

Antimicrobial Activity of *Terminalia bellerica* Leaf and Stem Collected from Two Different Sites

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ABSTRACT

Terminalia bellerica belonging to the family Combretaceae is frequently used as traditional medicine by tribal folk to get remedies from several ailments. In this study, the antimicrobial potential of different extracts of leaf and stem of *T. bellerica* collected from two different sites was investigated against five Gram positive, five Gram negative and four fungi. The extraction was done by cold percolation method. The dry powder was first defatted by hexane and then extracted in acetone. The acetone extract was fractionated into two fractions by solvent-solvent partition method. The antimicrobial activity was done by disc diffusion assay. The MIC and MBC was also evaluated. The *T. bellerica* extracts showed more antibacterial activity than antifungal activity and antibacterial activity was more towards Gram negative bacteria than Gram positive bacteria. The extracts are especially good against Gram positive bacteria like *Corynebacterium rubrum* and *Staphylococcus epidermidis* and Gram negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*. *T. bellerica* extracts can be used as effective antibacterial agents in the search for new drugs.

Keywords: *Terminalia bellerica*, antimicrobial activity, Gram negative bacteria, agar well diffusion method, solvent extracts.

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest sources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for

introducing and domesticating new exotic plant varieties. In India, the use of medicinal plants is centuries-old tradition and approximately two million traditional health practitioners still use medicinal plants for curing various ailments.

Herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly¹. They are also cheap, easily available and affordable. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Plants produce wide array of bioactive principles and constitute a rich source of medicines. Herbal medicines are prepared from a variety of plant materials as leaves, stems, roots, bark etc. They usually contain biologically active ingredients and are used primarily for treating mild or chronic ailments. In India 45,000 plant species have been identified and out of which 15-20 thousand plants are of good medicinal value. According to World Health Organization (WHO) estimates, more than 80% of the people in developing countries depend on the traditional medicine for their primary health needs. It is generally estimated that over 6000 traditional plants in India are in use folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries².

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this, problems are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction; this situation forced scientists to search for new antimicrobial substances. There is an increase in the number of infectious diseases, including bacterial infections with various levels of drug resistance. Almost 50,000 people are dying worldwide every day because of infectious diseases. The continued evolution

of infectious diseases and the development of resistance by pathogens to existing pharmaceuticals have led to the intensification of the search for new novel leads, against fungal, parasitic, bacterial, and viral infections³. Bacterial resistance is an increasing threat to the successful treatment of infectious diseases. As bacterial resistance continues to evolve, some pathogens that were once considered routine to treat are developing, or have developed, resistance to almost every antibacterial agent currently available.

This alarming situation imposes the need for a search and development of new drugs and discovery of new medicinal agents with novel modes of activity. There is an urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. One of the most promising areas is herbal medicines. Natural products are known to play an important role in drug discovery and chemical biology. In fact, higher plants are the only source, which can provide compounds with new structural features and new mechanism of biological activity. Herbal remedies are readily available, have minimal side effects and economical as compared to synthetic drugs. There has been considerable interest in the use of plant extracts as multi-drug complex as an alternative method to control pathogenic microorganisms^{4,5}. There are many reports that different plant parts such as bark, leaves, peel, seed, stem, may potentially possess antimicrobial property^{6,7,8}. Most of the medicinal and aromatic plants and essences are rich sources in antibacterial compounds which can be an alternative to combat bacterial diseases.

The Combretaceae is a large family of plants, the most commonly occurring genera of which are *Combretum* and

*Terminalia*⁹, both widely used in traditional medicine. The genus *Terminalia* is widely distributed and is known as a rich source of secondary metabolites^{10,11,12} and flavonones and chalcones¹³. *Terminalia bellerica* Roxb. belonging to the family Combretaceae, commonly known as myrobalan, is a deciduous tree found throughout the Indian forests and plains. It is known as Bahera in India and has been used for centuries in Ayurveda, a holistic system of medicine originating from India. The tree is about 30-40 m. in height and 2-3 m. in girth. The stem is straight and the leaves are broadly elliptic clustered near the end of the branches. The flowers are simple, solitary in axillary spikes. The fruit is ovoid 1-2 cm in diameter drupe of grey to dark brown in colour. Fruit extract used as astringent, antiseptic, rejuvenative, brain tonic, expectorant and laxative. It is used in coughs and sore throat. Its pulp used in dysentery, diarrhoea and liver disorders. It is also useful in leprosy, fever and hair care¹⁴. This plant exhibits several pharmacological effects including antibacterial, antimalarial, antifungal, anti HIV, antioxidant and antimutagenic effects¹⁵. The fruit possesses antidiabetic and antioxidant activities¹⁶.

