Antidiarrheal Activity of the 80% Methanol Root Extract of *Thalictrum ryhnchocarpum* Dill. & A. Rich and its major constituent Against Castor Oil-Induced Diarrhea in Mice

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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

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This is to certify that the thesis prepared by Kebede Feyisa, entitled: **"Antidiarrheal Activity of the 80% Methanol Root Extract of *Thalictrum ryhnchocarpum* Dill. & A. Rich and Its Major Constituent Against Castor Oil-Induced Diarrhea in Mice"** and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Pharmacognosy) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Antidiarrheal Activity of the 80% Methanol Root Extract of *Thalictrum ryhnchocarpum* Dill. & A. Rich and Its Major Constituent Against Castor Oil-Induced Diarrhea in Mice.

Kebede Feyisa

Addis Ababa University, 2019

Diarrhea is one of the main causes of infant mortality in Ethiopia and in many other developing countries, causing about 4 to 8 million deaths annually worldwide. Despite recent reductions in morbidity and mortality worldwide, diarrhea still remains one of the leading causes of death in developing countries. Like many other developing countries, people in Ethiopia strongly rely on the therapeutic benefits of traditional medicine to treat diarrhea. One such plant is *Thalictrum ryhnchocarpum* Dill. & A. Rich. Thus, in the present study, the 80% methanol extract of the root of *T. ryhnchocarpum* and the compound isolated thereof have been evaluated for their antidiarrheal activity. Phytochemical investigation of the 80% methanol root extract of *T. ryhnchocarpum* by preparative TLC over silica gel resulted in the isolation of one major alkaloid unequivocally identified as berberine using various spectroscopic techniques, including MS, ^{1}H, ^{13}C-NMR and DEPT spectral data.

Both the total extract and berberine were tested for their *in vivo* antidiarrheal activity on three models namely, castor oil-induced diarrhea, charcoal meal and enteropooling models in mice. It was shown that both the extract and berberine possess significant antidiarrheal activity in a dose-dependent manner on all the tested models. Particularly, the root extract showed the maximum activity on castor oil-induced diarrhea model, with 71.4%, 74.0% and 78.0 % ($P < 0.01$) and berberine displayed even higher activity with 81.0%, 87.6 % and 88.3% at tested concentration of 100, 200 and 400 mg/kg, respectively. Antidiarrheal activity of the root extract and berberine was also noted on the other two models. When administered up to a dose of 2000 mg/kg, neither the root extract nor berberine showed acute toxicity in Swiss albino mice. In view of the present results, it can be concluded that the root extract of *T. ryhnchocarpum* and berberine could serve for the treatment of diarrhea thereby supporting the traditional claim of the plant against diarrhea.

Keywords: *Thalictrum ryhnchocarpum,* Acute toxicity, Antidiarrheal activity, Berberine, Castor oil-induced diarrhea, Anti-enteropooling, Gastro-intestinal transit.

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List of abbreviations and Acronyms

^{13}C NMR	Carbon thirteen Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
ABTS	2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)
ADI	Antidiarrheal Index
ANOVA	Analysis of Variances
DEPT	Distortional Enhancement by Polarization Transfer
DMSO	Dimethyl Sulfoxide
DPPH	2, 2 –diphenyl-1-picrylhydrazyl
FOP	Fecal Output
GIT	Gastro Intestinal Tract
IBD	Inflammatory Bowel Disease
LD50	Median Lethal Dose
MRETR	80 % Methanol Root Extract of *Thalictrum ryhnchocarpum*
MIC	Minimum Inhibitory Concentration
MTT	3-(4, 5) – dimethylthiazoyl-3, 5- diphenyl tetrazolium bromide
MVSIC	Mean Volume of Small Intestine Contents
MWSIC	Mean Weight of Small Intestine Contents
NO	Nitric Oxide
NSAID	Non-Steroidal Anti-inflammatory Drugs
OECD	Organization of Economic Co-operation and Development
R*f*	Retention Factor
SEM	Standard Error of the Mean
SPSS	Statistical Package for Social Science
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TOF MS	Time of flight Mass Spectrometer
TR	*Thalictrum ryhnchocarpum*
UNICEF	United Nation International Children's Emergency Fund
WHO	World Health Organization

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1. INTRODUCTION

1.1 Background

Diarrhea is a common condition frequently seen in clinical practice. Strict definition of diarrhea is difficult due to the individual differences in defecating habits, but it is generally defined as: frequent defecation more than 3 times a day, loose and formless stool, or an abnormal increase in the total amount and water content of stool (Elisha *et al.*, 2013; Almeida and Kamath, 2017). However, it is the consistency of the stools rather than the number is most important (WHO, 2005).

Diarrhea is the second leading cause of mortality among children under five years of age next to respiratory infections and kills more young children than AIDS, malaria, and measles combined, causing about 4 to 8 million deaths annually (Balekar *et al.,* 2012; Adeniyi *et al.,* 2014; Zaman *et al.,* 2015; Tenório *et al.,* 2016). Ethiopia's pneumonia and diarrhea mortality rate are the 5th highest in the world next to India, Nigeria, Pakistan and the Democratic Republic of Congo (Tadesse *et al.,* 2017). Diarrhea is the 2nd leading cause of death across all ages next to lower respiratory infections and the two-week prevalence of diarrhea among children under five years of age was 13% in Ethiopia (Tadesse *et al*., 2017).

Diarrhea can include gastrointestinal complications such as fever, abdominal pain, or vomiting. It is related to an imbalance in the regulation of absorption and secretion in the intestine accompanied by hypermotility resulting in the excess loss of body fluids and electrolytes in feces (Mo and Ou, 2010; Sharma *et al.,* 2015) and its causes are diverse. It is a leading cause of malnutrition and death among children in the developing countries of the world today (Karthik *et al.,* 2011; Begum *et al.,* 2013).

Based on the duration of diarrheal episodes, diarrhea could be classified as acute, persistent, and chronic diarrhea. Acute diarrhea is defined as an abnormally frequent discharge of semisolid or fluid fecal matter from the bowel lasting less than 14 days, while persistent diarrhea is an episode of diarrhea, with or without blood that lasts at least for 14-30 days. However, diarrhea is termed as chronic when it lasts more than 30 days (Riddle *et al.,* 2016). Acute infectious diarrhea is generally associated with other clinical features suggesting enteric involvement including abdominal pain and cramps, bloating, and fecal urgency (Riddle *et al.,* 2016) whereas, chronic

diarrhea is most likely due to inflammatory bowel disease (IBD). These include ulcerative colitis or Crohn's disease.

The common reason for causing diarrhea is gastrointestinal infection by various types of bacteria such as *Campylobacter*, *Escherichia coli*, *Salmonella, Shigella* and *Vibrio cholerae* (Tenório *et al.*, 2016), viruses like *Cytomegalovirus, Norovirus*, *Rotavirus* and fungi such as *Candida albicans* (Kotloff *et al.*, 2013). This infection can be spread out through food, drinking water, and unhygienic environment (Maniyar *et al.*, 2010; Pérez-Gutiérrez *et al.*, 2013).

Despite the multiplicity of etiologies, the four major mechanisms responsible for the pathophysiology in water and electrolytes transport are increased luminal osmolarity (osmotic diarrhea), increased electrolytes secretion (secretory diarrhea), decreased electrolytes absorption, and deranged intestinal motility causing a decreased transit time (Maniyar *et al.*, 2010; Umer *et al.*, 2013; Rahman *et al.*, 2015).

To control diarrhea disease, different types of treatments are carried out such as: fluid and electrolytes (oral rehydration solution (ORS)) (Munos *et al.*, 2010), zinc supplement (Nichter *et al.*, 2008), probiotics (non-pathogenic organisms) and prebiotics (oligosaccharides) (Anand *et al.*, 2016), antisecretory agents such as loperamide, diphenoxylate and bismuth subsalicylate which act by stimulating absorption and reducing secretion of water in gastrointestinal tract (Casburn-Jones and Farthing, 2004), antiperistaltics agents (loperamide and diphenoxylate) (Thiagarajah *et al.*, 2015) and antimicrobial agents (Kotloff, 2017).

During the last two decades, much attention has been paid to the health-promoting effects of edible medicinal plants, because of multiple beneficial effects and negligible adverse effects. Therefore, recent research has focused on the beneficial role of medicinal plants in order to ascertain effective and safe therapeutic strategies for the treatment of human diseases (Nabavi *et al.*, 2016).

Like in other developing countries, a large percentage of the population in Ethiopia depends on therapeutic benefit of traditional medicine (Birru *et al.*, 2016). Several ethnobotanical survey studies conducted in Ethiopia indicate that there are a number of plants which are claimed to have antidiarrheal activity (Enyew *et al.*, 2014; Lulekal *et al.*, 2014; Woldeab *et al.*, 2018). However, many of these plants have not been scientifically investigated to confirm their claimed applications.

1.2 Medicinal plants for the treatment of diarrhea

Medicinal plants play an important role in human life for therapeutic purposes and popularized worldwide due to great contribution by traditional practitioners (WHO, 2003). In developing countries, up to 80% of populations depend on plants for their primary healthcare (Sasidharan *et al*., 2011; Pérez-Gutiérrez *et al.*, 2013) and the value of medicinal plants to human livelihoods is essentially infinite. Medicinal plants have source for the invention of novel drugs and 25% of modern drugs contain one or more active principles of plant origin (Enyew *et al.,* 2014).

As shown in Appendix I, there are numerous reports in the literature concerning the folklore use of medicinal plants as antidiarrheal agents; examples include *Bersama abyssinica* Fresen. (Melianthaceae), *Calpurnia aurea* (Lam)Benth. (Fabaceae), *Rumex nepalensis* Spreng. (Polygonaceae), (Lulekal *et al.,* 2014). *Allamon daneriifolia* (Apocynaceae), *Bruguiera cylindrical* (Rhizophoraceae), *Crinum latifolium* (Amaryllidaceae) (Ashrafuzzaman *et al.,* 2016).

1.3 Ethiopian medicinal plants used for the treatment diarrhea

Ethiopia is a home of many languages, cultures and believes which in turn have contributed to the high diversity of traditional knowledge and practices of the people which, among others, include the use of medicinal plants. Traditional medicine still remains the main resource for a large majority (80%) of the people in Ethiopia for treating health problems (Giday *et al.,* 2010) and a traditional medical consultancy including the consumption of medicinal plants has a much lower cost than modern medical attention (Teklehaymanot and Giday, 2007).

Several studies have shown that Ethiopian medicinal plants possess genuine antidiarrheal activity. These include *Calpurnia aurea* (Ait.) Benth. (Umer *et al*., 2013), *Salvia schimperi* and *Zehneri ascabra* (Linni. F) Sond (Tadesse *et al*., 2014), *Discopodium penninervum* (Hochst.) (Derebe *et al*., 2018)**,** *Croton macrostachyus* Hochst.ex Delile (Degu *et al*., 2016) and *Lantana camara* Linn (Tadesse *et al*., 2017).

1.4 The Genus *Thalictrum*

The genus *Thalictrum* belongs to the family Ranunculaceae and is commonly known as "meadow rue" (Erdemgil *et al.,* 2003). Members of the genus are perennial herbaceous plants distributed in the temperate and tropical regions of the world including China, India, Japan, and Russia (Erdemgil *et al.,* 2003, Bajpai *et al.*, 2017). The genus contains above 120 species

(Khamidullina *et al.,* 2006) of which *Thalictrum minus* L; (Lesser meadow rue) is an important species distributed in many parts of the world (Mushtaq *et al.,* 2016).

1.4.1 Ethnomedicinal uses

Several *Thalictrum* species are used traditionally as stomachic, purgative, diuretic, tonic, bitter, aperient, and for the treatment of snake bite, jaundice, rheumatism and as an antiseptic (Kupchan and Chakravarti, 1963; Erdemgil *et al.,* 2003; Gurunathan *et al.,* 2013; Jiang *et al.,* 2013). They are also claimed to have antitumor, antimicrobial, antituberculosis, antimalarial and anti-inflammatory activities and also used in veterinary medicine (Mushtaq *et al.,* 2016; Bajpai *et al.,* 2017; Yan *et al.,* 2018).

All parts of the plants are used in traditional medicine for a variety of purposes in the regions where they grow. Plant parts including shoots, leaves, aerial parts stem and root barks and fruits have been used traditionally to cure various ailments (Chen *et al.,* 2003; Khamidullina *et al.,* 2006; Li *et al.,* 2016).

1.4.2 Phytochemistry

The chemicals and bioactive components of the different parts of genus *Thalictrum* have been investigated. Phytochemical screening studies revealed that, the presence of diverse beneficial class of compounds such as alkaloids, cycloartane triterpene glycosides, triterpeniods, cyanogenic glycosides, oleanane glycosides, saponins, flavonoids, steroids and organic acids supporting the use of the genus for culinary and therapeutic purposes (Erdemgil *et al.,* 2003; Zhang *et al.,* 2011; Mayeku *et al.,* 2014; Li *et al*.,2016; Mushtaq *et al*., 2016; Bajpai *et al*., 2017; Meng *et al*., 2017; Yan *et al.,* 2018).

Alkaloids, mainly bisbenzylisoquinolines are the major constituents of the genus *Thalictrum*. These compounds showed a remarkable role in various pharmacological activities including antiallergic, anti-inflammatory, antirheumatic, antiseptic (Erdemgil *et al*., 2003; Gurunathan *et al.,* 2013; Zhang *et al.,* 2013), antitumor, antimicrobial, anti-tuberculosis and antimalarial as well as application in veterinary medicine (Mushtaq *et al.,* 2016; Bajpai *et al*., 2017; Yan *et al*., 2018).

The Phytochemistry of this genus has attracted much attention and to date more than 200 alkaloids almost all of the isoquinilone group have been reported from approximately 100

species of the genus (Erdemgil *et al.,* 2003; Gao *et al.,* 2005; Mayeku *et al.,* 2014; Kumar *et al*., 2016; Mushtaq *et al*., 2016; Bajpai *et al.,* 2017). The major classes of compounds which have been isolated from *Thalictrum* species are listed in Appendix II.

1.4.3 Biological and pharmacological activities

Perusal of literature reveals that several secondary metabolites that are found in *Thalictrum* species are endowed with diverse biological effects. Among these, the most biologically active constituents are alkaloids, triterpenoid glycosides, triterpene saponins, cycloartane triterpene glycosides and flavonoids (Erdemgil *et al*., 2003; Ropivia *et al*., 2010; Zhang *et al*., 2011; Gurunathan *et al.*, 2013; Mushtaq *et al.*, 2016).

These compounds were shown to exhibit antimicrobial, antitumor, antimalarial, anti-inflammatory, antiamebic, antiallergic, antiarrhythmic, antiseptic, and antiviral activities (Kupchan and Chakravarti, 1963; Chen *et al.,* 2003; Erdemgil *et al*., 2003; Gurunathan *et al.,* 2013; Mushtaq *et al.,* 2016; Bajpai *et al*., 2017; Jiang *et al.,* 2017; Yan *et al.,* 2018).

1.4.3.1 Antimicrobial activity

Extracts obtained from the genus *Thalictrum* were shown to possess antimicrobial properties both *in vitro* and *in vivo* (Gurunathan *et al*., 2013; Mayeku *et al*., 2013). Gurunathan *et al.* (2013) evaluated the antibacterial activity of the various alcoholic extracts of *T. javanicum* against *Bacillus subtillus, Proteus mirabilis* and *Streptococcus faecalis* and found out that they possess a potent activity. The activity of the extracts was attributed to high polarity of the methanol which might have dissolved a variety of phytochemicals like alkaloids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids (Gurunathan *et al.,* 2013).

