FORMULATION OF *CASSIA DIDYMOBOTRYA* LEAF LAXATIVE TABLETS

By

SINIDU MEKONNEN WENDEMAGEGN

U53/75434/2014

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Pharmacy in Industrial Pharmacy of the University of Nairobi.

November 2016

DECLARATION

This dissertation is my original work and has not been presented for a degree award in any university or published anywhere else.

Principal investigator:

Sinidu Mekonnen Wendemagegn, B.Pharm

U53/75434/2014

Department of Pharmaceutics and Pharmacy Practice,

University of Nairobi

Signature ……Date…….

Supervisors

This dissertation has been submitted for examination with our approval as university supervisors:

1. Prof. Kimani A. M. Kuria, PhD
   Department of Pharmaceutics and Pharmacy Practice,
   University of Nairobi

   Signature……Date……

2. Dr. Lucy J. Tirop, PhD
   Department of Pharmaceutics and Pharmacy Practice,
   University of Nairobi

   Signature…….Date……

TABLE OF CONTENTS

DECLARATION OF ORIGINALITY

Name of student	Sinidu Mekonnen Wendemagegn
Registration Number	U53/75434/2014
College	Health Sciences
School	Pharmacy
Department	Pharmaceutics and Pharmacy Practice
Course Name	Master of Pharmacy in Industrial Pharmacy

DECLARATION

1. I understand what plagiarism is and I am aware of the University's policy in this regard.
2. I declare that this dissertation is my original work and has not been submitted anywhere for any examination, award of a degree or publication. Where other people's work has been used, it has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.
3. I have not used the services of any professional agencies to produce this work.
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her work.
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature…….Date……

ACKNOWLEDGEMENT

First and foremost, all the glory goes to the almighty God for making all things possible for me to reach this point in life.

My most sincere gratitude goes out to my supervisors Prof. Kimani A.M. Kuria and Dr. Lucy J. Tirop; this work would not have been possible without your reinforcement, dedication, guidance, and mentorship you provided throughout my study period. Thank you very much.

My sincerer thanks also go to all the following people for their invaluable contribution and support, without which this work would not have become a success;

- To my sponsor, the German Academic Exchange Service (Deutscher Akademischer Austauschienst/DAAD), for all the financial support and facilitation.
- Dr. Maru, chairman of the department of pharmaceutics and pharmacy practice.
- Regal Pharmaceuticals Ltd. who provided me with all the inactive raw materials that I needed to carry out this study.
- National Quality Control Laboratory (NQCL) director, Dr. Hezekiah K. Chepkwony for giving me the permission to use their laboratory to carry out my work.
- Dr. E. Mbae (Deputy Director NQCL), for facilitating everything that I required to carry out the study.
- Miss. Sara (NQCL-analyst) for supporting me while I was in the NQCL lab doing part of my work.
- Laboratory technical support staff members especially Lily, Agnes, Richard, Francis, Josephat, Emmanuel, and Raphael for supporting me while I was working in the different laboratories.
- My classmate Dr. Atebe, for all his support, motivation and for being a good friend. It was a pleasure learning together.
- Finally, to my family and dear friends for their continued love, support and motivation.

DEDICATION

This work is dedicated to my wonderful husband, Yoseph Atilaw, who has been a constant source of support and encouragement during my study and life in general. I am truly thankful for having you in my life.

ABBREVIATIONS AND ACRONYMS

A	Absorbance
API	Active Pharmaceutical Ingredients
BP	British Pharmacopoeia
CI	Compressibility Index
D_{av}	Average Particle Diameter
D_b	Bulk Density
D_T	Tapped Density
F	Friability
ISO	International Standard Organization
n	Number of test(s)
MCC	Microcrystalline Cellulose
Q.S.	Sufficient Quantity
SD	Standard Deviation
USP	United States Pharmacopoeia
UV	Ultra Violet
Ɵ	Angle of repose

LIST OF PLATES

LIST OF TABLES

DEFINITION OF TERMS

Pre-formulation: it is a research and development process phase where physical, chemical and mechanical properties characterization of a new drug substance is done in order to develop a stable, safe and effective dosage form.

Tablets: are solid dosage forms containing a single active substance or more together with other excipients which are in powder form and compressed into a dosage form.

Compressibility: it is the capacity of a bed of powder to decrease in volume as a result of the application of force/pressure.

Bulk density: The bulk density of a powder is the ratio of the mass of an untapped powder sample to its volume, including the contribution of the inter-particulate void volume.

Tapped density: The tapped density is an increased bulk density attained after mechanically tapping a receptacle containing the powder sample.

Tablet hardness test: is a laboratory technique used to test the breaking point and structural integrity of a tablet under conditions of storage, transportation, and handling before usage.

Friability: is the tendency for a tablet to chip, crumble or break following compression. This tendency is normally confined to uncoated tablets and surfaces during handling or subsequent storage. (or include additional: to test the durability of tablets during transit)

Disintegration test: it is a test carried out to determine whether tablets or capsules disintegrate within the prescribed time when placed in a liquid medium under the given experimental conditions.

Assay: is an analytic procedure carried out for qualitatively assessing or quantitatively measuring the presence of a substance and the amount of that substance.

ABSTRACT

Cassia didymobotrya belongs to the genus *Cassia* and family Fabaceae. *C. didymobotrya* is a shaggy shrub and found in Eastern and Central Africa. Different parts of the plant are used in various countries, namely Kenya, Ethiopia, Rwanda, Tanzania, Angola, Mozambique, Sudan and Uganda for treating variety of disease conditions. The leaves are traditionally mainly used as laxatives.

The leaves of *C. didymobotrya* contain chrysophanol, physcion, aloe-emodin, fallacinol, rhein, parietinic acid, torosachrysone, sennoside B, C and D, flavonoids, α-amyrin, β-amyrin, arachidonic acid, chrysophanic acid, catechinic tannins, kaempferol, lauric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, rhein, glycoside, β-sitosterol stearic acid5, 1,4-anthroquinone chrysophanic acid, daucosterol, physcion, knipholone and several anthroquinine derivatives.

The aim of this work was to formulate and evaluate tablets from the leaf extracts of *C. didymobotrya* to serve as an alternative laxative tablet.

In this study 80% ethanolic extracts of the dried leaves of *C. didymobotrya* were prepared following appropriate cold maceration method. The extract was tested for presence of hydroxyanthracene glycosides by carrying out Bontrager's test which gave a positive result. Total content of hydroxyanthracene glycosides in the dry leaves as well as in the leaf dry extracts was determined using UV-visible spectrophotometric method as stated in BP 2016, which was calculated as sennoside B. The total hydroxyanthracene glycosides percentage content was detected to be 1.077%w/w and 3.6%w/w in the dry leaf powder and leaf extract respectively.

The dried extract of *C. didymobotrya* was observed to be very hygroscopic and it tends to become sticky and liquefied. Thus handling of the extract even at normal room temperature and relative humidity was challenging. To address this problem the *C. didymobotrya* leaf dry extract was premixed with colloidal silicon dioxide. The tablet formulation was then carried out by wet granulation using various excipients (lactose, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, colloidal silicon dioxide, talc and magnesium stearate) with different functions. Two different formulation studies were done by varying the order of addition of the disintegrant sodium starch glycolate. In formulation I, equivalent amount of the disintegrant was

added both intra-granularly and extra-granularly, while in the case of formulation II, the disintegrant was added only extra-granularly.

