KWAME NKRUMAH UNVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH AND ALLIED SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACOGNOSY

QUALITY ASSESSMENT AND CLINICAL EVALUATION OF THE SAFETY AND EFFECTIVENESS OF A GHANAIAN HEPATORESTORATIVE POLYHERBAL PRODUCT (HPK) AT THE WA REGIONAL HOSPITAL

BY

ALFRED OFORI AGYEMANG

MAY, 2019

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BY

ALFRED OFORI AGYEMANG

(BSc. HERBAL MEDICINE)

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD DEGREE OF

MASTER OF PHILOSOPHY IN PHARMACOGNOSY

MAY, 2019

DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Kwame Nkrumah University of Science and Technology, Kumasi or any other educational institution, except where due acknowledgement is made in the thesis.

Alfred Ofori Agyemang (**PG 9590017**) …… Date: …….

Certified by:

Professor Merlin Lincoln Kwao Mensah …… Date: …….
(Supervisor)

Dr. Rodríguez Celia Yamile …… Date: ……...
(Co-Supervisor)

Professor Abraham Yeboah Mensah …… Date: ……
(Head of Department)

DEDICATION

To my wife, Owusua, children and parents for all the inspirations and sacrifices.

To Prof. Merlin. L. K. Mensah for all the motivation and support.

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ACKNOWLEDGEMENT

To the most gracious God, I am forever grateful for the strength and guidance.

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To my wife Yaa Owusua, Joseph and Mary my kids and Mr and Mrs Amoabeng Agyemang my lovely parents.

Ethical Clearance

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Ref: CHRPE/AP/038/18

6th February, 2018.

Mr. Alfred Ofori Agyemang
Upper West Regional Hospital
Post Office Box 6
WA.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: ***"Quality Assessment and Evaluation of the Safety and Effectiveness of Heptonica a Polyherbal Product for Treating Liver Disease."***

Proposed Site: ***Upper West Regional Hospital (Herbal Medicine Unit)***

Sponsor: ***Principal Investigator.***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter of 13th November, 2017 from the Upper West Regional Hospital (study site) indicating approval for the conduct of the study in the Hospital.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 6th February, 2018 to 5th February, 2019 renewable thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Yours faithfully,

Rev. Prof. John Appiah-Poku.
Honorary Secretary
FOR: CHAIRMAN

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

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ABSTRACT

Liver diseases are increasing globally and many clients in developing countries tend to use herbal products for their treatment but most of these products have not been studied scientifically. The study aimed at assessing quality, safety and effectiveness outcomes of the treatment of patients with deranged liver functions using a Ghanaian polyherbal product, HPK. HPK which is used as a hepatorestorative is a decoction prepared from *Bidens pilosa*, *Citrus aurantifolia* and *Trema orientalis*. Quality parameters of HPK were assessed through physicochemical, microbiological, spectroscopic and chromatographic analysis. Atomic Absorption Spectroscopy (AAS) was used for heavy metals analysis. Fifty clients with laboratory-confirmed deranged liver function gave informed consent and were recruited for the study at the Upper West Regional Hospital. Participants took HPK (30 mL 8 hourly) for 28 days.
Laboratory investigations (LFT, RFT, FBC and Urine R/E) were undertaken at day 0 (baseline), 14 and 28 to assess for safety and effectiveness of HPK. HPK exhibited DPPH free radical scavenging activity with IC_{50} of 23.39µg. HPK contains reducing sugars, alkaloids, tannins, saponins, coumarins, flavonoids and phytosterols. HPLC, TLC, UV and IR fingerprints have been established. Microbiological cultures did not show any observable contamination of the HPK. Heavy metal analysis: Lead, Mercury, Nickel, Cadmium and Arsenic yielded content levels within the safe WHO reference ranges. Compared to baseline values, HPK showed no observable toxicity on renal function, haematological parameters and urinalysis in humans after 28 day period. However, HPK significantly improved the liver function of the participants: AST (p - 0.0001), ALT ($p < 0.0001$), GGT (p - 0.0017), total bilirubin (p - 0.0146), direct bilirubin ($p < 0.0001$) and alkaline phosphates (p -0.0295). The other parameters showed no significant differences in 28 days. Within the limits of the study, HPK had hepatorestorative action with no observable toxicity and can be used with confidence as indicated on its label as a liver tonic.

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LIST OF ABBREVIATIONS

AAE	Ascorbic Acid Equivalent
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
CYP3A	Cytochrome P4503A
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediamine tetraacetic acid
FBC	Full blood count
FPPS	Faculty of Pharmacy and Pharmaceutical Sciences
FTIR	Fourier-Transform Infrared Spectroscopy
GGT	Gamma-glutamyltransferase
Hb	Haemoglobin
HCT	Haematocrit
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
IQR	Inter Quartile Range
LFT	Liver Function Test
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean platelet volume
NAFLD	Non Alcoholic Fatty Liver Disease
NASH	Nonalcoholic Steatohepatitis
PCT	Plateletcrit
R_f	Retardation factor

RFT	Renal Function Test
TAC	Total Antioxidant Capacity
UV	Ultraviolet
WBC	White Blood Cells
RBC	Red Blood Cells
PDW	Platelet distribution width
PLT	Platelet
RDW-CV	Red blood cell distribution width (coefficient of variation)
RDW-SD	Red blood cell distribution width (Standard deviation)

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

'Traditional medicine is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness' (WHO, 2013). Traditional medicine is very important globally for the majority of the people who use it for treating diseases and improving their health. Traditional Chinese Medicine (TCM), Unani and Ayurveda are all traditional medicines practices in Asia (Liu, 2011). In Africa, traditional herbal medicine practice, bone setting, circumcision, spiritualism and psychic healing, are some of the traditional medicine practices (Mahamoodally, 2013).

Some benefits of Traditional medicine practice are acceptability, availability, affordability and it deals with treating the whole individual and not only disease presentation. Traditional medicine is associated with some challenges which include varied means of practice some of which cannot be explained scientifically and this makes it very difficult to regulate the practice and practitioners of Traditional medicine. Secondary, therapeutic agents used in traditional medicines are not mostly standardized.

Natural products which are used in traditional medicine are obtained from plant, animal and mineral sources with the majority being plant-based (Anulika *et al*., 2016; Yuan, 2016). Despite all the criticisms of Traditional medicine, majority of the world population depend on Traditional medicine mainly herbal medicine for their primary healthcare (Pambazuka, 2002; WHO, 2003; Ekor, 2014). The use of plants and their products for medicinal purposes (herbal medicines) have been practised from time immemorial to date (Theophile *et al*., 2006; Abe and Ohtani, 2013 and Okaka 2019). There are many disease areas for which plants and their products are used as remedies (Shosan *et al*., 2012).

Herbal medicines are categorized into herbs, herbal material, herbal preparations and finished herbal products depending on the level of processing (WHO, 2007a, 2007b). Commercial production of herbal medicines escalated around 100 years ago (Mahomoodally, 2013). The worldwide natural product trade experiences an annual growth rate of 7% and now generating about US $ 107 billion dollars to the global economy (Oguntade *et al*., 2013). This cost of global phytomedicine is expected to increase to US $ 5 trillion by the year 2050 (Rajani and Kanaki, 2010). Africa as a continent depend heavily on natural products mainly from plants for healthcare and other purposes (Mahomoodally, 2013). Ghanaians have been using traditional medicine for health before the advent of allopathic medicine and the World Health Organization (WHO) estimates about 70% of Ghanaians use traditional medicine as primary healthcare (Yarney *et al*., 2013; Gyasi *et al*., 2015).

Affordable and readily available herbal medicines are highly acceptable means of treatment in Ghana, making the practice of traditional medicine flourishing. The general perception of herbal products for improving health has not changed (Sarma *et al*., 2011). Herbal products are used in the prevention of diseases, diagnosis, and treatment and for improving the general wellbeing of humans (Sofowora *et al*., 2013). Advancement in science especially, with research methods and technology, has contributed massively to the quality of herbal medicine practice. Traditional uses of many plants have been confirmed with modern science and there is increased demand for more plant medicines which are the backbone of traditional medicine (Hosseinzadeh *et al.*, 2015; Singh, 2015).

Years back in Ghana, herbal medicines were prepared extemporaneously according to the individual's need and were not meant to be stored for longer periods. Herbal medicine in the years past was mostly practised by the informally trained indigenous people using herbs and herbal materials with emphasis on effectiveness but not product quality (Boadu and Asase, 2017). Currently, herbal medicines are produced in commercial quantities in Ghana and are meant for longer term storage and use by the general public (Komlaga *et al*., 2015).

The practice of herbal medicine in Ghana has been elevated further since the training of Medical Herbalists began at Kwame Nkrumah University of Science and Technology in 2001. The application of modern science to herbal medicine practice through higher learning have encouraged further public confidence and acceptability

of herbal medicine practice as well as the use of herbal products. The inception of the process of integration of herbal medicine services into the national healthcare system in 2011, has further improved the practice of herbal medicine in Ghana. Treatment outcomes from the use of the herbal products in the pilot centres have increased curiosity among clinicians and other health workers as to the scientific basis of such products. There is an increased demand by both clients and other health professionals for detailed knowledge of the uses of medicinal plants and plant products in the diagnosis, treatment and prevention of diseases and the improvement of health. (Agyei-Baffour *et al*., 2017).

1.2 PROBLEM STATEMENT

The liver is a very robust organ with auto-regeneration properties but liver (hepatic) diseases are not so easy to treat (Jones *et al*., 2018). Hepatic diseases comprise a wide range of complex conditions or disorders many of which can affect and compromise the functions of the liver leading to morbidity and mortality in people of all races, ages and sex in different environments.

Globally, there are limited therapeutic agents for preventing and managing liver diseases. The few therapeutic options for treating liver diseases are very expensive and not accessible to many people (Sofowora *et al*., 2013, Uhl *et al*., 2014, WHO, 2013). Developing and less developed countries cannot afford the few expensive drugs and products for treating liver diseases (WHO, 2016). Diseases of the liver need more attention, resources and research globally in the area of diagnosis, prevention, management and cure to reduce the disease burden (WHO, 2016).

Lack of affordable and accessible therapeutic agents for the treatment of liver diseases is impeding the quality of life of patients as well as increasing mortality (Asrani *et al*., 2013). There is a great need to search for more agents that are able to protect the liver and also manage and treat liver diseases. Although herbal medicines are the most dominant treatment option for liver diseases in developing and underdeveloped countries, challenges are raised against their use. Most herbal products have not been subjected to standardization or clinical studies to prove their safety and effectiveness in humans (Dhiman and Chawla, 2005).

1.3 JUSTIFICATION FOR RESEARCH

There are few allopathic medicines for the treatment of liver diseases and these are mostly not accessible in less developed countries. Some of the few allopathic drugs for treating liver diseases are also limited in efficacy and compounded by toxic side effects that people complain about (Kumar *et al.*, 2017). The interferons which are used in treating liver diseases are examples of drug groups people react to. The interferons are naturally occurring soluble glycoproteins produced and released by cells to interfere with viral replication. (Sugawara *et al*., 2010). In spite of the high advances in modern science, it is still a challenge getting safe and efficacious agents to improve hepatic protection and enhance hepatic functions (Madrigal-santillán *et al.*, 2014). Although there are claims of many years of using herbal medicines for liver diseases, little evidence is available concerning their safety and effectiveness in human clinical studies (Ahmad & Raza, 2004; Stickel *et al*., 2007). A Ghanaian polyherbal product given a code as HPK being investigated is already being used by the general public.

HPK is a finished liquid herbal product manufactured by the Centre for Plant Medicine Research (CPMR) which is located at Akuapem Mampong of the Eastern Region of Ghana. HPK is a decoction produced from the dried leaves of *Citrus aurantifolia* (Rutaceae), the dried aerial part of *Bidens pilosa* (Compositae) and the dried bark of *Trema orientalis* Linn. Blume (Ulmaceae). It is an orally administered preparation for adults only at the dose of 30 mL, eight hourly after meals. HPK has been used for over 30 years at the CPMR and for the past five years by Medical Herbalists at some of the 22 public hospitals which are pilot centres for the integration of herbal medicine across Ghana. The three plant components of HPK have ethnobotanical evidence of being in the management of liver diseases (Lawal *et al*., 2016, Mukazayire *et al*., 2011). No published scientific work is available on HPK despite its long use in Ghana. This study, it is hoped, will provide scientific data on the quality, safety and effectiveness of the finished herbal product already used by people with deranged liver function.

1.4 RESEARCH QUESTION

Can HPK restore deranged liver function in humans to normalcy?

1.5 AIM

The aim of this study is to establish the quality parameters and assess the safety and effectiveness of HPK, a hepatorestorative polyherbal product on the Ghanaian market.

1.6 SPECIFIC OBJECTIVES

1. To establish the quality parameters of HPK:
    - Organoleptic and physicochemical parameters of HPK (and its component plants)
    - Chromatographic characteristics of HPK (and its component plants)
    - Determine the lead, mercury, cadmium, arsenic and nickel metal content of HPK
    - Determine microbial content of HPK
    - To evaluate the antioxidant activity of HPK and component plants using DPPH and Total Antioxidant Capacity (TAC)

2. Clinical assessment of HPK
    - Assess the safety of HPK on renal function, haematological and urine parameters in study participants
    - To assess the effectiveness of HPK as a hepatorestorative in study participants

CHAPTER 2

LITERATURE REVIEW 2.1

THE LIVER

The liver (Figure 2.1) is the largest internal organ in the human body weighing about 1.5 kilograms; it makes up about 2.5% of the adult body weight and it is located at the right hypochondrium of the abdomen (Ozougwu, 2017).

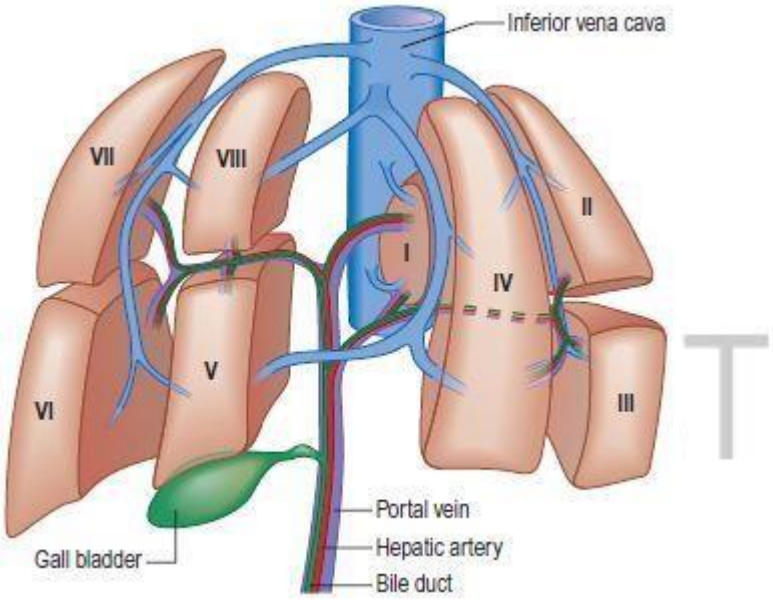


Figure 2.1 Segmental anatomy of the liver showing the eight hepatic segments.
I, caudate lobe; II–IV the left hemiliver; V–VIII the right hemiliver (Kumar, 2009)

The liver is responsible for many physiological functions within the human body including metabolic, circulatory and defense. The synthetic functions of the liver include the production of all circulating plasma proteins except γ-globulins. Plasma proteins, albumin, binding proteins (e.g. haptoglobin), acute phase proteins and clotting factors are all formed in the liver. (Madrigal-Santillán *et al.*, 2014). Blood coagulation factors like prothrombin, fibrinogen, and factors V, VII, IX, X and XIII except for factor VIII are made in the liver. The composition of the complement system, Proteins S, C, and antithrombin are all produced by the liver (Moradi *et al*., 2016).

The liver has immunological functions also and they are mediated through the reticuloendothelial system. The liver performs phagocytosis through the Kupffer cell and removes haemolytic products from the body. Secretion of interleukins, collagenase, tumour necrosis factor (TNF) and lysosomal hydrolases are immunological functions of the liver (Gao, 2016, Robinson *et al*., 2016).

The endocrine functions of the liver include angiotensinogen secretion and the conversion of thyroxine (T_4) to Triiodothyronine (T_3). The liver is responsible for the breakdown of hormones including glucagon, insulin, glucocorticoids, growth hormones and parathyroid hormone (Ozougwu, 2017). The liver is also responsible for the metabolism of carbohydrate, proteins and lipids. It undertakes gluconeogenesis and

the conversion of fructose/galactose to glucose. On lipids, the liver synthesizes cholesterol/lipoproteins, bile acid, bilirubin, fatty acids and ketoacids. Amino acids are produced by the liver. Drug metabolisms, as well as detoxification, are all vital functions of the liver. The liver is involved in the acidbase regulation within the body. Storage of iron, copper, vitamins (A, D, E, K and B12), blood and glycogen, are key functions of the liver (Plaats, 2005; Chiang, 2017).

2.2 LIVER DISEASES

2.2.1 Classification of liver diseases

Liver or hepatic diseases comprise a wide range of complex conditions that affect the liver. Liver diseases are classified according to the type, cause and duration of the liver disease. There are many diseases and agents that affect the liver resulting in either acute or chronic liver damages and may be classified as communicable and non-communicable. Liver diseases fall under both communicable and noncommunicable diseases depending on the cause. Alcoholic liver diseases, nonalcoholic liver diseases (NALD), drug-induced liver diseases, autoimmune diseases, liver cancers and many more are all considered as non-communicable. Infectious causes like viral hepatitis are the communicable causes of liver disease. Based on duration, there are acute and chronic liver diseases (Albillos and Garcia-Tsao, 2011).

2.2.2 Causes of liver diseases

All agents that cause the production of reactive oxygen species, DNA methylation and oxidative stress are all capable of causing liver disease (Ali *et al.*, 2017). Free radicals and oxidants causing oxidative stress have been implicated in the initiation, cytokine-induced liver inflammation and progression of liver diseases (Ahmad and Raza, 2004). Oxidative degradation of hepatocytes indirectly manipulates protease/ antiprotease balance (Valaskova *et al.*, 2016).

Infectious agents like viruses, fungi, bacteria and helminths are major causes of liver diseases. Among the infections, viruses account for high morbidity and mortality worldwide. Cytomegalovirus, Epstein-Barr, herpes simplex and hepatitis A, B, C, D, and E are some of the viruses responsible for viral liver diseases. Hepatitis A is the most common type of viral hepatitis occurring worldwide. (WHO, 2009, 2016, 2017). Hepatitis C is the most common cause of liver cirrhosis followed by alcohol.

Liver diseases from autoimmune causes include primary biliary cirrhosis (PBC), autoimmune hepatitis, cystic fibrosis and primary sclerosing cholangitis (PSC). Wilson's disease (α_1 antitrypsin deficiency), non-alcoholic steatohepatitis (NASH) and hemochromatosis (iron deposition) are some metabolic and genetic factors leading to liver damages (Stournaras and Tziomalos, 2015).

Some allopathic chemotherapeutic agents (e.g. acetaminophen, diclofenac, rifampicin, and isoniazid) and some natural drugs (e.g. Heliotropium) have long been associated with liver toxicity (Björnsson, 2016). Other chemicals like carbon tetrachloride, chlorinated hydrocarbons and alcohol also cause liver injury mostly through lipid peroxidation (Ahmad and Raza, 2004). Some poisons also exhibit their lethal effect by liver damage. Vascular obstruction e.g. Budd Chiari and portal hypertension also result in liver malfunction (Lin *et al.*, 2017).

Alcohol is one of the hugely abused recreational substances worldwide and it is responsible for fatty liver, alcoholic liver disease, non-alcoholic fatty liver diseases (NAFLD), liver cirrhosis and liver cancer. High alcohol consumption leads to liver damage as well as non-alcoholic fatty liver diseases (NAFLD) (Singal and Anand, 2013). Nutritional disorders like central obesity which are increasing globally are linked to non-alcoholic liver disease (NAFLD) (Wang *et al.*, 2016).

2.2.3 Epidemiology of liver diseases

Most liver diseases from the varied causes end up in liver cirrhosis and liver cancers. In the year 2000, both cirrhosis and liver cancer resulted in 3% of global mortality but the rate has increased to 3.5% in 2015. Liver cancer and cirrhosis are consistently part of the 20 top causes of mortality globally (WHO, 2016).

About 840 million people are living with different forms of chronic liver diseases and over 2 million deaths from liver diseases are expected to rise across the world (Ahsan *et al*., 2009; Marcellin and Kutala, 2018). Liver cirrhosis from harmful use of alcohol accounted for more than 2.3 million deaths globally in 2008 (WHO, 2010). An estimated 844 million people worldwide have chronic hepatic diseases. Globally, hepatitis B and C are estimated to affect about 420 million people. Every year about 6-10 million people are newly infected with viral hepatitis.

About 1.4 million people die each year from hepatitis alone (WHO, 2009, 2016, 2017). The global prevalence of chronic hepatitis B (CHB) is 3.6% with Sub-Saharan Africa

having the highest rate of 8% and European countries with the lowest prevalence rate of 0.5% (Hope *et al*., 2013). The average global prevalence of chronic hepatitis C (CHC) is 2.5% with Africa having the highest prevalence of 5.6% and the lowest rate of 1.8% in the United States of America (Vernon *et al*., 2011).

Alcoholic liver disease (ALD) has a global prevalence of 8.5% with European countries and the United States of America has the highest rate of about 12% (EASL, 2012). It is estimated that the global prevalence rate of NAFLD is about 25% with the highest rate been identified in the Western countries. USA has the highest prevalence of NASH of 16% while the global prevalence is 3-8% (Vernon *et al*., 2011). Ghana reported 7,833 mortalities due to liver diseases in the year 2017 that translated to 3.72% of the total annual mortality (WHR, 2017). In 2015, liver diseases caused 459 deaths among admitted patients in Ghana and the figure increased to 573 in 2016 (GHS, 2017).

2.2.4 Signs and symptoms of liver diseases

Liver disease may present as compensated or decompensated liver disease. Compensated liver disease occurs when the liver is compromised but it is able to perform many essential functions thereby not showing early symptoms. Liver diseases are mostly asymptomatic, especially in compensated chronic liver diseases. In decompensated liver disease, the liver is extensively affected and it is unable to undertake regular functions. Compensated liver disease is a systemic disorder that is associated with multi-organ involvement and normally present with symptoms, signs and complications (EASL, 2018).

The symptoms of liver diseases depend on the type and stage of the liver damage and may be asymptomatic or present the following: nausea, vomiting, fatigue, anorexia, diarrhoea, ascites, ankle oedema, pyrexia, jaundice, impotence, itches, steatorrhea, dark urine and cholestasis. Other signs of liver diseases: hepatomegaly, thrombocytopenia, splenomegaly, hepatic flap and pleural effusion (Beckingham, 2001). Chronic liver disease shows the following pathophysiological presentation: hepatic insufficiency, portal hypertension, hyperdynamic circulation, necrosis, hepatitis and fibrosis (Ribeiro *et al*., 2013). Spider naevi, palmar erythema, clubbing, Dupuytren's contracture, parotid enlargement, gynaecomastia, testicular atrophy, fever, loss of body hairs and xanthomas are seen in compensated chronic liver diseases.

Loss in bone mass and density, palpable liver, menstrual disorders, hepatic flap, fetor hepaticus, oedema and neurological disorders like coma and disorientation are seen in decompensated liver disease (Grattagliano *et al.*, 2009).

2.2.5 Diagnosis of liver disease

Liver diseases can be diagnosed by history taking, physical examination and side room investigation. Some of the side room investigations are liver biochemistry, ultrasonography, computed tomography (CT) scan and liver biopsy for histopathology.

Liver biochemistry including aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), bilirubin (total, direct and indirect), total proteins, albumin, alkaline phosphatase and internationalized normalized ratio (INR) are used in determining the status of liver and the type of liver disease. Determination of hepatic cancer biomarkers like alpha-fetoprotein is another means of assessing liver health (Cujic *et al*., 2010). Staging of chronic liver diseases is done with the following: clinical presentations of clients, duration of disease, liver biopsy, AST to platelet ratio index (APRI), form index (depends on the age, platelets, GGT and cholesterol), FibroTest (FT), FibroMeter (FM), Hepascore (HS) and Ultrasound e.g. Transient elastography (TE) (Ribeiro *et al*., 2013).

