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## Phytochemical profile and antimicrobial potential of methanolic extracts of bark and leaf of *Quassia indica* (Gaertn.) Nootb.

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### ABSTRACT

The study comprises the results of phytochemical analysis and antimicrobial evaluation of extracts from bark and leaf of *Quassia indica* (Gaertn.) Nootb. – a medicinal plant used in traditional healing owing to its analgesic, anti-inflammatory, antifeedant and antimicrobial properties. A preliminary qualitative analysis was carried out successively in five different solvents with increasing order of polarity-Petroleum ether, Chloroform, Ethyl acetate, Methanol and Water to document the nature and yield of phytochemicals. The extracts were evaluated for antimicrobial effect using two strains of bacteria – *Escherichia coli* and *Staphylococcus aureus* and fungi – *Aspergillus niger* and *Candida albicans*. Among solvents methanol and water were found as effective extractants in which most of the secondary metabolites - alkaloids, flavonoids, terpenoids, phenolics, tannins, phytosterols were released. Quantitative analysis of the methanolic and aqueous extracts was carried out to estimate the quantity (mg/g tissue) of the phytoconstituents. The alkaloid content was much higher in leaf extract (5.7 mg/g) than in bark (3.5 mg/g). The phenolic content expressed as mg/ g GAE was determined in the methanolic extract, bark (24.38) > leaf (10.44) and the aqueous extract does not show much phenolic content. Flavonoid were maximum in methanolic leaf extract (1.085 mg/g) and minimum in aqueous bark extract (0.305 mg/g) and the terpenoid content was detected in methanolic extracts of leaf (0.4016 mg/g) and bark (0.4224 mg/g). The leaf extract indicated more tannin content (1.536 mg/g) than bark (1.328 mg/g). Evaluation of antimicrobial activity suggested leaf extract as an effective antibacterial and antifungal agent at a concentration of 1000 µg/ml with inhibition zones- 24 mm (*S.aureus*), 22 mm (*E.coli*) and 14 mm (*A.niger*), 14 mm (*C. albicans*). The bark extract was comparatively lesser efficient in resisting microbial growth (*E. coli* – 20 mm; *S. aureus* – 22 mm; *A. niger* – 12 mm; *C. albicans* – 10 mm).

**Keywords:** *Quassia indica*, Phytochemical analysis, Antibacterial activity, Antifungal activity.

### INTRODUCTION

The genus *Quassia* belongs to Simaroubaceae and consists of three species of small trees - *Q. africana* found in Congo, *Q. amara* found in tropical America such as Costa Rica, *Quassia indica* (Gaertn.), distributed throughout India. *Quassia indica* (Gaertn.) Nootb. (earlier name – *Samadera indica*) is a locally available medicinal plant mainly used by local folklore practitioners and tribal people in India to treat various diseases. The plant parts - bark, leaves, fruits and seeds are employed in Ayurvedic preparations. The bark and leaf possess strong bitter taste and exhibits medicinal properties against various disorders and skin diseases. *Quassia* is used to treat diseases such as vata, kapha, arthritis, constipation and skin diseases like leprosy, scabies, pruritus, erysipelas [1].

Bark of *Q. indica* is used in the treatments of fever. The juice of pounded bark is considered as a cure for skin diseases. After maceration, the bark and wood are used as tonic, stomachic and emmenagogue [2]. Decoction of leaves taken to relieve cough. The leaves are bruised and then applied externally as a treatment of erysipelas. The macerated leaves, mixed with coconut oil are used to kill head lice. Leaves are bruised and applied over skin eruptions. Pounded leaves are rubbed into scalp to treat dandruff [3]. Infusion of leaves used as insecticide, especially against white ants in the Congo and Madagascar. It is also used for treatment of malaria [4].

Quassinoids are a group of diterpenoids found in plants from the Simaroubaceae family and are considered as taxonomic marker of the family [5, 6]. Four quassinoids, indaquassin C1, samaderins C2, B3 and A4, isolated from the seeds and bark of *Q. indica*, were tested for insect antifeedant and growth regulatory activities against the tobacco cutworm, *Spodoptera litura*. Indaquassin C was the most effective antifeedant. Samaderin C increased pupal duration and induced pupal mortality [7].

