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Study of effect of betel leaves (*Piper Betel*) on β-lactamase producing pathogens

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Abstract

The increasing resistance to antimicrobials among pathogens demands new strategies for management of infectious diseases. In this respect, use of combination therapy and/or use of herbal compounds are more likely to result in successful therapeutic outcomes. In the current study, solvent extracts of *Piper betel* leaves were tested for antibacterial activity against pathogenic β-Lactamase producing strains as well as common laboratory cultures. The bioactive compounds from betel leaves were extracted with the help of Soxhlet apparatus at 60°C to obtain a semi-solid mass using different solvents such as ethanol, methanol, acetone and water. The antibacterial activity of solvent extracts of *P. betel* leaves was evidently confirmed by the observed zones of inhibition in the range of 11-26mm. The Minimum bactericidal concentration of methanol extract of *P. betel* leaves was also determined and it was found to be 10mg/ml. Moreover, the sub-inhibitory concentrations of methanol extracts of *P. betel* leaves successfully lowered the MBC of ampicillin from 10mg/mL to 300µg/ml, indicating a synergistic activity. Furthermore, they also showed a moderate anti-oxidant property suggested by the calculated DPPH scavenging activity. In addition, the GC-MS analysis of methanol extract of *P. betel* leaves was carried out and it showed the presence of various bioactive compounds including benzoic acid, pyrazine, coumaran and 4-vinyl-gluacol. These results suggest the potential of *P. betel* leaves as a source of remedy towards the problem of antimicrobial resistance.

Keywords: MBC; Soxhlet; β-Lactamase; Piper Betel; GC-MS; Antimicrobial Resistance

1. Introduction

Exploring the basic characteristic of bacteria introduces us to their adaptive nature and ability to overcome the obstacles in their cell cycle; thus enabling them to survive in unfavorable environments [1]. In view of this fact, the emergence of antimicrobial resistance among pathogens is certainly inevitable. However, the lack of consciousness regarding the long-term exposure of antibiotics on the bacterial community has posed a serious health threat to the common population. This is especially true due to the lack of awareness regarding the harmful consequences of antibiotic abuse [2]. The unnecessary use of antibiotics in sub-minimal doses has led to the emergence of Extended-Spectrum β-Lactamase (ESBL) and Metallo β-Lactamase (MBL) producing pathogens. These β-lactamase enzymes are evolved comparatively later in the timeline of antimicrobial resistance and target the newer generations of antibiotics. The ESBLs are plasmid mediated enzymes which are capable of hydrolyzing and inactivating a wide variety of β-lactams including third-generation cephalosporin's, penicillins and aztreonam [3]. The MBL producers on the other hand characteristically show resistance to the last resort carbapenem antibiotics like imipenem, ertapenem and meropenem [4].

Although conquering the bacterial system is difficult, certain strategies like use of combination therapy have worked effectively in the past in treating infectious diseases [5]. However, the treatment protocols may present themselves with serious antibiotic overdose and dependence leading to decreased immunity, in cases of highly infectious cases [6, 7].

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More recently, attempts are made towards the use of herbal remedies in treating infections caused by drug-resistant strains of pathogens [8-10].

Piper betel leaves, commonly known as Paan or betel leaves is one such example of an herbal source that is explored for its activity against infections caused by drug-resistant pathogens. It belongs to the *Piperaceae* family and is traditionally used in India as a mouth freshener [11, 12]. Dental caries results due to the bacterial fermentation of carbohydrates and decreased salivary flow in the oral cavity. The antibacterial nature of *P. betel* leaves has shown effectiveness against the same [13, 14]. It is also useful against other problems of the oral cavity like bad breath [15].

The ayurvedic medicine has evidently considered this plant for its antiseptic properties for hundreds of years [16]. Moreover, the essential oils extracted from betel leaf find applications in a variety of industrial products including perfumes, mouth fresheners, tonics, food additives as well as certain medicines [17]. Other properties of betel leaves such as a carminative, stimulant, antimicrobial, antioxidant, antidiabetic, immunomodulatory, anti-inflammatory, and radioprotective are also well documented [11, 17, 18].

