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Research Article

Evaluation of Antimicrobial Activity Determination and Phytochemical Investigation in Selected Plants

Singh Nitu, Patidar K C*

School of Botany, Devi Ahilya University, Indore (M.P.), India

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ABSTRACT

The work was intended to investigate the antibacterial activity of the various medicinal plants *viz. Calotropis procera*, *Jatropha curcas*, *Cryptostegia grandiflora*, *Clerodendron inermis* and *Cassia tora*, collected from Indore (Madhya Pradesh, India) region. The aim of the study, with an objective, was to evaluate the antimicrobial potentials and to determine the zone of inhibition of extracts of selected plants on some medically important bacterial strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. Various plant extracts were tested for the antibacterial activities against *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Corynebacterium diphteriae*. Zone of inhibition of extracts were compared with that of standards like streptomycin. The outcomes illustrated that the noteworthy inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were also carried out. The microbial activity of the selected plant was due to the presence of various secondary metabolites. Hereafter, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Antibacterial activity, plant extract, minimum inhibitory concentration (MIC), Phytochemicals, Zone of inhibition (ZOI).

INTRODUCTION

Infectious diseases may be an unavoidable fact of life, however, there are many strategies available to help us protect ourselves from infection and to treat a disease once it has developed. Today the control of microbial action through the properties of naturally occurring components has become an important field of research[1]. The resistances against bacteria and microorganism have been increased due to random use of commercial antimicrobial medicines widely used in the treatment of infectious diseases. These situations forced scientists to explore for new anti-microbial and therapeutic antioxidant efficacious substances from various sources, such as medicinal plants[2].

Despite the extensive use of antibiotics, infectious diseases continue to be a leading cause of morbidity and mortality worldwide[3]. Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems[4]. The discovery of antibiotics has also decreased the spread and severity of a wide variety of inferior diseases. However, and as a result of their uncontrolled use, the efficiency of many antibiotics is being threatened by the emergence of microbial resistance to existing chemotherapeutic agents. This started the scientists worldwide to take the interest in natural antimicrobial compounds available from plant sources[5].

For many decades, phytochemicals, the secondary metabolites of plants, have been used throughout the world, as drug and remedies for various diseases *viz.* chronic anti-viral, anti-inflammatory[6], anti-ulcer[7], antianxiety[8], antinociceptive[9], hepatoprotective[10], antioxidants[11] and antimicrobials[12]. Off late, the use of natural antimicrobial compounds has gained much attention by the researchers and consumers. This is due to two primarily major factors, first, the misuse and mishandling of antibiotics has resulted in the dramatic rise of a group of microorganisms including foodborne pathogens that are not only antibiotic resistant but also more tolerant to several food processing and preservation methods[13]. In addition, increasing consumers awareness of the potential negative impact of synthetic preservatives on health versus the benefits of natural additives has generated interest among researchers in the development and use of natural products[13]. This has prompted the researchers to look for alternative that can enhance the safety and efficacy of infection therapy. Compounds derived from natural sources have the potential to antimicrobial properties against a broad range of pathogens[14].

This work was intended to explore the antibacterial activity of natural components from different plant sources. The vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the local flora for antibacterial and antifungal activity from *Calotropis procera*, *Jatropha curcas*, *Cryptostegia grandiflora*, *Clerodendron inermis* and *Cassia tora*.

*Author for Correspondence: singh.neetu.dav@gmail.com

Table 1: Antibacterial activity of different parts of *Cassia tora* against different bacteria.

Name of bacteria	Zone of inhibition (diameter, in mm)				
	Root	Bark	Leaf	Streptomycin	Negative control
Corneybacterium diphteriae	++	++	++	++	-
Salmonella typhi	+	-	+	++	-
Escherichia coli	++	++	+	+++	-
Staphylococcus aureus	+	+	+	+++	-
Bacillus subtiillis	+	+	+	++	-

(-) No zone of inhibition; (+) Zone of inhibition (5-10 mm in diameter); (++) Zone of inhibition (11-19 mm in diameter) (+++) Zone of inhibition 20-25 mm in diameter)

