Research Article

Prevention of Red Complex Oral Pathogen Using Phytochemical Derived from Poly Spice Extract an *in vitro* and *in silico* Analysis

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ABSTRACT

Background: Major periodontal disease pathogens include *S. mutans* and *Porphyromonas gingivalis* a major challenging bacterial infection among dentist. Traditional Indian ayurveda medicine has employed spices to treat many inflammatory illnesses. Examining the antibacterial activity against oral pathogen and anti-inflammatory activity are the study's primary goals. **Materials and Methods:** 9 different spices were mixed and extracted by soxhlet method. Phytochemical analyzed by qualitatively and evaluated by GC-MS. Antibacterial activity of spice extract done by disc diffusion against oral pathogens. *In silico* analysis of phytochemical performed by Autodock. **Results:** Ethanol extract reveals absence of saponin and acetone extract shows absence of saponin and anthroquinones. GC-MS data reveals that the ethanol extract have more different compounds than acetone extract. Both extracts showed presence of derivatives of fatty acid, carboxylic acid, phenol and piperidine. Anti-inflammatory study reveals that acetone extract found to be potent than ethanol extract and equal to standard with least IC_{50}. All the three tested pathogens were sensitive to poly spice extract. Based on zone of inhibition and MIC ethanol extract was found to be best than acetone extract. Molecular docking of piperdine derivative interacted with active site of Arg-gingipain and peptidyl arginine deaminase of *P. gingivalis*. These docking results and *in vitro* studies confirms that the polyspice extract have broad spectrum antibacterial compounds found to be effective against oral pathogens and thus has a lot of potential for being developed into novel, secure antibacterial agents.

Keywords: Antibacterial, Antioxidant, Periodental, Piperdine, Spice.

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INTRODUCTION

Infectious infections were responsible for one-third of the 55 million deaths reported worldwide in 2011 by the World Health Organization.[1] Drug resistance among pathogens thought to be major factor to elevate the infective and death ration since they can proliferate under many exposed to antibiotic drugs as well as transmit resistance after which are one of the therapies during infectious state.[2] The problem of antibiotic resistance is made worse by the decline in the discovery and development of novel antimicrobial agents, which are unable to inhibit bacteria like *Staphylococcus aureus* that are resistant to antibiotics.[3] Natural medicines should be given a lot of attention in order to properly treat human ailments due to their great efficacy against infections and relatively low adverse effects.[4] The US Food and Drug Act and other regulatory bodies have designated spices, herbs and their constituents as Generally Recognized as Safe (GRAS) substances. In the Indian food, several number of spices are frequently used, including ajowan, clove, ginger, black pepper, cumin and asafetida.[5-7] Numerous writers' works on the antibacterial properties of plants against a variety of bacteria, yeasts and moulds have been referenced in the literature. Numerous studies in the literature demonstrate that a variety of spices have broad-spectrum antibacterial activity. For example

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allicin, ajoene, capsaicin, dihydrocapsaicin, curcumin, 6-gingerol, 6-shogaol and piperine, responsible for their antimicrobial activities.[8-11] When anaerobic Gram-negative bacteria in subgingival plaques become polymicrobially infected, periodontitis develops as an inflammatory immunological reaction. The two most prevalent protozoa, *Entamoeba gingivalis* and *Trichomonas tenax*, are primarily saprophytic. The most common fungus found in the mouth cavity is the species Candida. *Candida, Cladosporium, Aureobasidium, Saccharomycetales, Aspergillus, Fusarium* and *Cryptococcus* were the most frequently observed species. This inflammatory condition harms the periodontal tissues and frequently causes the alveolar bone that surrounds the teeth to deteriorate.[12,13] Dental plaque biofilms containing anaerobic Gram-negative rods and spirochetes cause chronic periodontitis.[14] most complicate and difficult to treat infections are caused by *Treponema denticola and Porphyromonas gingivalis* associate chronic periodontitis. In this work, we studied the impact of spice extract components to prevent oral harmful microorganisms.

