Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: September 30, 2023

Citation: Susila, A. V., et al., 2023. Evaluation of antimicrobial and Matrix metalloproteinase inhibitory properties of onion peel extracts - An *in vitro* study. Medical Research Archives, [online] 11(9). https://doi.org/10.18103/mra.v11i9.4225

DOI: https://doi.org/10.18103/mra.v11i9.4225

ISSN: 2375-1924

RESEARCH ARTICLE

Evaluation of antimicrobial and Matrix metalloproteinase inhibitory properties of onion peel extracts - An *in vitro* study

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ABSTRACT

Matrix metalloproteinase inhibition and antimicrobial properties are important in many applications in the field of Conservative Dentistry and Endodontics to preserve the quality of dentin in a bond or to prevent reinfection and failure in root canal treated teeth. Many natural products are being explored for the above properties as they are safer compared to synthetic ones.

Aim: Present study aims to check for the antibacterial action of small onion and large onion peel extracts against Enterococcus faecalis (E. faecalis), Streptococcus mutans (S. mutans), and their Matrix metalloproteinase inhibitory action.

Materials and methods: Ethanolic extracts of small onion and large onion peels were prepared and their Minimum inhibitory concentration, Minimum bactericidal concentration, and zone of inhibition determined. Computational Drug Designing and Characterization of Phytocompounds present in the extract was done by Gas Chromatography-Mass Spectrometry Profiling. Ultraviolet-visible spectrophotometric analysis and Fourier transform infrared spectroscopy were performed for the characterization. Molecular docking (in-silico) was performed using AutoDock open source free software by using editing options and other default parameters for enzymatic interactions and affinities.

Results: Both onion peel extracts had good antibacterial properties against both S mutans and E faecalis as determined by their Minimum inhibitory concentration, Minimum bactericidal concentration, and zone of inhibition. They were found to have many phytochemicals notably, β-sitosterol and quercetin. Small onion peel extract had greater quantities of β-sitosterol and quercetin than large onion peel extract in Gas Chromatography-Mass Spectrometric analysis. Ultraviolet-visible spectrophotometry revealed that the extracts were transparent in the wavelength range studied and had the characteristic peaks. Fourier transform infrared Spectroscopy confirmed the presence of benzene derivative and anhydride. Molecular docking for enzymatic inhibition using in-silico docking study found that both the extracts have Matrix metalloproteinase 2 & 9 inhibition equivalent to control Galardin.

Keywords: Antibacterial property, Onion peel extracts, MMP inhibition, Quercetin

Introduction

Recent advances in adhesive dentistry has led to improved physicomechanical and bonding properties of composite resin. However, failure occurs due to loss of retention, marginal discoloration, microleakage, nanoleakage and bond degradation. Dentin is a composite structure containing inorganic apatite crystal embedded in extracellular matrix. During secretion of dentin matrix odontoblast produces Matrix metalloproteinases (MMPs), which get trapped into the calcified matrix. Acid production by cariogenic bacteria stimulates MMPs, which play an important role in the destruction of dentine during caries formation[1]. Matrix metalloproteinases play a significant role in extracellular matrix destruction and bone remodelling as they are associated with morphogenesis, tissue repair and wound healing. The destruction of the inflamed pulp is controlled to some extent by TIMPs (Tissue Inhibitors of MMP)[2]. During pulpitis, bacteria and inflammatory cells such as macrophages, monocytes and neutrophils release interleukin 1 (IL -1) and tumour necrosis factor (TNF); they also induce MMP-1, MMP-2 and TIMP-1[3,4]. MMPs are also involved in the resorption of bone in the periradicular area; MMPs-2, 3, 8, 9 and 13 are expressed in periapical lesions[5,6].

The micromechanical bonding and longevity of composite resin depends on hybrid layer. Hybrid layer comprises of demineralized collagen that is infiltrated by adhesive monomer[7]. Degradation of resin dentin bond occurs due to: a) degradation of collagen fibrils due to activation of MMPs and cysteine cathepsin; b) acid etching technique; if the etching is aggressive it would expose collagen below the hybrid layer leaving a zone of weak dentin that is susceptible to long term degradation; c) water sorption resulting in hydrolysis of resin[8]; Nanoleakage is around 20-100nm wide which is very much smaller compared to microleakage and occurs in the absence of gap formation. These spaces may not allow bacterial penetration but water and host/microbe-derived proteolytic or hydrolytic enzymes could enter through this space resulting in bond degradation. MMP expression would compromise the longevity of the dentin bond and negatively affect the mechanical properties of non-vital root dentin. Hence its inhibition is critical for maintaining the quality of dentin-resin bonds and stabilizing the mechanical properties of no-vital dentin.

In the past few years growing interest is focussed on screening different sources for MMP inhibitor activity, caries prevention, ability to maintain the integrity of hybrid layer, remineralization[9] and improve bond strength[10]. One such effort is exploring plant sources for medicinal components with specific antimicrobial and anti-MMP activity. Natural collagen crosslinking agents like proanthocyanidin, hesperidin, riboflavin and quercetin have shown to improve mechanical properties of demineralized collagen matrix. 2% green tea extract is a natural MMP inhibitor which is made from leaves of Camellia sinensis and it contains polyphenol epigallocatechin which increases bond durability[11]. Chitosan a natural hydrophilic polycationic biopolymer derived from chitin improves the durability of resin-dentin interface when used with adhesive system[12]. Combination of Chitosan and riboflavin also stabilizes collagen network. Aloevera has also been found to have MMP inhibitory activity[13].

