UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL SURVEY AND IN VITRO ANTIBACTERIAL ACTIVITY OF ETHNOMEDICINAL PLANTS TO TREAT GASTROINTESTINAL AILMENTS IN YOBE STATE, NIGERIA

ABDALLAH MUHAMMAD SALIHU

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By

ABDALLAH MUHAMMAD SALIHU

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2021

DEDICATION

I dedicated this work to my late father (Salihu Abdallah Gamoji) and my lovely mother (Hajiya Maryam Abubakar Bello).

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in Fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairperson : Rusea Go, PhD
Faculty : Science

Many plants in Yobe State, Nigeria have potentialities in curing many diseases. Rural and urban people made use of medicinal plants as their curative measures with the fact that, conventional drugs are quite expensive as well as contribute to the antimicrobial drug resistance. Abundant chemical constituents played vital roles which enabled the fight against any disease and ailments. This study aim to: Assess the most commonly used plant extracts in curing gastrointestinal ailments across Yobe State, Nigeria; evaluate their antibacterial efficacy against some enteric isolates using disk and well diffusion; determine phenolic and flavonoid contents of these plants extracts (*Vachelia nilotica* pods extract, *Sclerocarya birrea* stem (bark) extract, *Guiera senegalensis* leaves extract and *Leptadenia hastata* leaves extract) and lastly, determine the bioassay guided fractionating substances of the selected plant stem (bark), and detect compounds present in *Sclerocarya birrea* (A.Rich.) Hochst stem (bark) fraction capable of actions against the isolate using LC-MS. Thus, a semi structured questionnaires was used to collect initial information on the plants. Their antibacterial efficacy were tested using isolates; *Escherichia coli, Salmonella typhi, Bacillus cereus* and *Staphylococcus aureus*, where inhibition zones were measured. Folin-Ciocalteu's reagent procedure was adopted for the phenolic content, absorbance recorded at 760 nm that of flavonoid measured at 510 nm spectrophotometrically. The data was subjected to Chi-square (x^2) comparisons using SPSS version 22 and Graph pad prism version 8. The surveyed plants, were mostly Fabaceae. It has also recorded that 41-50 years were many into practice with their *P-value* 0.13. The majority of the respondents, were illiterates (*P-value* 0.06). However, ailments were ranged 0.69 – 0.75 informant consensus factors. Moreover, plant species with 0.34, 0.27 values were the highest Relative frequency citations. The (Methanolic and ethanolic) stem (bark) extracts reveal sound inhibition zones; *S. typhi* (16.3 mm); *E. coli* (15 mm); *B. cereus* (17 mm), on crude *S. birrea* stem (bark) where, 50mg/ml was the MIC without MBC. *S. typhi* (18 mm); *E. coli* (15 mm) were inhibited with *V. nilotica* (L.) Willd. ex Delile pods ethanolic extract, and exerted its MICs on (12.5 and 25 mg/ml & 50mg/ml).Nevertheless, organisms were resistant to aqueous extracts using Ciprofloxacin (19.7 mm - 33 mm) as the positive control, where by, no inhibition zones

procured from *Leptadenia hastata* (Pers.) Decne leaves extracts against the isolates. Extracts were significantly different according to Tukey at $p \geq 0.05$. Nevertheless, Gallic acid increased tremendously in *V. nilotica* pods extracts with a curve ($R^2 = 0.9958$). While a large Catechin increase noticed in *S. birrea* stem (bark) extracts and followed by *V. nilotica* pods extracts with a curve ($R^2 = 0.9993$), all were significantly different at *P-value* < 0.0001 across the extracts which turned to have low contents especially *L. hastata* leaves extracts as compared. Their respective correlations were clearly denote phenolic contents with a curve ($R^2 = 0.5025$) and flavonoid contents ($R^2 = 0.7089$). Subsequently, 10mg/ml of the *S. birrea* stem (bark) ethyl acetate fraction extract was the MIC and no MBC on the fraction extract, therefore, inhibited the growth of the *S. typhi*. Statistically showed that, the isolate was susceptible to the positive control (Ciprofloxacin 30.33±0.0) as the highest inhibition zone followed by the ethyl acetate fraction extract at 10 mg/ml (9.7 ±0.0) and resistant as well at P< 0.0001 Tukey. Subsequently, LC-HRMS results of the most active fraction identified 16 compounds with various structures include:1,8-Diazabicyclo[5.4.0]undec-7-ene;Epigallocatechingallate;2,Amino1,3,4octadecanetriol;Gentisic acid; Vidarabine as well as DL-Isoleucine among others. Lastly, reasonable amount of chemical compounds determined the actions of individual plants, notably towards development of many valuable pharmaceutical products. *S. birrea* stem (bark) extract was found to be very useful in Yobe State, which is in accordance with the present study by having very active compounds for the efficacy including Vidarabine as the novel one.