Considering the increasing antibiotic resistance among pathogens, the objective of our study was to investigate the efficacy of *P. betel* leaf extract as a possible alternative source of medicine by exploring its antioxidant, antibacterial, as well as resistance reversal activities against ESBL and MBL producing clinical isolates as well as common laboratory cultures.

2. Material and methods

2.1. Plant material

Piper betel leaves were obtained from a local shop and authenticated by an expert botanist.

2.2. Test organisms

Gram-negative pathogens isolated and characterized for β-lactamase production in a previous study was used as test organisms [19-21]. A total of forty-three representative β-lactamase producing pathogens including *K. pneumoniae* (11), *Escherichia coli* (17), *P. aeruginosa* (5), *Proteus mirabilis* (6) and *Citrobacter diversus* (4) were used in the current study. In addition, seven common laboratory cultures including *E. coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Staphylococcus aureus 6538, Shigella* sp., and *Vibrio cholerae* were also used. The β-lactamase producing isolates were maintained on Nutrient Agar (NA) slants supplemented with 100µg/mL of ampicillin whereas the laboratory cultures were maintained on regular NA slants. The cultures were stored at refrigerated conditions until further use.

2.3. Preparation of Plant Extract

The leaf samples were washed with distilled water, dried in shade for 8 days, and ground to fine powder with the help of a mechanical blender. The extraction of bioactive compounds was carried out from a 100g sample in 200mL solvents (viz., ethanol, methanol, acetone and water) using soxhlet apparatus, over a period of 12h. The extract was further concentrated at 40°C on a water bath to obtain a semisolid mass. This mass was re-suspended in the respective solvents to get the required concentration of the extract for carrying out further analysis. These concentrates were prepared in large volumes and stored at 4°C until further use in order to avoid batch to batch variations in our study [22, 23].

2.4. Sterility testing of plant extract

The sterility of the extracts was confirmed by checking for bacterial or fungal growth after spot inoculating them on a sterile Nutrient Agar (NA) and Sabouraud's Agar (SAB) plate respectively [24]. The NA plates were incubated at 37°C and SAB plates at 30°C for an extended duration of 7 days to confirm the absence of contaminants.

2.5. A qualitative study of the inhibitory activity of *P. betel* leaf extracts against test cultures

The antibacterial effect of *P. betel* leaf extracts against test pathogens was determined by the agar well diffusion method [25]. Sterile molten NA butt was seeded with 0.4mL of 24h old test pathogens (0.1 OD_{540nm}) and poured into sterile petri-plates. After solidification, wells were punched into the medium using a sterile cork-borer and 50µL of various plant extracts were added to the same. It was then allowed to diffuse through the wells during its incubation at 37°C for 24h, after which the resulting zones of inhibition were measured. Control wells were also set up using 50µL of solvents used in the study, for each isolate.

2.6. Determination of MBC of *P. betel* leaf extracts against test cultures

Agar dilution method was carried out to determine the minimum bactericidal concentration (MBC) of *P. betel* leaf extracts against the test pathogens. Different concentrations of solvent extract ranging from 5-50mg/mL with an interval of 5mg/mL were supplemented into molten NA butts cooled to around 40°C. The test isolates were spot inoculated (5µL) on solidified medium and incubated at 37°C for 24h. MBC was defined as the lowest concentration of *P. betel* leaf extracts that completely inhibited the growth of test cultures [26].

2.7. Evaluation of the synergistic activity of *P. betel* leaf extracts and ampicillin against test cultures

The agar dilution method was similarly used to determine the synergistic activity between *P. betel* leaf extracts and ampicillin. It was carried out by incorporating sub-lethal (½MBC) concentrations of *P. betel* leaf extracts into molten NA butt which were cooled to around 40°C along with 100-500µg/mL of ampicillin with an interval of 100µg/mL [26].

