᵃ	14.4±0.1ᵃ	14.5±0.1ᵃ
	10	13.1±0.2ᵇ	18.5±0.2ᵃ	15.6±0.1ᵃ	17.1±0.2ᵃ	15.8±0.1ᵃ	16.6±0.2ᵃ
	20	14.5±0.2ᵇ	19.5±0.1ᵃ	16.7±0.1ᵃ	18.7±0.4ᵃ	18.1±0.3ᵃ	17.5±0.1ᵃ
	30	14.9±0.2ᶜ	20.7±0.1ᵃ	18.2±0.3ᵃ	20.6±0.1ᵃ	19.4±0.1ᵃ	18.5±0.1ᵇ
	40	16.2±0.2ᶜ	22.7±0.2ᵃ	22.4±0.3ᵃ	22.1±0.3ᵇ	20.6±0.1ᵇ	19.4±0.1ᵇ
	50	18.6±0.1ᵇ	24.5±0.2ᵃ	24.2±0.3ᵃ	23.0±0.2ᵇ	22.6±0.3ᵇ	20.6±0.1ᵇ
Methanol extract	2.5	7.8±0.2ᶜ	9.4±0.1ᶜ	12.6±0.1ᵃ	9.5±0.2ᶜ	11.5±0.2ᵇ	12.5±0.1ᵃ
	5	9.1±0.3ᶜ	10.6±0.1ᶜ	13.5±0.2ᵇ	10.4±0.1ᶜ	13.5±0.1ᵇ	14.6±0.1ᵃ
	10	11.8±0.3ᶜ	12.1±0.4ᶜ	14.6±0.1ᵇ	11.6±0.1ᶜ	14.6±0.1ᵇ	15.5±0.2ᵇ
	20	13.8±0.3ᵇ	14.2±0.2ᶜ	16.5±0.2ᵃ	13.5±0.1ᵇ	15.6±0.2ᶜ	16.5±0.2ᵇ
	30	16.4±0.3ᵇ	15.6±0.1ᶜ	17.5±0.2ᵃ	15.5±0.1ᵇ	16.5±0.2ᵇ	17.4±0.3ᶜ
	40	18.0±0.4ᵇ	16.9±0.3ᶜ	18.4±0.1ᵇ	16.5±0.2ᶜ	17.5±0.2ᶜ	18.3±0.1ᶜ
	50	19.5±0.5ᵇ	18.1±0.4ᵇ	21.4±0.2ᵇ	18.6±0.1ᶜ	18.5±0.2ᶜ	19.5±0.1ᶜ
Erythro	10	25.5±0.3ᵃ	8.5 ±0.5ᵇ	15.4±0.9ᵃ	9.3±0.3ᵇ	9.5±0.2ᶜ	22.8±0.1ᵃ
CHX	10	8.4 ±0.4ᵇ	9.6±0.2ᵇ	#NI	#NI	7.5±0.5ᵇ	8.1±0.1ᵇ

Identical small letters refer to not significant differences between bacteria at probability of 5% (P≤0.05) by Duncan's multiple range test (Duncan, 1955). Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate
#NI = No Inhibition observed; Resistant; Resistant; Intermediate; Susceptible

3.2. Minimum Inhibitory Concentration (MIC)

The leaf, stem and bark extracts of *D. viscosa* have been studied to determine the antibacterial activity against *S. mutan, L. casei, P. gingivalis, P. intermedia, F. nucleatum* [13] and *C. canimorsus* [14] as well as studying the antifungal activity against *Aspergillus flavus*, *Drechslera turcica* and *Fusarium verticillioides* [15]. High antimicrobial activity against *Staphylococcus aureus* using methanol and ethanol extracts for *Hopea parviflora* has been reported [16]. The crude extract of *D. viscosa* has inhibitory effects against *Staphylococcus aureus, Streptococcus pyogenes and Corynebacterium diphtheriae* [16, 17]. The bioassay MIC test for *D. angustifolia* plant extracts showed that there was no inhibition of bacterial growth by the leaf, bark and stem extracts of diethyl ether, ethanol and methanol of concentration of 2.5mg/ml, 5mg/ml and 10mg/ml. However higher concentration of these plant extracts of 15mg/ml and above showed significant inhibition in all the tested bacterial species. Methanol extracts had the least effective inhibition in all the extracts. This may be due to its higher polarity as compared to other solvents used in the extraction of the extracts. The high polarity reduces the penetration of the extracts into the molecular membranes of the bacteria and hence reduces their pharmacological and toxic effects against the pathogens. Hence higher concentrations of extracts of 30mg/ml, 40mg/ml and 50mg/ml were effective against all the tested oral and periodontal pathogenic bacterial. The high molecular weight of most phytochemicals makes their isolation in the purest form poses some practical difficulties and as a result reduces their antimicrobial activity [13, 15].These results are illustrated in tables 4, 5 and 6 below. *P. intermedia* species was the most susceptible to inhibition by *D. angustifolia* extracts as compared to other bacterial species tested; while *P. gingivalis* and *L. casei* were more resistant to inhibition. *P. intermedia* and *S. mutan* bacterial species showed resistance to inhibition to the positive control treatments used in the experiment i.e. 10mg/ml of erythromycin and 10mg/ml of Chlorhexidine gluconate solution.