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Pharmacological information of Quassia suggests that this plant possesses a wide spectrum of biological activity such as antimalarial, antiplasmodial, antihelminthic, and antioxidant [8]. The objective of this study was to analyse the types of secondary metabolites in bark and leaves and to assess the potency of the extracts as antimicrobial agents.

## MATERIALS AND METHODS

### Preparation of Extracts

The bark and leaf were washed thoroughly in tap water, chopped into slices, shade dried and ground into fine powder. The dry powder (25 g) of both samples was re extracted successively with 100 ml each of petroleum ether, chloroform, ethyl acetate, methanol and water. These five samples were placed in a water bath at a temperature of 45°C for 24 hours and then placed in a gyratory shaker at 120 rpm for 48 hours. Each type of extract was filtered using whatman No.1 filter paper, dried to attain constant weight and the yield and quality of extract was recorded. The final residues were stored in a refrigerator at 4°C till further use.

### Preliminary Qualitative Analysis

Extract from each solvent was used to prepare a stock solution (mg/ml) using the respective solvent. These extracts along with blanks were analyzed qualitatively for the presence of various phytochemicals. Phytochemical examinations were carried out for all the extracts of bark and leaf of *Q. indica* (Gaertn.) Nooteb. as per the standard methods [9].

#### Test for Alkaloids

Approximately 50mg of extract was dissolved in 5 ml of distilled water followed by 2ml of hydrochloric acid to initiate an acid reaction and filtered. The filtrate was subjected to qualitative analysis to determine the presence of alkaloids as detailed below.

- i) **Dragendorff's test:** Dragendorff's reagent (1ml) was added to 2ml of the filtrate. Presence of alkaloid was indicated as a red precipitate formation.
- ii) **Wagner's test:** Two drops of Wagner's reagent was added to 1ml of the test solution. The formation of brown precipitate confirmed the test as positive for alkaloids

#### Test for flavonoids

About 100 mg of each extract was heated with 10 ml of ethyl acetate in boiling water bath for 3 minutes. The mixture was filtered and the filtrate was used for the following tests.

- i) **Ammonium test:** the filtrate was shaken with 1 ml of dilute ammonia solution (1%, v/v). The layers were allowed to separate. A yellow colour observed at ammonia layer suggested the positive result.
- ii) **Alkaline reagent test:** Few drops of 20% (w/v) NaOH solution was mixed with 2 ml of extract. Formation of intense yellow color, which becomes colourless on addition of dilute HCl, indicated the presence of flavonoids.
- iii) **Shinoda test:** A few magnesium turnings and 0.5 ml of concentrated hydrochloric acid was added drop wise to 1ml of test solution. A crimson red colour appeared confirmed the test.

#### Test for Phytosterols/ terpenoids

**Liebermann-Burchard's test:** 2 mg of the extract was dissolved in 2ml of acetic acid anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the side test tube. A brown ring formation at the junction confirmed the test for the presence of phytosterols.

#### Test for Tannins

- i) **Ferric chloride test:** A few drops of 5% (w/v) ferric chloride solution were added to 2ml of the test solution. Formation of bluish black colour indicated the presence of hydrolysable tannin.

#### Test for Cardiac glycosides

**Keller-Killiani test:** A few drops of 5% ferric chloride solution and 0.4 ml of glacial acetic acid were added to 5 mg dry extract. Then 0.5ml of concentrated sulphuric acid was added along with the side of the test tube carefully. The appearance of blue colour in acetic acid layer confirmed the test.

#### Test for Phenol

Extracts were treated with 3-4 drops of 10% (w/v) ferric chloride solution. Formation of greenish black colour indicates the presence of phenol.

#### Test for Triterpenoids

**Salkowski test:** Approximately 2mg of dry extracts was shaken with 1ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.

#### Test for Anthraquinone glycosides

**Hydroxyanthraquinone test:** A few drops of 10% (w/v) potassium hydroxide solution were added to 1ml of the extract. The formation of red colour confirmed the test.

