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Research Article

Phytochemical Analysis and Effect of Solvents on Antibacterial Activity of *Tamarindus Indica* Leaf and Stem

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Abstract

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern and multidrug resistance bacteria on rise. So, there is an urgent need to identify novel and potent active antimicrobial agents. In the present work, phytochemical analysis and antimicrobial activity of Tamarindus indica leaf and stem was done . The extraction was done by individual cold percolation method using six different solvents viz., petroleum ether, toluene, ethyl acetate, acetone, methanol and water. Phenolic and flavonoid content of different extracts was determined using Folin-ciocalteu assays and aluminium chloride colorimetric method respectively. The antimicrobial activity was evaluated by agar well diffusion method against eight different pathogenic species of Gram negative bacteria and Gram positive bacteria. The leaf and stem had various phytochemicals like tannins, flavonoids, triterpenes. Maximum extractive yield was in aqueous extract both in leaf and stem. Maximum phenol content was in polar solvent methanol and acetone extracts both in leaf and stem. Maximum flavonoid content was in toluene extract and followed by ethyl acetate extracts both in leaf and stem. Acetone extract of T. indica leaf showed good antimicrobial activity against all the bacteria investigated.

Keywords: *Tamarindus indica, leaf and stem, Total phenol and flavonoid content, Antimicrobial activity, Phytochemical, solvents*

Introduction

Antibiotics resistance is a life threatening problem against pathogens which is increasing over the last decades. Antibiotics have an effect on a particular molecular target in pathogens which enables bacteria to expand corresponding target site resistance (Hamouda *et al*., 2014). The speed of antibiotic use is increasing the frequency of antibiotic resistance development is also increases. Thus the efficacy of antibiotics becomes reduced (Livermore, 2004)

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators. Plant-derived substances have recently become of great interest owing to their versatile applications (Shelar *et al*., 2012). Plant have natural constituents can be derived from any part of the plant like leaves, bark, roots, fruits, fruit, rind seeds, etc. Secondary metabolites represent a complex of several compounds with different pharmacological properties and it can affect several targets (Wink 2008). Extraction of bioactive compounds from medicinal plants shows physiological activity, which facilitates pharmacological studies leading to synthesis of a more potent drug (Manna and Abalaka, 2000)

Plants are rich in a wide variety of phytochemicals like flavonoids, tannins, terpenoids, alkaloids, that have been found to have antimicrobial activities (Weimann and Heinrich, 1997). Phytochemicals have protective or disease preventive properties to protect itself. Though, some phyto-chemicals demonstrate a protective effect for humans against different diseases (Shelar *et al*., 2012).

Tamarindus indica Linn (Caesalpiniceae) is commonly known as tamarind. It grows as a large tree and with a short massive trunk, ferny pinnate leaves, small yellow flowers and flat reddish brown pods. It is widely used as a food and medicine (Morton, 1987; Raimondi *et al*., 2003) originated from India. The tree can grow up to 20 meters (60ft) in height and stay evergreen in regions without dry (Burkill, 1985) and now it is one of the most important plant resources as food material. It is cultivated mainly for the pulp in the fruit, which is used to prepare a beverage and to flavor confections curries and sauces and it is accepted as herbal medicine in parts of the world. The flower and leaf are eaten as vegetables.

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The leaves have antihelmintic and vermifuge properties, destroying intestinal parasites (Pampaloma, 1999). The leaves have a proven hepatoprotective activity associated with the presence of polyhydroxylated compounds, with many of them are flavonolic in nature **(**Jouyex *et al*., 1995). Fruit is regarded as a digestive carminative, laxative, expectorant and blood tonic (Komutarin *et al*., 2004). Other parts of plant present antioxidant (Tsuda *et al*., 1994), antihepatotoxic (Joyeux *et al*., 1995), Anti-inflammatory (Rimbau *et al*., 1999), Antimutogenic (Ramos *et al*., 2003), etc.

In the present work, various solvent extracts of leaf and stem of *Tamarindus indica* L. were evaluated for its phenol and flavonoid content and antibacterial potential.