MATERIALS AND METHODS

Extraction of spice Material

Each 5 g of *Cinnamomum verum*, *Piper longum*, *Nigella sativa, Cinnamomum tamala, Piper nigrum*, *Illicium verum, Elettaria cardamomum, Syzygium aromaticum* and dried *Zingiber officinale* finely powdered and packed in to soxhlet column. The study material was put into the Soxhlet apparatus's thimble chamber. After being heated in the bottom flask, the extraction solvent chloroform vaporized into the sample thimble, condensed in the condenser and then dripped back. The round bottom flask is filled with 200 mL solvent (1:2 W/V) and kept under 50ºC for 6 hr. Then the mixture was subsequently filtered and concentrated at 40ºC. The concentrated extract is diluted and subjected to GCMS at Bisopheber College, Tiruchirappalli for phytochemical detection. A combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer was used for the GC-MS study. As the carrier gas, helium gas is set to a column rate flow rate of 1.0 mL/min. After 5 min, the internal temperature in the column was increased to 150°V at a rate of 4ºC/min. At a rate of 20ºC/min, the temperature was increased to 250°C and maintained there for 5 min. Elution of 47.5 min run was given to elute the fractions.

Qualitative phytochemical analysis.

Detection of Alkaloids

Spice extract were dissolved water at 1:9 ratio. 5 mL of sample and 3 mL of 1 N hydrochloric acid reacted and filtered. To this filtrate 1 mL of Mayer's reagent was applied. Formation of yellowish white precipitate recorded as positive.[15]

Test for Anthraquinones

About one mL of extract and 1 mL of (V/V) 10% ammonia solution was mixed gently in a test tube. The bright pink colouration considered as presence of anthraquinones.

Detection of betacyanin

One part of 2N alkaline reagent was mixed with two part of extract and kept at 100f for 5 min. Formation of yellow color indicates the test is positive.

Coumarin test

The extract diluted with ethanol is test tube containing filter paper that had been pretreated with NaOH (1N) solution. The filter exposed in the boiling water and development of yellow fluorescence under a UV recorded as positive.

Flavonoid test

Extract was combined with 2 mL of a 2.0% NaOH solution before the combination received 2 drops of diluted hydrochloric acid. When yellow turns colourless the test is positive.

Phenol detection

Few drops of ten percent ferric chloride was added to the test tube contain 2 mL of extract. Changes of colour of extract from green to blue or black recorded as positive.

Test for Saponins

In a test tube, 5.0 mL of distilled water and extract were thoroughly combined. When the liquid was vigorously stirred with a few drops of olive oil, saponins were visible in the surface as stable foam.

Identification of Tannins

Diluted spice extract treated with ferric chloride reagent. A blue-green precipitate indicates presence of tannins.

Antibacterial activity disc diffusion method

Sterile disc were loaded with known concentration of (100 µg/mL) sample from stock of crude extract (1 mg/mL) and allowed to air dry. Sterile Muller Hinton agar plate are prepared and inoculated with test pathogen *Streptococcus mutans, P. gingivalis* and *Candida albicans* using sterile swab. Sample loaded disc were placed over the agar surface and gently pressed to fix on the agar surface. Chlorohexidine used as positive control and respective solvent kept as vehicle. Growth inhibition was recorded

followed by 24 hr incubation at 37±2ºC under incubator. Plates of PG were incubated under anaerobic jar. Other plates were incubated under aerobic condition.[16]

Determination of Minimum Inhibitory Concentration (MIC)

The activity of extract (1 mg/5 mL) against the *Sreptococcus mutans, P. gingivalis* and *Candida albicans* were determined using standard broth dilution technique. Series of test tube filled with 1000 µL nutrient broth. One mL of extract (200 µg/mL) added to first tube and serially diluted to obtain range of 100 to 1.5 µg/mL medium. To this 20 µL of test pathogen (1×10^6 CFU/mL) was added. The tube was incubated at 37°C for 24 hr. After incubation, 20 µL of resazurine (1 (1zurinencubation, ºall tubes and further incubated 4 hr at 37ºC. The MIC was determined as the highest dilution which showed no oxidation of dye indicates absence of visible growth.