MATERIALS AND METHODS

Collection of the plant material

T. bellerica stem and leaf were collected from two sites i.e. one from Rajkot and the other from Jamnagar districts of Gujarat, India in July, 2012. The plant was compared with voucher specimen (voucher specimen number PSN290) deposited by Dr. PS Nagar at the department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The plant parts (stem and leaf) were washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottle.

Extraction

Cold percolation extraction method

The dried stem and leaf powder was extracted by cold percolation method¹⁷. Ten grams of dried powder was taken in 100 ml of hexane in a conical flask, plugged with cotton wool, and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, it was filtered through eight layers of muslin cloth, centrifuged at 5,000 rpm in a centrifuge for 15 min and the supernatant was collected and the solvent was evaporated using a rotary vacuum evaporator to dryness. Hexane was evaporated from the powder. This dry powder was then taken in 100 ml of acetone and was kept on a rotary shaker at 120 rpm for 24 h. The procedure followed was same as above and finally the residues were weighed to obtain the extractive yield of all the extracts and were stored in air-tight bottles at 4°C. In all four extracts for each part (stem and leaf) were obtained JHE, JAC, RHE and RAC. JHE and RHE represent hexane extract of stem and leaf collected from Jamnagar and Rajkot site respectively. Similarly, JAC and RAC represent acetone extract of stem and leaf collected from Jamnagar and Rajkot site respectively.

Isolation of *Terminalia bellerica* acetone extract

Fractionation of the acetone extracts (JAC and RAC) was done by solvent-solvent partition¹⁸. Five grams of acetone extract of *T. bellerica* stem/leaf was dissolved in hot methanol (200 ml). Slight precipitation obtained was discarded as methanol insoluble matter. The methanol soluble fraction was filtered and collected. It was concentrated to about 50 ml volume and ethyl acetate was added to it till faint turbidity was obtained. Then it was allowed to settle down in a refrigerator. The settled gelatinous reddish mass and supernatant was separated and collected separately. The supernatant was further concentrated and ethyl acetate step was repeated till reddish gelatinous mass

obtained. All the settled mass was collected together and dissolved in methanol. It was concentrated further to dryness and designated as Fraction I (FS I i.e. JAC I and RAC I). The collected supernatant was concentrated further to near dryness and then dissolved in methanol. Then chloroform was added to it and cooled. Light yellow waxy sediment was separated and light buff coloured supernatant was collected. This supernatant was concentrated further to dryness and designated as Fraction II (FS II i.e. JAC II and RAC II).

Antimicrobial activity

Microorganisms tested

Five Gram positive bacteria viz. *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Listeria monocytogens* ATCC 19112, *Corynebacterium rubrum* ATCC 14898 and *Staphylococcus epidermidis* ATCC 12228; five Gram negative bacteria viz. *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* NCIM 2241, *Salmonella typhimurium* ATCC 23564, *Escherichia coli* NCIM 2931 and *Klebsiella pneumonia* NCIM 2719; and three fungi *Candida albicans* ATCC 2091, *Candida glabrata* NCIM 3448 and *Cryptococcus neoformans* NCIM 3542 were obtained from National Chemical Laboratory, Pune, India. The bacteria and fungi were maintained on nutrient agar and MGY medium (Hi Media, India) respectively while *L. monocytogenes* and *E. coli* were maintained on Brain heart infusion agar and Luria medium (Hi Media, India) respectively at 4°C and sub-cultured before use. The microorganisms studied are clinically important ones causing several infections, food borne diseases, spoilages, skin infection and it is essential to overcome them through some active therapeutic agents.

Antimicrobial assay

The antimicrobial activity of extracts and fractions was done by agar disc diffusion method^{19, 20}. The Petri dishes were prepared by pouring 150 ml sterilized molten Muller Hinton Agar for bacteria and Sabouraud Dextrose Agar for fungal strains, which was then seeded with 1 ml of test culture containing 1×10^8 cfu/mL for bacteria and 2.0×10^5 spores/mL for fungal strains as McFarland 0.5 turbidity standard. The media were allowed to solidify. Crude acetone extract and its Fraction I and Fraction II of both stem and leaf were dissolved in 100% DMSO (Dimethyl sulfoxide) at concentration of 20 mg/ml. These drugs were used to evaluate antimicrobial activity. Sterile filter paper disks (6 mm) were impregnated with 20 µl of each drug separately and allowed to saturate for 30 min, and were then placed on the surface of the agar plates, which had previously been inoculated with test microorganisms. All plates were incubated for 24 and 48 hours at 37°C and 28°C for the bacterial and fungal strains, respectively. Results were recorded by measuring the zone of inhibition appearing around the disks. All the tests were performed in triplicate and the mean values are presented. Pure DMSO was used as the negative control.

