CHARACTERIZATION OF PHENOLIC COMPOUNDS FROM LEAF EXTRACTS OF *Bidens pilosa* L. var. Radiata AND EVALUATION OF THEIR ANTACID POTENTIAL IN ARTIFICIAL STOMACH MODEL

MERAB LILIAN NDIEGE

BSc.

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Analytical Chemistry of the Department of Chemistry and Biochemistry, School of Sciences and Aerospace Studies, Moi University

2021

DECLARATION

Declaration by the candidate

I, Merab Lilian Ndiege, duly declare that this thesis is my original work and has not been presented for a degree in any other University for any other award. No part of this thesis may be reproduced without the prior written permission of the author and/ Moi University.

Merab Lilian Ndiege _____ ___ Date: 15/6/2021

(MSC/ACH/3/18)

Declaration by supervisors

This thesis has been submitted for examination with our approval as Moi University Supervisors

Prof. Fredrick O. Kengara Date: 12/6/2021

_____

Bomet University College, Bomet, Kenya

Dr. Geoffrey K. Maiyoh Date: 13/6/2021

_____

Moi University, Eldoret, Kenya

DEDICATION

This thesis is dedicated to the Almighty God, my parents Mr. Bernard Ndiege and Mrs. Margaret Ouma for their support and encouragement throughout the study. My siblings and all friends for their support and my classmates in the MSc in Analytical Chemistry class of 2018 for their support and loyalty all throughout the study.

ABSTRACT

Several studies reveal that Gastric Ulcer Disease (GUD) is one of the most prevalent gastrointestinal diseases affecting 5-10% of the population worldwide. Antacids have been used in the management of GUD. However, due to the side effects of synthetic antacids such as hypersensitivity reactions, nutritional deficits and bone marrow suppression, an alternative antacid with fewer side effects which is eco-friendly is needed. Therefore the aim of this study was to characterize phenolic compounds in extracts of *Bidens pilosa* leaves and evaluate the antacid potential in a modified artificial stomach model. Specific objectives were to: determine phytochemical composition of extracts of *B. pilosa* leaves, characterize the phenolic compounds from *B. pilosa* leaves and to evaluate the neutralizing effect of extracts of *B. pilosa* leaves. Extraction was done using soxhlet apparatus. Phytochemicals present were determined using calorimetric method. Classical column chromatography was used for fractionation and thin layer chromatography (TLC) was used to merge the fractions with the same retention factors, while solid phase extraction (SPE) was used for the clean-up. FTIR with a range between (4000-400 cm^{-1}), UV-visible spectroscopy and GC-MS/MS were used for characterization. The pH of *B. pilosa* fractions with concentrations 800 and 400 $mgkg^{-1}bw^{-1}$, representing high dose (HD) and low dose (LD) respectively, and their neutralizing effects on artificial gastric acid were determined and compared to water and sodium bicarbonate (standard). A modified model of Vatier's artificial stomach was used to determine the time taken to neutralize the artificial gastric acid. The neutralization capacity *in vitro* was determined with a classical titration method based on Fordtran's model. Phytochemical analysis of each extract showed the presence of saponins, alkaloids, phenols, tannins, cardiac glycosides and flavonoids. Dichloromethane: methanol extract (1:1) showed the highest total phenolic and flavonoid content of 288.7 ± 1.27 mg GAE/g and 274.7 ± 2.02 mg QE/g extract respectively. Phenolics were in abundance while the other compounds were in low yields thus were not considered further. FT-IR spectral data showed a broad peak absorption in the range 3300-3200 cm^{-1} and was attributed to O-H stretch, 1150-1085 cm^{-1} C-O stretch, 1000-650 cm^{-1} C=C bend, 1440-1395 cm^{-1} O-H bend, 1650-1580 cm^{-1} N-H bend. Some of the phenolic compounds identified by GC-MS/MS were; 2,4-Di-tert-butylphenol, 2,6-Bis (tert-butyl)phenol, 3,5-ditert-butylphenol and 2,5-bis(1,1-Dimethylethyl)phenol. All treatments including HD and LD *B. pilosa* fractions and sodium bicarbonate showed significant acid neutralizing effects with ($P<0.01$ and $P<0.05$) when compared with water. The duration for consistent neutralization and neutralization capacities of fractions of *B. pilosa* HD and LD were significantly higher with ($P<0.01$) than those of water. HD and LD *B. pilosa* fractions were consistently active in the artificial stomach model and possessed potent antacid effects. In conclusion, the results obtained showed the presence of phenolic compounds

in the *B. pilosa* leaf extracts which were responsible for the observed antacid activity. This study recommends *B. pilosa* leaf extract as an effective alternative to sodium bicarbonate that is commonly used and known to have side effects like alteration in systematic pH.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

ACKNOWLEDGMENT

All praises, glory and honor to the almighty God for His guidance and grace up to this point. I would like to express my deep sense of gratitude to both my supervisors Prof. Fredrick O. Kengara and Dr. Geoffrey K. Maiyoh for their endless pieces of advice, support, corrections, encouragement and guidance during the course of this study. It was a great honor and privilege to have worked with such knowledgeable and caring supervisors, may the almighty God bless you.

My appreciation also goes to Africa Center of Excellence in Phytochemical, Textile and Renewable Energy (ACEIIPTRE) for funding this work without which it would have been impossible to finish this work on time. To Prof. Ambrose Kiprop (ACEIIPTRE center leader and Dean, School of Science and Aerospace Studies), Dr. Jackson Cherutoi (Chairman, Department of Chemistry and Biochemistry) and Dr. Sarah Chepkwony for always responding to my calls and requests, you really helped me keep on toes with this research.

I equally appreciate my MSc lecturers Dr. K'Owino and Dr. Phanice Wangila for helping me come up with a research study and instrumentations which were developed during the class lectures. To lab technicians of Moi University School of Science and Aerospace studies for their guidance at the preliminary and lab work stages of this research, you tirelessly ensured that I got the point of every step and procedure. I wish to thank the members of staff at Moi University Department of Chemistry and Biochemistry and the Department of Chemical Engineering for their assistance as I was carrying out this study.

Finally, to my course mates, MSc Analytical Chemistry class of 2018, I am grateful for your support, pieces of advice and co-operation.

DEFINITION OF KEY TERMS/CONCEPTS

Antacid activity **:** The ability to maintain the pH value above 3

Prevalence **:** A frequently used epidemiological measure of how commonly a disease or condition occurs in a population. Prevalence measures how much of some disease or condition there is in a Population at a particular point in time

Infection **:** Is the invasion of a host organism's bodily tissues by disease-causing organisms, their multiplication, and the reaction of host tissues to these organisms and the toxins they produce. Infections are caused by microorganisms such as viruses, bacteria, and larger organisms like macro parasites and fungi.

Hydrochloric acid in the stomach:

Is an acid that provides an optimum pH for normal functioning of the enzymes present there. For example, hydrochloric acid helps convert pepsinogen to pepsin, which is responsible for breaking down proteins in the stomach.

Non-Steroidal Anti- Inflammatory Drugs (NSAIDs):

Are drugs used to reduce inflammation and relieve fever and pain by blocking enzymes and proteins made by the body, they relieve pain and fever. They also reduce swelling and inflammation caused by an injury or a disease. Anti-Inflammatory refers to the property of a substance or treatment that reduces inflammation.

Gastrointestinal tract **:** Is the stomach and intestine, sometimes including all the structures from the mouth to the anus. (The "digestive system" is a broader term that includes other structures, including the accessory organs of digestion).

Phytochemicals **:** Are non-nutritive plant chemicals that have protective (or) disease preventive properties.

ABBREVIATIONS

GUD : Gastric ulcer disease

PPI : Proton pump inhibitors

NSAIDS : Non-Steroidal Anti-Inflammatory Diseases

PDC : Peritoneal dialysis

HCl : Hydrochloric acid

H. pylori : *Helicobactor pylori*

UV : Ultra-violet detector

WHO : World health organization

B. pilosa : *Bidens pilosa*

ESD : Endoscopic sub mucosal dissection

SEM : Standard error mean

P^H : Potential of hydrogen

H^+/K^+ ATPase: Hydrogen potassium adenosine triphosphatase

P_{450} : Cytochrome at 450nm

SB : Sodium Hydrogen carbonate

TPC : Total phenolic compounds

GC-MS : Gas chromatography- mass spectrometry

FTIR : Fourier transform infrared spectrometry

SPE : Solid phase extraction

R_f : Retention factor

LD : Lower dose

HD : Higher dose

NIST : National institute of standards and technology

CHAPTER ONE

INTRODUCTION

1.1. Gastric ulcers

Gastric ulcers is a sore on the lining of the stomach. Gastric ulcer disease (GUD) is one of the most prevalent gastrointestinal diseases affecting 5-10% of population worldwide (Graham, 2014). It is characterized by mucosal damage with inflammatory cell infiltration and coagulation necrosis (Mahdy, Shehab, & Bayoumi, 2018). GUD is a complex disease with multiple contributing factors and unclear etiology (Cheng, He, Ai, Huang, & Lu, 2017). Many believe that GUD is mainly due to imbalance between aggressive and defensive factors affecting gastric mucosa leading to ulcerative damage (El-Meligy, Awaad, Soliman, Kenawy, & Alqasoumi, 2017). Pepsin, hydrochloric acid, reactive free radicals, and refluxed bile are some of the aggressive factors (Farzaei, Abdollahi, & Rahimi, 2015). On the other hand, defensive factors include mucus secretion, bicarbonate production, gastro protective prostaglandin synthesis, endogenous nitric oxide and normal tissue (Miraglia et al., 2018). Several other factors contribute to the etiology of ulcer progression, e.g, *Helicobacter pylori* (*H. pylori*) infection (Miftahussurur, Yamaoka, & Graham, 2017), smoking (Chuang et al., 2017), excessive use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) (Miraglia et al., 2018) or alcohol (Chuang et al., 2017), and psychological stress (Levenstein, Rosenstock, Jacobsen, & Jorgensen, 2015).

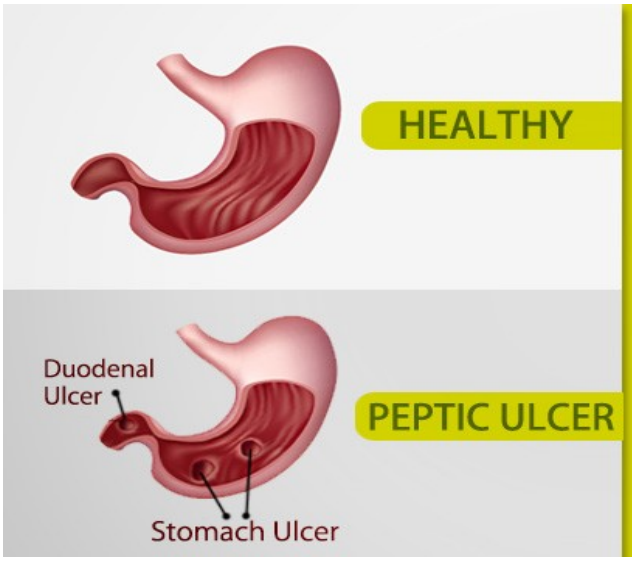


Figure 1.1: Healthy stomach and (peptic ulcer)

Drugs used currently for treatment of gastric ulcers include antacids (aluminum hydroxide and magnesium trisilicate) which neutralize gastric acid, acid blockers - ranitidine (Zantac), famotidine (Pepcid), cimetidine (Tagamet HB) and nizatidine (Axid AR) (Jacobs & Richter, 2016) which reduce gastric acid secretion for a prolonged duration, proton pump inhibitors (omeprazole and lansoprazole), tissue-lining protecting agents (sucralfate and misoprostol) and antibiotics for hyperacidity caused due to *Helicobacter pylori* infection. Apart from being expensive for the general population, there are numerous side effects associated with the drugs used in the treatment of ulcers, including arrhythmia, impotence, gynaecomastia and hematopoietic changes and ulcer relapse (Jacobs & Richter, 2016; Schulman, Chan, Devery, Ryan, & Thompson, 2017; Sethuraman, Hedden, & Leskow, 2017).

1.2. Use of medicinal plants in treatment of gastric ulcer

Attempts have been made to know about some plants - and their constituents - which may be used in treatment of gastric ulcer. Various plants like *Anogeissus latifolia* (Kala, 2016), *Alchornea castaneaefolia, Utleria salicifolia (Tripathy & Afrin, 2016), Solanum nigrum (Geetha, Harathi, Giribabu, & Naidu, 2017), Ocimum sanctum, Asparagus racemosus*, Scoparia *dulcis, Byrsonima crassa, Allophylus serratus Kurz*, *Aloe vera* (L.) Burm.f., *Butea frondosa* Roxb etc. and their phyto-constituents proved active in antiulcer therapy (Jivad & Bahmani, 2016).

Previously, a number of other plants have been tested for antacid activity. The root of *Tephrosiapurpurea (L.) Pers* was tested *in vitro* with the modified Vatier's artificial stomach model in order to measure the antacid activity in methanol, ethyl acetate and chloroform extracts. The methanolic extract had a higher antacid activity than sodium bicarbonate, whereas the other two extracts showed moderately good response, but less than the standard (Sandhya, Venkata, Vinod, & Rsnakk, 2012). Aqueous extracts of the fruit rind of *Garcinia indica* were tested for their antacid activity, also using the modified Vatier's artificial stomach model. The extracts showed potent antacid activity when compared to water, but not as good as the standard sodium bicarbonate (V. Panda & Khambhat, 2013)

1.3. Medicinal applications of *Bidens pilosa*

Bidens pilosa is an important traditional medicine in South Africa that has been used by various cultural groups for a wide range of treatments. The whole plant including the

root, stem, leaf and flower are used in various folk medicines and as a popular herbal tea ingredient (Bhatt, Sharma, & Pandey, 2009). For instance, a leaf decoction is used to treat headaches, ear infections, kidney problems, and flatulence (Bum et al., 2011). It has been proven to be effective for curing infectious hepatitis (Lee, Peng, Chang, Huang, & Chyau, 2013), and diabetes mellitus (Hsu, Lee, Chang, Huang, & Yang, 2009), (Yang, 2014). The leaf extract is also used to cure malaria (Kaushik et al., 2015), stomach and mouth ulcers (Buhner, 2012), diarrhea (Bartolome, Villaseñor, & Yang, 2013), hangover (Khanh et al., 2009), and the whole plant is also used as a poison antidote. In sub-Sahara Africa, both fresh and dry shoots as well as young leaves of *B. pilosa* are sometimes used as human food, although they are believed to contribute to the etiology of human esophageal cancer (Abdou, Scherlach, Dahse, Sattler, & Hertweck, 2010). Despite the medicinal applications of *Bidens pilosa*, including its reported use for ulcer treatment by traditional healers, there is no scientific basis for using *Bidens pilosa* for gastric ulcer treatment. Hence, this study mainly aims at phytochemical screening for secondary compounds, characterization of phenolic compounds from *Bidens pilosa* leaves and establishing their antacid activity potential.

1.4. Statement of the Problem

Gastric ulcer disease represents a serious medical problem in the world (Sung J.J.Y et al., 2009). In Kenya approximately 7.5% new cases are reported each year (Kayamba et al., 2015). Modern medicines have their own limitations, especially against ulcers with a complex pathology (Thorsen K, et al., 2011). A number of drugs - including antacids, proton pump inhibitors, prostaglandins analogues, cytoprotective agents and histamine receptor antagonists – are currently available for the treatment of gastric ulcers (Kavitt,

Lipowska, Anyane-Yeboa, & Gralnek, 2019). However, most of these drugs produce several adverse reactions including toxicities and may even alter biochemical mechanisms of the body upon chronic usage indicating a need for substitute medication from an alternative system of medicine.

The clinical evaluation of these drugs has showed the development of tolerance and incidence of relapses and side effects that make their efficacy arguable (Etkin, 2017). Moreover, the cost of ulcer treatment drugs is high (Mohiuddin, 2019) and cannot be afforded by all the groups – including the poor and the old - that have this disease. Despite the medicinal applications of *Bidens pilosa* and its ulcer treatment reported by traditional healers, there is no scientific basis for using *Bidens pilosa* for the treatment of gastric ulcers. To fill this knowledge gap, there is need for a study to provide the lacking information on *B. pilosa* phenolic compounds responsible for gastric ulcer treatment.

1.5. Justification

This study will lead to the development of a more readily available, cheaper and safer alternative for gastric ulcer treatment. The society will, therefore, be able to get treated at a low cost without fear of the high cost of medicine for treatment of gastric ulcer. The study will add to scientific knowledge on the chemical composition of *B. pilosa*, and this will form a baseline for future studies. Moreover, some of the compounds identified could act as precursors for formulation of modern medicine (Heinrich & Anagnostou, 2017).

1.6. Main objective

To characterize phenolic compounds of *B. pilosa* leave extracts and evaluate their antacid potential in a modified artificial stomach model.

1.7. Specific objectives

- To determine the phytochemical composition of *B. pilosa* leaf extracts.
- To characterize phenolic compounds from *B. pilosa* leaf extracts.
- To evaluate the neutralizing effect, duration and neutralization capacity of phenolic compounds of *B. pilosa* leaf extracts on gastric acid.

1.8. Research questions

- What are the types and proportions of phytochemicals present in *Bidens pilosa* leaf extracts?
- What are the properties of the phenolic compounds present in the leaf extracts of *B. pilosa?*
- Do the phenolic compounds of *Bidens pilosa* leaves possess neutralizing effect on artificial gastric acid? What is the duration and capacity of phenolic compounds to neutralize the gastric acid?

1.9. Significance of the study

Findings from this study may lead to value addition in that *B. pilosa*, a plant considered as a weed may now be used for health benefits and will thus be a source of income. If the study is not carried out, ignorance on the medicinal use of the plant will persist and it will continue to be seen as a weed - hence its potential health benefits will remain unexploited. *B. pilosa* grows in many parts of the country (Africa), therefore, GUD

patients in remote and marginalized areas, who do not have access to modern medical facilities will confidently opt to using *B. pilosa* leaf extracts based on scientific data efficacy provided here. Benefits of this study may also extend to patients in urban setting who are experiencing challenges related to toxicity, lack of efficacy and side effects associated with modern GUD medicines.

LITRATURE REVIEW

2.1. Gastric ulcer description

Acidity is a common gastrointestinal disease which may not be necessarily caused by a pathogenic infection. It is attributed to a functional disorder that can result due to a variety of reasons which are related to heartburn and gas formation in the stomach (Kumar et al., 2017). In acidity, gastro esophageal reflux disease which is commonly known as urdhva gata amalpitta in Ayurveda, there is a movement of gastric juices from the stomach into the lower esophagus (Sandhya et al., 2012). This is a condition which occurs when acidic contents in the stomach move upward into the esophagus and make it dysfunctional.

Gastric acid is a digestive fluid formed in the stomach having a pH of 1 to 2 (Mehta et al., 2016). It is a mixture of hydrochloric acid, large quantities of potassium chloride and sodium chloride (Li et al., 2017). The acid in the stomach plays a significant role in the digestion of proteins, by activating digestive enzymes and making ingested proteins unravel so that digestive enzymes can break down the long chain amino acids (Somaratne et al., 2020). General symptoms found in children are respiratory problems, inadequate weight, vomiting, coughing and turning down food (Tang et al., 2018). The symptoms shown by adults include long heartburn, chest and stomach pain, gas formation in the stomach, inflammation in the chest, gastro esophageal reflux, voice change and formation of ulcer in esophagus, pain during muscular contractions and pain in the ears (Bansod, Bansod, Galande, Shamkuwar, & Singh, 2016).

Gastric acid secretion is a complex process that involves neuronal, hormonal, and endocrine pathways, all of which have one common target: the parietal cell (Engevik, Kaji, & Goldenring, 2020). The parietal cell is responsible for secreting concentrated hydrochloric acid into the gastric lumen. Gastric ulcer can be complicated by upper gastrointestinal bleeding (Manno et al., 2016).

The secretion of gastric acid (HCl) is intimately related to GUD disease. Antacids heal ulcers through elimination of gastric acid by neutralization and have been used in the treatment of GUD for many years.

- Antacids act by neutralizing gastric acid

eg; neutralization reaction of sodium bicarbonate (standard) in equations below;

$$HCl_{(aq)} + NaHCO_{3(aq)} \longrightarrow NaCl_{(s)} + H_2O_{(l)} + CO_{2(g)} \quad \textit{Equation 2.1}$$

- Neutralization reaction of extracts would be;

$$HCl_{(aq)} + XOH_{(aq)} \longrightarrow XCl_{(s)} + H_2O_{(l)} \quad \textit{Equation 2.2}$$

Common antacid preparations include; sodium bicarbonate (SB), calcium carbonates and salts of aluminum and magnesium. Since some people cannot use chemosynthetic drugs because of their side effects, plant medicines that have less side effects are studied (V. Panda & Khambhat, 2013).

2.1.1. Diagnosis of gastric ulcer

Medical physicians use information from the patient's medical history, a physical exam, and laboratory tests for diagnosis. The presence of an ulcer can only be determined by

looking directly at the stomach with endoscopy or an X-ray test, from medical history, physical examination, and laboratory tests (Allison et al., 2016).

2.1.2. Risk factors of gastric ulcer

Some risk factors for GUD include; being 70 years or older (Matsunaga et al., 2020), drinking alcohol, having a history of peptic ulcers (Oh, Lee, Giovannucci, Keum, & Control, 2020), smoking, severe injury or physical trauma. It's a myth that spicy foods can increase your risk for ulcers or cause them (Zibima, Oniso, Wasini, & Research, 2020). But certain foods can irritate the stomach further in certain individuals.

2.1.3. Complications of gastric ulcer

Some of the complications and causes of gastric ulcers are bleeding, perforation, peritonitis and blockage caused when the ulcer wears away the stomach or small intestine and breaks the blood vessels there (Bellorin-Marin & Pomp, 2018), when ulcer breaks through the lining and stomach wall, causing bacteria, acid, and food to leak through (Altieri & Spaniolas, 2019), by inflammation and infection of the abdominal cavity due to perforation (Machado et al., 2019) and when scar tissue forms as a result of the ulcers and keep food from leaving the stomach or duodenum (Kavitt et al., 2019).

2.2. Management of gastric ulcer using conventional drugs

Antacids, Histamine Type-2 Receptor Antagonists (H2 blockers) and proton pump inhibitors (PPI) are synthetic medicines that have been in use to prevent and manage gastric ulcers (Lin et al., 2017).

H2 receptor blockers are a subclass of antihistamines that include cimetidine, ranitidine, famotidine, and nizatidine (Werbel & Cohen, 2018). H2 antihistamines are effective in

the treatment of gastric ulcer disease, gastroesophageal reflux disease, and hypersecretory conditions by indirectly reducing gastric acid secretion (Contreras-Zentella, Olguín-Martínez, Sánchez-Sevilla, & Hernández-Muñoz, 2017) . Cimetidine and ranitidine have been used as off-label therapeutic interventions for warts (Werbel & Cohen, 2018). Although less common, there have been reports of ranitidine-associated extrapyramidal symptoms, loss of color vision, mania, late-onset depression and sleep disturbance (Werbel & Cohen, 2018).

Treatment with this family of drugs has been associated with many side effects, from minor - including gastrointestinal disturbance (such as constipation, diarrhea, and nausea), headache, and skin rash (Cohen, Bueno de Mesquita, & Mimouni, 2015), to more consequential complications - including hypersensitivity reactions, nutritional deficits, bone marrow suppression, bone fractures, neurotoxicity, hepatotoxicity and gastric tumors (Pérez-Fontan et al., 2016). However, central nervous system adverse effects may not be common. Similarly, H1 antihistamines have been reported to cause neuropsychiatric reactions, particularly in the elderly (Cecere et al., 2017). Due to these side effects of H2 receptors, PPI was suggested for use because it was detected of less side effects (Suzuki et al., 2019).

Unlike anticholinergics and histamine2-receptor blockers, PPIs inhibit the final common pathway of acid secretion (the H/K ATPase) in response to any and all stimulation of the parietal cell (Strand, Kim, & Peura, 2017). PPIs are highly protein bound and subject to degradation by hepatic P_{450} cytochromes (Mrudula, Caroline, Kingsley, & Jaiswal, 2017). Although the CYP2C19 pathway (CYP2C19 catalyzes the metabolism of several drugs) is dominant overall, individual agents have variations which have led to concerns over

efficacy and drug-drug interaction (Zhou, Pedersen, Dawed, & Pearson, 2016). Omeprazole and its stereo-isomer esomeprazole are metabolized almost entirely by CYP2C19, thereby offering the greatest potential for interaction with other drugs (Corsonello et al., 2018). Rabeprazole and lansoprazole/dexlansoprazole are also metabolized by CYP2C19, but they possess significant affinity for CYP3A4 which oxidizes small foreign organic molecules (xenobiotics), such as toxins or drugs, so that they can be removed from the body (Mitrasinovic & Dynamics, 2021). Pantoprazole, is primarily degraded by CYP2C19 O-demethylation and sulfate conjugation which results in the lowest potential for cytochrome induction or inhibition among the benzimidoles (Strand et al., 2017). PPIs have been reported to be effective in promoting healing, as well as preventing bleeding complications from gastric ulcers of varying etiologies, including post- Endoscopic Submucosal Dissection (ESD) (Kataoka et al., 2016).

Mucoprotective agents are optional medication types used in gastric ulcer prevention that accelerates gastric ulcer healing. Mucoprotective agents exert their effects through two major mechanisms: (i) independent of any prostaglandin, for example, sucralfate (aluminium salt) agonist effect and (ii) related to prostaglandin activity (e.g. misoprostol [prostaglandin E_1 analogue], rebamipide [endogenous prostaglandin inducer], and ecabet sodium [prostaglandin E_2 inducer]) (Rapat Pittayanon, Myriam Martel, & Alan Barkun, 2018). Misoprostol has been approved for preventing ulcer and ulcer complications in patients taking antiplatelet/non-steroidal anti-inflammatory drugs. However, misoprostol is currently not widely adopted in daily clinical practice because of concerns with its side effects (Taha, McCloskey, McSkimming, & McConnachie, 2018). Some endoscopists

routinely use combination that includes a PPI and a mucoprotective agent for such patients (R Pittayanon, M Martel, & AN Barkun, 2018).

All currently approved PPIs are benzimidazole derivatives: heterocyclic organic molecules that include both a pyridine and benzimidazole moiety linked by a methylsulfinyl group (Strand et al., 2017). The prototypical example of this structure, omeprazole, was the first clinically useful PPI (Benito-Garcia, Chambel, & Morais-Almeida, 2018). Subsequently used PPI drugs include lansoprazole, pantoprazole, rabeprazole and the stereo-isomeric compounds esomeprazole and dexlansoprazole. Although each of these drugs has different substitutions on their pyridine and/or benzimidazole rings, in general they are remarkably similar in their pharmacological properties (Saniee, Shahreza, & Siavoshi, 2016). Although previous systematic reviews and meta-analyses reported that adding such a mucoprotective agent to a PPI results in better outcomes compared with using a PPI alone, there were some concerns in searching methodology and included trials (R Pittayanon et al., 2018).

