

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF *ISATIS TINCTORIA* L. (BRASSICACEAE) USING THE MICRO-PLATE METHOD

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Abstract

Isatis tinctoria L. has well-documented history as conventional therapeutic herb. In present study its crude extract was examined for broad-spectrum antimicrobial activity using micro-titer plate method. Four different plant parts were extracted with 14 different solvents. All fractions were analyzed against seven bacterial and four fungal strains. Ethyl acetate, chloroform, n-hexane and acetone showed maximum antibacterial activity with minimum IC₅₀ value (≤ 200 $\mu\text{g/ml}$). Leaves>branches> roots>flower is the order of different parts based on antibacterial activity. Although, in some cases like against *Klebsiella pneumonia* and *Micrococcus luteus* the flower showed better results as compared to other parts. Roots showed better results against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Extracts showed better antimicrobial activity as compared to antibiotics (cefotaxime). The activity of the extracts against gram positive was better than gram negative. For antifungal activity, ethyl acetate > n-hexane-ethyl acetate (1:1) > chloroform> acetone was the order of the fraction with increasing growth inhibition rate. All the parts (except branches) were observed having antifungal activity. The most resistant strains found in this study were *Mucor mycosis*, none of the fraction have more than 30% inhibition on used concentration. Plant crude extract being having broad spectrum antimicrobial activity is suggested for pre-clinical and clinical trials.

Key words: Anti-bacterial, Anti-fungal, *Isatis tinctoria* L., Resistant strains, Therapeutics agents

Introduction

Isatis tinctoria L. is a well-known medicinal plant commonly known as Woad, belongs to family of Brassicaceae. It is distributed in Europe, Russia, China, Japan, Iran, Afghanistan, Pakistan, Germany and England. Woad is locally known as "Tora panrra" is well known for its insecticidal effects; it is placed around the natural products as a barrier to protect them from insects and reptiles. It was prescribed for the cure of inflammatory problem, snake bites, tumors, injuries, ulcers and hemorrhoids; Mohsenzadeh *et al.*, 2009). The compounds isolated from *Isatis tinctoria* L. have antifungal and anti-mycobacterial activity (Honda *et al.*, 1980).

Several classes of modern antibiotics are available for killing these bacterial strains to get rid of the diseases caused by them, but there is also exponential increase in the resistant of these bacteria strains to drugs. To solve the resistant problem, the best way is to use herbal preparation and phytochemicals (Gilani *et al.*, 2007; Shinwari *et al.*, 2009). Other secondary metabolites are carboxylic acid both aliphatic and aromatic, different derivatives of glycosides, peptides, isoprenoids (Hartleb *et al.*, 1995), flavonoids and anthranoids (Wu *et al.*, 1997). The procedure used for the separation of pharmaceutically active compound from a complex mixture of metabolites by using a suitable solvent is called extraction. The solvent penetrate mixed and dissolve the same polarity containing compounds. These extracts can be used as solution, suspension, semi-solid, or as a powder (Ncube *et al.*, 2008). The quality of extract mainly depends up on: type and part of plant used, solvent (nature, concentration and polarity) used for extraction and appropriate procedure (Ncube *et al.*, 2008). Different parts of the plant like branches, flowers, leaves, roots,

fruits, seeds and barks etc. are selected either randomly or on the basis of conventional and traditional knowledge (Parekh *et al.*, 2006; Gilani *et al.*, 2010). The compounds that can slow down or completely stop oxidative damage by scavenge free radicals are called anti-oxidants. These antioxidants are reducing agents and donate hydrogen by receiving oxygen (Anokwuru *et al.*, 2011; Yamagishi *et al.*, 2011). Fruits, vegetables and cereals are a rich source of antioxidants (Mertens-Talcott *et al.*, 2006). Synthetic antioxidants are available but natural source having no or less side effect are preferred (Meenakshi *et al.*, 2011). Phenols, flavonoids and tannins are medicinal plants based antioxidants being focused by the scientists (Upadhyay *et al.*, 2010). In the present study we used fourteen different solvent extraction systems to find out active fraction as anti-microbial from four different parts of *Isatis tinctoria* L.