Material and methods

Collection of plant materials

The leaf and stem of *Tamarindus indica* L. (PSN 263) was collected in the month of August 2013 from Saurashtra University Campus, Rajkot, Gujarat, India. The plant was compared with voucher specimen (voucher specimen PSN 263) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. They were thoroughly washed, separated and dried under shade. The dried plant parts (leaf and stem) were crushed to fine powder and stored in air tight bottles which were later used for further studies.

Qualitative phytochemical analysis

The crude powder of leaf and stem of *T. indica* was subjected to qualitative phytochemical analysis (Harborne, 1973) to identify the presence or absence of different phytoconstituents.

Extraction

The dried powder of the leaf and stem of *T. indica* was extracted individually by the cold percolation method (Parekh and Chanda 2007) using different organic solvents like petroleum ether (PE), toluene (TO), ethyl acetate EA), acetone (AC), methanol (ME) and water (AQ). Ten grams of dried powder was added to 100 ml of hexane in a conical flask, which was plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 ml of solvents (toluene, ethyl acetate, acetone, methanol and water) in different conical flasks, which were plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvents were evaporated; the dry extract was stored at 4 °C in airtight bottles. The extracts were weighed to obtain the extraction yield.

Quantitative phytochemical analysis

Total phenol and total flavonoid content were estimated in all the solvents extract of *T. indica* leaf and stem.

Determination of total phenol content

The amount of total phenol content of different solvent extracts of *T. indica* leaf and stem was determined by Folin- ciocalteu's reagent method (Mc Donald *et al*., 2001). The extract (0.5) ml and 0.1ml of Folin-ciocalteu's reagent (0.5N) were mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of sodium carbonate (2M) solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm (Systronic, India), against a blank sample. The calibration curve was made by preparing Gallic acid (10 to 100 µg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g^{-1} of extracted compounds).

Determination of total flavonoid content

The amount of flavonoid content of different solvent extracts of *T. indica* leaf and stem was determined by aluminium chloride colorimetric method (Chang *et al*., 2002). The reaction mixture (0.3 ml) consisted of 1.0 ml of sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made by preparing quercetine (5 to 60 µg ml^{-1}) solution in methanol. The flavonoid content is expressed in terms of standard equivalent (mg g^{-1}) of extracted compound.

Antimicrobial susceptibility test

Test microorganisms

The microorganisms used were obtained from the National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4 °C. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Staphylococcus aureus 2* (SA2) ATCC29737, *Listeria monocytogenes* (LM) ATCC19112 and *Corynebacterium rubrum* (CR) ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa (PA)* ATCC27853, *Klebsiella pneumoniae* (KP) NCIM2719 and *Salmonella typhimurium* (ST) ATCC23564.

Antimicrobial activity (Agar well diffusion assay)

In vitro antimicrobial activity of different solvent extracts of leaf and stem of *T. indica* was determined by standard agar well diffusion assay (Perez *et al*., 1990;

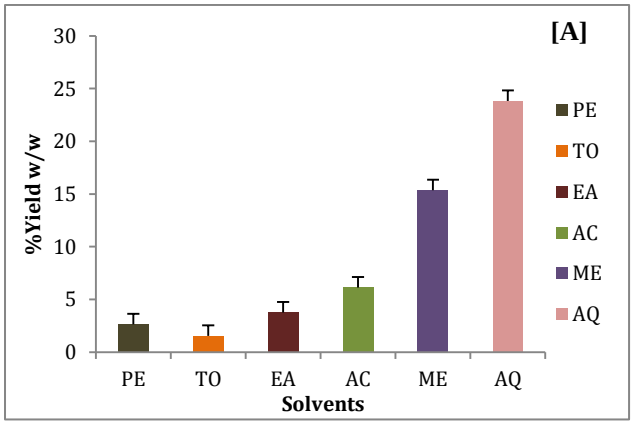
Kaneria and Chanda, 2012). Mueller Hinton agar and Sabouraud dextrose agar media was used for antibacterial and antifungal activity respectively. Molten Mueller Hinton agar / Sabouraud dextrose agar (40-42°C) were seeded with 200 µl of inoculums (1 × 10^8cfu/ml) and poured into Petri dishes. The media were allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Different extracts were dissolved in 100% DMSO at a concentration of 20 mg/ml, from this 100µl of different extracts were added into the sterile 8.5 mm diameter well. The plates were incubated at 37°C for 24h bacteria. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