Onion (Allium cepa L.) is a biennial plant belonging to the Liliaceae family, one of the most important vegetable crops with a global production of about 55 million tonnes annually. It contains many phytochemicals, carotenoids, copaenes, phenols, terpenoids, vitamins and flavonoids. Among them sitosterol, quercetin[14], apigenin and fisetin are notable ones. β-Sitosterol has antiviral, antinociceptive[15], anti-inflammatory, analgesic and immunomodulatory[16] properties. Quercetin shows anti-inflammatory, antibacterial and antioxidant properties[17,18] , antihistaminic, antiallergic, anticancer and antiviral activities[19]. Onion extracts have an inhibitory effect on MMPs 2 and 9. Inhibition of MMPs could be a useful approach for the treatment of various diseases. It could be a potential additive for dentin bonding agents and also prevent dental caries[20]. In a previous study, the glycoside quercetin was identified and quantified in 3 onion varieties (red, yellow and yellow-green). It was found to be one of the most important compounds in all varieties, with the quercetin-3'-glucoside content being higher in the red onion[21]. Another study showed that cariogenic bacteria were more sensitive to quercetin than periodontally associated bacteria[22]. Since onion peels are a rich source of quercetin, investigating their MMP inhibition and antibacterial activity is worthwhile. In-silico molecular docking is a promising approach in the field of computational drug design, where small molecules are screened by aligning and evaluating them at the binding site of a protein. This proves to be a reliable predictor in advanced testing and clinical application.

Though onion bulb extracts are widely studied and reported for beneficial properties both in Medicine and Dentistry, the peels are mostly discarded and never utilized to their full potential. Eco-friendly healthcare aims to salvage treasure from trash. Thus what is being discarded as waste in food industry can be efficiently recycled for medicinal properties and the burden of waste also can be reduced. The present study aims to investigate the antibacterial activity of small and large onion peel extract against *E. faecalis* which is the most common organism in asymptomatic persistent endodontic infections and *S. mutans*, the pioneer organism in dental caries as well as their MMP inhibitory activity.

Materials and methods:

MICROBIOLOGICAL ANALYSIS:

Medium and cultivation of *E. faecalis* and *S. mutans strains*

An ampoule of freeze-dried *E. faecalis* (ATCC 29212) procured from American Type Culture Collection and Gene Bank, (ATCC), (CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh - 160036, INDIA) was revived in Mueller-Hinton Agar (MHA, Himedia, India) and purity was subsequently assessed by Gramme staining. An overnight culture of *E. faecalis* ATCC 29212 and the clinical isolate from the department of conservative dentistry & endodontics, were inoculated in 100 ml Mueller-Hinton broth (MHB, Himedia, India) and incubated at 37°C for 18 hours. Then the concentration was adjusted with a spectrophotometer to an optical density corresponding to the McFarland standard scale turbidity of 0.5

S. mutans (MTCC 890) procured from Microbial Type Culture Collection and Gene Bank, (MTCC), (CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh - 160036, INDIA) was revived on sheep blood agar (7% sheep blood + nutrient agar, pH - 7.4 ± 0.2). Gramme staining confirmed purity. An overnight culture of *S. mutans* MTCC 890 was inoculated into 10 ml of brain heart infusion broth (Himedia, India) and incubated at 37°C for 1 hour. Then the concentration was adjusted with the spectrophotometer to an optical density corresponding to the McFarland standard scale turbidity of 0.5.

PREPARATION OF EXTRACTS:

Small and large onions were purchased and authenticated from recognised botanist. They were peeled, the peels were dried, ground into powder and was mixed with 96.9% ethanol in a conical flask. The mixture was stirred thoroughly with a glass rod. Conical flask was intermittently shaked for 72 hrs. The solution was filtered with fine muslin cloth and Whitman No1 filter paper. The filtrate was concentrated using evaporator at 40 °C.

DETERMINATION OF THE MINIMUM INHIBITORY CONCENTRATION (MIC) WITH THE MICROBROTH DILUTION ASSAY:

The MIC of ethanolic extracts (extracts of small and large onion peels) was determined as follows. A gradually increasing dilution of the extracts was added to the appropriately labelled wells of a microtitre plate. The last well of each row served as control. 10 µl of *E. faecalis* (ATCC 29212) or*S. mutans* (MTCC 890) were added to the wells. The plates were incubated overnight at 37°C. The MIC was reported as the lowest concentration of the test solution that did not allow growth (visible turbidity) of *E. faecalis* ATCC 29212/*S. mutans* MTCC 890. The MIC value was confirmed by performing the MBC

MINIMUM BACTERICIDAL CONCENTRATION (MBC):

The MBC of ethanolic extracts of small and large onion peels was determined by spot inoculation. 5 µl of the culture from each well of the microtitre plate were added to the MHA plate for *E. faecalis* and the MSA plate for *S. mutans*. The minimum concentration of the test solution that completely inhibited colony growth (~99%) was scored as the MBC of the respective test solution.

AGAR WELL DIFFUSION TECHNIQUE FOR ZONE OF INHIBITION:

The lawn culture of S. mutans (MTCC 890) was grown on mutans sanguis agar (Himedia). Wells with a diameter of 8 mm were punched with a sterile cork borer and 150 µl of the ethanolic extracts (small onion and large onion peels) and NaOCl were added to the respective wells. The plates were incubated at 37°C for 24 - 48 hours in a candle jar (3% to 5% CO2). The test was carried out in triplicate.

The lawn culture of *E. faecalis* ATCC 29212 was grown on Mueller Hinton Agar (MHA) (Himedia). Wells with a 8 mm diameter wells punched with a sterile cork borer, 150 µl of the extracts, NaOCl added to the respective wells and the plates were incubated at 37°C for 24 hours. The assay was conducted in triplicate. After the incubation period, the zone of inhibition measured for both microorganisms.

COMPUTATIONAL DRUG DESIGN AND CHARACTERISATION OF ONION PEEL EXTRACTS

1. Characterisation of phytocompounds by Gas Chromatography-Mass Spectrometry (GC-MS) profiling.

Gas Chromatography-Mass Spectrometry analyses of small and large onion peel extracts were performed. Pure helium gas (99.99%) with a constant flow rate of 1 ml/min was used as the carrier medium. Electron ionization energy was used for GC-MS spectral detection. High ionization energy of 70 eV (electron volts) and a scan time of 0.2 s were used for detection. The fragments ranged from 40 to 600 m/z. The injection volume was 1 µl (split ratio 10:1), and the injector temperature was 250 °C (constant). The column oven temperature was 50 °C for 3 min, then increased by 10 °C per minute up to 280 °C, and the final temperature was 300 °C for 10 min. The phytochemicals present in the extracts were identified by comparing the retention time (min), peak area, peak height and mass spectral patterns with the spectral database of existing compounds available in the National Institute of Standards and Technology (NIST-2020) library.

