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Comparative Study on the Antibacterial Activity of Some Selected Medicinal Plants on *Staphylococcus aureus* & *Escherichia coli*

O.B. Tojola[1,2], L. Lajide[1], B.J. Owolabi[1], M.T. Olaleye[3]

[1]Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria
[2]Federal Institute of Industrial Research Oshodi, Lagos State, Nigeria.
[3]Department of Biochemistry, Federal University of Technology, Akure, Ondo State
*Corresponding author: greatexploit2014@gmail.com

ABSTRACT

In this research work some medicinal plants were investigated for antibacterial activity against *Staphylococcus aureus and Escherichia coli* using agar diffusion method. The dried plant materials were grinded, extracted using three different solvents and they were tested against the organisms at different concentrations. *Escherichia coli* and *Staphylococcus aureus* have been the serious causes of a variety of community and hospital acquired infections. Infectious diseases caused by bacteria and fungi are one of the leading causes of death globally today. The ethyl acetate and methanol extracts of *Hilleria latifolia* showed better antibacterial activity against the two organisms than the extracts of the other plants. The *Hilleria latifolia* extracts exhibited highest zone of inhibition of 27.00±1.00 mm against *Staphylococcus aureus* and they also exhibited the highest zone of inhibition of 26.00±0.00 mm against *Escherichia coli* at 100.00 mg/ml concentration. All the plants showed antibacterial activity against all the organisms at all concentrations. The study shows that *Annona muricata*, *Hilleria latifolia, Heliotropium indicum* and *Parinari curatellifolia* have significant antimicrobial activity and that they are promising source of antimicrobial drugs for the treatment of infections caused by *Staph*ylococcus *aureus* and *Escherichia coli.*

Keywords: Medicinal plants, Infectious diseases, Zone of inhibition, Antimicrobial drugs, Agar diffusion method

1.Background of the Study

Antibiotic resistance is rising to dangerously high levels in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of infections such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and food borne diseases are becoming harder, and sometimes impossible, to treat as antibiotics become less effective (WHO, 2020). A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Iwu *et al*., 1999). *Maytenus senegalensis* is a synonym of *Celastrus senegalensis* and it is one of the species in Celastraceae family. *Maytenus senegalensis* is known for its antiplasmodial (El Tahir *et al*., 1999; Malebo *et al*., 2015); anthelmintic (Zangueu *et al*., 2018) and antibacterial properties (Lindsey *et al.,* 2006, Jain *et al.,* 2008). Its leaves and roots have been shown to possess anti-inflammatory activity *in vitro* and in eodema models induced by carrageenan or croton oil (Sosa *et al*., 2007; da Silva *et al*., 2011; Makgatho *et al*., 2018).

The plant *Hilleria latifolia* occurs in tropical Africa, Guinea to Ethiopia, Africa, Mozambique and North Africa. It also occurs in Madagascar and Srilanka. It can also be found in along the West African coast from Sierra Leone to Cameroun. It is native to South-America, naturalized in tropical Africa and Sri-lanka (Dakosi, 1998). *Parinari curatellifolia* is a good source of some macronutrients, especially Vitamin C, and might contribute significantly to the mineral requirements of the rural populace (Muchuweti *et al*., 2013). It was reported that *Parinari curatellifolia* phytochemical screening showed that it contained some antioxidant compounds such as polyphenol and other secondary metabolites, such as alkaloid, anthraquinones, and glycosides (Ogbonnia *et al*., 2008). Extracts from *Annona muricata* (also known as graviola) are among a myriad of botanical products which have shown promising medicinal value (Nwokocha *et al*., 2012; Somsak *et al*., 2016; Lee *et al*., 2016). Study evaluated an alcoholic extract of *Heliotropium indicum* for antimicrobial activity against four strains each of gram positive and gram negative bacteria, and three strains of fungi and two yeast. Results showed dose-dependent antimicrobial activity to all the test organisms (Rao *et al*., 2002).

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2. Materials & Methods

2.1 Plant Collection

The fresh aerial part of *Annona muricata, Heliotropium indicum, Hilleria latifolia,* stem part of *Maytenus senegalensis*, seeds of *Parinari curatellifolia* were obtained locally from farmlands in Lagos State and Ogun State, South West, Nigeria. The plant materials were air dried under shade, grinded to

coarse powder and stored in a closed container until use.

