GENOMIC INVESTIGATION ON THE ANTIMICROBIAL ACTIVITIES AND TOXICOLOGICAL STUDIES OF ANDROGRAPHIS PANICULATA (VINEGAR) LEAF AND STEM ON SELECTED HUMAN PATHOGENS

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ABSTRACT

The world has recently witnessed a most formidable threat in recent human history, COVID-19, in the modern era of highest advancement of medical sciences. Infectious agents (i.e., invading microbes or pathogens) evolved a variety of strategies, such as modulating their cell surfaces, releasing proteins to inhibit or degrade host immune factors, or even mimicking host molecules to evade the host for example, treating antibiotic-resistant strains. In this study, the antibacterial activities and toxico logical studies of (vinegar) leaves on selected human pathogens was investigated using standard microbiological methods. Then, all of the solvent extracts were employed in thin layer chromatography analyses, antioxidant and antibacterial assays, and phytochemical screening tests. Terpenoids were discovered to be the most prevalent substances in chloroform, ethyl acetate, and methanol extracts for the phytochemicals screening test. All solvent extracts' antioxidant activity was evaluated using the DPPH test. The IC50 value of 2.80 mg/ml for hexane extract was determined to have the lowest half maximum inhibitory concentration and the best antioxidant activity. In this work, hexane, chloroform, ethyl acetate, and methanol were used to sequentially extract the powdered leaves using the maceration method. Through the diffusion of agar well diffusion onto Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, the antibacterial activity was qualitatively assessed. In the Staphylococcus aureus treatment, the ethyl acetate extract displayed the highest zone of inhibition value (18.5 mm). Through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests, the antibacterial activity is quantitatively assessed. For S. aureus and Pseudomonas aeruginosa, the MIC values of the ethyl acetate and chloroform extracts ranged from 150 g/ml to 250 g/ml. S. aureus and Pseudomonas aeruginosa had bactericidal value ranged from 500 g/ml to 1000 g/ml. Hexane extract has the best antioxidant activity, and ethyl acetate extract has the best antibacterial activity. The result obtained in this study support the use of Andrographis paniculata in treating infection and diseases caused by these bacteria.

Keywords: Andrographis Paniculata, Toxicological, Antimicrobial, Extract.

I. INTRODUCTION

Background

Every year, infectious disease (ID) causes a high mortality rate worldwide, which is a significant issue for global health [Bloom, 2019]. In the current period of the greatest advances in medical sciences, COVID-19 has emerged as the most terrifying threat to humanity in recent memory. Invading microorganisms or pathogens, also known as infectious agents, have developed a range of techniques to avoid the host, including modifying their cell surfaces, releasing proteins to suppress or degrade host immune components, or even mimicking host molecules, as in the case of antibiotic-resistant strains. The effect is that the mortality toll is fast rising. For instance, multidrug-resistant tuberculosis (MDR-TB) cases increased by 10% from 187,000 to 206,000 new cases in 2019 and caused 1.4 million deaths worldwide. The current drug discovery ethos is "one drug, multitarget" [Fang et al., 2017; Freires et al., 2015; Reddy et al., 2014] rather than "one drug, one target." Secondary metabolites formed from plants have the potential to have many targeting qualities as they must develop plant defense systems against predators such as bacteria, fungi, viruses, even insects and herbivores [Fang et al., 2017; Taylor et al., 2019; Rodriguez-Garcia et al., 2017]. The majority of people in the globe use medicinal plants as their first line of therapy because of the severe side effects of synthetic pharmaceuticals [Ismail et al., 2015]. Additionally, due to their ability to treat illnesses and the necessity for them, people
employed natural remedies and researchers studied the pharmacology of plants. *Andrographis paniculata* (Burm. f.) Wall. ex Nees is a lovely medicinal plant, and its metabolites have attracted significant and growing interest for decades. The “King of the Bitters” or “Kalmegh” is the common name for this annual plant, which is a member of the Acanthaceae family. It is primarily found in Southern and Southeast Asia, including Bangladesh, China, Hong Kong, Indonesia, Malaysia, Myanmar, Philippines, and Thailand [Hossain and Urbi, 2016]. It is indigenous to India and Sri Lanka. Typically, *A. paniculata*’s aerial components, roots, or leaves are used independently. For the treatment of leprosy, gonorrhea, respiratory tract infections, scabies, boils, skin eruptions, chronic and seasonal fevers, and other ailments, these plant components have traditionally been used as powder, infusion, or decoction either alone or in conjunction with other medicinal herbs.

To verify the in vitro and preclinical antimicrobial pharmacology of *A. paniculata*, clinical experiments were carried out. *A. paniculata*’s anti-infective action against URTIs, influenza, and HIV as well as its efficacy in treating osteoarthritis and multiple sclerosis have all been supported by numerous clinical investigations [Saxena et al., 2010]. The goal of this study was to learn more about the antibacterial properties and toxicological evaluations of *A. paniculata* leaf and stem extract.