2.2.6 Management of liver diseases

Liver diseases are mainly managed on a supportive basis, removal of causative agents and treating the underlying causes. There are limited chemotherapeutic interventions from both allopathic and also herbal products (Hussain *et al*., 2017). The choice of therapy depends on many factors including the type of liver disease, the stage and intensity and the availability of professionals and healthcare facility for specific intervention services (e.g. liver transplant). Radiotherapy and surgical interventions (e.g. liver transplant) are also interventions for treating liver diseases (Kim and Jung, 2017). Monitoring is key to prevent complications in the management of liver diseases (Bittencourt, *et al*., 2015). Herbal medicines have been used e.g. in Burkina Faso in the management of liver diseases (Hong *et al.*, 2015; Sombie *et al*., 2018).

2.3 HERBAL MEDICINES

There are many concerns on the use of herbal medicines with respect to the standardization of product, safety and effectiveness outcomes for patients.

Standardization of natural products including herbal medicine is a very challenging task globally that needs creative and innovative means to address it from multiple angles. Because of the challenges, the World Health Organization (WHO) and other bodies have proposed measures to ensure the quality of natural products, especially for medicinal purposes (WHO, 2013). The means to standardize herbal products are still facing regional and national challenges. Countries who have included herbal products in their national pharmacopoeias still do not have the most efficient set standards for natural products (Prakash *et al.*, 2017).

2.3.1 Quality of Herbal Medicines

Quality involves all measures and procedures that ensure products meet a set standard for the intended purpose. It is the degree to which a process, systems and the herbal product meet established requirements (EMEA, 2011). Quality of a drug is determined by its identity, content, purity, levels of contaminants (e.g. microbial and heavy metals), as well as other physical, biochemical properties or by its manufacturing procedures (Borba *et al.*, 2014; WHO, 2007a). The quality of herbal medicines has a direct effect on its safety and efficacy (Gupta, 2016).

A poor quality product will not be safe for human use because of possible toxicities from contamination and substandard contents. High quality products can be assured of achieving their therapeutic purpose and their safety will be guaranteed. Plants contain complex compounds that afford them the synergistic therapeutic or toxic effects and can be used as markers for quality (Rasoanaivo *et al.*, 2011). Because so many factors influence the presence and concentration of secondary metabolites, selecting an analytical method or marker compounds for quality assessment becomes extremely difficult especially getting commercially available reference compounds. Marker compounds are used in identification and standardization of herbal products. Identifying marker compounds in herbal products is difficult especially for compounds in polyherbal products. Reference compounds for polyherbal products are very difficult to obtain and in case they are available, they are extremely costly (Rajani and Kanaki, 2010). The complexity and nature of compounds in plants and herbal products, the absence of trained personnel, equipment and inadequate funds make their standardization and characterization extremely difficult. Herbal product quality will be compromised by using inferior raw material, poor processing and storage conditions (Pauli *et al.*, 2012).

2.3.1.1 Microbial contamination of Herbal products

Microbial contamination of herbal products is common and it affects the stability and overall quality and activity of the product (Agarwal *et al.*, 2014). Microbes can affect the secondary metabolites and reduce the activity of products or can convert the secondary metabolites into toxic compounds (Idu *et al.*, 2010).

2.3.1.2 Heavy metals in Plants

Some minerals low concentrations are needed by the human body for growth, development and improvement of body functions (Soetan *et al*., 2010). Heavy metals form part of environmental pollutants responsible for liver disease. There are about fifty-three (53) elements that are categorized as heavy metals and some are of medical importance to the human body. Dietary elements that are required for humans in more than 100 mg per day are termed macrominerals (e.g. Calcium, Phosphorus, Magnesium, Sodium) but those required in less than 100 mg per day are referred to as trace elements e.g. zinc (Sarma *et al.*, 2011). The World Health Organization and some countries have set safe limit ranges for some heavy metals that may be present in food and medicinal products like herbal medicines (FSA, 2009; FAO/WHO, 2011).

Plants absorb some of these heavy metals from the soil and the environment in which they grow. Some plants have a higher tendency to accumulate heavy metals from the soil than others. Plants with higher tendency to accumulate heavy metals from the soil are termed hyperaccumulators. Different plants have different affinities to accumulate specific heavy metals. For example, *Bidens pilosa* is a hyperaccumulator plant for cadmium (Bernard, 2008). Contamination of herbal products have become a global concern due to complex human activities leading to excessive deposition of metals in soils (WHO, 2007a; Jung, 2008; Annan *et al*., 2013; Karayil and Ch, 2014).

There is some evidence of contamination from heavy metals (e.g. Cd and Pb) in medicinal plants that end up in the finished products (Chan, 2003; Ekeanyanwu *et al*, 2013; Okem *et al*., 2014). But some heavy metals are so toxic they should not be present in herbal products for human consumption. Heavy metals like zinc, copper, iron, cobalt and chromium are only toxic above recommended concentrations but cadmium, mercury and lead are exclusively toxic elements to humans (Jaishankar *et al.*, 2014). Contaminants and heavy metals are taken up by the plants from the environment, vehicular emission, atmospheric pollution, soil, artificial fertilizers, pesticides, water for irrigation and mining and some end up in herbal products

(Gajalakshmi *et al.*, 2012; Shaban *et al*., 2016). Heavy metals in herbal products can be from the plants that were used in production, processing (harvesting, drying, storage), equipment, water or reagents used, containers or closures, packaging and product storage conditions (Street, 2012; Okem *et al.*, 2014).

2.3.2 Hepatoprotectives and Hepatorestoratives

Hepatoprotective or anti-hepatotoxic substances are agents with the ability to prevent hepatic injury in structure and function when the liver is exposed to hepatotoxic hazardous agents. Hepatorestorative substances, however, are agents with the capability to restore health, vitality and function to hepatocytes after the liver have been subjected to malfunction from hazardous agents (Adewusi and Afolayan, 2010).

Plants have been used over the years as a remedy for liver diseases or to restore normal functions of the liver. These products are either mono or polyherbal products made from varied plant parts. There are some plants that have been associated with hepatoprotective effect especially from the Asteraceae family e.g. *Tridax procumbens* (Pawar *et al*., 2012). Hepano tablet is a polyherbal product from India in public use that has undergone randomized control clinical study as a hepatoprotective (Kumar *et al*., 2017). 'Liv 52' and 'Livomyn' are other polyherbal hepatoprotectives that have undergone animal studies in India to prove their efficacy (Sapakal *et al*., 2008). These two products are registered by the Food and Drugs Authority and are used in Ghana for the management of liver diseases.

Most of the specific mechanisms of action by which plants and plant products offer hepatic protection and therapy are not well elucidated. Some of the mechanisms of hepatoprotection are achieved through modulation of the immune system, inhibition of oxidation of hepatocytes by reducing reactive oxygen species within cells (Ker *et al*., 2014). Some plants offer hepatoprotection also by improving the hepatic synthesis of DNA, mimicking the functions of glutathione reductase and superoxide dismutase. Inhibition of lipid peroxidation, down-regulation of translation and transcription of viral mRNA (antiviral plants), reducing levels of growth factor beta1 leads to the reduction of hepatic fibrosis are all means to restore hepatic functions (Polyak *et al*., 2013; Vargas-Mendoza *et al*., 2014). Reduction in the concentration of cytochrome P450 1A1 by suppressing pyridine activation and inhibition of fibrogenesis and tumorigenesis are other means of achieving hepatoprotection.

Most of the studies on plants for therapeutic and protective effects on the liver have been done in animals and so the outcomes cannot be wholly extrapolated to humans. (Ali *et al.*, 2017). Some plants used in treating liver diseases are listed in Table 2.1. Over 100 plants are claimed to possess hepatoprotective effects (Parmar *et al*., 2010).

Table 2.1 Some plants used in treating liver diseases

Botanical source	**Common name**	**Plant part used**	**Dosage form/preparation**
Tithonia diversifolia (Asteraceae)	Tithonia	Leaves	Tea from leaves (Hirt and M'Pia, 2008)
Carica papaya (Caricaceae)	Pawpaw	Roots	Boil a handful of roots in 1L of water. Dosage: drink whole the liquid in a day (Hirt and M'Pia, 2008)
Azadirachta indica (Meliaceae)	Neem tree	leaves	Boil 2 bundles of the fresh leaves in 6L of water. Dosage: drink a small amount each time. (Mshana *et al.*, 2000)
Phyllanthus nururi (Euphorbiaceae)	Phyllanthus	Aerial part	Decoction. (Harish and Shivanandappa, 2006)
Alstonia boonei (Apocynaceae)	Alstonia	Root bark and leaves	Decoction. (Onwusonye *et al*., 2017)
Annona muricata (Apocynaceae)	Soursop	Leaves	Boil the leaves in water. Dosage: 1 teacup 3 times daily. (Mshana *et al.*, 2000)
Ananas comosus (Bromeliaceae)	Pineapple	Fruit	Boil the pineapple peels in water. Dosage: glassful 3 times daily. (Mshana *et al.*, 2000)
Catharanthus roseus (Apocynaceae)	Vinca	Leaves and roots	Boil dried roots and leaves in water. Dosage: 1 teacup daily (Mshana *et al.*, 2000)

Jatropha curcas (Euphorbiaceae)	Jatropha	Roots	Boil roots of Jatropha and stem bark of *Mangifera indica*. Dosage: drink decoction 3 times daily (Mshana *et al.*, 2000)
Tridax procumbens (Asteraceae)	Tridax	Whole plant	Boil 10g of the whole plant in 1L of water. Dosage: take 100mL 4 times daily. (Mshana *et al.*, 2000)

2.3.3 Hepatotoxicity of plant extracts

Although there are many medicinal properties from plants, some plants are also toxic to humans. Much knowledge on the traditional use of herbal medicine abound but not much knowledge have been compiled on the potential toxicities from plant use in medicine. Liver damage from plants sources may result from interactions of herbal products with other agents. Herb-herb, herb-drug or herb-food interactions can also result in liver damages. Some plants have also been scientifically identified to contain toxic phytochemicals. For example, Heliotropium and Crotalaria plants have been reported to contain some toxic pyrrolizidine which causes hepatitis (Patel *et al.*, 2013).

Not much has been done on research and documentation of toxicities from plants sources because the toxicities are mostly not reported and recorded. Some of the known and documented toxic plants exert their effects by localized and systemic reactions (Nelson *et al*., 2007). Plants exert poisonous effects due to the nature and class of phytoconstituents and cell inclusions present and also depends on how the plants are treated or processed (Wink, 2015).

The liver is responsible for metabolizing drugs with enzymes like microsomal cytochrome P450, glutathione-S-transferases, mixed-function monooxygenases, sulfotransferases, and UDP-glucuronosyltransferase. Any substance whether natural or synthetic that can influence any of these enzymes may interfere with drug metabolism that may lead to toxicities of the liver and possibly other organs (Kaplowitz *et al*., 2013; Zgheib and Branch, 2016).

Although some plants have been established to be hepatotoxic, it is difficult to associate most liver toxicities to herbal products because of multiple factors. A study showed 62.5% of drug-induced liver injuries were from plant products (Suk *et al.*, 2012). In Western countries, herbal medicines are the second most common cause of

drug-induced hepatotoxicity. Plant-based dietary supplements for enhancing sexual performance, muscular body-building, weight reduction (*Camellia sinensis*), anxiety, depression (*Cimicifuga racemosa*) and analgesics were mostly responsible for herbinduced liver injuries. *Teucrium chamaedrys* (Germander), *Ephedra sinica* (Ma Huang) and *Viscum album* (mistletoe) are some very toxic herbs especially to the liver, leading to various degrees of injuries or herb-induced liver injuries (HILI) (Teschke *et al*., 2013; Stournaras and Tziomalos, 2015).

Herbal induced injuries are caused in three ways; hepatocellular, cholestatic or a combination of the two. R-value which is a ratio of alanine transaminase to alkaline phosphatase (ALT/ALP) is used as an indicator to predict the possible cause of the liver injuries. When R is > 5, the liver injury is classified as hepatocellular, when R is < 2 it is cholestatic, and when R between 2-5 it is a combination (Chalasani *et al.*, 2014; Dalal *et al*., 2017). It was observed from a study that women were more associated with a higher probability of developing drugs and herbal induced liver toxicities compared to men (Kessler *et al.*, 2001)

It is difficult to associate a liver injury to a herbal product if it was taken once because the patient cannot be given the suspected product again. A wrongfully identified plant used in formulation may lead to liver injuries. Irrational or inappropriate use of herbal medicines using the wrong dose or indication can all contribute to potential liver injuries. A contaminant in the product due to bad manufacturing practices can also be associated with liver damage and not necessarily be from the plant. Other external contaminations of the herbal products due to poor storage of the product may be responsible for some liver toxicities after herbal product consumption (Teschke *et al.*, 2013).

Biochemical transformation of pyrrolizidine alkaloids into pyrrole derivatives by cytochrome P450 enzymes acts as alkylating agents have been associated with acute liver toxicity in animals studies (Newman *et al*., 2017; Schramm *et al*., 2019). Eugenol found in oil of clove causes hepatic necrosis and *Lycopodium serratum* causes acute and chronic hepatitis (Stickel *et al*., 2005). Other studies showed activities of cytochrome P450 3A enzymes on diterpenoids led to the production of toxic metabolites in mice experiments. Glutathione depletion and induction of Cytochrome P450 3A enhanced the formation of the toxic diterpenoid metabolites which can activate apoptosis of hepatocytes (Stickel *et al*., 2005).

2.3.4 Product (HPK) under test

HPK is the code given to the product under study which is a polyherbal decoction produced by the Centre for Plant Medicine Research (CPMR) at Akuapem Mampong of the Eastern Region of Ghana. It is composed of extracts of the dried leaves of *Citrus aurantifolia* Christm. (Rutaceae), the dried aerial part of *Bidens pilosa* Linn. (Asteraceae) and the bark of *Trema orientalis* Linn. Blume (Ulmaceae).

2.3.4.1 *Bidens pilosa* (Asteraceae)

Bidens pilosa (Asteraceae) commonly known as Bur Marigold, Blackjack, devils needles, farmers' friends, beggar's ticks, broomsticks (Fig 2.2). It is locally called Ananse mpanie, Gyinantwi, Ofonnena and Praaduro (Twi), Piladura (Ga) and Ofumrena (Fante). It is widely distributed worldwide especially in the tropical and temperate zones (Mshana *et al.*, 2000; Busia, 2007).

Figure 2.2 Picture of the aerial part of *Bidens pilosa*

A. Morphological description of *Bidens pilosa*

It is a perennial erect herb that grows to about 60-150 cm in height. The leaves are glabrous in nature, dentate, serrate and dissected. *B. pilosa* has yellow or white flowers. It has long narrowed ribbed achenes as seeds (Bartolome *et al.*, 2013).

B. Traditional uses of *Bidens pilosa*

Bidens pilosa serves as a treatment for many disorders in different parts of the world. For example, the aerial part of *B. pilosa* is used to treat pulmonary tuberculosis, catarrh, cough, bronchial asthma and influenza in Cuba, China, Korea and other countries (Bunalema *et al.*, 2013). The whole plant is used in treating dysmenorrhea, hyperemesis gravidarum (morning sickness) and irregular menstruation (Oliveira *et al.*, 2004).

Peptic ulcers, intestinal worms, enteritis, dysentery, diabetes mellitus, hypotension, malnutrition, musculoskeletal pains, diarrhoea, colic and acute appendicitis constipation are all diseases treated with *B. pilosa* in Ghana (Mshana *et al.*, 2000; Pan *et al.*, 2013). Decoctions, tinctures and powders of *B. pilosa* are used in treating eye infections, otitis and pharyngitis in Africa and China (Bartolome *et al*., 2013; Borges *et al*., 2013). The whole plant is used to treat snakebite envenomation and jaundice (Mshana *et al.*, 2000; Lai *et al.*, 2015). *Bidens pilosa* can be formulated into many dosage forms such as teas, powders, decoctions, and tinctures either alone or with other plants (Bartolome *et al*., 2013). The leaves of *B. pilosa* is used to treat acute infectious hepatitis, renal infections, wounds, burns, snake bites, malaria and skin diseases (Oliveira *et al.*, 2004).

C. Constituents of *Bidens pilosa*

Phytochemical constituents of *B. pilosa* include; flavonoids, alkaloids, terpenoids, porphyrins and phenylpropanoids (Bartolome *et al*., 2013). Some compounds identified in *B. pilosa* are; 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 2--D-glucopyranosyloxy-1-hydroxy-5(*E*)tridecene-7,9,11-triyne, 2-D-glucosepyronosyloxy-1-hydroxytrideca-5,7,9,11tetrayne, cytopiloyene and 3-D-glucopyranosyloxy-1-hydroxy-6(*E*)-tetradecene-8, 10, 12-triyne (Lee *et al.*, 2013). Quercetin **[1]** (Fig 2.4), Luteolin, Centaurein and Cytopiloyne have been identified in *B. pilosa* (Bairwa *et al*., 2010).

D. Biological activities of *Bidens pilosa*

Ethanolic extracts of *Bidens pilosa* have been used as anticancer agents and as antimalarial (Prakash *et al.,* 2013). *B. pilosa* have immuno-modulatory, gastric antisecretary and antiulcer and anti-mycobacterium activities (Bairwa *et al.*, 2010). Other pharmacological activities confirmed in scientific studies are; antioxidant effect, (Bartolome *et al*., 2013), anti-inflammatory, antifungal and antibacterial (Ashafa *et al.*, 2009). *Bidens pilosa* causes suppression of histamine release thereby acting as a potent antihistamine (Ajagun-ogunleye *et al*., 2015).

2.3.4.2 *Citrus aurantifolia* (Rutaceae)

Citrus aurantifolia Christm. (Rutaceae) (Figure 2.3) commonly known as sour lime, lime, key lime and Mexican lime. It is locally known in Ghana as Ankaatware and Ankaadwea (Twi), Ankama (Fante), Nyamsa (Dagbani), Lumburi (Ewe), Domumni (Nzema), Leemu (Mole) (Mshana *et al.*, 2000). *Citrus aurantifolia* is a plant that grows worldwide especially in the tropical and subtropical zones (Busia, 2007).

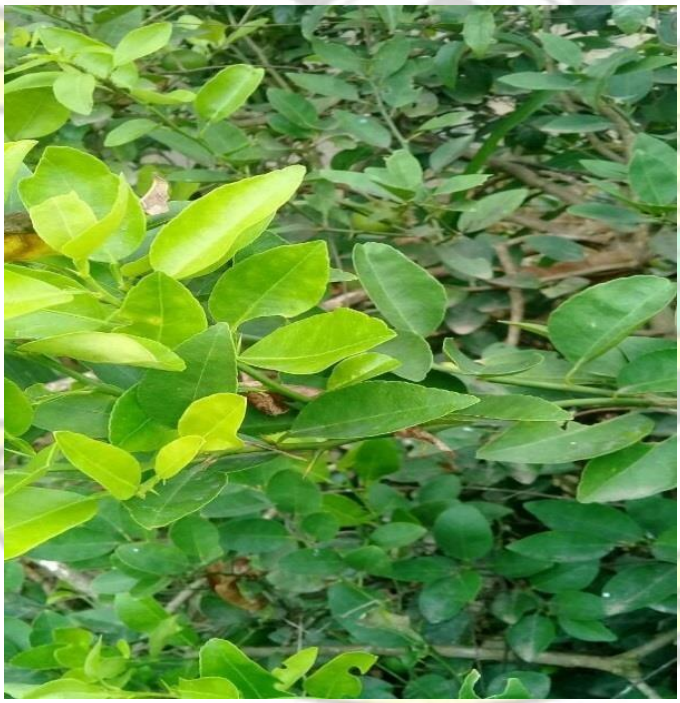


Figure 2.3 *Citrus aurantifolia* branches and leaves

A. Morphological description of *Citrus aurantifolia*

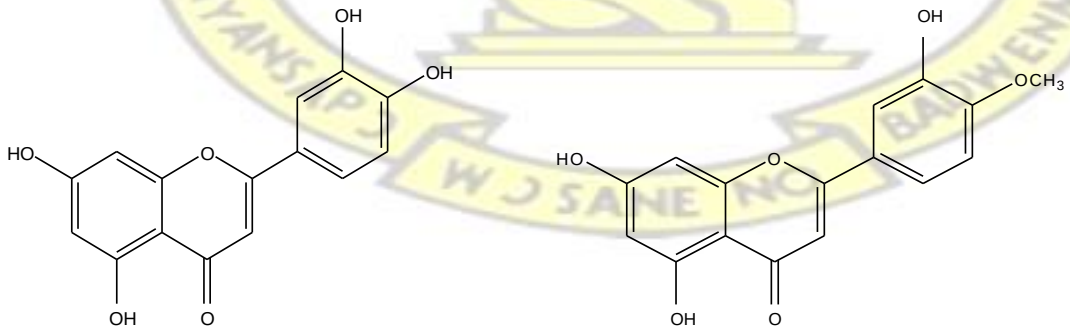
Citrus aurantifolia is a small tree that grows to about 5 meters in height. It bears green leaves and fruits throughout the season. The leaves are elliptical in shape and arranged alternately with crenulate margin and petiolated. The flowers are yellow measuring about 2.5 cm in diameter (Enejoh *et al*., 2015).

B. Traditional uses of *Citrus aurantifolia*

Citrus aurantifolia is used as medicine for many people in Africa and Asia (Rafiq *et al.*, 2016). The leaves of lime are used to treat jaundice and infective hepatitis in Ghana (Mshana *et al.*, 2000). *C. aurantifolia* is used as an appetite stimulant (Nakajima *et al*., 2014). Other traditional uses of *C. aurantifolia* are; antiulcer, antidiarrheal, laxative, anthelmintic, treat sore throat and cold as well as reducing cholesterol (Ross, 2005). In Finland, *C. aurantifolia* is used to treat asthmatic attacks. Externally, it is used as antiseptic, and insect repellant against mosquito bites (Sandoval-Montemayor *et al*., 2012). *Citrus aurantifolia* have antidiuretic properties and it is also for the treatment of headaches (Enejoh *et al*., 2015). *Citrus aurantifolia* is used to treat cough and facial pimples (Ibukun *et al*, 2007).

C. Constituents of *Citrus aurantifolia*

The aqueous extract of *Citrus aurantifolia* leaf contains these secondary metabolites; alkaloids, saponins, tannins, proteins, phytosterols, flavonoids, and glycosides. Some compounds isolated from *C. aurantifolia* include; quercetin **[1]**, Hesperidin **[2]** (Figure 2.4), neohespiridin, naringin, narirutin, eriocotin, didymin, ascorbic acid, limonoids and rutin (Okwu, 2006; Nakajima *et al*., 2014; Rafiq *et al.*, 2016).



Quercetin **[1]** Hesperidin **[2]**

Figure 2.4 Chemical structures of Quercetin and Hesperidin (Loizzo *et al.*, 2012)

D. Biological activities of *Citrus aurantifolia*

Citrus aurantifolia have anticancer, anti-inflammatory, immune stimulant properties (Tichy *et al*., 2008). It has a very high concentration of vitamins (e.g. ascorbic acid) and minerals which serves as potent antioxidants. The leaf extract of *Citrus aurantifolia* had activity against breast cancer cell lines (Rooprai *et al*., 2001). No toxicity was observed when the extract of *Citrus aurantifolia* at 1200 mg/kg per day was subjected to acute and subchronic toxicity studies in rats (Chunlaratthanaphorn *et al.*, 2007).

Hepatoprotective enzymes are induced by *Citrus aurantifolia* as well as blocking genetic material damage in humans (Okwu, 2006). Studies in mice proved that *Citrus aurantifolia* was able to reverse cholestatic liver fibrosis caused by bile duct ligation (Lim *et al.*, 2016). In addition to this, *C. aurantifolia* is anti-inflammatory, regulates metabolic syndrome, and can delay the onset of Alzheimer's disease as well as prevent cancers. (Rawson *et al*., 2014). *C. aurantifolia* was found to be anticoagulant, antibacterial, antilipidemic, antifungal and antidiabetic (Narang and Jiraungkoorskul, 2016).

2.3.4.3 *Trema orientalis* (Ulmaceae)

Trema orientalis (Ulmaceae) commonly known as Pigeonwood, charcoal tree, hop out, Indian charcoal tree, gun powder tree, and indigo nettle tree are common names of *T. orientalis*. In Ghana, it is called Sesea (Twi), Wadzawadza (Ewe), Osesea (Fante) and Seazealera (Nzema) (Busia, 2007). *Trema orientalis* is found mostly in the savanna low land areas of tropical countries especially in Africa and Asia (Folashade *et al*., 2012).