1.4.3.2 Immunosuppressive activity

Recent pharmacological research has shown that some triterpene saponins from *Thalictrum* plants hold potential immunosuppressive activity. Acutiaporberinen (**5**) (Appendix II), a bisalkaloid derived from *T. acutifolium*, showed apoptosis-inducing activity for human non-small cell lung cancer (NSCLC) cell line, PLA-801 and a cultured highly metastatic human lung cancer cell line 95-D (Chen *et al*., 2003).

1.4.3.3 Antiparasitic activity

In vitro antileishmanial activity of northalrugosidine **(13),** a bisbenzyltetrahydroisoquinoline alkaloid isolated from *T. alpinum*,was studied against *L. donovani* promastigotes and showed strong potency at a concentration of 0.28 μM with highest selectivity (29.3-fold) (Naman *et al*., 2014). Northalrugosidine (**13**) was tested *in vivo* using a murine model of visceral leishmaniasis, resulting in the observation of a dose-dependent reduction of the parasitic burden in the liver and spleen without overt toxicity effects (Naman *et al*., 2014).

Ropivia *et al.* (2010) isolated alkaloids from *T. flavum* and were evaluated for their antiparasitic potential as well as their cytotoxicity activity. Tertiary isoquinolines from *T. flavum* roots, particularly bisbenzylisoquinolines, were found to be leishmanicidal against *Leishmania major*. The bisbenzylisoquinoline thaligosidine (**16**) and aporphine alkaloid preocoteine (**20**) were also found to possess antiplasmodial activity against malaria causing parasites but their activity was ten times less potent than the reference drug chloroquine (0.06 μg/ml) at a concentration of 1.2 and 0.5 μg/ml, respectively.

1.4.3.4 Cytotoxic effect

Cytotoxicity evaluation of northalrugosidine (**13**) isolated from *T. alpinum* was carried out against HT-29 human colon adenocarcinoma cells and showed promising cytotoxic activity at a concentration of 0.28 μM (Naman *et al*., 2014). Li *et al.* (2016) isolated two rare chlorine containing benzylisoquinoline alkaloids, thalfoliolosumines A (**46**) and B (**47**) from the whole plant of *T. foliolosum* and evaluated their antiproliferative effects by MTT assay against MCF-7, PC-3, and U937 cells, and trypan blue assay against HL-60 cells. The two compounds exhibited moderate *in vitro* antiproliferative activity against MCF-7, PC-3, and HL-60 cells, and good inhibitory effects against U937 cells with IC_{50} values of 7.50 and 6.97 μM, respectively.

1.4.3.5 Antioxidant activity

Karyagina *et al*. (2011) evaluated the antioxidant activity of *T. minus* in cell extracts and culture medium and was expressed as total polyphenol content in ferulic acid equivalents. In these systems (cell extracts and culture medium), the inhibition of lipid oxidation and diphenyl picrylhydrazine reduction (EC_{50} = 12 - 15 μg/ml) were reported.

1.5 *Thalictrum ryhnchocarpum* Dill. & A. Rich

Thalictrum ryhnchocarpum is locally known as Mararree (Afan Oromo) and Sire bizu (Amharic). It is one of the *Thalictrum* species grown in Ethiopia. The species is a wild plant found in North shoa (Salale) and West shoa, Oromia region in Ethiopia (Enyew *et al*., 2014). It is somewhat scrambling perennial herb, up to 4 m tall. The leaves are up to 40 cm long and divided 3-4 times into elliptic to ovate, and the leaflets are 3-lobes. The flowers are small, green to purplish in colour with no petals. The fruits are asymmetrically spindle-shaped with a long beaked apex, pendent on long hair-like pedicels.

T. ryhnchocarpum is used in herbal medicine for the treatment of several human health problems in many parts of Africa (Mayeku *et al*., 2014). Aqueous extracts of different parts of this species is used to accelerate wound healing and treat stomach ulcers, breast cancer, snake bites, dysentery, diarrhea, skin rashes and used to control *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Salmonella gallinarum*, *Staphylococcus aureus* and *Candida albicans* (Mayeku *et al.,* 2014).

In Central Ethiopia around Fiche district the root of *T. ryhnchocarpum* is used for the treatment of diarrhea and additionally the fresh root is used for treatment of Rh-factor by tying on the pregnant women's neck with other plant leaf such as *Achyranthes aspera*, *Cucumis ficifolius* and *Gomphocarpus purpurascens* (Megersa *et al.,* 2013; Enyew *et al*., 2014).

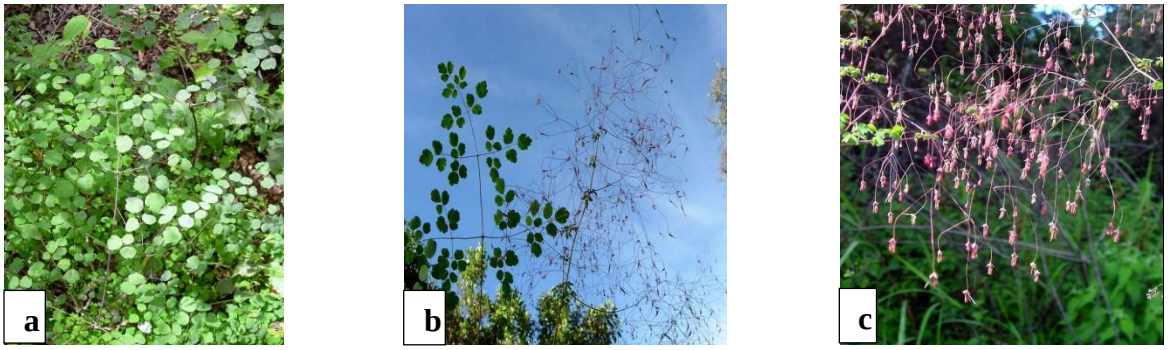

Figure 1: Leaves (a), Fruits (b) and Flowers (c) of *Thalictrum ryhnchocarpum* Dill. & A. Rich (Photograph taken by Kebede Feyisa around Gebre Guracha Town, Central Ethiopia, in September 2018).

1.6 Statement of the problem

Diarrhea is still one of the major causes of morbidity and mortality among children worldwide, particularly in sub-Saharan Africa (Umer *et al.*, 2013; Muhammad *et al*., 2016). Though, antibiotics are the indispensable remedy of infectious diarrhea, a significant surge of antibiotic resistance has been spreading globally and threatening our ability to combat common infectious diseases (WHO, 2014). According to Antimicrobial Resistance Global Report (WHO, 2014), some of diarrhea causing pathogens such as *E. coli*, *Neisseria gonorrhoeae*, and *Shigella* species, have developed resistance to the current drugs.

Even if the drugs are available for treating diarrhea, majority of the existing drugs suffer from adverse effects like the induction of bronchospasm, intestinal obstruction, constipation (loperamide), dependency (diphenoxylate), dry mouth and urinary retention (atropine) (Sagar *et al*., 2005). Therefore, these scenarios have forced researchers to search for new antidiarrheal substances, especially of medicinal plants origin. The roots and leaves of *Thalictrum* species are storehouses of many important secondary metabolites especially alkaloids (Erdemgil *et al.*, 2003; Mayeku *et al.*, 2013) which can be a possible source of new antidiarrheal drugs.

In view of the traditional use of the plant for treating diarrhea, the present study was undertaken to investigate extracts of the plant as well as its major component for their activity in experimentally induced diarrhea.

1.7 Significance of the study

The present study of the antidiarrheal activity of the root extract and its major constituent from *T. ryhnchocarpum* against the three models of diarrhea can provide important information on both the chemistry of the plant and its antidiarrheal activity. Thus, the research project's goal was designed to validate the traditional medicinal claim of the plant as antidiarrheal. Furthermore, the finding will lead and provide baseline information for those who are interested in natural product chemistry and antidiarrheal activity.

2. OBJECTIVES OF THE STUDY

2.1 General objective:

- The aim of the present study was to evaluate antidiarrheal activity of the 80% methanol root extract of *Thalictrum ryhnchocarpum* and its major constituent against experimentally induced diarrhea.

2.2 Specific objectives:

- To test the acute toxicity of the 80% methanol root extract of *T. ryhnchocarpum*;
- To isolate the major compound(s) of the extract using preparative thin-layer chromatography (PTLC);
- To characterize the isolated compound(s) by using spectroscopic techniques such as NMR and MS;
- To test the acute toxicity of the isolated compound(s) and
- To evaluate the *in vivo* antidiarrheal activity of the extract and isolated compound(s) against experimentally induced diarrhea using different models in mice.

3. MATERIALS AND METHODS

3.1 Study area

The study was carried out at facilities available at the Department of Pharmaceutical Chemistry and Pharmacognosy and Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia. NMR and MS data of the isolated compound were generated at the University of South Australia, Cancer Research Institute, Adelaide, SA 5000, Australia.

3.2 Materials

3.2.1 Chemicals, reagents and drugs

The chemicals and reagents used to perform the experiments were the following: castor oil (Amman Pharmaceutical Industries, Jordan), loperamide (Daehwa Pharmaceuticals, Republic of Korea), activated charcoal (Acuro Organics Ltd, New Delhi), silica gel for preparative thin layer chromatography GF_{254} (UNI-CHEM$^{(R)}$, India), Chloroform (Finkem Laboratory Reagent, India), methanol (Reagent Chemical Limited, UK), and ethyl acetate (Research-Lab-Fine, India). All chemicals were analytical grade and obtained from Pharmaceutical Fund and Agency and from the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University, Ethiopia.

3.2.2 Instruments

The following instruments were used for the experiment: Rota vapor (Buchi Rota Vapor R-200, Switzerland), UV spectrophotometer (Shimadzu Spectrophotometer MultiSpec-1501, Japan), nuclear magnetic resonance spectroscopy (Bruker Avance DMX 400, Germany), freeze dryer (JIANGSUZHENGJI, China), and high resolution mass spectrometer (AB SCIEX Triple TOF 5600 mass spectrometer, Concord, ON, Canada).

3.2.3 Plant material

The fresh root of *T. ryhnchocarpum* was collected from Gebre Guracha, North Shewa Zone of Oromia Region, about 156 km north of Addis Ababa in April 2018. The plant was identified and authenticated by a taxonomist and a voucher specimen (collection number KF-001) was

deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University. After collection, the roots were cut into pieces and gently washed using distilled water to remove dust materials. The cleaned roots pieces were dried under shade for 14 days to prevent the effect of the sun and finally ground into powder using a mechanical grinder.

3.2.4 Experimental animals

Healthy Swiss albino mice of either sex, weighing 20–35 g and aged 6–8 weeks were used for the experiments. Animals were obtained from the animal center of the Department Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University. The animals were housed in plastic cages at room temperature and on a 12 h light/dark cycle with free access to pellet food and water, and acclimatized for one week before the commencement of the experiment (Umer *et al.,* 2013). The care and handling were according to international guidelines for the use and maintenance of experimental animals (NRC, 2011).

3.3 Methods

3.3.1 Preparation of plant extract

Cold maceration technique was used for extraction of the plant material. Powdered root (400 g) was soaked in 80% methanol (1 L) in an Erlenmeyer flask with occasional shaking for 72 h at room temperature. The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding fresh solvent. The filtrates from each extraction were combined and methanol removed using rotary evaporator (BUCHI Rotavapor R-200, Switzerland) under reduced pressure at a temperature not exceeding 40℃. The remaining aqueous solution was then left overnight in a deep freezer and lyophilized using a freeze dryer (JIANGSUZHENGJI, China). The dried brownish powder was then stored in a refrigerator for future use.

3.3.2 Chromatographic techniques

Normal phase analytical thin layer chromatography (TLC) was performed using silica gel 60 F254 pre-coated plates (0.20 mm) (E-Merck, Darmstadt). Preparative TLC (PTLC) were prepared using glass plate measuring 20 cm x 20 cm in which a slurry of silica gel GF254 (UNI-CHEM$^{(R)}$, India) suspended in distilled water (1:2 w/v) was spread over the glass plates to obtain

0.5 mm thick layers. The plates were activated for 1 h at 105 ℃ and allowed to cool to room temperature and humidity before use. Chromatograms were developed using a mixture of chloroform and methanol (6:1 v/v).

3.3.3 Solvent system

The solvent system used for both analytical TLC and PTLC was a mixture of chloroform and methanol in the ratio of 6 to 1.

3.3.4 Visualization

The chromatographic zones were visualized in daylight and then under ultraviolet (UV) light of wavelengths 254 and 366 nm.

3.3.5 Isolation of compounds

Compounds were isolated by dissolving the extract in methanol and applied directly to PTLC plates over silica gel of 0.5 mm thickness. After development, two major bands designated TR_1 and TR_2 based on ascending order of R_f values, were scrapped off separately. The compounds were recovered first by washing the adsorbent with a mixture of methanol and ethyl acetate in a ratio of 1:1, followed by filtration and evaporation of the organic solvents. The isolated compounds were further purified by repeated PTLC using of 0.25 mm thick plates.

3.3.6 Spectroscopic techniques

3.3.6.1 NMR and MS

NMR spectra were recorded on a Bruker Avance DMX 400 FT-NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature using dimethyl sulfoxide (DMSO) as a solvent. A region from 0 to 12 ppm for ^{1}H and 0 to 205 ppm for ^{13}C was employed for scanning. Signals were referred to as internal standard tetramethylsilane (TMS). Chemical shifts are reported in δ units and coupling constants (J) in Hz. Multiplicities of ^{1}H NMR signals are indicated as *s* (singlet), *d* (doublet), and *t* (triplet). Masses of the isolated compounds were analyzed by the positive-ion mode time of flight mass spectrum (+mode-TOF-MS). High-resolution mass spectra were obtained from an AB SCIEX Triple TOF 5600 mass spectrometer (Concord, ON, Canada) equipped with ESI (electro spray ionization).

3.4 Acute oral toxicity test

Acute oral toxicity study was carried out using the limit test recommendations of OECD 425 Guideline (OECD, 2008). Ten healthy adult female Swiss albino mice were selected and assigned into two groups, comprised of five animals per group and weighing between 25 to 30 g. First, one mouse was fasted (with free access to water) for 3 h and then loaded with 2000 mg/kg of the test substance orally. The mouse was then fasted for further 1 h and strictly observed for the general signs and symptoms of toxicity, food, water intake and mortality within 24 h. Since no death was observed within 24 h, additional four mice were fasted for 3 h and administered the same dose of the test substance followed by 1 h fasting. The animals were observed continuously for 4 h with 30 min interval and then for 14 consecutive days with an interval of 24 h for the general signs and symptoms of toxicity or gross behavioral changes, food and water intake, tremors, convulsion, salivation, diarrhea, sleep, coma and mortality.

3.5 Experimental design

3.5.1 Grouping and dosing

In all models, animals were randomly divided into eight groups (negative control, positive control and six test groups) comprising of five animals in each group. Negative controls received vehicle (10 ml/kg, distilled water) and positive controls received loperamide (3 mg/kg) in all models. The test groups (group 3, 4 and 5) received different doses (100, 200 and 400 mg/kg, respectively) of the extract and (group 6, 7 and 8) received (100, 200 and 400 mg/kg, respectively) of isolated compound orally which were determined based on the acute oral toxicity test.