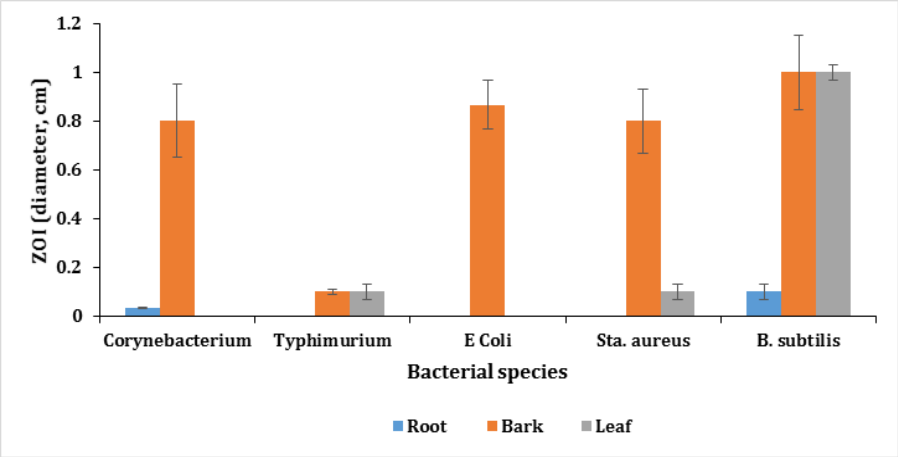

Figure 1: Antibacterial activity of different parts of *Cassia tora* against different bacterial species.

Table 2: Antibacterial activity of different parts of *Cleodendrum* against different bacterial species.

Name of bacteria	Zone of inhibition (diameter, in mm)				
	Root	Bark	Leaf	Streptomycin	Negative control
Corneybacterium diphteriae	+	+	++	++	-
Salmonella typhi	+	-	-	++	-
Escherichia coli	+	+	++	++	-
Staphylococu aureus	-	++	++	++	-
Bacillus subtiillis	-	+	+	+++	-

(-) No zone of inhibition; (+) Zone of inhibition (5-10 mm in diameter); (++) Zone of inhibition (11-19 mm in diameter) (+++) Zone of inhibition 20-25 mm in diameter)

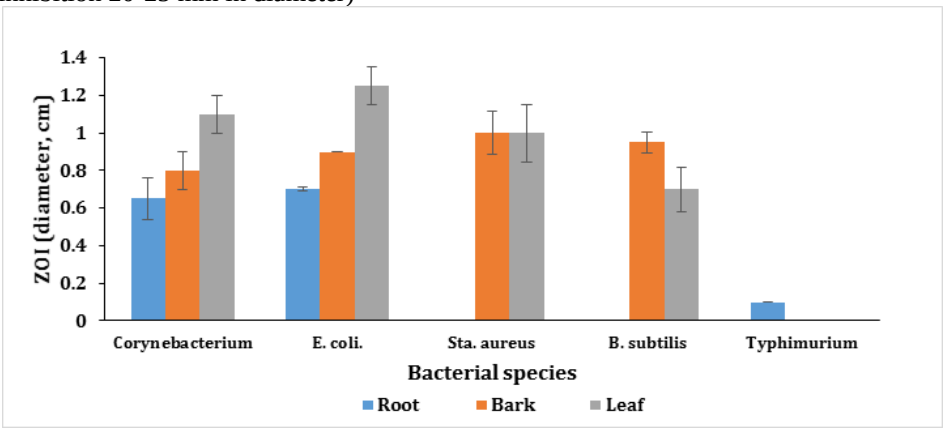

Figure 2: Graphical representation of the Antibacterial activity of different parts of *Cleodendrum* against different bacterial species.

MATERIALS AND METHODS

collection of plant material

Fresh root, bark and leaves of five different plants *viz. Calotropis procera, Jatropha curcas, Cryptostegia grandiflora, Clerodendron inermis* and *Cassia tora* free from disease were collected from Indore, Madhya Pradesh,

Table 3: Antibacterial activity of different parts of *Cryptostegia* against different bacterial species.

