

Full Length Research Paper

Antimicrobial activities and chemical properties of *Tamarindus indica* L. leaves extract

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Tamarindus indica L. of the family Fabaceae is known to be used in folk medicine. Among efforts to verify this scientifically, the antimicrobial activities of the leaves extract were investigated against Gram negative and positive bacteria. The results were supported by SM images. The phytochemical constituents of the dried powdered leaves were extracted using aqueous and organic solvents. The antimicrobial activity of this extract was evaluated by using inhibition zone diameter, of both Gram negative and positive bacteria and fungi using agar well diffusion method. The most pronounced effect was shown by the ethanol extract. Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the tested organisms showed that the lowest MIC and MBC were demonstrated against *Klebsiella pneumoniae* and *Micrococcus luteus*, but the highest MIC and MBC was exhibited against *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* (MRSA). The phytochemical analysis revealed the presence of four major compounds, identified as flavanoidal glycosides. Using the total ion chromatography (TIC) two major compounds were identified as Orientin and Vitexin. The present study suggested that *T. indica* could have wide spectrum antimicrobial activity, therefore a new classes of antibiotics could be useful for infectious disease chemotherapy and control.

Key words: *Tamarindus indica*, antibacterial, antifungal, phytochemical constituents, crude extract.

INTRODUCTION

Plants produce a good deal of secondary metabolites which have benefited mankind in various ways, including treatment of diseases (Elaine et al., 2002). *Tamarindus indica* L. (Tamarind) belongs to the dicotyledonous family leguminosae and subfamily caesalpiniaceae (Khanzada et al., 2008). Tamarind has been used for centuries as a medicinal plant; its fruits are the most valuable part which have often been reported as curative in several pharmacopoeias. The leaves have a proven hap to protective activity associated with the presence of polyhydroxylated compounds with many of them of a flavonolic nature (Joyeux et al., 1995). Leaves also present good levels of protein, fat, fiber, and some vitamins such as thiamine, riboflavin, niacin, ascorbic acid and B-carotene (El-Siddig et al., 2006).

Flavonoid and other polyphenols are metabolites that have been found in tamarind leaves. These compounds have recorded as antimicrobial agents in many other plants. Due to their antimicrobial, an antifungal and antiseptic effect, have an extensive ethnobotanical use in many areas (Shankar et al., 2005). The present study was designed to evaluate the phytochemical and antimicrobial properties of tamarind leaves extracts, material obtained from Jeddah city, Saudi Arabia.

MATERIALS AND METHODS

Collection and preparation of samples

Samples of *T. indica* L. leaves were collected during May 2011 from Jeddah city (21°24'3"N, 39°17'45"E), Saudi Arabia. Species status of this plant was ferved at Faculty of Sciences Herbarium, King Abdulaziz University, Jeddah, washed in running tap water and then rinsed in distilled water for five minutes.

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Preparation of *T. indica* extracts

Ten grams of dried *T. indica* leaves were thoroughly washed in running water prior to cutting into small pieces by blender 1-2 mm. Extraction was done by adding 100 ml of distilled water and organic solvents (ethanol, petroleum ether, diethyl ether, ethyl acetate, chloroform and methanol extract) (1:10W/V) under cold conditions for 48 h. The solvent extract was filtered through a filter paper and evaporated under reduced pressure at 40°C until dryness. The extract was diluted by dimethyl sulfoxide (DMSO) and stored in 20°C until analysis according to Boeru and Derevici (1978).

Phytochemical screening

LC MS conditions

The high performance liquid chromatography (HPLC) system consisted of an Agilent 1200 system, solvent delivery module, quaternary pump, auto sampler, and column compartment (Agilent Technology, Germany). The column effluent was connected with Agilent 6320 Ion Trap LC-ESI-MS. Column heater was set to 25±2°C. The control of HPLC system and data processing were performed by Chem Station (Rev. B.01.03-SR2, 204) and 6300 Series Trap Control version 6.2 Build No. 62.24 (Bruker Daltonik GmbH).

Analytes were separated on Agilent Zorbax Eclipse XDB-C18 (250 × 4.6 mm i.d., 5 μm particle diameter) protected with Agilent-Zorbax XDB-C18 pre-column (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was programmed to deliver 100% 20 mM ammonium formate (in water) for 30 min, and 30-40 min; 0 to 10% acetonitrile with 90-100% ammonium formate 20 mM (85% formic acid and 25% ammonium hydroxide), flow rate 0.5 ml/min. An authentic reference compounds for the MS ID of active compounds in the plant extracts were used, these are: Isovitexin (≥98% HPLC, lot no.: BCBF5376V), orientin (≥95% HPLC, lot no.: 060M1734), isoorientin (lot no.: 100M1508V), 4-O-caffeoylquinic acid (≥98% HPLC, lot no.: BCBF0423V), and chlorogenic acid (≥95% HPLC, lot no.: SLBB6914V) were purchased from Sigma, China. Other materials were of analytical grade.

ESI-MS ion trap conditions

Smart target ion 550 m/z, Nebulizer 36 psi, dry gas 12 L/min, dry temperature 350°C, Auto MS (2), fragmentation amplitude 1V, polarity +ve, ICC smart target 150000, max accumulation time 150 ms, and scan range 100-700 m/z.

Extraction and sample preparation

About 2 g *Tamarindus* dry leaves powder added to 10 ml (90% MeOH, in water), vortexed 1 min, sonicated at 70°C for 10 min, then Centrifuged at 5500 rpm for 10 min. The supernatant was filtered using 0.45 micron Nylon Syringe membrane filter. Clear filtrate was dried under N₂ gas (99.9999% purity) at 80°C. Residue with CHCl₃, sonicated 2 min, vortexed, filtered, and then dried, reconstituted in 0.5 ml methanol, injected 5 μl applying same LCMS method two times (positive and negative ionization mode). The non-soluble part CHCl₃ was dissolved in de-ionized water (0.5 ml), sonicated, filtered and 5 μl was injected.

