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RESEARCH ARTICLE

Screening of Potential plants from Kalimantan as an Antimicrobial agent for Coliform bacteria

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ABSTRACT:

The use of extract plants as a therapy method for bacterial illnesses is possible. Plants have antibacterial and antioxidant properties and include various chemicals that protect the human body from infections. And coliform bacteria are a major cause of public health issues. This study aimed to determine the potential of a variety of plants as an alternative antibacterial agent. The study was carried out with extract plants utilizing the disc diffusion method. The ultrasonic-assisted extraction procedure with n-hexane, DMSO and methanol to extract the leaf and stem bark of the plants. Zones of inhibition are measured using the Disc Diffusion Method, and the diameter of bacterial growth inhibition is measured in millimeters. At a 10 mg/mL dosage, all plant extracts had a growth-inhibitory impact against at least one of the three microorganisms tested, especially *Enterobacter aerogenes.* The extract of *Luvunga scandens* leaf had the strongest antibacterial action among the ten active plant extracts tested inhibiting the growth of *Escherichia coli*, *Klebsiella pneumonia*, and *Enterobacter aerogenes* at MICs of 10 mg/mL. It was concluded that all plants tested had potential as an antibacterial against coliform bacteria.

KEYWORDS: Antimicrobial. Coliform. Disc diffusion. Extract plants.

INTRODUCTION:

Infectious diseases cause a lot of morbidity and mortality, especially in low- and middle-income nations like Indonesia[1]. Bacteria have recently emerged on a global scale[2.3]. Inappropriate prescribing. irrational use and uncontrolled antibiotic access have all aggravated this trend in Indonesia[4]. Antibiotic resistance develops in pathogenic microorganisms, posing a serious threat to human health, particularly in individuals with weakened immune systems[5]. Negative bacteria specifically *Eschericia coli* and *Klebsiella pneumonia* were the most predominant pathogens and were multidrug resistance most antimicrobials[6].

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With various chemicals that can protect the human body against infections and cell oxidation, medicinal plants have the potential to be antibacterial and antioxidant. This has a lot to do with secondary metabolite molecules in these plants[7].

Antibiotic treatment effectiveness is influenced significantly by bacterial resistance and limited medication use. In the field of medicine. an alternative antimicrobial is required. Alternative antibacterial agents for disease-causing microorganisms are currently being researched. Much research has demonstrated that the compounds found in plants extract have antibacterial properties. Plant extracts can be employed as part of a current bacterial illness therapy strategy[8-10]. Indonesia has a diverse range of medicinal plants. making it a biodiversity hotspot. Natives have used more than 250 medicinal plants in West Kalimantan, Indonesia, representing 165 genera and 75 families. The Dayak

Kenyah group in Kalimantan, Indonesia, uses over 200 types of forest medicinal herbs. In addition, the Benuaq Dayak group in West Kutai, Indonesia, said to use 62 different types of medicinal plants. However, only about a third of the 6000 species known to the general population have been identified and given detailed data, including chemical and biological features[11].

Bioactive nutraceuticals, bio-pharmaceuticals, and food additives are all possible applications for natural antioxidants. In this regard, natural antioxidants are being extracted, characterized, and used extensively to identify promising candidates for countering the aging process[12]. The causative agent of the significant public health outbreaks are groups of coliform bacteria[13]. Bacterial agents belonging to the coliform group, such as *E. coli, Klebsiella sp.*, and *Enterobacter sp*. cause various infections in humans, including gastroenteritis, urinary tract infections, and other infectious diseases disorders[14]. Antibiotic resistance and its associated toxicity issues have recently reduced the use of antimicrobial medicines, prompting more research into the function of plant antimicrobials in the fight against resistant strains. Many researches on the potential of plants as antibacterials have been carried out, such as *Rubia cardifolia*[15] and *Cleome rutidosperma*[16] as antibacterials for *Eschericia coli*. Then *Curcuma longa*[17] and *Tectona grandis*[18] as antibacterials for *Klebsiella pneumonia* and *Enterobacter aerogenes*. As a result, The aim of this study is to determine in vitro antibacterial activity of plant species collected from Kalimantan against Coliform bacteria

MATERIALS AND METHODS:

Plant Material:

The stem bark and leaves of *Melicope glabra, Luvunga scandens, Artocarpus sericicarpus, Artocarpus anisophyllus,* and *Artocarpus dadah* were collected from Balikpapan Botanical Garden in East Kalimantan. Indonesia. The plant was identified at Purwodadi Botanical Garden. East Java, with a voucher specimen number 0074 IPH.06/HM/XII/2015.

