Some Qualitative and Quantitative Phytochemical Screening of Local Castor (*Ricinus communis* L.) Seeds and Leaves

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DEDICATION

To:

My kind family;

Respectable teachers;

Faithful friends;

all who wish success, and

gave me the hope to complete this work.

With gratitude and love..

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Abstract

The castor plant can vary greatly in its growth habit and appearance. The variability has been increased by breeders who have selected a range of cultivars for leaf and flower colors, and for oil production. This work aimed to study the phytochemical characteristics of *Ricinus communis* L. leaves and stems, through the detection of the polar and apolar content percentages, the presence of the main phytochemicals and the active ingredients via the TLC technique. Stems and leaves of castor plant were collected from the main campus, University of Gezira. The collected parts were cleaned, dried at room temperature under shade away from the direct sunlight. The dried parts were crushed to fine size particles and were then used for the extraction, thin layer chromatography test (TLC) and phytochemical screening in the Laboratory of Food Analysis, Faculty of Engineering and Technology, University of Gezira. The results of this study revealed that, the polar and apolar contents in the leaves were more than in the stems. According to the used materials, *R. communis* leaves contain more phytochemical classes than the stems. saponins, flavonoids and steroids were detected in the leaves, while, flavonoids and steroids were only detected in stems, whereas, tannins, alkaloids and glycosides were not detected in any tested part. And according to the used materials, *R. communis* leaves contains more phytochemical active ingredients than the stems which contain fewer number of active ingredients (specially, when methanolic extract was used). The study recommends using the materials and the methods that leads to harvest and detect more classes of phytochemicals. Similar phytochemical screening should be run for all the Sudanese aromatic and medicinal plants in order to establish local database. Also further studies using HPLC technique should be run to identify the obtained active ingredients and further antimicrobial activity tests using different parts (especially seeds and flowers) of this plant should be run.

بعض المسح الكيفي والكمي النباتي الكيميائي لبذور وأوراق نبات الخروع المحلي

هناء الدسوقي أحمد محمود

ملخص الدراسة

يمكن أن يختلف نبات الخروع كثيراً في بيئة نموه وفي مظهره. وقد تم زيادة هذا التباين بواسطة علماء الإكثار والذين يتخيرون مدي واسع من الاصناف من أجل ألوان الاوراق والازهار ولإنتاجية الزيت. هدف هذا العمل لدراسة الخصائص الكيميائية النباتية لأوراق وسيقان نبات الخروع، من خلال تحديد نسب المكونات القطبية واللاقطبية، تواجد المجاميع الكيميائية النباتية الرئيسية والمواد الفعالة عن طريق تقنية كروماتوغرافيا الطبقة الرقيقة. جمعت أوراق وسيقان الخروع من حدائق المجمع الرئيسي لجامعة الجزيرة. تم تنظيف العينات المجمعة وتجفيفها في درجة حرارة الغرفة تحت الظل بعيداً عن ضوء الشمس المباشر. تم سحن الأجزاء الجافة لجزيئات صغيرة الحجم، ومن ثم تم إستخدامها للإستخلاص ولإختبار الطبقة الرقيقة ولعمل المسح الكيميائي النباتي في معمل تحليل الأغذية، كلية الهندسة والتكنولوجيا، جامعة الجزيرة. أوضحت نتائج هذه الدراسة بأن المواد القطبية واللاقطبية في أوراق الخروع أكثر من تلك الموجودة في السيقان. وإعتماداً علي للمواد المستخدمة، وجد أن أوراق الخروع تحتوي علي مجاميع كيميائية نباتية رئيسية أكثر من السيقان. المواد الصابونية، الفلافونويدية والاسترويدية تم تحديدها في الأوراق بينما الفلافونويدات والاسترويديات فقط تم تحديدها في السيقان، بينما القلويدات، التانينات، والجلايكوسيدات لم يتم تحديدها في أي من أجزاء نبات الخروع. وإعتماداً علي المواد المستخدمة، إحتوت أوراق الخروع علي مكونات فعالة أكثر من السيقان والتي إحتوت علي أعداد أقل منها (خصوصاً عندما إستخدم المستخلص الميثانولي). توصي الدراسة بإستخدام المواد والطرق المؤدية للحصول علي أكبر قدر من المجاميع الكيميائية الرئيسية وللتحقق منها. مسوحات كيميائية نباتية مماثلة يجب إجراؤها علي كل النباتات الطبية والعطرية السودانية وذلك بغرض عمل قاعدة بيانات محلية. وكذلك ضرورة إجراء تقنية كروماتوغرافيا الطبقة الرقيقة عالية الأداء في البحوث اللاحقة لتحديد هوية المواد الفعالة التي تم الحصول عليها وكذلك يجب إجراء إختبارات للنشاط المضاد للميكروبات بإستخدام أجزاء مختلفة من هذا النبات، خصوصاً البذور والأزهار.

