Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.01.Art019

Exploration of Various Extracts of Adhatoda vasica for Use in Orthodontics

Murukesan S*, Kishore Kumar
Department of Orthodontics, Sree Balaji Dental College and Hospital, Chennai, India

Abstract

Fixed appliances used in orthodontics can alter the oral microbiota, leading to an increase in aggressive gram-positive and gram-negative bacterial species. These bacteria are strongly associated with enamel and dentin caries and periodontal disease. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for pain control during orthodontic treatment which can affect tooth movement and lengthen treatment time. The study evaluated the use of Adhatoda vasica in various aspects of orthodontics. The study involved the extraction of plant material, qualitative phytochemical analysis, GC-MS analysis, antibacterial activity testing, and in vitro toxicity testing on bone cell lines. The results showed that Adhatoda vasica extracts had antimicrobial activity against various pathogens and demonstrated cytocompatibility with bone cell lines. The hydroalcoholic and pet ether extracts showed the most promising results. The study highlights the impact of fixed appliances on oral microbiota, the potential role of Adhatoda vasica in orthodontics, and the need for further research in this area.

Keywords: *Adhatoda Vasica, Antibacterial Activity, Cytotoxicity, Orthodontics.*

Introduction

Fixed appliances used in orthodontics alter the oral microbiota and there is an increase in aggressive gram-positive and gram-negative bacterial species, that are strongly connected with enamel and dentin caries and periodontal disease. Oral microbiota changes observed in orthodontic patients appear to be in line with those observed in patients with poor oral hygiene who have gingivitis and/or periodontal disease [1, 2]. Further, during fixed appliance treatment, patients are exposed to the heavy metals released from corroded appliances, which may increase Reactive Oxygen Species (ROS) levels via metal-catalyzed free radical reactions [3]. Furthermore, during orthodontic treatment, various inflammatory mediators (i.e., cytokines) causing aseptic inflammation in the periodontal ligament are released, inducing a cascade of reactions in the periodontal tissue that leads to tissue remodelling and tooth movement [4]. Given that periodontal inflammation is one of the primary sources of ROS in the mouth, it is possible that aseptic inflammation is also associated with oxidative stress-induced damage.

While numerous pharmacological and non-pharmacological methods are used in pain control during orthodontic treatment including, low-level laser therapy, Transcutaneous Electrical Nerve Stimulation and vibratory stimulation, Nonsteroidal anti-inflammatory drugs (NSAIDs) are the preferred method of pain control. However, NSAIDs can affect tooth movement and lengthen treatment time [5].

Recent advent of phytochemicals as biological mediators has opened new vistas in aforementioned areas of orthodontics. Of various herbs, Adhatoda vasica has caught the attention of investigators due to multiple properties relating to orthodontics. Adhatoda vasica (L.) Nees (AV), also known as Malabar

Received:20.12.2023 ***Accepted:13.03.2024*** ***Published on:29.03.2023***
****Corresponding Author: murukesan.subramanian.1970@gmail.com***

nut tree, is a shrub native to the Indian peninsula. The plant is used in India's indigenous medical system and is a well-known expectorant in both the Ayurvedic and Unani systems of medicine [6, 7]. It has large number of uses including antibacterial, antipyretic and so on [8-12]. Vasicine, deoxyvasicine, vasicinone, vasicol, vasicinol and adhatodinine are among the alkaloids found in the plant [13]. While antimicrobial, anti-inflammatory, anti-oxidant and osteo-modulatory effects are known in adhatoda vasica, their effective use in orthodontics is hitherto unexplored till date, leaving a major lacuna in both experimental and clinical aspects. This study evaluated the use of Adhatoda vasica in various aspects of orthodontics, like prevention of gingivitis & periodontitis and promote swifter tooth movement.

Authors hypothesize the following:

1. "Adhatoda Vasica has potent action against cariogenic bacteria."
2. "It can be tolerated well by bone that undergoes remodelling"

Materials and Methods

Extraction of Plant material

The plant leaf powder was purchased from 3V products, Avadi, Tamil Nadu, India and used as received. 95 g of powder sample was macerated with 400 mL of solvent and kept 72 hours on a rotary shaker at 150 rpm at 25 °C temperature. The entire mixture was filtered and concentrated to dryness by evaporating on a vacuum evaporator as the slightly modified procedure of Pandey *et al.*, 2020 [14]. The extracted crude sample was kept in airtight glass vials and stored at 4 °C until use.

Qualitative Phytochemical Analysis

Phytochemical analysis was performed using standard methods as described earlier [15]. Tests were performed for alkaloids, saponins, tannins, cardiac glycosides, flavonoids, phenols, steroids, terpenoids, Quinones and Proteins.

