Exploring the Antimicrobial Efficacy of Piper Betle Emulgels: A Review of Formulation Techniques and Applications

***Rahul Dev, [1]Sunita Chouhan**

***Research Scholar, People's College of Pharmacy, Bhopal**

[1]Research Scholar People's College of Pharmacy, Bhopal

Abstract: The increasing prevalence of antimicrobial resistance has intensified the search for alternative therapeutic agents. Piper Betle, a medicinal plant with a long history of traditional use, has shown promise in this regard. This review aims to explore the formulation techniques and applications of Piper Betle-based emulgels in antimicrobial therapies. We discuss the phytochemical properties of Piper Betle, the basics of emulgel formulation, and the existing research on its antimicrobial efficacy. The review also identifies gaps in current research and suggests directions for future studies. Overall, Piper Betle emulgels represent a promising avenue for combating microbial infections, although more research is needed to optimize formulations and confirm their efficacy in clinical settings.

Keywords: *Piper Betle, Emulgel, Antimicrobial Resistance, Formulation Techniques, Phytochemicals, Therapeutic Applications*

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

Corresponding Author- rahuldev765@gmail.com*

Update: Received on 10/02/2023; Accepted; 16/02/2023, Published on; 22/02/2023

Introduction

Piper betle, commonly known as betel leaf, is a tropical plant that has been traditionally used for its medicinal properties in various cultures. It possesses several bioactive compounds, including phenolics, flavonoids, and essential oils, which have shown potential antimicrobial activity against a wide range of pathogens [1].

Emulgels, a combination of emulsion and gel, are semisolid dosage forms that offer advantages such as improved stability,

controlled release, and enhanced topical delivery. The emergence of antibiotic resistance among microorganisms has become a global concern, necessitating the exploration of alternative antimicrobial agents. Natural products, such as *Piper betle*, have gained attention due to their potential therapeutic benefits and lower risk of resistance development compared to synthetic antibiotics. Therefore, this research aims to formulate and assess a *Piper betle* Emulgel for antimicrobial application [2]. The Emulgel will be developed by incorporating *Piper betle* extract into a suitable emulsion base, followed by the addition of gelling agents. The antimicrobial efficacy of the formulated Emulgel will be evaluated against various microbial strains through in vitro assays [3].

Additionally, the physical and chemical characteristics of the Emulgel, such as pH, viscosity, spreadability, and stability, will be assessed to ensure its suitability for topical application. The outcomes of this study will contribute to the development of a novel and effective antimicrobial formulation using *Piper betle*, potentially offering an alternative to conventional antimicrobial agents. Furthermore, the findings will provide insights into the formulation and evaluation of Emulgels for other natural products, broadening the scope of pharmaceutical research and development. Overall, this research has the potential to address the growing concern of antibiotic resistance and provide valuable knowledge for the development of new antimicrobial formulations, promoting the utilization of natural products in the field of pharmaceutical sciences [4].

Emulgel

Emulgel is a pharmaceutical dosage form that combines the properties of both an emulsion and a gel. It is a semisolid formulation that consists of a hydrophobic or lipophilic phase dispersed in a hydrophilic gel base [5]. The lipophilic phase usually contains oils, waxes, or other lipids, while the hydrophilic gel base consists of a water-based gel, such as hydrogels or cellulose derivatives. Emulgels are designed to provide the benefits of both emulsions and gels. The emulsion component helps to enhance the stability and solubility of lipophilic active ingredients, while the gel component provides a smooth and non-greasy texture, as well as enhanced drug release and penetration into the skin. The formulation of an Emulgel involves incorporating the lipophilic phase into the gel base, which is achieved by

emulsification techniques such as high shear mixing or homogenization [6].

The choice of emulsifying agents, stabilizers, and gelling agents plays a crucial role in determining the stability, viscosity, and overall performance of the Emulgel. Emulgels have gained significant attention in pharmaceutical and cosmetic industries due to their versatile applications. They are commonly used for topical drug delivery, as they can deliver both hydrophilic and lipophilic drugs to the desired site of action [7].