PPIs are membrane permeable, acid-labile weak bases. In order to prevent premature activation and degradation by luminal gastric acid, these drugs are packaged in a variety of delivery systems. These include enteric-coated tablets, gelatin capsules, or coated granules supplied as a powder for suspension (Strand et al., 2017). They also may be packaged in combination with bicarbonate to confer temporary luminal pH neutralization (Stella, Entwisle, Newlon, & Naykki, 2017). Once clear of the stomach, PPIs are absorbed in the proximal small bowel. There are also intravenous (IV) formulations available for lansoprazole, pantoprazole, and esomeprazole, which provide immediate acid suppression and are well suited for hospitalized patients in whom the oral route of

administration is not appropriate (Al-Badriyeh, Alabbadi, Fahey, Al-Khal, & Zaidan, 2016).

Once absorbed, circulation transits the PPIs to activated gastric parietal cells where they concentrate within the acidic secretory canaliculi. Here, the PPI undergoes acid-catalyzed cleavage of a chiral sulfoxide bond (except esomeprazole and dexlansoprazole which are nonchiral) into active sulfenic acid and/or sulfonamide (Abe et al., 2017; Corsonello et al., 2018). These compounds then bind covalently to cysteine residues on the (H^+/K^+ ATPase) and act to inhibit acid secretion until replacement pumps can be synthesized (Wolfe & Lowe, 2015). Although frequently considered equivalently effective with respect to clinical parameters, the specific pharmacologic properties among individual PPIs are somewhat different e.g rabeprazol (Kim et al., 2017). PPIs require the active canaliculi expression of (H^+/K^+ ATPases) for binding which occurs in response to a meal (Kim et al., 2017). While controversial, there are suggestions that patients who are genetically rapid drug metabolizers, may be less likely to fully respond to their PPI treatment, especially *H. pylori* eradication than slower metabolizers of the drugs (Wilson & Nicholson, 2017), (Franciosi et al., 2018).

The PPIs represent the most potent inhibitors of gastric acid secretion available since, as noted above, they directly block the acid pump itself. Proton pump inhibitors are superior to H_2-receptor antagonists (H_2RAs, unlike proton pump inhibitors, are acid suppressants with no intrinsic antibacterial activity or specific pharmacokinetic interaction with antibiotics. Proton pump inhibitors have a higher antisecretory capacity than H_2RAs when prescribed at usual doses (Abourehab, 2017). But with extensive application, concerns are raised about serious adverse reactions in long-term use of PPIs (Laine &

Nagar, 2016). Although PPIs have had an encouraging safety profile, Several recent reports have raised concerns about a potential risk of serious infections among individuals treated with any of the two main groups of Invasive Group A Streptococcal Infections (IGAS), namely H2 receptor antagonists (H2) (Tabarean, 2016) and proton pump inhibitors (PPI) (Zhu & Hong, 2017), recent studies regarding the long-term use of PPI medications have noted potential adverse effects, including risk of fractures, pneumonia, *Clostridium difficile* diarrhea, hypomagnesemia, vitamin B_{12} deficiency, chronic kidney disease, and dementia (Freedberg, Kim, & Yang, 2017). These emerging effects have led to subsequent investigations to assess these potential risks in patients receiving long-term PPI therapy.

In the particular case of patients undergoing chronic peritoneal dialysis (PD), there is a specific concern that treatment with these drugs could promote peritoneal infections by enteric bacteria (Pérez-Fontan et al., 2016). However, most of these drugs produce several adverse reactions including toxicities and may even alter biochemical mechanisms of the body upon chronic usage (Daure, Ross, & Webster, 2017). There is need to study and come up with drugs of less side effects hence the need to study medicinal plants that have potential in the management and treatment of gastric ulcers.

2.3. Medicinal plants

Plants are of great importance in the environment as rural inhabitants depend on plants of their surroundings for food, and shelter (Aniama, Usman, & Ayodele, 2016). Wild plants supply medicines (Kunwar, Acharya, Chowdhary, & Bussmann, 2015), crafts and cosmetics to rural and urban communities (Etkin, 2017). Important herbal products are spices, herbal teas, functional food ingredients, medicinal raw materials, aromatic plants,

essential oils, flavoring, fragrant products and dietary supplements (Mehta et al., 2016). Plants have also been used as medicines for thousands of years all over the world. According to WHO estimates, it indicates that at least 80% of the population, mostly in developing countries still relies on plant-based medicines for primary care because they are readily available (Ahmad, Radotra, Singh, Verma, & Sultan, 2017).

Medicinal plants contain numerous biologically active compounds which are helpful in improving life and in treatment of disease: These include compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols, simple phenolic compounds and many more (Cseke et al., 2016). The presence of various life sustaining constituents in plants has made scientists to investigate many plants for their uses in treating certain infective diseases and management of diseases as well (Shakya, 2016). Medicinal plants are cheaper and more accessible to most of the population in the world. Thus, there is need to encourage the use of medicinal plants as potential sources of new drugs. Indeed, there has been a heightened interest in herbal remedies in several parts of the world (Thomford et al., 2015).

Since the beginning of this century, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world (Shakya, 2016). In spite of production of chemically synthesized drugs for a number of diseases, natural products of plant origin have their own importance and have remained one of the most important resource for developing new drugs to treat various diseases (Yuan, Ma, Ye, & Piao, 2016). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for discovering new drugs. Natural products from plants are a rich resource used for centuries to cure various ailments. Extracts of many plants are

highly efficient against parasitic as well as microbial infections (Mustafa, Arif, Atta, Sharif, & Jamil, 2017). The use of natural medicine in the treatment of various diseases like gastric ulcer disease is an absolute requirement of our time. The use of different parts of several medicinal parts to cure specific ailments has been in vogue from ancient times (Akman, Geçibesler, Kumar, Sayyed, & Zaid, 2019). Indigenous systems of medicine cater for the needs of the population residing in villages (Kunwar et al., 2015).

The use of phytoconstituents (plants) as drug therapy to treat major ailments has proved to be clinically effective and relatively less toxic than the conventional drugs (Tripathy & Afrin, 2016). No matter how much advance is made in allopathic medicine or chemotherapy, the adverse effects or side-effects cannot be ignored. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial in infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Balouiri, Sadiki, & Ibnsouda, 2016).

Natural products play an important role in drug development programs in the pharmaceutical industry (David, Wolfender, & Dias, 2015). However, World Health Organization (WHO) also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines (Hosseinzadeh, Jafarikukhdan, Hosseini, & Armand, 2015). Therefore there is need to find alternative therapies, which have negligible adverse impacts on the patient, for cure of diseases. This can be made possible by the use of traditional medicines and herbal remedies, given their low incidences of side-effects (Fu et al., 2018). Biochemical information on the role of plant drugs in preventing ulceration is of increasing

importance. The drugs from plants are fairly innocuous and relatively free of toxic effects (Palle, Kanakalatha, & Kavitha, 2018).

In recent years, secondary plant metabolites have been investigated as a source of medicinal agents (Sharma, Pramanik, & Agrawal, 2016). The phytochemicals with adequate antibacterial activity are used for the treatment of bacterial infections. Herbal medicines have great importance in maintaining the health of every person (A.-h. Zhang et al., 2018). Plants have several pharmacological roles such as an antioxidant, antiviral, anticancer, antimicrobial, antifungal and antiparasitic agent. Plants have free radical scavenging molecules, including flavonoids, phenolics, anthocyanins and vitamins, which show antioxidant like activity (Shakya, 2016). Demands of herbal medicines are increasing in both developed and developing countries due to growing recognition of natural plants having lesser side effects, are easily available in the immediate environment, and are of low cost (Ijaz et al., 2018).

2.4. Anti-ulcer medicinal plants

2.4.1. General information on the anti-ulcer plants

Natural products have been reported to mediate anti-gastric ulcer effects via various modes of action: neutralization of the gastric acid (Drake & Hollander, 1981; Jacob et al., 2016), increasing antioxidant activity (Al-Wajeeh et al., 2016; Boeing et al., 2016), metabolization of lipid peroxide (Bin-Jumah, 2019; Chen, Lu, Jin, Yu, & Shi, 2019; Koushki, Yekta, Amiri-Dashatan, Dadpay, & Goshadrou, 2019), reduction of gastric lesion (Kwenda, Rabley, & Canales, 2020), scavenging, endogenous hydrogen peroxide,

inhibition of pepsinogen production (Re et al., 2016), restoration of the epithelial cell (Ayla et al., 2019; Ottomann et al., 2010) and protection of the gastric wall.

Attempts have been made to know about some plants - and their constituents - which may be used in treatment of gastric ulcer. Various plants like *Anogeissus latifolia,(Kala, 2016) Alchornea castaneaefolia (Madan et al., 2018), Utleria salicifolia (Tripathy & Afrin, 2016), Solanum nigrum (Geetha et al., 2017), Ocimum sanctum, Asparagus racemosus,* Scoparia *dulcis, Byrsonima crassa, Allophylus serratus Kurz, Aloe vera* (L.) Burm.f., *Butea frondosa Roxb, Butea frondosa Roxb* etc. and their phyto-constituents have proved active in antiulcer therapy (Jivad & Bahmani, 2016).

Previously, a number of other plants have been tested for antacid activity. The root of *Tephrosiapurpurea (L.) Pers* was tested in vitro, with a modified Vatier's artificial stomach model, in order to measure the antacid activity in methanol, ethylacetate and chloroform extracts (C. B. Christensen, J. Soelberg, & A. K. J. J. o. e. Jäger, 2015b). The methanolic extract had a higher antacid activity than sodium bicarbonate, whereas the other two extracts showed moderately good response, but less than the standard (Sandhya et al., 2012). Aqueous extracts of the fruit rind of *Garcinia indica* was tested for its antacid activity also using the modified Vatier's artificial stomach model, and the extracts showed potent antacid activity when compared to water, but was not as good as the standard sodium bicarbonate (Panda and Khambat, 2013).

2.4.2. *Bidens pilosa*

Bidens pilosa L. is a species of a flowering plant of the Asteraceae family and belongs to the *Bidens* genus, which comprises approximately 280 species (Hao, Bhattacharya, Ma,

& Wang, 2018). *Bidens pilosa* is an annual plant and originated from South America, but it is now widely distributed in most tropical areas of the world (Ramadan, Amro, & Abd-Almoneim, 2018), (Ooka & Owens, 2018) and in African countries like Kenya. The weed is widespread throughout several counties in Kenya, including Migori. It's variants include pilosa var., radiata var., minor var., odorata var., alba var., bimucronata var., bisetosa,calcicola, and alausensis (Khanh et al., 2009).

Bidens pilosa species has been recognized as a serious weed as it has explosive reproductive potential and ability to thrive in diverse habitats, and is thus a potentially invasive weed in different parts of the world (Lemos et al., 2013),(Cui & He, 2009). This plant is a tall branched weed with thin white and yellow flowers, that develop into a cluster of barbed seeds (Rana, Badiyala, Sharma, & Kumar, 2015). It grows with numerous ridged branches, reaches over two meters high under favorable conditions, and is commonly called by many vernacular names such as hairy beggar tick, spanish needles, devil needles, black jack, railway daisy, famers friends, onyiego and pitchforks (Stevenson & Waite, 2011; Vilà et al., 2011; K.-m. Zhang et al., 2019). Leaves are opposite, petioled, pinnate, with 3–5 sharply serrated ovate leaflets (Xu & Chang, 2017; Xuan & Khanh, 2016) and are slightly hairy. *Bidens pilosa* is easily recognized by its elongated budlike achenes that are curved or hooked bristles, a device that insures its dissemination.

The branches and stems are marked with parallel lines or ridges that are smooth and green or with brown stripes (Malan, 2018) . The tiny inflorescence is a capitulum (congested head of flowers) with yellow centers and white ray petals and the achenes are blackish, narrow, ribbed, and sparsely bristled to smooth (Xuan & Khanh, 2016). The

seeds are dark brown or black, slender, reach 1 cm in length, and are clustered on the end of the stalk (Madzinga, Kritzinger, & Lall, 2018).

The characteristics of *Bidens pilosa* seeds allow them to be widely dispersed by wind. The seeds are like short, stiff hairs (Pyšek et al., 2017). They get stuck in feeders, fur or socks. This bur is widespread throughout the warmer regions of the world, the little black seeds hook unto cloths or horses and thereby the bur spread itself around. It is susceptible to hand weeding if small enough and thick mulches may prevent it from growing. Each seed has two to four barbed spines. A weed of gardens, woodlands and waste areas, a person who brushes against it will end up covered in the burs and need to pick them off one by one. Although this plant is considered a weed in many parts of the world, in other parts it is a source of medicine (L. Zhang, Smyrk, Young Jr, Stratakis, & Carney, 2010).

Figure 2.1. *Bidens pilosa* plant from (Source: Natural medicine facts)

CHAPTER THREE

METHODOLOGY

3.1. Chemicals, reagents and consumables

Hexane 85%, Dichloromethane 99.5%, Acetone 99.5%, Methanol 99.8%, Acetonitruile 99.9%, trifluoroacetic acid 98%, Gallic acid 99.5%, silica gel, Folin and ciocalteu's phenol reagent, quercetin, pepsin from porcine mucosa were obtained from Loba Chemie PVT.LTD, Pyrex Kenya. All were of analytical grade. TLC plates and filter papers were obtained.

3.2. Equipment

Hot plate and a hand-held UV light source (365nm) were obtained from Omicron Laserage Laserproducte GmbH, Germany. Centrifuge (80-1), Peristaltic pump was obtained from Zhengzhou Mingyi Instrument Equipment Co. Ltd, China. Classical column chromatography was obtained from Kobian, Kenya and Beckman coulter UV-Vis spectrophotometer, North America. US. GCMS/MS chromatography was obtained from Shimadzu Europa Analytical Instruments, Japan.

3.3. Sample collection, identification and coding

Fresh *Bidens pilosa* plant was identified by a botanist from Moi University and leaves were obtained from Moi University farm, Eldoret at coordinates (latitude 0^0, 17' 22.2" N, longitude 35^0, 17' 50.5" E).

3.4. Preparation of crude extract

The collected *B. pilosa* leaves were washed under running tap water to remove any dust particles, air dried at room temperature in the shade for two weeks, and then ground. 10 grams of *B. pilosa* leaf's powder was weighed and Soxhlet extracted with 200 ml of hexane for 8 hours (Mogana *et al*, 2011), followed by extraction with 200ml of dichloromethane, methanol and a mixture of dichloromethane and methanol in the ratio 1.1 with each of the 3 extracts collected separately.

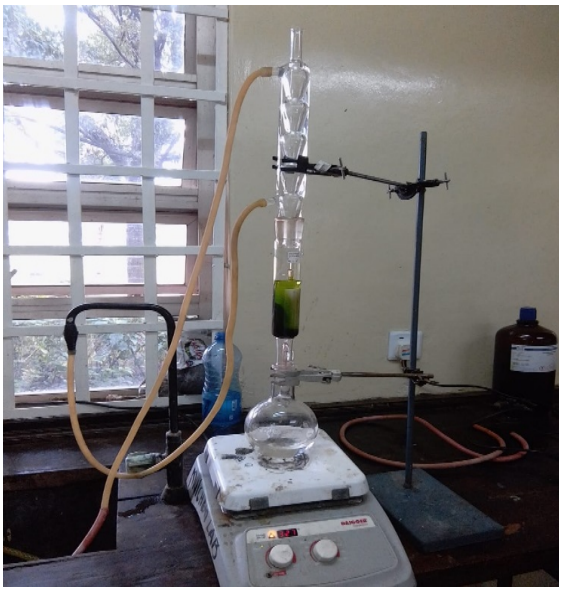


Figure 3.1. Soxhlet extraction system that I set in the laboratoty

The extracts were decanted and filtered using Whatman No. 1 Filter Paper, and concentrated using a rotary evaporator at 60 ^{0}C to remove volatile solvent from the nonvolatile solvent of interest, followed by drying under vacuum.

3.5. Phytochemical Analysis

Each of the three crude plant extracts was subjected to phytochemical screening. Phytochemical screening was done to assess the qualitative chemical composition of the

extracts by using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like flavonoids, saponins, tannins, phenols, alkaloids, terpenoids, anthraquinones, cardiac glycosides, steroids according to (Pandey & Tripathi, 2014).

3.5.1 Analysis of Flavonoids

The alkaline reagent test was used. A 2 ml plant extract was treated with two drops of 1 % sodium hydroxide. Intense yellow color solution became colorless on addition of 0.1M HCl and indicates the presence of flavonoids.

3.5.2 Analysis of Phenols

Ferric chloride test was used. 2 ml of plant extract was treated with two drops of neutral 5% ferric chloride solution and the formation of a dark green color indicates the presence of phenols.

3.5.3 Analysis of Alkaloids

Wagner’s reagent test was used. To 2 ml of plant extract, two drops of Wagner’s reagent were added along the sides of the test tube and the reddish- brown color shows the presence of alkaloids.

3.5.4 Analysis of Tannins

To 5 ml of plant extract, two drops of 1 % iron (III) chloride was added. Formation of a brown solution indicates the presence of tannins.

3.5.5 Analysis of Cardiac glycosides

Keller-kiliani test was used. 50 mg of plant extract was treated with 1ml glacial acetic acid and two drops of 5% ferric chloride. To this solution, two drops of

concentrated sulphuric acid was added, and the appearance of a greenish blue color within a few minutes indicates the presence of cardiac glycosides.

3.5.6 Analysis of Steroids

Liebermann-Burchard test was used. 1 ml of plant extract was treated with 2 ml acetic anhydride and then two drops of concentrated H_2SO_4 were added down the sides of the test tube. Formation of a blue green ring indicates the presence of steroids.

3.5.7 Analysis of Saponins

Frothing test was used. The plant extract (50 mg) was diluted with distilled water up to 20 ml and shaken for 15 minutes in a graduated cylinder. The formation of 2 cm thick foam shows the presence of saponins.

3.6. Total phenolic content (TPC) determination

Total phenolic content was determined spectrophotometrically by using Folin-ciocalteu method (Nile, Nile, & Keum, 2017)., which utilizes 10 % follin reagent 15 % sodium carbonate and gallic acid. To prepare the gallic acid stock solution, 1g of gallic acid was transferred into a 1000 ml volumetric flask and dissolved with distilled water. Then more distilled water was added up to the mark to give a 1000 µg/ml (ppm) stock solution. To prepare 10 % folin reagent, 10 ml of folin reagent was taken into a 100ml volumetric flask and 100ml of distilled water was added into it. Preparation of 15% sodium carbonate was done by dissolving 15g of sodium carbonate in 100 ml of distilled water.

3.6.1. Experimental procedure

An aliquot of 0.5 ml of each of the extracts (dichloromethane, methanol and dichloromethane : methanol (1:1)) was mixed with 2.5 ml of 10 % folin reagent and 2.5 ml of 15 % sodium carbonate. The mixtures were incubated at 25 ^{0}C for 20 minutes in the dark and their absorbances were measured using Beckman coulter UV-Vis spectrophotometer (USA) at a fixed wavelength of 725 nm. The 1000 µg/ml gallic acid stock solution was used to prepare working solutions of 20 µg/ml, 40 µg/ml, 75 µg/ml, 100 µg/ml, and 125 µg/ml. 500 µl of each working solution was measured and mixed with 2.5 ml folin reagent and 2.5 ml Sodium carbonate, followed by incubation at 25 ^{0}C for 20 minutes in the dark, and thereafter UV-Vis spectrophotometric analysis at 725 nm. The total flavonoid content was calculated using formula 3.1 below

Equation 3.1. Total phenolic content

$$TPC = \frac{Absorbance\ of\ galic\ acid \in mg}{Absorbance\ of\ standard \times Mass\ of\ extrcat \in mg}$$

…….Equation 3.1

The result was expressed as mg gallic acid equivalent (GAE) per gram of extract based on the standard curve of gallic acid.

3.7. Total Flavonoid Content (TFC) Determination

Total flavonoid content of leaf extracts was determined by aluminium chloride calorimetric method (Idris, Linatoc, Muhammad, Aliyu, & Bakar, 2018), which utilized quercetin, 10% aluminium chloride and 1M potassium acetate. To prepare the standard; 1mg of quercetin was dissolved into 1ml distilled water to make the concentration 1000µg/ml (ppm) stock solution. To prepare 10% aluminium chloride solution; 10g of

$AlCl_3$ was taken in 100ml volumetric flask and small amount of distilled water was added to dissolve and more distilled water was added to make it to the mark. To Prepare 1M potassium acetate solution; 9.8g of potassium acetate was taken in 100ml volumetric flask and small amount of distilled water was added to dissolve and more distilled water was added to make it to the mark.

3.7.1. Experimental procedure

1.0ml of extracts/standard of different concentrations (10µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml and 100 µg/ml were separately placed in a test tube, 3ml of methanol was added into the test tube, 200µl of 10% aluminium chloride and potassium acetate were added into the test tube, 5.6 ml of distilled water was added into the test tube. They were then incubated at 25^0C for 30 minutes to complete the reaction and thereafter UV-Vis spectrophotometric analysis at 420nm. The flavonoid content of the extract was expressed as mg of quercetin equivalent/g of dried extract. The total flavonoid content was calculated using formula 3.2 below.

Equation 3.2. Total flavonoid content

$$TFC = \frac{Absorbance\ of\ quercetin \in mg}{Absorbance\ of\ standard \times Mass\ of\ extrcat \in mg} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

Equation 3.2

3.8. UV-VIS Spectrum analysis

The concentrated extracts of dichloromethane, methanol and dichloromethane: methanol were centrifuged at 3000 rpm for 15 min and filtered through Whatmann No.1 filter paper. Aliquots of the concentrated extracts were diluted at a ratio of 1:10 with the respective corresponding extraction solvents (dichloromethane, methanol and

dichloromethane: methanol). The extracts were scanned at wave lengths ranging from 200 to 800 nm using UV-VIS Spectrophotometer according to (Omotoso, Olorunfemi, Mikailu, & Research, 2014) and the characteristic peaks were detected. The peak values of the UV-VIS were recorded.

3.9 Chromatographic analysis of phenolic compounds from *Bidens pilosa* leaf extract

Dichloromethane: methanol (1:1) extract was chosen for fractionation because it showed the highest total phenolic and flavonoid content. Classical column chromatography was used for the cleanup and TLC to merge the fractions.

3.9.1 TLC analysis

Using a pencil, a faint line was drawn at the edge of the TLC plate. The sample 1g containing the compounds to be separated was dissolved in a DCM: methanol (1:1) solvent mixture. A small amount of the solution was drawn up into the tip of the pipette glass and lightly spotted onto the baseline of TLC plate. The spotting of the compound was repeated in the same place to accumulate enough sample. The spotted TLC plate was then placed in the elution tank containing DCM: methanol (1:1). When the eluent neared the top of the plate, the plate was removed, the solvent front marked, and a UV lamp at 365nm was used to determine the spots according to (Meyers & Meyers, 2008).

3.9.2 Column Chromatography

3.9.2.1. Packing

C_{18} column was taken and supported by a clamp and stand, a cotton wool was put on the stop cock (Wang, Zhang, Yan, Han, & Sun, 2014), 180g of silica gel was dissolved in 600

ml of dichloromethane to form a slurry, which was delivered to 1/3 of the column and left for 24 hours to settle followed by draining of the solvent to circa 1 ml above the stationary phase (to prevent drying of the stationary phased). The mobile phase (dichloromethane) was added to clean up the stationary phase.

3.9.2.2. Loading

5ml of the concentrated sample was added gently along the column. Then serial elution was carried out using different solvent systems of increasing polarity as follows: 100 ml DCM, 100 ml DCM: acetone (1:1), 100 ml acetone, 100 ml acetone: methanol (3:1), 100 ml acetone: methanol (1:1) and 100 ml methanol. Each of the elution fractions was collected separately. The obtained fractions were subsequently subjected to TLC for further identification, and fractions with similar R_f values were pooled together into one fraction.

3.10 Clean-up (concentration) and characterization of phenolic compounds

3.10.1 Solid phase extraction (SPE) clean-up and concentration

Solid phase extraction clean up procedure used the Sep Pak C_{18} cartridge.

The cartridges were conditioned with 5 ml methanol followed by 5 ml Milli-Q water at 1.2 ml/min, using a water aspirator connected to the cartridge pack as vacuum source. 2 ml from each fraction was passed through the cartridges. The cartridges were then washed with 2 ml Milli-Q water. The compounds were eluted from the cartridges with 2 ml aliquots of Methanol: Acetonitrile (1:1 v/v).The SPE elutes were lyophilized to dryness and the dried residues were reconstituted in 1 ml methanol for subsequent characterization.

3.10.2 Characterization of phenolic compounds

Characterization was based on the approach of (Bi, Zhou, & Row, 2012).This included Fourier Transform Infrared Spectroscopy (FT-IR) analysis of KBr pellets at 400-4000cm^{-1} and Gas chromatography-Mass Spectrometry (GC-MS) for quantitative analysis.

3.10.2.1. FT-IR analysis

Potassium bromide (KBr) based pellets were prepared by establishing pressure of 10 kg/cm^2 for about 30 seconds. A pure KBr tablet was used as a blank for background subtraction. Loopfuls from the isolates were placed on the KBr disk and read at wave numbers ranging between 4000-400cm^{-1}. Polystyrene infrared was used as the standard.

3.10.2.2 GC-MS/MS Screening

Sample Preparation

The dichloromethane: methanol (1:1) fraction samples were diluted in dichloromethane: methanol (1:1) mixture of solvents, then filtered through 0.22 µm PTFE syringe filters and transferred to 2 ml vials for GC-MS analysis.

A Shimadzu QP 2010-SE GC-MS coupled to an auto sampler was used for the analysis. Ultrapure Helium was used as the carrier gas at a flow rate of 1ml / minute. A BPX5 non polar column, 30m; 0.25 mm ID; 0.25 µm film thickness, was used for separation. The GC temperature program was: 50 ^{0}C (1 minute); 10 ^{0}C/min to 180 ^{0}C (1 min); 3 ^{0}C/min to 250 ^{0}C (22 min). Total run time was 60 minutes. Only 1 µL of the sample was injected at 200 ^{0}C in split mode (10:1). The interface temperature was 250 ^{0}C and the EI ion source temperature was 200 ^{0}C. Mass analysis was done in full scan mode, 50-700 nm (to accommodate high molecular-weight-compounds), with an initial solvent-delay time of 3

minutes. Raw mass spectra were matched against the NIST 2014 library of mass spectra for possible identification of compounds.