Keywords: gastrointestinal, Informant consensus factor, medicinal plants, Bioassay guided fractionation, solvent partitioning, *Salmonella typhi*, *Sclerocarya birrea*, LC-MS

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TINJAUAN FITOKIMIA DAN AKTIVITI ANTIBAKTERIA IN *VITRO* TUMBUHAN ETHNOMEDICINAL UNTUK MERAWAT PENYAKIT GASTROINTESTINAL DI NEGERI YOBE, Nigeria

Oleh

ABDALLAH MUHAMMAD SALIHU

Julai 2021

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Banyak tanaman di Negeri Yobe, Nigeria berpotensi menyembuhkan banyak penyakit. Penduduk desa dan bandar menggunakan tanaman ubat sebagai langkah penyembuhan mereka dengan fakta bahawa, ubat konvensional cukup mahal dan juga menyumbang kepada ketahanan terhadap ubat antimikrob. Kandungan bahan kimia yang banyak memainkan peranan penting yang membolehkan memerangi penyakit dan penyakit. Tujuan kajian ini adalah untuk: menilai ekstrak tumbuhan yang paling biasa digunakan dalam menyembuhkan penyakit gastrointestinal penyakit di seluruh Negeri Yobe, Nigeria; menilai keberkesanan antibakteria mereka terhadap beberapa isolat enterik menggunakan cakera dan penyebaran dengan baik; tentukan kandungan fenolik dan flavonoid ekstrak tumbuhan ini (ekstrak polong *Vachelia nilotica*, ekstrak batang *Sclerocarya birrea* (kulit kayu), ekstrak daun *Guiera senegalensis* dan ekstrak daun *Leptadenia hastata*) dan terakhir, tentukan bahan pecahan berpandu bioassay tumbuhan terpilih batang (kulit kayu),dan menentukan kandungan fenolik dan flavonoid ekstrak tumbuhan ini dan terakhir; menentukan bahan pecahan berpandu bioassay dari batang tanaman terpilih (kulit), dan mengesan sebatian yang terdapat dalam *Sclerocarya birrea* (A.Rich.) Batang Hochst (kulit) pecahan yang mampu bertindak terhadap isolat menggunakan LC MS. Oleh itu, soal selidik separa berstruktur digunakan untuk mengumpulkan maklumat awal mengenai tanaman. Keberkesanan antibakteria mereka diuji menggunakan isolat; *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus* dan *Staphylococcus aureus*, di mana zon penghambatan diukur. Prosedur reagen Folin Ciocalteu diadopsi untuk kandungan fenolik, serapan direkodkan pada 760nm dari flavonoid yang diukur pada 510 nm secara spektrofotometrik. Data tersebut dilakukan perbandingan Chi square (x2) menggunakan SPSS versi 22 dan grafik prisma versi 8. Tumbuhan yang disurvei, kebanyakannya adalah Fabaceae. Ia juga mencatatkan bahawa 41-50 tahun banyak berlatih dengan nilai P mereka 0,13. Majoriti responden, buta huruf (nilai *P*- 0.06). Walau bagaimanapun, penyakit berjumlah 0.69 - 0.75 faktor permuafakatan informan. Lebih-lebih lagi, spesies tumbuhan dengan nilai 0.34, 0.27 adalah petikan frekuensi Relatif tertinggi. Ekstrak batang (Metanol dan etanol) kulit kayu menunjukkan zon penghambatan bunyi; *S. typhi* (16.3 mm*); E. coli* (15mm); *B. cereus*