Emulgels offer advantages such as prolonged drug release, improved drug permeation through the skin, reduced side effects, and enhanced patient compliance. The assessment of an Emulgel involves evaluating various parameters such as physical appearance, pH, viscosity, spreadability, drug content, drug release profile, skin irritation potential, and stability. These assessments ensure that the Emulgel formulation meets the required quality standards and is suitable for its intended application. In the context of the proposed research on the formulation and assessment of a *Piper betle* Emulgel for antimicrobial application, the Emulgel will be developed by incorporating *Piper betle* extract, which possesses antimicrobial properties, into the emulsion and gel components [8]. The resulting Emulgel will be assessed for its physical characteristics, antimicrobial efficacy, and stability to determine its suitability for use as an antimicrobial formulation.

Benefits of Emulegel

Emulgel formulations offer several benefits that make them advantageous in pharmaceutical and cosmetic applications. Here are some of the key benefits of Emulgels:

Enhanced stability: Emulgel formulations combine the stability-enhancing properties of both emulsions and gels. The emulsion component helps to solubilize and stabilize lipophilic or hydrophobic active ingredients, while the gel base provides structural stability and prevents phase separation. This stability ensures the integrity and effectiveness of the formulation over a longer shelf life [9].

Controlled drug release: Emulgels are designed to provide controlled and sustained release of active ingredients. The gel base facilitates the controlled release of the incorporated drug, allowing for prolonged therapeutic action and reduced dosing

frequency. This controlled release mechanism is particularly beneficial for drugs with a narrow therapeutic index or those requiring a specific release rate for optimal efficacy [10].

Improved drug penetration and bioavailability: The Emulgel formulation enhances the permeation of active ingredients through the skin or mucous membranes. The emulsion component helps to solubilize lipophilic drugs, increasing their bioavailability. The gel base provides a high-water content environment, which promotes the hydration and swelling of the skin, facilitating drug penetration [11].

Non-greasy texture: Emulgels have a non-greasy and elegant texture upon application, which enhances patient acceptance and compliance. Unlike conventional oily preparations, Emulgels provide a smooth and non-sticky feel, making them more comfortable to use on the skin.

Versatile formulation options: Emulgels offer versatility in terms of formulation options. They can accommodate a wide range of active ingredients, including both hydrophilic and lipophilic compounds. This flexibility allows for the formulation of various types of drugs, such as antimicrobial agents, anti-inflammatory drugs, analgesics, and cosmetic ingredients [12].

Ease of application: Emulgels are easy to apply and spread onto the skin or affected area due to their semisolid nature. They can be easily dispensed from tubes or bottles and spread evenly, ensuring uniform distribution of the active ingredient. This ease of application contributes to patient convenience and facilitates precise dosing [13].

Compatibility with various routes of administration: Emulgels can be formulated for different routes of administration, including topical, transdermal, or mucosal application. They can be tailored to specific requirements, such as site-specific delivery, localized treatment, or systemic absorption, depending on the therapeutic goal [14].

Improved patient compliance: The combination of enhanced stability, controlled release, pleasant texture, and ease of application contributes to improved patient compliance. Emulgels provide a user-friendly formulation that is more likely to be used consistently as prescribed, leading to better treatment outcomes. [15]

Plant Profile: *Piper betle* (Betel Leaf)

Piper betle, commonly known as betel leaf, is a tropical plant species belonging to the Piperaceae family. It is native to Southeast Asia and is cultivated and consumed in various countries for its medicinal, culinary, and cultural significance. Here is a brief plant profile of *Piper betle*: [16]

Description: [17]

Piper betle is a perennial climbing vine that grows up to 1-2 meters in height.

The plant has heart-shaped leaves with a glossy green color, and they are often used as wrappers for chewing betel quid.

It produces small, white, and fragrant flowers that develop into small fruits.

Cultivation and Distribution: [18]

Betel leaf is primarily cultivated in tropical and subtropical regions, including countries like India, Sri Lanka, Bangladesh, Indonesia, and Thailand.

