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 Escherichia 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, nutrient 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).
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)}{\varepsilon^2} (Arzai et al., 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 Soxhlet 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 coagulate 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

Isolates Sensitivity Screening using NZ25N And NZ53P
Discs
Nutrient agar powder were prepared using distilled water,
stereilized 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 37ºC 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

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>
<thead>
<tr>
<th>Table 1: Extraction, Isolation, Antimicrobial activity, MIC and MBC of <em>L. hastata</em> leaf methanolic extracts against <em>E. coli</em>.</th>
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<tr>
<th>Extraction and Isolation of Leaf components of the <em>L. hastata</em> (stat.)</th>
<th>Antimicrobial activity of leaf methanolic extracts of <em>L. hastata</em> concentration mg/mL</th>
<th>MIC &amp; MBC of <em>L. hastata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>50g/500 ml Weight of Methanolic extract = 5.0 g</td>
<td>“T. org. <em>E. coli</em> 1.88±2.39 10.75±2.55</td>
<td>“125 500 1000” + + +</td>
</tr>
<tr>
<td>Characteristics Solid: Greenish methanolic: Dark Greenish</td>
<td></td>
<td>125 500 1000 + + -</td>
</tr>
<tr>
<td>Percentage Yielded 0.5 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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.
ANTIBACTERIAL ACTIVITIES OF LEAF... Yaro et al., FJS

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 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 H. hispidus, saba dariffa markedly inhibited the growth of E. coli, K. pneumoniae and P. aeruginosa 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, flavonoids, 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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REFERENCE


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