Prior to compression, the prepared granules of both batches were assessed for bulk and tap density, Hausner's ratio, compressibility and angle of repose according to official methods. Then the compressed tablets were assessed for appearance, hardness, friability, thickness, uniformity of weight, disintegration and content assay. Formulation I was found to have an average disintegration time of 17.75 minutes and formulation II had an average disintegration time of 23.33 minutes. The assay for the percentage content of hydroxyanthracene glycosides, stated as sennoside B was determined using UV-vis spectrophotometer (Shimadzu-1800, Japan) and it was found to be 94.44% for formulation I and 91.78% for formulation II. The value obtained for percentage content was found to lie within the acceptance range; reference was made to the value stated for *Senna* tablets since the method for assay of *Senna* tablets in BP was used. In addition, the tablets obtained from the two formulations also passed tests done for uniformity of weight, hardness, and friability.

1.0 INTRODUCTION

1.1 Background

Herbal drugs have been used since the time of the existence of life on earth. Even now in the modern world, about 80% of the world population, herbal drugs still plays a significant part in satisfying the primary health care requirements of the world population. This is mainly attributed to the cost constraints or inaccessibility of modern drugs to the larger population (Chawla *et al.*, 2013).

Currently there are a number of herbal drugs from different plant species. Plants belonging to the genus *Cassia* are well known for their medicinal use in the traditional system. The leaves and seeds of these plants are traditionally used for treating constipation, cough, flatulence, ringworm, colic, dyspepsia, bronchitis, cardiac disorders and many more. Different parts of the plant consist of various types of phytochemical constituents. The leaf mainly consists of anthraquinone glycosides and flavonoids. Of all the anthraquinone glycosides sennosides are well known for their medicinal importance as a laxative agent (Alemayehu *et al.*, 1989; Singh *et al.*, 2013).

Senna is one such herbal drug that is available in the market for use as a laxative for a long period of time. *Senna* contains the dried leaflets or fruits of *Cassia senna* (*Cassia acutifolia*/ *Alexandrian senna*) and of *Cassia angustifolia* (*Tinnevelly senna*). The constituent responsible for the pharmacological activity of *Senn*a was isolated and identified to be sennosides (A and B), which belongs to the anthraquinone family (Agarwal & Bajpai, 2010; Franz, 1993; Upadhyay *et al.*, 2011).

Cassia didymobotrya belongs to the genus cassia and family Fabaceae. *C. didymobotrya* is a shaggy shrub and found in Eastern and Central Africa (Alemayehu *et al.*, 1989). It is favors open sunny location. It is known as “Popcorn Cassia” due to the characteristic a buttered popcorn like aroma produced by it flowers (Reddy *et al.*, 2010). It is largely found along lakeshores, streams, rivers, deciduous, bush land and old plantations (Swamy *et al.*, 2014).

The leaves of *C. didymobotrya* are used in various countries, namely Kenya, Ethiopia, Rwanda, Tanzania, Angola, Mozambique, Sudan and Uganda for variety of disease conditions such as treating malaria, gastrointestinal problems, pneumonia, and also as a purgative (Mahadevan *et al.*, 2002).

Different secondary metabolites have been reported from the plant, such as sennoside B, C and D, emodin, chrysophanol, physcion, knipholone, alo-emodine, rhein, catechinic tannins, dianthroneemodin, dianthrone aloe-emodin, flavonoids and aloe-emodin B-glucoside (Alemayehu *et al.*, 1989; H.S.Irwin & Barneby, 2008).

Most herbal preparations are liquids which are associated with challenges of accurate dose administration to the consumer, and stability, manufacturing and as well as packaging challenges to the manufacturer. However, solid preparations like tablets on the other hand present the consumer the convenience of administration, and low-cost and high production speed to the manufacturer (Qusaj *et al.*, 2012; Verloop *et al.*, 2004).

Therefore, in this study the leaf extract of *C. didymobotrya* was formulated as tablet dosage form using wet granulation method.

1.2 Problem Statement

Herbal drugs are becoming favored over the currently used modern drugs. The genus cassia is popular for its medicinal use and a number of herbal preparations have been introduced to the market for use by the general population. One of such species that has gained a wide acceptance is official *Senna*. These species are well known for their use as laxatives. These species are predominantly found in Somalia, near the river Nile, the Arabian Peninsula, south India and Pakistan. *C. didymobotrya* also belongs to the same family and traditionally it is reported to have the same use as laxative like the official *Senna* and it is predominantly found in central and East Africa. However, so far no effort has been made to formulate the plant as an official dosage form to serve as an alternative laxative herbal drug.

Therefore, in this study the leaf extracts of *Cassia didymobotrya* were formulated into tablets.

1.3 Objectives

1.3.1 General Objective

The general objective of this study was to extract *Cassia didymobotrya* leaves and formulate it into tablets.

1.3.2 Specific objectives

The specific objectives of this study were

-Extraction of the leaves of *C. didymobotrya*

-Determination of hydroxyanthracene glycosides content in the leaf extract

-Formulation of the leaf extract into tablets

-Evaluation of tablets

1.4 Significance and anticipated outcome

C. didymobotrya is traditionally used as a laxative. The tablets that will be formulated from the leaf extract will allow delivery of the plant in conventional dosage. This will avoid the need to administer crude leaf powder, decoction or other inconvenient traditional preparation forms of the plant to patients. This in turn will increase acceptance of this herbal preparation as an alternative laxative formulation.

1.5 Delimitation

Preformulation study was limited to chemical analysis for the determination of the percentage content of hydroxyanthracene glycosides in the *C. didymobotrya* leaf dry extract in order to determine the dosage.

1.6 Limitation

The study was not done on leaves that were collected from an old plant.

2.0 LITERATURE REVIEW

2.1 Standardization of Herbal drugs

Herbal medicines contain active ingredients that are present in complex mixtures formulated as crude fractions of plants or combination of plants. Herbal drugs are widely accepted as an alternative treatment for primary health care needs in both developing and developed populations. However, herbal medicines have a range of limitations including; lack of evidences of safety, efficacy, standardization, varying production practices and absence of regulatory standards and implementation protocols (Chawla *et al.*, 2013; Mosihuzzaman & Choudhary, 2008).

Standardization of herbal medicines is the process by which herbal preparation is modified to a definite content of a constituent or group of constituents of known therapeutic activity. This is achieved by recommending a set of standards or intrinsic characteristics, definitive qualitative and quantitative values, and constant parameters that carry an assurance of safety, quality, efficacy, and reproducibility (Folashade *et al.*, 2012; Mosihuzzaman & Choudhary, 2008).

The process of standardization involves starting from authentication of the natural flora, collection of plant material, extraction, separation of components of the extract by bioassay guided fractionation and development of herbal drugs exploiting current technical standards. Numerous factors affect the quality of herbal drugs making standardization a requisite. Some of the factors are; active principles in herbal drugs are unknown, herbal drugs are mixtures of complex constituents resulting in a complex physiological response, raw material source and quality variability, absence of selective analytical methods or reference standards for developing standard finger prints to regulate efficacy among various batches, and more (Chawla *et al.*, 2013; Sahil *et al.*, 2011).

The quality issue of herbal dugs can be ensured by conducting some important tests like; micro and macroscopic investigation, moisture content, exclusion of foreign organic matter, extractive values, ash value, qualitative and quantitative chemical tests, chromatographic characterization, toxicological test, phytochemical evaluation, and microbial tests (Chawla *et al.*, 2013; Sahil *et al.*, 2011; Yadav *et al.*, 2011).