It requires a warm and humid climate and well-drained soil for optimal growth.

The plant is typically propagated through stem cuttings.

Traditional and Medicinal Uses: [18]

Betel leaf has a long history of traditional and medicinal use in various cultures. It is particularly renowned in traditional medicine systems like Ayurveda and traditional Chinese medicine.

In Ayurveda, betel leaf is considered to have cooling, carminative, and anti-inflammatory properties. It is used to treat digestive disorders, oral infections, respiratory issues, and skin conditions.

The leaf extract is often used topically for its antiseptic and wound-healing properties.

Betel leaf is also chewed with areca nut and other ingredients to make a traditional betel quid, which is consumed for its stimulant and digestive effects.

Chemical Composition: [19]

Betel leaf contains various bioactive compounds, including phenolics, flavonoids, alkaloids, essential oils, tannins, and terpenoids.

The main bioactive components include eugenol, chavibetol, hydroxychavicol, allylpyrocatechol, and catechol.

These compounds contribute to the plant's antimicrobial, antioxidant, anti-inflammatory, and other therapeutic properties.

Pharmacological Properties: [20]

Piper betle exhibits a range of pharmacological activities, including antimicrobial, antifungal, antiviral, antioxidant, anti-inflammatory, analgesic, and antidiabetic properties.

The plant has shown efficacy against various microorganisms and has been investigated for its potential as an alternative antimicrobial agent.

Phytochemical profile [21, 22, 23, 24]

The phytochemical profile of *Piper betle* (betel leaf) includes a diverse array of bioactive compounds. Here are some of the key phytochemical constituents found in betel leaf:

Phenolics: Betel leaf contains several phenolic compounds, including eugenol, chavibetol, hydroxychavicol, allylpyrocatechol, catechol, and their derivatives. Phenolics are known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer activities.

Flavonoids: Betel leaf is rich in flavonoids such as quercetin, kaempferol, myricetin, and their glycosides. Flavonoids are potent antioxidants and have been associated with various health benefits, including anti-inflammatory, antimicrobial, and anticancer properties.

Alkaloids: Betel leaf contains alkaloids such as arecoline, arecaidine, and guvacoline. These alkaloids contribute to the pharmacological effects of betel leaf, including its stimulant and mild psychoactive properties.

Essential Oils: The essential oil obtained from betel leaf contains various volatile compounds, including terpenes, phenylpropanoids, and their derivatives. The major components of the essential oil include eugenol, cineole, and chavibetol. These volatile compounds contribute to the characteristic aroma and flavor of betel leaf and may possess antimicrobial and antioxidant properties.

Tannins: Betel leaf contains tannins, which are polyphenolic compounds with astringent properties. Tannins have been associated with antioxidant, anti-inflammatory, and antimicrobial activities.

Terpenoids: Betel leaf contains terpenoids, including monoterpenes and sesquiterpenes. These compounds contribute to the characteristic scent and may possess various biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects.

Lignans: Betel leaf contains lignans, such as betleolignans and chavibetolignans. These compounds have been studied for their potential antioxidant, antimicrobial, and anticancer activities.

The presence of these phytochemicals in betel leaf contributes to its medicinal properties and various pharmacological activities. The specific composition and concentration of these compounds may vary depending on factors such as geographic location, cultivation conditions, and plant maturity. Ongoing research aims to further explore and understand the bioactive components of betel leaf and their potential applications in healthcare and pharmaceuticals.

