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Invitro Assesment of Antibacterial Activity of *Calotropis Procera and Coriandrum Sativum* Against Various Pathogens

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Subject: Biotechnology:

Abstract
The present study is aimed to focus on antimicrobial activity of two plant samples which are collected from the Lucknow region. The plant extract are prepared in five solvents (methanol, acetone, petroleum ether and ethyl acetate) to check the antibacterial activity against bacterial pathogens. The pathogens are *P.aeruginosa, S.typhi, S.aureus, E.coli, B.subtilis.* This process are done by the Agar Well Diffusion Method. The phytochemical analysis of *Calotropis procera* and *Coriandrum sativum* parts exhibited the presence of secondary metabolites which were saponin, terpenoids, cardiac glycosides, reducing sugars, quinines, phlobatannins, tannins, Polyphenol and glycosides. Among the different types of tested extract, the stem of methanol extract of *Coriandrum sativum* with different solvents showed the least MIC value at concentration ranging 0.19 at conc. of 45µg/ml was obtained against *S.aureus.* the leaves of ethyl acetate extract of *Coriandrum sativum* showed the least MIC value at concentration ranging 0.29 at conc. of 40µg/ml against *S.typhi.* The maximum zone of inhibition was recorded in case of leaves of methanol extract of *Calotropis procera* against *E.coli* ranging 25.5 mm of zone of inhibition. In case of stem ethyl acetate extract of *Calotropis procera* against *S.aureus* ranging 20mm of zone of inhibition.

Key words: *Agar well diffusion method, Secondary metabolites and Zone of inhibition.*

Introduction
An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan. Antimicrobial agents either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Various parts of medicinal plants like the leaves, flowers, fruits, roots and the bark extract, infusion, decorations and powders have proven useful in curing a wide range of health related issues[29]. Medicinal plants possess potent medicinal value that is due to the presence of variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body. Very few of these chemicals are toxic[30]. Novel molecules obtained in many cases are highly active against microbes.

Calotropis procera belongs to Asclepideacea family. The Hindi names of this plant are Madar, Aak. The stem of this plants are erect, soft. This plant is abundant in warm climate areas having dry, sandy and alkaline soils. *Coriandrum sativum* belongs to Apiaceae family. The Hindi name of this plant is Dhaniya. The English name of this plant is coriander. The herbs of this plant are used to make the delicious common Indian chutney. The fruit of this plant are used in baking, pickles, soups, sausage, candies, sauces and act as a flavouring agent. The dried stem parts of this plant are used as a fuel. It has eleven components of essential oils, six types of acids, minerals and vitamins, each having a number of beneficial properties. It is considered to be a stimulant, diuretic, carminative and tonic. It is very helpful in treatment of different types of digestive dysfunctions. It helps in case of indigestion, ulcerative colitis, typhoid fever and diarrhea. Coriander juice can help in treatment of dry skin and could be effective in removal of pimples and blemishes. A decoction made from dried plant is an excellent eye tonic. It contains antioxidant, antibacterial and antimicrobial effect. Both the plants are herbal and medicinal plants.

Methodology

Sample Collection
The samples of leaves and stems of *Calotropis procera* and *Coriandrum sativum* were collected near MRD Life Science Lab, Lucknow and brought from local market of Gomti nagar Lucknow. The plant samples were identified on the basis of botanical identity and standard description. The samples were properly washed, dried under sunlight and also dried in hot air oven at 40^{o} C-50^{o}C for 1-2 days. The dried samples were grinded by mixer and converted into the powdered form.

Preparation of plant extract

The plant extract of leaves and stems of *Calotropis procera* and *Coriandrum sativum* were prepared with the help of organic solvents. Organic solvents have a common structure (at least 1 carbon and 1 hydrogen atom), low molecular weight, lipophilicity and volatility and they exist in liquid form at room temperature. The solvents are acetone, methanol, petroleum ether, ethyl acetate. These solvents were employed in the ratio of 1:10. In a container, the powdered form of samples were dissolved in the solvents and kept into a dark room for 48 hrs. After completion of 48 hrs, the solvents were filtered through a whatmann filter paper and evaporate the filter up to thick solid residue in the hot air oven at 40°C-50°C for 24 hrs. Then the used of DMSO for the secondary metabolites preserved in extracts. These extracts were used for screening.