#### Test for carbohydrates

- i) **Molisch's test:** To 1ml of the test solution added a few drops of 1% alpha naphthol followed by 2 ml concentrated sulphuric acid along the side of test tube. A reddish violet ring at the junction of two liquids confirmed the test.
- ii) **Fehling's test:** About 2 mg dry extract was dissolved in 1ml of distilled water and added 1ml each of Fehling's (A and B) solution, shaken and heated on a water bath for 10 minutes. Formation of brick red precipitate confirmed the test.

#### Test for proteins

**Biuret test:** To 2ml of the test solution added 5 drops of 1% (w/v) copper sulphate solution and 2ml of 10% NaOH. Mix thoroughly. Formation of purple or violet color confirmed the test.

**Test for fats and fixed oils:** To 5 drops of the sample was added 1ml of 1% copper sulphate solution and a few drops of 10% NaOH. The formation of clear blue solution confirmed the test.

### Test for saponin

**Foam test:** 5ml of the test solution taken in a test tube and shaken well for five minutes. Formation of stable foam confirmed the test.

### Quantitative Analysis of Phytochemicals

#### Determination of alkaloids - Harborne (1973) method <sup>[10]</sup>

To 5g of powdered samples 200ml of 20% acetic acid was added and kept for 40h. It was filtered and the volume was reduced to 50 ml using water bath. To this sample, concentrated ammonium hydroxide was added drop wise until precipitation was complete. The precipitate was allowed to settle, collected by filtration and weighed <sup>[11]</sup>. The percentage of total alkaloid content was calculated as:

$$\text{Percentage of total alkaloids (\%)} = \frac{\text{weight of residue}}{\text{weight of sample taken}} \times 100$$

#### Determination of total phenolics

About 0.1ml of the extract was blended with 3.9ml distilled water and 0.5ml Folin's reagent. The solution was kept at room temperature for 3 minutes and then added 2ml of 20% (w/v) sodium carbonate solution. After incubation in boiling water bath for 1 minute, the solution was cooled and the absorbance read at 650 nm. Gallic acid was used as the standard to express the total phenol content <sup>[12]</sup>.

#### Determination of total flavonoids

The aluminium chloride colorimetric assay was used to estimate the content of total flavonoids <sup>[13]</sup>. About 1ml of extracts was diluted with 200 µl of distilled water followed by the addition of 150 µl of sodium nitrite (5%) solution. This mixture was incubated for 5 minute and then 150 µl of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes. Then 2ml of sodium hydroxide (4%) solution was added and made up to 5ml with distilled water. The mixture was shaken well and left for 15 minutes at room temperature. The absorbance was measured at 510 nm.

#### Determination of total tannin

**Reagents - Colouring agent:** dissolve 1.6221gm of ferric chloride (0.1M), 0.9 ml of hydrochloric acid (0.1N) and 263.4 mg of potassium ferrocyanide (0.008M) in 100 ml of water.

**Working standard solution:** Dissolved 10 mg of tannic acid in 100 ml of distilled water.

**Procedure:** 1gm of each sample was boiled with 100 ml of distilled water for 30 minutes, cooled, filtered through a whatman no.1 filter paper and made up to 100ml with distilled water. Added 0.5ml of coloring agent. After 30 minutes incubation at room temperature the blue colour developed was read at 760nm against reagent blank. Added colouring reagent to the standard (tannic acid at concentrations 20-100µg) Express The tannin content was calculated and expressed as mg of tannic acid equivalent (TE)/gm of dried sample <sup>[14]</sup>.

#### Determination of total terpenoid

Estimation of total terpenoid content was carried out following the protocol of Ghorai *et al* <sup>[15]</sup>. 1gm of each extract was dissolved in 10

ml methanol and 15 ml water. Mixture was shaken well and centrifuged at 10000 rpm for 10 minutes. Filtrate was taken. To 1ml of extracts, added 2ml of chloroform followed by 3ml of concentrated sulphuric acid. Formation reddish brown colour indicated the presence of terpenoids and estimated by reading the absorbance at 538nm against blank of 95% methanol.