Plant Extraction:

The stem bark and leaves of *M. glabra, L. scandens, A. sericicarpus, A. anisophyllus,* and *A.dadah* were dried and ground into powder form. The powder (100g) was extracted using 500mL n-hexane as a solvent by ultrasonic-assisted extraction. The extract was filtered. and filtrate was then evaporated using a rotary evaporator to obtain n-hexane extract. The residue was further extracted using 500mL dichloromethane as a solvent to obtain dichloromethane extract. Then the residue was again extracted using 500mL methanol as a solvent to obtain methanol extract. All extracts were tested against Coliform bacteria.

Stock Preparation of Plant Extracts:

The stock extract solution was used as a concentration with DMSO solution 1%. and methanol was used as a solubilizing solvent for test samples and used as a control to evaluate the antibacterial assay. The concentration of plant extracts used were 2.5 mg/mL, 5 mg/mL, and 10 mg/mL.

Test Organisms and inoculums preparation:

The coliform bacterial used for the test were *Escherichia coli, Klebsiella pneumonia*, and *Enterobacter aerogenes*. All the stock cultures were obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga, Indonesia. Bacterial cultures were cultured on MCA (Mac Conkey Agar) and NA (Nutrient Agar) (MERCK). Bacterial cells in the suspension were measured according to standard 0.5 Mac Farland.

Antibacterial activity of plant extract:

The disk diffusion method is used to evaluate antimicrobial activity of the each plant extract. Concisely, 15ml of sterilized Mueller Hinton Agar (MERCK) (pH 7.2±0.2) were applied in to the surface of sterile petri dishes (9cm in diameter) and allowing them to settle for base plate preparation. 100µl of bacterial test suspension (0.5 Mac Farland) were poured into each base plate and cotton swab (Himedia). 100µl of different test sample concentrations were soaked with sterile paper discs (6mm). The air-dried discs were placed on each base plate and incubated at 37±2°C for 24h. Methanol and Ampicillin (20mg/disc) was used as positive treatment, taken as an average of three measurements at different directions. The inhibition of zones around the discs was determined as the diameter (mm) of bacterial growth inhibition.

Statistical Analysis:

Data expressed as mean Inhibition zone diameter ±SEM. The results recorded were by Microsoft excel ver. 2013. and the result obtained was analyzed descriptively.

RESULT:

In this study, 10 extracts from five different plant species were tested in vitro for antibacterial activity. Using the maceration ultrasonic method, the maximum extract was obtained from the *Artocarpus dadah* plant with a bark sample of 14.44%, while the lowest was *Melicope glabra* with a bark sample of 2.02% (Table 1). Using the disc diffusion method, the extract was used to calculate the MIC value. The study's findings revealed that all of the plant extracts tested had antibacterial activity against the microorganisms (Table 2).

At a 10mg/mL dosage, all plant extracts had a growth-inhibitory impact against at least one of the three microorganisms tested. The extract of *Luvunga scandens* leaf had the strongest antibacterial action among the ten active plant extracts tested, inhibiting the growth of *Escherichia coli, Klebsiella pneumoniae,* and *Enterobacter aerogenes* at MICs of 10mg/mL. The growth of *Enterobacter aerogenes* bacteria was inhibited by all of the plant extracts tested. Extracts from the *Melicope glabra* and *Artocarpus dadah* plant were unable to inhibit the growth of *Klebsiella pneumoniae* bacteria, and only two extracts, *Luvunga scandens* leaf extract and *Artocarpus dadah* bark extract, were able to inhibit the growth of *Escherichia coli* bacteria.