Pathogens Used

The Antibiogram analysis of *Calotropis procera* and *Coriandrum sativum* were performed against 5 bacterial pathogens. These all pathogenic cultures were originally procured from IMTECH, Chandigarh, India. Their sub-culturing were maintained in MRD Life Sciences Lab, Lucknow (U.P). The MTCC no. for used cultures is as follows –

Bacterial culture

P.aeruginosa : MTCC 741, *B.subtilis :* MTCC 6633, *S.aureus :* MTCC 96
E.coli : MTCC 1304, *S.typhi:* MTCC 53

Antibacterial activity against various pathogens

Antibiotic Sensitivity Tests by Agar Well Diffusion Method

AST refers Antibiotic Sensitivity Technique. AST is employed to check the sensitivity of antibiotic against various pathogens. Antibiotic are chemicals which inhibit the growth of bacterial pathogens. Antibiotics are ample nontoxic to the host and are used as chemotherapeutic agents in the treatment of infectious diseases. To check the activity of AST by calculating the diameter of Zone of Inhibition in mm. After preparation of nutrient agar. The nutrient agar were poured into the petriplates and allowed to solidify the gel. After solidifying the gel, the spreading of the culture on the agar gel and punched with a sterile tips to make the open wells. The plant extract were loaded into the open wells. Then incubate at 37° C for 24 hrs. Next day measure the zone of inhibition in mm.

Phytochemical Analysis: Phytochemicals are types of metabolites which were present in plants and responsible for antibacterial and antifungal activity.

Tannins :

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60°C for 2 hrs. To filtrate the boiled extract. Add 10µl ferric chloride in filtrate solution. The results were brownish green colour to the solution. This colour showed that the presence of tannin in the plant.

Saponnins :

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60° C for 2 hrs. To filtrate the boiled extract. Add 2-3 drops of olive oil in the filtrate mixture and then shaken vigorously. The result was emulsion. This emulsion showed the presence of saponins in the plant.

Flavonoids:

The powdered samples were dried and dissolved in the ethyl acetate in the ratio 1:10 then boiled in the water bath at 100° C for 10 minutes. To filtrate the boiled extract. Add dilute NH_3 in the filtrate mixture. The result was yellowish colour. This colour showed that the presence of flavonoids in the plant.

Terpenoids:

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60°C for 2 hrs. To filtrate the boiled extract. Add chloroform in the filtrate mixture after that mix well. The test tubes were placed in a water container to keep cool. Add concentrate H_2SO_4 from the side of the test tube. The colour was observed a clear reddish brown colour at the interface. This proves the presence of terpenoids in the plant extract.

Cardiac Glycosides:

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60° C for 2 hrs. To filtrate the boiled extract. Add glacial acetic acid and some drops of ferric chloride solution. Then add concentration H_2SO_4 from the side of test tube. The results were brown ring formed at the interface.

Reducing Sugars:

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60° C for 2 hrs. To filtrate the boiled extract. Add de-ionised distilled water. Then after addition of Fehling's solution A&B. The mixtures were warmed at 40° C for half an hr in water bath. The colour was observed brick red precipitate at the bottom of the test tube. It was indicate the presence of a reducing sugar.

Polyphenol :

The powdered samples were dried and dissolved in the ethanol in the ratio 1:10 then boiled in the water bath at 60^0C for 2 hrs. To filtrate the boiled extract. Add Folin Ciocalteu reagent and add distilled water. After add sodium carbonate solution, vortex to mix the solution.

Then after incubate at room temperature in dark room for 30 minute. After 30 minute take O.D. at 680 nm.

Phlobatannins :

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60^oC for 2 hrs. To filtrate the boiled extract. Add few drops of HCl. The result was deposition of red precipitate. It was indicate the presence of phlobatannins.

Quinones :

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60^oC for 2 hrs. To filtrate the boiled extract. Add few drops of sodium hydroxide in the solution and shaken the solution vigorously. The colour was observed blue green red colour. It was indicate the presence of quinines.

Glycosides :

The powdered samples were dried and dissolved in the distilled water in the ratio 1:10 then boiled in the water bath at 60^oC for 2 hrs. To filtrate the boiled extract. The extract was hydrolysed with HCl solution and neutralized with NaOH solution. Add few drops of Fehling's solution A&B. The result was observed red precipitate. It was indicate the presence of glycosides.

Minimum Inhibitory Concentration

Principle: Minimum inhibitory concentration is an important diagnostic laboratory test which is generally performed in microbiology. It is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As much as the value of MIC will be low it will show that antimicrobial agent have better potential against microorganisms. It is the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms (or organisms).