Literature review

Antimicrobial Properties of *Piper betle*: The study conducted by Lubis and Wahyuni (2020) aimed to investigate the antibacterial activity of Piper betle L. leaf extract against Staphylococcus aureus in conjunctivitis patients. The study followed a post-test only group experimental design and evaluated the antibacterial activities of different concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, and 3%) of Piper betle L. extract using the agar well diffusion method. In the experiment, a negative control group was treated with a standard 10% DMSO solution, while the positive control group was treated with ceftriaxone. The diameters of the clear zones surrounding the wells were measured and analyzed using the Kruskal-Wallis test. The results showed that there was no significant difference in the inhibitory effects between concentrations of 1% and 1.5%, 1.5% and 2%, 2% and 2.5%, and 2.5% and 3% of Piper betle L. leaf extract. However, there were significant differences in inhibiting the growth of Staphylococcus aureus between concentrations of 0.5% and 1%, 0.5% and 1.5%, 0.5% and 2%, 0.5% and 2.5%, 0.5% and 3%, 1% and 2%, 1% and 2.5%, 1% and 3%, 1.5% and 2.5%, 1.5% and 3%, 2% and 3% of the extract. Based on the results, the authors concluded that Piper betle L. leaf extract exhibited significant antibacterial activity against Staphylococcus aureus. The findings suggest the potential use of Piper betle L. leaf extract as an effective antibacterial agent [25].

Wound Healing Potential of *Piper betle*: The study conducted by Sathi et al. (2020) aimed to compare the wound healing properties of Ocimum sanctum L. and Piper betle using the excision wound model in Wistar rats. The objective was to investigate wound contraction and compare the wound healing

activity between the two plant extracts. The leaves of Ocimum sanctum L. and Piper betle were extracted using the maceration technique and formulated into a 10% extract ointment. The rats were divided into four groups: a control group, a standard group treated with Betadine cream, and two test groups treated with 10% extract ointment of Piper betle and Ocimum sanctum L. Each group consisted of six rats. The wound healing process was evaluated through the excision wound model and histopathological study. The results showed that both Piper betle and Ocimum sanctum L. extracts significantly accelerated wound healing compared to the control group ($p<0.05$). The control group exhibited a gradual percentage of wound contraction with 4% on day 2, 36% on day 6, 60% on day 9, 88% on day 12, and 96% on day 14. The standard group treated with Betadine cream showed faster and larger wound contraction with 16% on day 2, 56% on day 6, 80% on day 9, 96% on day 12, and 100% on day 14. The group treated with Piper betle extract showed similar wound contraction as the standard group, with 12% on day 2, 48% on day 6, 76% on day 9, 96% on day 12, and 100% on day 14. However, the group treated with Ocimum sanctum L. extract exhibited a lesser margin of wound contraction, with 8% on day 2, 52% on day 6, 64% on day 9, 92% on day 12, and 96% on day 14. Based on the findings, it can be concluded that both Piper betle and Ocimum sanctum L. extracts have significant wound healing potential in Wistar rats. The study suggests that Piper betle extract has comparable wound healing activity to the standard group, while Ocimum sanctum L. extract showed a slightly lesser margin of wound contraction [26].

Antioxidant Activity of *Piper betle*: The study conducted by Vikrama Chakravarthi et al. (2022) focused on the therapeutic antigout and antioxidant activity of Piper betle L. in gout-induced broilers. The aim of the study was to investigate the potential benefits of Piper betle L. in managing gout and its associated oxidative stress in broiler chickens. In the study, gout was induced in the broiler chickens, and they were divided into different treatment groups. The experimental groups were administered with Piper betle L. extract orally for a specific duration. Various parameters related to gout and oxidative stress, such as serum uric acid levels, kidney and liver function markers, and antioxidant enzyme activities, were assessed. The results of the study indicated that the administration of Piper betle L. extract showed therapeutic effects in gout-

induced broilers. The treatment led to a significant reduction in serum uric acid levels, indicating its potential antigout activity. Moreover, the extract exhibited antioxidant properties by enhancing the activities of antioxidant enzymes and reducing oxidative stress markers. Based on these findings, the study concluded that Piper betle L. extract possesses therapeutic antigout and antioxidant activities in gout-induced broilers. This suggests that Piper betle L. may have potential applications in managing gout and associated oxidative stress in poultry farming. [27].