A. Morphological description of *Trema orientalis*

The tree grows rapidly to a height of 18 meters and branches to 4-8 meters high. The bark is grey in colour, pubescent with dotted lenticels with its alternative distichous simple leaves having unequal sides and shapes, subcordate base, acuminate apex. The leaf margin is serrated and the surface is pubescent and it has small and whitish axillary cluster flowers (Busia, 2007). The fruit is a drupe, sub-globose, about 3 mm in diameter when fully matured and black when it is dry (Hawthorne and Jongkind, 2006; Ugwoke *et al.*, 2017).

B. Traditional uses of *Trema orientalis*

The leaves of *Trema orientalis* are used to treat jaundice, oliguria, pharyngitis, bronchitis, cough, malaria, diabetes mellitus, hematuria, oliguria and cardiovascular diseases in many African countries (Mshana *et al.*, 2000; Adinortey *et al*., 2013).

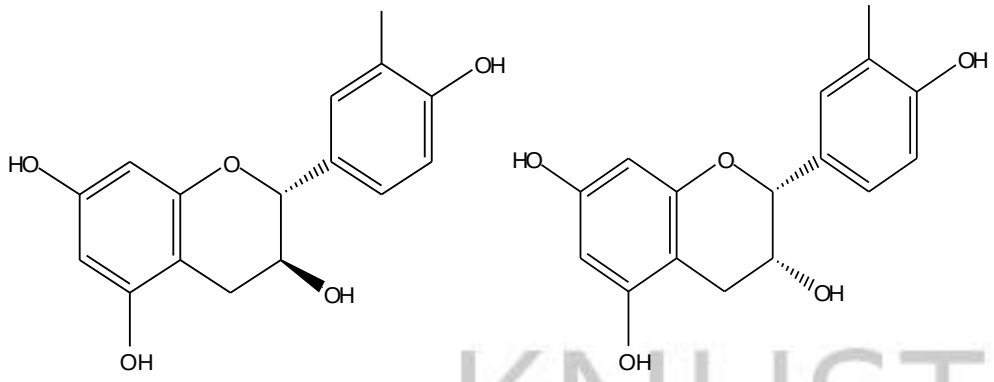
The leaves are used as laxative, vermifuge, antiemetic and also for treating infections, paralysis, convulsions and general poison antidote (Uddin, 2008). The decoction of leaves and bark are used in treating yellow fever, chicken pox, gonorrhoea, malaria and anaemia (Folashade *et al*., 2012; Moshi *et al*., 2012).

The stem bark of *Trema orientalis* is used in the treatment of malnutrition, sexually transmitted diseases, renal disease and musculoskeletal pains (Aboaba and Choudhary, 2015). The bark is also used for the treatment of dysentery, diarrhoea, hematuria, epilepsy, pleurisy, sickle cell disease and musculoskeletal pains (Kuo *et al.*, 2007; Mpiana *et al.*, 2011; Panchal *et al.*, 2015; Ugwoke *et al.*, 2017). The stem bark stimulates lactation and also possesses febrifuge activity. The bark combined with the leaves is locally used as a diuretic. The roots are used in treating bronchial asthma, hematuria and gastrointestinal bleeding (Uddin, 2008; Adinortey *et al*., 2013). *Trema orientalis* is also used in the treatment of sore throat, toothache, yellow fever, asthma, diabetes mellitus and intestinal worms (Tchamo *et al.*, 2001; Tuo *et al.*, 2015; Gabriel *et al*., 2016; Jiji *et al.*, 2016).

C. Constituents of *Trema orientalis*

Trema orientalis contains tannins, reducing sugars, alkaloids, flavonoids triterpenoids, phytosterols and saponins as secondary metabolites (Adinortey *et al*., 2013; Panchal *et al.*, 2015). The stem bark of *Trema Orientalis* have been found to contain Ampelosin F, quercetin **[1]**, Catechin **[3]**, Epicatechin **[4]** (Figure 2.5), Syringaresinol, N-(trans-p-Coumaroyl) tyramine, N-(trans-p-Coumaroyl) octapamin, trans-4-Hydroxy-cinnamic acid scopoletin, lupeol, β-sitosterol, hexacosanoid acid, 3,4-dihydrobenzoic acid, p-hydrobenzoic acid, sweroside, and 3,5-Dimethyl-4hydroxyphenyl-1-O-β-D-glucoside (Tchamo *et al.*, 2001; Kuo *et al.*, 2007).

OH OH



Catechin **[3]** Epicatechin **[4]**

Figure 2.5 Chemical structures of Catechin and Epicatechin (Kuo *et al.*, 2007)

D. Biological activities of *Trema orientalis*

Some of the pharmacological properties of *Trema orientalis* include; analgesic, laxative, diuretic, anti-inflammatory, anticonvulsant, antisickling, anthelmintic, antiplasmodial and antioxidant activities (Njayoy *et al.*, 2008; Adinortey *et al*., 2013). The stem bark of *Trema orientalis* exhibited hypoglycemic effects (Theophile *et al.*, 2006). The bark of *Trema orientalis* has been established to have antioxidant and an inhibitor of α-amylase that synergistically control hyperglycemia (Trono *et al*., 2016). The *Trema orientalis* has febrifuge, galactogogue, antipyretic, antisecretory effect on the gastrointestinal tract and diuretic properties (Babatunde *et al*., 2015; Sayeed *et al.*, 2017). The stem bark of *Trema orientalis* possesses antibacterial activity against medically important organisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus subtillis* (Rout *et al.*, 2012).

2.4 CLINICAL RESEARCH ON FINISHED HERBAL PRODUCTS

Clinical research is a study of medical science that deals with the safety and effectiveness of devices, medications, diagnostic items and treatments intended for human use for the diagnosis, prevention, treatment and improvement of disease symptoms (FDA, 2018). It is not easy to apply the same conventional method of clinical research to assess traditional medicine for safety and efficacy because of many complexities and challenges when it comes to natural products (Liu, 2011; Singh *et al.*, 2015).

A clinical study can be a retrospective or prospective study; retrospective studies models use already existing data on past and current patients to answer scientific

questions. A prospective clinical studies use new data on current and future patients over a time period during which medically relevant events occur, mostly using methods specific to the particular research. Prospective study can either be noninterventional (observational) or interventional (experimental) by manipulating study-related treatment (Euser *et al*., 2009; Wylie, 2015).

In conventional medicine, potential drugs undergo four phases of clinical trials to be accepted into clinical use. A clinical trial is a prospective biomedical or behavioural research study of human subjects that is set up to answer specific questions about biomedical or behavioural interventions (drugs, vaccines, medical devices, or new ways of using known drugs, treatments, or devices). The purposes of clinical trials are used to assess whether new biomedical or behavioural interventions are safe, efficacious, and effective (WHO, 2019). Clinical trials are conducted in four phases:

2.4.1 Phase 1

Phase 1 of clinical trials deals with few healthy people between 20 to 100 who use the intervention for the first time as outpatients to elucidate the safe dose and identify side effects (safety and efficacy) (WHO, 2019).

2.4.2 Phase 2

Phase 2 of clinical trials are conducted on hundreds of patients with the disorder to be prevented, diagnosed and treated for a short period. Assessment is made for safety, efficacy, dose-response and dose frequency. This study may qualify as a phase two trial in people with liver disease in limited people (WHO, 2019).

2.4.3 Phase 3

Phase 3 of clinical trials deals with a large number (thousands) of human subjects by comparing the intervention product to a standard for adverse effects (WHO, 2019).

2.4.4 Phase 4

Phase 4 of clinical a trial is for post-marketing surveillance of the intervention product. These studies are designed to monitor the effectiveness of the approved intervention in the general population and to collect information about any adverse effects associated with widespread use (WHO, 2019).

2.5 QUALITY OF LIFE ASSESSMENT

Quality of life assessment explores and measure ways to enhance comfort and the quality of life for people for chronic disease. Quality of life assessment are tools used to ascertain how comfortable people are in many aspects of life. It is an individuals' perceptions of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards and concerns.

It is a broad-ranging concept affected in a complex way by the persons' physical health, psychological state, level of independence, social relationships and their relationship to salient features of their environment. There are many scales to assess the quality of life of people with disease conditions in terms of health. Karnofsky scale is one which grades quality of life on a ten point scale with 100 being the maximum impact and zero for death. Adding up each score produces the total score for a client (Burckhardt and Anderson, 2003).

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 HPK

Two batches of HPK with batch numbers HT 17002 and HT 18002 were obtained from the Centre for Plant Medicine Research (CPMR) at Akuapem Mampong in the Eastern Region of Ghana. Details of HPK have been given in Table 3.1. The products were kept at the storeroom of the Pharmacy Department of the Upper West Regional Hospital, Wa, at a room temperature of 28^{o}C.

Table 3.1 Details of HPK

Batch No.	Man Date	Expiry Date	Date of Collection
HT 17002	13/9/2017	13/9/2018	21/11/2017
HT 18002	19/7/2018	19/7/2019	21/7/2018

3.1.2 Plant material collection and authentication

The three plants which are components of HPK were collected for quality studies. The aerial part of fresh *Bidens pilosa* L. and leaves of *Citrus aurantifolia* were collected at Obuasi in the Ashanti region of Ghana on 21st, October 2017 within geographical coordinates of 6^{0}14'38.3"N, 1^{0}40'15.9"W and 6^{0}14'39.0" N, 1^{0}40'15.9"W respectively.

The fresh bark of *Trema orientalis* was harvested at Kwahu Asakraka in the Eastern region of Ghana on 20th November 2017 within geographical location coordinates of 6^{0}18'06.5"N; 0^{0}31'36.7"W.

All the plants were authenticated by Dr George Henry Sam and Mr Clifford Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS), KNUST. Voucher specimens have been kept at the herbarium of the Department of Herbal Medicine with details as shown in Table 3.2.

Table 3.2 Voucher numbers of plant components of HPK

Name	Plant part	Voucher number
Bidens pilosa	Aerial part	KNUST/HM1/2019/L002
Citrus aurantifolia	Leaf	KNUST/HM1/2019/L001
Trema orientalis	Bark	KNUST/HM1/2019/SB/008

3.2 METHOD

3.2.1 Preparation of plant material

The plant materials were cleaned thoroughly under running tap water to remove soil residues and other foreign materials. *Bidens pilosa* aerial parts and *Citrus aurantifolia* leaves were air dried at room temperature for two weeks while the stem bark of *Trema orientalis* was cut into smaller pieces and was sun-dried for two weeks. All the dried plant materials were milled to coarse powders with a mechanical milling machine and stored in airtight amber glass containers and well covered.

3.2.2 Quality assessment

The quality assessment conducted on HPK included the following: organoleptic characters, test for secondary metabolites, physicochemical analysis (pH, relative density and weight per mL), Infra-red and Ultraviolet spectroscopy, and chromatography (thin layer and high-performance liquid). Total antioxidant capacity (TAC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) bioassays were done on HPK, *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*. Other parameters assessed were: heavy metals content and microbial load determination in HPK.

3.2.2.1 Organoleptic characters

The sensory organs were used to determine the organoleptic characteristics (taste, colour and odour) of HPK, *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*.

3.2.2.2 Phytochemical screening

Qualitative phytochemical screening for secondary metabolites present in HPK, *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* were done using standard procedures (Evans, 2009).

A. Test for Alkaloids

Five (5) mL of 1% H_2SO_4 was added to an equal volume of HPK and filtered. It was rendered distinctly alkaline with dil. Ammonia and partitioned with chloroform using a separating funnel. The chloroformic extract was evaporated to dryness and the residue was dissolved in 1% H_2SO_4. Few drops of Dragendorff's reagent (Potassium bismuth iodide solution) were added. An orange-red precipitate indicates alkaloids are present.

Each 0.5 g of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* powdered sample was extracted with 50 mL ammoniacal alcohol (1 part strong ammonia: 9 parts 95% ethanol) and filtered. The filtrate was evaporated to dryness and the residue was then extracted with 1% H_2SO_4 and filtered. The filtrate was made distinctly alkaline by adding dilute ammonia (NH_3) solution and partitioned with chloroform in the separating funnel. The chloroformic layer was separated and evaporated to dryness on the water bath. The residue was dissolved in 1% H_2SO_4 and few drops of Dragendorff's reagent were added.

B. Test for Saponins

Five (5) mL of HPK was mixed with an equal volume of water in a test tube and shaken. The test tube was allowed to stand for 10 minutes and observed. A froth which persists for more than 10 minutes indicates saponins.

For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*, 0.5 g of each was mixed with 10 mL water and was shaken vigorously in a test tube. A forth which persist for more than 10minutes indicates the presence of saponins.

C. Test for Tannins

A: About 5 mL water was added to 1 mL of HPK and few drops of 1% Lead acetate solution was added. For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*, 0.5 g of each was boiled with 25 mL water for 30 minutes, allowed to cool and filtered. To each 1 mL of filtrate, 5 mL of water was added, then 2 to 5 drops of 1% Lead acetate solution was added. The presence of coloured precipitates indicates the presence of tannins.

B: HPK (1 mL) was diluted with 5 mL of water and a few drops of 1% Ferric chloride solution in drops and then excess. An olive-green, brownish green or dark green colour observed both in drops and excess indicates the presence of condensed tannins whilst blue-black colour in drops and excess indicates hydrolysable tannins.

For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis,* 0.5 g of each was boiled with 25 mL water for 30 minutes, allowed to cool and filtered. To each 1mL of filtrate, 5 mL of water and a few drops of 1% Ferric chloride solution in drops and then excess.

D. Test for Reducing Sugars

To 5 mL of HPK, 5 mL of dilute hydrochloric acid (HCl) was added and heated on a water bath for 2 minutes. The extract was made distinctly alkaline with drops of 20% NaOH. 1 mL each of Fehling's solutions A and B were added and the extract and was heated on a water bath. Formation of a brick red precipitate proves the presence of reducing sugars.

To 0.2 g each of the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis,* 10 mL of dilute hydrochloric acid (HCl) was added and boiled on the water bath for 5 minutes and filtered. One mL each of Fehling's solutions A and B were added and the extract and was heated on a water bath.

E. Test for Flavonoids

A strip of filter paper was dipped in HPK and was allowed to dry. The dried filter paper was exposed to the fumes of strong ammonia and observed for intense yellow colour which disappears when exposed to conc. HCl.

To 0.2 g each of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* powders, was shaken with 25 mL water and filtered. A strip of filter paper was dipped in the extract and was allowed to dry. The dried filter paper was exposed to the fumes of strong ammonia and observed.

F. Test for Triterpenoids

Five (5) mL of HPK was partitioned with chloroform and concentrated sulphuric acid (H_2SO_4) was added carefully down the test tube of the chloroformic extract. Triterpenoid presence is seen as a reddish-brown ring at the interphase (Salkowski test).

For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*, 0.5 g of each was shaken with chloroform and filtered. To the extract, concentrated sulphuric acid (H_2SO_4) was added carefully down the test tube.

G. Test for Phytosterols

Liebermann-Burchard test for sterols was used by shaking HPK with Chloroform in a separating funnel. To 5 mL of the chloroformic extract, acetic anhydride and conc. sulphuric acid (H_2SO_4) was added down the test tube wall to form the lower layer. The presence of blue colour at the interface indicated the presence of the steroidal ring.

For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*, 0.2 g of each was shaken with chloroform and filtered. To 5 mL of the chloroformic extract, acetic anhydride and conc. sulphuric acid was added down the test tube wall to form the lower layer.

H. Test for Coumarins

HPK was partitioned with Chloroform and evaporated to dryness. The residue was dissolved in hot distilled water, filtered and allowed to cool down. The sample was divided into two (1 and 2). 0.5 mL of ammonia solution was added to extract 1 in the test tube and the other left as a control. Sample 1 was then observed under UV light. Sample 2 is a negative control for observation. Observation of a blue fluorescence under UV lamp was indicative of coumarins.

For the powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis*, 0.2 g of each was shaken with chloroform, filtered and evaporated to dryness. The residue was dissolved in hot distilled water, filtered and allowed to cool down. The sample was divided into two (1 and 2). 0.5 mL of ammonia solution was added to extract 1 in the test tube and the other left as a control. Sample 1 was then observed under UV light.

3.2.2.3 Physicochemical methods

A. pH determination

The pH of 20 mL of two samples each from the two batches of HPK were determined using a digital pH meter (Mettler Toledo fine Go) at room temperature of 29.6 ℃.

B. Relative Density Determination

The relative density of HPK was determined by the ratio of the weight of 1 mL of HPK to the weight of 1 mL of water.

C. Residue on drying

The weight of 300 mL of HPK was accurately determined and it was placed in an oven at 60 ℃ to evaporate until a constant weight was obtained. Duplicate determinations were done and the average was calculated to determine the weight per millilitre of HPK on drying.

3.2.2.4 Chromatographic Analysis

A. Thin Layer Chromatography (TLC)

HPK was partitioned with chloroform and the chloroformic extract was evaporated to dryness. Decoctions were made from the powders of *Bidens pilosa*, *Citrus aurantifolia* and *Trema aurantifolia* by boiling 1g each with 300 mL of water for 30 minutes.

Each aqueous extract was partitioned with chloroform and the chloroformic extracts obtained were evaporated to dryness. Each dried extract was dissolved in chloroform and spotted on aluminium pre-coated silica gel plates GF254 (0.25mm thick) for analytical thin layer chromatography (TLC) obtained from Alpha laboratory (UK). The plates were allowed to dry and developed in the pre-saturated chromatank containing petroleum ether and ethyl acetate (6:4) as the mobile phase. Short wavelength (254 nm) and (long wavelength (365 nm) Ultra Violet lamp was used to visualize the chromatograms to observe for fluorescent compounds. The plates were sprayed with Anisaldehyde reagent followed by heating a hair-dryer.

B. High Performance Liquid Chromatography (HPLC)

HPLC Instrumentation and Condition

The chromatographic system composed of Dionex Ultimate 3000 RS Pump HPLC, degasser, Dionex Ultimate XRS Autosampler and a Dionex Ultimate RS diode array multiple wavelength detector. BDS Hypersil C_{18} reversed phase column (120A pore size, Ø 4.6 mm x 150 mm) was used as the stationary phase.

Chromatographic method development

Three solvents i.e. Acetonitrile (ACN), methanol (MeOH) and acetic acid (CH_3COOH) were selected as the mobile phase for the chromatographic separation. This was based on the separation, retention time, the height of the peak and the area produced. Detection wavelength for the samples was selected after analyzing fingerprints produced by the multiple wavelength detector. The wavelength of 345 nm was chosen as it was most suitable for the purpose. Flow rate and injection volume were set at 1.0 mL/min and 20 µL respectively. The column temperature was also kept at an ambient temperature of 26 ℃. Isocratic elution mode was used in HPLC method development.

Validation of the HPLC method

The HPLC method used for the assessment was validated according to the guidelines of the International Conference on Harmonization (ICH). Parameters validated included the linearity and range, recovery/accuracy, limits of detection (LOD), the limit of quantitation (LOQ) and system suitability (ICH, 2005).

Preparation of Standard

Quercetin was chosen as a biomarker because it has been identified in *B. pilosa, C. aurantifolia* and *T. orientalis* and it is commercially available. Quercetin solution was constituted by dissolving it in methanol to produce 1 mg/mL concentration. The solution was sonicated for 30 minutes and filtered through PTFE membrane syringe filters before injection into the HPLC machine. The determination was done in triplicates.

Preparation of Sample

HPK and plant extracts were evaporated in an oven at 60 ℃. Each was then dissolved in methanol to a concentration of 100 mg/mL because quercetin was readily soluble in methanol during the method development.

These methanol extracts were filtered through Polytetrafluoroethylene (PTFE) membrane syringe filters (0.45µm). All injections into the HPLC machine were done in triplicate.

3.2.2.1 Spectroscopic analysis A. Infrared Spectroscopy

Fifty (50) mL of HPK was evaporated at 40^{o}C in the oven until a residue of constant weight was obtained. Sample powders of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* were used for the analysis.

All Fourier transform infrared (FTIR) analysis were done at the Central Laboratory of KNUST using PerkinElmer Spectrum Version 10.03.09 model which was scanned from 400 to 4000 cm^{1} spectra range.

B. Ultraviolet Spectroscopy

A decoction made from 0.2 g each of *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* in 50 mL of water. Two percent ($^{v}/_{v}$) solution was prepared from the decoctions and HPK for the UV analysis at the Central Laboratory of KNUST. The slit width of 1 nm, the scan speed of 480 nm per minute was used to scan the samples between 200 and 700 nm wavelengths. The UV spectrum was recorded using the Evolution 300 UV-VIS machine.

3.2.2.2 Microbial Load Determination A. Blood agar for Aerobic Microorganisms

Two batches of HPK were taken through bacterial load determination using blood agar. Already prepared commercial plate of blood agar was taken from the refrigerator and dried in the oven at 30 ℃. The dried plate was marked and labelled appropriately as 1 and 2 for the two separate batches of HPK. A wire loop was flamed, allowed to cool and used to pick a sample of HPK to make the initial inoculum. Straight line streaking out of the initial inoculum was done after heating and cooling the wire loop. Sideways streaking out in straight lines were done from the first streak. A third streaking was made in a zigzag manner from the second streaking. The blood agar was incubated

under an aerobic condition at 37 ℃ for 24 hours. The agar plate was taken out of the incubator after 24 hours and examined for bacterial growth (Russell *et al.*, 2006).

B. MacConkey agar for Aerobic Microorganisms

Already prepared plate of MacConkey agar was taken from the refrigerator and dried in the oven at 30 ℃. The dried plate was marked and labelled appropriately as 1 and 2 for the two separate HPK batches. A wire loop was flamed, allowed to cool and used to pick a sample of HPK to make the initial inoculum. Straight line streaking out of the initial inoculum was done after heating and cooling the wire loop. Sideways streaking out in straight lines were done from the first streak. A third streaking was made in a zigzag manner from the second streaking. MacConkey agar was incubated in an aerobic condition at 37 ℃ for 24 hours. The agar plate was taken out of the incubator after 24 hours and examined for bacterial growth (Lau *et al.*, 2003).

C. Chocolate agar for Anaerobic Microorganisms

Already prepared plate of chocolate agar was taken from the refrigerator and dried in the oven at 30 ℃. The dried plate was marked and labelled appropriately as 1 and 2 for the two separate HPK batches. A wire loop was flamed, allowed to cool and used to pick a sample of HPK to make the initial inoculum. Straight line streaking out of the initial inoculum was done after heating and cooling the wire loop. Sideways streaking out in straight lines were done from the first streak. A third streaking was made in a zigzag manner from the second streaking. Chocolate agar was immediately incubated in an anaerobic atmosphere at 37 ℃ for 24 hours. The agar plate was taken out of the incubator after 24 hours and examined for bacterial growth (Deschler *et al*., 2012).

3.2.2.7 Heavy metals analysis

Total dissolved heavy metal determination for lead (Pb), Arsenic (As), Cadmium (Cd), Nickel (Ni) and Mercury (Hg) using wet digestion were done using M5 Atomic Absorption Spectrophotometer (AAS) with an Autosampler and granite furnace at the Faculty of Agriculture, Soil Science Department, KNUST. Nitric and perchloric acids were used for the digestion to determine total dissolved heavy metals present.
The test was replicated and the mean results were used for data analysis (Gajalakshmi *et al.*, 2012).

3.2.3 Antioxidant studies

3.2.3.1 DPPH free radical scavenging activity

Microtitre based DPPH assay (Brand-Williams *et al*., 1995) was used to assess the scavenging capacities of HPK and its plant components. Five concentrations were made ranging from 100% to 6.25% of HPK and its plant components. L-Ascorbic acid was used as the reference drug. To each 1 mL of the test sample, 3 mL of DPPH (0.4 mM) was added. This was incubated in the in a box placed in a dark locker for 30 minutes. The antioxidant activity was measured by reading the absorbance at 517 nm with the aid of microplate reader using UV-VIS Spectrophotometer (PerkinElmer). Methanol was used as the blank and all readings were made in triplicates. The DPPH percentage scavenging activity was calculated based on the equation;

$$\% \text{ DPPH radical scavenging activity } (\%) = \frac{(A_o - A_1)}{A_0} \times 100,$$

Where: A_o – absorbance of the control and A_1 – absorbance of the test extracts.