2. UltraViolet-visible (UV-Vis) Spectrophotometer Analysis

UltraViolet-visible spectrophotometric analysis was done for characterizing the ethanolic extracts of small and large onion peels using a UV-visible spectrophotometer (UV-Vis, Shimadzu - UV 3600 Plus Model) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. It was diluted to 1:10 with the same solvent for the above analysis (Donkor, S et al., 2019). The extract was examined under visible and UV light in the wavelength ranging from 200-800nm for proximate analysis. The qualitative UV-VIS profile was taken due to the sharpness of the peaks and proper baseline.

3. Fourier Transform-Infra Red Spectroscopy (FT-IR) Analysis

The Fourier Transform-Infra Red spectra of crude plant extracts were recorded in FTIR instrument (Model/Make: IRTracer-100; Michelson interferometer (30° incident angle) Equipped with Advanced Dynamic Alignment system, Shimadzu), with PC-based software controlled instrument operation and data processing. A small number of powdered test samples were made into pellets using KBr and a thin film was prepared by applying pressure. Data of infrared transmittance was collected over a wave number ranging from 4000 cm−1 to 400 cm−1. The samples analyzed with plain KBr pellets as blank as comparison and subtracting relevant noise. The spectral data were compared with a reference to identify the functional groups existing in the sample (Lestyo Wulandari et al., 2016).

4. M2 Protein Modelling

4.1 Amino Acid Sequence of MMP2 retrieved from UniprotKB Database:
>sp|P08253|MMP2_HUMAN 72 kDa type IV collagenase OS=Homo sapiens OX=9606 GN=MMP2 PE=1 SV=2

MEALMARGALTGPLRALCLLGCLLSHAAAAP
SPIIKFPGDVAPKTDKELAVQYLNTFYGCPKES
CNLFVLKDTLKKMQKFFGLPQTGDLDQNTIE
TMRKPRCGNPDVANYNFFPRKPKWDKNQIT
YRIIGYTPDLDPETVDDAFARAFQVWSDVTPL

RFSRIHDGEADIMINFGRWEHGDGYPFDGK
DGLLAHAFAPGTGVGGDSHFDDDELWTLGE
GQVVRVKYGNADGEYCKFPFLFNGKEYNSC
TDTGRSDGFLWCSTTYNFEKDGKYGFCPHE
ALFTMGGNAEGQPCKFPFRFQGTSYDSCTT
EGRTDGYRWCGTTEDYDRDKKYGFCPETAM
STVGGNSEGAPCVFPFTFLGNKYESCTSAGR
SDGKMWCATTANYDDDRKWGFCPDQGYS
LFLVAAHEFGHAMGLEHSQDPGALMAPIYTY
TKNFRLSQDDIKGIQELYGASPDIDLGTGPTP
TLGPVTPEICKQDIVFDGIAQIRGEIFFFKDRFI
WRTVTPRDKPMGPLLVATFWPELPEKIDAVYE
APQEEKAVFFAGNEYWIYSASTLERGYPKPLT
SLGLPPDVQRVDAAFNWSKNKKTYIFAGDKF
WRYNEVKKKMDPGFPKLIADAWNAIPDNLD
AVVDLQGGGHSYFFKGAYYLKLENQSLKSVK
FGSIKSDWLGC[23].

Amino Acid Sequence of MMP9 retrieved from UniprotKB Database:

>sp|P14780|MMP9_HUMAN Matrix metalloproteinase-9 OS=Homo sapiens OX=9606 GN=MMP9 PE=1 SV=3

MSLWQPLVLVLLVLGCCFAAPRQRQSTLVLFP
GDLRTNLTDRQLAEEYLYRYGYTRVAEMRGE
SKSLGPALLLLQKQLSLPETGELDSATLKAMR
TPRCGVPDLGRFQTFEGDLKWHHHNITYWI
QNYSEDLPRAVIDDAFARAFALWSAVTPLTFT
RVYSRDADIVIQFGVAEHGDGYPFDGKDGLL
AHAFPPGPGIQGDAHFDDDELWSLGKGVVV
PTRFGNADGAACHFPFIFEGRSYSACTTDGR
SDGLPWCSTTANYDTDDRFGFCPSERLYTQD
GNADGKPCQFPFIFQGQSYSACTTDGRSDG
YRWCATTANYDRDKLFGFCPTRADSTVMGG
NSAGELCVFPFTFLGKEYSTCTSEGRGDGRL
WCATTSNFDSDKKWGFCPDQGYSLFLVAAH
EFGHALGLDHSSVPEALMYPMYRFTEGPPLH
KDDVNGIRHLYGPRPEPEPRPPTTTTPQPTAP
PTVCPTGPPTVHPSERPTAGPTGPPSAGPTGP
PTAGPSTATTVPLSPVDDACNVNIFDAIAEIG
NQLYLFKDGKYWRFSEGRGSRPQGPFLIADK
WPALPRKLDSVFEERLSKKLFFFSGRQVWVYT
GASVLGPRRLDKLGLGADVAQVTGALRSGR
GKMLLFSGRRLWRFDVKAQMVDPRSASEVD
RMFPGVPLDTHDVFQYREKAYFCQDRFYWR
VSSRSELNQVDQVGYVTYDILQCPED

4.2 Search for templates

Basic Local alignment Search Tool (BLAST) and Hidden Markov Models-Hidden Markov Models (HMM-HMM) based lightning fast iterative sequence (HHblits) template search was performed on the SWISS-MODEL template library (SMTL). 549 different templates were found when the target sequence and primary amino acid sequences of the SMTL were compared to BLAST. After an initial HHblits profile was generated, the method of Steinegger et al. was used, and an iteration of HHblits against Uniclust30 was performed (Mirdita, von den Driesch, et al.). The resulting profile was compared with all SMTL profiles. A total of 839 templates were discovered.

