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**Manjulata Vyas**
Assistant Professor in Department of Chemistry Shree Shree Laxmi Narayan Govt. Pench valley P. G. college, Parasia, Chhindwara, Madhya Pradesh, India

**Munesh Kumar Sharma**
Assistant Professor in Department of Microbiology, Rajshree Medical Research Institute, Bareilly, Uttar Pradesh, India

**Yogendra Singh Chouhan**
Assistant Professor in Department of Chemistry, Govt. P.G. Degree College Khargone, Madhya Pradesh, India

**CS Goswami**
Professor, Department of Chemistry, Govt. KRG College Gwalior, Madhya Pradesh, India

**Correspondence**
**Manjulata Vyas**
Assistant Professor in Department of Chemistry Shree Shree Laxmi Narayan Govt. Pench valley P. G. college, Parasia, Chhindwara, Madhya Pradesh, India

# Phytochemical investigation and antimicrobial activity of plant constituents of *Piper betle* leafs

**Manjulata Vyas, Munesh Kumar Sharma, Yogendra Singh Chouhan and CS Goswami**

**Abstract**
*Piper betle* Linn. (Paan) belongs to family Piperaceae. It predominantly found in India, Srilanka, Thailand, and Southeast Asian countries. It's leaf contain phytochemical Which tested in Ethyl acetate (EA), acetone, methanol and petroleum ether extract of leaf with having related antimicrobial activity for medicinal use. Except Petroleum ether extract carbohydrate test were positive. Ethyl Acetate extracts for proteins, acetone extract for Anthroquinone Glycosides, Ethyl Acetate and Acetone extracts for Saponins, EA extract for Flavanoids were positive. Tannins, Phenolic Compounds, Amino Acid and Alkaloids were positive except Petroleum Ether extract. The antimicrobial susceptibility tested by well and disc diffusion method for pathogenic bacteria as *Pseudomonas vesicularis, Streptococcus faecalis, Escherichia coli* etc. *Escherichia coli* showed the strong resistance to extract of the Petroleum Ether, Methanol and Ethyl acetate. Methanol extract showed maximum inhibition against all the bacteria. Study proved that the extract of leaf useful against infectious diseases.

**Keywords:** *Piper betle* Linn. phytochemical, antimicrobial activity, metabolites, pathogenic bacteria

## 1. Introduction
India is an endowed with a rich wealth of medicinal plants. Herbal remedies of plants are very old in history. And it is useful for the treatment of diseases in traditional medicine systems from thousands of year and continue to play a major role in the primary health care of about 80% of the world's inhabitants (World Health Organization statistic) [1]. As such attention is focused on natural sources of lead compounds in which exists a wealth of more complex compound structures and novel modes of action (Lesney, 2004). While there are many different compounds that can be found within the 250000 flowering plant species worldwide, they can be classified into distinct classes of compounds based on similar characteristics [2]. The main classes of bioactive compounds from plants include flavonoids, terpenoids, terpenes, alkaloids, saponins and coumarins (Cowan, 1999).
Isolation and characterization of biologically active compounds of medicinal herbs were an unexpectable approach in drug discovery which lead to the isolation of pharmacologically active drugs such as cocaine, codeine, digitoxin, quinine and morphine (Newman *et al*., 2000). It is after the compound has been characterized (both chemically and biologically) that it can be assessed in terms of its potential as a lead compound and indeed if the compound is actually novel and worthy of further investigations [3]. To be considered as a pharmaceutical lead, the active compound must have a reasonably simple structure so it can be easily synthesized or have a novel mode of action that is more efficient than current drugs [4]. The determination useful for the potential and promote the use of herbal based medicine, it is essential to intensify the study of medicinal plants that find place in folklore [5-7]. So the study was done to investigate the phytochemical characteristics and in-vitro antimicrobial susceptibility of extract from *Piper betle* plant's leaf against the different micro-organisms.

## Materials and methods
Collection and Authentication of *Piper betle* leafs of *Piper betle* were purchased from the local market of Narsinghpur District (Madhya Pradesh ),Vouchers specimen were deposited at the Herbarium, Head of Botany Dept. Govt. M. L. B. College Bhopal.