Quality, purity and authenticity of herbal drugs can be established by references specified in pharmacopoeia, like structural, analytical, physical standards; process control standards and poly-herbal reference standards for the drugs (Bele & Khale, 2011; Chawla *et al.*, 2013).

Two different approaches can be employed for the standardization of a herbal preparation; First, based on active principle, in cases where the herbal preparation contains known active constituents with known therapeutic values, standardization can be done based on the known active principle, and second, based on marker compounds, a specific compound is used as a marker in cases where the active principle is not known to evaluate the existence of other compounds with therapeutic values in the herbal preparation. "Marker compounds are chemically defined constituents of a herbal drug which are useful for control purposes, independent of whether they have any therapeutic activity or not" (Bele & Khale, 2011; Yadav *et al.*, 2011).

Standardization is the key to ensure batch to batch consistency in efficacy, safety, effectiveness and acceptability of herbal products. This is now possible due to the advance in technology where different approaches, like chemical, chromatographic, botanical, chemical, and biological methods can be employed for estimating active principles present in the crude extract (Bele & Khale, 2011).

2.2 Cassia didymobotrya

C. didymobotrya belongs to the family of Fabaceae, which includes over 300 species.

Plate 1: Growing *Cassia didymobotrya* plant

http://www.stridvall.se/flowers/gallery/album21/D80A3963

2.2.1 Description

Cassia didymobotrya is a "deciduous shrub or small tree up to 4.5 meters tall. The leaves are arranged spirally, paripinnately compound with 8-18 pairs of leaflets; stipules broadly ovate-cordate, 1-2.5 cm × 0.1 cm, acuminate, persistent; Petiole 1-8 cm long; leaflets oblong-elliptical, 2-6 cm × 0.5-2.5cm, mostly rounded to obtuse at apex, mucronate, shortly hairy on both sides, in florescence an erect, axillary raceme 10-50 cm long, 20-30 flowered; bracts 1-2.5 cm long" (H.S.Irwin & Barneby, 2008).

2.2.2 Distribution and climatic conditions required for growth

Cassia didymibotrya is natural found in Kenya, Ethiopia, Sudan, Ethiopia, Uganda, Tanzania, Congo, and other tropical African countries. It was introduced to other parts of the world as an ornamental, a fodder, green manure, and cover crop (H.S.Irwin & Barneby, 2008).

Cassia didymobotrya flowers two times in a year in the tropics. It is grown by seed and by cuttings. The seeds germinate easily, but exhibits dormancy due to its hard seed coat. This can be resolved by different approaches like; soaking in water for 24 hours, dipping in strong sulfuric acid, mechanical scarification, or immersion in boiling water. Germination can take place in dark and light conditions but require an optimum temperature of 20 – 25^{o}C for good germination rate. Harvesting can be done several times in a year, but the leaves have the highest content of constituents if harvested when flowering (H.S.Irwin & Barneby, 2008).

2.2.3 Traditional medicinal use

Cassia didymobotrya is broadly used in Central and East Africa as a traditional medicine to treat various human and livestock diseases. In the treatment of abdominal pain the leaves, stem and root decoction or infusion are used for their laxative and purgative benefits. While in countries like Uganda, Burundi and Rwanda the same decoction is taken to banish intestinal worm and cure ring worm. In some cases a larger quantity of the same decoction is taken as an emetic. The decoction of the leaves is given together with food to children in order to boost appetite. It is also drunk to manage back ache and gonorrhea in women. The liquid from boiled leaves is used for bathing children with food poisoning and also patients with measles. The roots and leaves decoction or root infusion is drunk to treat fever from malaria or other fevers, headache and jaundice. The plant, especially the roots decoction, is used as an antidote for general poison for its purgative and emetic effect. It is also used to treat livestock diseases. Leaf, stem and root

decoction is used for treating cattle skin disease. However, like any other cassia species the plant is poisonous if overdosed resulting in violent vomiting and diarrhoea and could be lethal. Other than its medicinal use, in some communities the inside of gourds used for milk storing is coated with ash of burnt twigs in order to improve digestibility and palatability the milk and to preserve it for more than a year. The plant leaf and root is also used as fish poison (H.S.Irwin & Barneby, 2008; Kokwaro, 2009; Nyamwamu *et al.*, 2015; Thangiah & Ngule, 2013).

2.2.4 Phytochemical profile

The leaves of *Cassia didymobotrya* contain chrysophanol, physcion, aloe-emodin, fallacinol, rhein, parietinic acid, torosachrysone, sennoside B, α-amyrin, β-amyrin, sennoside C, sennoside D, catechinic tannins, lauric acid, flavonoids, arachidonic acid, chrysophanic acid, kaempferol, myristic acid, rhein, myristoleic acid, oleic acid, β-sitosterol stearic acid, palmitic acid, , glycoside, , 1,4-anthroquinone chrysophanic acid, daucosterol, physcion, knipholone and several anthroquinine derivatives (Alemayehu *et al.*, 1989; Mahadevan *et al.*, 2002).

3.0 MATERIALS AND METHODS

3.1 Study design

This was an experimental study.

3.2 Study Location

The study was carried out in the laboratory at the Department of Pharmaceutics and Pharmacy Practice, University of Nairobi, Kenya.

3.3 Equipment/ Apparatus

The following equipment were used for the experimental study; Rotavapor (RII, Buchi, Switzerland), UV-spectrophotometer (Shimadzu -1800, Japan), single tablet press machine (iNWEKA, type Iep-1, India), disintegration tester apparatus (Erweka ZT3-1, GmbH, Germany), hardness tester machine (Scheuniger-2E, Switzerland), friability tester apparatus (Erweka, Gmbh, type TA3R, Germany), drying oven (memmert, Germany), Vernier caliper and weighing balance.

3.4 Plant Material

The plant material (*Cassia didymobotrya* leaf), was gathered from Gatitu village, Kiambu County, Kenya. The other materials required for the study were obtained from the University of Nairobi, School of Pharmacy and from collaborators of the University, Regal Pharmaceuticals Ltd and National Quality Control Laboratory.

3.5 Extraction

The collected leaves of *C. didymobotrya* were dried under shade in the Pharmaceutics and Pharmacy Practice department laboratory. The dried leaves milled with an electronic mill and sieved through a180µm sieve. The dried leaves of *C. didymobtrya* were extracted by maceration procedure using 80% ethanol.

Using maceration method, the powdered leaves of *C. didymobotrya* (150 g) was macerated with 80% ethanol for 72 hours. The extraction with 80% ethanol was repeated three times until exhausted. The extract thereof was combined, filtered and concentrated to dryness by adding acetone using a rotary evaporator. The obtained crude extract yield from the maceration extraction procedure was weighed and percentage yield determined.

3.6 Evaluation of *Cassia didymobotrya* leaf and dry extract

3.6.1 Identification of Anthraquinone glycodsides

Bontrager's reaction was used for detecting presence of anthraquinone glycosides in the extract. In this reaction 5mL of dilute sulphuric acid was added to the sample of 0.5g of *C. didymobotrya* leaf extract, heated for 5 minutes on a water bath and filtered while still hot and allowed to cool. The resulting filtrate was shaken with an equal volume of tetra hydro chloride. Then an equal volume of ammonia was gently added in tilted manner. The formation of a rose pink color in the ammonia layer was taken as a positive result.

