Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 5(3): 89-92
Received: 13-03-2016
Accepted: 15-04-2016

Shahid Aziz
Department of Chemistry, Mirpur University of Science & Technology (MUST), Mirpur, AJK. Pakistan.

Asma Rabniwaz
A) Department of Chemistry, Mirpur University of Science & Technology (MUST), Mirpur, AJK. Pakistan.
B) Department of Chemistry, Quid-e-Azam University Islamabad, Islamabad, Pakistan.

Habib-ur-Rehman
Department of Chemistry, Mirpur University of Science & Technology (MUST), Mirpur, AJK. Pakistan.

Kh. Shakil Ghani
Mohtrama Benazir Bhutto Shaheed Medical College Mirpur, Mirpur, AJK. Pakistan

Correspondence
Shahid Aziz
Department of Chemistry, Mirpur University of Science & Technology (MUST), Mirpur, AJK. Pakistan.

Phytochemical and Biological Screening of *Riccinus communis* Seed Oil Grown Wild in Jammu & Kashmir

Shahid Aziz, Asma Rabniwaz, Habib-ur-Rehman, Kh. Shakil Ghani

Abstract
The aim of the present study was to investigate the phyto-constituents present within the seed oil of *Ricinus communis* and to estimate its antibacterial, antifungal and antioxidant activity. Phytochemical studies confirmed the presence of alkaloids, terpenoids, cardiac glycosides, tannins, steroids and saponins whereas flavonoids, anthraquinone and reducing sugars were found absent. Eight phyto-components were identified from the seed oil of *R. communis* by using GC-MS. The *R. communis* seed oil showed moderate antibacterial and antifungal activity. Furthermore, the antioxidant activity was determined by using *in-vitro* model namely, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid was used as reference. The sample oil showed almost no antioxidant activity.

Keywords: *Ricinus communis*, phytochemical screening, phyto-constituents, antibacterial, antifungal and antioxidant activity.

1. Introduction
The plant of *Ricinus communis* is a type of blooming plant of the family Euphorbiaceae. This plant has special place with subtribe Ricininae and monotypic genus Ricinus. The development of plant and its connection to different plant species are at present considered utilizing current hereditary tools [1].
The seed is actually not a correct bean. Plant is native to India, the southeastern Mediterranean Basin, and Africa, until now is far reaching all around tropical locales (and broadly become somewhere else as a fancy plant [2].
The castor bean is the wellspring of castor oil that has a large mixed bag of importance. Castor beans hold about 40 to 60% oil that is affluent in fatty acid triglycerides, predominantly the component called ricinolein. The castor bean holds a poison called as ricin, which is additionally display within more level focuses all over the plant.
There are many compounds reported in *Ricinus communis* including triterpenoids, flavonoids, lignin, tannins, alkaloids, glycosides etc. Out of these compounds alkaloids have significant importance. Its major portion is present in leaves, root and seeds.
Ricinus communis seeds oil is a valuable triglyceride fatty acid commonly called as vegetable oil got through the crushing and then mechanically pressing of seeds of the *Ricinus communis* [3]. The color of *R. communis* oil is light or pale yellow having gentle otherwise little bit smell or flavor. The boiling point of seeds oil is about 313°C and thickness or density is high that is 961 kg/m^3 [4]. While talking about its composition *R. communis* seed oil is triglyceride in which ricinoleate is its major part covering more or less 90 percent of fatty acid chain while linoleates and oleate and are also other important composition of castor oil.

Fig 1: Seeds of *R. Communis*

R. communis seed oil and its compounds are utilized as a part of the making of cleansers, oils, brake solvents, paints, inks, and color pigments, coatings, anti-low temperature safe plastics, polishes, nylon fiber, medicines and in aroma. *R. communis* oil is well known because it is a major contributor of ricinoleic acid which is 18 carbons containing fatty acid having unsaturation at single point. Ricinoleic acid is uncommon around the list of fatty acids in a sense that it holds a -OH group on the carbon at 12th number. Due to the presence of -OH group on ricinoleic acid *R. communis* seed oil shows more polarity as compared to other fats. The chemical reactivity of *R. communis* seed oil due to alcoholic -OH allows the chemical binding to other functional groups present which is not conceivable with many seed oils. On account of presence of ricinoleic acid substance, oil is offering a higher cost than other seeds oils and is thus a profitable chemical in feedstock. *R. communis* seed oil is composed of various compounds like ricinoleic acid (95 to 85%) oleic acid (6 to 2%) linoleic acid (5 to 1%), stearic acid (1 to 0.5%), palmitic acid (1 to 0.5%), linolenic acid (1 to 0.5%), ecosanoic acid (0.5 to 0.3%), dihydroxystearic acid (0.5 to 0.3%) and others 0.5 to 0.2% [5].