3.6 Determination of antidiarrheal activity

3.6.1 Castor oil-induced diarrhea

The method described by Umer *et al.* (2013) was followed for this study. Swiss albino mice of either sex were fasted for 18 h with free access to water and randomly allocated to five groups of five animals each. Vehicle treated group received distilled water (10 ml/kg) (negative control); Group 2 (positive control) received the standard drug loperamide 3 mg/kg, orally. Animals in groups 3, 4 and 5 received the extract or isolated compound at doses of 100, 200 and 400 mg/kg by mouth, respectively. One hour after treatment, diarrhea was induced by oral administration of

0.5 ml castor oil to each mouse. The animals were then housed in a separate transparent cage in which the floor is lined with white paper. The paper was changed every hour for a total of four hours. During the observational period, the onset of diarrhea, number and weight of wet stools, total number and the total weight of fecal output were recorded. Finally, the percentage of fecal output (% FOP) and diarrheal inhibition (% inhibition of defecation) were calculated by using the formulas described below.

$$\%\ \text{of fecal output} = \frac{\text{Mean faecal weight of each treatment group}}{\text{Mean faecal weight of negative control group}} \text{X } 100$$

$$\%\ \text{inhibition of defecation} = \frac{\text{Mo} - \text{M}}{\text{Mo}} \times 100$$

Where, Mo is mean defecation of negative control and M stands for mean defecation of test sample/standard drug.

3.6.2 Charcoal meal (gastrointestinal motility) test

Mice were fasted for 18 h with free access to water and grouped and treated as described under grouping and dosing section. One hour after treatment each mouse received 0.5 ml of castor oil for castor oil-induced intestinal motility test. One hour after castor oil administration, all mice received 1 ml of 5% activated charcoal suspension. The animals were then sacrificed by cervical dislocation after 30 min of administering activated charcoal and the entire length of the intestine (from the pylorus to the cecum) was removed and placed lengthwise on a white paper. The distance travelled by the charcoal meal and the total length of the intestine was then measured. The peristaltic index and percentage of inhibition were calculated by using the following formula (Meite *et al.*, 2009).

$$\text{Peristaltic index(PI)} = \frac{\text{Mean distance travelled by charcoal meal}}{\text{Mean length of small intestine}} X100$$

$$\%\ \text{Inhibition} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100$$

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

3.6.3 Anti-enteropooling test

Intraluminal fluid accumulation was determined using the method described by Rouf *et al.* (2003). Animals were fasted for 18 h and grouped and treated, as described under grouping and dosing section, 1 h before oral administration of castor oil (0.5 ml/mouse). One hour after castor oil administration, the mice were sacrificed by cervical dislocation. The abdomen of each mouse was open and the whole length of the intestine, from the pylorus to the caecum, was ligated, dissected and carefully removed. The small intestines were weighed and the intestinal contents were collected by milking into a graduated tube to measure the volume. The empty intestines were reweighed and the difference between the two weights was calculated. Finally, the percentage of reduction of intestinal secretion and weight of intestinal contents was determined by using the following formulas (Rouf *et al.*, 2003).

$$\%\ \text{inhibition by using MVSIC} = \frac{\text{MVICC} - \text{MVICT}}{\text{MVICC}} \times 100$$

Where, MVICC is the mean volume of the intestinal content of the negative control group and MVICT is the mean volume of the intestinal content of the test group.

$$\%\ \text{inhibition by using MWSIC} = \frac{\text{C} - \text{T/D}}{\text{C}} \times 100$$

Where C is the mean weight of intestinal content of the control and T is the mean weight of intestine content of the test/drug group.

3.6.4 *In vivo* antidiarrheal index

In vivo antidiarrheal index (ADI) of the positive control and test substance treated groups was determined using the different data obtained from the above tests using the formula developed by Than *et al.* (1989).

$$\text{ADI } in\ vivo = \sqrt[3]{\text{D}}\text{freq x Gmeq x Pfreq}$$

Where Dfreq is the delay in defecation time or diarrhea onset obtained from castor oil induced diarrheal test which is:

$$\text{Dfreq} = \frac{\text{Mean onset of diarrhea in the test group} - \text{Mean onset of diarrhea in the control group}}{\text{Mean onset of diarrhea in the control group}}\ \text{X } 100$$

Gmeq is the gut meal travel reduction (as % of control) obtained from charcoal meal test (% inhibition), and Pfreq is the purging frequency or reduction in the number of wet stools (as % of control) obtained from castor oil diarrheal model (% inhibition of defecation).

3.7 Data analysis

All data were entered and analyzed using SPSS (statistical Package for Social Science) software version 20.0. One-way analysis of variance (ANOVA) was done to determine statistical differences among all groups of the study. Pairwise comparisons were conducted by Tukey post hoc multiple comparison tests. The results of the data were presented as mean ± standard error of the mean (SEM). The *p*-values < 0.05 were considered statistically significant.

3.8 Ethical approval

Ethical approval will be obtained from the Scientific and Ethics Committee of the Department of pharmaceutical chemistry and Pharmacognosy, School of Pharmacy, Addis Ababa University. All experimental activities were carried out in accordance with recommendations from the declaration of nationally and internationally conventional standards for the employment of experimental animals, and code of ethics of animal experiments, which comply with scientific and ethical guidelines.

4. RESULTS AND DISCUSSION

4.1 Antidiarrheal activity tests of crude extract

4.1.1 Acute oral toxicity tests

Acute toxicity studies revealed that up to 2000 mg/kg, both 80% methanol root extract and the isolated compound (TR_1) from *T. ryhnchocarpum* did not cause any mortality in mice within the first 24 h as well as for the following 14 days, signifying that the oral LD_{50} is greater than 2000 mg/kg. Gross physical and behavioral observations of the experimental mice also showed no visible sign of overt toxicity like loss of appetite, tremors, hair erection, salivation and diarrhea. Thus, the observations that no death has occurred up to an oral dose of 2000 mg/kg indicate that the root extract is safe to mice and may explain the safety of the plant when used by the local people for the treatment of diarrhea.

4.1.2 Effects on castor oil-induced diarrhea in mice

In this study, antidiarrheal activity of the 80% methanol root extract of *T. ryhnchocarpum* was evaluated based on its effect against castor oil-induced diarrhea, gastrointestinal transit of charcoal meal and castor oil-induced enterpooling with reference to actions of loperamide which reduces gastrointestinal transit, fluid accumulation and secretory diarrhea.

Diarrhea may occur when there is a change in active ion transport by decreased sodium absorption or increased chloride secretion, change in intestinal motility, increase in luminal osmolarity; and/or increase in tissue hydrostatic pressure (Birru *et al.*, 2016). From all these mechanisms castor oil through its active compound ricinoleic acid induces diarrhea by stimulating secretory processes and intestinal motility secondary to irritation and inflammation that leads to the release of prostaglandin (Balekar *et al.,* 2012; Umer *et al.,* 2013; Birru *et al.,* 2016).

Castor oil is hydrolyzed in the upper small intestine to ricinoleic acid, which stimulates fluid secretion, inhibit water and electrolyte absorption, reduce active sodium and potassium absorption and decrease Na, K-ATPase in the small intestine and colon. Castor oil also increases the peristaltic activity and produces permeability changes in the intestinal mucosa membrane to electrolytes and water. Furthermore, ricinoleic acid can also lead to the release of endogenous

prostaglandins, which play an important role in the modulation of GIT, stimulate motility, secretion and cause diarrhea (Muhammad *et al.,* 2016).

Results of the present study showed that the 80% methanol root extract of *T. ryhnchocarpum* reduces castor oil-induced diarrhea as well as the number of diarrheal feces and total weight of feces, which could be taken as antidiarrheal activities. Loperamide is one of the most efficacious and widely used antidiarrheal drugs. Loperamide effectively antagonizes diarrhea induced by castor oil (Ay *et al.,* 2016). The therapeutic effect of loperamide is believed to be due to its anti-motility and anti-secretory activity. Loperamide is a synthetic opiate analog developed specifically for use in diarrhea. All opiate agonists have effects on intestinal smooth muscle. Loperamide regulates the gastrointestinal tract by inhibiting the propulsive motor activities, predominantly in the jejunum and this effect is partially inhibited by opiate antagonists. Other effects on intestinal motility may be mediated through inhibition of prostaglandin stimulation of gut motility and/or through calcium antagonist action (Ay *et al.,* 2016).

In folk medicine the aqueous root extract of *T. ryhnchocarpum* is used for treatment of diarrhea. However, in the present study 80% methanol was used because hydroalcoholic solvents (especially 80% methanol) are usually better and more efficient in extracting the most important bioactive constituents of plant materials due to their expanded polarity range. It was also confirmed that many compounds which are otherwise insoluble individually in pure methanol could be extracted quite easily with hydroalcoholic solvents due to co-solubility (Wojcikowski *et al.,* 2009).

As shown in Table 1, in the castor oil-induced diarrhea model, the 80% methanol root extract of *T. ryhnchocarpum* produced a significant effect on all parameters measured: onset of diarrhea, the number of wet and total stools and weight of wet stools in a dose-dependent manner. For example, within the 4 h period of post castor oil administration, all animals treated with 100, 200 and 400 mg/kg extract doses, the frequency of defecation and fluid contents of the feces were significantly different (71.4%, $p < 0.01$, 74.0%, $p < 0.01$, and 78.0%, $p < 0.01$, respectively) from mice exposed to distilled water only.

Table 1:- Effect of the 80% methanol root extract of *Thalictrum ryhnchocarpum* (MRETR) on castor oil-induced diarrhea in mice

Treatment	Dose (mg/kg, p.o)	Onset of diarrhea (min)	Total no of feces Output	Total no of wet feces	Total weight of feces (g)	Total weight of wet feces (g)	%Inhibition of defecation
Vehicle	-	43.4±1.54	17.2±1.77	15.4±1.63	1.67±0.27	1.58±0.25	-
Loperamide	3	101.6 ± 20.28^{a1c1d2e1}	4.2 ± 0.66^{a2c2d1}	2.0 ± 0.63^{a2}	0.43 ± 0.11^{a2c1d2}	0.29 ± 0.14^{a2}	87.0
MRETR	100	55.0 ± 3.99^{b1}	13.8 ± 0.49^{b2}	4.4 ± 0.40^{a2}	1.27 ± 0.08^{b1}	0.44 ± 0.06^{a2}	71.4
MRETR	200	87.2±13.81	11.8 ± 1.24^{b1}	4.0 ± 0.71^{a2}	1.71 ± 0.23^{b2}	0.84 ± 0.22^{a1}	74.0
MRETR	400	98.4 ± 8.71^{a1}	9.6 ± 2.29^{a1}	3.4 ± 0.60^{a2}	0.94 ± 0.20^{a1}	0.41 ± 0.14^{a2}	78.0

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data were analyzed by 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, e compared to 400 mg/kg of MRETR; 1p < 0.05, 2 p < 0.01, 3p < 0.001; Mice in the control group received distilled water (10 ml/kg).

Even though diarrhea has been defined over time by various scientific groups and organizations in different ways, greater emphasis is given on the consistency of stools than the frequency (WHO, 2005; Sisay *et al.,* 2017). Therefore, in the present work, determination of percentage inhibition was based on the reduction of frequency of wet fecal outputs which is a good marker of antidiarrheal activity. Percentage inhibition of frequency of defecation for the 400 mg/kg dose of the extract was 78.0 % ($p < 0.01$) which is close to the 87.3% achieved by the positive control (loperamide). Previous reports have also indicated that some plant extracts show antidiarrheal activity in a dose-dependent manner (Balekar *et al.,* 2012; Degu *et al.,* 2016; Sisay *et al.,* 2017; Tadesse *et al.,* 2017).

Antidiarrheal properties of medicinal plants have been attributed to the presence of diverse class of compounds such as alkaloids, terpenes, glycosides, saponins, flavonoids, steroids and organic acids (Erdemgil *et al.,* 2003; Mayeku *et al.,* 2014; Meng *et al.,* 2016; Mushtaq *et al.,* 2016). Non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to inhibit castor oil induced diarrhea apart from their inhibition of prostaglandin synthesis (Awouters *et al.,* 1978). Similarly, the 80% methanol root extract of *T. ryhnchocarpum*, which is rich in alkaloids, showed anti-inflammatory activities like those of NSAIDs (Erdemgil *et al.,* 2003; Gurunathan *et al.,* 2013; Zhang *et al.,* 2013). Therefore, it is possible that the hydro alcoholic extract of *T.*

ryhnchocarpum exerts its antidiarrheal activity by inhibiting castor oil-induced prostaglandin synthesis.

In addition, the antidiarrheal effect of the extract might be also due to the inhibition of active secretion of ricinoleic acid, resulting in the activation of Na^+, K^+ ATPase activity that promotes absorption of Na^+ and K^+ in the intestinal mucosa. This effect could probably be linked to the presence of terpenoids, tannins and flavonoids in the extract which have been shown to increase colonic absorption of water and electrolytes (Palombo and Wiley, 2006; Mayeku *et al.*, 2013).

As depicted in Figure 2, there was a reduction in the percentage of mean fecal output, with 400 mg/kg of crude extract displaying the maximum effect (56.3 %, $p < 0.05$) as compared with the negative control. As compared to the standard drug, the extract at doses of 100 and 200 mg/kg showed a good effect to lessen the percentage of mean fecal output. However, at a concentration of 200 mg/kg did not show significant inhibition of percentage of mean fecal output (102%) as compared to the negative control.

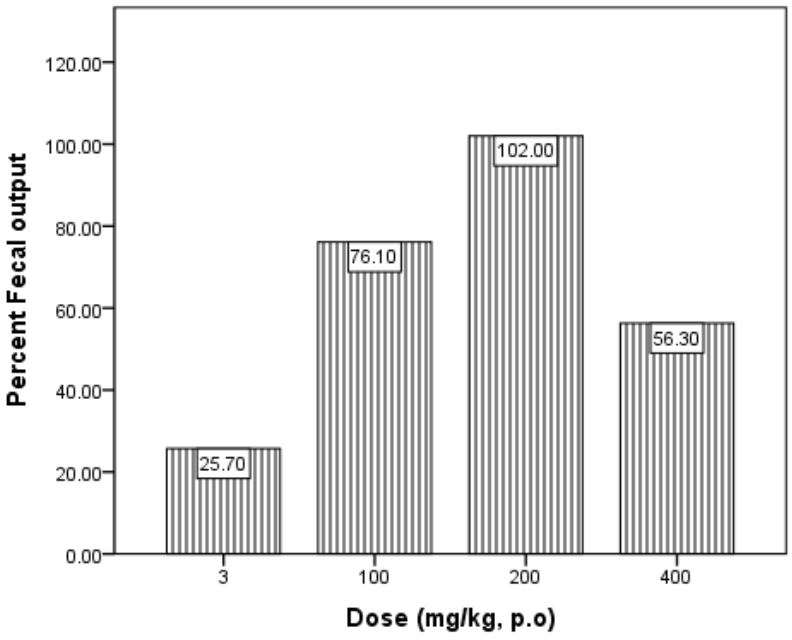

Figure 2: Percentage mean fecal output of the 80% methanol roots extract of *Thalictrum ryhnchocarpum* (MRETR) on castor oil-induced diarrheal model in mice

4.1.3 Effect on castor oil-induced gastrointestinal transit

The reduction of gastrointestinal motility is one of the mechanisms by which antidiarrheal agents can act. Studies made on activated charcoal showed that it prevents the absorption of drugs and chemicals into the system by adsorbing them on the surfaces of the charcoal particles. Thus, activated charcoal was used in the gastrointestinal motility test to find out the effects of the extracts on the peristaltic movement (Meite *et al*., 2009).

