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Study of Antioxidants, Phytochemicals and Heavy Metals in Crude Leaf Extract in Different Solvents of Castor (*Ricinus communis*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author BBT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GR and SG managed the analyses of the study. Author SG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The castor oil plant *Ricinus communis* is a member of the family Euphorbiaceae. This plant is a member of the genus Ricinus which is traditionally called castor bean. Castor seed is the source of castor oil, which has a number of uses. The leaves were dried in an oven at 50 – 60ºC for 72 h. The moisture content was calculated. The dried leaves were grounded and extracted in chloroform, ethanol, ethyl acetate and methanol solvents. Each solvent extract was collected separately and evaporated on rotavapour. The semi-solid liquid extract was made to the desired volume by the addition of the respective solvent. Antioxidant power estimation and phytochemical screening of leaves extract for alkaloids, carbohydrates, Saponin, proteins & amino acids, tannins, flavonoids,

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glycosides and terpenoids were done by standard test procedures reported in the literature. Atomic absorption spectrophotometric study revealed the presence of the heavy metals Cu, Ca, Cr, Zn, Mn, Fe and Mg in decreasing order of their concentrations. Carbohydrate was tested positive in four solvent extracts. Alkaloids and Saponin were tested negative in each of the four solvent extracts. All four leaf extracts were found to have antioxidant potential.

Keywords: Ricinus communis (castor); antioxidant; phytochemicals; chloroform; ethanol; ethyl acetate; methanol; heavy metals.

1. INTRODUCTION

Ricinus communis 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 [1]. *R. communis* can vary greatly in its growth habit and appearance. It is a fast growing suckering perennial shrub that can reach the size of a small tree (around 12 meters or 39 feet). The glossy leaves are 15-45 cm long, long stalked, alternate and palmate with 5-12 deep lobes with coarsely toothed segments.

Three terpenoids and a tocopherol- related compound have been found in the aerial parts of *R. communis*. Compounds named (3E, 7Z, 11E) – 19 hydroxycasba – 3, 7, 11 –trien-5-one, 6α– hydroxyl – 10β – methoxy - 7α, 8α-epoxy-5-oxocasbane-20, 10-olide, 15α-hydroxylup-20(29)-en-3-one, and (2R, 4aR, 8aR – tetrahydro – 4a – hydroxyl-2, 6, 7 8a – tetramethyl-2 (4, 8, 12 – trimethyltridecyl)- 2H – chromene – 5, 8-dione were isolated from the methanol extracts of *R. communis* by chromatographic methods [2]. Partitioned n-hexane fraction of *R. communis* root method extract resulted in enrichment of two triterpenes lupeol and urs-6-ene-3, 16-dione (erandone). Crude methanolic extract, enriched in hexane fraction and its isolates at doses 100 mg/kg P.O. exhibited significant ($P< 0.001$) anti-inflammatory activity in carrageenan-induced hind paw edema model [3].

Ebers papyrus is an ancient Egyptian medical papyrus of herbal knowledge which describes castor oil as a laxative [4]. Castor oil is well known as a source of ricinoleic acid, a monounsaturated, 18-carbon fatty-acid. Among fatty acids, ricinoleic acid is unusual in that it has a hydroxyl functional group on the 12^{th} carbon. This functional group causes ricinoleic acid (and castor oil) to be more polar than most fats. The chemical reactivity of the alcohol group also allows chemical derivation that is not possible with most other seed oils. Because of its ricinoleic acid content, castor oil is a valuable chemical in feed stocks, commanding a higher price than other seed oils.

Singh and Geetanjalie [5] have described pharmacological (e.g. anti-inflammatory, anti-diabetic, anti-tumor, anti-asthmatic potential and other medicinal properties of extracts of different plant parts of *R. communis*. They have also investigated the presence of important phytochemical constituents such as flavonoids, glycosides, alkaloids, steroids, terpenoids, etc. and their possible structure in the same extract. Anti-dandruff activity of *R. communis* L. methanol, aqueous, chloroform and petroleum ether leaf extracts, against Malassezia spp, causative agent of dandruff in people who have overactive sebaceous gland was presented by Sibi et al. [6].

Gupta et al. [7] demonstrated strong antioxidant potential of the methanolic extract of *R. communis* leaves. Antioxidant, anti-microbial and free radical scavenging potential of various extracts of aerial parts of *R. communis* were examined by Iqbal et al. [8]. The present study provides evidence that *R. communis has* proven to be a potential source of natural antioxidants that could replace synthetic antioxidants. Vandita et al. [9] have investigated the effects of tannins, alkaloids, cardiac glycosides, terpenoids, flavonoids and steroids of *R. communis* on antibacterial, fungal and cytotoxic activities. The cytotoxic effects of selected plants were tested against HEK 293T (Human embryonic kidney cell line) and C2C12 (Mouse, muscle cell line) by MTT assay.

