Antidiarrheal Activity of Crude Extract and Coumarins from *Psydrax schimperiana* (A. Rich.) Bridson. Roots in Swiss Albino Mice, and Evaluation of Nutritional Composition and Anti-nutritional Factors of Its Fruits

A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacognosy

By: -Getahun Damissie (B. Pharm)

Under the supervision of

Prof. Ariaya Hymete (PhD)

Dr. Solomon Tadesse (PhD)

Dr. Ketema Tolossa (PhD)

January 2023

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Getahun Damissie, entitled: **"Antidiarrheal activity of crude extract and coumarins from *Psydrax schimperiana* (A. Rich.) Bridson. roots in Swiss albino mice, and evaluation of nutritional composition and antinutritional factors of its fruits,"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacognosy complies with the regulations of the university and meets the accepted standards for originality and quality.

Signed by the Examining Committee:

Prof. Ariaya Hymete (Advisor): Signature_____ Date_____

Dr. Solomon Tadesse (Advisor): Signature_____ Date_____

Dr. Ketema Tolossa (Advisor): Signature_____ Date_____

Mr. Biniam Paulos (Internal Examiner): Signature_____ Date_____

Dr. Workineh Shibeshi (External Examiner): Signature_____ Date_____

Abstract

Despite considerable improvements in the accessibility of medical services, diarrheal illnesses continue to claim the lives of children, accounting for up to 9% of all pediatric fatalities worldwide. The roots of *Psydrax schimperiana* is traditionally used to treat diarrhea and its fruits are consumed for nutritional purposes in Ethiopia. This study aimed to investigate *in vivo* antidiarrheal activity of crude extract and coumarins isolated from the roots of *P. schimperiana* to provide a pharmacological basis for its traditional use as diarrhea agent and to evaluate the nutritional composition and anti-nutritional factors from its fruits.

Maceration with 80% methanol was used to prepare the crude extract from the powdered roots of *P. schimperiana*. The crude roots extract of *P. schimperiana* was tested for *in vivo* antidiarrheal efficacy in mice utilizing castor oil-induced diarrhea, gastrointestinal transit time and enteropooling models at doses of 100, 200, and 400 mg/kg. Phytochemical investigation of the crude roots extract led to the isolation of two coumarins, isoscopoletin and scoparone. The structures of these compounds were established by interpretation of their spectral data, and by comparison with data reported in the literature. Isoscopoletin and scoparone were evaluated for antidiarrheal activity against castor oil-induced diarrhea model at 10 mg/kg and 20 mg/kg doses.

Moisture content, ash value, crude protein, crude fibers, crude fat, phytate, tannin, and oxalate contents of *P. schimperiana* fruits were determined according to the standard analytical procedure. All generated data were analyzed using SPSS software version 26.

The crude root extract of *P. schimperiana*, at doses of 100, 200, and 400 mg/kg, inhibited defecation by 37.5%, 46.2%, and 61.2%, respectively. At a dose of 20 mg/kg, scoparone and isoscopoletin reduced defecation by 61.2% and 66.6%, respectively.

Fruits from *P. schimperiana* contained 31% fiber, 9.19% crude protein, 12.40% moisture, 6.38% ash, and 0.99% fat. The fruits also contained the anti-nutritional factors phytate (1.16%), oxalate (0.72%), and tannin (22.96%). The study provides a scientific basis for further development and usage of the roots of *P. schimperiana,* and warrants continued investigation of isoscopoletin and scoparone toward development as a novel treatment for diarrheal diseases. The fruits of *P. schimperiana* can be considered as a beneficial source of vital nutrients such as carbohydrate, protein, and crude fibers. However, the higher tannin content may interfere with the absorption of essential minerals and inhibit effective utilization of proteins.

Keywords: Antidiarrheal activity, Traditional medicine, *Psydrax schimperiana*, Isoscopoletin, Scoparone, proximate analysis, antinutritional factors.

Acknowledgments

First and foremost, glories, praises, and thanks be to God, the Almighty, for His infinite mercy and inexpressible help. I am extremely grateful to my supervisors, Prof. Ariaya Hymete, Dr. Solomon Tadesse, and Dr. Ketema Tolossa for their invaluable advice, committed assistance, and genuine help with incredible encouragement and provision of constructive comments.

I would also like to express gratitude to Mr. Belachew Borento for his support during plant material collection, Mr. Melaku Wondafrash for plant material authentication, Mr. Molla Wale for his consistent care of the laboratory animals, Mr. Debebe Zelalem for his support during nutritional and anti-nutritional analysis, and all members of Department of pharmaceutical chemistry and pharmacognosy, School of Pharmacy, Collage of health science, Addis Ababa University, for their assistance. I would also like to express my sincere gratitude to Salale University and Addis Ababa University for sponsoring my studies. I am grateful for the financial support of the 'Thematic Research Project on Wild Edible Plant' to complete my thesis work successfully. I'm extremely grateful to Mr. Sean Chin Chan, Department of Drug Discovery, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida 33612, United States for his invaluable assistance by performing Nuclear magnetic resonance spectroscopy analysis.

To my family, particularly my parents and sisters, thank you for your love, support, and unshakable faith in me. Above all, I would like to thank my wife, Lelistu Tibebu for her unconditional love and support, and for all the late nights and early mornings, without her tremendous understanding and encouragement, it would be impossible for me to complete my study.

Finally, my appreciation also goes out to my friends for their encouragement and support throughout my studies.

Table of Contents

List of acronyms and abbreviations

^{1}H NMR: Proton Nuclear Magnetic Resonance

^{13}C NMR: Carbon Thirteen Nuclear Magnetic Resonance

ADI: Antidiarrheal Index

DEPT: Distortionless Enhancement by Polarization Transfer

ESITOFMS: Electrospray Ionization Time-Of-Flight Mass Spectrometry

IC_{50}: Half Maximal Inhibitory Concentration

LC-MS: Liquid Chromatography-Mass spectrum

MREPS: Methanol Roots Extract of *P. Schimperiana*

MIC: Minimal Inhibitory Concentration

MVSIC: Mean Volume of Small Intestinal Content

MWSIC: Mean Weight of Small Intestinal Content

PTLC: Preparative Thin Layer Chromatography

SPSS: Statistical Package for Social Science

TNF: Total Number of Feces

TNWF: Total Number of Wet Feces

TWF: Total Weight of Feces

TWWF: Total Weight of Wet Feces

TOF: Time of Flight

UNICEF: United Nations International Children's Emergency Fund

WEPs: Wild Edible Plants

WHO: World health organization

List of tables page

List of figures page

1. INTRODUCTION

1.1 Overview of diarrhea

Diarrhea is defined as the passage of three or more loose stools with an abnormal increase in water content and volume (Elisha *et al.,* 2013). It is marked by increased gastrointestinal motility, secretion, and disruption in regular bowel movements as well as a decrease in fluid and electrolyte absorption (Meite *et al.,* 2009). Diarrhea can be divided into three categories; acute diarrhea (lasting less than two weeks), persistent diarrhea (lasting two to four weeks), and chronic diarrhea (lasting more than four weeks) (Nguyen *et al.,* 2006). Diarrhea can be caused by protozoa, viruses, and bacteria pathogens. *E. coli, Shigella, Campylobacter* and *Salmonella* are common bacterial pathogens and *Cryptosporidium* has been the most common protozoal pathogen in immunocompromised children (Ahs *et al.*, 2010).

Acute diarrhea is caused by enteric infections such as viruses, bacteria, and parasites. Whereas chronic diarrhea can be caused by inflammatory bowel problems, malabsorption syndromes, and drugs (Nguyen *et al.*, 2006). Pathogenic agents such as *Cryptosporidium* and *Giardia lamblia* are the main causes of persistent diarrhea (Nguyen *et al.*, 2006). According to World Health Organization reports, diarrheal disease is responsible for about 370,000 deaths and this is nearly 9% of all mortality among children under the age of five globally (WHO, 2020).

1.2 Malnutrition and wild edible plants

Malnutrition is a severe global health issue that affects rural populations, particularly children and pregnant women in developing nations (Ogungbenle and Anisulowo, 2014). Currently, global food security and economic growth depends on a declining number of plant species. Underutilized plants can also meet nutritional requirements due to their better nutritional value compared

to some optimally utilized plants (Fokou *et al.*, 2004). One of the most prevalent dietary issues in children and infants is a protein-energy deficiency (FAO, 1994). The presence of anti-nutritional factors such as phytate, oxalate and tannin in plants restricts the utilization of plant-based diets (Kathirvel and Kumudha, 2011). Antinutritional factors can adversely interfere with the absorption of proteins and minerals including calcium, zinc and iron (Kruger *et al*., 2013).

Wild edible plants (WEPs) are the available food in the wildlife but are not cultivated as a crop in the agricultural fields (Getachew *et al*., 2013). Ethiopia is rich in WEPs, about 413 wild plant species are known to be consumed in parts of Ethiopia and they are widely utilized in meeting dietary needs, particularly for vitamins and other minerals (Duguma, 2020). Some WEP distributed in Ethiopia, contained large amounts of antinutritional components. For instance, *Ximenia caffra, Amaranthus graecizans,* and *Portulaca quadrifida* contained high quantities of anti-nutritional components (Getachew *et al*., 2013). *Ensete ventricosum* and *Moringa stenopetala* showed significant phytate content (Gizachew *et al*., 2019).

1.3 Medicinal plants for the treatment of diarrhea

For many years, medicinal plants have been a vital source of natural ingredients for sustaining human health. Since prehistoric times plants like *Aloe harlana* were used for treatments of diarrhea and colon cleaner (Belayneh and Bussa, 2014). Nowadays, the use of traditional medicine is becoming popular in the developed world (Andarge *et al.,* 2014). Medicinal plants like *Vernonia amygdalina* and *Lepidium sativum* known as sources for traditional antidiarrheal agents (Woldeab *et al.,* 2018). Traditional medicines are the most essential and sometimes sole sources of treatments for approximately 80% of Ethiopians and 95% of the preparations are made from plants (Abebe, 2001). Ethiopian extensive usage of traditional medicine can be linked to cultural acceptability, efficacy, physical accessibility and economic affordability (Abebe, 2001). Several

Ethiopian plants have been reported in the literature to have been used in traditional medical practice for the treatment of diarrhea (Appendix 1).

1.4 Statement of the problems

Diarrhea disease is still a major public health problem in the world and is responsible for about 370,000 deaths among children under the age of five globally (Walker *et al*., 2013; WHO, 2020). The majority of deaths occurred in underdeveloped countries. It is one of the most common health concerns in tropical and subtropical nations, resulting in around 688 million illnesses and 499,000 deaths among children under the age of five (Kotloff *et al.*, 2017). According to the 2016 Ethiopia Demographic and Health Survey, 13% of children under the age of five had diarrheal disease and it is the second leading cause of death among Ethiopian children under the age of five next to lower respiratory infections (Tadesse *et al.*, 2017; Alebel *et al.*, 2018).

Malnutrition is a severe global health issue that affects children and pregnant women in developing nations. According to UNICEF (2022) reports, about 39 million children under the age of five are overweight, 149 million have stunted and 45 million children under the age of five suffer from wasting (UNICEF, 2022). In 2019, the continental accountability scorecard launched by the African Union and the Africa Leaders for Nutrition reported about 58.7 million stunting in Africa (Africa Union, 2022). There are approximately 22.6 million people are undernourished in Ethiopia (WFP, 2022). Currently, global food security and economic growth depends on a declining number of plant species. Underutilized plants can also meet nutritional requirements due to their better nutritional value compared to some optimally utilized plants. Ethiopia is endowed with a number of wild edible plants; however, little is known about their nutritional benefits due to insufficient attention given to research on the dietary values and anti-nutritional factors of wild nutraceutical plant (Getachew *et al*., 2013).

Antidiarrheal drugs available have side effects.

Although *P. schimperiana* roots is traditionally used to treat diarrhea, its efficacy and safety have not been investigated. The fruits of *P. schimperiana* is consumed as a food source, however, its health benefit is not examined. Herein, we discuss the *in vivo* antidiarrheal efficacy of *P. schimperiana* 80% methanol root extract (crude extract) and compounds that were isolated from crude roots extract to provide pharmacological rationale for its traditional use in the management of diarrhea as well as to quantitatively determine the nutritional compositions and anti-nutritional factors of *P. schimperiana* fruits to evaluate their health benefits.

1.5 Genus *Psydrax*

1.5.1 Botanical description and geographical distribution of genus *Psydrax*

Psydrax is a Greek word meaning a blister or bump. It is a genus of flowering plants in the Rubiaceae family also called the madder family, bedstraw family, or coffee family. The family which is made up of trees, shrubs and some lianas, comprises 13,143 species grouped under 611 genera (Barbhuiya *et al.*, 2014; Mahyuni *et al.*, 2018; Feenna *et al.*, 2020).

Psydrax is a monophyletic genus of the tribe Vanguerieae in Rubiaceae having coriaceous leaf blades, keeled stipules, reflexed anthers, cartilaginous seeds, and a very shallow to nearly inconspicuous apical crest without a lid-like area in the pyrene (Arriola *et al*., 2017). The genus comprises more than 130 species of trees, shrubs and lianas. Plants belonging to *Psydrax* are widely distributed in tropical Africa, Asia, Arabian and Australia. There are around 31 *Psydrax* species across tropical and East Africa. Seven species from the genus are found in Madagascar (Arriola *et al*., 2017). *P. parviflora* and *P. schimperiana* are the two species distributed in Ethiopia (Hedberg I and Edwards S, 2003). Despite being a member of the Rubiaceae family, which is a pharmacologically and phytochemically significant plant family, the genus *Psydrax* has received limited scientific attention (Chukwudulue *et al*., 2022).

1.5.2 Ethnomedicinal uses

Several *Psydrax* species have been used in traditional medicine in different parts of Africa, Asia and Oceania. This genus is used in ethnomedical treatment of fever, diabetes, ulcers, inflammation, cardiovascular disease, epilepsy, and stomach ailments (Chukwudulue *et al*., 2022).

Their leaves, aerial parts, stems, roots, barks and fruits are reported to have been used to treat a variety of diseases such as malaria, diarrhea, stomachache, purgative and for the treatment of snakebite (Norhajaret *et al*., 2010; Catarino *et al*., 2016; Manya *et al*., 2020).

The leave of *P. dicoccos* is traditionally used in India for the treatment of fever, cough, asthma, and inflammation (Kalaichelvi and Dhivya, 2016). The roots and stem barks of *P. subcordata* are widely utilized in Ghana, Nigeria, Cameron and Côte d'Ivoire for the treatments of malaria, epilepsy, diabetics, hypertension, hemorrhoids, pain, stomach ulcer, inflammation and cardiovascular problems (Appiah *et al.*, 2018; Agyare *et al.,* 2009). *P. livida*, *P. dicocco* and *P. odorata* are traditionally used as purgative (Nyahangare *et al.,* 2015). The aqueous decoction of *P. umbellate* and *P. nitida* is used orally for the treatment of diarrhea (Eswani *et al*., 2010; Raja *et al*., 2011). In Ethiopia's traditional medicine, the roots of *P. schimperiana* are used to treat, diarrhea and its leaves is used for the treatment of stomach ache and snake bite (Dalle, 2019; Asfaw *et al.*, 2021). Although many *Pysdrax* species are used in traditional medicine, phytochemical research is limited. A small number of species have so far yielded several secondary metabolites, with iridoids serving as chemotaxonomic markers of the genus (Chukwudulue *et al*., 2022).

1.5.3 Phytochemistry

The chemicals and bioactive components of the different parts of the genus *Psydrax* have been investigated. Phytochemical studies were conducted on *P. subcordata, P. acutiflora, P. montigena, P. livida, P. odorata, P. dicoccos, P. puberula* and *P. schimperiana.* Diverse beneficial classes of compounds such as alkaloids, terpenoids, flavonoids, cyanogenic glycosides and miscellaneous compounds were isolated from the root, leaves, bark and fruits of *Psydrax* species (Joubouhi *et al*., 2015; Yang, 2016).

Secondary metabolite such as alkaloids were isolated from *P. odorata, P. lamprophyll, P. arnoldiana* and *P. schimperiana,* flavonoids from *P. montigena* and *P. subcordata* and terpenoids from *P. subcordata, P. odorata, P. lamprophylla* and *P. arnoldiana* (Schwarz *et al*. 1996; El seedi *et al.*, 2005; Joubouhi *et al.*, 2015; Yang, 2016; Poullain and Caledonia, 2016). 3,5-dicaffeoylquinic acid **[1]**, 3,4-dicaffeoylquinic acid **[2]** and cyanogenic glycoside **[42]** were isolated from the fruits of *P. schimperiana* (Schwarz *et al*., 1996). Major classes of compounds that have been isolated from *Psydrax* species listed in appendix-II.

1.5.4 Biological and pharmacological activities

Different pharmacological activities have been reported from different plants of the species in the genus *Psydrax.* Out of the 130 species in this genus, (*P. subcordata, P. palma, P. acutiflora, P. montigena, P. schimperiana, P. livida, P. horizontalis,* and *P. dicoccos*) have tested for their pharmacological activity. *P. dicoccos* and *P. subcordata* were tested for their antibacterial and antifungal activity (Chukwujekwu *et al*., 2005; Umaiyambigai *et al*., 2016; Akoto *et al*., 2019). *P. acutifolia* was pharmacologically evaluated for the treatments of malaria and inflammation (Ilboudo *et al*., 2013). During these reviews, no literature was found on *Psydrax* species antidiarrheal activity.

1.5.4.1 Antibacterial

Umaiyambigai *et al* (2016) reported that the antibacterial activity of methanol leaves extract of *P. dicoccos* showed the highest activity against *Staphylococcus aureus* followed by gram-positive bacteria *Streptococcus pyogenes* and *Enterococcus faecalis*, *E. coli, Pseudomonas aeruginosa, Proteus vulgaris,* and *Vibrio cholera* have the highest zone of inhibition of 22.5 mm and the lowest minimum inhibitory concentration (MIC) values of 125 µg/ml, and minimum bactericidal concentration (MBC) value of 250 µg/ml (Umaiyambigai *et al.,* 2016).

The stem bark methanol extract of *P. subcordata* showed concentration-dependent bacterial growth inhibition. The methanol extract was active against *S. aureus, S. pyogenes, E. coli* and *P. aeruginosa* (Anokwah *et al*., 2016). Antibacterial activity of Linearin (**23**) isolated from the fruits of *P. subcordata* performed by microdilution method and showed activity against *S. aureus*, with a MIC value of 8 µg/ml, where ampicillin (MIC 8 µg/ml) and ciprofloxacin (MIC 2 µg/mL) were used as standard drugs (Joubouhi *et al*., 2017).

1.5.4.2 Antifungal activity

Umaiyambigai *et al* (2016) reported the antifungal activity of methanol leaves extract of *P. dicoccos*. Significant antifungal activities against *Candida albicans, C. parapsilosis* and *C. tropicalis* were reported (Umaiyambigai *et al*., 2016).

1.5.4.3 Anti-malarial activity

Aqueous leave extract of *P. acutifolia* showed antimalarial activity with half maximal inhibitory concentration (IC_{50}) of 50 mg/ml. while di-isopropyl ether extract showed greater inhibition of parasite growth with an IC_{50} of 9.5 mg/ml. This is due to dose-dependent inhibition in the production of the pyrogenic cytokine interleukin-1 beta (IL-1β) (Ilboudo *et al*., 2013).

1.5.4.4 Anti-inflammatory activity

P. acutifolia aqueous extract revealed significant inhibition of IL-1β production at the dose of 500 mg/ml. IL-1β is one of the most potent pro-inflammatory cytokines responsible for fever (Ilboudo *et al*., 2013). Another study conducted by Anokwah *et al* (2016) on the anti-inflammatory activity of steam bark extracts of *P. subcordata* on carrageenan-induced footpad edema in seven days, showed the dose-dependent edema inhibition of (61.21, 53.00 and 49.34 %) at (300, 100 and 30 mg/kg), respectively. Dexamethasone (0.3, 1 and 3 mg/kg) and diclofenac (10, 30 and 100 mg/kg) were used as a positive control (Anokwah *et al*., 2016).

1.5.4.5 Anti-oxidant activity

Anokwah *et al* (2016) reported the antioxidant activities of the methanol extract of the stem bark extract *P. subcordata*. The extract exhibited radical scavenging activity with an IC_{50} of 226.5 ± 0.86 µg/ml (Anokwah *et al.,* 2016).

1.5.4.6 Anticonvulsant properties

Daanaa *et al* (2017) reported anticonvulsant activities of *P. subcordata* hydroethanolic leave extract. It delayed the onset and decreased the duration and frequency of pentylenetetrazole and picrotoxin-induced convulsions. Its anticonvulsant activity is demonstrated by delaying the onset of seizures as well as by reducing the severity of seizures and mortality of mice. This effect may be mediated through the GABAergic mechanism (Daanaa *et al.,* 2017).

1.5.5 *Psydrax schimperiana* (A. Rich.) Bridson

1.5.5.1 Botanical description of *P. schimperiana*

Psydrax schimperiana (A. *Rich.) Bridson*, previously known as *Canthium schimperianum (*A. Rich.) is a shrub or small tree up to 6 m tall. Its leaves are 2-10.5×1-5 cm decussate, evergreen, elliptic to ovate-lanceolate, more or less acute at apex, narrowed to base, glabrous, glossy above, drying green to brownish petioles up to 5 mm long. Stipules are 7-10 mm long. Flowers are 4-5 parts and grow in sessile cymes with 7-50 flowers, pedicels are pubescent. The calyx limb has a short rim of teeth. White corolla with lobes that are sharp and have a tube length of 2.5-3.5 mm, lobes 4–6 mm long. Its fruits are 5–6.5×6–8 mm and black when ripe (Diane, 1985; Hedberg and Edwards, 2003).