In the evaluation of castor oil-induced gastrointestinal transit model using charcoal meal, results showed that the hydroalcoholic root extract of *T. ryhnchocarpum* significantly reduced the intestinal propulsive movement of charcoal meal at 100 (34.1%, $p< 0.05$), 200 (66.7 %, $p< 0.01$) and 400 mg/kg (71.1%, $p < 0.05$) (Table 2) as compared to the control indicating the presence of an anti-motility activity.

Terpenoids and alkaloids have been reported to prolong the time for absorption of water and electrolytes by hampering the peristaltic movement of the intestine (Palombo and Wiley, 2006; Mayeku *et al.,* 2013; Zhang *et al.,* 2013). The presence of such secondary metabolites in the studied plant might therefore be the reason for the antidiarrheal effect due to their ability to inhibit intestinal motility. Furthermore, the significant antimotility effect of the extract could be due to the synergistic inhibitory effect of such secondary metabolites on castor oil-induced gastrointestinal motility (Degu *et al.,* 2016; Tadesse *et al.,* 2017).

Table 2: Effect of the 80% methanol root extract of *Thalictrum ryhnchocarpum* (MRETR) on castor oil-induced gastrointestinal transit in mice

Treatment	Dose (mg/kg, p.o)	Mean length of small intestinal (cm)	Mean distance traveled by charcoal (cm)	Peristalsis index (%)	Percent of inhibition
Vehicle	-	59.40±1.44	27.00±3.91	45.50	-
Loperamide	3	57.60±2.56	4.40±0.81 ^{a3c1}	7.64^{a2e2}	83.7
MRETR	100	56.40±1.63	17.80±2.13 ^{a1}	31.60^{a1}	34.1
MRETR	200	56.60±2.14	9.00±1.92 ^{a2}	16.00^{a2}	66.7
MRETR	400	57.00±2.38	7.80±3.79 ^{a2d2}	13.70^{a2d2}	71.1

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data was analyzed by 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, e compared to 400 mg/kg of MRETR; 1 $p < 0.05$, 2$p < 0.01$, 3$p < 0.001$; Mice in the control group received distilled water (10 ml/kg).

4.1.4 Effect on castor oil-induced enteropooling

The enteropooling model was aimed to assess the secretary components of diarrhea. In this model, the intestinal fluid secretion induced by castor oil was blocked by the test extract in a dose-related manner (Table 3). Maximum percentage inhibition of the volume of the intestinal contents was observed at 400 mg/kg (67.2 %, $p < 0.01$), but the weight of intestine contents was not reduced significantly even at a higher dose. There was no statistically significant difference in the volume of intestinal fluid and weight of intestine contents when each dose of the crude extract was compared with the standard drug.

It is known that castor oil induces alteration in intestinal electrolyte transport. Results of the present study suggest that the effects of *T. ryhnchocarpum* extract may be due to an increase in the absorption of electrolytes and/ or inhibition of hypermotility of the intestine, thereby increasing its capacity to retain fluids, an action similar to that of loperamide.

It has been reported that ricinoleic acid, the active metabolite of castor oil, might activate the nitric oxide pathway and induce nitric oxide (NO) dependent gut secretion (Mascolo *et al.*, 1994). Phytochemical constituents such as flavonoids and terpenoids are implicated in attenuation of NO synthesis (Jang *et al.*, 2004). The pronounced inhibition of castor oil-induced enteropooling exerted by the hydroalcoholic extract of *T. ryhnchocarpum* might possibly be

related to the presence of those constituents that increase the absorption of electrolytes and water by inhibiting castor oil mediated NO synthesis.

Table 3: Effect of the 80% methanol root extract of *Thalictrum ryhnchocarpum* (MRETR) on castor oil induced enteropooling in mice

Treatment	Dose (mg/kg, p.o)	MWSIC (g)	% inhibition by using MWSIC	MVSIC (ml)	% inhibition by using MVSIC
Vehicle	-	2.55±0.29	-	0.58±0 .06	-
Loperamide	3	1.84±0.11	27.8	0.18±0.04[a2]	68.9
MRETR	100	1.88 ±0.19	26.3	0.38±0.04	34.5
MRETR	200	1.82±0.21	28.6	0.26±0.07[a2]	55.2
MRETR	400	1.98±0.14	22.4	0.19±0.06[a2]	67.2

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data was analyzed by one-way ANOVA followed by Tukey post hoc test; a compared to the control, b compared to the standard drug, c compared to 100 mg/kg, d compared to 200 mg/kg, e compared to 400 mg/kg; 1 $p < 0.05$, 2 $p < 0.01$, 3$p < 0.001$; MWSIC: Mean weight of small intestinal content, MVSIC: Mean volume of small intestinal content; Mice in the control group received distilled water (10 ml/kg).

4.1.5 *In vivo* antidiarrheal index

The *in vivo* antidiarrheal index (ADI) was measured by considering the delay in defecation (time of onset, Dfreq), gut meal travel distance (Gmeq) and purging frequency in a number of wet stools (Pfre) as major parameters (Than *et al.*, 1989). The highest the ADI value, the more effective the extract is at curing diarrhea (Prasad *et al.*, 2014). The finding revealed that ADI increased in a dose-dependent manner and at the dose of 400 mg/kg of 80% methanol extract (88.91%) showed highest ADI value as compared to the other doses, reinforcing the notion that this dose is endowed with best antidiarrheal activity (Table 4).

Table 4: *In vivo* antidiarrheal indices (ADIs) of the 80% methanol root extract of *Thalictrum ryhnchocarpum* (MRETR).

Treatment	Dose (mg/kg, p.o)	Delay in defecation (min), Dfreq (%)	Gut meal travel distances (Gmeq) (%)	Purging frequency in number of wet feces (%)	*In vivo* antidiarrheal index (ADI)
Vehicle	-	-	-	-	-
Loperamide	3	134.1	60.7	87.0	89.13
MRETR	100	26.73	34.1	71.4	40.22
MRETR	200	100.92	66.7	74.0	79.26
MRETR	400	126.73	71.1	78.0	88.91

p.o: orally

4.2 Isolation of compounds

Two major compounds, with R_f values of 0.3 (TR_1) and 0.5 (TR_2) (Figure 3), were isolated from the 80% methanol root extract of *T. ryhnchocarpum* by repeated PTLC over silica gel using chloroform: methanol (6:1) as a solvent system. TR_1 showed characteristic bright yellow, dark brown and yellow colors, when viewed under daylight, UV light of 254 nm and UV 366 nm, respectively. TR_2 did not absorb visible light (daylight). When viewed under UV light of 254 and 366 nm, TR_2 appeared as dark and brown spot.

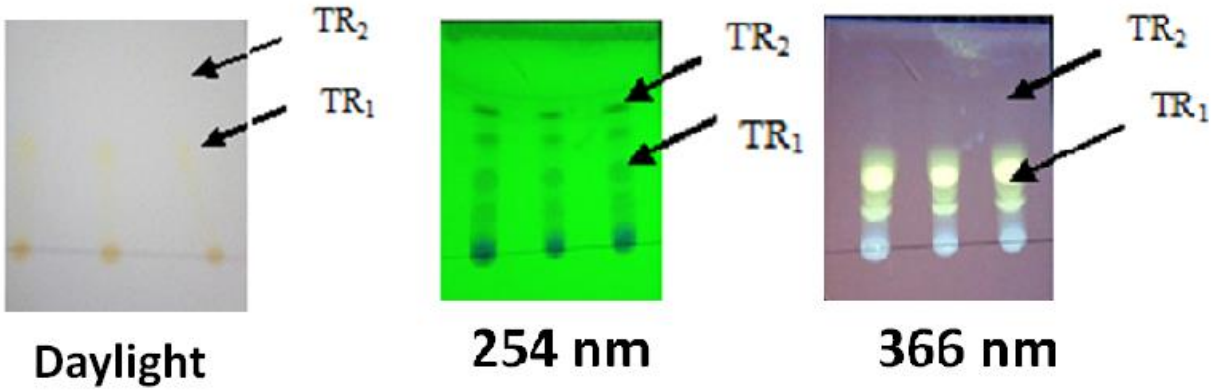

Figure 3: Silica gel TLC chromatograms of the 80% methanol root extract of *Thalictrum ryhnchocarpum* (MRETR) when viewed in daylight and under UV light of 254 and 366 nm (solvent system chloroform: methanol in a ratio of 6:1).

4.2.1 Characterization of the isolated compounds

TR_1 was fully characterized by using various spectroscopic techniques as indicated below. However, due to paucity of TR_2, it was not possible to characterize it.

4.2.2 TR_1

TR_1 was obtained as a yellow solid crystal (powder) with R*f* value of 0.3 in $CHCl_3$: CH_3OH (6:1). A molecular formula of $C_{20}H_{18}NO_4^+$ was deduced for TR_1 by a high-resolution Time of Flight mass spectrometry (+ve HR-TOF-MS) (obtained mass *m/z* 336.1471 $[M+H]^+$, calc. exact mass *m/z* 336.1236 $[M+H]^+$) (Figure 4).

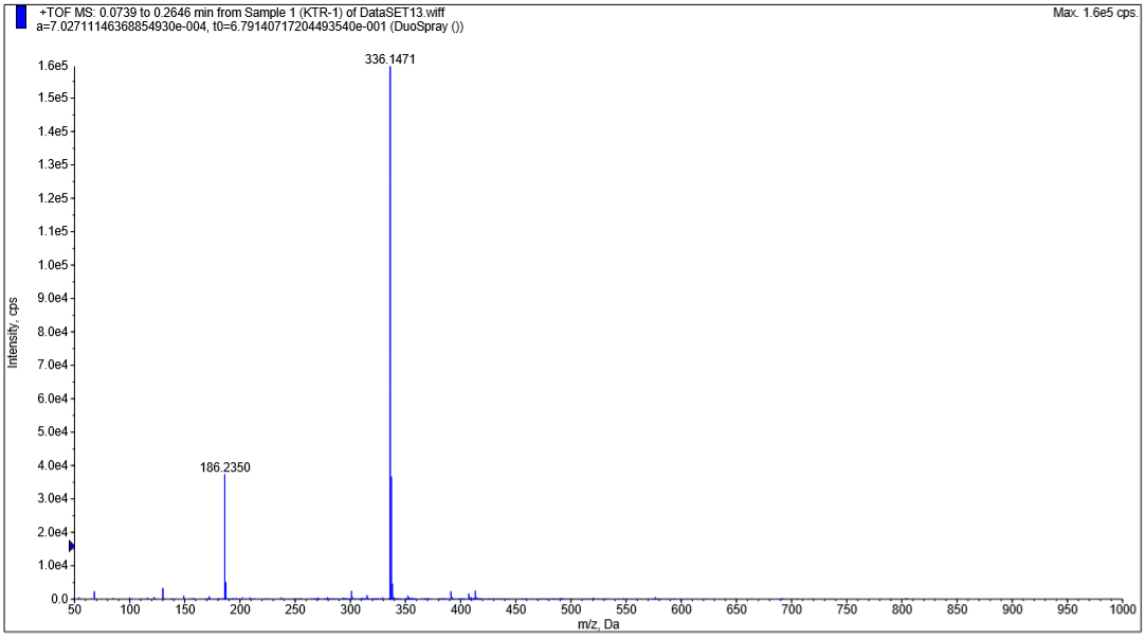

Figure 4: Positive mode time of flight mass spectrum (TOF-MS) of TR_1.

Analysis of ^{1}H-NMR spectrum of TR_1 revealed the presence of six aromatic protons assignable to δ 7.79 (1H, *s*, H-1), δ 7.08 (1H, *s*, H-4), δ 9.90 (1H, *s*, H-8), δ 8.20 (1H, *d*, *J* = 9.2Hz, H-11), δ 8.00 (1H, *d*, J = 9.2Hz, H-12), and δ 8.94 (1H, *s*, H-13). In addition, the presence of six protons in the two methoxy groups attached to C-9 and C-10 was supported by the ^{1}H NMR spectrum (Table 5), which showed two singlets at δ 4.11 (3H, *s*, 9-OCH_3) and δ 4.21 (3H, *s*, 10-OCH_3). It was also noted that one methylene (CH_2) protons signal of methylenedioxy group at δ 6.17 (2H, s, O-CH_2-O) and triplets at δ 4.94 (2H, *t*, J = 6.08 Hz, H-6)

and δ 3.21 (2H, *t*, *J* = 6.2Hz, H-5) for methylene protons attached to C-6 and C-5. The signal that appeared at δ 3.49 was due to solvent residual signal of DMSO (Figure 5).

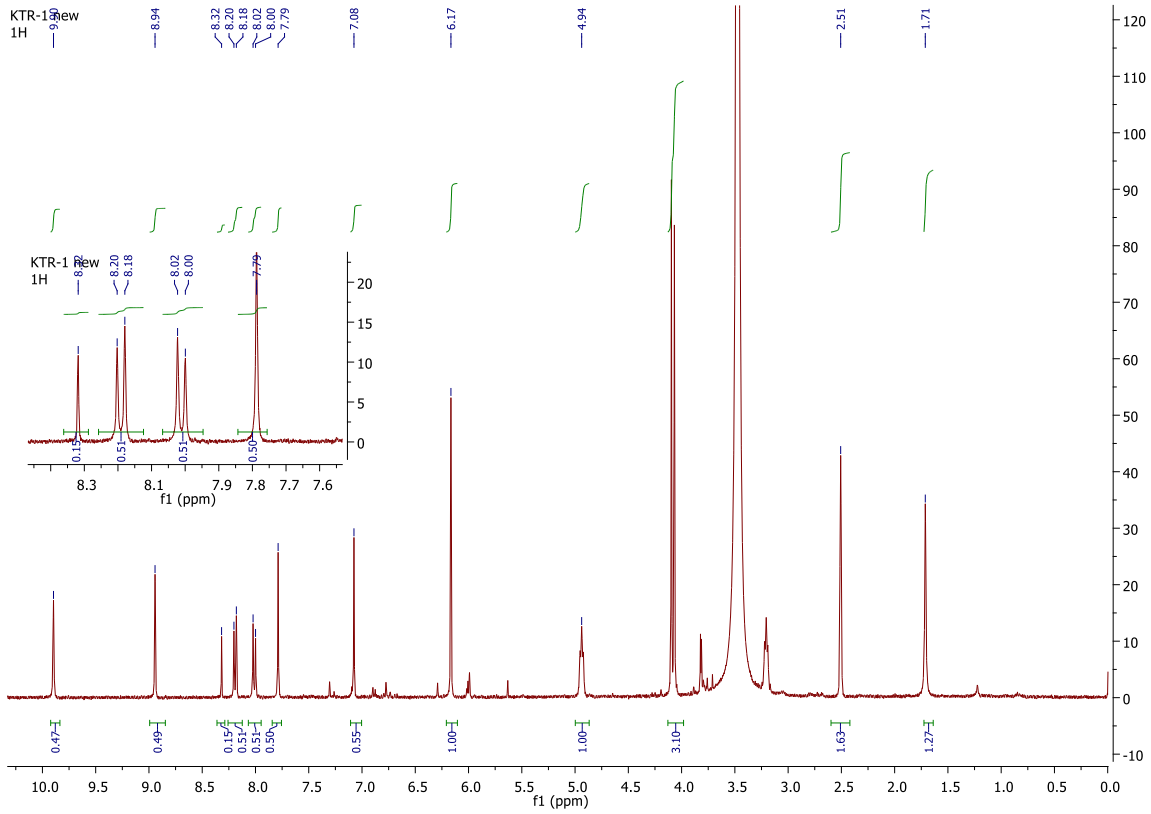

Figure 5:[1]H-NMR spectrum of TR_1.