3.11. Preparation of standard solutions for gastric acid neutralization experiment

Sodium bicarbonate was dissolved in distilled water to make 400 mg/kg and 800 mg/kg solutions and these were used as the control standards for the neutralizing capacity of low dose (LD) and high dose (HD) *B. Pilosa* phenolic fractions, respectively. Preparation of artificial gastric acid was done according to the method of (Wu, Chen, & Chen, 2010). Briefly, 2 g of NaCl and 3.2 mg of pepsin were dissolved in 500 ml distilled water. Hydrochloric acid (7.0 ml) and adequate water were added to make a 1000 ml solution. The pH of the solution was adjusted using 12.8 M HCl to 1.2, the pH of stomach gastric acid. This gastric acid was then used in the artificial stomach model as described in section 3.14 and illustrated in figure 3.2.

3.12. pH determination of phenolic compound fractions of *Bidens pilosa* leaves

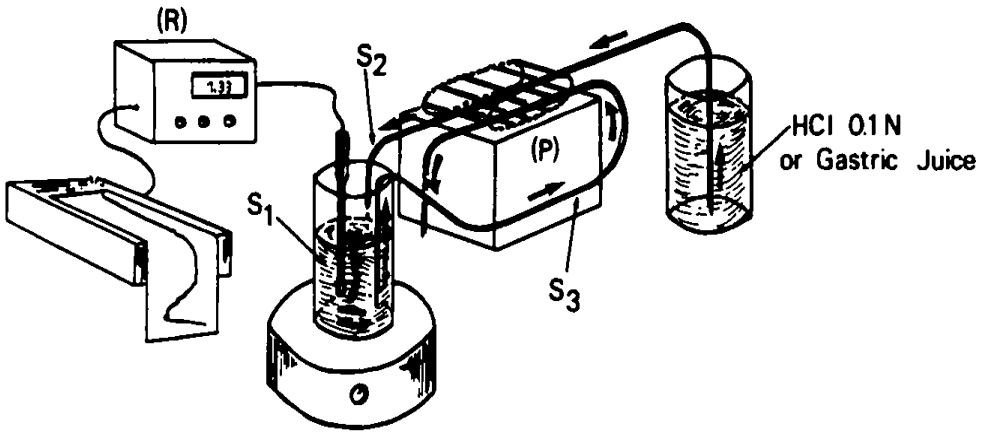
The pH of 400 mg/kg (LD) and 800 mg/kg (HD) of the fractions were determined at temperatures ranging 25 ^{0}C-37 ^{0}C. The pH of sodium bicarbonate (400 mg/kg (LD) and 800 mg/kg (HD)) and water were also determined using a pH meter for comparison.

3.13. Determination of the neutralizing effect of the extracts on artificial gastric acid.

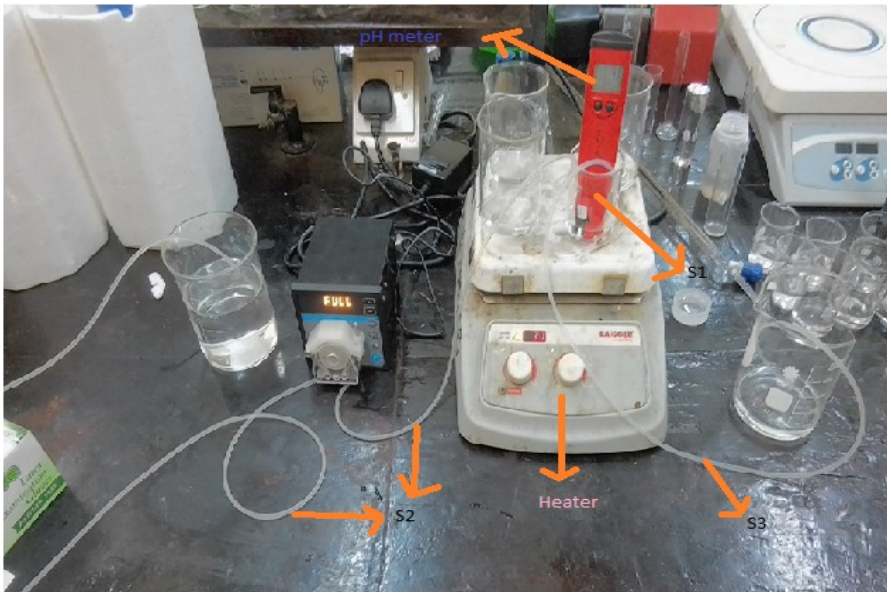
The two sodium bicarbonate standards and *B. Pilosa* phenolic fractions solutions (400 mg/kg for LD and 800 mg/kg for HD), as well as water (control), each were added separately to artificial gastric acid at pH 1.2 in the ratio 9:10. The pH values of the

resultant solutions were evaluated to examine the neutralizing effect of the *B. Pilosa* phenolic fractions.

3.14. Determination of the duration of consistent neutralization on artificial gastric acid using the modified artificial stomach model



(a)



(b)

Figure 3.2. (a) Structure of modified artificial stomach (Castela-Papin et al., 1999), (b) artificial stomach I modelled in the laboratory.

- The apparatus of the modified artificial stomach model was made up of;
    a) A pH meter (R)
    b) Stomach (S)
    c) A peristaltic pump (P)

A sketch of an artificial stomach is provided in figure 3.2a. It is made up of three parts; S1- reservoir container, S2- modeled secretory flux (F-IN) and S3- modeled gastric emptying flux (F-OUT). Figure 3.2b shows the artificial stomach that was set up in the laboratory.

Each freshly prepared test solutions were added to artificial gastric acid at pH of 1.2 in the ratio 9:10 in the container of the artificial stomach at 37 ^{0}C and continuously stirred at 30 rpm with 2.5 cm magnetic stirring apparatus. Artificial gastric acid at pH of 1.2 was pumped at 3 ml/min into the container of the artificial stomach S_1 through S_2 and simultaneously pumped out at 3 ml/min by S_3. A pH meter was connected to continuously monitor the changes of pH in the container of artificial stomach. The duration of the neutralization effect was determined when the pH value returned to its initial value of 1.2.

3.15. Determination of the neutralizing capacity using the titration method.

Each of the freshly prepared test solution was placed in a 250 ml beaker and warmed to 37 ^{0}C. A magnetic stirrer was continuously run at 60 rpm to imitate the stomach movement. The samples were titrated with artificial gastric acid to the end point of pH 3.

The consumed volume of artificial gastric acid was measured. The total consumed H^+ (mmol) was measured as 0.063096 mmol/ml × V (ml).

3.16. Quality assurance and quality control

For UV-Vis analysis, external linear standard calibration curves were used for the quantification of total phenolic content and total flavonoid content of the *B. pilosa* extracts. The limit of quantification (LOQ) was 20 ppm because it gave an absorbance of 0.1 (figure 3.3) and 20ppm with an absorbance of 0.04 (figure 3.4) respectively. Only samples with absorbance between 0.1 and 1 were quantified: Those with absorbance above 1 were diluted and re-analyzed. Those with absorbance below 0.1 were concentrated or reported as 'not detected'. The calibration curves were generated every day that analysis was carried out. The integrity of the calibration curve was confirmed after every 10 samples by analyzing the calibration standards.

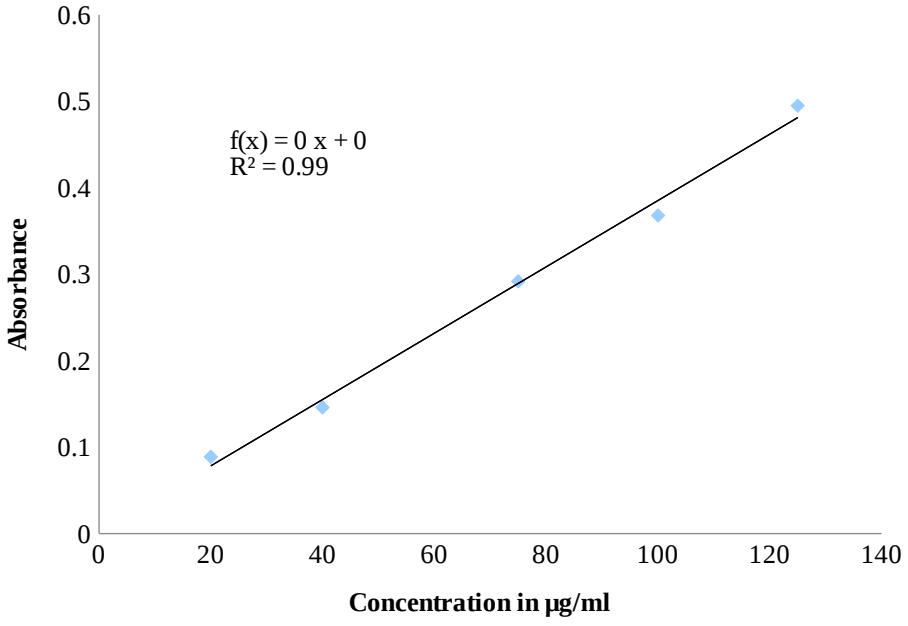


Figure 3.3.

Standard curve of Gallic acid for total phenol content determination

As shown in figure 3.3, a good correlation coefficient (r^2 = 0.9936) was obtained from the standard curve.

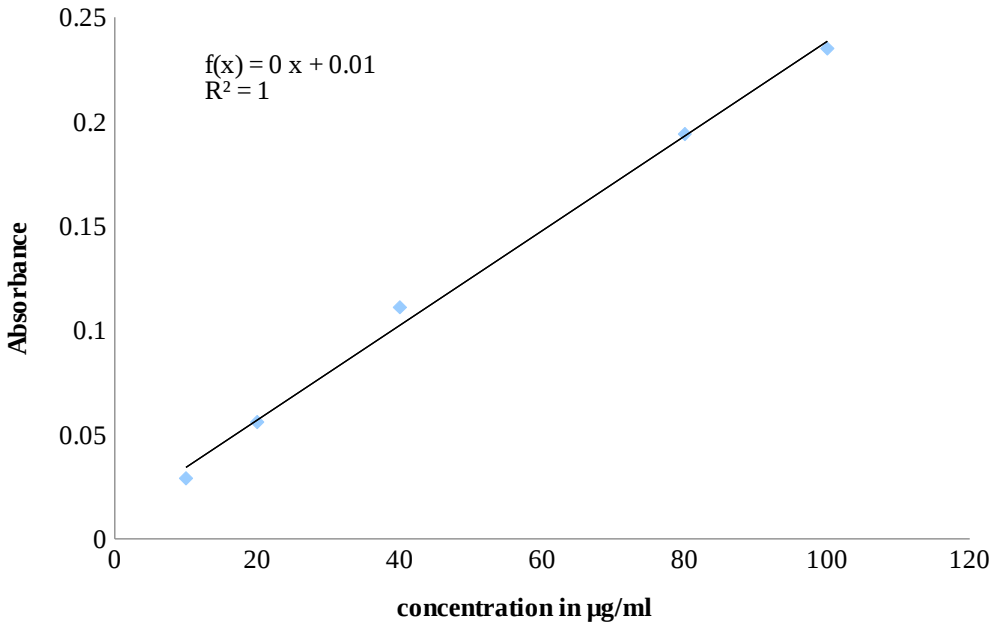


Figure 3.4. Standard curve of Quercetin for total flavonoid determination

As shown in figure 3.4, a good correlation coefficient (r^2 = 0.9962) was obtained from the standard curve.

For FT-IR analysis, FT-IR spectra were analyzed for the quantification of functional groups of phenolic compounds in *B. pilosa* extracts. The limit of quantification (LOQ) was 493.41 which was identified in fraction 6. For GC-MS/MS analysis, A Shimadzu QP 2010-SE GC-MS coupled to an auto sampler was used for the analysis and compared to NIST library, the molecular weight, retention time and peak numbers were considered for comparison between the phenolic compounds that were identified. The limit of quantification (LOQ) was 3.085 for all the fractions' retention times. The septum was at a

rate of 1ml/minute for all the samples, the column was conditioned after every 3 minutes. The GC-MS was kept running throughout the analysis to ensure stability.

3.17. Statistical analysis

All data were analyzed by Microsoft Excel. The data obtained was presented as means and standard error of means (mean ± SEM). Comparisons between the groups were analyzed by one-way analysis of variance (ANOVA). Student's t-test was performed to check whether two sets of data were statistically different from each other. The differences were considered to be statistically significant when $P < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Phytochemical screening of *B. pilosa* leaf extracts

The *B. Pilosa* leaf extracts were analyzed for identification of phenolic compounds and other phytochemicals. Results are shown in table 4.1 below.

Table 4.1. Qualitative analysis of phytochemicals extracted from *B. pilosa* leaves

Phytochemical	Chemical test	Solvents		
		DCM	Methanol	DCM: Methanol
Phenols	Ferric chloride test	++	+++	+++
Flavonoids	Alkaline reagent test	++	+++	+++
Alkaloids	Wagner's reagent test	+	++	++
Tannins		-	++	++
Cardiac glycosides	Keller-kiliani test	++	++	++
Saponins	Frothing test	+	++	++
Steroids		-	-	-

Key: dichloromethane (DCM) '+++' (abundant); '++' (present); '+' (trace amounts); '-'(not detected).

Phenols and flavonoids were the most abundant (Table 4.1). Other than phenols and flavonoids, various phytochemical compounds including cardiac glycosides, tannins, alkaloids and saponins were also present, while steroids were not detected at all. For phenols and flavonoids, the highest yields were in the methanol and dichloromethane: methanol (1:1) extracts. This can be attributed to phenol's high solubility in methanol, as well as in the polar: nonpolar solvent mixture, while the low amounts in dichloromethane

can be attributed to the low solubility of phenol in the solvent (Le Floch, Tena, Rıos, & Valcarcel, 1998).

Alkaloids, saponins and tannins showed higher yields in the methanol and dichloromethane: methanol (1:1) extracts compared to the dichloromethane extract, and this can also be attributed to the solubility of these compounds in the solvents. Cardiac glycosides yields were comparable in all the extracts and this can be explained by reports which indicate that the compound has uniform solubility in organic solvents (Vilas Boas, 2017). These results are comparable to findings reported on the phytochemical constituents of *Areca catechu* leaf extracts that were tested on ethanol/HCl and indomethacin induced gastric ulcer in ICR mice, and showed gastro-protective effects (Kang Pa Lee et al., 2016). These six phytochemicals have been shown to have gastroprotective, anti-inflammatory and antibiotic activity (Oyinloye, Olooto, Kosoko, Alabi, & Udeh, 2019; Ugwah, Ugwah-Oguejiofor, Etuk, Bello, & Aliero, 2019) and may be responsible for gastro-protective effects of *B. pilosa* leaves.

4.2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Determination

The total phenolic contents (in mg of gallic equivalent per gram dry weight) and total phenolic content (in mg of quercetin equivalent per gram dry weight) of the leaves of *Bidens pilosa* for all crude extracts obtained are shown in Table 4.2. The highest amounts of phenols and flavonoids were found in the dichloromethane: methanol extract at 288.7± 1.27 mg GAE/g and 274.7 ± 2.02 mg QE/g (of dry weight of extract) respectively while the lowest were recorded in the dichloromethane extract at 83.1 ± 0.29 mg GAE/g and 51.5 ± 0.58 mg QE/g (of dry weight of extract) respectively. The results show that

dichloromethane: methanol *B. pilosa* leaves extract contained a high phenolic and flavonoid content related to more phenolic compounds, which may contribute to their antiulcer activity hence guiding the selection of the extract for the chromatographic analysis.

Table 4.2. Total Phenolic Contents and Total Flavonoid Content for Crude Extracts

Sample	**Total phenolic (mg/g)**	**Total Flavonoid (mg/g)**
Dichloromethane	83.1 ± 0.29	51.5 ± 0.58
Methanol	268.4 ± 0.73	233.5 ± 0.58
Dichloromethane: methanol	288.7 ± 1.27	274.7 ± 2.02

(±) means standard error of the mean

4.3. UV–Visible absorption spectrum *Bidens pilosa* leaf extracts

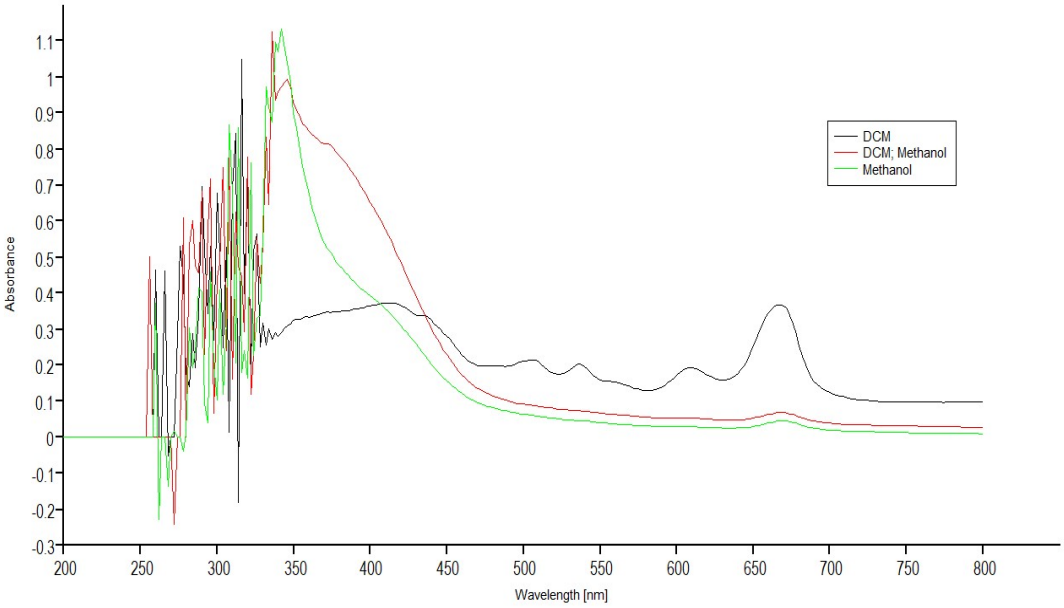


Figure 4.1. UV–Visible absorption spectra of *Bidens pilosa* leaf extracts in different solvent systems (DCM, DCM: Methanol, Methanol)

Figure 4.1 shows the UV-VIS spectra of the *Bidens pilosa* leaf extracts. The dichloromethane extract had absorption peaks at 316, 412, 506, 668 and 786 nm with absorbance of 1.049, 0.373, 0.123, 0.367 and 0.099 respectively. The dichloromethane: methanol (1:1) extract had absorption peaks at 336 and 666 nm with absorbance of 1.126 and 0.068, respectively while the methanol extract had absorption peaks at 342 and 667 nm with absorbance of 1.133 and 0.045, respectively. Phenolic and other compounds are usually classified on the basis of their characteristic UV-Vis spectra (Dankowska & Kowalewski, 2019). For instance, these absorption bands are characteristic for hydroxycinnamic acid derivatives (phenols) at 300-320 nm, jervine (alkaloids) at 330-360 nm (Sangster & Stuart, 1965), tannic acid (tannins) at 520-550 nm (Lopes,

Schulman, & Hermes-Lima, 1999) and anthocyanins (a flavonoid) at 500-520 nm, (Aaby, Ekeberg, Skrede, & Chemistry, 2007) which are attributed to phenolic compounds.

4.4. Chromatographic analysis of phenolic compounds from *Bidens pilosa* leaf extract.

The dichloromethane: methanol (1:1) extract gave thirty-nine fractions. The obtained fractions were subsequently subjected to TLC for further identification, and fractions with similar R_f values, were pooled together into one fraction which resulted into 8 fractions which are shown in figure 4.2.

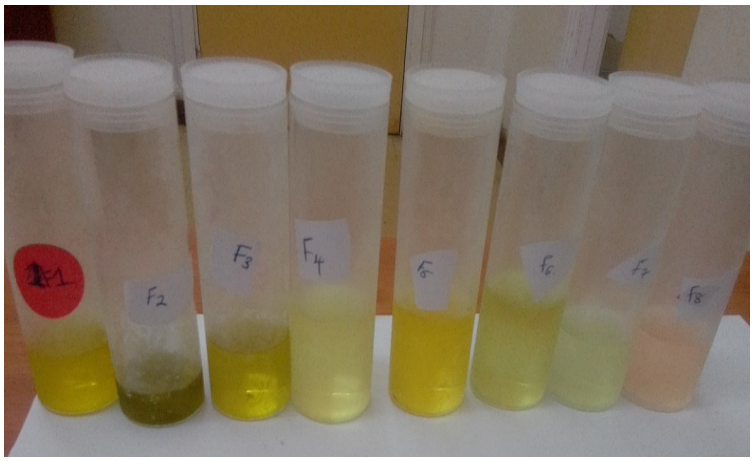


Figure 4.2. Combined fractions using Rf values

4.5. Functional groups identification by FTIR

Fourier Transform Infrared Spectroscopy (FT-IR) spectroscopy was used to identify the functional groups of the active components present in the fractions. The resultant spectra (appendix 1 to 9) were studied and the identified peaks are presented in table 4.3 to 4.11.

Table 4.3. FTIR peak values of polystyrene (standard)

S. No.	Peak values	Possible bond	Functional groups
1	3792.37		Phenols
2	3062.32	C=C	Aromatics
3	3027.05	C=C	Alkenes
4	2923.41	C-C	Alkanes
5	2852.00	C-C	Alkanes
6	1944.82		Unknown
7	1873.34		Unknown
8	1804.12	C=O	Acid chlorides
9	1743.41	C=O	Amides
10	1667.50	C=O	Amides
11	1598.97	N-H	Amines
12	1541.96	N=O	Nitro compounds
13	1491.54	C-O	Ethers
14	1448.53	C-C	Alkane
15	1369.23	N=O	Nitro compounds
16	1320.67	C=O	Acid
17	1185.90	N-C	Amines
18	1155.46	C-O	Ethers
19	1068.59	C-O	Ethers
20	1028.20	C=O	Esters (acyl)
21	969.26	C=C	Alkene
22	906.72	C=C	Alkene
23	842.63	C=C	Aromatics
24	755.24	C-H	Alkene
25	697.00	C-H	Alkene
26	542.47		Unknown

Table 4.4. FTIR peak values of fraction 1

S. No.	Peak value	Possible bond	Functional group
1	3943.16		Unknown
2	3883.42		Unknown
3	3827.39		Unknown
4	3783.55		Unknown
5	3473.77-3418.58	O-H	Alcohol
6	3254.81	O-H	Carboxylic acid
7	2315.95		Unknown
8	2089.18		Unknown
9	1593.57	N-H	Amines

10	1397.36	O-H	Carboxylic acid
11	1212.85	C=O	Acids(acyl)
12	1118.61	C-O	Ethers
13	992.16	C=C	Alkenes
14	912.22	C=C	Alkenes
15	702.94	C=C	Alkenes

Table 4.5. FTIR peak values of fraction 2

S. No.	Peak values	Possible bond	Functional group
1	3840.18		Unknown
2	3789.94		Unknown
3	3436.68	O-H	Alcohol
4	2929.50	C=O	Acids
5	2380.85		Unknown
6	2347.72		Unknown
7	1595.74	N=O	Nitro compounds
8	1396.51	O-H	Carboxylic acid
9	1121.95	C-O	Ethers
10	1069.51	C-O	Alcohol
11	914.54	C=C	Alkene
12	696.50	C=C	Aromatic
13	620.18	C≡C	Alkynes
14	494.84		Unknown

Table 4.6. FTIR peak values of fraction 3

S. No.	Peak value	Possible bond	Functional group
1	3789.70		Unknown
2	3769.35		Unknown
3	3424.97	O-H	Alcohol
4	1596.10	N=O	Nitro compounds
5	1395.69	O-H	Carboxylic acid
6	1120.92	C-O	Ethers
7	1069.12	C-O	Ethers
8	697.75	C=C	Alkenes

Table 4.7. FTIR peak values of fraction 4

S. No.	Peak value	Possible bond	Functional group
1	3823.04		Unknown
2	3770.23		Unknown
3	3447.88-3416.33	O-H	Alcohol
4	2354.06		Unknown
5	2318.44		Unknown
6	1595.15	N=O	Nitro compound
7	1394.80		Unknown
8	1121.08	C-O	Ethers
9	1068.55	C-O	Primary alcohol
10	618.30		Unknown

Table 4.8. FTIR peak values of fraction 5

S. No.	Peak value	Possible bond	Functional group
1	3789.90		Unknown
2	3430.85	O-H	Alcohol
3	1595.70	N=O	Nitro compounds
4	1394.54		Unknown
5	1121.68	C-O	Ethers
6	1069.57	C-O	Ethers
7	913.72	C=C	Alkenes

8	693.79	C=C	Alkenes
9	621.81	C≡C	Alkynes

Table 4.9. FTIR peak values of fraction 6

S. No.	Peak value	Possible bond	Functional group
1	3789.82		Unknown
2	3770.36		Unknown
3	3426.02	O-H	Alcohol
4	2351.13		Unknown
5	2316.34		Unknown
6	1595.31	C=O	Nitro compounds
7	1396.71	O-H	Carboxylic acid
8	1122.35	C=O	Esters
9	1069.39	S=O	Sulfoxide
10	914.97	C=C	Alkene
11	696.68		Unknown
12	620.69	C≡C	Alkyne
13	493.41		Unknown

Table 4.10. FTIR peak values of fraction 7

S. No.	Peak value	Possible bond	Function group
1	3776.17		Unknown
2	3422.71	O-H	Alcohol
3	2429.10	C=O	Acids
4	2315.83		Unknown
5	1594.56	C=O	Amides
6	1397.96	O-H	Carboxylic acid
7	1216.68		Unknown
8	1118.79		Unknown
9	1069.35		Unknown
10	992.70	C=C	Alkene
11	699.96		Unknown

Table 4.11. FTIR peak values of fraction 8

S. No.	Peak value	Possible bond	Functional group
1	3389.38	N-H	Amines
2	2351.24		Unknown
3	2320.77		Unknown
4	1556.49	N-H	Amines
5	1396.43	O-H	Carboxylic acid
6	1214.92	C=O	Acids
7	1121.34	C=O	Esters
8	1068.86		Unknown
9	992.97	C=C	Alkenes

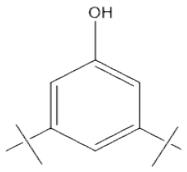
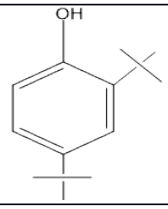
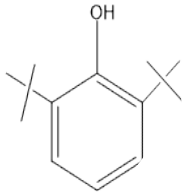
10	915.14	C=C	Alkenes
11	620.48	C≡C	Alkynes

The fractions gave bands with noticeable peaks that are characteristic of phenolic compound structures with ranges from 600-500cm^{-1} related to C-I stretching vibration for halo compounds identified in fractions F2, F4, F5, F6 and F8; 995-665cm^{-1} related to C=C bending for alkenes (flavonoids) in fractions F1, F2, F3, F5 and F8; 1470–1150 cm^{-1} could be due to C-H stretching vibration for phenols which were identified in all the fractions; 1250-1020 cm^{-1} related to C-N stretching vibration for amines identified in all the fractions; 1275-1200 cm^{-1} due to C-O stretching vibration for vinyl ether identified in F1, F7 and F8; 1440 - 1395 cm^{-1} related to O-H bending for alcohol and carboxylic acid identified in F1, F2, F3, F6, F7 and F8; 1650 - 1580cm^{-1} related to N-H bending for amines which shows the presence of alkaloids and were identified in all fractions except F8; 3500-3200cm^{-1} attributed to O-H stretching vibration for carboxylic acid and alcohols identified in all fractions; 1070-1030cm^{-1} could be due to S=O stretching vibration for sulfoxide identified in all fractions except F1; around 620cm^{-1} related to C-H bending for alkenes identified in F2, F5, F6 and F8 and around 3700cm^{-1} which can be attributed to the polysaccharides and/or lignins as reported by (Lucarini et al., 2020). The identified functional groups are similar to those in a study on characterization and quantification of phenolic compounds from the leaf of *Rhapis excels (Thunb) (Vanaja & Kavitha, 2016)*. Hence, the functional groups identified by the FT-IR analysis are found in phenolic compounds. This is consistent with the results from phytochemical screening - which showed phenols and flavonoids to be the most abundant, as well as from UV-Vis spectra (Fig. 4.1) - which showed peaks characteristic of phenolic compounds.