Bacterial and fungal strains

Cultures were prepared for *in vitro* antibacterial assay of seven bacteria, three Gram negative: *Escherichia coli* (ATCC8739);

Klebsiella pneumoniae (ATCC700603) and *Pseudomonas aeruginosa* (ATCC27853) and four Gram positive: *Bacillus subtilis* (ATCC11774); Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC977) and *S. aureus* (ATCC29213) *Micrococcus luteus* (ATCC4698). Those strains were provided by Microbiologics® USA. For the antifungal assay, four fungi (*Aspergillus flavus* (ATCC200026); *Aspergillus fumigatus* (ATCC204305); *Aspergillus niger* (ATCC1015) and *Candida albicans* (ATCC10231) were used. The tested organisms were subcultured on nutrient agar medium (Oxoid laboratories, UK) slopes for bacteria and Saborod dextrose agar slopes (Oxoid laboratories, UK) for fungi were the media used. These stock cultures were stored in the dark at 4°C during the survey.

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce (1994). DMSO was used as a negative control and streptomycin and ciprofloxacin (10 mg/disc) were used as a positive control for bacterial strains, Amphotericin B and Nystatin were used as a positive control for fungi.

The plates were done in triplicate. Bacterial cultures were incubated at 37°C for 24 h while the other fungal cultures were incubated at (30-32°C) for 48 h. Solutions of 10 mg /ml of streptomycin, ciprofloxacin, nystatin and Amphotericin B were used as standard for comparison. Antimicrobial activity was determined by measurement zone of inhibition (Agwa et al., 2000).

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was estimated for each of the tested organisms in triplicates. To 0.5 ml of varying concentrations of the extracts (0.05, 1, 3, 5, 7, 10, 15, 20, 25 and 30 mg/ml), 2 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard for all bacterial isolates was introduced to the tubes. A tube containing nutrient broth only was seeded with the test organisms to serve as control. Tubes containing bacteria cultures were then incubated at 37°C for 24 h. The tubes were then examined for bacterial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes, which did not show any growth and inoculated on sterile nutrient agar by streaking. After incubation the concentration at which there was no visible growth, was noted as the minimum bactericidal concentration (Doughari, 2006).

Scanning electron microscopy

The bacterial samples were slashed into small cubes of 5-8 mm³ and immersed in perfusion fixative for one hour. Fixation was continued in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2. After rinsing in phosphate buffer, samples were post fixed in aqueous 1% OsO₄ for 30 min. in 4°C. After washing in the same buffer three times for 5 min. Specimens were dehydrated in a graded series of 30 to 100% ethanol.

Air dry for a minimum of 15-30 min. Mount specimens, coating by gold and samples were photographed using a QUANTA FEG 450 scanning electron microscope at 20 kV. For SEM studies the samples of fungi were placed on the double side carbon tape on AL-Stub. The specimens were examined without coating by a field emission scanning electron microscope (QUANT FEG 450, Amsterdam, Netherland). The microscope was operated at an accelerating voltage of 20 kV and 10 mm work distance (Dykstra,

1993; Hayat, 1989).

Statistical analysis

For each experiment three replicates and three determinations were conducted. Means of variable, standard error and least significant differences were carried out using SPSS, to detect any significant differences between pathogenic microorganisms and extract type.

RESULTS and DISCUSSION

Phytochemical analysis

Four major compounds

Four new major flavanoidal glycosides were confirmed by matching with NIST2008 database (Figures 1, 2 and 3). The total ion chromatogram (TIC) was averaged to get the exact mass of detected compound. Extracted ion chromatogram was also applied for MS1 and MS2, and average MS spectra were deduced. All MS scan of MS2 were 100% matched with corresponding compounds as per NIST2008. Two major compounds were identified as orientin (42.58 min, m/z 448+H) (Figure 4) and vitexin (tR 44.97 min, m/z432+H). The MS2 spectrum was identical with completely separated peak at 43.69 min with variable intensities of major m/z fragments. Same case with MS2 spectrum of vitexin which show comparable m/z profile the peak eluted at 45.43 min, with variable m/z fragments intensities (Figure 6).

Minor compounds

Two minor compounds were confirmed by LCMSMS Ion Trap, namely: iso-orientin and isovetexin (Figures 5 and 7). In our results, the activity of *T. indica* extracts may include the presence of secondary metabolites for showing such biological effects. The phytochemical composition of *T. indica* extracts were analyzed by GC-MS and showed various components. *T. indica* extract revealed the presence of two major compounds identified as orientin and vitexin. In this context, other workers reported some other compounds, Bahatia et al. (2001) found two pairs of ring isomers vitexin, isovitexin (saponaretin), orientin and iso-orientin (homo-orientin) in the leaves of this plant. Also, Andreanus (2007) stated that the leaves contained sixtien, isovetexin, orientin, isoorientin, 1- malic acid, tannin, glycosides and peroxidase. Emmy et al. (2010) showed that *T. indica* leaves are a fair source of vitamin C and α Carotene, high mineral content, particularly P, K, Ca and Mg, anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity.

Prajapati et al. (2006) found that the fruit and roots of *T. indica* L. are rich in tartaric acid, citric acid, maleic acid,

potassium bitartrate, oxalic acid, Kernal, polysaccharides, flavonoid, glycoside, vetixen, orientin, homoorientin and hordenine.