**Plant Material**

The collected leafs were dried under shade and pulverized by mechanical grinder and the powder was passing through sieve. The powdered material was successfully extracted with Petroleum ether by hot continuous percolation method in soxhlet apparatus for 15 hours [8]. The defatted material was then extracted with Ethyl Acetate, Acetone and methanol to get different extracts. The extracts were concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests after performing the test the Petroleum Ether (PE), Acetone (AE), methanol (ME) & Ethyl Acetate (EA) and taken for the antimicrobial screening.

**Preparation of Extracts**

The leafs of *Piper betle* were collected from Narsinghpur district and these were made dry and powdered in a grinder. This powder was then sieved to isolate a fine powder soxhlet apparatus (30 cycles each), carried out first with petroleum ether (60-80 ºC) to defat the material (yield: 1.49% w/w). The defatted material was then extracted with alcohol acetone chloroform and water to get different extracts [9]. The extracts were concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests. Test extracts were then dried crushed to fine powder and dissolved in 10% aqueous dimethylsulfoxide (DMSO) for further study [10].

For extraction successive solvent extraction was performed using solvent as per their polarity order in soxhelet apparatus. Pet Ether (40:60) (PE), Acetone (AE), Methanol (ME) and Ethyl Acetate (EA) was selected as the solvents.

**Phytochemical Investigation**

**Test for carbohydrates**

The test solution is combined with a small amount of Molisch reagent, (α-naphthol dissolved in ethanol) in a test tube solution. Then concentrated $H_2SO_4$ was added through the sides of the test tube, without mixing, to form a bottom layer. A positive reaction was indicated by appearance of a purple ring at the interface between the acid and test layers.

**Test for reducing sugars**

1 ml. Fehling's A and 1 ml. Fehling's B solutions were mixed and it was boiled for 1 min. with equal volume of extract. It was heated in a boiling water bath for 5-10 min at 60 °C except Petroleum ether extract showed the presence of reducing sugar by changing the color of solution into brick red color precipitate.

**Test for monosaccharides**

Equal volume of freshly prepared Barfoed's reagents and different extracts mixed and it was heated for 1-2 minutes in a boiling water bath and then it was cooled which forms a brick-red precipitate.

**Test for proteins**

To 3 ml of extracts with Biuret reagent. The reagent turns from blue to violet in the presence of proteins. Only Ethyl Acetate extract showed the presence of proteins.

This technique is used to determine the presence of the amino acid tyrosine. Million's A, is added to the test solution. A yellow color indicates the presence of protein. A drop of Million's B, a sodium nitrite solution, is then added. A red color indicates a positive reaction.

**Test for steroids**

2 ml of extract, 2 ml of chloroform and 2 ml of concentrated $H_2SO_4$ were added and it was shaked well and the color was observed. The chloroform layer shows a red to blue color and the acid layer shows a green fluorescence.

**Test for glycosides**

50 mg of each extract sample added in to 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added. This solution was carefully transferred to the surface of 2 ml concentrated $H_2SO_4$ and the observation was noted down. Brown ring at interphase is characteristic of cardiac glycosides. All extracts showed negative test.

3 ml of extract, dilute $H_2SO_4$ was added and it was boiled and filtered. To the cold filtrate, equal volume of benzene or chloroform was added it was shaked well. The organic solvent layer was separated and ammonia was added to it and the color of ammonical layer was observed. Only Acetone extract showed the change in color from rose red to intense red which showed the presence of Anthroquinone Glycosides.

Extracts was shacked vigorously, with water. The foam was observed. The presence of lumps in the foam showed Presence of Saponin Glycosides Ethyl Acetate and Methanol extracts showed the positive test and remaining two showed negative test.

**Test for flavonoids**

Extract 5 ml of 95% ethanol was added then few drops of concentrated HCl and 0.5 gm of magnesium turnings were added and the change was observed. Pink color obtained into small quantity of residue lead acetate solution was added & the color change was observed in Ethyl Acetate extract other three extracts showed negative test.

**Test for fats and oils**

**A.** To 2-3 ml of the alcoholic solution of extract, add few ml of chloroform and solubility was observed.

**B.** To 2-3 ml of the alcoholic solution of extract, add few ml of 90% ethanol and solubility was observed.

**Test for tannins and phenolic compounds**

To 2-3 ml of alcoholic solution of extract, few drops of 5% w/v ferric chloride solution was added and the color change was observed. A Green color obtained showed the presence of Tannins in all three extracts except Petroleum Ether.