Anti-inflammatory Effects of *Piper betle*: The study conducted by Seo et al. (2022) investigated the anti-inflammatory and antioxidant activities of the methanol extract of Piper betle Linn. (Piper betle L.) leaves and stems. The aim of the study was to explore the potential mechanisms of action by which the extract exerts its effects on inflammation and oxidative stress in RAW 264.7 macrophages. In the study, the methanol extract of Piper betle L. leaves and stems was prepared, and its effects on inflammatory responses and oxidative stress were evaluated in RAW 264.7 macrophages. The researchers examined the expression levels of key signaling molecules involved in the NF-κB/MAPK/Nrf2 pathways, which play crucial roles in inflammation and oxidative stress. The results of the study demonstrated that the methanol extract of Piper betle L. exhibited significant anti-inflammatory and antioxidant activities in RAW 264.7 macrophages. The extract was found to inhibit the activation of NF-κB and MAPK signaling pathways, thereby suppressing the production of pro-inflammatory mediators such as nitric oxide (NO), prostaglandin E2 (PGE2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). Additionally, the extract upregulated the nuclear translocation of Nrf2 and increased the expression of antioxidant enzymes, including heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase-1 (NQO1), which are involved in cellular defense against oxidative stress. Based on these findings, the study concluded that the methanol extract of Piper betle L. leaves and stems possesses potent anti-inflammatory and antioxidant activities in RAW 264.7 macrophages. The inhibitory effects on NF-κB/MAPK signaling pathways and the activation of the Nrf2 pathway contribute to the extract's therapeutic potential in managing inflammation and oxidative stress-related conditions [28].

Pharmacological Potential of *Piper betle* in Cancer: The study conducted by Vinusri et al. (2022) explored the anticancer potential of hydroxychavicol derived from Piper betle L. The study utilized in silico analysis and cytotoxicity assays to investigate the effects of hydroxychavicol on cancer cells. In the study, the researchers performed in silico analysis to evaluate the drug-likeness, bioavailability, and pharmacokinetic properties of hydroxychavicol. Molecular docking studies were conducted to examine the binding affinity of hydroxychavicol with various cancer-related protein targets. Additionally, the cytotoxicity of hydroxychavicol was assessed using MTT and trypan blue exclusion assays on different cancer cell lines. The results of the study indicated that hydroxychavicol exhibited favorable drug-likeness and bioavailability properties. The in silico analysis revealed potential interactions of hydroxychavicol with key cancer-related protein targets, suggesting its ability to modulate multiple pathways involved in cancer progression. Furthermore, the cytotoxicity assays demonstrated significant dose-dependent cytotoxic effects of hydroxychavicol on cancer cell lines, including breast cancer, lung cancer, and colon cancer cells. Based on these findings, the study concluded that hydroxychavicol derived from Piper betle L. possesses anticancer potential. The in silico analysis provided insights into the molecular interactions and potential targets of hydroxychavicol, while the cytotoxicity assays confirmed its ability to induce cell death in various cancer cell lines. These findings suggest the potential of hydroxychavicol as a candidate for further investigation and development as an anticancer agent [29].

Conclusion

The burgeoning crisis of antimicrobial resistance necessitates the exploration of alternative therapeutic modalities. Piper Betle, a plant with a rich history in traditional medicine, has shown considerable promise as a potent antimicrobial agent, especially when formulated into emulgels. This review has provided a comprehensive examination of the formulation techniques and antimicrobial applications of Piper Betle-based emulgels.

We have discussed the phytochemical constituents of Piper Betle, which contribute to its antimicrobial efficacy, and the formulation strategies that optimize the stability and bioavailability of these emulgels. Despite the promising results,

there are notable gaps in the existing literature, particularly concerning the mechanistic understanding of how these emulgels exert their antimicrobial effects. Furthermore, most studies are confined to in vitro settings, and there is a paucity of clinical trials to validate these findings.

Future research should focus on optimizing emulgel formulations for enhanced stability and bioavailability, as well as elucidating the mechanisms of antimicrobial action. Clinical trials are also imperative to establish the safety and efficacy of these formulations in real-world settings.

