Research article

In-vitro and in-silico approach to assess the bacterial collagenase inhibiting property of andrographolide

Madhumathi D.[1], Ram Sabarish[1], Srikanth J.[2], S.K. Balaji[1]

[1]Department of Periodontics, Sri Ramachandra Dental College and Hospital,
[2]Department of Pharmacology, Sri Ramachandra Faculty of Pharmacy,
Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai, Tamil Nadu, India

(Received: March 2022 Revised: December 2022 Accepted: January 2023)

Corresponding author: **S. K. Balaji.** Email: hod.periodontology@sriramachandra.edu.in

ABSTRACT

Introduction and Aim: Andrographolide is one of the main constituents of *Andrographis paniculata*. An anti-collagenase property of andrographolide is yet to be explored. Thus, the primary goal of study is to assess the anti-collagenase property of andrographolides against standard bacterial collagenase. The secondary objective is to evaluate the molecular docking between andrographolide and proteins of periodontal pathogens [*P. gingivalis* (Arg and Lys Gingipains), *P. intermedia and A. actinomycetecomitans*].

Materials and Methods: Anti-collagenase property of an andrographolide was determined using a UV spectrophotometer at four different concentrations (0.5%, 1%, 2.5% and 5%) against bacterial collagenase. Furthermore, an *in-silico* molecular docking study was performed between andrographolide and the proteins of periodontal pathogens using the Molegro Virtual Docker (MVD) software.

Results: Different concentrations of andrographolide had a collagenase inhibiting property of upto 65% at an IC50 value of 2.4µg/ml at the end of 20mins. Based on molecular docking, the proteins exhibited high binding energy to the bacterial enzymes.

Conclusion: Within the limitations of this study andrographolides can inhibit bacterial collagenase in an *in-vitro* model. Also, Andrographolide was successfully docked in the protein binding sites of *P. gingivalis* (Arg and Lys Gingipains), *P. intermedia* and *A. actinomycetecomitans* indicating its ability to interact with these proteins.

Keywords: Andrographolide; collagenase; immunomodulation; periodontitis; molecular docking.

INTRODUCTION

Periodontal disease results due to an unperturbed interaction between the host defence system and the pathogenic oral microbiota. In addition, various genetic and environmental factors can influence the disease outcome. The virulence factors released by the subgingival microbiota initiates the periodontal tissue injury. The vicious interplay between bacterial products with host response propagates disease pathogenesis resulting in progression periodontal attachment loss and bone loss. The initiation of periodontal disease is primarily by microorganisms. Numerous cell types, such as neutrophils, macrophages, osteoblasts, osteoclasts, macrophages, epithelial cells and fibroblasts produce collagenases which are proteolytic enzymes. Also, microorganisms are involved in collagenase production. These enzymes have a significant impact on tissue remodelling and the extracellular matrix protein breakdown. Matrix metalloproteinase (MMP) overexpression has been remarkably linked to periodontal disease. The balance between MMPs and their tissue inhibitors are crucial for maintaining the homeostasis of the periodontium. Any abnormal alterations can lead to periodontal disease which can be treated with host modulating agents in addition to conventional periodontal treatment (2).The purpose of host modulating therapy (HMT) is to treat the host side of the host-bacteria interaction thereby it reduces tissue destruction. HMT consists of systemically or locally delivered synthetic agents which are used as an adjuncts to conventional periodontal treatment. Although these synthetic agents are more effective as a host modulating agent, certain adverse effects has been reported (3). Currently, the usage of herbal products has increased in treating periodontitis in the form of locally delivered host modulating agent. Hence this study focuses on the use of phytomedicine as an adjunct to conventional periodontal therapy.

Traditional Medicinal System (TMS) is a time-tested practice to counter diseases and to lead a healthy life. It consists of Ayurveda, Siddha, Unani, Naturopathy and Homoeopathy (4). Many plants are being studied for their potential as phytonutrients or phytotherapy materials. It has been suggested that several plants have anti-inflammatory, antibacterial, antioxidant, astringent and other useful properties (5). The application of these properties are currently being tried in the treatment of gingival and periodontal diseases (5).