Antimicrobial activity of leaves extracts of *T. indica*

The antimicrobial activities of leaves extracts of *T. indica*, obtained with six different organic solvents and aqueous extracts, the ethanol extracts showed the highest activity against the test organisms followed by the methanol extracts and ethyl acetate extracts against the tested bacterial strains (Table 1). Ethanol extracts in this study might have had higher solubility for more phyto-constituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases. Ethanol extracts showed best activity against *Micrococcus luteus* > *Staphylococcus aureus* > MRSA > *Bacillus subtilis* among the Gram+ve bacteria. The ethanol extracts of leaves of *T. indica* were also tested on three Gram-ve bacteria; *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The results showed a strong activity against *K. pneumoniae* followed by *E. coli* and *P. aeruginosa*. The aqueous extracts have the least antibacterial activity compared to other solvent extracts. Similar results have been reported in other studies. Daniyan and Muhammad (2008) recorded that the ethanol extracts produce strong antibacterial activity against *E. coli*, *K. pneumoniae*, *Salmonella paratyphi* and *P. aeruginosa*. The aqueous extracts have the least antibacterial activity compared to ethanol extract against *P. aeruginosa*. However, it has been reported that the acetone extracts showed the highest activity against the test organisms, followed by the ethanol extract and water extracts (Doughari, 2006).

Antimicrobial activities of standard antibiotics showed an inhibitory effect against all the tested bacteria. The results also showed that the ciproflaxocin is more effective than streptomycin. The present results go in the line with Shital (2010). The aqueous extract of *T. indica* has been reported to show the presence of alkaloids, which are formed as a metabolic by product, having antibacterial activity against all the tested bacteria in the order of sensitivity *S. aureus*, *E. coli* and *P. aeruginosa* except for *Salmonella typhimurium*. Similar results have been reported by Doughari (2006).

The antifungal activity of aqueous and organic extracts of *T. indica* leaves extracts compared to standard antibiotics are shown in (Table 2). The data showed that the ethanol extracts exhibited strong activity followed by methanol extracts against all tested fungi except *A. flavus*. The ethanol extracts exhibited remarkable antifungal activities against the tested fungi in the order of sensitivity as *A. fumigatus* > *C. albicans* and equal effect on *A. niger* and *A. flavus*. The aqueous extracts of *T. indica* leaves were in active against all the tested fungi.

The antifungal activities of standard antibiotics

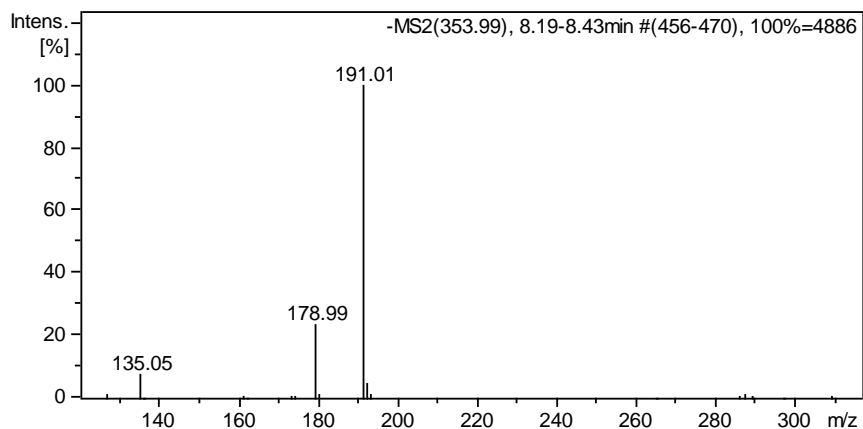


Figure 1. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as 3-O-Caffeoylquinic acid (chlorogenic acid). The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.

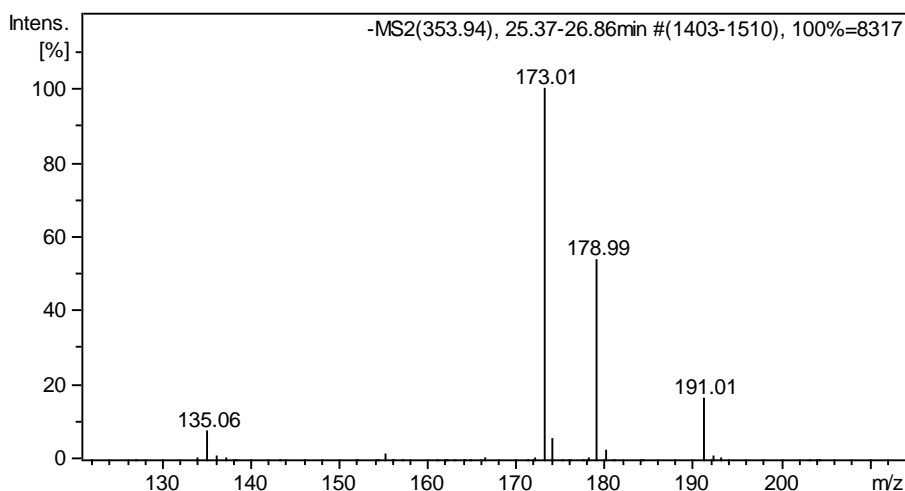


Figure 2. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as 4-O-Caffeoylquinic acid (chlorogenic acid). The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.

showed that the Amphotericin is less effective than nystatin.

