Evaluation of Antimicrobial Efficacy of Piper betle Linn Leaf Extract against Growth of Microorganisms

Dhani Ram Deka, Pranjoli Das, Probin Phanjom and Manash Pratim Sarma

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 02

Res. Jr. of Agril. Sci. (2022) 13: 354–359

CARAS

Res. Jr. of Agril. Sci. (Mar-Apr 2022)
13(2): 354–359

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

www.rjas.org

Full Length Research Article

Evaluation of Antimicrobial Efficacy of *Piper betle* Linn Leaf Extract against Growth of Microorganisms

Dhani Ram Deka[1], Pranjoli Das[2], Probin Phanjom[3] and Manash Pratim Sarma[4]

Received: 07 Dec 2021 | Revised accepted: 20 Feb 2022 | Published online: 08 Mar 2022
© CARAS (Centre for Advanced Research in Agricultural Sciences) 2022

A B S T R A C T

Piper betle L. leaf a heart shaped evergreen medicinal herb is traditionally used for the treatment of various diseases. The raw leaves as well as its extract shows significant antimicrobial activity, bactericidal activity, antifungal activity, immunosuppressive activity, anticancer activity, antiseptic, anti-inflammatory and anti-helminthic effects and are also used to prepare many Ayurvedic medicines. Ehanol extract of *Piper betle* L. leaves were prepared and its antimicrobial activity against gram positive and gram-negative bacteria were investigated. It was found that the leaf extract was effective against gram positive and negative microbes. The phytochemical analysis showed the presence of phytochemical compounds Flavinoids, Tannins, Steroids and Terpenoids. The presence of these phytochemical compounds suggests the possibility that phenolic compounds proved to be bactericidal. The experiment relating *Piper betle* L. leaves may be taken further in assessing the phytochemical component structure and properties and their role in bactericidal activity.

***Key words*:** *Piper betle* L. plants, Leaf extract, Antimicrobial activity

Traditional or herbal medicines are solely dependent by majority of the world's population for treatment of various diseases.in developed and developing countries as well as under developing countries. Medicinal plants are the "backbone" of traditional or herbal medicines and have been a resource worldwide as an alternative or a complementary medicine for thousands of years for healing in local communities. In most of the developing countries, the medicinal plants recognized for medical use have been adopted as traditional medical practice which became an integral part of their cultures. Indigenous knowledge systems are passed on from generation to generation in all cultures around the globe. In the world, nearly 2000 ethnic groups are available and almost every group has its own experience and traditional medical knowledge. The major systems of indigenous medicines practised all over the world are Ayurveda, Unani, Siddha and Folk (tribal) medicines. Ayurveda and Unani Medicine are widely practised in India among these systems. Recently, it was estimated by World Health Organization that about 80% of the population in this world rely on traditional or herbal medicines for their primary healthcare needs and solving the health issues [1-5].

Human race totally depends on various plants parts such as fruit, flower, bulb, gel, leaves, roots, barks peels, buds, etc. for their existence and survival not only for food but also for its medicinal properties like acting as blood purifier, inhibiting allergic reactions, healing burns and wounds, preventing various diseases, stimulating digestion and appetite, possessing anticancer activity, etc. From ancient times, forests are well known to be a large reservoir of medicinal plants as well as aromatic plant used for manufacturing drugs and perfumery products. In the quest of developing novel drugs, modern researchers as well as pharmaceutical companies are making use of all the information related to medicinal plants and herbal medicines which are considered to be a source of bioactive agents in the preparation of synthetic medicine [6-8].

With the evolution of wide range of antimicrobial resistant bacterial strains Gram-positive and Gram-negative bacteria it has become vital to develop novel antimicrobial compounds to fight these superbugs. The phytochemical components present in medicinal plants such as flavonoids, alkaloids, tannins, and terpenoids possess antimicrobial and antioxidant properties [9-11]. The plants extract of various medicinal plants exhibit antibacterial, antifungal and antiviral activities. The heart shaped *Piper betle* Linn (Piperaceae) leaf is well known for its heart-related curative properties, regulates irregular heart beat and blood pressure, digestive and pancreatic lipase stimulant activities, smooth and skeletal muscles relaxant actions. It is applicable in many ayurvedic preparations and treatment of various diseases like bad breath, boils and