**Test for alkaloids**

To 2-3 ml of filtrate, few drops of Dragendorff's reagent were added and precipitate was observed. Occurrence of orange red precipitate showed the presence of alkaloids in all three extract other then Petroleum Ether extract.

**Test for amino acids**

3 ml of the test solution was heated with 3 drops of 5% ninhydrin solution in a boiling water bath for 10 min and then the color change from blue to blue – violet was observed in all three extracts showed positive test except Petroleum Ether extract.

**Test for terpenoids**

2-3 ml of test sample, add piece of tin, and 2 ml thionyl chloride then color change observed in all three extracts except Petroleum Ether extract.

**Table 1:** Phytochemicals of *Piper betle* leaf

| Tests | | Piper Ethyl Actetate | Piper acetone | Piper Methanol | Piper Petroleum Ether |
|---|---|---|---|---|---|
| Carbohydrate | | Positive | Positive | Positive | Negative |
| cardiac glycoside | | Negative | Negative | Negative | Negative |
| anthro glycoside | | Negative | Positive | Negative | Negative |
| Saponin | | Positive | Positive | Negative | Negative |
| Flavanoid | | Positive | Negative | Negative | Negative |
| Alkaloid | Dragandr off | Positive | Positive | Positive | Negative |
| | Wagner | Positive | Positive | Positive | Negative |
| Tannin & phenols | 5% FeCl3 | Positive | Positive | Positive | Negative |
| | lead acetate | Positive | Negative | Positive | Negative |
| Protein | Biurate | Positive | Negative | Negative | Negative |
| sulpher containing protein | | Positive | Positive | Positive | Negative |

**Screening for Antimicrobial Activity**

All the extracts and fractions from *Piper betle* show antibacterial activity against all tested strains. Zone of inhibition were test for concentration ranging from 12.5mg/ml to 50mg/ml. (12.5mg/ml, 25mg/ml, 37.5mg/ml, 50mg/ml). Antibacterial activity tested for two methods such as well diffusion method and Disc diffusion method.

**Screening of Antibacterial activity**

The agar well diffusion method technique (Bauer *et al*., 1966) was used to determine the antibacterial activity of the plant extracts. Inoculate the different culture on Mueller Hinton Agar plate. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labeled accordingly; 50mg/ml, 37.5mg/ml, 25mg/ml, 12.5mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48hours in upright condition. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

The disc diffusion method (Bauer *et al*., 1966) was used to test antimicrobial activity of the extractives against 7 bacteria. Solutions of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (5mm diameter) were then impregnated with known amount of the test substances using micropipette and the residual solvent were completely evaporated. Dried and sterilized filter paper discs (5mm diameter) were then impregnated with known amount of the test substances using micropipette and the residual solvent were completely evaporated. Discs containing the test material were placed on Mueller Hinton agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30ug/disc) and blank discs (impregnated with solvent followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4ºC) for 24 h to allow maximum diffusion. These were a gradual change of test material concentration in the media surrounding the discs. The plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms with the bacterial control strains. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition included 5 mm disc expressed in millimeter.

The test extracts were individually tested against a panel of microorganisms including *Pseudomonas vesicularis, Streptococcus faecalis, Aeromonas hydrophila, Salmonella typhi, Staphylococcus cohni, Serratia ficaria, Escherichia coli (E. coli).*

**Table 2:** Zone of inhibition (mm) of bacterial growth by varying concentration of extracts of *Piper betel* by agar well diffusion method.

**A. Petroleum Ether extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 6.95±0.77 | 7±1.42 | 8.15±1.05 | 8.90±1.02 |
| Streptococcus faecalis | 7.45±0.6 | 7.65±0.54 | 7.75±0.44 | 7.85±1.25 |
| Aeromonas hydrophila | 7.00±1.44 | 7.05±0.33 | 8.57±0.66 | 6.87±0.44 |
| Salmonella typhi | 6.75±0.44 | 6.95±1.01 | 7.05±0.66 | 7.23±1.6 |
| Staphylococcus cohni | 6.15±0.55 | 6.52±0.65 | 7.25±0.75 | 8.93±0.46 |
| Serratia ficaria | 6.01±0.2 | 6.04±0.21 | 7.34±0.15 | 8.25±0.25 |
| Escherichia coli | 8.34±0.55 | 9.44±0.45 | 11.02±0.15 | 12.67±0.15 |