Andrographolide (AG) is a one of the major constituent of *Andrographis paniculata* , a member of

the acanthaceae family and a commonly used as a traditional medicine in India, China and Southeast Asia (6). It exhibits a broad range of biological activities such as anti-microbial(6), anti-inflammatory (7), antioxidant (7, 8), anti-diabetic (9), anti-cancer (10) and anti-viral (11) activities. This plant has been reported for the treatment of various diseases in India, Asia and Africa. In an animal model, AL Batran *et al.*, examined andrographolide's antimicrobial potential against periodontal pathobionts. He assessed its impact on alveolar bone resorption induced by *Porphyromonas gingivalis* (Pg) in rats and concluded that it inhibits Pg-induced alveolar bone resorption (8). However, anti-collagenase property of andrographolides is yet to be explored.

Thus, the primary objective of this study is to evaluate the anti-collagenase property of andrographolides against standard bacterial collagenase and the secondary objective is to evaluate the molecular docking between andrographolide and proteins of periodontal pathogens (*P. gingivalis* (Arg and Lys Gingipains), *P. intermedia and A. actinomycetecomitans*).

MATERIALS AND METHODS

The study employed an *in vitro* and *in silico* experimental design. Ethical clearance was obtained from the Institutional Ethics Committee of Sri Ramachandra institute of higher education and research (CSP/21/SEP/99/492).

Materials and reagents

All the materials (Andrographolide, the main component of *Andrographis paniculata;* Clostridium collagenase enzyme; FALGPA substrate; Tricine buffer and a Positive control(Epigallectocatechin gallate) were procured from Sigma Aldrich. Sample size [Different concentrations (0.5%, 1%, 2.5%, and 5%) of an extract and the experiment was triplicated] was calculated based on the previous article (12).

Collection of ligand and proteins

Three dimensional (3D) structure of ligand i.e., andrographolide (CID: 5318517) was downloaded from pubchem database (https://pubchem.ncbi.nlm.nih.gov). 3D structure of *P.gingivalis* (Arginine specific gingipain (PDB ID 1CVR) and Lysine specific Gingipain (PDB ID 4TKX), *P. intermedia* (PDB ID 3BBA) and *A.actino-mycetecomitans* (PDB ID 4U10) was downloaded from RCSB protein database (http://www.rcsb.org/pdb/home/home.do) (Fig. 1).

Collagenase inhibition assay

The anti-collagenase test was based on the methodology proposed by Van wart et al (1981) using a UV spectrophotometer(13). Dilution series of an extract (different concentrations of 0.5%, 1%, 2.5%, and 5%) of extract was prepared and mixed with a 50µL of an enzyme in a 96 well microtitre plate. The assay was performed in 50mM Tricine buffer (pH 8.0). Collagenase from *Clostridium histolyticum* (ChC) was dissolved in buffer. According to the supplier information, the synthetic substrate FALGPA (N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala) was dissolved in the buffer. Prior to substrate addition, Andrographolide was dissolved and incubated with the enzyme for 30 mins. The final reaction mixture contains tricine buffer, 0.8mM FALGPA, 0.1 unit of ChC and andrographolide.

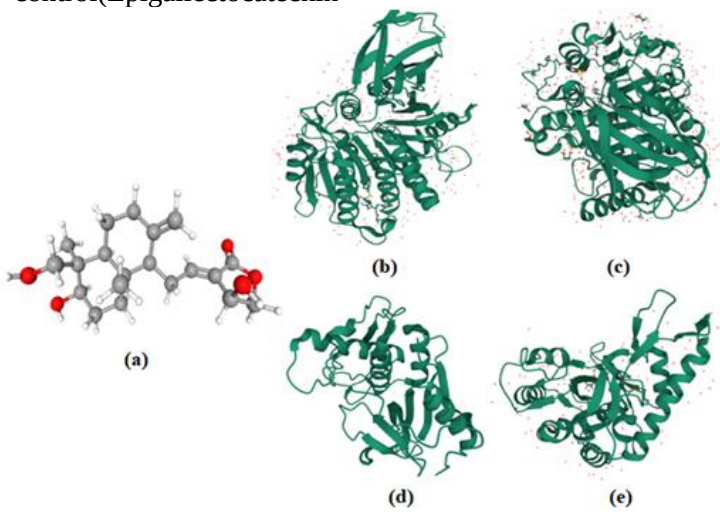

Fig.1: 3D structure of (a) Andrographolide used as ligand for molecular docking with collagenase enzyme of the following microorganisms i.e. (b) *Arg gingipain* (c) *Lys gingipain* (d) *Prevotella intermedia* (e) *Aggregatibacter actinomycetemcomitans.*