(17mm), pada batang *S. birrea* kasar (kulit kayu) di mana, 50mg / ml adalah MIC tanpa MBC. *S. typhi* (18 mm); *E. coli* (15 mm) dihambat dengan ekstrak etanolik pod *nilotica V.*, dan menggunakan MIC pada (25 mg / ml &50 mg / ml).Walau bagaimanapun , organisma tahan kepada ekstrak akueus. Siprofloksasin (19.7 mm-33 mm) sebagai kawalan positif, di mana, tiada zon perencatan mendapat dari *Leptadenia hastata* (Pers.) Decne, ekstrak daun terhadap penyisihan. Ekstrak nyata sekali berbeza menurut Tuki pada $p \geq 0.05$. Meskipun asid galik sungguh bertambah dalam lenggai *V. nilotica* ekstrak dengan satu lengkungan ($R^2 = 0.9958$). Manakala Catechin besar bertambah dalam ekstrak batang (kulit) *S. birrea* dan diikuti oleh lenggai *V. nilotica* ekstrak dengan satu lengkungan ($R^2 = 0.9993$), semua nyata sekali berbeza pada Nilai $P < 0.0001$ merentasi ekstrak yang bertukar untuk mempunyai isian rendah khususnya ekstrak daun *L. hastata* seperti yang jika dibandingkan.menghubung kait mereka dengan jelas menunjukkan kandungan fenolik dengan satu lengkungan ($R^2 = 0.5025$) dan kandungan flavonoid ($R = 0.7089$). Berikutnya, 10 mg / ml etil asetat batang (kulit) *S. birrea* pecahan ekstrak ialah MICs dan tiada MBCs oleh itu, menghalang pertumbuhan *S. typhi*. Secara statistik menunjukkan bahawa, mengasingkan rentan kepada kawalan positif (Ciprofloxacin 30.33±0.0) apabila zon perencatan tertinggi diikuti oleh pecahan ekstrak etil asetat pada 10 mg / ml (9.7 ±0.0) dan tahan juga pada $P< 0.0001$ Tuki. Kemudiannya, LC-HRMS menyebabkan pecahan yang paling aktif mengenal pasti 16 sebatian dengan pelbagai struktur merangkumi: 1,8 Diazabicyclo [5.4.0] undec 7 ene; Epigallocatechingallate;2Amino1,3,4octadecanetriol;Asid Gentisic; Vidarabine dan juga DL Isoleucine antara lain. Akhir sekali, sebilangan besar sebatian kimia menentukan tindakan setiap tumbuhan, terutamanya ke arah pengembangan banyak produk farmaseutikal yang berharga. Ekstrak batang *S. birrea* (kulit kayu) didapati sangat berguna di Negeri Yobe, yang sesuai dengan kajian ini dengan mempunyai sebatian yang sangat aktif untuk keberkesanannya termasuk Vidarabine sebagai yang baru.

Kata Kunci: gastrousus, faktor konsensus Informant, tumbuhan ubatan, Bioassay berpandukan pemeringkatan, pemetakan pelarut, *Salmonella typhi, Sclerocarya birrea*, LC-MS

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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AGE	acute gastroenteritis
BCAAs	branched-chain amino acids
CcpA	Responds to a preferred carbon source
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acids
ET OH	Ethanolic
ETEC	Enterotoxigenic *Escherichia coli*
FL	Fidelity level
FtsZ	Filamenting temperature-sensitive mutant Z
GIT	Gastrointestinal
HUS	haemolytic uremic syndrome
HPLC	High performance liquid chromatography
ICF	Informant consensus factor
LB Agar	Luria Broth Agar
LC-MS	Liquid chromatography mass spectrometry
MBC	Minimum bactericidal concentrations
MHA	Mueller Hinton agar
MG	Milligram
MIC	Minimum inhibitory concentrations
MLSB	Macrolides, lincosamide and streptogramin B antibiotics
ML	Milliliter
MRSA	Methicillin resistant *Staphylococcus aureus*
MSA32	Muhammad Salihu Abdallah voucher number 32
NANTMP	National Association of Nigerian Traditional Medicine Practitioners