Adeola et al. (2010) stated that antimicrobial activities of *T. indica* the methanol and hexane crude extracts obtained from it pulps were evaluated to determine their inhibition active. The methanol and hexane crude extracts obtained from it pulps were evaluated to determine their inhibition activities on human pathogenic microorganisms. Only the hexane extract exhibited intrinsic antifungal properties on *Penicillium* species. *A. niger* and *C. albicans* were resistant to all the extracts from all the locations except for the methanol extract of the pulp that showed activity on *A. niger*. The methanol extract showed very weak activities on *Penicillium* species. Generally, methanol extract exhibited higher

antimicrobial activity than hexane extract.

Doughari (2006) reported that the tested *T. indica* extracts did not show any antimycotic activity against four fungi tested *A. flavus*, *A. fumigatus*, *A. niger* and *C. albicans*. The present results go in the line with Adeola et al. (2010) who reported that the bacterial pathogens were more sensitive to the *T. indica* crude extracts than fungal pathogens. Some studies have also shown that *T. indica* pulp extracts exhibited higher antimicrobial activities against *S. typhimurium*, *S. aureus* and lower activity against *A. niger* (Dipali et al., 2010).

The antimicrobial activity of the pure essential oil was higher than those found in the aqueous or hydro alcoholic extracts. *B. subtilis* was the most sensitive bacteria against all kinds of *Tamarindus* extract, whereas *P.*

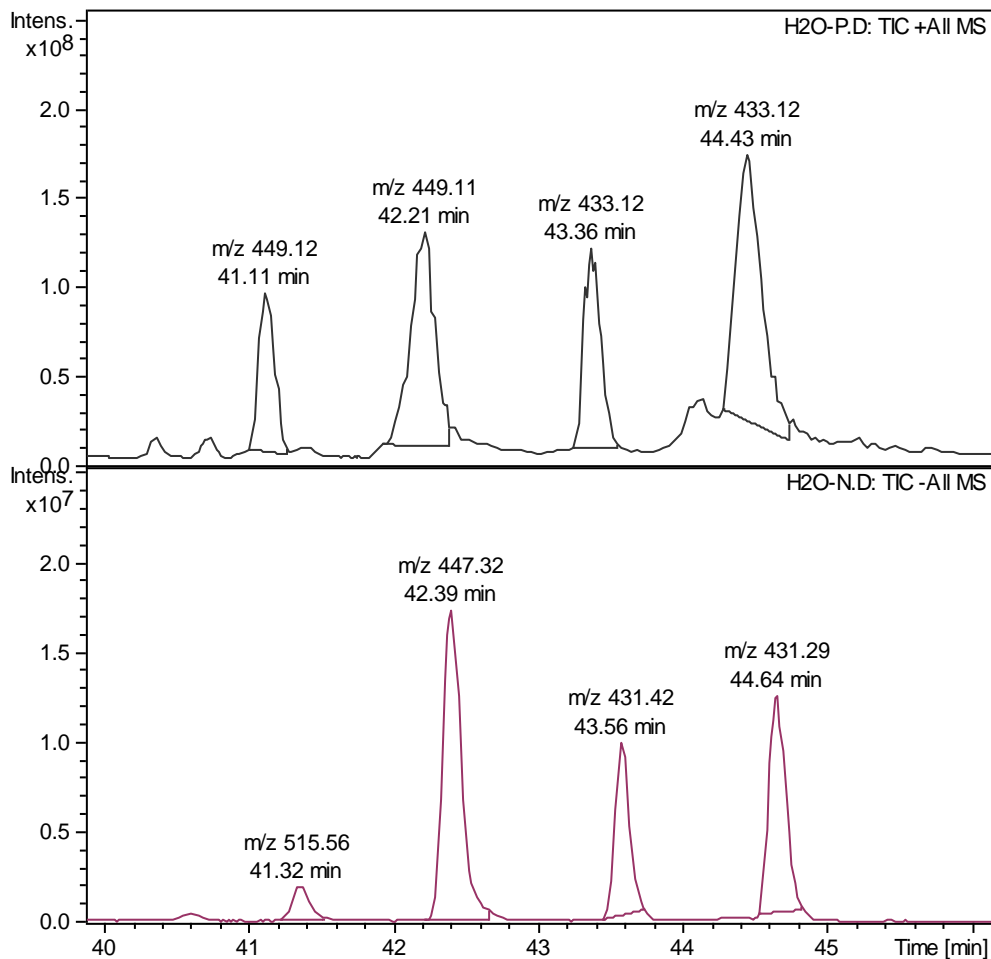


Figure 3. Representative TIC-MS chromatogram of aqueous extract of tamarindus leaves powder monitored by positive (upper) and negative (bottom) polarization MS modes. Orientin (41.12 min), iso-orientin (42.22 min), vitexin (43.36 min), and iso-vitexin (44.44 min).