* **Dhani Ram Deka**
✉ deka.dhan@gmail.com

[1,4] Assam down town University, Panikhaiti, Guwahati - 781 027, Assam, India

[2] Centre for Nanotechnology, IIT Guwahati - 781 039, Assam, India

[3] Assam Don Bosco University, Sonapur, Tepesia - 782 402, Assam, India

abscesses, conjunctivitis, constipation, headache, itches, mastitis, mastoiditis, leucorrhoea, otorrhoea, swelling of gum, rheumatism, bronchitis, cuts and injuries, respiratory catarrhs and antiseptic. The *Piper betle* L. (Piperaceae) leaves and its extract shows significant antimicrobial activity, bactericidal activity, antifungal activity, immunosuppressive activity, anticancer activity, antiseptic, anti-inflammatory and antihelminthic effects against broad spectrum of micro-organisms, urinary tract pathogenic bacteria, ringworms, many fungal infections and autoimmune disorders such as rheumatoid arthritis, systemic lupus erythomatous or emphysema. The bioactive molecule sterol present in *Piper betle* L. (Piperaceae) leaf extracts is observed to be responsible for anti-bacterial activity [12-14].

Piper betle L. leaf is a green-coloured smooth heart shaped (Fig 1) continuing climber belonging to the Family Piperaceae (Table 1). *Piper betle* L. (Piperaceae) leaves are grown in humid conditions of relative high humidity in well-drained fragile and clayey soils of pH 7-7.5 that are rich in rich in organic matter It is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. In India they are cultivated in the states of Assam, Andhra Pradesh, Bihar, Gujarat, Odisha, Karnataka, Madhya Pradesh, Rajasthan, West Bengal and Maharashtra [15-18].

Table 1 *Piper betle* L. leaves its botanical name, taxonomy and vernacular name

Botanical name		Taxonomy
Kingdom	:	Plantae
Division	:	Magnoliphyta
Class	:	Magnolipsida
Order	:	Piperales
Family	:	Piperaceae
Genus	:	Piper
Species	:	Betle
Binomial name	:	*Piper betle* L.

Vernacular name		
Sanskrit	:	Nagavallari, Nagini, Nagavallika, Tambool, Saptashira, Varnalata Mukhbhushan,
English	:	Betel, Betel pepper, Betel-vine
Hindi	:	Pan

Fig 1 *Piper betle* Linn. leaves

The various constituents of *Piper betle L.* leaf is shown in (Table 2). In the present study, ethanol extracts of *Piper betle* L. leaves were prepared and qualitative analyses of their phytochemical constituents and antimicrobial studies were done to analyze the various phytochemical components of the plant; examine the antimicrobial effect towards some bacterial pathogens *Escherichia coli*, *Bacillus subtillis* and *Klebsiella* sp. and also to assess Minimum Inhibitory Concentration (MIC) on these bacterial pathogens.

Table 2 Constituents of the *Piper betle* L. leaf

Constituents	Percentage	Minerals		Vitamins	
Water	85-90%	Constituents	Percentage	Constituents	Percentage
Proteins	3-3.5%	Calcium	0.2-0.5%	Vitamin-C	0.005-0.01%
Carbohydrates	0.5-6.1%	Iron	0.005-0.007	Nicotinic acid	0.63-0.89mg/100gms
Fat	0.4-1%	Iodine	3.4µg/100gms	Thiamine	10-70µg/100gms
Fibre	2.3%	Phosphorus	0.05-0.6%	Vitamin-A	1.9-.9mg/100gms
Phenol		Potassium	1.1-4.6%	Riboflavin	1.9-30µg/100gms
Terpene		Alkaloid		Essential oil	0.08-0.2%
Tannin	0.1-1.3%	Bitter compounds	0.7-2.6%		

MATERIALS AND METHODS

Piper betle L. leaves, test organisms *Escherichia coli*, *Bacillus subtillis* and *Klebsiella* sp.

Solvent

Ethanol, Peptone**,** Beef extract**,** Sodium Chloride**,** Distilled water**,** Agar Agar.