4.3 Template selection

The quality of each identified template was predicted based on the characteristics of the target-template alignment. The best templates were selected for the model-building process.

4.4 Model building

Based on the target template alignment (Studer et al.), ProMod3 was used to build models[24]. The template and the model copy conserved coordinates between the target and the template. Insertions and deletions were modified using a fragment library. The side chains were then rebuilt. Finally, the geometry of the model was regularized using a force field.

4.5 Model Quality Estimation

The qualitative model energy analysis (QMEAN) scoring function was used to assess global and per-residue model quality (Studer et al.)[25].

4.6 Conservation of the oligomeric state

Annotation of the quaternary structure of the template was used to model the target sequence in its oligomeric form (Bertoni, et al). The approach is based on Support Vector Machines (SVM), a supervised machine learning algorithm that combines interface preservation, structural clustering, and other features of the template to produce a quaternary structure of quality estimate (QSQE)[26]. The QSQE score is a number between 0 and 1 for a model built with a given orientation and template. It indicates the expected precision of contacts between chains. Higher numbers indicate a higher level of reliability. This complements the accurate estimate of the tertiary structure provided by the Global Model Quality Estimate (GMQE) score.

4.7 Optimization and validation of protein model structure

The technique of structure determination was to validate the three-dimensional structures. Before molecular docking, the local validation criteria such as Ramachandran plot outliers, clashing contacts, and deviations from the ideal bond length and bond angles were checked (Pražnikar, J., et al 2019)[27].

4.8 Prediction of the protein active site

The ligand binding site of the modelled protein was performed using the CastP server (http://sts.bioe.uic.edu/castp/calculation.html) for MMP2 and (https://www.uniprot.org/uniprotkb/P14780/entry) for MMP9.

Prediction of Absorption, distribution, metabolism, excretion & toxicity (ADMET) properties.

The physicochemical properties of the selected ligands were calculated using the Absorption, distribution, metabolism, excretion SwissADME server (http://www.swissadme.ch/index.php) and the toxicity prediction was calculated using the ProTox- II web server (https://tox-new.charite.de/protox_ II /index.php?site=compound_input). The prepared ligand was subjected to molecular docking with a selected target protein. The physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness score, and medicinal chemistry were predicted.

MOLECULAR DOCKING

Docking was performed with the free open-source AutoDock software using the editing options and other standard parameters. The downloaded protein data bank (pdb) file of the protein was loaded and water molecules were removed from the native protein structure. Polar hydrogen bonds and Kollman charges were added manually. Then, the pdb file was saved in pdb partial charge (q) atom type (t) (pdbqt) format to proceed with the docking process in AutoDock 4.2 software. Then, the ligand file was loaded and the torsion root angles were determined. The ligand file was also saved in pdbqt format. The AutoGrid maps were then used by the AutoDock docking calculations to determine the total interaction energy of a ligand with a macromolecule. The grid box was calculated using the Autogrid box detecting option to

create a grid parameter file (gpf). The stored pdbqt files of protein and ligand were processed for docking using the Lamarckian genetic algorithm without changing the default parameters to generate a docking parameter file (dpf). For these two files, the root mean square deviation (RMSD) table was automatically generated using the command prompt.

MOLECULAR VISUALIZATION

Interactive visualization and analysis of protein-ligand interaction analysis of molecular structures was performed using Biovia Discovery Studio open-source software. Protein-ligand interaction is based on hydrogen bond formation, hydrophobic interaction, pi-pi interaction, van der wall interaction and salt bridge formation. All these analyses were performed using molecular visualization software. The corresponding data were collected and summarized[28].

STATISTICAL ANALYSIS:

Statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows, version 26.0, Armonk, NY: IBM Corp. Released 2019). Descriptive statistics were performed to determine the mean and standard deviation. To determine the difference in means, a one-way analysis ANOVA was performed, followed by a post hoc test. The P value < 0.05 was considered statistically significant

Results

The MIC, MBC, and zones of inhibition (ZOI) for small and large onions peel extracts are shown in Table 1. There was no statistically significant difference between the values for the two onion types and the control.

Table 1: Mean Value of MIC and MBC and ZOI

	Mean Value of MIC and MBC(mg/mL)								Mean Value of ZOI(mm)					
	Large Onion				Small onion				Large Onion		Small onion		3% NaoCl (control)	
	MIC		MBC		MIC		MBC		ZOI					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S. mutans	6.25	0	6.25	0	6.25	0	6.25	0	18.3	0.57	18	1	17.3	0.57
E.faecalis	12.5	0	12.5	0	12.5	0	12.5	0	18	2	17.6	0.57	16.6	0.57

Characterization of phytocomponents by GC-MS (Fig 1)

The peaks of 1, 2-dimethyl-3-isopropyldiaziridine, acetonitrile, bromo-, 1-butanethiol, 4-(9-borabicyclo o[3.3.1]non-9-yl)oxy, silanethoxytrimethyl-, l-alanine, n-propargyloxycarbonyl-, hexadecyl ester, butane, 1,1-diethoxy-, propanamide, N,N-dimethyl-s, 1-ethyl-3-methylcyclohexane (c,t), pyrimidine-2,4,6-trione, 5-[1-(2-aminocyclohexylamino) propylidene]-1-cyclohexyl, heptanoic acid, ethyl ester, 5,6-epoxy-6-methyl-2-heptanone, 2-fluoro-5-methylaniline, 7.etaHydoxy-tetrahydrosolasodine, D-fructose, 1-O-methyl, nonanoic acid, diethyl phthalate, betaD-glucopyranoside, methyl, d-glycero-l-gluco-heptose, pentadecanoic acid, n-hexadecanoic acid, dodecanoic acid, 2,8-dimethyl, methyl ester, octadecanoic acid, 2-

