IJMPR 2022, 6(8), 58-62

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

EVALUATION OF ANTIMICROBIAL PROPERTY OF *OCIMUM SANCTUM* AND *PIPER BETLE* COMBINATION

Md Hasnat Jahan Ali, Nabaneeta Ghosh, Niharika Gupta, Moumita Tambuli, Nayanika Dey, Namrata Thakur, Ananya Bhattacharjee*

[1,2,3,4,5,6]Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim-737136, India.
[7]Assistant Professor, Department of Pharmacology, Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim-737136, India.

Received on: 11/06/2022
Revised on: 01/07/2022
Accepted on: 21/07/2022

***Corresponding Author**
Ananya Bhattacharjee
Assistant Professor, Department of Pharmacology, Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim-737136, India.

ABSTRACT

Piper betle and *Ocimum sanctum* leaf has long been used traditionally for its various pharmacological activities like analgesic, antidiabetic, anti-caries, cold & cough reliving as well as for its antimicrobial activity. In this study we tried to evaluate synergistic effect of combined leaf extract of both leaf. Ethanolic extract of *Piper betle* and *Ocimum sanctum* was performed using rotary evaporator. Gram Positive organism, *Staphylococcus aureus* andgram negative organism, *E.coli* was used for the evaluation of antimicrobial activity.*Piper betle*, *Ocimum sanctum* alone leaf extract and both leaves combined extract was used to check the zone of inhibition and minimum inhibitory concentration (MICs) against both gram negative and gram positive micro-organism. Alone *Piper betle* and *Ocimum sanctum* leaf extract showed significant zone of inhibition when compared to control group, whereas combined leaf extract showed moderately significant increase in zone of inhibition than the alone treated leaf extracts of *Piper betle* and *Ocimum sanctum*. Different concentration (31.25µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml, 1000µg/ml) of combined leaf extract were used for evaluation of MICs. However there was no zone of inhibition in 31.25µg/ml conc. and the minimum inhibitory concentration exhibiting zone of inhibition was at 62.5µg/ml concentration. The combined leaf extract showed extremely significant antimicrobial activity in our study.

KEYWORDS: Tulsi, betel, antimicrobial, antimicrobial combination, zone of inhibition.

INTRODUCTION

Food-borne illnesses associated with microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis* present a major public health concern throughout the world.[1]

Food may get contaminated due to the presence and growth of microorganism and it may affect the quality and quantity. Further, microbial contamination of food still poses important public health and economic concerns for human society.[2]

In addition to passive transfer of pathogens to food, active growth of a pathogen may also occur in foods, because of improper storage, leading to marked increases in microbial load.[3]

The Antimicrobial resistance has been increasing with passing years, the herbal medicines are coming into play more often than synthetic or chemical drugs. Herbs are traditionally being used since ancient times for treatment of many diseases since ancient times for many medicinal treatments and with developing world. Herbs because of its medicinal value, these are widely used to maintain and provide a good health.[4]

Some herbs that possess Antimicrobial property are *Tephrosia purpurea*, *Solanum surattense*, *Solanum surattense*, *Saraca asoca*, *Allium salivum*, *Indianbael*, *Piper nigrum*, *Bitter melon*, *Piper betle*, *Ocimum sanctum*, *Thymus vulgaris*, *Calotropis procera*, *Curcuma longa* and many more.[4]

Ocimum sanctum, commonly known as tulsi, traditionally used in Ayurveda due to its healing property. Due to its various uses it is often termed as 'queen of herbs'. *Piper betle* or Paan have long been using in the Indian traditional medicine system for its antioxidant and antibacterial properties. Their antimicrobial properties has already been discovered and studied.[5,6,7]

As the antimicrobial properties has already been discovered and studied, these plants were taken together

for evaluation of antimicrobial activity of the combination.

MATERIALS AND METHODS

Collection of plants: Fresh betel leaves were collected from Kalimpong, D.S Gurung road bazaar area, India, and fresh tulsi leaves were collected from Matigara, Siliguri, India.

Preparation of leaf extract

- Preparation of betel leaf extract: First the betel leaves were shed dried following washing. Then air-dried leaves of Piper betel were pulverized into powdered form. Then extraction process was in maceration process where the dried powder (20 g) was dissolve into 96% ethanol (80ml) at room temperature for 5 days, and then it was filtered with filter paper to obtain the extract. The solvents from the extracts were evaporated by rotary evaporator.[8]

The obtained semisolid extract was weighed and stored in refrigerator in an airtight container and used for the current studies. Yield was also calculated.