The absorbance at λ=340 nm was measured immediately after the substrate incorporation. Enzyme activity was evaluated by a decrease in absorbance and expressed as a percentage, according to the following formula:

Anti-collagenase activity (%) = 100 × [(Ac– As)/Ac]

where Ac is the Activity of control and As is the Activity of sample.

Drug likeliness scoring

Drug likeliness property of an andrographolide was done using drulito software. The ligand structure (Andrographolide) was downloaded from Pubchem database either in *.mol or *.sdf file format as input file for DruLiTo software. The drug likeliness scoring of a ligand was done by following Lipinski's rule.

Molecular docking

Molecular docking is one of the techniques that is most frequently utilised in structure-based drug design because it can anticipate the conformation of small-molecule ligands within the suitable target binding site with a high degree of accuracy (14). In this study, a molecular docking procedure was performed using the Molegro Virtual Docker (MVD) software, version 6.0.

RESULTS

Anti-collagenase activity

Immediately after substrate addition, a 5 µg/mL concentration of an andrographolide showed 44% enzyme inhibition, whereas concentrations of 0.5, 1, and 2.5 µg/mL of an extract caused 38%, 40%, and 40% enzyme inhibition, respectively with an IC50 value of 3.01µg/ml (Figure 2). At the end of 20 mins, a concentration of 5 µg/mL of an extract showed 65% enzyme inhibition, whereas lower concentrations of 0.5, 1, and 2.5 µg/mL of an extract caused 55%, 60%, and 62% enzyme inhibition, respectively with an IC50 value of 2.4 µg/ml (Figure 2).There is no statistical correlation was found between the concentrations immediately and at the end of 20 mins after substrate addition as shown in Table 1.

Lipinski score from DruLiTo analysis

DruLiTo software was used to analyse the Lipinski's properties of an andrographolide. The result of the Molecular Weight, xLog p, H Bond Acceptor, H Bond Donor and topological polar surface area (TPSA) were shown in Table 2.

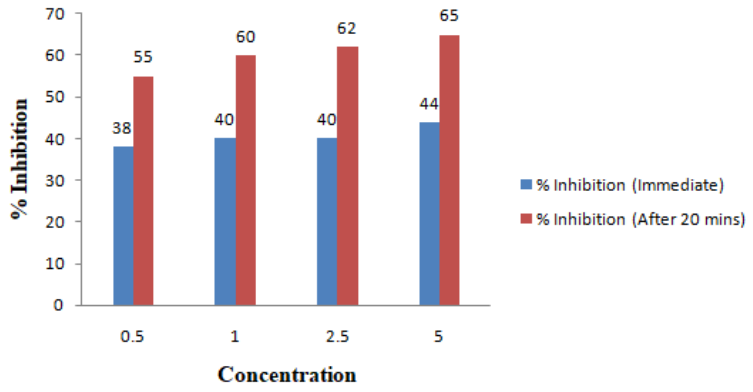

Fig. 2: The percentage of collagenase inhibition at different concentrations of an andrographolide immediately and at the end of 20 mins after substrate addition

Table 1: Statistical correlation between the concentrations immediately and at the end of 20 mins after substrate addition

Sample	Immediate			After 20 mins		
	Mean ± SD	P value	95% CI	Mean ± SD	P value	95% CI
5%	0.536±0.069	.149	.364213±.707987	0.522±0.083	.351	.315682±.728918
2.5%	0. 530±0.036	.149	.461692±.644374	0.546±0.050	.351	.420728±.672205
1%	0. 562±.0120	.149	.532025±.592109	0.563±0.019	.351	.515177±.611889
0.5%	0. 585±0.258	.149	.520952±.649515	0.591±0.049	.351	.467995±.714405
+ve control	0. 488±0.005	.149	.475076±.501324	0.506±0.013	.351	.472093±.541573
Total	0. 544±0.046	.149	.519374±.570479	0.546±0.052	.351	.517076±.575057

One way ANOVA, CI: Confidence interval.