(2-hydroxyethoxy)ethyl ester, n-decanoic acid, 2-ethyl-5-(12-tridecenyl)pyrrolidine, 15-hydroxypentadecanoic acid, quinoxaline, 4H-1-benzopyran-4-one, 6,7-dimethoxy-3-phenyl(quercetin), 2,3-diphenyl-, beta-sitosterol, benzo[h]quinoline, 2,4-dimethyl, 1,2-bis(trimethylsilyl)benzene, 5-methyl-2-phenylindolizine, were found in small onion peel extracts (Fig 1a)

Cholestan-6-en-3-ol, O-acetyl-24-methyl-5,8-[tetrahydrofuran-2,5-dione-3,4-diyl)-, benzene, 1,2,3,4-tetramethyl, alphaPhellandrene, cyclohexene, 1-methyl-4-(1-methylethylidene)-, 1-(4-amino-furazan-3-yl)-5-piperidin-1-ylmethyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester, Quinoline, 6-methyl, phenol, 3,5-bis(1,1-dimethylethyl)-, 1,3-propanediol, 2-amino-1-(4-nitrophenyl)-, D-glycero-D-manno-heptitol, Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis, 2H-pyran, 2-(2,5-hexadiynyloxy) tetrahydro, heptadecafluorononanoic acid, allyl ester, phthalic acid, di(2-methylallyl)ester, nonanoic acid, 2,4,6-trimethyl, methyl ester, 2-[3-(4-tert-butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethylnicotinonitrile, 5-hydroxy-7-methoxy-2-methyl-3-phenyl-4-chromenone, formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo [1,5-a]pyrimidin-5-yl)-, methyl ester, 1,2-benzisothiazol-3-amine tbdms, 2-[3-(4-tert-butyl-phenoxy)-2-hydroxy-propylsulfanyl]-4,6-dimethyl-nicotinonitrile,6H-benzofuro[3,2-c][1]benzopyran (quercetin), 1,2-bis(trimethylsilyl) benzene, silica, diethylbis(trimethylsilyl)ester, cyclotrisiloxane, hexamethyl, cyclobarbital, cyclotrisiloxane, hexamethyl, cyclotrisiloxane, hexamethyl, were detected in the large onion peel extracts (Fig 1b).

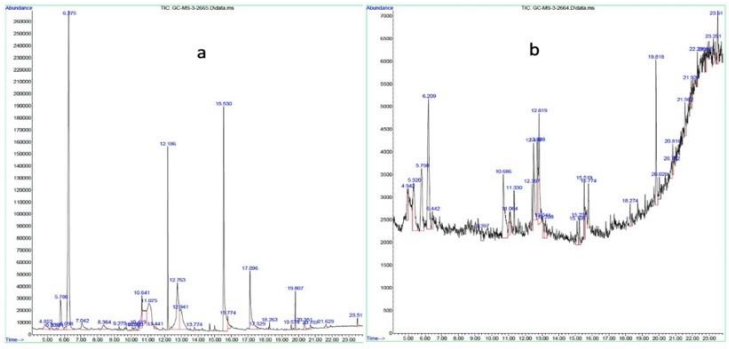

Fig 1 GC-MS : a) Small onion b) Large onion

UV-Vis profile and FTIR characterization(Fig 2)

The UV-Vis profile of the large onion peel extract showed peaks at 210 to 308 nm. (Fig 2a). The UV-Vis profile of the small onion peel extract showed peaks at 208 to 382 nm (Fig 2b). The absorption spectrum of the both the extracts was almost transparent in the wavelength range of 200-800 nm. The spectrum was compared with the reference table to assign the obtained peaks to a functional group. The large onion peel extract showed functional groups of benzene derivatives at 430 nm (Fig 2c) and the small onion peel extract showed functional groups of anhydrides at 1043 nm (Fig 2d).

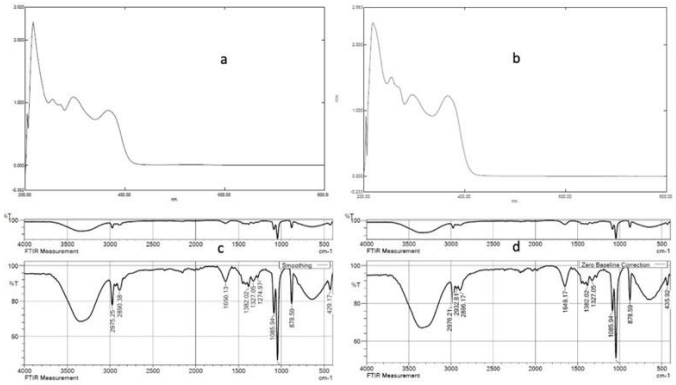

Fig 2 The UV-Vis profile of : a) Large onion peel extract, b)Small onion peel extract. FTIR spectrum: c) Large onion peel extract, d) Small onion peel extract.

Prediction of ADMET properties

The physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness score, and `medicinal chemistry of the two most active constituents of the extracts are given in Table 2.