Gas Chromatography-Mass Spectroscopy (GC-MS) Procedure

The plant extract was used for the GC-MS analysis. 2µl of the plant extract was dissolved in HPLC grade methanol and subjected to GC-MS. JEOL GCMATE II GC-MS (Agilent Technologies 6890-N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min, at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio is 5:4. The sample (1 µl) is evaporated in a split less injector at 300°C. Run time is 22 min. The compounds are identified by gas chromatography coupled with mass spectrometry. The molecular weight and structure of the compounds of test materials were ascertained by the interpretation of mass spectrum of GC-MS using the database of National Institute Standard and Technology (NIST). The mass spectrum of the unknown component is compared with the spectrum of the known components stored in the NIST library. By making use of this the name, molecular weight and structure of the components of the test materials were ascertained.

Antibacterial Activity

The antibacterial activity of the test samples was carried out by the disc diffusion method [16]. The targeted microorganism was cultured in Mueller-Hinton broth and incubated for 24 hrs. The Petri dishes containing Mueller Hinton agar (MHA) medium were cultured with diluted bacterial strain. The prepared discs were placed on the culture medium. Test samples (500 µg, 1000 µg and 2000µg) were injected to the sterile disc. Standard drug Streptomycin (20µg) was used as a positive reference standard to determine the sensitivity of microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimetres as its antibacterial

activity. Organisms used for the study were *Streptococcus mutans, Streptococcus sobrinus*, Lactobacillus acidophyllus and Staphylococcus aureus.

In vitro Toxicity Testing on Bone Cell Lines

SaOS-2 cell line was procured from NCCS, stock cell was cultured in DMEM medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (1 x 10^4 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µL of different concentrations of test samples were added onto the partial monolayer in microtiter plates. The plate was then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 20 µL of MTT (2 mg/1 mL of MTT in PBS) was added to each well. The plate was incubated for 4 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage of viability was calculated using the following formula, % viability = 100 x Sample absorbance/Control absorbance

Results

Table 1. Extract Quantity

Solvent system	Powder weight (g)	Extract weight (g)
Petroleum Ether	95	2.718
Ethanol	95	6.445
Hydroalcoholic	95	9.891
Aqueous	95	7.946

Table 2 Phytochemical Analysis of Extract

Sample	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
PEE	+	+	-	-	+	+	+	+	+	-
ETE	+	+	-	-	+	+	+	+	+	-
HAE	-	+	-	-	+	+	+	+	+	+
AQE	-	+	-	-	+	+	+	+	+	+

+ Present; - Absent; PEE – Petroleum Ether, ETE – Ethanol, HAE – Hydroalcoholic; AQE - Aqueous

Table 3. Folding Endurance of fibers used in the study

Samples	**Zone of Inhibition (mm)**											
	Streptococcus mutans			***Streptococcus sobrinus***			***Lactobacillus acidophyllus***			***Staphylococcus aureus***		
Concentration	500	1000	2000	500	1000	2000	500	1000	2000	500	1000	2000
ETE	-	8	10	-	-	10	-	-	10	-	-	8
AQE	10	12	-	-	-	10	-	-	8	-	-	-
HAE	-	-	10	-	-	12	-	8	8	-	8	12
PEE	-	8	10	-	-	10	-	8	10	-	10	12
Streptomycin (20µg)	21			28			28			26		

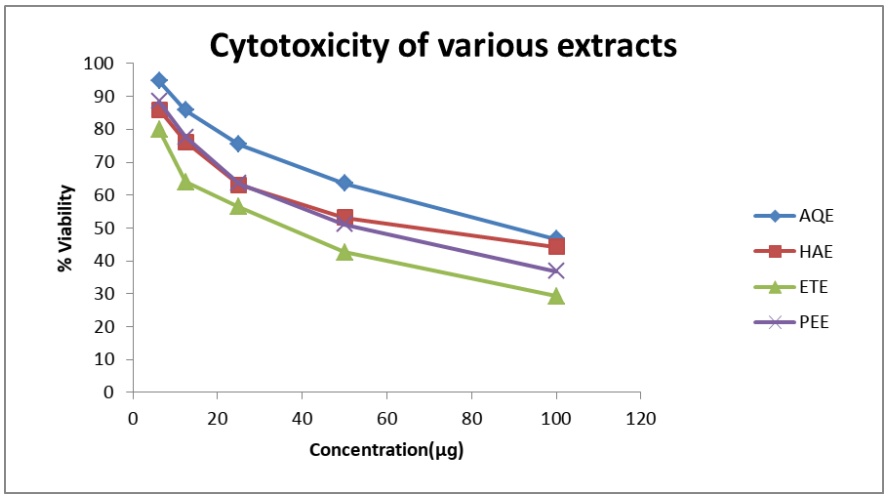

Figure 1. Cytotoxicity of various extracts

Discussion

The potential benefits of using herbal compounds in orthodontic treatments have gained attention in recent years, and while research is ongoing, some herbal compounds have demonstrated certain properties that may contribute to improved outcomes and patient comfort. Antimicrobial effects are essentially needed to control and reverse the dysbiosis caused during orthodontic therapy [17]. Some currently researched compounds are Neem, tea tree oil, and eucalyptus oil [18-20]. It helps in reducing plaque buildup leading to maintenance of good oral hygiene during orthodontic treatment.

