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Anti-bacterial, anti-oxidant and cytotoxicity of aqueous and organic extracts of *Ricinus communis*

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The present study aimed to examine anti-microbial, anti-oxidant and cytotoxicity in leaf extract of *Ricinus communis* extract (in different solvent). The leaf powder of *R. communis* was extracted using different solvents. The anti-bacterial activity of the extracts was determined by agar well and disc diffusion method. The extracts were also subjected to phytochemical analysis. The anti-oxidant activity of the extracts was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), alkaline DMSO, deoxyribose and nitric oxide scavenging assay. The cytotoxicity of the extracts was estimated using MTT cell proliferation assay. The photochemical qualitative analysis of methanollic plant extracts revealed the presence of alkaloid, flavonoid, tannins, glycoside, reducing sugar, anthraquinones and saponins. The methanollic extract showed zone of inhibition of 15 mm each against *Bacillus subtilis, Staphylococcus epidermis* and *Saccharomyces cereviceae* by using well diffusion method, whereas *S. cereviceae* gave 12 mm zone of inhibition by disc diffusion at a concentration of 40 mg/mL. The anti-oxidant activity by different methods gave IC_{50} value of 102.1 ± 4.16, 30.27 ± 3.85 and 382.6 ± 3.30 µg/mL in aqueous, benzene and ethyl acetate extract respectively by using DPPH method. The acetone extract gave IC_{50} value of 357.1 ± 4.96 µg/mL by nitric oxide method. The aqueous and acetone extract gave IC_{50} value of 860.1 ± 7.73 and 626.7 ± 2.25 µg/mL, respectively by deoxyribose method. The chloroform and ethyl acetate extract showed cytotoxicity in A549 cell line having IC_{50} value of 687 ± 3.92 and 957 ± 4.46 µg/mL respectively by MTT cell proliferation assay whereas, aqueous extract in Jurkat cell line gave IC_{50} value of 918 ± 2.05 µg/mL. This study demonstrates that the *R. communis* extracts are potential source for anti-microbial, anti-oxidant and anti-cancer agent. Further study is needed to identify the specific bioactive compounds, their mode of action and their non-toxic nature in *in vivo* condition.

Key words: *Ricinus communis*, (3-(4, 5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide) (MTT) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

INTRODUCTION

The herbal medicines and products have been used in the pharmaceuticals company for the production of medicines since time unknown. *Ricinus communis* (Euphorbiaceae) is commonly known as Arand in India.

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The name Arand indicates the property of the plant to drive out diseases. The castor oil is a reputed remedy for all kinds of rheumatic affections. It has been reported to cure dyspnoea, hydrocele, flatulence, dysentery, ascites, piles, cough, lumbago, headache, leprosy, arthritis, calculus, disuria, elevates phantom tumor, spleen disorders, impurity of blood, dyspepsia and worm troubles (Jena and Gupta, 2012). The studies carried out by various workers have shown anti-microbial activity against *Salmonella typhimurium*, *Proteus vulgaris*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*, *Pseudomonas aeruginosae*, *Escherichia coli* (Jombo and Enenebeaku, 2008; Kota and Manthri 2011; Verma et al., 2011; Dastagir et al., 2012; Khursheed et al., 2012; Kensa and Yasmin, 2011) and *Enterococcus faecalis* (Lekganyane et al., 2012) and anti-oxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and reducing power assay (Singh et al., 2010; Kadri et al., 2011). The n-butanol extract of *R. communis* showed 0.625 and 2.50 µg/mL anti-bacterial activities against Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *Shigella flexneri* respectively (Iqbal et al., 2012). A wide spectrum of *R. communis* as an anti-microbial agent has been reported. It has found to inhibit secondary infection in oral cancer patients (Panghal et al., 2011). In one of the study using *R. communis,* leaf extract showed zone of inhibition of 15.90 ± 2.13 mm against *Staphylococcus aureus*. The MIC values against the strain ranged from 1.95 to 250 mg/mL for different leaves extracts (Bereket et al., 2014). The hot and cold methanol and ethanol extracts showed inhibition on both *S. aureus* and *E. coli*.

The hot and cold ethanol extracts revealed inhibition of *S. aureus* with MIC and MBC values of 5 and 10 mg/mL, respectively. *E. coli* was inhibited by hot extracts of both ethanol and methanol having the MIC and MBC values of 40 and 80 mg/mL, respectively (Jeyaseelan and Jashothan, 2012). The *R. communis,* petroleum ether and acetone extracts showed anti-microbial activities against dermatophytic and pathogenic bacterial strains, *Streptococcus progenies, Staphylococcus aureus* as well as *Klebsiella pneumoniae* and *Escherichia coli* (Islam et al., 2010). The hexane and ethanolic leaf extract of *R. communis* also showed anti-bacterial activity against *B. subtilis* and *E. coli* (Bais, 2014). The phytochemicals alkaloids and cardiac glycosides were found in high concentration in the leaves and stem extracts of *R. communis.* It was found to give 11.2 and 63.60% yields respectively and was also responsible for its antioxidant and anti-hemolytic activities. Furthermore, extracts of these two phytochemicals also showed a decrease in the growth and proliferation of pathogenic *Klebsiella pneumonia* and *Staphylococcus aureus* (Ibraheem and Maimako, 2014). The essential oil of *R. communis* showed cytotoxicity in HeLa cell lines and anti-microbial activity against *B. subtilis*, *S. aureus* and *Enterobacter cloacae* (Zarai et al., 2012).