Optimization of Culture Conditions

Principle: Optimization is basically done to check growth activity of different microorganisms by inoculating them in different carbon sources, nitrogen sources and applying different temperature and pH. In this method prepared broth is incubated at different temperature and pH, having different carbon and nitrogen sources and this incubated broth is used to check growth of microorganisms with the help of colorimeter by taking O.D. It involves suitable carbon, pH, nitrogen and temperature. Different 1% carbon sources such as Dextrose, Maltose, Starch, Lactose, Sucrose**.** Different 1% nitrogen sources such as Urea, NH_4Cl, Na_2HPO_4, $NaH_2P\ O_4$, Na_2CO_3**.**Different pH: 5,7,9,10 and Different temperature: Room temperature, 37^oC, 40^0C, 50^0C.

Results

Table 1: Pathogen results against solvents

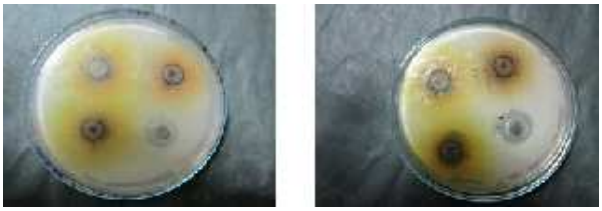
Pathogens	Ethyl Acetate	Methanol	Acetone	Petroleum Ether
E.coli	9.5	10.5	12	12
S.typhi	13	12	13	--
S.aureus	10.5	13	11.5	13
P.aeruginosa	14	12.5	14.5	16
B.subtilis	12.5	13	12.5	12.5

Antibacterial activity of Leaves of *C.procera*

This table show that the best results are obtained for Acetone and Petroleum Ether against *E.coli* and Ethyl Acetate and Acetone against *S.typhi* and Methanol and Petroleum Ether against *S.aureus* and Petroleum Ether against *P.aeruginosa* and Methanol against *B.subtilis*.

Figures 1 showed the antibacterial activity of leaves of *Calotropis procera* against various pathogens.

E.coli ***S.typhi***



S.aureus ***P.aeruginosa***

B.subtilis

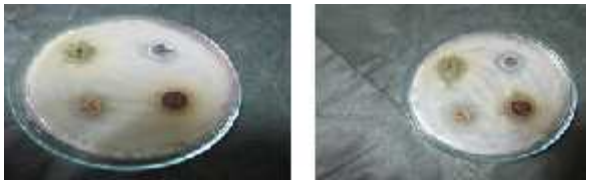
Figure: 1 Antibacterial activity of Leaves of *Calotropis Procera* against various pathogens

Table 2: Antibacterial activity of stem of *Calotropis procera* against various pathogens

Pathogens	Petroleum Ether	Methanol	Acetone	Ethyl acetate
E.coli	--	13	12.5	13.5
S.typhi	13.5	16.5	17.5	16.5
S.aureus	12	12.5	12	14
P.aeruginosa	12.5	15.5	15.5	16
B.subtilis	13	19	16	24.5

This table show that the best results are obtained for Ethyl acetate against *E.coli* and Acetone against *S.typhi* and Ethyl acetate against *S.aureus* and Ethyl acetate against *P.aeruginosa* and Ethyl acetate against *B.subtilis.*

E.coli ***S.typhi***



S.aureus ***P.aeruginosa***

B.subtilis

Fig 2: Shows the antibacterial activity of stem of *Calotropis procera* against these pathogens.

Table 3: Antibacterial activity of leaves of *Coriandrum sativum* against various pathogens

Pathogens	Petroleum ether	Methanol	Acetone	Ethyl acetate
E.coli	--	13	12.5	13.5
S.typhi	13.5	16.5	17.5	16.5
S.aureus	12	12.5	12	14
P.aeruginosa	12.5	15.5	15.5	16
B.subtilis	13	19	16	24.5

This table show that the best results are obtained for Ethyl acetate against *E.coli* and Acetone against *S.typhi* and Ethyl acetate against *S.aureus* and Ethyl acetate against *P.aeruginosa* and Ethyl acetate against *B.subtilis*

E.coli ***S.typhi***

S.aureus ***P.aeruginosa***

B.subtilis

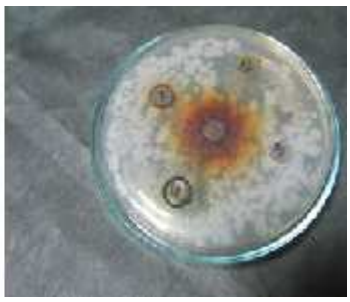
Fig 3: Shows the antibacterial activity of leaves of *Coriandrum sativum* against these pathogens. The 4th well is shown the distilled water against these pathogens.

