International Journal of Agriculture and Animal Production
ISSN 2799-0907
Vol : 02 , No. 04 , June-July 2022
http://journal.hmjournals.com/index.php/IJAAP
DOI: https://doi.org/10.55529/ijaap.24.1.8

Comparative Study on the Antimicrobial Activity of Some Selected Medicinal Plants on Klebsiella pneumonia & Candida albicans

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Received: 28 February 2022 **Accepted:** 10 May 2022 **Published:** 15 June 2022

Abstract: ***Medicinal plants have been the basis of treatment of various diseases by man over the years and a large portion of the world population are depending on the use of traditional medicine. Medicinal plants are known to be source of bioactive compounds that have therapeutic values, the World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because of the great potential they possess. In this research work five different medicinal plants were subjected to antimicrobial study against Klebsiella pneumonia and Candida albicans at different concentrations. The plants were extracted using hexane, ethyl acetate and methanol. The ethyl acetate extract of Parinari curatellifolia exhibited better antibacterial activity than the other extracts at all concentrations, having 26.00±0.00 mm zone of inhibition at 100.00 mg/ml concentration. The ethyl acetate and methanol extracts of Hilleria latifolia exhibited the highest zone of inhibition against Candida albicans, having zone of inhibition value of 20.00±0.00 mm at 100.00 mg/ml concentration. None of the extracts exhibited higher zone of inhibition than the standard drugs used. The study has shown that Parinari curatellifolia is a better antimicrobial agent against infections caused by Candida albicans and Klebsiella pneumonia.***

Keywords: ***Antimicrobial Study, Bioactive Compounds, Medicinal Plants, Therapeutic Values, Zone of Inhibition.***

1. INTRODUCTION

Medicinal plants are the backbone of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish, 2008). Medicinal plants are known to be source of bioactive compounds that have therapeutic

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values, the World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because of the great potential they possess (Amos et al., 2001). Currently, antimicrobial resistance (AMR) is one of the major threats to global health and factors such as global climatic change, globalization (increased international travel and food importation/exportation), and change in demographics are worsening the crisis (Cheng et al., 2016; Miranda et al., 2013; WHO, 2014). It is estimated that by 2050, the death rate due to AMR will balloon to 10 million lives per year at an expense of one hundred trillion dollars (O'Neill, 2016; De Kraker, 2016).

Each year, pneumonia affects about 450 million people globally and results in about 4 million deaths (Ruuskanem et al., 2011; Lodha et al., 2013). With the introduction of antibiotics and vaccines in the 20th century, survival has greatly improved (Ruuskanem et al., 2011). Nevertheless, pneumonia remains a leading cause of death in developing countries, and also among the very old, the very young, and the chronically ill (Ruuskanem et al., 2011; George, 2005). Pneumonia often shortens the period of suffering among those already close to death and has thus been called "the old man's friend" (Eddy, 2005). Candidiasis is a fungal infection caused by a yeast called Candida. Some species of Candida can cause infection in people; the most common is Candida albicans. Candida can cause infections if it goes out of control or if it enters deep into the body (for example, the bloodstream or internal organs like the kidney, heart, or brain) (Centers for Disease Control & Prevention, 2020). Invasive candidiasis has become a substantial threat to public health. It affects more than 250,000 people every year and is associated with a mortality rate exceeding 70 % (Pappas et al., 2018; Bassetti et al., 2013; Lai et al.,2008).

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 air tight 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.

2.3 Microorganisms

In this study, one bacteria strain and one fungal strain were used, the bacteria strain was Klebsiella pneumonia where the fungal strain was Candida albicans. The bacteria used was a clinical isolate obtained from Medical Microbiological Department of University College Hospital, University of Ibadan, Ibadan, Oyo State Nigeria. Single colony plates of nutrient agar medium of this organism was maintained at 4 ˚C and sub-cultured on to nutrient broth

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for 24 h prior to testing. The fungus was maintained on the prepared sterile Sabouraud dextrose agar medium.

2.4 Antibacterial Activity Assay:

Antibacterial activity of the Annona muricata, Heliotropium indicum, Hilleria latifolia, Maytenus senegalensis, 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 concentration 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 h to allow pre-dilution. The plates were incubated uprightly at 37 ºC for 18 - 24 h. Then antibacterial activity was determined by measuring the diameter of zone of inhibition (ZI) in millimeter.

