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Comparative Phytochemical Analysis and Antimicrobial Activity of Four Medicinal Plants

Sumanta Das[1], Shahid Jamal[1], Madhurima Dutta[1], Sharmistha Rej[1] and Sabyasachi Chatterjee[1*]

[1]Department of Biotechnology, the University of Burdwan, Rajbati, Bardhaman - 713104, West Bengal, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was aimed to evaluate the phytochemical constituents and antimicrobial activity of the leaf and stem extracts of *Ricinus communis*, *Solanum nigrum*, *Clerodendrum infortunatum* and *Calotropis gigantea*. The selected plants were made a comparative study of medicinal value. The result of phytochemical analyses revealed that chemical diversity in aqueous and ethanol leaf extracts of *R. communis* which showed the presence of flavonoids, steroids, carbohydrates, proteins, tannins, glycosides, terpenoids, alkaloids, phenols, glycosides and anthraquinones. Phytochemical compound is comparative less in ethyl acetate extracts of leaf from *S. nigrum*, *C. infortunatum* and *C. gigantea*. In Total Phenol Content assay (TPC), the highest concentration of phenols was found in ethanol leaf extracts of *R. communis* (800 mg GAE/100 g) and the less amount of TPC recorded in aqueous extracts of *C. infortunatum* (20 mgGAE/100 g). The Total Flavonoids Content assay (TFC) value followed similar trend the highest concentration was found

**Corresponding author: E-mail: sabsach@rediffmail.com, sumantad1991@gmail.com;*

in ethyl acetate leaf extracts of *R. communis* (450 mg CE/100 g) and the absent in aqueous extracts of *C. gigantea* and *C. infortunatum*. The antimicrobial activities of aqueous, methanol and ethanol extracts leaf and stem were determined on selected bacteria, *Escherichia coli, Bacillus subtilis* and the fungus, *Curvularia lunata*. The result indicates that the aqueous and methanol leaf extracts of *R. communis* were showed strong antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* at a concentration of 100 mg/ml respectively. Even ethanol leaf extracts of *Ricinus communis* showed better zone of inhibition diameter 45±0.65 mm compared to other plants extract against fungus, *Curvularia lunata*.

Keywords: Phytochemical constituents and antimicrobial activity; leaf and stem extracts; bacteria and fungus; total phenol and flavonoids content assay.

1. INTRODUCTION

The World Health Organization has estimated that 80% of the inhabitants of the world rely mainly on traditional plants for their primary health care needs and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles. Medicinal plants have been traditionally used for different kinds of infection diseases [1]. Many natural substances of plant origin reported biologically active, endowed with antimicrobial properties [2]. The frequency of life threatening infection caused by pathogenic organisms has increased worldwide. Although a large number of antimicrobial agents have been discovered, pathogenic microorganism constantly developing resistance to these agents [3]. Antibiotics are sometimes associated with side effects whereas there are some advantages of using antimicrobial compound of medicinal plants. Such as often fewer side effects, better tolerance and relatively less expensive [4]. Antibacterial constituents of medicinal plants and their use for the treatment of the microbial infection as possible alternatives to synthetic drugs to which many infectious microorganism have become resistant seem to very much promising [5]. However, several studies have indicated that medicinal plants contain compounds, e.g. peptides, unsaturated fatty acid, aldehydes, flavonoids, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are significant therapeutic application against pathogens, including bacteria, fungus and viruses [6]. These secondary metabolites produced by plants are organic chemicals of high structural density which play different functions including chemotherapeutic, bactericidal, bacteriostatic and antimicrobial functions [7]. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant.