Preparation of Inoculums

From the stock slants bacteria were inoculated in Muller Hinton Broth (MHB) and fungi were inoculated in Sabouraud Dextrose Broth (SDB). All flasks are incubated for 24 hrs in shaking condition.

Preparation of the extracts and/or antibiotics for MIC and MBC study

The extracts and fractions dissolved in 100% of DMSO were first diluted to highest concentration ($1250 \mu\text{g ml}^{-1}$) to be tested, and then serial two-fold dilution was made in a concentration range (0.605 to $1250 \mu\text{g ml}^{-1}$).

Chloramphenicol and ceftazidime were used as a positive control (0.0156 to 32 $\mu\text{g ml}^{-1}$).

Preparation of bacterial inocula for MIC and MBC study

The inocula of the test organisms were prepared using the colony suspension method²¹. Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspension of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile Muller Hinton broth before use to yield 6×10^5 CFU ml^{-1} .

Determination of the minimum inhibitory concentrations (MIC)

The MIC was determined by the micro well dilution method²² with some modifications. This test was performed in sterile flat bottom 96 well micro test plates (Tarsons Products Pvt. Ltd.). 150 μl volume of Mueller Hinton broth was dispensing into each well and 20 μl of varying concentrations of the extract was added in decreasing order along with 30 μl of the test organism suspension. The final volume in each well was 200 μl (150 μl Mueller Hinton broth, 30 μl of the test organism suspension, and 20 μl plant extract/antibiotic). Three control wells were maintained for each test batch; the positive control (CH/CF, MHB and test organism), sterility control (MHB and DMSO) and organism control (MHB, test organism and DMSO). Plates were then incubated at 37°C for 24 h. Experiments were carried out in duplicate. After incubation, 40 μl of INT solution (0.2 mg ml^{-1}) dissolved in sterile distilled water was added to each well²³. The plates were incubated for further 30 min and estimated visually for change in color to pink indicating reduction of the dye due to bacterial growth. The highest dilution

(lowest concentration) that remained clear corresponded to the MIC.

Determination of the minimum bactericidal concentration (MBC)

MBC was determined from all wells showing no growth as well as from the lowest concentration showing growth in the MIC assay for all the samples. Bacterial cells from the MIC test plate were sub cultured on freshly prepared solid nutrient agar plates by making streaks on the surface of the agar. The plates were incubated at 37°C for 24 h overnight. Plates that did not show growth were considered to be the MBC for the extract or drug used²⁴. The experiment was carried out in duplicate.

Statistical analysis

Each sample was analyzed individually in triplicate and the results are expressed as the mean value ($n = 3$) \pm Standard Error of Mean (S.E.M.).

RESULT AND DISCUSSION

There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases²⁵. Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures

Extractive Yield

The extractive yield of acetone extract and fraction of *T. bellerica* stem of Jamnagar and Rajkot regions is shown in Fig. 1. In Jamnagar region, maximum extractive yield was in JAC II. JAC and JACI showed

extractive value which was lower than that of JACII. JHE showed lowest extractive value (Fig. 1a). Unlike Jamnagar region, in Rajkot region, maximum extractive yield was in RAC I. The extractive yield of RAC II was much less than RAC I but more than crude extracts (RHE and RAC).

The extractive yield of acetone extract and fraction of *T. bellerica* leaf of Jamnagar and Rajkot regions is shown in Fig. 2. In Jamnagar region, maximum extractive yield was in JAC II. JAC and JAC I had similar extractive value which was lower than that of JAC II. The hexane extract (JHE) showed lowest extractive value (Fig. 2a). In Rajkot region, maximum extractive yield was in RAC II similar to that of JAC II. Here also, RAC and RAC I had almost similar extractive values but were very much less than RAC II. RHE like JHE had minimum extractive yield (Fig. 2b). From the above results, it can be concluded that leaf and stem collected from two different sites showed different trends. The extractive yield of stem was different in stem collected from two different sites but it did not affect the yield of leaf. The extractive yield depends on many factors like plant part, solvent used, temperature, soil conditions, climatic conditions, etc. There are many reports where different solvents showed different extractive yield²⁶⁻³⁰.

Antimicrobial activity

The antibacterial activity of *T. bellerica* stem acetone extract and its fractions from Jamnagar and Rajkot regions against Gram positive bacteria are shown in Figs. 3-4. The antibacterial activity of *T. bellerica* stem acetone extract and its fractions from Jamnagar and Rajkot regions against Gram positive bacteria is shown in Fig. 3. (JAC, JAC I, JAC II showed activity against *C. rubrum* and *S. epidermidis* while *B. cereus*, *B. subtilis* and *L. monocytogenes* were resistant towards all the four extracts. JAC and JAC II showed highest zone of inhibition

against *S. epidermidis* (Fig. 3a). RAC, RAC I, RAC II showed similar trend of activity like that of JAC and its fractions (Fig. 3b). The antibacterial activity of acetone extract and its fractions of stem collected from both sites showed almost similar activity towards Gram positive bacteria.