Materials and Methods

Antimicrobial activity of *Isatis tinctoria* L. was performed using micro-plate method. A total of 56 fractions, extracted from four different parts (branches, flowers, leaves and roots) with fourteen different solvents were analyzed against seven bacterial and four fungal strains.

Bacterial culture preparation: A bacterial colony was picked from the starter culture and placed in freshly prepared nutrient broth. The culture was incubated for 12 hours. After incubation, the culture was stored in the refrigerator at 4°C. Before each assay the culture was transferred to incubator for 14 hrs. For assay the culture was diluted in the ratio of 1 to 10 in nutrient broth (Sultanbawa *et al.*, 2009).

Bacterial test strains used

Gram positive: (1) *Bacillus subtilis* (2) *Micrococcus luteus* (3) *Staphylococcus aureus*.

Gram negative: (4) *Escherichia coli* (5) *Klebsiella pneumoniae* (6) *Pseudomonas aeruginosa* (7) *Salmonella typhimurium*.

Assay procedure: The assay was performed in 96-well plate. In the first row 7.5µl (4mg/ml) of each sample was added and then diluted down in the column using three fold serial dilution to get the final concentration of 100, 33.3, 11.1 and 3.7µg/ml (Fig. 1). The Dimethyl sulfoxide (DMSO) and cefotaxime were taken as negative and positive control respectively. After samples dilutions, 195µl inoculums (of each strain mentioned earlier) freshly diluted were added to each well containing either sample, DMSO or cefotaxime. Three empty wells were inoculated only with culture. The plate were incubated for 30 minutes and reading were taken using (ELX800, BIOTEK) micro-plate reader at 630nm (Sultanbawa *et al.*, 2009). After calculating percent inhibition, IC₅₀ was also calculated for each sample by using method of Anderson *et al.* (2001).

Antifungal assay: The antifungal activity was carried out using micro-titer plate method with a little modification in the micro-spectrophotometric assay (Broekaert *et al.*, 1990). Fungal strains selected for assay were; *Mucor mycosis*, *Aspergillus nigar*, *Aspergillus fumigatus* and *Aspergillus flavus*. Crude extracts (20mg/ml) at the rate of 20µl were added to each well in micro-plate. Then 20µl of amphotericin (an antifungal) as positive control, DMSO and only culture were used as negative control. Then 100µl of the freshly diluted culture was added to each well, containing either, amphotericin or DMSO (Satish *et al.*, 2007). Plate were incubated for 30 minutes at 27°C, and readings were taken using (ELX 800, BIOTEK) micro-titer plate reader at 630 nm. Percent inhibition was calculated using formula;

$$\% \text{ Inhibition} = [(\Delta C - \Delta T) / \Delta C] \times 100.$$

ΔC represent corrected (48 hours reading – 30 min reading) absorbance of control, ΔT is corrected absorbance of test sample (Broekaert *et al.*, 1990).

Results

An antibacterial activity was evaluated against 14 different extracts each from leaves, flower, branches, and flowers of *Isatis tinctoria* L. All the extracts were analyzed against human pathogens. All the pathogens were clinical isolates in which two strains, *Pseudomonas* and *Klebsiella* were isolated from burn samples of patient under treatment in Pakistan institute of medical sciences (PIMS), Islamabad, Pakistan. Assays were performed using modified technique of microtiter 96-well plate method. The readings were taken on 630nm wave length rays using (ELX 800, BIOTEK) micro-plate reader. All the experiments were performed in triplicate and IC₅₀ was calculated using Excel sheet 2013 for each bacterial strain. Using Origin-Pro 8.5 graphs were prepared from IC₅₀ calculated for each strain.