Based on the results of the measurement of the inhibition zone on the test bacteria (table 3). The lowest value was leaf extract of *Luvunga scandens* against *Escherichia coli* with an inhibitory value of 6.22+0.32 mm, and the highest value was leaf extract of *Melicope glabra* against *Enterobacter aerogenes*, with an inhibitory value of 8.75+0.14mm. Among the 10 plant extracts tested, the leaf extract of *Luvunga scandens* produced the best antimicrobial effect to inhibit the growth of all test bacteria (*Escherichia coli, Klebsiella pneumonia*, and *Enterobacter aerogenes*) at an inhibition zone of 6.22+0.32mm; 6.41+0.42mm; and 8.23+0.33mm, respectively.

Table 1. Ethnobotanical data on Kalimantan medicinal plants tested

Botany Name	Local Name	Family	Code specimen	Part used	Traditional use	Extract Yield (%)
Melicope glabra	Bebangun / kotep/ wawangun	Rutaceae	BP01-F	Leaf	diarrhea. fever. high blood pressure. and cancer	6.54
			BP01-SB	Stem Bark	antioxidant	2.02
Luvunga scandens	Saluang belum	Rutaceae	BP02-F	Leaf	gout. kidney diseases. fatigue. urinal disorders. and malaria. acting as an antidote against snake bite and ringworm	3.41
			BP02-SB	Stem Bark	Boost stamina. backache. kidney pain. treat malaria	3.86
Artocarpus sericicarpus	Peluntan/pedalai	Moraceae	BP03-F	Leaf	None known	7.89
			BP03-SB	Stem Bark	None known	4.62
Artocarpus anisophyllus	Mentawa / Puatn	Moraceae	BP04-F	Leaf	treatment for boils and itch	3.88
			BP04-SB	Stem Bark	None known	2.10
Artocarpus dadah	Selanking / Daraak	Moraceae	BP05-F	Leaf	None known	8.71
			BP05-SB	Stem Bark	None known	14.44

Table 2. Antimicrobial activity of methanol extracts from Kalimantan medicinal plants

Plant name	Part used	Microorganisms/minimum inhibitory concentrations (mg/mL)		
		Eschericia coli	***Klebsiella pneumonia***	***Enterobacter aerogenes***
Melicope glabra	Leaf	-	-	10
	Bark	-	-	10
Luvunga scandens	Leaf	10	10	10
	Bark	-	10	10
Artocarpus sericicarpus	Leaf	-	-	10
	Bark	-	10	10
Artocarpus anisophyllus	Leaf	-	10	10
	Bark	-	10	10
Artocarpus dadah	Leaf	-	-	10
	Bark	10	-	10

Table 3. Antibacterial activity of plant extracts with well diffusion assay

Plant species	Part used	Inhibition zone (mm) in 10 mg/mL of extract concentration		
		Eschericia coli	***Klebsiella pneumonia***	***Enterobacter aerogenes***
Melicope glabra	Leaf	-	-	7.03 ± 0.12
	Bark	-	-	8.75 ± 0.14
Luvunga scandens	Leaf	6.22 ± 0.32	6.41 ± 0.42	8.23 ± 0.33
	Bark	-	6.67 ± 0.32	7.08 ± 0.45
Artocarpus sericicarpus	Leaf	-	-	8.23 ± 0.62
	Bark	-	6.52 ± 0.82	6.72 ± 0.20
Artocarpus anisophyllus	Leaf	-	6.92 ± 0.77	8.17 ± 0.19
	Bark	-	7.39 ± 0.67	7.25 ± 0.40
Artocarpus dadah	Leaf	-	-	6.55 ± 0.28
	Bark	6.32 ± 0.14	-	6.87 ± 0.92
Methanol	-	-	-	-
Ampicillin 20mg/disc	-	-	-	-

Data are means of three replicates (n = 3) with standard deviations; the antibacterial inhibition category based on the inhibition zone diameter (<8 mm: weak. 8-11 mm: moderate. 11-20 mm: strong.> 20 mm: very strong)[19]

The leaf extract of *Luvunga scandens* and the bark of *Artocarpus dadah* showed low inhibitory activity against *Escherichia coli*. Leaf and bark extract of *Luvunga scandens*, bark extract of *Artocarpus sericicarpus*, leaf and bark extract of *Artocarpus anisophyllus* also had low inhibitory activity against *Klebsiella pneumoniae*. Meanwhile, the bark extract of *Melicope glabra*, leaf extract of *Luvunga scandens*, *Artocarpus sericicarpus* and *Artocarpus anis*ophyllus showed moderate inhibitory activity against *Enterobacter aerogenes*, and other plant extracts had low inhibitory activity against *Enterobacter aerogenes*. The diameter of the inhibition zone formed was related to the antimicrobial compounds in each plant extract.