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CHAPTER ONE
INTRODUCTION

A natural product is a chemical compound or substance produced by a living organism-found in nature (Cutler and Cutler, 2000). In the broadest sense, natural products include any substance produced by life. The term natural product has also been extended for commercial purposes to refer to cosmetics, dietary supplements, and foods produced from natural sources without added artificial ingredients (Samuelson, 1999).

Plants have always been a rich source of pharmacologically active nature products (e.g. alkaloids, morphine, cocaine, digitalis, quinine, tubocurarine, nicotine, and muscarine). Many of these compounds are useful drugs in themselves (e.g. alkaloids, morphine and quinine), and others have been the basis for synthetic drugs (e.g. local anaesthetics developed from cocaine. Clinically useful drugs which have been recently isolated from plants include the anticancer agent paclitaxel (Taxol) from the yew tree, and the anti-malarial agent artemisinin from *Artemisia annua* (Peter and Kaufman, 1999).

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome (FDA, 2000).

The castor oil plant can vary greatly in its growth habit and appearance. The variability has been increased by breeders who have selected a range of cultivars for leaf and flower colors, and for oil production. It is a fast-growing, suckering perennial shrub that can reach the size of a small tree (around 12 m or 39 feet), but it is not cold hardy. The glossy leaves are 15–45 cm (5.9–17.7 in) long, long-stalked, alternate and palmate with 5–12 deep lobes with coarsely toothed segments. In some varieties they start off dark reddish purple or bronze when young, gradually changing to a dark green, sometimes with a reddish tinge, as they mature (Christopher, 1996).

Castor oil has many uses in medicine and other applications. An alcoholic extract of the leaf was shown, in lab rats, to protect the liver from damage from certain poisons (Kalaiselvi *et al*., 2003). Methanolic extracts of the leaves of *Ricinus communis* were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The extract was not toxic. The pericarp of castor bean showed central nervous system effects in mice at low doses. At high doses mice quickly died. A water extract of the root bark showed analgesic activity in rats. Antihistamine and anti-inflammatory properties were found in ethanolic extract of *Ricinus communis* root bark (Lomash *et al*., 2010).

The preliminary phytochemical study of *R. communis* revealed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides. The dried leaves of *R. communis* showed the presence of alkaloids, ricinine, flavones, glycosides, monoterpenoids, and ellagic phenolic compounds. The seeds contain 45% of glycosides and also lipases and a ricinine. The castor oil showed the presence of palmitic, stearic, arachidic, oleic, and stearic acids. The castor oil obtained from the seed of the plant is still widely used traditionally and herbally as a medicine (tendraJi and Ashishkumar, 2012).

The objective of this study

This work aimed to study the phytochemical characteristics of *Ricinus communis* L. leaves and stems, through the detection of the polar and apolar content percentages, the presence of the main phytochemicals classes and the active ingredients via the TLC technique.

CHAPTER TWO
LITERATURE REVIEW

2.1 Castor plant

The castor plant (*Ricinus communis*) is a species of flowering plant in the spurge family, Euphorbiaceae. It belongs to a monotypic genus, *Ricinus*, and subtribe, Ricininae. The evolution of castor and its relation to other species are currently being studied using modern genetic tools. Its seed is the castor bean, which, despite its name, is not a true bean. Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India, but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant). Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed contains ricin, a toxin, which is also present in lower concentrations throughout the plant (Phillips and Rix, 1999).

2.1.1 Scientific classification:

Kingdom: Plantae

Class: Angiosperms

Subclass: Eudicots

Superorder: Rosids

Order: Malpighiales

Family: Euphorbiaceae

Subfamily: Acalyphoideae

Tribe: Acalypheae

Subtribe: Ricininae

Genus: *Ricinus* L.

Species: *R. communis,* (Everitt *et al*., 2007).

2.1.2 Description

The castor oil plant can vary greatly in its growth habit and appearance. The variability has been increased by breeders who have selected a range of cultivars for leaf and flower colors, and for oil production. It is a fast-growing, suckering perennial shrub that can reach the size of a small tree (around 12 m or 39 feet), but it is not cold

hardy. The glossy leaves are 15–45 cm (5.9–17.7 in) long, long-stalked, alternate and palmate with 5–12 deep lobes with coarsely toothed segments. In some varieties they start off dark reddish purple or bronze when young, gradually changing to a dark green, sometimes with a reddish tinge, as they mature. The leaves of some other varieties are green practically from the start, whereas in yet others a pigment masks the green color of all the chlorophyll-bearing parts, leaves, stems and young fruit, so that they remain a dramatic purple-to-reddish-brown throughout the life of the plant. Plants with the dark leaves can be found growing next to those with green leaves, so there is most likely only a single gene controlling the production of the pigment in some varieties. The stems (and the spherical, spiny seed capsules) also vary in pigmentation. The fruit capsules of some varieties are showier than the flowers.

