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ANTIBACTERIAL ACTIVITIES OF LEAF METHANOLIC EXTRACTS OF *Leptadenia hastata* AGAINST *Escherichia coli* CLINICAL ISOLATE

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ABSTRACT

The research aims at potential activities of leaf methanolic extracts of *Leptadenia hastata* against clinical *Escherichia coli* and extraction, phytochemicals and biochemical tests to the plant and organism. *Escherichia coli* is Gram negative, non-capsulated, bacillus, clinically important bacteria mostly isolated clinically and capable to cause gastro enteritis, urinary tract infections, diarrhea among others. *L. hastata* is a climbing shrub that produces flexible latex which become woody at the base. Potential activities of *L. hastata* leaf methanolic extracts against clinical *Escherichia coli* were observed. *L. hastata* leaf were sampled, Phyto-chemically screened using methanol and processed using Di-MethylSulfurOxide (DMSO). Clinical Bacterial Isolates were collected from Murtala Muhammed Specialist Hospital Kano, Kano State, Nigeria, investigated and screened for *E. coli* using Gram's staining technique and some biochemical tests procedure for confirmation. *L. hastata* leaf methanolic extracts were processed against the isolates. Four (4) *E. coli* were found and tested against leaf methanolic extracts of *L. hastata* at different concentrations of 125, 250, 500 and 1000 mg/mL respectively. Activity was observed directly proportional to the concentrations, where the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were at 500 and 1000 mg/mL accordingly. The research may lead to effective drug development against the clinical *E. coli* to address its pathogenicity, for the Public Health benefit. It is recommended that further research should be carried out to determine bioactive components of *L. hastata* leaf, toxicity level tests and Molecular characterization to the isolate *(E. coli)*.

Keywords: Potential*, Leptadenia hastata, E. coli,* Methanolic Extracts, Isolates

INTRODUCTION

Bacteria are divided into pathogenic and non-pathogenic in terms of disease causing capability, therefore, the pathogenic here are significantly considered, for their clinical importance. Pathogenic bacteria are the one that have ability to cause disease to their host (both plant and animal). Most of the clinical bacteria are often to be pathogenic like *Eschericia coli*, *Staphylococcus aureus* among others (Yusha'u and Kawo*,* 2018). *Escherichia coli,* is Gram negative, non-capsulated, bacillus and clinically important bacteria mostly isolated clinically (Tham, *et al*., 2010). *L. hastata* survives well under dry, harsh and non-fertile soil status and it resists drought (AJDDD.; 2018). The plant is used for supplement, nutriment and antimicrobial, this is for its medical promoting properties and it is found in both Jigawa and Kano State, Nigeria (Bayala *et al.,* 2012). The plant is applied broadly for controlling and treatment of various disease conditions (Ishaac *et al*., 2018). Methanolic extracts of *L. hastata* antimicrobial components as source of potential remedies against pathogenic *E. coli* exist for several years. So, as at now greater percentage of populations in Africa use combine and or single medicinal herbs for day to day medical care demand (WHO, 2008). Plants like *L. hastata* are medically employed in some developing and developed states for various treatment (Rekha *et al.*, 2013). Many disease conditions such hypertension, trypanosomiasis, hypertension, acute rhinopharyngitis, sexual impotence among others are treated with *L. hastata* leaf extracts in qualitative and quantitative application (Yaro, *et al.,* 2022).

Plate 1: *Leptadenia hastata* climbing leaf

MATERIALS AND METHODOLOGY

The plant (*L. hastata*) Leaf were handily picked, identified and given certified accession number at the Department of plant Biology, Bayero University, Kano, Nigeria. The Sample Size is calculated according to Arzai *et al* standard $N = \frac{Z^2(P \times Q)}{d^2}$ (Arzai *etal.,*2014). The characteristics of *L. hastata* Leaf methanolic extracts were obtained using phytochemical screenings procedure according to Cheesebrough, (2000; 2005 & 2010) and Evans (2020). As the plant Leaf materials collected, were air dried at room temperature, as done and been doing by traditional medicine practitioners, then were made into small pieces and grinded to powder form using clean laboratory motor and pestle. Extraction of the antimicrobial/bio-components was done by percolation method at room temperature using methanol (solvent) according to Cheesebrough, (2000; 2005 & 2010); CLSI (2005); Evans (2020) and Kontagora, *et al*., (2020). 100g of the coarse plant leaf was dissolved in 1 L of methanol (solvent), then were packed into a thimble and transferred into a Soxhlex extractor. The extraction was carried out with 1 L of 95% methanol until there was no colour change in the methanol, which indicated that the extraction has been completed. The methanolic extracts harvested were filtered using a rotary evaporator, where the methanol extracts were separated. The remaining methanol in the extracts were separated using the oven set at 80ºC until the weight was constant. The extracts were collected in plastic container, closed tightly (air and water tight) and stored in a refrigerator at 4ºC to the required time, that was sensitivity test time.