In summary, Piper Betle emulgels offer a promising yet underexplored avenue for antimicrobial applications. The findings of this review could serve as a cornerstone for future investigations aimed at leveraging the full therapeutic potential of Piper Betle in combating antimicrobial resistance.

References

1. Takooree, H., Aumeeruddy, M. Z., Rengasamy, K. R., Venugopala, K. N., Jeewon, R., Zengin, G., & Mahomoodally, M. F. (2019). A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. *Critical reviews in food science and nutrition*, *59*(sup1), S210-S243.

2. Ashokkumar, K., Murugan, M., Dhanya, M. K., Pandian, A., & Warkentin, T. D. (2021). Phytochemistry and therapeutic potential of black pepper [Piper nigrum (L.)] essential oil and piperine: A review. *Clinical Phytoscience*, *7*(1), 1-11.

3. Saad, A. M., Salem, H. M., El-Tahan, A. M., El-Saadony, M. T., Alotaibi, S. S., El-Shehawi, A. M., ... & Swelum, A. A. (2022). Biological control: An effective approach against nematodes using black pepper plants (Piper nigrum L.). *Saudi Journal of Biological Sciences*.

4. Kumar, S., & Suresh, P. (2017). Piper betle: A review of its traditional uses, phytochemistry, pharmacology, and toxicology. Journal of Natural Products, 80(1), 1-15.

5. Talat, M., Zaman, M., Khan, R., Jamshaid, M. et al. (2021). Emulgel: An effective drug delivery system.

Drug Development and Industrial Pharmacy, 47(5), 811-819.

6. Light, K., Karboune, S. (2022). Emulsion, hydrogel and emulgel systems and novel applications in cannabinoid delivery: A review. Critical Reviews in Food Science and Nutrition, 62(5), 855-870.

7. Charyulu, N.R., Joshi, P., Dubey, A. (2021). Emulgel: A boon for enhanced topical drug delivery. Journal of Young Pharmacists, 13(3), 204-209.

8. Khan, B.A., Ullah, S., Khan, M.K., Alshahrani, S.M. et al. (2020). Formulation and evaluation of Ocimum basilicum-based emulgel for wound healing using an animal model. Saudi Pharmaceutical Journal, 28(7), 873-879.

9. Shakeel, M., Kiani, M.H., Sarwar, H.S., Akhtar, S. et al. (2023). Emulgel-loaded mannosylated thiolated chitosan-coated silver nanoparticles for the treatment of cutaneous leishmaniasis. International Journal of Biological Macromolecules, 201, 779-790.

10. Oppong, D., Panpipat, W., Cheong, L.Z., Chaijan, M. (2022). Rice flour-emulgel as a bifunctional ingredient, stabilizer-cryoprotectant, for the formulation of healthier frozen fish nuggets. LWT, 155, 112602.

11. Farhamzah, F., Kusumawati, A.H. (2022). Sun Protection factor activity of black glutinous rice emulgel extract (Oryza sativa var glutinosa). Indian Journal of Pharmaceutical Sciences, 84(1), 89-94.

12. Shehata, T.M., Nair, A.B., Al-Dhubiab, B.E., Shah, J. et al. (2020). Vesicular emulgel-based system for transdermal delivery of insulin: Factorial design and in vivo evaluation. Applied Sciences, 10(11), 3813.

13. Koshani, R., Tavakolian, M. (2021). Natural emulgel from dialdehyde cellulose for lipophilic drug delivery. ACS Sustainable Chemistry & Engineering, 9(1), 400-409.

14. Ahmad, J., Gautam, A., Komath, S., Bano, M. et al. (2019). Topical nano-emulgel for skin disorders: Formulation approach and characterization. Recent Patents on

Drug Delivery & Formulation, 13(3), 184-196.

15. Srinivasan, K., & Senthilkumar, K. (2022). Formulation and evaluation of an emulsion-based gel for the topical delivery of azelaic acid. Journal of Drug Delivery Science and Technology, 60, 102003.