- Preparation of tulsi leaf extract: First the tulsi leaves were washed and shed dried. The air-dried leaves of Ocimum sanctum were pulverized into powdered form. Then extraction process was by maceration where the dried powder (20 g) was dissolved into 96% ethanol (80ml) at room temperature for 3 days then filtered with filter paper to obtain the extract and the solvents from the extracts were evaporated by rotary evaporator. Yield was also calculated.[6]

Microorganism: *Escherichia coli* (gram negative) and *staphylococcus aureus*(gram positive) were used as test microorganism. Cultures of each bacterial strain were obtained from the stock of microbiology laboratory.

Antimicrobial activities

Antimicrobial activity assessment

The agar diffusion method was used to determine antimicrobial property of *Piper betle*, *Ocimum sanctum* and their combination group. Petri plates were prepared by adding 20ml of nutrient agar media and it was allowed to solidify. After that 0.1ml of standard inoculum of staphylococcus and E.coli was added and spreaded uniformly, and allowed to dry for 5min. In a ready-made sterile filter paper disc of 6mm diameter the leaf extract of *Piper betle*, *Ocimum sanctum* and their combination group were added at a conc. of 2500µg/ml and placed on the petriplate.[48] The plates were kept in the B.O.D Incubator at 37^0C for 24 hours and after 24 hour zone of inhibition formed around the disc were calculated in mm.[9,10]

Determination of minimum inhibitory concentration (MICs)

The minimum inhibitory concentration of the combination group was determined by agar diffusion method.[9] We have inoculated agar plates by standardized inoculum of the test microorganism in this procedure. Then, filter paper discs (about 6 mm in diameter) are dipped into the test compound at a desired concentration and are placed on the surface of nutrient agar media. The Petri dishes are incubated under suitable and controlled conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism. Then the diameters of growth of zone of inhibition are measured.[10]

In this procedure, the loopful of gram positive as well as gram negative organisms from the stock culture were taken and transferred to the petri plates. Then the plates were allowed to incubate at 37^0C for 24 hour. The combined leaf extract of *Piper betle* and *Ocimum sanctum* of equal quantity were made upto the concentration of 2000µg/ml and serially diluted to get 1000,500,250,125 and 62.5µg/ml. The control Petri plates containing only bacterial suspension were incubated at 37^0C for 24 hour. The lowest concentration of the combined extract which does not allow any microbial growth after evaluation was determined as MIC, Which were expressed in µg/ml.[10]

Statistical analysis

Data were expressed as the mean and SEM of the means and statistical analysis was carried out employing one-way ANOVA followed by tukey cramer multiple comparison test.