2.5 Antifungal Activity Assay

Antifungal activity of the ethyl acetate extract and methanol extract of Annona muricata, Heliotropium indicum, Hilleria latifolia, Maytenus senegalensis, Parinari curatellifolia were determined by using surface plate method (agar diffusion) on a sterile Sabourand Dextrose Agar. A sterile Sabourand Dextrose Agar was prepared accordingly and aseptically poured into the sterile petri dishes in duplicates and allowed to set properly, 0.2 ml of the diluted organism (10^{-2}) was spread on the agar using a sterile cork borer of the 6 mm diameter. Tioconajole (70 %) was used as the positive control while the solvent of extraction was used as the negative control. In each of the well the graded concentrations of the sample were introduced into the wells including the controls. The plates were then left on the bench for 2 h. so as to allow the sample to diffuse properly into the agar. The plates were incubated uprightly in the incubator for 48 h at 26 - 28 ˚C. The fungi plates were observed after 48 h of incubation and the clear zones of inhibition were measured in millimeter.

3. RESULTS & DISCUSSION

Table 1: The Plants Antibacterial Activity Against K. pneumonia

S/N	Plant	Extract	Gentamicin	Zone of Inhibition (mm) at Different Concentrations		
				100.00 (mg/ml)	50.00 (mg/ml)	25.00 (mg/ml)
1	Maytenus senegalensis	EAEMS	37.00 ±1.00	17.00 ±1.00	14.00 ±0.00	12.00 ±0.00
		MEMS	37.00 ±1.00	15.00 ±1.00	13.00 ±1.00	10.00 ±0.00
2	Parinari curatellifolia	EAEPC	37.00 ±1.00	26.00 ±0.00	22.00 ±0.00	19.00 ±1.00
		MEPC	37.00 ±1.00	17.00 ±1.00	14.00 ±0.00	12.00 ±0.00

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3	Heliotropium Indicum	**EAEHI**	36.00 ±0.00	14.00 ±0.00	12.00 ±0.00	10.00 ±0.00
		MEHI	37.00 ±1.00	21.00 ± 1.00	18.00 ±0.00	16.00 ±0.00
4	Hilleria Latifolia	**EAEHL**	38.00 ±0.00	23.00 ±1.00	19.00 ± 1.00	17.00 ±1.00
		MEHL	38.00 ±0.00	25.00 ±1.00	21.00 ±1.00	18.00 ±0.00
5	Annona muricata	**EAEAM**	38.00 ± 0.00	25.00 ±1.00	21.00 ±1.00	17.00 ±1.00
		MEAM	37.00 ±1.00	21.00 ±1.00	18.00 ±0.00	16.00 ±0.00

EAEMS - Ethyl acetate extract of Maytenus senegalensis, **MEMS** - Methanol extract of Maytenus senegalensis, **EAEPC**- Ethyl acetate extract of Parinari curatellifolia, **MEPC** - 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

Table 2: The Plants Antifungal Activity Against Candida albicans

S/N	**Plant**	**Extract**	**Tioconajole (70 %)**	**Zone of Inhibition (mm) at Different Concentrations**		
				100.00 (mg/ml)	**50.00 (mg/ml)**	**25.00 (mg/ml)**
1	Maytenus senegalensis	**EAEMS**	28.00 ±0.00	20.00 ±0.00	18.00 ±0.00	16.00 ±0.00
		MEMS	28.00 ±0.00	17.00 ±1.00	14.00 ±0.00	10.00 ±0.00
2	Parinari curatellifolia	**EAEPC**	28.00 ±0.00	20.00 ±0.00	18.00 ±0.00	16.00 ±0.00
		MEPC	28.00 ± 0.00	17.00 ±1.00	14.00 ±0.00	12.00 ± 0.00
3	Heliotropium indicum	**EAEHI**	28.00 ±0.00	18.00 ±0.00	16.00 ±0.00	14.00 ±0.00
		MEHI	27.00 ±1.00	18.00 ±0.00	16.00 ±0.00	14.00 ±0.00
4	Hilleria latifolia	**EAEHL**	27.00 ±1.00	20.00 ±0.00	18.00 ±0.00	16.00 ±0.00
		MEHL	32.00 ±6.00	20.00 ±0.00	18.00 ±0.00	16.00 ±0.00
5	Annona muricata	**EAEAM**	27.00 ±1.00	19.00 ±1.00	17.00 ±1.00	15.00 ±1.00
		MEAM	28.00 ±0.00	15.00 ±1.00	13.00 ±1.00	10.00 ±0.00

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EAEMS - Ethyl acetate extract of Maytenus senegalensis, **MEMS** - Methanol extract of Maytenus senegalensis, **EAEPC**- Ethyl acetate extract of Parinari curatellifolia, **MEPC** - 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