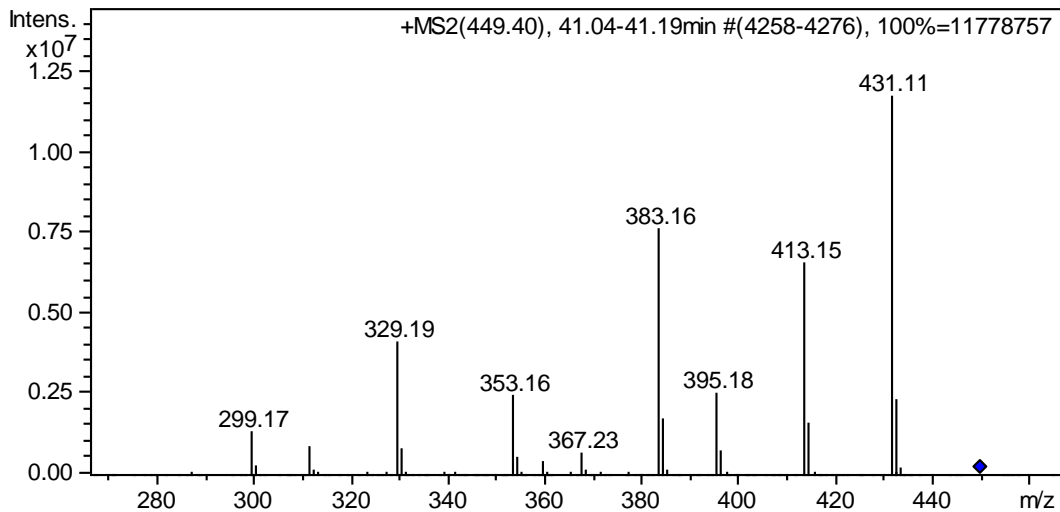


Figure 4. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as orientin. The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.

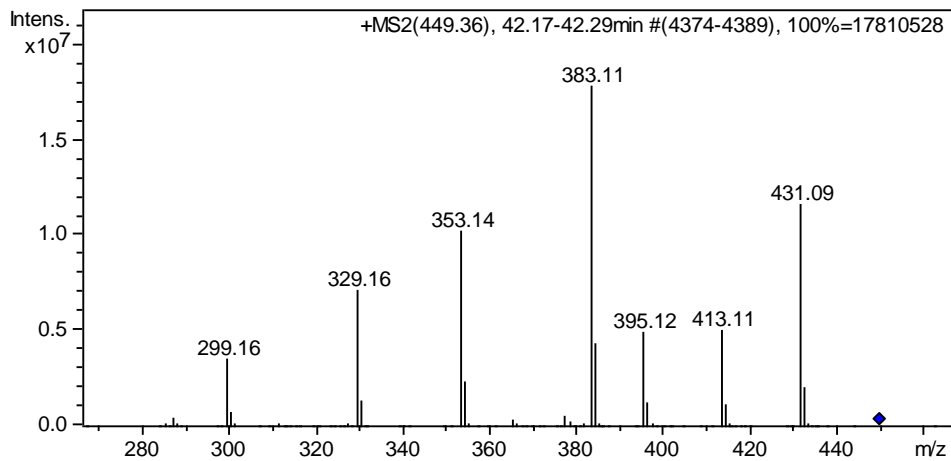


Figure 5. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as isoorientin. The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.

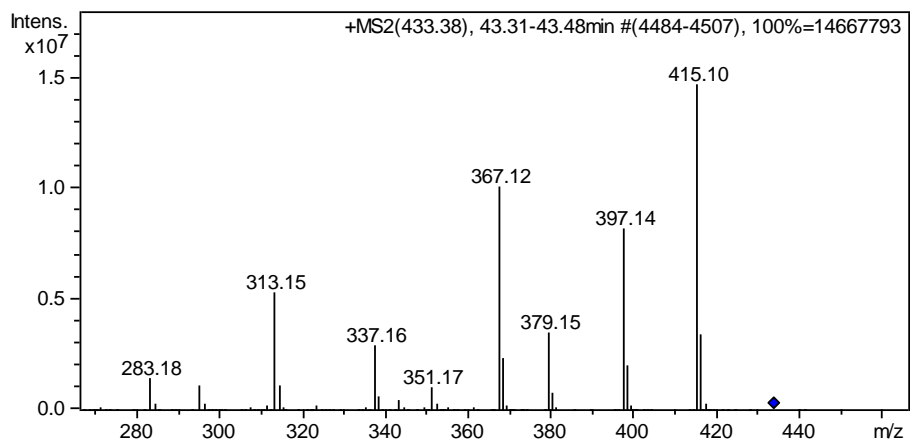


Figure 6. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as vitexin. The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.

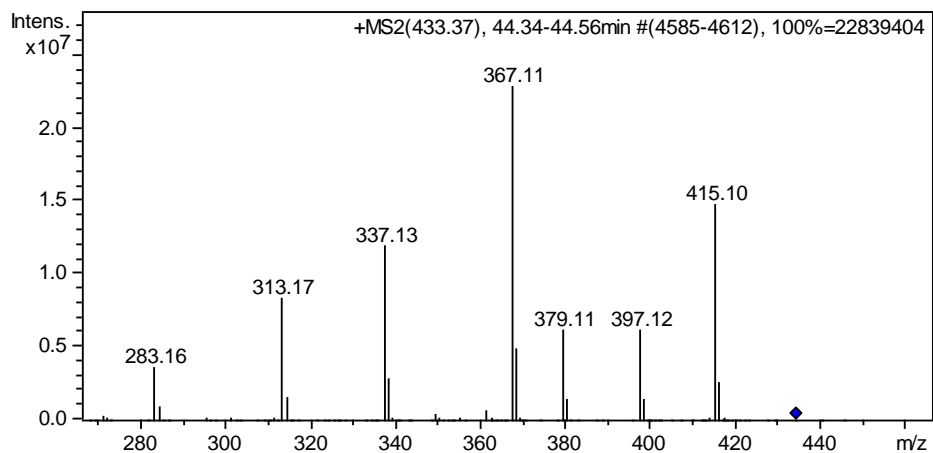
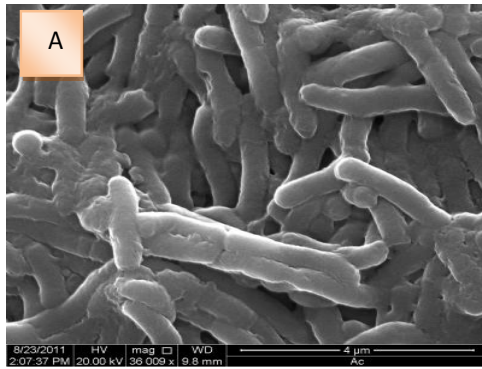
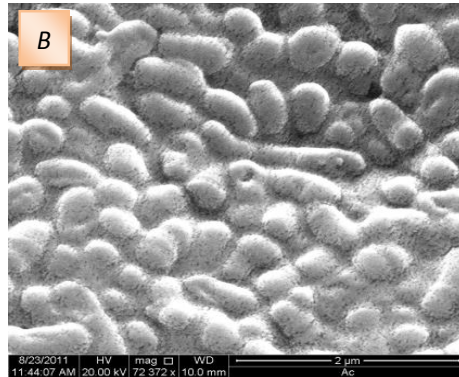