Preparation of *Piper betle* L. extracts

Fresh and mature contamination free *Piper betle* L. leaves were picked up from Bhomolahati village of Bezera nearby Baihata Chariali of Kamrup (Rural) district, Assam, India. The leaves collected were first washed and cleaned with distilled water; air dried at room temperature for one week and then kept in hot air oven for 2 days at 50°C. These dried leaves were then grinded into fine powder using Grinder mixer; sieved and kept in Borosil vials and finally stored in the desiccator for further use. The *Piper betle* L. leaf extract was prepared by mixing 50g of powdered *Piper betle* L. leaf with 400ml of ethanol (60%) for 1 hour with frequent stirring followed by reflux heating for 4 hours at a maximum temperature of 75°C with continuous stirring. This solution was cooled to room temperature and then filtered using Whatmann's filter paper 4. The extract was concentrated by evaporating it at ≈70°C to remove the excess amount of alcohol [19-22]. This extract was preserved at room temperature (Fig 2).

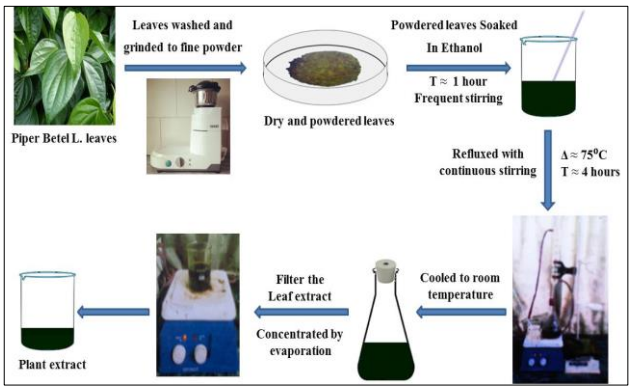


Fig 2 Schematic representation of preparation of *Piper betle* L. leaf extract

Characterization

Qualitative investigation

Phytochemical screening of crude extract

Phytochemical screenings the *Piper betle L.* extracts were carried out for to determine the bioactive compounds as per standard methods [23-27].

Detection of flavonoids

Ammonia test: A few drops of 1% ammonia solution were added to extract of leaves in a test tube, the formation of yellow colour indicates the presence of flavonoids.

Detection of steroids

Leibermann Burchard test: 1ml of extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by side of the test tube. Chloroform layer turned red and sulphuric layer showed yellow with green fluorescence which indicates the presence of steroids.

Detection of terpenoids

Salkowski test: 5ml of ethanol extract was mixed with 2ml of chloroform in a test tube. Then 3ml of concentrated sulphuric acid was added to the mixture to form a layer. An interface with a reddish-brown coloration indicates the presence of terpenoids.

Detection of tannins

Ferric chloride test: 0.5 g of powdered leaves was boiled in 20ml distilled water on a test tube and then was filtered. Then 0.1% of Ferric Chloride was added to the filtered sample. The formation of a Brownish green or blue-black coloration is an indication for the presence of tannins.

Quantitative investigation

Preparation of thin layer chromatography (TLC) plates

Dry, clean glass plates were placed over a plane surface. Slurry of the adsorbent was prepared in water in the ratio 1:2 (W/V). The slurry was stirred thoroughly for 2 minutes and was poured onto the head glass plates. The slurry was coated over the glass plates at a thickness of 0.25mm for quantitative analysis and the plate was left at room temperature for half an hour and was shifted to the hot air oven for 30 minutes at 100 to 120°C to remove the moisture and active the absorbent on the plate.

a) Sample application

25cm was left from one end of the glass plate and it was in equal distances from the edges. The leaf extract was applied by a capillary tube as a small spot marked as the origin. The Sample was allowed to dry so that spotting can be done at least twice for proper resolution.

b) Chromatograph run

The plate was then transferred into the chromatographic jar with content 40ml of the solvent, chloroform: ethanol (9:1). The solvent was allowed to run until the last 2mm of the plate. The plate was then taken out and air dried and the spots were observed under the UV trans-illuminator (360nm).