The Research Ethical Consideration

The research ethical consideration and clearance is issued by Kano State Ministry of Health. Where Murtala Muhammad Specialists Hospital Kano was the hospital used for the research clinical samples.

Isolation and Screening of the Clinical Isolates

The test organism is *Escherichia coli.* The clinical bacterial isolates were clinically and aseptically collected from the clinic (Murtala Muhammad Specialists Hospital Kano), in the Petri dishes, wrapped in the nylon bag, enclosed in the professionalized clinical samples transportation iced box, aseptically taken to Department of Microbiology, Bayero University, Kano Nigeria, where the organism was screened and identified, successfully. The colonies from different culture media were examined physically for their characteristics appearance that include colony size, shape, pigmentation, odor, reaction

to Gram's staining for revealing their morphology microscopically and biochemically using the standard procedure of Cheesebrough (2000 & 2010). The biochemical tests employed include: citrate utilization test, Triple Sugar Iron (TSI) tests, urease test, indole test, motility test, catalase and coagulase tests. The samples source areas are: Skin wound, throat/sputum swabs, urinary/gastrointestinal tract (urine deposit), High Virginal Swab among others as in accordance with Clinical Laboratory Standard Institute (CLSI), 2005 and Cheesebrough, (2000 & 2005).

Isolates Sensitivity Screening using NZ25N And NZ53P Discs

Nutrient agar powder were prepared using distilled water, sterilized by autoclaving according to (R.S.Yaro Yalwa., 2016 and 2018). Then aseptically dispensed in twenty milliliter (20 ml) into Petri dishes and solidified. The isolates were aseptically emulsified on the face of prepared culture medium, using sterile swab stick and allowed for 1h proper diffusion. The discs were aseptically mounted on the emulsified and diffused isolates in the Petri dishes, labelled and inverted at 37°C for 24 h, for Zones of inhibitions reports after 24 h using Vernier Caliper in accordance with Clinical Laboratory Standard Institute (CLSI), (2005) and Cheesebrough, (2010).

Gram's Staining to the Isolates

The 24 h old cultured test organisms, aseptically smeared on cleaned, sterile, grease free microscopic glass slides and fixed by flaming for 5 times for 5 S each, then they were allowed to cool and full steps of Gram's staining technique were observed, bloated and air dried and examined microscopically using oil immersion objective lenses (x100 power) for the Gram's staining reaction results, that is Gram's positive and negative respectively as in accordance with Clinical Laboratory Standard Institute (CLSI), (2005) and Cheesebrough, (2000; 2005 & 2010).

Gram's Staining Results

Four (4) organisms with characteristics of Gram's negative reaction, bacillus, and non-capsulated in nature were

suspected to be *E. coli,* out of sixteen (16) tested isolates, then confirmed biochemically.

Biochemical Tests Against the Isolates

After Gram's staining technique, some of the following biochemical tests were employed against the clinically isolated *E. coli*: citrate utilization test, Triple Sugar Iron (TSI) tests, urease test, indole test, motility test, catalase and coagulase tests. as in accordance with Clinical Laboratory Standard Institute (CLSI), (2005) and Cheesebrough, (2000 & 2005) among others.

Triple Sugar Iron (TSI) tests

65 g/l of Triple Sugar Iron was prepared, aseptically dispensed into test tubes, closed, boiled to completely dissolve, processed and sloped according to (R.S.Yaro Yalwa., 2016 and 2018). The organisms were aseptically inoculated, processed within 24 h at 37°C. The report showed the hydrogen sulphide production which is one of the confirmations of *E. coli* as per gram negative bacilli isolate.

Catalase

Drops of Hydrogen peroxide were placed on grease free microscopic glass slide, a loop-full of the isolate(s) was emulsified aseptically using sterile wire loop, the result showed no frothings after 60 s, this is also one of the confirmatory of *E. coli* as gram negative bacilli isolate as in accordance to Cheesebrough, (2000).

Coagulase test

Blood serum drops were aseptically placed onto grease free microscopic glass slide, a loop-full of the isolate(s) was emulsified aseptically using sterile wire loop, the precipitates as a result of clumping or coagulation were not observed from the isolates after 60 s, so this confirmed the organism as Coagulase negative and as *E. coli*, tested Indole positive, MR negative, VP negative, and tests Motility positive among others as in accordance with Cheesebrough, (2000). The result tables are in the appendix pages as obtained.