Results and discussion

The results of qualitative phytochemical analysis of the crude powder of *T. indica* leaf and stem are shown in Table 1. The leaf had maximum amount of flavonoids content followed by tannins; steroids, triterpens and cardiac glycosides were present in trace amount while saponins and phlobatanins were absent. The stem had maximum amount of tannins while flavonoid, triterpens and alkaloids were present in minimal amount; phlobatanins, steroids, saponins and cardiac glycosides were absent.

Extractive yield

The extractive yield leaf and stem of *T. indica* in different solvents is given in Fig.1. The extractive yield was distinctly more in leaf than in stem. In leaf, AQ extract had maximum yield followed by ME extract. In all other solvent extracts, the yield was quite less (Fig. 1A). They can be ranked as AQ> ME> AC>EA>PE>TO. In stem, the yield was quite less and the rank of their yield was almost similar to that of leaf (Fig. 1B).



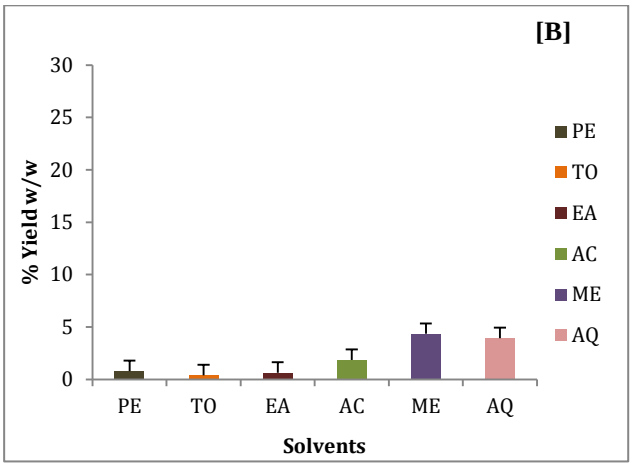


Fig. 1: Extractive yield of different solvents extracts of *T. indica* in leaf [A] and stem [B]

Quantitative phytochemical analysis

Total phenol content

Total phenol content of different solvent extracts of *T. indica* is shown in Fig. 2. In *T. indica* leaf, maximum TPC was in polar solvent ME followed by AC extract (Fig. 2A).