3.6.2 Total content of hydroxyanthracene glycosides in *C. didymobotrya* leaf

The determination of the total content of hydroxyanthracene glycosides in the dried leaves of *C. didymobotrya* was done as per the pharmacopoeial method for the *Senna* leaf (British Pharmacopoeia, 2016).

In this method, of the powdered leaves 0.15 g was placed in a 100mL flask and 30 mL of water was added to it, weighed, and heated for 15 minutes under reflux condenser in a water bath. This was cooled, weighed and the weight was adjusted to the initial weight with water. Then this was centrifuged and to the 20 mL of the supernatant liquid transferred in to a 150 mL separating funnel a 0.1 mL of dilute hydrochloric acid was added and shaken with 3 quantities, each of 15 mL, of chloroform. This was allowed to separate and the chloroform layer was discarded. 0.10 g of sodium hydrogen carbonate was added and shaken for 3 min. This was centrifuged and 10.0 mL - of the supernatant liquid was transferred to a 100 mL round-bottomed flask with a ground-glass neck. To this 20 mL of ferric chloride solution was added and mixed. Then the resulting mixture was heated in a water-bath for 20 min under a reflux condenser and 1 mL of hydrochloric acid was added and heated for a further 20 min, with frequent shaking, to dissolve the precipitate. The mixture was then cooled, transferred to a separating funnel and shaken with 3 quantities, each of 25 mL, of ether previously used to rinse the flask. The 3 ether layers were combined and washed with 2 quantities, each of 15 mL, of water. The ether layer was then transferred to a volumetric flask and diluted to 100.0 mL with ether. From this, 10.0 mL was taken and carefully evaporated to dryness and the resulting residue was dissolved in 10.0 mL of a 5 g/L solution of magnesium acetate in methanol. Then finally, the absorbance was measured at 515 nm, using methanol as the compensation liquid.

As per the BP 2016 the percentage content of hydroxyanthracene glycosides is expressed as sennoside B, which was calculated using the equation below (Equation 1): i.e. taking the specific absorbance of sennoside B to be 240.

$$\frac{A \times 1.25}{m} \qquad Equation\ (1)$$

Where,

A is absorbance at 515 nm,

M is mass of the substance to be examined, in grams.

3.6.3 Total content of hydroxyanthracene glycosides in *C. didymobotrya* leaf dry extract

The determination of total content of hydroxyanthracene glycosides in *C. didymobotrya* leaf dry extract was done as per the method stated in BP 2016 for the standardized *Senna* leaf dry extract (British Pharmacopoeia, 2016).

In this method, of the dry extract of the leaves of *C. didymobotrya* 0.15 g was placed in a 100mL flask, dissolved in water and diluted to 100 mL with water. The resulting solution was filtered and the first 10 mL of the filtrate was discarded. 20 mL of the filtrate was transferred to a 150 mL separating funnel and 0.1 mL of dilute hydrochloric acid was added and shaken with 3 quantities, each of 15 mL, of - ether. The layers were allowed to separate and the ether layer was discarded. Then 0.10 g of sodium hydrogen carbonate was added to the aqueous layer and shaken for 3 min. The mixture was centrifuged and 10.0 mL of the supernatant liquid was transferred to a 100 mL round-bottomed flask with a ground-glass neck. 20 mL of ferric chloride solution was added and mixed. Then the resulting mixture was heated in a water-bath for 20 min under a reflux condenser and 3 mL of hydrochloric acid was added and heated for a further 20 min, with frequent shaking, to dissolve the precipitate. The mixture was then cooled, transferred to a separating funnel and shaken with 3 quantities, each of 25 mL, of ether previously used to rinse the flask. The 3 ether layers were combined and washed with 2 quantities, each of 15 mL, of water. The ether layer was then transferred to a volumetric flask and diluted to 100.0 mL with ether. 10.0 mL carefully evaporated to dryness and the residue was dissolved in 10.0 mL of a 5

g/L solution of magnesium acetate in methanol. Then finally, the absorbance was measured at 515 nm, using methanol as the compensation liquid.

As per the BP 2016 the percentage content of hydroxyanthracene glycosides is expressed as sennoside B, which was calculated using the equation below (Equation 2): i.e. taking the specific absorbance of sennoside B to be 240.

$$\frac{A \times 4.167}{m} \qquad Equation\ (2)$$

Where,
A is absorbance at 515 nm,
m is mass of the substance to be examined, in grams

3.7 Preparation of tablets from *Cassia didymobotrya* leaf dry extract

3.7.1 Composition

The raw materials for this study were selected based on identifying the function necessary to formulate the extract into a tablet dosage form. Excipients necessary to aid the formulation and processing of tablet dosage form were included; filler, binder, glidant, adsorbent, lubricant, and disintegrant. The required quantity of each excipient in the formulation was within the recommended range published in the Handbook of pharmaceutical excipients (Rowe *et al.*, 2006). The overall plant extract and excipients proportion used in the formulation are illustrated in the table below (Table 1).

The use of alcoholic solvent for the extraction tends to give herbal extracts that are hygroscopic in nature. This in turn makes it challenging for such herbal extracts to be processed into a solid dosage form and maintain stability under normal storage conditions. This was also evident in the 80% ethanolic extract of *C. didymobotrya* leaf which was very hygroscopic and it tends to become sticky and liquefied even at normal room temperature and relative humidity. Taking this in mind the *C. didymobotrya* leaf dry extract was premixed with colloidal silicon dioxide in a 1:1 ratio to take advantage of the absorbent property of colloidal silicon dioxide; to aid ease of

handling of the extract during processing. This specific ratio of extract to colloidal silicon dioxide was chosen based on a previous study done on an API that exhibited similar hygroscopic behavior like this extract (Choudhari *et al.*, 2015; Hughes *et al.*, 2006; Jeon *et al.*, 2010)

Table 1: Materials and quantity per tablet

Sr. No.	Material	Function	Composition	
			Unit (% w/w per tablet)	Unit (mg per tablet)
Dry extract- Colloidal silicon dioxide premix				
1.	*Cassia didymobotrya* leaf dry extract (equivalent to 3.6 mg sennoside B)	Active ingredient	20	100.00
2.	Colloidal silicon dioxide	Absorbent	20	100.00
Other Excipients				
3.	Lactose	Filler	34.30	171.50
4.	Microcrystalline Cellulose	Filler	14.70	73.50
5.	Acacia	Binder	5	25.00
6.	Sodium starch glycolate	Disintegrant	4	20.00
7.	Purified Water	Granulating Fluid	q.s.	q.s
8.	Sodium lauryl sulfate	Wetting agent	0.5	2.50
9.	Talc	Glidant	1	5.00
10.	Magnesium stearate	Lubricant	0.5	2.50
Total Tablet Weight			100%	500 mg

**Q.s. – Sufficient quantity.

3.7.2 Preparation of Granules

C. didymobotrya leaf dry extract was formulated in to tablets, by following two different types of formulations, by varying the order of addition of the disintegrant in the formulations. In the first formulation, equal amount of the disintegrant was added both intra-granular and extra-granular. In the second formulation, the disintegrant was added only extra-granular. The composition of the two formulation batches is given in Table 2.

The tablets were prepared by wet granulation method using fillers (lactose and microcrystalline cellulose), binder (acacia), absorbent (colloidal silicon dioxide), disintegrant (sodium starch glycolate), wetting agent (sodium lauryl sulfate), glidant (talc), and lubricant (magnesium stearate).