Ricinus communis belongs to the family Euphorbiceae commonly known as Castor oil plant having many medicinal uses in antioxidant, anti-inflammatory, antimicrobial, antifungal, wound healing, insecticidal and many other medicinal properties [8]. *Solanum nigrum* belonging to the family Solanaceae commonly known as Black nightshade having medicinal use as laxative, antifungal, antimicrobial, anticancer and also used in antioxidant and antipyretic [9]. *Clerodendrum infortunatum* belongs to the family Lamiaceae commonly known as Bharaangi. The leaves and roots are used as herbal remedy for asthma, cough, skin diseases and antimicrobial activities [10]. *Calotropis gigantea* belongs to the family Apocynaceae commonly known as Madar having many medicinal uses in asthma, diarrhea, nausea, vomiting, antimicrobial and many other medicinal properties [11].

In this study, different fractions (aqueous, ethanol, methanol and ethyl acetate) of leaf and stem extracts *of R. communis, S. nigrum, C. infortunatum and C. gigantean* were prepared for phytochemicals analyses and antimicrobial activities.

2. MATERIALS AND METHODS

2.1 Collection of Plants

Ricinus communis and three selected plants i.e. *Solanum nigrum, Calotropis gigantea and Clerodendrum infortunatum* were collected in 20th March, 2014 at different areas of the University of Burdwan, India. The plants were identified and authenticated at the herbarium unit of the Department of Botany, University of Burdwan, India. Then leaf and stem parts of the four plants were cut and washed in tap water.

2.2 Preparation and Extraction of Leaf Extracts

The leaf extracts of four selected plants were prepared separately using aqueous, ethanol, methanol and ethyl acetate for comparative study of phytochemical analyses and antimicrobial activity. The plant leaves were carefully washed with tap water, rinsed with distilled water, and immediately spread over tissue paper for air dried under shade for 15 to 20 minutes at room temperature. 2 g of plant leaves were submerged in 20 ml distilled water or solvent (methanol, ethanol, ethyl acetate) and crushed by using morterpestle. The extract was filtered using filter paper. A greasy material (crude extract) obtained for each plants was transferred to screw- cap tube, labeled and stored under refrigerated (4ºC) condition until further experiment was carried out.

2.3 Preparation and Extraction of Stem Extracts

The stem extracts of four selected plants were prepared by using ethanol for comparative study of antimicrobial activity. The plant stems were carefully washed with tap water, rinsed with distilled water, and immediately spread over tissue paper for air dried under shade for 15 to 20 minutes at room temperature. 2g of plant stems were submerged in 20 ml of 95% ethanol and crushed by using morterpestle. The extract was filtered using filter paper. A greasy material (crude ethanol extracts) obtained for each plants was transferred to screw- cap tube, labeled and stored under refrigerated (4ºC) condition until use.

2.4 Preparation of Mix Extracts

The mixed extracts were prepared by using stem and leaf (1:1) of four selected plants. The plant stems and leaves were carefully washed with tap water, rinsed with distilled water, and immediately spread over tissue paper for air dried under shade for 15 to 20 minutes at room temperature. 2 g of plant stems & leaves (1:1) were extracted in 20 ml of 95% ethanol and crushed by using morterpestle. The extract was filtered using filter paper. A greasy material (crude ethanol mix extracts) obtained for each plants was transferred to screw- cap tube, labeled and stored under refrigerated (4ºC) condition until use.

2.5 Bacterial Culture

The microbial cultures *Escherichia coli* and *Bacillus subtilis* were procured from Department of Biotechnology, Burdwan University.

2.6 Fungal Culture

Curvularia lunata was used for fungal susceptibility test. The fungal culture was supplied by Department of Botany, the University of Burdwan, India.

2.7 Phytochemical Analysis

Each solvent fraction of all the plants leaf extract were analysed for its phytochemical components [12-14]. The extracts were screened for the following phytochemical compounds: Flavonoids, alkaloids, tannin, steroids, anthraquinones, saponin, terpenoids, phenol, proteins, glycosides, as well as carbohydrates.

3. QUALITATIVE TEST

3.1 Test for Flavonoids

Leaf extract (0.5 ml) of four selected plants were taken and were heated with 10 ml of ethyl acetate over a steam bath for 3 min. Then 1 ml of dilute ammonia solution and few drops of concentrated H_2SO_4 were added.