2.2 Preparation of Extracts

The grinded plant material was sequentially extracted using hexane, ethyl acetate and methanol respectively using the method of maceration at normal room temperature for a period of three days according to Handa *et al*., 2008. The extract was filtered and then distilled off the extracting solvent by drying it on an evaporating dish under a mild temperature.

Microorganisms

Two strains of bacteria were used for this study, one was gram positive, which was *Staphylococcus aureus* (ATCC 29213) and the second was gram negative, which was *Escherichia coli* (ATCC 35218). Single colony plates of nutrient agar medium of these organisms were maintained at 4˚C and sub-cultured on to nutrient broth for 24 hours prior to testing.

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Antibacterial activity assay

Antibacterial activity of the *Annona muricata, Heliotropium indicum, Hilleria latifolia, Maytenus senegalensis* and *Parinari curatellifolia* extracts were determined by using pour plate method (agar diffusion) on sterile nutrient agar medium. Nutrient agar medium was poured into the sterile petri-plate and the medium was allowed to solidify for about 45-60 minutes. Gentamicin (10 µg/ml) was used as positive control while the solvent of extraction was used as the negative control. Using a sterile cork borer of 6 mm diameter, the wells were made according to the number of graded concentrations of the sample. In each well, the different graded concentrations of the sample were prepared, this was done in duplicates. The plates were allowed to stay on the bench for 2 hrs to allow pre-dilution. The plates were incubated uprightly at 37 ºC for 18-24 hrs. Then antibacterial activity was determined by measuring the diameter of zone of inhibition (ZI) in millimeter.

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3. Results & Discussion

Table 1: The Plant Extracts Antibacterial Activity Against *Staphylococcus aureus*

S/N	Plant	Extract	Gentamicin	Zone of Inhibition at Different Concentrations (mg/ml)		
				100.00	50.00	25.00
1	*Maytenus senegalensis*	EAECS	39.00± 1.00 mm	15.00± 1.00 mm	13.00± 1.00 mm	10.00± 0.00 mm
		MECS	37.00± 1.00 mm	17.00± 1.00 mm	14.00± 0.00 mm	12.00± 0.00 mm
2	*Parinari curatellifolia*	EAEPC	37.00± 1.00 mm	26.00 ± 0.00 mm	24.00± 0.00 mm	20.00 ± 0.00 mm
		MEPC	37.00± 1.00 mm	19.00± 1.00 mm	15.00 ±1.00 mm	13.00± 1.00 mm
3	*Heliotropiumindicum*	EAEHI	38.00± 0.00 mm	22.00± 0.00 mm	18.00± 0.00 mm	16.00± 0.00 mm
		MEHI	37.00± 1.00 mm	25.00± 1.00 mm	22.00± 2.00 mm	19.00± 1.00 mm
4	*Hilleria latifolia*	EAEHL	37.00± 1.00 mm	27.00± 1.00 mm	24.00± 0.00 mm	20.00± 0.00 mm
		MEHL	37.00± 1.00 mm	27.00± 1.00 mm	23.00± 1.00 mm	21.00± 1.00 mm
5	*Annona muricata*	EAEAM	36.00± 0.00 mm	25.00± 1.00 mm	21.00± 1.00 mm	18.00± 0.00 mm
		MEAM	39.00± 1.00 mm	15.00± 1.00 mm	13.00± 1.00 mm	10.00± 0.00 mm

EAECS - Ethyl acetate extract of *Maytenus senegalensis,* **MECS** - Methanol extract of *Maytenus senegalensis,* **EAEPA** - Ethyl acetate extract of *Parinari curatellifolia*, **MEPA** - Methanol extract of *Parinari curatellifolia,* **EAEHl** - Ethyl acetate extract of *Heliotropium indicum*, **MEHI** -

Methanol extract of *Heliotropium indicum,* **EAEHL** - Ethyl acetate extract of *Hilleria latifolia,* **MEHL** - Methanol extract of *Hilleria latifolia,* **EAEAM**- Ethyl acetate extract of *Annona muricata,* **MEAM -** Methanol extract of *Annona muricata*