Table 2: ADME Properties of beta sitosterol and Quercetin

Physicochemical properties		
	beta sitosterol	Quercetin
Formula	C29H50O	C15H10O7
Molecular weight	414.71 g/mol	302.24 g/mol
Num. heavy atoms	30	22
Num. arom. heavy atoms	0	16
Fraction Csp3	0.93	0.00
Num. rotatable bonds	6	1
Num. H-bond acceptors	1	7
Num. H-bond donors	1	5
Molar Refractivity	133.23	78.04
TPSA	20.23 Å²	131.36 Å²
Lipophilicity		
Log $P_{o/w}$ (iLOGP)	4.79	1.63
Log $P_{o/w}$ (XLOGP3)	9.34	1.54
Log $P_{o/w}$ (WLOGP)	8.02	1.99
Log $P_{o/w}$ (MLOGP)	6.73	-0.56
Log $P_{o/w}$ (SILICOS-IT)	7.04	1.54
Consensus Log $P_{o/w}$	7.19	1.23
Water Solubility		
Log *S* (ESOL)	-7.90	-3.16
Solubility	5.23e-06 mg/ml ; 1.26e-08 mol/l	2.11e-01 mg/ml ; 6.98e-04 mol/l
Class	Poorly soluble	Soluble
Log *S* (Ali)	-9.67	-3.91
Solubility	8.90e-08 mg/ml ; 2.15e-10 mol/l	3.74e-02 mg/ml ; 1.24e-04 mol/l
Class	Poorly soluble	Soluble
Log *S* (SILICOS-IT)	-6.19	-3.24
Solubility	2.69e-04 mg/ml ; 6.49e-07 mol/l	1.73e-01 mg/ml ; 5.73e-04 mol/l
Class	Poorly soluble	Soluble

Pharmacokinetics		
GI absorption	Low	High
BBB permeant	No	No
P-gp substrate	No	No
CYP1A2 inhibitor	No	Yes
CYP2C19 inhibitor	No	No
CYP2C9 inhibitor	No	No
CYP2D6 inhibitor	No	Yes
CYP3A4 inhibitor	No	Yes
Log K_p (skin permeation)	-2.20 cm/s	-7.05 cm/s
Druglikeness		
Lipinski	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation
Ghose	No; 3 violations: WLOGP>5.6, MR>130, #atoms>70	Yes
Veber	Yes	Yes
Egan	No; 1 violation: WLOGP>5.88	Yes
Muegge	No; 2 violations: XLOGP3>5, Heteroatoms<2	Yes
Bioavailability Score	0.55	0.55
Medicinal Chemistry		
PAINS	0 alert	1 alert: catechol_A
Brenk	1 alert: isolated_alkene	1 alert: catechol
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5	Yes
Synthetic accessibility	6.30	3.23

Molecular Docking

3D molecular model of MMP 2 (Fig 3a) and MMP 9 (Fig 3b) is given. The selection of ligands is shown in Table 3. In the present study, the minimum binding energy of the phyto-compound beta-sitosterol (-8.3) and quercetin (-9.1) was compared with that of the standard drug galardin (-8.0) to determine the anti-MMP 2 potential; their molecular interaction with the known MMP 2 protein model is listed. The 3D computational interpretation of binding and interactions using Autodock software for Beta-sitosterol (Fig 4a), Galardin (Ilomastat) (Fig 4b), Quercetin (Fig 4c), to MMP 2 is given. The anti-MMP 9 potential as evidenced by the minimum binding energy of beta-sitosterol (-8.5), quercetin (-9.1), obtained were compared with the standard drug galardin (-9.1); their molecular interaction with the known MMP 9 protein model is listed in Table 4. The 3D computational interpretation of binding and interactions using Autodock software for Beta-sitosterol (Fig 5a), Galardin (Ilomastat) (Fig 5b), Quercetin (Fig 5c), to MMP 9 is given

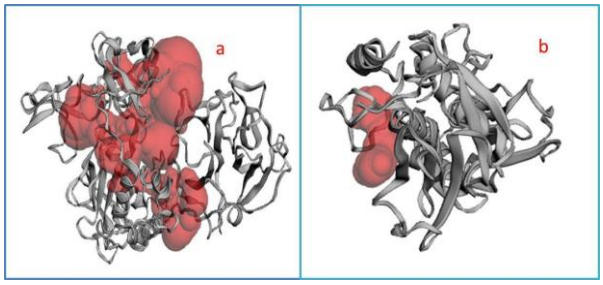

Fig 3: 3D molecular model of a) MMP 2, b) MMP 9

Table 3: Ligands selected for Molecular Docking.

S. No.	Phyto-compounds	Abundance (%)
1.	Flupentixol	2.04
2.	beta-D-Ribopyranoside	6.50
3.	D-Galactonic acid	5.08
4.	Mitozolomide	1.54
5.	Phthalic acid	2.91
6.	7-Chlorocinchoninic acid	1.24
7.	Quinoxaline	6.19
8.	3H-Pyrazol-3-one	2.91
9.	beta-l-Arabinopyranoside	9.56
10.	Isosorbide Dinitrate	5.57
11.	n-Hexadecanoic acid	14.15
12.	beta-Sitosterol	0.73
13.	Quercetin	1.23

Table 4: Molecular Docking of Phyto-Compounds against Matrix Metalloproteinase2 (MMP2) and Matrix Metalloproteinase 9 (MMP9).

Matrix Metalloproteinase 2 (MMP2)		
S. No.	Phyto-compounds	Minimum Binding Energy
1.	Flupentixol	- 7.8
2.	beta-D-Ribopyranoside	- 5.0
3.	D-Galactonic acid	- 6.3
4.	Mitozolomide	- 6.8
5.	Phthalic acid	- 6.9
6.	7-Chlorocinchoninic acid	- 7.5
7.	Quinoxaline	- 5.7
8.	3H-Pyrazol-3-one	- 4.4
9.	beta-l-Arabinopyranoside	- 5.9
10.	Isosorbide Dinitrate	- 6.4
11.	n-Hexadecanoic acid	- 6
12.	Beta-Sitosterol	- 8.3
13.	Quercetin	-9.1
14.	Galardin (Standard Drug)	- 8.0
Matrix Metalloproteinase 9 (MMP9)		
15.	Beta-Sitosterol	- 8.5
16.	Quercetin	- 9.1
17.	Galardin (Standard Drug)	- 9.1