Anti-*Escherichia coli* activity: Four different concentrations of crude extracts: 100, 33.34, 11.1 and 3.7µg/ml were tested against each strain. IC₅₀ values were calculated using MS Excel Sheet 2013 shown in (Fig. 1). The best activity even better than Cefotaxime (used as a standard) reported on used concentrations was of ethyl acetate's extracts from branches, flowers, leaves. Chloroform's extracts from roots were observed having maximum activity with lower IC₅₀ value. Acetone-water (1:1) combination's extracts from branches and flowers; methanol-water (1:1) combination's extracts from leaves and ethanol's extracts from roots were reported having poor activity on the concentrations used (Fig. 2).

Anti-*Bacillus subtilis* activity: Cefotaxime used as standard caused maximum inhibition with only 70µg/ml IC₅₀ value. The chloroform fraction of branches, leaves, roots and ethyl acetate fraction of branches and leaves showed maximum inhibition with 117, 111, 110, 110 and 111 (µg/ml) IC₅₀ values respectively (Fig. 2).

Anti-*Salmonella typhi* activity: On the basis of IC₅₀ value many of the extracts resulted better inhibition than Cefotaxime (used as a standard) on different concentration used in assay. The IC₅₀ value for present standard was calculated 100 µg/ml. Some of the extracts from each part showed lower than 100 µg/ml IC₅₀ value (Fig. 3).

Anti-*Staphylococcus aureus* activity: Very diverse results were reported from the activity against *S. aureus* with an IC₅₀ value ranges from less than 100µg/ml to more than 5000µg/ml. Cefotaxime (used as positive control) was found having an IC₅₀ value of 200µg/ml, which greater than many extracts. The significantly (p<0.05) maximum inhibition with lowest IC₅₀ value from branches was noted with ethyl acetate fraction. The best fraction is represented with subscript (g). Other fraction are also nominated with same letter, that shows similar activity with no significant (p<0.05) difference. Significantly (p<0.05) maximum inhibition form flower, leaves and root was reported for ethyl acetate fraction. The minimum inhibition with maximum IC₅₀ values was reported: methanol's fraction from branches with more than 800 µg/ml; methanol's fraction from flowers with more than 1000µg/ml; methanol-ethyl acetate (1:1) combination's fraction from leaves and water's fraction with more than 5000 µg/ml from roots (Fig. 4).

Anti-*Klebsiella pneumoniae* activity: In the present study the *K. pneumoniae* showed resistant to Cefotaxime (3rd generation broad spectrum antibiotic) used as positive control. The IC₅₀ value calculated for Cefotaxime was more than 700µg/ml, except branches and flowers. Cefotaxime showed very weak activity whereas highest IC₅₀ values were reported in leaves and roots. The significantly (p<0.05) best inhibitory activities were reported for: ethyl acetate's fractions from all plant parts (branches, flowers, leaves and roots). The significantly (p<0.05) minimum inhibitory activities with maximum IC₅₀ values were reported for: acetone-water (1:1) combinations' fractions and Cefotaxime from branches; methanol-water (1:1) combinations' fraction from flowers; acetone-water (1:1) combinations' fraction after Cefotaxime from leaves and methanol-water (1:1) combinations' after Cefotaxime from roots (Fig. 5).