The flowers are borne in terminal panicle-like inflorescences of green or, in some varieties, shades of red monoecious flowers without petals. The male flowers are yellowish-green with prominent creamy stamens and are carried in ovoid spikes up to 15 cm (5.9 in) long; the female flowers, borne at the tips of the spikes, have prominent red stigmas. The fruit is a spiny, greenish (to reddish-purple) capsule containing large, oval, shiny, bean-like, highly poisonous seeds with variable brownish mottling. Castor seeds have a warty appendage called the caruncle, which is a type of elaiosome. The caruncle promotes the dispersal of the seed by ants (Christopher, 1996).

2.1.3 Medicinal uses

Castor oil has many uses in medicine and other applications. An alcoholic extract of the leaf was shown, in lab rats, to protect the liver from damage from certain poisons (Joshi *et al*., 2004; Sabina *et al*., 2009; Kalaiselvi *et al*., 2003). Methanolic extracts of the leaves of *Ricinus communis* were used in antimicrobial testing against eight pathogenic bacteria in rats and showed antimicrobial properties. The extract was not toxic (Oyewole *et al*., 2010). The pericarp of castor bean showed central nervous system effects in mice at low doses. At high doses mice quickly died. A water extract of the root bark showed analgesic activity in rats (Williamson, 2002). Antihistamine and anti-inflammatory properties were found in ethanolic extract of *Ricinus communis* root bark (Lomash *et al*., 2010).

2.1.4 Other uses

Extract of *Ricinus communis* exhibited acaricidal and insecticidal activities against the adult of *Haemaphysalis bispinosa* Neumann (Acarina: Ixodidae) and

hematophagous fly *Hippobosca maculata* Leach (Diptera: Hippoboscidae). The Bodo tribals of Bodoland, Assam (India) used the leaves of this plant to feed and rear the larvae of muga and endi silkworms. Castor oil is an effective motor lubricant and has been used in internal combustion engines, including those of World War I airplanes, some racing cars and some model airplanes. It does not mix with petroleum products. It has been largely replaced by synthetic oils that are more stable and less toxic (Zahir *et al*., 2010).

2.1.5 Other modern uses

In Brazil, castor oil (locally known as mamona oil) is now being used to produce biodiesel. In rural areas, the abundant seeds are used by children for slingshot balls, as they have the right weight, size, and hardness. The attractive castor seeds are used in jewelry, mainly necklaces and bracelets. Castor oil was traditionally used on the skin to prevent dryness. This is now used as a base for many cosmetics.

Castor oil in a processed form, called Polyglycerol polyricinoleate—or PGPR, is currently being used in chocolate bar manufacturing as a less expensive substitute for cocoa butter. Castor oil is often used in the USA to repel moles and voles for lawn care. In Mexico this plant is known as Grilla (Everitt *et al*., 2007).

2.1.6 Toxicity

The toxicity of raw castor beans is due to the presence of ricin. Although the lethal dose in adults is considered to be four to eight seeds, reports of actual poisoning are relatively rare (Wedin *et al*., 1986). According to the 2007 edition of *Guinness World Records*, this plant is the most poisonous in the world. Despite this, suicides involving ingestion of castor beans are unheard of in countries like India where castor grows abundantly on the roadsides. The aversion to the use of the beans in suicide could be due to the painful and unpleasant symptoms of overdosing on ricin, which can include nausea, diarrhea, tachycardia, hypotension and seizures persisting for up to a week. However, the poison can be extracted from castor by concentrating it with a fairly complicated process similar to that used for extracting cyanide from almonds. If ricin is ingested, symptoms may be delayed by up to 36 hours but commonly begin within 2–4 hours. These include a burning sensation in mouth and throat, abdominal pain, purging and bloody diarrhea. Within several days there is severe dehydration, a drop in blood pressure and a decrease in urine. Unless treated, death can be expected

to occur within 3–5 days, however in most cases a full recovery can be made (Soto-Blanco *et al*., 2002).