RESULTS AND DISCUSSION

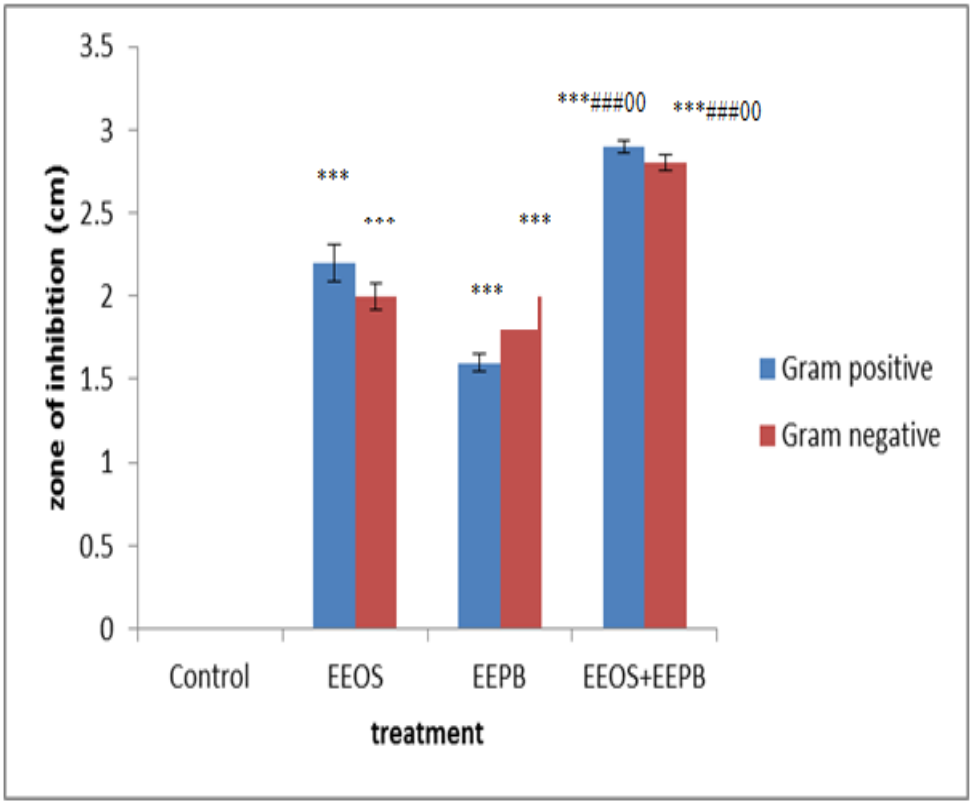
Antibacterial activity assay

The zone of inhibition formed around the disc were calculated and it was found that there was no zone of inhibition in the control group, EEOS (Ethanolic extract of *Ocimum sanctum*) and EEPB (Ethanolic extract of *Piper betle*) exhibited extremely significant zone of inhibition compared to control group, where as the combination of both EEOS+EEPB exhibited moderately significant increase in the zone of inhibition compared to EEOS and EEPB alone treated groups.

Table 1: Assay of Antimicrobial Activity.

microorganisms	Zone of inhibition			
	Control	EEOS	EEPB	EEOS+EEPB
E.coli	-	$1.96\pm0.08^{***}$	$2\pm0.17^{***}$	$2.9\pm0.05^{***###00}$
S.aureus	-	$2.2\pm0.11^{***}$	$1.6\pm0.05^{***}$	$2.9\pm0.05^{***###00}$

All values are mean ± SEM, of three parallel measurements, ***P <0.001 when compared to control; ###P <0.001 when compared to EEOS treated group, 00P <0.01 when compared to EEPB treated group. EEOS= Ethanolic extract of *Ocimum sanctum*, EEPB= Ethanolic extract of *Piper betle*, – means no zone of inhibition.



All values are mean ± SEM, of three parallel measurements, ***P <0.001 when compared to control; ###P <0.001 when compared to EEOS treated group, 00P <0.01 when compared to EEPB treated group. EEOS= Ethanolic extract of *Ocimum sanctum*, EEPB= Ethanolic extract of *Piper betle*, – means no zone of inhibition.

Determination of minimum inhibitory concentration (MICs)

According to the results, the minimum inhibitory concentrations (MICs), defined as the lowest concentrations of the combined leaf extract of *Piper betle* and *Ocimum sanctum* of equal quantity that resulted in complete growth inhibition of the tested pathogens, were found to be in the range of 62.5 to 1000µg/mL.

There was no zone of inhibition in 31.25µg/mL. for both gram positive as well as gram negative organism. In 62.5 µg/mL concentration showed first zone of inhibition for both gram positive as well as gram negative organism, so the concentration 62.5 µg/mL is the minimum inhibitory concentration of both gram positive as well as gram negative organisms.

In this study, the Gram-positive bacteria were found to be more susceptible to plant extracts than Gram-negative bacteria.

Table 2: Determination of minimum inhibitory concentrations.

microorganisms	Combination of EEOS+ EEPB					
	1000µg/ml	**500µg/ml**	**250µg/ml**	**125µg/ml**	**62.5µg/ml**	**31.25µg/ml**
E.coli	15.66±0.08	14.43±0.12**	12.23±0.08**	10.23±0.08**	8.33±0.12**	0
S.aureus	12.3±0.11	11.13±0.14**	10.53±0.12**	8.03±0.06**	7.4±0.06**	0

All values are mean ± SEM, of three parallel measurements, **P <0.01 when compared to 1000µg/ml, EEOS= Ethanolic extract of *Ocimum sanctum*, EEPB= Ethanolic extract of *Piper betle*.