2.8. Evaluation of antioxidant activity of *P. betel* leaf extract by DPPH method

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay was carried out with an aim to measure the scavenging activity of any antioxidant towards DPPH, which is stable organic nitrogen radical. The electrons in DPPH pair with a suitable antioxidant present in the plant extract and lose its color. The disappearance of the violet color of DPPH is proportional to the antioxidant activity of plant extract. In our study, a 2mL volume of various diluted aliquots of *P. betel* solvent extracts and ascorbic acid (control) was mixed with 2mL of 0.2mM DPPH prepared in ethanol. The mixture was kept in dark for 30min after vigorous shaking. The absorbance of DPPH was measured at 520nm using a spectrophotometer. Ascorbic acid was used as a standard antioxidant. The capability to scavenge the DPPH radical of each solution was calculated using the following equation:

$$\text{Scavenging effect (\% inhibition)} = [(\text{Control} - \text{Test})/\ \text{Control})] \times 100.$$

The IC_{50} was calculated by plotting a graph of the %inhibition versus various concentrations of extract in µg/mL [27-29].

2.9. Gas Chromatography-Mass Spectrophotometry analysis

The bioactive components from *P. betel* leaf extracts were analyzed by GC-MS HP 7890 system (Agilent technologies). Capillary column with dimensions 30m X 0.25mm X 0.25µm was equipped. The program used for GC oven temperature was 5min isothermal at 300ºC, followed by 90º-260ºC at a rate of 10ºC/min, then held at 260ºC for 5min. The injection port temperature was 240ºC. Along with that a Joel, AccuTOF GCV MS system, with a time of flight analyzer, was used [30]. The entire analysis was carried out at IIT Bombay, Mumbai 400076. The compounds of the *P. betel* leaf extracts were identified by comparison of their retention indices (RI) and mass spectra fragmentation with those on the stored library available with IIT, Bombay.

2.10. Statistical analysis

All experiments were carried out in triplicates and represented as mean values.

3. Results and discussion

3.1. Sterility testing

The solvent extracts obtained from *P. betel* leaves were found to be free of any bacterial or fungal contamination as confirmed by the absence of growth on NA and SAB plates respectively. The extended incubation time confirmed the absence of slow-growing contaminants and stressed cells that may have survived the processing of solvent extracts.

3.2. A qualitative study of the inhibitory activity of *P. betel* leaf extracts against test cultures

Table 1 represents the antibacterial activities of methanol and ethanol extracts of *P. betel* leaves carried out by agar well diffusion method. It showed zones of inhibition in the range of 11-26mm for methanol extracts against both β-lactamase producers as well as laboratory cultures. Ethanol extracts showed very less or no antibacterial activity against the test isolates. The acetone and water extracts did not show any activity against the test cultures. Consequently, no further studies were carried out using these extracts. The solvent controls used in our study also did not show any zone of inhibition against test cultures.

Similar findings were reported in another study where methanolic extracts of *P. betel* leaves were found to be more effective against pathogenic strains of *E. coli*, *P. aeruginosa* and *S. aureus* with zones of inhibition of 25mm, 17.5mm and 15mm respectively [22]. In another study, the ethanolic extract of *P. betel* leaves showed considerable inhibitory activity ranging from 12.33mm to 14.67mm against all the test organisms in their study. The maximum zone of inhibition was observed against *S. aureus* (14.67mm) and minimum against *S. dysenteriae* (12.33mm) [17]. Ethanol extracts were also found to be effective against pathogenic drug-resistant strains of *E. coli, S. aureus* and *P. aeruginosa* in a previous study and showed zones of inhibition in the range of 16-30mm [31]. A recent study carried out using the crude solvent extracts of *P. betel* leaves reported lower activity on gram-negative bacteria as compared to the gram-positive bacteria. The most sensitivity was found against *S. aureus* followed by *B. subtilis* in their study [32].