**B. Acetone extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 6.27±0.22 | 6.54±0.44 | 7.89±0.22 | 8.1±0.36 |
| Streptococcusfaecalis | 5.9±0.22 | 6.1±0.44 | 6.52±0.45 | 7.12±0.66 |
| Aeromonas hydrophila | 5.9.±0.22 | 6.0 ±0.2 | 6.1±0.12 | 6.30±0.44 |
| Salmonella typhi | 7.00±0.67 | 7.67±0.54 | 8.72±0.96 | 9.35±0.86 |
| Staphylococcus cohni | 6.07±0.44 | 6.21±0.34 | 6.36±0.45 | 6.7±0.61 |
| Serratia ficaria | 5.9±0.66 | 6.11±0.44 | 7.2±0.18 | 7.89.±0.44 |
| Escherichia coli | 6.56±0.63 | 8.34±0.18 | 9.68±0.95 | 11.01±1.08 |

**C. Methanol Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 5.98±0.67 | 6.14±0.34 | 8.22±0.75 | 9.25±0.39 |
| Streptococcus faecalis | 6.12±0.48 | 7.89±0.43 | 8.43±0.77 | 9.89±0.84 |
| Aeromonas hydrophila | 7.85±0.77 | 8.34±0.43 | 9.65±0.45 | 9.78 ±0.87 |
| Salmonella typhi | 9.22±0.92 | 9.47±0.67 | 10.09±0.65 | 12.98±0.26 |
| Staphylococcus cohni | 6.01±0.22 | 6.10±0.44 | 6.21±0.21 | 6.25±0.77 |
| Serratia ficaria | 6.89±0.22 | 6.99±0.55 | 7.25±0.56 | 7.99±0.65 |
| Escherichia coli | 10.87±0.45 | 13.75±0.43 | 15.32±0.68 | 16.81±0.25 |

**D. Ethyl Acetate Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 5.71±0.22 | 6.95±0.72 | 9.57±0.25 | 10.15±0.65 |
| Streptococcus faecalis | 6.21±0.28 | 6.55±0.68 | 8.95±0.46 | 9.75±0.46 |
| Aeromonas hydrophila | 6.11±0.25 | 7.25±0.42 | 7.58±0.93 | 7.95±0.55 |
| Salmonella typhi | 6.13±0.5 | 7.35±0.44 | 8.65±0.28 | 11.17±0.57 |
| Staphylococcus cohni | 6.09±0.23 | 7.18±0.63 | 8.15±0.74 | 9.35±0.23 |
| Serratia ficaria | 5.98±0.16 | 6.87±0.19 | 7.98±0.32 | 8.82±0.76 |
| Escherichia coli | 11.42±0.33 | 13.21±0.55 | 14.27±0.28 | 16.42±0.27 |

**Table 3:** Zone of inhibition (mm) of bacterial growth by varying concentration of extracts of *Piper betel* by agar disc diffusion method.

**A. Petroleum ether Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 5.89±0.55 | 5.97±0.44 | 6.01±0.25 | 6.05±0.72 |
| streptococcus faecalis | 6.78±0.44 | 7.89±1.07 | 9.11±0.49 | 9.37±0.15 |
| Aeromonas hydrophila | 6.21±0.12 | 6.45±0.89 | 6.95±0.99 | 7.98±0.25 |
| Salmonella typhi | 6.56±0.53 | 7.25±0.22 | 7.97±0.44 | 8.97±0.89 |
| Staphylococcus cohni | 7.12±0.12 | 7.37±0.66 | 8.27±0.79 | 9.43±0.55 |
| Serratia ficaria | 6.01±0.12 | 6.05±0.22 | 6.1±0.39 | 6.12±0.65 |
| Escherichia coli | 8.21±0.21 | 9.15±0.35 | 10.45±0.57 | 10.560.85 |

**B. Acetone Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 8.12±0.56 | 9.25±0.44 | 10.04±0.33 | 11.25±1.01 |
| Streptococcus faecalis | 6.35±0.15 | 6.54±0.45 | 6.65±0.46 | 6.76±1.01 |
| Aeromonas hydrophila | 7.07±0.95 | 8.01±0.14 | 8.98.±0.66 | 9.07±0.22 |
| Salmonella typhi | 09.22±0.67 | 10.15±0.55 | 11.21±0.89 | 12.17 ± 0.29 |
| Staphylococcus cohni | 7.35±0.69 | 7.87±0.67 | 8.98±0.75 | 9.1±0.29 |
| Serratia ficaria | 6.12±0.55 | 6.65±0.34 | 7.25±0.21 | 8.78±0.68 |
| Escherichia coli | 9.77±0.66 | 9.95±0.86 | 10.85±0.17 | 14.75±0.13 |

