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Research Article

Medicinal Efficacy of *Tinospora cordifolia, Caesalpinia bonduc,* and *Quercus infectoria* Extracts

Abdullah Maqbool, Rida Batool*, and Nazia Jamil

Institute of Microbiology and Molecular Genetics, University of the Punjab,
Quaid-e-Azam Campus, Lahore, Pakistan

Abstract: Medicinal plants have therapeutic value because of the chemical substances present in them. This study was performed to explore the antibacterial and antioxidant potential of three medicinally important plants, such as *Caesalpinia bonduc* (karanjuwa), *Tinospora cordifolia* (Giloy), and *Quercus infectoria* (Majuphal) extract; that are locally used for the cure of various diseases, particularly dengue fever. To study the anti-bacterial, phytochemical and antioxidative potential of these plants, plant extracts were made in ethanol, methanol, and ethyl acetate. Antibacterial assay revealed that all the selected plant extracts of *C. bonduc, T. cordifolia,* and *Q. infectoria* variably inhibited the growth of test bacterial strains. The maximum antibacterial activity observed for the ethyl acetate extract of *T. cordifolia* was against *Bacillus* (41 mm) and *Pseudomonas* (38 mm). Phytochemical analysis of extracts of *C. bonduc, T. cordifolia,* and *Q. infectoria* revealed the presence of alkaloids, quinones, flavonoids, phenols, and reducing sugars. Antioxidative potential of the extracts of *C. bonduc, T. cordifolia,* and *Q. infectoria* was revealed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (66%, 62% and 77%), phosphomolybdate (480, 520 and 490 µg/ml), showed ferric reducing antioxidant power (FRAP) assay (266%, 371% and 265%), and total flavonoid content (75%, 170% and 155%) assay. Due to significant antibacterial and antioxidant potential, the extract of *T. cordifolia* was subjected to Thin-layer chromatography (TLC) analysis that confirmed the presence of different bioactive components in the extract of *T. cordifolia* (Giloy). Gas Chromatography-Mass spectrophotometry (GCMS) analysis of the bioactive fractions revealed Phthalic acid and Benzene acetic acid as the compounds responsible for imparting good antibacterial and antioxidative potential to the ethyl acetate extract of *T. cordifolia*. These findings highlight the potential of *T. cordifolia* as a valuable medicinal plant for the development of future antimicrobial drugs.

Keywords: *Tinospora cordifolia*, *Caesalpinia bonduc*, *Quercus infectoria,* Antibacterial Activity, Phytochemicals, Antioxidant Potential, Phthalic Acid, Benzene Acetic Acid.

1. INTRODUCTION

Human history reveals that the utilization of different plants for the curing of various diseases started thousands of years ago. Plants that can cure infectious diseases are called medicinal plants [1]. Local people in several countries, like China and Egypt, started to use herbal medicines for curing infectious and non-infectious diseases [2]. Various synthetic drugs from the bioactive compounds of these remedial plants were extracted that play a very important part in the development of modern medicines. This is the reason that the focus of research has been on folk medicine to treat infectious diseases [3]. It is crucial to comprehend the significance of the new medication and make an effort to isolate numerous bioactive compounds found in specific medicinal plants. In this study, three medicinal plants: *Caesalpinia bonduc, Quercus infectoria and Tinospora cordifolia* were used. *C. bonduc* plant is a member of the Caesalpiniaceae family [4]. Chemical analysis of this plant has highlighted its significance in the pharmacological field, owing to its diverse range of bioactive properties. Widely found in hot and humid climates, this therapeutic plant, also known as nicker or fever nut, has been extensively utilized in traditional medicine to cure numerous ailments [5]. *Q. infectoria* is a member of the family Fagaceae. It is a small-sized tree that is widely spread in Iran, Asia, and different areas of Greece. On this tree, galls arise on young branches when it is attacked

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* Corresponding Author: Rida Batool <rida.mmg@pu.edu.pk>

by the gall-wasp. In Malaysia, the galls are known as *manjakani* and *majuphal* locally. It also has great importance in Indian folk medicines, where it is extensively used in powder form for the cure of various tooth-related diseases [6, 7].