Poisoning occurs when animals, including humans, ingest broken seeds or break the seed by chewing: intact seeds may pass through the digestive tract without releasing the toxin. Toxicity varies among animal species: four seeds will kill a rabbit, five a sheep, six an ox or horse, seven a pig, and eleven a dog. Ducks have shown far more resistance to the seeds: it takes an average of 80 to kill them. The toxin provides the castor oil plant with some degree of natural protection from insect pests such as aphids. Ricin has been investigated for its potential use as an insecticide. The castor oil plant is also the source for undecylenic acid, a natural fungicide. Commercially available cold-pressed castor oil is not toxic to humans in normal doses, either internal or externally (Irwin, 1982).

2.1.7 Chemistry

Three terpenoids and a tocopherol-related compound have been found in the aerial parts of *Ricinus communis*. Compounds named -19-hydroxycasba-3, 7, 11-trien-5-one, 6α-hydroxy-10β-methoxy-7α, 8α-epoxy-5-oxocasbane-20, 10-olide and 15α-hydroxylup-20(29)-en-3-one. Other compounds (-3,4,4a,8a-tetrahydro-4a-hydroxy-2,6,7,8a-tetramethyl-2-(4,8,12-trimethyltridecyl)-2*H*-chromene-5,8-dione were also isolated from the methanol extracts of *Ricinus communis* by chromatographic methods (Tan *et al*., 2009). Partitioned h-hexane fraction of *Ricinus communis* root methanol extract resulted in enrichment of two triterpenes: lupeol and urs-6-ene-3, 16-dione (erandone). Crude methanolic extract, enriched n-hexane fraction and isolates at doses 100 mg/kg p.o. exhibited significant ($P < 0.001$) anti-inflammatory activity in carrageenan-induced hind paw oedema model (Pooja *et al*., 2013).

2.1.8 Phytochemical Constituents

The preliminary phytochemical study of *R. communis* revealed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides. The dried leaves of *R. communis* showed the presence of alkaloids, ricinine (0.55%) and N-demethylricinine flavones glycosides kaempferol-3-Okaempferol-3-O-β-D-glucopyranoside, quercetin xylopyranoside, quercetin-3-O-β-D-glucopyranoside, kaempferol O-β-rutinoside and quercetin-3-O-β-monoterpenoids (1, 8-cineole, camphor and α-sesquiterpenoid (β-caryophyllene), gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid

are the major phenolic compounds isolated from leaves. Indole-3-acetic acid has been extracted from the roots.

The seeds contain 45% of glycosides of ricinoleic, isoricinoleic, stearic and dihydroxystearic acids and also lipases and a ricinine. The castor oil showed the presence of palmitic (1.2%), stearic (0.7%), arachidic (0.3 hexadecenoic (0.2%), oleic (3.2%), linoleic (3.4 ricinoleic (89.4%) and dihydroxy stearic acids. The castor oil obtained from the seed of the plant is still widely used traditionally and herbally as a medicine. The seed of the plant is used as fertilizer after the oil was extracted from the seed and cooked to destroy the toxin and incorporated into animal feeds. The principal use of castor oil is as a purgative and laxative. It is also used as a lubricant, lamp fuel, a component of cosmetics, and in the sink, plastics, fibers, hydraulic fluid, brake fluid, varnishes, paints, embalming fluid, textile dyes, leather finishes, adhesives, waxes, and fungicides. In India, the leaves are used as food for silk worms and the stalks are used for fuel This species has been planted for its dune stabilization R. communis revealed the, alkaloids, flavonoids, and glycosides. Seed showed the presence of two demethylricinine (0.016%), and six O-β-D-zylopyranoside, glucopyranoside, quercetin-3-O-β-D-glucopyranoside, kaempferol-3-Rutinoside. The cineole, camphor and α-pinene) and a gallic acid, quercetin, gentisic ellagic acid are the major phenolic acetic acid has been. The seeds contain 45% of fixed oil, of ricinoleic, isoricinoleic, stearic and also lipases and a crystalline alkaloid, The GLC study of castor oil showed the presence of ester), arachidic (0.3%), linoleic (3.4%), linolenic (0.2%), and dihydroxy stearic acids (4%). The stem also contains ricinine. The ergost-fucosterol; and one probucol isolated from ether extract of seeds.The GC-MS analyses of *R. communis* columns are identified compounds cineole (30.98%), α-pinene (16.88%), and camphor camphene (7.48%) (Jitendra and Ashishkumar, 2012). Phytochemical analysis of *R. communis* extracts was presented in Table (2.1).

2.1.9 Phyto-pharmacology

Antioxidant activity It is concluded that *R. communis* antioxidant activity by using lipid method and free radical scavenging effect on 2,2 picrylhydrazyl radical (DPPH) and hydroxyl hydrogen peroxide. The high antioxidant activity of the seed of *R. communis* at low concentration shows that it could be very useful for the treatment of disease resulting from oxidative stress (Jitendra and Ashishkumar, 2012).