Antimicrobial study

Bacterial cultures

Pathogenic bacteria species were isolated and from clinical specimens such as body fluids like blood, urine, pus, and ET fluid and diarrhoea samples maintained in our research laboratory.

Microbial strains and inoculums preparation

Active cultures of bacterial strains were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures (maintained at 4°C on nutrient agar medium) for overnight at 37°C. The three Bacterial strains *Escherichia coli*, *Bacillus subtillis* and *Klebsiella sp.* used in our experiment was provided by our research laboratory.

Preparation of nutrient media

The nutrient media for bacterial cultures was prepared by mixing Peptone (5 g/L), Beef extract 3 g/L, Sodium Chloride 5 g/L, Distilled water 1 L, Agar agar (for solidification) at pH 6.5 to revive the bacterial species. The solid nutrient media was made to plate the bacteria cultures and assess the activity of leaf extract.

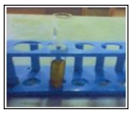
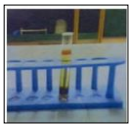
Preparation of Agar plates

The test tubes containing the nutrient media, petri-plates, cotton and micropipette tips were autoclaved at 121°C for 15 minutes. The autoclaved media (with agar) was poured into the Petri-plates and kept in the laminar air flow until it is solidified. For revival of bacterial cultures, the media (without agar) was taken in the test tubes and then inoculated with a loopful of the bacterial colonies and incubated in a shaker at 37°C for 2 days until the media turns turbid indicating microbial growth. The solidified agar plates were swabbed with revived bacteria cultures using autoclaved cotton. The leaf extract were punctured on those plates with the help of a micro tip and incubated at 37°C for 48 hours to observe the zone of inhibition [28-33].

Antibacterial assay

Antibacterial activity of leaf extract was carried out against bacterial strains by well diffusion method. The enriched cultures were swabbed over Agar plates using sterile cotton swabs. With the help of cork borer fine wells of 7mm diameter were made in the solidified agar medium. Each well was loaded with 25 µL of leaf extracts and incubated at 37°C for 24h after thirty minutes of pre-diffusion time. The diameter of the inhibition zones were measured and classified as resistant, intermediate and sensitive after the incubation period.

Table 3 Qualitative analysis of secondary metabolite of leaves extract by phytochemical test

Phytochemicals/ Test name	Observations	Results	Results for secondary metabolite analysis
	Flavonoids		
H_2SO_4 Test	Reddish brown or orange colour precipitate	+ +	
	Steroids		
Leibermann Burchard Test	Violet to Blue or Green colour formation	+ +	
	Terpenoids		
Salkowski Test	Reddish brown precipitate	+ +	
	Tannins		
Ferric Chloride Test	Brownish green colour	+	

+ indicates presence; - indicates absence

Determination of MIC by visual investigation

Different concentrations of leaf extract were added to test tubes containing nutrient broth and antibiotic. Freshly, grown bacterial strains 100 µL (106 cells/mL) in nutrient broth were inoculated into those test tubes and incubated for 24hours at 37°C. The presence of microorganism in the test tube after

incubation period is indicated by the occurrence of turbidity, whereas complete inhibition of microbial growth is due to non-appearance of any turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC. The MIC was calculated for the individual bacterial species.

RESULTS AND DISCUSSION

Phytochemical investigation

The phytochemical analysis of the *Piper betle* L. leaf extract revealed the presence of important bioactive components such as flavonoids, tannins, steroids and terpenoids as shown in (Table 4).

Qualitative secondary metabolite screening by TLC

The presence of phenolic compounds in *Piper betle* L. leaf extract was analyzed by conducting qualitative secondary metabolite screening by TLC using Chloroform: Methanol solvent system in the ratio of 9:1. The TLC plate showed 5 separate bands under UV light of 360nm with Rf values (in cm) 0.75, 0.56, 0.38, 0.25, 0.18 which indicated the presence of different phenolic compounds (Fig 3).