Kadri et al. [10] have investigated in vitro antioxidant properties of essential oil of *R. communis* L. The essential oil from the aerial parts of *R. communis* was obtained by hydro-distillation and analyzed by GM-MS. Antioxidant activity of the investigated essential oil was evaluated by different test systems: 1, 1-diphenyl – 2 picrythydrazyl (DPPH) assay, β-carotene bleaching test and reducing power assay. The

essential oil exhibited a potential antioxidant activity.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The plant material, leaves of *R. communis*, was collected from the Institute of Applied Science and Technology (IAST), University of Guyana, Turkeyen Campus, Georgetown, Guyana.

2.2 Preparation of Plant Materials

The collected leaves material of *R. communis* was weighed on Citizen CTG 3000E electronic balance. The leaves were dried in an oven (Gallenhamp Incubator Model IH – 150) at 50-60°C. The dried leaves were cooled at room temperature and weighed again on the same Citizen electronic balance.

2.3 Extraction and Preparation of Test Solutions

The ground leaves of *R. communis* were extracted in respective amounts of ethyl acetate, ethanol, ethanol and chloroform solvents. Each time 20 grams of pulverized leaves was soaked with 200 ml of solvent for 48 hours. The solvent was decanted each time and residue again soaked with the same solvent for 24 hours. All the extracts were then combined and filtered. The evaporation of solvent was done on rotavapour (Buchi). The respective solvent was added to viscous semisolid liquid extract to make up the derived volume of extract solution.

2.4 Reducing Antioxidant (Protective) Power

Potassium ferricyanide, trichloroacetic acid, butylated hydroxyl anisol, sodium dihydrophosphate, ferric chloride, ammonium thiocyanate, ferric chloride, linoleic acid (99.5%), thiobarbituric acid, sodium monohydrophosphate, and potassium dihydrophosphate were obtained from Aldrich, USA. All chemicals were used without further purification. All aqueous solutions were prepared in double distilled water.

The reducing antioxidant or protective power of the ethanolic, ethyl acetate, chloroform and methanolic *R. communis* leaf extracts were determined by the method reported in the literature. The different concentrations of leaf extracts (100-1000 µL) in 1 m L of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 pH 6.6) and potassium ferricyanide $K_3Fe\ (CN)_6$ (2.5 mL 1%). The mixture was incubated at 50°C for 20 minutes. Then 2.5 m L of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 minutes at 3,000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water 92.5 mL and $FeCl_3$, 0.5 mL 1%. The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Phillips X 500). Increased absorbance of the reaction mixture indicates increase in the reducing power.

2.5 Phytochemical and Heavy Metals Analysis of the Plant Extracts

Phytochemical analysis of ethanolic, methanolic, ethyl acetate and chloroform leaves extract was carried out by suitable methodologies in search of active ingredients responsible for antimicrobial toxicity. The phytochemicals investigated were Saponin, terpenoids, alkaloids, glycoside, carbohydrates, protein and amino acids, tannins and flavonoids. The phytochemical analysis was carried out according to the method reported in the literature by Edeoga et al. [11].

The leaves (2g) was treated with 10 cm^3 aqua regia (75% vol hydrochloric acid and 25% vol nitric acid) and heated to dryness. Distilled water (20 cm^3) was added and the mixture stirred and filtered. The filtrate was subjected to analysis using Xplor AA – GOC Scientific Equipment Atomic Absorption Spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Potential of *R. communis* Leaves Extract

Antioxidant potential is the measure of the reducing ability of the antioxidant. Antioxidant potential is evaluated by measuring the transformation of iron (III) to iron (II) in the presence of the sample extract [12]. The ability to reduce iron (III) may be the results of hydrogen donation from phenolic compounds], which is also related to the presence of some reducing agent [13]. In addition, the number and position of hydrogen group of phenolic compounds also affect their antioxidant potential [14]. The increase in concentrations of leaves extract may also cause deviation from increase in its reducing power which may be due to decrease in

hydrogen donor ability of phenolic compounds. The reducing power of chloroform, ethanolic, ethyl acetate and methanolic *R. communis* leaf extracts are given in Tables 1, 2, 3 and 4 respectively.

Table 1. Reducing antioxidant power of chloroform leaf extract of *R. communis* (Castor)

S. no.	Volume of leaf extract (µL)	Absorbance of leaf extract (nm)
1	Chloroform (control)	0.000
2	1.00	0.001
3	2.00	0.004
4	3.00	0.004
5	4.00	0.005
6	5.00	0.006
7	6.00	0.006
8	7.00	0.007
9	8.00	0.006
10	9.00	0.005
11	10.00	0.009

Table 2. Reducing antioxidant power of ethanol leaf extract of *R. communis* (Castor)

S. no.	Volume of leaf extract (µL)	Absorbance of leaf extract (nm)
1	Ethanol (control)	0.000
2	1.00	0.001
3	2.00	0.003
4	3.00	0.004
5	4.00	0.002
6	5.00	0.007
7	6.00	0.007
8	7.00	0.008
9	8.00	0.009
10	9.00	0.006
11	10.00	0.007