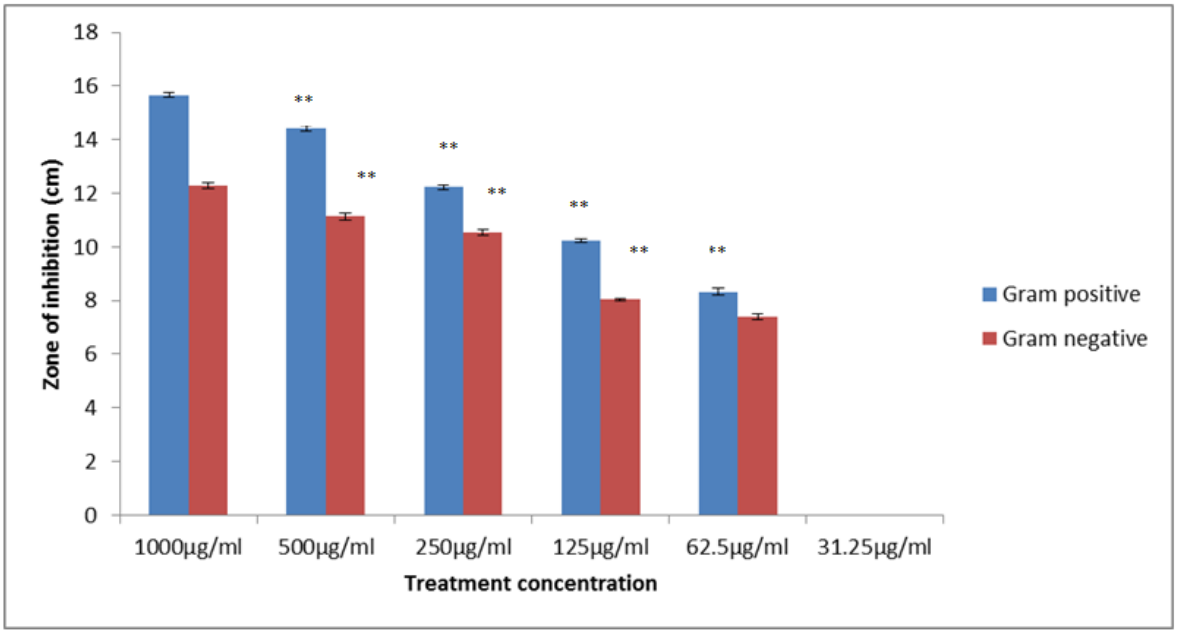


Figure 2: Determination of minimum inhibitory concentrations.

All values are mean ± SEM, of three parallel measurements, **P <0.01 when compared to 1000µg/ml, EEOS= Ethanolic extract of *Ocimum sanctum*, EEPB= Ethanolic extract of *Piper betle.*

DISCUSSION

Piper betle belongs to the family Piperaceae and is most commonly found in Southeast Asian region. It is widely used as mouth freshener for its aromatic and pungent flavor. It is also used for its antibacterial activity against Plaque and dental caries forming bacteria, e.g. *Streptococcus mutans*, *Staphylococcus aureus*, Fungal Pathogen e.g. *Candida albicans.*[11]

Ocimum sanctum belongs to family Lamiaceae and is found in Indian subcontinent as well as Southeast Asian region. In Ayurveda tulsi is denoted as "Mother medicine of Nature" and is often referred as "Elixir of life". It is most widely used for its activity in reliving cough, cold, Asthma as well as for its wound healing activity.[12]

Ethanolic extract of *Piper betle* as well as *Ocimum sanctum* leaves separately showed significantamount of antimicrobial activity against gram +ve & gram –ve micro-organism. The antimicrobial activity of *Piper betle & Ocimum sanctum* has been reported in the past as well.[8,6]

As the antimicrobial property is known to be existed for both *Piper betle & Ocimum sanctum*, so to check the synergistic effect, ethanolic extract of *Piper betle & Ocimum sanctum* was taken to carry out the combined study.

The assay of antimicrobial activity was done by the agar diffusion method which was used to determine antimicrobial property of *Piper betle*, *Ocimum sanctum* and of their combination. In our study both the ethanolic extract of *Piper betle* and *Ocimum sanctum* showed an extremely significant zone of inhibition against both gram +ve (Staphylococcus aureus) and gram –ve (Escherichia Coli) bacteria, whereas the combination of *Piper betle* and *Ocimum sanctum extract* showed moderately significant increase in inhibitory zone as compared to *Piper betle* and *Ocimum sanctum* alone treated group.

For the evaluation of minimum inhibitory concentration (MICs) different concentration of combined leaf extract ranging from 31.25 to 1000µg/ml were taken. At the conc. of 31.25µg/ml there was no zone of inhibition however from the conc. 62.5µg/ml the first zone of inhibition appeared for both gram positive and gram negative bacteria. So 62.5µg/ml conc. is the minimum inhibitory concentration of both gram positive as well as gram negative micro-organism.

The phytochemical analysis of *Piper Betle* shows that it contains some active compounds like alkaloids, phenols, flavonoids, saponins, glycosides, tarpenoids and steroids. Alkaloids and flavonoids contribute in *Piper betle* leaf extract antibacterial activity.[8,13]

Tulsi consist many essential oils like eugenol, rosmarinic acid, eucalyptol, apigenin, myretenal, luteolin, β-sitosterols, and carnosic acid, which possess antibacterial, antifungal, antioxidant and anti-inflammatory activities. It is evident that alkaloids and flavonoids present in it show a very good antimicrobial property.[14,15,16]

As both *Piper Betle* and *Ocimum Sanctum* already possess antimicrobial property, and are well used as

antimicrobial agent, the combined effect of the both leaf extract has enhanced anti-microbial activity may be due to the presence of both flavonoids and tannins, so in future the combined extract can be used and planned for evaluation of antimicrobial formulation through a detailed clinical study.

