ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

PHYTOCHEMICAL SCREENING & IN-VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY FROM STEM EXTRACT OF PLANT PIPER BETEL

Amandeep Singh[1], Nisha A Bhatt [2], Mohd. Adnan[3]

[1-] Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun
[2-] Asso. Professor, Dev Bhoomi Institute of Pharmacy & Research, Dehradun
[3-] Research Scholar, Dev Bhoomi Institute of Pharmacy & Research, Dehradun

Abstract: The objective of the present project work was to investigate the active constituent of plant Piper betel characterized for its antimicrobial activity. For the research analysis the stem powder subjected to phytochemical screening and was found to contain carbohydrate, protein, polyphenolic compounds, flavonoid, alkaloids and total antioxidant. The estimation and evaluation of antimicrobial activity was done by disc diffusion method. For this the different concentration ethanolic extract (20, 40, 60 & 80%) was prepared and from which initial concentration of 1mg/ml were loaded on 6mm dimension filter paper which was sterilized previously by autoclaving. The loaded disc was placed on surface of medium and the compound was allowed to diffuse for 5 minutes and all the plated were incubated at 37°C for 24 hrs. The end of incubation, inhibition zones formed around the disc in few mm, measurement done by using the ruler. As, a result the maximum inhibition was showed by the concentration of 60% dilution. The extract confirmed significant antimicrobial activity against the bacterial strains test.

Introduction: Piper betel Linn (Piperaceae) stem is widely used as a post meal mouth freshener and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Due to strong pungent aromatic flavour betel stem are used as masticatory by the Asian people. Its common names are betel (in English), paan (in Indian), phlu (in Thai) and sirih (in Bahasa Indonesian). Grown abundantly in many parts of India, betel is an evergreen dioecius herb that needs warm and moist growth conditions for its growth. Stem of betel vine are used with various condiments such as areca nut (kattha), cloves, cardamom, arecanut, candied rose and fennel for chewing purposes (Verma *et al*., 2004). Indian system of medicine and health has adopted the use of betel stem in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. This particular property has paved way for further experimental studies, which have established paan extract to have antimicrobial and antileshmian properties (Sarker *et al.,* 2008). Fresh juice of betel stem is also used in many ayurvedic preparations. Betel stem have long been studied for their diverse pharmacological actions.

Traditional healers from different remote communities in India claim that their medicine obtained from these betel stem is cheaper and more effective than modern medicine. Patients belonging to these communities have a reduced risk of acquiring infectious diseases from resistant pathogens than the people from urban areas who may be treated with regular antibiotics. A novel approach to the prevention of antibiotic resistance of pathogenic species is the use of new compounds that are not based on existing synthetic antimicrobial agents (Shah *et al*., 2005). It is imperative that evaluation of the potential use of folkloric medicine for the treatment of infectious diseases produced by common pathogens be performed on a scientific base. Many plants are thus becoming probable sources of important drugs and pharmaceutical industries, nowadays, have come to consider this traditional medicine as a source of bioactive agents which can be used in the preparation of synthetic medicine. Furthermore, they are possible source for new as well as potent antibiotics to which the pathogenic strains are not resistant. Reports of various researches show that betel extract and betel oil exhibit antimicrobial and antioxidant activities in model systems (Salleh et al., 2002; Lei *et al.,* 2003; Bhattacharya et al., 2006).

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

The objective of this study includes the evaluation of the phytochemical constituents of the ethanol extract of the betel leaf and investigating the efficacy of the same as an antimicrobial agent on the four pathogenic bacteria species.

Modern medication

The incidence of antimicrobial resistance is on continued rise with a threat to return to the "pre-antibiotic" era. This has led to emergence of such bacterial infections which are essentially untreatable by the current armamentarium of available treatment options. Various efforts have been made to develop the newer antimicrobials with novel modes of action which can act against these multi-drug resistant strains. This review aims to focus on these newly available and investigational antibacterials approved after year 2000, their mechanism of actions/resistance, and spectrum of activity and their phases of clinical trials. Newer unexploited targets and strategies for the next generation of antimicrobial drugs for combating the drug resistance and emerging pathogens in the 21st century have also been reviewed in the present article.