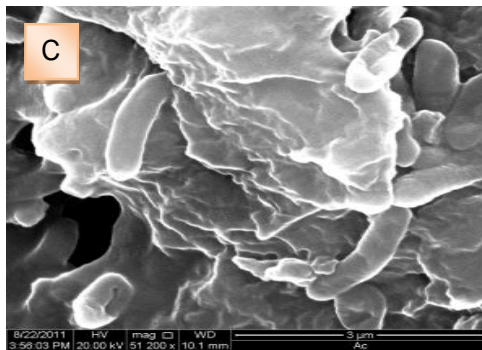
Figure 7. Average MS2 of negative m/z 353.5 eluted at 8.3 min. Confirmed as isovitexin. The MS2 peak abundance was matched with the reference standard and by matching with NIST2008 database.



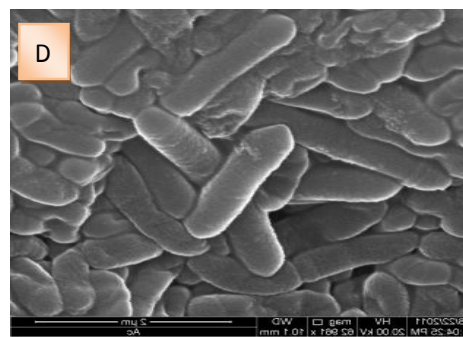
E. coli treated with *T. indica* extract



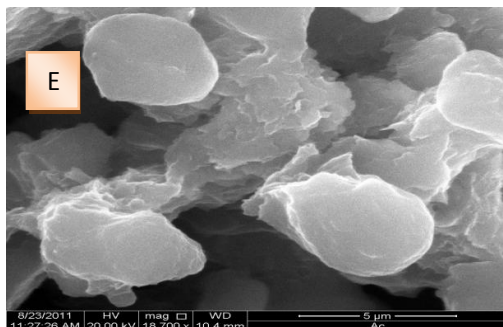
Untreated *E. coli* as control



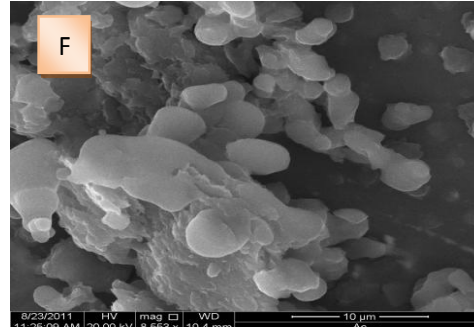
K. pneumoniae treated with *T. indica* extract