The [13]C-NMR spectrum (Figure 6) of TR_1 displayed a total of 20 carbon atoms which, along with DEPT-135 experiment, revealed two CH_3, three CH_2, six CH and nine quaternary carbons (Table 5). Six aromatic CH carbon signals at δ 105.91, δ 108.87, δ 144.15, δ 127.21, δ 124.01, and δ 120.68 were assigned to C-1, C-4, C-8, C-11, C-12, and C-13, respectively (Table 5). The presence of oxygenated CH_3 carbons at δ 62.37 (9-OCH_3) and δ 57.53 (10-OCH_3) were also noted. The signals at δ 120.89 (C-14a), δ 121.88 (C-8a), δ 131.10 (C-4a), δ 133.49 (C-12a), δ 137.95 (C-14), δ 145.90 (C-9), δ 148.16 (C-2), δ 150.30 (C-3) and δ 150.85 (C-10) were attributed to the non-protonated quaternary carbons. In addition, the DEPT-135 spectrum displayed three downward peaks at δ 26.82 (C-5), δ 55.68 (C-6) and δ 102.53 (OCH_2O) which showed the carbon atoms of a methylene group (Figure 7).

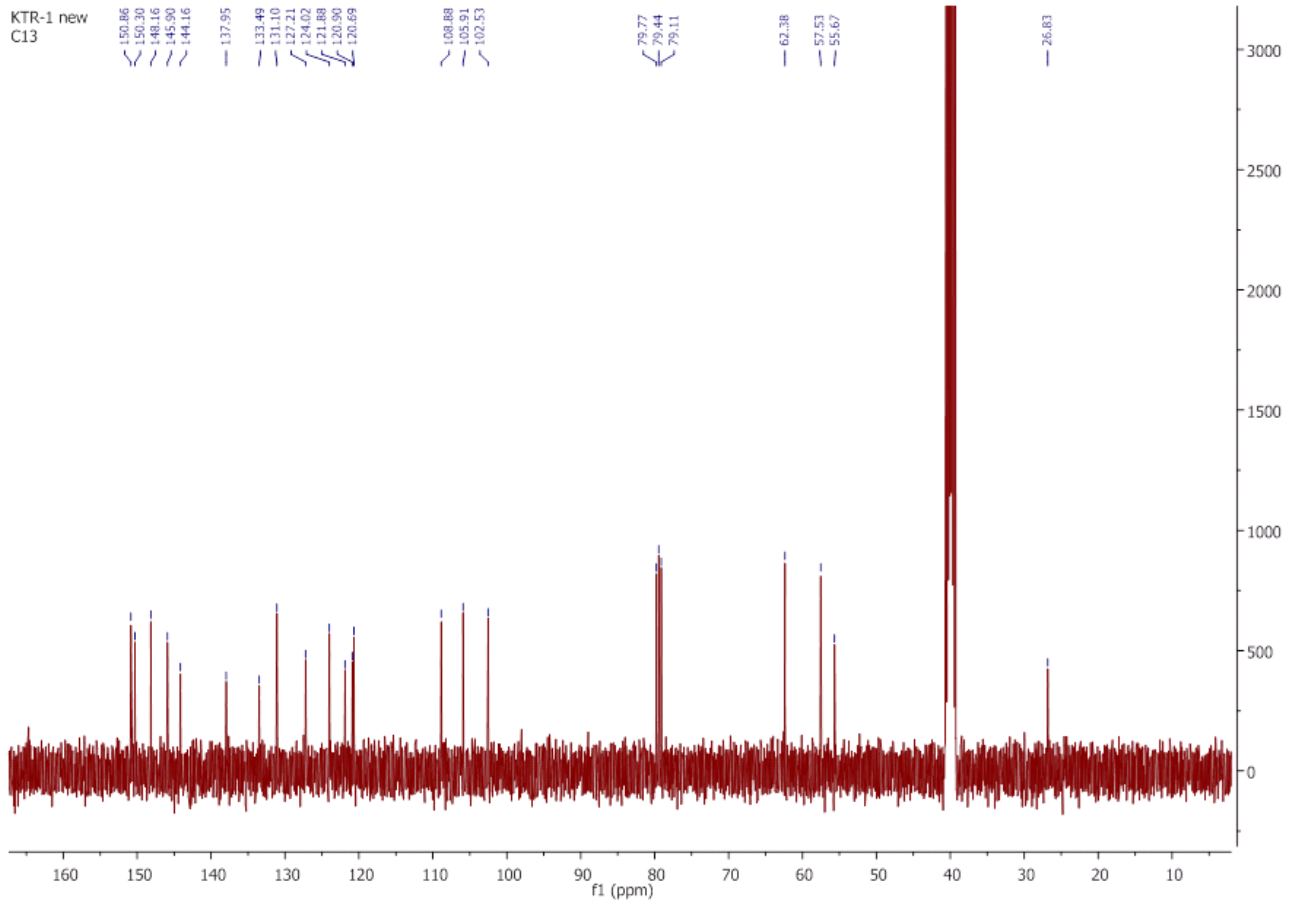

Figure 6: ^{13}C-NMR spectrum of TR_1.

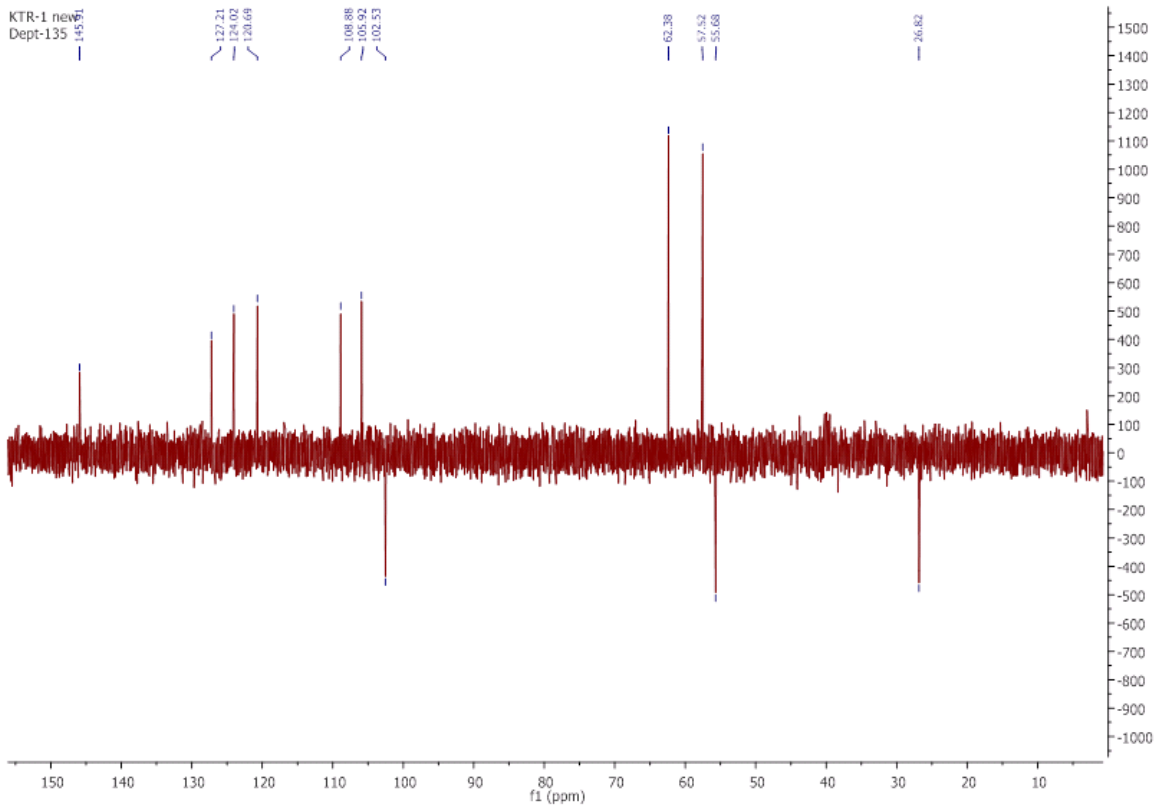

Figure 7: DEPT-135 spectrum of TR_1.

The complete assignments of ^{1}H and ^{13}C chemical shifts are listed in Table 5. From the data presented above and by comparing the ^{1}H and ^{13}C-NMR data of TR_1 with the compound reported by Meng *et al.* (2017), the chemical structure of TR_1 was established as berberine (Figure 8).

Table 5: Comparison of the ^{1}H NMR and ^{13}C NMR spectral data of the TR_1 with ^{1}H NMR and ^{13}C NMR of berberine reported by Meng *et al*. (2017).

Assignments	^{1}H (delta, ppm)		^{13}C (delta, ppm)	
	TR_1	Berberine	TR_1	Berberine
1	7.79, *s*	7.77, *s*	105.91	105.4
2	-	-	148.16	147.7
3	-	-	150.30	149.8
4	7.08, *s*	7.08, *s*	108.87	108.4
4a	-	-	131.10	130.6
5	3.21, *t* (6.08 Hz)	3.21, *t* (6.2 Hz)	26.83	26.3
6	4.94, *t* (6.2 Hz)	4.93, *t* (6.2 Hz)	55.67	55.2
7	-	-	-	-
8	9.90, *s*	9.87, *s*	144.15	143.7
8a	-	-	121.88	121.4
9	-	-	145.90	145.4
10	-	-	150.85	150.4
11	8.19, *d* (9.2Hz)	8.19, *d* (9.1Hz)	127.21	126.8
12	8.01, *d* (9.2Hz)	7.89, *d* (9.1Hz)	124.01	123.5
12a	-	-	133.49	133.0
13	7.94, *s*	8.91, *s*	120.68	120.1
14	-	-	137.95	137.5
14a	-	-	120.89	120.4
$-OCH_2O-$	6.17, *s*	6.17, *s*	102.53	102.5
9-OCH_3	4.11, *s*	4.10, *s*	62.37	61.37
10-OCH_3	4.21, *s*	4.07, *s*	57.53	57.00

Spectra run in DMSO-d_6 at 400 MHz, *J* in parenthesis (Hz), *s* = singlet, *d* = doublet (^{1}H-NMR), and at 100 MHz (^{13}C-NMR spectra).

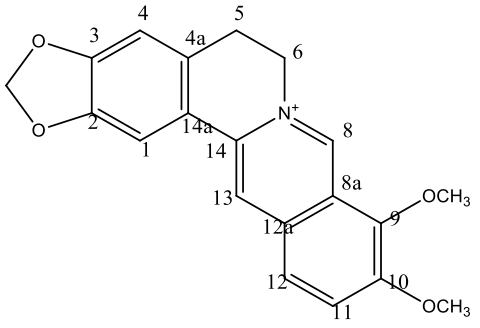

Figure 8: Structure formula of TR1, berberine.

4.3 Antidiarrheal effects of berberine

4.3.1 Effects on castor oil-induced diarrhea in mice

Antidiarrheal activity of the isolated compound, berberine was studied on castor oil-induced diarrheal model in Swiss albino mice. The results showed that berberine at all the tested doses significantly delayed the time of diarrheal onset and the frequency of stooling (number of wet feces and the total number of feces). Data from the experiment showed that the percentage of diarrheal inhibition compared to negative control was 81.0% ($p < 0.05$), 87.6 % ($p < 0.001$), and 88.3% ($p < 0.001$) at doses of 100, 200 and 400 mg/kg, respectively. These effects were comparable with the standard drug, loperamide 87.0% ($p < 0.05$) (Table 6).

In addition to inhibition of prostaglandin synthesis, NSAIDs inhibit castor oil-induced diarrhea (Awouters *et al.*, 1978). Similarly, berberine has been reported to have anti-inflammatory activity like NSAIDs (Zhang *et al*., 2011; Gurunathan *et al*., 2013). Thus, it is possible that berberine exerts its antidiarrheal effect by inhibition of castor oil-induced prostaglandin synthesis which is effected through inhibition of active secretion of ricinoleic acid, resulting in the activation of Na^+, K^+ ATPase activity that promotes absorption of Na^+ and K^+ in the intestinal mucosa (Palombo and Wiley, 2006; Mayeku *et al.*, 2013).

Table 6: Effect of berberine isolated from the 80% methanol root extract of *Thalictrum ryhnchocarpum* on castor oil-induced diarrhea in mice

Treatment	Dose (mg/kg)	Onset of diarrhea (min)	Total # of feces	Total # of wet feces	Total weight of feces (g)	Total weight of wet feces (g)	Percent inhibition of defecation
Vehicle	-	43.40±1.54	17.20±1.77	15.40±1.63	1.67±0.27	1.58±0.24	-
Loperamide	3	101.6±20.28^{a1}	4.20±0.66^{a3}	2.00±0.63^{a3}	0.43±0.11^{a2}	0.29±0.14^{a3}	87.0
Berberine	100	90.40±12.17^{a1}	6.00±1.67^{a3}	3.00±0.71^{a3}	1.01±0.29	0.76±0.26^{a1}	81.0
Berberine	200	162.20±29.79 ^{a2}	3.40±1.03^{a3}	1.91±0.75^{a3}	0.46±0.17^{a2}	0.27±0.14^{a3}	87.6
Berberine	400	178.80±13.14^{a3c1}	5.40± 1.69^{a3}	1.80±0.66^{a3}	0.67±0.21^{a1}	0.33±0.13^{a3}	88.3

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data were analyzed by one-way ANOVA followed by Tukey post hoc test; a compared to the control, b compared to the standard drug, c compared to 100 mg/kg, d compared to 200 mg/kg, e compared to 400 mg/kg; 1 $p < 0.05$, 2 $p < 0.01$ 3$p < 0.001$; Mice in the control group received distilled water (10 ml/kg).

As depicted in Figure 9, there was a dose independent reduction in the percentage of mean fecal output, at doses of 200 mg/kg (27.5 %, $p < 0.01$) and 400 mg/kg (40.1 %, $p < 0.05$) of berberine compared to the negative control but, not at the lower dose (100 mg/kg).

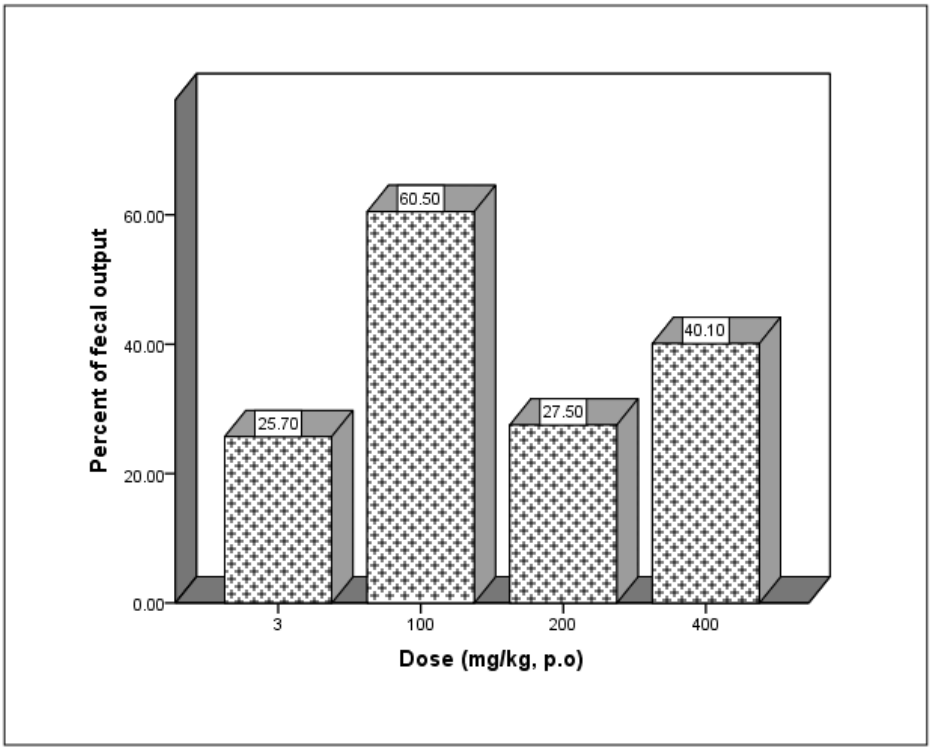

Figure 9: Percentage mean fecal output of berberine isolated from the 80% methanol root extract of *Thalictrum ryhnchocarpum* on castor oil-induced diarrheal model in mice.