Initial, in both formulations, the *C. didymobotrya* leaf dry extract was pre-mixed with colloidal silicon dioxide in a mortar using pestle till a homogeneous mixture was obtained. To the resulting pre-mix fillers (lactose and MCC), disintegrant (sodium starch glycolate, only in formulation I) and binder (acacia) were added and dry mixed prior to moistening with the granulating solvent (water). Wet massing was done by adding sufficient quantity of water while mixing until a homogeneous wet mass was produced. The wet mass produced was forced through a 1.7 μm sieve as part of the wet screening process and dried at 55^{o}C in hot air oven for 1 hr. Then, the dry granules were passed through a 710 μm and 250 μm sieves to break up agglomerates and to remove excess fine powders respectively, to result in granules with uniform size distribution. The dry screened granules were transferred into a plastic container and the remaining extra-granular ingredients, disintegrant (sodium starch glycolate), wetting agent (sodium laurayl sulfate), and glidant (talc), were added and mixed for 5 minutes prior to adding the lubricant (magnesium stearate). The granules further mixed for 3minutes after addition of the lubricant. The lubricated granules was then kept in a plastic container and kept in a desiccator protected from moisture and direct light. Finally, following the completion of the granulation process physical characterization of the formulated granules was done by carrying out tests.

Table 2: Tablet Formulations

Sr. No.	Material Name	Formulation I		Formulation II	
		%w/w per tablet	Mg/tablet	%w/w per tablet	Mg/tablet
Dry extract- Colloidal silicon dioxide premix					
1.	*Cassia didymobotrya* leaf dry extract (equivalent to 3.6 mg sennoside B)	20	100.00	20	100.00
2.	Colloidal silicon dioxide	20	100.00	20	100.00
Intra-granular Excipients					
3.	Lactose	34.30	171.50	34.30	171.50
4.	Microcrystalline Cellulose	14.70	73.50	14.70	73.50
5.	Acacia	5	25.00	5	25.00
6.	Sodium starch glycolate	2	10.00	--	--
7.	Purified water	Q.s.	Q.s.	Q.s.	Q.s.
Extra-granular Excipients					
8.	Sodium starch glycolate	2	10.00	4	20.00
9.	Sodium lauryl sulfate	0.5	2.50	0.5	2.50
10.	Talc	1	5.00	1	5.00
11.	Magnesium stearate	0.5	2.50	0.5	2.50
Total Tablet Weight		**100 %**	**500 mg**	**100 %**	**500 mg**

3.7.3 Quality assessment of granules

Prior to compression, the lubricated granules was evaluated for flow character and compressibility by carrying out tests like; bulk density, tap density, angle of repose, compressibility index and Hausner's ratio according to official methods.

3.7.3.1 Bulk density

This is the ratio of total mass of powder to the bulk volume of the powder. The bulk density was determined by placing a weighed powder into a measuring cylinder and recording the volume. The bulk density was calculated as follows:

$$D_b = \frac{M}{V_b} \qquad \text{----- Equation (3)}$$

Where D_b is bulk density; M, is mass of powder and V_b is bulk volume of powder.

3.7.3.2 Tap density

This is the ratio of the total mass of powder to the tap volume of powder. The tap density was determined using a Stamp Volumeter, where a weighed quantity of sample was subjected to a mechanical tapping and the volume of the powder after tapping was determined. Then tap density was calculated as follows:

$$D_T = \frac{M}{V_T} \qquad \text{----- Equation (4)}$$

Where D_T is tap density, M is mass of powder, and V_T is tap volume of powder.

3.7.3.3 Hausner's ratio

This is the indirect index for measuring the ease of powder flow (flowability). This was calculated as follows:

$$Hausner's\ ratio = \frac{D_T}{D_b} \qquad \text{----- Equation (5)}$$

Where D_T is tap density and D_b is bulk density.

3.7.3.4 Compressibility index (CI)

Compressibility index is a measure that is obtained from bulk density measurement. The percentage compressibility will be calculated as follows:

$$\%CI = \frac{D_T - D_b}{D_T} \times 100 \qquad \text{------- Equation (6)}$$

Where D_T is tap density and D_b is bulk density.

3.7.3.5 Angle of repose

This was determined using funnel method, where the powder was placed in the funnel and allowed to flow through the funnel orifice freely on to the surface covered with graph paper. Then the height and diameter of the powder cone was measured and angle of repose was calculated as follows:

$$\tan\theta = \frac{h}{0.5 \times d} \qquad \text{------ Equation (7)}$$

Where Θ is angle of repose, h is height of cone powder and $d$is diameter of cone powder.

3.7.4 Preparation of Tablets

Compression was carried out on the obtained lubricated granules by using a single flat faced round punch with 10 mm diameter. Initially, compression machine adjustment for fill weight and compression force was done manually; this was confirmed by taking sample of compressed tablets and testing for the physical parameters, i.e. aesthetic appearance, weight, hardness, disintegration time and friability. In the compression process the machine engine was not engaged because of the small size of each batch. Thus, die filling and punch rotation was done manually. After completion of the compression process, the compressed tablets were collected in a plastic container and kept in desiccation chamber protected from moisture and direct light at room temperature.

3.7.5 Quality assessment of compressed tablets

The compressed tablets were evaluated for both physical and chemical quality attributes such as; appearance, uniformity of weight, hardness, friability, thickness, disintegration, and assay.

3.7.5.1 Appearance

The compressed tablets were visually inspected for properties like color, shape, smell, and texture.

3.7.5.2 Uniformity of Weight

This was determined by measuring the individual and average weight of 20 tablets that are randomly selected. Each individual tablet weight deviation from the average value was calculated.

3.7.5.3 Hardness test

The hardness test was carried out on 10 tablets that were randomly selected from each batch using an electronic hardness tester machine (schleuniger-2E, Switzerland).

3.7.5.4 Friability test

20 randomly selected tablets were pre weighed and placed in a friability tester machine (Erweka, Gmbh, type TA3R, Germany) that was operated at 25 revolutions for 4 minutes. After completion of the revolution period tablets were removed from the tester, de-dusted and weighed. The loss on weight of tablet following the friability study was calculated using the equation stated below. The tablets should not lose more than 1% of their original weight to pass the friability test.

$$F\% = \frac{(W_o - W)}{W_o} \times 100 \quad -------- Equation\ (9)$$

Where F is friability, W_o is initial weight of tablets and W is final weight of tablets (after the friability test).

3.7.5.5 Disintegration time

The disintegration test was done using a disintegration testing machine (Erweka ZT3-1, Gmbh, Germany). According to the method described in the BP 2013, 6 tablets from each batch were place in each tubes of the disintegration tester and immersed in a water bath maintained at 37 ± 2 °C. The time elapsed for each tablet to disintegrate was observed and recorded for each batch.