MATERIALS AND METHODS

Collection and identification of plant

The leaves of plant were collected from in and around the campus of Integral University, Lucknow, India. The plants were authenticated and sample vouchers were stored in NBRI, Lucknow. The identified plant parts were washed and air dried at room temperature and was powdered with the help of mortar and pestle. The plant extracts prepared using Soxhlet apparatus in different solvents.

Plant extracts preparation using different solvents

A 20 g of finely ground dry plant parts were weighed and kept in a muslin cloth. The muslin cloth containing dry extract were placed in extraction chamber of the soxhlet apparatus. The extraction solvent in boiling flask was heated and its vapour condenses in the condenser. The condensed solvent strips into the thimble containing the crude dry plant and extracts it by contact. When the level of liquid in extraction chamber rises to the top of siphon tube, the liquid contents of thimble siphon in flask A. This process is continous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated (Handa et al., 2008). The aqueous and organic solvents were used for crude extract preparation. The organic solvents used in the present study were cyclohexane benzene, chloroform, acetone, ethyl acetate, ethanol and methanol. All the extracts were air dried in petri plates and the extracts were weighed and kept in eppendroff at -20° C until used.

Determination of extraction yield in plant extract (% yield)

The yield (% w/w) from all dried extracts was calculated by the formula:

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0 \times 100}$$

Where, W_2 is the weight of the extract and the container, W_1 weight of the container alone and W_0 the weight of the plant powder (Anokwuru et al., 2011).

Phytochemical analysis (qualitative) of plant extract

The qualitative phytochemical analysis was estimated on the basis of color formation by standard methods (Tiwari et al., 2011; Mir et al., 2013; Trease and Evans, 1983; Kokate et al., 1997; Hegde and Joshi, 2010). They are as follows:

Alkaloids

The plant extract was prepared by taking 500 mg of dry plant material in 500 mL of methanol on a water bath at 37°C for 20 min, the extract was filtered and allowed to cool and few drops of Wagner's reagent added (2 g iodine and 6 g of potassium iodide in 100 mL distilled water). A reddish brown colored precipitate indicated the presence of alkaloids.

Anthraquinones (Borntrager's test)

The 0.5 g of dry plant material was boiled with 10% hydrochloric acid (HCl) in a water bath, filtered and allowed to cool and equal volume of chloroform ($CHCl_3$) and few drops of 10% ammonia was

added and heated. Formation of rose-pink color indicated the presence of anthraquinones.

Flavonoids

The crude methanolic extract was heated with 10 mL of ethyl acetate for 3 min, filtered and 1 mL of ammonia solution was added to 4 mL filtrate, formation of yellow color indicated the presence of flavonoids.

Phlobatannins

An aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid (HCl) and the deposition of red precipitate showed the presence of phlobatannins.

Glycosides (Fehling's test)

To the 2 mL of methanolic extract, 10 mL of 50% hydrochloric acid (HCl) was added and heated in water bath for 30 min and then 5 mL of Fehling's solution was added. After 5 min, formation of brick red precipitate indicated the presence of glycosides.

Saponins (Frothing test)

The 0.2 g of the methanolic extract was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy mist of small bubbles) showed the presence of saponins.

Steroids (Salkowski test)

The methanolic extract was dissolved in methanol and to it 5 drops of concentrated sulphuric acid (H_2SO_4) was added. The formation of red color indicated the presence of steroids.

Tannins (Ferric chloride test)

The 0.5 g of methanolic extract was dissolved in 10 mL of distilled water, filtered and ferric chloride reagent was added, a blue-black precipitate was taken as evidence for the presence of tannin.

Terpenoids (Salkowski test)

The 0.2 g of dried plant material was mixed with 2 mL of chloroform and 3 mL of concentrated hydrochloric acid (HCl) was added carefully to form a layer. A reddish brown coloration of the interface formed indicated the presence of terpenoids.

Reducing sugar

The 0.5 g of methanolic extract dissolved in 1 mL of distilled water and 2 to 8 drops of Fehling solution was added and boiled for few minutes. The presence of brick red precipitate indicated presence of reducing sugar.