The extraction procedure was successful in all four solvents, however, the hydroalcoholic extract had the maximum number of solids (9.8gm yield per 95gm of powder) as shown in Table 1. About phytochemical analysis, all solvents extracted Phenols, Cardiac glycosides, Steroids, Terpenoids and flavonoids. Saponins and tannins were not detected in any extract. Alkaloids were found in PEE and ETE extracts. On the contrary, proteins were detected only in aqueous and hydro-alcoholic extracts (Table 2).

In this study, four cariogenic species were selected to be studied against AV. As a result of the current study, against all pathogens, ethanolic, hydroalcoholic and pet ether extracts showed good antimicrobial activity at 2000 µg/ml (Table 3). However, the aqueous extract did not show sufficient activity against *S. mutans and A. viscosus.* Previously, citronellol, cinnamic acid and trans-cinnamaldehyde have been shown to act against *S. mutans* [21].

Adhatoda vasica has not yet been widely tested against *S. mutans*, this study has shown promising activity of AV againt *S. mutans*.

Cytotoxicity of AV has been demonstrated against various cancer cell lines [22] and its cytoprotective activity against various toxins on normal cells [23]. In the current study, for orthodontic applications, In MTT assay, aqueous extract was the most compatible, followed by Pet ether and hydroalcoholic, and the most toxic was ethanolic extract (Fig. 1). There was a dose-dependent increase in toxicity in $SaOS_2$ cells showing that at low doses they can be used to modulate bone growth.

From the above observations, it is clear that though aqueous extract is shown as cytocompatible, it showed lesser antimicrobial and other activities. From the results of the study, it can be suggested that PEE and HAE are suitable for clinical studies in orthodontics.

Conclusion

From the results of the study, it is shown that extracts of AV are putative agents for preventing caries, gingivitis/ periodontitis during orthodontic treatment. Further, correlating with computational studies and in vitro studies, at proper doses, AV extracts can influence the bone for accelerated tooth movement.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

The authors thank Dr. S. Rangachari, retired professor of Orthodontics, Madras Dental College for his kind encouragement and guidance.

References

[1] Freitas, Marquezan, M., Nojima, M. daC., Alviano, D. S., Maia, L. C., Maia, L. C., et al. 2014. The influence of orthodontic fixed appliances on the oral microbiota: A systematic review. *Dental Press Journal of Orthodontics*, *19*(2), 46–55. https://doi.org/10.1590/2176-9451.19.2.046-055.oar.

[2] Contaldo, Lucchese, M., A., Lajolo, C., Rupe, C., Di Stasio, D., Romano, A., Petruzzi, M., & Serpico, R. 2021. The oral microbiota changes in orthodontic patients and effects on oral health: An overview. *Journal of Clinical Medicine*, *10*(4), 780. https://doi.org/10.3390/jcm10040780.

[3] Primožič, Poljšak, J., B., Jamnik, P., Kovač, V., Čanadi Jurešić, G., & Spalj, S. 2021. Risk assessment of oxidative stress induced by metal ions released from fixed orthodontic appliances during treatment and indications for supportive antioxidant therapy: A narrative review. *Antioxidants*, *10*(9), 1359. https://doi.org/10.3390/antiox10091359.

[4] Kovac, Poljsak, B., Perinetti, G., Primozic, J., Primozic, J et al. 2019. Systemic level of oxidative stress during orthodontic treatment with fixed appliances. *BioMed Research International*, *2019*, 5063565. https://doi.org/10.1155/2019/5063565.

[5] Hussain, A. S., Al Toubity, M. J., & Elias, W. Y. 2017. Methodologies in Orthodontic Pain Management: A Review. *Open Dentistry Journal*, *11*, 492–497. https://doi.org/10.2174/1874210601711010492.

[6] Chopra, R. N., & Nayar, S. L. (1956). *Glossary of Indian medicinal plants.* Council of Scientific and Industrial Research.

[7] Kapoor LD. CRC Handbook of ayurvedic medicinal plants. 2018. *CRC Press*.