Table (2.1): Phytochemical analysis of *Ricinus communis* extracts

Item	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
Steroids	+	-	+	-	-
Alkaloids	-	-	-	-	-
Phenolic groups	-	+	+	+	+
Flavonoids	-	-	+	+	-
Saponins	-	-	+	-	-
Amino acids	-	-	-	-	-
Anthraquinones	-	-	-	-	-

Source: Narayani *et al*., (2012)

The responsible chemical constituents of antioxidant activity are Methyl ricinoleate, Ricinoleic acid, 12 octadecadienoic acid and methyl ester stem and leave extracts also produce antioxidant activity due to the presence of flavonoids in their extracts Antinociceptive activity The methanolic leaves extract of antinociceptive activity against formalin induced paw licking and The antinociceptive activity showed due to the presence preliminary Phytoconstituents like saponins, steroids and alkaloids, stigmasterol, Y-sitosterol, fucosterol; and one probucol isolated from ether extract of seeds.R. communis essential oil using capillary compounds like α-thujone (31.71%) and 1,8-pinene (16.88%), camphor (12.92%) and Lupeol and 30-Norlupan-3β-ol-20-one are obtained from coat of castor bean (Jitendra and Ashishkumar, 2012).

2.2. Phytochemicals

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome (FDA, 2000).

Compounds in plants (apart from vitamins, minerals, and macronutrients) that have a beneficial effect to the human body are termed phytonutrients. There are over 10,000 of them, and they have effects such as antioxidant, boosting the immune system, anti-inflammatory, antiviral, antibacterial (Table, 2.2), and cellular repair. Highly colored vegetables and fruits tend to be highest in these chemicals, but tea, chocolate, nuts, flax seeds, and olive oil are all excellent sources as well. Various families of plants tend towards certain families of phytonutrients, for example, orange foods tend to have the caretenoid group (Brown and Arthur, 2001and Papp *et al*., 2007).

Table (2.2): Activities of various phytochemicals from plants

Phytochemicals	**Activities**
Phenols and Polyphenols	Antimicrobial, Anthelmintic, Antidiarrhoeal
Flavones	Antimicrobial
Flavonoids	Antimicrobial, Antidiarrhoeal
Tannins	Antimicrobial, Anthelmintic, Antidiarrhoeal
Terpenoids	Antimicrobial, Antidiarrhoeal
Alkaloids	Antimicrobial, Anthelmintic
Glycosides	Antidiarrhoeal
Saponins	Antidiarrhoea

Source: Kumar *et al*., 2010

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans. The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). For aromatic plants, hydrodistillation techniques, hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) may be employed. Some of the latest extraction methods for aromatic plants include headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermomicrodistillation and molecular distillation (Handa *et al*., 2008).

The basic parameters influencing the quality of an extract are (Ncube *et al*., 2008):

1- Plant part used as starting material

2- Solvent used for extraction (Table, 2.3)

3- Extraction procedure

Effect of extracted plant phytochemicals depends on

1- The nature of the plant material

2- Its origin

3- Degree of processing

4- Moisture content

5- Particle size

The variations in different extraction methods depend upon:

1- Type and time of extraction

2- Temperature

3- Nature and concentration of solvent

4- Polarity (Ncube *et al*., 2008)

Phytochemical screening for castor leaf was investigated by Kensa and Syhed (2011), and the obtained results were presented in Table (2.4)

Table (2.3): Solvents used for active component extraction from castor leaf

Water	Ethanol	Methanol	Chloroform	Ether	Acetone
Anthocyanins	Tannins	Anthocyanins	Terpinoids	Alkaloids	Phenol
Starch	Polyphenols	Terpinoids	Flavonoids	Teroinoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonols	Tannins		Fattyacids	
Terponoids	Terinoids	Xanthoxylenes			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			

Source: Cowan (1999)

Table (2.4) The phytochemical screening for castor leaf

Item	Status
Tannins	++
Saponins	++
Flavonoids	+++
Steroids	++
Carbohydrates	+
Alkaloids	+++++
Phenol	++
Resins	+++

Source : (Kensa and Syhed, 2011)

2.3. Chromatography:

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the *mobile phase*, which carries it through a structure holding another material called the *stationary phase*. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the Thin-layer chromatography (TLC) plate at different rates, separation is achieved (Vogel *et al*., 2003).

TLC can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. Specific examples of these applications include: analyzing ceramides and fatty acids, detection of pesticides or insecticides in food and water, analyzing the dye composition of fibers in forensics, assaying the radiochemical purity of radiopharmaceuticals, or identification of medicinal plants and their constituents (Reich and Schibli, 2007).

A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis. This method is referred to as HPTLC, or "high-performance TLC".