Anti-microbial activity

The anti-microbial activity was screened in extracts by disc diffusion and well diffusion method. The bacterial strains used were *S. aureus* 2079, *E. coli* 2065, *Proteaus vulgaris* 2027, *B. cereus* 2156, *B. subtilis* 296, *S. epidermis* 2493 and *S. cereviceae* 3090. All the strains were obtained from the National Chemical Laboratory (NCL), Pune, India. Dried filter paper discs (4 mm) impregnated in known amount of test samples and for well diffusion method, the extracts were inoculated in well prepared using well cutter (0.6 cm). The dried plant extracts were prepared in dimethyl sulfoxide at a concentration of 40, 30, 20, 10 and 5 mg/mL. The plates were incubated at 37° C for 24 h. Anti-microbial activity was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which dimethyl sulfoxide was used as a negative control and the discs of Tetracycline (30 mcg/disc), Penicillin G (10 units/disc), Streptomycin (10 mcg/disc) and Amoxicillin (30 mcg/disc) were used as a positive control. The experiment was done three times and the mean values were presented (Kamaraj et al., 2012).

Anti-oxidant assay

The different anti-oxidant assays were used for the study of DPPH method, superoxide radical with the alkaline DMSO (dimethyl sulfoxide) method, nitric oxide radial inhibition assay and hydroxyl radical in the deoxyribose method. L-ascorbic acid, butylated hydroxy toluene and quercetin were used as standard, while methanol or dimethyl sulfoxide was used in place of plant extract as control. The crude plant extracts were prepared at different concentrations varying from 1000 µg/mL to 0.46 µg/mL.

DPPH (1, 1 – Diphenyl – 2- Picryl Hydrazyl) radical scavenging activity method

DPPH radical scavenging activities of all the fractions were determined by the method of Blois (1958) with some modification. The crude plant extracts of 10 µl was mixed with 200 µl of 100 mM DPPH (dissolved in methanol). The reaction mixtures were incubated for 30 min at 37°C under dark condition. The absorbance was measured at 490 nm spectrophotometrically (Ara and Nur, 2009).

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract)}}{\text{Absorbance of control}} \times 100$$

Scavenging of superoxide radical with the alkaline DMSO (Dimethyl sulfoxide) method

Alkaline DMSO radical scavenging assay were determined by the method of Kunchandy and Rao (1990) with slight modification (Sanja et al., 2009; Vaijanathappa et al., 2008). The reaction was prepared by mixing 0.1 mL of nitro blue tetrazolium (1 mg/mL in DMSO) and 1 mL of alkaline DMSO (1 mL of DMSO containing sodium hydroxide 5 mM in 0.1 mL of water). To the reaction mixture 0.3 mL of the crude extract prepared in DMSO was added. The absorbance was measured at 560 nm spectrophotometrically.

$$\text{Percentage super oxide scavenging activity} = \frac{\text{Test absorbance} - \text{Control absorbance}}{\text{Test absorbance}} \times 100$$

Anti-oxidant assay by nitric oxide radial inhibition assay

The plant extracts (1 mL) was mixed with 1 mL phosphate buffer saline and 4 mL (10 mM) sodium nitroprusside and was kept for incubation at room temperature at 25°C for 150 min. After incubation,

0.5 mL of reaction mixture and 1 mL sulphanilic acid reagent (0.33% sulphanilic acid in 20% glacial acetic acid) were added and incubated for 5 min at room temperature (for diazotization reaction). Then 1 mL N-(1-naphthyl) ethylene-di-amine di-hydrochloride was added and kept in diffused light for 30 min and absorbance was measured at 540 nm (Badami et al., 2005).

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Scavenging of hydroxyl radical in the deoxyribose method

The scavenging of hydroxyl free radical was measured by the method of Halliwell et al. (1987) with minor changes. The reaction mixture prepared containing deoxyribose (3 mM) 0.2 mL; ferric chloride (0.1 mM) 0.2 mL; ethylene diamine tetra acetic acid disodium salt (EDTA) (0.1 mM) 0.2 mL; ascorbic acid (0.1 mM) 0.2 mL and hydrogen peroxide (2 mM) 0.2 mL in phosphate buffer (pH, 7.4, 20 mM). To the reaction mixture, 0.2 mL of various concentrations of the extract or standard in DMSO was added to form a final volume of 1.2 mL. The solution was then incubated for 30 min at 37°C. After incubation, ice-cold tri-chloro acetic acid (0.2 mL, 15% w/v), and thio-barbituric acid (0.2 mL, 1% w/v) in 0.25 N hydrochloric acid were added. The reaction mixture was then kept in a boiling water bath for 30 min, cooled, and the absorbance was measured at 532 nm (Hinneburg et al., 2006).

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity by MTT cell proliferation assay

Cell proliferation were measured by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide) assay that colorimetrically measures a purple formazan compound produced by viable cells (Mosmann, 1983). The cell lines used for the present study were Jurkat (human lymphoblastic leukeamia), Hek 293 (human kidney), A549 (human alveolar adenocarcinoma) and MRC-5 (human lung). The cells were plated 0.5 x 10^4 cells for A549, Hek 293 and 1 x 10^4 cells for MRC-5 and Jurkat in 96 well plates. After 24 h of plating, cells were treated with crude plant extracts of different solvents at the concentrations of 1000, 500, 250, 125 and 62.5 µg/mL. The treated cells were incubated for 24 h at 37°C. After 24 h of treatment, MTT was added and again the cells were kept for 4 h at 37°C. The formation of purple formazan compound produced by viable cells was dissolved in dimethyl sulphoxide and the plates were read at 570 nm wavelength using ELISA reader. All the assays were performed in triplicate. The percentage inhibition was calculated in cancerous and normal cell lines and IC_{50} values were determined.