3.7.5.6 Assay of *Cassia didymobotrya* tablets

This was done as per the method stated in the BP 2016 for assay of *Senna* tablets. In this procedure, 20 tablets were weighed and powdered. The powdered tablet containing equivalent of 7.5 mg of total senossides was taken and to that 30 mL of water was added, weighed, and heated for 15 minutes on a water bath under a reflux condenser. Then this was cooled, weighed and weight adjusted to initial weight with water. The mixture was centrifuged and 20 mL of the supernatant liquid was transferred to a separating funnel, to this 0.1 mL of 2M hydrochloric acid was added and shaken with two 15 mL quantities of chloroform. This was allowed to separate and the chloroform layers are discarded. Then 0.10 g of sodium hydrogen carbonate was added and shaken for 3 min; centrifuged and 10.0 mL of the supernatant liquid was transferred to a

round-bottomed flask with a ground-glass neck. To this a mixture of 8 mL of iron (III) chloride solution and 12 mL of water was added and mixed. Then the resulting mixture was heated in a water-bath heat for 20 min under a reflux condenser and 1 mL of hydrochloric acid was added and heated for further 20 min, with frequent shaking, to dissolve the precipitate. The mixture was then be cooled, transferred to a separating funnel and extracted with three 25 mL quantities of ether previously used to rinse the flask. The combined ether extract layer was washed with two 15 mL quantities of water and sufficient quantity of ether was added to the ether layer to produce 100mL. 10mL of the resulting solution was evaporated to dryness on a water-bath and the resulting residue was dissolved in 10.0 mL of 1M potassium hydroxide. Then finally, the absorbance of the resulting solution was measured at the maximum at 500 nm. The content of total sennosides was calculated as sennoside B, taking 200 as the value of A (1%, 1 cm) at the maximum at 500 nm.

4.0 RESULTS AND DISCUSSION

4.1 Plant extraction

Cassia didymobotrya contains hydroxyanthracene glycosides which are soluble in alcoholic solvent, thus the extraction was done using 80% v/v ethanol.

Extraction of 150 g of *C. didymobotrya* leaf powder was done with 80% ethanol and concentrated to dryness by adding acetone using a rotary evaporator. 30.2 g crude extract was obtained from the extraction procedure and the percentage yield was calculated to be 20.13%.

The dried extract of *C. didymobotrya* leaf was deep dark brown colored, with a characteristic smell, shiny and crystalline like in appearance. But tend to become sticky and liquefied when left in the open air at room temperature and relative humidity. Thus, the extract was kept in a sealed container that was placed in a desiccation chamber.

4.2 Evaluation of *Cassia didymobotrya* leaf and dry extract

The presence of hydrooxyanthracene glycosides in *C. didymobotrya* crude extract was detected by carrying out Bontrager's reaction. This was confirmed positive by the formation of a rose pink color in the ammonia layer.

The total content of hydroxyanthracene glycosides in the dried leaflets of *C. didymobotrya* was determined using UV-visible spectrophotometer. The method specified on BP for *Senna* leaf was used. Following this method the total content of hydroxyanthracene glycosides, expressed as sennoside B, was calculated to be 1.077% w/w (Table 3).

The total content of hydroxyanthracene glycosides, expressed as sennoside B, in the dry extracts produced form *C. didymobotrya* leaf was determined using the method specified on BP for standardized *Senna* leaf dry extract, and it was calculated to be 3.6%w/w (Table 3).

Table 3: Total percentage content of hydroxyanthracene glycosides

Preparation	Percentage content of hydroxyanthracene glycosides (n=3)		
	% content w/w	Standard Deviation	%RSD
Powdered *C. didymobotrya* leaf	1.077	0.008	0.743
C. didymobotrya leaf dry extract	3.60	0.047	1.314

The total content of hydroxyanthracene glycosides, expressed as sennoside B, in the *C. didymobotrya* leaf dry extract was determined to be in the range of 3.56 - 3.65 % w/w (average 3.6% w/w). This value is low when compared to standardized *Senna* leaf dry extract. Senna is reported to contain hydroxyanthracene glycosides, calculated as sennoside B, in a range between 5.5% - 8.0%. *C. didymobotrya* leaf extract contained about 64.7% to 66.6% of hydroxyanthracene glycosides in the standardized *Senna* extract (British Pharmacopoeia, 2016). This lower value may be due to the time of collection of the leaves. As per a study done on *Senna* (*Cassia angustifolia Vahl.*) to determine the influence of leaf picking time on sennosides content, it was found that the best time to collect the leaves in order to get the highest percentage content of sennosides was 90 days after sowing. That is when the plant is still very young. So the study recommended that the collection of *Senna* leaves should be done 90 days after sowing to get maximum sennosides contents (Upadhyay *et al.*, 2011). But in the case of the *C. didymobotrya* leaves collected for this particular study, the plant was a very old. So this could be one reason why the percentage content for sennosides was very low. The other reason could be the geographical location where the plant was grown. The leaves of *C. didymobotrya* used for this study were collected only from a single location, Kiambu County, Kenya, but before making any conclusion about the plant further studies should be done on the plant collected from different geographical locations and comparison should be done to identify the best environmental condition under which the plant gives higher sennosides content. If all these studies are done, then *C. didymobotrya* could become a competitive source of sennosides.

4.3 Granulation

In this study, *C. didymobotrya* tablets were formulated using wet granulation method and tablets were evaluated for bulk density, tap density, Hausner's ratio, compressibility index, ad angle of repose following methods in official monograph procedures.

The granules obtained from the different formulations (Formulation 1 and Formulation 2) had bulk density ranging from 0.53 g/cm^3 to 0.56 g/cm^3 and tap density ranging from 0.59 g/cm^3 to 0.63 g/cm^3 (Table 4).

Characterization of granules flow was done by determining Hausner's ratio and compressibility index based on the values obtained for bulk density and tap density. The values obtained range from 1.12 to 1.13 for Hausner's ratio and 10.17% to 11.11% for compressibility index (Table 4). In reference to official monograph, a material with compressibility index value ranging between 11-15% and Hausner's ratio ranging between 1.12-1.18 is considered to possess good flow character (British Pharmacopoeia, 2013).

Angle of repose was also used to characterize the flow character of the granules. The values obtained for angle of repose range from 31.5^o to 42.3^o (Table 4). A value of angle of repose ranging between 31-35° indicates 'good' flow whereas a value between 41-45° indicates 'passable' flow and may hang up (British Pharmacopoeia, 2013).

Thus, based on the results obtained for compressibility index and Huasner's ratio, the formulated granules from both formulations were rated as granules with 'good' flow character. On the contrary, based on the values obtained for angle of repose, the granules of formulation I have better flow character than formulation II. The discrepancy observed in the results obtained for the scale of flow character may not be attributed to the formulation difference in the two batches, since in both cases equivalent proportion of both the extract and the other excipients were used and the only difference made was in the order of addition of the disintegrant. Rather this may be as a result of relying on manual mixing and sieving which makes it hard to standardize and make it consistent throughout. The other reason could be, the nature of the testing methods used for flow characterizations being qualitative method can itself be a source of discrepancy in the values obtained (Shah *et al.*, 2008).

Table 4: Granules Micromeretic Property

Micromeretic property	Formulations	
	Formulation I	**Formulation II**
Bulk Density	0.53g/cm^3	0.56g/cm^3
Tap density	0.59g/cm^3	0.63g/cm^3
Hausner's ratio	1.12	1.13
Compressibility index	10.17	11.11
Angle of repose	31.5^o	42.3^o

4.4 Tableting

Two batches (Formulation I and Formulation II) of *C. didymobotrya* tablets of batch size 120 tablets were made at uniform fill weight of 500 mg and compression force of about 81N. The tablet pressing machine used is shown in Plate 2 bellow.