Table 1 Antibacterial activity of methanol extracts of *P. betel* leaves against β-lactamase producers and common laboratory cultures

Test organisms	No. of isolates	Zones of inhibition of solvent extracts in mm		Mean MBC in mg/mL
		Methanol	Ethanol	
β-lactamase producers				
K. pneumoniae	11	16.33-21.48	8.67-11.33	10
E. coli	17	15.67-20.56	9.42-11.67	10
P. aeruginosa	05	11.82-16.78	-	10
P. mirabilis	06	14.33-19.93	-	10
C. diversus	04	14.67-26.33	9.50-10.67	10
Laboratory cultures				
E. coli	01	15.33	-	10
S. typhi	01	18.67	11.33	10
S. paratyphi A	01	17.56	10.5	10
S. paratyphi B	01	16.22	12.67	10
S. aureus 6538	01	13.33	-	10
Shigella sp.,	01	18.67	-	10
V. cholera	01	17.5	-	10

3.3. Determination of MBC of *P. betel* leaf extracts against test cultures

The ineffectiveness of acetone, ethanol and water extracts, and the antibacterial activity of methanol extracts of *P. betel* leaves against the test cultures were further confirmed by determination of it MBCs. Table 1 represents the MBC of the methanol extracts of *P. betel* leaves carried out by agar dilution method. It was found to be 10mg/mL. Other solvent extracts of *P. betel* leaves showed very high MBC compared to that of methanol extract.

A similar study reported MIC values of 0.625%w/v for *E. coli* ATCC 25922, *V. cholerae* ATCC 6395 and *S. aureus* ATCC 25923. It further revealed that the antibacterial activity was highest at around neutral pH and moderate temperature [17]. Another study reported the MIC of methanol extract of *P. betel* leaves to be in the range of 1125 to 2250 µg/mL [32]. A recent study reported MBC values of ethanol extracts of *P. betel* leaves in the range of 2.5-10%w/v against *E. coli* (5%w/v), *P. aeruginosa* (5%w/v), *S. aureus* (10%w/v) and *C. albicans* (2.5%w/v) [33].

3.4. Evaluation of the Synergistic activity of *P. betel* leaf extracts and ampicillin against test cultures

Table 2 represents the synergistic effect of methanol extract of *P. betel* leaves and ampicillin against the test cultures. Interestingly, the MBC value of ampicillin was found to reduce from 10mg/mL to 300µg/mL when used in combination with methanol extract of *P. betel* leaves.

The current era faces increasing problems associated with bacterial resistance to antibiotics. Hence several medicinal plants are explored for its antibacterial activity against enteric pathogens, clinical isolates, oral flora mutants as well as

drug-resistant pathogens [17, 22, 34]. More often, the independent use of medicinal plants may be ineffective in highly infectious cases. Instead, their application in combination therapies to reverse the existing resistance towards the antibiotics is a more practical approach given the present scenario. To this effect, our current study holds immense value in the screening of valuable medicinal plant like *P. betel*, which is not only antibacterial in nature but also shows potential in reversing the already developed resistance to common antibiotics like ampicillin.

Table 2 Synergistic activity of methanol extract of *P. betel* leaves and ampicillin

Test Pathogens	**MBC of Ampicillin**	**MBC of methanol extract of *P. betel* leaves mg/mL**	**Sub-lethal concentration used**	**MBC of ampicillin in presence of methanol extract of *P. betel* leaves (µg/mL)**
β-lactamase producers				
K. pneumoniae	More than 10mg/mL	10	5	300
E. coli				300
P. aeruginosa				300
P. mirabilis				300
C. diversus				300