1.5.5.2 Geographical distribution and traditional use of *P. schimperiana*

P. schimperiana can be distributed in an area of 900-2600 m along *Acacia* and woodlands of Ethiopia, Somalia, Congo, Rwanda, Burundi, Malawi and Arabia (Hedberg and Edwards, 2003). In Ethiopia, it is distributed in Tigray, Amhara, Oromia, and Southern Nations, Nationalities and Peoples Region (SNNPR) and locally known as Gaalloo (Afan Oromo), Tsehag (Tigrinya), Sagad (Amharic) and Kahelta (Konso) (Kidane *et al.*, 2014; Bogale *et al.*, 2017; Dale, 2019; Asfaw *et al.*, 2021). The root of *P. schimperiana* is crushed and mixed with water and used to treat diarrhea in the West Arsi zone (Dalle, 2019; Asfaw *et al.*, 2021).

The fruits of *Psydrax schimperiana* is consumed by Konso, Maale and Ari ethnic communities in Southern Nations, Nationalities, and Peoples' Regional State and Borena pastoralists in Oromia Regional State as a source of food (Gemedo-Dalle *et al*., 2005; Addis *et al*, 2013; Kidane, 2014). The stem bark of *P. schimperiana* is also made into a decoction and paste for the treatment of breast cancer in Kenya (Ochwang e*t al.*, 2004, 2018).

Figure 1. Arial parts of *Psydrax schimperiana* A. Rich. Bridson (Photograph taken by Getahun Damissie at Mesoya village, Konso zone, SNNPR, Ethiopia in December 2021.

2. OBJECTIVES OF THE STUDY

2.1 General objective

To evaluate *in vivo* Antidiarrheal activity of crude extract and compounds isolated from the roots of *P. schimperiana* and to determine the nutritional composition and anti-nutritional factors of *P. schimperiana* fruits.

2.2 Specific objectives

- To evaluate the antidiarrheal activity of crude extract from roots of *P. schimperiana* against experimentally induced diarrhea using different models in mice.
- To isolate compounds from the crude extract of *P. schimperiana* roots and to elucidate the structures of isolated compounds.
- To evaluate the antidiarrheal activity of isolated compounds from the crude extract of *P. schimperiana* against the castor-oil-induced diarrhea model.
- To determine the nutritional composition and antinutritional factors from the fruits of *P. schimperiana*

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

The roots of the *P. schimperiana* plant were collected from Mesoya village, Konso zone (5° 14' 60.00" N, 37° 28' 59.99" E), Southern Nations, Nationalities, and Peoples' Region, 607 kilometers to the south of Addis Ababa in December 2021. The arial part of the plant was authenticated by Mr. Melaku Wondafrash from the Department of Biology, College of Natural and Computational Sciences, Addis Ababa University, and a voucher specimen (GD-001) was deposited at the National Herbarium for future reference. To remove dust material, the roots were gently washed with water, cut into pieces, dried under shade, and powdered using a mortar and pestle. The fruits were collected from Mesoya village, in July 2022. The fruits were washed with clean water and dried under shade at room temperature and then powdered to uniform size by the mechanical grinder. The powder was stored for further analysis.

3.1.2 Chemicals and reagents

The following chemicals, solvents and drugs were used during this study: distilled water (AAU pharmaceutics laboratory), Petroleum ether, n-hexane, chloroform, ethyl acetate (LOBA-Chemie, India) and methanol (Sheba Pharmaceutical PLC, Ethiopia), silica gel HF_{254} (LOBA-Chemie, India), castor oil (Amman Pharmaceutical Industries Co.), loperamide (Daehwa Pharmaceuticals, Republic of Korea) and activated charcoal (LABMERK, India). All chemicals and regents were analytical grade.

3.2.3 Instruments

Rotavapor (Heidolph Instruments GmbH and Co., Germany), and uv-visible spectrophotometer NMR spectra were recorded using Bruker Avance DM×400 FT-NMR 500 MHz for ^{1}H and 126 MHz for ^{13}C. Agilent LC-MS system with diode array detector, Agilent 11005 series system (Agilent system, USA), and ESI-API were used for the characterization of isolated compounds. digital balance, capillary tube and measuring cylinder, oral gavage, test tube, ruler and metabolic gage were also utilized during antidiarrhea activity determination.

3.2 Methods

3.2.1 Extraction and phytochemical screening

3.2.1.2 Extraction

The plant material was extracted using 80% methanol maceration. The powdered roots (1 kg) were soaked for 72 hours at room temperature in 80% methanol (3.5 L) with occasional shaking and then filtered through a muslin cloth and Whatman No.1 filter paper. The marc was re-macerated two times with fresh solvents and filtrated in the same manner. Filtrates from each extraction were combined and concentrated using rotary evaporator under reduced pressure at a temperature of not more than 40^{o}C. The remaining solvent was removed in an oven at 40^{o}C over two weeks. The dried extract was weighed and transferred to a beaker, covered with aluminum foil, and kept in the locked cabinet protected against light and moisture at room temperature for the entire period of the experiments

3.2.1.3 Preliminary phytochemical screening

Preliminary phytochemical screening of the crude extract of *P. schimperiana* was carried out to test the presence/absence of alkaloids, flavonoids, tannins, terpenoids, phenol, steroids, cardiac glycosides, anthraquinone and saponins using standard chemical tests and procedures (Zohra *et al.*, 2012; Hosahally *et al.*, 2012; Nasrin, 2013).

3.2.2 Isolation and characterization of compounds

3.2.2.1 Fractionation

Fractionation was carried out using pressurized flash chromatography using pre-packed cartridge (silica gel, 230–400 mesh) with n-hexane, chloroform, ethyl acetate and methanol. Each fraction was collected separately and was analyzed using normal phase analytical thin-layer chromatography (TLC) plates and visualized under ultraviolet light at 254 and 366 nm. On the basis of the TLC profile, the chloroform and ethyl acetate fractions were combined and prepared for further purification by PTLC.

3.2.2.2 Preparative thin layer chromatography (PTLC)

Compounds were isolated by subjecting combined fractions of chloroform and ethyl acetate to PTLC (20 cm×20 cm plates). Silica gel 60 HF was used as the stationary phase and chloroform and methanol (9.5:0.5) were used as the mobile phase. Bands on PTLC were visualized under ultraviolet light of wavelength 254 and 366 nm and the desired bands were scraped off individually, and washed with chloroform and methanol (1:1) mixture, filtered, concentrated, labeled as GDS-1 and GDS-2 and prepared for further spectral analysis and biological assay.

3.2.2.4 Characterization of isolated compounds

^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on Bruker Avance DMx400 FT-NMR spectrometer using TMS as internal standard. Agilent LC-MS system with diode array detector was used to assess the purity of GDS-1 and GDS-2, and to determine their masses. Mass spectrometry was performed using an Agilent 11005 series system (Agilent system, USA), and ionization of sample was carried out using ESI-API (capillary voltage, 4000 V; fragmentor, 160 V; drying gas temperature, 350 ºC; gas flow (N_2), rate, 10l/min; nebulizer pressure, 50 psig).

3.2.3 Determination of antidiarrheal activity

3.2.3.1 Experimental animals

Ninety-five healthy Swiss albino mice weighing 20-30 g and aged 6-8 weeks were obtained from the animal house of the department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University. Mice were held in stainless steel cages at room temperature and on a 12 h light/dark cycle with free access to water and food pellets *ad libitum* in the animal house.

3.2.3.2 Acute oral toxicity test

Acute toxicity study was conducted according to the Organization for Economic Cooperation and Development (OECD) guidelines 425 (OECD, 2008). A limit dose of 2000 mg/kg of the extract was used involving five mice. Each mouse was treated with a single oral dose of 2000 mg/kg of extract in sequence at 48 h intervals. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h, and daily thereafter, for a total of 14 days for any clinical signs of toxicity or mortality.

3.2.3.3 Castor oil-induced diarrhea

Swiss albino mice (45) were fasted for 18 hours with free access to water and randomly divided into nine groups of five animals. One hour after administration of an extract (100, 200, 400 mg/kg), GDS-1 (10 mg/kg, 20 mg/kg) or GDS-2 (10 mg/kg, 20 mg/kg), castor oil (0.5 ml) was given orally to each mouse to induce diarrhea. The vehicle (10 ml/kg of distilled water) and loperamide 3 mg/kg were administered to negative and positive control groups, respectively. The animals were housed in a clear metabolic gage and the onset of diarrhea, the number and weight of wet stools, and the total number and weight of fecal output were documented. The percentage of mean fecal output and inhibition of defecation was calculated as shown below (Shoba and Thomas, 2001).

$$\text{Mean fecal output (\%)} = \frac{\text{Mean fecal weight of each treatment group}}{\text{Mean fecal weight of the control group}} \times 100$$

$$\text{\% inhibition of defecation} = \frac{\text{Total number of feces in the negative control} - \text{Total number of feces in the treated group}}{\text{Total number of feces in the negative control}} \times 100$$

3.2.3.4 Gastrointestinal motility test

To test the effect of the extract on gastrointestinal motility, the test animals (25 mice) were starved for 18 hours before the experiment having free access to water. Mice were divided into control and test groups containing five mice in each group. Test groups received the extract at doses of 100, 200, or 400 mg/kg while the negative control group received 10 ml/kg of distilled water and the positive control group received 3 mg/kg of loperamide. Each mouse was given 0.5 ml of castor oil one hour after treatment and 1 ml of a 5% activated charcoal suspension in 5 ml of distilled water after 30 minutes of castor oil administration.

Thirty minutes after administering activated charcoal, mice were sacrificed and the small intestine (from the pyloric sphincter to caecum) was removed and its length as well as the distance traveled by charcoal was measured. The intestinal charcoal transit was expressed as a percentage of the distance moved by charcoal to the total length between the pylorus and the caecum. The peristaltic index and percentage of inhibition were calculated using the formula shown below (Uddin *et al.,* 2005).

$$\text{Peristaltic index (PI)} = \frac{\text{Mean distance traveled by charcoal meal (negative control} - \text{test)}}{\text{Mean length of the small intestine}} \times 100$$

$$\%\ \text{Inhibition of motility} = \frac{\text{Mean percent of distance traveled by the charcoal meal (negative control} - \text{test)}}{\text{Mean percent of distance traveled by the charcoal meal negative control}} \times 100$$

Where Dc is the distance traveled by the negative control and Dt stands for the distance traveled by the test group.

3.2.3.5 Castor oil-induced enteropooling activity

Intraluminal fluid accumulation was determined using the method described by Islam *et al* (2013). Twenty-five mice were fasted for 18 hours and grouped. The test groups received the extract at doses of 100, 200, or 400 mg/kg while the negative control group received 10 ml/kg of distilled water and the positive control group received 3 mg/kg of loperamide. One hour after administering castor oil, each mouse was sacrificed, its abdomen opened, the small intestine removed from the pyloric sphincter to the ileocecal junction, ligated at both ends, and then carefully dissected out. The volume of intestinal contents was measured after each small intestine weighed and its contents gently milked into a graduated tube. Each intestine was re-weighed and the difference between the full and the empty intestines were calculated. Finally, the percentage inhibitions of the volume and weight of intestinal contents were calculated using the formula given below (Islam *et al*., 2013).

Inhibitions of volume (%) = $\frac{\text{MVICC}-\text{MVICT}}{\text{MVICC}} \times 100$

Where MVICC = Mean volume of the intestinal content of the control group; MVICT = Mean volume of the intestinal content of the test group.

Inhibitions of weight (%) = $\frac{\text{MWICC}-\text{MWICT}}{\text{MWICC}} \times 100$

Where, MWICC = Mean weight of the intestinal content of the control group; MWICT = Mean weight of the intestinal content of the test group

3.2.3.6 *In vivo* antidiarrheal index

In vivo antidiarrheal index (ADI) of the standard drug and test substances were determined using the formula shown below (Okpo *et al.*, 2011).

In vivo ADI = $\sqrt[3]{\text{Dfreq} \times \text{Gmeq} \times \text{Pfreq}}$

Where D_{freq} = Delay in defecation time or diarrheal onset (in % of control); G_{meq} = Gut meal travel reduction (in % of control) and P_{freq} = purging frequency as the number of wet stool reduction (in % of control).

3.3 Determination of nutritional and antinutritional composition

The determination of moisture content, ash value, crude protein, crude fiber, crude fat, tannin, phytate, and oxalates was performed according to the standard analytical procedure.

3.3.1 Determination of total moisture content

Two grams of the sample were weighed (W_1) put into pre-weighted crucibles (W_0), and dried in an oven at 105°C overnight. The dried sample was cooled in a desiccator for 30 min and weighed to a constant weight (W_2). The percentage loss in weight was expressed as percentage moisture

content on a dry weight basis and this was repeated three times to obtain triplicate values (AOAC, 2006).

$$\% \text{ Total moisture content } = \frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where W_0 = weight of the empty crucible (g)

W_1 = weight of fresh sample + empty crucible (g)

W_2 = weight of dried sample + empty crucible (g)

3.3.2 Determination of total ash value

Two grams of the dried and powdered sample were weighed (W_1) and placed in pre-weighed empty crucibles (W_0), and then ashed for three hours at 600°C in a muffle furnace. The hot crucibles were weighed (W_3) after being cooled in a desiccator and the percentage residual weight was expressed as ash value. The experiments were performed in triplicate (AOAC, 2006).

$$\% \text{ Total ash value} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

3.3.3 Determination of total crude protein

Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed in triplicate and put into a digestion flask, and then 10 ml of concentrated H_2SO_4 was added and for 2 h, the material was heated on a thermostatically controlled micro digestion bench until it become colorless. After heating, the content of the flask was left to cool and was transferred to a round bottom flask with distilled water. 10 ml of 40% NaOH solution was carefully added, stirred, and then subjected to distillation until all the ammonia evaporated and added sulfuric acid solution. 5 ml of 4% boric acid is added to avoid pumping (Kirk, 1950).

Standard solution 0.01M HCl was used to titrate it until the end of experiment. The conversion factor 6.38 was used to get the percentage of protein contents (AOAC, 2006).

% Total crude protein = % nitrogen × conversion factor

3.3.4 Determination of total crude fat

Two grams of the powdered sample were used to extract the crude lipid for 5 h in a soxhlet extractor with petroleum ether at (60 to 80°C) (AOAC, 2006).

$$\% \text{ Total crude fat} = \frac{W_2 - W1}{S} \times 100$$

3.3.4 Determination of total crude fiber

Two grams of the powdered sample were employed in triplicates to quantify the crude fiber by acid and alkaline digestion methods (20% H_2SO_4 and 20% NaOH solutions). Dried samples were boiled for 30 minutes in 200 ml of H_2SO_4. The solution was then passed through a muslin cloth and heated until the washings were acid-free. The leftovers were then cooked for 30 minutes in 200 ml of NaOH. Following a muslin cloth filter, it was once more washed with 25 ml of boiling H_2SO_4, 50 ml of water and 25 ml of ethanol. The residue was taken out and placed in a dish with pre-weighed ashing (w_1). The residue was cooled in a desiccator, dried for 2 h at 130°C, and weighed (w_2). Following a 30-minute combustion at 600 ±15°C, it was cooled in a desiccator and reweighed (w_3) (James, 1995).

$$\% \text{ Total crude fiber contents} = \frac{W_2 - W3}{W_1} \times 100$$

3.3.5 Total carbohydrate determination

The carbohydrate content was calculated using the following formula:

Total carbohydrate (%) = 100 – [protein (%) + moisture (%) + ash (%) + crude fiber (%) + crude Fat (%)] (Sarkiyayi *et al*., 2013).

3.3.6 Determination of phytate content

The phytate content in the sample was determined according to the method described by Adeniyi (2009). A 0.08 gm dry sample was extracted for 1 h at room temperature with 10 ml of 0.2 NHCI before being centrifuged at 3000 rpm for 30 minutes. The phytate estimation was performed using the clear supernatant. The mixture was then centrifuged after 2 ml of Wade reagent (0.03% solution of $FeCl_3$; $6H_2O$, containing 0.3 % sulfosalicylic acid in distilled water) was added. The spectrophotometer was used to measure the absorbance at 500 nm. The phytate concentration was calculated from the difference between the absorbance of the control (3 mm of 0.2 NHCI and 2 ml of wade reagent) and that of the assayed sample (Adeniyi *et al*., 2009).

$$\text{Phytate (\%)} = \frac{[(\text{AB} - \text{AS}) - \text{ intercept}]}{\text{Slope x W x 3}} \times 100$$

Whereas, AB = absorbance of the blank, AS = absorbance of the sample, W = fresh sample weight, Slope = slope of the calibration curve, Intercept = intercept of the calibration curve

3.3.7 Determination of tannin content

The technique described by Embaby (2010) was used to quantify the amount of tannin. About one gram of the sample was mixed with 10 ml of concentrated HCl in methanol solution and put on a mechanical shaker for 24 h at room temperature. The extracts were centrifuged for 20 min and 1 ml of the supernatant was placed in a test tube along with 5 ml of the Vanillin-HCl reagent.

After 20 minutes, the tannin content was measured at 500 nm against an experimental blank adjusted to zero absorbance.

$$\text{Tannin } (\%) = \frac{[(\text{As} - \text{Ab}) - \text{ intercept}]}{\text{Slope } \times \text{D } \times \text{W}} \times 100$$

Whereas, As = sample Absorbance Ab = blank Absorbance D = Density of solution (0.791 g/ml) W = Weight of' sample in gram

3.3.8 Determination of oxalate content

Oxalate was determined by using the method of Oke (1969). One gram of the sample was placed in a 250 ml volumetric flask, and 190 ml of distilled water and 10 ml of 6M HCl were added. The mixture was warmed in a water bath at 90°C for 5 h and the digested sample was centrifuged at a speed of 2,000 rpm for 5 min. 50 ml aliquots of the supernatant were reduced by evaporation to 25 ml, the brown precipitate was filtered off and washed. With drops of strong ammonia solution, the combined solution and washings were titrated until the methyl orange's salmon-pink color turned pale yellow. The solution was heated to 90°C in a water bath, and 10 ml of a 5 % calcium chloride ($CaCl_2$) solution was used to precipitate the oxalate. The solution was centrifuged after being allowed to stand for the full night. After being heated to 90°C and titrated against 0.05 M $KMnO_4$, the precipitate was washed into a beaker with hot 25% sulphuric acid (H_2SO_4) diluted with 125 ml of distilled water.

$$\text{Oxalate}(\%) = \frac{\text{The volume of Kmno4 consumedx} \times 0.00630}{\text{weight of sample}} \times 100$$

3.3 Data analysis

Statistical Package for Social Science (SPSS) software version 26 was used to analyze all data. The results were presented as mean with a standard error of the mean (±SEM). ANOVA (one-way analysis of variance) was used to compare groups, followed by a post hoc Tukey's multiple comparison test. The difference between the compared groups was considered statistically significant at a 95% confidence interval used for the antidiarrheal activity. The data of nutritional composition and anti-nutritional factors were presented as mean with standard deviation.

3.4 Ethical approval

All the experimental protocols related to the use of mice were approved by the Institutional Ethics Committee of the School of Pharmacy, College of Health Sciences, Addis Ababa University with Approval Number: = ERB/SOP/457/15/2022. All experimental activities were carried out by respecting the declaration of nationally and internationally accepted customary standards for the use of experimental animals, as well as the code of ethics for animal experiments, which followed scientific and ethical norms (OECD, 2008). Animal handling, care, and use during all experiments followed the internationally accepted laboratory guideline (NRC, 2011).

4. RESULTS AND DISCUSSION

4.1 Extraction and phytochemical screening

4.1.1 Extraction

In Ethiopia's traditional medicine, the roots of *P. schimperiana* are crushed, mixed with water, and taken orally to treat diarrhea (Dalle, 2019). However, in this investigation, the crude roots extract of *P. schimperiana* was prepared by maceration with 80% methanol to obtain polar, moderately polar, and non-polar components. The final yield (w/w) of the extract was about 3.5%.

4.1.2 Phytochemical screening

Bioactive secondary metabolites available in medicinal plants such as alkaloids, phenol, terpenoids, steroids, quinones, coumarin and saponins are responsible for the therapeutic effects of medicinal plants (Zohra *et al.*, 2012; Hosahally *et al*., 2012; Nasrin, 2013). Preliminary phytochemical screening was carried out to determine the composition of secondary metabolites in the roots of *P. schimperiana*. According to our findings terpenoids, saponin, phenols, tannin, flavonoid, alkaloids and anthraquinone glycoside were found in the roots of *P. schimperiana*, and cardiac glycosides and steroids were absent (Table 1).

Table 1. Secondary metabolites from the crude extract *P. schimperiana* roots.

Secondary metabolites	**Crude extract**
Terpenoids	++
Saponins	+
Phenols	++
Tannins	+
Flavonoids	+
Cardiac glycosides	–
Steroids	–
Alkaloids	+
Anthraquinones	+

4.2 Isolation and characterization of compounds

Purification of the crude extract on PTLC with methanol and chloroform (9.5:0.5), offered two compounds (GDS-1 and GDS-2) (Figure 2). Both GDS-1 (R*f* = 0.35) and GDS-2 (R*f* = 0.52) appeared as fluorescent blue spot under ultraviolet light at 254 nm and 366 nm.

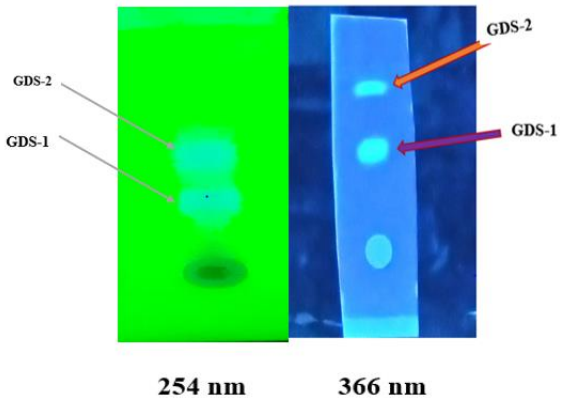


Figure 2. Normal phase analytical TLC of the crude extract of *P. schimperiana*.