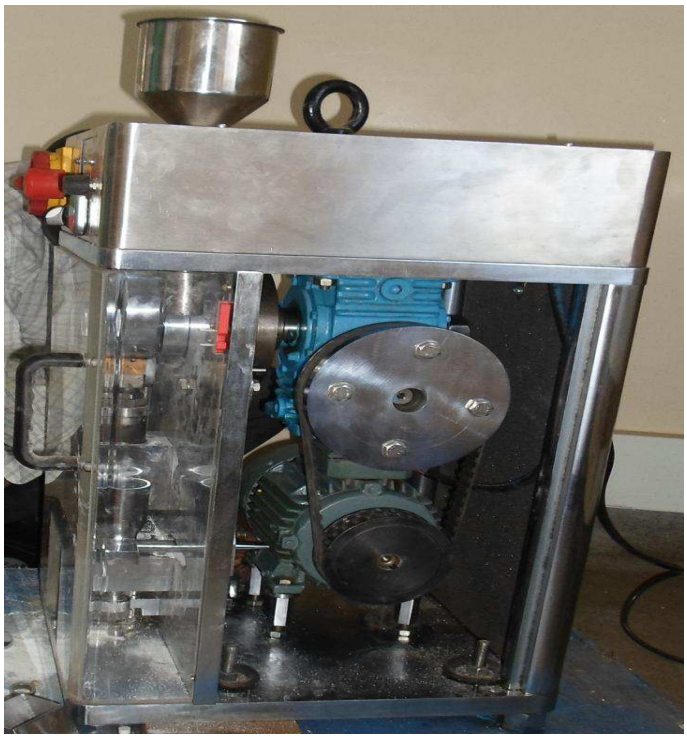

Plate 2: Tableting machine

4.5 Quality assessment of compressed tablets

Appearance: tablets were flat faced; light green, smooth, and shiny in appearance with an average diameter of 10 mm and 5mm thickness.

Uniformity of Weight: Randomly selected 20 tablets from each batch were weighed, mean weight and standard deviation was calculated. The results for uniformity of weight test are presented in Table 5.

Table 5: Results of Uniformity of weight test

Formulation	No of tablets weighed	Mean weight g *Weight*	STDEV	Min Dev. Form the Mean weight	Max. Dev. From the mean weight
Formulation I	20	0.50	0.010	-4.19%	+1.80%
Formulation II	20	0.49	0.012	-2.83%	+3.24%

As per BP 2013, the acceptance limit for uncoated tablets with tablet weight 250 mg or more is ±5% deviation from the average weight. This means, not more than two of the individual tablet weight should deviate from the average weight by more than 5% and none should deviate by more than 10% from the average weight (British Pharmacopoeia, 2013).

In reference to this monograph, all batches of tablets have passed the uniformity of weight test and there was no single tablet that has deviated from the mean weight by more than 5%.

Hardness test: Randomly selected 10 tablets form each batch was weighed individually and hardness test was done using hardness tester machine (Scheuliger-2E, Switzerland). The hardness testing machine used and the results for hardness test are presented in Plate 3 and Table 6 respectively.

Plate 3: Hardness testing machine

In this study a compression force of average 85N and 81.5N was used for formulation I and formulation II respectively. A relatively higher compression force was chosen because tablets formulated at 40-70 N compression force failed the friability test during machine setting process and the appearance of the tablets was not smooth and shiny. However, the choice of higher compression force has not affected the disintegration time significantly and the disintegration time was still within range when compared to the values stated for *Senna* tablets in BP, which is "maximum time, 60 minutes" (British Pharmacopoeia, 2016). Thus, a decision was made to use higher compression force/harder tablet after confirming that the higher hardness value does not affect the other quality parameters.

From the results presented for hardness test in Table 6 it can be seen that since a higher proportion of the values for individual tablets compression force lie far from the mean value for hardness test, a larger value for standard deviation was obtained. This shows that hardness test done for both formulations have low precision. One reason for this higher value of standard deviation could be the fact that the compression of the tablets was done manually without engaging the engine, thus there could be a possible inconsistency in the compression force applied on the powder fill in the dies resulting in a variation in the force required to break the tablets. Second reason could be the fact that the equipment used to test hardness was very old and hence my not guarantee repeatable results.

Friability test: A measurement of a tablet tendency to powder, chip, and fragment during handling and transport was made on 20 randomly selected tablets from each batch. The sampled tablets were subjected to mechanical stress using friability tester (Erweka, Gmbh, Type TA3R, Germany) and the loss on weight following the applied mechanical stress was calculated.

Friability values for both types of formulations were within the specified limit of acceptance (i.e. not more than 1%) (British Pharmacopoeia, 2013). Friability values ranged from 0.4 to 0.70 %. Hence, both formulation I and formulation II have passed the test for friability. However, in comparison to one another formulation I has better tendency to withstand powdering, chipping and fragmentation of tablets during handling and transport. The Friability testing machine used and the results for friability test are presented in Plate 4 and Table 6 respectively.

Plate 4: Friability testing machine

Disintegration test: Disintegration test was done on randomly selected six tablets from each batch using a disintegration testing machine (Erweka ZT3-1, Gmbh, Germany). The Disintegration testing machine used and summary of the results for disintegration test are presented in Plate 5 and Table 6 respectively.

Plate 5: Disintegration testing machine

From the two formulations, formulation I displayed better disintegration time profile with an average of 17.5 minutes. However, formulation II displayed longer disintegration time profile with an average of 23.3minutes. This can be attributed to the mode of addition of the super-disintegrant, sodium starch glycolate. In formulation I, equal distribution of super-disintegrant in both intra-granular and extra-granular phases was made. Conversely, in formulation II, the same disintegrant was added only extra-granular. The addition of a disintegrant both intra-granular and extra-granular will serve as dual function; the portion added extra-granular quickly breaks down the tablet to granules, and the portion added intra-granular further breaks the granules into smaller particles. This in turn resulted in fast disintegration and hence, short disintegration time (Remington *et al.*, 2006). However, when compared to the acceptance limit stated for *Senna* tablets on BP, which is “maximum minutes, 60 minutes”, both formulations have a disintegration time that was still within range (British Pharmacopoeia, 2016).

Table 6: Results of Quality assessment of tablets

Test Parameter		Unit of measure	Sample size	Mean	Standard deviation	%RSD
Formulation I	Uniformity of weight	g	20	0.50	0.010	1.932
	Hardness	N	10	85.30	9.900	11.61
	Friability	%	20	0.40	--	--
	Disintegration time	Min	6	17.75	0.420	2.357
Formulation II	Uniformity of weight	g	20	0.49	0.012	2.404
	Hardness	N	10	81.50	8.440	10.35
	Friability	%	20	0.70	--	--
	Disintegration time	Min	6	23.33	2.880	12.322

Assay of *Cassia didymobotrya* tablets: The method stated in BP for assay of *Senna* tablets was used to determine the percentage content of hydroxyanthracene glyscosides in *C. didymobotrya* tablets. In this procedure 20 tablets were weighed and powdered. The powdered tablet containing equivalent of 7.5 mg of total senossides was taken through the procedure. Summary of the results for assay test are presented in Table 7 below.

Table 7: Assay results of *C. didymobotrya* tablets

Test Parameter		Unit of measure	n	Mean	Content in mg	Standard deviation	%RSD
Formulation I	Assay (% content)	%	3	94.44 %	3.4	1.019	1.079
Formulation II	Assay (% content)	%	3	91.77 %	3.3	0.387	0.421

Following this procedure the percentage content of hydroxyanthracene glycosides, in the two tablet formulations of *C. didymobotrya*, expressed as sennoside B was determined using UV-vis spectrophotometer by measuring the absorbance at 500nm maximum and it was found to be 94.44% for formulation I and 91.78% for formulation II. Both values obtained for percentage content lie within the acceptance range; in comparison to the value stated for *Senna* tablets (i.e. 85% to 115% of the stated amount) since the method for assay of *Senna* tablets on BP was used (British Pharmacopoeia, 2016).