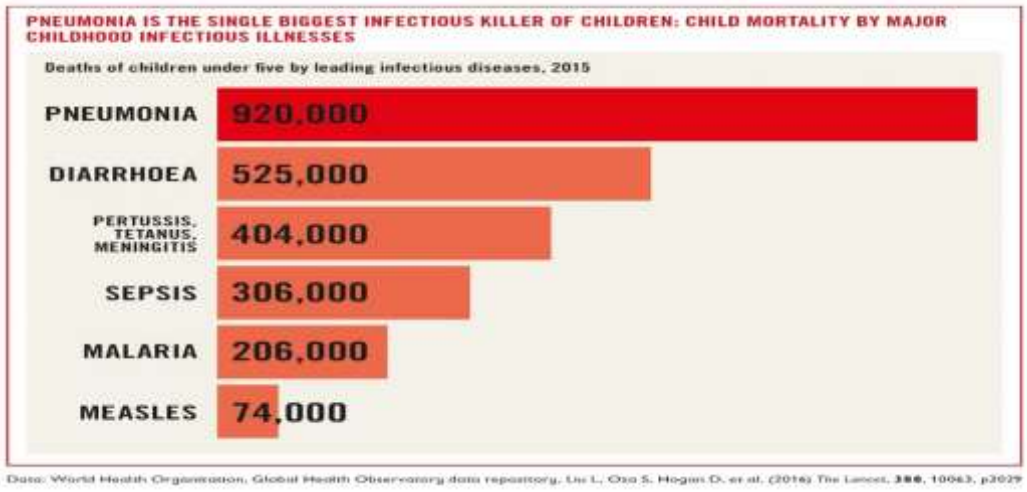

Fig. 1: Child Mortality by Major Childhood Infectious Illnesses

Antimicrobial Activity

The antimicrobial activity of the ethyl acetate extract and methanol extract of five medicinal plants were investigated against Klebsiella pneumonia and Candida albicans at different concentrations. The results showed that the antibacterial activity and antifungal activity of the plant extracts are concentration dependent, having better antimicrobial activity at higher concentrations. In Table 1, the ethyl acetate extract of Parinari curatellifolia exhibited the highest antibacterial activity against Klebsiella pneumonia in all the concentrations. The ethyl acetate extract of Heliotropium indicum exhibited the least antibacterial activity against Klebsiella pneumonia, its zone of inhibition ranged from 10.00±0.00 mm to 14.00±0.00 mm. None of the plant extracts had higher zone of inhibition than the standard drug used (Gentamicin). In Figure 1, it is shown that the highest death of children under five was caused by pneumonia, as reported by the World Health Organization. In Table 2, the results of the antifungal activity of the plant extracts against Candida albicans are shown using Tioconajole (70.00 %) as standard drug. The ethyl acetate extracts of Maytenus senegalensis and Parinari curatellifolia exhibited the same antifungal activity against Candida albicans at all concentrations, having their highest zone of inhibition as 20.00±0.00 mm. The methanol extract of Annona muricata showed the least antifungal activity against Candida albicans, having the lowest zone of inhibition at all concentrations.

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4. DISCUSSION

A large portion of the world population (more than 85.00 %) especially in developing countries according to a World Health Organization (WHO) report depend on traditional systems of medicine for treatment of variety of diseases (WHO, 1993). In this study, the antimicrobial activity (antibacterial and antifungal) of five different medicinal plants were determined and the results compared with standard drugs. In Table 1, the Parinari curatellifolia ethyl acetate extract exhibited the highest antibacterial activity against K. pneumonia at all concentrations. In Table 2, the ethyl acetate of Heliotropium indicum and Hilleria latifolia exhibited the same antifungal activity with their respective methanol extracts against Candida albicans at all concentrations. Maytenus heterophylla and Maytenus senegalensis are two African shrubs or trees that go under the common name of spike thorn, which belong to the Celastraceae family. Different plant parts of this species are largely used in traditional medicine for infectious and inflammatory diseases treatment (da Silva et al., 2011). Maytenus senegalensis is a synonym of Celastrus senegalensis and it has been reported to have antibacterial property (Lindsey et al., 2006; Jain et al., 2008). Parinari curatellifolia has various documented uses in ethnomedicine including treatment of wound infections, cancer, pneumonia, fever, bacterial infections, and inflammation (Kraft et al., 2003). The methanolic 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 (Schmelze and Gurib-Fakim, 2008). The leaves of A. muricata has been found to exhibit a significant inhibition against some selected groups of fungi as Alternaria solani, Alternaria albicans, Aspergillus fumigatus and Penicillum chrysogenum (Abubacker and Deepalakshmi, 2013). A. muricata leaf extract exhibits a broad spectrum of activity against a panel of bacteria (B. subtilis, Staph. aureus, K. pneumonia, P. vulgaris, etc.) responsible for common bacterial diseases like pneumonia, diarrhea, UTIs and skin infections (Gbeassor et al., 1990).

5. CONCLUSION

These results showed that the plant extracts exhibited antimicrobial activity against K. pneumonia and Candida albicans, this is due to the presence of active compounds in the plants. Therefore, for further studies it is recommended to isolate and characterize the active compounds responsible for the antimicrobial activity.

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