NC	Negative control
NUC	number of used citations
NS	number of used species for each citation
PC	Positive control
PCR	Polymerase chain reaction
PUFAs	Polyunsaturated fatty acids
PWD	Post weaning diarrhoea
QE	Quercetin equivalent
R&D	Research and Development
RFC	Relative frequency of citation
RNA	Ribonucleic acids
RpiR	responds to pentose phosphate pathway intermediates
SCFAs	Short-chain fatty acids
SF	Specific frequency for a specific ailment.
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxins
TF	Total number of citations of that very species
TFC	Total flavonoids contents
TPC	Total phenolic contents
UV	Ultraviolet light
µ L	Microliter
µ g	Microgram
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Rich biodiversity that Nigeria has and the number of plants used in traditional medicine of certain ailments were still not documented, such as on antimalarial plants covered in some part of Africa, as such traditional knowledge also need to be documented by conserving reports of valuable resources contained in the plants for the medicinal purposes (Tefera & Kim, 2019). Majority of the traditional herbal medications used in Africa are provided by practitioners who live within the populations. They trusted the system over time and are often willing to assist the patients with their knowledge and skills. Most of these herbal medicines are procured in their crude forms although some pharmaceutical pre-packaged forms also exist. Interestingly, unaffected interest in various traditional practices now exists among practitioners of traditional, indigenous or alternative systems (Aliyu et al., 2015).It has been proved that a certain reasonable number has been documented to be the rate on how indigenous traditional medicines were made from plants' natural products. Despite the fact that some of the traditional knowledge were not used in a larger society.

Many important compounds found in plants were identified through phytochemical analysis so as to know the suitable one for a proper medication. Plants species played important roles and due to that, they need to be conserved for the betterment of populace to properly attack the problems, serious destruction may lead to extinction of the plants species. Thus, many plants were tested on different health problems and worked wonderfully due to having sound natural products (Kumari et al., 2017).

1.2 Problem statements

Many plants in Nigeria have potentialities in curing many diseases. Moreover, many researches have been conducted scientific studies. With the advent of many orthodox, it has been difficult to utilize them due to the cost implication in curing different ailments. Plants became useful being the reservoir of many ingredients to alter the actions of microbial pathogens, which is being earlier stated by Madara et al. (2018). Based on the many researches surfaced in the world of traditional medicine, African traditional systems of medication have come up with a model so as to cure many diseases in a cheaper rate and total submission from the populace (Mahomoodally, 2013). The ethno medicinal plants survey has been revitalized in many aspect of screening the compounds for an alternative medication (Ashidi et al., 2010).

However, some of the Nigerian medicinal plants are helpful to different organs of the body in curing so many disorders and infections that do affect them. Generally, they do possessed ; fats, proteins, vitamins, carbohydrates and other bioactive compounds,

among them include: (*Aspilia africana, Bryophyllum pinnatum, Garcinia kola, Spondias mombim* and *Uvaria chamea*) they have been used also in curing tumor viruses as highlighted by (Okwu, 2007). It has been proved that ortho, cyanins, lignans, flavonoids and lignins were the phytoconstituents that made up of the phenolic groups which contributed towards; anticlotting, anti-inflammatory, immune enhancers as well as antioxidants, which were active as disease preventives as well as protect plants and humans damage (Okwu, 2007). The *G. kola* showed successful inhibitory activities against microbes, especially bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) and fungi (*Candida albican*), which was in similar with some antibiotics (Okwu, 2003) and such in conformity with that of (Akoachere *et al*. 2002).