DISCUSSION:

Pathogen assay using the disc diffusion test method and has been carried out with the aim of comparing the activity of the extract with standard antibiotics[20]. The antibacterial activity report of the ethanolic extracts from the present study's plants against coliform bacteria is limited. Several research have found that *Luvunga scandens* contains antimalaria[21], antifungal[22], insecticidal[23], anti-inflammatory, and antibacterial activities[24]. Another study reported that ethyl acetate, dichloromethane, and methanol extracts of *L. scandens* in DPPH assays showed moderate anti-oxidative activity as radical scavengers with IC_{50} values of 94g/mL, 445 g/mL, and 480g/mL, respectively. *Luvunga scandens* extracts sensitivity was found in all five bacterial strains. Ethyl acetate extract showed antibacterial activity against *Escherichia coli* (TISTR 780), *Staphylococcus aureus* (TISTR 1466), *Bacillus cereus* (TISTR 687) and *Enterococcus faecalis* (TISTR 379) with MIC values ranging from 3.125 to 25mg/mL. Methanol extract showed moderate antibacterial activity with MIC value of 25mg/mL. *Staphylococcus aureus* (TISTR 1466) and Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300) both showed antibacterial activity against dichloromethane extract with MIC values of 6.25 and 12.5mg/mL, respectively[23].

Melicope sp. contains alkaloids, flavonoids, lignin, terpenoids[25], steroids, tannins and saponins[26], glabranin, umbelliferone, scopoletin, and sesamin[27]. With MIC values of 208.3±90.6µg/mL, the isolated chemicals umbelliferone and sesamin demonstrate strong inhibitory activity against *S. mutans*. Another study reported the antibacterial activity of the plant extracts against bacteria. The ethyl acetate extract had greater antibacterial activity against *E. faecalis* (4166.7± 1443.47µg/mL) and *S. mutan* (833.3±360.8µg/mL) compared to the methanolic extract with MIC value 8333.3±2886.8µg/mL and 1041.7±360.8µg/mL[28].

In traditional medicine, the Genus *Artocarpus* is commonly used. Phenolic chemicals are abundant in *Artocarpus* species in general. *Artocarpus* extracts and metabolites particularly leaves, barks, stems, and fruits have been demonstrated to be beneficial bioactive substances. *Artocarpus* has been shown to be effective in treating inflammation. malaria fever, diarrhea, diabetes, and tapeworm infections in several pharmacological investigations. The n-hexane, dichloromethane, and methanol extracts of *Artocarpus sericicarpus* demonstrated antimalarial activity with IC50 values of >4, 2.11, and >4µg/mL, respectively[29]. However, there were no previous reports about the antibacterial activity of the plant. The inhibitory capacity of the *Artocarpus anisophyllus* leaf extract on Gram-positive bacteria (*S. aureus*) was considerably stronger than Gram-negative bacteria (*E.coli*)[30]. Another study showed that the methanolic extract of *Artocarpus anisophyllus* has antibacterial activity against other bacteria including *Salmonella typhi* with MIC value was 1.25-2.5% at concentration. *Streptococcus mutans* (0-0.625%), *Streptococcus sobrinus* (1.25-2.5%), and *Propionibacterium acnes* (0.625-1.25)[31]. *Artocarpus dadah* is an endemic plant in Indonesia, and only a few people have studied it. There were no reports of the antibacterial activity of the plant.

In addition, the solvent used also has an effect on the content of plant extracts. Methanol solvents can give positive results for many phytochemical groups such as alkaloids, flavonoids, tannins, steroids, proteins, polyphenols and terpenoids. Plant extracts with methanol as a solvent showed better results than other solvents such as water, ether, ethanol, and DMSO[32-34].

CONCLUSION:

It was concluded that all extracts Kalimantan medicine plant had a growth-inhibitory impact against at least one of the three microorganisms tested. The *Luvunga scandens* leaf extract has more potential for inhibiting coliform bacteria growth, including *Escherichia coli*, *Klebsiella pneumonia*. and *Enterobacter aerogenes* with MIC values of 10 mg/mL. And the highest value was leaf extract of *Melicope glabra* against *Enterobacter aerogenes* with an inhibitory value of 8.75 + 0.14 mm. It was concluded that all extracts of Kalimantan medicine plants tested had potential as an antibacterial against coliform bacteria.