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials:

Samples of leaves and seeds of castor plant were collected from El Nishishiba area, and were then immediately cleaned and transferred to the Laboratory of Food Analysis, Faculty of Engineering and Technology, University of Gezira, were the phytochemical analysis were run.

The collected parts of *R. communis* plants were firstly divided into leave and seed parts. The leaves were cut into small pieces. The two parts were dried separately, at room temperature away from direct sunlight, and then grounded to fine particles by using mortar and pestle. One part of the prepared plant materials were subjected to phytochemical screening (presence of some main components such as alkaloids, glycosides, etc) and the other part to the thin layer chromatography test, using recommended methods.

3.2 Methods:

The phytochemical tests (for terpenoids, flavonoids, tannins, saponins and alkaloids) were performed according to the method of Nidhi *et al*., (2013).

3.2.1 Test for Terpenoids:

To 0.5 g each of the extract was added to 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. Reddish brown coloration of the interface indicated the presence of terpenoides.

3.2.2 Test for Flavonoids:

About 4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red color was observed for flavonoids and orange color for flavones.

3.2.3 Test for Tannins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

3.2.4 Test for Saponins:

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously, and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

3.2.5 Test for Alkaloids:

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent. The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

3.2.6 Physical test for Resin:

On heating at a comparatively low temperature, resins soften and finally melt forming sticky or adhesive fluids, without decomposition, but when heated in the air, resins burn readily with a smoky flame (AOAD, 1988).

3.2.7 Test for sterols*:*

The test is carries by adding one drop of concentrated H_2SO_4 to a solution of the glycoside in glacial acetic acid. A change in color occurs from red, through violet and blue to green was taken as an indication of the presence of sterols (AOAD, 1988).

3.2.8 The polar and apolar percentages

Considering the dried weight of the sample product was (W_1) and the dried weight of the filter paper that used to enrolled the sample was (x), the sample weight before the first extraction (using petroleum ether) was ($W_1 + x$), and the dried weight after the first extraction was ($W_2 + x$). Considering also the dried weight of the sample after the second extraction (using methanol) was ($W_3 + x$).

The polar and apolr percentages were calculated from the following formulas:

% Apolar = $[(W_1+ x) – (W_2+ x)$ X 100 /($W_1)$

% Polar = $[(W_2+ x) – (W_3+ x)$ X 100 /($W_1)$

3.2.9 Thin layer chromatography test

In this test, the procedure described by Vogel *et al*., (2003) was carefully followed to run a test for the polar extract (ethanolic extract) and the apolar extract (hexane extract) for each part of the *R. communis* plant.

Identification of the individual components of the crude polar or apolar by thin layer chromatography started by dissolving of about 5 ml of the crude extract in a small volume in ethanol or n-hexane, respectively. The solution was applied as a band, using a micro-syringe on a TLC plate coated with silica gel (0.5 mm thickness), the plate was developed in a tank containing the solvent mixture (chloroform: methanol; 2:1) for about 45 minutes. After solvent drying at room temperature, the developed plate was covered with another clean glass plate exposing a silica gel zone of about 2 cm at one edge, the two plates were clamped together and a provision was made to ensure that only this narrow zone was been reached by the detection reagent.

3.3. Statistical analysis

Microsoft office, Excel program, 2007, was used to present and analyze the obtained data. Simple descriptive statistics and ANOVA single factor were also used to clear the differences observed in the values of the two parts of castor plant.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. The polar and apolar contents of *R. communis* parts

The detection of the polar and apolar contents in the different parts of each plant, will help to understand which part is rich in such contents. Some are prefer to use plant seeds while other are preferring the leaves, stems, roots, bods, or even the exudates, according to their own purpose.

The polar content (methanoilc extract) of *R. communis* leaves and stem were presented in Table (4.1). Stem showed the lowest polar contents (8.1, 8.8 and 9.4%) with a mean of 8.67%, while the % polar content in the leaves were 11.3, 12.4 and 13.0%, with a mean of 12.23%, whereas, the % apolar content (petroleum ether extract) in leaves were 16.8, 18.2 and 13.2%, with a mean of 16.1%, while the % apolar content in the stems were 7.9, 8.2 and 5.3%, with a mean of 7.13%.

The observed difference here was significant (f= 18.19; f-crit=4.06). It was clear that, the % mean of polar and apolar contents in the leaves and stems of *R. communis* were not similar, hence, the leaves contains more polar and apolar constituents than the stems.

Dastagir *et al*., (2013) found that, the oil was 9.2% in castor stem while it was 12.9% in castor leaves.

The choice of solvent is influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay. The choice will also depend on the targeted compounds to be extracted.