The IC_{50} which is the sample concentration needed to inhibit 50% of the DPPH free radical was calculated by plotting a graph of percentage inhibition of DPPH against concentration.

3.2.3.2 Total Antioxidant Capacity Determination

To 1 ml of extract, 3 mL of the reagent (0.4 mM ammonium molybdate, 28 mM Sodium phosphate and 0.6 M sulphuric acid) was added. The samples were incubated for 90 minutes at 60 ℃ after which they were allowed to cool to room temperature and the absorbance was measured at 695 nm against water as the blank. This was then assayed by the method used by (Prieto *et al*., 1999; Dasgupta and De, 2004). The total antioxidant capacity was calculated as the number of equivalents of Ascorbic acid (Scalzo *et al.*, 2005).

3.2.4 Clinical evaluation of HPK

3.2.4.1 Study Area

The study was conducted at the Upper West Regional Hospital (UWRH) at Wa, a Municipality in the Upper West Region of Ghana. The Upper West Region constitutes

12.7% of the total land size of the Republic of Ghana. It shares boundaries to the south with Northern Region, north, with the Republic of Burkina Faso, east with the Upper East Region and west with la Cote d'Ivoire.

The region has 11 Districts/Municipalities with Wa as the regional capital with a total population of 702,110 (GSS, 2012). Wa Municipality lies between latitude 1º40'N to 2º45'N and longitude 9º32'W to 10º20'W. It is 326 meters above sea level. Wa Municipality shares boundaries to the north with Nadowli, District, Wa East District to the east and Wa West District to the south and west (GSS, 2014). The geographical map of Wa Municipal showing health facilities is shown in Figure 3.1.

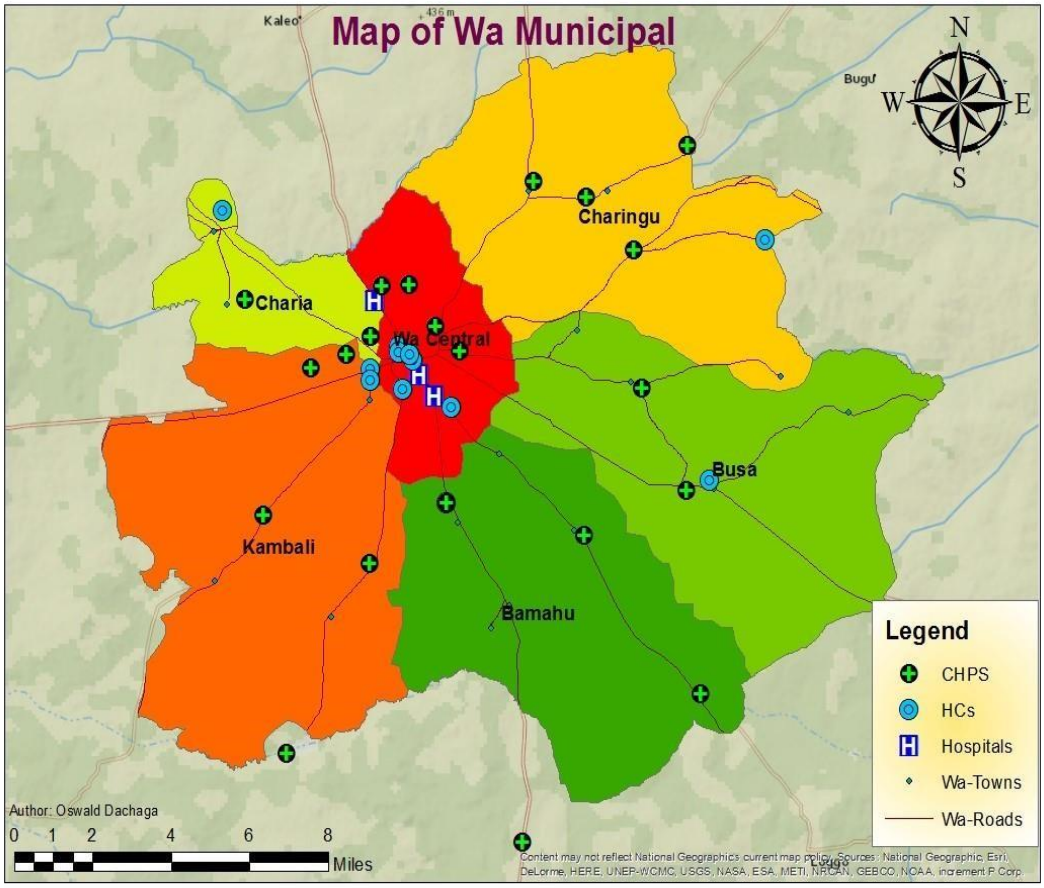


Figure 3.1 Geographical Map showing the health facilities of Wa Municipality

The Upper West Regional Hospital is the main referral facility for the people of Upper West Region, surrounding communities from other regions and sometimes foreign nationals from Burkina Faso and la Cote d'Ivoire. The Upper West Regional Hospital provides both primary healthcare as well as specialized healthcare services to clients. The departments in the hospital include; Public health, Medical, Surgical, Pediatric,

Neo-Natal Intensive Care Unit (NICU), Ear, Nose and Throat (ENT), Eye, Mental health, Obstetrics and Gynaecology, Dental, Herbal Medicine, Physiotherapy, Radiology and Medical Laboratory. The health teams in the UWRH are the Internal Medicine, Surgical, Obstetrics and Gynaecology, Psychiatric, Dental and Paediatric teams.

Figure 3.2 Geographical Map of Upper West Regional Hospital, Wa

3.2.4.2 Ethical Clearance

Ethical approval for the study was given by the Institutional Review Board (Committee on Human Research, Publication and Ethics-CHRPE) at the School of Medical Sciences (SMS) KNUST and Komfo Anokye Teaching Hospital (KATH) with reference: CHRPE/AP/038/18 in February 2018. Approvals were also given by the Regional Health Administration Training and Research Unit of Upper West Region and the Upper West Regional Hospital. The ethical approval letter is attached (page IV).

3.2.4.3 Period of Study

The study took place at the Herbal Medicine Department of the Upper West Regional Hospital (Figure 3.2), Wa from July to November, 2018.

3.2.3.4 Target Population and Sample Size

The study was targeted at clients who patronize the services of the Herbal Medicine Department of the Upper West Regional Hospital, Wa. Clients with suspected liver diseases were asked to do the liver function test, renal function test, full blood count (FBC) and routine urine examination. A sample size of fifty (50) clients (males and females) determined according to the formula below were recruited:

$$sample\ size\ (SS) = \frac{Z^2 \times (P) \times (1 - P)}{C^2}$$

Where: **Z** = Z value (e.g. 1.96 for 95% confidence level); **p** = percentage picking a choice expressed as a decimal (0.5 used for sample size needed)
c = confidence interval, expressed as decimal (e.g., .04 = ±4)

Convenience sampling technique (Elfil and Negida, 2017) was used in selecting the clients for the study based on the inclusion and exclusion criteria.

3.2.4.5 Inclusion and exclusion criteria

A. Inclusion criteria

- Clients with deranged liver disease confirmed by biochemistry laboratory test
- Adults above 18 years
- Outpatients both males and females
- Clinically stable with no life-threatening conditions
- Ability to come for follow up
- Willing to sign consent forms

Adults above eighteen (18) years who attended the Herbal Medicine Department of Upper West Regional Hospital as out-patients that qualified were recruited and enrolled on the study.

B. Exclusion criteria

- People below 18 years
- Critically ill clients
- Clients on admission
- Clients with a high temperature above 39^{o}C
- Signs of severe liver disease
- People with co-morbidities
- Clients on admission
- Clients on other hepatoprotectives
- People on recreational drugs or other drugs 14days before the study period
- Pregnant women and breastfeeding mothers
- Inability to come for follow up
- Clients with any condition that might compromise any of the biochemical parameters

People with the above characteristics were excluded from the study.

3.2.4.6 Participant withdrawal/enrolment

A participant was to be withdrawn if he/she clinically deteriorated or worsened during the study period. Any individual who developed adverse drug reaction to HPK was to be withdrawn from the research. Clients had the liberty to withdraw from the study if they wish with or without explanation to the researcher and standard treatment options were made available to them.

3.2.4.7 Participant's selection

Clients who voluntarily access herbal medicine services and those referred by allopathic Medical Officers to the Herbal Medicine Department of Upper West Regional Hospital were screened for possible liver diseases. Clients with signs and symptoms suggestive of liver disease (e.g. nausea, vomiting, ascites, itches, jaundice, dark urine, clubbing, palmar erythema, spider naevi, etc.) were sent to the laboratory to do liver function test, renal function test, full blood count and urine routine examination. The clients who qualify based on the criteria were given information and the option if they want to be part of the study. Those who voluntarily agreed to be part

were made to sign the patient consent forms and enrolled in the study. A total of 50 participants were recruited.

3.2.4.8 HPK administration and follow up

Nine (9) bottles of HPK were dispensed to the 50 clients to be taken 30 mL three times daily (8 hourly) after meals according to the manufacturer's instruction. Five bottles of HPK were given for the first two weeks and 4 bottles given on the 14th day review for the last two weeks. Most clients were called on the phone daily to check for compliance, to assess any possible adverse drug reaction and to remind them of the next hospital visit. Home visits were made to clients whose houses could be identified to physically see some patients on HPK and to encourage compliance. The 50 clients were seen at the hospital on day 0, 14, and 28 days and each was client was clinically assessed and taken through laboratory investigations (liver function test, renal function test, full blood count and urine routine examination) per each visit. The clients were always clinically assessed to ascertain their progress or deterioration. Clients who did not respond well to HPK within the study period were referred to the allopathic Medical Officers of the Upper West Regional Hospital for further assessment and care.

3.2.4.9 Study design

The open-label prospective non-comparative clinical method was used in this study. There were no positive and negative controls for the study (Casteels and Flamion, 2008; Ballou *et al*., 2017).

3.2.4.10 Data Collection

Clients' data were captured once they were enrolled in the study. Codes were given to clients and data entered with the codes to guarantee anonymity. Template for data collection captures demographics (age, sex, marital status and education), clients contact and residential address, signs, symptoms, adverse reactions and quality of life. All laboratory results were also recorded in a data capturing template (Appendix 1).

3.2.4.11 Assessment of Safety of HPK

Aspartate transaminase (AST), Alanine transaminase (ALT), Bilirubin (direct and total), Gamma-glutamyltransferase (GGT), Alkaline phosphatase (ALP), Proteins

(total and albumin), Urea, Creatinine, Sodium, Chloride, Potassium reagents and urine dipsticks (URIT 10V) were all purchased from Fortress Diagnostic Limited, UK.

Vital signs (body weight, blood pressure and axillary temperature) of all recruited clients were taken on day 0, 14 and 28. Venous blood samples were taken from each client on day 0 (baseline), 14 and 28. All blood samples were collected in EDTA containers and then divided into two portions for haematology and biochemistry analysis. Full blood count was done using Mindray 3000 Haematology analyzer while Mindray 3000 Biochemistry analyzer was used to run the liver and renal function tests.

Renal function test (RFT), full blood count (FBC) and urinalysis baseline parameters were compared to the end of 28 days of the study period. Any significant negative change (abnormally low or high parameters) in the compared results in relation to the recommended reference ranges was classified as a toxic effect.

3.2.4.12 Assessment of effectiveness of HPK

Serum Aspartate transaminase (AST), Alanine transaminase (ALT), Bilirubin (direct and total), Gamma-glutamyltransferase (GGT), Alkaline phosphatase (ALP), Proteins (total and albumin) were determined for each patient on day 0 (baseline), 14 and 28. The effectiveness of HPK was assessed by comparing the liver function test results. Significant improvement of the baseline results compared to the end of the study period (28 days) was considered effective. The secondary endpoint of HPK studies was assessed using the Karnofsky quality of life assessment scale. The type of liver disease was classified using the R-value formula;

$$R = \frac{ALT}{ALP}$$

Where ALT = Alanine transaminase
ALP = Alkaline phosphatase

CHAPTER 4
RESULTS AND DISCUSSION

The data from the clinical assessment, laboratory work and questionnaire administered to clients on the use of HPK on careful analysis yielded the following results presented here.

4.1 QUALITY ASSESSMENT OF HPK

4.1.1 Organoleptic properties

The organoleptic characteristics of HPK, *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* using the sensory organs are as listed in Table 4.1.

Table 4.1 Organoleptic characteristics of HPK and its plant components

Characteristics	**HPK**	***B. pilosa***	***C. aurantifolia***	***T. orientalis***
Form	Decoction	Powder	Powder	Powder
Taste	Bitter	Bland	Sweet	Bland
Colour	Brown	Green	Green	Brown
Odour	Characteristic	Odourless	Aromatic	Odourless

The bitter taste, brown appearance and characteristic odour are all parameters that can be used to identify and check adulteration of HPK. The use of the sensory organs (eye, skin, tongue, ear and nose) are employed in the organic and qualitative evaluation of herbal products to establish its sensory profile (Shulammithi *et al*., 2016). The taste of HPK and its components are different because each contains different phytoconstituents and at varying concentrations.

Organoleptic characteristics become distinct tools for identification of herbal medicines.

4.1.2 Phytochemical constituents of HPK and its component plants

The results of screening for secondary metabolites in HPK compared to *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* are as presented in Table 4.2. All the test samples contained tannins, flavonoids, triterpenoids, phytosterols, coumarins, reducing sugars and saponins. Only *Trema orientalis* tested negative for alkaloid which is in contrast to the presence of alkaloid in methanolic and aqueous extracts of the stem bark (Trono *et al*., 2016).

The phytoconstituents detected indicated that the product may contain the plants listed on its label. These secondary metabolites can be used as fingerprint and also check adulteration. The presence and concentration of secondary metabolites are

characteristic of the plant parts being used, source of plants, method of preparation and the amount of substance in the formula.

Table 4.2 Phytoconstituents present in HPK and its plant components

Phytoconstituents	**HPK**	***B. pilosa***	***C. aurantifolia***	***T. orientalis***
Alkaloids	+	+	+	-
Reducing sugars	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Phytosterols	+	+	+	+
Coumarins	+	+	+	+
Saponins	+	+	+	+

Key: + means detected, - means not detected

4.1.3 Physicochemical properties of HPK

The physicochemical properties of the two batches of HPK are presented in Table 4.3. The pH of two batches of HPK was found to be 5.27 ± 0.01 and 5.25 ± 0.02 at the temperature of 29.6 ℃ and fall within the acceptable pH range of 4.0 – 10.0 for oral liquids intended for oral administration (Kumar and Yagnesh 2016). The relative density of HPK was determined to be 0.985 ± 0.005. The mean volumes of two batches of HPK were measured to be 315 ± 15 mL and 325 ± 5 mL which were below the 330 mL volume stated on the product label (Table 4.3).

Accurate dosing is key to the therapeutic outcome of every medicinal product (Peck, 2015). The inconsistent and inaccurate volume of the product makes dosage regimen a challenge since one cannot be sure of the right quantities of the herbal products that are dispensed to the patient for the required duration of treatment. The inadequate dosage regimen will ultimately have a negative impact on the effectiveness of the product because each product have therapeutic doses and for a specific duration to elicit a response. Clients were given more bottles of HPK to make up for the less volume to achieve a therapeutic effect.

Table 4.3 Physicochemical properties of HPK

Physicochemical parameters	Batch A	Batch B	Difference
pH	5.27 ± 0.02	5.27 ± 0.01	0.0 ± 0.015
Relative Density	0.985 ± 0.005	0.985 ± 0.005	-0.01 ± 0.007
Weight per ml (mg/mL)	53.28 ± 0.08	53.25 ± 0.05	-0.38 ± 0.44
Volume per bottle (mL)	315.0 ± 15.0	325.0 ± 5.0	-10.00 – 15.81

Batch A and B are two batches of HPK presented as mean ± SD

4.1.4 Thin Layer Chromatographic (TLC)

The chromatographic fingerprint for HPK and the individual plants were obtained by developing silica gel plates using petroleum ether and ethyl acetate (6:4) as the solvent system. Ultraviolet light was used to visualize and detect spots. Details of the chromatogram and R_f values for HPK and its plant components are stated in Table 4.4 and Figure 4.1.

Table 4.4: R_f values of HPK and its plant components

TLC Spot	R_f values			
	HPK	*B. pilosa*	*C. aurantifolia*	*T. orientalis*
1	0.20	0.17	0.17	
2	0.42	0.42	0.42	0.42
3	0.58		0.57	
4			0.67	0.71
5	0.80	0.83	0.83	0.83

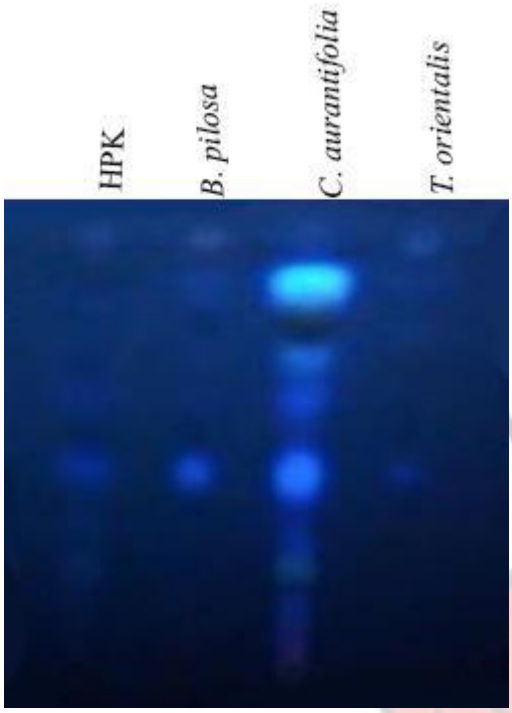


Figure 4.1 Thin layer chromatogram of HPK and its component plants viewed under 365nm UV

Thin layer chromatography (TLC) of HPK, *Bidens pilosa*, *Citrus aurantifolia* and *Trema orientalis* showed 4, 3, 5 and 3 spots respectively. These spots represent compounds with different colours, shapes and R_f values (Table 4.4). The R_f value shows the movement of a substance over that of the solvent.

Two common R_f values of 0.42 and 0.8 were identified for HPK and its component plants and can be used for identification and purity assays to check adulteration (Evans, 2009). From the R_f values, it can be deduced that the plants stated on the manufacturer's primary label may be present in HPK.

4.1.5 HPLC

Validation of the HPLC method

Linearity and Range:

A strong relationship was established between the peak area and the concentrations injected. Correlation coefficients for all the samples were > 0.998. Regression equations are also reported in Table 4.5.

System Suitability:

The HPLC system was found to be efficient and reliable given the theoretical plate numbers and the relative standard deviation (RSD) for the area and retention times obtained Table 4.6.

Precision:

An inter-day precision was performed for the HPLC method developed. Results indicated the system as being robust to random variations in environmental conditions. Percentage RSD for all the samples are reported as Table 4.7.

Accuracy and Recovery:

The accuracy of the method was within the ICH recommendation which permits an RSD < 2% (ICH, 2005). The method was found to be ideal given the results in Table 4.8.

Limits of Detection (LOD) and Limits of Quantitation (LOQ):

LOD and LOQ were determined using the signal to noise ratio. LOD was determined as 3.3 times the signal to noise ratio and LOQ also as 10 times the signal to noise ratio. Concentrations obtained are indicated as Table 4.9.

Table 4.5 Calibration of the standard compound

Standard Compound	**Regression Equation**	**Correlation Coefficient (r^2)**	**Linearity and Range (mg/mL)**
Quercetin	y = 579.72x -1.599	0.9996	0.0625 – 1.0000

Table 4.6 System suitability analysis for the standards used

Standard Compound	**Theoretical Plates (>2000)**	**Injection Precision for Area (% RSD)**	**Injection Precision for Retention Time (% RSD)**
Quercetin	2002.847	0.75	0.27

Table 4.7 Inter-day repeatability test reported as the %RSD of the HPLC method

Standard Compound	**Precision for Area (% RSD)**	**Precision for Retention Time (% RSD)**

Quercetin	0.21	0.01

Table 4.8 Percentage recovery for the standard compounds using the HPLC method

Standard Compound	**Peak Area (n=3)**	**% RSD**	**% Recovery**
Quercetin	173.56 ± 0.44	0.31	100.55 ± 0.19

Table 4.9 Limits of Detection (LOD) and Limit of Quantitation (LOQ) for the HPLC method

Standard Compound	**LOD (mg/mL)**	**LOQ (mg/mL)**
Quercetin	0.013	0.015

Table 4.10 Concentrations of Quercetin in tested sample

	HPK	***Bidens pilosa***	***Citrus aurantifolia***	***Trema orientalis***
Quercetin (mg/mL)	ND	0.014	0.022	ND

Key: ND- not detected

The chromatographic method was developed and validated using quercetin as standard for robustness, precision, accuracy/recovery, LOD, LOQ according to ICH. Quercetin was chosen for the study because it is present in all the plants and it is commercially available (Bairwa *et al*., 2010; Nakajima *et al*., 2014). Quercetin which is a flavonoid and found to be contained in *Bidens pilosa, Citrus aurantifolia* and *Trema orientalis* were confirmed except in *Trema orientalis* (Table 4.10). The chromatogram for quercetin is shown in Appendix 5. Concentration of quercetin found was 0.014mg/mL and 0.022mg/mL for *Bidens pilosa* and *Citrus aurantifolia* respectively (Table 4.10). The quercetin concentrations detected and quantified in *B. pilosa* and *C. aurantifolia* were higher than the validated LOD and LOQ (Table 4.9; Appendix 6-8).

Quercetin was not found in HPK and *T. orientalis* using the retention time of the method developed and validated even though all the component plants contain quercetin. Quercetin is stable under high temperatures above 120^{o}C and should be detectable in a decoction (Vaishnav-hota *et al*., 2012). Quercetin may be present in the product but could not be detected if the concentration is below the lowest detectable

limit (LOD) of the method used. Quercetin, therefore, cannot be used in the quality assessment of HPK.

4.1.6 Spectroscopy Results

4.1.6.1 Ultraviolet Spectroscopy Results

The λmax and UV-Vis spectrum are used to determine the characteristic fingerprint for HPK and its plant components. Details of the UV-Vis spectrum report have been shown in Table 4.11 and Appendix 9.

Table 4.11 Peaks and wavelength for HPK and its plant component

Name	**Bands/Peaks**	
	No. of Peaks	**λmax**
HPK	1	256
Bidens pilosa	1	269
Citrus aurantifolia	1	269
Trema orientalis	1	280

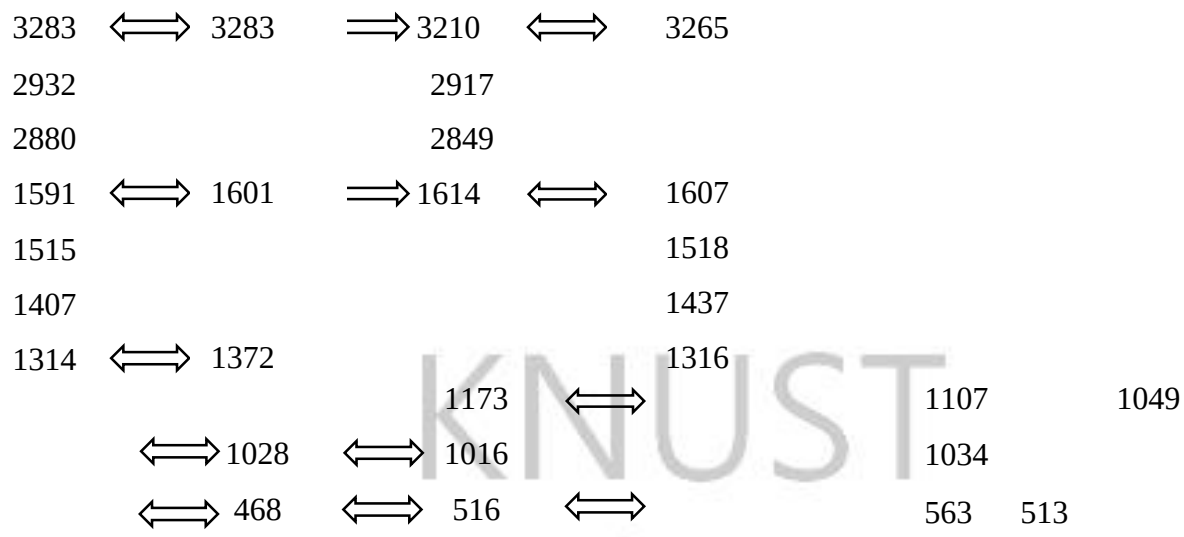
The spectroscopic results of HPK gave specific λmax which can be used to confirm the identity of the product and to also check adulterations. The height, nature and other characteristics of the peaks seen are markers for identification for HPK.