4.3.2. Effect on castor oil-induced gastrointestinal transit

In the small intestinal transit test, berberine was able to inhibit intestinal motility; a rising tendency of the inhibitory effect on the gastrointestinal motility was observed in all the tested doses. The charcoal meal method was selected to follow displacement of the gastrointestinal content because the reduction of gastrointestinal motility is one mechanism by which many antidiarrheal agents act (Tadesse *et al.*, 2017). Berberine significantly reduced intestinal transit as observed by the decrease in GI motility of the charcoal meal. As presented in Table 7, berberine significantly inhibited gastrointestinal transit time of charcoal meal as compared to the negative control. At all the tested doses its effect was not significantly different from that of the

standard drug, loperamide at a dose of 3 mg/kg. These results suggest that berberine acts on all parts of the intestine.

Table 7: Effect of berberine isolated from the 80% methanol root extract of *Thalictrum ryhnchocarpum* on castor oil-induced gastrointestinal transit in mice.

Treatment	Dose (mg/kg, p.o)	Mean length of small intestinal (cm)	Mean distance traveled by charcoal (cm)	Peristalsis index (%)	Percent inhibition
Vehicle	-	59.4±1.43	27.00 ±3.90	45.50	-
Loperamide	3	57.6±2.56	5.00±0.84[a3]	8.68[a3]	81.8
Berberine	100	59.2±2.00	10.80 ±2.67[a3]	18.20[a3]	60.6
Berberine	200	57.2±1.46	9.40±6.27[a3]	16.40[a3]	65.7
Berberine	400	57.8±1.59	8.40 ± 1.20[a3]	14.53[a3]	69.3

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data were analyzed by one-way ANOVA followed by Tukey post- hoc test; compared to the control, b to the standard drug, c to 100 mg/kg, d to 200 mg/kg, e to 400 mg/kg; [1]$p < 0.05$, [2]$p < 0.01$; Mice in the control group received distilled water (10 ml/kg).

4.3.3. Effect on castor oil-induced enteropooling

In the castor oil-induced enteropooling test, treatment of mice with different doses of berberine produced a significant decline in the intestinal fluid accumulation. Maximum percentage inhibition of the volume of the intestinal contents was observed at 200 (58.6 %, $p < 0.001$) and 400 mg/kg (58.6 %, $p < 0.001$), but the compound did not significantly inhibit the weight of intestinal contents. However, percentage inhibition of both the intestinal fluid and the weight of intestinal contents was observed at 100 (51.7%, $p < 0.01$) and (31.7%, $p < 0.05$) mg/kg, respectively (Table 8).

According to Mascolo *et al*. (1994), ricinoleic acid which is the active metabolite of castor oil might activate the NO pathway and induce NO dependent gut secretion. The fact that intestinal fluid accumulation and Na^+ secretion induced by castor oil is attenuated by pretreatment of mice with NO synthesis inhibitors (Degu *et al.,* 2016) reinforces the notion that the anti-enteropooling effect of berberine could probably be by interfering with the NO pathway. Previous reports indicate that several alkaloids possess inhibitory effect on NO synthesis (Kondo *et al*., 1993). Therefore, the activity of berberine might be due to inhibition of NO synthesis pathway.

Table 8: Effect of berberine isolated from the 80% methanol root extract of *Thalictrum ryhnchocarpum* on castor oil induced enteropooling in mice.

Treatment	Dose (mg/kg, p.o)	MWSIC (g)	% inhibition by using MWSIC	MVSIC (ml)	% inhibition by using MVSIC
Vehicle	-	2.55±0.29	-	0.58±0.07	-
Loperamide	3	1.84±0.11	27.8	0.18±0.04[a3]	68.9
Berberine	100	1.74±0.14[a1]	31.7	0.28±0.03[a2]	51.7
Berberine	200	1.84±0.12	28.0	0.24±0.05[a3]	58.6
Berberine	400	1.80±0.21	29.4	0.24±0.05[a3]	58.6

All values are expressed as mean ± standard error of the mean (SEM); (n = 5); Data was analyzed by one-way ANOVA followed by Tukey post hoc test; a compared to the control, b compared to the standard drug, c compared to 100 mg/kg, d compared to 200 mg/kg, e compared to 400 mg/kg; 1 $p < 0.05$, 2 $p < 0.01$ 3$p < 0.001$; MWSIC: Mean weight of small intestinal content; MVSIC: Mean volume of small intestinal content; Mice in the control group received distilled water (10 ml/kg).

4.3.4 *In vivo* antidiarrheal index

The *in vivo* antidiarrheal index (ADI) was measured by taking the delay in defecation (time of onset, Dfreq), gut meal travel distance (Gmeq) and purging frequency in number of wet stools as major parameters. The greatest ADI of the berberine was achieved at the maximum dose of 400 mg/kg (121.6%) (Table 9).

Table 9: *In vivo* antidiarrheal indices of berberine isolated from the 80% methanol root extract of *Thalictrum ryhnchocarpum*.

Treatment	Dose (mg/kg, p.o)	Delay in defecation (Min), Dfreq (%)	Gut meal travel distances (Gmeq) (%)	Purging frequency in number of wet feces (%)	*In vivo* antidiarrheal index
Vehicle	-	-	-	-	-
Loperamide	3	142.7	81.8	87.0	100.5
Berberine	100	99.1	60.6	81.0	78.6
Berberine	200	257.3	65.7	87.6	114.0
Berberine	400	293.8	69.3	88.3	121.6

Berberine is an isoquinoline alkaloid previously isolated particularly from the roots, rhizomes, stem, and bark of a number of important medicinal plants, such as *Berberis aristata*, *B. aquifolium, B. vulgaris*, and *Coptis chinensis*, (Kulkarni and Dhir, 2007; Liu *et al.*, 2013),*Cortex phellodendri, Hydrastis canadensis Mahonia fortune* and *Rhizoma coptidis* (Kulkarni and Dhir, 2007),(Cernakova and Kostalova 2002; Hsieh *et al.*, 2007; Yuan *et al.*, 2017), *Thalictrum foliolosum* (Kumar *et al.*, 2016), *T. orientale* (Erdemgil *et al.*, 2001) and *T. ramosum* (Meng *et al.*, 2017).

It belongs to the structural class of protoberberines which includes a quaternary base and contains many derivate and analogues, such as berberine hydrochloride, berberine sulfate, and berberine citrate or phosphate, contributing to its multiple pharmacological and biochemical effects (Liu *et al.*, 2013).

Berberine is widely used as an antibacterial (Cernakova and Kostalova 2002; Otani *et al.*, 2005; Yuan *et al.*, 2017), antifungal, antiprotozoal, antitrypanosomal (Cernakova and Kostalova 2002) and antimalarial drug (Otani *et al.*, 2005). It possesses antidepressant (Kulkarni and Dhir, 2007), cytotoxic (Orfila *et al.*, 2000), anti-inflammatory, antioxidant, cardio protective and antialzheimer (Rackova *et al.*, 2004; Mak *et al.*, 2014) properties. Hsieh *et al*. (2007) reported that berberine displays immune system-stimulating properties as well as ameliorating effects on hyperlipidemia and hyperglycemia.

5. CONCLUSION

The present study showed that the 80% methanol root extract of *T. ryhnchocarpum* and its major component berberine possess antidiarrheal activity in experimental animal model. The results confirm that the activity of the studied plant is at least in part attributed to the presence of the berberine. In view of the relative safety of the crude extract as well as berberine, the potential of the studied plant for the management of diarrhea cannot be overemphasized. From the results of the present work, the use of the root extract of the plant in traditional medicine for the treatment of diarrhea appears to be well founded. However, further work, such as chronic toxicity studies have to be performed to evaluate possible therapeutic values of the plant.

6. RECOMMENDATIONS

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

- Isolation and characterization of minor compounds from the root extract of *T. ryhnchocarpum* and study their antidiarrheal and antimicrobial activities;
- Evaluation of the antidiarrheal activity of the crude extract and berberine against another chemical induced diarrhea and
- Determination of sub-acute and chronic toxicities of the total extract and berberine.

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APPENDIXES

Appendix I: List of some antidiarrheal ethnomedicinal plants (Rouf *et al.,* 2003; Megersa *et al.,* 2013; Umer *et al.,*2013; Elisha *et al.,* 2013; Tadesse *et al.,* 2014; Enyew *et al.,* 2014; Zaman *et al.,*2015; Asrie *et al*., 2016; Ay *et al.,* 2016; Yacob *et al*., 2016; Birru *et al.,* 2016; Degu *et al.,* 2016; Muhammad *et al.,* 2016; Sisay *et al*., 2017; Molla *et al*., 2017; Woldeab *et al*., 2018; Derebe *et al.,* 2018).

	Scientific name	**Family**
1	*Acacia albida Del*	Fabaceae
2	*Ajuga alba Robyns*	Lamiaceae
3	*Ajuga integrifoliaBuch.Ham*	Lamiaceae
4	*Ajuga remotaBenth*	Lamiaceae
5	*Allamondaneriifolia*	Apocynaceae
6	*Allium sativum L.*	Alliaceae
7	*Artemisia rehan L.*	Asteraceae
8	*AsystasiaguttataBrummitt*	Acanthaceae
9	*BarleriaargenteaBalf.fil.*	Acanthaceae
10	*Bersama abyssinicaFresen.*	Melianthaceae
11	*Bidenspilosa L.*	Asteraceae
12	*Boscia angustifolia A. Rich.*	Capparidaceae
13	*Boswellia papyrifera (Del.) Hochist.*	Burseraceae
14	*Bruceaantidysenterica J. F. Mill.*	Simaroubaceae
15	*Bruguiera cylindrical*	Rhizophoraceae
16	*CadabarotundifoliaForssk.*	Capparaceae
17	*Calpurnia aurea (Ait.) Benth.*	Fabaceae
18	*Capparis tomentosa Lam*	Capparaceae
19	*Carica papaya L.*	Caricaceae
20	*Carissa spinarum L.*	Apocynaceae
21	*Carmona retusa*	Boraginaceae
22	*Cissampelosmucronata A. Rich.*	Menispermaceae
23	*Cissampelospareira L.*	Menispermaceae

24	*Citrus limon (L.) Burm.fil.*	Rutaceae
25	*Clematis hirsutaGuill. & Perr.*	Ranunculaceae
26	*Clematis simensisFresen.*	Ranunculaceae
27	*Cleome gynandra L.*	Capparidaceae
28	*Clutia lanceolate Forssk*	Euphorbiaceae
29	*ClutiaabyssinicaJaub. & Spach*	Euphorbiaceae
30	*Coffea arabica L.*	Rubiacea
31	*ConyzapyrrhopappaA.Rich*	Asteraceae
32	*Cordia Africana*	Boraginaceae
33	*Cordia myxa,*	Boraginaceae
34	*Cordia rothii*	Boraginaceae
35	*Cordia americana,*	Boraginaceae
36	*Crinum latifolium*	Amaryllidaceae
37	*Croton macrostachyusHochst.exDelile*	Euphorbiaceae
38	*Cyathula cylindrical Moq.*	Amaranthaceae
39	*DiscopodiumPenninervum (Hochst.)*	Solanaceae
40	*Dodonaea angustifolia L.f.*	Sapindaceae
41	*Echinopscacrocheatus L.*	Asteraceae
42	*Eleusinecoracana (L.) Gaertn.*	Poaceae
43	*Entada abyssinicaA.Rich.*	Fabaceae
44	*EmbeliaschimperiVatke*	Myrsinaceae
45	*Ficussagittatavhal*	Moraceae
46	*Idigofera spicata Forssk.*	Fabaceae
47	*Jasminum abyssinicum R.Br.*	Oleaceae
48	*Khaya senegalensis A. Juss*	Meliaceae
49	*Lantana camara L.*	Verbenaceae
50	*Lantana ukambensisVerdc.*	Verbenaceae
51	*Lepidium sativum L.*	Brassicaceae
52	*MaesalanceolataForssk.*	Myrsinaceae
53	*Mangifera sylvatica Roxb.*	Anacardiaceae
54	*Maytenus senegalensis (Lam.)*	Celastraceae

55	*Melia azedarach L.*	Meliaceae
56	*Mimusops kummel A.DC.*	Sapotaceae
57	*Momordica charantia*	Cucurbitaceae
58	*Moringa oleifera Lam*	Moringaceae
59	*Myrtuscommunis Linn*	Myrtaceae
60	*Nigella sativa L.*	Ranunculaceae
61	*Otostegiaintegrifolia (Forssk.)*	Lamiaceae
62	*Otostegiatomentosa A. Rich.*	Lamiaceae
63	*OliniarochetianaA.Juss.*	Oliniaceae
64	*Pandanus foetidusRoxb.*	Pandanaceae
65	*Podocarpusfalcatus C.N. Pag*	Podocarpaceae
66	*RubussteudneriSchweinf.*	Rosaceae
67	*Rumexmaritimus Linn.*	Polygonaceae
68	*RumexnepalensisSpreng.*	Polygonaceae
69	*Salvia ballotiflora Benth*	Acanthaceae
70	*Salvia schimperi*	Myrsinaceae
71	*Sapiumellipticum (Hochst)*	Euphorbiaceae
72	*Schinusmolle L.*	Anacardiaceae
73	*Senna didymobotry (Freser) Irowin&Barinely*	Fabaceae
74	*Senna obtusifolia (L.) Irwin and Barneby*	Fabaceae
75	*Senna singueana (Delile) Lock*	Fabaceae
76	*Solanaciogigas (Vatke) Jeffrey*	Asteraceae
77	*Solanum giganteum Jacq.*	Solanaceae
78	*Solanum nigum L.*	Solanaceae
79	*Thalictrum rhynchocarpumQuart. Dill. & A. Rich.*	Ranunculaceae
80	*Urenasinuata L. (Borss)*	Malvaceae
81	*VerbascumsinaiticumBenth.*	Scrophulariaceae
82	*Vernonia amygdalina Del.*	Asteraceae
83	*Vernonia auriculiferaHiem*	Asteraceae
84	*Zehneriascabra (Linni. f) Sond*	Cucurbitaceae
85	*Viscum tuberculatum A. Rich*	Viscaceae

86	*Ziziphusmauritiana*	Rhamnaceae
87	*Withaniasomnifera (L.) Dunal.*	Solanaceae

Appendix II: Some compounds isolated from the genus *Thalictrum* and their structures.