4.2.2 Structural elucidation of GDS-1 and GDS-2

4.2.2.1 Characterization of GDS-1

Structural elucidation of GDS-1 was based on spectroscopic data and a comparison of its spectroscopic characteristics with that reported in the literature. GDS-1 was obtained as an amorphous colorless powder. Electrospray ionization time-of-flight mass spectrometry (ESITOFMS) showed a pseudo-molecular ion peak at *m*/*z* 193.0 $[M+H]^+$ (Figure 3).

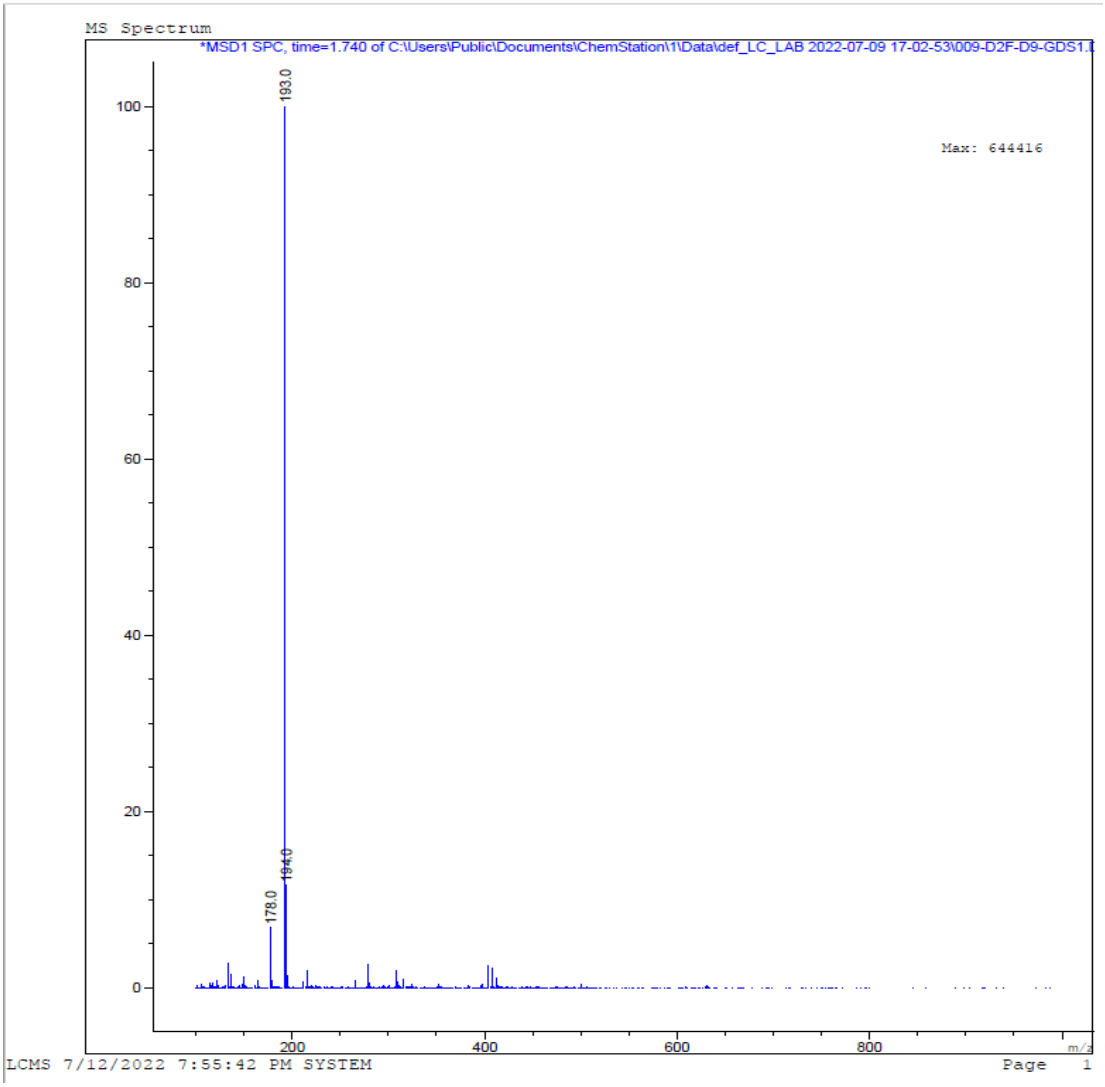


Figure 3. Mass spectrum of GDS-1.

^{1}H NMR (500 MHz, CDCl3) spectrum of GDS-1 showed the presence of four aromatic protons assignable to δ 7.59 (d, *J* = 9.4 Hz, 1H), 6.91 (s, 1H), 6.84 (s, 1H), 6.26 (d, *J* = 9.4 Hz, 1H), and a methoxy group at δ 3.95 (s, 3H) (Figure 4). The signal at 7.26 and 2.1 occurred due to deuterated chloroform (solvents) and water residue respectively. As indicated in table 2, the ^{1}H NMR data was compared to and agreed with that of the reference compound isoscopoletin (Suksungworn *et al*., 2017).

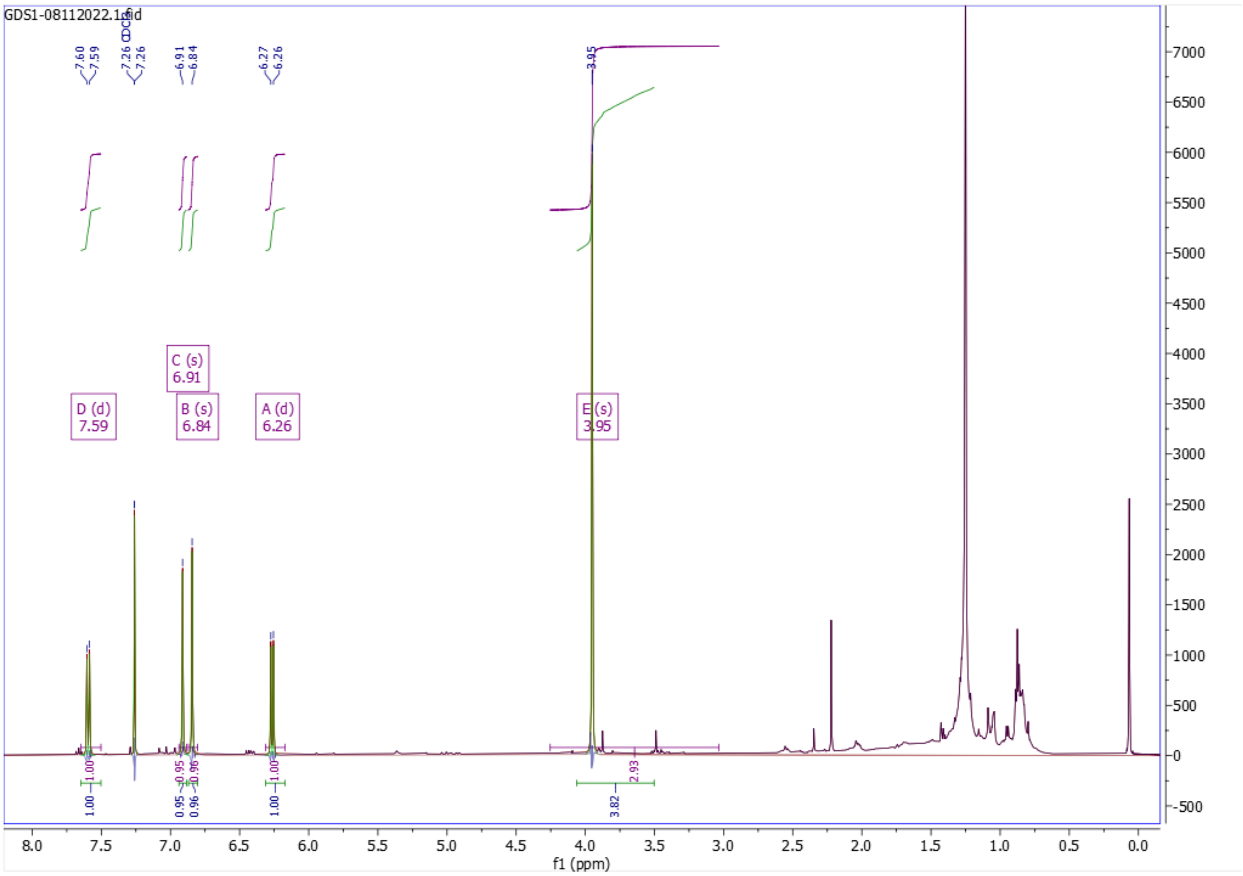


Figure 4. ^{1}H-NMR spectrum of GDS-1.

^{13}C NMR (126 MHz, DMSO) spectral data revealed 10 carbons at δ 161.83, 151.99, 147.56, 145.01, 111.96, 111.06, 108.68, 107.53, 103.07 and 56.05 (Figure 5), DEPT spectrum analysis was performed based on combined information from DEPT-45 (Figure 6) DEPT-90 (Figure 7) and DEPT-135 (Figure 8).

The aromatic CH carbon signals were assigned at δ 111.96 (C-3), 144.30 (C-4), 108.71 (C-5) and 145.01 (C-8). Furthermore, there were also quaternary carbons peak at 161.83 (C-2), C-6 (107.63), C-7 (147.56), C-9 (103.08) and C-10 (152.00), and methoxy at 56.06 (7-OCH_3). Based on the spectral information the structure of GDS-1 was deduced based on the above data as 6-hydroxy-7-methoxy coumarin (Figure 9). GDS-1 NMR data agreed with isoscopoletin, which was previously reported in the literature (Table 2) (Suksungworn *et al*., 2017).

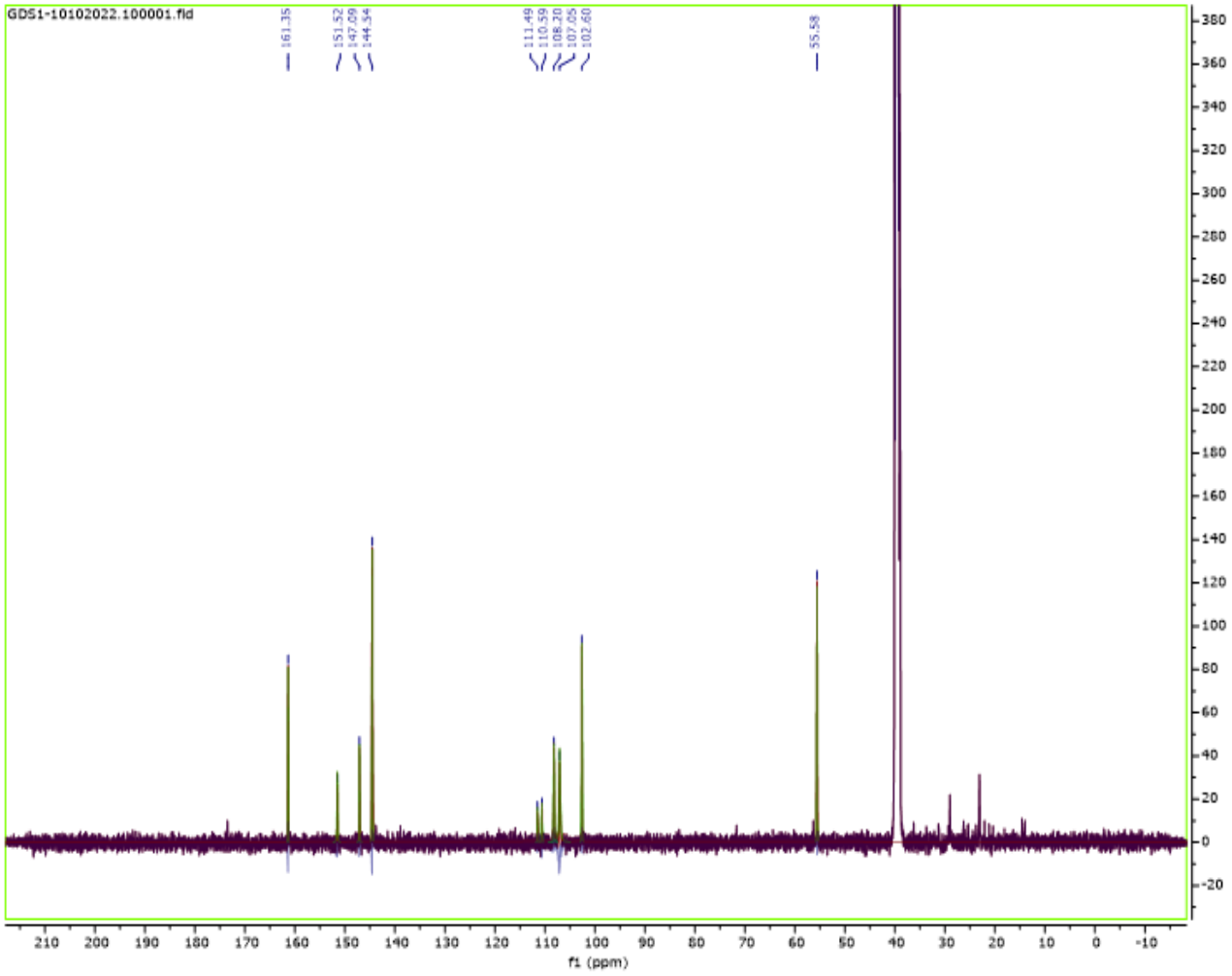


Figure 5. ^{13}C NMR spectrum of GDS-1.

Table 2. ^{1}H NMR and ^{13}C NMR data of GDS-1 and reference compound.

Present data			Reference (isoscopoletin) (Suksungworn *et al*., 2017)	
Position	^{1}H NMR (500 MHz, $CDCl_3$) GDS-1 δ (ppm, mult., J Hz)	^{13}C NMR (126 MHz, DMSO) of GDS-1 δ (ppm)	^{1}H NMR δ (ppm) (mult., J Hz)	^{13}C NMR of δ(ppm)
2	-	161.83	-	161.47
3	6.26 (d, 9.5)	111.96	6.26 (d, 9.5)	113.36
4	7.59 (d, 9.5)	144.30	7.59 (d, 9.5)	143.31
5	6.84 (s)	108.71	6.84 (s)	111.48
6	-	107.63	-	107.50
7	-	147.56	-	149.72
8	6.91 (s)	145.01	6.91 (s)	144.03
9	-	103.08	-	103.18
10	-	152.00	-	150.24
7 – OCH_3	3.95 (s)	56.06	3.95 (s)	56.41

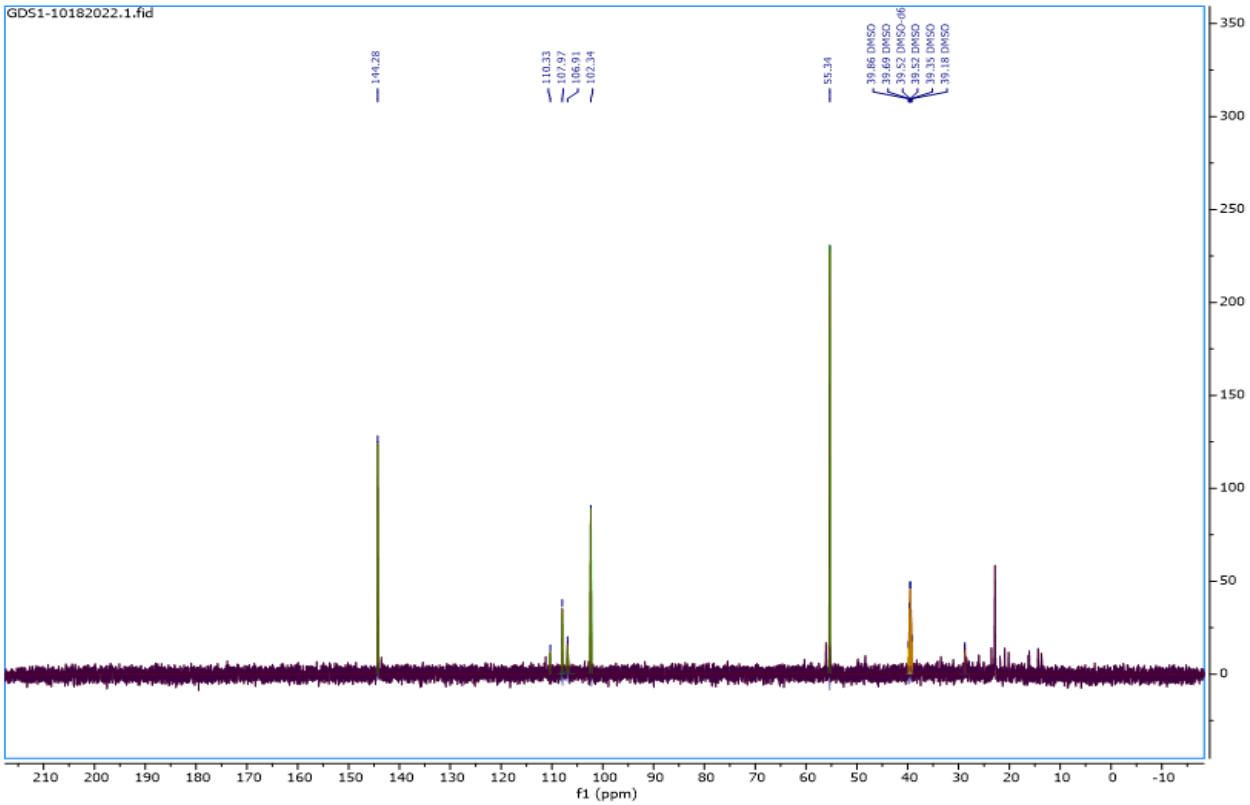


Figure 6. DEPT-45 spectrum of GDS-1.

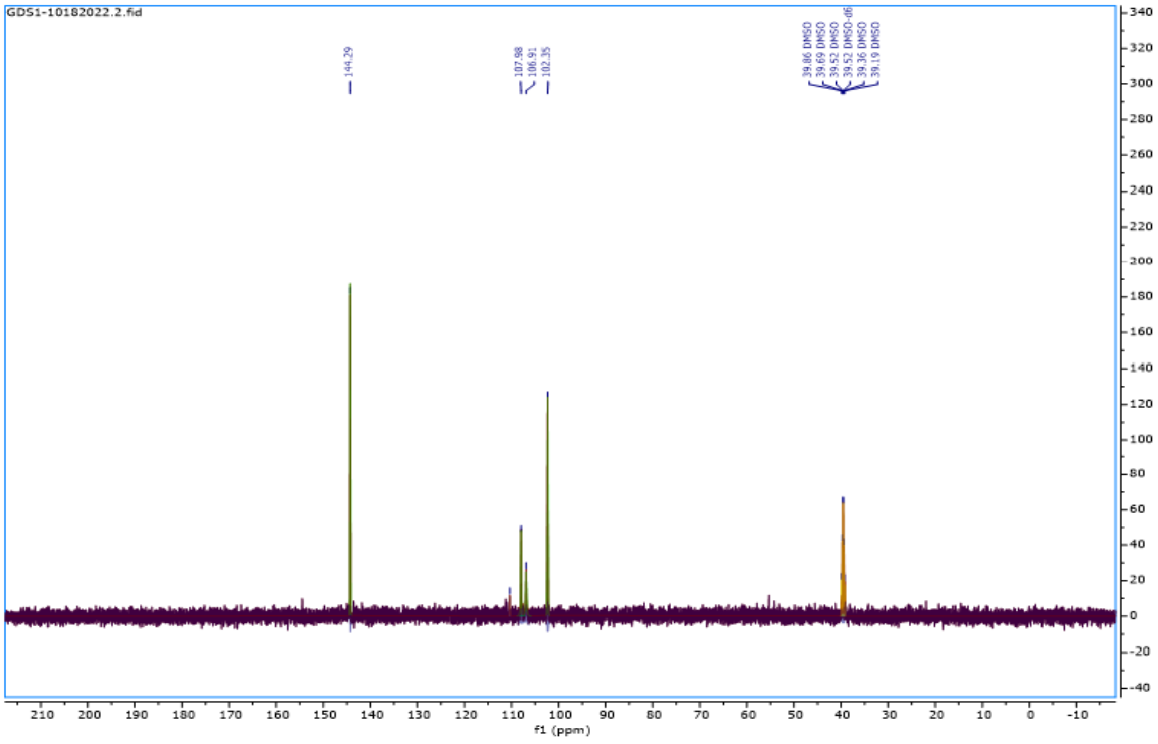


Figure 7. DEPT-90 spectrum of GDS-1.