RESULTS

The present study shows that medicinal plants possess anti-microbial, anti-oxidant and cytotoxic properties that support *R. communis* value in herbal medicine for the treatment of different diseases. The presence of alkaloid, flavonoid, tannins, glycoside, reducing sugar, anthraquinones and saponins which were estimated qualitatively were found and may be responsible for its anti-microbial, anti-oxidant and cytotoxicity of *R. communis* extracts. The initial weight of the dried plant was taken as 20 g in 200 mL of solvent. The percentage yield in aqueous extract is 10.7%, cyclohexane extract 0.9%, benzene extract 1.85%, chloroform extract 3.5%, acetone extract 6.15%, ethyl acetate extract 0.9%, ethanol extract 0.4% and methanol extract 0.9%. A wide range of the yields among extracts was observed depending on the extraction solvent.

Anti-microbial activity of *R. communis* extract

The *R. communis* aqueous and organic extracts showed significant anti-microbial activity against *B. subtilis, E. coli*, *S. epidermis*, *S. cereviceae*, *P. vulgaris*, *B. cereus* and *S. aureus*. The anti-bacterial activity by well diffusion method was found to be in the order of methanol > aqueous > benzene > ethyl acetate > acetone extract. The cyclohexane, chloroform and ethanolic extracts did not show activity against any of the strains used. The results of anti-microbial activity of *R. communis* showing zone of inhibition by well diffusion method are given in Table 1. The disc diffusion method showed anti-bacterial activity in the order of methanol > ethyl acetate > aqueous > benzene > acetone extract whereas, cyclohexane, chloroform and ethanol extract did not show anti-microbial activity against any of the strains used. The results of anti-microbial activity of *R. communis* showing zone of inhibition by well diffusion method are given in Table 2 and Figure 1. The plant extracts were compared with the standard antibiotics as a positive control and dimethyl sulphoxide (DMSO) as negative control against different bacterial strains. The plant extracts when compared with the antibiotics for their anti-bacterial activity showed significant activity and zone of inhibition in them were found to be equivalent to the standard anti-biotics. The zone of inhibition of standard antibiotics and negative control dimethyl sulfoxide are given in Table 3.

Comparative IC_{50} values of aqueous and organic extracts by different anti-oxidant assay

The IC_{50} values of the aqueous and organic extracts were calculated by different anti-oxidant assay that is DPPH, alkaline DMSO, nitric oxide scavenging assay, and hydroxyl radical assay in the deoxyribose method. The aqueous and organic extracts of *R. communis* showed 50% inhibition against the above mentioned anti-oxidant assays as shown in Table 4, Figures 2, 3, 4 and 5. The results given show that benzene extract of *R. communis* with IC_{50} value of 30.27 ± 3.85 µg/mL possesses strong anti-oxidant activity as compared to other extracts used for the present study.

Cytotoxicity in different cell lines by crude plant extracts using MTT assay

R. communis aqueous leaf extract showed maximum

Table 1. Zone of inhibition (mm) of *Ricinus communis* extract in different solvents by agar well diffusion method.

Plant extract	Conc. of extract (mg/mL)	Zone of Inhibition (mm)						
		B. subtilis	***E. coli***	***S. epidermis***	***S. cereviceae***	***P. vulgaris***	***B. cereus***	***S. aureus***
Aqueous	40	13 ±2.33	NZ	14±0.5	NZ	NZ	9 ±1	NZ
	30	12 ±4.51	NZ	13 ±0.55	NZ	NZ	6 ±4.44	NZ
	20	11 ±3.78	NZ	12 ±0.05	NZ	NZ	4 ±3.21	NZ
	10	10 ±0	NZ	11 ±0.05	NZ	NZ	2 ±0	NZ
	5	2 ±0	NZ	NZ	NZ	NZ	NZ	NZ
Cyclohexane	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Benzene	40	NZ	NZ	13 ±0	NZ	NZ	NZ	9 ±0.11
	30	NZ	NZ	12 ±2.56	NZ	NZ	NZ	6 ±0.1
	20	NZ	NZ	11 ±1.09	NZ	NZ	NZ	4 ±0
	10	NZ	NZ	10 ±0.1	NZ	NZ	NZ	2 ±5.6
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Chloroform	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Acetone	40	NZ	NZ	NZ	NZ	NZ	NZ	9 ±5.5
	30	NZ	NZ	NZ	NZ	NZ	NZ	8 ±0
	20	NZ	NZ	NZ	NZ	NZ	NZ	6 ±1
	10	NZ	NZ	NZ	NZ	NZ	NZ	4 ±0
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethyl acetate	40	16 ±0.5	NZ	NZ	NZ	NZ	NZ	12 ±1
	30	15 ±0.1	NZ	NZ	NZ	NZ	NZ	10 ±1.2
	20	14 ±0.5	NZ	NZ	NZ	NZ	NZ	9 ±0
	10	12 ±0.5	NZ	NZ	NZ	NZ	NZ	8 ±4.5
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethanol	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Methanol	40	15 ±0	12 ±1	15 ±0.1	15 ±4.4	NZ	10 ±0.5	NZ
	30	14 ±2.33	10 ±0.9	14 ±4.55	14 ±2.30	NZ	8 ±0.05	NZ
	20	13 ±0.1	8 ±0.1	13 ±6.0	13 ±0	NZ	7 ±0.05	NZ
	10	12 ±0.5	7 ±0.2	12 ±2.33	12 ±3.1	NZ	5 ±0	NZ
	5	9 ±0.5	NZ	4 ±0	2 ±9.08	NZ	NZ	NZ