Table 2: The values of drug-likeness properties of andrographolide

Sl. no	Ligand	Molecular weight	xlog p	H bond acceptor	H bond donor
1	Andrographolide (5318517)	350.21	2.913	5	3

Table 3: The MolDock score and Rerank score for the ligand with all the four target enzymes

Ligand (Andrographolide)	Target collagenase enzymes	MolDock score	Rerank score
[00]5318517	*Arg specific gingipain*	-94.476	-47.705
[00]5318517	*Lys specific gingipain*	-53.414	-52.442
[00]5318517	*Prevotella intermedia*	-104.214	-74.767
[00]5318517	*Aggregatibacter actinomycetecomitans*	-128.030	-77.583

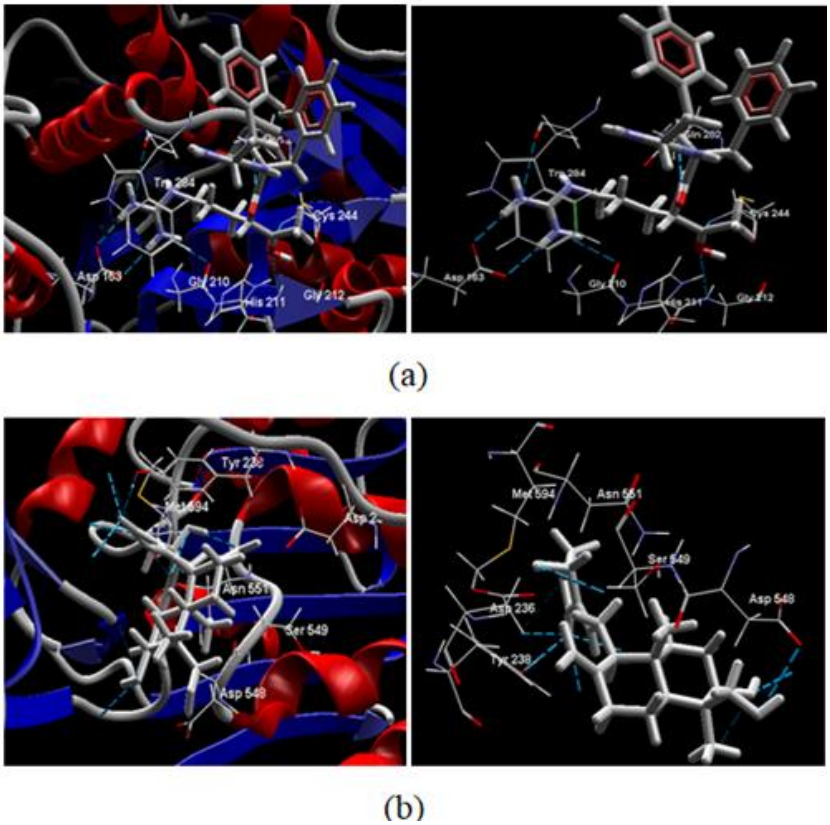

Fig. 3: 3D molecular docking poses of andrographolide and (a) Arginine specific gingipain (b) Lysine specific gingipain

Docking

The docking analysis of the ligand with all the four target enzymes generated total energy which was shown in Table 3 as MolDock score and Rerank score.

Interaction of andrographolide with arginine specific gingipain

The results show the molecular docking of ligand (Andrographolide) with the target arginine specific gingipains enzyme and the best docking position of andrographolide with arginine specific gingipain(Fig. 3(a)). The ligand binds with 6 residues namely Gly 212, His 211, Asp 163, Cys 244, Thr 284 and Gln 282 as shown in Fig. 3(a).

Interaction of andrographolide with lysine specific gingipain

The results show the binding energies of the selected ligand (Andrographolide) with the target lysine specific gingipain enzyme and the best docking pose of andrographolide with arginine specific gingipain[Fig. 3(b)]. The ligand binds with 6 residues namely Asp 548,Tyr 238, Ser 549, Asn 551, Asp 236 and Met 594 as shown in Fig. 3b.