4.1.6.2 Infrared Spectroscopic Characterization Results

Subjecting plants and products through FTIR produces characteristic peaks based on the state/medium of preparation. HPK, *Bidens pilosa*, *Citrus aurantifolia* and *Trema orientalis* all had characteristic FTIR spectra (Appendix 10). Details and possible common wave numbers indicating functional groups between the plants and the product have been shown in Table 4.12.

Table 4.12 IR Spectroscopic Characterization of HPK and its plant components

Wave number (cm^{-1})			
HPK	***B pilosa***	***C. aurantifolia***	***T. orientalis***



The FTIR results show that there are common wavenumbers representing functional groups in the product and its component plants. Six wavenumbers were common between HPK and at least two plants. Three wave number (468-563, 1591-1614 and 3210-3283) were common in all the plants and HPK. Inference can be made that all the plants may be present in HPK. The IR spectra can be used as a fingerprint for HPK for identity and for checking adulteration.

4.1.7 Results of Heavy Metals of HPK

HPK was found to contain nickel (Ni), cadmium (Cd), mercury (Hg), lead (Pd) and arsenic (Ar) in concentrations that have been presented in Table 4.13. The mean concentrations of Ni, Cd, Hg, Pd and Ar are 0.0103, 0.0021, 0.0008, 0.0065 and 0.0005 respectively.

Table 4.13 Heavy metals present in HPK

Metal	Mean ± SEM (mg/kg)	WHO Permissible limit (mg/kg)

Nickel	**0.0103 ± 0.0070**	1.68
Cadmium	**0.0021 ± 0.0019**	0.20
Mercury	**0.0008 ± 0.0008**	1.0
Lead	**0.0065 ± 0.0035**	10.0
Arsenic	**0.0005 ± 0.0132**	10.0

Permissible limits of heavy metals (WHO, 2007a)

Some heavy metals have been associated with oxidative stress liver diseases in animal studies (Arroyo *et al.*, 2012). Nickel, cadmium, mercury, lead and arsenic tested for were all present in HPK (Table 4.13). All the heavy metals tested are toxic to human life and their presence in herbal products should strictly be monitored to ensure public safety. If these metals are present in concentrations higher than the recommended reference range, they negatively impact on health when ingested. For example, cadmium is known to be nephrotoxic, harmful to bones and DNA (Shin *et al.*, 2013). The 5 heavy metals tested were all present in concentrations within the permissible limits of the World Health Organization. These 5 elements are toxic and as such, their concentrations within acceptable limits (WHO, 2007a) implies HPK may be safe in humans.

4.1.8 Microbial load determination of HPK

4.1.7.1 Blood agar for aerobic microorganisms

There was no bacterial growth observed at 37 ^{o}C on the blood agar after 24 hours of incubation of HPK. This means aerobic bacterial are absent in the finished herbal product (HPK).

4.1.7.2 MacConkey agar for aerobic microorganisms

There was no bacterial growth observed at 37 ^{o}C on the MacConkey agar after 24 hours of incubation of HPK.

4.1.7.3 Chocolate agar for anaerobic microorganisms

There was no bacterial growth observed at 37 ^{o}C on the chocolate agar after 24 hours of incubation of HPK.

Microbial presence in liquid herbal products possess health risk to consumers and also increases the rate of product degradation. Observing no bacterial growth using MacConkey and blood agar showed the absence of aerobic bacteria in HPK. Chocolate agar produced no bacterial growth also proving that HPK no anaerobic bacteria. This implies HPK may have been produced under good manufacturing practice (GMP), well preserved with preservatives and stored because these are essential for excluding microbial load in products (Noor *et al.*, 2013). These will ensure high quality product with longer shelf life and safety. Some herbal products have been found to contain pathogenic facultative anaerobic bacteria like; *Salmonella typhi*, *Shigella* species, *Escherichia coli* and *Staphylococcus aureus* (Abba, 2009).

4.2 ANTIOXIDANT RESULTS OF HPK AND ITS PLANT COMPONENTS

The standard drug, Ascorbic acid, HPK, *B. pilosa*, *C. aurantifolia* and *Trema orientalis* all had scavenging effects on DPPH radical with different IC_{50} values stated in Table 4.14 and Figure 4.2.

Citrus aurantifolia leaves had the highest activity with IC_{50} of 21.79 followed by HPK and they were lower than ascorbic acid the standard with IC_{50} of 2.29.

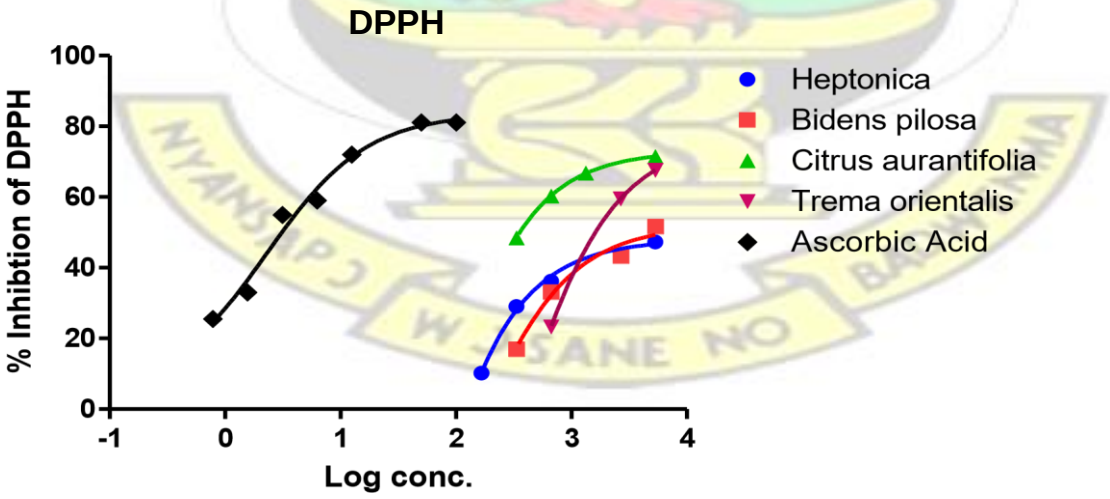


Figure 4.2 Log concentration versus percentage DPPH scavenging of HPK, its component plants and Ascorbic acid.

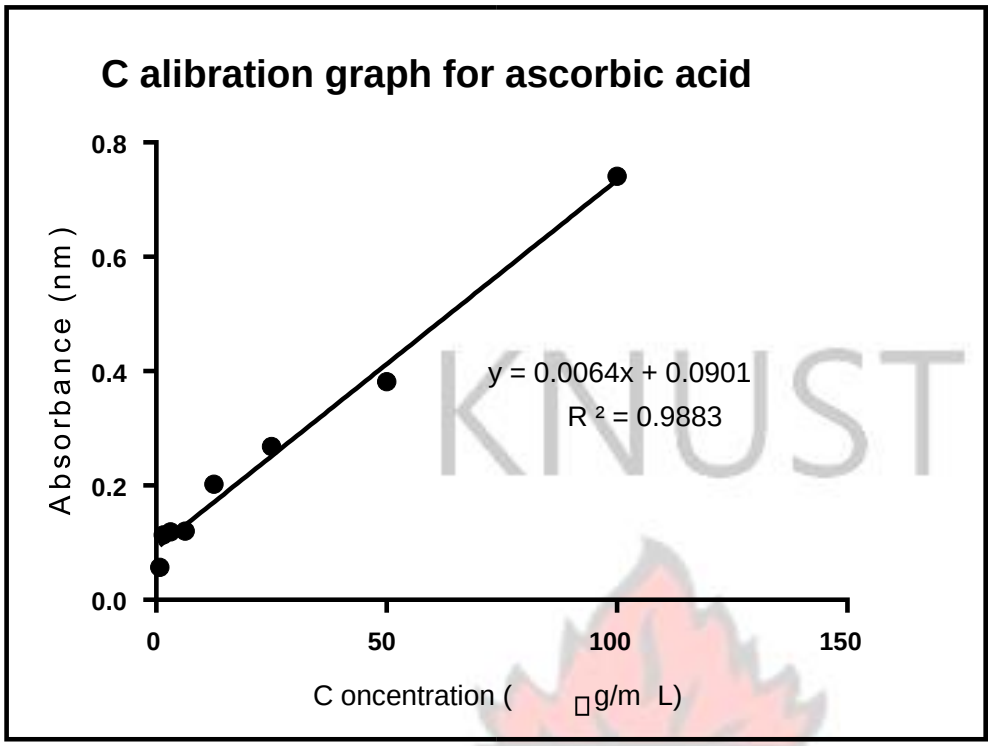


Figure 4.3 Calibration curve for Ascorbic acid

Table 4.14 Antioxidant results of HPK and its plant components

Sample	**DPPH (IC_{50}, µg/mL)**	**TAC (AAE mg/g)**
HPK	23.39	37.99 ± 0.23
Bidens pilosa	113.80	18.52 ± 4.15
Citrus aurantifolia	21.79	841.5 ± 2.18
Trema orientalis	274.5	4.37 ± 0.29
Ascorbic acid	2.29	-

Data entered as mean ± SEM

Oxidative degradation of hepatocytes indirectly manipulates protease/ antiprotease balance (Valaskova *et al*., 2016). HPK had antioxidant activity using DPPH and TAC models but its antioxidant activity was lower compared to ascorbic acid. HPK may

have improved the liver function of participants because of its free scavenging activity. *Bidens pilosa*, *Citrus aurantifolia* and *Trema orientalis* all have antioxidant activities and therefore have the capability to reduce hepatic oxidation (Uddin and Akond, 2016) and oxidative liver disease.

4.3 RESULTS OF CLINICAL EVALUATION OF SAFETY AND EFFECTIVENESS OF HPK

4.3.1 Demographics of Patients

Out of the 50 total participants, 39 (78%) were males while the remaining 11 representing 22% were females. The majority were between the age group of 20-29 with deranged LFT followed by 30-39 years with the least being the 50-59 years group. Details of participants' demographic data are presented in Table 4.15.

Table 4.15 Age and sex distribution of clients

Gender	Age (years)						Total
	< 19	20-29	30-39	40-49	50-59	>60	
Male	4(8%)	11 (22%)	14(28%)	3(6%)	2(4%)	5(10%)	39 (78%)
Female	0(0.0%)	5(10%)	0(0.0%)	3(6%)	1(2%)	2(4%)	11 (22%)
Total	4(8%)	16(32%)	14(28%)	6(12%)	3(6%)	7(14%)	50 (100%)

In both males and females, increasing in age was associated with higher of a deranged liver function up to 40-49 years and then the numbers start to rise again. In all the age groups, males were having higher deranged liver functions compared to women.

4.3.1.1 Marital status and aetiology of liver disease of clients

Married people 27 (54%) had the highest percentage with deranged liver function followed by singles 20 (40%) and the widow group 3 (6%) as presented in Table 4. 16.

It was observed that 15 (30%) of the singles had hepatitis B followed by the married with 13 (26%). Many singles with viral hepatitis if not treated may imply a higher chance of sharing with future partners and couples leading to more married participants with liver disease.

Table 4.16 Marital status of clients and aetiology of liver disease

	Marital status			
Aetiology	**Single**	**Married**	**Widow**	**Total**
Hepatitis B	15	13	1	29
Hepatitis C	0	3	1	4
ALD	1	3	0	4
Unknown	4	6	1	11
HCC	0	2	0	2
Total	20 (40%)	27 (54%)	3 (6%)	50 (100%)

Key: ALD (Alcoholic liver disease), HCC (Hepatocellular carcinoma)

4.3.1.2 Age of clients and aetiology of liver disease

Viral hepatitis was the major cause of deranged liver function representing 64% (32) of the total population. People of the ages between 20–29 years had the highest ratio of 32% of the respondents having deranged liver function followed by the 30-39 years age group as shown in Table 4.17. Old age has been associated with certain liver diseases including Nonalcoholic Steatohepatitis (NASH), Nonalcoholic liver diseases (NAFLD) and hepatocellular carcinoma because, ageing results in accumulation of agents that lead to inflammation (Sheedfar, 2013).

Viral hepatitis was responsible for 64% of all the participants consisting of 29 (58%) hepatitis B and 4 (8%) hepatitis C. The cause of deranged liver function could not be found for 11 (22%) of the participants' while hepatocellular carcinoma (HCC) was responsible for 2 (4%) of the cases (Table 4.17).

Table 4.17 Aetiology of liver disease versus age distribution

	Aetiology of Liver disease					
Age (yrs)	**Hepatitis B**	**Hepatitis C**	**ALD**	**Unknown**	**HCC**	**Total**
10-19	3	0	0	1	0	4

20-29	14	0	0	2	0	16
30-39	8	2	1	2	1	14
40-49	1	0	1	3	1	6
50-59	0	2	0	1	0	3
>60	3	0	2	2	0	7
Total	29 (58%)	4 (8%)	4 (8%)	11 (22%)	2 (4%)	50 (100%)

4.3.1.3 Aetiology of liver disease and Educational Status of Clients

Among the 50 people sampled with deranged liver functions, 24 (48%) had been educated to the tertiary level. Clients with secondary education 10 (20%) and no formal education 9 (18%) had the respective percentages as seen in Table 4.18.

Table 4.18 Aetiology versus Educational status of clients

Aetiology	**Educational status**					**Total**
	Nil	**Primary**	**JHS**	**SHS**	**Tertiary**	
Hepatitis B	5	1	2	9	12	29
Hepatitis C	2	0	0	0	2	4
ALD	0	0	0	1	3	4
Unknown	2	2	1	0	6	11
HCC	0	0	1	0	1	2
Total	9	3	4	10	24	50

A sample of 50 people comprising 39 (78%) males and 11 (28%) females with the ages of 20-29 and 30-39 having the highest and second highest frequencies of deranged liver function respectively (Table 4.15). On marital status, the married had the highest percentage of 54 and the least were widows with 3 (6%) (Table 4.16).

The educational background of participants showed that those with tertiary education was the highest with 24 (48%) deranged liver function (Table 4.18). That was followed

up by 10 (20%) of people with no secondary education. The least was 3 (6%) having primary education. It is that the educated people engage in very risky lifestyle exposing them to viral hepatitis as seen with 24 (48%) of the educated participants or they are ignorant about their health issues even though educated to the tertiary level. Some may also have contracted viral hepatitis through vertical transmission (Kumar, 2009).

4.3.2 Type of liver disease of participants classified by R-values

It was observed that 90% of the participants had R-value less than 2 indicating cholestatic liver disease. The remaining 10% had a combination of cholestatic and hepatocellular (liver cell) (Table 4.19).

Table 4.19 Classification of liver disease based on R-values

Type	R-value	Frequency	Percentage
Hepatocellular	> 5	0	0
Cholestatic	< 2	45	90
Combination	2-5	5	10

Viral hepatitis, hepatocellular carcinoma and alcoholic disease identified among participants were causing more cholestasis than hepatocellular damage. That means participants had challenges with the formation and secretion of bile for metabolic activities. Bile is formed from bilirubin and so inadequate bile formation will lead to accumulation of bilirubin (Sedensky and Dufour, 2011).

4.3.3 Evaluation of symptoms

Participants reported different symptoms associated with their liver diseases. Symptomatic evaluations of participants were done on day 0, 14 and 28. These recorded symptoms were based on the compilation and frequency of all the complaints of the 50 participants.

4.3.3.1 Symptoms on first presentation (Day 0)

Clients who qualified and were enrolled in this study had many presenting complains among which fatigue and abdominal pains were the majority while pedal oedema and ascites had the least as presented in Figure 4.4.

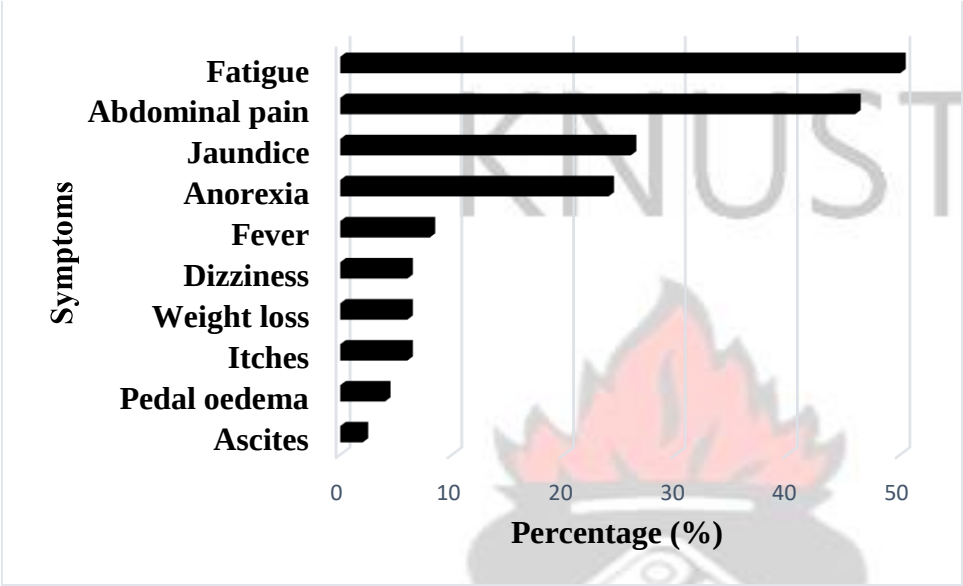


Figure 4.4 Symptoms of liver disease as stated by participants on day 0

From the annual report of the Herbal Medicine Department of the Upper West Regional Hospital, more females (64.03%) report to the Department compared 35.97% males. Ironically, more male participants (78%) were sampled compared to 22% of females with deranged liver function. This was found to be similar to other studies showing gender differences in liver diseases (Mishra *et al*., 2009; Durazzo *et al.*, 2014; Sagnelli *et al*., 2017). The demographic data of participants also show more youth (60%) with deranged liver function.

The high rate of the deranged liver function may be attributed to the risky lifestyle of most males. Multiple sexual partners, alcohol and abuse of other drugs are more common in males than females (Mutinta, 2014). In all, the pattern of the presentation shows more males, more youth and more married people had deranged liver function compared to the other groups.

4.3.3.2 Symptoms after 14 days

It was observed at day 14 that the majority of the clients had a symptomatic improvement in their conditions with jaundice, abdominal pain, fatigue and body

itches having moderately high improvements compared to the baseline complaints. All the symptomatic observations on day 14 review have been presented in Table 4.20.

Table 4.20 Assessed symptoms of liver disease on day 14

Symptoms	No Improvement (%)	Mild Improvement (%)	Moderate Improvement (%)	Maximum Improvement (%)
Jaundice	16.67	8.33	66.67	8.33
Abdominal pain	0	21.05	68.42	10.53
Fatigue	0	21.05	68.42	10.53
Anorexia	0	40	30	30
Ascites	0	100	0	0
Pedal oedema	0	0	100	0
Itches		33.3	66.7	0
Weight loss	0	50	50	0
Fever		75	25	0
Dizziness	0	50	50	0

4.3.3.3 Symptoms of liver disease after 28 days

After 28 days of taking HPK, most clients' experienced maximum improvement compared to their presenting complaints on day 0.

Jaundice and fever had the highest recovery of 75% each while abdominal pain had the least recovery of 45.83%. All the results of symptomatic review after clients taking HPK after 28 days have been presented in Table 4.21.

4.21 Assessed symptoms of liver disease on day 28

Symptoms	No Improvement (%)	Mild Improvement (%)	Moderate Improvement (%)	Maximum Improvement (%)
Jaundice	0	0	25	75
Abdominal pain	0	20.83	33.33	45.83
Fatigue	0	5.26	36.84	57.89
Anorexia	0	0	40	60
Ascites	0	100	0	0
Pedal oedema	0	0	100	0
Itches	0	0	33.33	66.67
Weight loss	0	50	0	50
Fever	0	0	25	75
Dizziness	0	0	50	50

Participants for this study presented with varied symptoms as stated in Figure 4.4. On day 0 fatigue, abdominal pain, jaundice were the highest complains in descending order and ascites was the least complaint. Almost all the symptoms had some level of improvement with pedal oedema having 100% moderate improvement and jaundice with 16.7% having no improvement. The symptoms and the laboratory tests results were satisfactory and all participants continued for the next two weeks of treatment. All parameters had some level of improvement after 28 days.

Fever and jaundice had 75% maximum improvements of symptoms while ascites had the lowest changes at 100% mild improvement. HPK had relieved most of their symptoms of especially fever and jaundice but less effect on ascites. The relief of symptoms follows the pattern of improvement seen from the laboratory results of participants. The relief of symptoms of participants could be suggestive of possible

Table
modes of action of HPK. By clearing jaundice, it may be acting as a choleretic reducing hyperbilirubinemia responsible for the yellow colouration and pruritus. HPK may also possess antipyretic activity because participants said it was relieving their fever. *Bidens pilosa* and *Citrus aurantifolia* have been proven to be antipyretics (Widowati *et al*., 2012; Ezeonwumelu *et al*., 2018).

4.3.4 Safety Profile of HPK

4.3.4.1 Effects of HPK on vital signs of patients

The means of axillary temperature, weight and blood pressure of respondents with standard error mean has been presented in Table 4.22 for day 0, 14 and 28. All the parameters on day 0, 14 and 28 were within the recommended reference range for adults.

Table 4.22 Effect of HPK on participants' vital signs

Parameter	**Day 0**	**Day 14**	**Day 28**	**P value**
Temperature (ºC)	36.10 ± 0.65	36.00 ± 0.50	36.00 ± 0.5	0.22
Weight (kg)	60.50 ± 12.25	60.50 ± 11.25	61.00 ± 11.25	0.98
Systolic BP (mmHg)	110.0 ± 25.50	110.5 ± 20.00	110.0 ± 20.00	0.15
Diastolic BP (mmHg)	70.00 ± 10.00	70.00 ± 0.00	70.00 ± 0.00	0.11

Data is presented as median ± Interquartile range (IQR) (n=50). $p \leq 0.05$ was considered significant when compared to baseline (Kruskal-Wallis test). P values are between day 0 and day 28

Although the participants complained about fever, measures temperatures were normal before the study. There were no significant changes in the assessed vital signs of patients before and after administration of HPK over the 28 day period of study. HPK had no significant effect on the measured temperature, weight and blood pressure of the patients at the Hospital. HPK did not cause any adverse effects on the measured vital signs of participants.

4.3.4.2 Effects of HPK on Renal function

As a measure of safety, the renal functions tests for all 50 participants in this study were done the summary results stated in Table 4.23.

4.23 Effects of HPK on Renal function of participants

RFT	Ref. Range	Day 0	Day 14	Day 28	P value
Na (mmol/L)	135-150	138.8 ± 5.6	137.7 ± 6.3	136.6 ± 8.3	0.1861
K (mmol/L)	3.1-5.3	4.0 ± 0.4	3.9 ± 0.4	4.1 ± 0.6	0.1869
Urea (mmol/L)	1.8-8.6	5.7 ± 2.9	5.4 ± 1.8	5.45 ± 2.1	0.13
Creat. (µmol/L)	44-123	81.3 ± 16.9	79.4 ± 18.5	81.5 ± 21.0	0.3123
BUN (mg/dL)	< 20	15.3 ± 9.9	15.7 ± 6.4	15.2 ± 4.3	0.1315
Cl (µmol/L)	97-107	105.5 ± 5.8	104.0 ± 3.2	106.1 ± 3.9	**0.012**

Data is presented as median ± IQR (n=50). Key: Na (Sodium), K (Potassium), Cl (Chloride), Creat. (Creatinine). P≤ 0.05 was considered significant when compared to baseline (Kruskal-Wallis test). P= P value for day 0 and 28

Urea, creatinine, blood urea nitrogen (BUN) and electrolytes (sodium, potassium and chloride) were kidney function test conducted on all participants on day 0, 14 and 28. The presence of abnormally deranged biochemistry levels indicates renal injury (Uchino *et al*., 2012). This was done to assess the safety of HPK in humans. The mean renal function test results show that all the parameters were within the reference range on day 0 (baseline).

