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In vitro control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L.

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ABSTRACT

Objective: To evaluate antibacterial activity of hot and cold ethanol and methanol leaf extracts of *Ricinus communis* L (*R. communis*) against *Staphylococcus aureus* (*S. aureus*) (NCTC 6571) and *Escherichia coli* (*E. coli*) (ATCC 25922). **Methods:** Leaf powder of *R. communis* L. was extracted with hot (in Soxhlet) and cold ethanol and methanol, separately. The antibacterial activity of the extracts was determined by agar well diffusion and macro broth dilution methods. The extracts were also subjected to phytochemical analysis. **Results:** All the four test extracts showed inhibition on both *S. aureus* and *E. coli*. Hot and cold ethanol extracts revealed significantly ($P<0.05$) higher inhibition on *S. aureus* than methanol extracts, and the hot ethanol extract had the lowest minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values (5 mg/mL and 10 mg/mL, respectively). *E. coli* was highly inhibited by hot extracts of both ethanol and methanol with the MIC and MBC of 40 mg/mL and 80 mg/mL, respectively. Phytochemical analysis revealed the presence of saponins, cardiac glycosides, tannins, flavonoids and terpenoids in all test extracts. **Conclusions:** This study demonstrates that the hot and cold methanol and ethanol extracts are potential sources for control of *S. aureus* and *E. coli*. Especially, the hot and cold extracts of ethanol are more inhibitive against *S. aureus* even at lower concentration. Further study is needed to identify the specific bioactive compounds, their mode of action and their nontoxic nature *in vivo* condition.

1. Introduction

Ricinus communis (*R. communis*) L. (in English-Castor oil plant, in Tamil-Ammanakku) is member of the family Euphorbiaceae. It is probably of African origin, now naturalized and cultivated near habitations in all tropical countries. This is a very variable plant both in habit and appearance. The plant grow 3.5 m to 13.5 m tall with hollow stems, simple and alternate leaves on long curved, cylindrical, purplish or green petioles. Flowers are monoecious, large, arranged on the thick rachis of an oblong panicle. Fruit is greenish, deeply grooved, tricoccus capsules, dehiscing longitudinally and septicidally into six valves. Seeds are ovoid, flattened and smooth shining[1].

This plant has been used in classical Egyptian and Greek medicine and its uses have been described in Ayurveda as early as sixth century[2]. Almost all parts of the plant are used for medicinal purpose; the fresh leaves are used externally for headache and as a poultice for boils and rheumatism, a decoction of the leaves acts as an emmenagogue. Juice from the leaves is used against dropsy, abscesses, ringworms, and warts. The root bark is purgative and it is also effective in skin diseases. Castor seeds contain a high percentage of oil that causes the purgative action and also it is prescribed for infestation of intestinal worms. The seeds contain a high proportion of fixed oil, an active principle ricin and an alkaloid ricinine. Ricinine is also found in the leaves[1,3].

The emergence of new infectious diseases and development of drug resistance in pathogenic microorganisms prompts the scientists to discover novel bioactive compounds. Medicinal plants are found to be better choice for wide range of bioactive compounds, because since ancient time they have been used for medicinal purposes[4]. Therefore, medicinal plants are nowadays widely screened to determine their bioactivity and

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to isolate novel bioactive compounds. *R. communis* also have been studied by various researchers from different countries; anti-inflammatory activity[5], antidiabetic activity[6], antimicrobial activity of root extracts[7], antimicrobial activity of seed protein extract[8], antimicrobial activity of oil paste[9] have been reported.

The present study was aimed to determine the antibacterial activity of ethanol and methanol extracts of leaves of *R. communis*, extracted by both hot and cold extraction methods, against standard bacterial cultures *Staphylococcus aureus* (*S. aureus*) (NCTC 6571) and *Escherichia coli* (*E. coli*) (ATCC 25922).

2. Materials and methods

2.1. Collection of plant material

R. communis was identified based on morphological characters, and healthy leaves were collected (on September, 2011) from the botanical garden of Department of Botany, University of Jaffna, Sri Lanka. The voucher specimen was also deposited at the Department of Botany, University of Jaffna, Sri Lanka.