Antimicrobial activity

The antimicrobial activity of *Piper betle* L. leaf extract were examined for three bacteria *E. coli Bacillus subtilis* and *Klebsiella species* using well diffusion method. It was observed that crude extract shows maximum antibacterial activity. In the antimicrobial experiment, 150µl of *Piper betle* L. leaf extract each of 20%, 40%, 60% and 100% along with the control (100% ethanol) were added to the wells of agar plates inoculated with three bacterial strains.in the prepared and inoculated agar plates for each of the three microorganisms. The results show that the *Piper betle* L. leaf extract inhibited the growth of micro-organisms by producing a clear zone around the well. The zones of inhibition of the plant extract at different concentration are shown in (Table 4, Fig 4-5).

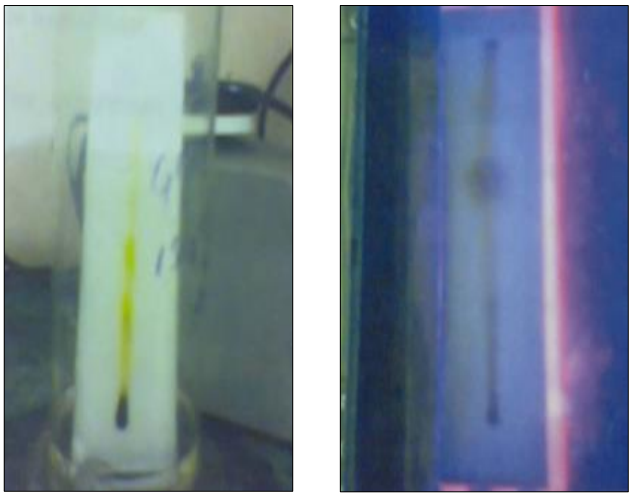


Fig 3 TLC plates showing the separation of phenolic components from *Piper betle* L. extract using Chloroform: Methanol (9:1) as a solvent system

Table 4 Antibacterial activity of Betel leaves extract (Zone of inhibition along with well diameter 0.3cm)

Bacterial strain	Zone of inhibition along with well diameter 0.3cm (in cm)				
	Control (100% ethanol)	20% of leaf extract	40% of leaf extract	60% of leaf extract	100% of leaf extract
E. Coli	0	1.8	2.4	2.9	3.0
Bacillus Subtilis	0	1.6	2.0	2.7	2.9
Klebsiella sp.	0	1.2	1.6	1.9	2.0

Control: 100% ethanol; 20% leaf extract: 20µL of the extract was added to 80 µL of ethanol; 40% leaf extract: 40µL of the extract was added to 60 µL of ethanol; 60% leaf extract: 60µL of the extract was added to 40 µL of ethanol; 100% leaf extract: 100µL of the extract was added to 0 µL of ethanol

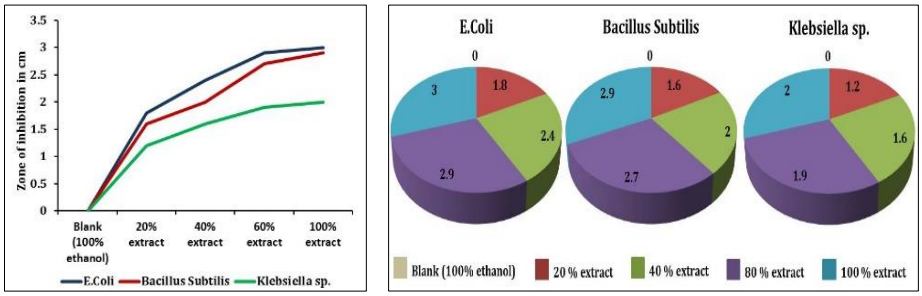


Fig 4 Plot and Pie diagram showing antibacterial activity of *Piper betle* L. leaf extract against bacterial strain *E. coli., Bacilus subtils* and *Klebsiella* sp. in different concentrations using agar well diffusion technique

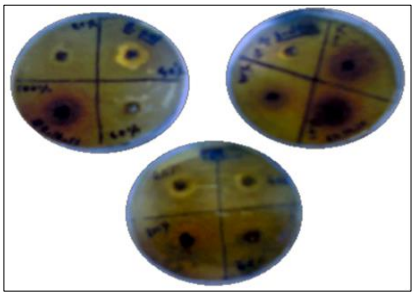


Fig 5 Plates showing zone of inhibition of *E. coli., Bacilus subtils* and *Klebsiella* sp. using agar well diffusion technique by *Piper betle* L. leaf extract

The MIC values of the leaf extract against the three microbes were found to be 40µl for *E. coli* at 2% of leaf extract; 70µl for *Bacillus subtilis* at 3.5% of leaf extract; 80µl for *Klebsiella sp*. at 4% of leaf extract as shown in (Table 5, Fig 6). Hence the increasing order of the betel leaves affectivity against the three microbes are found to be *Klebsiella sp.*< *Bacillus subtilis* < *E. coli*.