Susceptibility of the *L. hastata* Leaf Methanolic Extracts Against tested Clinical *E. coli*

The Bioassay was carried out as followed:

McFarland standard Application

The turbidity of the *E. coli* suspension to be tested was standardized using the McFarland standard.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using Agar Dilution Method

Procedure: 21 g of Mueller Hinton Broth powder were prepared in 1L of distilled water, dissolved and distributed in sterile 5ml bijou bottles, processed according to (R.S.Yaro Yalwa., 2016 and 2018). Isolated colonies from grown test organism plates were picked and inoculated into the prepared Mueller Hinton Broth (in 5 ml batches). Bacterial inoculum was prepared in Mueller Hinton Broth (in 5 ml batches) and compared with the 0.5 prepared McFarland standard. 38 g of Mueller Hinton Agar powder were prepared in 1L of distilled water, dissolved and autoclave and dispensed in 20 mL and allowed to solidify according to (R.S.Yaro Yalwa., 2016 and 2018). The grown test organism was aseptically processed. The swabbed plates were allowed for 1 h diffusion. Holes were punched with the aid of Cork's borer. The Leaf methanolic extracts of *L. hastata* were aseptically introduced into punched holes respectively using sterile syringe each in triplicate. Allowed to diffuse for 1h, then processed at 37°C for 24h. The reports were taken in mm, where the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) were obtained at 500 and 1000 mg/mL in accordance with Clinical Laboratory Standard Institute (CLSI), (2005); Akinyemi *et al.,* (2005); Valleycobia *et al.,* (2001) and Cheesebrough (2010) among others.

RESULTS AND DISCUSSION

Results

Table 1: Extraction, Isolation, Antimicrobial activity, MIC and MBC of *L. hastata* leaf methanolic extracts against *E. coli*.

Extraction and Isolation of Leaf components of the *L. hastata*		**Antimicrobial activity of leaf methanolic extracts of *L. hastata* concentration mg/mL (stat.)**				**MIC & MBC of *L. hastata***		
Leaf extract		**"T. org.**	**125**	**500**	**1000"**	**"125**	**500**	**1000"**
50g/500 ml	Weight of Methanolic extract = 5.0 g	*E. coli*	1.88±2.39 10.75±2.55	1.87±2.39 11.5±0.33	1.88±2.39 10.5±0.33	+	+	-
Characteristics	Solid: Greenish methanolic: Dark Greenish					**125** +	**500** +	**1000** -
Percentage Yielded	0.5 %							

Key: *L. hastata*, = *Leptadenia hastata,* g =gram, % = percentage, T. org. = test organism, + = present of activity, - = lack of activity, *E. coli = Escherichia coli,* Stat.= statistical analysis, µg/ml =microgram per milliliter.

Discussion

The leaf Methanolic extracts of *L. hastata* have reasonable inhibitory and bactericidal activities on the clinically isolated *E. coli* at 500 and 1000 mg/mL concentrations each respectively.

Therefore, the finding reveals that leaf methanolic extracts of the *L. hastata* are effective against clinical pathogenic *E. coli* similarly compared to the study of Aquino *et al*., (2011) where their results revealed that the stem bark aqueous extracts of *L. hastata* markedly inhibited the growth of *P. aeruginosa* at 600mg/mL and *E. coli* and Yusha'u (2011) as his results revealed that the stem bark of ethanolic extracts of *Hibiscus, sabdariffa* markedly inhibited the growth of *E. coli*, *K. pneumoniae* and *P. auruginosa* at 4000 mg/disc as reported that the metabolites in the plant were reported to possess antimicrobial components and be responsible for antimicrobial activity associated with some ethno-medicinal plants. The finding is also similar to the study conducted by Bello *et al*.; (2012) and Aliero *et al.,* (2011) respectively on *L. hastata* leaf extracts that indicated the presence of triterpenes, cardiac glucosides, phenolic glycosides, tannins, flavanoids, total flavonoid, alkaloids and saponins and Bayala *et al.,* (2011) comparatively and respectively.

CONCLUSION

Conclusively, *L. hastata* against clinical pathogenic *E. coli* should be essentially and easily accessible, as per Development of Public Health, to Kano state, Nigeria and Africa as well as the world-wide in general as the plant is widely and abundantly available.

RECOMMENDATION

Further investigation should be done for the plant (*L. hastata*) essential bioactive components. Tests for Gas chromatography mass spectrometer (GCMS) and toxicity level for human consumption. The isolate (*E. coli*) molecular characterization should be carried out, for the right drug development of the organism treatment, as per Public Health matter.

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