From Tables 1 to 4 regarding antioxidant power of chloroform, ethanolic, ethyl acetate and methanolic leaf extracts of *R. communis, results* reveal that the antioxidant powers of chloroform, ethanolic, ethyl acetate and methanolic leaf extract of *R. communis* were found to be nearly equal. In the chloroform extract (Table 1) highest absorbance was observed at 10.0 µL concentration, while the absorbance was lowest at 1.0 µL concentration of leaf extract; thus, lowest observance indicates maximum antioxidant power. In ethanolic extract (Table 2) highest absorbance was recorded at 8.0 µL concentration, while lowest was at 1.0 µL concentration. Antioxidant power decreases after 8.0 µL concentration. This may be due to decrease in hydrogen donor ability of phenolic compounds. In ethyl acetate extract (Table 3) minimum difference in the absorbance of leaves extract and control was observed at 1.0 µL concentration, while highest and same (0.009 nm) difference in absorbance was noted at 5.0 µL, 6.0 µL, 8.0µL and 9.0 µL concentrations. Methanolic leaves extract (Table4) was found to have same antioxidant power or difference in absorbance from control as ethyl acetate. The maximum difference in absorbance (0.009 nm) was found for each solvent extracts. That is no definite order of increase or decrease in anti-oxidant power (from 1.0 µL to 10.0 µL concentration of extract) was observed in all solvent systems.

Table 3. Reducing antioxidant power of ethyl acetate leaf extract *R. communis* (Castor)

S. no.	Volume of leaf extract (µL)	Absorbance of leaf extract (nm)
1	Ethyl acetate (control)	0.000
2	1.00	0.005
3	2.00	0.006
4	3.00	0.007
5	4.00	0.008
6	5.00	0.009
7	6.00	0.009
8	7.00	0.008
9	8.00	0.009
10	9.00	0.009
11	10.00	0.007

Table 4. Reducing antioxidant power of methanol leaf extract of *R. communis* (Castor)

S. no.	Volume of leaf extract (µL)	Absorbance of leaf extract (nm)
1	Methanol (control)	0.000
2	1.00	0.005
3	2.00	0.006
4	3.00	0.007
5	4.00	0.008
6	5.00	0.009
7	6.00	0.009
8	7.00	0.008
9	8.00	0.009
10	9.00	0.009
11	10.00	0.007

Table 5. Phytochemical analysis of Castor (*R. communis*) leaves extract

S. no.	Phyto constituents	Ethanol	Methanol	Ethyl acetate	Chloroform
1	Alkaloids	-	-	-	-
2	Carbohydrate	+	+	+	+
3	Saponin	-	-	-	-
4	Protein and amino acids	-	+	-	-
5	Tannins	+	+	-	+
6	Flavonoids	+	-	+	-
7	Glycosides	-			
8	Terpenoids	+	-	-	+

Note: - = Absent; + = Present

From the Table 5 the results reveal that Phyto - constituent, alkaloids are absent in each of the four solvents (chloroform, ethanol, ethyl acetate and ethanol) extracts. Carbohydrate is present in the leaves extract of each solvent. Saponin is found to be negative in each of the four leaves extracts. Protein and amino acids are found to be present in methanolic extract while absent in chloroform, ethyl acetate and methanolic extract. Tannins were found to be positive in ethanolic, methanolic and chloroform extracts, while negative in ethyl acetate leaves extract. Flavonoids were found to be present in ethanolic and ethyl acetate leaves extract, while absent in methanolic and chloroform leaves extracts. Glycosides were found negative in ethanolic extract and could not be detected in methanolic, ethyl acetate and chloroform extracts. Terpenoids are found to be present in ethanolic and chloroform extracts and absent in ethyl acetate and methanolic extract.

Table 6. Heavy metal analysis in Castor *(R. communis)* leaves in mg/ kg

Name of plant	Used part	Metals	Mg/kg
Castor	Leaves	Zn	30.87
(R. communis)		Cu	2.14
		Ni	Nd
		Mn	21.64
		Fe	128.20
		Ca	84.42
		Mg	247.10

Nd = Not detected

Heavy metal analysis was done in leaves of *R. communis* by using Xplor AA – GOC Scientific Equipment Atomic Absorption Spectrophotometer. It shows the highest amounts was that of Mg (247.10 mg/kg), followed by Fe (128.20 mg/kg), whereas the least amount was that of Cu (2.14 mg/kg), but there was no detection of Ni metal.

4. CONCLUSION

This study scientifically validates the use of the leaves of *Ricinus communis* as antioxidant power of chloroform, ethanolic, ethyl acetate and methanolic leaf extracts of *R. communis*. Results reveal that the antioxidant power of chloroform, ethanolic, ethyl acetate and methanolic leaf extract of *R. communis* were found to be nearly equal. Phyto - constituent alkaloids are absent in each of the four solvents but carbohydrate was present in the extract of each solvent. In this study, we detected seven components of heavy metals not previously reported, and confirmed the high Mg and Fe presence in *R. communis* leaves. This information gives light to the present intention to find chemical proof that supports the pharmacological activities of *R. communis* leaves.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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