2.2. Preparation of plant extracts

Collected plant leaves were rinsed under running tap water, dried under shade on paper towel, then ground into fine powder using electric blender, and stored in air tight bottles. The powder was extracted using ethanol and methanol in two different methods.

For the cold extraction, 20 g powder was soaked in 100 mL of methanol (BDH-AnalaR, England) and ethanol (BDH-AnalaR, England), separately in airtight container, and kept for 24 h with intermittent shaking. The mixture was filtered through filter paper (Whatman No 1). Then the solvents were completely removed from the filtrate at reduced pressure and 40 ℃ temperature in rotavapor (BUCHI, Switzerland)[10]. The dried extracts were stored at 4 ℃ until used for further study.

For the hot extraction, 20 g powder was extracted with 100 mL of ethanol and methanol in Soxhlet apparatus for 24 h, separately. The resulting extracts were concentrated and the solvent was completely removed by using rotavapor at reduced pressure and 40 ℃ temperature[11]. The dried extracts were stored at 4 ℃ until used for further study.

2.3. Phytochemical analysis of test extracts

A portion of each test extracts were subjected to determination of the presence of following phytochemicals as described by Trease and Evans[12].

2.3.1 Test for tannins

One millilitre of distilled water and one to two drops of ferric chloride solution were added to 0.5 mL of extract solution and observed for brownish green or a blue black coloration.

2.3.2. Test for terpenoids

Five millilitre of extract was mixed with 2 mL of chloroform in a test tube. Three millilitre of concentrated sulfuric acid was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration indicated the presence of terpenoids.

2.3.3. Test for saponins

Five millilitre of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with three drops of olive oil and observed for the formation of an emulsion, which indicated the presence of saponins.

2.3.4. Test for flavonoids

A few drops of 1% ammonium solution were added to the 2 mL of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

2.3.5. Test for cardiac glycosides

One millilitre of concentrated sulfuric acid was taken in to a test tube. Five millilitre of extract was mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride. The above mixture was carefully added to the 1 mL of concentrated sulfuric acid. Presence of cardiac glycosides was detected by the formation of a brown ring.

2.3.6. Test for phlobatannins

Ten millilitre of extract was boiled with 1% hydrochloric acid in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins.

2.3.7. Test for alkaloids

One millilitre of 1% hydrochloric acid was added to the 3 mL of extract in a test tube. Then it was treated with a few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids.

2.3.8. Test for resins

Five millilitre of copper solution was added to the 5 mL of extract. The resulting solution was shaken vigorously and allowed to separate. A green precipitate indicated the presence of resin.

2.3.8. Test for glycosides

Ten millilitre of 50% sulfuric acid was added to the 1 mL of extract in a boiling tube. The mixture was heated in a

boiling water bath for 5 min. 10 mL of Fehling's solution (5 mL of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

2.3. Test bacteria

The standard test bacteria *S. aureus* (NCTC 6571) and *E. coli* (ATCC 25922) used in the current study were obtained from Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka. The cultures were stored on nutrient agar slants at 4 ℃ temperature, and before the antibacterial testing they were sub cultured on nutrient agar medium (Oxoid, England).

2.4. Determination of antibacterial activity

Antibacterial activity of the test extracts was tested by agar well diffusion method. Nutrient agar plates were prepared by incorporating 1 mL of test bacteria (0.5 McFarland standards) into 20 mL of molten nutrient agar. After solidification of the medium, wells were made using 8 mm diameter of sterile cork borer, and 100 μL of each of the test extracts (500 mg/mL), Streptomycin (1 mg/mL) and control (methanol and ethanol) were added into the wells, separately. Plates were incubated at 37 ℃ for 24 h. The antibacterial activity of the test samples was determined by measuring the diameter of clear zone around the well[13]. Three replicates were maintained for each experiment.

2.5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC was determined by the macro broth dilution method. Test extracts were diluted to 320.00, 160.00, 80.00, 40.00, 20.00, 10.00, 5.00, 2.50, and 1.25 mg/mL as two fold dilution in nutrient broth. The tubes were inoculated with 1.0 mL (0.5 McFarland standards) of test bacteria and incubated at 37 ℃ for 24 h. The MIC was taken as the lowest concentration of test samples that did not permit any visible growth. For the determination of MBC, two loops full of culture were taken from each of the broth tubes that showed no growth in the MIC tubes and inoculated onto fresh nutrient agar plates. After 24 h incubation, the plates were observed for the growth of bacteria. The concentrations of the extracts that showed no growth was recorded as the MBC[14]. Each experiment was repeated thrice.