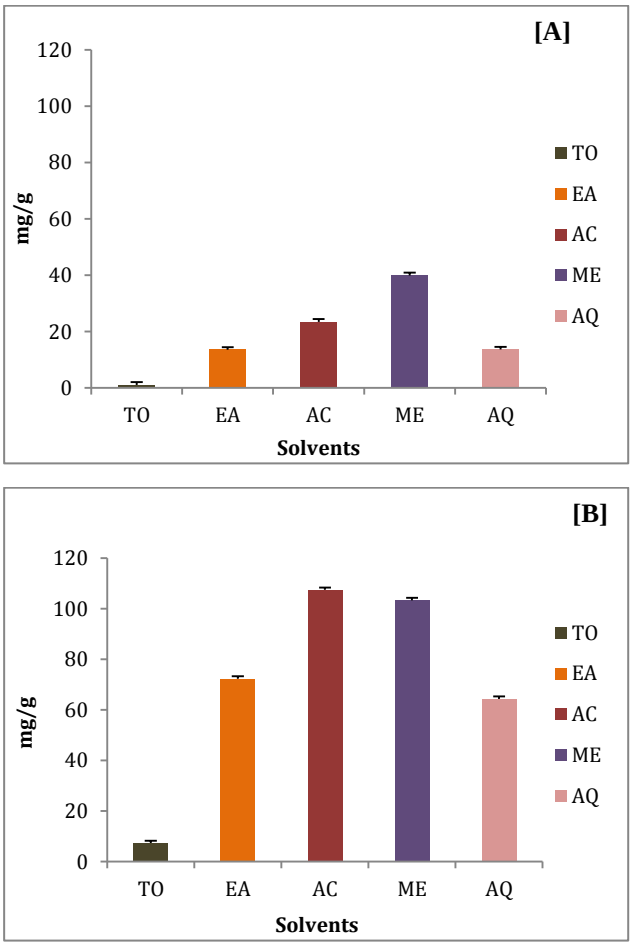


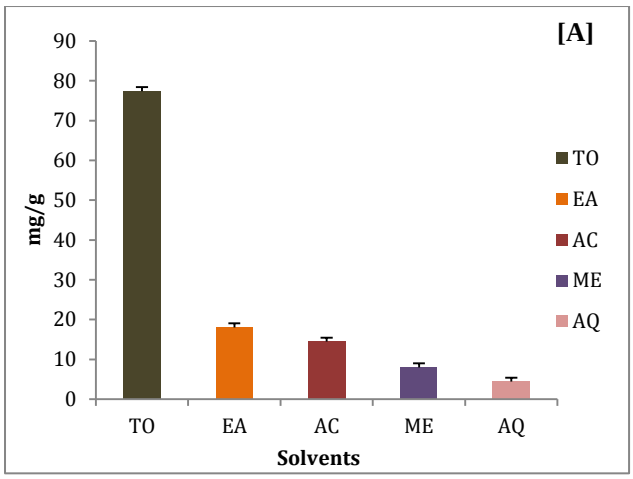
Fig. 2: Total phenol content of different solvents extract of *T. indica* leaf [A] and stem [B]

The TPC content of both semi polar solvents was less than polar solvent extracts. Here also, the TPC of TO extract was very much less than that of EA extract. TPC of AQ extract was similar to that of EA extract. In *T. indica* stem, maximum phenol content was in AC and ME extracts (Fig. 2 B). The TPC of EA extract was less than AC and ME extracts while TO had lowest TPC, very much less than all the solvent extracts. TPC of AQ extract was moderate.

In *T. indica* also, AC and ME extracts of leaf and stem had maximum TPC but the content was many fold higher in stem than in leaf. In all the other solvent extracts also, TPC was more in AC extract of stem. When both the plants are compared, maximum TPC was in AC extract of *T. indica* stem. It is established fact that the extraction yield of phenols is greatly influenced by the polarity of the solvent (Turkmen *et al*., 2006; Jakopic *et al*., 2009; Rakholiya *et al*., 2014).

Total flavonoid content

In *T. indica* leaf, maximum flavonoid content was in TO extract and minimum in ME in organic solvent extracts (Fig.3 A).