Antibacterial activity of extracts and fractions of *T. bellerica* stem from Jamnagar and Rajkot regions against Gram negative bacteria is shown in (Fig.4). All the four extracts of stem collected from both the regions showed antibacterial activity against *K. pneumonia*. JHE and RHE did not show activity against any other Gram negative bacteria (Fig. 4). The remaining three extracts from both the regions showed similar type of inhibition against all the other bacteria. *P. aeruginosa* was resistant to all the extracts of both the sites. *K. pneumoniae* was the most susceptible Gram negative bacteria towards all the extracts and fractions (Fig. 4b). From these results it can be concluded that Gram negative bacterial were more susceptible than Gram positive bacteria but the susceptibility was not very much different from stem collected from two different sites.

The antibacterial activity of *T. bellerica* leaf acetone extract and its fractions from Jamnagar and Rajkot regions against Gram positive bacteria are shown in Figs. 5-6. Antibacterial activity of *T. bellerica* leaf acetone extract and its fractions from Jamnagar and Rajkot regions against Gram positive bacteria is shown in Fig. 5. JHE and RHE did not show any activity against any of the Gram positive bacteria studied (Fig. 5). JAC and RAC and their fractions showed similar type of antibacterial activity against *C. rubrum* and *S. epidermidis* i.e. leaf collected from two different sites showed same type of inhibition. *B. cereus*, *B. subtilis* and *L. monocytogenes* were resistant towards all the extracts of both the sites. The antibacterial activity of acetone extract and its fractions of leaf collected from both sites showed almost

similar activity towards Gram positive bacteria. These results are very much similar to that shown by stem (Fig. 3a).

Antibacterial activity of extracts and fractions of *T. bellerica* leaf from Jamnagar and Rajkot regions against Gram negative bacteria is shown in Fig. 6. Fig. 6a shows the activity of leaf collected from Jamnagar region. Only JAC, JAC II showed activity against *K. pneumoniae*. All other extracts showed no activity against any of the Gram negative bacteria (Fig. 6a). Fig. 6b shows the activity of leaf collected from Rajkot region. Here an entirely different trend was observed. RHE showed activity against *K. pneumoniae* and *P. mirabilis*. RAC showed activity against all tested Gram negative bacteria and also showed maximum zone of inhibition against *S. typhimurium*. RAC II showed more activity than RAC I. *K. pneumoniae* was the most susceptible Gram negative bacteria towards all the extracts and fractions (Fig. 6b). The antibacterial activity of leaf extracts was different than that of stem extracts. The leaf extracts collected from Rajkot site showed better activity than leaf extracts collected from Jamnagar site. Thus stem extracts collected from different sites showed similar activity but the leaf extracts showed different activity. The trend was same but more Gram negative organisms were susceptible to leaf extracts of Rajkot site. The extracts did not show any antifungal activity.

The results suggest that antibacterial activity of plant extracts depends not only on solvent, temperature, method of extraction but also on soil type and climatic conditions. The same plant collected from one place may not show same activity collected from another place. Overall, the antibacterial activity of *T. bellerica* stem and leaf acetone extracts and its fractions showed better activity towards Gram negative bacteria than Gram positive bacteria. This is very promising because it is reported that plant extracts show more activity towards Gram positive bacteria than

Gram negative bacteria^{31,32,33,34}. This difference in activity is because of the difference in the cell wall structure of Gram positive and negative bacteria. The susceptibility of Gram negative bacteria towards plant extracts is reported by^{35,36,37}.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration)

Minimum inhibitory concentration refers to the lowest concentration of the antimicrobial agent which is required for the inhibition of visible growth of the tested microorganism. The MIC value of the drug has to be determined as these values provide the reference point for determining the interactions. MIC values were calculated using INT dye on a 96 well micro-titre plate. The MBC is interpreted as the lowest concentration that can completely kill the microorganisms. Experiments were performed in duplicate. MIC and MBC are expressed in terms of $\mu\text{g/ml}$. The MIC and MBC values of stem extracts and its fractions for Gram positive bacteria Gram negative bacteria and yeast are shown in Tables 1-3 while that of leaf are shown in Tables 4-6.

