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Response Of Hydroalcoholic Extract of Plumeriaalba Leaves Against Periodontal Disease Triggering Microbiota

Omji Porwal[1,*], Jonie Lee Ju Nee[2], Shivkanya Fuloria[2], Mehmet ozdemir[1], Duran kala[1], Esra Tariq Anwer[1], Neeraj KumarFuloria[2*]

[1] Department of Pharmacognosy,Faculty of Pharmacy, Tishk International University, Erbil, 44001, KRG, Iraq.

[2] Pharmaceutical chemistry Unit, Faculty of Pharmacy, AIMST University, Bedong, Kedah 08100 Malaysia.

Correspondence*
Omji Porwal, Shivkanya Fuloria,Neeraj Kumar Fuloria

Abstract

Disturbance of humanmicrobiotamanifests in periodontitis that is connected with many other diseases.Adjustment of human microbiota by herbs intended present study to determinethe response of hydroalcoholic extract of Plumeriaalbaleaves (HEPAL) against periodontal disease(PD) triggering bacteria. Current study involvedpreparation of HEPAL via Soxhletextraction, followed by phytochemical screening and evaluation of inhibitory potential of HEPAL against PDtriggering bacteria such as P. aeruginosa, S. pyogenes, S. aureus and E. faecalis. The phytochemical screening of HEPAL exhibited presence of alkaloid, flavonoids, glycosides, carbohydrates, proteins, and amino acids. The HEPAL exhibited its best inhibition activity against S. aureus and E. faecalis. Present study concludes that HEPAL possess high inhibitoryactivity against PD triggering bacteria.High inhibitory potential of HEPALagainst PD could be attributed topresence of flavonoidal and alkaloidal constituents.

Key Words: Periodontal disease,antimicrobials, phytochemical, Plumeriaalba, bacteria

1. Introduction

Human body comprises pathogenic and non-pathogenic bacteria population in symbiotic relationship. Small disturbance in this population may lead to various infections or diseases [1]. Periodontal disease (PD)is a chronic inflammation disease of the tissues of teeth, that is triggered by complex interaction between immuno-response and different micro-organisms and [2].PD is known to be connected with various other systemic diseases, such as cardiovascular diseases, diabetes, rheumatoid arthritis, respiratory disease, chronic kidney disease, obesity, and cancer. Fact suggestP. aeruginosa, S. pyogenes, S. aureus and E. faecalis to trigger PD [3-5].The extensive and inappropriate use of antibiotics may lead to emergence of antibiotic-resistance. Development of microbe's resistance and patient tolerance reduces the efficacy of commercial antibiotics and increases the demand of alternative antibiotics [7-10]. Plants are known to possess various bio-constituents, such as alkaloids, phenols, polyphenols, terpenoids, flavonoids, flavonols, and glycosides; that are responsible for several biological and antimicrobial activities [11-15].Plumeriaalba (Apocynaceae) is a famous, large evergreen plant, which is widely used in the treatment of various diseases. Found in various parts of South East Asia, an aromatic shrub with white flowers and a yellowish centre. In

Malaysia, it is also commonly known as 'kemboja' or temple tree, but several names such as 'pokokkubur' and 'bungakubur' have also been used [16].Occurrence of bacterial resistance to conventional antibiotics creates the necessityto search antibacterial agents from different sources [17]. Hence the present study was done to explore the antibacterial potential of the Plumeriaalba leavesagainst the microbiotabacteria that triggers PD.

2. Material and methods

The leaves of Plumeriaalbaleaves were collected in AIMST University campus located in Kedah, Malaysia and authenticated in University Sains Malaysia, Penang. The leaves were plucked from each branch, carefully chosen and washed for the removal of impurities and dirt. The leaves were then air-dried for seven daysuntil the leaves were completely dried without the presence of moisture. Upon drying, the leaves were crushed and later homogenized into powder form using an electronic blender and stored in refrigerator at 4 °C for further studies[18].

2.1. Preparation of hydroalcoholic extract of Plumeriaalba leaves (HEPAL)

100g of powdered Plumeriaalba leaves were weighed and transferred into a 500mL conical flask. 300mL of solvent containing 95% of ethanol and distilled water was added into the conical flask containing powdered leaves, in the ratio of 60:40 respectively and stirred with a glass rod. The conical flask was then sealed with a cotton plug and aluminium foil and was allowed to stand in a mechanical shaker for the maceration process for seven days.After seven days, the liquid was strained off and clarified by filtration using Whatman filter paper No.1. The filtrate was then collected and evaporated using a water bath.The HEPALwas obtained, labelled and stored in the refrigerator for further studies [19-27].