Serious infections caused by microorganisms resistant to commonly used antimicrobials have become a major healthcare problem worldwide in the 21st century. This is responsible for the significant increase in morbidity and mortality, longer hospitalization and increased health care costs. Keeping in view the seriousness of this problem, the World Health Organization (WHO) has selected "Antimicrobial resistance: No action today no cure tomorrow" as the theme for World Health Day 2011 as a preventive measure.

In recent years, the number of availability of new antimicrobials for human use across the globe has been lower than in the recent past. No new classes of antimicrobials were developed in the thirty seven years between the introduction of nalidixic acid (1962) and linezolid (2000) and all antimicrobials that entered the market during this time period were modifications of the existing molecules. The development of new antimicrobial agent is very expensive and time consuming, leading to diminishing interest of pharmaceutical industries in it. On an average, research and development of anti-infective drugs takes around 15-20 years, and can cost more than $1000 million.[1] The cost of bringing a new product to the market is increasing at a rate of 10% per annum. The majority of large pharmaceutical companies have terminated their anti-infective research programs altogether.

In the present review, all new antibacterial agents which have been approved after the year 2000 have been described along with their mechanism of action, development of resistance, spectrum of activity and the stage of developmental in case of yet to be approved drugs. Some newer unexploited targets and strategies for combating drug resistance have also been reviewed.

Materials and Methods

Collection of plant material

The stem of *Piper betel* L. was collected from and identified by the institute. Plant Materials Fresh betel vine stem were collected from the nearby village in Bhauwala Dehradun, India. The stem was shade dried and crushed into fine powder with electric blender. The powdered sample was sealed in polythene bags and was stored in desiccators until further uses.

. **Preparation of extracts by cold maceration**

- **Aqueous extract:**
  Weight accurately about 20 gram of the coarsely powdered drug was washed with distilled water and, it was allowed to macerate for 7 days with occasional shaking. After a week the liquid was filtered with the help of the muslin cloth and the drug material was pressed to liberated more menstruum from the marc. Both the extracts were mixed and liquid was evaporated without heating to get aqueous extracts.
- **Ethanolic extract**
  Accurately weight about 20 gm of the coarsely powered drug was washed with ethanol and it was allowed to macerate for 7 days with occasional shaking , After a week of the liquid was filtered with the help of the muslin cloth and the drug material was pressed to liberate more menstruum from the marc. Both of the extracts were mixed and the liquid was evaporated without heating to get ethanolic extracts.
- **Hydroalcoholic extract**
  Weight accurately about 20 gm of coarsely powdered drug was washed with hydroalcoholic solution (50:50) and it was allowed to macerate for 7 days with occasional shacking. After a week the liquid was filtered with the help

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

of muslin cloth material was pressed to liberated more menstrum from the marc. Both the extracts were mixed and the liquid was evaporated without heating to get hydroalcoholic extract.

Procedure

The plant material was collected and herbarium file was created including flower, leaf, Flower

1. Stem was dried in open air for 10 to 15 days to remove moisture from the leaves.
2. After drying the Stem was crushed into fine coarse particles.

The fine particles of Stem of plant was taken and put into maceration to obtain the extract.

Phytochemical investigation (Khandelwal, 2008)

- **Test for carbohydrates**
  1. **Molish's test (general test):**Take 2-3ml of extract was taken and few drop of alpha napthol solution in 95%alcohol were added .Then it was shaken continuously min 2 min and then conc. H2SO4 was added from sides of the test tube, and then violet colour was formed at the junction of two liquids.
- **Test for reducing sugar**
  2. **Fehling's solution test:** rbed1 ml of fehling's (A) and 1ml of fehling's (B) solution was mixed and boiled for 1 min .Equal volume of extract solution was added then it was heated in boiling water bath for 5-10 min. First yellow, then brickred colour ppt was absorbed.
  3. **Benedict test:** 2 ml of Benedict solution and test solution was mixed in test tube. It was heated in boiling water bath for 5 min. Solution appeared green in colour which indicated the presence of reducing sugar in test solution.
- **Test for proteins**
  1. **Biuret test (general test):** 3ml of test solution was added to 2 ml of 4%NAOH and few drops of 1%CUSO4 solution. Violet colour was to be appeared.
  2. **Million's test (for proteins):** Mix 3ml of test solution with million's reagent. Finally white ppt was formed.
- **Test for amino acid:**
  1. **Ninhydrin test:** 3 ml of test solution was heated with 3 drops of 5%ninhydrin solution on boiling water bath for 10 min. Purple coloured were appeared.
  2. **Test for tyrosine:** heated 3ml test solution and 3 drops milion's reagent red colour was obtained.
- **Test for alkaloids:**

  Evaporatedthe aquous Alcoholic and chloroform extracts separately reduce added diluted HCL shake well and filtered. With filtrate, performed following tests.