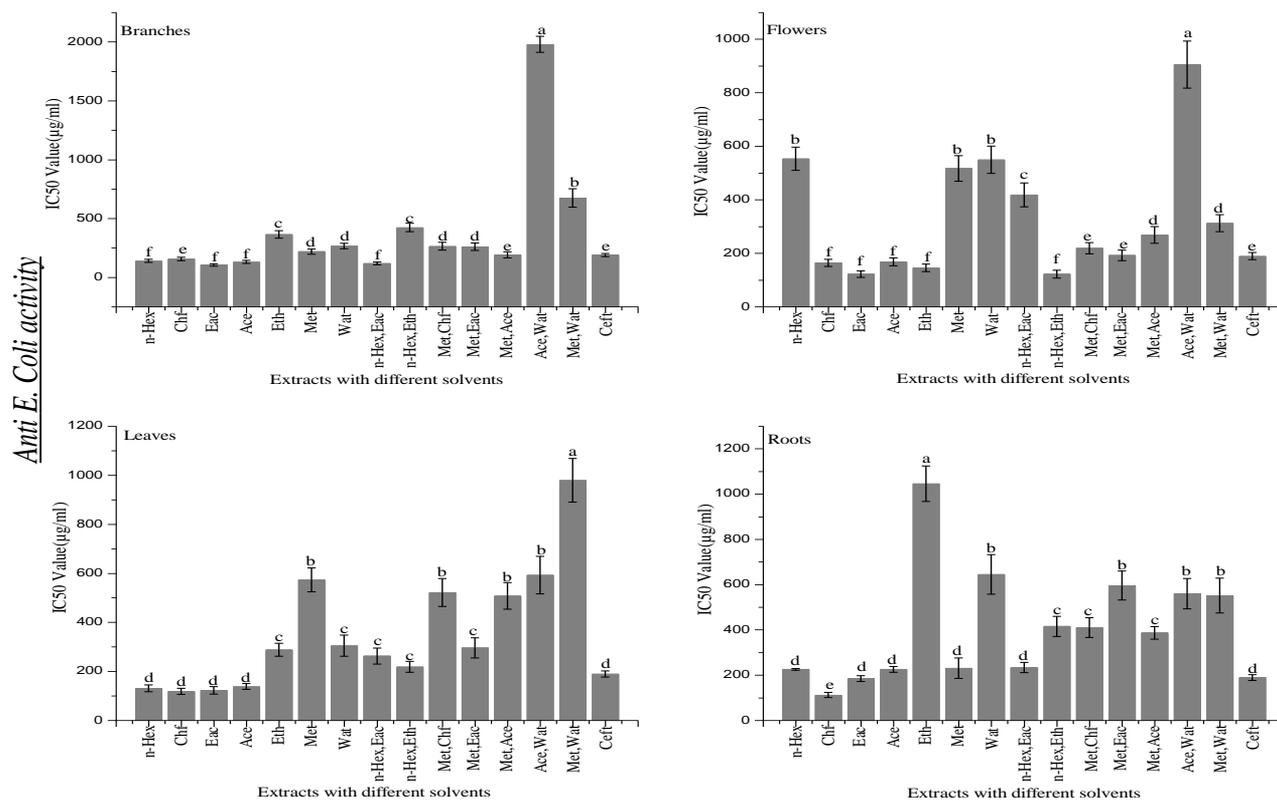


Fig. 1. Anti-*Escherichia coli* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