Table 4: Antibacterial activity of stem of *Coriandrum sativum* against various pathogens

Pathogens	Petroleum ether	Methanol	Acetone	Ethyl acetate
E.coli	10	Partial	15	16.5
S.typhi	11	15.5	6	15
S.aureus	13	17	--	17.5
P.aeruginosa	10.5	Partial	Partial	18.5
B.subtilis	17	11.5	Partial	13.5

This table show that the best results are obtained for Ethyl acetate against *E.coli* and Methanol against *S.typhi* and Ethyl acetate against *S.aureus* and Ethyl acetate against *P.aeruginosa* and Petroleum ether against *B.subtilis.*

E.coli



S.typhi

S.aureus

P.aeruginosa

B.subtilis

Fig. 4: Shows the antibacterial activity of stem of *Coriandrum sativum* against these pathogens. The 4th well is shown the ethyl acetate of leaves of *Coriandrum sativum* against these pathogens.

S.typhi

P.aeruginosa

E.coli

S.aureus

B.subtilis

Fig. 5: Shows the antibacterial activity of stem of petroleum ether of *Coriandrum sativum* against these pathogens.

Phytochemical Analysis

Table 5:- Phytochemical Analysis of *Calotropis procera.*

S.NO.	Phytochemicals	Colour indications	Result (Stem) (+ve or -ve)	Result (Leaves)
1	Tannins	Brownish green colour	Partially positive	Partially positive
2	Saponin	Got emulsion	Positive	Positive
3	Flavonoids	Yellow colour	Negative	Negative
4	Terpenoids	Reddish brown colour	Positive	Positive

5	Cardiac Glycosides	Light green colour	Positive	Positive
6	Reducing Sugars	Brick red colour	Negative	Positive
7	Phlobatannins	Red precipitate	Negative	Negative
8	Quinones	Blue green red colour	Positive	Partial Positive
9	Glycosides	Red precipitation	Negative	Negative
10	Polyphenol	O.D at 680 nm	0.40	0.73

Table: 6 Phytochemical analysis of Coriandrum *sativum.*

S.NO.	Phytochemicals	Colour indications	Result (Leaves) (+ve or -ve)	Result (Stem)
1	Tannins	Brownish green colour	Negative	Positive
2	Saponin	Got emulsion	Positive	Positive
3	Flavonoids	Yellow colour	Negative	Negative
4	Terpenoids	Reddish brown colour	Positive	Positive
5	Cardiac Glycosides	Light green colour	Positive	Negative
6	Reducing Sugars	Brick red colour	Negative	Negative
7	Phlobatannins	Red precipitate	Positive	Negative
8	Quinones	Blue green red colour	Positive	Negative
9	Glycosides	Red precipitation	Negative	Negative
10	Polyphenol	O.D at 680 nm	1.03	0.25

Minimum Inhibitory Concentration (MIC)

Table :- 9 MIC results of acetone leaf extract of *Calotropis procera.*

T.T. No.	Leaves + Acetone Extract O.D	Concentration (µg/ml)
4	**0.04**	0.0051

Table :-10 MIC results of Petroleum ether leaf extract of *Calotropis procera.*

T.T. No.	Leaves + Petroleum Ether Extract O.D	Concentration (µg/ml)
5	**0.00**	0.00032

Table :-11 MIC results of Ethyl Acetate leaf extract of *Calotropis procera.*

T.T. No.	Leaves + Ethyl Acetate Extract O.D	Concentration (µg/ml)
4	**0.05**	0.00357

Table :-12 MIC results of Methanol leaf extract of *Calotropis procera.*

T.T. No.	Leaves + Methanol Extract O.D	Concentration (µg/ml)
3	**0.12**	0.077

Table :-13 MIC results of Methanol stem extract of *Calotropis procera.*

T.T. No.	Stem + Methanol Extract O.D	Concentration (µg/ml)
4	**0.29**	0.0128

Table :-14 MIC results of Petroleum Ether stem extract of *Calotropis procera.*

T.T. No.	Stem + Petroleum Ether Extract O.D	Concentration (µg/ml)
4	**0.23**	0.002

Table :-15 MIC results of Ethyl Acetate stem extract of *Calotropis procera.*

T.T. No.	Stem + Ethyl Acetate Extract O.D	Concentration (µg/ml)
3	**0.15**	0.020

Table :-16 MIC results of Acetone stem extract of *Calotropis procera.*

T.T. No.	Stem + Acetone Extract O.D	Concentration (µg/ml)
4	**0.26**	0.0056

Table :-17 MIC results of Acetone Leaf extract of *Coriandrum sativum*.

T.T. No.	Leaves + Acetone Extract O.D	Concentration (µg/ml)
6	**0.11**	0.00015

Table :-18 MIC results of Ethyl Acetate Leaf extract of *Coriandrum sativum*.