Table 5 Results for minimum inhibitory concentration (MIC)

Organisms	Concentration of the leaf extract (%)	MIC value (µl)	Diameter of the zone of inhibition (cm)
E. coli	2.0	40	0.8
Bacillus subtilis	3.5	70	1.1
Klebsiella sp.	4.0	80	1.3

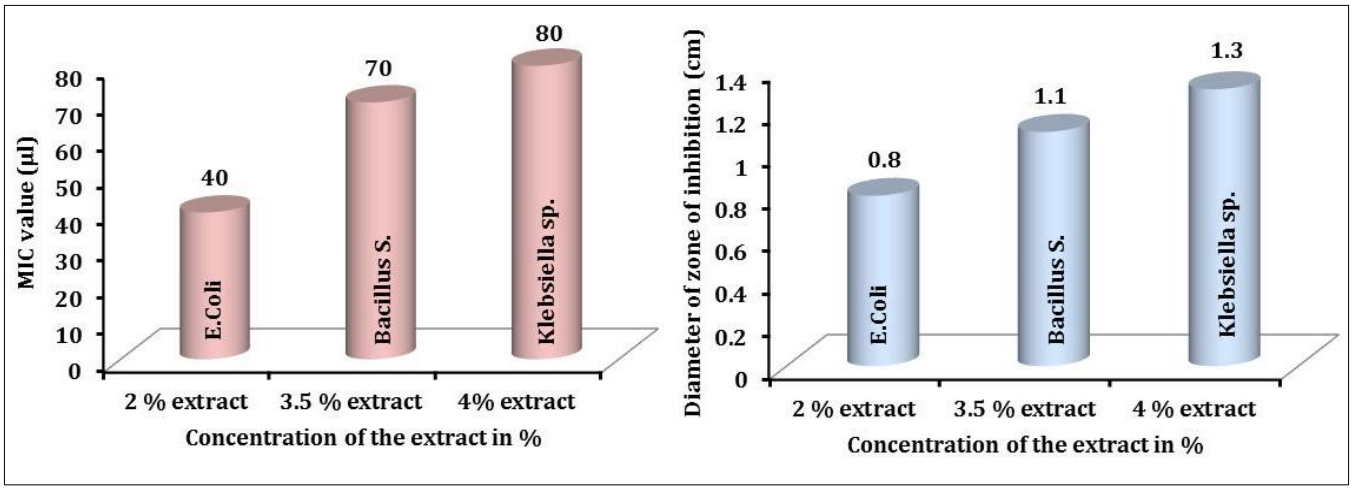


Fig 6 Bar graph showing MIC value and diameter of zone of inhibition of bacterial pathogens at different concentration of *Piper betle* L. leaf extract

CONCLUSION

Piper betle L. extract has potential application as a traditional natural medicine and helps in treating many diseases. The phytochemical compounds and different effective bioactive compounds present in this natural herb inhibit the growth of micro-organisms. Due to their antibacterial properties, it has been recognized for many pharmacological activities and is in great demand. It plays a vital role for microbiological safety of the human health. In future, essential oil obtained from leaf extract will be used to treat dental pathogens; phenolic compounds present in the leaf will act as a good antioxidant. The findings of the present study lay the foundation for future large scale prospective studies from North East India. Also, it adds scientific merit to long term use of *Piper betle* L. in as traditional herbal medicine.

Acknowledgement

The Authors are thankful to the Central Instrumentation Facility (CIF) and Department of Biotechnology, Assam Down Town University, Guwahati, Assam, India for providing the infrastructure facility.