3.5. Evaluation of antioxidant activity of *P. betel* leaf extract by DPPH method

Figure 1 represents the DPPH radical scavenging activity of methanol extract of *P. betel* leaves and ascorbic acid. It was observed that the scavenging activity of the extract increased with its increasing concentration. It was found to be 32.32% at 10µg/mL concentration and increased to 80.56% at 2mg/mL concentration. The DPPH radical scavenging activity of the standard ascorbic acid, on the other hand, was found to be 78.81% at 10µg/mL concentration and 95.74% at 2mg/mL concentration. The IC_{50} value was calculated to be 292.077µg/mL for the *P. betel* extracts and 13.89µg/mL for the ascorbic acid. Hence indicating that the *P. betel* leaves possess moderate anti-oxidant activity as compared to the control. In another study, the DPPH radical scavenging activity of various solvent extracts of 200µg/mL *P. betel* leaves were studied. The hexane, chloroform and methanol extract showed 69.14% (IC_{50} 50µg/mL), 92.25% (IC_{50} 17.50µg/mL) and 90.94% (IC_{50} 22.50µg/mL) activities respectively. The control sample (i.e., ascorbic acid) showed a DPPH radical scavenging activity of 83.67% (IC_{50} 20µg/mL) in their study [35]. In a similar study, the IC_{50} value of 9.362µg/mL was reported for *P. betel* leaves by DPPH assay [36].

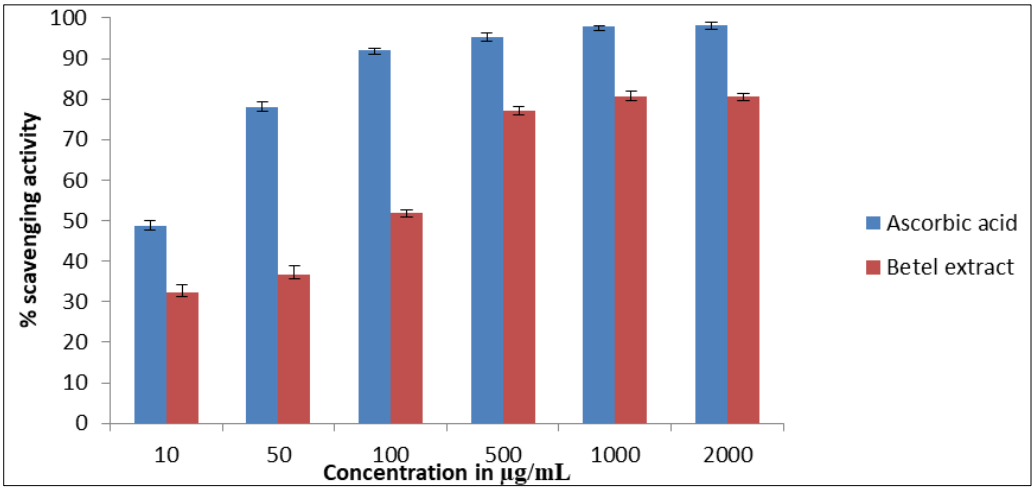

Figure 1 The DPPH radical scavenging activity of methanol extract of *P. betel* leaves and ascorbic acid

3.6. Gas Chromatography-Mass Spectrophotometry analysis

The GC-MS chromatogram (Figure 2) showed the presence of 3 major and 5 minor peaks. Table 3 represents the bioactive compounds identified in our study. The highest peak observed at retention time 7.90mins was identified as 2-Methoxy-4-vinyl-phenol, making it a major constituent of methanol extract of *P. betel* leaves. It is a flavouring agent also

known for its antioxidant, antimicrobial and anti-inflammatory properties. In another study, the GCMS analysis revealed the presence of 4 chromanol (27.81%) in aqueous extract, 2 methoxy 4–(-2propenyl) acetate (61.15%) in ethanol extract and squalene (21.78%) in aqueous extracts as major constituents [37]. The composition of bioactive components in *P. betel* leaves may vary depending on various factors like cultivation soil, temperature, climate, soil fertility, choice of solvents used in laboratory for extraction etc. In general, the leaves contain water (85-90%), protein (3-3.5%), fat (0.4-1%), carbohydrates (0.5-6.1%), fibers (2.3%), essential oil (0.08-0.2%) and Tannin (0.1-1.3%) [38]. The presence of vitamins and minerals like calcium, phosphorus, potassium, iron, iodine, carotene, nicotinic acid, thiamine, riboflavin, vitamin C and amino acids are also reported [39].