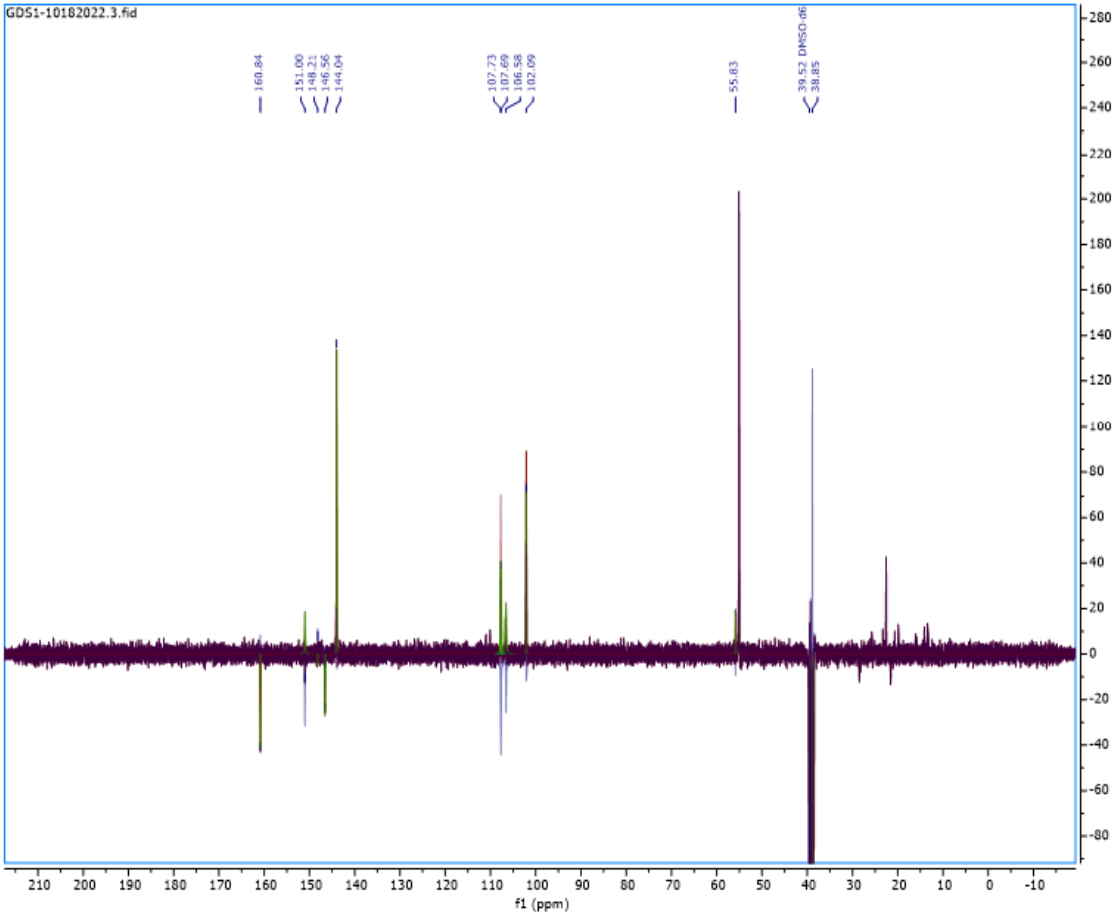
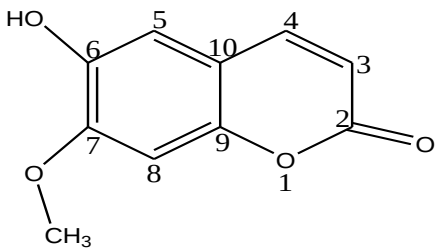


Figure 8. DEPT-135 spectrum of GDS-1.



Isoscopoletin (GDS-1)

Figure 9. Chemical structure of compounds isolated from *P. schimperiana* roots (GDS-1).

The calculated exact mass of GDS-1 is 192.9874u with a molecular formula of $C_{10}H_8O_4$.

4.2.2.2 Characterization of GDS-2

GDS-2 was obtained as an amorphous colorless powder. ESI-TOF-MS exhibited a pseudo-molecular ion peak at *m*/*z* 207 $[M-H]^+$ (Figure 10).

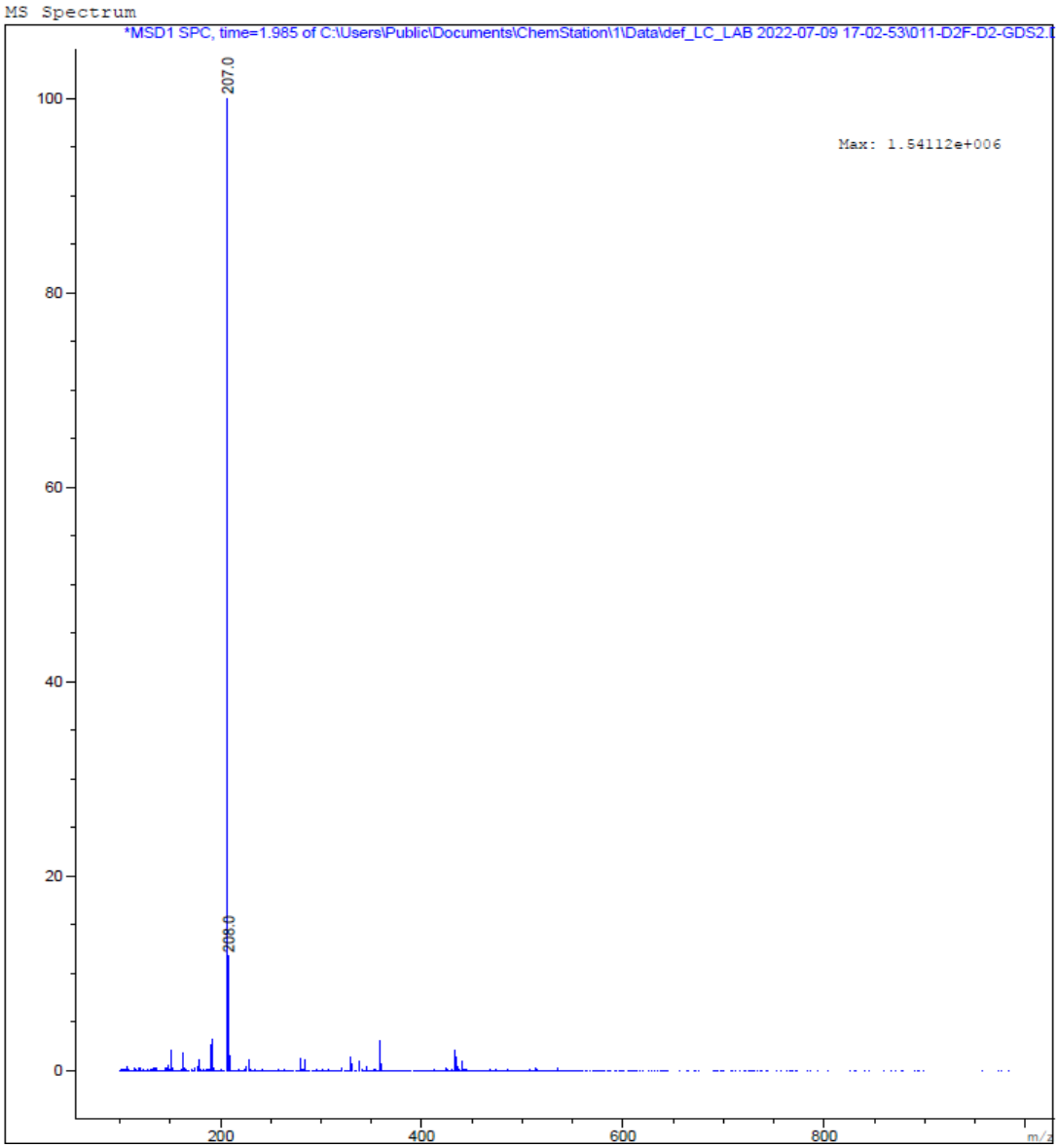


Figure 10. Mass spectrum of GDS-2.

Elucidation of the structure of GDS-2 was performed by combining data from ^{1}H NMR (500 MHz, CDCl3) and ^{13}C NMR (126 MHz, DMSO, DEPT-45, DEPT-90 and DEPT-135).

The ^{1}H NMR spectra of GDS-2 showed at δ, 7.62 (1H, d, J 9.42 Hz, H-4), 6.85 (1H, s, H-5), 6.85 (1H, s, H-8), 6.29 (1H, d, J 9.44 Hz, H-3), 3.92 (3H, s, 7-OCH3), 3.95 (3H, s) **(**Table 3 and Figure 11).

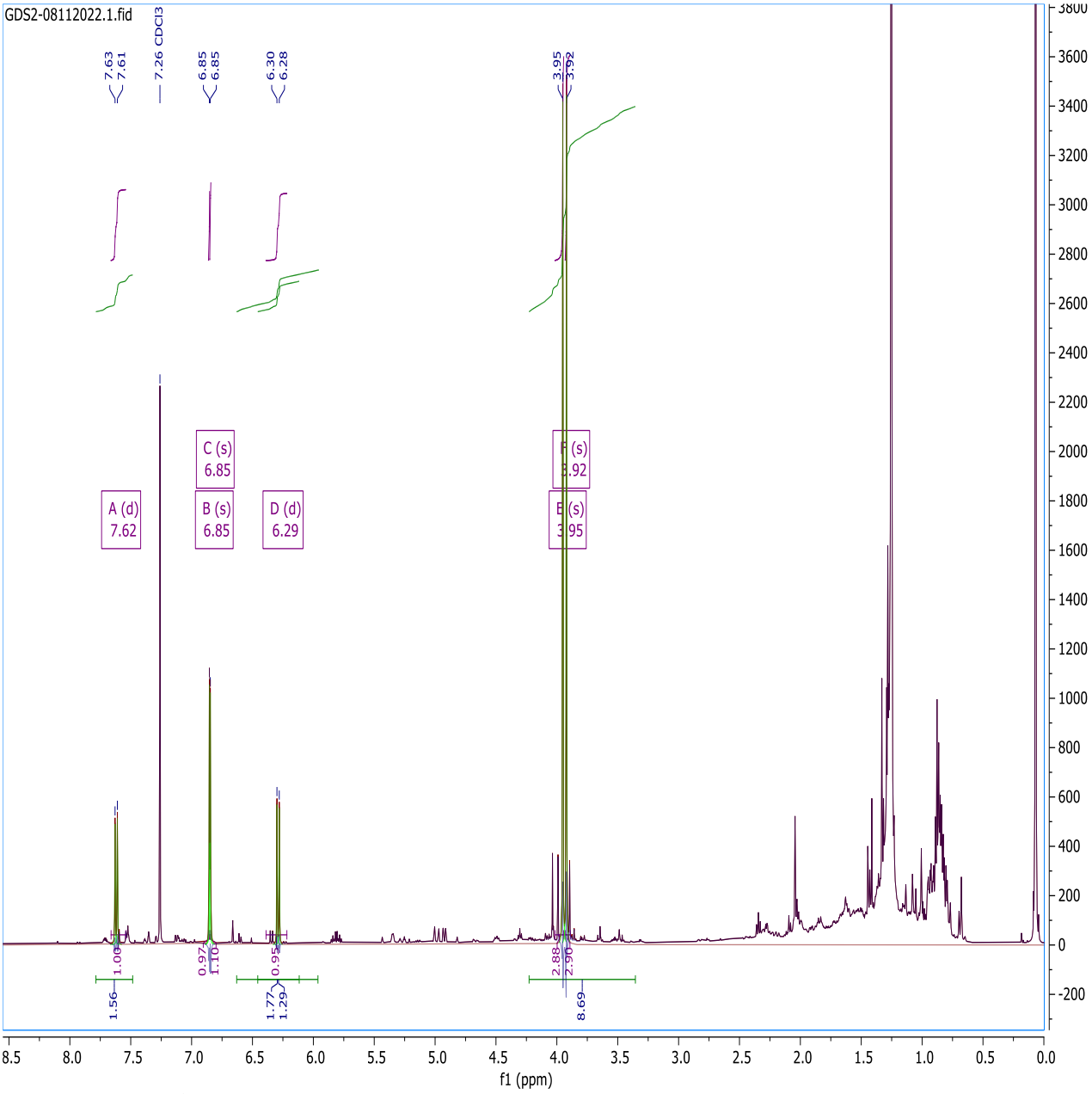


Figure 11. The ^{1}H-NMR spectrum of GDS-2.

Data from ^{13}C NMR (126 MHz, DMSO) DEPT-135 showed at δ=161.03 (C-2), 153.02 (C-7), 149.91 (C-6), 146.34 (C-9), 144.83 (C-4), 113.14 (C-10), 111.67 (C-3), 109.43 (C-5), 100.53 (C-8), 56.65 (CH3O-), 56.38 (CH3O-) (Table-3). The signals at δ 111.67, 144.83, 109.43 and 100.53 represents aromatic CH carbon of C-3, C-4, C-5 and C-8, respectively. The signal at δ 161.03 (C-2), 149.91 (C-6), 153.02 (C-7), 146.34 (C-9) and 113.14 (C-10) were attributed to the non-protonated quaternary carbons. DEPT-45 (Figure 12), DEPT-90 (Figure 13), and DEPT-135 (Figure 14) were also utilized in the structural elucidation of GDS-1. Spectroscopic data were carefully analyzed and in comparison, with those reported in the literature and the structure of the compound, GDS-2 was suggested to be 6,7-dimethoxy coumarin as shown in figure 15. GDS-2 NMR data agreed with scoparone, which was previously reported in the literature (Table 3) (Waight *et al*., 1987; Ma *et al*., 2006).

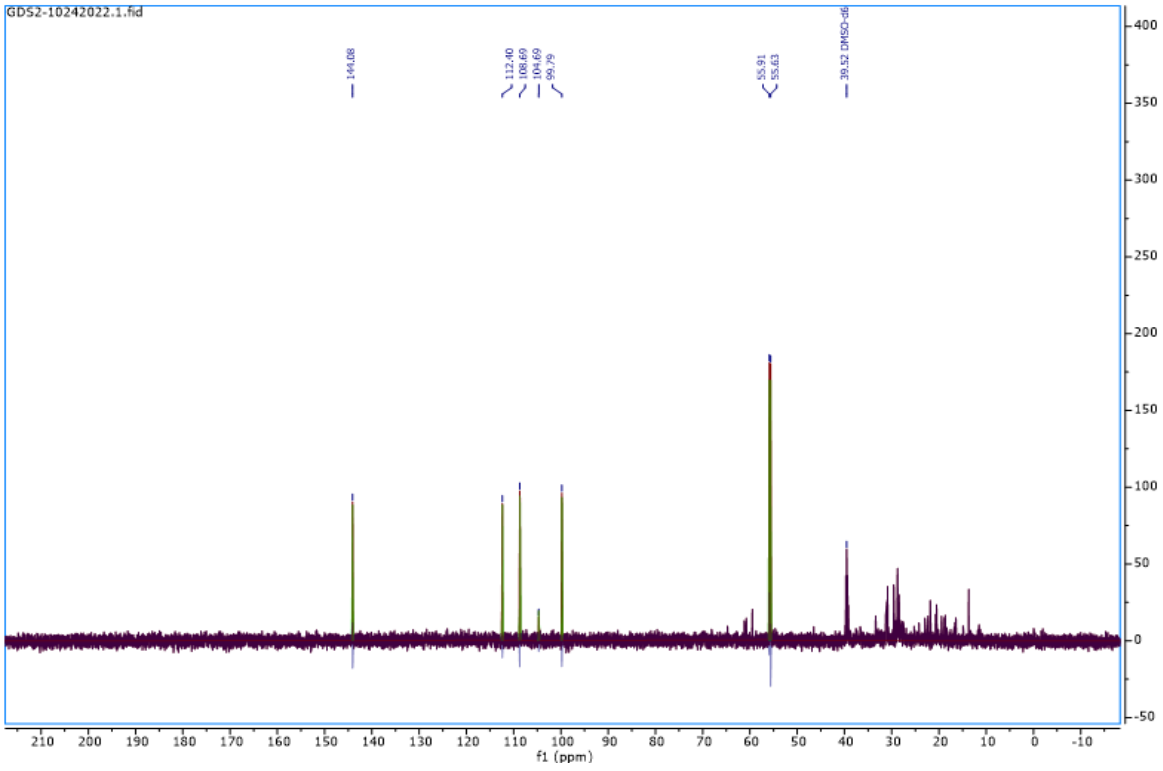


Figure 12. DEPT-45 spectrum of GDS-2.

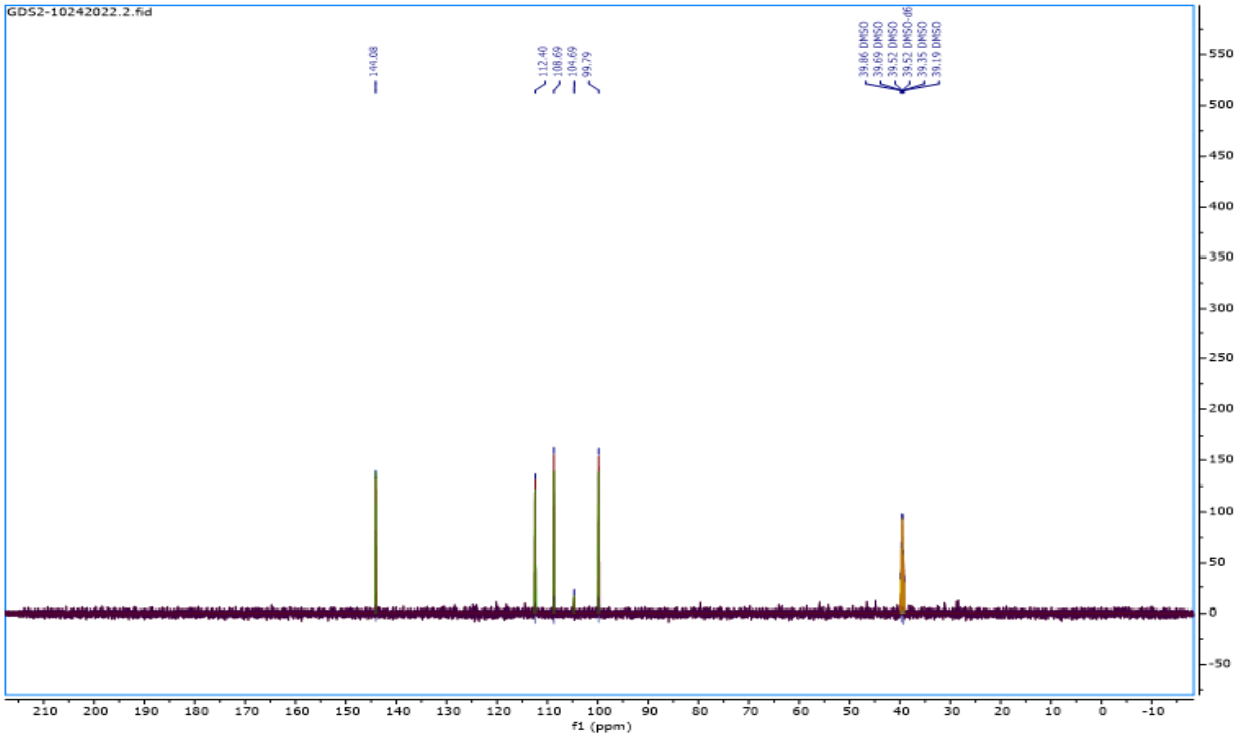


Figure 13. DEPT-90 spectrum of GDS-2.

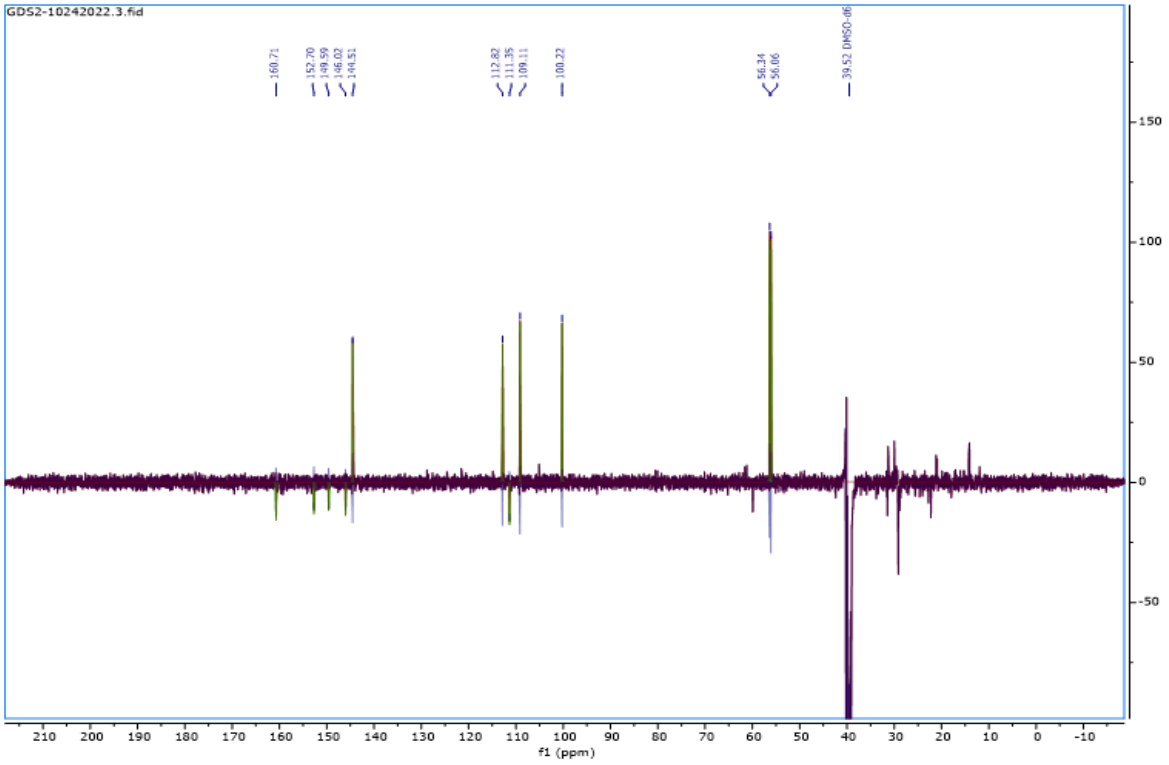
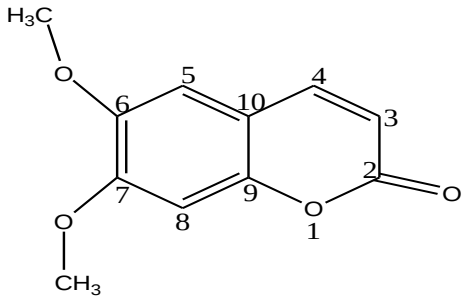


Figure 14. DEPT-135 spectrum of GDS-2.



Scoparone (GDS-2) (molecular formula of $C_{11}H_{10}O_4$ and calculated exact mass = 206.0576u)

Figure 15. Chemical Structure of 6,7-dimethoxycoumarin (GDS-2).