The bacterial strains used were *B. subtilis*, *E. coli*, *S. epidermis*, *S. cereviceae*, *P. vulgaris*, *B. cereus* and *S. aureus* at the concentration of 40, 30, 20, 10 and 5 mg/mL, respectively (NZ- No Zone). The values are mean ± standard deviation (n=3).

Table 2. Zone of inhibition (mm) of *Ricinus communis* extract in different solvents by disc diffusion method.

Plant extract	Conc. of extract (mg/ml)	Zone of Inhibition (mm)						
		B. subtilis	***E. coli***	***S. epidermis***	***S. cereviceae***	***P. vulgaris***	***B. cereus***	***S. aureus***
Aqueous	40	10 ± 0	NZ	12 ± 0.08	NZ	NZ	NZ	NZ
	30	9 ± 0.2	NZ	10 ±0.03	NZ	NZ	NZ	NZ
	20	8 ± 0.2	NZ	8 ± 0	NZ	NZ	NZ	NZ
	10	NZ	NZ	6 ± 0.22	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Cyclohexane	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Benzene	40	NZ	NZ	10 ± 0	NZ	NZ	NZ	NZ
	30	NZ	NZ	9 ± 3.2	NZ	NZ	NZ	NZ
	20	NZ	NZ	7 ± 3.35	NZ	NZ	NZ	NZ
	10	NZ	NZ	4 ± 0.5	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Chloroform	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Acetone	40	NZ	NZ	NZ	NZ	NZ	NZ	9 ± 0.45
	30	NZ	NZ	NZ	NZ	NZ	NZ	8 ± 0.21
	20	NZ	NZ	NZ	NZ	NZ	NZ	6 ± 5.0
	10	NZ	NZ	NZ	NZ	NZ	NZ	4 ± 1.30
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethyl acetate	40	12 ± 3.2	NZ	NZ	NZ	NZ	NZ	12 ± 0
	30	10 ± 3.4	NZ	NZ	NZ	NZ	NZ	10 ± 3.2
	20	9 ± 7.5	NZ	NZ	NZ	NZ	NZ	9 ± 1.2
	10	NZ	NZ	NZ	NZ	NZ	NZ	8 ± 0
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ethanol	40	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	30	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	20	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	10	NZ	NZ	NZ	NZ	NZ	NZ	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Methanol	40	10 ± 0.05	7 ± 0.5	NZ	12 ± 3.22	NZ	8 ± 0	NZ
	30	9 ± 0.5	4 ± 0.5	NZ	10 ± 0	NZ	8 ± 1	NZ
	20	6 ± 0.5	2 ± 0.1	NZ	8 ± 0.5	NZ	7 ± 0.5	NZ
	10	4 ± 0.1	NZ	NZ	5 ± 0.05	NZ	5 ± 0.2	NZ
	5	NZ	NZ	NZ	NZ	NZ	NZ	NZ

The bacterial strains used were *B. subtilis*, *E. coli*, *S. epidermis*, *S. cereviceae*, *P. vulgaris*, *B. cereus* and *S. aureus* at the concentration of 40, 30, 20, 10 and 5 mg/mL, respectively (NZ- No Zone). The values are mean ± standard deviation (n=3).

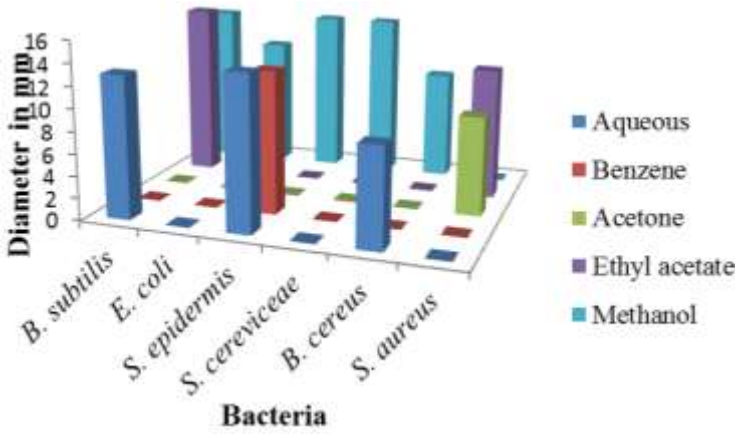

Figure 1. Comparative anti-microbial activity in *R. communis* extracts against selected bacteria.