3.2 Test for Alkaloids

Leaf extract (0.5 ml) of four selected plants were taken in each tube was treated with 5 ml of 1% hydrochloric acid on a boiling water bath for 20 min. Then few drops of Wagner's reagent were added.

3.3 Test for Tannin

Leaf extract (0.5 ml) of four selected plants were taken in each tube and 10 ml of distilled water was added. Then the mixtures were added with 1M ferric chloride reagent.

3.4 Test for Steroids

Leaf extract (1 ml) of four selected plants taken in each tube were treated with 2 ml of acetic anhydride and cooled on ice. The mixtures were mixed with 0.5 ml of chloroform and 1ml of concentrated H_2SO_4.

3.5 Test for Anthraquinones

Leaf extract (0.5 ml) of four selected plants taken were taken in each tubes and were shaken with 10ml of benzene. 5 ml of ammonia solution was added and mixture was shaken well.

3.6 Test for Saponin

Leaf extract (0.5 ml) of four selected plants were taken in each tube and were boiled in 2.5 ml of distilled water and shaken vigorously. Then few drops of olive oil was added and shaken vigorously in water bath for formation of emulsion.

3.7 Test for Terpenoids

Leaf extract (1 ml) of four selected plants was taken in each tube. Then mixtures was treated with 1 ml of 2, 4-dinitrophenyl hydrazine dissolved in 100 ml of 2 M HCl.

3.8 Test for Phenols

Leaf extract (0.5 ml) of four selected plants were taken in each tubes and were boiled with 2 ml of distilled water on the water bath. Then 10% $FeCl_3$ reagent was added in each tube.

3.9 Test for Proteins

Leaf extract (0.5 ml) of four selected plants were taken in each tube. Then 0.1 ml of Millon's reagent was added.

3.10 Test for Glycosides

Leaf extract (0.5 ml) of four selected plants were taken in each tube and was dissolved in pyridine and few drops of 20% sodium nitropruside together and few drops of NaOH was added.

3.11 Test for Carbohydrates

Leaf extract (0.5 ml) of four selected plants were taken in each tube and few drops of Molisch's reagent was added. Then 1 ml of conc. H_2SO_4 was added.

4. QUANTITATIVE TEST

The total phenol and flavonoids in the plants extracts were quantitatively determined [15].

4.1 Estimation of Phenol

The amount of total phenol content, in various solvent extracts of leaf was determined by Folin-Ciocalteau's reagent. 1 ml of each extract in each tube was taken. 2 ml of distilled water and 0.5 ml of Folin-ciocalteau's reagent was added. After 3 minutes, 2 ml of 20% sodium carbonate solution was added. The tubes are kept in boiling water bath for 1 minute and then cooled. Absorbance (650 nm) was measured in colorimeter. Gallic acid was used as a standard positive control. The total phenol content in the extracts were calculated from the standard curve and the results expressed as gallic acid equivalent per 100 g dry weight of the (mgGAE/100 g) extract.

4.2 Estimation of Flavonoids

One milliliter of each extract in each tube was taken. Then 4 ml of distilled water and 0.3 ml of 5% sodium nitrite were added. After 5 minutes, 0.3 ml of 10% of aluminum chloride was added. Again after 5 minutes, 2 ml of 1 M sodium hydroxide was added. Volume was made up to 10 ml and absorbance (540 nm) was measured in colorimeter. Catechin was used as a standard positive control. The total flavonoids content in the extracts were calculated from the standard curve and the results expressed as catechin equivalent per 100 g dry weight of the (mg CE/100 g) extract.