4.6. GC-MS/MS results of the fractions of *Bidens pilosa* leaf extract

The chromatograms generated from GC-MS analysis (Appendix 10 to 12) and total ion chromatograms of the identified phenolic compounds (Appendix 13) were studied and the results, along with possible identities (based on their fragmentation patterns and retention times matched to NIST 2014 library), are presented in tables 4.4 to 4.6. The tables contain results of fractions 1, 2, and 3 which include the identified phenolic compounds with their respective molecular masses, retention times and structures. No phenolic compounds were identified in fractions 4, 5, 6, 7 and 8 hence were not further analyzed.

Table 4.12. Phenolic compounds and structures identified in fraction 1 extract of *B. pilosa* by GC-MS

Peak no.	Retention time	Molecular weight	Formula	Structure	IUPAC name	Class of the compound	Activity	Reference
9	14.923	206	$C_{14}H_{22}O$		3,5-ditert-butylphenol	Phenols	Antioxidant and pro-oxidant	(Monowar, Rahman, Bhore, Raju, & Sathasivam, 2019; Norell, 1975)
9	14.923	206	$C_{14}H_{22}O$		2,4-Di-tert-butylphenol	Phenols	Antibacterial, Antioxidant	(Aissaoui et al., 2019; Choi et al., 2013)
9	14.923	206	$C_{14}H_{22}O$		2,6-Bis(tert-butyl)phenol	Phenols	Antifungal	(Das, Romi, Das, Sharma, & Thakur, 2018)

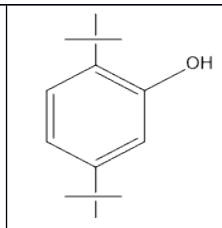
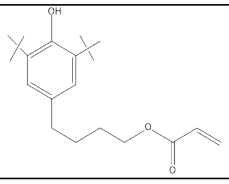
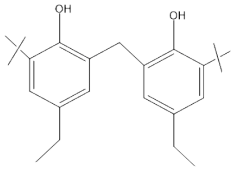
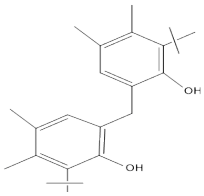
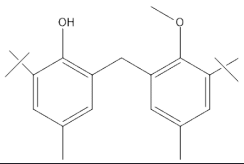
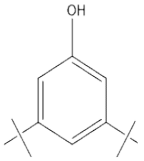
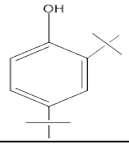
9	14.923	206	$C_{14}H_{22}O$		2,5-bis(1,1-Dimethylethyl)phenol	Phenols	Antioxidant	(Karimi & Jaafar, 2011; Khusro, Aarti, Salem, Pliego, & Rivas-Caceres, 2020)
24	21.988	332	$C_{21}H_{32}O_3$		4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate	Phenols	Antimicrobial	(Jeevitha, Deepa, & Michael, 2018)
38	33.100	368	$C_{25}H_{36}O_2$		2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)	Phenols	Antibacterial	(Omran, Fatthalah, El-Gendy, El-Shatoury, & Abouzeid, 2013)
38	33.100	368	$C_{25}H_{36}O_2$		2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)	Phenols	Not reported	
38	33.100	354	$C_{24}H_{34}O_2$		2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol	Phenols	Not reported	

Table 4.13. Phenolic compounds and structures identified in fraction 2 extract of *B. pilosa* by GC-MS

Peak no.	**Retention time**	**Molecular weight**	**Formula**	**Structure**	**IUPAC name**	**Class of the compound**	**Activity**	**Reference**
6	14.923	206	$C_{14}H_{22}O$		3,5-ditert-butylphenol	Phenols	Antioxidant and pro-oxidant	(Monowar et al., 2019)
6	14.923	206	$C_{14}H_{22}O$		2,4-Di-tert-butylphenol	Phenols	Antibacterial, Antioxidant	(Aissaoui et al., 2019; Choi et al., 2013)

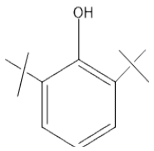
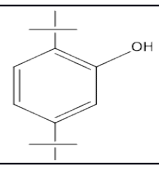
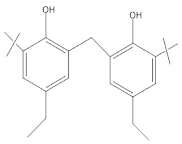
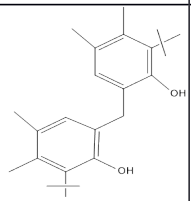
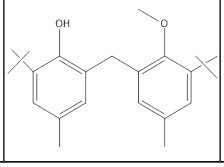
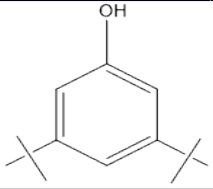
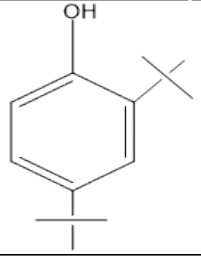
6	14.923	206	$C_{14}H_{22}O$		2,6-Bis(tert-butyl)phenol	Phenols	Antifungal	(Das et al., 2018)
6	14.923	206	$C_{14}H_{22}O$		2,5-bis(1,1-Dimethylethyl)phenol	Phenols	Antioxidant	(Karimi & Jaafar, 2011; Khusro et al., 2020)
20	33.100	368	$C_{25}H_{36}O_2$		2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)	Phenols	Antibacterial	(Omran et al., 2013)
20	33.100	368	$C_{25}H_{36}O_2$		2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)	Phenols	Not reported	
20	33.100	354	$C_{24}H_{34}O_2$		2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol	Phenols	Not reported	

Table 4.14. Phenolic compounds identified in fraction 3 extract of *B. pilosa* by GC-MS

Peak no.	**Retention time**	**Molecular weight**	**Formula**	**Structure**	**IUPAC name**	**Class of the compound**	**Activity**	**Reference**
6	14.929	206	$C_{14}H_{22}O$		3,5-ditert-butylphenol	Phenols	Antioxidant and pro-oxidant	(Monowar et al., 2019)
6	14.929	206	$C_{14}H_{22}O$		2,4-Di-tert-butylphenol	Phenols	Antibacterial, Antioxidant	(Aissaoui et al., 2019; Choi et al., 2013)

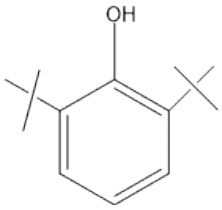
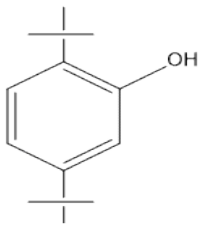
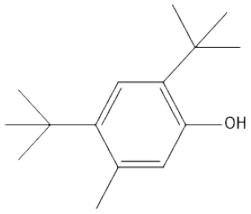
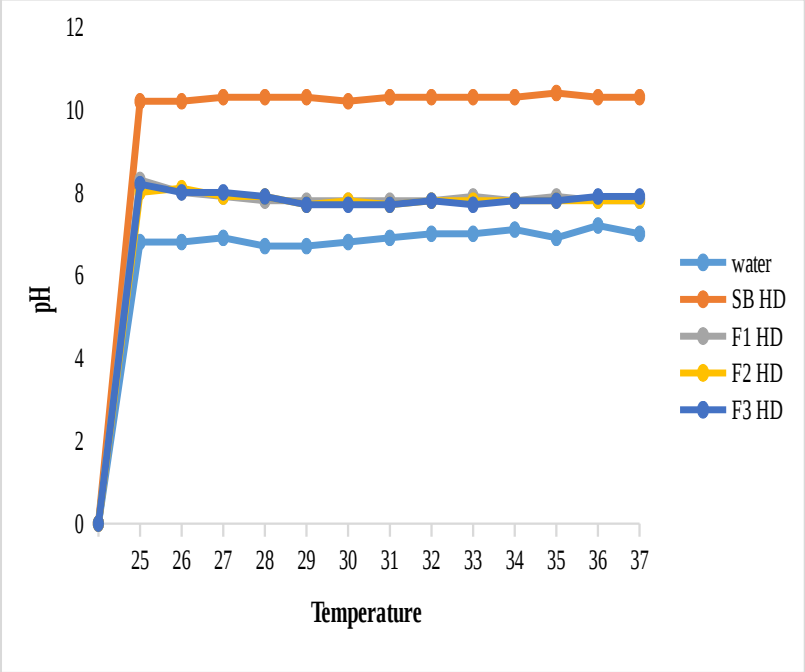
6	14.929	206	$C_{14}H_{22}O$		2,6-Bis(tert-butyl)phenol	Phenols	Antifungal	(Das et al., 2018)
6	14.923	206	$C_{14}H_{22}O$		2,5-bis(1,1-Dimethylethyl)phenol	Phenols	Antioxidant	(Karimi & Jaafar, 2011; Khusro et al., 2020)
9	21.993	220	$C_{15}H_{24}O$		4,6-di-tert-Butyl-m-cresol	Phenols	Antibacterial	(Dar, Ganai, Yousuf, Bhat, & Bhat, 2012; Wei, Ma, Zhang, & Chemicals, 2012)

Table 4.15. Precursor ions and main product ions obtained by GCMS/MS for the phenolic compounds

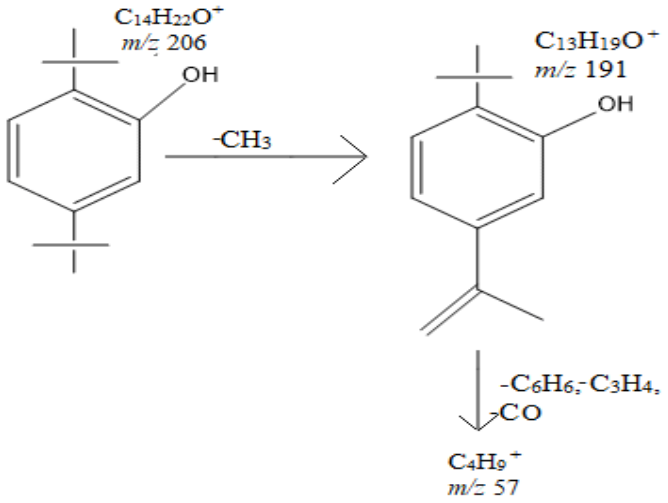
Name	**Formula**	**$M^{+\cdot}$**	**Fragmentation ions**
2,4-ditert-butylphenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}$-($^{\cdot}CH_3$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)-($^{\cdot}CH_3$)-$^{\cdot}CO]^+$
2,6-Bis(tert-butyl)phenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}$-($^{\cdot}CH_3$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)-($^{\cdot}CH_3$)-$^{\cdot}CO]^+$
2,5-bis(1,1-Dimethylethyl)phenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}$-($^{\cdot}CH_3$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)-($^{\cdot}CH_3$)-$^{\cdot}CO]^+$
3,5-ditert-butylphenol	$C_{14}H_{22}O^{+\cdot}$ *m/z* 206	206	191$[M^{+\cdot}$-($^{\cdot}CH_3$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)-($^{\cdot}CH_3$)-$^{\cdot}CO]^+$
2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)	$C_{25}H_{36}O_2^{+\cdot}$ *m/z* 368	368	191$[M^{+\cdot}$-($^{\cdot}C_5H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CO$)]$^+$ 175$[M^{+\cdot}$-($^{\cdot}C_5H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)]$^+$ 163$[M^{+\cdot}$-($^{\cdot}C_6H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_6H_6$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)$_2$]$^+$
2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)	$C_{25}H_{36}O_2^{+\cdot}$ *m/z* 368	368	312$[M^{+\cdot}$-($^{\cdot}CH_3CCH_2$)-($^{\cdot}CH_3$)]$^+$ 191$[M^{+\cdot}$-($^{\cdot}C_5H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CO$)]$^+$ 178$[M^{+\cdot}$-($^{\cdot}C_6H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}HCO$)]$^+$ 163$[M^{+\cdot}$-($^{\cdot}C_6H_7$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_6H_6$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)$_2$]$^+$
2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol	$C_{24}H_{34}O_2^{+\cdot}$ *m/z* 354	354	178$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}HCO$)]$^+$ 163$[M^{+\cdot}$-($^{\cdot}C_5H_5$)-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}CH_3$)-($^{\cdot}HCO$)]$^+$ 57$[M^{+\cdot}$-($^{\cdot}C_6H_6$)$_2$-($^{\cdot}C_3H_5$)$_2$-($^{\cdot}HCO$)$_2$]$^+$
4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl	$C_{21}H_{32}O_3^{+\cdot}$ *m/z* 332	332	219 $[M^{+\cdot}$-($^{\cdot}CH_3CH_3CCH_2$)-$^{\cdot}HCO$-$^{\cdot}CO]^+$ 203 $[M^{+\cdot}$-($^{\cdot}CH_3CH_3CCH_2$)-($^{\cdot}CH_3$)-

acrylate	2,4-Di-tert-butylphenol		$(\cdot HCO)_2]^+$ 189 $[M^{+\cdot}-(\cdot C_2H_5)-(\cdot CH_3CH_3CCH_2)-(\cdot HCO)_2]^+$
4,6-di-tert-Buty			$)]^+$



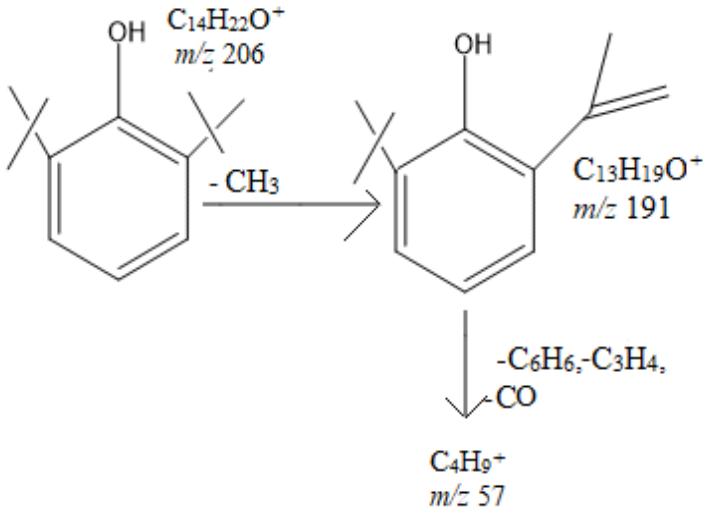
a)

2,5-bis(1,1-Dimethylethyl)phenol



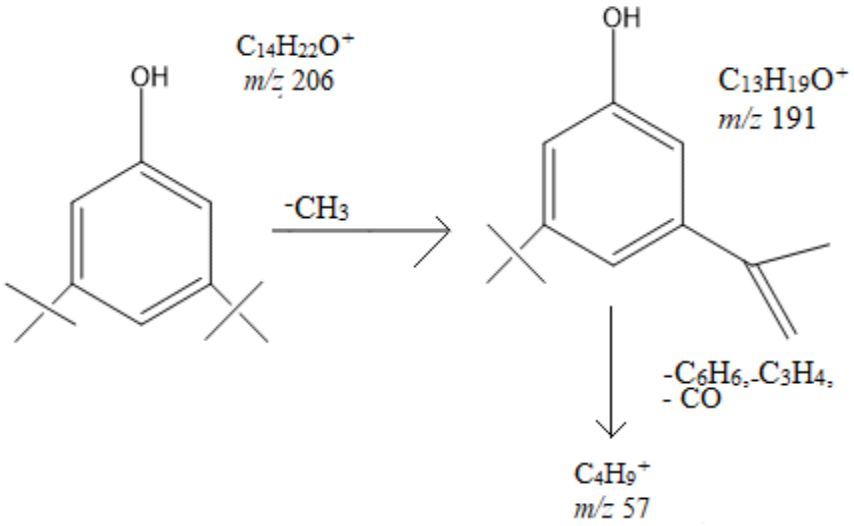
b)

2,6-Bis(tert-butyl)phenol



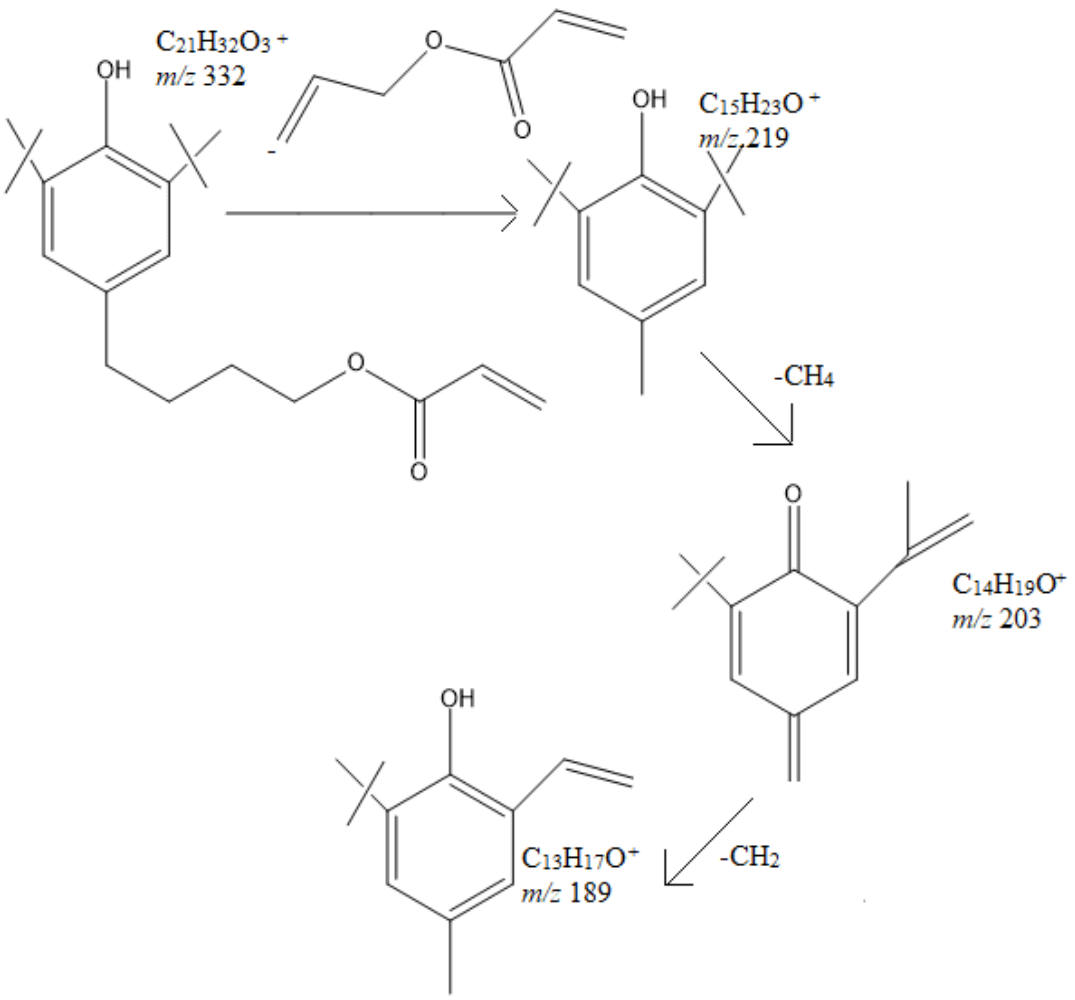
c)

3,5-ditert-butylphenol



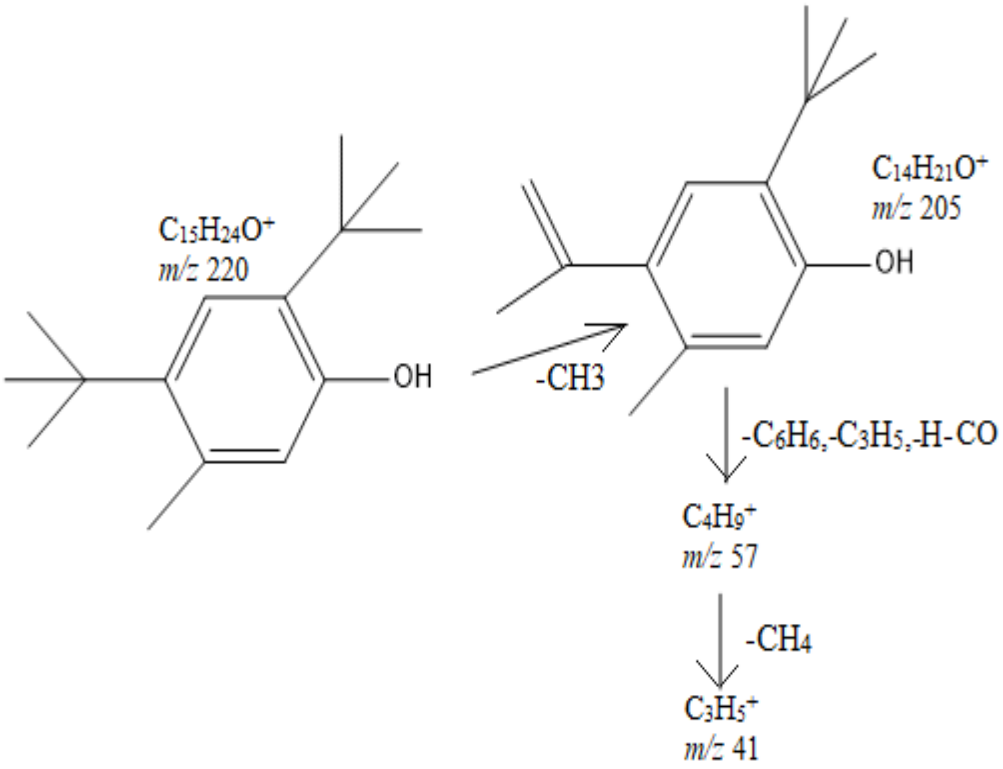
d)

4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate



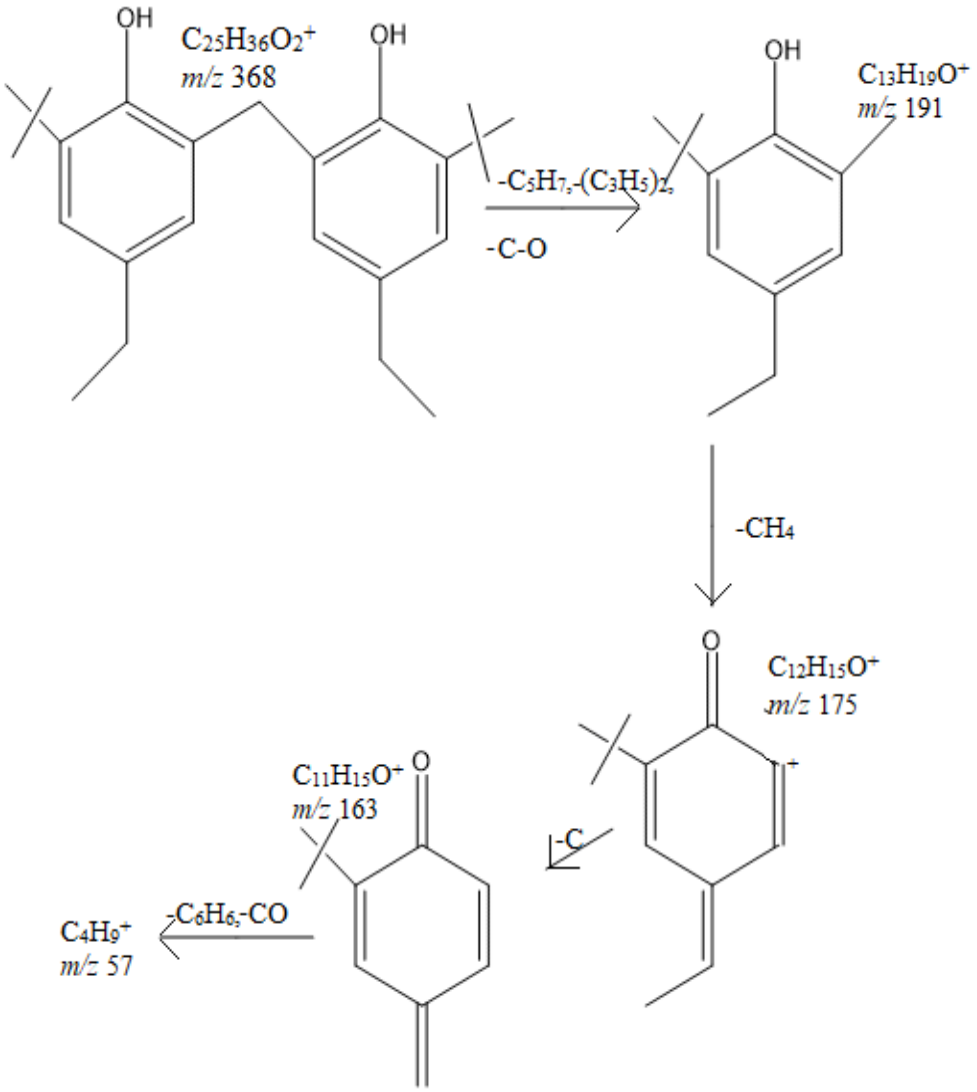
e)