Anti-inflammatory effect of extract

The test combination contains total volume of 5 mL prepared using 0.5 mL of 1% bovine serum albumin, 4 mL of Phosphate Buffered Saline (PBS, pH 6.4) and 4x concentration of 0.5 mL of extract was added to obtain 25, 50, 75 and 100 µg/mL. After mixing the components, it was allowed to stand for 15 min at 37ºC then heated for 5 min at 70ºC. A spectrometer was used to determine the visibility at 660 nM after cooling of extract (A1). 2-[(2,6-Dichlorophenyl)amino] benzeneacetic acid sodium salt (sigma Aldrich) was used as standard (5 mg/mL). As a control, phosphate buffer solution was employed (A2). This equation was used to determine the percentage inhibition of protein denaturation:[17]

$$\%\ \text{inhibition of denaturation} = 100\times\left(\frac{1-A2}{A1}\right),$$

Molecular docking

Smiles of piperdine derivative retrieved from pubchem and used for ADME. Preparation of Arg-gingipain (1CVR) and Peptidylarginine Deaminase (PDB ID: 4YTB) protein file were retrieved from the Protein Data Bank (http://www.rcsb.org/pdb) in pdb format. Cavity must be determined to find the residues in the receptor. To locate the compounds in the active site of target, the binding pocket was identified. Receptor processed for docking followed by deleting unwanted solvents, ligands, nonstandard residues. Hydrogen bonds were added using the offline Autodock (4.2.6) which was downloaded from https://www.cgl.ucsf.edu/chimera/. This allowed for the determination of the cavity. The Discovery Studio 4.0 software was utilized to separate macromolecules from superfluous molecules. The ligand structure design of the resulting from GCMS obtained from PubChem website search (http://pubchem.ncbi.nlm.nih.gov./). Mol format was used to store this file using smiles of ligand. In order to achieve desirable docking configurations, the x, y and z dimensions have been configured at 60×60×60 with a resolution of 0.500 Å and grid box centered for docking to occur The AutodockVina (4.2.6) application, run through an input request, was used to carry out the interaction between target and ligand. Examining the binding docking results' free energy value and viewing the output in log.txt format allowed for the completion of the structural interaction study.

RESULTS

This study is the first report on polyspice based extraction and its effectiveness on oral pathogens. The analysis includes nine different spices were selected and extracted with polar solvent system done by soxhlet method. Preliminary phytochemical screening of extracts of Polyherbal formulations determined qualitatively. The ethanol extraction of the number of co spice revealed the presence of metabolites such flavonoids, alkaloid, phenol, coumarins and betacyanins, anthraqinine. Acetone extract reveals that most of the phytochemical tests were positive except saponin and anthroquinones. The compounds detected by qualitative phytochemical is given in Table 1. Saponins are absent in both extract and anthroquinones present only on ethanol extract. Further the Figure 1 represent gas chromatogram of acetone extract reveals presence of 15 different peaks and their corresponding retention time. The identified compounds are listed on Table 2. Compound 2-oxabicyclo[2.2.2] octane, 1,3,3-trimethyl (RT 7.691 min) is first eluted identified and Bis(2-ethylhexyl) phthalate (RT34.909 min) was the last eluted one.3-allyl-6-methoxyphenol (12.02%),Bis(2-ethylhexyl) phthalate and benzene

Table 1: Qualitative phytochemicals.

Test name	Ethanol extract	Acetone extract
Alakaloid	Positive	Positive
Anthroquinones	Positive	Negative
Betacyanin	Positive	Positive
Coumarins	Positive	Positive
Flavonoids	Positive	Positive
Phenol	Positive	Positive
Saponin	Negative	Negative
Tannin	Positive	Positive

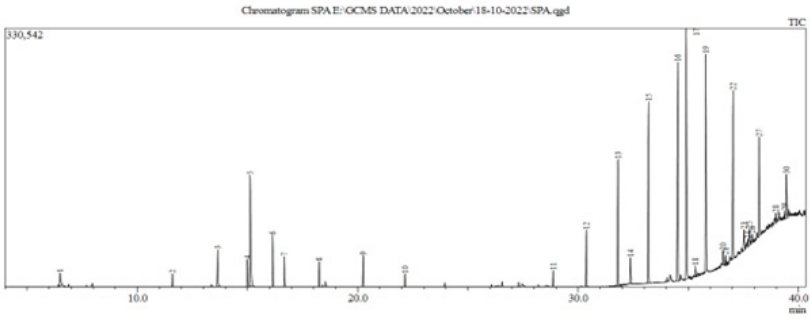

Figure 1: GCMS analysis of spice extracted with acetone.