**C. Methanol Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| Pseudomonas vesicularis | 11.57±0.75 | 12.09±0.77 | 12.92±0.65 | 14.07±0.87 |
| Streptococcus faecalis | 8.98±0.22 | 9.93±0.78 | 10.07±0.23 | 10.92±0.87 |
| Aeromonas hydrophila | 8.85±0.60 | 9.89±0.65 | 10.9±0.23 | 11.29±0.65 |
| Salmonella typhi | 9.09±1.23 | 10.34±1.48 | 12.45±0.75 | 14.25±0.91 |
| Staphylococcus cohni | 6.89±0.78 | 7.85±0.93 | 8.11±0.94 | 9.1±0.23 |
| Serratia ficaria | 7.01±0.65 | 7.43±0.85 | 7.99±0.24 | 8.25±0.32 |
| Escherichia coli | 12.51±0.44 | 15.45±0.24 | 16.15±0.22 | 16.19±0.31 |

**D. Ethyl Acetate Extract**

| Microbial Strains | Extracts Concentration | | | |
|---|---|---|---|---|
| | 12.5 mg/ml | 25 mg/ml | 37.5 mg/ml | 50 mg/ml |
| *Pseudomonas vesicularis* | 6.01±0.25 | 6.08±0.88 | 6.11±0.94 | 6.17±0.19 |
| *Streptococcus faecalis* | 5.78±0.85 | 5.85±0.97 | 6.11±0.18 | 6.13±0.5 |
| *Aeromonas hydrophila* | 6.1±0.12 | 6.11±0.25 | 6.18±0.92 | 6.32±0.45 |
| *Salmonella typhi* | 7.±0.18 | 7.11±0.87 | 7.22±0.13 | 7.43±0.25 |
| *Staphylococcus cohni* | 6.12±0.85 | 6.21±0.82 | 6.43±0.54 | 7.05±0.94 |
| *Serratia ficaria* | 5.87±0.12 | 5.97.1±0.98 | 6.12±0.13 | 6.21±0.54 |
| *Escherichia coli* | 10.01±0.74 | 11.65±0.61 | 13.12±0.6 | 13.99±0.87 |

**Results and Discussions**

The Phytochemical tests of Ethyl acetate, Acetone, Methanol and Petroleum ether extracts from *Piper betle* leaves were performed for carbohydrate, cardiac glycoside, anthroglycoside, Saponin, flavanoid, Alkaloid, Tannin & phenols, Protein, sulpher containing protein and the results were presented in Table 1. The Petroleum ether extract showed the absence of carbohydrate other three showed positive test. Only Ethyl Acetate extracts showed the presence of proteins. All four extracts of *Piper Betle* showed negative test for Cardiac Glycoside. Only Acetone extract showed the test for Anthroquinone Glycosides. Ethyl Acetate and Acetone extracts showed the positive test of Saponins. EA extract showed the positive test for Flavanoids. The Flavanoids showed antimicrobial effect with other medicinal properties [11]. All three extracts showed positive test for Tannins and Phenolic Compounds except Petroleum Ether extract. About tannin which may have the toxic to different microorganisms (Bacteria [12], yeast and filamentous fungus, viruses [13]) so it shows antimicrobial activity [14, 15]. All three extract other showed positive test for Alkaloids other then Petroleum Ether extract. All three extracts showed presence of Amino Acid except Petroleum Ether extract. The antimicrobial properties of extract of *Piper betle L.* in the presence of interfering substances tested by *in vitro* technique for *Pseudomonas vesicularis, Streptococcus faecalis, Aeromonas hydrophila, Salmonella typhi, Staphylococcus cohni, Serratia ficaria, Escherichia coli* bacterial species which is presented in Table. 2 to 3. In well diffusion *Escherichia coli* exhibited the strong resistance to the Petroleum Ether extract of *Piper betle* L. in the presence of interfering substances. For the results of the Petroleum ether extract of *Piper betle* L. showed a good antimicrobial activity against *Escherichia coli*. At the higher concentration 50mg/ml, shows larger zone of inhibition and at the lowest concentration, 12.5mg/ml, smaller zone of inhibition [16, 17]. Methanol extract showed the highest antimicrobial property against at the concentration of 50 mg/ml for *Escherichia coli* and Ethyl acetate extract showed the highest inhibition against *E. coli* at the concentration of 50 mg/ml. In disc diffusion method the methanol extract showed highest inhibition for the growth of *E. coli* at 50mg/ml concentration.