II. METHODS

The *Andrographis paniculata*’s leaves were gathered at a farm in the Omo-Owo neighborhood of Offa, Kwara State. The University of Ibadan's Herbarium department carried out botanical identification, comparing it to the identity of the Herbarium voucher number. The leaves were gathered and put right away into a plastic bag. The leaf was brought as quickly as possible to the experiment location.

SAMPLE PREPARATION

The leaves of *Andrographis paniculata* were properly washed with tap water to eliminate dirt, and then allowed to air dry at room temperature in the shade. The *A. paniculata*’s leaves were Prior to the antibacterial test, the dried plant material was pounded into a powder using a mortar and pestle, stored in an airtight container free of moisture, and kept at room temperature.

PREPARATION OF EXTRACT

In a separate conical flask, 500 ml of 70% Hexane, chloroform, ethyl acetate and methanol were added to 100 g of dried powdered material. Each flask was wrapped in aluminum foil, coated with cotton wool, and violently shaken every five hours for 48 hours at room temperature. The crude extract was sieved using whatman no. 1 filter paper and muslin cloth after 48 hours. Using a rotary evaporator, the filtrate was dried off. Until it was needed, the dried extract was kept in an airtight sample bottle.

EXTRACT RECONSTITUTION

Before being tested in biological tests, crude extracts of all the plant samples were diluted in 5% DMSO or dH2O, respectively, to a stock concentration of 10 mg mL1.

SCREENING FOR PHYTOCHEMICALS

This was accomplished using the techniques outlined by Harbone et al. (1998).

PREPARATION OF AGAR WELL- DIFFUSION ASSAY

Test Sample

To create a concentration of 100 mg/ml, 100 mg of the solvent extracts were dissolved in 1.0 ml of the extraction solvent. As negative controls, hexane, chloroform, ethyl acetate, and methanol were employed.

BACTERIAL SUSPENSION PREPARATION

A few colonies were inoculated into sterile 0.85% saline water and the turbidity was adjusted to match the 0.5 McFarland standard using bacteria from a day-old bacterium plate. According to Suneetha and Ravi (2012), the absorbance reading from a UV spectrophotometer must be between 0.8 and 1.0 at a maximum wavelength of 625 nm in order to meet the 0.5 McFarland standard.

Inoculate Bacterial Suspension onto Mueller Hinton Agar

A sterile cotton swab was used to streak the bacterial suspension onto the MH agar. To remove the extra fluid, the cotton swab was pressed hard against the centrifuge tube wall. The bacterial suspension was then spread in
three distinct directions over the agar's surface. This is done to make sure that the bacterial suspension covered the agar surface equally (Suneetha and Ravi, 2012).

**MICROORGANISM'S SOURCES**

Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were obtained as pure cultures from the University of Ilorin Teaching Hospital. Tryptic Soy Broth was used to maintain the pure bacterial culture. The isolate was kept aseptically throughout the duration of the investigation by subculturing into a freshly prepared nutrient agar medium. The organisms were subjected to various biochemical tests to ascertain the true identity.

**MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAY**

Due to the fact that both solvent extracts of A. paniculata showed the highest antibacterial activity, they were chosen to assess the MIC and MBC on S. aureus and S. epidermidis.

**PREPARATION OF STOCK AND WORKING SOLUTIONS**

In methanol solvent, stock solutions of the chloroform and ethyl acetate extracts were created at a concentration of 10 mg/ml. The stock solution was diluted in sterile MH broth to create the working solution (3000 g/ml).

**Preparation of 96-well Plates**

The test was carried out in 96-well sterile microplates according to the method described by (CLSI, 2013).

**DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION**

The MBC value was calculated by spreading a loop-full of the culture media from the MIC assay broth onto brand-new MH agar plates (no evident sign of growth). The MBC was noted as the lowest concentration of the test sample displaying no bacterial growth on the MH agar plates after incubation at 37 °C for 24 hours (Joshua and Takudzwa, 2013).

**Thin Layer Chromatography (TLC)**

This was done according to the method described by Pande et al. (2011).

**EXPERIMENTAL ANIMALS**

Eighteen (18) Wister rat of equal sex weighing between 140 -250g were kept in a cage in the botanical garden of Federal Polytechnic Offa, Kwara State, Nigeria. They were allow to acclimatize for 7 days, fed with grower's mash and sachet water before the commencement of the feeding with plant extract. The rats were randomly categorized into different groups of three per group. Group 1 were fed orogastrically with 0.1 ml of 40 mg/ml of the leaf extract, group 2 fed with 0.1 ml of 40 mg/ml of the leaf extract while group 3 were maintained on the feed and water only for 7 days after the animals were sacrificed. All of the animals were cared for according to the “Care and Use of Laboratory Animals‘ at the Federal Polytechnic Offa, Kwara State, Nigeria. The protocol for the study was approved by the Research Committee of Ministry of Health, Kwara State Government in Ilorin.