(62.08%) 1-methoxy-4-(2-propenyl)-(6.34%) were most predominant compound found in acetone extract. octadecanepiperidin and -oxabicyclo[2.2.2]octane were least identified compounds (less than 1%). Figure 2 represents GCMS analysis of spice extracted with ethanol and the identified compounds are given in Table 3. Spectrum showed 30 different retention peaks. Hexatriacontane (RT 35.796 min 20%), Phthalic acid (RT 34.907/ 13.42%) Eicosane (32%) and phenol, 2-methoxy-4-(2-Propenyl)-(RT15.121/5.96%). (2E, 4E, 8E)-9-(2H-1,3-benzodioxol-5-yl)-1-(piperidin-1-yl) nona-2,4,8-trien-1-one is a natural product found in *Piper retrofractum, Piper nigrum* and *Piper longum* were also found in this study. Though the acetone extract is less significant on qualitative analysis the GCMS data found to be more effective than ethanol extract contains more fatty acid and phenolic derivatives. Acetone extraction had the highest yield of secondary metabolites. This is the first report revealing the phytochemistry profiling of polyspice preparation.

Table 2: Ethanol extract of spice Compounds identified from NIST library.

Peak	Retention time	Height %	Name
1	7.691	0.76	2-oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-
2	11.32	1.82	Azulene
3	13.641	6.34	benzene, 1-methoxy-4-(2-propenyl)-
4	14.989	3.12	3-cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate
5	15.116	12.02	3-allyl-6-methoxyphenol
6	16.138	1.16	undecane, 4,7-dimethyl-
7	16.67	1.63	Caryophyllene
8	18.247	0.99	Octadecane
9	18.532	2.93	phenol, 2-methoxy-4-(2-propenyl)-, acetate
10	20.247	0.77	Octadecane
11	26.546	1.84	1,2-benzenedicarboxylic acid, dibutyl ester
12	26.662	1.34	1,3,2-dioxaborinane, 2-(1-methylbutoxy)-
13	26.951	1.72	9(10h)-anthracenone, 10-[[9-(2-chloro-2-phenylethenyl)-9,10-dihydro-10-oxo-9-anthracenyl]o xy]-10-(phenylethynyl)-
14	32.374	0.77	(2E,4E,8E)-9-(Benzo[d][1,3] dioxol-5-yl)-1-(piperidin-1-yl) nona-2,4,8-trien-1-one
15	34.909	62.8	Bis(2-ethylhexyl) phthalate

Antibacterial assay

The effect of extract on control of pathogen reveals that it could be active against both bacteria and yeast such as *Candida albicans, Porphyromonas gingivalis* and *Streptococcus mutans* (Table 4). The antimicrobial effect of ethanol extract was recorded as 16±0, 17.3±0.57 and 17.3±1.15 millimeter (mM) in diameter (DM) on *C. albicans, P. gingivalis* and *S. mutans*. Ethanol extract of poly spice extract showed more are less equal inhibitory zones against both bacteria and yeast (Figure 3). Acetone extract exhibited 14.6±0.57, 17.6±0.57, 16.6±0.57 mm against tested pathogens. Among the pathogens *P. gingivalis* and *C. albicans* were found to be highly sensitive. Whereas *S. mutans* was moderately sensitive (Figure 4). In addition, Minimum Inhibitory Concentration (MIC) showed satisfactory susceptibilities to ethanol extract found as 25, 6.25 and 3.125 µg/mL among pathogenic strains *C. albicans*, *P. gingivalis* and *S. mutans*. The *a*cetone extract MIC was 25 µg for *C. albicans* and 12.5 µg for bacterial pathogen (Table 5).