  1. **Dragondroff's test:** 2-3 ml of test solution was mixed with 3ml of Draggondroff's reagent. Orange brown ppt was formed.

  2.Mayer's test: 2-3ml of test solution was mixed with 3 ml of mayer's reagent. White colour ppt was formed.

  3.Hager's test: 2-3 ml of test solution was mixed with 3 ml of Hager;s reagent. Yellow colour ppt was formed.

- **Test for Glycoside:**
  1. **Test for Deoxy sugar (Killer Killani test):** 2 ml of extracts, added glacial acidic acid, 1 drop of 5%FECL3and concentrated H_2SO_4 reddish brown colour appeared at the junction of the two liquid layer and upper layer appeared bluish green.
  2. **Test for sapponins glycosides (Foam test):** shaken the drug extract vigorously with water. Persistent foam observed.
- **Test for flavonoids:**

  1. Shinoda test: dry powdered or extract, added 5ml of 90% ethanol, then few drop of conc. HCL and 0.5 gm magnesium turnings. Pink coloured were observed.

  2. To small quantity of residue, added lead acetate solution. Yellow coloured ppt, were formed.

  3. Addition of increasing amount of sodium hydroxide to the residue show yellow colour.
- **Test for steroids:**

  1. Salkowski test: 2ml of extract, edited 2 ml of chloroform, and 2 ml of concentrated H2SO4 and shaken well and then chloroform layer appeared red and acid layer showed greenish yellow fluorescence.
- **Test for tannins and phenolic compounds:**

  2-3 ml of aqueous and alcoholic extract, added few drops of following reagents:
  1. 5% Ferric chloride solution: Deep blue black coloured were appeared.
  2. Lead acetate solutions: White ppt.
  3. Acetic acid solutions: Red coloured solutions.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

4. Diluted iodine solutions: Transient red colour.
5. Dil.HNO3: Reddish to yellow colour.

In-vitro antimicrobial screening

In vitro antimicrobial activity of the ethanol extracts of betel vine was screened against a total of four above mentioned bacterial strains.

Disc diffusion method

The antimicrobial activity of ethanol extract of betel vine was screened using disc diffusion technique. The agar plates were prepared by pouring 15 ml of molten nutrient agar media into sterile petriplates. The plates were allowed to solidify and 0.1 % inoculum suspension was swabbed uniformly with sterile cotton and was allowed to stand for 15 minutes. The different dilutions of extracts (0, 20, 40, 60 and 80 %) from initial concentration of 1 mg/ml were loaded on 6 mm autoclaved filter paper discs. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were incubated at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with ruler in millimeter. These studies were performed in triplicate.

MIC by visual analysis

The ethanol extract was later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial strain. Freshly, grown bacterial strains 100 µL (106 cells/ml) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (10–500 µl) from the stock extract (1 mg/ml) and antibiotic, respectively and incubated for 24 h at 37 °C. Presence of turbidity denoted presence of microorganism in the test tube after the period of incubation whereas the complete absence of any turbidity indicates complete inhibition of microbial growth. 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.

Time- kill Kinetics of the ethanol extract Determination

The ethanol extract was later tested to determine the time-kill kinetics for each bacterial strain. Freshly grown bacterial strains 100 µl (about 106 cells/ml) in nutrient broth was inoculated in tubes with nutrient broth supplemented with different concentrations (0, 25 µg, 50 µg and 100 µg) of the extract at 37 °C and optical density was recorded at 1h intervals up to 15 h. Graphs were plotted on the basis of the turbidity varying over a period of time. The growth rate thus obtained was studied for any signs of bactericidal
effects of the plant extract.