4.3 Antibacterial Evaluation Leaf Extracts

The leaf extracts were screened for antimicrobial activity using agar well diffusion method [16]. A total of 30 ml molten Müller-Hinton (M.H) agarwere poured into sterile petriplates. 500 µl of inoculum of each bacteria were spread on the surface of Müller-Hinton (M.H) agar (pH 7.3) petriplates. Using Sterile metal cup borer (10mm diameter), wells were made into the set agar containing the bacterial culture. In each plates two well were made, one used for extract of leaves and another used for solvent as a positive control. 800 µl of leaf extract of four selected plants at concentration 100 mg/ml were poured into wells in each petriplates. Control were set up by using solvent viz., distilled water, methanol and ethanol which were used to prepare the respective leaf extracts. The plates were allowed to keep at room temperature for 20 minutes to allow excess prediffusion of extracts. Thereafter, the plates were incubated for 24 hours at 37ºC temperature.

4.4 Antibacterial Evaluation of Stem Extracts

The ethanol stem extracts were screened for antimicrobial activity using agar well diffusion method. A total 30 ml molten Müller- Hinton (M.H) agar (30 ml) were poured into sterile petriplates. 500µl of inoculum of each bacteria were spread on the surface of Mueller Hinton agar (pH 7.3). By using metal sterile cup borer (10 mm diameter), wells were made into the set agar containing the bacterial culture. In each plates three wells were made i.e. one used for ethanol as a positive control, one used for stem extracts and another used for mixture of extracts (stem and leaves 1:1). The plates were allowed to keep at room temperature for 20 minutes to allow for diffusion. Thereafter, the plates were incubated for 24 hours at 37ºC temperature.

4.5 Antifungal Evaluation of Leaf Extracts

The leaf extracts of four selected plants were screened for antifungal activity by using agar well diffusion method. A total of molten 30ml Potato Dextrose Agar (PDA) was poured into sterile petriplates. A metal sterile cup borer was used to cut a deep uniform well in the medium at the center of the petriplates. A loopful fungal cultured was taken and transferred it near the well. Ethanol leaf extracts (0.8 ml) was poured in the well. The plates were incubated for 48 hours at 30ºC temperature.

5. STATISTICAL ANALYSIS

The data were statistically analyzed and results were expressed as means± Standard Error (S.E).

6. RESULTS and DISCUSSION

6.1 Phytochemical Analysis

In this study, the phytochemical components analysis as shown in Tables 1-4. *Ricinus communis* showed maximum degree of chemical diversity (metabolites presence in extracts).

6.2 Antimicrobial Activity

Antimicrobial activity was observed in leaf extracts (100 mg/ml) of aqueous, methanol and ethanol. The aqueous leaf extracts of *Solanum nigrum* was inhibited more as compared to other. The inhibition zone against *Bacillus subtilis* was highest in leaf extract of *Solanum nigrum* and least in *Clerodendrum infortunatum.*

6.3 Comparative Study in between Ethanol Leaf and Stem Extracts

The ethanol mixed extracts (stem & leaf) of *Ricinus communis* showed highest zone of inhibition zone occurred in *Ricinus communis* and least in *Solanum nigrum.*

6.4 Antifungal Activity

The leaf extracts of *Ricinus communis* was found to be a much better antifungal, exhibiting broad range of antifungal activity against *Curvularia lunata* than other selected plant leaf extracts.

7. DISCUSSION

The result of the phytochemical analysis, Table1, reveals that flavonoids, tannin, terpenoids, protein, glycosides, carbohydrate, anthraquinones, steroids, saponin and alkaloids were present in the leaf extracts *of Ricinus communis* while all the secondary metabolites components were not present or partial present in the leaves extract of *Solanum nigrum*, *Calotropis gigantea* and *Clerodendrum infortunatum* respectively (Tables 2-4).

Biological and antimicrobial activities of *Ricinus communis* extracts have been attributed to chemical compounds. Furthermore, antimicrobial activities of the medicinal plant have been attributed to phenolic compounds such as flavonoids, phenolic acids and tannins present in them.

Total phenol content of selected plants, using four solvents: methanol, ethanol, aqueous and ethyl acetate. Among the four plants, ethanol extract of *Ricinus communis* was found to have highest TPC (800 mgGAE/100 g), followed by ethanol extracts of *Calotropis gigantea* (350 mg GAE/100 g), ethanol extract of *Solanum nigrum* (150 mgGAE/100 g), and ethanol extract of *Clerodendrum infortunatum* (150 mg GAE/100 g).The result showed that among all the solvent extracts, the ethanol extract using had highest TPC. This may be due to fact that phenolic are often extracted in higher amount using such as ethanol, methanol and aqueous (Table 5).