Table 3. ^{1}H NMR and ^{13}C NMR data of GDS-2 and reference compound.

Present data			Reference (scoparone) (Ma *et al*., 2006)	
Position	^{1}H NMR (500 MHz, $CDCl_3$) GDS-2 δ (ppm, mult., J Hz)	^{13}C NMR (126 MHz, DMSO) GDS-2 δ (ppm)	^{1}H NMR δ (ppm) (mult., J Hz)	^{13}C NMR of δ (ppm)
2	-	161.03	-	161.7
3	6.29 (d, 9.4)	111.67	6.28(d, 9.6)	111.7
4	7.62 (d, 9.4)	144.83	7.65 (d, 9.6)	143.6
5	6.85 (s)	109.43	6.88 (s)	108.2
6	-	149.91	-	150.2
7	-	153.02	-	153.0
8	6.85(s)	100.53	6.83 (s)	100.2
9	-	146.34	-	146.6
10	-	113.14	-	113.7
6– OCH_3	3.95(s)	56.65	3.96(s)	56.6
7– OCH_3	3.92 (s)	56.38	3.94(s)	56.6

4.3 Determination of antidiarrheal activity

4.3.1 Acute oral toxicity of the crude extract

Oral administration of 2000 mg/kg crude extract of *P. schimperiana* roots resulted in no mortality and overt toxicities such as anorexia, erectile hairs, tremors, diarrhea, and salivation during a total follow-up of 14 days. Thus, the lethal dose of 50% (LD_{50}) of the crude extract is estimated to be greater than 2000 mg/kg in mice. The results of this study suggest that for traditional medicinal purposes, a dose of 2000 mg/kg of *P. schimperiana* roots extract could be considered safe. However, further subchronic and chronic toxicological research is needed to investigate the safety characteristics of *P. schimperiana* roots.

4.3.2 Antidiarrheal activity of the crude extract

4.3.2.1 Effects of crude extract on castor oil-induced diarrheal model

The crude extract showed antidiarrheal activities with different doses. The crude extract from the roots of *P. schimperiana* with three doses at 100, 200, and 400 mg/kg delayed the onset of diarrhea and the total amount of fecal output during 4 hours of observation when compared to the negative control. The finding reveals that at test doses of 100, 200, and 400 mg/kg, the percentages of defecation inhibitions were 37.5%, 46.2% and 61.2%, respectively. The 400 mg/kg dose of the extract significantly reduced the number and weight of total and wet fecal output when compared to the negative control (Table 4). Our findings showed that *P. schimperiana* crude extract may contain substance(s) with strong antidiarrheal activity. In a dose-dependent approach, the extract significantly protected mice from castor oil-induced diarrhea. Loperamide 3 mg/kg was used as a positive control in this study, the therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory properties (Coupar, 1987).

Table 4. Effect of crude root extract of *P. schimperiana* (MREPS) against castor oil-induced diarrhea in mice.

Treatment	Dose (mg/kg, P.O)	The onset of diarrhea (min)	TNF	TNWF	TWF (gm)	Total WWF (gm)	% Inhibition of Defecation
Vehicle	*	56.60±2.77	8.40±0.51	6.00±0.55	0.53±0.4	0.32±0.03	-
Loperamide	3	149.25±4.23^{a1c1d1e1}	2.7±0.62^{a1}	0.75±0.25^{a1}	0.11±0.02^{a1}	0.06±0.01 ^{a1}	88.8
MREPS	100	93.4 ± 2.84^{a1b1d1e1}	5±0.45 ^{a1b2}	3.4±0.51^{a1b2}	0.23±0.019 ^{a1b2}	0.16±0.04 ^{a1}	37.5
MREPS	200	114.75 ±5.54^{a1b1c1}	4±0.41^{a1}	2.6±0.5^{a1}	0.23±0.016^{a1b2}	0.15±0.02^{a1}	46.2
MREPS	400	127.75 ±3.42^{a1b1c1}	3.5±0.29^{a1}	2.0±0.41^{a1}	0.17±0.014 ^{a1}	0.08±0.01^{a1}	61.2

[1]

[1] Data are expressed as mean ± standard error of the mean (SEM); (n=5); Analysis was done by using One-Way ANOVA followed by Tukey post hoc test; [a] compared to the control, [b] compared to loperamide, [c] compared to 100 mg/kg of MRETR, [d] compared to 200 mg/kg of MRETR, [c]om-pared to 400 mg/kg of MREPS; 1p < 0.01, 2 p < 0.05; * = Mice in the control group received distilled water (10 ml/kg); MREPS = Methanol Root Extract of *P. schimperiana*, TNF = Total Number of Feces, TNWF = Total Number of Wet Feces, TWF = Total Weight of Feces, and TWWF = Total Weight of Wet Feces.

Loperamide was used as a positive control in this study, it is an opioid phenylpiperidine that reduces intestinal transit time, stimulates opioid receptors in the myenteric plexus, possesses antisecretory properties, and blocks calcium channels (Vannueten *et al*., 1979). The 400 mg/kg dose of the extract showed 32% of mean total fecal output and 25% of mean wet fecal output. Whereas, the percentage means fecal output of 100 mg/kg and 200 mg/kg, did not show any difference (43.4%) (Figure 16). The therapeutic effect of loperamide is believed to be due to its antimotility and antisecretory properties (Coupar, 1987).

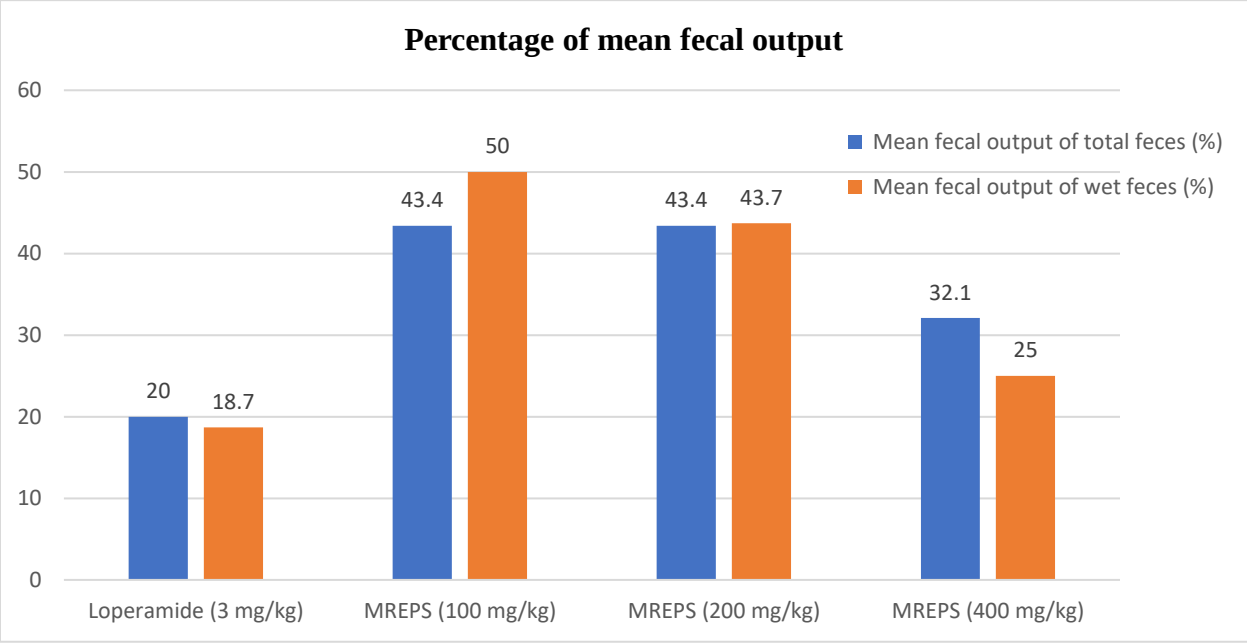


Figure 16. Percentage of the mean fecal output of crude extract from the roots of *P. schimperiana*.

4.3.2.2 Effect of crude extract on gastrointestinal motility model

The activated charcoal meal was used as a marker to study the effect of medicinal plants on the gastrointestinal motility model. Antidiarrheal activity of plant secondary metabolites was investigated by their ability to inhibit intestinal motility and prevent the secretion of intestinal contents (Tadesse *et al.*, 2017). The crude extract from the roots of *P. schimperiana* reduced intestinal

transit time significantly, as evidenced by a decrease in the distance traveled from pylorus to caecum by charcoal meal. At doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, the percentage reduction of gastrointestinal transit of charcoal was 18.71%, 35.90% and 58.95% respectively. The extract significantly decreased the gastrointestinal movement of charcoal meal caused by castor oil, at a dose of 100, 200 and 400 mg/kg when compared to a negative control (Table 5). The mean distance traveled by activated charcoal at 400 mg/kg test dose (12.28±1.15 cm) showed significant reduction compared with a negative control with 58.95% percent of inhibition in mice.

Table 5. Effect of crude extract from the roots of *P. schimperiana* (MREPS) against gastrointestinal motility test model in mice.

Treatment	Dose (mg/kg, P.O)	Mean length of the small intestine (cm)	Mean distance traveled by charcoal (cm)	Peristalsis index (%)	% Inhibition
Vehicle	*	56.86±1.22	29.92±1.08	52.62	
Loperamide	3	54.66±0.93	7.16±0.86[a1]	13.09	76.06
MREPS	100	58.70±1.19	24.32±1.3[a1]	41.43	18.71
MREPS	200	53.80±1.63	19.18±0.96[a1]	35.65	35.90
MREPS	400	56.02±1.21	12.28±1.15[a1]	21.92	58.95

[2]

[2] Data are expressed as mean ± standard error of the mean (SEM); (n=5); Analysis was done by using One-Way ANOVA followed by Tukey post hoc test; [a] compared to the control, 1p < 0.01, * = Mice in the control group received distilled water (10 ml/kg); MREPS = Methanol Root Extract of *P. schimperiana*.

4.3.2.3 Effect of crude extract on castor oil-induced enteropooling

In the castor oil-induced enteropooling model, intestinal contents volume and weight were significantly reduced by the crude extract as compared to the negative control at all doses tested. The crude extract at doses of 100, 200, and 400 mg/kg produced 16.7%, 24%, and 44.5% inhibition of volume of intestinal content and 10.7%, 14.7%, and 20.9% inhibition of intestinal content weight. The standard drug loperamide (3 mg/kg) also significantly inhibited intestinal fluid accumulation (59.7%) (Table 6). Probably due to its antimotility and antisecretory effects, which are similar to those of loperamide, the crude extract effectively counteracted the action of castor oil and greatly reduced intraluminal fluid accumulation when compared to the negative control (Coupar, 1987). Castor oil and its active metabolite ricinoleic acid are well known for their diarrhea inducing properties by reducing active Na^+ and K^+ absorption in stomach as well as the activity of Na^+, K^+ ATPase in the small intestine and colon (Meite *et al.*, 2009).

Table 6. Effect of crude extract from the roots of *P. schimperiana* (MREPS) against castor oil-induced enteropooling in mice.

Treatment	Dose (mg/kg, P.O)	MVSIC (ml)	% Inhibition using MVSIC	MWSIC (gm)	% Inhibition using MWSIC
Vehicle	*	0.72±0.016	-	1.77±0.026	-
Loperamide	3	0.29±0.015^{a1}	59.7	1.30±0.027^{a1}	26.5
MREPS	100	0.60±0.016 ^{a1b1}	16.7	1.58±0.018^{a1b1e1}	10.7
MREPS	200	0.54±0.008 ^{a1b1d2e1}	24	1.51±0.021^{a1b1e2}	14.7
MREPS	400	0.40±0.014 ^{a1b1e1}	44.5	1.40±0.019^{a1b2}	20.9

[3]

4.3.2.4 *In vivo* antidiarrheal index

An extract's effectiveness to treat diarrhea is measured by its antidiarrheal activity index (ADI), the higher the ADI value, the greater the effectiveness (Than *et al*., 1989). The crude extract showed increments in ADI in a dose-dependent manner and the maximum ADI (78.72%) was observed at 400 mg/kg. However, this is lower than the ADI of 3 mg/kg loperamide (98.78%) (Table 7).

[3] Data are expressed as mean ± standard error of the mean (SEM); (n=5); Analysis was done by using One-Way ANOVA followed by Tukey post hoc test; [a] compared to the control, [b] compared to loperamide, [c] compared to 100 mg/kg of MREPS, [d] compared to 200 mg/kg of MREPS, [e] compared to 400 mg/kg of MREPS; 1p < 0.01, 2p < 0.05; MREPS = Methanol Root Extract of *P. schimperiana*, MWSIC: Mean Weight of Small Intestine Content: MVSIC: Mean Volume of Small Intestine Content; * = Mice in the control group received distilled water (10 ml/kg).

Table 7. *In vivo* antidiarrheal index of crude root extract of *P. schimperiana*.

Treatment	Dose (mg/kg, P.O)	Delay in defecation (%)	Gut meal travel distances (%)	Purging frequency in a number of wet feces (%)	*In vivo* antidiarrheal index (ADI)
Vehicle	-	-	-	-	-
Loperamide	3	163.70	76.06	78.5	98.78
MREPS	100	65.12	18.71	40.5	36.54
MREPS	200	102.74	35.9	52.4	57.58
MREPS	400	125.71	58.95	66.7	78.72

MREPS = Methanol Root Extract of *P. schimperiana*.

4.3.3 Antidiarrheal activity of isoscopoletin and scoparone on castor oil-induced diarrheal model

Isoscopoletin is the major primary metabolite of scoparone formed via O-demethylation (Fayyaz *et al*., 2018). Isoscopoletin and scoparone were tested at doses of 10 mg/kg and 20 mg/kg against the castor oil-induced diarrhea model. After oral administration of 20 mg/kg isoscopoletin and scoparone, normal defecation was inhibited by 61.2% and 66.6% in an average time of 4 hours, while loperamide 3 mg/kg showed an 88.8% inhibition (Table 8). Mice treated with 20 mg/kg of isoscopoletin had a mean total and wet fecal output of 24.5 and 40.6%, but mice treated with scoparone (20 mg/kg) showed a mean total and wet fecal output of 43.4 and 53.1%, respectively (Figure 17).

Ricinoleic acid, the major constituent of castor oil, promotes the release of prostaglandins and nitric oxide (Shettima *et al*., 2016). Therefore, the antidiarrheal action exerted by scoparone and isoscopoletin might be associated with the inhibition of nitric oxide synthase and prostaglandin formation. By inhibiting cyclooxygenase-2 and inducible nitric oxide synthase, scoparone has been shown to have anti-inflammatory properties (Jang *et al*., 2005). In addition, scoparone showed antibacterial activity against *Shigella dysenteriae* (MIC = 0.2 g/ml) and *E. coli* (Zone of inhibition = 17.5 mm at 30 mg/ml), which are known causes of diarrheal (DuPont, 2009; Yang *et al*., 2010; Kiplimo and Koorbanally, 2012). However, further studies are needed to elucidate the antidiarrheal molecular mechanisms of action and targets of isoscopoletin and scoparone. Scoparone is rapidly absorbed and distributed in the extravascular system in rat after oral administration (dose = 90 mg/kg, Cmax = 14.67 mg/L, AUC = 81.15 mg*h/L, CL = 1.23 L/h) (Yin *et al*., 2012; Hsueh and Tsai, 2018).

Table 8. Effect of Isoscopoletin and Scoparone isolated from the roots of *P. schimperiana* (MREPS) against castor oil-induced diarrhea in mice.

Treatment	Dose (mg/kg, P.O)	The onset of diarrhea (min)	TNF	TNWF	TWF (gm)	TWWF (gm)	Defecation inhibited (%)
Vehicle	*	56.60±2.77	8.40±0.51	6.00±0.55	0.53±0.4	0.32±0.03	-
Loperamide	3	149.25±4.23^{a1}	2.67±0.62^{a1}	0.75±0.25^{a1}	0.11±0.02^{a1}	0.06±0.01 ^{a1}	88.8
Isoscopoletin	10	104.3±4.7^{a1b1}	3.67±0.33^{a1}	3.0±0.58^{a1}	0.12±0.018^{a1}	0.09±0.03^{a1}	48.3
Isoscopoletin	20	127.6±5.04 ^{a1}	2.67 ±0.33^{a1}	2.33±0.3^{a1}	0.13 ±0.01^{a1}	0.13 ±0.01^{a1}	61.20
Scoparone	10	103.3±12.14 ^{a1b1}	4.67±1.20^{a2}	3.00±0.58^{a1}	0.23±0.03^{a1b2}	0.17±0.02^{a2}	48.3
Scoparone	20	134.5±6.43 ^{a1}	3.5±0.645^{a1}	2.00±0.5^{a1}	0.14±0.04^{a1}	0.12±0.04^{a1}	66.6

[4]

[4] Data are expressed as mean ± standard error of the mean (SEM); (n=5); Analysis was done by using One-Way ANOVA followed by Tukey post hoc test; [a] compared to the control, [b] compared to loperamide; 1p < 0.01, 2 p < 0.05; * = Mice in the control group received distilled water (10 ml/kg).

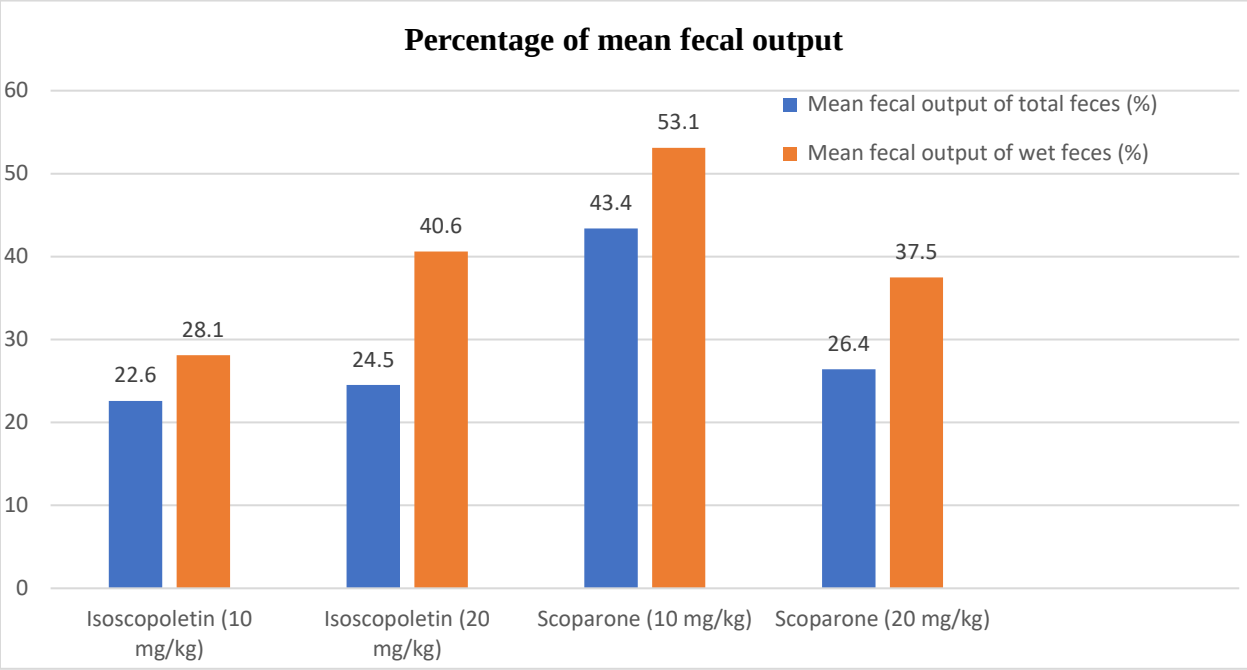


Figure 17. Percentage of mean fecal output isoscopoletin and scoparone from the roots of *P. schimperiana*

To the best of our knowledge, this is the first study to isolate the antidiarrheal compounds isoscopoletin and scoparone from *P. schimperiana* roots.

4.4 Nutritional and anti-nutritional composition of *Psydrax schimperiana* fruits

The findings from proximate analyses revealed that *P. schimperiana* fruits contained high carbohydrate content (40.03±0.15%), followed by crude fibers (31±0.0%) and low crude fat (0.99±0.01%) content (Table 9). Dietary fiber is the edible part of a plant or its extracts or analogous carbohydrates that are not easily digested or absorbed in the human small intestine but are partly or completely fermented in the large intestine. High consumption of fiber helps protect against colon cancer, cardiovascular diseases, and constipation. Adequate intake of dietary fiber is therefore beneficial to good health (McRae, 2017).

Table 9. Proximate composition and anti-nutritional factors from the fruits of *P. schimperiana*.

Proximate composition	
Parameter	**Percentage composition (%)**
Moisture content	12.40 ±000
Ash value	6.38 ±0.02
Crude fat	0.99 ±0.01
Crude fiber	31.0 ±0.00
Crude protein	9.19 ±0.12
Total carbohydrate	40.03 ±0.15

Antinutritional factors	
Parameter	**Percentage composition (%)**
Tannin	22.96 ±0.04
Phytate	1.157 ±0.004
Oxalate	0.72 ±0.04

The values are means of triplicate determinations ± Standard deviation

The fruits of *P. schimperiana* exhibited high tannin content (22.96±0.04%) followed by phytate (1.157±0.004%) (Table 9). High tannin levels prevent the small intestine from absorbing digestive end products, and high phytate contents in the food impair the body's ability to absorb calcium and iron. Oxalates also inhibit calcium absorption by binding to the calcium ion to form complexes which may also cause kidney stones (Getachew *et al*., 2013). Food containing 1-6% phytate over a prolonged time reduces the bioavailability of minerals, particularly divalent and trivalent ions of zinc, magnesium, calcium, copper and manganese (Agbaire, 2012; Abbas and Ahmed, 2018). Phytate is also associated with nutritional diseases such as rickets and osteomalacia in children and adults (Erdman, 1979).