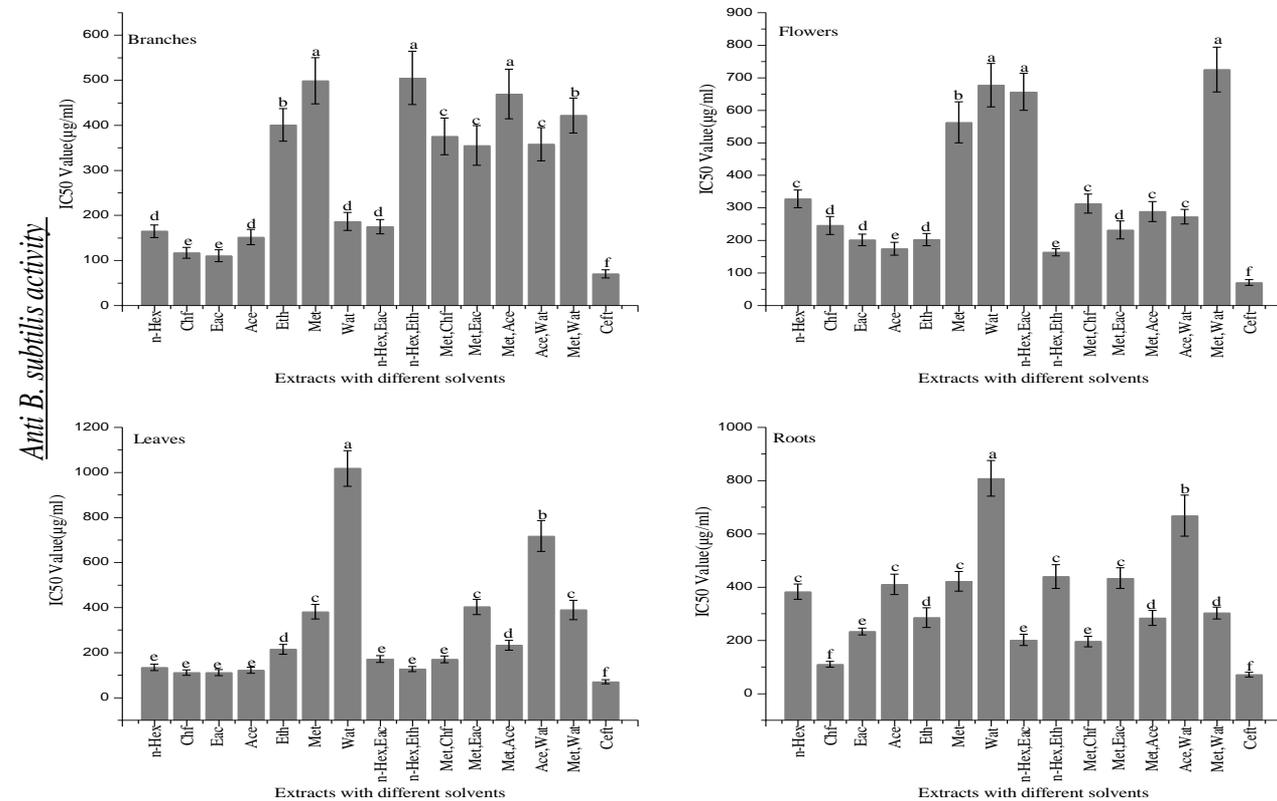


Fig. 2. Anti-*Bacillus subtilis* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