Table 2: The Plant Extracts Antibacterial Activity Against *Escherichia coli*

S/N	Plant	Extract	Gentamicin	Zone of Inhibition at Different Concentrations (mg/ml)		
				100.00	50.00	25.00
1	*Maytenus Senegalensis*	EAECS	36.00± 0.00 mm	15.00± 1.00 mm	13.00± 1.00 mm	10.00± 0.00 mm
		MECS	37.00± 1.00 mm	15.00± 1.00 mm	13.00± 1.00 mm	10.00± 0.00 mm
2	*Parinari Curatellifolia*	EAEPC	36.00± 0.00 mm	25.00 ± 1.00 mm	22.00 ± 2.00 mm	19.00 ± 1.00 mm
		MEPC	37.00± 1.00 mm	17.00± 1.00 mm	14.00± 0.00 mm	12.00± 0.00 mm
3	*Heliotropium Indicum*	EAEHI	36.00± 0.00 mm	18.00± 0.00 mm	16.00± 0.00 mm	14.00± 0.00 mm
		MEHI	37.00± 1.00 mm	25.00± 1.00 mm	21.00± 1.00 mm	18.00± 0.00 mm
4	*Hilleria Latifolia*	EAEHL	38.00± 2.00 mm	26.00± 0.00 mm	21.00± 1.00 mm	18.00± 0.00 mm
		MEHL	36.00± 0.00 mm	26.00± 0.00 mm	23.00± 1.00 mm	19.00± 1.00 mm
5	*Annona Muricata*	EAEAM	36.00± 0.00 mm	24.00± 0.00 mm	20.00± 0.00 mm	18.00± 0.00 mm
		MEAM	36.00± 0.00 mm	21.00± 1.00 mm	18.00± 0.00 mm	16.00± 0.00 mm

EAECS - Ethyl acetate extract of *Maytenus senegalensis,* **MECS** - Methanol extract of *Maytenus senegalensis,***EAEPA** - Ethyl acetate extract of *Parinari curatellifolia*, **MEPA** - Methanol extract of *Parinari curatellifolia,* **EAEHI** - Ethyl acetate extract of *Heliotropium indicum*, **MEHI** - Methanol extract of *Heliotropium indicum,* **EAEHL** - Ethyl acetate extract of *Hilleria latifolia,* **MEHL** - Methanol extract of *Hilleria latifolia,* **EAEAM**- Ethyl acetate extract of *Annona muricata,* **MEAM -** Methanol extract of *Annona muricata*

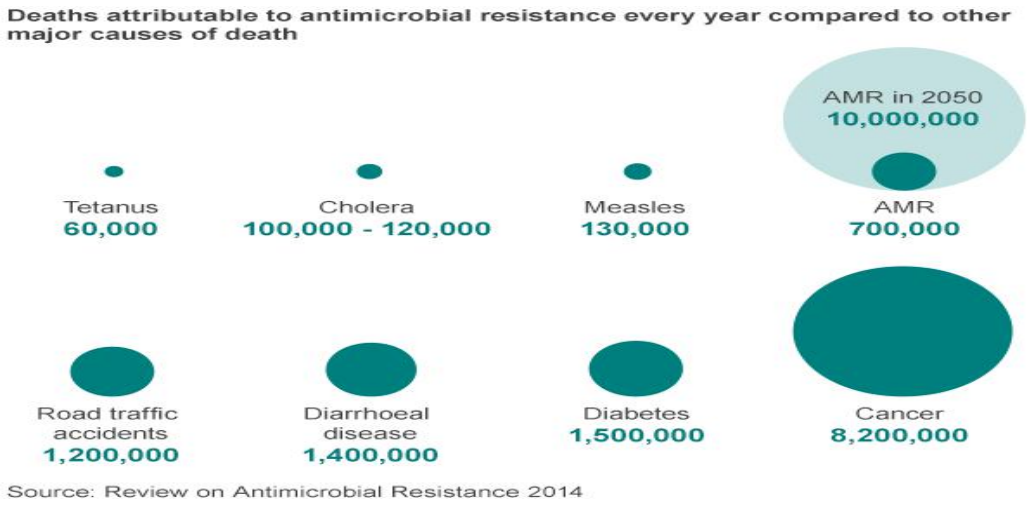

Figure 1: Death Attributable to Antimicrobial Resistance

Antibacterial Activity

The antibacterial activity of the ethyl acetate extract and methanol extract of five medicinal plants namely, *Annona muricata*, *Maytenus senegalensis*, *Parinari curatellifolia*, *Heliotropium indicum* and *Hilleria latifolia* were investigated against *Staphylococcus aureus* and *Escherichia coli*. The extracts were tested at different concentrations and were found to exhibit varying degrees of antibacterial properties. The results in Table 1 and 2 showed that the antibacterial activity of the plant extracts are concentration dependent, they exhibited better activity at higher concentrations. In Table 1, the ethyl acetate extracts of *Parinari curatellifolia* and *Hilleria latifolia* showed better antibacterial activity against *Staphylococcus aureus* than the ethyl acetate