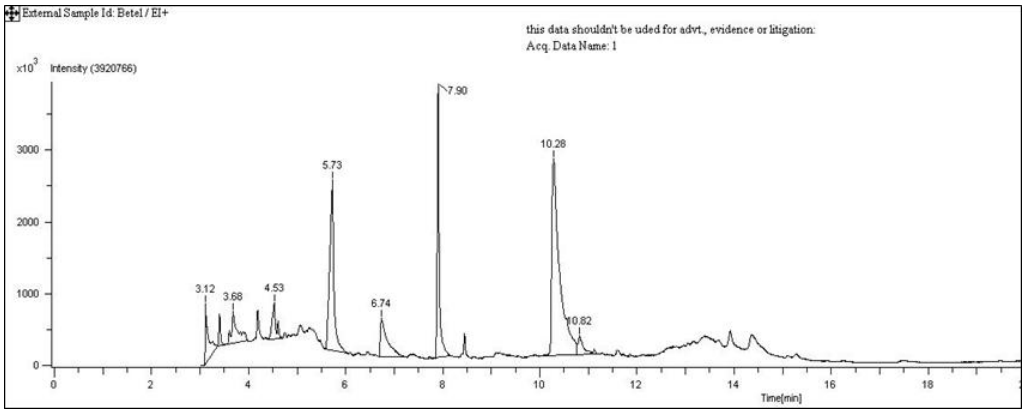

Figure 2 GC-MS chromatogram of methanol extract of *P. betel* leaves

Table 3 Bioactive constituents identified in the methanol extract of *P. betel* leaves by GCMS analysis

SR No.	Retention Time (min)	Peak Area (10^3)	Name	Compound Nature	Function
1.	5.73	64495	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid	Antimicrobial, Anti-inflammatory, Antioxidant agent
2.	6.74	83555	Benzofuran, 2,3-dihydro-	Coumaran	Antimicrobial, Anti-inflammatory
3.	7.90	460653	2-Methoxy-4-vinylphenol	Phenolic compound	Antioxidant, Antimicrobial, Anti-inflammatory
4.	10.28	235338	Benzoic acid, 3,4-dimethyl-	Aromatic carboxylic acid	Antimicrobial,
5.	13.92	37486	2,2,6-trimethyl-1-[3-methylbuta-1,3-dienyl]-7-oxabiylo[4.1.0]heptan-3-ol		Antimicrobial

4. Conclusion

The current study clearly suggests the antibacterial as well as anti-oxidant activity of *P. betel* leaf extracts against common laboratory as well as drug resistant pathogens like β-lactamase producers. Moreover, the resistance reversal activity of these extracts on common antibiotics like ampicillin may present us with hopeful insights to explore and fight the problem of antibiotic resistance.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

References

[1] Ernst RK, Guina T, Miller SI. How intracellular bacteria survive: surface modifications that promote resistance to host innate immune responses. Journal of Infectious Diseases. 1999; 179(2): 326-330.

[2] Selim SA. Antimicrobial, anti-plasmid and cytotoxicity potentials of marine algae Halimeda opuntia and Sarconema filiforme collected from Red Sea Coast. International Journal of Medical Health, Biomedicine, Bioengineering and Pharmaceutical Engineering. 2012; 6(1): 1154-1159.

[3] Giske CG, Monnet DL, Cars O, Carmeli Y, on behalf of ReAct-Action on antibiotic resistance. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrobial Agents and Chemotherapy. 2008; 52(3): 813-821.

[4] Fritsche TR, Sader HS, Toleman MA, Walsh TR, Jones RN. Emerging Metallo-β-Lactamase-mediated resistances: A summary report from the worldwide SENTRY antimicrobial surveillance program. Clinical and Infectious Diseases. 2005; 41(4): 276-278.