### Analysis of Antimicrobial activity

#### Microbial strains

The microorganisms used for the evaluation of antibacterial activity were one Gram negative strain – *Escherichia coli* and one Gram positive strain – *Staphylococcus aureus*. The fungal strains selected were *Aspergillus niger* and *Candida albicans*.

#### Culture Medium and Inoculation

The stock cultures of microorganisms used in this study were maintained in Mueller Hinton Agar slants (for bacteria) and Potato-Dextrose Agar (for fungi) at 4°C.

#### Mueller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Mueller Hinton Agar Medium (Himedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The sterile medium was poured onto 100 mm petri plates (20ml/plate) while still molten.

#### Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (Himedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Streptomycin** (standard antibacterial agent, concentration: 1mg / ml)

#### Procedure

Petriplates containing 20 ml Mueller Hinton Agar Medium were seeded with bacterial culture - *E. coli* and *S. aureus* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 5 mm was bored using a well cutter and sample of 250, 500, and 1000 µg/ml concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well <sup>[16]</sup>. Streptomycin was used as a positive control.

The inoculum was prepared by suspending a loopful of culture from stock slant into 25 ml of sterilized nutrient broth (prepared by dissolving 5 g Nutrient broth – readymade Himedia in 200 ml distilled water), maintained in gyratory shaker at 120 rpm for 48 hours to get uniform growth of bacteria.

#### Medium for culture plate

11.2g Nutrient agar was dissolved in 400 ml distilled water, as per the instruction provided by the suppliers (Himedia) and heated gently to dissolve the Nutrient agar completely. The medium was autoclaved at

121°C and 15 lb/in<sup>2</sup> pressure for 20 minutes. The sterilized Nutrient agar medium was cooled to 45 – 50°C and 30 ml was poured into each culture plate. The plates were examined after 24 hours for any contamination before carrying out the antibacterial analysis of the extracts.

#### Preparation of Antibiotic (Positive Control)

The positive controls employed for comparison of antibacterial potential of the extracts was the standard streptomycin. 1g powder of streptomycin was dissolved in saline solution and made up to 10ml with distilled water, this solution was used as stock1(100 mg/ml). 0.1 ml solution was taken from stock1 and made up to 10ml with distilled water, it was used as stock 2 (mg/ml). 0.1 ml solution was taken from stock 2 and used as positive control (100 µg/ml).

#### Antibacterial Analysis

Determination of antibacterial potential of the extracts was carried out using the agar well diffusion method [17]. The solidified nutrient agar in the petriplates was inoculated by dispensing 0.2 ml of the inoculum using a sterilized pipetting device and spread evenly with sterile cotton swabs. Four wells were prepared in each plate with the help of a sterile cork-borer of 10mm diameter. The extract was then introduced into each well to get concentrations of desirable levels. Well introduced with 0.1 ml of streptomycin served as control. All the plates containing loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and antibiotic control were measured by calculating the difference between diameter of cork-borer and diameter of inhibition. The same method of experiment was carried out in the triplicates and the mean values were reported for the final

consideration.

#### Antifungal Activity

In order to assess the significance of the extract samples to resist fungal growth, the antifungal activity was determined by Agar well diffusion method.

**Preparation of Inoculum** – The fungal strains – *Aspergillus niger* and *Candida albicans* were grown at 27°C on Potato Dextrose Agar (PDA) slants. After 7 days, spores were collected from the stock slants and suspended in 0.85% saline. A loop full of cells from the suspension was transferred to sterilized Potato Dextrose broth medium to get fungal suspension of each strain.

The 48 hours old cultures grown in potato dextrose broth were used for inoculation of fungal strains on PDA plates. An aliquot (0.02ml) of inoculum was introduced to the solidified PDA in petri dishes and swabbed. Appropriate wells (5 mm) were made on agar plate by using sterile cork borer and samples of different concentration (250; 500 and 1000 µg/ml) were added. The zone of inhibition was measured after overnight incubation and compared with that of standard antimycotic agent – Clotrimazole (100 µg/ml).

### RESULTS AND DISCUSSION

The successive extracts of bark and leaf of *Q. indica* (Gaertn.) Nooteb. Were compared for their quality and yield. The yield of crude extract was obtained by measuring its dry weight. The yield was found to be low in petroleum ether due to its low polarity and more in methanol and aqueous phases. The yield and quality of extract in various solvent systems is shown in the Table.1.