For stem extracts and its fraction, MIC varied from <19.5 to $>2500 \mu\text{g ml}^{-1}$ while MBC was $>2500 \mu\text{g ml}^{-1}$ for the Gram positive bacterial strains (Table 1). *C. rubrum* was the most susceptible bacterial pathogen to JAC I (MIC: $<19.5 \mu\text{g ml}^{-1}$). For the Gram negative bacterial strains the MIC varied from <39 to $>2500 \mu\text{g ml}^{-1}$ and MBC was 625 to >2500 (Table 2). *P. aeruginosa* was the most susceptible bacterial pathogen to almost all the extracts except RHE. For the fungal strains, MIC varied from 78 to $>2500 \mu\text{g ml}^{-1}$ while MBC was $>2500 \mu\text{g ml}^{-1}$ (Table 3). *C. albicans* was susceptible to JAC, *C. glabrata* was susceptible to RAC II and *C. neoformans* was susceptible to both fractions of JAC and RHE and RAC (MIC: $78 \mu\text{g ml}^{-1}$). For the standard antibiotics (CH and CF) MIC and MBC ranged from 1 to $32 \mu\text{g ml}^{-1}$ and 8 to

>32 $\mu\text{g ml}^{-1}$ respectively (Tables 1-3). Amongst all the tested extracts, JACI showed least MIC value against CR – Gram positive bacteria; while JACI, JACII and RAC and RACII showed least MIC value against PA – Gram negative bacteria; Amongst fungal strains, JAC showed least MIC value against CA, RHE and RACII against CG and JACI, JACII, RHE and RAC against CN. The stem collected from Jamnagar site showed better activity i.e. lower MIC values than that collected from Rajkot site.

For leaf extracts and its fraction MIC varied from 78 to >2500 $\mu\text{g ml}^{-1}$ while MBC was >2500 $\mu\text{g ml}^{-1}$ for the Gram positive bacterial strains (Table 4). *C. rubrum* was susceptible bacterial pathogen to JAC and its both fractions and RAC and its both fractions (MIC: 78 $\mu\text{g ml}^{-1}$). *L. monocytogenes* was susceptible to RAC and its both fractions (MIC: 78 $\mu\text{g ml}^{-1}$) i.e. both *C. rubrum* and *L. monocytogenes* showed same MIC values but their susceptibility was towards different extracts. For the Gram negative bacterial strains the MIC varied from <39 to >2500 $\mu\text{g ml}^{-1}$ while MBC was >2500 $\mu\text{g ml}^{-1}$. *P. aeruginosa* was the most susceptible bacterial pathogen to RAC II (MIC: <39 $\mu\text{g ml}^{-1}$) closely followed by *P. mirabilis*; it was the susceptible pathogen to RAC and RAC I (MIC: 78 $\mu\text{g ml}^{-1}$). For the fungal strains, MIC varied from 39 to >2500 $\mu\text{g ml}^{-1}$ while MBC was >2500 $\mu\text{g ml}^{-1}$. *C. glabrata* was susceptible to RHE (MIC: 39 $\mu\text{g ml}^{-1}$). For the standard antibiotics (CH and CF) MIC and MBC ranged from 1 to 32 $\mu\text{g ml}^{-1}$ and 8 to >32 $\mu\text{g ml}^{-1}$ respectively. Amongst all the tested extracts, JAC and RAC and their fractions showed least MIC value against *C. rubrum* - Gram positive bacteria; while RAC and RACI showed least MIC value against *P. mirabilis* - Gram negative bacteria. Antibacterial activity was better than antifungal activity. The leaf collected from Rajkot site showed better activity i.e. lower MIC values than that collected from Jamnagar

site. Gibbons³⁸ suggested that isolated phytochemicals should have MIC < 1000 $\mu\text{g ml}^{-1}$. However, Madikizela³⁹ found in their study, antibacterial MIC values equal to or less than 1000 $\mu\text{g ml}^{-1}$ for crude extracts were considered active. China⁴⁰ found in their study the petroleum ether extract of *S. grandiflora* flower showed MICs values ranged from 13-250 $\mu\text{g ml}^{-1}$ against the tested pathogenic strains.