Many other plant species showed activities on both gram positive and other gram negative bacteria, such as; *E. coli*, *S. typhi*, *S. aureus*, *Klebsiella* spp, *Shigella flexneri*, *B. subtilis* etc., due to the presence of phenolic compounds which were used widely for the purpose of curative and prevention of many ailments and disorders (Ofokansi *et al*., 2005). Both primary and secondary metabolites with disparity in their action on microbes and other disorders since in the olden days (Kumari et al., 2017). During AIDS pathogenesis and diarrheic diseases, gastrointestinal tract plays a vital role in the worsening the system, where such an issue mostly happened in a rural areas as well as some part of cities in African region (Cimerman et al., 1999). More so, more than a billion people worldwide in under developing countries are affected with parasitic infections specifically with *Ascaris lumbricoides* which triggered not only adult but to even children by causing malnutrition as pinpointed by (Ijaz, 2013).

The gastrointestinal tract infections in form of gastroenteritis, enteritis or enterocolitis which most common intestinal parasites such as *Giardia lamblia* (*Giardia duodenalis*, *Giardia intestinalis*), *Cryptosporidium hominis* or *Cryptosporidium parvum* as well as *Entemoeba histolytic*. Infections caused by the parasitic organisms manifest with an abdominal pain, diarrhoea, dysentery, watery diarrhoea, dysentery or bloody stool may be caused by the parasites as well (Garcia et al., 2018). It has been known through ancient knowledge that, *Moringa oleifera* cures so many diseases and disorders due to the fact that, possessed so many natural products that do away with the abnormalities, such as anaemia, asthma, rheumatism, diarrhoea, among others as pinpointed by (Gupta et al., 2018). It has been compounded that, herbal formulations in traditional medicine were active due to the presence of bioactive compounds (Mustapa et al., 2018).

1.3 Research Justification

Medicinal plants are regarded as valuable and most useful natural resources used for the invention of new novel drugs. Many compounds such as; saponins, terpenoids, flavonoids, coumarins etc., were better known as attribute to the efficacies of the used medicinal plants in treating many ailments caused by microbes. Infectious diseases caused by bacterial pathogens have caused many deaths toll across the globe which was a major health-threat, approximately millions were killed. Some Gram- negative bacteria (*Escherichia coli* and *Klebsiella* spp.) were recorded to be responsible, as such, many metabolites inhibited their growths (Hajrah et al., 2018).

Nevertheless, much screened compounds from the plants extracts have shown anticancer activities for more than forty years, whereby, African plants leads some part of other continents like; China and India. Nigerian government has recently set aside US$1billion for the development of drugs from the medicinal plants, this came up due to the fact that, some part of the country like south-western part, used plants for their medications by Ashidi et al. (2010). Report has shown that, many plants species were destroyed in search of medicine as well as for the fire wood. There will be a time the valuable medicinal plants will be disappeared if care is not taking, with such actions on the plants, so many diseases may lead to death of a larger number of both local and urban populace (Salisu et al., 2015).

Polyherbal formulations of medicinal plants is in line with synergistic effect of phytochemicals, where did a tremendous effort in curing many ailments, Mustapa (et al., 2018). Generally people in Nigeria and specifically Yobe State, made use of medicinal plants as their curative measures as well as raw materials for their day to day activities as shown in appendix A2, more importantly, combat various microbial and other disorders for their well-being (Elekwa et al., 2017). Moreover, some plant species have shown a larger portion of percentages for having the anti-gastrointestinal characteristics. The most common one include; Parasitosis, diarrhoea, constipation, colic, stomach ulcer, vomiting and lack of appetite (Ouachinou et al., 2019). It has been assessed in India and other regions that plants species revealed a myriad number of benefits.

1.4 Research objectives

This study aims to:

1. To assess the most commonly used plant extracts in curing gastrointestinal ailments across Yobe State, Nigeria.

2. To evaluate their antibacterial efficacy against some enteric isolates using disk and well diffusion.

3. To determine the phenolic and flavonoid contents of the four selected plants extracts (*V. nilotica* pods extract, *S. birrea* stem (bark) extract, *G. senegalensis* leaves extract and *L. hastata* leaves extract).

4. To determine the bioassay-guided fractionating substances of the selected plant stem (bark), and detect compounds present in *Sclerocarya birrea* (A. Rich.) Hochst stem (bark) fraction capable of actions against the isolate using LC-MS.

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