The results as shown in Table 4.21 indicate that there were no significant changes in the baseline measure (day 0) of kidney function compared to the end of the study period (day 28). The effect of this chloride change was not clinically important because it was within the expected reference range for humans. This observation is in line with other works on the plants used in the preparation of HPK. It has been established in animal studies that *Bidens pilosa* improves the structure and function of the kidney by enhancing regeneration of renal tubule (Pegoraro *et al.*, 2018). *B. pilosa* effectively

Table
protected the kidney of animals exposed to carcinogenic substances (El-Kabany and Ibrahim, 2013). *Citrus aurantifolia* have been proven to be nephroprotective in animal studies by preventing alteration of kidneys under duress (Aprioku and Obianime, 2014; Dosoky and Setzer, 2018). *Trema orientalis* in animal studies did not provide any evidence of renal injury in animal toxicity studies and so it is expected not to cause renal damage in humans (Hemalatha *et al*., 2019).

4.3.4.3 Effects of HPK on Full Blood Count (FBC)

The blood is the medium through which most substances are transported within the body. The mean haematological test results show that all the parameters were within the normal reference range for African adult on day 0 (baseline) (Teferi *et al*., 2005). Administration of HPK led to significant increases in haemoglobin, MCV, MCH, platelet count and lymphocyte percentage at days 14 and 28 when compared to baseline values. HPK may be a haematinic in addition to its intended actions on the liver. The liver is responsible for secreting 10% of erythropoietin in adults which is for erythropoiesis (Lombardero, 2011). Any product capable of improving renal function may also enhance erythropoiesis in adults (Zivot *et al*., 2018). Only the monocytes showed significant reductions at days 14 and 28 when compared to baseline values. Haematological analysis for participants during the 28-day study has been presented in Table 4.24.

After taking HPK for 28 days, haematological assessment of patients showed that it caused no observable toxicity in humans. There was no significant change in platelets and its differentials but there were significant changes in haemoglobin, MCV and MCH. It has been established that *Bidens pilosa* exhibited an antioxidant effect on human red blood cells by reducing protein/lipid peroxidation and oxidative hemolysis (Yang *et al*., 2006). By improving haemoglobin concentration and MCV, HPK can be explored for possible haematinic activity. There was a significant change in monocytes represented as Mid (Table 4.22). All the haematological changes after 28 days were within the recommended reference ranges for humans and that proves its safety on haematological parameters within the 28-day study period.

4.24 Assessment of HPK on FBC parameters

Haematology	Ref Range	Day 0	Day 14	Day 28	P value
WBC	2.5-8.5	5.2 ± 0.2	5.2 ± 0.2	5.1 ± 0.2	0.2697
Lymph #	0.8-4	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	0.2655
Mid #	0.1-1.5	0.5 ± 0.0	0.5 ± 0.0	0.45 ± 0.0	**0.046**
Gran #	2.0-7.0	2.8 ± 0.2	2.7 ± 0.1	2.8 ± 0.2	0.4248
Lymph %	20-50	39.6 ± 1.5	42.6 ± 1.4	41.3 ± 1.5	0.1371
Mid %	3.0-15	9.6 ± 0.3	9.0 ± 0.3	8.8 ± 0.3	**0.031**
Gran %	40-70	49.4 ± 1.3	48.8 ± 1.2	50.3 ± 1.5	0.2735
Hb	12.0-18	13.3 ± 0.3	13.7 ± 0.3	13.7 ± 0.3	**0.0269**
RBC	4.0-5.5	4.7 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	0.2845
MCV	76-96	85.9 ± 1.2	87.4 ± 1.0	87.6 ± 0.9	**0.0213**
MCH	27-34	29.4 ± 0.6	30.9 ± 0.5	30.8 ± 0.4	**< 0.0001**
MCHC	32-36	33.4 ± 0.3	33.9 ± 0.2	34.0 ± 0.4	0.2495
RDW-CV	11.0-16	14.6 ± 0.3	14.6 ± 0.3	14.8 ± 0.3	0.1565
RDW-SD	35-56	49.6 ± 0.9	48.7 ± 0.8	49.3 ± 0.8	0.4059
PLT	100-400	224.6 ± 11.5	237.6 ± 12.3	231.3 ± 12.2	0.2135
MPV	6.5-12	8.5 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	0.3006
PDW	9-17	15.1 ± 0.2	15.0 ± 0.3	15.18 ± 0.2	0.3661
PCT	0.11-0.28	0.3 ± 0.1	0.2 ± 0.0	0.20 ± 0.0	0.2061

Key: Data is presented as mean ± SEM (n=50) (#, number; %, percentage). $P \leq 0.05$ was considered significant when compared to baseline (one-way ANOVA followed by a Dunnett's multiple comparison test). P= P value for day 0 and 28. **Mid** cells: monocytes, **Granulocytes**: Basophils, Eosinophil's and Neutrophils

Table

4.3.4.4 Effects of HPK on Urinalysis

Oral administration of HPK had the following outcome on the urine parameters after assessing clients' urine using urine dip stick and urine microscopy with the results shown in Table 4.25 and Table 4.26.

Table

4.25 Effects of HPK on Quantitative Urinalysis Results

Urine	**Day 0**	**Day 14**	**Day 28**	**P value**
pH	6.50 ± 0.12	6.48 ± 0.13	6.82 ± 0.14	**0.0243**
Specific gravity	1.02 ± < 0.001	1.02 ± < 0.001	1.02 ± < 0.001	0.2071
Pus cell	2.96 ± 0.45	2.84 ± 0.53	1.86 ± 0.18	**0.0109**
RBC	0.16 ± 0.09	0.12 ± 0.08	0.1 ± 0.08	0.3221
Epithelial cells	2.76 ± 0.35	2.76 ± 0.28	2.74 ± 0.24	0.4818

Data is presented as mean ± SEM (n=50). P≤ 0.05 was considered significant when compared to baseline (one-way ANOVA followed by a Dunnett's multiple comparison test). P= P value for day 0 and 28

The urine is a good diagnostic tool to detect many disease states within the body (WHO, 2005). Assessment of urinalysis for the 50 participants for the 28 day study period revealed a significant rise in urine pH (6.5 ± 0.12 to 6.82 ± 0.14). Average pus cell concentrations reduced from 2.96 ± 0.45 to 1.86 ± 0.18 (Table 4.23). HPK was able to improve the urine appearance from cloudy to clear state. All 4% of the participants that had blood in urine and urine bilirubin got cleared at the 28 day period. Calcium oxalate crystals in the urine as well as the 2% of urine leucocytes, were all reduced within the 28 day period. HPK may have facilitated the excretion of the calcium oxalate crystals in urine by making it more soluble. This observation is similar to another human study using only *Citrus aurantifolia* fruit juice decreasing calcium in clients with renal stones (Sja'bani *et al*., 2007).

Table

4.26 Effects of HPK on Qualitative Urinalysis Results

Parameter	**Details**	**Day 0**	**Day 14**	**Day 28**
		%	**%**	**%**
Urine colour	Straw	98	84	90
	Amber	2	16	10
Urine appearance	Clear	68	68	78
	Hazy	26	18	16
	Cloudy	6	14	6
Urine proteins	Negative	88	84	90
	Trace	8	14	6
	Positive	4	2	4
Urine glucose	Negative	100	100	100
Urine Ketones	Negative	100	100	100
Urine Blood	Negative	96	98	100
	Trace	2	2	0
	Positive	2	0	0
Urine Bilirubin	Negative	96	100	100
	Positive	4	0	0
Leucocytes	Negative	78	84	96
	Trace	10	8	4
	Positive	12	8	0
Urobilinogen	Normal	92	98	98
	Increased	8	2	2
Cast	Not seen	100	100	96

	Seen	0	0	4
Crystals	Not seen	96	98	98
	Calcium oxalate	4	2	2

4.3.5 Assessment of Effectiveness of HPK

Each client was enrolled on the study because they had a deranged liver function. A total of fifty (50) patients were assessed for AST, ALT, GGT, Bilirubin (total and direct), proteins (total and albumin) and alkaline phosphatase (ALP) on day 0, 14 and 28 (Table 4.27). Figure 4.5 showed the clearance of jaundice in the eyes of a participant within a period of two weeks. Details of the effects of HPK are shown in Figure 4.5.

At day 0, AST, ALT and GGT (inflammatory enzymes) were all increased denoting hepatic injury (Table 4.27). Bilirubin (direct and indirect) were all elevated above the reference range for adults implying cholestatic liver disease and decreased excretory function. Liver function test is a measure to assess the liver and it's anabolic, catabolic, excretory and other functions. AST and ALT are enzymes mostly found in the cytoplasm of hepatocytes but the former is also present in the brain, heart and skeletal muscles (Al-Saeed and Alawami, 2013; Alam and Farooqui, 2014).

With respect to liver diseases, the need to administer therapeutic agents is to halt the progression of the disease, stimulate and improve hepatocyte integrity and regeneration capacity, liver function and to reduce complications. It is also to treat the underlying cause of liver disease and improve the quality of life of clients (Luedde *et al*., 2015). There were significant improvement in the AST, ALT, GGT, bilirubin (total and direct) and alkaline phosphatase after 28 days compared to the baseline results. HPK may be having activity against viral hepatitis which was a major cause of liver diseases of participants. The biochemical improvement was supported by the clinical improvement seen on the participants. As can be seen in Figure 4.5, a participant with jaundiced eyes got cleared in two weeks after starting treatment with HPK.

HPK may be responsible for the anti-inflammatory effect on the liver by reducing inflammatory enzymes AST and ALT. *B pilosa* and *C. aurantifolia* which are claimed components of HPK have been established to possess anti-inflammatory activity on the liver by reversing hepatic fibrosis and blocking genetic damage to hepatocytes (Ashafa *et al*., 2009; Kviecinski *et al.*, 2011; Rawson *et al*., 2014). HPK by reducing

Table
direct and total bilirubin may possess choleretic activity by causing more bile secretion or improving bile flow through the hepatic ducts. *B. pilosa* protected

the liver against cholestatic liver disease in animal studies (Suzigan *et al.*, 2009). Total proteins and albumin showed no significant differences between the baseline values and after 28 days. HPK was not able to improve the production of albumin and other plasma proteins thereby not improving total proteins. It maintained the normal levels of protein production with no adverse effects.

Table 4.27 HPK on LFT of general population

LFT Parameters	Ref.	Day 1	Day 14	Day 28	P value
AST (U/L)	< 37	40.5 ± 46.5	33.1 ± 26.0	27.8 ± 19.4	**0.0001**
ALT (U/L)	< 42	44.0 ± 45.6	27.1 ± 29.4	25.8 ± 65	**< 0.0001**
GGT (U/L)	7.0 - 50	58.6 ± 119.5	64.1 ± 85.27	40.1 ± 101.6	**0.0017**
Total Bilirubin (umol/L)	< 17	25.9 ± 38.2	18.2 ± 11.8	15.2 ± 17.6	**0.0146**
Direct Bilirubin (umol/L)	< 3.5	7.6 ± 13.5	5.1 ± 4.2	3.8 ± 2.9	< **0.0001**
Total Proteins (g/dl)	66-87	70.8 ± 10.5	75.0 ± 9.4	74.8 ± 9.3	0.0929
Albumin (g/dl)	35-55	42.0 ± 7.3	42.0 ± 5.5	42.0 ± 6.0	0.4482
ALP (U/L)	< 270	176.9 ± 175.6	171.3 ± 153.6	171.4 ± 153.5	**0.0295**

Data is presented as median considered significant (IQR) (n = 50). $p \leq 0.05$ was (Wilcoxon matched pairs test).

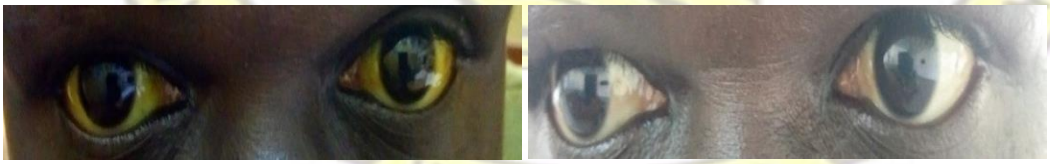


Figure 4.5 Participant with jaundiced eyes (left) and after 2 weeks of treatment (right)

4.3.5.1 Effect of HPK on Cholestatic liver disease

Table 4.28 Effect of HPK on Cholestatic liver disease

LFT Parameters	Reference	Day 0	Day 28	P value
AST (U/L)	< 37	41.00 ± 47.15	28.00 ± 19.25	**0.0006**
ALT (U/L)	< 42	43.00 ± 47.10	25.00 ± 27.90	**0.0006**
GGT (U/L)	7.0 – 50	47.30 ± 106.30	37.00 ± 51.05	**0.0094**
T. Bil. (umol/L)	< 17	27.80 ± 48.30	16.40 ± 19.90	**0.0111**
D. Bil. (umol/L)	< 3.5	8.00 ± 12.70	3.70 ± 3.00	**<0.0001**
T. Proteins (g/dl)	66-87	70.80 ± 12.00	75.00 ± 9.50	0.1565
Albumin (g/dl)	35-55	42.00 ± 7.00	41.00 ± 5.40	0.3936
Alkaline Phos. (U/L)	< 270	192.40 ± 161.90	176.20 ± 142.70	**0.0334**

Data is presented as median ± Interquartile range (IQR) (n=45). P≤ 0.05 was considered significant when compared to baseline (Wilcoxon matched pairs test). T. Bil.: Total bilirubin, D. Bil.: Direct Bilirubin

Table 4.29 Effect of HPK on Hepatocellular-Cholestatic liver disease

LFT Parameters	Reference	Day 0	Day 28	P value
AST (U/L)	< 37	49.02 ± 13.80	30.98 ± 8.75	**0.0318**
ALT (U/L)	< 42	46.36 ± 5.81	31.56 ± 6.50	**0.0204**
GGT (U/L)	7.0 – 50	213.30 ± 42.84	161.70 ± 49.99	**0.0475**
T. Bil. (umol/L)	< 17	11.04 ± 3.80	11.02 ± 0.56	0.9959
D.Bil. (umol/L)	< 3.5	6.56 ± 2.29	4.82 ± 0.94	0.5636
T. Proteins (g/dl)	66-87	65.68 ± 5.35	74.00 ± 2.92	0.2716
Albumin (g/dl)	35-55	43.24 ± 2.37	43.40 ± 1.29	0.9595
Alkaline Phos (U/L)	< 270	14.08 ± 1.40	13.22 ± 1.30	0.7062

Data is presented as mean ± SEM (n=5). P≤ 0.05 was considered significant when compared to baseline (Paired t-test). T. Bil.: Total bilirubin, D. Bil.: Direct Bilirubin

Cholestasis is a disorder characterized by decrease bile flow resulting from impaired secretion by hepatocytes or to obstruction of bile flow through the intra and extrahepatic bile ducts. Cholestatic injury is defined as a disproportionate elevation of alkaline phosphatase level as compared with AST and ALT levels. Mixed pattern of injury is defined as the elevation of both alkaline phosphatase and AST/ALT levels (Kwo *et al*., 2016). These conditions may have accounted for jaundice seen clinically on patients. Bilirubin is eliminated from the body by secretion into bile and then into

stools. (Jones *et al*., 2018). Hyperbilirubin can be manifested as jaundice and can also cause pruritus as were reported by patients. There was no participant in the hepatocellular damaged group but 10% were in the mixed section. From the Rvalues, the results show the cholestasis is more from hepatic duct obstruction and less from the hepatocytes damage. HPK had more activity in cholestatic liver disease compared to the mixed cause as seen in Table 4.28 and Table 4.29. It is possible HPK may have improved the hepatocytes function, increased bile formation as well as improving bile flow through the hepatic ducts.

4.3.5.2 Effects of HPK on LFT of Males

The general data was further analyzed to ascertain if there was any trend with respect to response to HPK based on the sex of patients. The analyzed results of 39 males who participated in the study are as stated in Table 4.29. The liver function test results of the participants had elevated AST, ALT, GGT and Bilirubin (total and direct) levels were elevated above normal reference levels. It was observed that the males had more significantly improved liver function compared to the women. Within the male population that was 78% of the sample, there was a significant improvement in the levels of AST, ALT, direct bilirubin and GGT with p values 0.011, 0.028, 0.0066 and 0.0001 respectively (Table 4.30). All the elevated parameters reduced to the normal reference ranges by the 28-day period.

Table 4.30 Effects of HPK on the LFT of males

LFT Parameters	P value	Day 0 Day 28
AST (U/L)	**0.011**	

	41.00 ± 51.4	28.70 ± 23	
ALT (U/L)	51.15 ± 122.7	38.55 ± 73.4	**0.0028**
GGT (U/L)	55.00 ± 119.9	39.20 ± 101.4	**0.0066**
Total Bilirubin (umol/L)	27.80 ± 38.5	18.50 ± 22.4	0.0516
Direct Bilirubin (umol/L)	7.80 ± 13.6	3.70 ± 3.0	**0.0001**
Total Proteins (g/dl)	70.80 ± 11.5	74.00 ± 8.0	0.3319
Albumin (g/dl)	44.00 ± 19.5	41.00 ± 11	0.2294
Alkaline Phosphatase (U/L)	204.4 ± 187.7	176.2 ± 158.5	0.0752

Data is presented as median ± Interquartile range (IQR) (n=39) . P≤ 0.05 was considered significant when compared to baseline (Wilcoxon matched pairs test).

4.3.5.3 Effects of HPK on LFT of Females

The effects of HPK on the female participants only in the 28-day study have been shown in Table 4.31.

Table 4.31 Effects of HPK on the LFT of females

LFT Parameters	**Day 0**	**Day 28**	**P value**
AST (U/L)	40.00 ± 18.7	25.00 ± 18.2	**0.0163**
ALT (U/L)	41.60 ± 39.7	20.00 ± 8.2	0.0998
GGT (U/L)	87.80 ± 119	43.00 ± 109.9	0.0830
Total Bilirubin (umol/L)	18.40 ± 19.1	11.20 ± 8.1	0.0830
Direct Bilirubin (umol/L)	7.30 ± 7.1	3.90 ± 1.5	0.2130
Total Proteins (g/dl)	66.00 ± 14.4	77.00 ± 25	0.1547
Albumin (g/dl)	40.00 ± 9.2	42.00 ± 8	0.4765
Alkaline Phosphatase (U/L)	128.6 ± 174.7	101.0 ± 166.3	0.1230

Data is presented as median ± Interquartile range (IQR) (n=11). P≤ 0.05 was considered significant when compared to baseline (Wilcoxon matched pairs test).

The 22% female population only showed a significant difference in AST with a pvalue of 0.0163 comparing baseline to 28 day period (Table 4.31). The rest of the parameters had no significant difference between the baseline and the end of the 28day study. This may have happened because, some animal and human studies have shown more

females metabolize drugs faster than males (Sakuma *et al.*, 2009; Yang *et al*., 2012). It is possible the females were breaking down HPK and eliminating them faster thereby having lower activity compared to the males.

4.3.5.4 Effects of HPK on participants with viral hepatitis

The data was further analyzed based on the aetiology of the deranged liver function and viral hepatitis (hepatitis B and C) contributed to 66% of the causes of liver disease (Table 4.15 and Table 4.16). It was imperative to find out whether the product will have any effect on that aetiology. The activity of HPK on the participants with viral hepatitis was analyzed and presented below in Table 4.32.

Table 4.32 Effects of HPK on the LFT of participants with viral hepatitis

LFT Parameters	Day 0	Day 28	P value
AST (U/L)	38.9 ± 42.40	29.3 ± 13.8	**0.0003**
ALT (U/L)	47.0 ± 44.75	33.8 ± 31.5	**0.0021**
GGT (U/L)	55.0 ± 121.50	39.2 ± 101.45	**0.0255**
Total Bilirubin (umol/L)	27.9 ± 39.45	18.9 ± 21.6	**0.0366**
Direct Bilirubin (umol/L)	8.0 ± 15.65	3.7 ± 3.0	**0.0009**
Total Proteins (g/dl)	70.8 ± 13.25	74 ± 11.0	0.2878
Albumin (g/dl)	42.96 ± 8.20	42 ± 5.5	0.3348
Alkaline Phosphatase (U/L)	204.4 ± 167.8	176.2 ± 133.3	**0.0179**

Data is presented as median ± Interquartile range (IQR) (n=33) . P≤ 0.05 was considered significant when compared to baseline (Wilcoxon matched pairs test).

There were very significant improvement of the liver function with respect to AST, ALT, GGT, total and direct bilirubin comparing the end of 28 days to the baseline with the following p values 0.0003, 0.0021, 0.0255, 0.0366 and 0.0009 respectively. It is a possibility HPK may possess antiviral properties by the extent it significantly improved participants liver function that was deranged by viral hepatitis. HPK may have achieved the observed improved liver effectiveness by being an adaptogen stimulating and promoting hepatocytes regeneration and improving hepatocyte

integrity. The normal functioning liver can now conjugate bilirubin and transport it actively into bile as a choleretic (improving bile flow) and excretion thereby reducing plasma bilirubin concentration (Valaskova *et al*., 2016).

4.3.5.5 Effects of HPK on participants with unknown aetiology of liver disease

Data from eleven participants (22%) whose cause of liver disease could not be identified were also analyzed to evaluate the effect of HPK on their deranged liver function.

There were no significant differences between the baseline and the end of the 28-day study period. Details of the results have been presented in Table 4.33.

Table 4.33 Effects of HPK on the LFT of participants with unknown aetiology

LFT Parameters	Ref. Day 0	Day 28	P value range	
AST (U/L)	< 37	25.5 ± 27	22 ± 10.6	0.328
ALT (U/L)	< 42	22.6 ± 29.4	19.7 ± 5.0	0.067
GGT (U/L)	7.0 - 50	24 ± 114.3	23.4 ±24.0	0.102
Total Bilirubin (umol/L)	< 17	21.9 ± 19.7	11.9 ± 9.8	0.142
Direct Bilirubin (umol/L)	< 3.5	7.8 ± 7.1	3.7 ± 3.2	0.075
Total Proteins (g/dl)	66-87	66 ± 18.4	75 ± 9	0.142
Albumin (g/dl)	35-55	44.8 ± 7	43 ± 5	0.534
ALP (U/L)	< 270	128.6 ± 121.3	135 ± 186.9	0.824

Data is presented as median ± Interquartile range (IQR) (n=11) . P≤ 0.05 was considered significant when compared to baseline (Wilcoxon matched pairs test).

To this group of participants with unknown aetiology, HPK had no significant difference on the LFT on day 0 and day 28. It is possible HPK could not manage what was the actual cause of the derangement of their liver function.

4.3.5.6 Effects of HPK on LFT of alcohol-induced liver disease

The results of the liver function tests of participants who had deranged functions due to alcohol were analyzed and there were no significant differences between the results of the baseline and the 28-day period. Details of the results are shown in Table 4.34.

The proportion of participants who had alcoholic liver disease were 4 representing 8% of the population and there was no significant difference between the baseline and the end of the 28-day study period. It is also possible that, by its mechanism of action, HPK does not have much influence on the liver biochemistry of alcohol induced liver diseases.

Table 4.34 Effects of HPK on the LFT of alcohol-induced liver disease

LFT Parameters	**Ref. range**	**Day 0**	**Day 28**	**P value**
AST (U/L)	< 37	87.9 ± 20.7	49.0 ± 16.2	0.341
ALT (U/L)	< 42	87.1 ± 17.2	44.7 ± 8.8	0.096
GGT (U/L)	7.0 - 50	250.6 ± 155.3	107.5 ± 59.1	0.237
Total Bilirubin (umol/L)	< 17	18.4 ± 11.3	10.7 ± 2.1	0.476
Direct Bilirubin (umol/L)	< 3.5	6.5 ± 3.3	5.7 ± 0.9	0.765
Total Proteins (g/dl)	66-87	71.5 ± 5.9	70.5 ± 1.8	0.860
Albumin (g/dl)	35-55	37.25 ± 2.1	39.00 ± 0.7	0.457
ALP (U/L)	< 270	145.2 ± 40.2	78.00 ± 26.1	0.135

Data is mean ± SEM (n=4) P≤ 0.05 was considered significant when compared to baseline (paired t-test).

4.3.6 Reported side effects of HPK

Out of the fifty participants who were enrolled on this study, two males representing 4% reported side effects after taking HPK; the first client experienced mild diarrhoea for the first two days but it resolved when treatment continued without the administration of any other agent. The second client reported itching and macular rashes on the left elbow which stopped after 24 hours of taking HPK without any other intervention. Upon assessment, those effects could not be directly associated with the product under study since the complaints were self-limiting even after continuation of the HPK. They were all minor cases that were self-limiting and terminated without any

intervention. This could possibly be due to idiosyncratic reactions to the product or possible interaction with other substances they may have taken (Chalasani *et al.*, 2014).