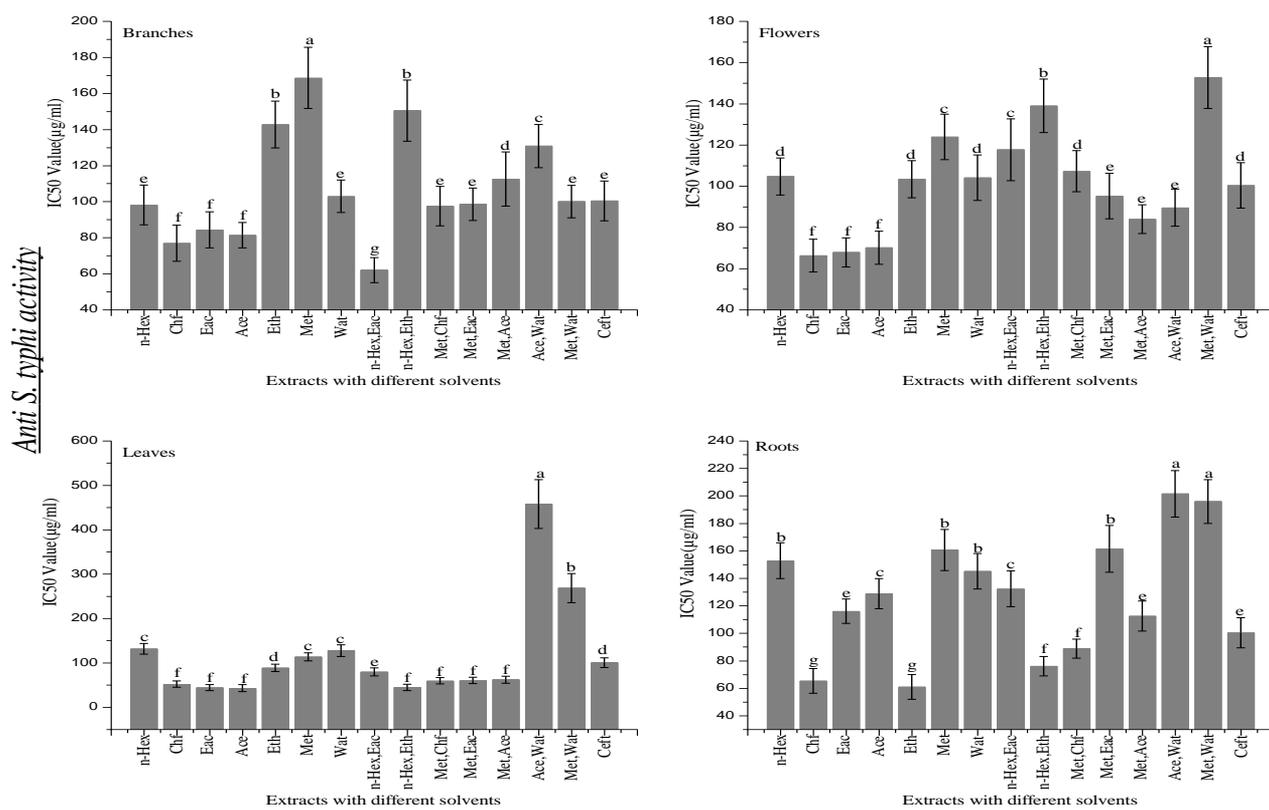


Fig. 3. Anti-*Salmonella typhi* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

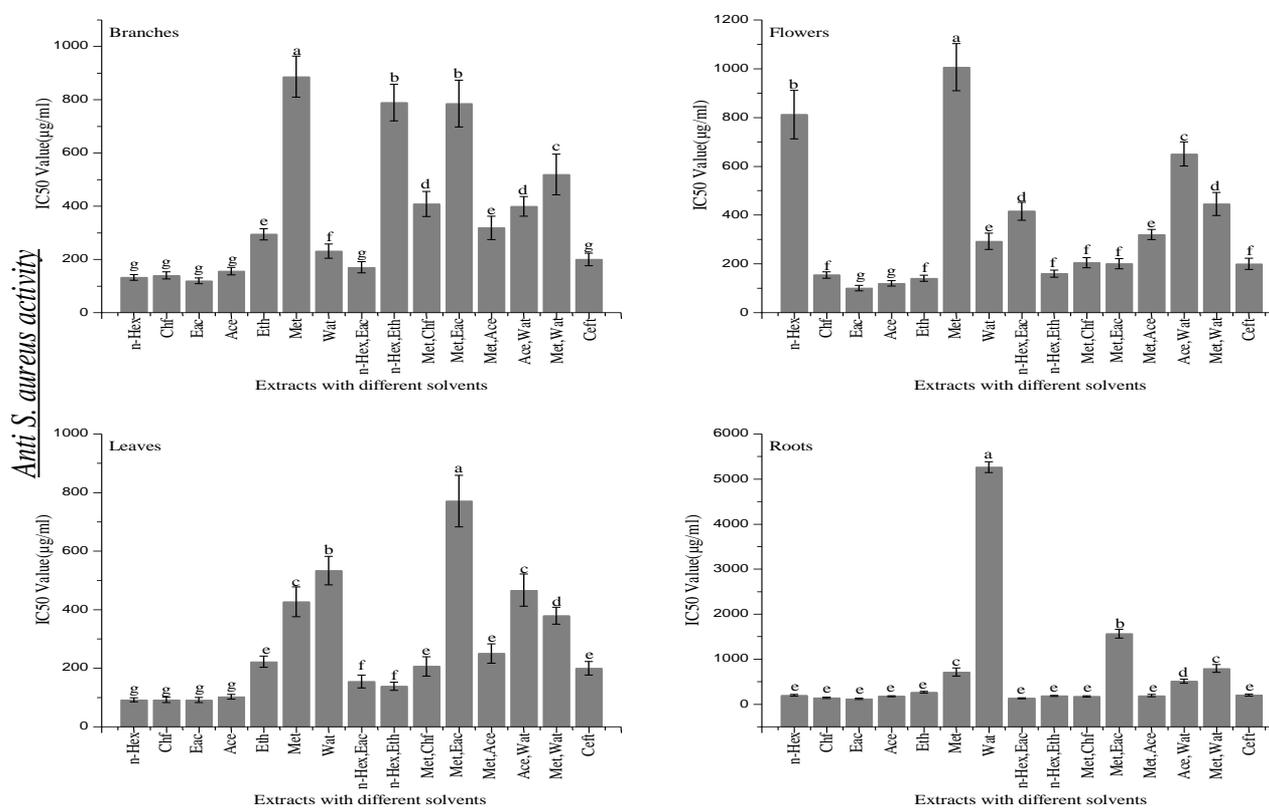


Fig. 4. Anti-*Staphylococcus aureus* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