In total flavonoids content assay of selected plants, ethyl acetate extract of *Ricinus communis* was found to be highest (450 mg CE/g), followed by ethyl acetate extract of *Solanum nigrum* (430 mg CE/100 g), ethyl acetate extract of *Calotropis gigantea* (320 mg CE/100 g) and ethyl acetate extract of *Clerodendrum infortunatum* (10

mgCE/100 g). The result showed that among all the solvent extract, ethyl acetate extract had highest TFC (Table 6). This may due to fact that flavonoids are often extracted higher amount in ethyl acetate.

Antimicrobial activity of methanol and aqueous leaf extracts of *Ricinus communis* was significantly different from *Solanum nigrum*, *Calotropis gigantea* and *Clerodendrum infortunatum*. In this study, aqueous and methanol leaf extracts of *Ricinus communis* on test organism at the concentration of 100 mg/ml showed zone of inhibition of 35±0.4 and 16±0.52 mm respectively against *Bacillus subtilis* and the least in *Clerodendrum infortunatum* (Table 7). Ethanol leaf extract of *Solanum nigrum* at the concentration of 100 mg/ml showed that highest zone of inhibition of 45±2.12 mm when compared with three tested plants against *Escherichia coli*. The activity of ethanol extract of *Ricinus communis* stem was significantly different from three tested plants. *Ricinus communis* had highest zone of inhibition of 21±0.97 mm at concentration of 100 mg/ml and least in *Clerodendrum infortunatum* against *Escherichia coli* (Table 8). The ethanol mixed extracts of *Ricinus communis* at the concentration of 100mg/ml inhibited more as compared to three tested plants against *Escherichia coli* (Table 8).

The ethanol leaf extracts from *Ricinus communis, Solanum nigrum, Calotropis gigantea and Clerodendrum infortunatum* were significance difference among antifungal effect against *Curvularia lunata.* Ethanol extracts of *Ricinus communis*at the concentration of 100 mg/mlstrongly inhibited the fungus with inhibition diameter of 45±0.65 mm, on the other ethanol extract of leaf from *Solanum nigrum, Calotropis gigantea and Clerodendrum infortunatum* had no activity against *Curvularia lunata* (Table 9).

Table 1. Leaf extracts of *Ricinus communis*

TEST	A.E	M.E	E.E	E.A.E
Flavonoids	-	-	++	+
Tannin	++	++	++	++
Steroids	++	++	++	-
Alkaloids	++	++	++	++
Phenol	++	++	++	++
Terpenoids	++	++	++	++
Saponin	++	-	-	++
Anthraquinone	++	-	-	-
Carbohydrate	++	++	++	++
Protein	++	++	++	++
Glycosides	++	++	++	++

A.E= Aqueous Extracts, M.E= Methanol Extracts, E.E= Ethanol Extracts, E.A.E= Ethyl Acetate Extracts, ++= strongly present, += partial present, -= absent

Table 2. Leaf extracts of *Clerodendrum infortunatum*

TEST	A.E	M.E	E.E.C	E.A.E
Flavonoids	-	+	++	-
Tannin	++	++	++	++
Steroids	++	++	-	-
Alkaloids	++	++	++	++
Phenol	+	++	+	+
Terpenoids	++	++	++	++
Saponin	++	-	-	+
Anthraquinone	++	-	-	-
Carbohydrate	++	++	++	++
Protein	++	++	++	++
Glycosides	++	++	++	++

A.E= Aqueous Extracts, M.E= Methanol Extracts, E.E= Ethanol Extracts, E.A.E= Ethyl Acetate Extracts, ++= strongly present, += partial present, -= absent