From the above, it can be concluded that *T. bellerica* leaf and stem showed promising antibacterial activity especially towards Gram negative bacteria. This is very promising because it is reported that plant extracts are more active against Gram positive bacteria and the search is always going on for plants extracts which are able to inhibit the dangerous Gram negative bacteria. However, yield and antibacterial activity of *T. bellerica* leaf and stem was affected by the site of collection. More work is needed to confirm its reason. Work in this direction is in progress.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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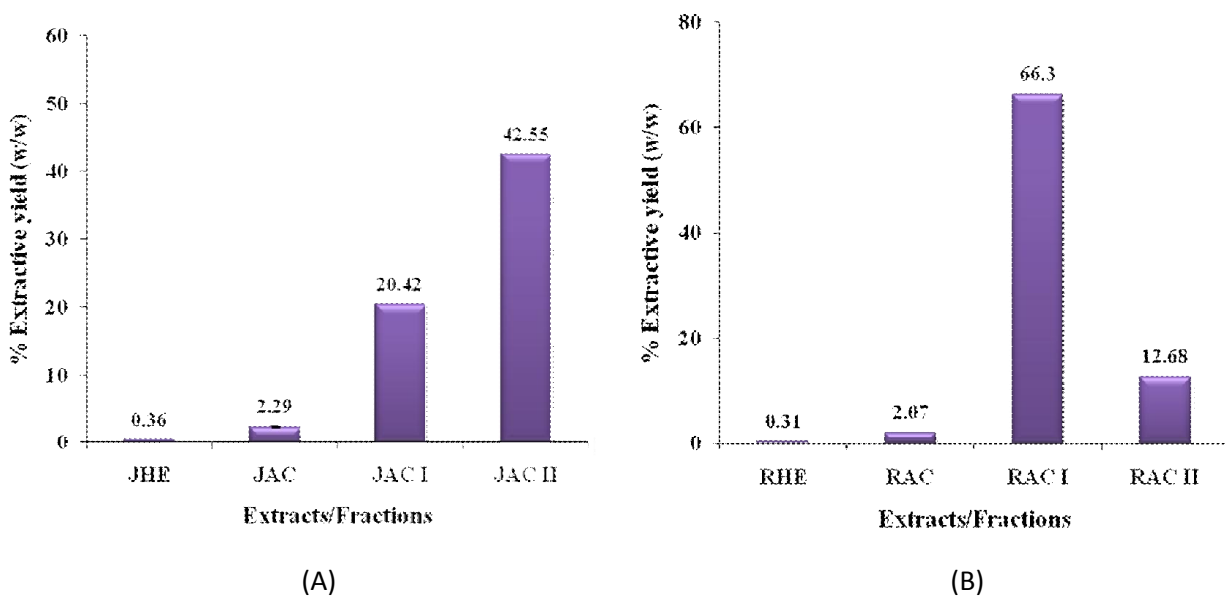


Figure 1. Extractive yield of extracts and fraction of *T. bellerica* stem of Jamnagar and Rajkot regions. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)

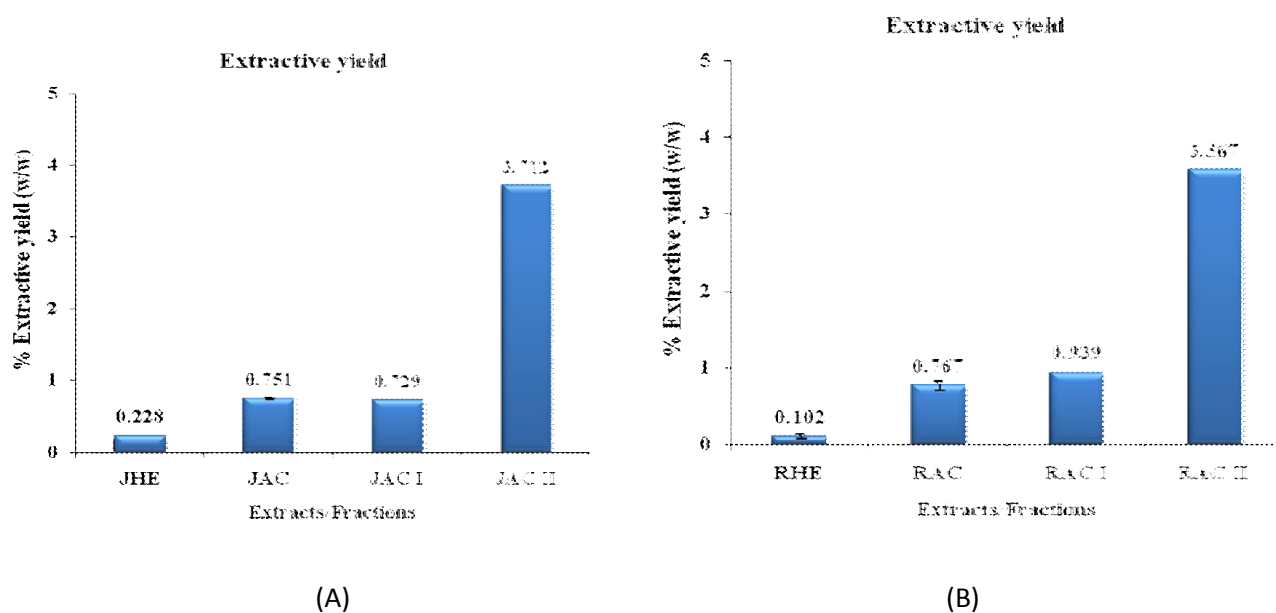


Figure 2. Extractive yield of extracts and fraction of *T. bellerica* leaf of Jamnagar and Rajkot regions. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)