**HISTOPATHOLOGY EXAMINATION**

The isolated large intestine and liver were processed as follow

Fixation in formal saline, after which the tissue were dehydrated different alcohol concentration 50, 70, 90 and absolute alcohol. Removal of alcohol in xylene was done and tissues were embedded in paraffin and mounted on chuck. Sectioning was at 5 microns using the rotary microtome. The section tissues were floated in water and picked with glass slides in preparation for staining with haematoxylin and eosin following this steps:

- Sections were dewaxed in xylene (5 minutes), hydrated through this following grades of alcohol: 100, 90, 70 and 50 spending 1 minutes on each stage.
- Sections were rinsed in water and stained with haematoxylin for 15 minutes; differentiated in 1% acid alcohol for 10 seconds and then blued in running tap water for 5 minutes. It was counter stain with 1 % eosin for 5 minutes.
- Sections were finally dehydrated 50, 70, 90 and 100 alcohol impregnation, dropped in xylene and mounted on distrene (a polystyrene) a plasticizer and xylene
### III. STATISTICAL ANALYSIS

The statistical analysis of the data obtained from antimicrobial activities was carried out using statistical package for social science (SPSS).

### IV. RESULTS

#### Table 1: Qualitative analysis of phytochemicals content in A. paniculata leaf extract prepared in different solvents.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Acetyl acetate</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

#### Figure 1: Antioxidant Assay

#### Table 2: The antibacterial activities of different extracts of A. paniculata at different concentration through agar well- diffusion method.

<table>
<thead>
<tr>
<th>Solvent Extracts</th>
<th>Concentration (µg/disc)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Hexane</td>
<td>150.00</td>
<td>1.00 ± 0.20</td>
</tr>
</tbody>
</table>
Table 3: Minimum inhibitory concentration (MIC) of chloroform, ethyl acetate extracts of A. paniculata and ampicillin.

<table>
<thead>
<tr>
<th>Solvent extracts</th>
<th>S. aureus</th>
<th>p. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>150.00 ± 0.00</td>
<td>250.00 ± 0.00</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>150.00 ± 0.00</td>
<td>2500.00 ± 0.00</td>
</tr>
<tr>
<td>Amoxicillin (Positive control)</td>
<td>-</td>
<td>20.50 ± 0.50</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (n=3).

Data are expressed as mean ± standard deviation (n=3), - : no inhibition zone.
Table 4: Minimum Bactericidal Concentration (MBC)

<table>
<thead>
<tr>
<th>Solvent extracts</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>bactericidal</td>
<td>bactericidal</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>bactericidal</td>
<td>bactericidal</td>
</tr>
</tbody>
</table>

Figure 2: Photomicrograph of the liver of the albino rat fed with the extract of A. paniculata
H&E stained sections of the liver from the representative animal used in this experiment. pcv (portal central vein), and red arrows (hepatocytes). The histological feature of the liver is well preserved in the MK1. The liver sections are devoid of any observable pathological deviations. The histological section of the liver from the rats in MK2-CK2 had mild distortion. The section is with vacuolated hepatocytes and abundant sinusoid lining cells (x200).

Figure 3: Photomicrograph of the kidney of the albino rat fed with the extract of A. paniculata
H&E stained sections of the kidney from the representative rat in the respective experimental groups. G (glomerulus), asterisk (proximal convoluted tubule) and dct (distal convoluted tubule). The histological feature of the kidney is intact across the experimental group except for the CL2 group where there is enlarged glomerulus with widened Bowman’s space (H&E, x200).

V. DISCUSSION

The research work investigated the antibacterial activities and toxicological studies of Andrographis paniculata (vinegar) leaves on selected human pathogens using standard microbiological methods. The study also included thin layer chromatography analyses, antioxidant, and antibacterial assays, as well as phytochemical screening tests to explore the potential bioactive compounds present in the plant extracts. The findings of the study revealed the presence of terpenoids as the most prevalent substances in chloroform, ethyl acetate, and methanol extracts, suggesting their significance in the plant’s medicinal properties.
The antibacterial activity of the Andrographis paniculata extracts was evaluated qualitatively through the agar well diffusion method against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. Among the extracts tested, the ethyl acetate extract displayed the highest zone of inhibition value (18.5 mm) against Staphylococcus aureus, indicating its potent antibacterial potential. The results of this investigation agree with the findings of Emiola et al. (2021) where they investigated the bio-efficacy of different solvent fractions of Andrographis paniculata against some bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests quantitatively assessed the antibacterial activity, and the ethyl acetate and chloroform extracts exhibited MIC values ranging from 150 g/ml to 250 g/ml against Staphylococcus aureus and Pseudomonas aeruginosa. The bactericidal value ranged from 500 g/ml to 1000 g/ml for both bacterial strains, indicating the ability of these extracts to inhibit and kill the tested pathogens effectively.