[5] Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clinical and Microbiology Reviews. 2012; 25(3): 450-470.

[6] Scarpignato C, Gatta L, Zullo A, Blandizzi C. Effective and safe proton pump inhibitor therapy in acid-related diseases – A position paper addressing benefits and potential harms of acid suppression. BMC Medicine. 2016; 14(1): 179.

[7] Mohle L, Mattei D, Heimesaat MM, Bereswill S, Fischer A, Alutis M, French T, Hambardzumyan D, Matzinger P, Dunay IR, Wolf SA. Ly6Chimonocytes provide a link between antibiotic-induced changes in gut microbiota and adult hippocampal neurogenesis. Cellular Reports. 2016; 15(9): 1945-1956.

[8] Sharmeen R, Hossain N, Rahman M, Foysal J, Faruque M. *In-vitro* antibacterial activity of herbal aqueous extract against multi-drug resistant *Klebsiella* sp. isolated from human clinical samples. International Current Pharmacy Journal. 2012; 1(6): 133-137.

[9] Noumedem JA, Mihasan M, Kuiate JR, Stefan M, Cojocaru D, Dzoyem JP, Kuete V. *In Vitro* antibacterial and antibiotic-potentiation activities of four edible plants against multidrug-resistant gram-negative species. BMC Complementary and Alternative Medicine. 2013; 13: 190.

[10] Voukeng IK, Kuete V, Dzoyem JP, Fankam AG, Noumedem JAK, Kuiate JR, Pages JM. Antibacterial and antibiotic-potentiation activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes. BMC Research Notes. 2012; 5: 299.

[11] Nagori K, Singh MK, Alexander A, Kumar T, Dewangan D, Badwaik H. Piper betle L: A review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART-MS (Direct Analysis in Real Time Mass Spectrometry). Journal of Pharmaceutical Research. 2011; 4(9): 2991-2997.

[12] Pradhan D, Suri KA, Pradhan DK, Biswasroy P. Golden Heart of the Nature: Piper betle, Journal of Pharmacognosy and Phytochemistry. 2013; 1(6): 147-167.

[13] Caburian AB, Osi MO. Characterization and evaluation of antimicrobial activity of the essential oil from the leaves of Piper betle L. International Science and Research Journal. 2010; 2(1): 1-13.

[14] Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology, 2003; 149(2): 279-294.

[15] Gururaj HB, Giridhar P, Ravishankar GA. Traditions in oral hygiene: Chewing of betel (Piper betle L.) leaves. Current Science. 2006; 92(1): 26-28.

[16] Chauhan ES, Aishwarya J, Singh A, Tiwari A. A Review: nutraceutical properties of Piper betel (Paan), American Journal of Phytomedicine and Clinical Therapy. 2016; 4(2): 28-41.

[17] Hoque M, Ratila S, Bari M. Antibacterial activity of ethanol extract of betel leaf (*Piper betle*). Bangladesh Journal of Microbiology. 2011; 28(2): 58-63.

[18] Sripradha S. Betel leaf- the green gold. Journal of Pharmacy and Scientific Research. 2014; 6(1): 36-37.

[19] Aruna K, Tariq M. Prevalence of extended-spectrum beta-lactamase production among uropathogens in south Mumbai and its antibiogram pattern. EXCLI. 2012; 11: 363-372.

[20] Tariq M, Aruna K. Phenotypic and Molecular characterization of MBL genes among uropathogens isolated in Mumbai city. British Microbiology Research Journal. 2015; 5(4): 368-383.

[21] Tariq M, Aruna K. Molecular detection of co-production of ESBL, AmpC and Integrons among uropathogens in a study from Mumbai. European Journal of Biomedical and Pharmaceutical Sciences. 2016; 3(4): 377-391.

[22] Khan J, Kumar N. Evaluation of antibacterial properties of extracts of *Piper betel* leaves, Journal of Pharmaceutical and Biomedical Sciences. 2011; 11(1): 1-3.