T. cordifolia has a common name, Gilroy belonging to the family Menispermaceae. It has a woody shrub that was native to India and is found in Sri Lanka and Burma in great numbers [8]. Male flowers are usually bunched, and the female flower is isolated in the racemes or racemose panicles. The flowering season expands over summers and winters [9]. *T. cordifolia* has different local names like Gilroy, Guduchi, etc. *T. cordifolia* has phytochemicals that belong to diverse classes of compounds, like phenols, flavonoids alkaloids, and terpenoids [10]. Numerous kinds of bioactive compounds have been extracted worldwide from different parts of *T. cordifolia* plant.

Dengue fever is a mosquito borne viral disease. Nature has a massive pool of bioactive materials that can be used directly as pharmaceuticals, or their derivatives can be utilized as effective agents to combat dengue. Presently, there are no specific treatments. But there are few medicinal herbs, such as *T. cordifolia,* which have presented some good pharmacological properties against dengue [11]. Hence, the present study was devised to investigate the phytochemicals, antibacterial, and antioxidative potential of extracts from three medicinal plants (*T. cordifolia* (wild), *C. bonduc*, and *Q. infectoria*) commonly used for dengue fever treatment. This exploration aims to lay the groundwork for their potential application in future studies focused on developing effective antiviral medications.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Extracts

Seed, bark, and galls of three different plants, i.e., *C. bonduc, T. cordifolia* (wild), and *Q. infectoria,* were collected from the local market in Lahore, Pakistan. These plant samples were stored in Institute of Microbiology and Molecular Genetics (IMMG) under the voucher numbers MMG-IM-30, MMG-IM-31, MMG-IM32 and MMG-IM-33. Seed, bark, and galls were thoroughly washed by sterilized distilled water 3-4 times, air-dried under shade, and crushed in powdered form. The powered material (20 grams) of each plant was dipped in the respective solvent (100 ml) for 48 hours; ethyl acetate was used for *T. cordifolia,* and ethanol was used for *C. bonduc* and *Q. infectoria.* The extracts were filtered and finally dried with the help of a rotary evaporator to make them concentrated for further use.

2.2. Antibacterial Activity of Plant Extracts

The antibacterial potential of ethyl acetate and ethanol plant extracts can be measured by an agar well-diffusion assay [12]. For this purpose, 100 µl of 24 hours old culture (OD_{600nm} 0.5) of *Bacillus* (JQ013099) and *Pseudomonas* (KC881031) were spread on Muller Hinton agar (MH) plates to check the antibacterial activity of selected plant extracts. In the wells, 50 µl of the previously prepared plant extract (20%) was added. These plates were incubated at 37 °C for 24 hours and zones of inhibition (mm) were measured. Ethanol and ethyl acetate were used as negative controls, whereas ampicillin (10 µg/ml) was a positive control**.** The experiment was done in triplicate.

2.3. Phytochemical Analysis

Selected plant extracts were subjected to the following tests for the screening of various phytochemicals present in these extracts [13].

2.3.1. Alkaloids

Three to four drops of the Wagner reagent were added to one ml of plant extracts. The development of a reddish-brown color indicated the alkaloids.

2.3.2. Phenols

To 1 ml of plant extract, a few drops of ferric chloride solution (5%) were added as a reagent. The appearance of the deep blue color confirmed the phenols.

2.3.3. Flavonoids

Flavonoids are a group of polyphenols that are especially present in plants. A 20% sodium hydroxide solution (0.5 ml) was mixed with plant extract (1 ml). Yellow coloration showed a positive test that became colorless when diluted

hydrochloric acid was added.

2.3.4. Terpenoids

The addition of one ml of plant extract to two ml of chloroform, followed by the careful incorporation of three ml of concentrated H_2SO_4, resulted in the appearance of a red color, indicating the presence of terpenoids [14].

2.3.5. Reducing sugars

For this, 100 µl of the Benedict reagent was added to one ml of plant extract and then boiled for five minutes in a water bath. The appearance of red precipitates confirmed the occurrence of reducing sugars in plant extracts [15].