Class of compounds	Structure No	Source	References
Alkaloids			
6, 6', 7', 12 - tetramethoxy -5'-hydroxy - 2, 2'- dimethyloxycanthan	**1**	*T. foliolosum*	(Kumar *et al.,* 2016)
6, 5', 6', 7', 12- pentamethoxy -5'-hydroxy -2, 2'- dimethyloxyethane	**2**	*T. foliolosum*	(Kumar *et al.,* 2016)
Thalifendine	**3**	*T. foliolosum*	(Kumar *et al.,* 2016; Meng *et al.,* 2017)
Berberine	**4**	*T. foliolosum*	(Kumar *et al.,* 2016; Meng *et al.,* 2017)
Acutiaporberine	**5**	*T. acutiafolium*	(Chen *et al.*,2003)
Isothalisopavine	**6**	*T.* minus	(Sidjimo*et al.,* 1998)
5'hydroxythalidasine,	**7**	*T. minus*	(Mushtaq *et al.,* 2016)
Thalrugosaminine	**8**	*T. minus*	(Mushtaq *et al.,* 2016)
O-methylthalicberine	**9**	*T. minus*	(Mushtaq *et al.,* 2016)
Neothalfine	**10**	*T. atriplex*Finet et	(Gao *et al.,* 2005)
Thaliatrine	**11**	*T. atriplex*Finet et	(Gao *et al.,* 2005)
Northalidasine	**12**	*T. flavum* L	(Chen *et al.*,2003; Ropivia *et al.,* 2010)
Northalrugosidine	**13**	*T. flavum* L	(Ropivia *et al.,* 2010)
Thalfoetidine	**14**	*T. flavum* L	(Ropivia *et al.,* 2010)
Northalfoetidine	**15**	*T. flavum* L	(Ropivia *et al.,* 2010)
Thaligosidine	**16**	*T. flavum* L	(Ropivia *et al.,* 2010)

Columbamine	**17**	*T. ramosum*	(Meng *et al.*, 2017)
Thalidastine	**18**	*T. ramosum*	(Meng *et al.*, 2017)
Magnoflorine	**19**	*T. ramosum*	(Meng *et al.*, 2017)
Aporphines	**20**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Bisbenzylisoquinoline dimers	**21**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Aporphine-benzylisoquinoline dimers	**22**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Protopines	**23**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Pavines	**24**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Diterpene alkaloids	**25**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Phenanthrenes	**26**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
6aR-2′-methoxycarbonyl-thaliadin	**27**	*T. cirrhosum*	(Yan *et al.*, 2018)
6aR-2′-carboxylthaliadin	**28**	*T. cirrhosum*	(Yan *et al.*, 2018)
6aR-3-methoxy-hernandalinol	**29**	*T. cirrhosum*	(Yan *et al.*, 2018)
6aS-1, 3, 10-trimethoxy-natalamine	**30**	*T. cirrhosum*	(Yan *et al.*, 2018)
3-methoxy-2′methoxycarbonyl-oxohernandalincin	**31**	*T. cirrhosum*	(Yan *et al.*, 2018)
Thaliadine	**32**	*T. cirrhosum*	(Yan *et al.*, 2018)
Predicentrine	**33**	*T. cirrhosum*	(Yan *et al.*, 2018)
N-methyllaurotetanine	**34**	*T. cirrhosum*	(Yan *et al.*, 2018)
3-methoxy-oxohernandaline	**35**	*T. cirrhosum*	(Yan *et al.*, 2018)
Xopurpureine	**36**	*T. cirrhosum*	(Yan *et al.*, 2018)
Oxophoebine	**37**	*T. cirrhosum*	(Yan *et al.*, 2018)
Laudanosine	**38**	*T. cirrhosum*	(Yan *et al.*, 2018)
Pseudolaudanine	**39**	*T. cirrhosum*	(Yan *et al.*, 2018)

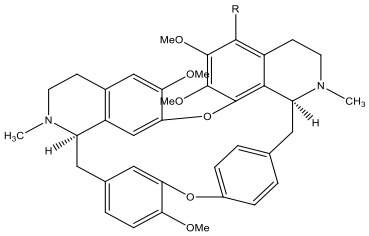
Rugosinone	**40**	*T. cirrhosum*	(Yan *et al.*, 2018)
O-methylflavinantine	**41**	*T. cirrhosum*	(Yan *et al.*, 2018)
Dihydroglaziovi	**42**	*T. cirrhosum*	(Yan *et al.*, 2018)
Linearisine	**43**	*T. cirrhosum*	(Yan *et al.*, 2018)
6,7,12-trimethoxy-2-methyl-13-hydroxy-11-(4′-formylphenoxy) benzylisoquinoline	**44**	*T. wangii*	(Jin *et al.*, 2018)
5,6-(methylenedioxy)-7,12-dimethoxy-2-methyl-10-(4′formylphenoxy) benzylisoquinoline	**45**	*T. wangii*	(Jin*et al.*, 2018)
Thalfoliolosumines A	**46**	*T. foliolosum*	(Li *et al*., 2016)
Thalfoliolosumines B	**47**	*T. foliolosum*	(Li *et al.*, 2016)
Flavonoids			
7,40-di-O-b-allosyl-apigenin	**48**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
7-O-(6acetyl-b-allosyl)-40-O-(b-allosyl) apigenin	**49**	*T. squarrosum* *T. minus*	(Chen *et al.*, 2003)
Kaempferol-3-O-[acetyl-a-L-arabinosyl-(1-6)]-b-D-glucoside	**50**	*T. atriplex,*	(Chen *et al.*, 2003)
40-methoxyl-apigenin-7-O-[4-acetyl-a-L-rhamnosyl-(1-6)-b-D-glucosyl-(1-3)]-6acetyl-b-D-glucoside	**51**	*T. atriplex,*	(Chen *et al*., 2003)
Kaempferol	**52**	*T. atriplex,* *T. smithii* *T. przewalskii*	(Li *et al.*, 2016)
Isoquercitin	**53**	*T. atriplex,* *T. smithii* *T. przewalskii*	(Li *et al*., 2016)
40-methoxyl-apigenin-7-O-[a-L-rhamnosyl-(1-6)]-b-D-glucoside	**54**	*T. atriplex,* *T. smithii*	(Li *et al.*, 2016)

		T. przewalskii	
Kaempferol-3-O-[b-D-glucosyl-(1-3)-a-L-rhamnosyl-(1-2)]-b-D-glucoside	**55**	*T. atriplex*, *T. smithii* *T. przewalskii*	(Li *et al.*, 2016)
40-methoxyl-apigenin-7-O-[4-acetyl-a-L-rhamnosyl-(1-6)]-b-D-glucoside	**56**	*T. atriplex*, *T. smithii* *T. przewalskii*	(Li *et al.*, 2016)
Kaempferol-3-O-b-D-glucoside	**57**	*T. atriplex*, *T. smithii* *T. przewalskii*	(Li *et al*., 2016)
Triterpene saponins (Cycloartane glycoside)			
Thalictosides	**58**	*T. thunbergii*	(Chen *et al.*,2003; Yan *et al.*, 2018)
Foetoside C	**59**	*T. foetidum*	(Chen *et al.*, 2003; Yan *et al.*, 2018)
Thalicoside B	**60**	*T. minus*	(Chen *et al.*, 2003; Yan *et al.*, 2018)
Thalicoside D	**61**	*T. minus*	(Chen *et al*., 2003; Yan *et al.*, 2018)
Squarroside II	**62**	*T. squarrosum*	(Chen *et al.*, 2003; Yan *et al.*, 2018)
Squarroside III	**63**	*T. squarrosum*	(Chen *et al.*, 2003; Yan *et al.*, 2018)
Squarroside IV	**64**	*T. squarrosum*	(Chen *et al.*, 2003; Yan *et al.*, 2018)
3-O-b-D-xylopyranosyl-(1-6)-b-D-glucopyranosyl-(1-4)-b-D-fucopyranosyl (22S,24Z)-cycloart-24-en-3b,22,26-triol 26-O(6-O-acetyl)-b-D-glucopyranoside	**65**	*T. fortune*	(Zhang *et al.*, 2013)
3-O-a-L-arabinopyranosyl-(1-6)-b-D-	**66**	*T. fortune*	(Zhang *et al.*, 2013)

glucopyranosyl-(1-4)-b-D-fucopyranosyl (22S,24Z)-cycloart-24-en-3b,22,26-triol 26-O-(6-Oacetyl)-b-D-glucopyranoside			
3-O-b-D-glucopyranosyl (24S)-cycloartane-3b,16b,24,25,30-pentaol 25-O-b-D-glucopyranosyl-(1- 6)-b-D-glucopyranoside	**67**	*T. fortune*	(Zhang *et al.*, 2013)
3-O-b-D-glucopyranosyl (24S)-cycloartane-3b,16b,24,25,30-pentaol 25-O-b-D-glucopyranosyl-(1-4)-b-D-glucopyranoside	**68**	*T. fortune*	(Zhang *et al.*, 2013)
Cycloramosides	**69**	*T. ramosum* B. Boivin	(Meng *et al*., 2016)
3-O-alpha-L-arabinopyranosyl-3-betta, 16beta, 24s, 25, 30-pentaolcycloartane-24-O-Betta-D-glucopyranoside	**70**	*T. ramosum* B. Boivin.	(Meng *et al.,* 2016)
3-O-alpha-L-arabinopyranosyl-3B, 26B, 24S, 25-tetrahydroxycycloartane-16-O-B-D-glucopyranoside	**71**	*T. ramosum* B. Boivin	(Meng *et al.,* 2016)
Glycosides			
3-O-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl (22S,24Z)-cycloart-24-en-3β,22,26- triol 26-O-β-D-glucopyranoside	**72**	*T. fortune*	(Zhang *et al.,* 2011)
3-O-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl(22S,24Z)- cycloart-24-en-3β,22,26-triol 26-O-α-L-arabinopyranosyl-(1→6)-β-D-	**73**	*T. fortune*	(Zhang *et al.,* 2011)

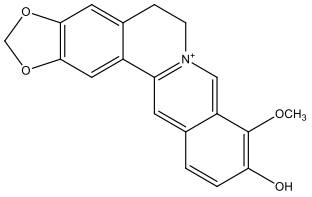
glucopyranoside			
3-O-β-D- glucopyranosyl-(1→4)-β-D-fucopyranosyl(22S,24Z)-cycloart-24-en-3β,22,26-triol 26-O-β-D-xylopyranosyl- (1→6)-β-D-glucopyranoside	**74**	*T. fortune*	(Zhang *et al.*, 2011)
3-O-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl(22S,24Z)- cycloart-24-en-3β,22,26-triol 26-O-β-D-quinovopyranosyl-(1→6)-β-D-glucopyranoside	**75**	*T. fortune*	(Zhang *et al.*, 2011)
(24S)- cycloartane-3β,16β,24,25,30-pentaol 3,25-di-O-β-D-glucopyranoside	**76**	*T. fortune*	(Zhang *et al.*, 2011)
24-O-acetyl-(24S)-cycloartane-3β,16β,24,25,30-pentaol 3,25-di-O-β-D-glucopyranoside	**77**	*T. fortune*	(Zhang *et al.*, 2011)
3-O-β-D-glucopyranosyl-24-O-acetyl-(24S)- cycloartane-3β,16β,24,25,30-pentaol 25-O-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside	**78**	*T. fortune*	(Zhang *et al.*, 2011)
3-O- β-D-glucopyranosyl-(24S)-cyclo-artane-3β,16β,24,25,30-pentaol 25-O-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside	**79**	*T. fortune*	(Zhang *et al.*, 2011)
Thalictricoside	**80**	*T. orientale*Boiss.	(Erdemgil *et al.*, 2003)
Lithospermoside	**81**	*T. orientale*Boiss.	(Erdemgil *et al.*, 2003)
Citroside B	**82**	*T. ramosum*	(Meng *et al.*, 2017)
Glochidionionoside	**83**	*T. ramosum*	(Meng *et al.*, 2017)

Pigenin 6, 8-di-C-β-D-xylopyranoside	**84**	*T. ramosum*	(Meng *et al.*, 2017)
Hydrangeifolin I	**85**	*T. ramosum*	(Meng *et al.*, 2017)

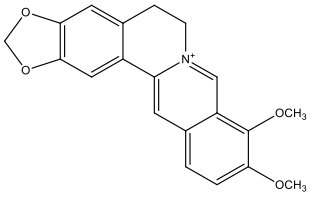


(**1**) R = OH

(**2**) R = OMe



(**3**)



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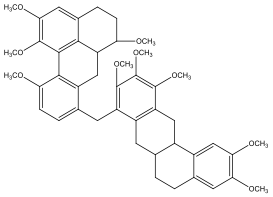

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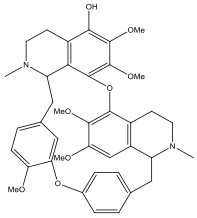

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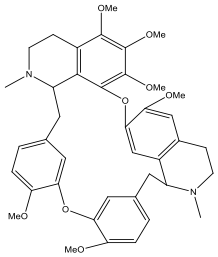

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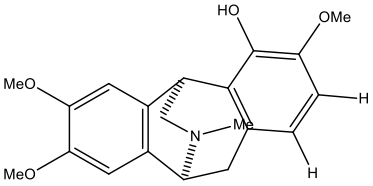

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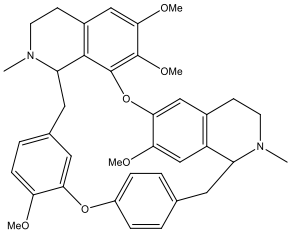

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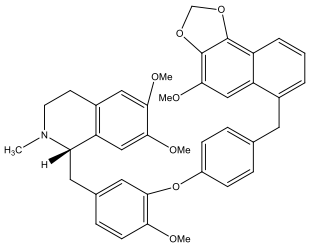

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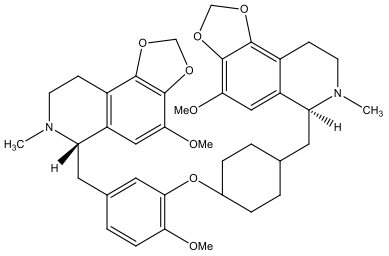

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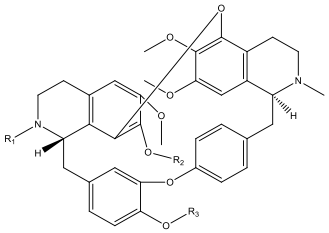


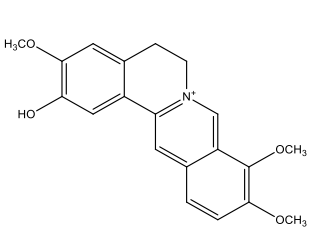
(**12**) R_1 = H, R_2 = Me, R_3 = Me

(**13**) R_1 = H, R_2 = H, R_3 = Me

(**14**) R_1 = Me, R_2 = Me, R_3 = H

(**15**) R_1 = H, R_2 = Me, R_3 = H

(**16**) R_1 = Me, R_2 = H, R_3 = H



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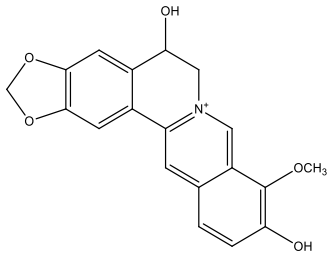

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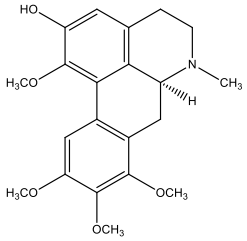

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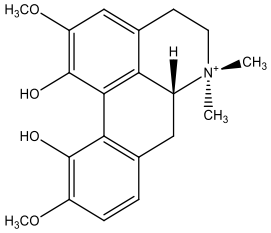

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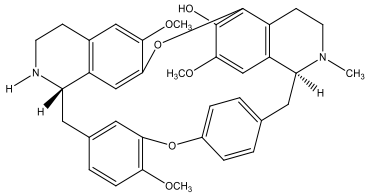