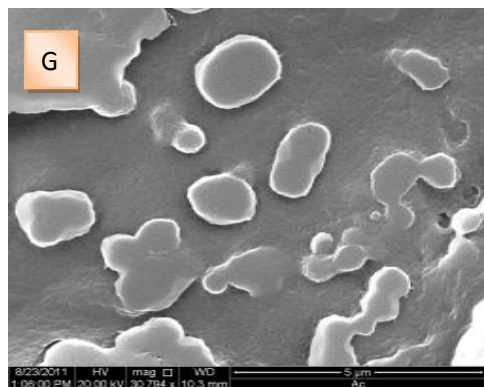
Untreated *K. pneumoniae* as control



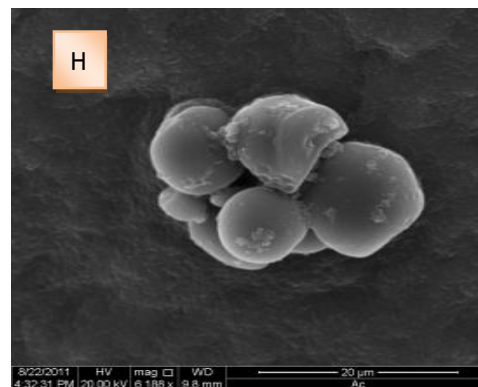
MRSA treated with *T. indica* extract



Untreated MRSA as control

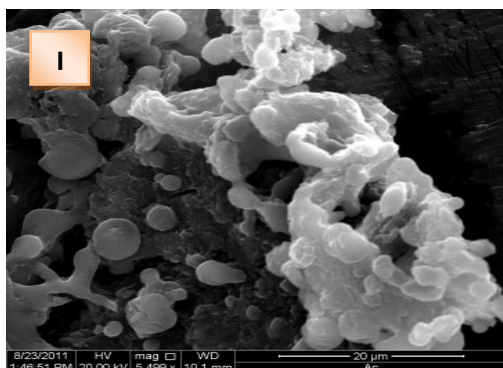


S. aureus treated with *T. indica* extract

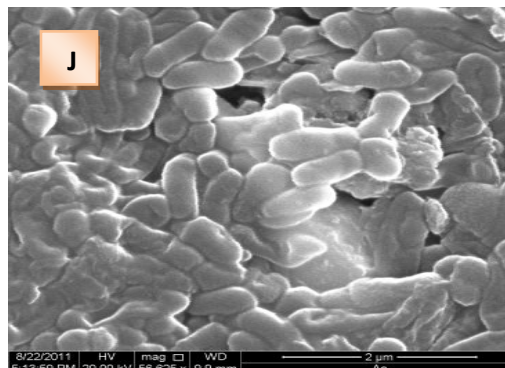


Untreated *S. aureus* as control

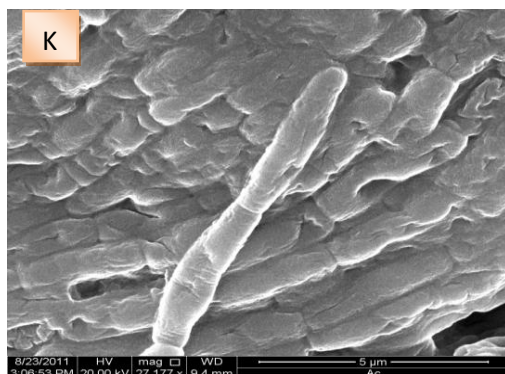
Figure 8. Morphological changes in some tested bacteria treated with *T. indica* ethanol extract using scanning electron microscope.



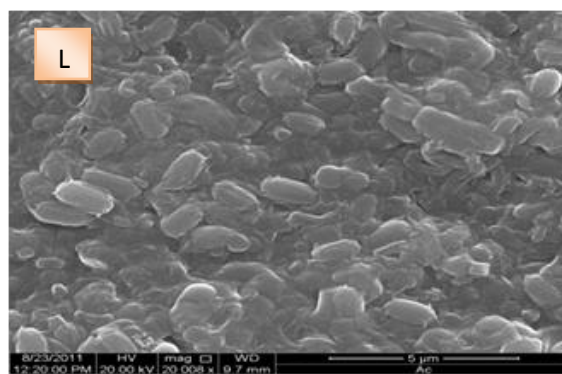
P. aeruginosa treated with *T. indica* extract



Untreated *P. aeruginosa* as control



B. subtilis treated with *T. indica* extract



Untreated *B. subtilis* as control

Figure 8. Contd.

aeruginosa and *C. albicans* remained resistant to a large part of the natural extracts evaluated (Julio et al., 2010).

Mode of antimicrobial action

Results of MIC and MBC are shown in Table 3. The results showed that *P. aeruginosa* had the highest MIC (25 mg/ml) and MBC (20 mg/ml). The highest MIC and MBC values of *P. aeruginosa* indicated that either the plant extracts are less effective on some bacteria or that the organism has the potential of developing antibiotic resistance. The obtained results are in agreement with those reported by Doughari (2006) who observed that the highest MIC and MBC values of *P. aeruginosa* was 14 and 20 mg/ml respectively of the ethanolic extracts of *T. indica*. The lowest MIC and MBC values was 10 and 5 mg/ml were found for *K. pneumoniae* while *S. aureus* had MIC and MBC values of 15 mg/ml. The MIC and MBC values were generally lower for the ethanol extracts, being 10-25 and 5-20 mg/ml respectively against the tested organisms. The low MIC and MBC values for other bacteria are indication of the efficacy of the plant extracts

and the obtained results are in same line with those reported by Doughari (2006) and Julio et al. (2010).

The mechanism of action of these phase phytochemicals may be via lysing the cell, increasing permeability of the cell wall and membrane, inhibition of protein and DNA synthesis and/or by inhibiting the transport of nutrient across the cell wall or membrane (Stewart and Beswick, 1979). This inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some phytochemicals that were found in the plant extract. The demonstration of antibacterial activity against both Gram (+ve) and Gram (-ve) bacteria may be indicative of the presence of broad spectrum antibiotic (Doughari, 2006). The optimal effectiveness of the medicinal plant may not be due to one main active constituent, but may be due to the combined action of different compounds originally present in the plant (Bai, 1990).

Scanning electron microscopy

The SEM analyses of treated and untreated cell with *T.*

Table 1. The antibacterial activity of aqueous and organic extract of *T. indica* concentration 100 mg/ml compared to antibiotics against the tested bacteria.

Types of bacteria	Bacterial species	Zone of inhibition (mm)							Positive control		The mean of bacteria
		Type of the extract							Antibiotics		
		Water	Ethanol	Petroleum ether	Diethyl acetate	Ethyl acetate	Chlorofom	Methanol	Streptomycin (10 µg/disc)	Ciprofloxacin (10 µg/disc)	
Gram positive bacteria	<i>B. subtilis</i> (ATCC11774)	15.00 ± 0.00	32.33 ± 0.33	11.67 ± 0.33	14.33 ± 0.33	21.67 ± 0.33	15.33 ± 0.33	21.67 ± 0.33	25.00 ± 0.00	34.00 ± 0.00	21.22
	MRSA (ATCC977)	15.33 ± 0.33	33.67 ± 0.33	12.33 ± 0.33	15.33 ± 0.33	16.67 ± 0.33	13.67 ± 0.33	22.67 ± 0.33	20.00 ± 0.00	36.00 ± 0.00	20.63
	<i>S. aureus</i> (ATCC29213)	30.67 ± 0.67	35.67 ± 0.33	21.67 ± 0.33	21.00 ± 0.58	32.67 ± 0.33	23.33 ± 0.33	33.33 ± 0.33	19.00 ± 0.00	38.00 ± 0.00	28.37
	<i>M. luteus</i> (ATCC4698)	36.33 ± 0.33	44.67 ± 0.33	0.00 ± 0.00	24.33 ± 0.33	40.00 ± 0.00	20.33 ± 0.33	43.33 ± 0.33	27.00 ± 0.00	46.00 ± 0.00	31.33
Gram negative bacteria	<i>E. coli</i> (ATCC8739)	21.33 ± 0.33	40.00 ± 0.58	17.33 ± 0.33	22.33 ± 0.33	34.67 ± 0.33	20.33 ± 0.33	35.67 ± 0.33	23.00 ± 0.00	44.00 ± 0.00	28.74
	<i>K.pneumoniae</i> (ATCC700603)	28.33 ± 0.33	41.67 ± 0.33	22.33 ± 0.33	22.33 ± 0.33	36.00 ± 0.58	22.00 ± 0.58	40.33 ± 0.33	25.00 ± 0.00	42.00 ± 0.00	31.11
	<i>P. aeruginosa</i> (ATCC27853)	30.00 ± 0.00	38.33 ± 0.33	21.67 ± 0.33	21.67 ± 0.33	34.33 ± 0.33	20.33 ± 0.33	36.33 ± 0.33	22.00 ± 0.00	42.00 ± 0.00	29.63
Mean of type of the extract		25.29	38.05	15.29	20.19	30.86	19.33	33.33	23.00	40.29	