Table 3. Leaf extracts of *Solanum nigrum*

Test	A.E	M.E	E.E	E.A.E
Flavonoids	+	+	-	++
Tannin	++	++	++	++
Steroids	+	++	-	-
Alkaloids	++	++	++	++
Phenol	++	++	+	++
Terpenoids	++	++	++	++
Saponin	++	-	-	+
Anthraquinone	++	-	-	-
Carbohydrate	++	++	++	++
Protein	++	++	++	++
Glycosides	++	++	++	++

A.E= Aqueous Extracts, M.E= Methanol Extracts, E.E= Ethanol Extracts, E.A.E= Ethyl Acetate Extracts, ++= strongly present, += partial present -= absent

Table 4. Leaf extracts of *Calotropis gigantean*

TEST	A.E	M.E	E.E.C	E.A.E
Flavonoids	+	-	+	++
Tannin	++	++	++	++
Steroids	++	++	-	-
Alkaloids	++	++	++	++
Phenol	+	+	++	+
Terpenoids	++	++	++	++
Saponin	++	-	-	+
Anthraquinone	++	-	-	-
Carbohydrate	++	++	++	++
Protein	++	++	++	++
Glycosides	++	++	++	++

A.E= Aqueous Extracts, M.E= Methanol Extracts, E.E= Ethanol Extracts, E.A.E= Ethyl Acetate Extracts, ++= strongly present, += partial present, -= absent

Table 5. Estimation of total phenol content assay (mg GAE/100g dry weight of extracts)

PLANTS	Ethyl acetate extract	Ethanolextract	Aqueous extract	Methanol extract
Ricinus communis	250	800	720	650
Solanum nigrum	370	150	640	640
Calotropis gigantea	170	350	160	170
Clerodendrum infortunatum	20	150	340	790

Table 6. Estimation of total flavonoids content assay (mg CE/100g dry weight of extracts)

PLANTS	Ethyl acetate extract	Ethanol extract	Aqueous extract	Methanol extract
Ricinus communis	450	15	25	35
Solanum nigrum	430	450	35	20
Calotropis gigantea	320	45	NIL	45
Clerodendrum infortunatum	150	340	NIL	70

Table 7. Determination of antimicrobial activity of leaf by agar well diffusion method

Leaf extracts	Zone of inhibition (mm)				Bacteria
	Ricinus communis	***Solanum nigrum***	***Calotropis gigantea***	***Clerodendrum infortunatum***	
Aqueous extract	35±0.43	40±1.8	15±0.46	12±2.3	*Bacillus subtilis*
Methanol extract	16±0.52	15±.0.2	14±0.54	12±1.10	
Ethanol extract	30±.97	45±2.12	28±1.72	33±0.97	*E. coli*

Data expressed as Mean zone of inhibition mm±SE

Table 8. Comparative study in between ethanol leaf and stem extracts against *E. coli*

Plants extract	Zone of inhibition(mm)		
	Ethanol stem extracts	Ethanol leaf extracts	Mixture of stem & leaf (1:1)
Ricinus communis	21±0.97	20±1.22	28±3.6
Solanum nigrum	15±0.53	17±0.36	15±0.67
Calotropis gigantea	17±0.45	14±0.5	16±0.21
Clerodendrum infortunatum	15±0.57	12±1.0	22±1.94

Data expressed as Mean zone of inhibition mm± SE

Table 9. Determination of antifungal activity of leaf by agar well diffusion method against *Curvularia lunata*

Plant leaf extract	Zone of inhibition (mm)
Ricinus communis	45±0.65
Solanum nigrum	NIL
Calotropis gigantea	NIL
Clerodendrum infortunatum	NIL

Data expressed as Mean zone of inhibition mm± SE

8. CONCLUSION

It can be concluded that all the plants screened in this study had some phytochemicals in common while antimicrobial activity was more with *Ricinus communis* compared to other three plants. However, further works need to be done on the isolation and identification antimicrobial components present in the *Ricinus communis* for its application in both animal and human pharmaceutical industry.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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