5. CONCLUSIONS

Generally, the finding of this study revealed that the crude extract from the roots of *P. schimperiana* possessed antidiarrhea activity on castor oil-induced diarrheal, gastrointestinal transit time, and castor oil-induced enteropooling models in mice. Isoscopoletin, the major primary metabolite of scoparone formed via O-demethylation, and scoparone exhibited antidiarrheal activity at the dose of 20 mg/kg and 10 mg/kg on castor oil-induced diarrheal model in mice. Therefore, this finding may be used as a reference in the study of natural products and will help future research that gear toward the development of antidiarrheal drugs of medicinal plants origin. The findings of this study also validated the traditional healer's claims and supported the ethnomedicinal use of *P. schimperiana* roots for the treatment of diarrhea. The proximate composition analysis result revealed the presence of crude fiber, crude fat, crude protein, total ash, moisture, carbohydrate, and anti-nutritional factors such as tannin, phytate, and oxalate in *P. schimperiana* fruits.

6. RECOMMENDATIONS

Based on the findings of the present study, the following recommendations are forwarded.

- Isolation of other compounds and investigation of their antidiarrheal activity as well as other biological activities on roots of *P. schimperiana* is required to identify existing chemical compounds and their biological activity.

REFERENCES

Abbas Y, Ahmad A (2018). Impact of processing on nutritional and antinutritional factors of legumes. *Annals Food Science and Technology*, **19**(2): 199-215.

Abebe D (2001). The role of medicinal plants in health care coverage of Ethiopia: the possible benefits of integration. https://agris.fao.org.

Abera B (2014). Medicinal plants used in traditional medicine by Oromo people, Ghimbi district, Southwest Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **10**(1): 1-15.

Addis G, Asfaw Z and Woldu Z (2013). The role of wild and semi-wild edible plants to household food sovereignty in Hamar and Konso Communities, South Ethiopia. *Ethnobotany Research and Applications*, **11**: 251-271.

Adeniyi S A, Orjiekwe C L and Ehiagbonare J E (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. *African Journal of Biotechnology*, **8**(1): 110-112.

Africa Union. (2022) Malnutrition. https://au.int/en/theme/2022/year-nutrition.

Agbaire P O (2012) Levels of anti-nutritional factors in some common leafy edible vegetables of southern Nigeria. *African Journal of Food Science and Technology*, **3**(4): 99-101.

Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A and Hensel A (2009). An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *Journal of Ethnopharmacology*, **125**(3): 393-403.

Ahs J W, Tao W, Löfgren J and Forsberg B C (2010). Diarrheal diseases in low-and middle-income countries; incidence, prevention and management. *The Open Infectious Diseases Journal*, **4**(1): 113-124.

Akoto C O, Acheampong A, Boakye Y D, Takyi S and Garba R (2019). Phytochemical screening and in vitro antioxidant and antimicrobial activities of the extracts of the stem-bark of *psydrax peruviana*. *Journal of Medicinal Plants Studies* **7**(5): 28-34.

Alebel A, Tesema C, Temesgen B, Gebrie A, Petrucka P and Kibret G D (2018). Prevalence and determinants of diarrhea among under-five children in Ethiopia: a systematic review and meta-analysis. *Plos one*, **13**(6): 1-20.

Andarge E, Shonga A, Agize M and Tora A (2015). Utilization and conservation of medicinal plants and their associated indigenous knowledge in Dawuro Zone: An ethnobotanical approach. *International Journal of Medicinal Plant Research*, **4**(3): 330-337.

Appiah K S, Oppong C P, Mardani H K, Omari R A, Kpabitey S, Amoatey C A, Onwona-Agyeman S, Oikawa Y, Katsura K and Fujii Y (2018). Medicinal plants used in the Ejisu-Juaben Municipality, southern Ghana: an ethnobotanical study. *Medicines*, **6**(1): 1-27.

Anokwah D, Mensah A Y, Amponsah K I, Mireku A E and Mintah D N (2016). Antiinflammatory, antioxidant and antimicrobial activities of the stem bark of *Psydrax subcordata*. *Der Pharmacia Letter*, **8**(20): 21-28.

AOAC (Association of Official Analytical Chemists) (2006). Official Methods of Analysis, 15th ed. AOAC Press, Washington DC., The USA. Pp. 78-90.

Arriola A H, Cobangkiat A B and Alejandro G J (2017). *Psydrax multiflora* sp. (Rubiaceae) from Palawan, Philippines. *Nordic Journal of Botany*, **35**(5): 182-184.

Asfaw A, Lulekal E, Bekele T, Debella A, Abebe A and Degu S (2021). Ethnobotanical investigation on medicinal plants traditionally used against human ailments in Ensaro district, north Shewa zone, Amhara regional state, Ethiopia. *Research Square*, **1**(1): 1-27.

Barbhuiya H A, Dutta B K, Das A K and Baishya A K (2014). The family Rubiaceae in southern Assam with special reference to endemic and rediscovered plant taxa. *Journal of Threatened Taxa*, **6**(4): 5649–5659.

Belayneh A, Bussa N F (2014). Ethnomedicinal plants used to treat human ailments in the pre-historic place of Harla and Dengego valleys, eastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **10**(1): 1-17.

Belayneh A, Asfaw Z, Demissew S and Bussa N F (2012). Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of BabileWereda, Eastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **8**(1): 1-11.

Bekalo T H, Woodmatas S D and Woldemariam Z A (2009). An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **5**(1): 1-15.

Birhanu T, Abera D and Ejeta E (2015). Ethnobotanical study of medicinal plants in selected Horro Guduru Woredas, Western Ethiopia. *Journal of Biology, Agriculture and Healthcare*, **5**(1): 83-93.

Bogale T, Datiko D and Belachew S (2017). Floristic composition and community analysis of Berbere forest, Bale Zone, South East Ethiopia. *Journal of Agriculture, Forestry and Fisheries*, **6**(6): 206-213.

Catarino L, Havik P J and Romeiras M M (2016). Medicinal plants of Guinea-Bissau, therapeutic applications, ethnic diversity and knowledge transfer. *Journal of Ethnopharmacology*, **183**: 71-94.

Chukwudulue U M, Attah A F, and Okoye F B (2022). Linking phytochemistry to traditional uses and pharmacology of an underexplored genus *Psydrax*: a review. *Phytochemistry Reviews*, **21**: 1577-1604.

Chukwujekwu J C, Vanstaden J and Smith P (2005). Antibacterial, anti-inflammatory and antimalarial activities of some Nigerian medicinal plants. *South African Journal of Botany*, **71**(4): 316-325.

Coupar I M. (1987). Opioid action on the intestine: The importance of the intestinal mucosa. *Life sciences*, **41**(8): 917-925.

Daanaa S, Eric B G and Eric W (2017). Anticonvulsant effect of the hydroethanolic leave extract of *Psydrax subcordata* in Murine Models. *Journal of Ethnopharmacology*. **124**(1): 103-110.

Dalle M (2019). Ethnobotanical study of medicinal plants in Nagelle Arsi district, West Arsi Zone of Oromia, Ethiopia. *Journal of Natural Research*, **9**(13): 1-19.

Diane M Bridson (1985). The Reinstatement of *Psydrax* and a Revision of African species. *Kew Bulletin*, **40**(4): 687-725.

Duguma H T (2020). Wild edible plant nutritional contribution and consumer perception in Ethiopia. *International Journal of Food Science*, *International Journal of Food Science,* pp. 1-16

Dupont H L (2009). Bacterial diarrhea. *New England Journal of Medicine*, **361**(16): 1560-1569.

Elisha I L, Makoshi M S, Makama S, Dawurung C J, Offiah N V, Gotep J G, Oladipo O O and-Shamaki D (2013). Antidiarrheal evaluation of aqueous and ethanol stem bark extracts of *Khaya senegalensis*. Juss (Meliaceae) in Albino rats. *Pakistan Veterinary Journal*, **33**(1): 32-36.

El-seedi H R, Backlund A and Larsson S (2005). Chemosystematic value of cyclopeptide alkaloids from *Heisteria nitida*. *Journal of Biochemical Systematics and Ecology,* **33**(5): 831-839.

Embaby, H E (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Science and Biotechnology*, **19**: 1055-1062.

Erdman J N (1979). Oily seed phytates. Nutritional implications. *Journal of the American oil chemists' society*, **56**: 736-741

Eswani N, Abd Kudus K, Nazre M, Noor A A and Ali M (2010). Medicinal plant diversity and vegetation analysis of logged over hill forest of Tekai Tembeling Forest Reserve, Jerantut, Pahang. *Journal of Agricultural Science*, **2**(3): 189-195.

Fayyaz A, Makwinja S, Auriola S, Raunio H and Juvonen R O (2018). Comparison of in vitro hepatic scoparone 7-O-demethylation between humans and experimental animals. *Planta Medica*, **84**(5): 320-328.

FAO (1994). The state of food and Agriculture, FAO Agricultural Services. 27. FAO/UN. Rome.

Feenna O P, Estella O U and Obodike E C (2020). Phytochemical analysis and anti-diabetic activity of leave extract of *Psydrax horizontalis* Schum and Thonn (Rubiaceae). *Pharmacognosy Journal,***12**(1): 95-102.

Fokou E, Achu M B and Tchounguep F M (2004). Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. *African Journal of Food, Agriculture, Nutrition and Development*, **4**(1): 43-48.

Gidey M, Beyene T, Signorini M, Bruschi P and Yirga G (2015). Traditional medicinal plants used by Kunama ethnic group in Northern Ethiopia. *Journal of Medicinal Plants Research*, **9**(15): 494-509.

Gedif T, Hahn H (2003). The use of medicinal plants in self-care in rural central Ethiopia. *Journal of ethnopharmacology*, **87**(3): 155-161.

Gemedo-Dalle T, Maass B L and Isselstein J (2005). Plant biodiversity and ethnobotany of Borana pastoralists in southern Oromia, Ethiopia. *Economic botany*, **59**(1): 43-65.

Getachew G A, Asfaw Z, Singh V, Woldu Z, Baidu-Forson J J and Bhattacharya S (2013). Dietary values of wild and semi-wild edible plants in Southern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, **13**(2): 1-19.

Gizachew W N, Tileye F, Sebsebe D, Kassahun T and Ashagrie Z W (2019). Comparison of proximate, mineral, and phytochemical composition of *Ensete ventricosum* (Welw.) Cheesman landraces used for a different purpose. *African Journal of Agricultural Research*, **14**(30): 1326-1334.

Hedberg I, Edwards S (2003). Flora of Ethiopia and Eritrea Volume 4, part 1 (2003) Apiaceae to Dipsacaceae. National Herbarium, Biology Department, Science Faculty, Addis Ababa University.

Hsueh T P, Tsai T H (2018). Preclinical pharmacokinetics of scoparone, geniposide and rhein in an herbal medicine using a validated LC-MS/MS method. *Molecules*, **23**(10): 2-15.

Hosahally V R, Seru G, Sutar S P, Joshi G V, Sutar P K and Karigar A A (2012). Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity. *International Current Pharmaceutical Journal*, **1**(8): 213-216.

Ilboudo D P, Nicoletta B, Silvia P and Yolanda C (2013). Antiplasmodial and anti-inflammatory activities of *Psydrax acutifolia* (Hiern), a plant used in traditional medicine in Burkina Faso. *Journal of Ethnopharmacology*. **148**(3): 763-769.

Islam A M, Uddin M E, Chowdhury M A, Rahman M M, Habib M R and Rahman M A (2013). *In vivo* antidiarrheal and cytotoxic potential of different fractions of *Pandanus foetidus* leaves. *American Journal of Biomedical Sciences*, **5**(3): 208-16.

Jang S I, Kim Y J, Lee W Y, Kwak K C, Baek S H, Kwak G B and Chai K Y (2005). Scoparone from *Artemisia capillaris* inhibits the release of inflammatory mediators in RAW 264.7 cells upon stimulation cells by interferon-γ plus LPS. *Archives of pharmacal research*, **28**(2): 203-208.

James C S (1995). Analytical Chemistry of food. Chapman and Hall. London. pp. 64-65.

Joubouhi C, Florence D M, Perrin L, David N, Dominique H and Laurence VN (2015). Five new iridoïd dimers from the fruits of *Canthium subcordatum* DC (syn . *Psydrax subcordata* DC). *Phytochemistry Letters*, **13**(15): 348-354.

Joubouhi C, Tamokou J, Ngnokam D and Kuiat J (2017). Iridoids from *Canthium subcordatum* iso-butanol fraction with potent biological activities. *BMC Complement Alternative Medicine*, **17**(17): 1-8.

Kalaichelvi K, Dhivya S M (2016). Ethno-medicinal knowledge of plants used by Irula tribes of Nellithurai Neat, Karamadai range, Western Ghats and phytochemical screening of selected *Lamiaceae* species. *Advanced Journal of Pharmacy and Life Science Research*, **4**(2): 54-64.

Kathirvel P, Kumudha P (2011). A comparative study on the chemical composition of wild and cultivated germplasm of *Phaseolus lunatus* L. *International Journal of Applied Biology and Pharmaceutical Technology*, **2**: 296-305.

Kidane B, Asfaw Z and Andel T (2014). Ethnobotany of wild and semi-wild edible fruits species used by Maale and Ari ethnic communities in Southern Ethiopia. *Ethnobotany Research and Applications,* **12**(3): 455-471.

Kirk P L (1950). Kjeldahl method for total nitrogen. *Analytical chemistry*, **22**(2), 354-358.

Kotloff K L, Platts-Mills J A, Nasrin D, Roose A, Blackwelder W C and Levine M M (2017). Global burden of diarrheal diseases among children in developing countries: Incidence, etiology, and insights from new molecular diagnostic techniques. *Vaccine,* **35**(49): 6783-6789.

Kruger J, Taylor J R, Du X, De Moura F F, Lonnerdal B and Oelofse A (2013). Effect of phytate reduction of sorghum, through genetic modification, on iron and zinc availability as assessed by an in vitro dualizability bio accessibility assay, Caco-s cell uptake assay, and suckling rat pup absorption model. *Food Chemistry,* **141**: 1019-1025.

Lulekal E, Asfaw Z, Kelbessa E and Van Damme P (2013). Ethnomedicinal study of plants used for human ailments in Ankober District, North Shewa Zone, Amhara region, Ethiopia. *Journal of Ethnobiology and Ethnomedicine,* **9**(1): 1-13.

Ma C H, Ke W, Sun Z L, Peng J Y, Li Z X, Zhou X, Fan G R and Huang C G (2006). Large-scale isolation and purification of scoparone from herbal artemisia scoparone by high-speed counter-current chromatography. *Chromatographia,* **64**(1): 83-87.

Mahyuni R, Chikmawati T, Ariyanti NS and Wong KM (2018). The *Psydrax dicoccos* complex (Rubiaceae) in malesia. *Floribunda,* **5**(8): 322-331.

Manya M H, Keymeulen F, Ngezahayo J, Bakari S, Kalonda M E, Kahumba B J and Lumbu S J (2020). Antimalarial herbal remedies of Bukavu and Uvira areas in D.R. Congo, an ethnobotanical survey. *Journal of Ethnopharmacology,* **249**: 1-59.

McRae M P (2017). Dietary fiber is beneficial for the prevention of cardiovascular disease: an umbrella review of meta-analyses. *Journal of chiropractic medicine,* **16**(4): 289-299.

Meite S, Nguessan J D, Bahi C, Yapi H F, Djaman A J and Guinea F G (2009). Antidiarrheal activity of the ethyl acetate extract of *Morinda morindoides* in rats. *Tropical Journal of Pharmaceutical Research*, **8**(3): 201-207.

Mengesha, G G (2016). Ethnobotanical survey of medicinal plants used in treating human and livestock health problems in Mandura Woreda of Benishangul Gumuz, Ethiopia. *Advancement in Medicinal Plant Research***, 4**(1): 11-26.

Meragiaw M (2016). Wild useful plants with emphasis on the traditional use of medicinal and edible plants by the people of Aba'ala, North-eastern Ethiopia. *Journal* of *Medicinal Plant and Herbal Therapy Research*, **4**(1): 1-16.

Mesfin F, Demissew S and Teklehaymanot T (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *Journal of Ethnobiology and Ethnomedicine,* **5**(1): 1-18.

Nahrstedt A, Rockenbach J and Wray V (1995). Phenylpropanoid glycosides, a furanone glucoside and geniposidic acid from members of the *Rubiaceae*. *Phytochemistry*, **39**(2): 375–378.

Nasrin F (2013). Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems. *International Current Pharmaceutical Journal*, **2**(2): 33-37.

Nyahangare E T, Mvumi B M and Mutibvu T (2015). Ethnoveterinary plants and practices used for ectoparasite control in semi-arid smallholder farming areas of Zimbabwe. *Journal of ethnobiology and ethnomedicine,* **11**(1): 1-16.

Nguyen T V, Le Van P, Le Huy C, Gia K N and Weintraub A (2006). Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. *International Journal of Infectious Diseases,* **10**(4): 298-308.

Norhajar E, Kamziah A K and Nazre M (2010). Medicinal plant diversity and vegetation analysis of logged over hill forest of Tekai Tembeling forest reserve, Jerantut, Pahang. *Journal of Agricultural Science,* **2**(3): 189-210.

NRC (2011). National Research Council. Guide for the care and use of laboratory animals. 8th edition. Washington: The National Academic Press.

Ochwang'i D O, Kimwele C N, Oduma J A, Gathumbi P K, Mbaria J M, Kiama S G (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of Ethnopharmacology*, **151**(3):1040–1055.

Ochwang'i D O, Kimwele C N, Oduma J A, Gathumbi P K, Kiama S G, Efferth T (2018). Cytotoxic activity of medicinal plants of the Kakamega County (Kenya) against drug-sensitive and multidrug-resistant cancer cells. *Journal of Ethnopharmacology*, **215**:233–240OECD (2008). Guidelines for the testing of chemicals; Acute oral toxicity: Up and down procedures. OECD Publishing, No. 425.

Ogungbenle H N and Anisulowo Y F (2014). Evaluation of Chemical and fatty acid Constituents of Flour and Oil of Walnut (*Juglans regia*) seeds. *British Journal of Research,* **1**(3):113-119.

Oke, O L (1969). Oxalic acid in plants and in nutrition. *World review of nutrition and dietetics,* **10**: 262-303.

Okpo S O, Ching F P and Ekeleme I C (2011). Evaluation of the anti-diarrheal activity of the aqueous extract from leaves of *Pterocarpus santalinoides*. *Research Journal* of *Pharmaceutical, Biological* and *Chemical* Sciences, **2**(3): 590-597.

Ojo M A (2022). Tannins in Foods: Nutritional Implications and Processing Effects of Hydro-thermal Techniques on Underutilized Hard-to-Cook Legume Seeds–A Review. *Preventive Nutrition and Food Science,* **27**(1): 14-19

Pandey A, Tripathi S (2014). Concept of standardization, extraction and pre-phytochemical screening strategies for the herbal drug. *Journal of Pharmacognosy and Phytochemistry*, **2** (5): 115-119.

Raja R N, Rama L S and Muthuchelian K (2011). GC-MS analysis of bioactive components from the ethanolic leave extract of *Canthium dicoccum* (Gaertn.) Teijsm and Binn. *Journal of chemical and pharmaceutical research*, **3**(3): 792-798.

Rockenbach J, Nahrstedt, A and Wray V (1992). Cyanogenic glycosides from *Psydrax* and *Oxy-anthus* species. *Phytochemistry*, **31**(2): 567-570.

Sarkiyayi S, Mohammed M and Yakubu A (2013). Comparative analysis of nutritional and anti-nutritional contents of some varieties of Mango (*Mangifera indica*) in Kaduna Metropolis-Nigeria. *Research journal of applied sciences, Engineering, and technology,* **5**(4): 387-391.

Schwarz B, Wray V and Proksch P (1996). A cyanogenic glycoside from *Canthium schimperia-num. Phytochemistry,* **42**(3): 633-636.

Sharma D K, Gupta V K, Kumar S, Joshi V, Mandal R S, Prakash A B and Singh M (2015). Evaluation of the antidiarrheal activity of ethanolic extract of *Holarrhena antidysentery* seeds in rats. *Veterinary World,* **8**(12): 1392-1395.

Shettima A Y, Sanda A F, Ali H, Bello R F, Modu B and Tijjani Y (2016). Antidiarrheal effects of aqueous leave extract of *Ziziphus mauritiana* in Wistar Strain Albino Rats. *Journal of Pharmaceutical and Chemical,* **3**: 323-328.

Shoba F G, Thomas M (2001). Study of antidiarrheal activity of four medicinal plants in castor-oil induced diarrhea. *Journal of Ethnopharmacology,* **76**(1): 73-76.

Suksungworn R, Srisombat N, Bapia S, Soun-Udom M, Sanevas N, Wongkantrakorn N, Kermanee P, Vajrodaya S and Duangsrisai S (2017). Coumarins from *Haldina cordifolia* lead to programmed cell death in giant mimosa, potential bio-herbicides. *Pakistan Journal of Botany,* **49**(3): 1173-1183.

Tadesse E, Engidawork E, Nedi T and Mengistu G (2017). Evaluation of the antidiarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC Complementary and Alternative Medicine,* **17**(1): 1-8.

Teklehaymanot T and Giday M (2007). Ethnobotanical study of medicinal plants used by people in Zegie peninsula, Northwestern Ethiopia. *Journal of ethnobiology and Ethnomedicine*, **3**(1): 1-11.

Than A, Kulkarni H J, Hmone W and Tha S J (1989). Antidiarrheal efficacy of some Burmese indigenous drug formulations in experimental diarrhea test models. *International Journal of Crude Drug Research*, **27**(4): 195-200.

Uddin S J, Shilpi J A, Alam S M, Alamgir M, Rahman M T and Sarker S D (2005). Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus moluccensis* in castor oil and magnesium sulfate-induced diarrhea models in mice. *Journal of Ethnopharmacology*, **101**(3): 139-143.