4,6-di-tert-Butyl-m-cresol



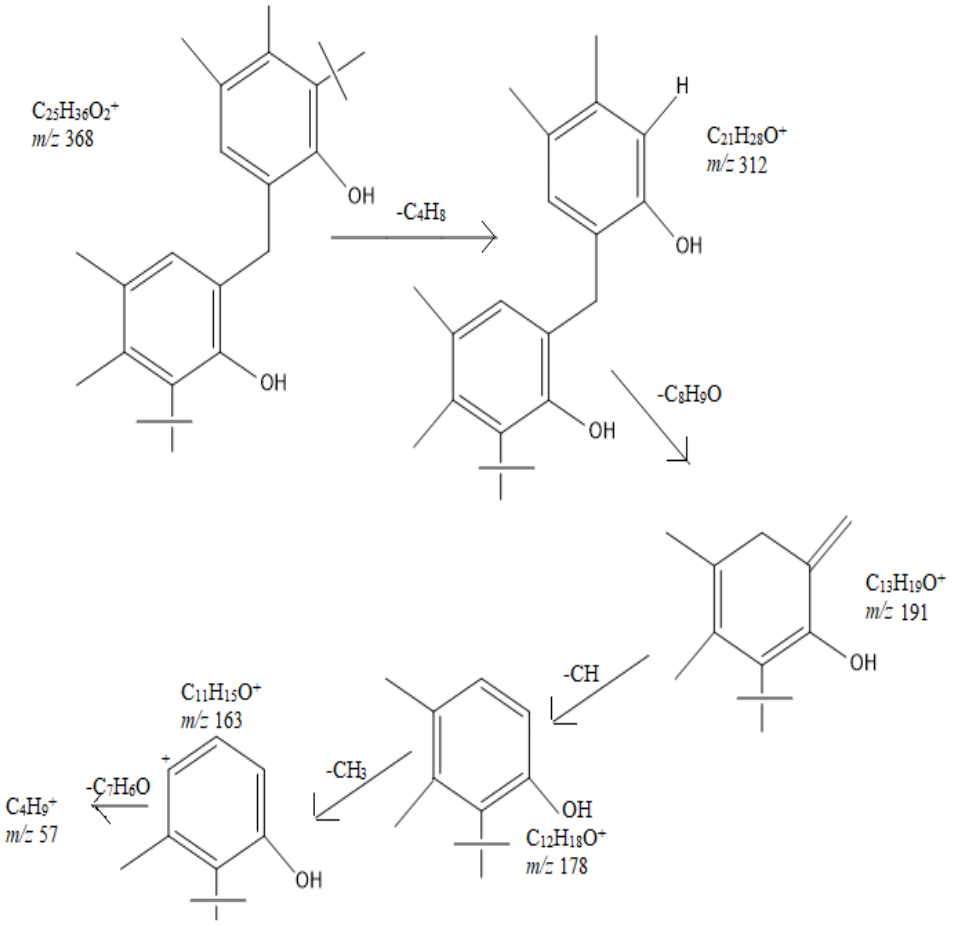
f)

2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)



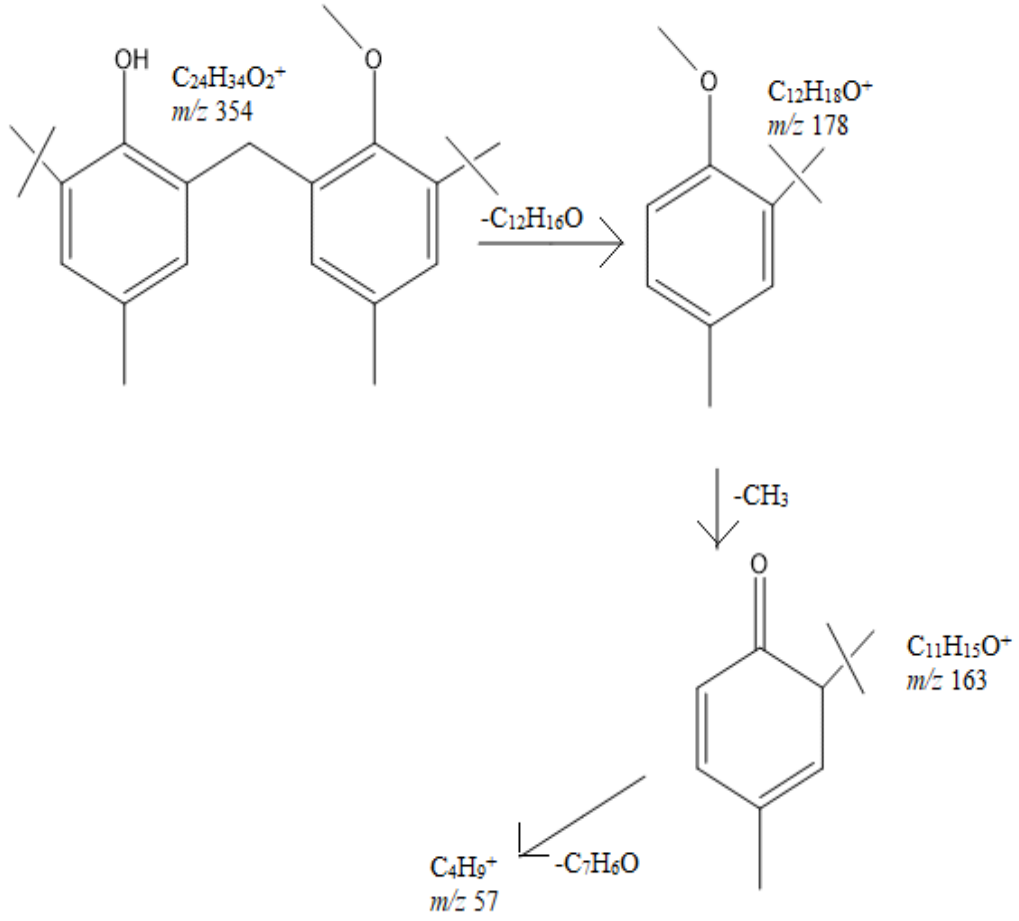
g)

2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol)



h)

2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol



i)

Figure 4.3 (a-i). Fragmentation patterns of the phenolic compounds of *Bidens pilosa leaves* studied by GC/MS–MS.

In fraction 1, 40 peak numbers were identified, the phenolic compounds identified were; 3,5-ditert-butylphenol (*m/z* 57, 191, 206) entry 51536, 2,4-ditert-butylphenol (*m/z* 41,57, 191, 206) entry 51535, 2,6-Bis(tert-butyl)phenol (*m/z* 41, 57, 131, 191, 206) entry 51537, 2,5-bis(1,1-Dimethylethyl)phenol (*m/z* 57, 191, 192, 206) entry 51541, 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate (*m/z* 41, 55, 57, 147, 189, 203, 219, 332) entry 160098, 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol) (*m/z* 57, 91, 119, 135, 141, 163, 175, 191, 368) entry 187878, 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) (*m/z*

57, 135, 163, 178, 191, 312, 368) entry 187879 and 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol (*m/z* 41, 57, 119, 163, 178, 354) entry 178091.

Fraction 2, 20 peak numbers were identified and phenolic compounds identified were; 2,4-ditert-butylphenol (entry 51535), 2,6-Bis(tert-butyl)phenol (entry 51537), 3,5-ditert-butylphenol (entry 51536), 2,5-bis(1,1-Dimethylethyl)phenol (entry 51541), 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol) (entry 187878), 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) (entry 187879) and 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol (entry 178091). Fragmentation pattern of fraction 2 phenolic compounds are the same as fraction 1.

Fraction 3, 10 peak numbers were identified and from the peak numbers, the phenolic compounds identified were; 3,5-ditert-butylphenol (entry 51536), 2,4-ditert-butylphenol (entry 51535), 2,6-Bis(tert-butyl)phenol (entry 21537), 2,5-bis(1,1-Dimethylethyl)phenol (entry 51541) having fragmentation patterns as in 1 and 2 and 4,6-di-tert-Butyl-m-cresol (*m/z* 29, 41, 57, 205, 220) entry 62571. Other compounds that were identified in all the 8 fractions were; 1-propenylpropyl ether (entry 2075) and 3-Hexen-2-one (entry 1726).

The GC-MS results are consistent with earlier indications - from phytochemical screening, UV-Vis spectrophotometry and FTIR analysis - that phenolic compounds are the major constituents in *B. Pilosa* leaf extracts A number of the identified compounds contained structural characteristics of phenolic compounds associated with antiulcer effects (Norell, 1975; Sumbul, Ahmad, Mohd, Mohd, & Sciences, 2011). These phenolic compounds have been associated with bioactive activities including; 2,4-ditert-butylphenol, 4,6-di-tert-Butyl-m-cresol and 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol)

as antibacterial (Dar et al., 2012; Selvakumar et al., 2007; Shunmugapriya, Vennila, Thirukkumar, Ilamaran, & Phytochemistry, 2017), 3,5-ditert-butylphenol and 2,5-bis(1,1-Dimethylethyl)phenol as antioxidant and antimicrobial (Karimi & Jaafar, 2011; Monowar et al., 2019), 2,6-Bis(tert-butyl)phenol (Darmadi, Sudirga, Suriani, & Wahyuni, 2019; Das et al., 2018) and 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) and 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol which have not been reported (Table 4.4-4.7). However, the antiulcer effects of these compounds have not been reported. Nevertheless, whereas individual compounds may not exhibit antiulcer effects, the effect could arise from synergistic interactions among the compounds (Sanhueza et al., 2017).

4.6.1. Other compounds identified

In fraction 1, other identified compounds were 1-propenylpropyl ether, 3-Hexen-2-one, 9-Eicosene, 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9 diene-2,8-dione and (1aS,4aS,7R,7aS,7bS)-1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol. In fraction 2, other identified compounds were 1-propenylpropyl ether, 3-Hexen-2-one, and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9 diene-2,8-dione. In fraction 3, other identified compounds were 1-propenylpropyl ether, 3-Hexen-2-one), 9-Eicosene, (E) and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9 diene-2,8-dione. These compounds also have no reports on antacid activity.

4.7. Antacid Assay

Antacid activity was evaluated in fractions 1, 2 and 3 since they were the only fractions that contained phenolic compounds as identified by GCMS/MS.

4.7. 1. pH determination of test solutions

pH values of water, standard and *Bidens pilosa* fractions (at concentrations of 400 and 800 $mgkg^{-1}bw^{-1}$) were determined over different temperatures and the results are shown in figures 4.3 and 4.4.

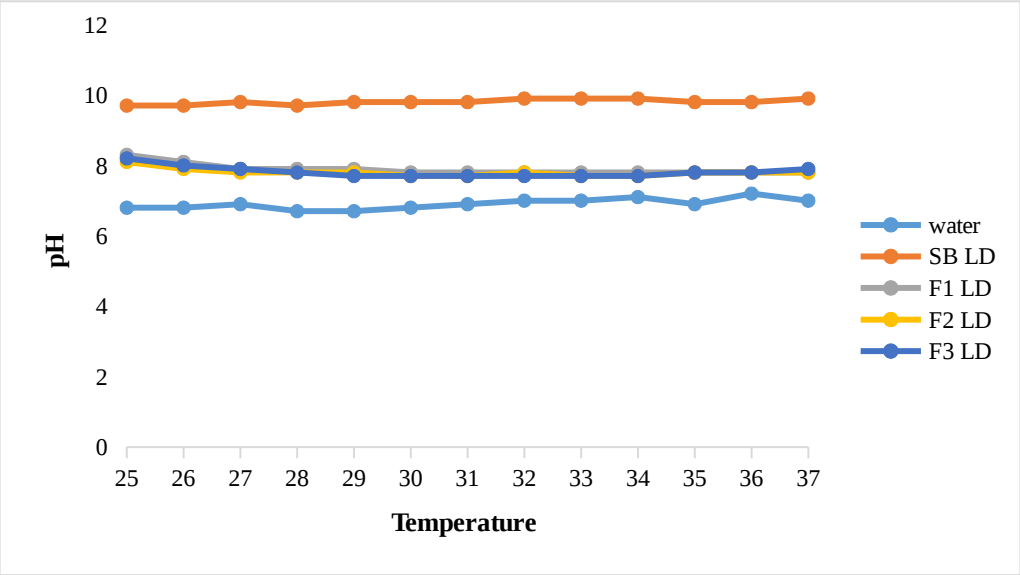


Figure 4.4. pH values of water and LD of standard and *Bidens pilosa* fractions ($400mgkg^{-1}bw^{-1}$).

SB: Sodium bicarbonate

F: Fraction

400 $mgkg^{-1}bw^{-1}$: Low dose (LD)

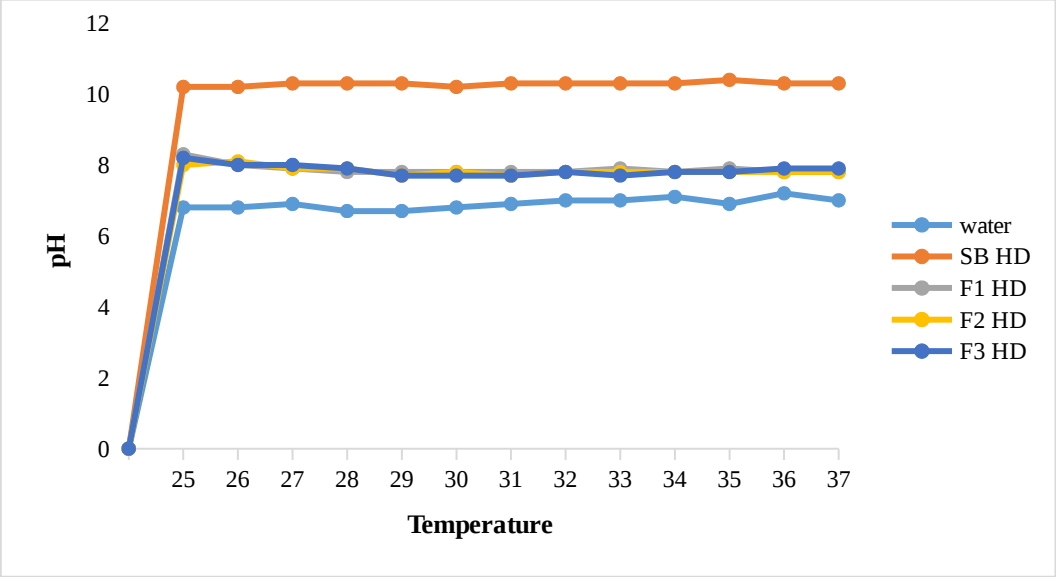


Figure 4.5. pH values of water and HD of standard and *Bidens pilosa* fractions (800mgkg^{-1}bw^{-1}).

SB: Sodium bicarbonate

F: Fraction

800 mgkg-^{1}bw^{-1}: High dose (HD)

It was observed that, the pH values of water, sodium bicarbonate and the fractions both high dose (HD that is 800mgkg^{-1}bw^{-1}) and low dose (LD that is 400mgkg^{-1}bw^{-1}) showed a stable pH at temperatures ranging from 25 ^{0}C to 37 ^{0}C, which confirms that temperature did not significantly affect the pH.

4.7.2. Neutralization effect of the fractions

Table 4.16. Effect of *Bidens pilosa* fractions (400mgkg^{-1}bw^{-1}and 800mgkg^{-1}bw^{-1}) on the pH of artificial gastric acid.

Drug		**P^H value**
Water		1.45±0.028868
Standard (SB)	400mgkg^{-1}bw^{-1}	2.1±0.08165**
	800mgkg^{-1}bw^{-1}	2.4±0.040825**
F1	400mgkg^{-1}bw^{-1}	1.925±0.025**
	800mgkg^{-1}bw^{-1}	1.975±0.047871**
F2	400 mgkg^{-1}bw^{-1}	1.8±0.040825*
	800 mgkg^{-1}bw^{-1}	1.825±0.047871*
F3	400 mgkg^{-1}bw^{-1}	1.875±0.025**
	800 mgkg^{-1}bw^{-1}	1.9±0.057735**

Data are presented as mean ± SEM (n = 4) *P < 0.05, ** p<0.01, when compared with water.

SB: Sodium bicarbonate

F: Fraction

800 mgkg-^{1}bw^{-1}: High dose (HD)

400 mgkg^{-1}bw^{-1}: Low dose (LD)

Upon addition of the concentrations (800 and 400) $mgkg^{-1}bw^{-1}$ of the fractions of *B. pilosa* leaf extracts and sodium bicarbonate to artificial gastric acid (pH 1.2), the results showed a significant difference with $P<0.05$ for fraction F2 and $P<0.01$ for SB and fractions F1 and F3. An increase in the pH values was observed in comparison to water (Table 4.8). The results showed that the high and low concentrations of fractions of *B. pilosa* leaf extracts and sodium bicarbonate have neutralizing effects that are significantly better than those of water but lower than the standard sodium bicarbonate and, therefore, are potent antacids. It was observed that sodium bicarbonate (in both concentrations) had the highest neutralizing effect followed by the fractions at concentrations of $800mgkg^{-1}bw^{-1}$ and then $400mgkg^{-1}bw^{-1}$, which confirmed that when the concentration of plant material is doubled, the antacid activity becomes more effective. This observation is consistent with findings in a recent study on antacid activity of *Laportea aestuans* (L.) Chew by (C. B. Christensen, J. Soelberg, & A. K. Jäger, 2015a).

4.7.3. Duration of consistent neutralization effect on artificial gastric acid

Table 4.17. Duration of antacid effect for consistent neutralization of gastric acid.

Drug		**Time in minutes at 37^0C**
water		20 ± 0.355903
Standard (SB)	400 $mgkg^{-1}bw^{-1}$	77 ± 0.398108**
	800 $mgkg^{-1}bw^{-1}$	80 ± 0.707107**
F1	400 $mgkg^{-1}bw^{-1}$	63 ± 0.408248**
	800$mgkg^{-1}bw^{-1}$	65 ± 0.476751**
F2	400 $mgkg^{-1}bw^{-1}$	35 ± 0.478714**
	800 $mgkg^{-1}bw^{-1}$	37 ± 0.25**
F3	400 $mgkg^{-1}bw^{-1}$	39 ± 0.314576**
	800 $mgkg^{-1}bw^{-1}$	43 ± 0.283945**

Data are presented as mean ± SEM (n = 4) **P < 0.01 when compared with water

SB: Sodium bicarbonate

F: Fraction

800 mgkg-$^{1}bw^{-1}$: High dose (HD)

400 $mgkg^{-1}bw^{-1}$: Low dose (LD)

Considering the duration for consistent neutralization of gastric acid, the neutralization duration of the HD (800 $mgkg^{-1}bw^{-1}$) and LD (400 $mgkg^{-1}bw^{-1}$) sodium bicarbonate and phenolic fractions were significantly longer than for water with P<0.01 for all the fractions (Table 4.9). According to (Christensen et al., 2015a; V. S. Panda & Shinde, 2016) when the concentration of the plant material is doubled, the antacid profile exhibits an ability to maintain the neutralizing activity on pH-unit higher for an extended period of time Therefore, the duration of antacid action of 800 $mgkg^{-1}bw^{-1}$ was the longest, followed by 400 $mgkg^{-1}bw^{-1}$, and these are comparable to the findings of (Dhanalakshmi

et al., 2018) who showed that the duration of antacid action of Sodium bicarbonate was the longest, followed by the *Hemidesmus indicus* 800 $mgkg^{-1}bw^{-1}$ and 400 $mgkg^{-1}bw^{-1}$, which were significantly higher than that for water.

4.7.4. *In vitro* physical neutralization capacity

Table 4.18. Consumed volume of artificial gastric juice and H+ in the titration of 9ml water, standard and *Bidens pilosa* fractions (400 $mgkg^{-1}bw^{-1}$and 800 $mgkg^{-1}bw^{-1}$) with artificial gastric juice (pH 1.2) to the end point pH 3.

Drug		**Consumed gastric acid in ml**	**Consumed H+ in ml**
Water		1.0 ± 0.05	0.06 ± 0.003
Standard (SB)	400$mgkg^{-1}bw^{-1}$	3.6 ± 0.025**	0.23 ± 0.002**
	800$mgkh^{-1}bw^{-1}$	4 ± 0.075**	0.25 ± 0.005**
F1	400$mgkg^{-1}bw^{-1}$	3.0 ± 0.047871**	0.19 ± 0.003**
	800$mgkh^{-1}bw^{-1}$	3.2 ± 0.040825**	0.20 ± 0.0025**
F2	400$mgkg^{-1}bw^{-1}$	1.6 ± 0.046615*	0.10 ± 0.0029*
	800$mgkh^{-1}bw^{-1}$	1.7 ± 0.057735**	0.11 ± 0.0036**
F3	400$mgkg^{-1}bw^{-1}$	1.8 ± 0.0225**	0.11 ± 0.0014**
	800$mgkh^{-1}bw^{-1}$	2.0 ± 0.0375**	0.12 ± 0.0023**

Data are presented as mean ± SEM (n = 4) *P < 0.05 and **P<0.01 when compared with water.

SB: Sodium bicarbonate

F: Fraction

800 mgkg-$^{1}bw^{-1}$: High dose (HD)

400 $mgkg^{-1}bw^{-1}$: Low dose (LD)

There was a significant difference on neutralization capacity with P<0.01 for SB and fractions F1, F2B and F3 and P< 0.05 for F2A when compared with water. The higher the

concentration, the higher the amount of gastric acid consumed during neutralization, with the sodium bicarbonate controls and *B. Pilosa* fractions (concentrations of 800 and 400 $mgkg^{-1}bw^{-1}$) consuming a higher quantity of the acid compared with water. These findings are similar to those in a study by (Christensen et al., 2015a) who showed that 1332 $mgkg^{-1}bw^{-1}$ consumed a higher quantity of neutralizing acid than 666 $mgkg^{-1}bw^{-1}$.

Efficacious, intensive antacid therapy is often unacceptable because of the common side effects, especially altered bowel functions (V. Panda & Khambhat, 2013). Aluminum salts may cause constipation and magnesium salts cause diarrhea (Maton & Burton, 1999). Sodium bicarbonate (SB) should be avoided even though it is a potent neutralizer of acid because it contains significant amounts of sodium and may alter the systemic pH (Carr et al., 2011). In addition, antacid-drug interactions have been frequently reported for synthetic antacids including Sodium bicarbonate. This makes this study to conclude that *B. pilosa* leaves are an alternative antacid to synthetic drugs.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

In conclusion, the results obtained showed the presence of important phytoconstituents in the *B. Pilosa* leaf extracts including phenols, tannins, cardiac glycosides, alkaloids, flavonoids and saponins. The identified phenolic compounds were; 3,5-ditert-butylphenol, 2,6-Bis(tert-butyl)phenol, 2,4-ditert-butylphenol, 2,5-bis(1,1-Dimethylethyl)phenol, 4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate, 2,2'-Methylenebis(6-tert-butyl-4-ethylphenol), 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) 2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol and 4,6-di-tert-Butyl-m-cresol. *B. pilosa* phenolic fractions of leaf extracts (both HD and LD) showed significant acid neutralizing effects that were comparable to the sodium bicarbonate standard, as was the duration for consistent neutralization and antacid capacities of *B. Pilosa* HD and LD were significantly higher than those of water. They were also consistently active in the artificial stomach model and possessed potent antacid effects. Hence *B. Pilosa* can be an effective alternative for sodium bicarbonate which is reported to have side effects like alteration in systemic pH.

5.2. Recommendations

This study provides data supporting the efficacy of *B. pilosa* leaf extract in gastric ulcer disease treatment and recommends it as an alternative to sodium bicarbonate. The study recommends an isolation of the identified phenolic compounds from *B. pilosa* leaves

It further recommends GUD patients to consider *B. pilosa* as viable option supported by scientific data, the traditional healers to use data to validate their claims, ministry of health to educate public on the importance of *B. pilosa* against GUD, farmers to consider cultivating *B. pilosa*.

REFERENCES

Aaby, K., Ekeberg, D., Skrede, G. J. J. o. A., & Chemistry, F. (2007). Characterization of phenolic compounds in strawberry (Fragaria× ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *55*(11), 4395-4406.

Abdou, R., Scherlach, K., Dahse, H.-M., Sattler, I., & Hertweck, C. (2010). Botryorhodines A–D, antifungal and cytotoxic depsidones from Botryosphaeria rhodina, an endophyte of the medicinal plant Bidens pilosa. *Phytochemistry, 71*(1), 110-116.

Abe, K., Shimokawa, J., Naito, M., Munson, K., Vagin, O., Sachs, G., . . . Fujiyoshi, Y. (2017). The cryo-EM structure of gastric H+, K+-ATPase with bound BYK99, a high-affinity member of K+-competitive, imidazo [1, 2-a] pyridine inhibitors. *Scientific reports*, *7*(1), 6632.

Abourehab, M. A. S. (2017). Peptic Ulcer: Mini Review.

Ahmad, S., Radotra, S., Singh, J., Verma, D., & Sultan, S. M. (2017). Ethnoveterinary uses of some important plants by pastoralists in Kashmir Himalaya. *SKUAST Journal of Research*, *19*(1), 121-128.

Aissaoui, N., Mahjoubi, M., Nas, F., Mghirbi, O., Arab, M., Souissi, Y., . . . Cherif, A. J. G. J. (2019). Antibacterial potential of 2, 4-di-tert-butylphenol and calixarene-based prodrugs from thermophilic Bacillus licheniformis isolated in Algerian hot spring. *36*(1), 53-62.

Akman, F., Geçibesler, I., Kumar, A., Sayyed, M., & Zaid, M. (2019). Evaluation of radiation absorption characteristics in different parts of some medicinal aromatic plants in the low energy region. *Results in Physics, 12*, 94-100.

Al-Badriyeh, D., Alabbadi, I., Fahey, M., Al-Khal, A., & Zaidan, M. (2016). Multi-indication pharmacotherapeutic multicriteria decision analytic model for the comparative formulary inclusion of proton pump inhibitors in Qatar. *Clinical therapeutics, 38*(5), 1158-1173.

Al-Wajeeh, N. S., Hajerezaie, M., Noor, S. M., Halabi, M. F., Al-Henhena, N., Azizan, A. H. S., . . . Abdulla, M. A. (2016). The gastro protective effects of Cibotium

barometz hair on ethanol-induced gastric ulcer in Sprague-Dawley rats. *bmc veterinary research, 13*(1). doi:10.1186/S12917-017-0949-Z

Allison, R., Lecky, D. M., Bull, M., Turner, K., Godbole, G., & McNulty, C. A. (2016). Audit of Helicobacter pylori testing in microbiology laboratories in England: to inform compliance with NICE guidance and the feasibility of routine antimicrobial resistance surveillance. *International journal of microbiology, 2016*.

Altieri, M. S., & Spaniolas, K. (2019). Gastric Ulcer Management. In *Clinical Algorithms in General Surgery* (pp. 149-152): Springer.

Aniama, S., Usman, S., & Ayodele, S. (2016). Ethnobotanical documentation of some plants among Igala people of Kogi State. *Int. J. Eng. Sci, 5*, 33-42.