[8] Wakhloo, R. L., Wakhloo, D., Gupta, O. P., & Atal, C. K. (1979). Vasicine hydrochloride, a new drug for interruption of pregnancy. *Journal of Obstetrics and Gynaecology of India*, *29*, 939-940.

[9] Classical uses of medicinal plants. Chaukhambha Visvabharati. *Journal of Obstetrics and Gynaecology of India.* 1979, *29*, 939–940.

[10] Venkatakrishna-Batt, K., Patel, V. K., Venkatakrishna-bhatt, H et al. 1983. Effect of Adhatoda vasica Massage in Pyorrhoea. *International Journal of Crude Drug Research*, *21*(4), 173–176. https://doi.org/10.3109/13880208309070638.

[11] Mathew A. S., Patel, K. N., & Shah, B. K. 1998. Investigation on antifeedant and anthelmentic potential of *Adhatoda vasica* nees. *Indian Journal of Natural Products and Resources, 14*, 11–16.

[12] Dhuley, J. N. 1999. Antitussive effect of Adhatoda vasica extract on mechanical or chemical stimulation-induced coughing in animals. *Journal of Ethnopharmacology*, *67*(3), 361–365. https://doi.org/10.1016/s0378-8741(99)00074-4.

[13] Claeson U. P., Malmfors, T., Wikman, G., Bruhn, J. G., Bruhn, J. G., et al. 2000. Adhatoda vasica: A critical review of ethnopharmacological and toxicological data. *Journal of Ethnopharmacology*, *72*(1–2), 1–20. https://doi.org/10.1016/s0378-8741(00)00225-7.

[14] Pandey, Adhikari, B. P., K., Pradhan, S. P., Shin, H. J., Lee, E. K., & Jung, H. J. 2020. In-vitro antioxidant, anticancer, and anti-inflammatory activities of selected medicinal plants from western Nepal. *Future Journal of Pharmaceutical Sciences, 6*, 1–12.

[15] Harborne, J. B., Harborne, J. B. 1973. *Methods of plant analysis. Phytochemical methods: A guide to modern techniques of plant analysis*. (1973):1–32.

[16] Elumalai, E. K., Ramachandran, M., Thirumalai, T., & Vinothkumar, P. 2011. Antibacterial activity of various leaf extracts of *MerremiaemarginataAsian Pac J of Trop Biomed*, *1*(5), 406–408.

[17] Amato, M., Di Spirito, F., D'Ambrosio, F., Boccia, G., Moccia, G. and De Caro, F. 2022. Probiotics in periodontal and peri-implant health management: Biofilm control, dysbiosis reversal, and host modulation. *Microorganisms*, *10*(11), 2289. https://doi.org/10.3390/microorganisms10112289.

[18] CAUSING. 2012. Antibacterial evaluation of azadirachta indica ethanolic leaf extract against selected acidogenic oral bacteria causing dental plaque in fixed orthodontic appliance patients–an invitro study. *International Journal of Botany and Research (IJBR)*. S.A.O.B., *1*, 30–40.

[19] Santamaria Jr, M., Petermann, K. D., Vedovello, S. A. S., Degan, V., Lucato, A. and Franzini, C. M. 2014. Antimicrobial effect of Melaleuca alternifolia dental gel in orthodontic patients. *American Journal of Orthodontics and Dentofacial Orthopedics*, *145*(2), 198–202. https://doi.org/10.1016/j.ajodo.2013.10.015.

[20] Marya, A., Karobari, M. I., Shah, P. K. and Rokaya, D. 2023. Use of natural oral care products in orthodontics. *Pharmacological Studies in Natural Oral Care*, 125–147.

[21] Ribeiro, M., Malheiro, J., Grenho, L., Fernandes, M. H., Simões, M. 2018. Cytotoxicity and antimicrobial action of selected phytochemicals against planktonic and sessile *Streptococcus mutans*. *PeerJ*, *6*, e4872. https://doi.org/10.7717/peerj.4872.

[22] Nirmala, S., Sabapathi, P. N., Sudhakar, M., Bathula, N. and Sravanthi, Y. 2019. Investigation of in vitro Anti-cancer property of Adhatoda vasica in Hela, HepG2, MCF-7, MDAMB-231 Cell Lines. *Research Journal of Pharmacognosy and Phytochemistry*, *11*(4), 212–216. https://doi.org/10.5958/0975-4385.2019.00036.0.

[23] Pant, M., & Basu, S. 2013. Cytoprotective Activity of Adhatoda Vasica Extract and Vasicine Against Tobacco Smoke Induced Cytoxicity. *Journal of Pharmaceutical Technology, Research and Management*, *1*(1), 109–117. https://doi.org/10.15415/jptrm.2013.11007