CONCLUSION

From the study it can be concluded that as both *Piper Betle* and *Ocimum Sanctum* already possess antimicrobial property, and are well used as antimicrobial agent, the combined effect of the both leaf extract has enhanced anti-microbial activity may be due to the presence of both flavonoids and tannins, the combination of both extract works wonders as an Antimicrobial agent, as it consist great natural source of many active therapeutic agent, for bacterial infections that works against both Gram positive and gram negative bacteria showing great Antibacterial activity, thus the combination study for both can be further investigated in future.

ACKNOWLEDGEMENTS

I acknowledge Dr. H.P. Chhetri, Director, Himalayan Pharmacy Institute, Sikkim and Dr. N.R. Bhuyan, Principal, Himalayan Pharmacy Institute, Sikkim for providing necessary facilities to carry out the research work.

REFERENCES

1. Hall RL. Foodborne illness: implications for the future. Emerging Infectious Diseases, 1997 Oct; 3(4): 555-9.
2. Soliman KM, Badeaa RI. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and chemical toxicology, 2002 Nov 1; 40(11): 1669-75.
3. Madigan MT, Martinko JM, Parker J. Brock biology of Microorganisms, 8th edn. Englewood Cliffs, NJ: Prentice-Hall, 1997; 986.
4. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, Nur H, Ismail AF, Sharif S, RamaKrishna S, Berto F. Antioxidant, antimicrobial and antiviral properties of herbal materials. Antioxidants, 2020 Dec 21; 9(12): 1309.
5. Yamani HA, Pang EC, Mantri N, Deighton MA. Antimicrobial Activity of Tulsi (Ocimum tenuiflorum) Essential Oil and Their Major Constituents against Three Species of Bacteria. Front Microbiol, 2016 May 17; 7: 681.
6. Mallikarjun SRA, Rajesh G, Shenoy R, Pai M. Antimicrobial efficacy of Tulsi leaf (Ocimum sanctum) extract on periodontal pathogens: An in vitro study. J Indian Soc Periodontol, 2016 Mar-Apr; 20(2): 145-50.
7. Lubis RR, Wahyuni DD. Antibacterial activity of betle leaf (Piper betle l.) extract on inhibiting Staphylococcus aureus in conjunctivitis patient. American journal of clinical and experimental immunology, 2020; 9(1): 1-5.
8. Das S, Ray A, Nasim N, Nayak S, Mohanty S. Effect of different extraction techniques on total phenolic and flavonoid contents, and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. 3 Biotech, 2019 Jan; 9(1): 37.
9. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis, 2016 Apr 1; 6(2): 71-9.
10. Bajpai VK, Rahman A, Shukla S, Mehta A, Shukla S, Arafat SY, Rahman MM, Ferdousi Z. Antibacterial activity of leaf extracts of Pongamia pinnata from India. Pharmaceutical biology, 2009 Dec 1; 47(12): 1162-7.
11. Chowdhury U, Baruah PK. Betelvine (Piper Betle L.): A Potential Source for Oral Care. Curr. Bot, 2020; 87-92.
12. Panchal P, Parvez N. Phytochemical analysis of medicinal herb (Ocimum sanctum). International Journal of Nanomaterials, Nanotechnology and Nanomedicine, 2019 Jul 22; 5(2): 008-11.
13. Akter KN, Karmakar P, Das A, Anonna SN, Shoma SA, Sattar MM. Evaluation of antibacterial and anthelmintic activities with total phenolic contents of Piper betel leaves. Avicenna J Phytomed, 2014 Sep; 4(5): 320-9.
14. Baliga MS, Jimmy R, Thilakchand KR, Sunitha V, Bhat NR, Saldanha E, Rao S, Rao P, Arora R, Palatty PL. Ocimum sanctum L (Holy Basil or Tulsi) and its phytochemicals in the prevention and treatment of cancer. Nutrition and cancer, 2013 Jan 1; 65(1): 26-35.
15. Gadiyar A, Ankola AV, Rajpurohit L. Evaluation of the antimicrobial activity of Ocimum sanctum L. (tulsi) extract against Streptococcus mutans and Lactobacillus acidophilus - An in vitro study. Int J Health Sci Res, 2017; 7(4): 224-228.
16. Borah R, Biswas SP. Tulsi (Ocimum sanctum), excellent source of phytochemicals. International Journal of Environment, Agriculture and Biotechnology, 2018; 3(5): 265258.