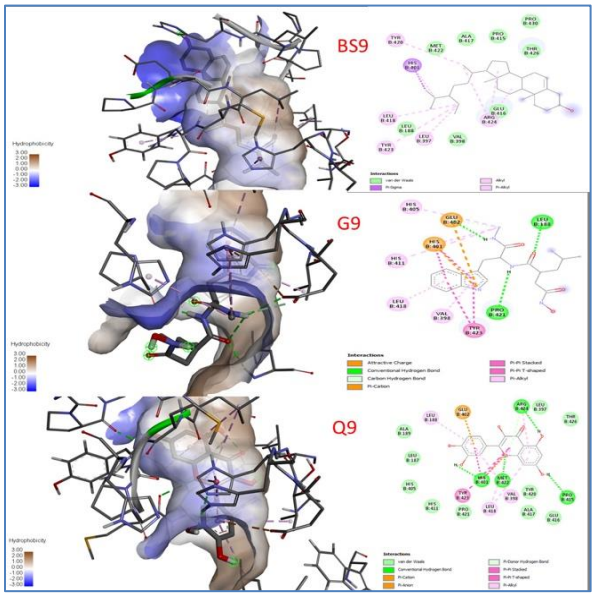

Fig 4: The 3D computational interpretation of binding to MMP 2 is given.Beta-sitosterol(BS2), b) Galardin (Ilomastat) (G2) , c) Quercetin(Q2)

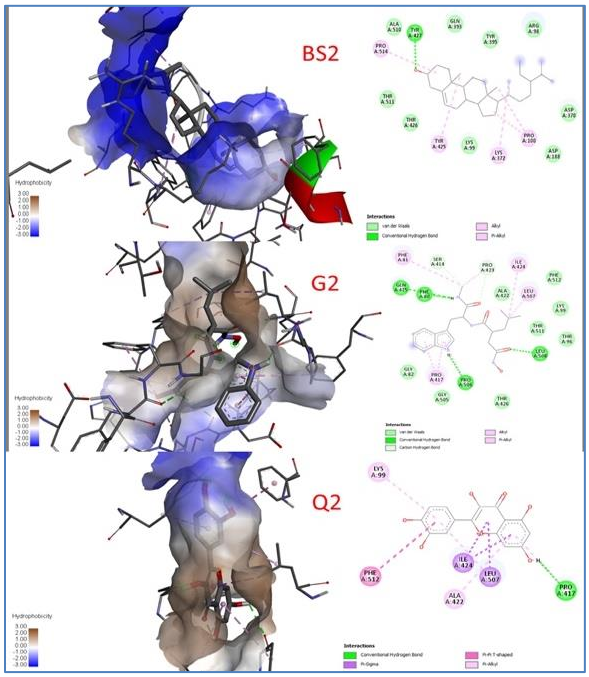

Fig 5: The 3D computational interpretation of binding to MMP 9 is given. a) Beta-sitosterol(BS9), b) Galardin (Ilomastat) (G9) , c) Quercetin(Q9)

Discussion

Bonding to dentin is more complicated than enamel due to its compositional and structural difference. Dentin is composed of approximately 45% vol of mineral phase, 33% vol of organic matrix, and the rest water[29]. The goal of bonded restoration is to achieve a close adaptation of restoration to tooth structure. In composite restoration resin interlocks with collagen fibres, inter-tubular structure, dentin surface structures to form hybrid layer. The integrity of the hybrid layer and the bond strength between resin and dentin are affected by MMP, cysteine cathepsin, and endogenous collagenolytic enzymes. Odontoblasts and pulp express significant amounts of MMPs and TIMPs[30]. MMPs are there in the form of collagenases, gelatinases, stromelysins, and matrilysins and can degrade the components of the extracellular matrix. MMP inhibition is achieved by collagen cross-linking[31], chelating cations[32], competitive inhibition for the active site. Inhibitors with multiple mechanisms of action are also available. Restorative procedures are many a time done on affected dentin in carious teeth which is a rich reservoir of cysteine cathepsins. These in turn activate latent MMPs in dentin matrix. This is termed as cysteine switch mechanism[33]. Adhesives with acidic constituents increase the gelatinolytic activity of host MMPs. During the immediate post-restorative phase, MMPs are very active due to this, lasting till 21 days[34].

In endodontics too, MMP inhibitors can preserve demineralized dentin collagen for the durability of resin-based root canal sealers. Both synthetic and natural substances are available with MMP inhibitory property. Endogenous enzymatic inhibitors like TIMPs control MMP activity, but the interaction has shorter half-life. Further, cessation of dentinal fluid flow in a non-vital tooth and acidic carious milieu in a vital tooth decrease the supply of TIMPs. Besides, acidic materials

used in restorative dentistry and endodontics remove TIMPs. It should be noted that organic acids (self-etch adhesives) more potently activate MMPs than inorganic acids (etch and rinse). Synthetic substances used for MMP inhibition include CHX-gluconate[32], EDTA, tetracycline, quaternary ammonium compounds and glutaraldehyde[31]. Natural substances used for MMP inhibition are riboflavin, grape seed extract, green tea polyphenol, proanthocyanidin, and quercetin. Phendione, a metal chelator, was used in endodontics as an anti-biofilm agent and it effectively inhibited MMP[35]. There was significant inhibition of dentin matrix-bound MMP by 17% EDTA at 1, 2, and 5 minutes[36]. Green tea polyphenol and its component epigallocatechin gallate showed strong inhibition of gelatinolytic activities of MMPs-2 and 9 and elastinolytic activity of MMP-12[37].

Quercetin is used as an antibiofilm and collagen stabilizing agent when used as an irrigant. In a CLSM study, quercetin was found to increase the percentage of dead bacteria in the biofilm of *E faecalis* and improved the flexural strength of dentin[38]. Li et al performed an in situ zymography assay and found that 0.5 and 1% quercetin inhibited MMP activity in dentin[20]. Quercetin decreased the production of inflammatory cytokines and there was a decrease in pathogenic bacteria such as *Enterococcus, Neisseria*, and *Pseudomonas* and an increase in the number of non-pathogenic *Streptococcus sp*[39]. Onions are rich sources of quercetin. Onion peels have a higher concentration of all health-promoting phytochemicals[40]; quercetin is reported to be almost 75 times higher in onion peel compared to onion bulb[41]. Sitosterol is the active ingredient found in many fruits and vegetables. It has antimicrobial, antioxidant, antienzymatic[42] properties. While the extracts of onion bulbs are widely studied, peels have gained only scant attention. Therefore, in the present study, we investigated the MMP inhibition and antibacterial properties of small and large onion peel extract.