CONFLICT OF INTEREST:

The authors declare no conflict of interest**.**

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REFERENCES:

1. World Health Organization (WHO). Indonesia: WHO statistical profile. 2015 [accessed 2021 August 01]. http://www.who.int/gho/countries/idn.pdf.
2. Golkar Z. Bagasra O. Gene Pace D. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. The Journal of Infection in Developing Countries. 2014; 8(2):129-36.doi.10.3855/jidc.3573.
3. Wright GD. 2014. Something old. something new: revisiting natural products in antibiotic drug discovery. Canadian Journal of Microbiology. 2014; 60(3):147-154. doi: 10.1139/cjm-2014-0063.
4. Abdulah R. Antibiotic abuse in developing countries. Pharmaceutical Regulatory Affairs Open Access. 2012; 1(2):1–3. doi.10.4172/2167-7689.1000e106 .
5. Nasrullah. Suliman. Rahman K. Ikram M. Nisar M. Khan I. Screening of Antibacterial Activity of Medicinal Plants. International Journal of Pharmaceutical Science Review and Reserch. 2012; 14(2):25-29.
6. Aljanaby AAJ. Israa AJA. Profile of Antimicrobial Resistance of Aerobic Pathogenic Bacteria isolated from Different Clinical Infections in Al-Kufa Central Hospital–Iraq During period from 2015 to 2017. Research Journal of Pharmacy and Technology. 2017; 10(10):3264-3270. doi.10.5958/0974-360X.2017.00579.0.
7. Wojdylo A. Oszmianski J. Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry. 2007; 105(3):940-949. doi.org/10.1016/j.foodchem.2007.04.038.
8. Gyles C. The growing problem of antimicrobial resistance. Can Vet J. 2011; 52(8):817–820.
9. Djeussi DE. Jaurès AKN. Jackson AS . Aimé GF . Igor KV . Simplice BT . Antoine HLN and Victor K. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine. 2013; 13:164. doi: 10.1186/1472-6882-13-164.
10. World Health Organization (WHO). Antimicrobial resistance: Global report on surveillance. vol. 2014. Geneva: WHO; 2014. [accessed 2021 August 01].
11. Okumura H. Kohei A. Ellyn KD. Ervizal AMZ. Elvira N. Yeni H. A Computer Aided System for Tropical Leaf Medicinal Plant Identification. Jurnal Bahan Alam Indonesia. 2013; 3(1):23-27.
12. Amoo SO. Aremu AO. Moyo M. Van Staden J. Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. BMC Complement. Alternative Medicine. 2012; 12:87–95. doi:10.1186/1472-6882-12-87.
13. Paterson DL. Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. Clinical Infectious Diseases. 2008; 15;47 Suppl 1:S14-20. doi: 10.1086/590062.
14. Peirano G. Multi resistant enterobacteriaceae new threat to an old problem: expect review of anti infective therapy. Expert Review of Anti-Infective Therapy. 2008; 6(5):657-69.doi: 10.1586/14787210.6.5.657.
15. Kulkarni A. Kale M. Sarode S. Firke S. Firke B. Warke P. Antimicrobial activity of several important Indian medicinal plants against several plant and human pathogens. Research Journal of Pharmacy and Technology. 2010; 3(3):924-926.
16. Prabha SB. Mohini R. MR Ramesh K. Evaluation of Antioxidant. Antibacterial and Anticancer Activity in vitro from *Cleome rutidosperma* leaf extract. Research Journal of Pharmacy and Technology. 2017;10(8):2492-2496.doi:10.5958/0974-360X.2017.00440.1.
17. Sandhya M. Nidhi R. Sudhanshu. Ekta M. Phytochemical and antimicrobial activity of *Tectona grandis* L. Research Journal of Pharmacy and Technology. 2012; 4(5): 188-191