Bacteria	Root	Bark	Leaf	Streptomycin	Negative control
Corneybacterium diptheri	-	+	-	+++	-
Salmonella Typhi	-	-	-	++	-
Escherichia coli	-	+	-	++	-
Staphylococus aureus	-	+	++	+++	-
Bacillus subtiillis	-	++	++	+++	-

(-) No zone of inhibition; (+)Zone of inhibition (5-10 mm in diameter); (++) Zone of inhibition (11-19 mm in diameter) (+++) Zone of inhibition 20-25 mm in diameter)

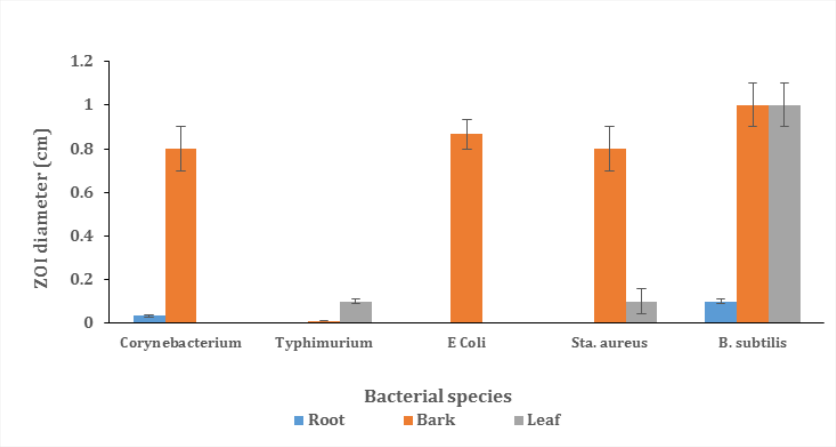

Figure 3: Graphical representation of the Antibacterial activity of different parts of *Cryptostegia* against different bacterial species.

Table 4: Antibacterial activity of different parts of *Calotropis* against different bacterial species.

Bacteria	Root	Bark	Leaf	Streptomycin	Negative control
Corneybacterium diptheri	-	-	+	++	-
Salmonella Typhi	-	+	-	++	-
Escherichia coli	+	-	-	++	-
Staphylococus aureus	-	-	-	++	-
Bacillus subtiillis	-	+	+	++	-

(-) No zone of inhibition; (+)Zone of inhibition (5-10 mm in diameter); (++) Zone of inhibition (11-19 mm in diameter) (+++) Zone of inhibition 20-25 mm in diameter)

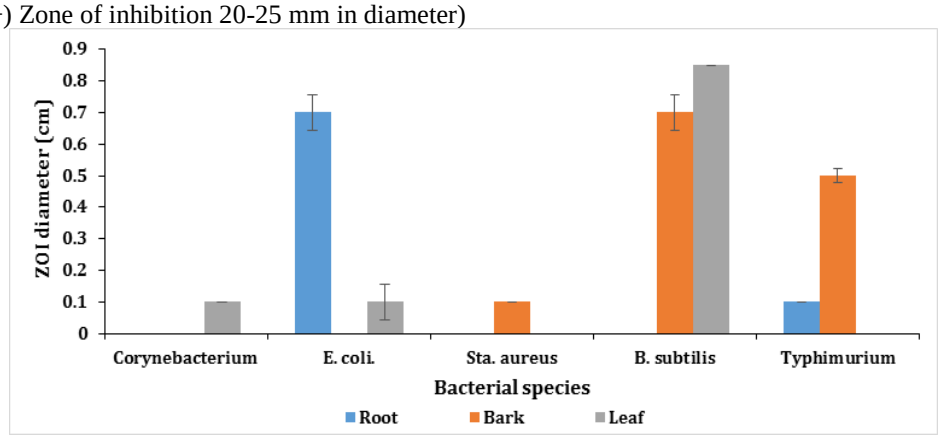

Figure 4: Graphical representation of Antibacterial activity of different parts of *Calotropis* against different bacterial species.

India. The parts of the plants will be washed thoroughly 2-3 times with running water and once with sterile distilled water, material will be then air-dried on sterile blotter under shade, ground, catalogued and stored.

Preparation of the plant Extracts

The extracts were prepared from air-dried samples of the whole plant or from the portion specified traditionally for treatment of diseases. About 50 *g* of the powdered plant material was taken in a dry 250 mL conical flask, then 100 mL methanol was added and allowed to macerate overnight[15]. Extracting plant powdered material at low speed for a longer period allows greater penetration of the solvent into the plant tissues which allows more of the plant compounds to be extracted[16]. The next day the

Table 5: Antibacterial activity of different parts of *Jatropha curcas* against different bacterial species.