Conflict of interest: None

LITERATURE CITED

1. Singh D, MSN. 2021. Phytochemistry, pharmacological property and medicinal uses of *Piper betle* L: A Review. *Journal of Natural Remedies* 21(11): 1.
2. Pradhan D, Suri KA, Pradhan DK, Biswasroy P. 2013. Golden heart of the nature: *Piper betle* L. *Journal of Pharmacognosy and Phytochemistry* 1(6): 147-167.
3. Ali A, Lim XY, Wahida PF. 2018. The fundamental study of antimicrobial activity of *Piper betle* extract in commercial toothpastes. *Journal of Herbal Medicine* 14: 29-34.
4. Amminbavi D, Lakshmi NP. 2020. Assessment of in vitro wound healing potential of Hibiscus leaf extract Emulgel. *Asian Jr. Pharm. Research* 10(2): 67-72.
5. Bala S, Saini M, Kamboj S, Upp G. 2011. Biodiversity of oxazolone derivatives in medicinal chemistry: A review. *Asian Journal of Research Chemistry* 4(2): 685-694.
6. Salam S, Rahmadani A, Haikal K, Febrina L, Anshory H, Kuncoro H. 2021. Novel amides derivative with antimicrobial activity of *Piper betle* var. nigra leaves from Indonesia. *Molecules* 26(2): 335.
7. Jalgaonwala RE, Mahajan RT. 2011. Isolation and characterization of endophytic bacterial flora from some Indian medicinal plants. *Asian Jr. Research Chemistry* 4(2): 296-300.
8. Paul S, Saha D. 2012. Comparative study of the efficacy of *Barleria prionitis* leaf extracts against bacteria. *Asian Jr. Pharm. Research* 2(3): 107-110.
9. Nayaka NMDMW, Sasadara MMV, Sanjaya DA, Yuda PESK, Dewi NLKAA, Cahyaningsih E, Hartati R. 2021. *Piper betle* (L): Recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. *Molecules* 26(8): 2321.
10. Valle Jr DL, Cabrera EC, Puzon JJM, Rivera WL. 2016. Antimicrobial activities of methanol, ethanol and supercritical CO_2 extracts of Philippine *Piper betle* L. on clinical isolates of gram positive and gram negative bacteria with transferable multiple drug resistance. *PloS One* 11(1): e0146349.
11. Tiwari P. 2014. Antimicrobial activity of draksharishta prepared by traditional and modern methods. *Asian Jr. Pharm. Tech.* 4(3): 131-133.
12. Lubis RR, Marlisa DDW. 2020. Antibacterial activity of betel leaf (*Piper betle* L.) extract on inhibiting *Staphylococcus aureus* in conjunctivitis patient. *American Journal of Clinical and Experimental Immunology* 9(1): 1.
13. Harjanti DW, Ciptaningtyas R, Wahyono F. 2019. Phytochemical properties and antibacterial activity of *Ageratum conyzoides*, *Piper betle*, *Muntinga calabura* and *Curcuma domestica* against mastitis bacteria isolates. *In*: *IOP Conference Series: Earth and Environmental Science* 247(1): 012049.
14. Syahidah A, Saad CR, Hassan MD, Rukayadi Y, Norazian MH, Kamarudin MS. 2017. Phytochemical analysis, identification and quantification of antibacterial active compounds in betel leaves, *Piper betle* methanolic Extract. *Pakistan Journal of Biological Sciences* 20(2): 70-81.