Ayla, S., Okur, M. E., Günal, M. Y., Özdemir, E. M., Polat, D. Ç., Yoltaş, A., . . . Karahüseyinoğlu, S. (2019). Wound healing effects of methanol extract of Laurocerasus officinalis roem. *biotechnic & histochemistry, 94*(3), 180-188. doi:10.1080/10520295.2018.1539242

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis, 6*(2), 71-79.

Bansod, A., Bansod, S. A., Galande, A. B., Shamkuwar, A. T., & Singh, K. H. J. I. S. J. (2016). Study of incidence of peptic ulcer perforation in young adults. *1*(3), 144-147.

Bartolome, A. P., Villaseñor, I. M., & Yang, W.-C. (2013). Bidens pilosa L.(Asteraceae): botanical properties, traditional uses, phytochemistry, and pharmacology. *Evidence-based complementary and alternative medicine, 2013*.

Bellorin-Marin, O. E., & Pomp, A. (2018). Gastric Bypass. In *Global Bariatric Surgery* (pp. 97-111): Springer.

Benito-Garcia, F., Chambel, M., & Morais-Almeida, M. (2018). Anaphylaxis due to proton pump inhibitors: current understanding and important clinical considerations. *Expert review of clinical immunology, 14*(8), 653-656.

Bhatt, K., Sharma, N., & Pandey, A. (2009). ‘Ladakhi tea’Bidens pilosa L.(Asteraceae): a cultivated species in the cold desert of Ladakh Himalaya, India. *Genetic resources and crop evolution, 56*(6), 879-882.

Bi, W., Zhou, J., & Row, K. H. (2012). Solid phase extraction of three phenolic acids from Saliconia herbacea L. by different ionic liquid-based silicas. *Journal of Liquid Chromatography & Related Technologies, 35*(5), 723-736.

Bin-Jumah, M. N. (2019). Antidiabetic Effect of Monolluma quadrangula Is Mediated via Modulation of Glucose Metabolizing Enzymes, Antioxidant Defenses, and Adiponectin in Type 2 Diabetic Rats. *oxidative medicine and cellular longevity, 2019*. doi:10.1155/2019/6290143

Boeing, T., da Silva, L. M., Somensi, L. B., Cury, B. J., Costa, A. P. M., Petreanu, M., . . . de Andrade, S. F. (2016). Antiulcer mechanisms of Vernonia condensata Baker: A medicinal plant used in the treatment of gastritis and gastric ulcer. *Journal of ethnopharmacology, 184*, 196-207.

Buhner, S. H. (2012). *Herbal antibiotics: Natural alternatives for treating drug-resistant bacteria*: Storey Publishing.

Bum, E. N., Taiwe, G., Moto, F., Ngoupaye, G., Vougat, R., Sakoue, V., . . . Rakotonirina, A. (2011). Antiepileptic medicinal plants used in traditional medicine to treat epilepsy. In *Clinical and genetic aspects of epilepsy*: IntechOpen.

Carr, A. J., Slater, G. J., Gore, C. J., Dawson, B., Burke, L. M. J. I. j. o. s. n., & metabolism, e. (2011). Effect of sodium bicarbonate on [HCO3−], pH, and gastrointestinal symptoms. *21*(3), 189-194.

Castela-Papin, N., Cai, S., Vatier, J., Keller, F., Souleau, C., & Farinotti, R. J. I. j. o. p. (1999). Drug interactions with diosmectite: a study using the artificial stomach–duodenum model. *182*(1), 111-119.

Cecere, R., Buquicchio, R., Girardi, B., Polimeno, R., Serviddio, G., Demoly, P., & Ventura, M. (2017). The basophil activation test in the diagnosis and management of adverse drug reactions in the elderly. *Official Journal of the Italian Society of Gerontology and Geriatrics*, 248.

Chen, Y., Lu, W., Jin, Z., Yu, J., & Shi, B. (2019). Carbenoxolone ameliorates hepatic lipid metabolism and inflammation in obese mice induced by high fat diet via regulating the JAK2/STAT3 signaling pathway. *international immunopharmacology, 74*. doi:10.1016/J.INTIMP.2019.03.011

Cheng, D., He, C., Ai, H.-h., Huang, Y., & Lu, N.-h. (2017). The possible role of Helicobacter pylori infection in non-alcoholic fatty liver disease. *Frontiers in microbiology, 8*, 743.

Choi, S. J., Kim, J. K., Kim, H. K., Harris, K., Kim, C.-J., Park, G. G., . . . Shin, D.-H. J. J. o. m. f. (2013). 2, 4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. *16*(11), 977-983.

Christensen, C. B., Soelberg, J., & Jäger, A. K. (2015a). Antacid activity of Laportea aestuans (L.) Chew. *Journal of ethnopharmacology, 171*, 1-3.

Christensen, C. B., Soelberg, J., & Jäger, A. K. J. J. o. e. (2015b). Antacid activity of Laportea aestuans (L.) Chew. *171*, 1-3.

Chuang, Y.-S., Wu, M.-C., Yu, F.-J., Wang, Y.-K., Lu, C.-Y., Wu, D.-C., . . . Wu, I.-C. (2017). Effects of alcohol consumption, cigarette smoking, and betel quid chewing on upper digestive diseases: a large cross-sectional study and meta-analysis. *Oncotarget, 8*(44), 78011.

Cohen, S., Bueno de Mesquita, M., & Mimouni, F. B. (2015). Adverse effects reported in the use of gastroesophageal reflux disease treatments in children: a 10 years literature review. *British journal of clinical pharmacology, 80*(2), 200-208.

Contreras-Zentella, M. L., Olguín-Martínez, M., Sánchez-Sevilla, L., & Hernández-Muñoz, R. (2017). Gastric Mucosal Injury and Oxidative Stress. In *Gastrointestinal Tissue* (pp. 65-79): Elsevier.

Corsonello, A., Lattanzio, F., Bustacchini, S., Garasto, S., Cozza, A., Schepisi, R., . . . Ticinesi, A. (2018). Adverse events of proton pump inhibitors: potential mechanisms. *Current drug metabolism, 19*(2), 142-154.

Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A., & Brielmann, H. L. (2016). *Natural products from plants*: CRC press.

Cui, Q. G., & He, W. M. (2009). Soil biota, but not soil nutrients, facilitate the invasion of Bidens pilosa relative to a native species Saussurea deltoidea. *Weed research, 49*(2), 201-206.

Dankowska, A., & Kowalewski, W. (2019). Tea types classification with data fusion of UV–Vis, synchronous fluorescence and NIR spectroscopies and chemometric analysis. *211*, 195-202.

Dar, S. A., Ganai, F. A., Yousuf, A. R., Bhat, T. M., & Bhat, F. A. J. A. J. o. M. R. (2012). Bioactive potential of leaf extracts from Urtica dioica L. against fish and human pathogenic bacteria. *6*(41), 6893-6899.

Darmadi, A. A. K., Sudirga, S. K., Suriani, N. L., & Wahyuni, I. G. A. S. (2019). *Antifungal Activities Of Cinnamon Leaf Extracts Against Sigatoka Fungus (Pseudocercospora Fijiensis).* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Das, R., Romi, W., Das, R., Sharma, H. K., & Thakur, D. J. B. m. (2018). Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. *18*(1), 71.

Daure, E., Ross, L., & Webster, C. R. (2017). Gastroduodenal Ulceration in Small Animals: Part 2. Proton Pump Inhibitors and Histamine-2 Receptor Antagonists. *Journal of the American Animal Hospital Association, 53*(1), 11-23.

David, B., Wolfender, J.-L., & Dias, D. A. (2015). The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews, 14*(2), 299-315.

Dhanalakshmi, R., Afrin, A., Akila, M., Alnoora, F., Dharani, R. J. J. o. P., & Phytochemistry. (2018). Preliminary phytochemical screening and in vitro antacid activity of Hemidesmus indicus leaves extract by modified artificial stomach model. *7*(4), 2546-2550.

Drake, D., & Hollander, D. (1981). Neutralizing Capacity and Cost Effectiveness of Antacids. *annals of internal medicine, 94*(2), 215-217. doi:10.7326/0003-4819-94-2-215

El-Meligy, R. M., Awaad, A. S., Soliman, G. A., Kenawy, S. A., & Alqasoumi, S. I. (2017). Prophylactic and curative anti-ulcerogenic activity and the possible mechanisms of action of some desert plants. *Saudi Pharmaceutical Journal, 25*(3), 387-396.

Engevik, A. C., Kaji, I., & Goldenring, J. R. J. P. r. (2020). The physiology of the gastric parietal cell. *100*(2), 573-602.

Etkin, N. L. (2017). Consuming a therapeutic landscape: a multicontextual framework for assessing the health significance of human-plant interactions. In *People-Plant Relationships* (pp. 61-81): Routledge.

Farzaei, M. H., Abdollahi, M., & Rahimi, R. (2015). Role of dietary polyphenols in the management of peptic ulcer. *World Journal of Gastroenterology: WJG, 21*(21), 6499.

Franciosi, J. P., Mougey, E. B., Williams, A., Suarez, R. A. G., Thomas, C., Creech, C. L., . . . Lima, J. J. (2018). Association between CYP2C19 extensive metabolizer phenotype and childhood anti-reflux surgery following failed proton pump inhibitor medication treatment. *European journal of pediatrics, 177*(1), 69-77.

Freedberg, D. E., Kim, L. S., & Yang, Y.-X. (2017). The risks and benefits of long-term use of proton pump inhibitors: expert review and best practice advice from the American Gastroenterological Association. *Gastroenterology, 152*(4), 706-715.

Fu, B., Wang, N., Tan, H.-Y., Li, S., Cheung, F., & Feng, Y. J. F. i. p. (2018). Multi-component herbal products in the prevention and treatment of chemotherapy-associated toxicity and side effects: a review on experimental and clinical evidences. *9*, 1394.

Geetha, G., Harathi, K., Giribabu, D., & Naidu, C. (2017). Influence of silver nitrate and different carbon sources on in vitro shoot development in Solanum nigrum (Linn)-an important antiulcer medicinal plant. *Science Spectru, 2*(1), 54-61.

Graham, D. Y. (2014). History of Helicobacter pylori, duodenal ulcer, gastric ulcer and gastric cancer. *World Journal of Gastroenterology: WJG, 20*(18), 5191.

Hao, J. H., Bhattacharya, S., Ma, L., & Wang, L. X. (2018). Breeding systems and seed production for six weedy taxa of Bidens. *Weed biology and management, 18*(1), 41-49.

Heinrich, M., & Anagnostou, S. J. P. m. (2017). From pharmacognosia to DNA-based medicinal plant authentication–pharmacognosy through the centuries. *83*(14/15), 1110-1116.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine, 6*(09), 635-642.

Hsu, Y.-J., Lee, T.-H., Chang, C. L.-T., Huang, Y.-T., & Yang, W.-C. (2009). Anti-hyperglycemic effects and mechanism of Bidens pilosa water extract. *Journal of Ethnopharmacology, 122*(2), 379-383.

Idris, A., Linatoc, A. C., Muhammad, S. M., Aliyu, A. M., & Bakar, M. F. A. (2018). Effect of Light Intensity on the Total Flavonoid and Total Phenolic Contents of Mikania Micrantha and Tridax Procumbens. *Journal of Science and Technology, 10*(4).

Ijaz, F., Rahman, I. U., Iqbal, Z., Alam, J., Ali, N., & Khan. (2018). Ethno-ecology of the Healing Forests of Sarban Hills, Abbottabad, Pakistan: An Economic and Medicinal Appraisal. In *Plant and Human Health, Volume 1* (pp. 675-706): Springer.

Jacob, S., Shirwaikar, A., Anoop, S., Khaled, R., Imtiaz, M., & Nair, A. (2016). Acid neutralization capacity and cost-effectiveness of antacids sold in various retail pharmacies in the United Arab Emirates. *hamdan medical journal, 9*(2). doi:10.7707/HMJ.452

Jacobs, J. W., & Richter, J. E. (2016). Role of H 2 RA and Proton Pump Inhibitor Therapy in Treating Reflux Disease. In *Diagnosis and Treatment of Gastroesophageal Reflux Disease* (pp. 71-91): Springer.

Jeevitha, T., Deepa, K., & Michael, A. J. A. J. o. M. R. (2018). In vitro study on the anti-microbial efficacy of Aloe vera against Candida albicans. *12*(40), 930-937.

Jivad, N., & Bahmani, M. (2016). A review of important medicinal plants native to Iran effective on recovery from peptic ulcer. *Der Pharm Lett, 8*(2), 347-352.

Kala, C. P. (2016). Medicinal plants used for gastrointestinal diseases in Garhwal region of Uttarakhand state in India. *Australian Journal of Herbal Medicine, 28*(1), 15.

Karimi, E., & Jaafar, H. Z. J. M. (2011). HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of Labisia pumila Benth. *16*(8), 6791-6805.

Kataoka, Y., Tsuji, Y., Sakaguchi, Y., Minatsuki, C., Asada-Hirayama, I., Niimi, K., . . . Fujishiro, M. (2016). Bleeding after endoscopic submucosal dissection: Risk factors and preventive methods. *World journal of gastroenterology, 22*(26), 5927.

Kaushik, N. K., Bagavan, A., Rahuman, A. A., Zahir, A. A., Kamaraj, C., Elango, G., . . . Marimuthu, S. (2015). Evaluation of antiplasmodial activity of medicinal plants from North Indian Buchpora and South Indian Eastern Ghats. *Malaria journal, 14*(1), 65.

Kavitt, R. T., Lipowska, A. M., Anyane-Yeboa, A., & Gralnek, I. M. (2019). Diagnosis and Treatment of Peptic Ulcer Disease. *The American journal of medicine*.

Kayamba, V., Sinkala, E., Mwanamakondo, S., Soko, R., Kawimbe, B., Amadi, B., . . . Mumbwe, C. J. B. g. (2015). Trends in upper gastrointestinal diagnosis over four decades in Lusaka, Zambia: a retrospective analysis of endoscopic findings. *15*(1), 1-9.

Khanh, T., Cong, L., Xuan, T., Uezato, Y., Deba, F., Toyama, T., & Tawata, S. (2009). Allelopathic plants: 20 hairy beggarticks (Bidens pilosa L.). *Allelopathy J, 24*, 243-254.

Khusro, A., Aarti, C., Salem, A. Z., Pliego, A. B., & Rivas-Caceres, R. R. J. J. o. E. V. S. (2020). Methyl-coenzyme M Reductase (MCR) Receptor as Potential Drug Target for Inhibiting Methanogenesis in Horses Using Moringa oleifera L.: An in Silico Docking Study. *88*, 102949.

Kim, M., Yu, S. K., Truong, Q.-K., Mai, X.-L., Chung, H. K., Kang, J.-S., & Kim, K. H. (2017). Determination of rabeprazole enantiomers in commercial tablets using immobilized cellulose-based stationary phase. *Archives of pharmacal research, 40*(3), 373-381.

Koushki, M., Yekta, R. F., Amiri-Dashatan, N., Dadpay, M., & Goshadrou, F. (2019). Therapeutic effects of hydro-alcoholic extract of Achillea wilhelmsii C. Koch on indomethacin-induced gastric ulcer in rats: a proteomic and metabolomic approach. *bmc complementary and alternative medicine, 19*(1), 1-16. doi:10.1186/S12906-019-2623-4

Kumar, S., Singh, J., Rattan, S., DiMarino, A. J., Cohen, S., Jimenez, S. A. J. A. p., & therapeutics. (2017). pathogenesis and clinical manifestations of gastrointestinal involvement in systemic sclerosis. *45*(7), 883-898.

Kunwar, R. M., Acharya, R. P., Chowdhary, C. L., & Bussmann, R. W. (2015). Medicinal plant dynamics in indigenous medicines in farwest Nepal. *Journal of ethnopharmacology, 163*, 210-219.

Kwenda, E. P., Rabley, A. K., & Canales, B. K. (2020). Lessons from rodent gastric bypass model of enteric hyperoxaluria. *current opinion in nephrology and hypertension, 29*(4), 400-406. doi:10.1097/MNH.0000000000000613

Laine, L., & Nagar, A. (2016). Long-term PPI use: balancing potential harms and documented benefits. *The American journal of gastroenterology, 111*(7), 913.

Le Floch, F., Tena, M., Rıos, A., & Valcarcel, M. J. T. (1998). Supercritical fluid extraction of phenol compounds from olive leaves. *46*(5), 1123-1130.

Lee, W.-C., Peng, C.-C., Chang, C.-H., Huang, S.-H., & Chyau, C.-C. (2013). Extraction of antioxidant components from Bidens pilosa flowers and their uptake by human intestinal Caco-2 cells. *Molecules, 18*(2), 1582-1601.

Lemos, J., Galvão, J., Silva, A., Fontanetti, A., Cecon, P., & Lemos, L. (2013). Management of Bidens pilosa and Commelina benghalensis in organic corn cultivation under no-tillage. *Planta Daninha, 31*(2), 351-357.

Levenstein, S., Rosenstock, S., Jacobsen, R. K., & Jorgensen, T. (2015). Psychological stress increases risk for peptic ulcer, regardless of Helicobacter pylori infection or use of nonsteroidal anti-inflammatory drugs. *Clinical Gastroenterology and Hepatology, 13*(3), 498-506. e491.

Li, M., Li, W., Lu, Y., Jameel, H., Chang, H.-m., & Ma, L. J. R. A. (2017). High conversion of glucose to 5-hydroxymethylfurfural using hydrochloric acid as a catalyst and sodium chloride as a promoter in a water/γ-valerolactone system. *7*(24), 14330-14336.

Lin, B. M., Curhan, S. G., Wang, M., Jacobson, B. C., Eavey, R., Stankovic, K. M., & Curhan, G. C. (2017). Prospective study of gastroesophageal reflux, use of proton pump inhibitors and H2-receptor antagonists, and risk of hearing loss. *Ear and hearing, 38*(1), 21.

Lopes, G. K., Schulman, H. M., & Hermes-Lima, M. J. B. e. B. A.-G. S. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *1472*(1-2), 142-152.

Lucarini, M., Durazzo, A., Kiefer, J., Santini, A., Lombardi-Boccia, G., Souto, E. B., . . . Gabrielli, P. J. F. (2020). Grape seeds: Chromatographic profile of fatty acids and phenolic compounds and qualitative analysis by FTIR-ATR spectroscopy. *9*(1), 10.

Machado, J., Cardoso, A. C., Gomes, I., Silva, I., Lopes, V., Peixoto, H., & Abelha, A. (2019). *Predicting the Length of Hospital Stay After Surgery for Perforated Peptic Ulcer.* Paper presented at the International Conference on Information Technology & Systems.

Madan, N. V., Sahu, M. S., Jambhulkar, Y. T., Mamidwar, A. S., Hingawe, N. T., & Maliye, A. N. (2018). A Review on Euphorbiaceae plants for peptic ulcer. *Research Journal of Pharmacognosy and Phytochemistry, 10*(4), 336-339.

Madzinga, M., Kritzinger, Q., & Lall, N. (2018). Medicinal plants used in the treatment of superficial skin infections: from traditional medicine to herbal soap formulations. In *Medicinal Plants for Holistic Health and Well-Being* (pp. 255-275): Elsevier.

Mahdy, A., Shehab, N., & Bayoumi, F. (2018). Protective effects of honey solution and Fagonia indica alcoholic extract against ethanol-induced gastric ulcer in rats. *International Journal of Clinical Pharmacology & Pharmacotherapy, 3*(1).

Malan, L. (2018). *Biology and germination characteristics of Urochloa mosambicensis and Urochloa panicoides.* North-West University,

Manno, M., Mangiafico, S., Caruso, A., Barbera, C., Bertani, H., Mirante, V. G., . . . Conigliaro, R. (2016). First-line endoscopic treatment with OTSC in patients with high-risk non-variceal upper gastrointestinal bleeding: preliminary experience in 40 cases. *Surgical endoscopy, 30*(5), 2026-2029.

Maton, P. N., & Burton, M. E. J. D. (1999). Antacids revisited. *57*(6), 855-870.

Matsunaga, T., Endo, H., Takamori, A., Shimada, F., Takedomi, H., Shirai, S., . . . Anzai, K. J. D. (2020). Lifestyle-and Comorbidity-Related Risk Factors Associated with Prescription of Gastric Acid Secretion Inhibitors to Japanese Patients Who Were Helicobacter pylori Negative and Had No Upper Gastrointestinal Lesions. 1-9.

Mehta, S., Bhardwaj, S., Bhatnagar, S., Mehta, S. S., Sudan, P., & Pandey, R. (2016). Herbal drugs: A boon in various pathological complications. *Journal of Innovations in Pharmaceuticals and Biological Sciences, 3*(3), 23-28.

Meyers, C., & Meyers, D. J. C. p. i. n. a. c. (2008). Thin-layer chromatography. *34*(1), A. 3D. 1-A. 3D. 13.

Miftahussurur, M., Yamaoka, Y., & Graham, D. Y. (2017). Helicobacter pylori as an oncogenic pathogen, revisited. *Expert reviews in molecular medicine, 19*.

Miraglia, C., Crafa, P., Franceschi, M., Barchi, A., Cambiè, G., Dal Bo, N., . . . Brandimarte, G. (2018). P. 01.29 Gastric ulcer: Risk factors, Helicobacter pylori infection & NSAIDS use in sample of 256 people. *Digestive and Liver Disease, 50*(2), e131.

Mitrasinovic, P. M. J. J. o. B. S., & Dynamics. (2021). On the inhibition of cytochrome P450 3A4 by structurally diversified flavonoids. 1-11.

Mohiuddin, A. (2019). Alternative Treatments of Minor GI Ailments. *International Journal of Health and Clinical Research, 2*(1), 29-60.

Monowar, T., Rahman, M., Bhore, S. J., Raju, G., & Sathasivam, K. V. J. B. r. i. (2019). Secondary metabolites profiling of Acinetobacter baumannii associated with chili (Capsicum annuum L.) leaves and concentration dependent antioxidant and prooxidant properties. *2019*.

Mrudula, G., Caroline, P., Kingsley, T., & Jaiswal, R. (2017). A Review on Drug Interactions in Oral Hypoglycemic Drugs by Mechanism of Cytochrome P450 Enzyme Inhibition. *Journal of Pharmaceutical Research, 16*(2), 154-159.

Mustafa, G., Arif, R., Atta, A., Sharif, S., & Jamil, A. (2017). Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan.

Nile, S. H., Nile, A. S., & Keum, Y.-S. (2017). Total phenolics, antioxidant, antitumor, and enzyme inhibitory activity of Indian medicinal and aromatic plants extracted with different extraction methods. *3 Biotech, 7*(1), 76.

Norell, J. R. (1975). Process for preparing 3, 5-dialkyl phenols. In: Google Patents.

Oh, H., Lee, D. H., Giovannucci, E. L., Keum, N. J. C. C., & Control. (2020). Gastric and duodenal ulcers, periodontal disease, and risk of bladder cancer in the Health Professionals Follow-up Study. *31*(4), 383-391.

Omotoso, A. E., Olorunfemi, E. O., Mikailu, S. J. N. J. o. P., & Research, A. S. (2014). Phytochemical Analysis of Cnidoscolus aconitifolius (Euphorbiaceae) leaf with Spectrometric Techniques. *3*(1), 38-49.

Omran, B. A., Fatthalah, N. A., El-Gendy, N. S., El-Shatoury, E. H., & Abouzeid, M. A. J. J. P. A. M. (2013). Green biocides against sulphate reducing bacteria and macrofouling organisms. *7*(3), 2219-2232.

Ooka, J. K., & Owens, D. K. (2018). Allelopathy in tropical and subtropical species. *Phytochemistry Reviews*, *17*(6), 1225-1237.

Ottomann, C., Hartmann, B., Tyler, J., Maier, H., Thiele, R., Schaden, W., & Stojadinovic, A. (2010). Prospective Randomized Trial of Accelerated Re-epithelization of Skin Graft Donor Sites Using Extracorporeal Shock Wave Therapy. *journal of the american college of surgeons*, *211*(3), 361-367. doi:10.1016/J.JAMCOLLSURG.2010.05.012

Oyinloye, O., Olooto, W., Kosoko, A., Alabi, A., & Udeh, A. J. A. J. o. B. R. (2019). Effects of Extracts of Daucus carota and Brassica oleraceae on Ethanol-induced Gastric Ulcer. *22*(1), 89-95.

Palle, S., Kanakalatha, A., & Kavitha, C. N. (2018). Gastroprotective and antiulcer effects of Celastrus paniculatus seed oil against several gastric ulcer models in rats. *Journal of dietary supplements*, *15*(4), 373-385.

Panda, V., & Khambhat, P. (2013). Evaluation of antacid activity of Garciniaindica fruit rind by a modified artificial stomach model. *Bulletin of Environment, Pharmacology and Life Sciences*, *2*(7), 38-42.

Panda, V. S., & Shinde, P. M. (2016). A comparative study of the antacid effect of raw spinach juice and spinach extract in an artificial stomach model. *Journal of Complementary and Integrative Medicine*, *13*(4), 387-391.

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *2*(5).

Pérez-Fontan, M., Lopes, D. M., Enríquez, A. G., López-Calviño, B., López-Muñiz, A., Falcón, T. G., & Rodríguez-Carmona, A. (2016). Inhibition of gastric acid secretion by H2 receptor antagonists associates a definite risk of enteric peritonitis and infectious mortality in patients treated with peritoneal dialysis. *PloS one*, *11*(2), e0148806.

Pittayanon, R., Martel, M., & Barkun, A. (2018). A 201 Efficacy of proton pump inhibitor plus mucoprotective agent for endoscopic submucosal dissection-derived ulcer; A

systematic review and meta-analysis of randomised control trial *Journal of the Canadian Association of Gastroenterology., 1*(suppl_2), 297-298.

Pittayanon, R., Martel, M., & Barkun, A. (2018). Role of mucoprotective agents in endoscopic submucosal dissection-derived ulcers: A systematic review. *Journal of gastroenterology and hepatology, 33*(12), 1948-1955.

Pyšek, P., Pergl, J., Essl, F., Lenzner, B., Dawson, W., Kreft, H., . . . Nishino, M. (2017). Naturalized alien flora of the world: species diversity, taxonomic and phylogenetic patterns, geographic distribution and global hotspots of plant invasion. Preslia 89: 203–274. In.

Ramadan, T., Amro, A., & Abd-Almoneim, M. S. (2018). Impact of Pollution on Weeds Phytosociology. *Egyptian Journal of Botany. , 58*(3), 423-436.

Rana, S. S., Badiyala, D., Sharma, N., & Kumar, R. (2015). Major weeds in the non-cropped lands of Himachal Pradesh. *Department of Agronomy, CSK Himachal Pradesh Krishi Vishvavidyalaya*, *Palampur*.