2.3.6. Steroids

One ml of chloroform was added to an equal volume of plant extract, and then one ml of conc. sulfuric acid was added at the end. A red color appeared, which indicated the steroids.

2.3.7. Tannins

For the detection of tannins, one ml of water is mixed with one ml of plant extract. Later on, a small amount of solution of $FeCl_3$ was added. Development of the green color showed the tannins.

2.4. Antioxidative Analysis of Plant Extracts

The presence of antioxidants in selected medicinal plants was confirmed by performing following four different assays:

1. Radical scavenging capacity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay
2. Phosphomolybdate assay
3. FRAP (Ferric Reducing Antioxidant Power) assay
4. Flavonoid content

2.4.1. Radical scavenging capacity by DPPH assay

Radical scavenging capacity was confirmed by following the method of Dilshad *et al.* [13]. Briefly, a standard solution of DPPH was prepared by the addition of DPPH (24 mg) in methanol (100 ml), and the optical density of the working solution was measured at 517 nm. After 60 min, the percentage radical scavenging capability was evaluated. Working solution (3 ml) was mixed with plant extract (100 µl), incubated in the dark, and optical density was measured.

2.4.2. Phosphomolybdate assay

The phosphomolybdate assay is commonly employed to evaluate the total antioxidant capacity of the extract. In this procedure, the plant extract (0.1 ml) was combined with a reagent solution containing 28 mM Na_3PO_4, 0.6 M sulfuric acid, and 4 mM $(NH_4)_6Mo_7O_{24}$. Subsequently, the test tubes were incubated in a water bath at 95 °C for one and a half hours. Following incubation, the test tubes were cooled, and the optical density was measured at 765 nm. Ascorbic acid was taken as the standard [15].

2.4.3. FRAP assay

Ferric reducing capacity of antioxidants present in plant extracts was confirmed by following the method of Jan *et al.* [15].

2.4.4. Flavonoid content

The estimation of flavonoid content was done by following the method of Dilshad *et al*. [16]. In this process, flavonoids form a complex with aluminum when reduced by antioxidants, resulting in color production. Rutin served as the standard in this assay, with concentrations ranging from 75 to 750 mg/L.

2.5. Thin Layer Chromatography (TLC)

In order to separate the different components of crude extract, TLC was employed by following the slightly modified method of Dilshad *et al.* [16]. A crude extract of *T. cordifolia* was spotted and developed in a solvent system (chloroform 8: methanol 1). Afterward, the TLC plate was taken out of the TLC tank and left to dry in the air. The separated components were then marked under both long and short UV rays. The spots observed were labeled from 1 to 7, and their *Rf* values were determined. The labeled spots were then scraped off from the TLC plate and placed in glass vials

containing ethyl acetate. After 24 hours, the extracts were filtered and the filtrate was allowed to evaporate until it dried.

2.6. Antibacterial Activity of TLC Spots

The antibacterial activity of these spots was checked against *Bacillus* (JQ013099) and *Pseudomonas* (KC881030). A disc diffusion assay was used to measure the antibacterial activity of the selected spots [13]. For this assay, ethyl acetate and ampicillin (10 µg/ml) were used as negative and positive controls, respectively.

2.7. Gas Chromatography Mass Spectrophotometry (GCMS) Analysis

GCMS analysis was performed to find the partially purified bioactive compound (spot numbers 2 and 5) that had antibacterial activity in the extract of *T. cordifolia*. The length of the column was 30 m, and helium was used as a carrier gas. The injection port temperature was maintained at 280 °C, and 2 µl of the sample was introduced at a flow rate of 1 ml/min. Subsequently, the temperature was increased to approximately 350 °C. The column was run for nearly 50 minutes leading to the detection of distinct peaks.

3. RESULTS

3.1. Selected Medicinal Plants

Medicinal plants, i.e., *C. bonduc (Karanjuwa), T. cordifolia (Giloy), and Q. infectoria (Majuphal),* which are being used locally for the treatment of flu and associated symptoms, particularly dengue fever, were procured from a local market in Lahore, Pakistan.