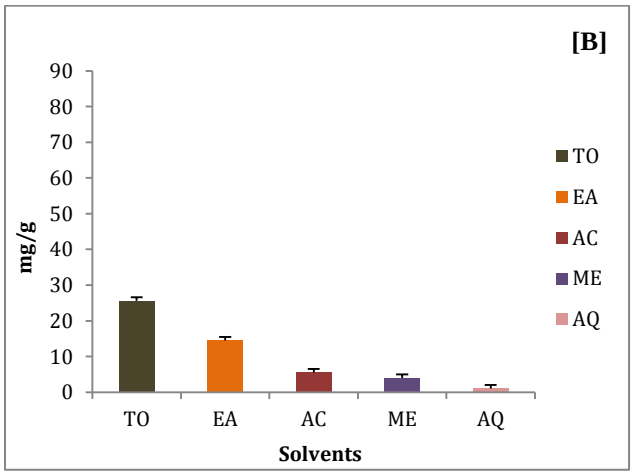


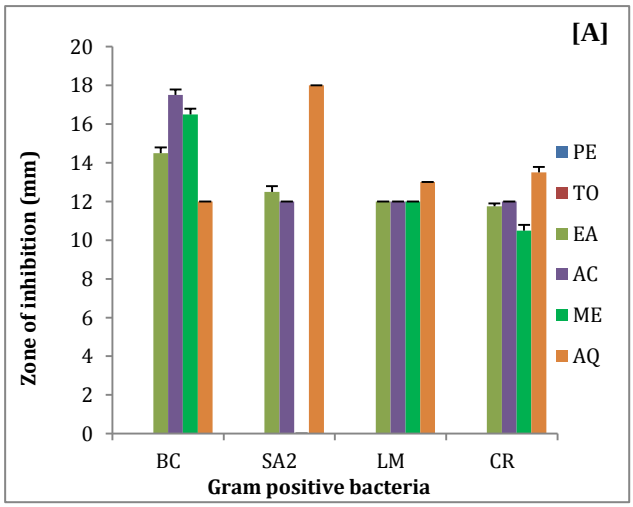
Fig. 3: Total flavonoid content of different solvents extract of *T. indica* leaf [A] and stem [B]

AQ and polar solvent ME extract had very less amount of flavonoid content, while EA and AC had similar amount of flavonoid content. In *T. indica* stem, maximum flavonoid content was in TO and minimum in AQ extract. The TFC was in the order TO > EA > AC > ME > AQ (Fig. 3B). In *M. emarginata*, when TFC of both leaf and stem are compared, maximum TFC was in EA and AC extracts of leaf (Fig. 17A) while in *T. indica*, maximum TFC was in TO extract of leaf. When both the plants are compared, maximum TPC was in TO extract of *T. indica* leaf.

T. indica leaf and stem showed different TPC and TFC in different solvent extracts and it is affected by the polarity of the solvents used for extraction. Total phenol content is directly correlated with antioxidant activity is reported for plant extracts by many researchers (Patel *et al*., 2011; Kaneria and Chanda, 2013).

Antibacterial activity

The antibacterial activity of different solvent extracts of *T. indica* leaf and stem against Gram positive bacteria is shown in Fig. 4.



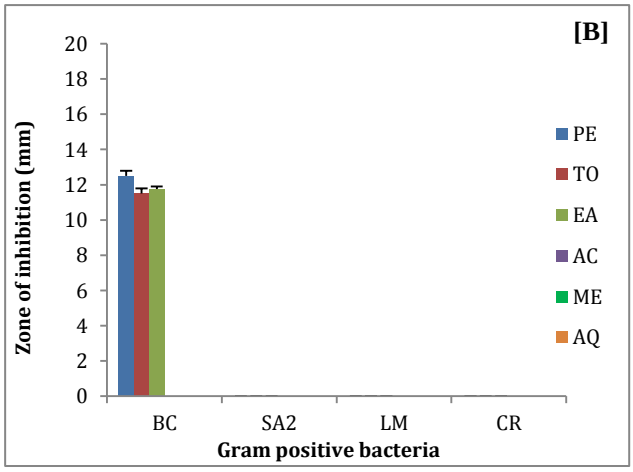


Fig. 4: Antibacterial activity of different solvent extracts of *T. indica* leaf (A) and stem (B) against Gram positive bacteria

Various solvent extracts of *T. indica* leaf showed more antibacterial activity than stem. In leaf, a varied level of antibacterial activity was envisaged. PE and TO extracts did not show any antibacterial activity against any of the 4 Gram positive bacterial strains investigated (Fig. 4A). All the other 4 solvent extracts showed antibacterial activity except ME extract against SA2. BC was the most susceptible bacteria; AC extract showed maximum antibacterial activity. An entirely different trend was observed in *T. indica* stem. Only BC was inhibited by PE, TO and EA extracts (Fig. 4B). None of the solvents showed any antibacterial activity against the other 3 Gram positive bacterial strains.

The antibacterial activity of different solvent extracts of T. *indica* leaf and stem against Gram negative bacteria is shown in Fig. 5. In leaf, PE and TO extract did not inhibit any of the four Gram negative bacteria investigated while AC extract inhibited all the bacteria (Fig. 5 A). KP was not inhibited by any other solvent extracts. PA was most susceptible bacteria which were inhibited by all the four solvent extracts. In stem, EC, ST and PA were not inhibited by any of the solvent extracts while *K. pneumoniae* was inhibited by PE, TO, EA and AQ extracts (Fig. 5B).