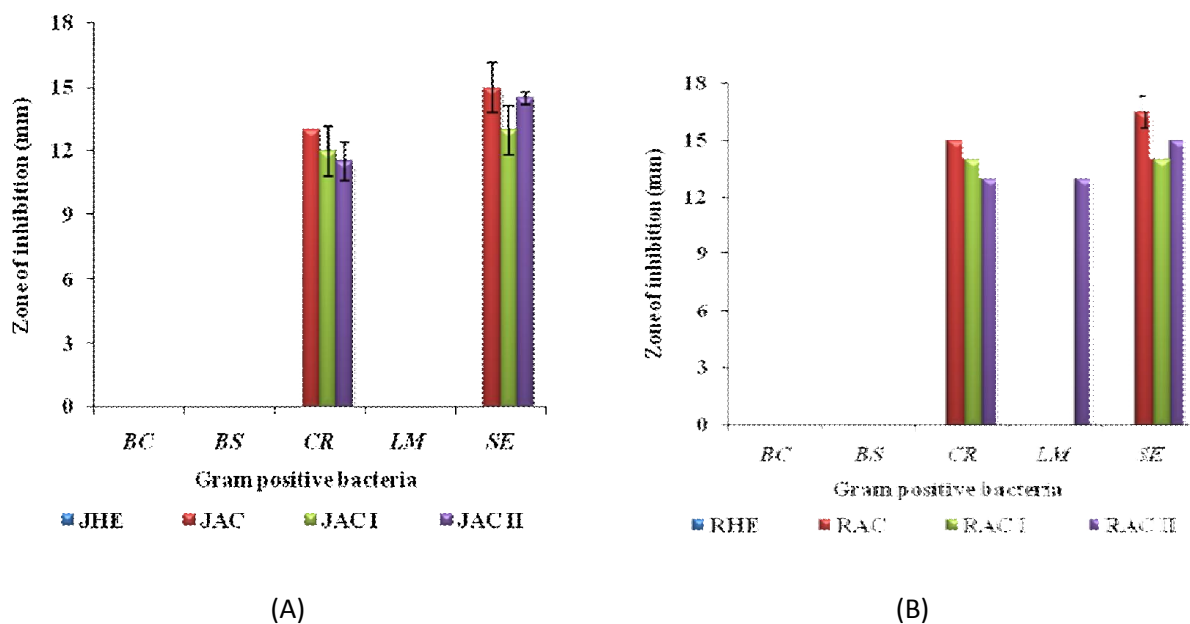


Figure 3. Antibacterial activity of extracts and fractions of *T. bellerica* stem from Jamnagar and Rajkot sites against Gram positive bacteria. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)

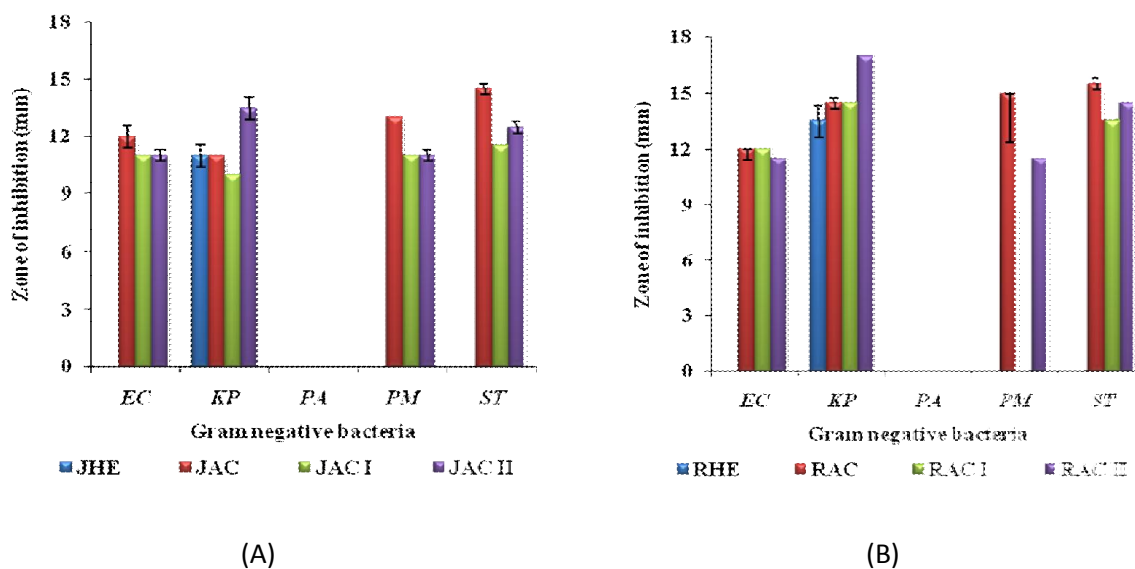
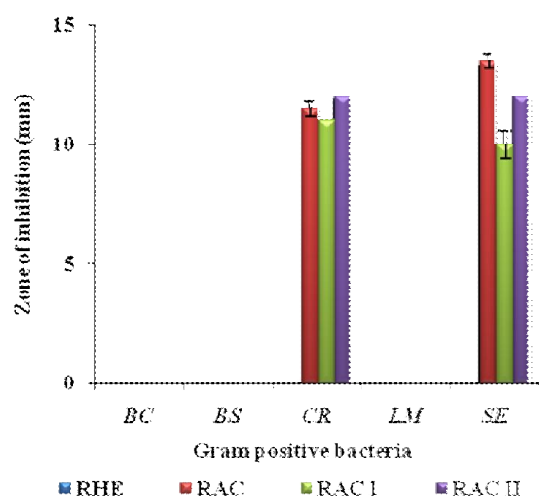
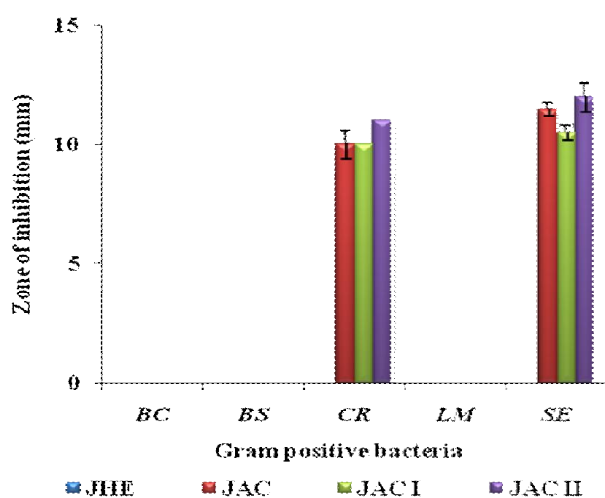