Ceftriaxone (commercial name Monocef) (1 mg/ml) was used as positive control. A solution of the solvent in which dried extract was dissolved served as negative control.

Statistical analysis all experiments were carried out in triplicate. Data points were represented by the mean of the measured values. Statistical analysis was carried out using MS-Excel software. Result and discussion

The relative efficiency of bactericidal activity by betel leaf ethanol extract to that of a broad-spectrum antibiotic such as ceftriaxone on the above mentioned microorganisms suggest the possibility of a more cost effective and potentially harmless antibacterial agent. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components and the toxicity of active components, their side effects and pharmacokinetic properties with the view of their use for in vivo studies.

The phytochemical analysis of the betel leaf extract revealed the presence of important bioactive components (Table1). From other scientific studies and researches it was observed that the presence of antioxidants in other medicinal plants imparted antimicrobial properties to those plats. These studies prompted us to study the antimicrobial properties of betel leaf extract. The antimicrobial effects were studied for four bacteria to start with viz Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris and Staphylococcus aureus. To study the antimicrobial properties disc diffusion test, determination of MIC by visual testing and time-kill kinetics was studied on the growth tested bacteria.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

Result and Discussion

This study provided that ethanolic extract of Piper betel stem having various phytochemical like alkaloids, saponin, protein, tannins, carbohydrates, flavonoid. which is essential for their pharmacological response.

The relative efficiency of bactericidal activity by betel stem part ethanol extract to that of a broad-spectrum antibiotic such as ceftriaxone on the above mentioned microorganisms suggest the possibility of a more cost effective and potentially harmless antibacterial agent. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components and the toxicity of active components, their side effects and pharmacokinetic properties with the view of their use for in vivo studies. The phytochemical analysis of the betel leaf extract revealed the presence of important bioactive components (Table 1). From other scientific studies and researches it was observed that the presence of antioxidants in other medicinal plants imparted antimicrobial properties to those plats. These studies prompted us to study the antimicrobial properties of betel leaf extract. The antimicrobial effects were studied for four bacteria to start with viz Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris and Staphylococcus aureus. To study the antimicrobial properties disc diffusion test, determination of MIC by visual testing and time-kill kinetics was studied on the growth tested bacteria.

Table No. 4.1 Result of Phytochemical Screening of Plant Extract

Phytochemical	**Present/absent**
Carbohydrate	++
Protein	++
Phenolic component	+++
Flavanoids	++
Total antioxidant	+++

Table No. 4.2 Phytochemical Screening &*In-vitro* Evaluation of Antimicrobial activity from Stem Extract of plant Piper betel

Extract	**Concentration (mg)**	**Disc size**	**Zone of inhibition (mm)**			
			Disc 1	**Disc 2**	**Disc 3**	**Disc 4**
Standard drug	250	5	9	8	10	14
Test 1	300	5	5	5	6	5
Test 2	400	5	5	5	5	6
Test 3	500	5	6	6	6	6
Test 4	600	5	7	6	6	7

CONCLUSION

The antimicrobial agents are very beneficial agents for the minimization of microbial activity. In the present study the relative efficiency of bactericidal activity by ethanolic extract of Piper betel stem to that of a broad-spectrum antibiotic such as ceftriaxone on the above mentioned microorganisms suggest the possibility of a more cost effective and potentially harmless antibacterial agent. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components and the toxicity of active components, their side effects and pharmacokinetic properties with the view of their use for in vivo studies.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 05, 2021