As per the discussion part Natural products form plants carried extensive bioactive components which have proved to be useful against different diseases including infectious diseases. It is proved that present extract of *Piper betle* leaves defined the antimicrobial activity. Antibacterial activity against *Bacillus* and *E. coli. Bacillus sp* showed maximum zone of inhibition [18]. Acetone extract showed zone of inhibition against the bacteria with maximum zone of inhibition was observed in *E. coli* [19]. In this present study, methanol extract showed maximum inhibition against all the bacteria and this may be due to alkaloids, tannin and phenols compound. Maximum zone of inhibition was observed in *E. coli*.

**Conclusion**

The study concluded bactericidal activity with relative efficiency of betel leaf methanol extract to that of a antibiotic such as Kanamycin 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, possible component that support antibacterial action, their side effects and pharmacokinetic properties with the view of their use for *in vivo* studies.

**References**


1. Farnsworth NR, Akerle O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bul. World Health Org. 1985; 63:965-981.
2. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure–activity relationships. Free Radic Biol Med. 1997; 22:749-760.
3. Kumar N, Gupta S, Tripathi AN. Gender-specific responses of Piper betle L. to low temperature stress: changes in chlorophyllase activity. Biol Plant. 2006; 50:705-708.
4. Ramji N, Ramji N, Iyer R, Chandrasekaran S. Phenolic antibacterials from Piper betle in the prevention of halitosis. J Ethnopharmacol. 2002; 83:149-152.
5. Cragg GM, Boyd MR, Cardellina JHII, Newman DJ, Snader KM, McCloud. Ethno botany and drug discovery: the experience of the US National Cancer Institute developmental therapeutics Program, National Cancer Institute, Bethesda. Ciba Found. Symp. 1994; 185:178-190, 190-196.
6. De Smet PA. The role of plant-derived drugs and herbal medicines in healthcare. Drugs. 1997; 54:801-840.
7. Vogel HG. Similarities between various systems of traditional medicine: Considerations for the future of ethnopharmacology. J Ethnopharmacol. 1998; 35:179-190.
8. Mahesh B, Satish S. Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. World J Agric Sci. 2008; 4 (S): 839-843.
9. Sarkar A, Kumar KA, Dutta NK, Chakraborty P, Dastidar SG. Evaluation of *In vitro* and *In vivo* antibacterial activity of dobutamine hydrochloride. Indian Journal of Medical Microbiology. 2003; 21(3):172-178.
10. Chatterjee A, Satyesh CP. The treatise on Indian medicinal plants. New Dehli. 2, 73-75.
11. Harborne JB, Willians CA. Advances in flavanoid research since 1992. Phytochemistry, Oxford. 2000; 55:481-504.
12. Harborne JB. Photochemical Methods. A guide to modern techniques of plant analysis. Chapman and Hall, London. 1973, 279.
13. Lin LU, Shu-wen L, Shi-bo J, Shu-guang W. Acta Pharmacol Sin. 2004; 25(2):213-218.
14. Akiyama H, Kazuyasu F, Yamasaki O, Oono T, Iwatsuki KJ. Antimicrobial Chemotherapy 2001; 48(48):487-491.
15. Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H *et al*. Microbiol Immunol. 2004; 48(4):251-261.
16. Kokate CK. Practical pharmacognosy. Vallabh prakashan, New Delhi. Preliminary phytochemical screening, Chapter, 6, 106-111.
17. Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A *et al*. Management of giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. J Ethnopharmacol. 1997; 56:233-236.
18. Kumar A, Rajput G, Srivastava G, Dhatwalia Vinod K. Phytocontent screening of Mucuna Seeds and Exploit in Opposition to Pathogenic Microbes. J Biol Environ Sci. 2009; 3(9):71-76.
19. Padma PN, Poonguzhali TV. Phytochemical screening and antibacterial property against human pathogenic bacteria from the leaf acetone extract of piper betle. L. Asian J Biochem Pharm Res. 2015; 1(5):251-259.