The values for percentage content of total hydroxyanthracene glycosides, calculated as sennosides B indicate that, 94.44% and 91.78% of the stated amount of sennosides are present in formulation I and Formulation II respectively. In other words, 3.4mg and 3.3mg of hydroxyanthracene glycosides, equivalent to sennosides B are present, from the label claim of 3.6mg of hydroxyanthracene glycosides, equivalent to sennoside B, in formulation I and formulation II respectively.

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study showed that, extraction of hydroxyanthracene glycosides from the leaves of *Cassia didymobotrya* can be done by maceration with ethanol. The dried extract was found to be hygroscopic. This hygroscopicity issue was overcome by incorporating an absorbent in the formulation. The leaf extract of *C. didymobotrya* contained 3.6%w/w of total hydroxyanthracene glycosides, expressed as sennoside B. This value is low when compared to that of *Senna* leaf extract.

In the formulation of the extract into a tablet, colloidal silicon dioxide was used effectively as an absorbent to overcome the hygroscopicity of the extract. It was also found that incorporation of the disintegrant both intra-granularly and extra-granularly resulted in tablets with better disintegration.

5.2 Recommendation

From the finding the content of hydroxyanthracene glycosides was low, and with the understanding that the leaves were sourced from an old plant *C. didymobotrya*. Further work should be done using leaves of the plant cultivated in line with commercially cultivated *Senna*. This will also allow studies using the pods of the plant. It is further recommended that the extract thereof be formulated into a capsule dosage form.

REFERENCES

Agarwal, V., & Bajpai, M. (2010). Pharmacognostical and biological studies on senna & its products: An overview. *International Journal of Pharma and Bio Sciences, 1*(2).

Alemayehu, G., Abegaz, B., Snatzke, G., & Duddeck, H. (1989). Quinones of senna didymobotry a. *Bulletin of the Chemical Society of Ethiopia, 3*(1).

Bele, A. A., & Khale, A. (2011). Standardization of herbal drugs: An overview. *International research journal of pharmacy, 2*(12), 56-60.

British Pharmacopoeia, C. (2016). *British pharmacopoeia* (Vol. I): TSO.

British Pharmacopoeia, C. (2013). *British pharmacopoeia* TSO.

Chawla, R., Thakur, P., Chowdhry, A., Jaiswal, S., Sharma, A., Goel, R., Sharma, J., Priyadarshi, S. S., Kumar, V., Sharma, R. K., & Arora, R. (2013). Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: A dreadful lifestyle disorder of 21st century. *Journal of Diabetes and Metabolic Disorders, 12*, 35-35. doi: 10.1186/2251-6581-12-35

Choudhari, Y., Reddy, U., McCarthy, W., & Monsuur, F. (2015). Mesoporous silica gel as a formulation aid for moisture sensitive drugs and excipients. *GRACE*.

Folashade, O., Omoregie, H., & Ochogu, P. (2012). Standardization of herbal medicines-a review. *International Journal of Biodiversity and Conservation, 4*(3), 101-112.

Franz, G. (1993). The senna drug and its chemistry. *Pharmacology, 47*(Suppl. 1), 2-6.

H.S.Irwin, & Barneby. (2008). *Plant resources of tropical africa, medicinal plants 1* (G. H. Schmelzer Ed.): PROTA foundation.

Hughes, K., Crake, N., Farrell, T., Do, N., Yu, F., & Vautravers, S. (2006). Protection and processing of a highly hygroscopic herbal extract by drug layering and film coating. *Abstract of Poster AAPS, 2006*.

Jeon, I., Kumar, A., Vandamme, T., & Betz, G. (2010). Dry granulation and tableting of st. John's wort dry extract: Effect of roll compaction variables, excipients, and tableting speed on granule and tablet properties. *Journal of drug delivery science and technology, 20*(2), 111-118.

Kokwaro, J. O. (2009). Medicinal plants of east africa. *3rd ed* (Kenya Literature Bureau,), 185-187.

Mahadevan, N., Upendra, B., Subburaju, T., Elango, K., & Suresh, B. (2002). Purgative and anti-inflammatory activities of cassia didymobotrya, fresen. *Ancient Science of Life, 22*(1), 9.

Mosihuzzaman, M., & Choudhary, M. I. (2008). Protocols on safety, efficacy, standardization, and documentation of herbal medicine (iupac technical report). *Pure and Applied Chemistry, 80*(10), 2195-2230.

Nyamwamu, L. B., Ngeiywa, M., Mulaa, M., & Lelo, A. E. (2015). Phytochemical constituents of senna didymobotrya fresen irwin roots used as a traditional medicinal plant in kenya.

Qusaj, Y., Leng, A., Alshihabi, F., Krasniqi, B., & Vandamme, T. (2012). Development strategies for herbal products reducing the influence of natural variance in dry mass on tableting properties and tablet characteristics. *Pharmaceutics, 4*(4), 501-516.

Reddy, L. J., Jose, B., Anjana, J., & Ruveena, T. (2010). Evaluation of antibacterial activity of trichosanthes cucumerina l. And cassia didymobotrya fres. Leaves. *Int J Pharm Pharm Sci, 2*(4), 153-155.

Remington, J. P., Troy, D. B., & Beringer, P. (2006). *Remington: The science and practice of pharmacy* (Vol. 1): Lippincott Williams & Wilkins.

Rowe, R. C., Sheskey, P. J., & Weller, P. J. (2006). *Handbook of pharmaceutical excipients* (Vol. 1): Pharmaceutical press London.

Sahil, K., Sudeep, B., & Akanksha, M. (2011). Standardization of medicinal plant materials. *International Journal of Research in Ayurveda & Pharmacy, 2*(4), 1100-1109.

Shah, R. B., Tawakkul, M. A., & Khan, M. A. (2008). Comparative evaluation of flow for pharmaceutical powders and granules. *Aaps Pharmscitech, 9*(1), 250-258.

Singh, S., Singh, S. K., & Yadav, A. (2013). A review on cassia species: Pharmacological, traditional and medicinal aspects in various countries. *American Journal of Phytomedicine and Clinical Therapeutics, 1*(3), 291-312.

Swamy, A. T., Ngule, M. C., Jackie, K., Edwin, A., & Ngule, M. E. (2014). Evaluation of in vitro antibacterial activity in senna didymobotrya roots methanolic-aqua extract and the selected fractions against selected pathogenic microorganisms. *Int. J. Curr. Microbiol. App. Sci, 3*(5), 362-376.

Thangiah, A. S., & Ngule, M. C. (2013). Phytopharmacological analysis of methanolic-aqua extract (fractions) of senna didymobotrya roots. *International Journal of Bioassays, 2*(11), 1473-1479.

Upadhyay, A., Nayak, P. S., & Khan, N. A. (2011). Sennoside contents in senna (cassia angustifolia vahl.) as influenced by date of leaf picking, packaging material and storage period. *Journal of Stored Products and Postharvest Research, 2*(5), 97-103.

Verloop, Q., Liebenberg, W., Marais, A. F., Lötter, A. P., & de Villiers, M. M. (2004). Compounded laxative formulations for substituting phenolphthalein with sennosides a & b in solid dosage forms. *Tropical Journal of Pharmaceutical Research, 3*(1), 265-277.

Yadav, P., Mahour, K., & Kumar, A. (2011). Standardization and evaluation of herbal drug formulations. *Journal of Advanced Laboratory Research In Biology, 2*(4).