It is recognized as a rumored solution in gastropathy i.e. amadosa, constipation, irritations, ascitis, strangury, fever, bronchitis, chest infection, skin maladies, coxalgia, colic, and lumbago. The leaves are suitable in blazes, talopia, and for showering particularly in rheumatoid joint swelling arthralgia and urodynia. Flowers of *Ricinus communis* are convenient in arthralgia and urodynia. Seeds are handy in ingestion and for making a medicinal cream to treat arthralgia. The seeds oil is an exceptionally viable laxative for all diseases brought on by vata [6]. Commercially it is used in lubricating agent formation, food preservatives and production of biodiesel [7].

2. Materials and Methods

2.1. Collection of plant material

Ricinus communis seeds were collected from the roadside of Mirpur Azad Kashmir during summer in mid May 2014.

2.2. Sample preparation

The fresh sample of *Ricinus communis* seeds were taken and were dried in shade at temperature of 30 to 35°C for 15 to 20 days. After that dried sample (seeds) were ground to fine powder separately with the help of electric grinder. The result of electric grinding was thick sticky mass which showed that a considerable amount of oil or fatty acid was present in seeds of *R. communis*.

The 80g of grinded seeds sample was taken and sunk in 250mL of *n*-Hexane for 15 to 20 days. The sample was shaken on daily basis for the convenience of thorough mixing and the release of undesired gases. The sample was then filtered through Whatmans filter paper No.1 by using Buckner funnel under high vacuum. The process was repeated for 3 to 4 times again and again by sinking of residue of seeds sample in and *n*-Hexane. Then the filtrate obtained as a result of filtration was condensed by using high vacuum rotary evaporator. The semisolid extract obtained was placed in open air at room temperature for complete drying.

2.3. Phytochemical Screening

Different chemical tests were carried out on seeds oil sample by using authentic or standard method for the identification of chemical constituents.

2.3.1. Detection of Alkaloids

The detection of alkaloid was done by method which is proposed by Trease and Evans [8]. Two mL of oil and 1 mL of Dragondroff's reagent was taken in china dish. Appearance of reddish brown coloration detects the presence of alkaloid.

2.3.2. Detection of Cardiac glycosides

Detection of cardiac glycosides test is also called as Keller-Kiliani test. 2.5 mL of seed oil was treated with 1 mL glacial acetic acid in a beaker. Few drops of ferric chloride was added in beaker. After that 1 mL of concentrated sulphuric acid was added, this gave a brown ring at interface. This indicates the presence of cardiac glycosides [9].

2.3.3. Detection of Tannins

This method was put forth by Edeoga [9]. According to this methodology, about 1mL of oil was boiled in 20 mL of distilled water, filtration was done after boiling and then few drops of 0.1% freshly prepared ferric chloride was added in filtrate. A bluish black or brownish black color indicates the presence of tannins.

2.3.4. Detection of Flavonoids

Appearance of yellow coloration indicates presence of flavonoids in each method. A portion of filtrate was taken in beaker and then few drops of aluminium chloride were added. A yellow color detects flavonoids in given sample [9].

2.3.5. Detection of Steroids

Two mL of acetic anhydride was added in 1mL of seed oil in beaker. 2 mL of concentrated sulphuric acid was added in it. The color changing from violet to blue or green detects presence of steroids.

2.3.6. Detection of Saponins

This method was also given by Edeoga [9]. According to this method 2mL of *R. communis* oil was boiled in 20 mL distilled water in water bath and filtered, after this, the 5 mL distilled water was added in filtrate and shaken for stable froth. The frothing was mixed with three drops of olive oil, froth formation indicate the presence of saponins.

2.3.7. Detection of Anthraquinone

This detection test was given by Trease and Evans [8]. Red, pink and violet color shows the presence of anthraquinone. According to this method, the seed oil was taken in china dish and 0.5 mL of ether was mixed well in this sample, then water was added and shaken with glass rod to detect anthraquinone.