UNICEF (2022). Malnutrition. https://data.unicef.org/resources/sofi-2022

Umaiyambigai D, Saravanakumar K and Raj G A (2016). Phytochemical profiles, antibacterial and antifungal activity of leaves from the *Psydrax dicoccos* (Gaertn). *Indo-Asian Journal of Multidisciplinary Research,* **2**(1): 443-452.

Vannueten J M, Helsen L, Michiels M and Heykants J J (1979). Distribution of loperamide in the intestinal wall. *Biochemical Pharmacology*, **28**(8): 1433-1434.

Waight E S, Razdan T K, Qadri B and Harkar S (1987). Chromones and coumarins from *Skimmia laureola*. *Phytochemistry*, **26**(7): 2063-2069.

Walker C L, Rudan I, Liu L, Nair H, Theodoratou E, Bhutta Z A, Brien K L, Campbell H and Black R E (2013). Global burden of childhood pneumonia and diarrhea. *The Lancet*, **381**(9875): 1405-1416.

Woldeab B, Regassa R, Alemu T and Megersa M (2018). Medicinal plants used for the treatment of diarrhoeal-related diseases in Ethiopia. *Evidence-Based Complementary and Alternative Medicine*. 1-20.

Wondimu T, Asfaw Z and Kelbessa E (2006). Ethnobotanical study of food plants around Dheeraa town, Arsi, Ethiopia. *Ethiopian Journal of Science*, **29**(1): 71-80.

World food programme (2022). Regional Food Security and Nutrition Update Eastern Africa Region. https://docs.wfp.org/api/.

World Health Organization (2020). Diarrhea. https://www.who.int/health-topics/diarrhoea/.

Yang B, Chen G, Song X, Chen Z, Song X and Wang J (2010). Chemical constituents and anti-microbial activities of *Canthium horridum*. *Natural product communications*, **5**(6): 913-14.

Yineger H, Yewhalaw D and Teketay D (2008). Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, **4**(1): 1-10.

Yin Q, Sun H, Zhang A and Wang X (2012). Pharmacokinetics and tissue distribution study of scoparone in rats by ultraperformance liquid-chromatography with tandem high-definition mass spectrometry. *Fitoterapia*, **83**(4): 795-800

Zhou J, Zhenlong W, Bamisaye O, Herbert A B, Olukemi A, Guangmin Y and Chun T (2019). Protein tyrosine phosphatase 1B inhibitory iridoids from *Psydrax subcordata*. *Journal of Natural Products*, **82**(10): 2916-2924.

Zohra S F, Meriem B, Samira S and Muneer M A (2012). Phytochemical screening and identification of some compounds from mallow. *Journal of Natural Product and Plant Resources*, **2**(4): 512-6.

APPENDICES

Appendix 1: -Plants used for the treatment of diarrhea in Ethiopian traditional medicine

No.	Name of plant	Family	Part used	Method of preparation	References
1	*Acacia albida* Del.	Fabaceae	Bark	Crushed, homogenized in water and taken orally	Wondimu *et al.*, 2006
2	*Acacia tortilis* (Forssk.) Hayne	Fabaceae	Leaves	Infusion took orally	Belayneh *et al.*, 2012
3	*Achyranthes aspera* L	Amaranthaceae	Leaves	Crushed and mixed with water taken orally	Birhanu *et al.*, 2015
4	*Ajuga integrifolia* Buch. Ham.	Lamiaceae	Root	Decoction of the fresh leave is drunk	Gidey *et al.*, 2015
5	*Allium sativum* L	Alliaceae	bulb	Chewed and swallowed	Gedif and Hahn, 2003.
6	*Bersama abyssinica* Fresen.	Melianthaceae	leaves	Boil and drink the decoction	Lulekal *et al.*, 2013
7	*Boscia angustifolia* A. Rich.	Capparidaceae	Root	Roots is chewed	Gidey *et al.*, 2015
8	*Cadaba rotundifolia* Forssk.	Capparidaceae	leaves	Fresh Leave crushed and mixed with water to drink the concoction	Meragiaw, 2016
9	*Cissampelosmucronata* A. Rich.	Menispermaceae	Root	Crushed and squeezed and taken orally	Mengesha, 2016
10	*Coffea arabica* L.	Rubiaceae	Seed	Roasted the seed pounded and mixed with honey and drunk	Adeniyi, 2014.
11	*Conyza pyrrhopappa* A. Rich.	Asteraceae	leaves	The decoction is taken orally	Andarge *et al.*, 2015
12	*Dodonaea angustifolia* L. f.	Sapindaceae	Root	Boiled and drink the decoction	Lulekal *et al.*, 2013
13	*Embeliaschimperi*	Myrsinaceae	Fruits	Crush and homogenize with	Lulekal *et al.*, 2013

	Vatke			cold water and drunk	
14	*Ficus thonningii* Blume.	Moraceae	Root	Chewed	Teklehaymanot and Giday, 2007
15	*Grewia villosa* Willd.	Malvaceae	leaves	Juice is taken orally.	Mesfin *et al.*, 2009
16	*Lantana ukambensis* Verdc.	Verbenaceae	leaves	Crushed and homogenized in water and taken orally	Bekalo *et al.*, 2009
17	*Leucas abyssinica* Briq.	Lamiaceae	Root	Crushed and mixed with water and taken orally	Andarge *et al.*, 2015
18	*Melia azedarach* L.	Meliaceae	Bark	Crushed, homogenized in water and drunk	Wondimu *et al.*, 2006
19	*Myrtus communis* L.	Myrtaceae	leaves	Juice is taken orally in the morning	Teklehaymanot and Giday, 2007
20	*Oryza sativa* L.	Poaceae	Leaves	Squeezed leaves are drunk	Gedif and Hahn, 2003.
21	*Syzygium-guineenses* (Willd) DC.	Myrtaceae	Bark	Decoction of powder taken orally.	Mengesha, 2016
23	*Thalictrum rhynchocarpon* Quart.	Ranunculaceae	Root	Boil the roots and drink the decoction	Lulekal *et al.*, 2013
24	*Tragia mitis* ex A. Rich.	Euphorbiaceae	Root	Crushed and mixed with water and sugar and taken orally	Yineger *et al.*, 2008
25	*Verbena officinalis* L.	Verbenaceae	Root	Powdered, mixed with water and taken orally	Gedif and Hahn, 2003.
26	*Vernonia auriculifera* Hiem	Asteraceae	Root	Crushed and squeezed and one cup is taken orally	Mengesha, 2016
27	*Ximenia americana* L.	Olacaceae	Root	Crushed, squeezed and taken orally	Mengesha, 2016
28	*Ziziphus spinachristi* L Desf.	Rhamnaceae	Steam bark	Steam bark is crushed, soaked in water and drunk	Mengesha, 2016

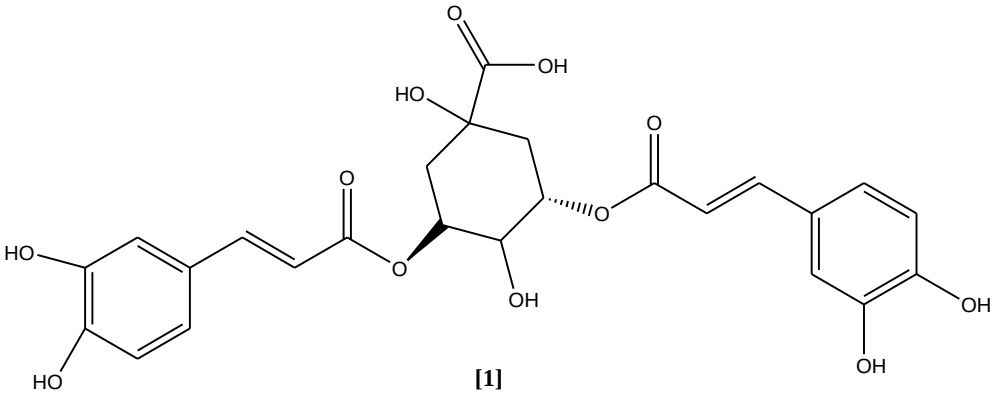
Appendix II: Some compounds isolated from *Psydrax* species and their structure

Class of compounds	Structure No	Source	References
Alkaloids			
3,5-dicaffeoylquinic acid	1	*P. schimperiana*	(Schwarz *et al*., 1996)
3,4-dicaffeoylquinic acid	2	*P. schimperiana*	(Schwarz *et al*., 1996)
Anorldianine	3	*P. arnoldiana*	(El-seedi *et al*., 2005)
Isoplectrodorine	4	*P. odorata*	(Poullain and Caledonia, 2016)
N-desmethylmyrianthine	5	*P. odorata*	(Poullain and Caledonia, 2016)
Plectrodorine	6	*P. odorata*	(Poullain and Caledonia, 2016)
Rhynchophylline	7	*P. lamprophylla*	(Yang, 2016)
10-hydroxycamptothecin	8	*P. lamprophylla*	(Yang, 2016)
Flavonoids			
Luteolin	9	*P. montigena*	(Yang, 2016)

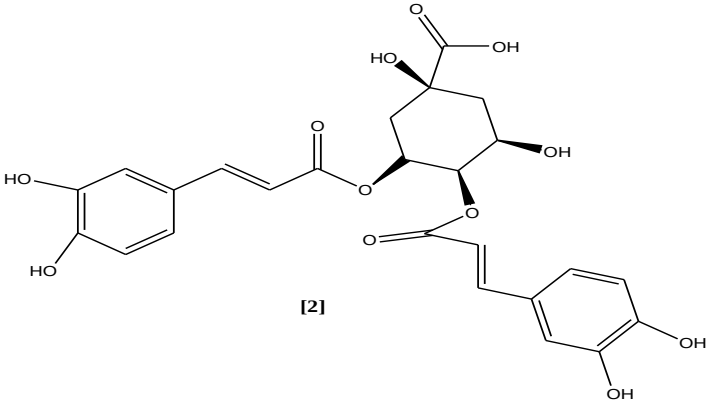
Myricitrin	10	*P. montigena*	(Yang, 2016)
Naringenin	11	*P. montigena*	(Yang, 2016)
Rutin	12	*P. montigena*	(Yang, 2016)
3',4', 7- trihydroxyflavone	13	*P. subcordata*	(Joubouhi *et al*., 2015)
Terpenes			
Betulinic acid	14	*P. subcordata*	(Joubouhi *et al*., 2015)
Canthiumoside 1	15	*P. subcordata*	(Joubouhi *et al*., 2015)
Canthiumoside 2	16	*P. subcordata*	(Joubouhi *et al*., 2015)
Canthiumoside 3	17	*P. subcordata*	(Joubouhi *et al*., 2015)
Canthiumoside 4	18	*P. subcordata*	(Joubouhi *et al*., 2015)
Canthiumoside 5	19	*P. subcordata*	(Joubouhi *et al*., 2015)
Genipin	20	*P. lamprophylla*	(Yang, 2016)
Geniposidic acid	21	*P. odorata*.	(Poullain and Caledonia, 2016)
linearin	22	*P. subcordata*	(Joubouhi *et al*., 2015)
Mussaenoside	23	*P. subcordata*	(Joubouhi *et al*., 2015)

Shanzhigenin methyl ester	24	*P. subcordata*	(Joubouhi *et al*., 2015)
1-epilinearin	25	*P. subcordata*	(Joubouhi *et al*., 2015)
1-epishanzhigenin methyl ester	26	*P. subcordata*	(Joubouhi *et al*., 2015)
Oleanolic acid	27	*P. subcordata*	(Joubouhi *et al*., 2015)
Shanzhiside methyl ester	28	*P. odorata*.	(Poullain and Caledonia, 2016)
Subcordatanol I	29	*P. subcordata*	(Zhou *et al*., 2019)
Subcordatanol II	30	*P. subcordata*	(Zhou *et al*., 2019)
Subcordatanol III	31	*P. subcordata*	(Zhou *et al*., 2019)
Subcordatanol IV	32	*P. subcordata*	(Zhou *et al*., 2019)
Subcordatanol V	33	*P. subcordata*	(Zhou *et al*., 2019)
Shanzhiside	34	*P. subcordata*	(Zhou *et al*., 2019)
Ursolic acid	35	*P. lamprophylla*	(Yang, 2016)
6-O-benzoylshanzhiside methyl ester	36	*P. odorata*.	(Poullain and Caledonia, 2016)
1-O-methylcrescentin I	37	*P. subcordata*	(Zhou *et al*., 2019)

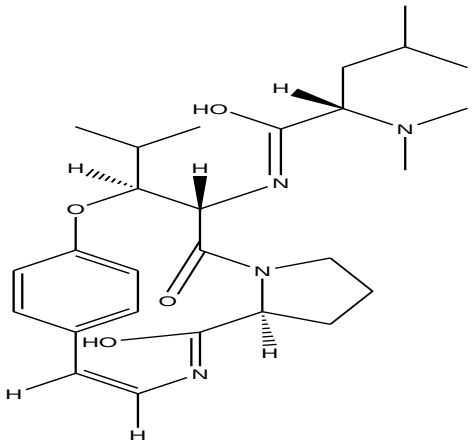
10-deoxyeucommiol	38	*P. subcordata*	(Zhou *et al*., 2019)
(Z)6, (Z)9-Pentadecadien-1-ol	39	*P. dicoccos*	(Joubouhi *et al*., 2015)
Coumarin			
Scoparone	40	*P. horridum*	(Yang *et al*., 2010)
Scopoletin	41	*P. horridum*	(Yang *et al*., 2010)
Miscellaneous compounds			
2R-[(2-Methoxybenzoylgenoposidyl)-5-O-β-D-apiofuranosyl-(1-6)-β-glucopyranosyloxy]-2-phenyl acetonitrile	42	*P. schimperiana*	(Schwarz *et al*., 1996)
Psydrin	43	*P. livida*	(Nahrstedt *et al*.,1995)
2R-b-D-glucopyranosyloxyphenylacetonitrile	44	*P. livida*	(Rockenbach *et al*., 1992



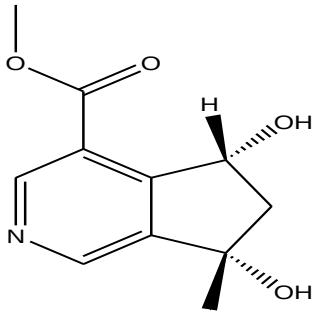
[1]



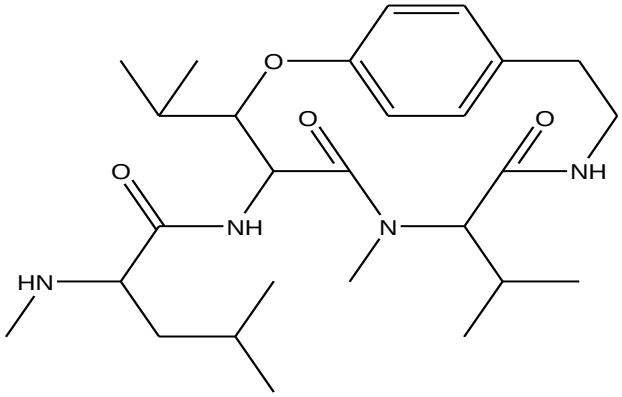
[2]



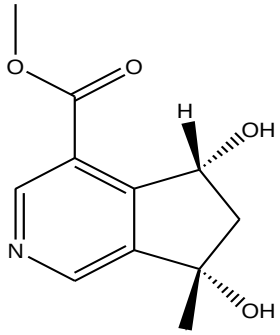
[3]



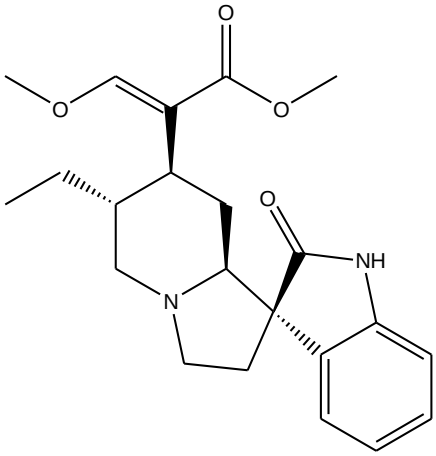
[4]



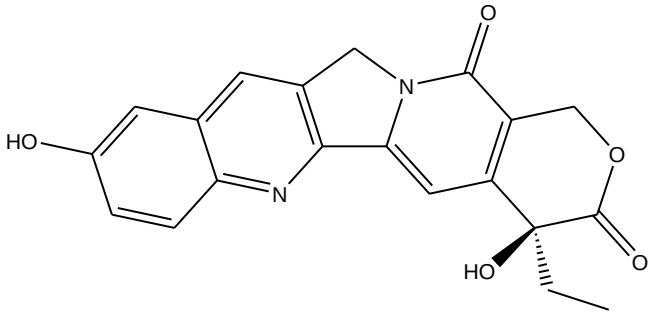
[5]



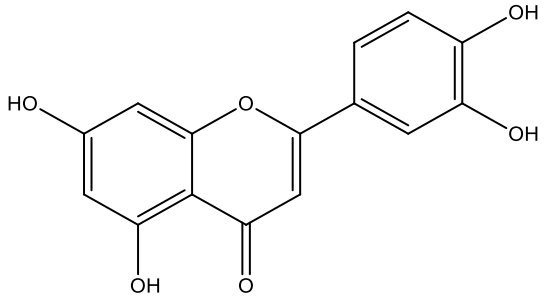
[**6**]



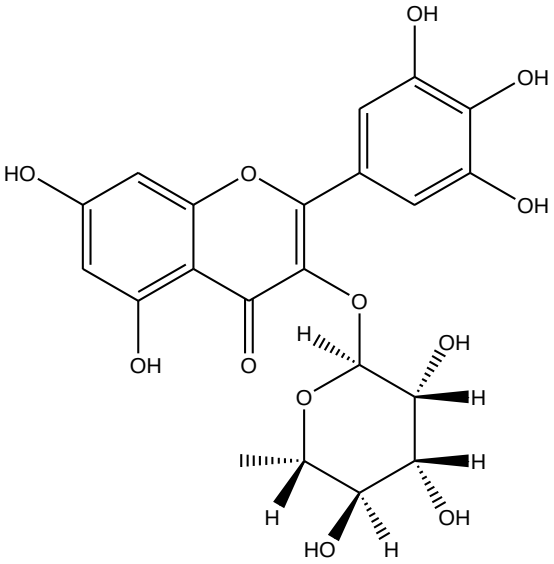
[7]



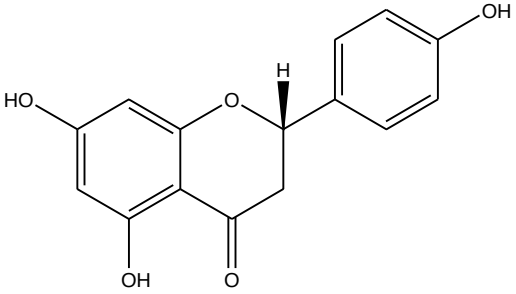
[**8**]


HO
O
OH
OH
O

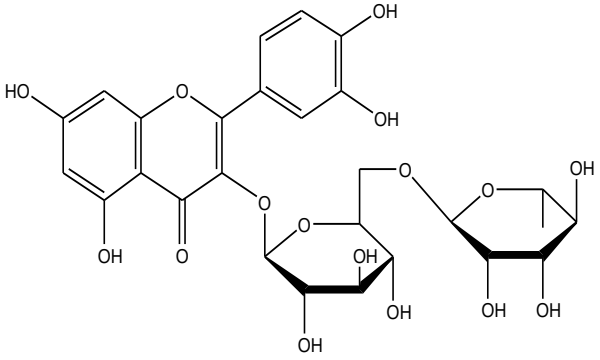
[**9**]


OH
OH
HO
O
OH
O
O
H
OH
H
H
H
OH
HO
H

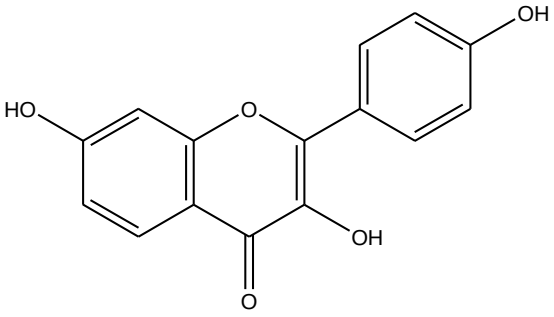
[**10**]


HO
O
H
OH
OH
O

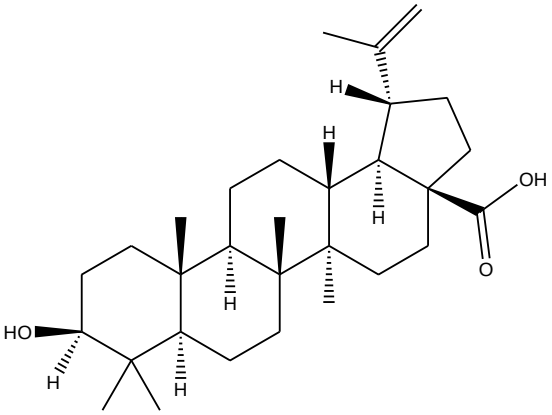
[**11**]


HO
O
OH
OH
OH
O
O
O
O
OH
OH
OH
OH
OH
OH

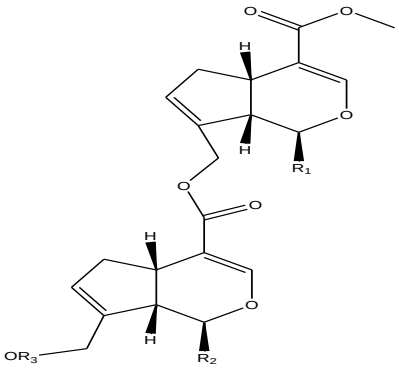
[**12**]