Interaction of andrographolide with *Prevotella intermedia* collagenase

The results showing the binding energies of selected ligand (Andrographolide) with the target *P. intermedia* enzyme and the best docking pose of andrographolide with arginine specific gingipain [Fig. 4(a)]. The ligand binds with 10 residues namely Thr 247, Ala 244, Gly 243, Cys 154, Gly 243, Ser 242, Tyr 264, Asp 350, Met 246 and Gly 304 as shown in Fig. 4(a).

Interaction of andrographolide with *Aggregatibacter actinomycetemcomitans* collagenase

The results show the binding energies of selected ligand (andrographolide) with the target *A. actinomycetecomitans* enzyme and the best docking pose of andrographolide with arginine specific gingipain (Fig. 4b). The ligand binds with 7 residues namely Lys 43, Thr 52, Gln 51, Pro 50, Pro 191, Thr 276 and Tyr 198 as shown in Fig. 4b.

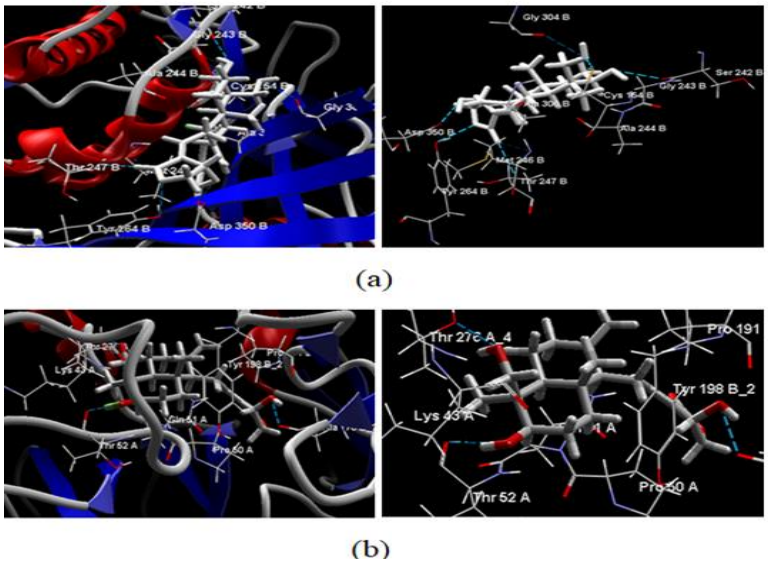

Fig. 4: 3D molecular docking poses of andrographolide and collagenase enzymes of (a) *Prevotella intermedia* (b) *Aggregatibacter actinomycetemcomitans*

DISCUSSION

The role of andrographolide in human health has been explored periodically and was found to be effective to treat various acute, chronic infections and systemic diseases (15). Li *et al.*, examined the effects of AG on in vivo animal models and discovered that daily gavage treatment with andrographolide (25, 50mg/kg) for 4 weeks reduced paw swelling, arthritis scores, leukocyte infiltration, cartilage erosion, and synovial hyperplasia in rats with complete Freund's adjuvant-induced arthritis (16). During the dengue outbreak in India (2006), in order to increase immunity, andrographolide has been administered in higher doses, where it has been shown to be successful in reducing infection (17). Additionally, Li *et al.*, evaluated andrographolide's antiviral activity against the Zika (ZIKV) and Dengue (DENV) viruses and discovered that it has the potential to be developed as a drug that is both anti-ZIKV and anti-DENV (18).