T.T. No.	Leaves + Ethyl Acetate Extract O.D	Concentration (µg/ml)
4	**0.29**	0.0040

Table :-19 MIC results of Petroleum Ether Leaf extract of *Coriandrum sativum*.

T.T. No.	Leaves + Petroleum Ether Extract O.D	Concentration (µg/ml)
4	**0.27**	0.0025

Table :-20 MIC results of Methanol Leaf extract of *Coriandrum sativum*.

T.T. No.	Leaves + Methanol Extract O.D	Concentration (µg/ml)
4	**0.18**	0.007

Table :- 21 MIC results of Methanol stem extract of *Coriandrum sativum*.

T.T. No.	Stem + Methanol Extract O.D	Concentration (µg/ml)
5	**0.19**	0.00045

Table :-22 MIC results of Acetone stem extract of *Coriandrum sativum*.

T.T. No.	Stem + Acetone Extract O.D	Concentration (µg/ml)
6	**0.10**	0.0001

Table :-23 MIC results of Ethyl Acetate stem extract of *Coriandrum sativum*.

T.T. No.	Stem + Ethyl Acetate Extract O.D	Concentration (µg/ml)
6	**0.13**	0.0001

Table :-24 MIC results of Petroleum Ether stem extract of *Coriandrum sativum*.

T.T. No.	Stem + Petroleum Ether Extract O.D	Concentration (µg/ml)
6	**0.17**	0.000064

Discussion

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such bacteria, fungi, or protozoan. Antimicrobial agents either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Various parts of medicinal plants like the leaves, flowers, fruits, roots and the bark extract, infusion, decorations and powders have proven useful in curing a wide range of health related issues[15]. The present study was carried out to analyse the antibacterial and antifungal activity of *Calotropis procera* and *Coriandrum sativum* with optimized conditions and to identify the presence of secondary compounds by phytochemical analysis.

Further work was only carried out the used parts of samples (*Calotropis procera* and *Coriandrum sativum*) were leaves and stems with five solvents i.e, methanol, acetone, petroleum ether and ethyl acetate which were organic solvents. The plant extracts with organic solvents showed better results as compared to distilled water.

The phytochemical analysis of *Calotropis procera* and *Coriandrum sativum* parts exhibited the presence of secondary compound which were saponin, terpenoids, cardiac glycosides, reducing sugars, quinines in leaves of *Calotropis procera* and saponin, terpenoids, cardiac glycosides, quinones in leaves of *Coriandrum sativum* and in stem of *Calotropis procera* and tannins, saponin, terpenoids in stem of *Coriandrum sativum.*

The maximum zone of inhibition was recorded in case of leaves of petroleum ether extract of *Coriandrum sativum* against *S.typhi* ranging 23mm of zone of inhibition. In case of stem of ethyl acetate extract of *Calotropis procera* against *B.subtilis* ranging 24.5 mm of zone of inhibition. So, finally it was found out that *Calotropis procera* stem parts have significantly best antibacterial activity as compared *Coriandrum sativum* leaves. Among the different types of tested extract, the stem of methanol extract of *Coriandrum sativum* with different solvents showed the least MIC value at concentration ranging 0.19 at conc. of 45µg/ml was obtained against *S.aureus.* the leaves of ethyl acetate extract of *Coriandrum sativum* showed the least MIC value at concentration ranging 0.29 at conc. of 40µg/ml against *S.typhi.*

The range was recorded in case of carbon sources in optimized condition of sucrose in *E.coli*, In nitrogen case, Na_2HPo_4 in *S.aureus*, In temperature case, 37^oC condition is suitable for all bacterias, In pH case, pH 7 in *P.aeruginosa*. The maximum zone of inhibition was recorded in case of leaves of methanol extract of *Calotropis procera* against *E.coli* ranging 25.5mm of zone of inhibition. In case of stem ethyl acetate extract of *Calotropis procera* against *S.aureus* ranging 20mm of zone of inhibition. So, finally it was found out that *Calotropis procera* leaves parts have significantly best antibacterial activity as compared stem of *Calotropis procera* stem.

Conclusion

The overall study conclude that plant samples can be used as a drug after purification.The present studies carried out by using coriander and Calotropis sample against bacterial pathogens. So because of this reason the maximum amount of herbal medicines can be prepared with the help of purification method because there are less chances of side effects. In present time, more than 80% plant species are unknown for their antibacterial and antifungal property which can be helpful for human welfare.

The future prospect for this work is the compound which is responsible for antibacterial activity can be characterized with the help of HPLC and through PCR analysis. The primer can be design and further drug can be validated.

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