Anti-inflammatory activity

The data given in Table 6 shows percentage of anti-inflammatory response reveals a concentration depended activity. The percentage of protein denaturation inhibition was low at least concentration and reached maximum at tested higher concentration (Figure 5). Acetone extract had the highest inhibition of protein denaturation at 100 µg (82%) and the lowest level at 25 µg (52%) and IC_{50} value was 144 µg (Figure 6). Ethanol extract had the highest inhibition

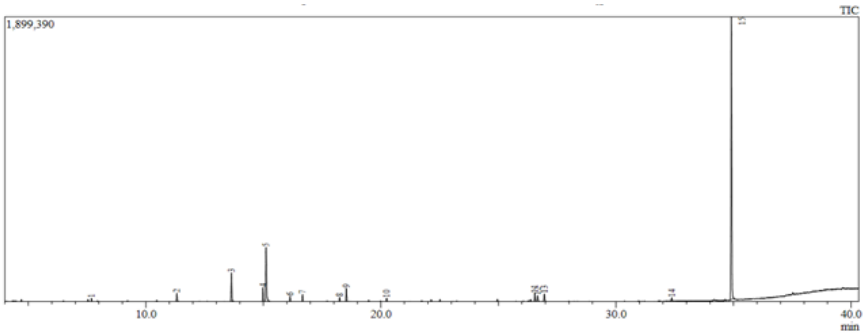

Figure 2: GCMS analysis of spice extracted with ethanol.

Table 3: Acetone extract of spice Compounds identified from NIST library.

Peak	Retention time	Height %	Name
1	6.491	0.71	phosphorous acid, triphenyl ester
2	11.596	0.71	Undecane
3	13.645	1.99	benzene, 1-methoxy-4-(2-propenyl)-
4	14.992	1.47	alpha.-Terpinyl acetate
5	15.121	5.96	phenol, 2-methoxy-4-(2-propenyl)-
6	16.14	2.75	Octadecane
7	16.673	1.62	Caryophyllene
8	18.249	1.35	Octadecane
9	20.249	1.68	Octadecane
10	22.147	0.7	Pentadecane
11	28.872	0.87	Octadecane
12	30.372	3.04	Tetracosane
13	31.808	6.77	Eicosane
14	32.375	1.4	(2E,4E,8E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)nona-2,4,8-trien-1-one
15	33.191	9.71	Eicosane
16	34.518	11.7	Eicosane
17	34.907	13.42	Phthalic acid, di(2-propylpentyl) ester
18	35.326	0.59	1-(2-hydroxyethoxy)tridecane
19	35.796	11.64	Hexatriacontane
20	36.573	0.77	spiro[cyclopentane-1,2'(1'h)-quinoxaline], 3'-(4-morpholinyl)-6',8'-dinitro-
21	36.699	0.55	Heptane, 2,4-dimethyl-
22	37.028	9.01	Hexatriacontane
23	37.528	1.12	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
24	37.705	0.43	pentane, 2,3,3-trimethyl-
25	37.777	0.88	Dodecane, 2,6,10-trimethyl-
26	37.91	0.47	2-Bromononane
27	38.218	5.31	Eicosane
28	38.971	0.55	Octane
29	39.37	0.44	Succinic acid, hexadecyl 2,3,4,5-tetrafluorobenzyl ester
30	39.451	2.37	Eicosane

Table 4: Antibacterial Activity of spice extract and zone of inhibition mm in diameter.

Test pathogen	Acetone extract	Ethanol extract
C. albicans	14.6±0.57	16±0
P. gingivalis	17.6±0.57	17.3±0.57
S. mutans	16.6±0.57	17.3±1.15

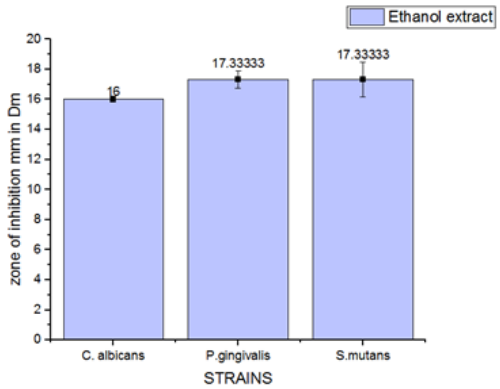

Figure 3: Zone of inhibition millimeter in diameter against pathogens by ethanolic extract.