2.2. Responseof HEPALAgainstPD triggering Pathogens

2.2.1. Preparation of bacterial culture

Bacterial strains of P. aeruginosa, S. pyogenes, S. aureus and E. faecaliswere used to evaluate the inhibitory potential of the HEPAL.The prepared stock culture of microorganism weremaintained at 4°C.Subcultures were prepared by transferring loopful of microorganismscolonies from stock cultures into the nutrient broth and incubated for 24 hours at 37˚C in the incubator. The broth turbidity indicated the microbial growth[28-30].

2.2.2. Well Diffusion Method

The inhibitory potential of HEPALagainst PD triggering pathogens was determined using well diffusion method. The experimental protocol was followed as per the standard references with slight modifications. Briefly, 20 µl of nutrient broth containing broth organism was poured into Muller Hinton agar plate, that was spread uniformly using L-shape rod. The wells were made on the agar medium with cork borer of 5 mm in diameter which was previously sterilized using autoclave at 121ºC for one hour. Each 50 µl of plant extracts were pipetted separately into the cup made on the agar plate. In the agar plate a few wells for extracts, standard and control. These plates contained the antibiotic Ciprofloxacin (standard) and tween 80 (control) solution for the purpose of comparison with the plant extracts. All the plates were incubated for 24 hours at 37ºC. The diameter of zone of inhibition around wells was measured in milliliters (mm) in triplicate and average values were calculated [31-37].

2.3. Preliminary Phytochemical screening of HEPAL

The HEPAL was subjected to preliminary phytochemical screening for the detection of various plant constituents. The prepared extract was screened for the presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins, tannins, and phenols as per the procedure given in standard references [38-46].

3. Results

3.1. Response of HEPALAgainstPD Triggering Pathogens

In present study, the HEPAL was evaluated for their inhibitory potential against PD triggering bacteria such as P. aeruginosa, S. pyogenes, S. aureus and E. faecalis. The prepared HEPAL was evaluated for their antimicrobial potential against various bacterial strains using well diffusion method. The results so obtained are given in table 1.

Table 1: Zone of inhibition of HEPAL

Microorganism	**Zone of inhibition (mm)**			
	P. aeruginosa	S. pyogenes	S. aureus	E. faecalis
Extract	10	8	22	21
Ciprofloxacin	25	24	24	25
Control	-	-	-	-

'-' indicates no zone of inhibition

3.2. Preliminary Phytochemical screening of HEPAL

The HEPAL was subjected to qualitative testing as per the procedure given in standard references. The list of compounds identified in HEPAL are given in table 2.

Table 1: Phytoconstituents of HEPAL

S. No.	**Phytoconstituents**	**HEPAL**
1	Carbohydrates	-
5	Proteins	+
6	Amino acids	+
7	Fats and Oils	-
8	Steroids	-
9	Volatile oils	+
10	Glycosides (Cardenoloids and Anthraquinone)	-
11	Saponin Glycosides	+
12	Cyanogenetic Glycosides	-
13	Alkaloids	+
14	1. Flavonoids	+
15	2. Tannins and Phenolic Compounds	-

Where, '+' indicates present and '-' indicates absent

4. Discussion

Facts suggests reports P. aeruginosa, S. pyogenes, S. aureus and E. faecalisto trigger PD. The incidences ofmicrobes resistance towards conventional antibiotics raises the demand for evaluation of alternative antimicrobials [7-10]. Reports suggestsuse of Plumeria albain the treatment of various diseases and to possess strong antimicrobial potential [16]. As per the literature available over different parts of Plumeriaalbaplant and very less literature was available over antimicrobial potential of Plumeria albaplant leaves. Hence, investigators of present study was planned to evaluate the in-vitro inhibitionpotential of HEPAL against PD triggering pathogens (P. aeruginosa, S. pyogenes, S. aureus and E. faecalis)using well diffusion method.The HEPALwere prepared byusing hydroalcoholic solvent. The HEPAL was investigated for inhibitory potential (using well diffusion method) and phytochemical screening. The HEPAL was investigated for its zone of inhibition against P. aeruginosa, S. pyogenes, S. aureus and E. faecalis. The HEPAL showed good inhibitory effect overgrowth of S. aureus and E. faecalis. The HEPAL when subjected to phytochemical testing to identify the nature of compound present in the extracts, exhibited presence of alkaloids, carbohydrates, glycosides and proteins.

5. Conclusion

The experimental results of inhibitory potential of hydroalcoholic extract of Plumeriaalba leavesagainst PD triggering pathogens, concludes that hydroalcoholic extract of Plumeria alba leavespossess good inhibitory activity against PD triggering pathogens especially S. aureus and E. faecalis. The high antimicrobial potential of hydroalcoholicextract of Plumeriaalba leavescan be attributed to the presence of alkaloidal and flavonoidal constituents.

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7. References

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