Table 3. The data represents zone of inhibition (mm) of the standards. The antibiotics used as a positive control are Amoxicillin, Penicillin G, Tetracycline and Streptomycin. Dimethyl sulphoxide was used as a negative control. The values are mean ± standard deviation (n=3).

Standards	*B. subtilis*	*E. coli*	*S. epidermis*	*S. cereviceae*	*P. vulgaris*	*B. cereus*	*S. aureus*
Amoxicillin	NZ	NZ	12 ±3.21	NZ	NZ	NZ	NZ
Penicillin G	NZ	NZ	NZ	NZ	20 ±0.91	NZ	NZ
Tetracycline	27 ± 0.05	24 ± 0.1	28 ±0	23 ±0.2	25 ±0.43	16 ±0.5	25 ±0.1
Streptomycin	20 ±0.5	16 ±0	NZ	20 ±0.05	20 ±0.1	21 ±0.63	15 ±0.11
Dimethyl sulfoxide	NZ	NZ	NZ	NZ	NZ	NZ	NZ

Table 4. Comparative chart of IC_{50} values of aqueous and organic extracts of *Ricinus communis* and standard L-ascorbic acid, BHT and quercetin. The plant extracts given were *R. communis* Unit for IC_{50} for all the activities are µg/mL. Data are expressed as mean ± SD (n=3). *p< 0.0001 vs 0 µg/mL.

Plant name	Extract in different solvents	IC_{50} values ± SD (µg/mL) of different anti-oxidant assay			
		DPPH	Alkaline DMSO	Nitric oxide	Deoxyribose
R. communis	Aqueous	102.1±4.16	-	-	860.1±7.73*
	Benzene	30.27±3.85	-	-	-
	Acetone	-	-	357.1±4.96*	626.7±2.25*
	Ethyl acetate	382.6±3.30*	-	-	-
L-ascorbic acid		61.4±1.55	537.7±14.33	54.97±4.73	865.2±1.50*
BHT		50.8±3.85	801.5±0	461.3±2.54*	958.8±0
Quercetin		27.9±1.55	316.5±1.21*	47.57±10.68	419.9±1.2*

cytotoxicity to Jurkat cells at a concentration of 1000 µg/mL, as compared to other extracts used whereas, the chloroform and ethyl acetate extracts showed maximum cytotoxicity on A549 cell line (Figures 6 and 7). The IC_{50} values of different extracts are listed in Table 5. The cytotoxicity on Jurkat and A549 cell lines indicates the anti-cancer activity of the crude plant extracts. The most significant activity against A549 cell line was showed by chloroform extract of *R. communis.*

DISCUSSION

In the present study, *R. communis* extracts (using different solvents) were tested to determine their inhibitory effect against standard bacteria, *S. aureus*, *E. coli, P. vulgaris, B. cereus, B. subtilis, S. epidermis* and *S. cereviceae*. The results demonstrated that these extracts had ability to control the bacteria *in vitro*. Different organic solvents beside aqueous solution were

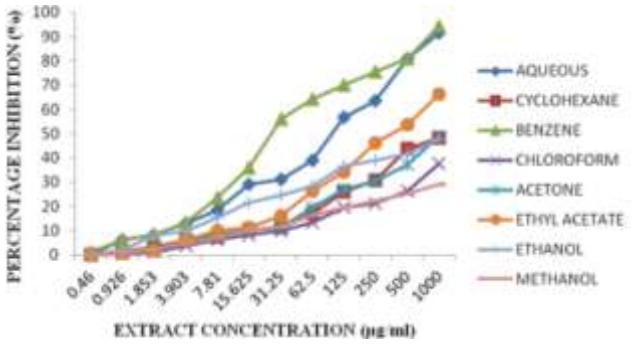

Figure 2. The scavenging effect of aqueous and organic extracts of *Ricinus communis* by DPPH method. The different concentrations of extracts used were 1000 to 0.46 µg/mL. The data represent the percentage DPPH inhibition Values are expressed as mean ± SD (n=3).