**Table 1:** The yield and color of extracts of bark and leaf samples after successive extraction.

Name of part	Weight of powder(g)	Name of solvent	Yield of extract (g)	Yield/100g	Color of extract
Leaf	25 g	Petroleum ether	0.126	2.52	Greenish yellow
		Chloroform	0.479	9.58	Dark green
		Ethyl acetate	0.259	5.18	Dark green
		Methanol	0.703	14.06	Dark green
		Water	1.157	23.14	Light brown
Bark	25 g	Petroleum ether	0.052	1.04	Colorless
		Chloroform	0.131	2.62	Yellowish green
		Ethyl acetate	0.579	11.58	Light green
		Methanol	0.257	5.14	Brown
		Water	0.939	18.78	Light brown

(Duration of extraction = 72 h; Treatment temperature = 45°C)

#### Phytochemical Analysis - Qualitative

The results of qualitative analysis of extracts of bark and leaf of *Q. indica* (Gaertn.) Nooteb. are illustrated in Tables 2 and 3 respectively. On basis of the intensity of the reaction product of qualitative tests, the data were graded as very high, (+++), high (++) , moderate (+) and nil (-). The phytochemical tests employed indicated that chloroform, methanolic and aqueous extracts contained most of the secondary

metabolites. However the extractions carried out with petroleum ether and ethyl acetate indicated little amount of phytoconstituents. The major phytoconstituents detected were alkaloids, flavonoids, phytosterols, saponins, fat and fixed oil and carbohydrates. Leaf extract in Chloroform contained higher degree of triterpenoids (+++). Chloroform, methanolic and aqueous extracts showed higher color indications for alkaloids, flavonoids, triterpenoids and phytosterols. Protein and anthraquinone were absent in all solvent extractions.

**Table 2:** Phytochemicals present in the successive extracts of Bark of *Q. indica* (Gaertn.) Nooteb.

Name of test	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
<b>Alkaloids</b>					
Wagner's test	-	++	+++	++	++
Dragendroff's test	-	-	-	++	+
<b>Flavonoids</b>					
Alkaline test	-	-	-	+	+
Shinoda test	-	+++	-	++	-
<b>Triterpenoids</b>					
Salkowskitest	-	++	-	+	-
<b>Tannin</b>					
FeCl <sub>3</sub>	-	-	-	+	+
<b>Phenol</b>					
	-	-	-	++	+
<b>Anthraquinone</b>					
Hydroxyanthraquinone	-	-	-	-	-
<b>Carbohydrates</b>					
Barfoed's test	-	-	-	+	-
Fehling's test	-	-	-	-	-
<b>Cardiac glycosides</b>					
Keller-Killiani	-	+	-	-	-
<b>Saponin</b>					
	-	-	-	-	-
<b>Protein</b>					
	-	-	-	-	-
<b>Fats &amp; Fixed oil</b>					
	++	+	+	-	-
<b>Phytosterol</b>					
	++	+++	-	++	+

(Duration of extraction = 72 h; Treatment temperature = 45°C)

(+++ High; ++ Moderate; + Low; - Nil)

**Table 3:** Phytochemicals present in the successive extracts of leaf of *Q. indica* (Gaertn.) Nooteb.

Name of test	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
<b>Alkaloids</b>					
Wagner's test	-	+++	+	+	+++
Dragendroff's test	-	++	+	+	+++
<b>Flavonoids</b>					
Alkaline test	-	-	-	+	++
Shinoda test	-	-	-	+++	+
<b>Triterpenoids</b>					
Salkowski test	-	+++	+	++	+
<b>Tannin</b>					
FeCl <sub>3</sub>	-	-	-	+	+
<b>Phenol</b>					
	-	-	-	++	+
<b>Anthraquinone</b>					
Hydroxy anthraquinone	-	-	-	-	-
<b>Carbohydrates</b>					
Barfoed's test	-	+	-	+++	++
Fehling's test	-	-	-	-	-
<b>Cardiac glycosides</b>					
Keller-Killiani test	-	-	-	-	-
<b>Saponins</b>					
	-	-	-	-	+
<b>Protein</b>					
	-	-	-	-	-
<b>Fats&amp;fixed oil</b>					
	-	++	-	-	-
<b>Phytosterol</b>					
Libermann-Burchards test	++	+++	-	++	-