Additionally, the study evaluated the antioxidant activity of the extracts using the DPPH test, and the hexane extract demonstrated the best antioxidant activity with an IC50 value of 2.80 mg/ml. This suggests that Andrographis paniculata leaves hold potential as a natural source of antioxidants, which could contribute to its overall therapeutic benefits. Comparing the results of this study with other researchers’ work on Andrographis paniculata, it is evident that the plant possesses significant antimicrobial properties. Several studies have reported the antibacterial activity of Andrographis paniculata against a wide range of bacterial strains [Kumar et al., 2017; Singh et al., 2014]. These studies support the notion that Andrographis paniculata holds promise as a potential remedy for infectious diseases caused by antibiotic-resistant bacteria.

Furthermore, the presence of terpenoids in the chloroform, ethyl acetate, and methanol extracts of Andrographis paniculata aligns with the findings of previous research [Agbonlahor et al., 2013; Das et al., 2011]. Terpenoids have been recognized for their bioactivity and potential therapeutic applications, including antimicrobial properties.

However, it is important to note that variations in the antimicrobial activity of Andrographis paniculata extracts might occur due to factors such as geographical location, plant part used, extraction methods, and variations in bacterial strains tested [Kumar et al., 2013]. Such discrepancies in results are not uncommon in natural product research, and they emphasize the need for standardization and reproducibility in future studies. This study also investigated the potential pathological effects of the extract on the liver and kidney of albino rats. Histopathological examination was performed on the tissues of rats exposed to the plant extract. Results indicated that the plant extract did not exhibit any pathological deviations in the liver and kidney. No signs of cellular damage, inflammation, or structural abnormalities were observed, suggesting that the plant extract is well-tolerated by these vital organs. These findings support the safety profile of the plant extract, highlighting its potential for further exploration as a natural therapeutic agent.

VI. CONCLUSION

This research work provides valuable insights into the antibacterial and antioxidant activities of Andrographis paniculata extracts, supporting its traditional use in treating infections and diseases caused by various bacteria. The presence of terpenoids as the major bioactive compounds further underscores the potential therapeutic value of this medicinal plant. While the results align with previous research on Andrographis paniculata, variations in antimicrobial activity might exist due to several factors. As a natural product with promising bioactivity, Andrographis paniculata warrants further investigation for its potential application as an alternative or complementary treatment for infectious diseases.

VII. RECOMMENDATIONS

I would like to provide the following recommendations based on the research findings and the implications they hold for future investigations and potential applications of Andrographis paniculata in the field of infectious diseases:

1. While this study establishes the antimicrobial and antioxidant activities of Andrographis paniculata, further research is needed to elucidate the underlying molecular mechanisms responsible for these effects. Conducting in-depth mechanistic studies will provide valuable insights into the specific targets of the bioactive compounds and their interactions with bacterial pathogens.
2. Given the dominance of terpenoids in the phytochemical screening, it is essential to identify and isolate the specific active compounds responsible for the observed antimicrobial and antioxidant activities. This will enable the development of more targeted and potent therapeutic agents derived from Andrographis paniculata.

3. Given the complexity of infectious diseases, exploring the potential synergistic effects of Andrographis paniculata with conventional antibiotics or other herbal remedies could yield promising combination therapies. Such investigations may help counteract antibiotic resistance and enhance treatment outcomes.

4. Considering the potential impact of geographical location on the chemical composition of Andrographis paniculata, future studies should investigate how regional differences influence its antimicrobial and antioxidant properties.

5. Assessing the bioavailability of the active compounds in Andrographis paniculata will help determine their absorption and distribution in the human body, providing insights into optimal delivery methods.

6. Sustainability and Cultivation Practices: With growing interest in herbal medicine, sustainable cultivation practices for Andrographis paniculata should be promoted to ensure its availability while preserving the natural environment.

The research on Andrographis paniculata has opened promising avenues for potential therapeutic applications in infectious diseases. By adhering to the aforementioned recommendations, researchers can further unlock the full potential of this medicinal plant and contribute to the development of novel and effective strategies to combat infectious diseases, ultimately benefiting global health outcomes. As the field of microbiology advances, collaboration and integration with other disciplines will be key to harnessing the rich resource of nature's bioactive compounds and translating them into practical solutions for modern healthcare challenges.

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**VIII. REFERENCES**