extracts of the remaining medicinal plants. The ethyl acetate extract of *Celastrus senegalensis* was found to exhibit the least antibacterial activity against *Staphylococcus aureus,* having the highest zone of inhibition of 15.00±1.00 mm. Also, in Table 1, the methanol extracts of *Heliotropium indicum* and *Hilleria latifolia* exhibited better activity than the other methanol extracts. The *Hilleria latifolia* methanol extract exhibited 27.00±1.00 mm zone of inhibition at 100.00 mg/ml concentration.

In Table 2, the antibacterial activity of the five medicinal plants against *Escherichia coil* are shown, while *Hilleria latifolia* extracts exhibited the highest zone of inhibition at 100.00 mg/ml concentrations. The *Maytenus senegalensis* extracts exhibited the least antibacterial activity against *Escherichia coil* at all concentrations. It has been reported that new resistance mechanisms are emerging, spreading globally, threatening our ability to treat common infectious diseases. Death attributable to antimicrobial resistance is at an alarming rate and it has been projected to increase to ten million death by the year 2050

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Discussion

Medicinal plants have been of age long remedies for human diseases because they contain component of therapeutic value (Nostro *et al*., 2000). In this study, the ethyl acetate and methanol extracts of five different medicinal plants were investigated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coil.* The plant extracts antibacterial activity were determined at different concentrations and were compared with Gentamicin as standard. In Table 1, the ethyl acetate and methanol extracts of *Hilleria latifolia* exhibited the highest antibacterial activity against *Staphylococcus aureus* at all concentrations. In Table 2, the methanol extract of *Hilleria latifolia* showed highest antibacterial activity against *Escherichia coli* at all concentrations but not higher than the standard drug used.

Stem-bark and root-bark extracts of *M. senegalensis* were tested against *Bacillus subtilus*, *Micrococcus luteus* and *Staphylococcus aureus*. (Khalid *et al*., 2007). Maytenonic acid-isolated rootbark has a proven antibacterial activity against *B. subtilus, Escherichia coli, Klebsiella pneumoniae* and *S. aureus*. (Lindsey *et al*., 2006). An acetone extract of the aerial parts of this species has been revealed to be active against a sensitive strain of *Mycobacterium tuberculosis* (H37Rv strain, 0.50 mg/mL) (Lall and Meyer, 1999). A number of studies have shown that extracts from *P. curatellifolia* have a number of pharmacological properties ranging from antibacterial activity to anticancer activity (Maroyi, 2013). The methaolic extract of aerial parts of *H. indicum* has broad spectrum of antibacterial activity against *S. aureus, Streptococcus pyogenes, S. pneumonia, Salmonella typhi, Corynebacterium ulcerans, E. coli* and *Klebsiella pneumonia* with the zones of inhibition 32.00, 35.00, 30.00, 0.00, 0.00, 28.00, 27.00 mm verified for these bacteria (Oluwatoyin *et al*., 2011).

H. latifolia is used extensively in traditional medicine for the treatment of diseases, especially as an anti-infective, anti-inflammatory and analgesic agent (Schmelzer and Gurib-Fakim 2008). The aerial part of the plant was used for this experiment and the previous works done on the plant showed that the leaf, twigs, root, stem and fruit seed extracts of *A. muricata* have several biological activities such as antibacterial (Viera *et al*., 2010), antitumor (Hamizah *et al*., 2012) and anti-malarial (Antoun *et al*., 1993).

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4.Conclusion

In conclusion the *Hilleria latifolia* plant aerial part showed better antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* than the other medicinal plants used in this study. The antimicrobial activity of the plant is due to the presence of active compounds and there is need for further studies to isolate and characterize the active compounds responsible for this activity.

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