Table (4.1): The polar and apolar (%) in *R. communis* stem and leaves

Rep.	Polar %		Apolar %	
	Leaves	Stem	Leaves	Stem
1	12.4	8.1	16.8	7.9
2	11.3	9.4	18.2	8.2
3	13.0	8.8	13.2	5.3

Anova: Single Factor

SUMMARY

Variance	*Average*	*Sum*	*Count*	*Groups*
0.74	12.23	36.7	3	**polar-Leaves**
0.42	8.67	26.3	3	**polar-Stem**
6.65	16.1	48.2	3	**apolar-Leaves**
2.54	7.13	21.4	3	**apolar-Stem**

ANOVA

F crit	*P-value*	*F*	*MS*	*df*	*SS*	*Source of Variation*
4.06	0.0006	**18.19**	47.12	3	141.36	**Between Groups**
			2.59	8	20.73	**Within Groups**
				11	154.8	**Total**

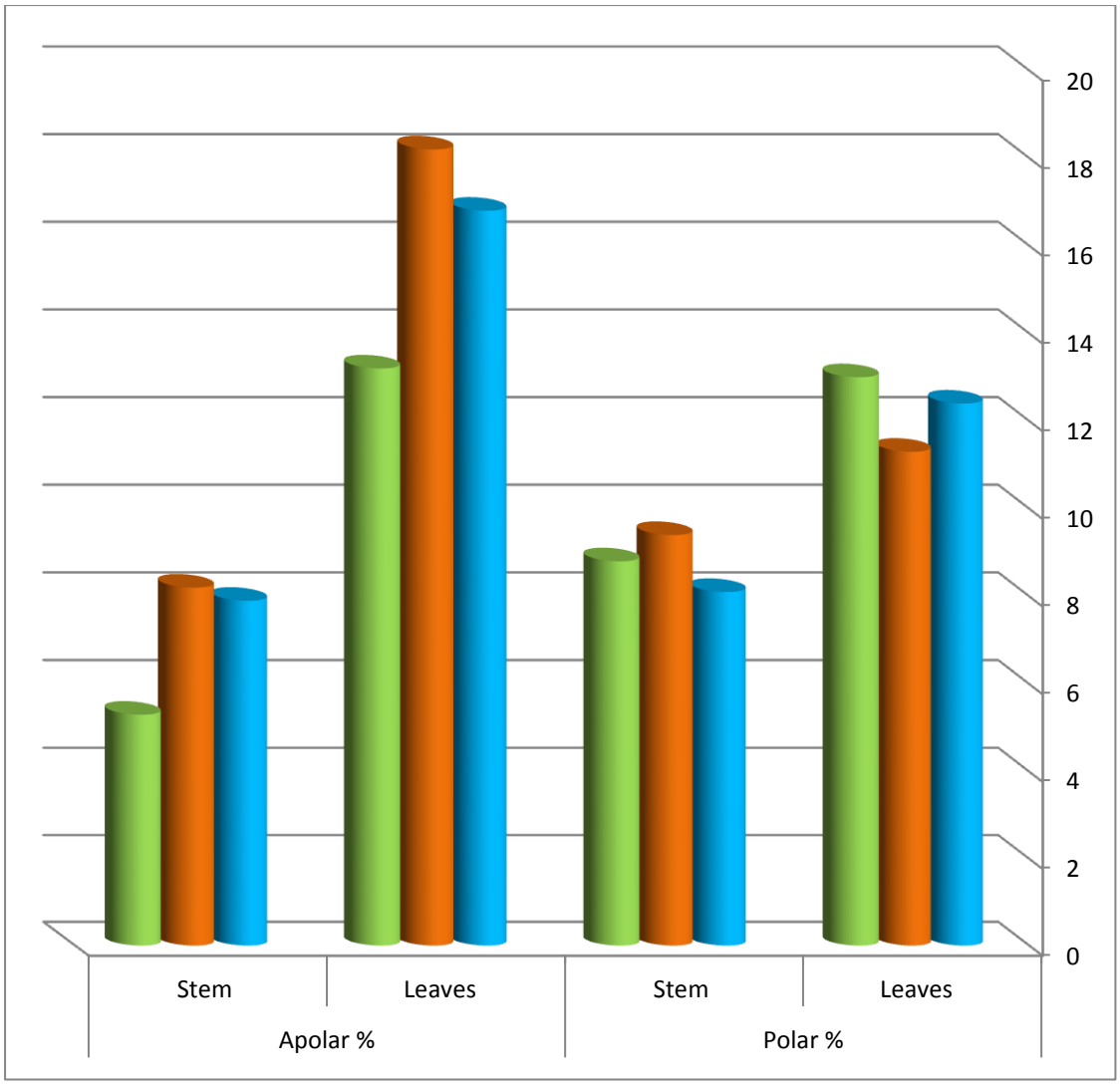

Figure (4.1): The polar and apolar (%) in *R. communis* stem and leaves

4.2 The phytochemical analysis of *R. communis* stem and leaves

The results obtained from the screening for the presence and absence of some phytochemicals in *R. communis* stems and leaves were presented in Table (4.2). The results showed that, saponins, flavonoids and steroids were detected in the leaves, while tannins, alkaloids and glycosides were not detected. Concerning stems, flavonoids and steroids were detected, while saponins, tannins, alkaloids and glycosides were not detected.