4.4 QUALITY OF LIFE ASSESSMENT USING THE KARNOFSKY SCALE

All clients who partook in the clinical study were assessed for their quality of life before they started taking HPK for their various liver disease and it was repeated at the end of the study. Details of the results have been presented in Table 4.35.

Table 4.35 Results of Karnofsky assessment between baseline and 28 days

	Day 0	**Day 28**	**P value**
Karnofsky scale	80.00 ± 0.40	91.40 ± 1.21	< 0.0001

On day 0, 80 ± 0.4 was the mean which improved significantly on day 28 to 91.40 ± 1.21 with a p-value of less than 0.0001. Some participants stated that they had better stamina, improved appetite and quality sleep apart from the improvement of baseline complaints.

4.5 REFERRALS

By the 14th day of the study, all clients were responding favourably to HPK and two cases of mild side effects were reported but they all resolved on the continuation of treatment without intervention. Furthermore, no client was referred out for any other intervention outside the study. Three clients (6%) who were not responding to treatment on day 28 were referred to the allopathic section of the Upper West Regional Hospital for further management. First was a case of hepatocellular carcinoma and the other two were on account of non-resolving ascites secondary to viral hepatitis. The treatment outcomes of participants have been summarized in Table 4.36.

Table 4.36 Outcome of treatment after 28 days

Clinical condition	**Medical Decision**		**Total**
	Not referred	**Referred**	
Improved	47 (94%)	0 (0%)	47 (94%)
No Change	0 (0%)	3 (6%)	3 (6%)

Total	47 (94%)	3 (6%)	50 (100%)

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

HPK, a finished polyherbal product contains reducing sugars, alkaloids, tannins, saponins, coumarins, flavonoids and phytosterols. It also contains Lead, Mercury, Nickel, Cadmium and Arsenic which were within the normal permissible range of WHO. The 2 batches of HPK used contains no bacteria. Fingerprints for HPK have been set using UV-Vis spectrum, FTIR, TLC and HPLC. Chromatographic parameters have been established for HPK and HPLC retention times as markers for quality assessment.

Safety assessment of HPK showed no observable toxicity within the 28 day period on urinalysis, renal function and haematological parameters.

On effectiveness, HPK improved AST and ALT to normal reference ranges whiles GGT, bilirubin (total and direct) which were all deranged were on day 0 before the administration of HPK. AST, ALT, GGT, Total Bilirubin, Direct Bilirubin and Alkaline Phosphatase all had significant improvement compared to the baseline. Deranged liver function caused by alcohol, hepatocellular carcinoma and unknown causes did not respond significantly to HPK comparing the baseline to the 28th day results. On aetiology, the group with viral hepatitis showed the most improvement of their liver function.

The group of participants with cholestatic liver disease had improvement of the liver function by reducing significantly AST, ALT, GGT, total bilirubin and alkaline phosphatase. HPK had no significant changes in the group with combined cholestatic and hepatocellular type of liver disease. The 50 participants had improved liver

function at the 28-day period compared to the baseline. There were significant changes in AST, ALT, total bilirubin and GGT to normalcy.

Male participants had better improvement of the deranged liver function through significant improvement in AST, ALT, GGT and Direct Bilirubin levels when compared with the baseline. The female group only had significant improvement in AST levels. There were symptomatic relief and the quality of life also improved comparing the day 0 to the 28-day period. HPK has been scientifically proven that it is an effective hepatorestorative in reversing deranged liver functions in humans with no observable toxicity and therefore safe for human clinical use.

5.2 RECOMMENDATIONS

Future studies should be considered in these areas:

1. Further work should be done on the chronic toxicity of HPK since most liver diseases develop into the chronic stage and a randomized control clinical trial in a large population.
2. Activity on viral load (hepatitis) should be carried out since participants in that group had significant improvement in their liver functions to ascertain if HPK has antiviral activity.
3. This study has shown that there is a hepatorestorative effect but HPK needs to be produced in a better dosage form to ensure accurate dose can be given to clients. The dosage form of the product should be improved to tablets or capsules to ensure product stability on storage and accurate dose delivery to patients to enhance the therapeutic effect.
4. The mechanism of action of HPK should be carried to provide details of how the product works.

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APPENDICES

APPENDIX 1: QUESTIONNAIRE FOR PARTICIPANTS

Participant Code: ……. HPK Batch: …..

A. PARTICIPANT DETAILS

Participant Initials …….. Educational status ……..

Date of birth: (D/M/Y) _ __ /_ _/_____ Age: ____ years

Gender: Male _____ Female _____ Weight: (Kg) _____

Temperature: _____^{o}C Blood Pressure: _____ mmHg

Residential Address/Nearest Landmark …….

Phone number: …… Preferred time to call……..

Contact person for patient follow up

Name: …… Phone number: ……

B. SYMPTOMS AND SIGNS AT PRESENTATION

……
……

Other medical conditions other than liver disease with the last two weeks

……
……

Other medical conditions

Condition	Current	Past

C. OTHER DRUGS PRESCRIBED

Drug name	Indication	Dose	Date started	Completion date

D. LABORATORY RESULTS

	Day 0	Day 14	Day 28
Liver Function Test			
Renal Function Test			
Full Blood Count			
Urine R/E			

APPENDIX 2: FOLLOW UP FORM FOR PARTICIPANTS PATIENT ASSESSMENT DIARY FOR THE TREATMENT PERIOD

A. Type of Follow-up

	MARK	DATE (dd/mm/year)
Home Visit		
Phone Calls		
Hospital Visit		
Lost to Follow-up		

B. Any other medicine taken during the study period

Medicine	Indication	Dose & Duration	Starting date	Completion date

Patient code:

A. Drug Compliance

Days/Date	Time for morning dose	Taken after meals?	Time for afternoon dose	Taken after meals?	Time for evening dose	Taken after meals?	Any adverse effects?
e.g. 11/8/18	e.g. 7am	e.g. Yes/No	e.g. 3pm	e.g. Yes/No	e.g. 11pm	e.g. Yes/No	Unusual effects
1							
2							
3							
7							

14							
28							

B. Post treatment outcome study

Treatment adherence: Compliance …… No compliance……. Reasons for non-compliance

_____
_____
_____

C. CLINICAL EVALUATION REPORT

Improved: _____ Deteriorated: _____ No change: _____

Referral: YES: _____ NO: _____

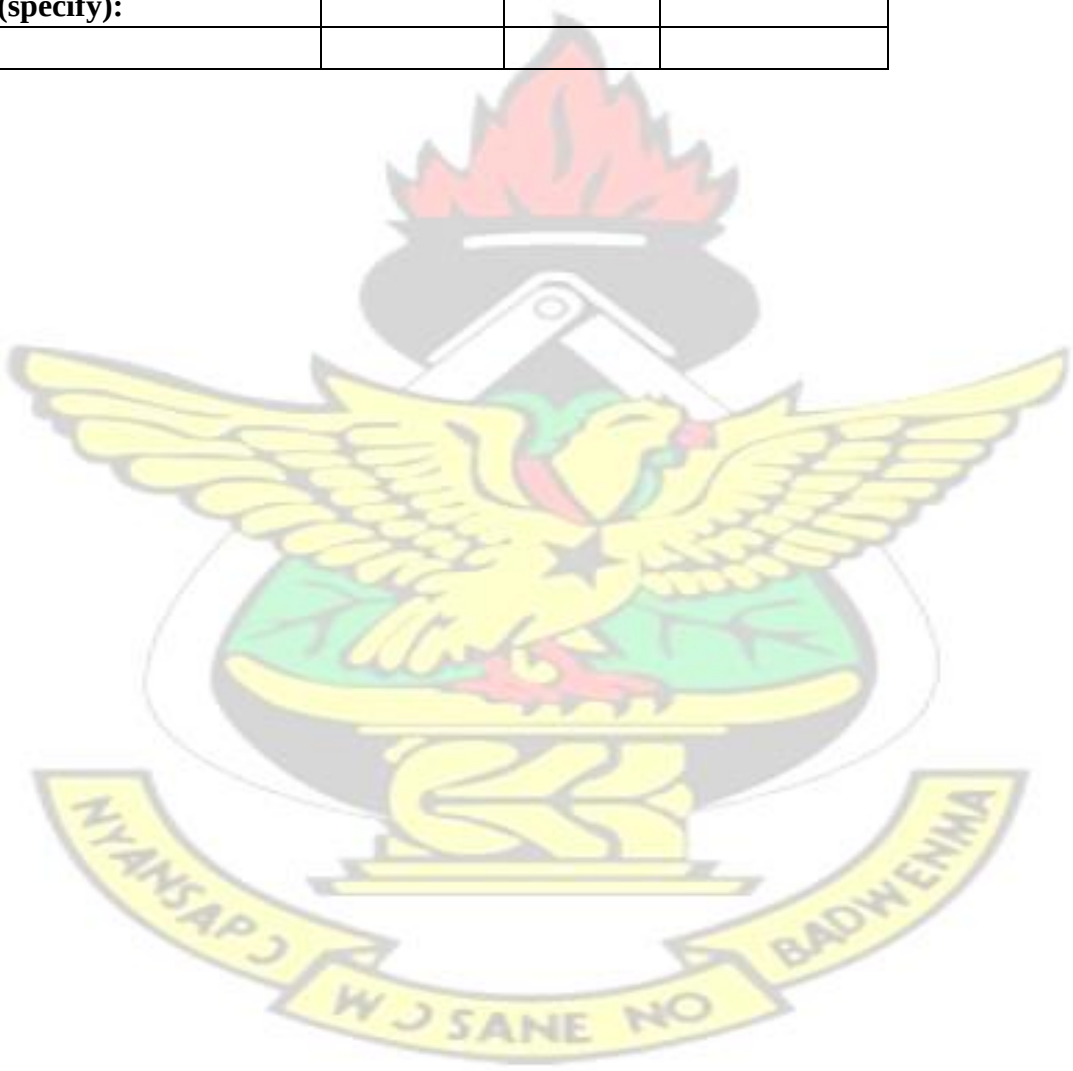
D. ADVERSE EFFECTS REPORTING SHEET

Starting Date	Description of events	Intervention given	Outcome	Resolved Date

E. ADVERSE DRUG REPORT SHEETCHECKLIST

Day:	Day 0	Day 14	Day 28
Nervous system			
Nervousness			
Drowsiness			
Numbness			
Insomnia			
Blurred vision			
Unpleasant taste			
Tinnitus			
Nightmares			
Cardiovascular:			
Abnormal heartbeat			
Chest pains			
Respiratory:			
Difficulty breathing			
Cough			

Gastrointestinal:			
Abdominal pain			
Nausea and Vomiting			
Heartburn			
Diarrhoea			
Genito-urinary:			
Difficulty urinating			
Change in sexual ability/desire			
Mucocutaneous:			
Pruritus			
Jaundice			
Others (specify):			



APPENDIX

3: KARNOFSKY SCALE FOR QUALITY OF LIFE ASSESSMENT

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

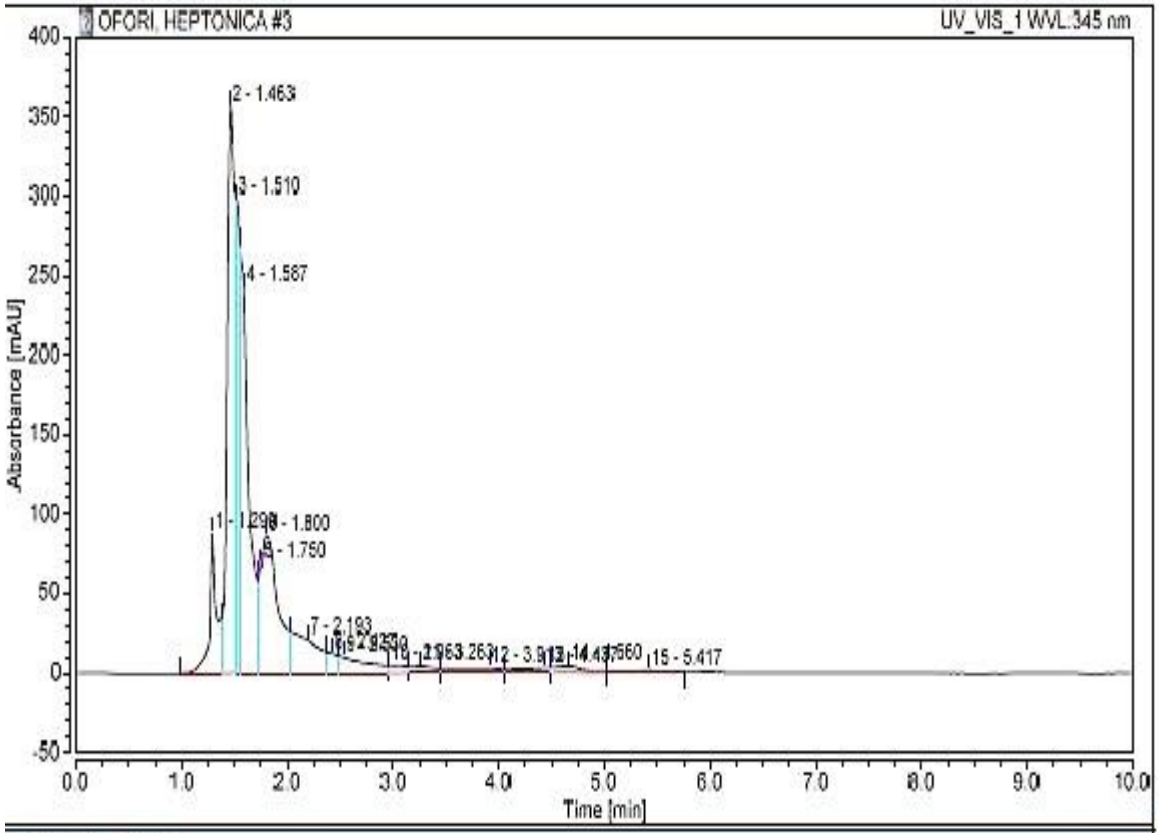
APPENDIX

4: HPLC CHROMATOGRAM FOR HPK

Injection Details

Injection Name:		*Run Time (min):*	10.00
Vial Number:	RA1	*Injection Volume:*	20.00
Injection Type:	Unknown	*Channel:*	UV_VIS_1
Calibration Level:		*Wavelength:*	345.0
Instrument Method:	Ofori, Heptonica	*Bandwidth:*	2
Processing Method:	Ofori, Heptonica	*Dilution Factor:*	1.0000
Injection Date/Time:	13/Feb/19 10:04	*Sample Weight:*	1.0000

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		1.290	6.513	87.681	6.12	7.72	n.a.
2		1.463	28.991	357.087	27.24	31.43	n.a.
3		1.510	11.633	298.465	10.93	26.27	n.a.
4		1.587	24.524	242.748	23.04	21.37	n.a.

APPENDIX

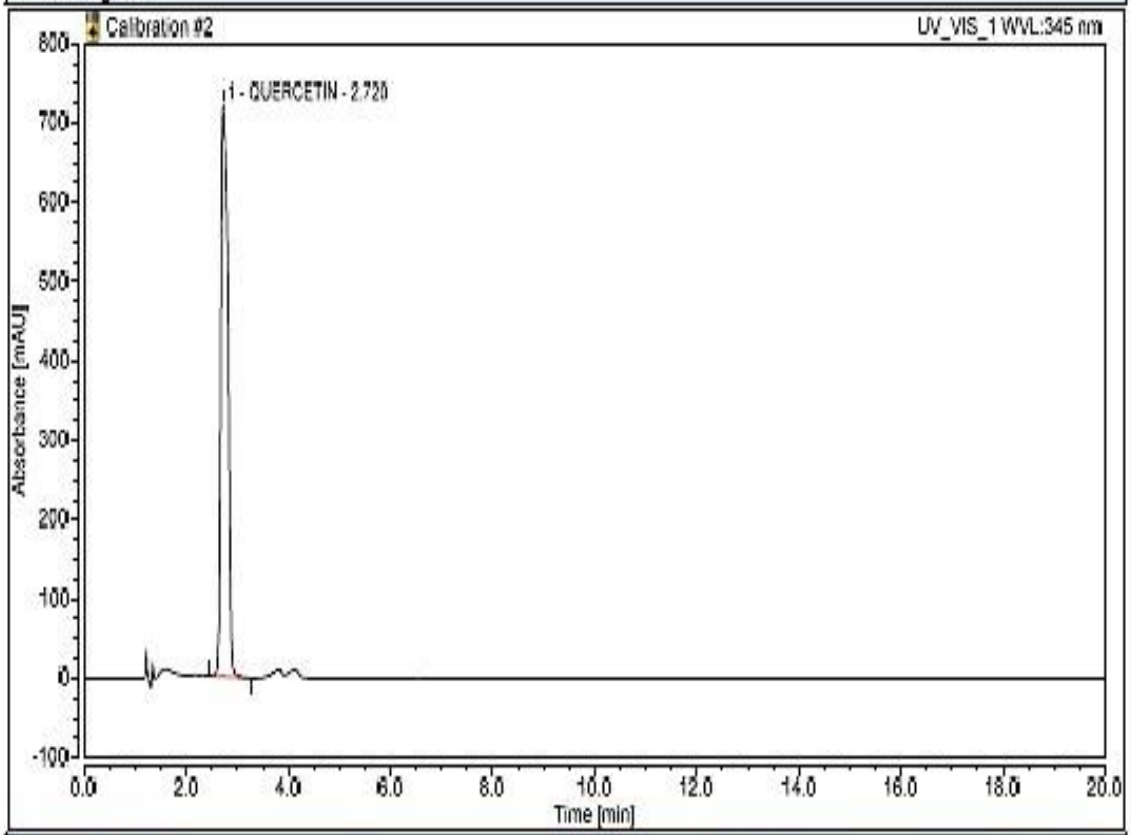
5: HPLC CHROMATOGRAM FOR QUERCETIN

Chromatogram and Results

Injection Details

Injection Name:		*Run Time (min):*	20.00
Vial Number:	RA1	*Injection Volume:*	4.00
Injection Type:	Calibration Standard	*Channel:*	UV_VIS_1
Calibration Level:	01	*Wavelength:*	345.0
Instrument Method:	Quercetin trial	*Bandwidth:*	2
Processing Method:	Quercetin trial	*Dilution Factor:*	1.0000
Injection Date/Time:	31/Jan/19 14:22	*Sample Weight:*	1.0000

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	QUERCETIN	2.720	116.214	721.360	100.00	100.00	0.9770
Total:			116.214	721.360	100.00	100.00	

APPENDIX

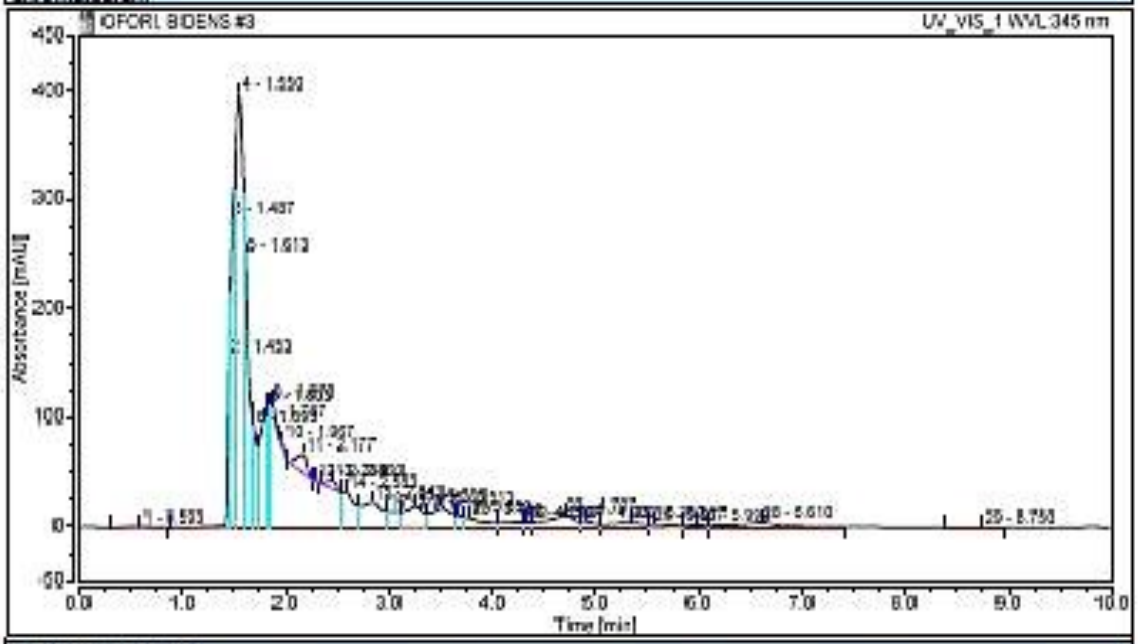
6: HPLC CHROMATOGRAM FOR *BIDENS PILOSA* AERIAL PART

Chromatogram and Results

Injection Details

Injection Name:		*Run Time (min):*	10.00
Vial Number:	RA2	*Injection Volume:*	20.00
Injection Type:	Unknown	*Channel:*	UV_VIS_1
Calibration Level:		*Wavelength:*	345.0
Instrument Method:	Ofori, Bidens	*Bandwidth:*	2
Processing Method:	Ofori, Bidens	*Dilution Factor:*	1.0000
Injection Date/Time:	13/Feb/19 10:42	*Sample Weight:*	1.0000

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a
1		0.593	0.144	0.527	0.09	0.03	n.a
2		1.453	5.580	157.040	3.66	9.24	n.a
3		1.487	10.217	284.082	6.70	16.71	n.a
4		1.550	31.502	397.689	20.67	23.40	n.a
5		1.613	14.841	249.904	9.74	14.70	n.a
6		1.693	4.696	92.285	3.08	5.43	n.a
7		1.787	7.820	98.113	5.13	5.77	n.a
8		1.833	4.413	111.302	2.89	6.55	n.a
9		1.870	37.234	115.667	24.43	6.80	n.a
10		1.967	0.177	4.525	0.12	0.27	n.a
11		2.177	1.896	16.881	1.24	0.99	n.a
12		2.293	0.012	0.322	0.01	0.02	n.a
13		2.460	0.686	7.093	0.45	0.42	n.a
14		2.583	4.680	33.086	3.07	1.95	n.a
15		2.843	5.501	23.120	3.61	1.36	n.a
16		2.977	2.137	14.733	1.40	0.87	n.a
17		3.290	3.877	19.195	2.54	1.13	n.a
18		3.513	3.970	17.442	2.60	1.03	n.a
19		3.680	0.896	10.663	0.59	0.63	n.a
20		3.790	1.887	7.930	1.24	0.47	n.a
21		4.290	0.950	4.495	0.62	0.26	n.a

Ofori, Bidens/Integration

Chromeleon (c) Dionex
Version 7.1.3.242

APPENDIX

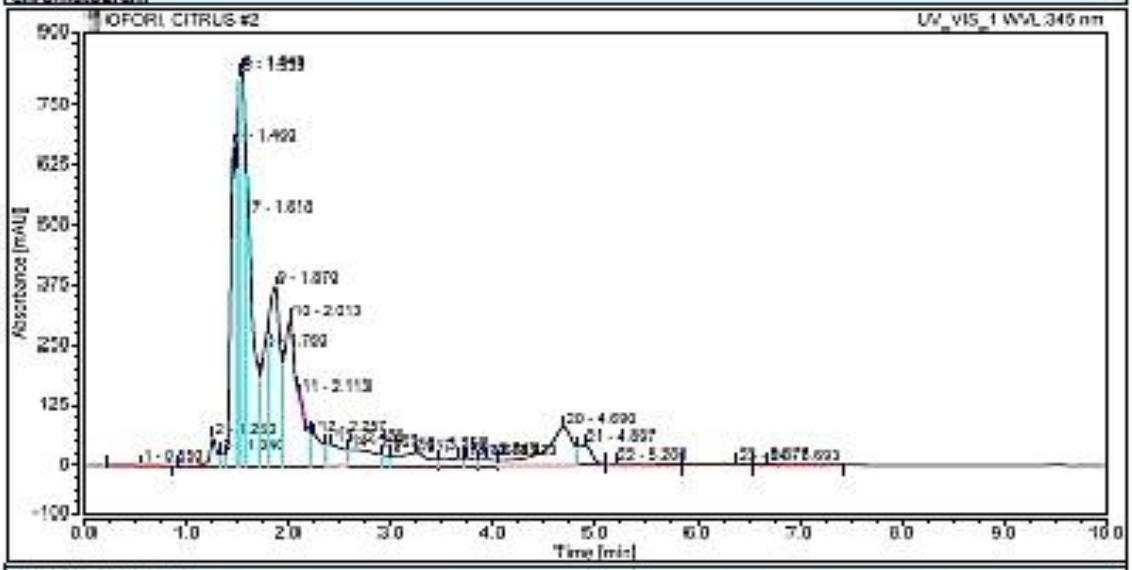
7: HPLC CHROMATOGRAM FOR *CITRUS AURANTIFOLIA* LEAVES

Chromatogram and Results

Injection Details

Injection Name:		*Run Time (min):*	10.00
Vial Number:	RA3	*Injection Volume:*	20.00
Injection Type:	Unknown	*Channel:*	UV_VIS_1
Calibration Level:		*Wavelength:*	345.0
Instrument Method:	Ofori, Citrus	*Bandwidth:*	2
Processing Method:	Ofori, Citrus	*Dilution Factor:*	1.0000
Injection Date/Time:	13/Feb/19 11:08	*Sample Weight:*	1.0000

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		0.560	0.387	1.254	0.11	0.03	n.a.
2		1.290	4.473	57.667	1.24	1.37	n.a.
3		1.340	1.297	24.570	0.36	0.58	n.a.
4		1.460	43.530	668.922	12.02	15.87	n.a.
5		1.533	30.406	815.622	8.42	19.35	n.a.
6		1.543	39.930	824.058	11.03	19.55	n.a.
7		1.610	46.173	520.458	12.75	12.34	n.a.
8		1.780	14.975	242.212	4.14	5.74	n.a.
9		1.870	46.960	373.634	12.89	8.86	n.a.
10		2.013	50.381	305.279	13.92	7.24	n.a.
11		2.113	0.613	14.644	0.17	0.35	n.a.
12		2.257	7.529	63.278	2.08	1.50	n.a.
13		2.423	8.602	44.134	2.38	1.05	n.a.
14		2.567	10.075	33.473	2.78	0.79	n.a.
15		2.917	1.759	22.857	0.49	0.54	n.a.
16		3.250	9.742	27.638	2.69	0.66	n.a.
17		3.650	3.474	14.915	0.96	0.35	n.a.
18		3.743	1.986	15.764	0.55	0.37	n.a.
19		3.923	2.516	13.133	0.70	0.31	n.a.
20		4.690	25.663	79.258	7.09	1.88	n.a.
21		4.897	6.698	42.995	1.85	1.02	n.a.