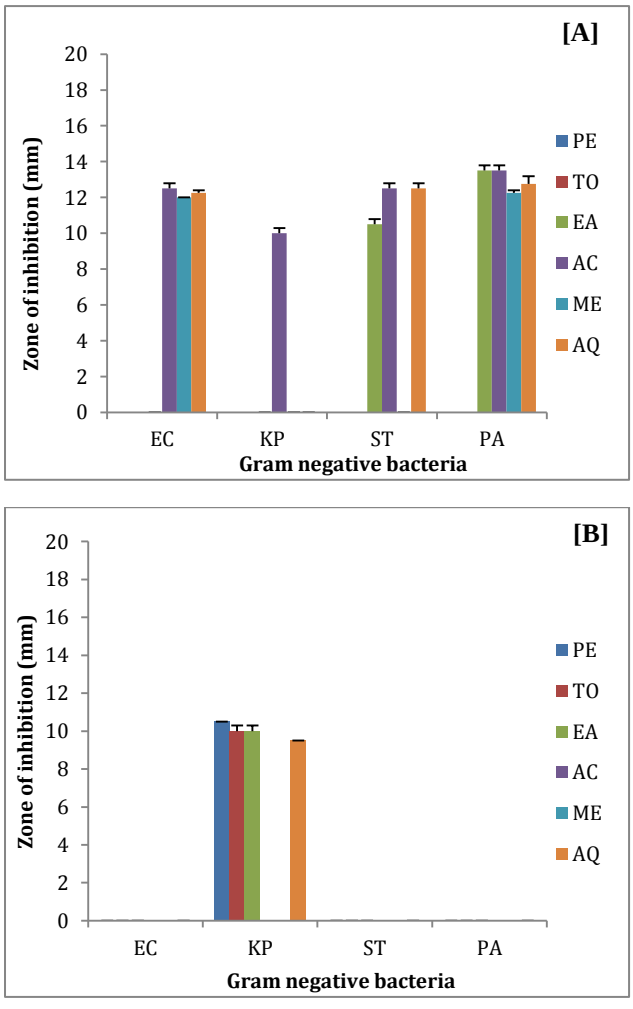


Fig. 5: Antibacterial activity of different solvent extracts of *T. indica* leaf (A) and stem (B) against Gram negative bacteria

In *T. indica*, the various solvent extracts of leaf showed best antibacterial activity towards both Gram positive and Gram negative bacteria and AC extract showed best antibacterial activity. The solvent extracts of stem showed very poor activity towards Gram positive and Gram negative bacteria. Further, there was no correlation between TPC and antibacterial activity. The plant part stem had maximum TPC in AC extract but maximum antibacterial activity was shown by AC extract of leaf. *T. indica* leaf and stem showed varied level of antibacterial activity because of different level of phytoconstituents present in both parts. The best activity was shown by AC xtract indicating the importance of the solvent used for evaluating any medicinal property of plants. Plants rich in phytoconstituents like flavonoids, tannins and terpenoids have antibacterial properties (Nogci *et al*. 2014). There is a growing interest in the antimicrobials plant origin for the treatment of bacterial infections or against multi drug resistant microorganisms.

Table: 1 Pytochemical analysis of *T. indica* leaf and stem

No.	Test	leaf	stem
1	Flavonoids	++	+
2	Tannins	++	+
3	Phlobotannis	-	-
4	Saponins	-	-
5	Steroid	+	-
6	Cardiac glycosides	++	-
7	Triterpenes	+	++
8	Alkaloids		
	(1)Mayer's	+	+
	(2)Dragondroff's	+	+
	(3)Wagner's	+	-

Conclusion

Finally it can be concluded that *T. indica* leaf and stem can be used as herbal source of antimicrobial agent against drug resistant pathogens. It has good phenol content and can be used for various pharmaceutical preparation. However, there is need for isolation of active constituents from crude extracts.

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