(Duration of extraction = 72 h; Treatment temperature = 45°C)

(+++ High; ++ Moderate; + Low; - Nil)

## Phytochemical Analysis - Quantitative

### Total alkaloid content

The total alkaloid content was recorded as high in leaf (5.7 mg/g) and low in bark (3.5 mg/g) and the observations are depicted in Fig. 1.

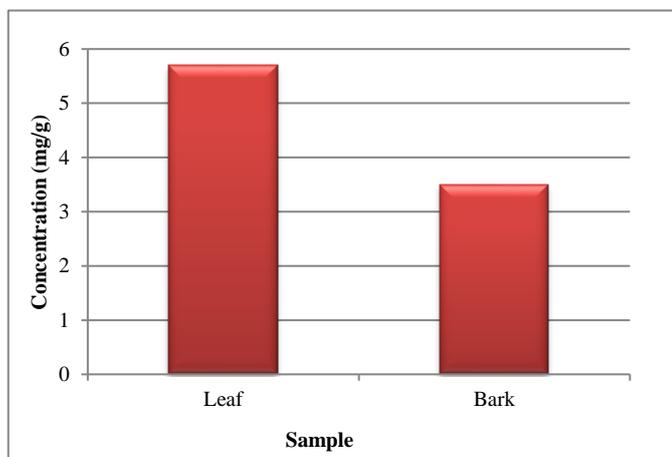


Figure 1: Total alkaloid content in Aqueous extract (mg/g)

The results on alkaloid level suggested that both leaf and bark are ideal sources of alkaloids and the bitterness of these parts could be correlated with the presence of alkaloids

### Total flavonoid content

The total flavonoid was highest in the methanolic extract than the aqueous (bark - 1.90 mg/g > leaf - 1.08mg/g). The flavonoid content was 0.54 mg/g in leaf and 0.30 mg/g in bark.

### Total Phenolic Content

An observation on comparative quantitative analysis of aqueous and methanolic extracts for flavonoids, phenol and terpenoids are depicted in Table 4. The total phenolic content was found to be much higher for methanolic extract and the phenol was absent in the aqueous extract. The highest phenolic content (GAE mg/g) of methanolic extracts was noted for bark (24.38 mg/g) > leaf (10.44 mg/g). However, among the aqueous extracts the phenolics were substantially low for bark (1.76 mg/g) > leaf (0.86 mg/g).

### Total Tannin Content

The concentration of tannin, estimated as tannic acid equivalent (TE mg/g) was recorded to be in the range of leaf (1.53 mg/g) and bark (1.32 mg/g) in methanolic extract and in the aqueous extract is 0.59 mg/g in leaf and 0.48 mg/g in bark.

### Total terpenoid Content

In comparison to alkaloids, flavonoids, phenolics and tannin, the total terpenoid content (mg/g) was detected as substantially low in methanolic and aqueous phases and were found to be in the order – bark (0.42 and 0.27) > leaf 0.34 and 0.278).

Table 4: Quantitative Data of Phytochemicals in Methanolic (ME) and Aqueous (AE) extracts of *Q. indica* (Gaertn.) Nooteb.

Name of part	Flavonoid (mg/g)		Phenol (mg/g)		Tannin (mg/g)		Terpenoid (mg/g)	
	ME	AE	ME	AE	ME	AE	ME	AE
Leaf	1.08	0.54	10.44	0.86	1.53	0.59	0.40	0.34
Bark	1.90	0.30	24.38	1.76	1.32	0.48	0.42	0.27

The observations from quantitative analysis suggested that methanol could effectively extract flavonoids and phenolics than water. The results also indicated that the bark of *Q. indica* is a comparatively richer source for flavonoids and phenolics. The metrics on tannin and terpenoid content in methanolic extracts of both leaf and bark did not indicate considerable variations.