REFERENCE

1. Verma A. et al, Genetic diversity amongst landraces medioecious of vegetively propagated plant, betel stem, 2004, Biological Sciences, Vol 29, PP 319-328.
2. Sarkar et al, an ethanolic extract of stem of piper betel linn mediates its antiheishmarial activity via apoptosis, parasitor. 2008,Vol 102(6), PP 1249-1255.
3. Sah et al, Clinical microbial,2005, Vol 11, PP 36-42.
4. Sallen et al;Inhibition of low density lipoprotein oxidation,Vol 50, PP 3693-3697
5. Lei D; Antioxidative and Antiplates,2003 Vol. 51;PP 2083-2088.
6. Bhattacharya et al; Radioprotective property of the ethanolic extract of piper betel,2005 Vol.46, PP 165-171.
7. Devmurari et al; Antibacterial activity and phytochemical study of ethanolic extract of piper betel.2012 Vol 2 ,PP 182-186
8. Archana B ,et al; In vitro study of Antimicrobial activity of betel. 2005, Vol 90, PP 727-733
9. Rojas JJ, Screening for antimicrobial activity of betel stem. 2006,Vol 6(2).
10. Shitut S, 1999 Antimirobial efficiency of piper betel; Vol 7(3) PP 137-139.
11. 29. Ghosh G, Subudhi BB, Badajena LD, Ray J, Mishra MK, Mishra SK. Antibacterial activity of Polyalthia longifolia var. angustifolia stem bark extract. Int J PharmTech Res 2011;3:256-60.
12. Phytochemical evaluation, antioxidant and antibacterial activity of Hopea ponga (Dennst) Mabberly extracts. Int J Pharm Pharm Sci 2014;4:326-30.
13. Kavitha PA, Pawan K, Narasimah MTP, Gopinath SM. Antibacterial activity of Polyalthia longifolia against hospital isolates of Bengaluru district. Int J Latest Res Sci Tech 2013;2:508-10.
14. Kabir S, Zahan R, Mosaddik A, Chowdhury AMS, Hasan CM, Rashid MA. Antioxidant and antitumor activities of Polyalthia simiarum (Buch.-Ham. Ex Hook. F and Thomson) Hook. F. and thomson turk. J Pharm Sci 2013;10:405-14.
15. Gowdhami M, Sarkar BL, Ayyasamy PM. Screening of phytochemicals and anti bactereal activity of Annona squamosa extracts. Int J Pharm Sci Invent
16. Ravindra CS, Kalaichelvan VK. Evaluation of antioxidant activity of leaf extracts of Holoptelea integrifolia (Roxb). Planch. Int J Appl Pharm 2014;6:6-8. 2014;3:30-9.
17. Kavita SM, Asha AK, Sucheta AG, Nirmala RD, Kashalkar RV. Evaluation of phenol, flavonoid contents and antioxidant activity of Polyalthia longifolia. J Chem Pharm Res 2011;3:764-9.
18. Mariod AA, Abdelwahab SI, Ahmed YM, Fauzi PNM, Chuen CS. Anti oxidant activity of different parts from Annona squamosa and Catunaregam nilotica methanol extract. Acta Sci Pol Technol Aliment 2012;11:249-57.
19. Saritha B, Karpagam, Sumathi. Studies on antioxidant activity, phenol and flavonoid content of Pisonia alba Span. Asian J Pharm Clin Res 2014;7:106-9.
20. Fewell AM, Roddick JG. Interactive antifungal activity of the glycoalkaloids, solanine and chaconine. Phytochemicals 1993;33:323–8.
21. Barnabas CG, Nagarajan S. Role of flavonoids and tannins in some medicinal plants: a review. Fitoterapia 1988;3:508-10.
22. Burapedjo S, Bunchoo A. Antimicrobial activity of tannins from Terminalia citrine. Planta Med 1995;61:365-6.
23. Uzuma D, Ahmadu R, Bwai DM, Thomas SA. Phytochemical screening and antibacterial activity of Polyalthia longifolia crude extracts. Asian J Pharm Biol Res
24. Sivashanmugam AT, Tapan KC. In vitro evaluation of free radical scavenging activity of medicinal plant Polyalthia longifolia (Sonner.) Thw. leaf extract. J Pharm Res 2011;4:3776-80. 2011;4:480-5.
25. Nandal, N. Impact of product innovation on the financial performance of the selected organizations: A study in indian context. Psychol. Educ. J. 2021, 58, 5152–5163.
26. Lalawmpuii PC, Malsawmtluangi C, Zothanpuia, Kakoti BB. Phytochemical screening and determination of antioxidant activity of Helicia nilagirica Bedd., an ethnomedicinal plant of Mizoram, India. Asian J Pharm Clin Res 2014;7:246-9.
27. Goveas SW, Abraham A. Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of Coscinium fenestratum. Asian J Pharm Clin Res 2013;6:218-21.