2.3.8. Detection of Reducing Sugar

Detection of reducing sugar is also called as Fehling test proposed by Khan *et al* [10]. According to this method the appearance of red or violet coloration indicates the presence of reducing sugar. Seed oil (1 mL) and 5 mL of distilled water were taken in beaker and a few drops of Fehling solution was added to it and heating is required for some time to detect presence or absence of reducing sugar.

2.4. Gas Chromatography-Mass Spectrometry analysis

The *R. communis* seed oil was subjected to GC-MS analysis on a GC- MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Restek RtxR – 5, (30 meter X 0.25 mm)

(5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1mL/min and an injection volume of 1.0 µl was employed(split ratio of 10:1); injector temperature 280 ^{0}C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 6 ^{0}C/ min to 280 ^{0}C, then ending with an isothermal for 15min at 280 °C. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes.

2.4.1. Identification of Compounds
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and technology (NIST).The mass spectra of the unknown components were compared with the spectra of the known components stored in the NIST library.

2.5. Biological Screening of *R. communis* seed oil
Biological screening included antibacterial, antifungal and antioxidant activity of *Ricinus communis* seed oil.

2.5.1 Antibacterial activity of *R. communis* Seed Oil.
Sample preparation: *Ricinus communis* seeds oil was taken and measured 10mL and dissolved in DMSO (Dimethyle sulphoxide) in such a way that solution formed mark up to 1mL.

Media preparation: 28g of agar was taken and put in 1litre of distilled water and heated to boil to dissolve completely. After that it was sterilized by autoclaving at 121^{o}C for 15 minute.

Culture used: Antibacterial activity of *Ricinus communis* seed oil was determined by using following cultures
i) *E. coli* Gram negative bacteria
ii) *S. aureus* Gram positive bacteria

Agar well method: Agar well method with small modifications was used for antibacterial activity determination [11].

2.5.2. Antifungal activity of *R. communis* Seed Oil.
Media preparation: For antifungal activity PDA was used. 39g of PDA was taken and put in 1litre of distilled water and heated to boil to dissolve completely. After that it was sterilized by autoclaving at 121 ^{o}C for 15 minute and mixed well before pouring.

Use of culture: Aspergillus was used as a culture.

Method: Same method was applied for antifungal activity as for antibacterial.

2.5.3. Antioxidant activity
DPPH method: Stable DPPH (2, 2 Diphenyl, 1-picrylhadrazylhydrate) was utilized as a chemical to measure the antioxidant activity of seed oil of *R. communis.* When DPPH starts reacting with an antioxidant the reaction progress with change in color of extract (from deep violet to light yellow), which can be measured at 518 nm by using UV spectrophotometer. The seed oil *R. communis* was tested in the DPPH free radical eliminating test by modified method described in literature [12]. Seed oil was first dissolved in DMSO to make solution up to 1mL. After that 80uL of DPPH was taken and 20 uL of sample also in a way to make a total of 100uL of test sample. After making solution sample was put in a 96 well plate and placed in a UV spectrophotometer to measure the antioxidant activity.

3.0 Results and Discussion
3.1 Phytochemical screening
The biological activity of natural products is concerned with the phytochemicals present in plant samples. Phytochemical screening plays an important role in the study of medicinal properties of plant sample.
Our observations revealed that *R. communis* seed oil contain alkaloids, terpenoids, cardiac glycosides, tannins, steroids and saponins whereas, flavonoids, anthraquinone and reducing sugars are absent in the seed oil. The preliminary phytochemical screening results are shown in Table 1.

Table 1: Phytochemical constituent analysis of *R. communis* Seed oil

Phytochemical constituents	*R. communis* (Seeds Oil)
Alkaloids	+
Terpenoids	+
Cardiac glycosides	+
Tannins	+
Flavonoids	-
Steroids	+
Saponins	+
Anthraquinone	-
Reducing sugar	-

3.3 GC-MS results of *R. communis* seeds oil
The compounds of *R. communis* seeds oil were isolated and characterized by using GC-MS technique. The major compounds which were identified from *R. communis* seed oil are listed in Table-2:

Table 2: GC-MS of *R. communis* seed oil

No	Constituents of *R. communis* Seed oil	Retention time (min)	Percentage area
1	Ricinoleic acid (**1**)	35.72	15.1
2	Phthalic acid (**2**)	36.95	12.6
3	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyle ester (**3**)	33.48	2.4
4	13-Hexyloxacyclotridec-10-en-2-one (**4**)	38.02	1.6
5	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (**5**)	31.42	1.1
6	Glycine,N-[(3a,5a)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-,methyl ester (**6**)	41.63	1.1
7	Ecosyl ester (**7**)	42.9	1.5
8	Oliec anhydride (**8**)	32.3	1.4

3.4. Biological Screening of *R. communis* seed oil
The *R. communis* seeds oil showed 60% activity against *Escherichia coli* and 72% activity against *Staphylococcus aureus* (table-3) whereas moderate activity was shown against *Aspergillus niger* (table-4). The *R. communis* seeds oil showed almost no antioxidant activity by using DPPH (table-5).

Table 3: Antibacterial activity of *R. communis* seed oil

Plant extract	Bacterial culture	Inhibition zone (mm)	%age Inhibition
Control	*Escherichia coli*	38	100
R. communis (seeds oil)		23	60
Control	*Staphylococcus aureus*	37	100
R. communis (seeds oil)		27	72

Table 4: Antifungal activity of *R. communis* seed oil

Plant extract	Fungal culture	Inhibition zone	% Inhibition
Control	*Aspergillus niger*	40	100
R. communis (seeds oil)		23	58

Table 5: Antioxidant activity of *R. communis* seed oil

Plant extract	DPPH inhibition	DPPH %age Inhibition
R. communis (seeds oil)	0.2	4
Gallic acid	5	100

4. Conclusion

Our observations revealed that seed oil of *Ricinus communis* contains alkaloids, terpenoids, cardiac glycosides, tannins, steroids and saponins whereas flavonoids, anthraquinone and reducing sugars were found absent. Eight phyto-components were identified from the seed oil by using GC-MS. The *R. communis* seed oil showed moderate antibacterial, antifungal activity and no antioxidant activity. The findings of the present study suggest that *Ricinus communis* could be a potential source of natural antibiotic. A detailed study on the role of different phyto-constituents which influences the antimicrobial activity further required to be investigated.

5. References

1. Rizzardo RA, Milfont MO, Silva EM, Freitas BM. Apis mellifera pollination improves agronomic productivity of anemophilous castor bean (Ricinus communis). Anais da Academia Brasileira de Ciencias. 2012; 84(4):1137-1145.
2. Sabina EP, Rasool MK, Mathew L, Parameswari. Studies on the protective effect of Ricinus communis leaves extract on carbon tetrachloride hepatotoxicity in albino rats. Pharmacologyonline. 2009; 2:905-916.
3. Joshi M, Waghmare S, Chougule P, Kanase A. Extract of Ricinus communis leaves mediated alterations in liver and kidney functions against single dose of CCl_4 induced liver necrosis in albino rats. Journal of Ecophysiology and Occupational Health. 2004; 4:169-173.
4. Kalaiselvi P, Anuradha B, Parameswari CS. Protective effect of *Ricinus communis* leaf extract against paracetamol-induced hepatotoxicity. Biomedicine. 2003; 23(2):97-105.
5. Aplin PJ, Eliseo T. Ingestion of castor oil plant seeds. The Medicinal Journal of Australia. 1997; 167:260-261.
6. Warrier PK, Nambiar VPK, Ramankulty C. Bioactive Food as Dietary Interventions for Diabetes. Journal of Ethanopharmacology. 1996; 104:129-131.
7. McGuire N. Taming the Bean. The American Chemical Society. 2007, 122-124.
8. Trease GE, Evans WC. Pharmacognosy 2nd Edition, Bailliere Tindall, Eastbourne, 1989, 74-78.
9. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4:685-688.
10. Khan AM**,** Qureshi RA, Ullah F, Gilani SA. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings, Journal of Medicinal Plants Research. 2011; 5:6017-6023.
11. Irshad S, Mahmood M, Perveen F. In-Vitro Anti-Bacterial Activities of Three Medicinal Plants Using Agar Well Diffusion Method. Research Journal of Biology. 2012; 2(1):1-8.
12. Bandoniene D, Markovic M, Pfannhauser W, Venskutonis PR, Gruzdiene D. Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC-DPPH methods. Eur. J Food. 2002; 214:143-147.