Name of bacteria	Zone of inhibition (diameter, in cm)				
	Root	Bark	Leaf	Streptomycin	Negative control
Corneybacterium diptheri	+++	-	+++	++	-
Salmonella Typhi	-	-	-	++	-
Escherichia coli	+++	+	++	+	-
Staphylococus aureus	+++	+	++	++	-
Bacillus subtiillis	+++	++	+++	+	-

(-) No zone of inhibition; (+)Zone of inhibition (5-10 mm in diameter); (++) Zone of inhibition (11-19 mm in diameter) (+++) Zone of inhibition 20-25 mm in diameter)

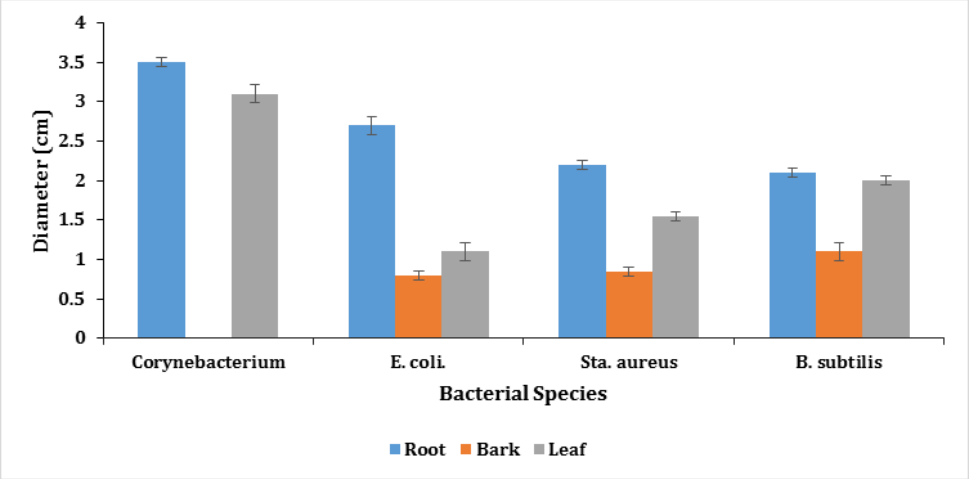

Figure 5: Graphical representation of Antibacterial activity of different parts of *Jatropha* against different bacterial species.

mixture was vigorously stirred for 10 minutes and allowed to settle. The supernatant was filtered and the residual plant material was extracted twice using 100 mL methanol. The three filtrates thus obtained were combined and the solvent was evaporated using rotary evaporator. The residual plant material was dried well and again extracted with methanol, using the same procedure as above. The crude extracts were kept at 4°C[17].

Phytochemical analysis

Qualitative phytochemical analysis of the crude powder of the 5 plants to be collected and determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red colour indicated the presence of flavonoids[18].

Procurement of microbial strains

Growth and maintenance of test microorganism for antimicrobial studies

Bacterial cultures of *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Corynebacterium diphteriae* were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. The bacteria were maintained on nutrient agar broth (NB) at 37°C. The agar media was prepared in accordane to previoudly reported method[19].

Maintenance of bacterial strains

Organisms were maintained by periodic subculture in nutrient agar slants. Briefly, 1 g beef extract, 2 g yeast extract, 5 g peptone and 5 g sodium chloride was dissolved in distilled water, heated slowly and then cooled. Subsequently, 15 g agar was dissolved in the above mixture by heating. The medium was then poured in 5 ml quantities in no. of test tubes. Sterilization was done by autoclaving at 121°C for 15 minutes[20]. The medium in the tubes was then allowed to solidify as slants.

Preparation of the inoculum

The bacteria were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min. The pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm)[21].

Anti-bacterial activity

The methanol extracts was tested by the disc diffusion method[22]. The test microorganisms were seeded into respective medium by spread plate method. After

Table 6: Phytochemical analysis of different plant extracts.

Name of the plant	Plant part extract	Phytochemical tested					
		Tannins	Alkaloids	Saponins	Glycosids	Terpenoids	Flavonoids
	Root	-	+	+	-	+	+
Cassia tora	Bark	-	+	-	-	-	+
	Leaf	+	+	-	+	-	+
Clerodendron	Root	+	-	-	+	+	-
inermis	Bark	-	+	-	+	+	-
	Leaf	+	+	-	+	+	+
Cryptostegia	Root	-	+	+	-	+	-
grandiflora	Bark	+	-	-	-	+	+
	Leaf	+	-	-	+	-	+
Calotropis	Root	+	+	-	-	+	-
procera	Bark	+	+	-	+	-	+
	Leaf	+	-	-	+	+	+
Jatropha	Root	+	+	+	+	+	-
curcas	Bark	-	+	-	-	+	-
	Leaf	+	+	+	+	+	-