[**13**]

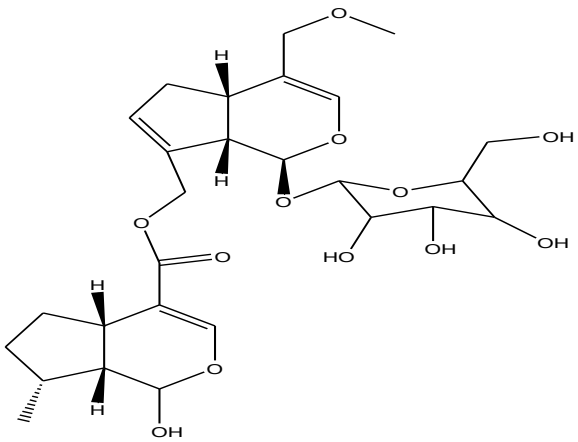


[**14**]

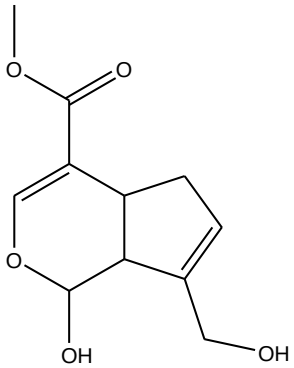


	R1	R2	R3
[15]	OH	OH	Ac
[16]	OH	O-Glc	Ac
[18]	O-Glc	O-Glc	Ac
[19]	O-Glc	O-Glc	H

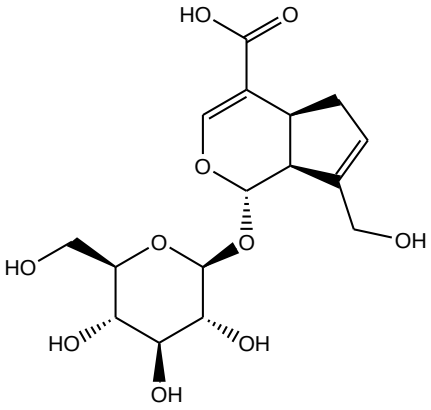
[**15-18**]



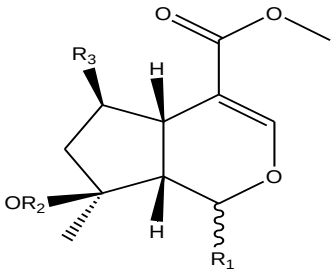
[**19**]



[**20**]

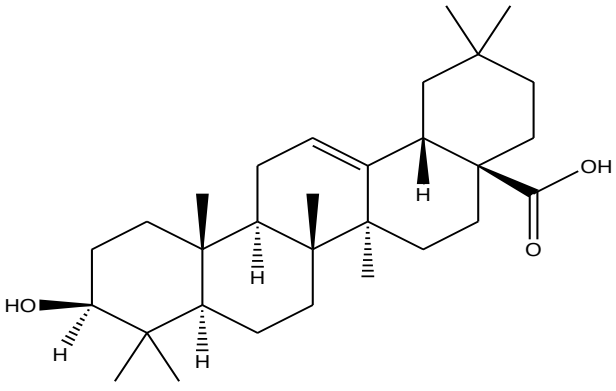


[**21**]

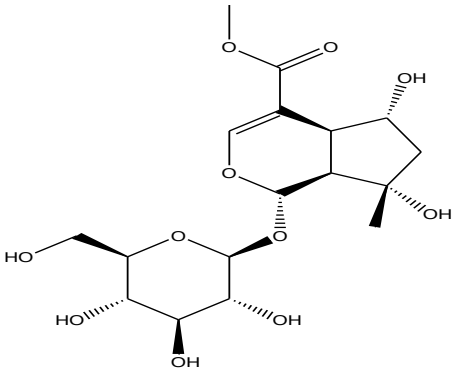


	R1	R2	R3
[22]	**B-OH**	**H**	**H**
[23]	**B-O-Glc**	**H**	**OH**
[24]	**B-OH**	**H**	**OH**
[25]	**a-OH**	**H**	**H**
[26]	**a-OH**	**H**	**OH**

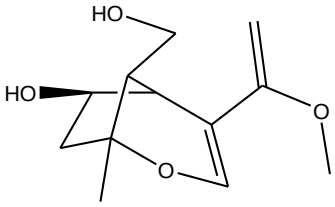
[**22-26**]



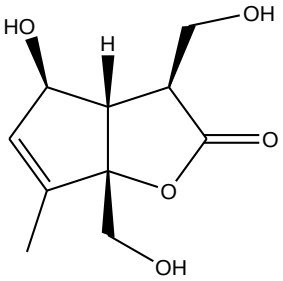
[**27**]



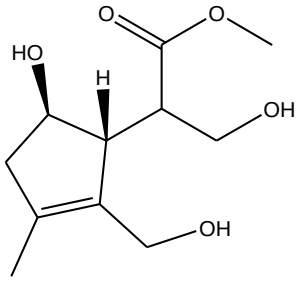
[**28**]



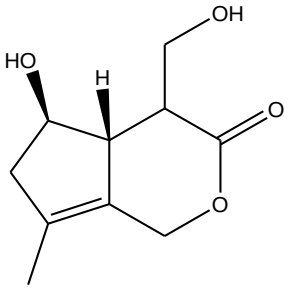
[**29**]



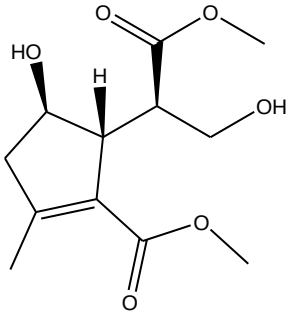
[30]



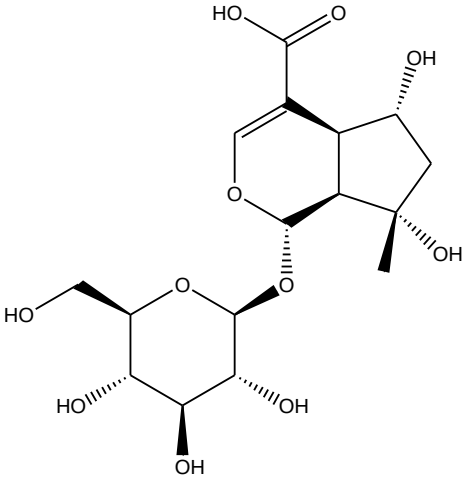
[31]



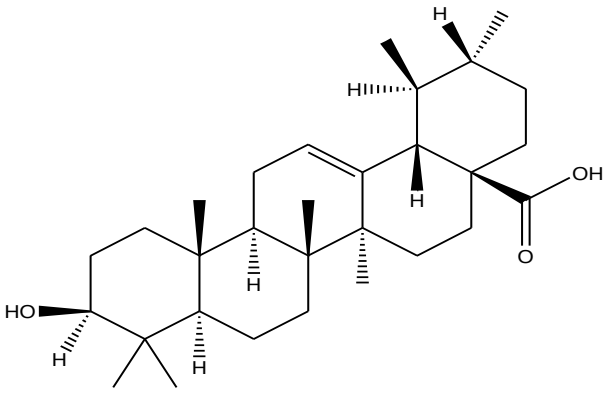
[**32**]



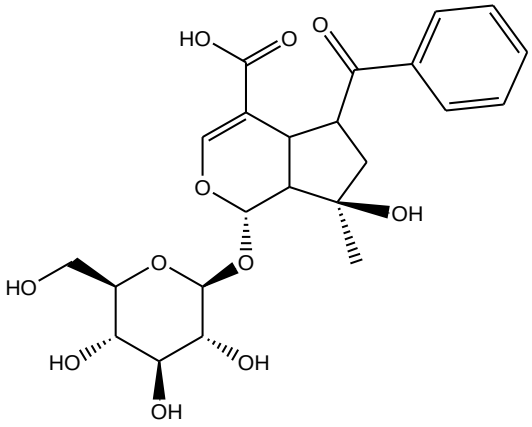
[**33**]



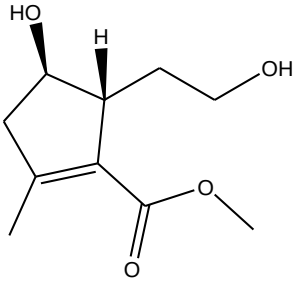
[**34**]



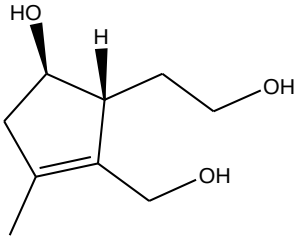
[**35**]



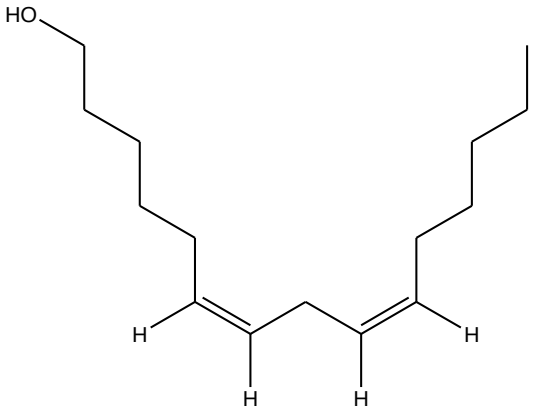
[**36**]



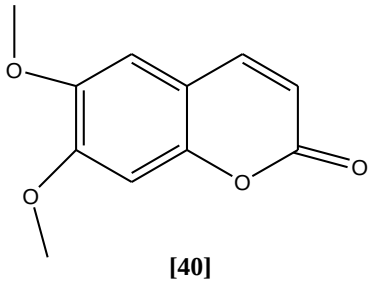
[**37**]



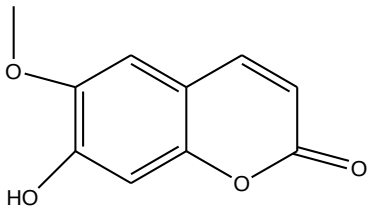
[**38**]



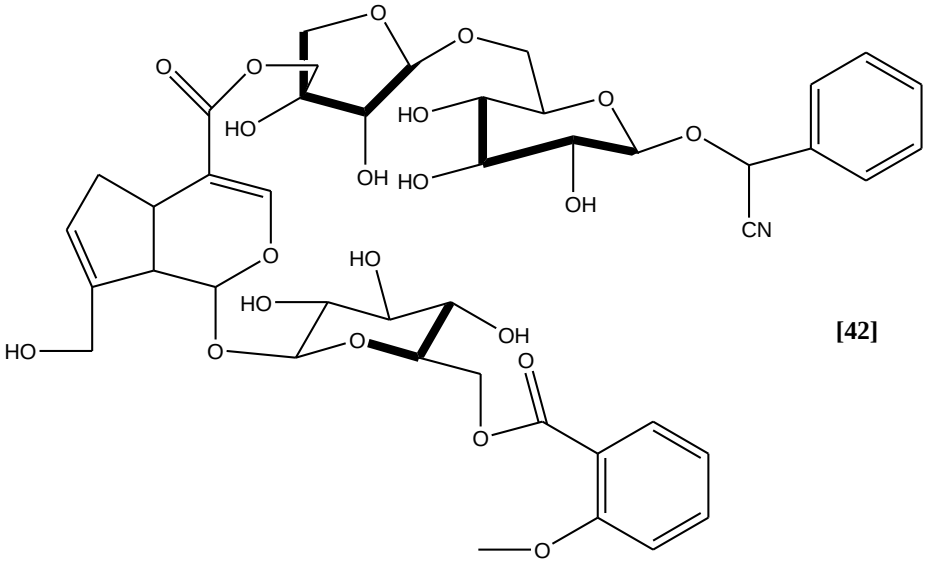
[**39**]



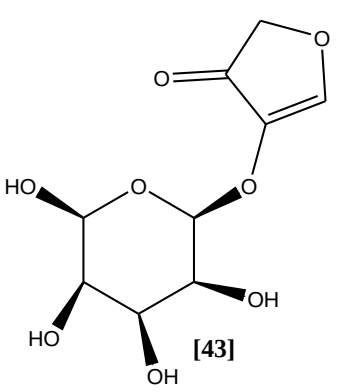
[40]



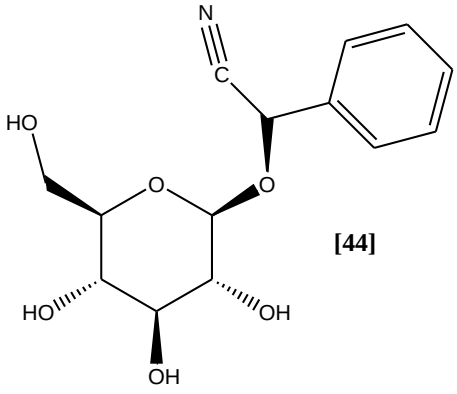
[41]



[42]



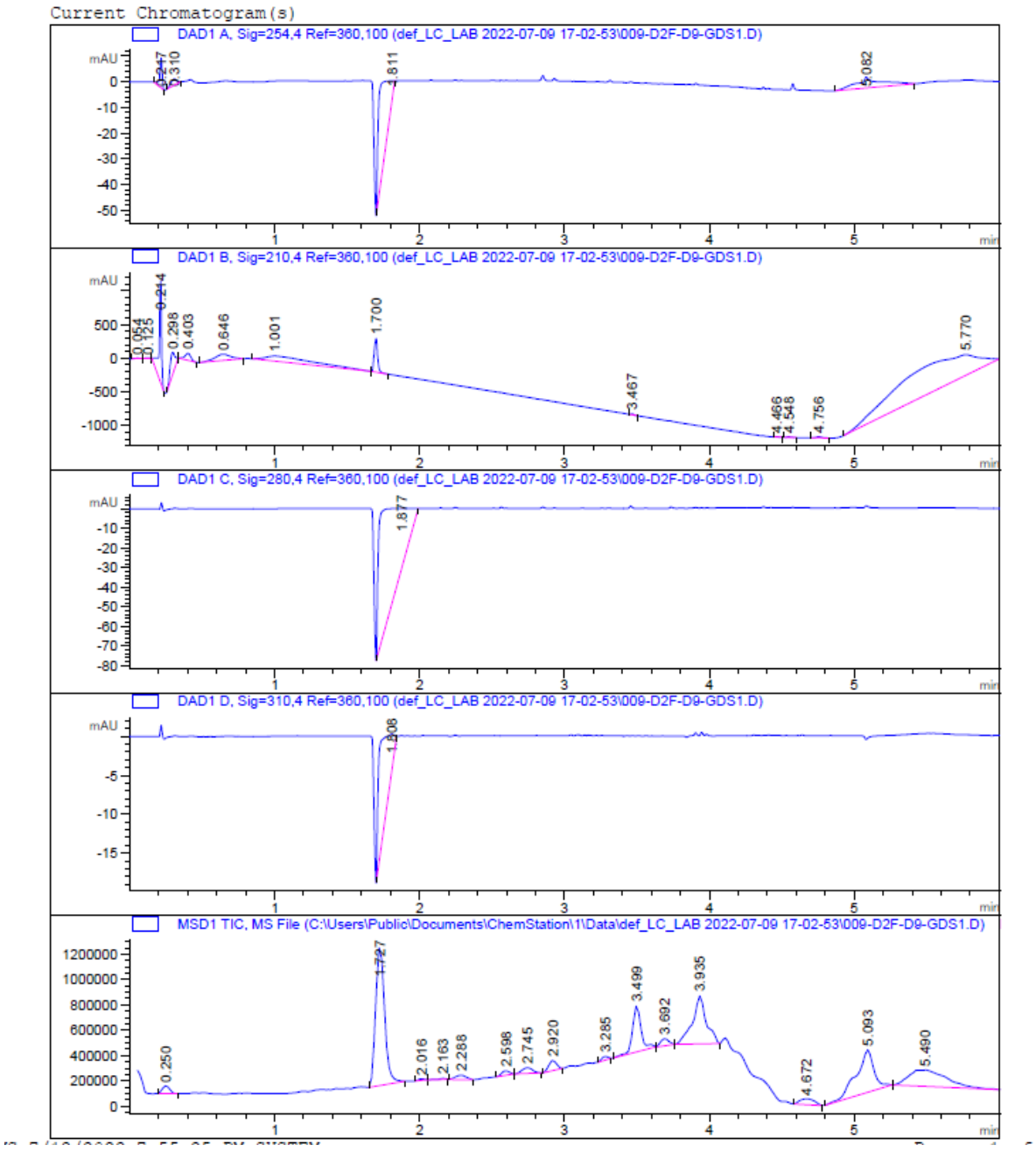
[43]



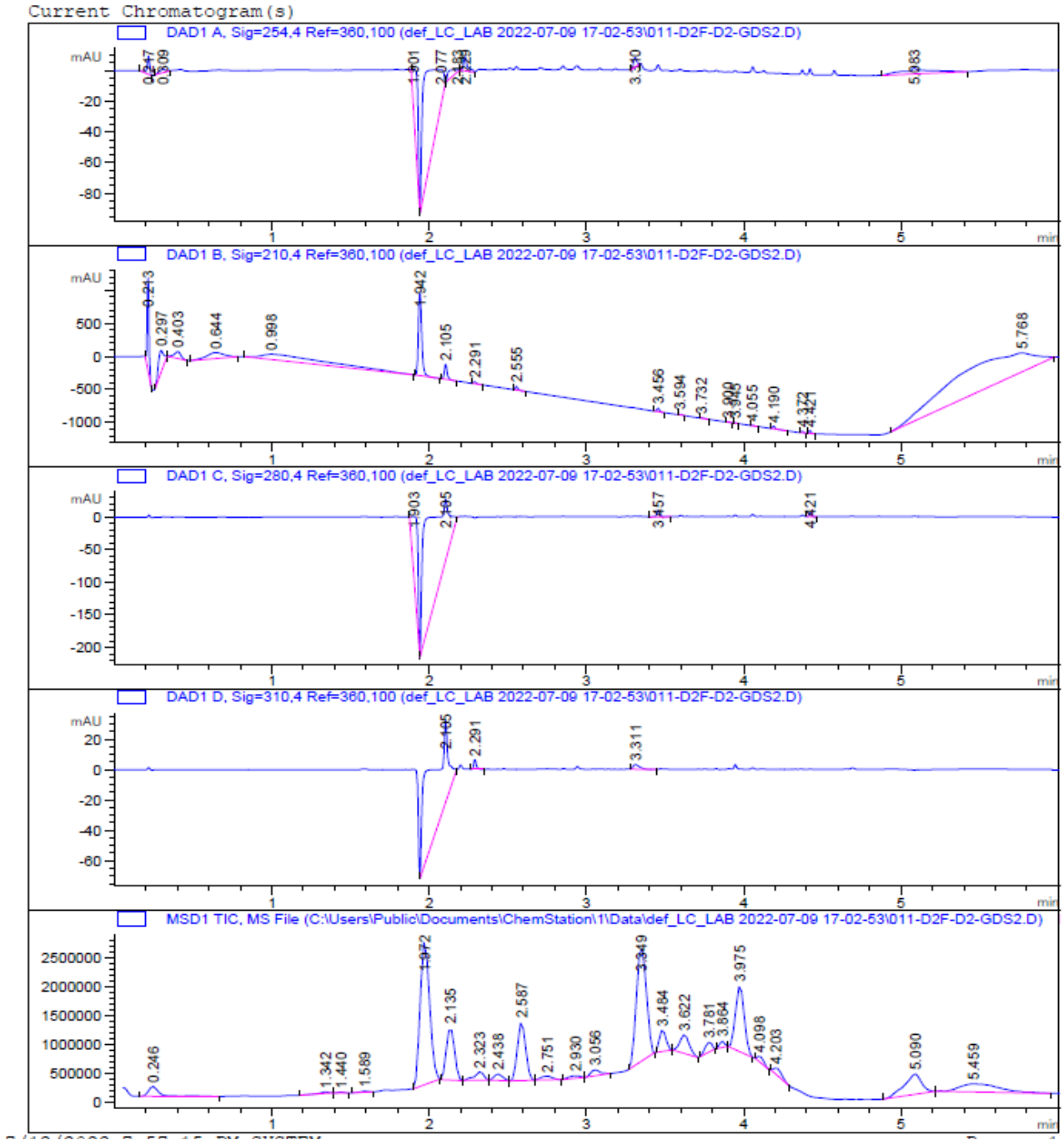
[44]

Appendix III: Liquid chromatography-Mass spectrometry (LC-MS) of GDS-1 and GDS-2

Appendix III$_a$: LC-MS Chromatogram of GDS-1 (Isoscopoletin)



Appendix III_b: LC-MS Chromatogram of GDS-2 (Scoparone)



አዲስ አበባ ዩኒቨርሲቲ
Addis Ababa University

በ ፋርማሲ ት/ቤት
የኢቲካል ሪቪው ኮምቴ

School of Pharmacy
Ethical Review Committee

ቀን Date: January 2, 2022

ቁጥር Ref. No.: ERB/SOP/457/15/2022

To: **Getahun Damissie**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled "**Evaluation of *in-vivo* antidiarrheal activity of 80% methanol extracts and compound isolated from the root of *Psydrax schimperiana* (A. Rich) Bridson in Swiss Albino Mice".** The committee thoroughly reviewed the proposal based on its operational guideline and found that, it fulfills all the ethical requirements stipulated in the guideline. This is, therefore, to inform you that the proposal is ethically approved for implementation.

With best regards,

Shemsu Umer (PhD)
Chairperson, ERC
School of Pharmacy
College of Health Sciences
Addis Ababa University

00251156 02 12 | 1176 | ቴሌክስ Telex: 21205 | ፋክስ Fax: 00251(11)1558566 | ቴሌግራም Cable: AAUNIV

Appendix IV: Scanned Letter for Ethical Clearance

Appendix V: Some photographs during laboratory works