Table 4: MIC effects of different concentrations of leaf extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Leaf extract	Conc. (mg/ml)	C. canimorsus	F. nucleatum	L. casei	P. gingivalis	P. intermedia	S. mutan
	100	-	-	-	-	-	-
Diethyl ether extract	2.5	-	-	-	-	-	-
	5	-	-	-	-	-	-
	10	-	-	-	-	+	-
	20	-	-	+	+	+	-
	30	+	+	+	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+

Ethanol extract	2.5	-	-	-	-	-	-
	5	-	-	-	-	-	-
	10	-	-	-	-	+	-
	20	-	-	-	-	+	+
	30	-	-	-	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Methanol extract	2.5	-	-	-	-	−	−
	5	-	-	-	-	−	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	-	-	-	-	+	-
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Erythro	10	+	+	-	-	-	-
CHX	10	+	+	+	-	-	-

(+) = Inhibition; (-) = No Inhibition; Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate

Table 5: MIC effects of different concentrations of bark extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Bark Extract	Conc. (mg/ml)	C. canimorsus	F. nucleatum	L. casei	P. gingivalis	P. intermedia	S. mutan
	100	-	-	-	-	-	-
Diethyl ether extract	2.5	-	-	-	-	-	-
	5	-	-	-	-	-	-
	10	+	+	+	+	+	+
	20	+	+	+	+	+	+
	30	+	+	+	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Ethanol extract	2.5	-	-	-	−	−	-
	5	-	-	-	-	-	-
	10	-	-	-	-	+	-
	20	+	+	+	-	+	+
	30	+	+	+	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Methanol extract	2.5	-	-	-	-	−	−
	5	-	-	-	-	−	-
	10	+	-	-	-	+	-
	20	+	+	+	+	+	+
	30	+	+	+	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Erythro	10	+	+	-	-	-	-
CHX	10	+	+	+	-	-	-

(+) = Inhibition; (-) = No Inhibition; Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate

Table 6: MIC effects of different concentrations of stem extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Stem extract	Conc. (mg/ml)	*C. canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
	100	-	-	-	-	-	-
Diethyl ether extract	2.5	-	-	-	−	−	-
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	+	-	-	-	-	-
	30	+	+	+	+	+	+
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Ethanol extract	2.5	-	-	-	−	−	-
	5	-	-	-	-	-	-
	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	+	+	+	+	+	-
	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Methanol extract	2.5	-	-	-	-	−	−
	5	-	-	-	-	−	-
	10	-	-	-	-	-	-
	20	-	+	+	-	-	+
	30	-	+	+	+	+	+

	40	+	+	+	+	+	+
	50	+	+	+	+	+	+
Erythro	10	+	+	-	-	-	-
CHX	10	+	+	+	-	-	-

(+) = Inhibition; (-) = No Inhibition; Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate

3.3 Minimum Bactericidal Concentration (MBC)

The minimum concentration of *D. angustifolia* extracts that killed selected oral and periodontal pathogens are shown in tables 7, 8 and 9 below. Higher concentrations of 30mg/ml, 40mg/ml and 50mg/ml of the plant extracts had bactericidal effects in most of the bacterial species. In all the cases, the susceptibility of the microorganisms to the extracts depends on the solvent used in the extraction and the concentration of the extract used against that microorganism. For example, 30mg/ml of diethyl ether leaf extract killed *C. canimorsus* bacteria while the same concentration of methanol leaf extract could not kill the same *C. canimorsus* species. The MBC value for diethyl ether leaf extract was 40mg/ml against *F. nucleatum* and *P. gingivalis* and 50mg/ml against *C. canimorsus*, *L. casei*, *P. intermedia* and *S. mutan*. *P. intermedia* was killed at a lower concentration of 20mg/ml of ethanol leaf extract while 30mg/ml of ethanol leaf extract killed *S. mutan*. *P. gingivalis* was killed by 40mg/ml of ethanol leaf extract while 50mg/ml of ethanol leaf extract killed *C. canimorsus*, *L. casei and F. nucleatum* species. The methanol leaf extract MBC was 30mg/ml against *P. intermedia* and *S. mutan*; and 40mg/ml against *F. nucleatum, P. intermedia* and *S. mutan*. The MBC value for diethyl ether bark extract was 30mg/ml against *C. canimorsus, F. nucleatum*, *P. intermedia* and *P. gingivalis*. *L. casei* was killed at a higher concentration of 40mg/ml of diethyl ether bark extract. *S. mutan* was resistant to all the diethyl ether bark extract concentrations tested. 30mg/ml of ethanol bark extract killed *C. canimorsus, F. nucleatum* and *P. gingivalis* while *L. casei* was killed by 40mg/ml of ethanol bark extract; and 50mg/ml of ethanol bark extract killed *P. intermedia* and *S. mutan* species. The methanol bark extract MBC was 30mg/ml for *P. gingivalis*; 40mg/ml against *C. canimorsus*, *L. casei*, *P, intermedia* and *S. mutan* while *F. nucleatum* was resistant against all the tested methanol bark extract concentrations. *P. gingivalis* was resistant to all the tested concentrations of both diethyl ether and ethanol stem extracts. The MBC value for diethyl ether stem extract was 40mg/ml against *F. nucleatum* and *P. gingivalis* and 50mg/ml against *C. canimorsus*, *L. casei*, *P, intermedia* and *S. mutan*. 30mg/ml of ethanol stem extract killed *S. mutan*. *P. gingivalis* was killed by 40mg/ml of ethanol stem extract. These results as compared with other studies shows that the type of solvents used in extraction of the *D. angustifolia* plant and other plants, and the part of the plant extracted significantly affects the rate of the biochemical components against bacterial species and therefore some antimicrobial effects may differ from other studies reported in some literature [18, 19, 20].

Table 7: MBC effects of different concentrations of leaf extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Leaf extracts	Conc. (mg/ml)	*C. canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
Diethyl ether extracts	10	N/A	N/A	N/A	N/A	-	N/A
	20	N/A	N/A	-	-	-	N/A
	30	-	-	-	-	-	-
	40	-	+	-	+	-	-
	50	+	+	+	+	+	+
Ethanol extracts	10	N/A	N/A	N/A	N/A	-	N/A
	20	N/A	N/A	N/A	N/A	+	N/A
	30	N/A	N/A	N/A	-	+	+
	40	-	-	-	+	+	+
	50	+	+	+	+	+	+
Methanol extracts	10	N/A	N/A	N/A	N/A	-	N/A
	20	N/A	N/A	N/A	N/A	N/A	-
	30	N/A	N/A	N/A	N/A	+	+
	40	-	+	-	-	+	+
	50	+	+	+	+	+	+
Erythro	10	+	-	N/A	N/A	N/A	N/A
CHX	10	+	-	-	N/A	N/A	N/A

(+) = Bactericidal Activity; (-) = No Bactericidal Activity; N/A = Test Not Applicable;
Erythro = Erythromycin antibiotic; CHX = Chlorhexidine HCl

Table 8: MBC effects of different concentrations of bark extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Bark extract	Conc. (mg/ml)	*C. canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
Diethyl ether Extracts	10	-	-	-	-	-	-
	20	-	-	-	-	-	-
	30	+	+	-	+	+	-
	40	+	+	+	+	+	-
	50	+	+	+	+	+	-
Ethanol Extracts	10	N/A	N/A	N/A	N/A	-	-
	20	-	-	-	N/A	-	-
	30	+	+	-	+	-	-
	40	+	+	+	+	-	-
	50	+	+	+	+	+	+
Methanol	10	-	N/A	N/A	N/A	-	N/A
	20	-	-	-	-	-	-