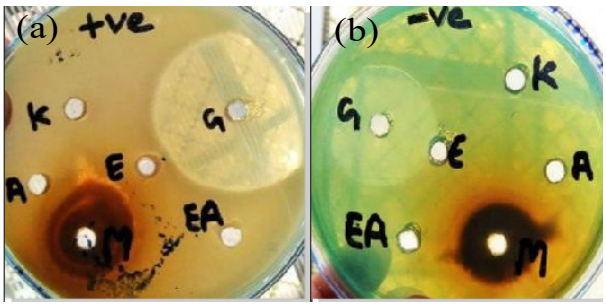


Fig. 1. Antibacterial activity of selected medicinal plant extracts: *T. cordifolia* (G); *Q. infectoria* (M) and *C. bonduc* (K) against (a) gram-positive and (b) gram-negative bacterial strain.

3.2. Antibacterial Activity of Plant Extracts

The maximum inhibition zone was shown by *T. cordifolia* (G) against both gram-positive (41 mm) and gram-negative (38 mm) strains. *Q. infectoria* (M) also inhibited the growth of both bacteria, as shown by their zones of inhibition against gram-positive (24 mm) and gram-negative (23 mm) bacteria (Figure 1). While, *C. bonduc* (K) demonstrated no inhibition potential (Table 1).

3.3. Phytochemical Analysis

Extracts of *T. cordifolia, C. bonduc, and Q. infectoria* were subjected to phytochemical analysis to find the different bioactive compounds present in them. All the plant extracts showed the presence of alkaloids, phenols, flavonoids, and sugars; whereas tannins, steroids, and pholobatanins were not found in *T. cordifolia* extract (Table 1).

3.4. Antioxidative Potential of Plant Extracts

The antioxidant potential of selected plant extracts was confirmed by different assays. Generally, the antioxidant ability of *T. cordifolia* was higher as compared to other selected plant extracts. The highest radical scavenging ability (DPPH) was detected in the extract of *Q. infectoria* (77%). While, the maximum amount of ascorbic acid (AA) (520 µg/ml) and highest ferric reduction potential (371%) were shown by the extract of *T. cordifolia*. The amount of total flavonoid concentration was also higher (170%) in the case of *T. cordifolia,* whereas *C. bonduc,* has shown the least (75%) concentration (Table 1). *T. cordifolia* exhibited significant antibacterial and antioxidative potential than *C. bonduc, and Q. infectoria;* therefore, it was selected for further analysis.

3.5. Thin-Layer Chromatography

Different bioactive components present in a crude plant extract can be separated with TLC. *T. cordifolia* had shown greater antibacterial and antioxidant potential than the rest of the tested extracts. The separated components were marked and treated with iodine. Seven spots were separated by means of TLC, as shown in Figure 2.

3.6. Antibacterial Activity of TLC Spots

Out of seven spots, two spot (2 and 5) gave the maximum antibacterial activity. Spot 2, gave a 10 mm inhibition zone with the *Rf* of 0.20, while spot 5, with *Rf* value of 0.55, showed 8 mm zone of inhibition. These two spots were further analyzed by gas chromatography mass spectrometry (Figure 3).

3.7. GC-MS Analysis

GC-MS analysis identified the compounds based on their mass. Analysis of these components presented two peaks at a retention time of 25.33 and 32.36 minutes. This analysis revealed that these components could be benzene acetic acid and phthalic acid, respectively (Table 2).

4. DISCUSSION

Throughout ancient history, plants have been utilized for their medicinal benefits across diverse cultures. The therapeutic properties of plants come from their bioactive constituents, which can be sourced from any part of the plant. Research has increasingly highlighted the potential of these medicinal plant extracts in uncovering innovative medicines, a need that continues to intensify with each passing day [17].

