Original Research Article

Study of antioxidant, phytochemicals and heavy metals in crude leaf extract in different solvents of Castor (*Ricinuscommunis*)

Abstract:

The castor oil plant *Ricinuscommunis* is a member of the family Euphorbiaceae. This plant is a member of the genus Ricinus which is traditionally called as castor bean. Castor seed is the source of castor oil, which has a number of uses. The sample leaves of *Ricinuscommunis* (castor) obtained from Institute of Applied Science and Technology (IAST), University of Guyana, Turkeyen Campus, Georgetown, Guyana. Leaves were dried in oven at 50 – 60 ºC for 72 h. The moisture content was calculated. The dried leaves were grounded and extracted in each chloroform, ethanol, ethyl acetate and methanol solvents. Each solvent extract collected separately and evaporated on rotavapour. The semi solid liquid extract was made to desired volume by the addition of respective solvent. Antioxidant, phytochemical screening of leaves extract for alkaloids, carbohydrates, saponins, proteins & amino acids, tannins, flavonoids, glycosides and terpenoids were done by standard test procedure reported in literature. Atomic absorption spectrophotometric study revealed the presence of the heavy metals Cu, Ca, Cr, Zn, Mn, Fe and Mg in the decrease order of their concentrations. Carbohydrate was tested positive in four solvent extracts. Alkaloid and saponins were tested negative in each four solvent extracts. All four leaves extract were found to have antioxidant potential.

Keywords: *Ricinuscommunis* (castor), antioxidant, phytochemicals, chloroform, ethanol, ethyl acetate, methanol, heavy metals.

INTRODUCTION:

Ricinuscommunis seed is the castor bean, which despites 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 is 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 physician 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 derivatization 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, steroid, 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 malasseziaspp, causative agent of dandruff in people who have over active 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* was examined by Iqbal et al. [8]. The present study evidence that *R. communis* proved to be potent natural antioxidants that could replace synthetic antioxidants. Vandita et al. [9] has investigated the effects of tannins, alkaloids, cardiac glycosides, terpenoids, flavonoids and steroids of *R. communis* on antibacterial, fungal and cytotoxic activities. The cytotoxic effect 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] has 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

MATERIALS AND METHODS:

Collection of Plant materials

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

Preparation of Plant Materials

The collected leaves material of *R. communis*was weighted on Citizen CTG 3000E electronic balance. The leaves were dried in oven (Gallenhamp Incubator Model IH – 150) at 50-60 ^{0}C. The dried leaves were cooled at room temperature and weighted again on same citizen electronic balance.

Extraction and Preparation of Test Solutions

The ground leaves of *R. communis*was extracted in each ethyl acetate, ethanol, ethanol and chloroform solvents. Each time 20 grams of pulverized leaves were soaked with 200 ml of solvent for 48 hours. The solvent is decanted each time and residue again soaked with same solvent for 24 hours. The all extract is 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.

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 plant ethanolic, ethyl acetate, chloroform and methanolic extract were determined by the method reported in literature [68, 69]. The different concentration of leaf extracts (100-1000 µL) in 1 m L of distilled water were mixed with phosphate buffer (2.5 m L, 0.2 pH 6.6) and potassium ferricyanide $K_3Fe(CN)_6$ (2.5 m L 1 %). The mixture was incubated at 50^0C for 20 mins. Then 2.5 m L of trichloroacetic acid (10 %) was added to mixture, which was then centrifuge for 10 mins at 3,000 rpm. The upper layer of solution (2.5 m L) was mixed with distilled water 92.5 m L and $FeCl_3$,0.5 m L 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.

Phytochemical and heavy metals analysis of the plant extracts

Phytochemical analysis of ethanolic, methanolic, ethyl acetate and chloroform leaves extract were carried out by suitable methodologies in search of active ingredients responsible for antimicrobial toxicity. The phytochemicals investigated were saponins, terpenoids, alkaloids, glycoside, carbohydrates, protein and amino acids, tannins and flavonoids. The phytochemical analysis was carried out according to the method reported in literature by Edeoga et al. [70].

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

RESULTS AND DISCUSSION:

Antioxidant potential of *R. communis* leaves extract

Antioxidant potential is the measure of reducing ability of the antioxidant. Antioxidant potential is evaluated by measuring the transformation of iron (III) to iron (II) in the presence of sample extract [11]. The ability to reduce iron (III) may be results from hydrogen donation from phenolic compounds [12], 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

130 **Table 2: Reducing antioxidant power of ethanol leaf extract of *R. communis***

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

131 **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

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133 **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

From Tables 1 to 4regarding 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. In chloroform extract (Table 1) highest absorbance was observed at 10.0 µL concentration, while lowest at 1.0 µL concentration of leaf extract was found to have maximum antioxidant power.In ethanolic extract (Table2) highest absorbance was recorded at 8.0 µL concentration, while lowest 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.There 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 5: Phytochemical analysis of Castor (*R. communis*) leaves extract

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

Note: - = Absent+ = Present

From the Table 5results reveal that Phyto - constituent, alkaloids is absent in each 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 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)* leavesin mg/ kg

Name of plant	Used part	Metals	Mg/kg
Castor *(R. communis)*	Leaves	Zn	30.87
		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 (128.20mg/kg**),** whereas the least amount was that of Cu (2.14 mg/kg), but did not detect Ni metal.

CONCLUSION:

This study scientifically validates the used of the levers of *Ricinuscommunis* 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 equalPhyto - constituent, alkaloids is absent in each four solvents but carbohadrate present extract 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 give light to the present intention to find chemical proof that supports the pharmacological activities of *R. communis* leaves.

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