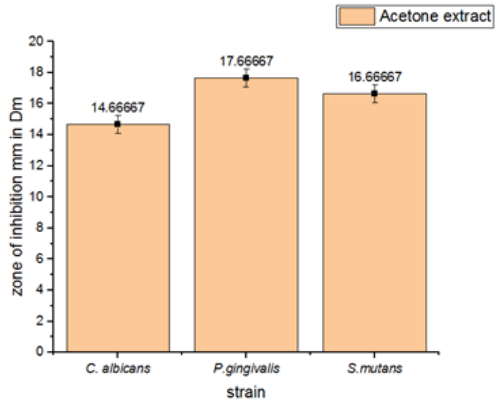

Figure 4: Zone of inhibition millimeter in diameter against pathogens by acetone extract.

of protein denaturation detected in 100 µg (78%) and the lowest in 25 µg (60%) and IC_{50} value was 209 µg. The anti-inflammatory assay of acetone extract were recorded as 52±1, 65±5.1,72±2, 82±0.6 and for

Table 5: Minimum inhibitory concentration (µg/mL) of poly spice extracts.

Test pathogen	Ethanol extract	Acetone extract
C. albicans	25	25
P. gingivalis	6.25	12.5
S. mutans	3.125	12.5

ethanol extract were 60±0.8, 64±0.6, 64±4.3, 78±0.8%. The independent T test between ethanol and acetone extract among different concentration was found to be significant at $p<0.05$ except 50 µg/mL ($p>0.05$). The percentage of anti-inflammatory of standard were 50±01.5, 58±1, 68±0.8, 80±0.8%. Was not found to be significant at 25 and 50 ug ($p>0.05$) but significant at 75 and 100 µg level ($p<0.05$). Similarly, p value of ethanol vs standard reveals at 75 ug the value is 0.132558 which is not found to be significant at $p<0.05$ and other concentrations were found to be significant (Table 7). The strong correlations we observed between acetone and diclofenac activities inhibitory effect and IC_{50} concentration indicate sample was found to be effective than standard.

Table 6: Anti-inflammatory activity of poly spice extract.

Concentration (µg/mL)	Acetone extract	Ethanol extract	*p* value	Diclofenac
25	52±1	60±0.8	0.000104	50±01.5
50	65±5.1	64±0.6	0.268379	58±1
75	72±2	64±4.3	0.052189	68±0.8
100	82±0.6	78±0.8	0.00533	80±0.8
IC_{50}	144	209		212

Docking study

The GC-MS have enormous phytocompounds with good Pharmacognostic property. The selected compound piperdine ($C_{21}H_{25}NO_3$) have good binding affinity with

Figure 6: IC_{50} value of spice extract anti inflammatory assay.

docking score was -7.973 kCal against gingipain (Table 8) and -6.355 kCal towards deaminase (Table 9). The corresponding hydrogen bonds formed by O2 and O1 atoms of ligand interacted with TYR LEU in Arg-gingipain (Figure 7). C7, C12, C10, C8 atoms formed hydrophobic interaction with tyr and arg of gingipain whereas C1, C16, C2 and C14 hydrophobically interacted with ser, leu, pro residues of deaminase (Figure 8). In both receptors Ionic interaction is formed by C18 atom of ligand with ARG and PRO. Weak hydrogen bond formed by O2 ligand with Tyr in gingipain. In peptidylarginine deaminase LEU interacted with C4 atom and produced a weak hydrogen bond. Compound piperine exhibited a strong negative binding energy among selected target by forming hydrogen, weak hydrogen and hydrophobic interactions. Though the

Table 7: Anti-inflammatory activity of poly spice extract.

Concentration (µg/mL)	Percentage of protein denaturation inhibition		
	Diclofenac	*p* value (acetone vs standard)	*p* value (ethanol vs standard)
25	50±01.5	0.15622	0.008708
50	58±1	0.126456	0.000739
75	68±0.8	0.026604	0.132558
100	80±0.8	0.006536	0.020353