Figure 4. Antibacterial activity of extracts and fractions of *T. bellerica* stem from Jamnagar and Rajkot sites against Gram negative bacteria. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)

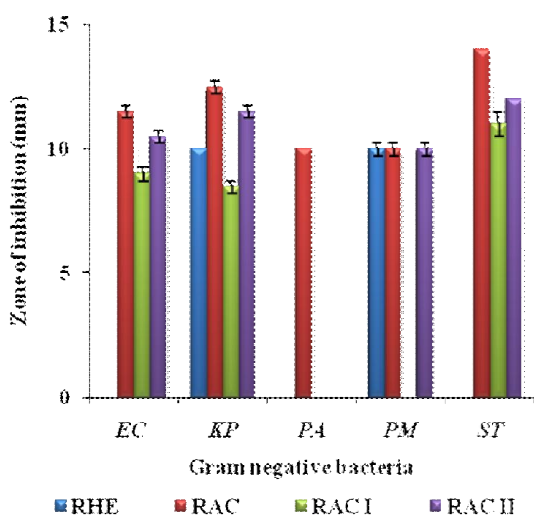


(A)

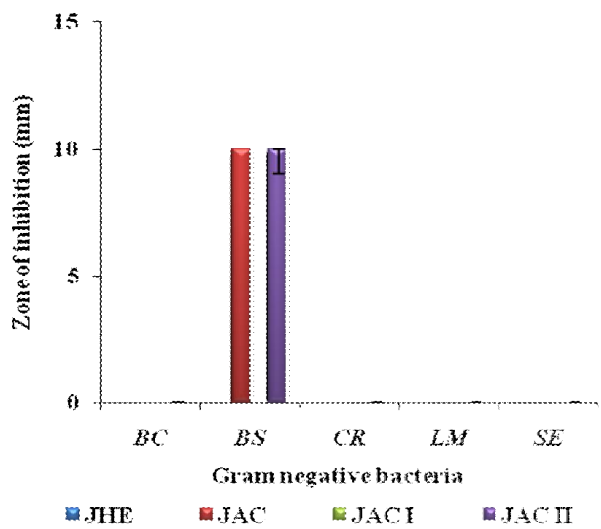


(B)

Figure 5. Antibacterial activity of extracts and fractions of *T. bellerica* leaf from Jamnagar and Rajkot sites against Gram positive bacteria. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)



(A)



(B)

Figure 6. Antibacterial activity of extracts and fractions of *T. bellerica* leaf from Jamnagar and Rajkot sites against Gram negative bacteria. (J = Jamnagar; R = Rajkot; HE = hexane extract; AC = acetone extract; ACI = Fraction I of acetone extract; ACII = Fraction II of acetone extract)