The effectiveness of mechanical periodontal therapy is limited due to the lack of accessibility in deep periodontal pockets. Host modulating therapy(HMT) decreases tissue destruction and stabilizes or even regenerate the periodontium by treating the host side of host bacterial interaction (19). In addition to conventional periodontal therapy, HMT consists of systemically or locally delivered agents which can be prescribed as a part of periodontal therapy. It can be used to reduce excessive levels of enzymes, cytokines and prostanoids (19). *Andrographis paniculata* has been studied for immunomodulatory activities and found that it is capable of enhancing both antigen-specific and nonspecific responses(20). In our study, it was observed *in-vitro* that andrographolide exhibited collagenase inhibiting property thereby having a host modulatory effect. Andrographolide inhibits the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signalling pathway and downstream transcription factors including nuclear factor kappa B (NF-B), which is the mechanism underlying the drug's anti-inflammatory and immunomodulatory actions (21). It implies that andrographolide, acting as a regulator of abnormal immunological responses, can have a various effects in immune disease models. Ambili *et al.*, evaluated the effectiveness of andrographolide in management of periodontal disease and reported that the adjunctive use of andrographolide as a host modulation agent for periodontal therapy by inhibiting NFκB and STAT3 activation and inhibition of inflammation and bone resorption related genes (22). In the present study, assessments were made immediately and at 20th min on considering its efficacy for topical application. Immediately and at the end of 20 mins after substrate addition, a concentration of 5µg/ml extract showed 44% and 65% enzyme inhibition respectively, with an IC_{50} value of 2.4µg/ml at the end of 20mins. Hence, topical application of andrographolide may be a promising adjunct to conventional periodontal treatment and may aid in improving periodontal treatment outcomes. However, the cell viability and cytotoxicity needs to be assessed to use as a locally delivered host modulating agent.

To study the receptor-ligand interaction in the inhibition of enzymes, molecular docking have been used. Megantara *et al.*, have reported that andrographolide could be developed as protease inhibitor for anti-malarial drug (23). In this study, andrographolide was docked successfully in the protein binding sites of periodontal pathogens(*P. gingivalis* (Arg and Lys Gingipains*), P. intermedia* and *A. actinomycetecomitans*). Drug likeness factors and Lipinski's rules were obeyed accordingly without any violation by this compound. This describes that the compound can used as a drug in the biological systems.

Locally delivered host modulatory system is based on the concept of controlled release drug delivery at the target site and should be considered as an adjunctive to mechanical debridement for the treatment of periodontal disease. Upon confirmation through various in-vitro and in-silico models for toxicity and lead molecular assessment on andrographolide, it may be used as a topically delivered host modulatory agent in successfully treating the periodontal diseases. However, more clinical studies are required to prove the host modulatory property of andrographolide in successfully managing the periodontally compromised patients.

CONCLUSION

Within the limitations of this study, andrographolides are capable of inhibiting bacterial collagenase in an *in-vitro* model. Also, Andrographolide was docked successfully in the protein binding sites of (*P.gingivalis* (Arg and Lys Gingipains), *P.intermedia* and *A.actinomycetecomitans*) indicating its ability to interact with these proteins.

ACKNOWLEDGEMENT

We greatly appreciate Dr. Ganesh Venkatraman and Ms. Roshini Ph.D scholar, Department of Human Genetics, Sri Ramachandra Institute of Health Education and Research for their excellent assistance.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