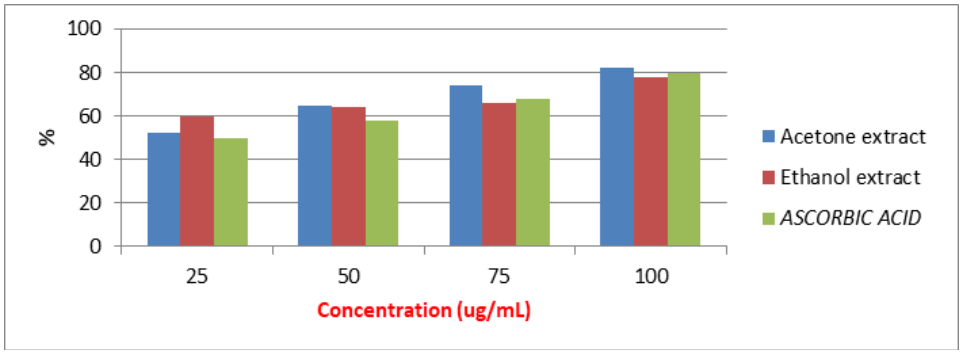

Figure 5: Percentage of anti-inflammatory activity.

data is significant this descriptive analysis further it need an in-depth view on molecular dynamics study.

Table 8: Docking score of ligands with Arg-gingipain.

Bond	Ligand	Amino acid	Docking Score kCal
Hydrophobic	C7, C12, C10, C8	Tyr and Arg	-4.742
Weak hydrogen	O2	Tyr	-13.236
Ionic interaction	C18	Arg	-3.556
Hydrogen bond	O2, O1	Tyr	-7.973

Table 9: Docking score of ligands with peptidylarginine deaminase.

Bond	Ligand	Amino acid	Docking Score kCal
Hydrophobic	C1, C16, C2, C14	Ser, Leu, Pro	-6.1
Weak hydrogen	C4	Leu	-6
Ionic interaction	C18	Pro	-5.6
Hydrogen bond	O1	Leu	-6.355

Extraction

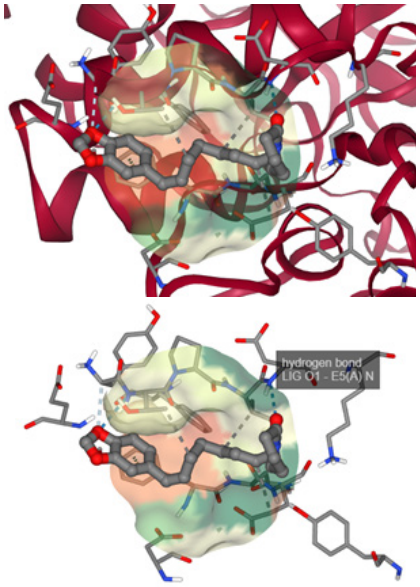

Figure 7: Interaction of piperdine with Arg-gingipain.