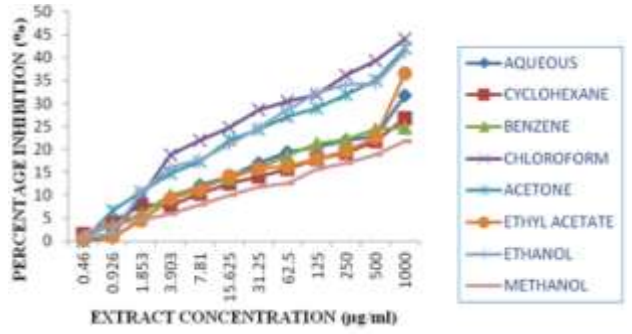

Figure 3. The scavenging effect of aqueous and organic extracts of *Ricinus communis* by Alkaline DMSO method. The different concentrations of extracts used were 1000 to 0.46 µg/mL. The data represent the percentage alkaline DMSO inhibition. Values are expressed as mean ± SD (n=3).

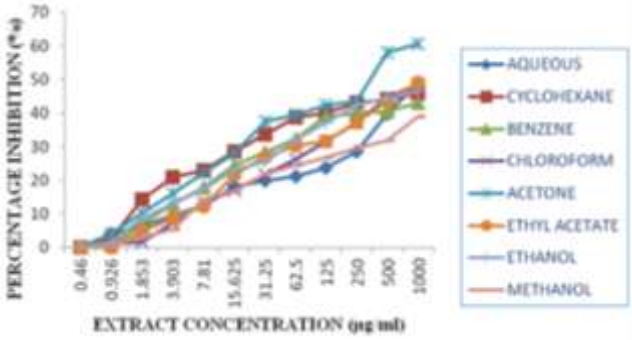

Figure 4. The nitric oxide radical scavenging activity of aqueous and organic extracts of *Ricinus communis*. The different concentrations of extracts used were 1000 to 0.46 µg/mL. The data represent the percentage nitric oxide inhibition. Values are expressed as mean ± SD (n=3).

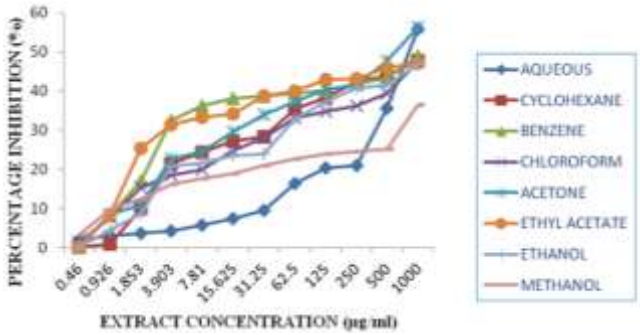

Figure 5. The hydroxyl radical scavenging activity of aqueous and organic extracts of *Ricinus communis* by deoxyribose method. The different concentrations of extracts used were 1000 to 0.46 µg/mL. The data represent the percentage inhibition values. Values are expressed as mean ± SD (n=3).

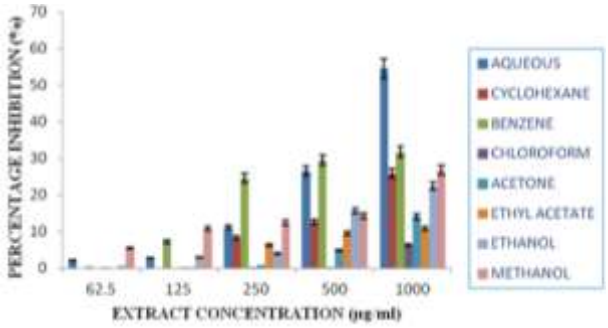

Figure 6. The cytotoxic effect of *Ricinus communis* extracts on Jurkat cell line using MTT assay. The different concentrations of extracts used were 1000 to 62.5 µg/mL. The data represent the percentage (%) inhibition. Values are expressed as mean ± SD (n=3).

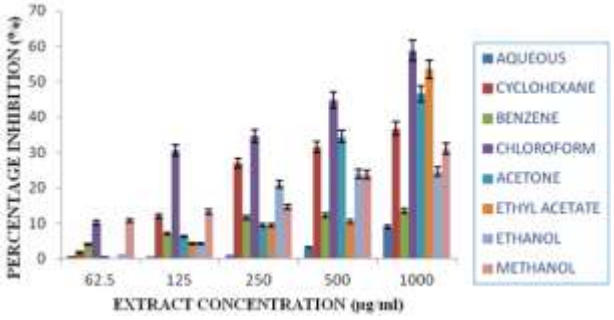

Figure 7. The cytotoxic effect of *Ricinus communis* extracts on A549 cell line using MTT assay. The different concentrations of extracts used were 1000 to 62.5 µg/mL. The data represent the percentage (%) inhibition. Values are expressed as mean ± SD (n=3).

used for extraction, but methanol extract showed maximum anti-microbial activity when compared to the other extracts. The methanol and ethanol are polar solvents but with different polarity, methanol has higher polarity than ethanol. The polarity of the solvents deter-

Table 5. Comparative chart of IC_{50} values of different cancerous and normal cell lines.