S. mutans colonizes the tooth and causes damage to the calcified structures in the presence of fermentable carbohydrates. They are acidogenic and aciduric bacteria with potential to adhere to the enamel surface and produce acidic metabolites, store sugars, and form extracellular polysaccharides. A study by Shu et al. on oral microorganisms showed that quercetin has an antibacterial effect against cariogenic bacteria such as *S. mutans, S. sobrinus, L. acidophilus, S. sanguis, A. actinomycetocomitans,* and *P. intermedia,* with MIC ranging from 1 to 4 mg/ml and MBC ranging from 8 to 16 mg/ml[22] . In the present study, the MIC and MBC of large and small onion peel extracts was found to be 6.25 mg/ml for *S. mutans. E. faecalis* invades dentinal tubules, resists nutrient deprivation, and competes with other microorganisms. In a previous study, it was found that the MIC of onion peel extract against *E. faecalis* ranged from 250-1000 µg/ml and MBC ranged from 250-2000 µg/ml in three different onion varieties[43]. In the present study, both the small and large onion peel extracts showed MIC and MBC at 12.5 mg/ml itself. Agar well diffusion test (for estimating ZOI) is a more practical and convenient method for simultaneous screening of antibacterial activity of reagents against culturable bacterial species. The antibacterial property of both the onion peel extracts against *S. mutans* and *E. faecalis* was further confirmed

by ZOI; against *S.mutans*, it was 18.3mm for large onion peel extract while for small onion peel extract it was 18mm; against *E. faecalis* it was 18mm for large onion peel extract while for small onion peel extract it was 17.6mm. Hence, both onion peel extracts had similar antimicrobial activity.

The search for potential therapeutic inhibitors of MMP2 was performed by computer-aided drug design and characterization of crude extracts of onion peel extracts by GC-MS profiling. The presence of health-promoting phytochemicals in the edible parts and peels of onion has been confirmed in other studies using GC-MS and HPLC[44,45]. In a previous study by Virginia Lanzotti et al[46], various bioflavonoids in onion extracts was found using GC-MS. In the present study, many phytochemicals and flavonoids were found in the ethanolic extracts of onion peels from both small and large onions using GC-MS. However, large onion peel extracts did not show detectable levels of β sitosterol.

UltraViolet-visible spectrophotometric analysis was performed to characterize the ethanolic extracts of small and large onion peels. The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the infrared region. FTIR and UV-visible spectrophotometric analysis peeks found in our study are conforming to those reported for onions by a previous study[47].

Molecular docking is an important tool in structural molecular biology and computational drug design. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand to a protein with a known three-dimensional structure. Successful docking methods effectively search high-dimensional spaces and use a scoring function that correctly ranks docking candidates for the protein's structure. AutoGrid is a program that precalculates grid maps of interaction energies for different atom types such as aliphatic carbons, aromatic carbons, hydrogen bonds and oxygen in a macromolecule such as a protein, DNA or RNA. In silico molecular studies of target proteins with drug candidates that have inhibitory activity are analyzed using the minimum binding energy and affinity score[48].

This computerized study is less time consuming and cost effective unlike Zymography, which is highly technique sensitive. A previous study by Sharma et al. on a methanolic onion extract and quercetin isolated from it showed their antienzyme activity against the enzymes of multidrug resistant bacteria [*E.coli and P.aeruginosa*]. This was confirmed by molecular docking as both showed higher binding energy with **β** lactamase, gyrase A, 2-trans-enoyl acyl carrier protein reductase (inhA) and topoisomerase 1V[49]. Onion extract was found to possess inhibitory potential against human serine protease [papain-like protease] and chymotrypsin-like protease by molecular docking[50]. In the present study, both onion peel extracts were found to inhibit MMP2 and MMP9 by in silico molecular docking. The control or reference used for our docking study was galardin. Of the phytochemicals present in the onion peel extracts, quercetin and **β** sitosterol had binding energies with MMPs 2 and 9. However, while quercetin had an identical binding energy to Galardin for both the MMPs, β sitosterol had slightly lower binding energy for both the MMPs.

Incorporating antimicrobial and MMP inhibitory constituents in adhesives, restorative materials, sealers and obturating materials would be value addition to these products as the longevity of the rehabilitative work is enhanced. In conservative dentistry and endodontics, this assumes significance as the tooth is pre-operatively compromised due to the carious process and activation of dormant enzymes in ECM. The highlight of the present study is that what is considered as trash (onion peels) can be efficiently salvaged to provide very useful substances with potential versatile functionalities. As a preliminary effort we have proven the MMP inhibitory activity of small (shallot) and large onion peel extracts by computational in-silico docking after confirming the molecular profile with GC-MS, FTIR, UV-VIS spectrophotometry and antimicrobial activity by agar well diffusion, MIC and MBC.

The limitations of this study arise from the methodology used to investigate MMP inhibitory potential. While in situ zymography and other direct methods are available, in silico molecular docking, a computational but reliable method, was chosen. Further studies using direct methods to investigate enzymatic inhibition potential should be performed to confirm the above results. In addition, the biocompatibility of these onion peel extracts should be evaluated before translating their *in vitro* benefits into clinical applications.

Conclusion

In conclusion, the small and large onion peels alcoholic extracts contained many phytochemicals and flavonoids, including quercetin and β sitosterol. The extracts had good antibacterial activity against S. mutans and E. faecalis. Their inhibitory potential on MMPs 2 and 9 was identical to the reference standard galardin. If this property is confirmed by direct methods and their biocompatibility is ensured, they can be used as potential agents in conservative dentistry and endodontics as antimicrobial and collagen stabilizing agents.

Conflict of Interest Statement:

There is no Conflict of interest.

Funding Statement:

None

Acknowledgement Statement:

None

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