Ofori, Citrus/Integration

Chromeleon (c) Dio
Version 7.1.3.2

APPENDIX

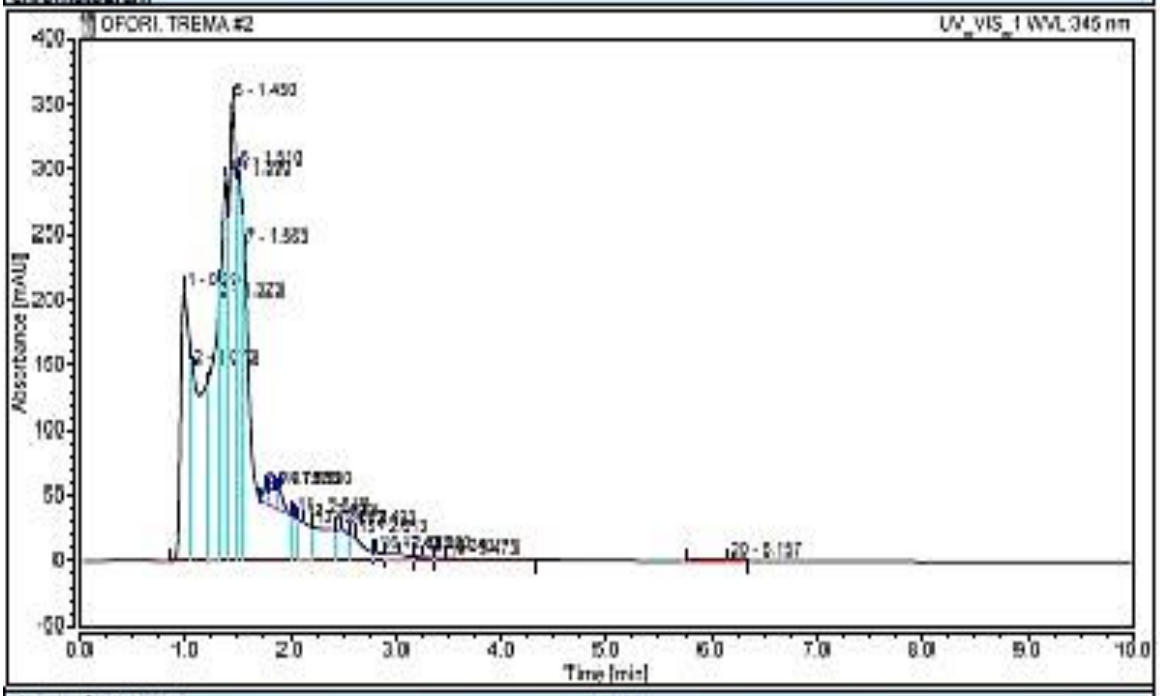
8: HPLC CHROMATOGRAM FOR *TREMA ORIENTALIS* BARK

Chromatogram and Results

Injection Details

Injection Name:		Run Time (min):	10.00
Vial Number:	RA4	Injection Volume:	20.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Calibration Level:		Wavelength:	345.0
Instrument Method:	Ofori, Trema	Bandwidth:	2
Processing Method:	Ofori, Trema	Dilution Factor:	1.0000
Injection Date/Time:	13/Feb/19 11:46	Sample Weight:	1.0000

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		0.990	20.064	208.083	11.41	10.78	n.a.
2		1.073	19.132	147.970	10.87	7.67	n.a.
3		1.323	21.461	199.426	12.19	10.34	n.a.
4		1.390	21.231	292.900	12.06	15.18	n.a.
5		1.450	24.433	353.095	13.88	18.30	n.a.
6		1.510	12.591	300.075	7.15	15.55	n.a.
7		1.583	34.371	240.487	19.52	12.46	n.a.
8		1.757	0.572	11.196	0.33	0.58	n.a.
9		1.853	1.049	14.379	0.60	0.75	n.a.
10		1.890	1.067	16.121	0.61	0.84	n.a.
11		2.040	2.108	33.443	1.20	1.73	n.a.
12		2.123	3.726	29.685	2.12	1.54	n.a.
13		2.203	5.178	25.472	2.94	1.32	n.a.
14		2.493	3.298	23.983	1.87	1.24	n.a.
15		2.613	2.833	18.040	1.61	0.93	n.a.
16		2.823	0.686	5.910	0.39	0.31	n.a.
17		3.023	1.060	4.868	0.60	0.25	n.a.
18		3.240	0.460	2.349	0.26	0.12	n.a.
19		3.473	0.597	1.537	0.34	0.08	n.a.
20		6.157	0.097	0.378	0.06	0.02	n.a.
Total:			176.037	1929.396	100.00	100.00	

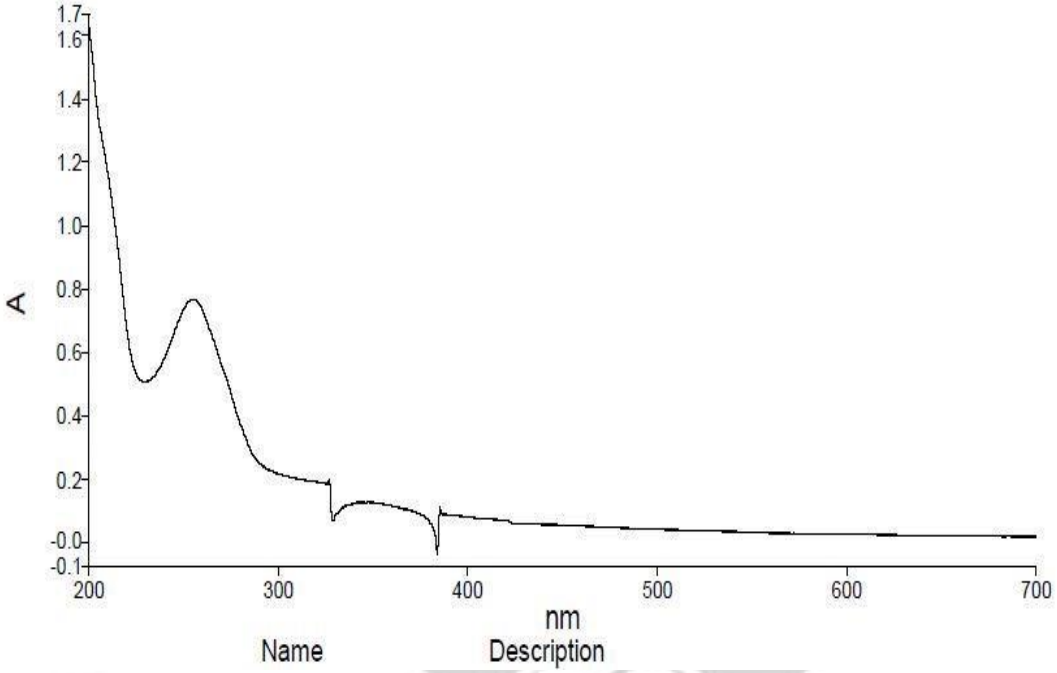
Ofori Trema Integrator

Chromeleon (c) Dion
Version 7.1.3.24

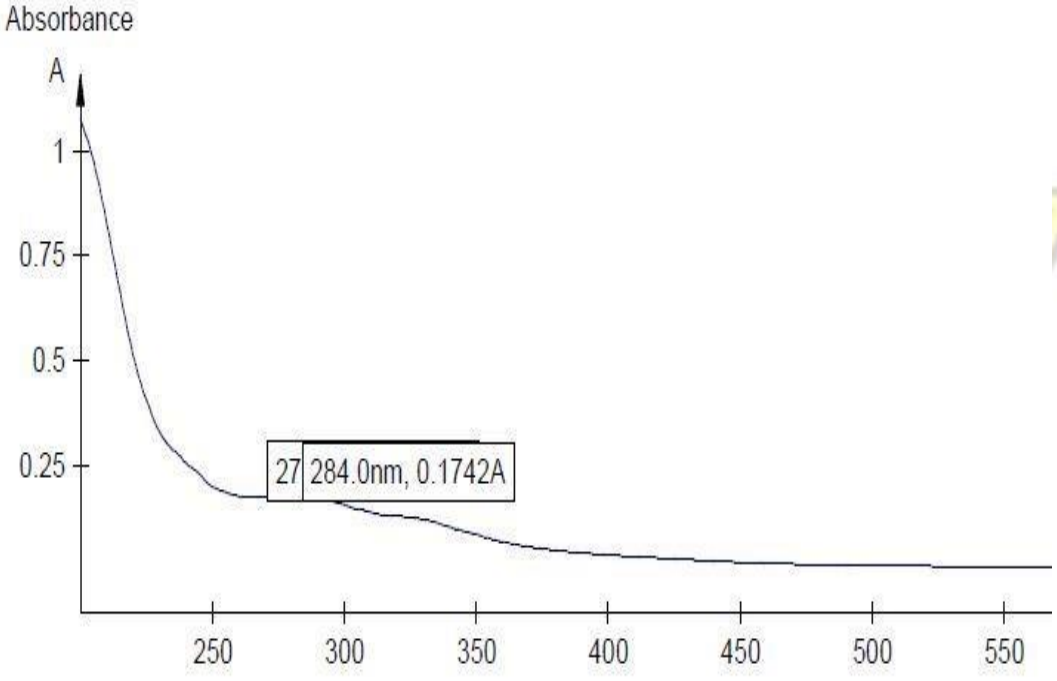
APPENDIX

9: UV RESULTS FOR HPK AND PLANT COMPONENTS A.

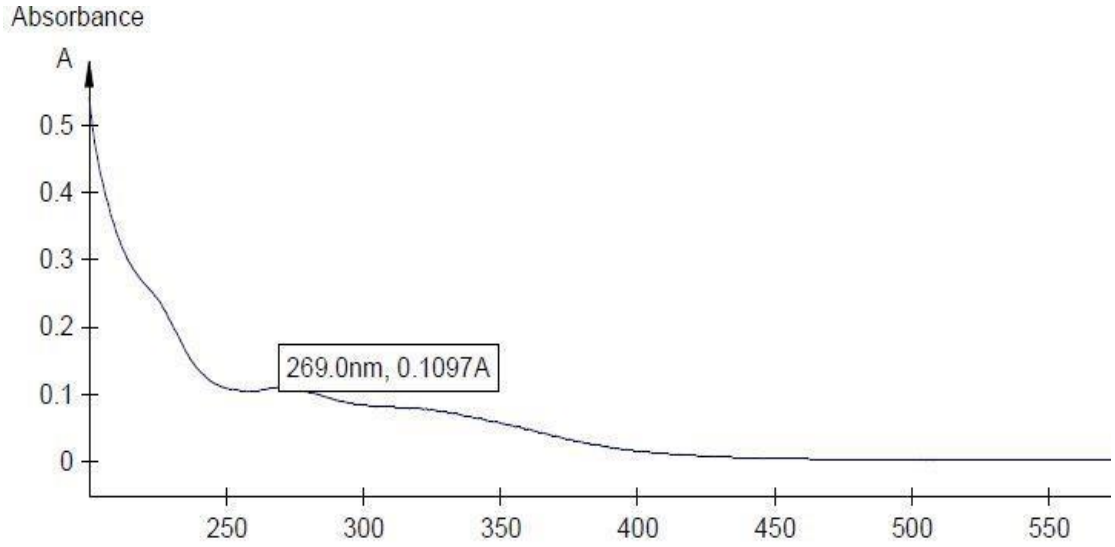
HPK



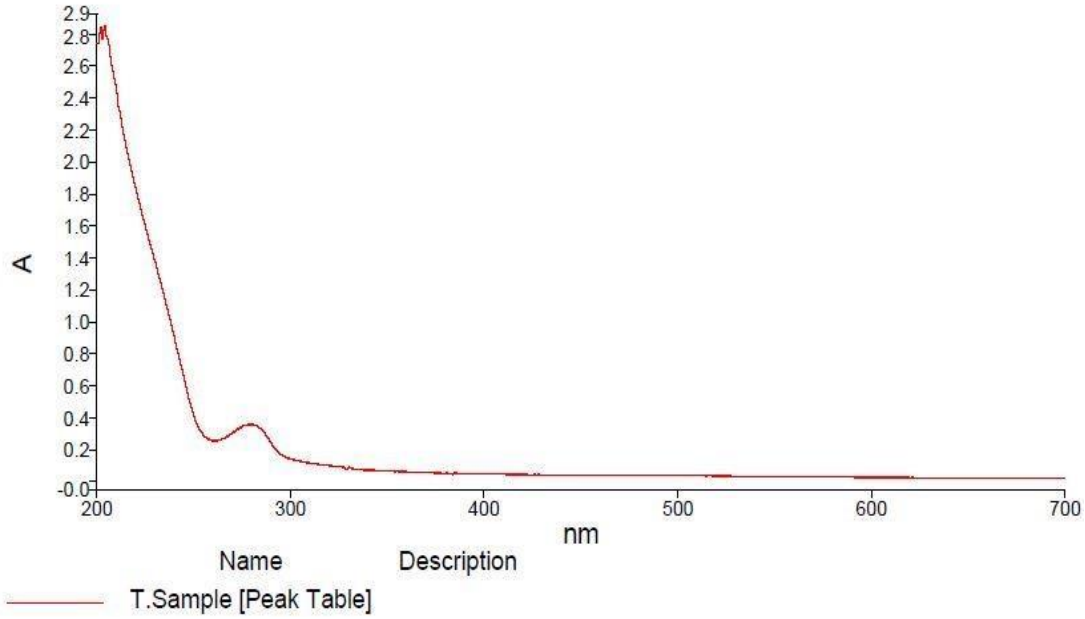
B. ***Bidens pilosa***



C. *Citrus aurantifolia*

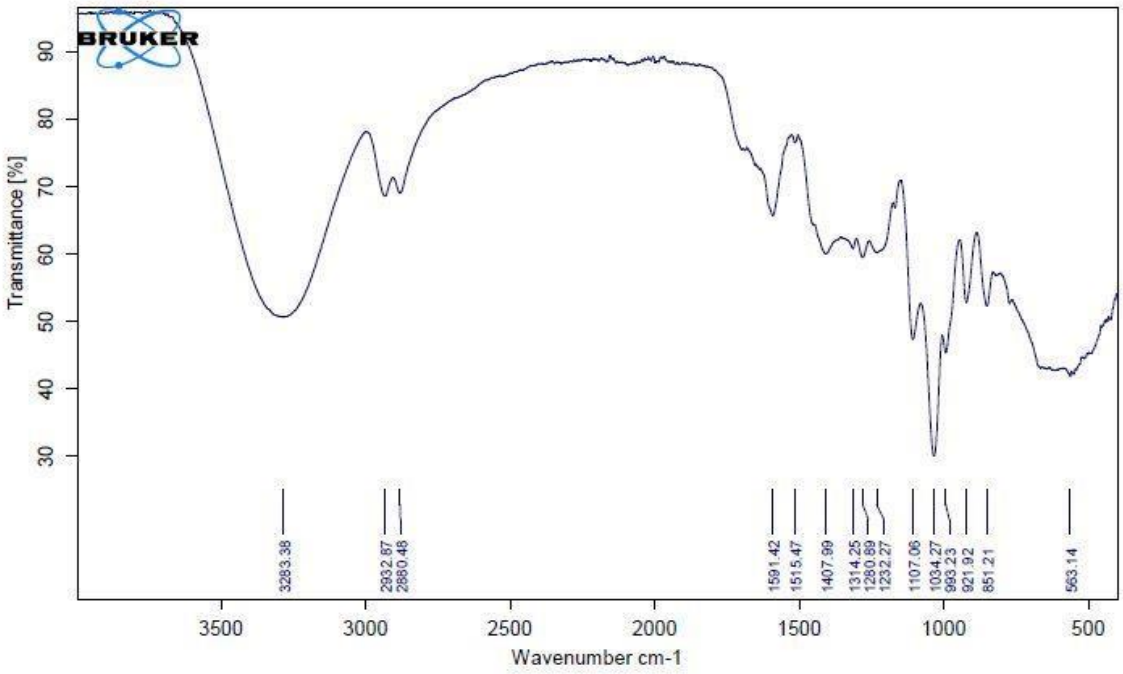


D. *Trema orientalis*

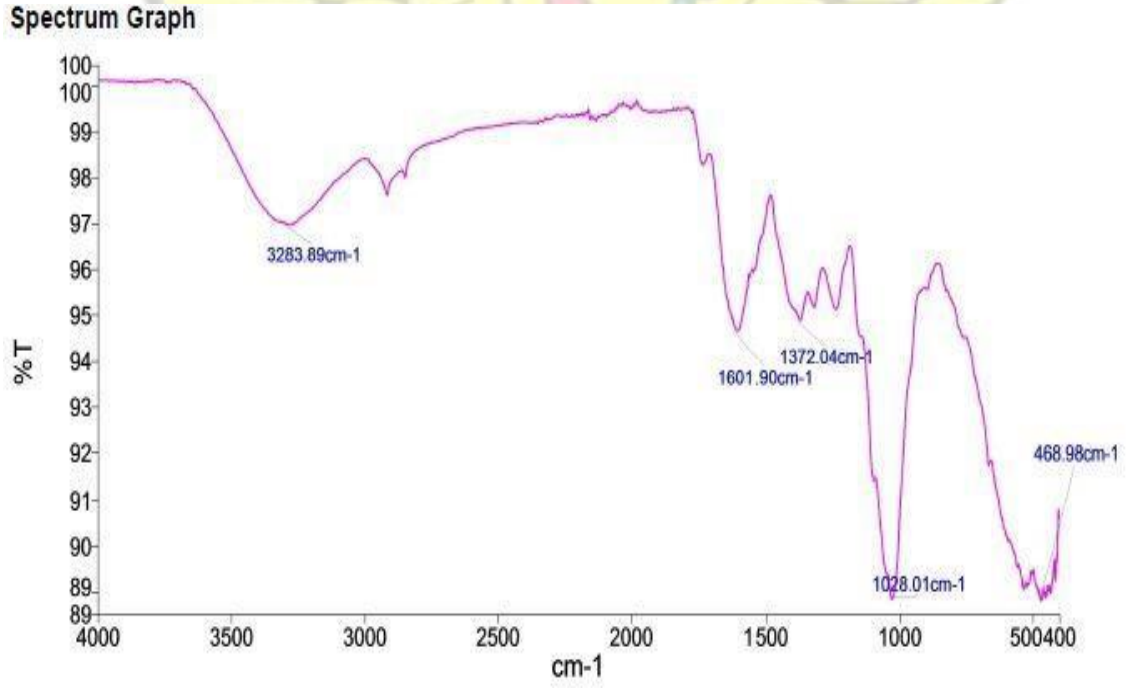


APPENDIX 10: FTIR SPECTROSCOPIC CHARACTERISTICS OF HPK AND PLANT COMPONENTS

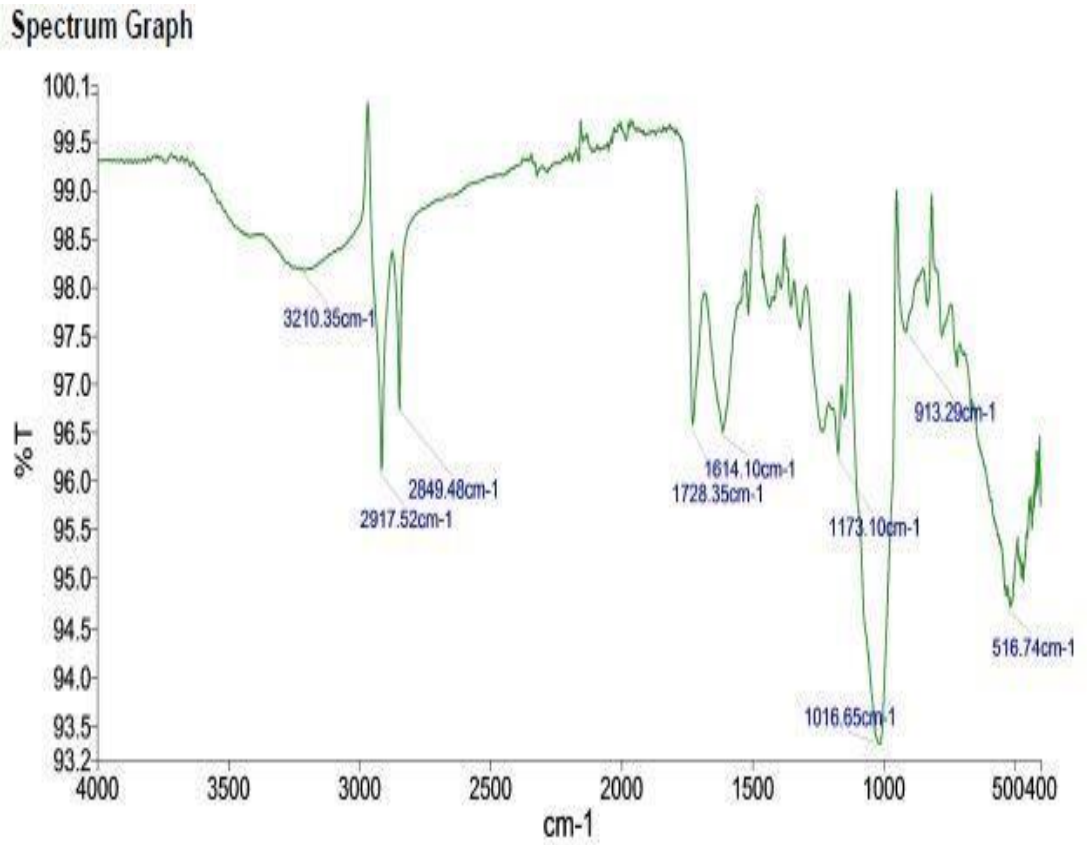
A. HPK



B. *Bidens pilosa*



C. *Citrus aurantifolia*



D. ***Trema orientalis***