In the present study, *T. cordifolia, C. bonduc,* and *Q. infectoria* extracts were explored for their antibacterial and antioxidative potential due to the local use of these plants for the treatment of dengue fever. Phytochemical analysis of these extracts revealed the presence of different metabolites, such

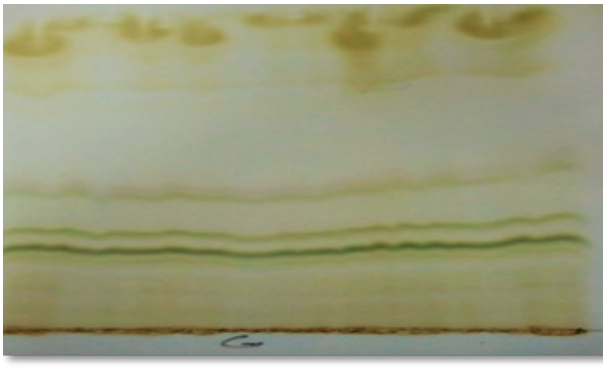


Fig. 2. TLC of the ethyl acetate extract of *T. cordifolia.*

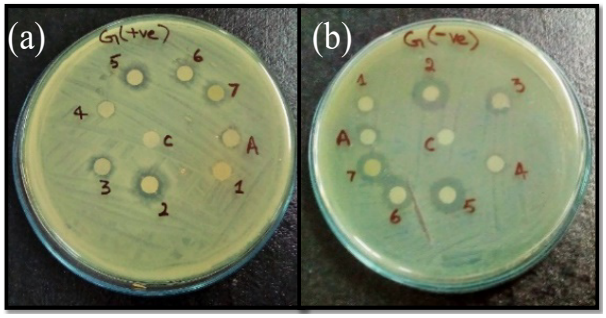


Fig. 3. Antibacterial activity of partially purified bioactive components of *T. cordifolia* extract against (a) gram positive (b) gram negative bacterial strain.

Table 1. Phytochemical analysis, antibacterial and antioxidant activity of selected medicinal plant extracts.

Extracts	Phytochemical Tests									Antibacterial Activity		Antioxidant Activity			
	Alkaloids	Phenols	Quinones	Terpenoids	Steroids	Phlobatanins	Sugars	Tannins	Flavonoids	Gram Positive (mm)	Gram Negative (mm)	DPPH (%)	Phosphomolybdate assay (µg/ml)	FRAP assay (%)	TFC (%)
T. cordifolia	+	+	+	-	-	-	+	-	+	41 ± 0.55	38 ± 0.32	62	520	371	170
C. bonduc	+	+	-	+	+	+	+	+	-	-	-	66	480	266	75
Q. infectoria	+	+	-	-	-	-	+	+	-	24 ± 0.17	23 ± 0.12	77	490	265	155

Mean of three replicates ± standard error of the mean.

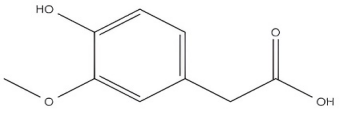
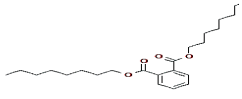
as saponins, alkaloids, amino acids, flavonoids, terpenoids, and tannins. The composition of different extracts was different because of the varied solvents in which extracts were made [18]. Though, ethanol is considered to be more polar as compared to ethyl acetate, which makes ethanol a better solvent to dissolve most of the bioactive compounds of plants in it as compared to ethyl acetate [19].

The antibacterial activity of extracts of *T. cordifolia* (Giloy), *C. bonduc* (Karenjuwa), and *Q. infectoria* (Majuphal) showed the presence of effective antibacterial compounds in them. Maximum growth inhibition for both strains (gram-positive and gram-negative) was observed by *T. cordifolia,* while minimum inhibition was shown by *C. bonduc*. The growth of gram-positive bacterial strain was inhibited slightly more than that of the gram-negative strain. The difference in inhibition potential for *Bacillus* and *Pseudomonas* is due to differences in the cell wall composition of both cell types. Gram-negative bacteria (*Pseudomonas*) have an extra layer outside of the cell wall, which makes them difficult to target by the different antibacterial compounds [20]. Another reason for this enhanced antibacterial activity could be the high sensitivity of the *Bacillus* strain to medicinal plant extracts as compared to the *Pseudomonas* bacteria. A study revealed that gram-negative bacteria are less prone to antibacterial drugs as compared to gram-positive bacteria [21].