Plant name	Extract in different solvents	Jurkat	Hek-293	A-549	MRC-5
Ricinus communis	Aqueous	918±2.05* µg/mL	-	-	-
	Chloroform	-	-	687±3.92* µg/mL	-
	Ethyl acetate	-	-	957±4.46* µg/mL	-

The concentrations of plant extract which reduced cell viability of cell lines to 50% were given in µg/mL. Data represents the mean ± SD (n=3). p <0.0001* versus 0 µg/mL.

mines the solubility of chemicals from plant powder (El-Mahmood and Doughari, 2008). The high polarity of methanol extract was found to be more effective against Gram positive bacteria *B. subtilis* and *S. aureus*, as well as Gram negative bacteria *P. aeruginosae* and *K. pneumoniae* as compared to ethanol and aqueous extracts (Naz and Bano, 2012). The Gram positive bacteria show more sensitivity to biomolecules present in plant extracts than the Gram negative bacteria. This difference is due to the cell wall composition of the two bacteria (Panda et al., 2009). The anti-bacterial activity of *R. communis,* reported earlier, against two strains that is *Enterobacter* sp. and *Bacillus subtilis* also correlated with the present study (Rao et. al., 2013). The n-hexane, chloroform, ethyl acetate and n-butanol extracts of *R. communis* also showed anti-bacterial activity against Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* as well as Gram negative bacteria, *E. coli* and *Shigella flexneri* (Iqbal et al., 2012).

The photochemical qualitative analysis of methanolic extracts showed the presence of alkaloid, flavonoid, tannins, glycoside, reducing sugar, anthraquinones and saponins. However, in the present study, the methanol extracts showed microbial growth inhibition in both agar well diffusion method and disc diffusion method. The presence of phytochemicals in the plants is responsible to protect them from infection of pathogenic micro-organisms (Cowan, 1999). Recent studies on biological activity of phytochemicals have demonstrated the value of phytochemicals in drug discovery. Flavonoids are hydroxylated phenolic substances and they are known to be synthesized by plants in response to microbial infection. Their activity is probably due to their ability to form complex with extracellular and soluble proteins and also with bacterial cell wall. The more lipophilic flavonoids may also disrupt microbial membranes (Cowan, 1999). Saponins interfere with or alter the permeability of the cell wall while the tannins act by coagulating the cell wall proteins (Jeyaseelan and Jashothan, 2012). The presence of saponins in *R. communis* is responsible for its anti-oxidant, anti-cancer and folklore remedies (Vandita et al., 2013). Polyphenols are anti-oxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers and some of the polyphenols also show metal chelation properties (Proestos et al., 2013). The phytochemicals may be responsible for anti-oxidant property of *R. communis* extract and showed significant activity by different methods.

The anti-oxidant activity in *R. communis* extracts using DPPH and nitric oxide scavenging assay correlates with the present study. The IC_{50} values of n-butanol and chloroform extracts by DPPH assay were found to be 140 ± 0.19 and 48560 ± 0.81 µg/mL, respectively. The IC_{50} values by nitric oxide assay of n-hexane, chloroform, ethyl acetate, and n-butanol extracts of *R. communis* was found to be 173.45 ± 0.84, 231.36 ± 0.91 and 109.77 ± 0.66 µg/mL, respectively (Iqbal et al., 2012).

Cytotoxicity was also showed by different plant extracts in human cell lines (Prakash and Gupta, 2013). The present study showed all the extracts possess cytotoxic activity against all the cancer cell lines used as compared to the normal cells, where no changes were observed. The methanolic plant extracts showed more activity and changes as compared to other extracts. The ethanolic extract of seed of *R. communis* showed 41% cytotoxicity against Colon 502713 cell lines, whereas the extract of stem showed 47% activity was against SiHa cell line using SRB assay (Prakash and Gupta, 2014). The extracts of *R. communis* leaves showed cytotoxicity against several human tumor cell lines having IC_{50} values ranging between 10-40 µg/mL and also showed apoptosis in SK-MEL-28 human melanoma cells (Darmanin et al., 2009). The *R. communis* leaf extract showed cytotoxic effect on A375 cell line with IC_{50} 48 µg/mL in concentration ranging between 25 to 100 µg/mL by MTT assay (Shah et al., 2015). The cytotoxic effect and anti-inflammatory activity of *R. communis* leaves extract showed percentage free radical ($ABTS^{+}$) scavenging activity of methanol 95%, acetone 91%, dichloromethane 62%, and hexane 50% at 2.50 mg/mL. The methanol extract had LC_{50} value of 784 µg/mL after 24-h exposure on Bud-8 cell line, whereas 629.3, 573.6 and 544.6 µg/mL in hexane, dichloromethane and acetone extract respectively (Nemudzivhadi and Masoko, 2014).

Conclusion

The methanollic extracts of *R. communis* in the present

study, were found to have the maximum activity and can be used as a therapeutic agent for curing number of microbial and cancers due to its anti-oxidant property. Therefore, all the extracts of *R. communis* studied were found to possess significant anti-bacterial, anti-oxidant and anti-carcinogenic activity. Further studies would be carried out for purification and characterization of the compounds.

Conflict of interests

The authors have not declared any conflict of interests

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