[23] Suppakul P. Sanla N. Phoopuritham P. Antimicrobial and antioxidant activities of betel oil. Kasetsart Journal of Natural Sciences. 2006; 40: 91-100.

[24] Sule LO, Agbabiaka TO. Antibacterial effect of some plant extracts on selected Enterobacteriaceae. Ethno botanical leaflets. 2008; 12: 1035-1042.

[25] Shareef AA. Evaluation of antibacterial activity of essential oils of *Cinnamomum* sp. and *Boswellia* sp. Journal of Basrah Research. 2011; 37(5): 1-13.

[26] Clinical and Laboratory Standards Institute, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, seventh edition: approved standard M07-A7, CLSI, Wayne, PA. 2006.

[27] Bendaoud H, Romdhane M, Souchard JP, Cazaux S, Bouajila J. Chemical composition and anticancer and antioxidant activities of *Schinus molle* L. and *Schinus terebinthifolius* Raddi berries essential oils. Journal of Food Sciences. 2010; 75(6): 466-472.

[28] Soni H, Sahu N, Singhai AK, Malik J. Radical scavenging activities and natural indicator activity of aqueous and ethanolic extract of Rosa damascene. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(5): 581-586.

[29] Ruiz JCR, Ordonez YBM, Basto AM. Antioxidant capacity of leaf extracts from two *Stevia rebaudiana* Bertoni varieties adapted to cultivation in Mexico. Nutricion Hospitalaria. 2015; 31(3): 1163-1170.

[30] Indian Pharmacopoeia 5th ed. Indian Pharmacopoeia Commission, Sector-23, Raj Nagar, Ghaziabad-201 002, India, 2007.

[31] Balaji K, Lisa TS, Kuan TS, Mirza B. Antibacterial activity of *Piper betel* leaves. International Journal of Pharmacy and Teaching Practice. 2011; 2(3): 129-132.

[32] Rahman S, Wadud A, Islam T, Hussain S, Esfat M, Bristy S, Tuhin AM. Evaluation of antibacterial activity of *Piper betel* Leaves and *Nigella sativa* seeds against Multidrug-Resistant food and water borne pathogenic bacteria: An in-vitro Study Model. Microbiology Research Journal International. 2017; 22(4): 1-11.

[33] Kusuma AF, Hendriani R, Genta A. Antimicrobial spectrum of red *Piper betel* leaf extract (*Piper crocatum* Ruiz & Pav) as natural antiseptics against airborne pathogens. Journal of Pharmaceutical Science and Research. 2011; 9(5): 583-587.

[34] Ghanwate N, Thakare P. Antimicrobial and synergistic activity of ingredients of betel quid on oral and enteric pathogens. Bioscience Discovery. 2012; 3(1): 47-51.

[35] Raghavan V, Sathish DK, Oommen PS. Phytochemical, antifungal and antioxidant properties of leaf extracts of *Thulasi vettila* an indigenous cultivar of *Piper betle* L. in kerala. International Journal of Advanced Research. 2018; 6(2): 1064-1069.

[36] Kavitha S, Perumal P. Antidiabetic and antioxidant activities of ethanolic extract of *Piper betle* L. leaves in catfish, *Clarias gariepinus*. Asian Journal of Pharmacy and Clinical Research. 2018; 11(3): 194-198.

[37] Deshpande SN, Kadam DG. GCMS analysis and antibacterial activity of *Piper betle* (linn) leaves against *Streptococcus mutans.* Asian Journal of Pharmacy and Clinical Research. 2013; 6(5): 99-101.

[38] Lakshmi BS, Naidu KC. Comparative Morphoanatomy of *Piper betle* L. cultivars in India. Annals of Biological Research. 2011; 1(2): 128-134.

[39] Umar RA. Sanusi NA, Zahari MN, Rohin MAK, Ismail S. Chemical composition and the potential biological activities of *Piper betel* – A Review. Malaysian Journal of Applied Sciences. 2018; 3(1): 1-8.