Re, V. D., Orzes, E., Canzonieri, V., Maiero, S., Fornasarig, M., Alessandrini, L., . . . Cannizzaro, R. (2016). Pepsinogens to Distinguish Patients With Gastric Intestinal Metaplasia and Helicobacter pylori Infection Among Populations at Risk for Gastric Cancer. *clinical and translational gastroenterology, 7*(7). doi:10.1038/CTG.2016.42

Sandhya, S., Venkata, K. R., Vinod, K., & Rsnakk, C. J. A. P. J. o. T. B. (2012). Assessment of in vitro antacid activity of different root extracts of Tephrosia purpurea (L) Pers by modified artificial stomach model. *2*(3), S1487-S1492.

Sangster, A. W., & Stuart, K. L. J. C. R. (1965). Ultraviolet spectra of alkaloids. *65*(1), 69-130.

Sanhueza, L., Melo, R., Montero, R., Maisey, K., Mendoza, L., & Wilkens, M. J. P. O. (2017). Synergistic interactions between phenolic compounds identified in grape pomace extract with antibiotics of different classes against Staphylococcus aureus and Escherichia coli. *12*(2), e0172273.

Saniee, P., Shahreza, S., & Siavoshi, F. (2016). Negative Effect of Proton-pump Inhibitors (PPI s) on Helicobacter pylori Growth, Morphology, and Urease Test and Recovery after PPI Removal–An In vitro Study. *Helicobacter, 21*(2), 143-152.

Schulman, A. R., Chan, W. W., Devery, A., Ryan, M. B., & Thompson, C. C. (2017). Opened proton pump inhibitor capsules reduce time to healing compared with intact capsules for marginal ulceration following Roux-en-Y gastric bypass. *Clinical Gastroenterology and Hepatology, 15*(4), 494-500. e491.

Selvakumar, N., Das, J., Trehan, S., Iqbal, J., Kumar, M. S., Rajagopalan, R., & Rao, M. N. V. S. (2007). Heterocyclic compounds having antibacterial activity: process for

their preparation and pharmaceutical compositions containing them. In: Google Patents.

Sethuraman, V., Hedden, D. B., & Leskow, K. M. (2017). Gastro-retentive sustained-release oral dosage form of a bile acid sequestrant. In: Google Patents.

Shakya, A. K. (2016). Medicinal plants: future source of new drugs. *International Journal of Herbal Medicine, 4*(4), 59-64.

Sharma, D., Pramanik, A., & Agrawal, P. K. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus Pestalotiopsis neglecta BAB-5510 isolated from leaves of Cupressus torulosa D. Don. *3 Biotech, 6*(2), 210.

Shunmugapriya, K., Vennila, P., Thirukkumar, S., Ilamaran, M. J. J. o. P., & Phytochemistry. (2017). Identification of bioactive components in Moringa oleifera fruit by GC-MS. *6*(3), 748-751.

Somaratne, G., Ferrua, M. J., Ye, A., Nau, F., Floury, J., Dupont, D., . . . nutrition. (2020). Food material properties as determining factors in nutrient release during human gastric digestion: A review. *60*(22), 3753-3769.

Stella, M. E., Entwisle, J. R., Newlon, J. W., & Naykki, C. L. (2017). Effervescent dosage form. In: Google Patents.

Stevenson, A., & Waite, M. (2011). *Concise Oxford English Dictionary: Book & CD-ROM Set*: Oxford University Press.

Strand, D. S., Kim, D., & Peura, D. A. (2017). 25 years of proton pump inhibitors: a comprehensive review. *Gut and liver, 11*(1), 27.

Sumbul, S., Ahmad, M. A., Mohd, A., Mohd, A. J. J. o. P., & Sciences, B. (2011). Role of phenolic compounds in peptic ulcer: An overview. *3*(3), 361.

Suzuki, M., Yokota, M., Matsumoto, T., Nakayama, M., Takemura, M., Kanemitsu, Y., . . . Murakami, S. (2019). Proton Pump Inhibitor Ameliorates Taste Disturbance among Patients with Laryngopharyngeal Reflux: A Randomized Controlled Study. *The Tohoku journal of experimental medicine, 247*(1), 19-25.

Tabarean, I. V. (2016). Histamine receptor signaling in energy homeostasis. *Neuropharmacology, 106*, 13-19.

Taha, A. S., McCloskey, C., McSkimming, P., & McConnachie, A. (2018). Misoprostol for small bowel ulcers in patients with obscure bleeding taking aspirin and non-steroidal anti-inflammatory drugs (MASTERS): a randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet Gastroenterology & Hepatology, 3*(7), 469-476.

Tang, Z., Shi, J., Ji, M., Shi, P., Huang, Z., & Huang, Y. J. S. j. o. g. o. j. o. t. S. G. A. (2018). The characteristics of 83 giant peptic ulcers in Chinese children: Evaluation and follow-up. *24*(6), 360.

Thomford, N., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., . . . Dandara, C. (2015). Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition. *Pharmaceuticals, 8*(3), 637-663.

Tripathy, S., & Afrin, R. (2016). Herbal treatment alternatives for peptic ulcer disease. *Journal of Drug Delivery and Therapeutics, 6*(3), 27-33.

Ugwah, M. O., Ugwah-Oguejiofor, C. J., Etuk, E. U., Bello, S. O., & Aliero, A. A. J. J. o. e. (2019). Evaluation of the antiulcer activity of the aqueous stem bark extract of Balanites aegyptiaca L Delile in Wistar rats. *239*, 111931.

Vanaja, D., & Kavitha, S. J. E. J. P. M. R. (2016). A study on phytochemicals, antioxidant activity and ft-ir analysis of Rhapis excelsa (thunb.) A. Henry. *3*(7), 390-394.

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., . . . Pyšek, P. (2011). Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology letters, 14*(7), 702-708.

Vilas Boas, S. A. M. (2017). *Studies on the solubility of phenolic compounds.*

Wang, X., Zhang, A., Yan, G., Han, Y., & Sun, H. (2014). UHPLC-MS for the analytical characterization of traditional Chinese medicines. *TrAC Trends in Analytical Chemistry, 63*, 180-187.

Wei, Z.-k., Ma, J.-w., Zhang, Q. J. F., & Chemicals, S. (2012). Study on the alkylation reaction of mixed P-cresol and m-cresol [J]. *6*.

Werbel, T., & Cohen, P. R. (2018). Ranitidine-Associated Sleep Disturbance: Case Report and Review of H2 Antihistamine-Related Central Nervous System Adverse Effects. *Cureus, 10*(4).

Wilson, I. D., & Nicholson, J. K. (2017). Gut microbiome interactions with drug metabolism, efficacy, and toxicity. *Translational Research, 179*, 204-222.

Wolfe, M. M., & Lowe, R. C. (2015). Gastric secretions. In *Yamada's Textbook of Gastroenterology* (pp. 399-419): Wiley-Blackwell.

Wu, T.-H., Chen, I.-C., & Chen, L.-C. J. W. J. o. G. W. (2010). Antacid effects of Chinese herbal prescriptions assessed by a modified artificial stomach model. *16*(35), 4455.

Xu, Z., & Chang, L. (2017). Asteraceae. In *Identification and Control of Common Weeds: Volume 3* (pp. 441-721): Springer.

Xuan, T. D., & Khanh, T. D. (2016). Chemistry and pharmacology of Bidens pilosa: an overview. *Journal of Pharmaceutical Investigation, 46*(2), 91-132.

Yang, W.-C. (2014). Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant Bidens pilosa L. *Evidence-Based Complementary and Alternative Medicine, 2014*.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules, 21*(5), 559.

Zhang, A.-h., Sun, H., Yan, G.-l., Han, Y., Zhao, Q.-q., & Wang, X.-j. (2018). Chinmedomics: A Powerful Approach Integrating Metabolomics with Serum Pharmacochemistry to Evaluate the Efficacy of Traditional Chinese Medicine. *Engineering*.

Zhang, K.-m., Shen, Y., Yang, J., Miu, X., Bhowmik, P. C., Zhou, X., . . . Xing, B.-s. (2019). The defense system for Bidens pilosa root exudate treatments in Pteris multifida gametophyte. *Ecotoxicology and environmental safety, 173*, 203-213.

Zhang, L., Smyrk, T. C., Young Jr, W. F., Stratakis, C. A., & Carney, J. A. (2010). Gastric stromal tumors in Carney triad are different clinically, pathologically, and behaviorally from sporadic gastric gastrointestinal stromal tumors: findings in 104 cases. *The American journal of surgical pathology, 34*(1), 53.

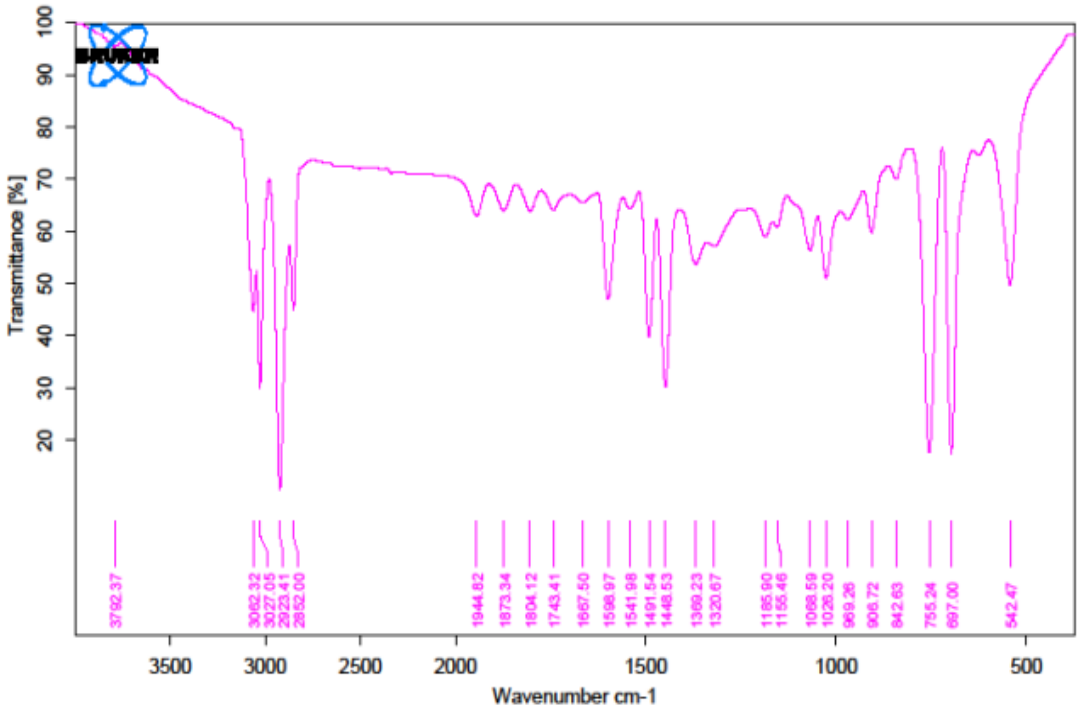
Zhou, K., Pedersen, H. K., Dawed, A. Y., & Pearson, E. R. (2016). Pharmacogenomics in diabetes mellitus: insights into drug action and drug discovery. *Nature Reviews Endocrinology, 12*(6), 337.

Zhu, W., & Hong, K. (2017). Potential cardiovascular risks of proton pump inhibitors in the general population. *International heart journal, 58*(2), 163-166.

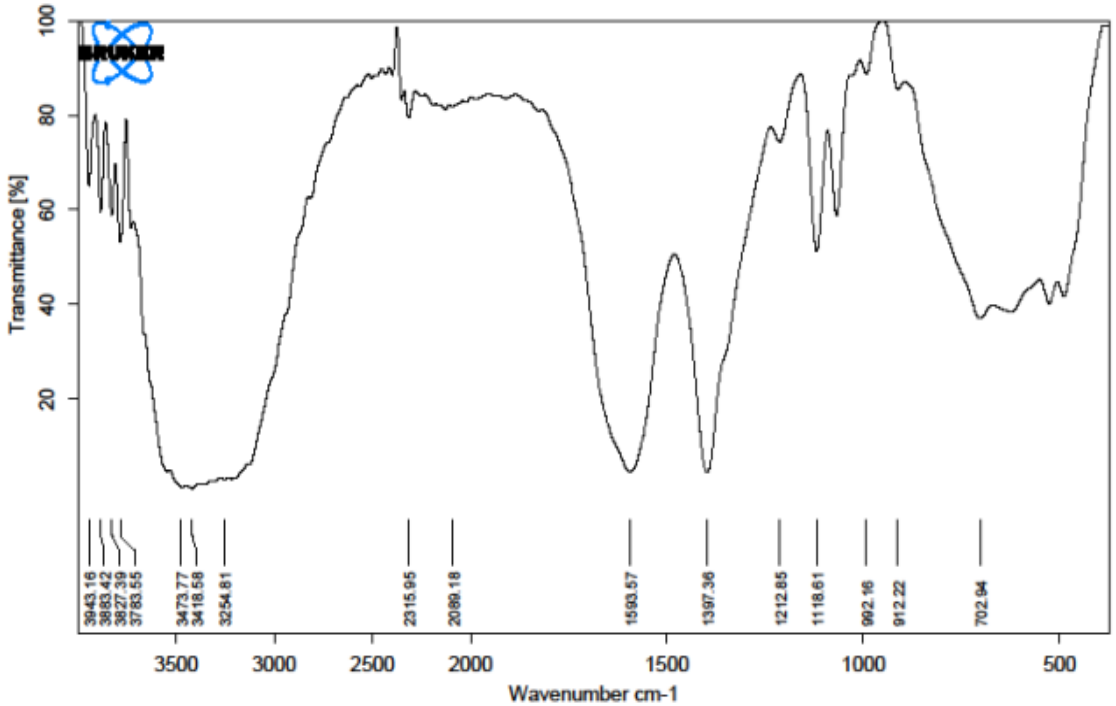
Zibima, S. B., Oniso, J. I., Wasini, K. B. J. I. J. o. H. S., & Research. (2020). Prevalence Trends and Associated Modifiable Risk Factors of Peptic Ulcer Disease among Students in a University Community South-South Nigeria. *10*(6), 97-105.

APPENDICES

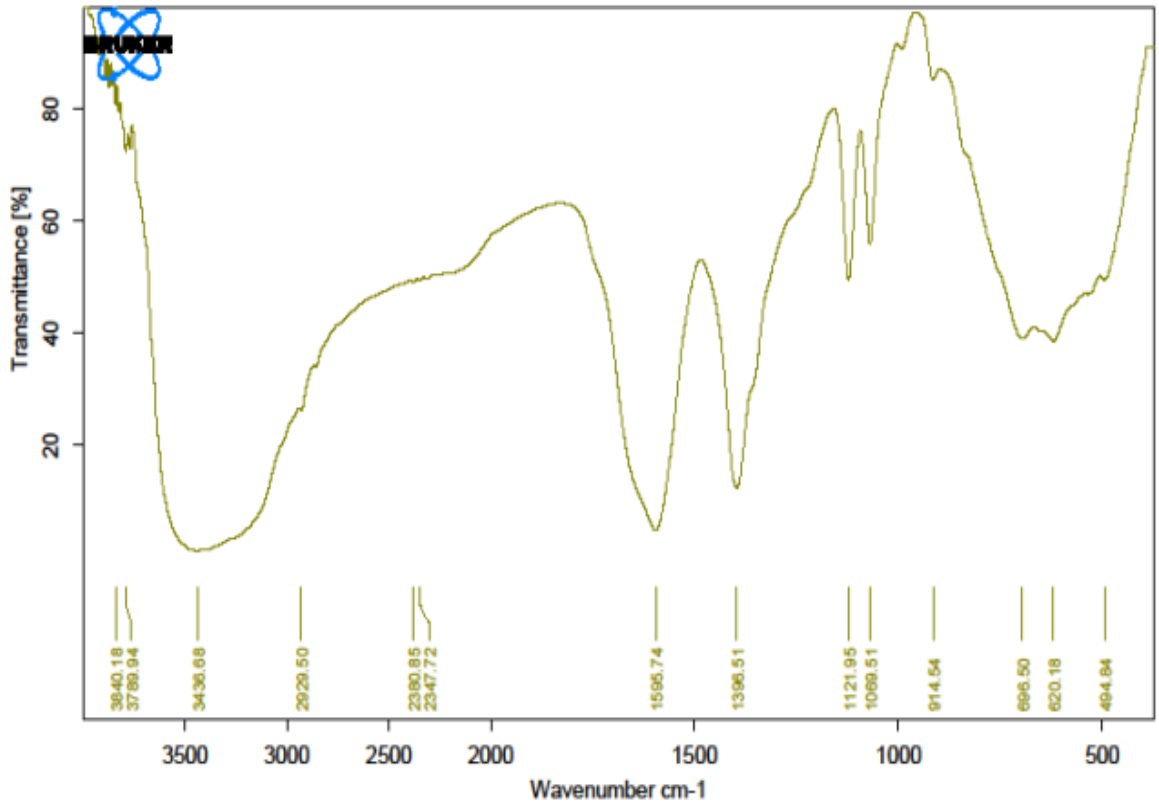
Appendix 1. FTIR spectrum of Polystyrene (FTIR standard)