15. Surjowardojo P, Saputra FT, Ridhowi A. 2019. Antimicrobial activity of *Piper betle* L. against some mastitis disease bacteria at different temperatures and extraction times. *Drug Invention Today* 11(10).
16. Saranya S, Anuradha V. 2020. Antibacterial activity of *Piper betle* leaf extracts against drug resistant bacteria of social relevance. *Gedrag and Organisatie Review* 33: 116-122.
17. Nasution H, Wulandari G. 2021. The effect of betel (*Piper betle*) leaf extract as antimicrobial agent on characteristics of bioplastic based on sago starch. In *IOP Conference Series: Materials Science and Engineering* 1122(1): 012098.
18. Ataguba GA, Dong HT, Rattanarojpong T, Senapin S, Salin KR. 2018. *Piper betle* leaf extract inhibits multiple aquatic bacterial pathogens and in vivo Streptococcus agalactiae infection in Nile tilapia. *Turkish Journal of Fisheries and Aquatic Sciences* 18(5): 671-680.
19.Nguyen LTT, Nguyen TT, Nguyen HN, Bui QTP. 2020. Simultaneous determination of active compounds in *Piper betle* Linn. leaf extract and effect of extracting solvents on bioactivity. *Engineering Reports* 2(10): e12246.
20. Kusuma SAF, Mita SR, Mutiara A. 2018. Antimicrobial lotion containing red *Piper betle* leaf (*Piper crocatum* Ruiz and Pav) ethanolic extract for topical application. *National Journal of Physiology, Pharmacy and Pharmacology* 8(1): 130-138.
21. Kusuma SA, Ami T, Gita S. 2017. Antibacterial effect of red *Piper betle* leaf (*Piper crocatum* ruiz and pav.) Ethanolic extracts to lactobacillus acidophilus and lactobacillus bifidus growth inhibition. *Asian Jr. Pharm. Clin. Research* 10: 65-68.
22. Kumar G, Badoni PP. 2018. Arisaema tortuosum leaf extract mediated synthesis of silver nanoparticles, characterization and their antibacterial activity. *Asian Jr. Research Chemistry* 419-422.
23. Betle Broth P. 2012. Phytofabrication and characterization of silver nanoparticles from *Piper betle* broth. *Research Journal of Nanoscience and Nanotechnology* 2(1): 17-23.
24. Alirsani Z, Aghazadeh M, Adibpour M, Amirchaghmaghi M, Pakfetrat A, Mozaffari PM, Zenooz AT. 2011. In vitro comparison of the antimicrobial activity of ten herbal extracts against streptococcus mutatis with chlorhexidine. *Journal of Applied Sciences* 11(5): 878-882.
25. Purba RAP, Paengkoum S, Yuangklang C, Paengkoum P. 2020. Flavonoids and their aromatic derivatives in *Piper betle* powder promote in vitro methane mitigation in a variety of diets. *Ciência e Agrotecnologia* 44.
26. Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F. 2015. Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. *Industrial Crops and Products* 71: 197-204.
27. Jose BE, PanneerSelvam P. 2018. Identification of phytochemical constituents in the leaf extracts of *Azima tetracantha* Lam using Gas Chromatography-Mass Spectrometry (GC-MS) analysis and antioxidant activity. *Asian Jr. Research Chemistry* 857-862.
28. Ramasubramaniaraja R. 2011. Pharmacognostical phytochemical including GC-MS investigation of ethanolic leaf extracts of *Abutilon indicum* (Linn). *Asian Jr. Pharm. Analysis* 88-92.
29. Surjowardojo P, Setyowati E, Ambarwati I. 2019. Antibacterial effects of green betel (*Piper betle* Linn.) leaf against *Streptococcus agalactiae* and *Escherichia coli*. *Agrivita, Journal of Agricultural Science* 41(3): 569-574.
30. Thirupatti C, Kumaravel P, Duraisamy R, Prabhakaran AK, Jeyanthi T, Sivaperumal R. 2013. Biofabrication of silver nanoparticles using *Cocculus hirsutus* leaf extract and their antimicrobial efficacy. Silver nanoparticles, Cocculus hirsut. *Asian Jr. Pharm. Tech*. 2013: 93-97.
31. Sugumaran M, Vetrichelvan T, Quine SD. 2009. Antidiabetic potential of aqueous and alcoholic leaf extracts of *Pithecellobium dulce*. *Asian Jr. Research Chem*. 83-85.
32. Gustiani S, Septiani W, Kasipah CICA, Sukardan D, Helmy Q. 2021. Antimicrobial effect of *Piper betle* leaves extract on cotton fabrics for vaginal discharge sanitary napkins. *Advanced Materials Research* 1162: 159-165.
33. Kasai D, Chougale R, Masti S, Gouripur G, Malabadi R, Chalannavar R, Dhanavant S. 2021. Preparation, characterization and antimicrobial activity of betel-leaf-extract-doped polysaccharide blend films. *Green Materials*. pp 1-20.