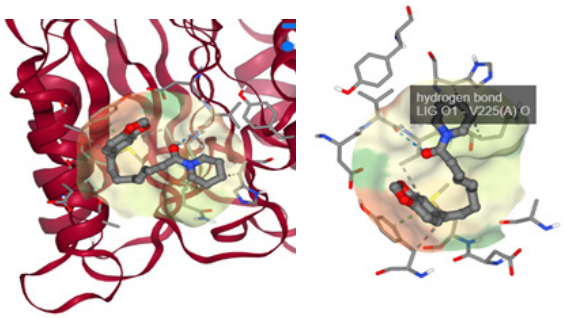

Figure 8: Interaction of piperdine with *peptidylarginine deiminase.*

DISCUSSION

Many different metabolites found in spice substance offer the ability for healing an extensive number of illnesses. The qualitative phytochemical of poly spice showed 87.5% positive on ethanol extract and 75% positive on acetone extraction. Several studies have already identified that the compounds found in these spices, including tannin, alkaloids, steroids, saponins and flavonoids.[18] The outcomes correlate with previous discoveries made by other researchers who explored these various spices.[19] the geographical site, soil nutrient, storage of spices may alter the qualitative phytochmical among spices which may differed from other reports. The phenolic compounds such as 3-(2-Hydroxyphenyl)-2-propenal, derivatives of Cinnamic aldehyde and Afzelechin.[20] Many spices have reported that galactoside, rosmarinic acid, kaempferol as major phenolics.[21] The GCMS reveals about 62% Bis(2-ethylhexyl) phthalate and 2% azulene was found only in ethanol extract where absent in acetone extract. Both the extract has piperdine and phenol, 2-methoxy. Both extract have had moderate to significant inhibitory zone among oral pathogens but differ on MIC does. Ethanol extract required least concentration 3.125 µg where as acetone extraction required 12.5µg. inhibition profiles and computational docking toward the PAD enzyme and gingipain reveals that piperdine has been act as potential pharmacological target. Most spices compounds had explored its inhibitory activity respectively of respiratory pathogenic infective agents.[22] The high bactericidal effect mainly recorded on cardamom, cinnamon and clove against gram negative pathogen and significantly over other strains.[23] Shan *et al.*[24] investigated the ability of phytochmical from five different medicinal spice active against *Salmonella enterica, S. aureus* and *L. monocytogenes*. Cui *et al.*[25] found that clove aqeous preparation exhibited potent antimicrobial property specially on anaerobic *Clostridium* sp. Keskin *et al.*[26] have reported 12 different spices extract control the growth of eight enteric pathogen. Park *et al.*[27] have isolated five ginger molecules with antibacterial impact on most common pathogenic oral bacteria. Dependent t-test of anti-inflammatory does not showed much variation on protein inhibition and found to be significant. The P value of independent t test of acetone preparation was not significant at least concentration where as ethanol shot not significant at higher end concentration. As in literature, denaturation of protein molecules has been well documented as being caused by an inflammatory process. NSAIDs' anti-rheumatic properties may be significantly influenced by their ability to inhibit protein denaturation.[28] Numerous

research have demonstrated the anti-inflammatory effects of spices using conventional laboratory studies. Compounds like phenols derivatives significantly isolated and reported as predominant plant compound with free radical, antifungal and capable to inhibit protein denaturation.[29] likewise The maximum anti-inflammatory activity was reported in with chili pepper.[30] Dose-dependent antibacterial activity of photochemical inhibitors, against arginine or lysine, which is consistent with previous reports.[31] investigations of molecular docking on novel piperidine capable to inhibit human yeast pathogen *Candida albicans* and found to be a potent lead compounds.[32] A combinatorial evaluation by *in silico* molecular modeling exhibited significant binding affinities on binding pocket growth of *P. gingivalis*, presumably through the mechanism of limiting the activity of pathogenic protein.[33]

CONCLUSION

Poly spice extracts an ever-reported study on oral pathogens was evaluated and the data confirms the effective antibacterial activity against oral bacterial pathogens and yeast infection. Phytochemicals found in extract showed inhibition of virulence factor of major pathogen *P. gingivalis* confirmed by docking. Poly spice extract found to be alternative ideal ayurvedic prophylaxis on deep tooth infection. The antibacterial, anti-inflammatory and high binding affinities of poly spice compound against oral infections were confirmed. The interaction profiles, emphasizing particular hydrophobic and hydrogen bonding interactions are essential for binding effectiveness. To verify the effectiveness and safety of these substances in biological systems, future studies should concentrate on validating these results both *in vitro* and *in vivo*.

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ETHICS APPROVAL

No animal studies involved and not supported by any funding grant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Polyherbal extraction was prepared using 9 different spices and its phytochmical explored by GCMS. Previous researchers focused on indigenous spice compounds against wound and UTI infection. This study aims to prepare polyspice and its activity on tooth infecting pathogens. This work provides evidence that in addition to the recognized benefits of polyspices on oral hygienic. Conventional qualitative test reports were identical among two extractions but a high degree of variation noted in GCMS. More compounds were detected in acetone extract than ethanol by GCMS but the activity was better in ethanol than acetone extract. The soxhlet extraction showed presence of major compounds phenol, flavonoids, alkaloids which exhibited promising biological activity. Further good antibacterial, anti inflammatory and antioxidant activity recorded in ethanol extract and found to effective similar to standard. Isolates *C. albicans, P. gingivalis* and *S. mutans* were highly sensitive to ethanol extract with least MIC 50μG which is higher in acetone extract and the result was vice versa in anti inflammatory studies. Maximum of 82±0.6% protein inhibition by acetone extract and 78±0.8% in ethanol extract were recorded. The choice of solvent does not affect the properties of phytochemical but differ on its concentration may affect the results. Further nanoparticle based studies and *in vivo* data is required to improve the efficacy of poly spice

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