Among the bacterial strains, the T. *cordifolia* extract exhibited the largest zone of inhibition. This significant inhibition may be due to the presence of flavonoids or tannins in the extract, known for their antibacterial properties. Conversely, extracts showing smaller zones of inhibition might have limited penetration through agar, rendering them less effective in generating larger inhibition zones. This observation aligns with research suggesting that the antibacterial efficacy of medicinal plants could hinge on factors such as the solubility of antibacterial compounds in solvents or the migration properties of plant extracts to the surrounding agar, thereby inhibiting bacterial growth. [22].

Antioxidative assays of the selected plant extracts were performed to check their antioxidative abilities. The ethyl acetate extract of *T. cordifolia* showed maximum antioxidative properties except for the DPPH, in which *Q. infectoria* shows the highest antioxidative potential, i.e., (77%). In the Phosphomolybdate assay, the addition of molybdenum to the plant extracts causes its reduction into Mo (V). This reduction produced a green-colored complex. *T. cordifolia* extract showed maximum phosphomolybdate reduction activity (520 µg/ml). FRAP reduction occurred through the action of antioxidants. By this, ferrous ions get reduced to ferric ions. The antioxidants present in the extracts of *T. cordifolia* caused the reduction of Fe^{3+} complex to the ferrous form, and thus proved to have reducing power (371 µg/ml). *T. cordifolia* extract showed greater flavonoid content (170 µg/ml). This revealed that *T. cordifolia* has a high percentage of phenolic content, which makes it a good antioxidative agent. The increased antioxidative efficacy observed in specific plant extracts might be attributed to the presence of phenolic compounds within them. Phenols have the capability to function as free radical terminators, thereby amplifying the capacity for radical scavenging. Consequently, it could be inferred that the extract from *T. cordifolia* harbors a substantial proportion of phenolic content.

Table 2. GC-MS analysis of a partially purified bio active components of extract of *T. cordifolia*.

Compound name	Molecular formula	Molecular weight (g/mol)	Retention time	Structure
Benzene Acetic Acid	$C_{20}H_{40}O_5Si_4$	472.9	25.33	
Phthalic Acid	$C_{24}H_{38}O_4$	390.6	32.36	

This observation is consistent with the findings of Martin et al., suggesting that the presence of phenolic compounds can elevate antioxidative potential [23].

Various components present in the extracts were separated with the help of the thin-layer chromatography (TLC) technique. All of the components were separated into different bands depending on their solubility in the solvent system. A brown band was observed on the TLC plate after its treatment with iodine. In compliance with the study of Chavan and coworkers, the brown spot indicates the presence of sugars in the extracts [24].

GCMS analysis of the antibacterial activity possessing fraction of *T. cordifolia* extract was done. Two fractions were found to have antibacterial properties, but their activity was less than the activity observed by the whole plant. The reason behind this difference could be due to the loss of some bioactive fraction during extraction or the synergistic effect of various components in the whole plant as compared to single fractions. Another reason could be the irreversible binding of the components to the chromatographic resins [25]. GCMS analysis of these compounds has shown them to be Benzene acetic acid and Phthalic acid. Phthalic acid is reported to have antibacterial potential [26]. Benzene acetic acid or phenyl acetic acid also possesses antimicrobial activities, as suggested by recent studies [27]. The antimicrobial activities of these two compounds (phthalic acid and Benzene acetic acid) can be a beneficial source for future drug-related studies.

5. CONCLUSIONS

In conclusion, the study found that the ethyl acetate extract of *Tinospora cordifolia* (Giloy) exhibited significant antibacterial and antioxidant properties, with the presence of bioactive compounds such as Phthalic acid and Benzene acetic acid. These findings highlight the potential applications of *T. cordifolia* as a valuable medicinal plant for the development of future antimicrobial drugs. Future research should focus on structurally analyzing the phytochemical components of these plants with anti-dengue properties. The isolation and utilization of phytochemicals with anti-dengue properties hold promise for pharmacological applications.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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