$F_{8,174} = 93.27.35$, $LSD = 2.53$. There is highly significant differences between bacteria. There is highly significant differences between type of the extract.

Table 2. The antifungal activity of aqueous and organic extract of *T. indica* concentration 100 mg/ml compared to antibiotics against the tested fungi.

Fungal species	Zone of inhibition (mm)							Positive control		Mean of fungi	
	Type of the extract							Antibiotics			
	Water	Ethanol	Petroleum ether	Diethyl acetate	Ethyl acetate	Chlorofom	Methanol	Amphotericin	Nystatin		
<i>A. flavus</i> (ATCC200026)	0.00 ± 0.00	25.33 ± 0.33	24.33 ± 0.33	23.33 ± 0.33	23.33 ± 0.33	22.67 ± 0.33	19.33 ± 0.33	29.00 ± 0.00	32.00 ± 0.00	22.15	
<i>A. fumigatus</i> (ATCC204305)	0.00 ± 0.00	28.33 ± 0.33	19.33 ± 0.33	20.67 ± 0.33	21.00 ± 0.00	23.33 ± 0.33	26.00 ± 0.00	26.00 ± 0.00	28.00 ± 0.00	21.41	
<i>A. niger</i> (ATCC1015)	0.00 ± 0.00	25.33 ± 0.33	17.00 ± 2.52	14.33 ± 0.33	16.00 ± 0.58	17.67 ± 0.33	23.67 ± 0.33	25.00 ± 0.00	30.00 ± 0.00	18.78	
<i>C. albicans</i> (ATCC10231)	0.00 ± 0.00	27.33 ± 0.33	24.67 ± 0.33	23.33 ± 0.33	17.33 ± 0.33	24.00 ± 0.58	24.33 ± 0.33	28.00 ± 0.00	29.00 ± 0.00	22.00	
Mean of type extract		0.00	26.58	21.33	20.42	19.42	21.92	23.33	27.00	29.75	

$F_{8,96} = 181.35$, $LSD = 1.80$. There is highly significant differences between fungi, there is highly significant differences between type of the extract.

indica ethanolic extract are shown in Figure 8. Antibacterial effects of the ethanolic extracts were seen on *E. coli*, *K. pneumoniae*, MRSA, *S. aureus*, *P. aeruginosa* and *B. subtilis*, *E. coli* showed blebs formation and indentations (Figure 8A) as compared to control (Figure 8B). This finding is in accordance with Heatl et al. (1999) who working on *Plantago major*.

The cell wall of *K. pneumoniae*, MRSA and *S. aureus* were crenate with shrunken and sticky cells (Figure 8C, E and G) compared to their control (Figure 8D, F and H). Our finding rhymes with those of Hoj et al., (2001) who reported the effect of some herbal plant and of Sharifa et al. (2008) working on *Plantago major*.

The cells of *P. aeruginosa* and *B. subtilis* were

shrunken, collapsed, perforated and became sticky (Figure 8I and K) as compared to control (Figure 8J and L). This could be due to the leak through the cell wall or perhaps some alteration in the membrane permeability. Similar observations were documented by Rand et al. (2011) using *Vignar adiata* L. Flavonoids activity is probably due to their ability to complex with extracellular

Table 3. MIC and MBC of ethanolic extracts of *T. indica*.

Types of bacteria	Bacterial species	Leaf extracts	
		MIC (mg/ml)	MBC (mg/ml)
Gram positive bacteria	<i>Bacillus subtilis</i> (ATCC11774)	20	10
	MRSA(ATCC977)	20	15
	<i>Staphylococcus aureus</i> (ATCC29213)	15	15
	<i>Micrococcus luteus</i> (ATCC4698)	10	7
	<i>Escherichia coli</i> (ATCC8739)	15	10
Gram negative bacteria	<i>Klebsiella pneumoniae</i> (ATCC700603)	10	5
	<i>Pseudomonas aeruginosa</i> (ATCC27853)	25	20

and soluble proteins along with bacterial cell wall (Tsuchiya et al., 1996). Modes of action reported for flavonoids are lyses and leakage of intracellular constituents, perturbation of cell homeostasis and inhibition of enzyme (Lambert, 2008). Indeed, it was surprising to us the promising results obtained in the present study, in spite of the immense previous research conducted on *T. indica* L. Therefore, they were strongly recommend more precise studies on this gifted plant.

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