2.6. Statistical analysis

The antibacterial effects of the extracts were expressed as mean ± standard deviation (SD) of three replicates. Statistical significance was determined using analysis of variance and Tukey test at $P = 0.05$ and t test (5% significant level) using statistical software SPSS Windows version 13.0.

3. Results

The qualitative phytochemical analysis showed that all the four test extracts of *R. communis* consisted saponins, cardiac glycosides, tannins, flavonoids and terpenoids. However, glycosides and phlobatannins were absent in the all test extracts. The resin was detected in both hot and cold ethanol extracts but not in both methanol extracts. The test for alkaloids showed positive results only in ethanol hot extract (Table 1). Overall, this study demonstrated that both hot and cold ethanol extracts was found as rich source for the presence of bioactive molecules compared to methanol extracts.

Table 1
Phytochemical constituents of test extracts of *R. communis*.

Phytochemicals	Ethanol hot extract	Ethanol cold extract	Methanol hot extract	Methanol cold extract
Glycosides	−	−	−	−
Alkaloids	+	−	−	−
Saponins	+	+	+	+
Cardiac glycosides	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	−	−	−	−
Resins	+	+	−	−
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+

+: presence, −: absence of phytochemical.

All the test extracts had inhibitory effect on both *E. coli* and *S. aureus* in agar well diffusion method, and their inhibitory effects differ significantly ($P<0.05$). The *E. coli* was highly inhibited by hot extracts of ethanol and methanol than the cold extracts of respective solvents, and there was no significant ($P>0.05$) difference between the inhibitory effect produced by the hot extracts of ethanol and methanol. Cold extract of ethanol had lower inhibitory effect on *E. coli* (Table 2).

Table 2
Antibacterial activity of test extracts (500 mg/mL), controls and standard against test bacteria (mm).

Test samples		Diameter of inhibition zone*	
		E. coli	*S. aureus*
Ethanol	Hot	13.7 ± 0.8^{ab}	20.3 ± 0.6^{a}
	Cold	10.7 ± 0.6^{c}	20.5 ± 0.5^{a}
Methanol	Hot	14.7 ± 0.6^{a}	10.5 ± 0.5^{c}
	Cold	12.3 ± 0.3^{b}	15.3 ± 0.6^{b}
Ethanol solvent		−	−
Methanol solvent		−	−
Streptomycin (1 mg/mL)		22. 5±0.7	15.2±0.4

−: No activity; *: Zone of inhibition includes the diameter of well (8 mm). Values with different superscript in the same column are significantly different ($P < 0.05$).

Hot and cold extracts of ethanol showed significantly (P<0.05) higher inhibition on *S. aureus* than both cold and hot extracts of methanol. However, cold methanol extract revealed significantly (P<0.05) higher inhibition on *S. aureus* compared to the hot extract of same solvent (Table 2).

When compared the sensitivity of *E. coli* and *S. aureus* to test extracts, *S. aureus* exhibited significantly (P<0.05) higher sensitivity to hot and cold ethanol extracts and cold methanol extract compared to *E. coli*. By contrast, the *E. coli* showed significantly (P<0.05) higher sensitivity to hot methanol extract than *S. aureus* (Table 2).

The control, ethanol and methanol solvents did not show any inhibition on test bacteria. The standard antibiotic, streptomycin revealed inhibition on both *E. coli* and *S. aureus*, and the inhibitory effect was significantly (P<0.05) higher on *E. coli* than *S. aureus* (Table 2). Nevertheless, among the test extracts, both hot and cold leaf extracts of ethanol possessed excellent antibacterial effects against S. aureus rather than standard, streptomycin.