R. communis leaves proved to contains more polar and apolar constituents and more phytochemicals compared to stem.

Nidhi *et al*., (2013), detected the presence of tannins and resins, while sterols, alkaloids, flavonoids and saponins were not detected in *R. communis*.

Tripathi *et al*., (2011), reported that, the ethanol extract of the dried, powdered hull portion of *R. communis* seeds indicated the presence of alkaloids, steroids, flavonoids, glycosides and phenolics.

In another experiment, terpenoids and alkaloids were not detected in *R. communis* leaves (Yadav and Munin, 2011).

Kehail (2004) proved that, *R. communis* seed powder were the best source of toxic against *Anopheles* and *Culex* mosquitoes amongst about 20 plant species from Wad Medani. Gezira State.

The enrichment of *R. communis* with several qualitative and quantitative phytochemicals makes it a potential source of medicine for several diseases and pest control program (since it exerted acaricidal and insecticidal activities) as was shown in Table (2.2; page 10 and 2.4; page 13).

Table (4.2): The phytochemical analysis of *R. communis* stem and leaves

Rep.	**Leaves**	**Stem**
Tannins	-	-
Saponins	+	-
Alkaloids	-	-
Flavonoids	+	+
Glycosides	-	-
Steroids	+	+

+ indicated the presence of the class,

– indicated the absence of the class.

4.3 The thin layer chromatography for *R. communis* seeds and leaves

The R_f values obtained from the spots that were separated in the TLC tests for *R. communis* leaves and stems were presented in Table (4.3).

In the petroleum ether extract, two spots were detected in the stems (R_f values were: 0.32 and 0.95), while 5 spots were separated in the leaves sample (R_f values were: 0.32, 0.44, 0.68, 0.88, and 0.95).

In the methanol extract, 5 spots were detected in the stems (R_f values were: 0.06, 0.50, 0.72, 0.94 and 0.97), also 5 spots were separated in the leaves sample (R_f values were: 0.69, 0.75, 0.84, 0.94 and 0.97).

It was clear that, *R. communis* leaves contain more active ingredients (10 spots) than stems (7 spots)

Obumselu *et al*., (2011) stated that, TLC for *R. communis* leaves, using development system of ethyl acetate: chloroform: methanol: water (15: 8: 4: 1), detected two spots from ethyl acetate extract (R_f value = 0.525 and 0.678) and two spots from ethanol extract (R_f value = 0.423 and 0.695), while Habib *et al*., (2011), detected 5 spots (R_f= 0.49, 0.50, 0.52, 0.71 and 0.88) and 4 spots (R_f= 0.59, 0.65, 0.68 and 0.88) from *R. communis* leaves and stems, respectively.

Table (4.3): The R_f values revealed by the TLC of *R. communis* stem and leave

Spot No.	Petroleum ether		Methanol	
	Stems	Leaves	stems	Leaves
1	0.32	0.32	0.06	0.69
2	0.95	0.44	0.50	0.75
3		0.68	0.72	0.84
4		0.88	0.94	0.94
5		0.95	0.97	0.97

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1- Polar and apolar contents in the leaves and stems of *R. communis* plant were significantly not similar i.e. *R. communis* leaves contains more polar and apolar contents than the stems.

2- According to the used materials, *R. communis* leaves contain more phytochemical classes than the stems. saponins, flavonoids and steroids were detected in the leaves, while, flavonoids and steroids were only detected in stems, whereas, tannins, alkaloids and glycosides were not detected in any tested part.

3- According to the used materials, *R. communis* leaves contain more phytochemical active ingredients than the stems which contain fewer number of active ingredients.

5.2. Recommendations

1- The materials and the methods that lead to harvest and detect more classes of phytochemicals, must be used.

2- Similar phytochemical screening should be run for all the Sudanese aromatic and medicinal plants inorder to establish local database.

3- Further studies using HPLC technique should be used to identify the obtained active ingredients.

4- Further antimicrobial activity tests using different parts (specially seeds and flowers) of this plant should be run.

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