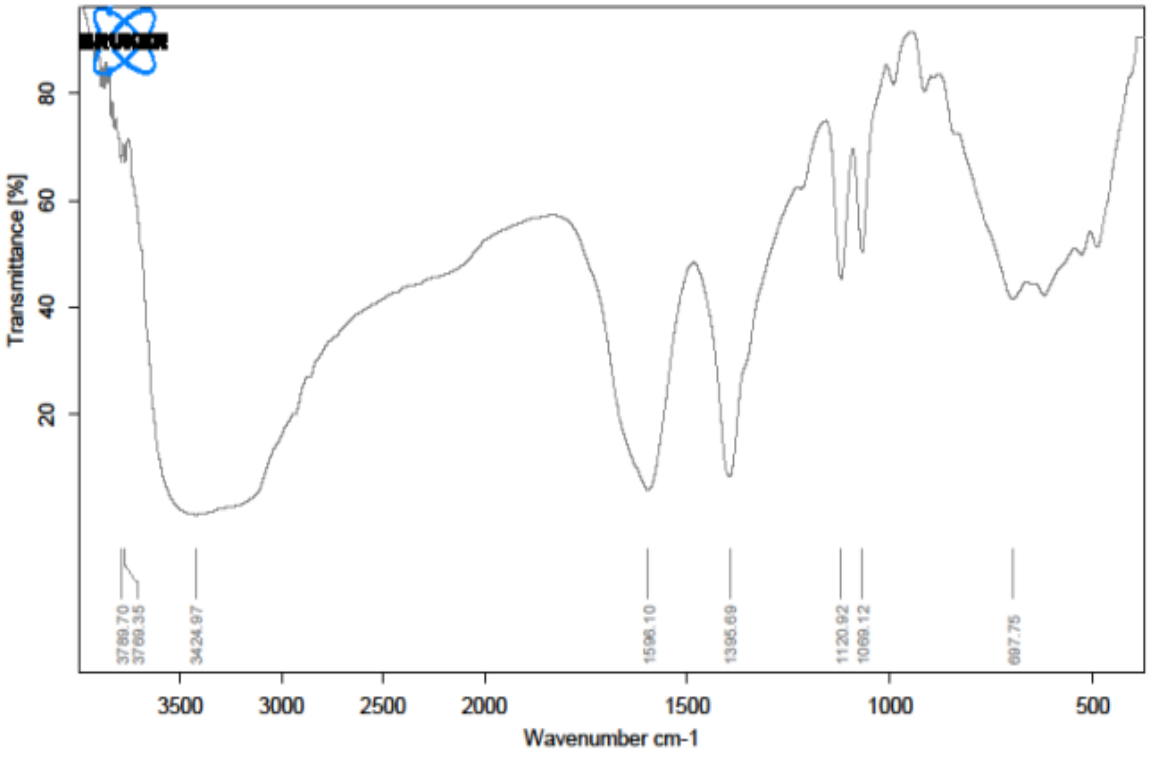
Appendix 2: FTIR spectrum of fraction 1



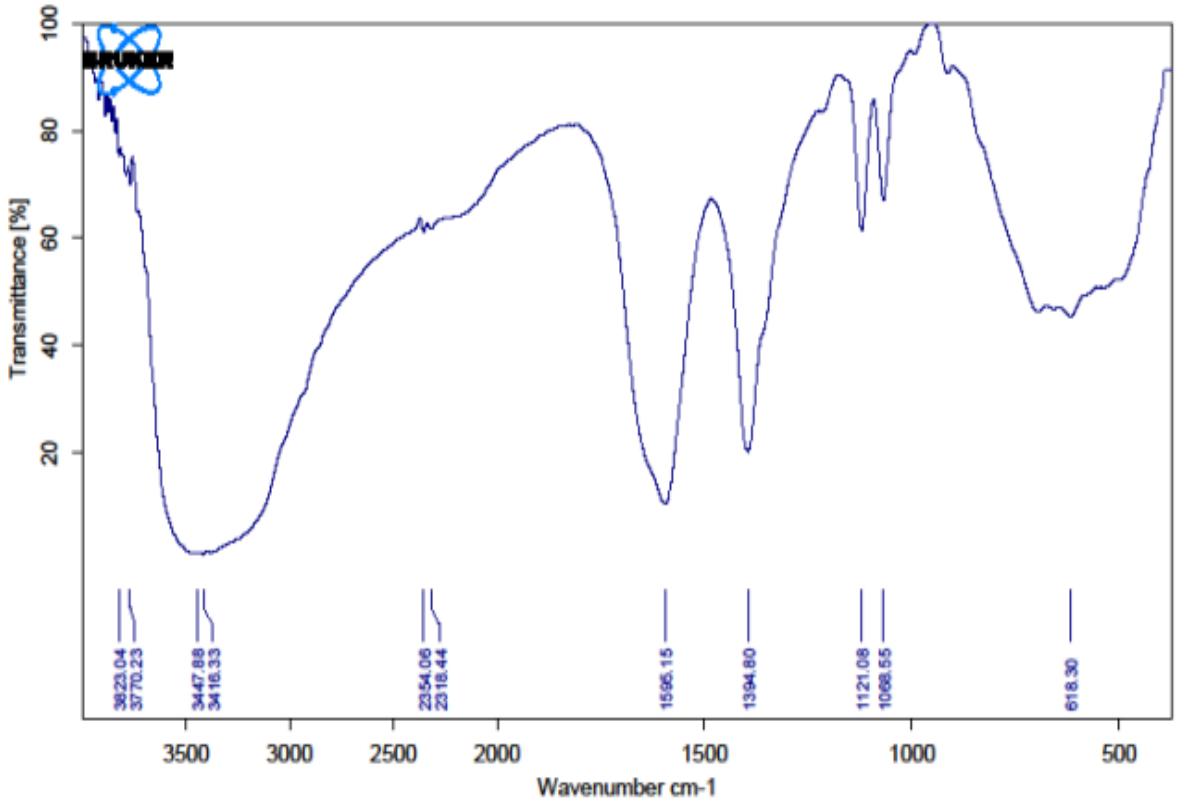
Appendix 3: FTIR spectrum of fraction 2



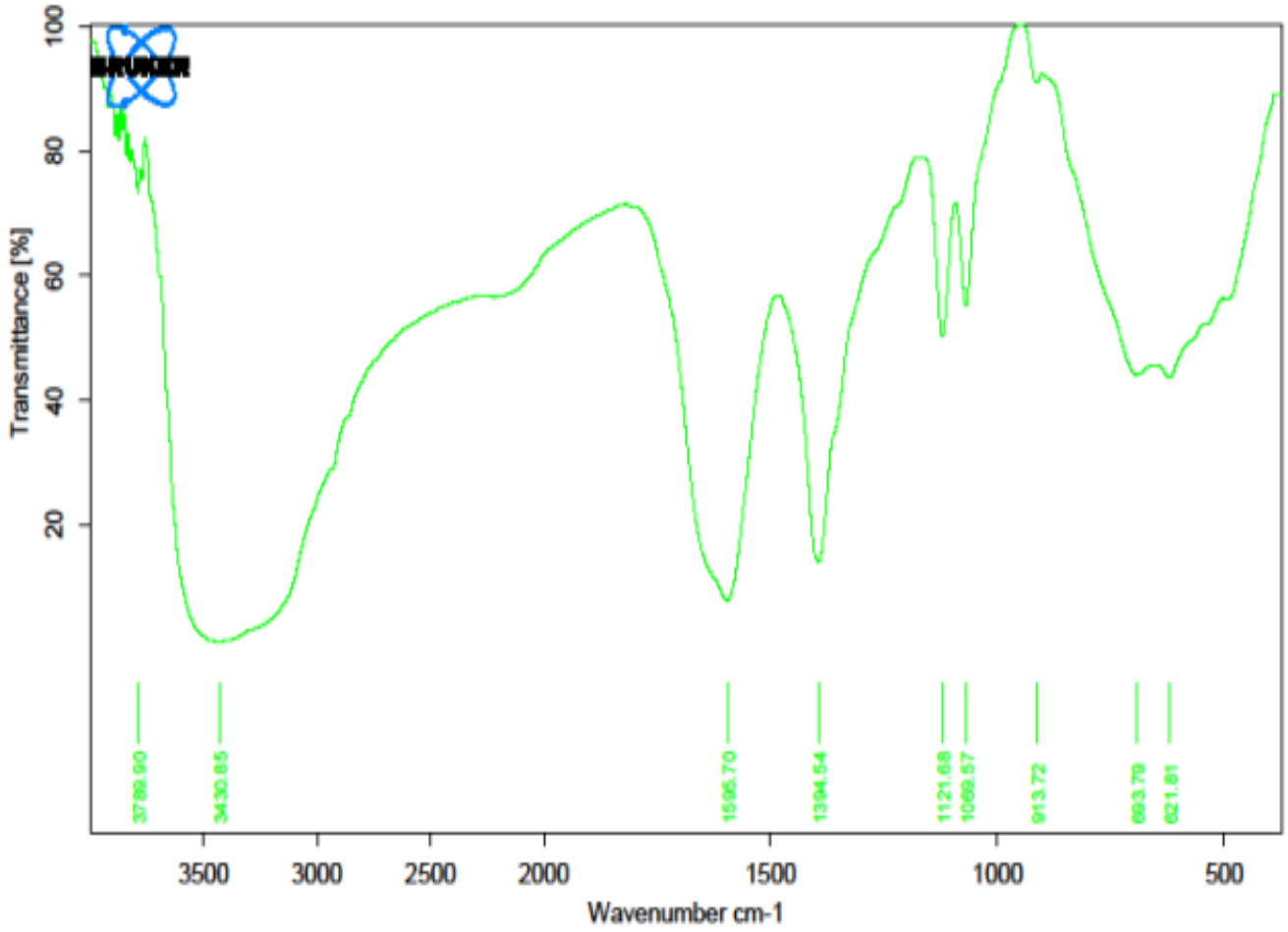
Appendix 4: FTIR spectrum of fraction 3



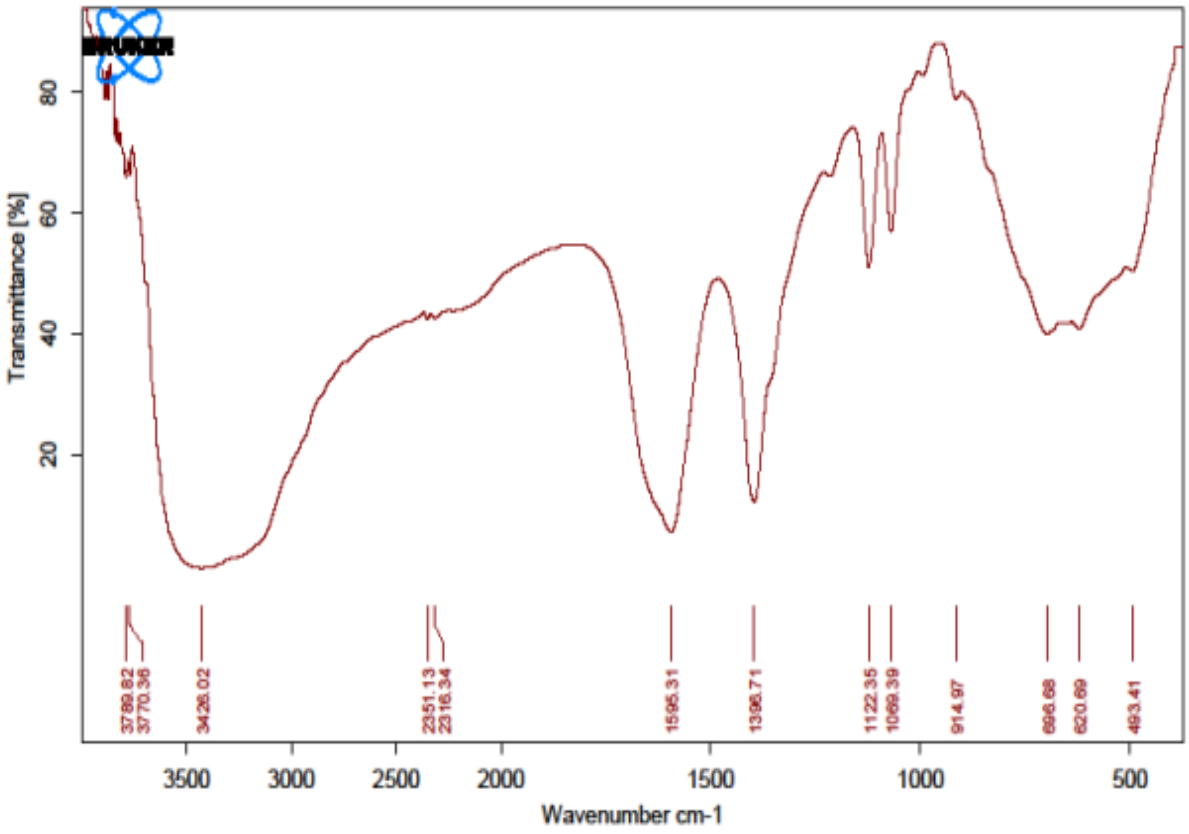
Appendix 5: FTIR spectrum of fraction 4



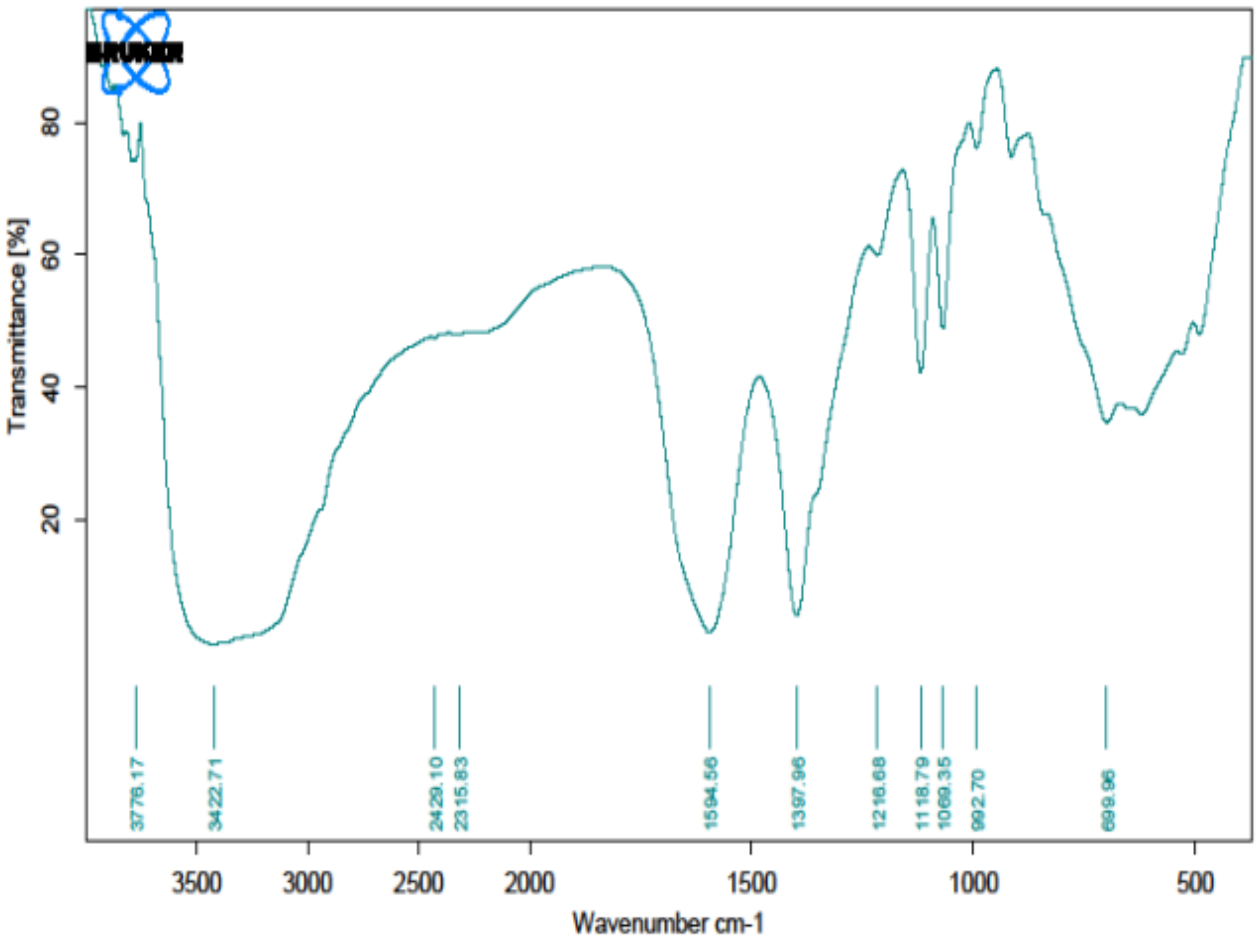
Appendix 6: FTIR spectrum of fraction 5



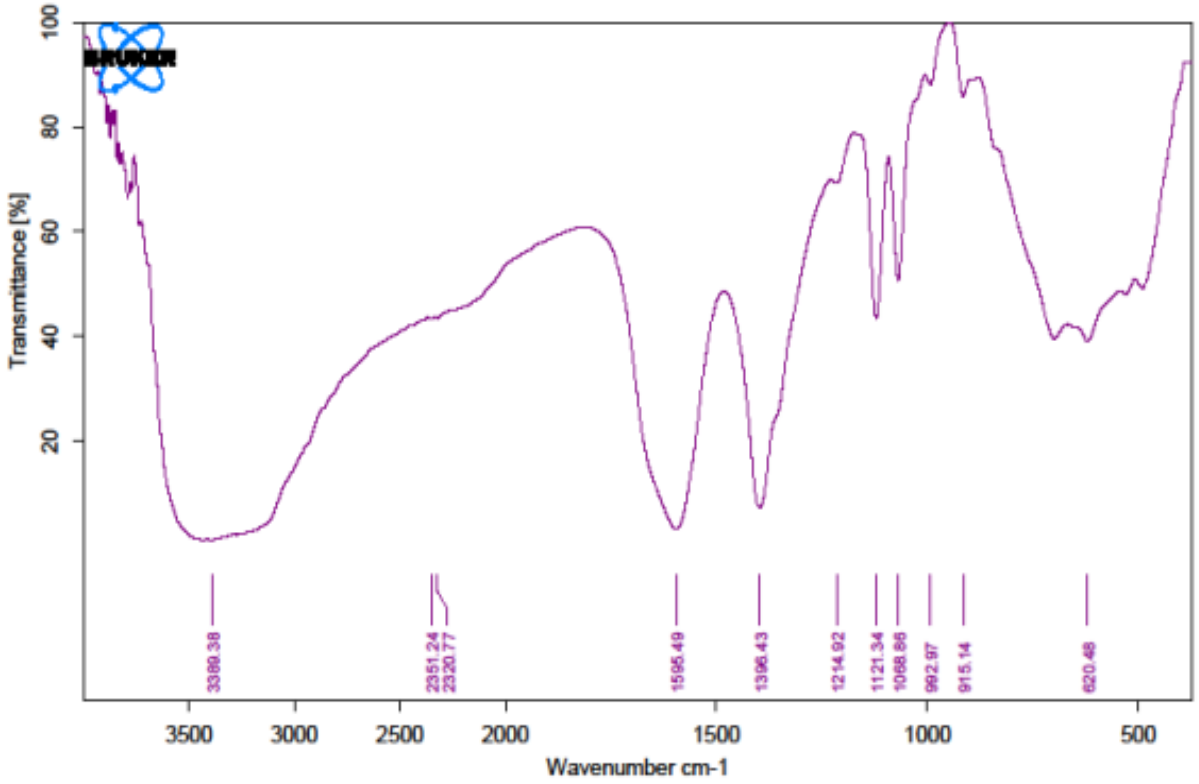
Appendix 7: FTIR spectrum of fraction 6



Appendix 8: FTIR spectrum of fraction 7

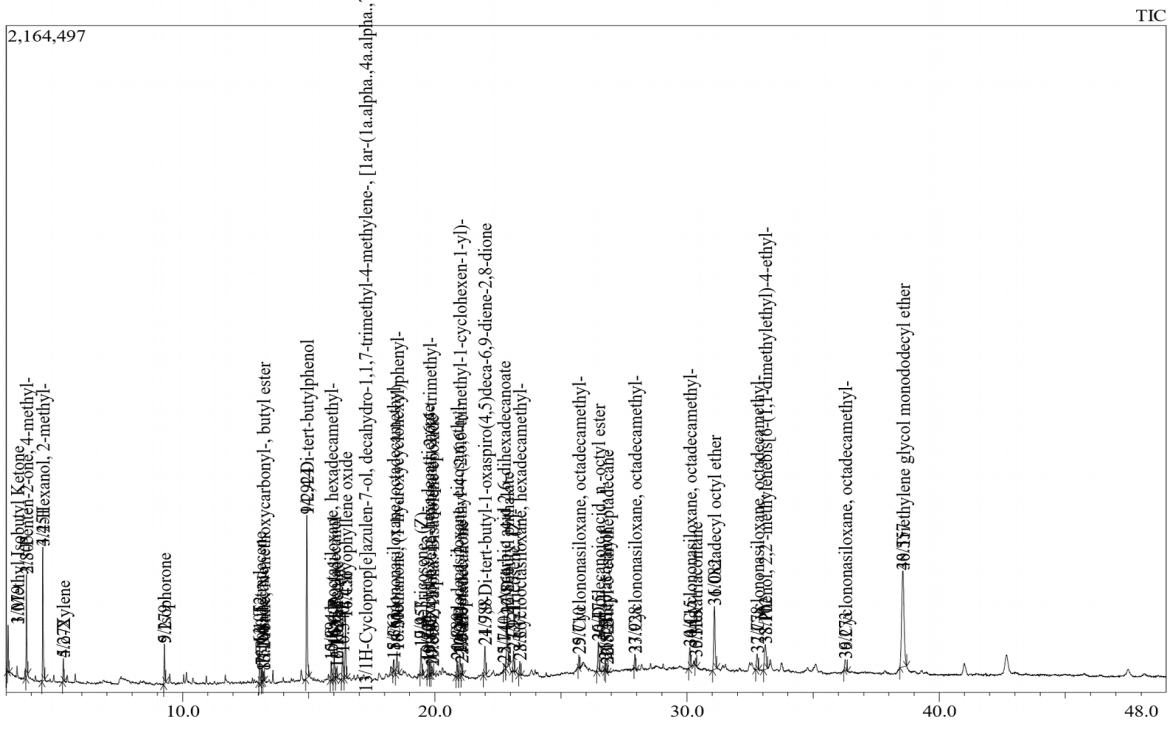


Appendix 9: FTIR spectrum of fraction 8

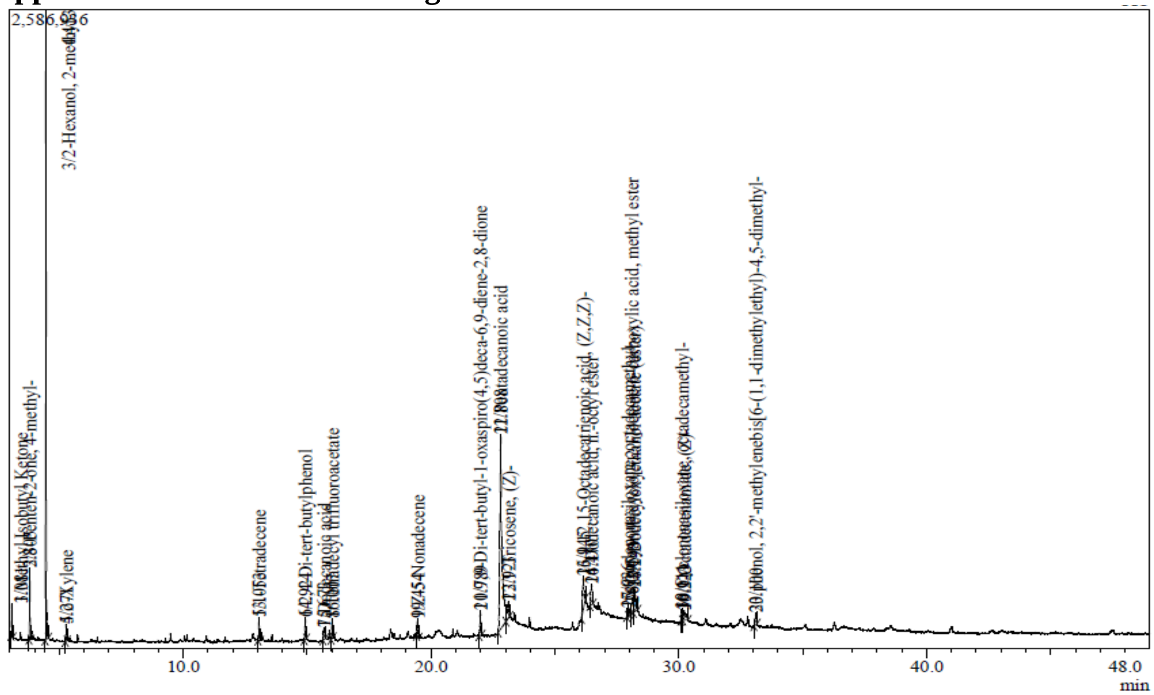


Appendix 10. GC-MS chromatogram of fraction 1

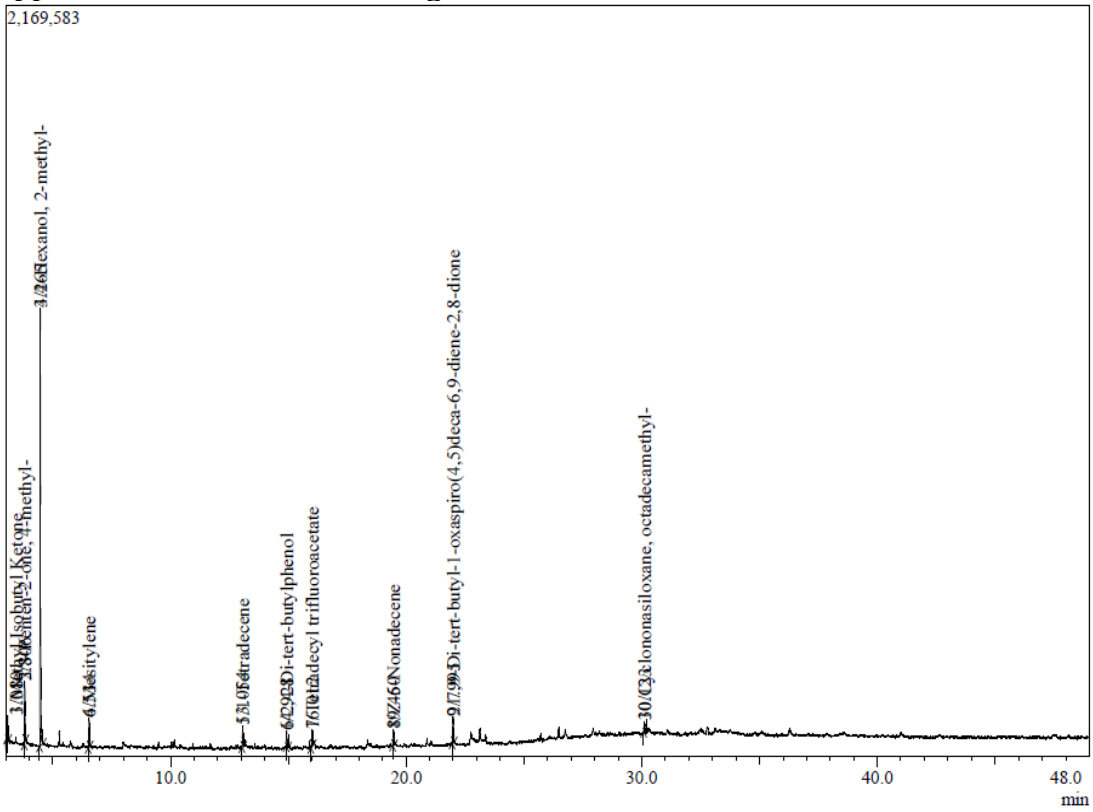
Org Method File : C:\GCMSsolution\Data\Project1\Martin\Volatiles_natproducts.qgm
Report File :
Tuning File : C:\GCMSsolution\System\Tune1\Default.qgt
Modified by : Admin
Modified : 31-Mar-20 1:37:56 PM



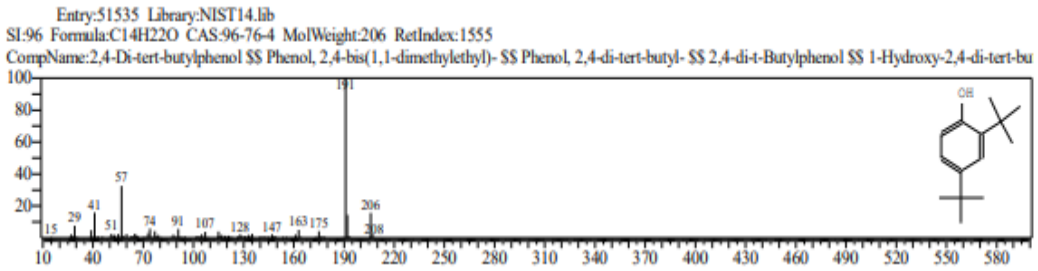
Appendix 11. GC-MS chromatogram of fraction 2



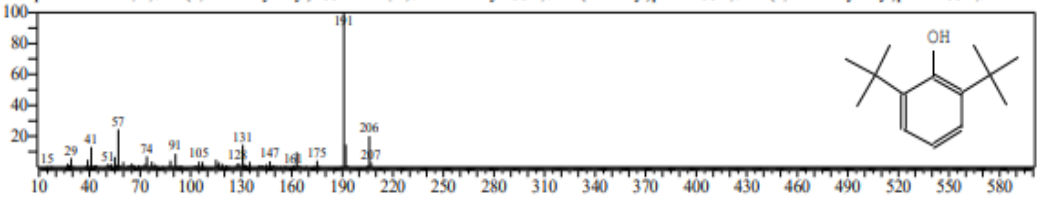
Appendix 12. GC-MS chromatogram of fraction 3



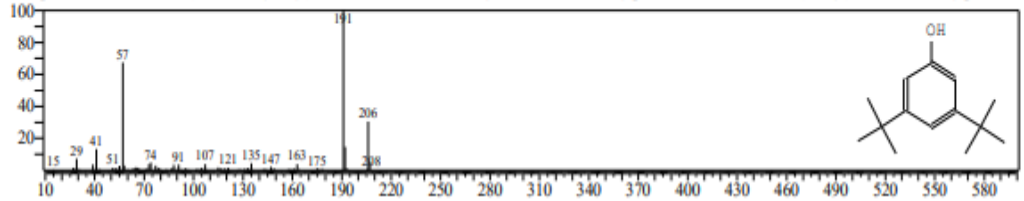
Appendix 13. Total ion chromatograms of identified phenolic compound from *Bidens pilosa* fractions



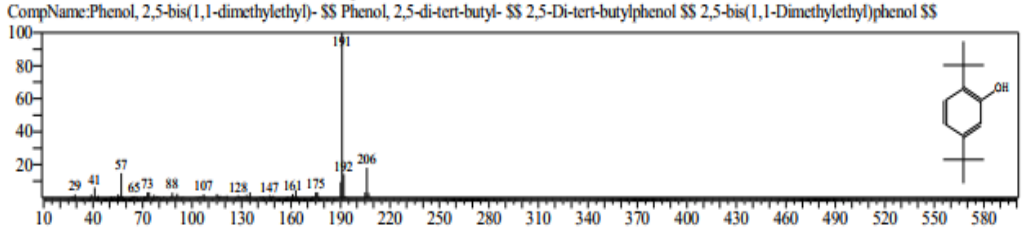
Entry:51537 Library:NIST14.lib
SI:92 Formula:C14H22O CAS:128-39-2 MolWeight:206 RetIndex:1555
CompName:Phenol, 2,6-bis(1,1-dimethylethyl)- $$ Phenol, 2,6-di-tert-butyl- $$ 2,6-Bis(tert-butyl)phenol $$ 2,6-Bis(1,1-dimethylethyl)phenol $$ 2,6-Di-tert



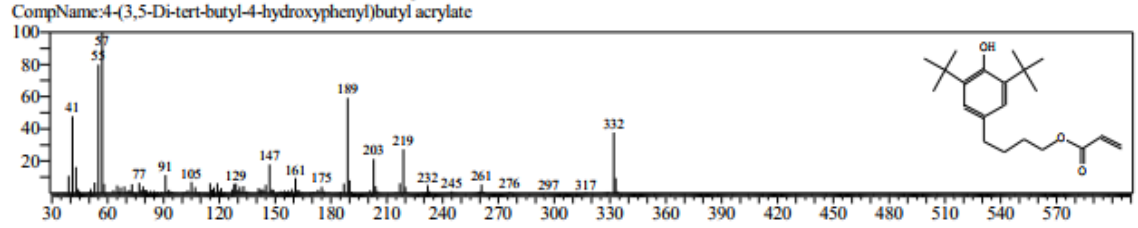
Entry:51536 Library:NIST14.lib
SI:90 Formula:C14H22O CAS:1138-52-9 MolWeight:206 RetIndex:1555
CompName:Phenol, 3,5-bis(1,1-dimethylethyl)- $$ Phenol, 3,5-di-tert-butyl- $$ 3,5-Di-tert-butylphenol $$ Phenol, 3,5-bis(t-butyl) $$ 3,5-Di-t-butylphenol $



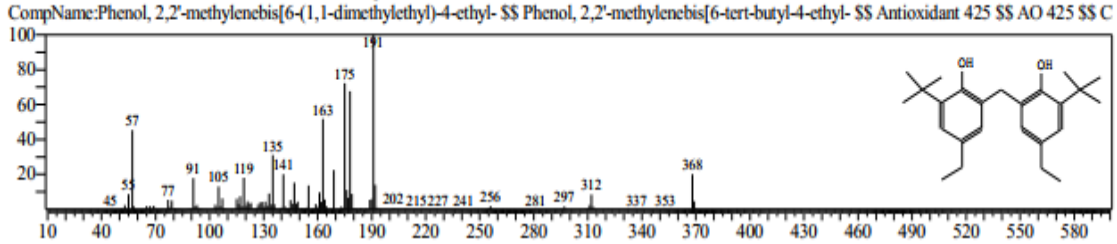
Entry:51541 Library:NIST14.lib
SI:89 Formula:C14H22O CAS:5875-45-6 MolWeight:206 RetIndex:1555
CompName:Phenol, 2,5-bis(1,1-dimethylethyl)- $$ Phenol, 2,5-di-tert-butyl- $$ 2,5-Di-tert-butylphenol $$ 2,5-bis(1,1-Dimethylethyl)phenol $$



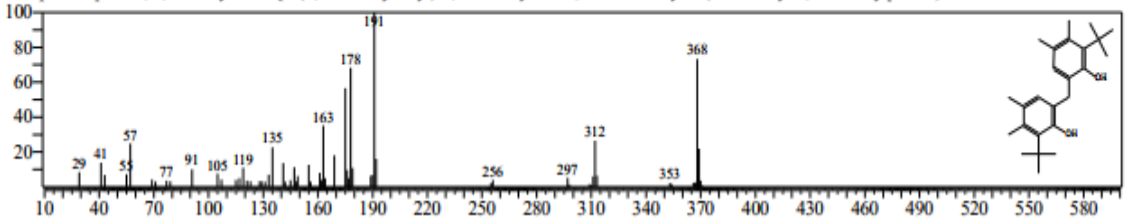
Entry:160098 Library:NIST14.lib
SI:62 Formula:C21H32O3 CAS:87033-84-9 MolWeight:332 RetIndex:2422
CompName:4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acrylate



Entry:187878 Library:NIST14.lib
SI:92 Formula:C25H36O2 CAS:88-24-4 MolWeight:368 RetIndex:2987
CompName:Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl- $$ Phenol, 2,2'-methylenebis[6-tert-butyl-4-ethyl- $$ Antioxidant 425 $$ AO 425 $$ C



Entry:187879 Library:NIST14.lib
SI:91 Formula:C25H36O2 CAS:0-00-0 MolWeight:368 RetIndex:0
CompName:phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4,5-dimethyl- $$ 2,2'-methanediylbis(6-tert-butyl-4,5-dimethylphenol) $$



Entry:178091 Library:NIST14.lib
SI:65 Formula:C24H34O2 CAS:27996-19-6 MolWeight:354 RetIndex:2757
CompName:2-tert-Butyl-6-(3-tert-butyl-2-methoxy-5-methylbenzyl)-4-methylphenol