Extracts	30	-	-	-	+	-	-
	40	+	-	+	+	+	+
	50	+	-	+	+	+	+
Erythro	10	+	-	-	N/A	N/A	N/A
CHX	10	+	+	-	N/A	N/A	N/A

(+) = Bactericidal Activity; (-) = No Bactericidal Activity; N/A = Test Not Applicable;
Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate

Table 9: MBC effects of different concentrations of stem extracts of *D. angustifolia* plant extracts against some oral and periodontal pathogens.

Stem extract	Conc. (mg/ml)	*C. canimorsus*	*F. nucleatum*	*L. casei*	*P. gingivalis*	*P. intermedia*	*S. mutan*
Diethyl ether extracts	20	-	N/A	N/A	N/A	N/A	N/A
	30	-	-	-	-	-	-
	40	-	+	-	-	-	-
	50	+	+	+	-	+	-
Ethanol extracts	30	+	+	+	-	+	-
	40	+	+	+	-	+	+
	50	+	+	+	-	+	+
Methanol extracts	10	-	N/A	N/A	N/A	N/A	N/A
	20	-	-	-	N/A	N/A	-
	30	-	+	+	-	-	+
	40	+	+	+	-	-	+
	50	+	+	+	+	+	+
Erythro	10	+	+	N/A	N/A	N/A	N/A
CHX	10	+	+	+	N/A	N/A	N/A

(+) = Bactericidal Activity; (-) = No Bactericidal Activity; N/A = Test Not Applicable;
Erythro = Erythromycin antibiotic; CHX = Chlorhexidine gluconate

4. Conclusion and Recommendations

4.1 Conclusion

There are three major oral diseases that commonly occur in all age populations. They are oral candidiasis, dental caries and the periodontal diseases. The pathogens coexist in the oral cavity as commensals. The beneficial effect of *D. angustifolia* has been established. Therapeutic agents may target the pathogenic organisms by eliminating them or their ability to cause infection by inhibiting the virulence factors. *D. angustifolia* if regularly used in the oral cavity, at high concentrations will kill cariogenic bacteria and the periodontal pathogens. As the saliva flow reduces the concentrations it will continue to kill periodontal pathogens and when the concentrations are even lower it will render these pathogens avirulent. At low concentrations the plant extract will not allow biofilm formation and acid production which are the major virulent factors in the development of dental caries.

The study can therefore be summarily concluded as follows;

- *D. Angustifolia* plant extracts contain phytochemicals that poses both bacteriostatic and bactericidal activities against oral and periodontal pathogens
- Leaves, bark and stem extracts had almost same types of phytoconstituents
- Leaves and Bark extracts had more potent phytoconstituents against oral pathogens than stem extracts
- The three solvents used in extraction – diethyl ether, ethanol and methanol produced sufficient yields and hence recommended as extraction solvents
- Synergistic effects from the association of different types of phytoconstituents in plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases.

4.2 Recommendations

1. *In vitro* analysis does not provide a complete representation of what happens in the oral cavity. Clinical trials would be ideal in testing the isolated compound by incorporating it into a mouth rinse or toothpaste and determining its efficacy in preventing dental caries.
2. Dental plaque is composed of a variety of organisms. Investigating the effect of the isolated compound on other microorganisms that are also found in dental plaque would be a step forward (Synergic Activities).
3. Structure-Activity Relationships (SAR) plays a key role in the efficacy of compounds against microorganisms. Determining how the isolated compound exerts its effect against cariogenic bacteria at the biochemical level would be an important study.
4. The isolated phytochemical compounds can be coated onto nanoparticles to enhance its activity and retentiveness.
5. Enzymes like Glycosyltransferase and ATPase contribute to the pathogenicity of some oral microbes like *S. mutans*. A study could be done to investigate the effect of the compound on such enzymes.
6. Phytochemical studies on *D. angustifolia* plants from different locations can be done as geographical variability can have an influence on chemical composition.
7. Extensive screening for phytochemicals in plants used in ethno-medicine for both human and veterinary drug development.
8. Sequencing and gene editing may provide clear biochemical pathway for formulations f more potent phytochemical Antimicrobials

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