1. Janakiram, C., Mehta, A., Venkitachalam, R. Prevalence of periodontal disease among adults in India: A systematic review and meta-analysis. J Oral Biol Craniofacial Res. 2020;10(4):800-806.
2. Checchi, V., Maravic, T., Bellini, P., Generali, L., Consolo, U., Breschi, L., Mazzoni, A. The role of matrix metalloproteinases in periodontal disease. International Journal of Environmental Research and Public Health. 2020; 17(14):4923; 1-13.
3. Bhatt, A. K., Govila, V., Sharma, M. Host modulatory agents in periodontics: A step towards the future. Journal of the International Clinical Dental Research Organization. 2015; 7(2): 130- 136.
4. Karunamoorthi, K., Jegajeevanram, K., Xavier, J., Vijayalakshmi, J., Melita, L. Tamil traditional medicinal system-siddha: an indigenous health practice in the international perspectives. CELLMED. 2012;2(2): 11-12.
5. Cruz Martínez, C., Diaz Gómez, M., Oh, M. S. (2017). Use of traditional herbal medicine as an alternative in dental treatment in Mexican dentistry: A review. Pharmaceutical biology. 2017; 55(1), 1992-1998.
6. Hossain, M. D., Urbi, Z., Sule, A., Rahman, K. M. *Andrographis paniculat*a (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal, 2014;2014:1-28.
7. Sheeja, K., Shihab, P. K., Kuttan, G. Antioxidant and anti-inflammatory activities of the plant Andrographis paniculata Nees. Immunopharmacology and immunotoxicology. 2006; 28(1): 129-140.
8. Al Batran, R., Al-Bayaty, F. H., Al-Obaidi, M. M. J. *In-vivo* effect of andrographolide on alveolar bone resorption induced by Porphyromonas gingivalis and its relationship with antioxidant enzymes. BioMed Research International,2013;2013: 1-5.
9. Subramanian, R., Asmawi, M. Z., Sadikun, A. In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochimica Polonica. 2008; 55(2):391-398.
10. Rajagopal, S., Kumar, R. A., Deevi, D. S., Satyanarayana, C., Rajagopalan, R. Andrographolide, a potential cancer therapeutic agent isolated from Andrographis paniculata. Journal of Experimental therapeutics and Oncology. 2003; 3(3), 147-158.
11. Ekalaksananan, T., Sookmai, W., Fangkham, S., Pientong, C., Aromdee, C., Seubsasana, S., *et al.*, Activity of Andrographolide and Its Derivatives on HPV16 Pseudovirus Infection and Viral Oncogene Expression in Cervical Carcinoma Cells. Nutr Cancer. 2015;67(4):687-696.
12. Thring, T. S., Hili, P.,Naughton, D. P. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complementary and Alternative Medicine. 2009; 9(1): 1-11.
13. Van Wart, H. E., Steinbrink, D. R. A continuous spectrophotometric assay for *Clostridium histolyticum* collagenase. Analytical biochemistry. 1981; 113(2): 356-365.
14. Ferreira, L. G., Dos Santos, R. N., Oliva, G., Andricopulo, A. D. Molecular docking and structure-based drug design strategies. Molecules. 2015; 20(7): 13384-13421.
15. Lim, J. C. W., Chan, T. K., Ng, D. S., Sagineedu, S. R., Stanslas, J., Wong, W. F. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. Clinical and Experimental Pharmacology and Physiology. 2012; 39(3): 300-310.
16. Luo, S., Li, H., Liu, J., Xie, X., Wan, Z., Wang, Y., *et al.*, Andrographolide ameliorates oxidative stress, inflammation, and histological outcome in complete Freund's adjuvant-induced arthritis. Chemico-Biological Interactions. 2020; 319:108984; 1-9.
17. Edwin, E. S., Vasantha-Srinivasan, P., Senthil-Nathan, S., Thanigaivel, A., Ponsankar, A., Pradeepa, V., *et al.*, Anti-dengue efficacy of bioactive andrographolide from *Andrographis paniculata* (Lamiales: Acanthaceae) against the primary dengue vector Aedes aegypti (Diptera: Culicidae). *Acta tropica.* 2016; 163: 167-178.
18. Li, F., Khanom, W., Sun, X., Paemanee, A., Roytrakul, S., Wang, D., *et al.*, Andrographolide and its 14-aryloxy analogues inhibit zika and dengue virus infection. Molecules. 2020; 25(21): 5037: 1-16.
19. Newman, M., Takei, H., Klokkevold, P. Carranza's Clinical Periodontology 11th edition St. Louis: Mosby, 2012; 414-418.
20. Puri, A., Saxena, R., Saxena, R. P., Saxena, K. C., Srivastava, V., Tandon, J. S. Immunostimulant agents from Andrographis paniculata. Journal of Natural products. 1993; 56(7): 995-999.
21. Low, M., Khoo, C. S., Münch, G., Govindaraghavan, S., Sucher, N. J. An *in vitro* study of anti-inflammatory activity of standardised *Andrographis paniculata* extracts and pure andrographolide. BMC Complementary and Alternative Medicine. 2015; 15(1): 1-9.
22. Ambili, R., Janam, P., Babu, P. S., Prasad, M., Vinod, D., Kumar, P. A., *et al.*, An *ex vivo* evaluation of the efficacy of andrographolide in modulating differential expression of transcription factors and target genes in periodontal cells and its potential role in treating periodontal diseases. Journal of Ethnopharmacology. 2017; 196: 160-167.
23. Megantara, S., Levita, J., Surantaatmadja, S. I. *In silico* study of andrographolide as protease inhibitors for antimalarial drug discovery. In 3rd International Conference on Computation for Science and Technology (ICCST-3). 2015; pp. 36-39. Atlantis Press.