The MIC for *E. coli* and *S. aureus* ranged from 5 mg/mL to 80 mg/mL, and the MBC ranged from 10 mg/mL to 160 mg/mL. The lowest MIC value, 5 mg/mL was exerted by cold and hot extracts of ethanol on *S. aureus* while the required MIC value of the hot and cold extracts of ethanol on *E. coli* were found to be 40 and 80 mg/mL respectively. However for *E. coli*, the required MIC values were equal in both hot and cold methanol extracts. Furthermore, hot and cold extracts of ethanol exhibited the lowest MBC against *S. aureus* were 10 and 20 mg/mL respectively. Simultaneously highest MBC 160 mg/mL was expressed by both hot and cold extracts of methanol on above organism (Table 3).

Table 3
MIC and MBC of test extracts (mg/mL).

Test extracts		*E. coli*		*S. aureus*	
		MBC	MIC	MBC	MIC
Ethanol	Hot	40	80	5	10
	Cold	80	160	5	20
Methanol	Hot	40	80	80	160
	Cold	40	160	40	160

4. Discussion

In the present study hot and cold extracts of ethanol and methanol extracts of *R. communis* were tested to determine their inhibitory effect against standard bacteria, *E. coli* and *S. aureus*. The results demonstrated that these extracts had ability to control the bacteria *in vitro*.

Different extraction techniques, cold and hot extraction, were carried out in this study, because, mode of extraction plays an important role on the amount and type of biomolecules present in the extract[15]. Cold extraction is the widely using technique than hot extraction. This is due to the reason that during hot extraction some volatile biomolecules escape from the extract because of high temperature[16]. In the present study, hot ethanol and methanol extracts showed significant effect on *E. coli* compared to cold extracts of respective solvents. The better activity of hot extracts may be due to the chemical changes caused by the hot treatment, and the resulting biomolecules may be more active than the biomolecules found in the cold extracts.

But this tendency was not observed against *S. aureus*, where hot and cold ethanol extracts showed better activity than methanol extracts. This variation in the inhibitory effect may be due to the difference in the amount or type of biomolecules in the extracts. Ethanol and methanol are polar solvents but with different polarity, methanol has higher polarity than ethanol. The polarity of the solvents determines the solubility of chemicals from plant powder[17].

Except hot methanol, all other extracts showed significant effect on *S. aureus* than *E. coli*. *E. coli* and *S. aureus* are Gram negative and Gram positive bacteria, respectively. Generally Gram positive bacteria show more sensitivity to biomolecules present in plant extracts than Gram negative bacteria. This is due to the differences in the composition of cell wall of the two bacteria[18].

Kota and Manthri[3] studied the antibacterial activity of hot ethanol extract of leaves of *R. communis* against *S. aureus* (MTCC740) and *E. coli* (MTCC41) by agar well diffusion method and the result correlates with the present study.

Oyewole *et al*[19] carried out a screening of antibacterial properties of cold methanol extract of *R. communis* leaves against *S. aureus* and *E. coli* by broth dilution method and they found absence of inhibitory effect of the extract on both of test bacteria. The phytochemical analysis showed the presence of tannins, phlobatannins, flavonoids, terpenoids and cardiac glucosides, but the saponins were absent. However, in the present study methanol extract showed inhibition in both agar well diffusion method and broth dilution method. Furthermore, the phytochemical study showed presence of saponins and absence of phlobatannins. The variation in the results may be due to the variation of plant variety or different geographical distribution of the plant[20].

In nature, phytochemicals are responsible to protect the plants from infection of pathogenic microorganisms[21]. In the present study phytochemical analysis of *R. communis* leaves revealed the presence of saponins, cardiac glycosides, tannins, flavonoids and terpenoids in all the test extracts. 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 complex with extracellular and soluble proteins and to complex with bacterial cell walls, more lipophilic

flavonoids may also disrupt microbial membranes. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds[21]. Saponins interfere with or alter the permeability of the cell wall. Therefore, this facilitates the entry of toxic materials or leakages of vital constituents from the cell, and the tannins act by coagulating the cell wall proteins[22].

In conclusion, this study demonstrates that the hot and cold methanol and ethanol extracts are potential sources for control of *S. aureus* and *E. coli*. Especially, the hot and cold extracts of ethanol are more suitable against *S. aureus* even at lower concentration. Further study is needed to identify the specific bioactive compounds, their mode of action and their nontoxic nature in vivo condition.

Conflict of interest statement

We declare that we have no conflict of interest.

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