18. Nidhi R. Sandhya M. An in vitro Evaluation of the Antimicrobial Activity of Curcuma longa against Selected Pathogenic Microorganisms. Research Journal of Science and Technology.. 2014; 6(2): 71-74.
19. Pelczar MJ and Chan ECS. Dasar-dasar mikrobiologi. Jakarta. UI Press Jakarta. 2005.
20. Srimanta KD. AS Dhake. A Nayak. NB Das. SN Pandeya. Antibacterial and antifungal activity of the Aerial Parts of Ammannia baccifera Linn. Research Journal of Pharmacy and Technology. 2011; 4(3): 430-432.
21. Eswani N. Kudus KA. Nazre M. Noor AGA. Ali M. Medicinal plant diversity and vegetation analysis of logged over hill forest of Tekai Tembeling Forest Reserve. Jerantut. Pahang. The Journal of Agricultural Science. 2010; 2(3):189-210. doi.10.5539/jas.v2n3p189.
22. Garg SC. Jain R. Antifungal Activity of *Luvunga scandens* against some Keratinophilic fungi. Indian Journal of Pharmaceutical Sciences. 1999; 61:248-249.
23. Singh G. Maurya S. Antimicrobial. antifungal and insecticidal investigations on essential oils. An overview. Natural Product Radiance. 2005; 4(3):179-192 .
24. Sundari AA. Dwi WI. Diyantoro. Exploration of Potential Moraceae as an Antimicrobial Agent for Coliform Bacteria. Malaysian Journal Of Medicine And Health Sciences.. 2020; 16 (SUPP16):24-28.
25. Suryati. Phytochemical and Antiinflammatory Activity of *Melicope ptelefolia* Champ ex Benth. Thesis. University Putra Malaysia. 2005.
26. Noorcahyati. Arifin Z. Ethnobotany of Efficacious Plants for Medicine of Ethnic Dayak Meratus Loksado. South Kalimantan. Prosiding Hasil Penelitian Balai Penelitian Teknologi Konservasi Sumber Daya Alam. Samboja. 2014.
27. Kassim NK. Rahmani M. Ismail A. Sukari MA. Ee GCL. Nasir NM. Awang K. Antioxidant Activity-guided Separation of Coumarins and Lignan from *Melicope glabra* (Rutaceae). Food Chemistry. 2013; 139(1-4):87-92. doi.10.1016/j.foodchem.2013.01.108.
28. Quek A. Mohd Zaini H. Kassim NK. Sulaiman F. Rukayadi Y. Ismail A. Abidin ZZ. Awang K. Oxygen radical antioxidant capacity (ORAC) and antibacterial properties of *Melicope glabra* bark extracts and isolated compounds. PLoS ONE. 2021; 16(5). doi.org/10.1371/journal.pone.0251534.
29. Tumewa L. A'yun LQ. Ilmi H. Hafid AF. Widyawaruyanti A. *Artocarpus sericicarpus* stem bark contains antimalarial substances against *Plasmodium falciparum*. Journal of Basic and Clinical Physiology and Pharmacology.. 2021; 32(4):853-858. doi: 10.1515/jbcpp-2020-0397.
30. Mulyani S. Ardiningsih P. Jayuska A. Antioxidant and antibacterial activity of Mentawa (*Artocarpus anisophyllus*) leaf extract. Jurnal Kimia Khatulistiwa. 2016; 5(1):36-43.
31. Pratama W. Saleh C. Astuti W. Phytochemical test and antibacterial activity of merhanol extract of Mentawa leaf. Jurnal Atomik. 2020; 5(2):114-118.
32. Killedar SG.. Harinath NM. Screening of Antimicrobial Potential and Phytoconstituents for Various Memecylon umbellatum Burm Flower Extracts. Asian Journal of Pharmaceutical Research. 2011; 1(4):114-118.
33. Mital NM. Antibacterial Activity of Leaves and Flowers of *Ipomoea aquatica* Forsk. (Convolvulacea). Asian Journal of Pharmaceutical Research. 2018; 8(2): 94-98.doi.10.5958/2231-5691.2018.00016.3.
34. Mohan KRK. Kiran BU. Ramesh C. Shaik M. Rakesh P. In-vitro Antimicrobial Activity of Four Indigenous Medicinal Plants Belonging to Bapatla. A.P. Research Journal of Pharmacy and Technology. 2010; 3(2): 461-465.