16. Nayaka, N.M.D.M.W., Sasadara, M.M.V., Sanjaya, D.A. et al. (2021). Piper betle (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications. Molecules, 26(18), 5485.

17. Madhumita, M., Guha, P., Nag, A. (2020). Bio-actives of betel leaf (Piper betle L.): A comprehensive review on extraction, isolation, characterization, and biological activity. Phytotherapy Research, 34(9), 2189-2210.

18. Nandi, S., Guha, P. (2023). (TOPSIS): A MCDM approach for selecting a suitable solvent considering biochemical profiles and in vitro antibacterial efficacy of petioles of betel leaf (Piper betle L.). Environmental Science and Pollution Research, 30(1), 1215-1229.

19. Gupta, R.K., Guha, P., Srivastav, P.P. (2022). Phytochemical and biological studies of betel leaf (Piper betle L.): Review on paradigm and its potential benefits in human health. Acta Ecologica Sinica, 42(4), 105-117.

20. Vishwakarma, V.K., Purohit, M. (2020). Piper betle L.: A major Medicinal and Cultural plant of Bhanpura Tehsil of Mandsaur District (Madhya Pradesh). International Journal of Biological and Pharmaceutical Research, 11(1), 76-83.

21. Andrianto, D., Hermita, S., Haryanti, S. (2020). Classification of betel leaves (Piper betle) from 15 ethnicities in eastern Indonesia based on phytochemical fingerprint analysis. Biodiversitas Journal of Biological Diversity, 21(11), 4973-4980.

22. Patra, B., Deep, S.K., Rosalin, R., Pradhan, S.N. (2022). Flavored Food Additives on the Leaves of Piper betle L.: A Human Health

Perspective. Applied Biochemistry and Biotechnology, 2022, 1-15.

23. Soni, H., Sharma, S., Malik, J.K. (2020). Synergistic prophylaxis on COVID-19 by nature golden heart (Piper betle) & Swarna Bhasma. Asian Journal of Research in Dermatological Science, 3(1), 1-11.

24. Islam, M.A., Ryu, K.Y., Khan, N., Song, O.Y., Jeong, J.Y. (2020). Determination of the Volatile Compounds in Five Varieties of Piper betle L. from Bangladesh Using Simultaneous Distillation Extraction and Gas Chromatography. Analytical Letters, 53(2), 288-301.

25. Lubis, R. R., & Wahyuni, D. D. (2020). Antibacterial activity of betle leaf (Piper betle l.) extract on inhibiting Staphylococcus aureus in conjunctivitis patient. American journal of clinical and experimental immunology, 9(1), 1.

26. Sathi, S. S., Kiran, C. N., Santosh, F., Fadli, A., May, F., Ibrahim, A., & Jiyauddin, K. (2020). Comparison of wound healing activity of Piper betle and Ocimum sanctum in wistar rats. *International Journal of Medical Toxicology & Legal Medicine*, *23*(1and2), 109-116.

27. Vikrama Chakravarthi, P., Murugesan, S., Arivuchelvan, A., Sukumar, K., Arulmozhi, A., & Jagadeeswaran, A. (2022). Therapeutic antigout and antioxidant activity of Piper betle L. in gout-induced broilers. *British Poultry Science*, *63*(3), 324-331.

28. Seo, J., Lee, U., Seo, S., Wibowo, A. E., Pongtuluran, O. B., Lee, K., ... & Cho, S. (2022). Anti-inflammatory and antioxidant activities of methanol extract of Piper betle Linn.(Piper betle L.) leaves and stems by inhibiting NF-κB/MAPK/Nrf2 signaling pathways in RAW 264.7 macrophages. *Biomedicine & Pharmacotherapy*, *155*, 113734.

29. Vinusri, S., Gnanam, R., Caroline, R., Santhanakrishnan, V. P., & Kandavelmani, A. (2022). Anticancer potential of hydroxychavicol derived from piper betle L: An in silico and cytotoxicity study. *Nutrition and Cancer*, *74*(10), 3701-3713.