(+ present; - Absent)

solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Corynebacterium diphteriae* were used for antibacterial test. 10µl plant extracts were added into each filter paper disc and Streptomycin sulphate (10 µg /disc) were used as positive control and methanol solvent (100 µg / ml) were used as negative control. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical studies revealed variations in the morphology and biochemical constituents. The screening of the plants extracts for the phytochemicals i.e tannins, saponins, flavonoids, etc, showed that the plants are abundant of the terpenoids except *Cassia tora*, in which alkaloids and flavonoids are more amount. Alkaloids are present in more amounts in leaf, root and bark extracts. Saponins were found to be only in root extracts of *Cassia tora, Cryptostegia grandiflora and Jatropa* curcus but absent in other plant.

Antibacterial activity of plants extract

Results obtained in the present study revealed that the tested five medicinal plants extracts possess potential antibacterial activity against tested bacteria viz. *Corynebacterium diphteriae, Salmonella typhi, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* (table no. 1 to 5). However the highest antibacterial activity was found with the root and leaf extracts of plant *Jatropha*. But it showed negligible antibacterial activity against *Typhimurium* (table 5). On the other hand, the plant extract of *Calotropis* showed least antibacterial activity (table 4). It was observed that the leaf extracts of all the plants showed more or less antibacterial activity.

The antibacterial activity of the extracts of different plant parts of *Cassia tora* is depicted in table 1 and the graphically presented in fig 1.

From fig 1 and table 1, it is clear that the bark extract of the plant has higher antibacterial activity than root and leaf extract. The highest antibacterial was seen against *B. subtilis*. However, leaf extract of the plant show the similar ZOI against *B. subtilis*. The lowest antibacterial activity is possessed by root extract. It is only active against *B. subtilis* and *Cornybacterim*, and giving small ZOI.

On the other hand, the leaf extract of the plant *Cleodendrum* exhibited maximum antibacterial activity (fig 2 and table 2). It showed maximum antibacterial activity against E. coli species, against which all the extracts i.e. root and bark extracts also active (fig 2). In this case also, root extract showed minimum antibacterial activity.

The plant extract of *Cryptostegia* showed little antibacterial activity. None of the extract showed any antibacterial against Salmonella *typhimurium*. The root extract is ineffective against any bacterial species (Table 3). Bark extract shown more or less antibacterial activity against all bacterial species except *Typhimurium.*

Similarly, the plant extract of *Calotropis* exhibited very little antibacterial activity (fig 4 and table 4). Here also, root extract was ineffective against all except E. coli, against which it showed slight antibacterial activity. The bark and leaf extract showed slight antibacterial against *B. subtilis*. Besides, *B. subtilis*, leaf extract and bark extract exhibited slight antibacterial activity against *Corneybacterium* and *Typhimurium* respectively.

In all the plants, Jatropa showed excellent antibacterial activity against all bacterial species, except *Salmonella typhimurium*, against which none of the extract was active. In contrast to earlier observations from previous plant, the root extract of *Jatropa* exhibited highest antibacterial activity followed by leaf extract. In this case, the bark extract exhibited minimal antibacterial activity (fig 5 and table 5). The maximum antibacterial activity of root and leaf extracts was found against *Corneybacterium*.

Phytochemical screening of plant extracts

The screening of the plants extrcts for the phytochemicals i.e tannins, alkaloids, saponins, flavonoids, etc, showed

that the plants are abundant of the flavonoids except *Cassia tora*, in which flavonoids are absent but tannins are present in more or less amount in leaf, root and bark extracts. Alkaloids were found to be present in bark extracts of Cleodendrum and Jatropa but absent in other plant. Saponins were found to be present in all the plants in more or less amount except in Calotropis. The anthocyanins were present in the leaf extracts of *Cassia tora* and Jatropa. Table 6 depicts the presence and absence of phytochemicals in different parts of tested plant extracts

CONCLUSION

It was concluded that the selected plants exhibit the antibacterial activity, due to the active phytochemicals present in them. It is hoped that these active constituents will provide useful information for discovering new compounds with better activity against various bacterial strains than agents currently available.

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