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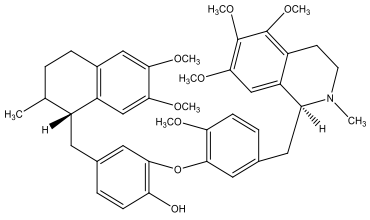

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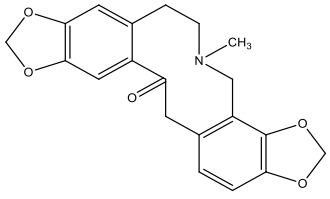

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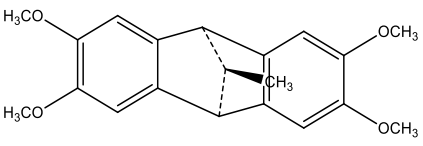

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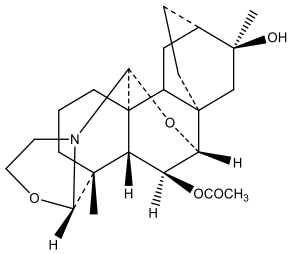

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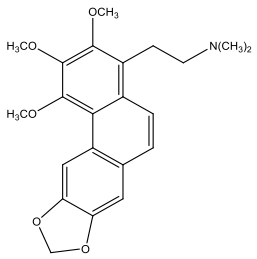

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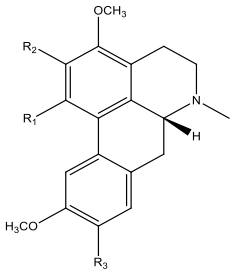
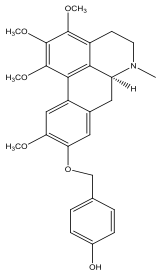
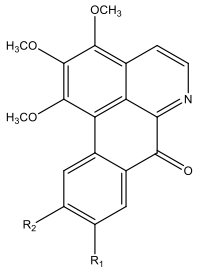
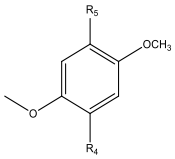
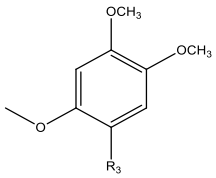






(**30**)

R_6 = 

R_1 = 

(**27**) $R_1 = R_2 = R_5 = OCH_3$, $R_3 = R_6$, $R_4 = COOCH_3$

(**28**) $R_1 = R_2 = R_5 = OCH_3$, $R_3 = R_6$, $R_4 = COOH$

(**29**) $R_1 = R_2 = R_5 = OCH_3$, $R_3 = R_6$, $R_4 = CH_2OH$

(**32**) $R_1 = R_2 = R_5 = OCH_3$, $R_3 = R_6$, $R_4 = CHO$

(**33**) $R_1 = H$, $R_2 = OH$, $R_3 = OCH_3$

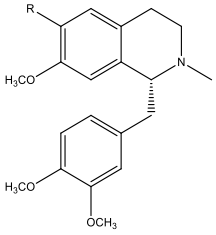
(**34**) $R_1 = R_2 = R_3 = OCH_3$

(**31**) $R_2 = OCH_3$, $R_3 = COOCH_3$

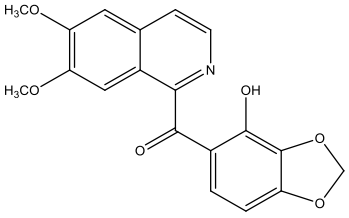
(**35**) $R_2 = OCH_3$, $R_3 = CHO$

(**36**) $R_1 = R_2 = OCH_3$

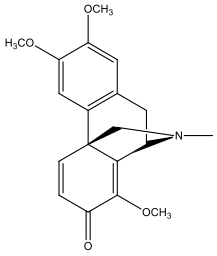
(**37**) $R_1 = R_2 = OCH_2O$



(**38**) R = OCH_3

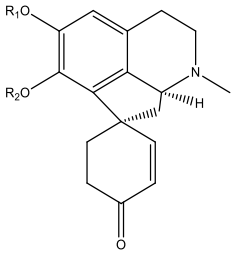


(**40**)

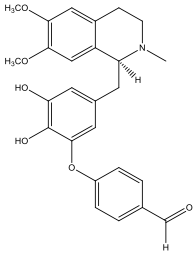


(**41**)

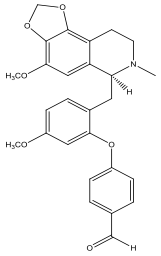
(**39**) R = OH



(**42**) $R_1 = CH_3$, $R_2 = H$

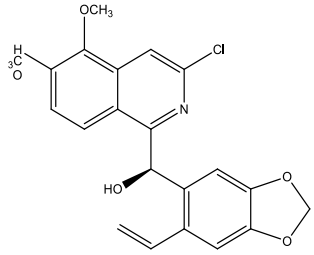


(**44**)



(**45**)

(**43**) $R_1 = H$, $R_2 = CH_3$



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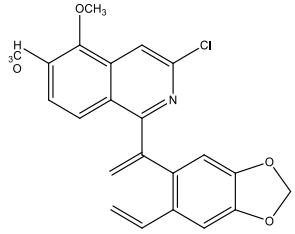
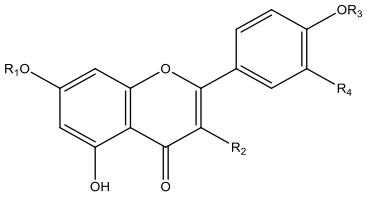


(**47**)



(**48**) $R_1 = R_3 = \beta$-all, $R_2 = R_4 = H$

(**49**) R_1 = 6-O-acetyl-β-all, R_3 =β-all, $R_2 = R_4$ =H

(**50**) $R_1 = R_3 = R_4 = H$, R_2 = O-3-O-acetyl-α-L-ara-(1-6)-β-D-glu

(**51**) R_1 = 4-acetyl-α-L-rham-(1-6)-D-glu-(1-3)-6-acetyl-β-D-glu, R_2 = R_4 = H, R*3* = Me

(**52**) R_1 = R_3 = R_4 = H, R_2 = OH

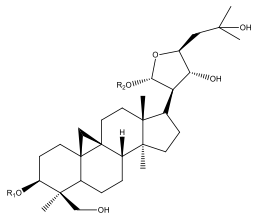
(**53**) R_1 = R_3 = H, R_2 = O-β-D-glu, R_4 = OH

(**54**) R_1 = α-L-rham-(1-6)-β-D-gluc, R_2 = R_4 = H, R_3 = Me

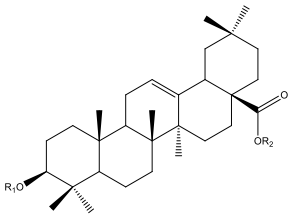
(**55**) R_1 = R_3 = R_4 = H, R_2 = O-β-D-glu-(1-3)-α-L-rham-(1-2)-β-D-glu

(**56**) R_1 = 2-acetyl-α-L-ham-(1-6)-β-D-glu, R_2 = R_4 = H, R_3 = Me

(**57**) R_1 = R_3 = R_4 = H, R_2 = O-β-D-glu



(**58**) R_1 = 6-deoxy-α-L-man-(1-2)-O-[6-deoxy-α-L-man-(1-6)]-β-D-glu, R_2 = β-D-glu-(1-2)-O-[β-D-xyl-(1-6)-β-D-glu



(**59**) R_1= β-D-xyl-(1-3)-α-L-rham-(1-2)-α-L-D-ara, R_2 = β-D-glu-(1-6)-β-D-glu

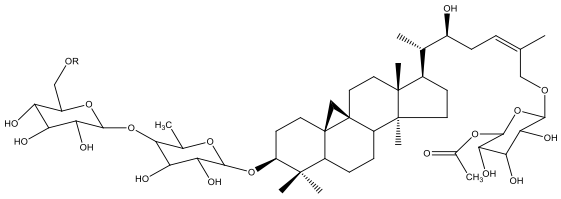
(**60**) R_1 = α-L-rham-(1-2)-β-D-glu-(1-3)-α-L-D-ara; R_2 = β-D-glu

(**61**) R_1 = α-L-rham-(1-2)-β-D-gluc-(1-4)-α-L-D-ara; R_2 = β-D-glu-(1-6)-β-D-glu

(**62**) R_1 = 6-deoxy-α-L-man-(1-2)-O-[β-D-glu-(1-4)]-β-D-xyl; R_2 = β-D-glu

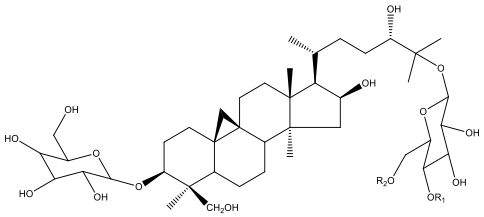
(**63**) R_1= 6-deoxy-α-L-man-(1-2)-O-[β-D-glu-(1-4)]-β-D-xyl; R_2 =β-D-glu-(1-6)-β-D-glu

(**64**) R_1 = β-D-glu-(1-4)-O-[β-D-glu-(1-3)-6-deoxy-α-L-man-(1-2)]-β-D-xyl; R_2 = β-D-glu



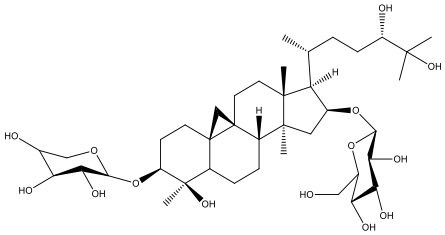
(**65**) R = xyl;

(**66**) R = ara

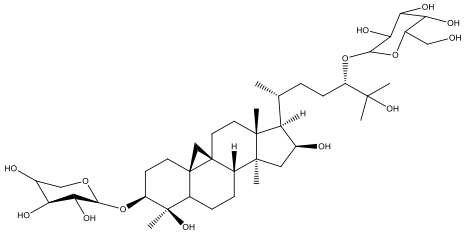


(**67**) R_1 = H, R_2 = glc

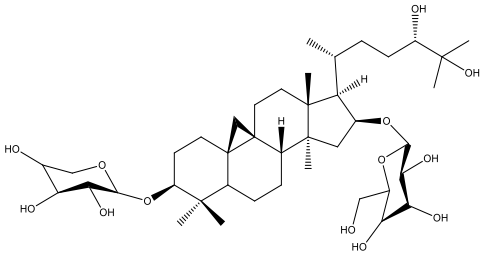
(**68**) R_1 = glc, R_2 = H



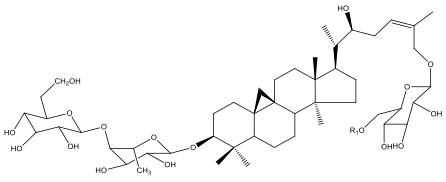
(**69**)



(**70**)



(**71**)

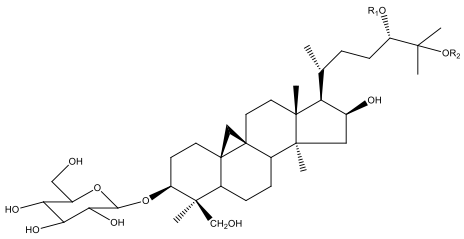


(**72**) R_1 = H

(**73**) R_1 = ara

(**74**) R_1= xyl

(**75**) R_1 = quin

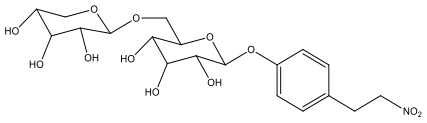


(**76**) R_1 = H, R_2 = glc

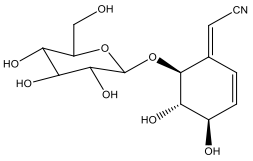
(**77**) R_1 = Ac, R_2 = glc

(**78**) R_1 = Ac, $_{R2}$ = glc(1-6)glc

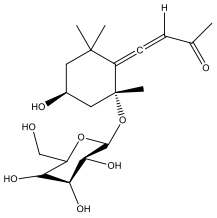
(**79**) R_1 = Ac, R_2 = glc(1-4)glc



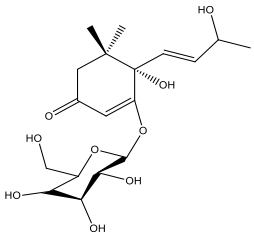
(**80**)



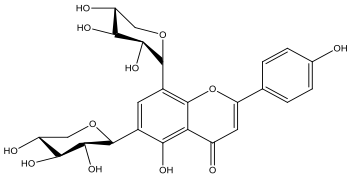
(**81**)



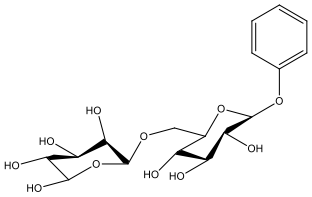
(**82**)



(**83**)

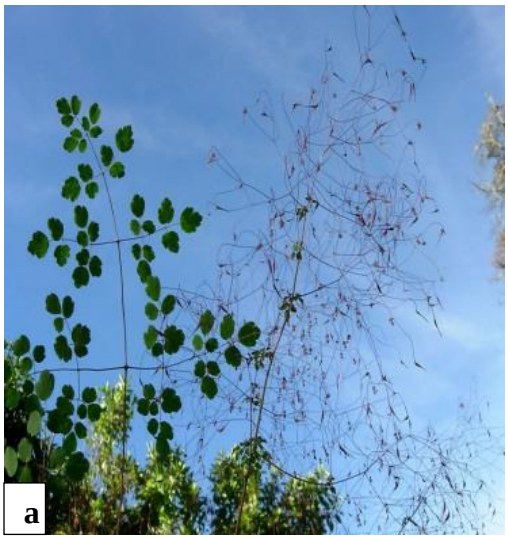


(**84**)

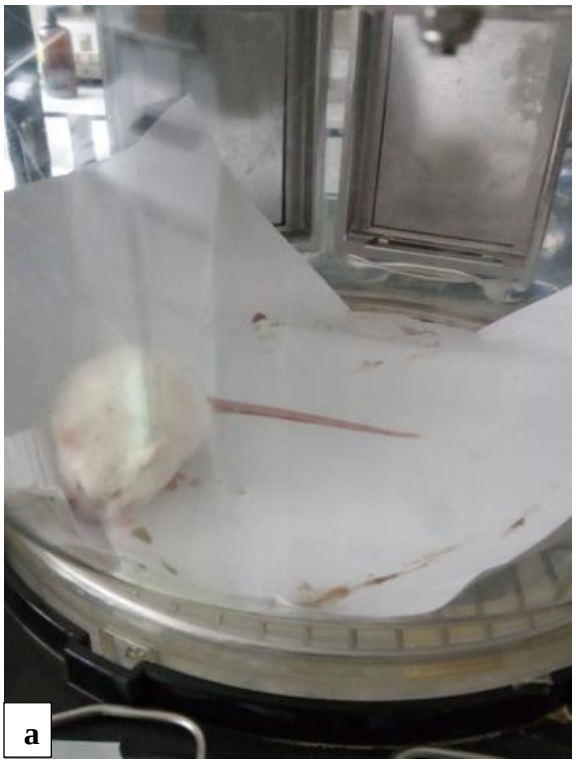


(**85**)

Appendix III: Leave and fruit (**a**), flower (**b**) and dried roots (**c**) of experimental plant (*Thalictrum ryhnchocarpum*).



Appendix IV: Some picture during laboratory work: mice during antidiarrheal test (a) and removal of small intestine from died mice (b).



Appendix V: Preparative thin layer chromatography