The relatively higher efficiency of methanol in extraction of flavonoids and phenolics could be attributed to the better solubility level of these constituents in methanol than in aqueous medium. Methanol may have more penetration capacity than water to release the phytochemicals from intracellular regions where they are stored.

All the secondary metabolites detected in the present study are reported to have many bioactive and therapeutic properties [18-21]. The medicinal property of the bark and leaf of *Q. indica* as evident from ethnobotanical and folkloric uses, could be attributed to the presence of these secondary metabolites.

Alkaloids from plant sources exhibit moderate to high levels of pharmacological activity. Alkaloids from natural sources with therapeutic value are 'Taxol' from *Taxus baccata*; 'Reserpine' from *Rauwolfia serpentina* and 'Vincristin' and 'Vinblastin' from *Catharanthus roseus*. Antibiotic activities are also attributed to alkaloids such as the use of "barberine" in ophthalmic and "sanguinerine" in tooth pastes [22]. The plant used in the present study is widely used to cure skin diseases in folkloric medicine. Bark and leaves of *Q. indica* are antihelmintic as well as antiseptic. This might be due to the antimicrobial properties owing to its high alkaloid content.

### Antimicrobial Analysis

The methanolic extracts of leaf and bark of *Q. indica* (Gaertn.) Nooteb. were investigated for *in vitro* antimicrobial activity against microorganism including Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) and two strains of fungi such as *Aspergillus niger* and *Candida albicans*. The antimicrobial sensitivity of the crude extracts and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates and the results are tabulated in Tables 5 a and 5 b.

The extracts of *Q. indica* (Gaertn.) Nooteb. showed moderate antimicrobial activity against all the microorganisms tested at 500 and 1000 µg/ml. The antimicrobial potential was detected as higher for leaf extract.

The antimicrobial activity of these extracts could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic

compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes. The comparatively poor antimicrobial profile indicated by the bark extract which contain lesser levels of alkaloids and terpenoids, as evident from the quantitative analysis supports this correlation.

The results of the present study were comparable with the earlier reports by Viswanad *et al* [1], which suggested good response by both Gram negative and Gram positive bacterial strains and *Candida albicans*, in their experiment with extracts of *Q. indica* (Gaertn.) Nootb. However, *Aspergillus niger* and *Aspergillus fumigatus* were resistant against the extracts. The study by Aiswarya and Pushpalatha [23] also reported the efficacy of the fruit and leaf extracts of *Q. indica* (Gaertn.) Nootb. in inhibiting the growth of *Bacillus thuringiensis*, *Klebsiella pneumoniae*, *Escherichia coli* and *Serratia* sp.

In general, the present study demonstrated significant antimicrobial potential of the bark and leaves of *Q. indica* (Gaertn.) Nootb. and could be explored for the treatment of various infections caused by these microbes.

**Table 5a:** Antibacterial activity of *Q. indica* (Gaertn.) Nootb. against different strains of bacteria.

	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)
<b>Control</b>	Streptomycin (100)	32	Streptomycin (100)	34
Leaf	250	Nil	250	Nil
	500	10	500	12
	1000	22	1000	24
Bark	250	Nil	250	Nil
	500	8	500	10
	1000	20	1000	22

(Extract - 20 µl; Period of incubation - 24 Hours; Control- Streptomycin (Antibiotic) Methanol; Well diameter – 5 mm)

**Table 5b:** Antifungal activity of *Q. indica* (Gaertn.) Nootb. against different fungal strains.

	<i>Aspergillus niger</i>		<i>Candida albicans</i>	
	Conc. (µg/ml)	IZ (mm)	Conc. (µg/ml)	IZ (mm)
<b>Control</b>	Clotrimazole (100)	16	Clotrimazole (100)	18
Leaf	250	Nil	250	Nil
	500	10	500	8
	1000	14	1000	14
Bark	250	Nil	250	Nil
	500	10	500	8
	1000	12	1000	10

## CONCLUSION

The study concluded that *Q. indica* (Gaertn.) Nootb. is an interesting source of secondary metabolites with potential for use as a medicinal plant. The bark and leaf extracts could control the growth of bacteria and fungi and possesses appreciable antimicrobial potential.

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