Anti-*Micrococcus luteus* activity: *M. luteus* was found susceptible to both cefotaxime (positive control) and many of the fractions from all parts. More than half fractions having an IC₅₀ value less than 100 µg/ml. IC₅₀ value for cefotaxime is only 78 µg/ml, while only 42 µg/ml in ethyl acetate's fraction from flowers were reported. The ethyl acetate's fraction from flowers showed significantly ($p < 0.05$) maximum inhibitory effect (Fig. 6).

Anti-*Pseudomonas aeruginosa* activity: The activities of some fractions against *P. aeruginosa* were not appreciable. These fractions had IC₅₀ values more than 700, 1000, 800 and 1200 (µg/ml) in branches, flowers, leaves and roots respectively. Besides having such high IC₅₀ values in some fraction more than half fraction having IC₅₀ values less 174 µg/ml, which is the IC₅₀ values for Cefotaxime. The chloroform fraction from leaves showed best inhibition on given concentrations that's why having 32 µg/ml IC₅₀ values. Methanol's and ethyl acetate's fractions from branches, (h)ethyl acetate fractions of flowers and chloroform's fraction from leaves and roots showed significantly maximum activities (Fig. 7).

Anti-fungal activity: Four fungal strains were selected to test the anti-fungal activity of *I. tinctoria* extracts. The response of different parts toward fungal strains was much diverged, over all fractions from braches were very poor, with only one fraction of methanol-acetone (1:1) combination showed more than 20% inhibition. The most resistant strains reported in this study were *Mucor mycosis*. None of the fraction has more than 30% inhibition on used concentration (Table 1).

All the values were nominated with alphabetical order from top to bottom. Fraction of ethyl acetate from flowers, acetone's fraction from leaves against *Aspergillus nigar*; ethyl acetate's fraction from leaves against *Aspergillus fumigatus*; n-hexane's, chloroform's and ethyl acetate's fractions from leaves, ethyl acetate's fraction from flowers against *Aspergillus flavus* are fraction with best activity (Table 1). The experimental fungal strains used in this study were observed having significant ($p < 0.05$) diversity in susceptibility toward used concentrations of (56) fractions. On the basis increasing susceptibility strains can be arranged like: (*Mucor mycosis* < *Aspergillus nigar* < *Aspergillus fumigatus* < *Aspergillus flavus*). On the basis of increasing (%) inhibition of fungal growth capability of plants parts can arranged as: (Branches < Roots < Flowers < Leaves). Just like antibacterial activity; chloroform's, ethyl acetate and acetone's fractions were very consistent as anti-fungal.

Discussion

Ethyl acetate fraction showed consistency except in branches against *Salmonella typhi*. Chloroform, acetone and n-hexane alone and combination were also consistent respectively. Leaves were the part with outstanding consistency, in the above consistent solvents. Branches, roots and flower were consistent respectively. Although in some cases like against *Klebsiella pneumoniae* and *Micrococcus luteus* the flower showed better results. Roots showed better results against *S. aureus* and *P.*

aeruginosa. The activity of positive control (cefotaxime) was very consistent against *B. subtilis* *M. luteus*, *S. typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* respectively. The worse activity of positive control was against *K. pneumoniae*. In all cases the extracts showed the best results as compared to antibiotics (cefotaxime). The activity of the extracts against gram positive was better than gram negative

Our results are similar to the findings Sartoratto *et al.* (2004) those reported that the aqueous and methanolic extracts of many Brazilian medicinal plants shows better antibacterial activity. The antibacterial activity against *E. coli* and other gram-negative have also been reported (Bakht *et al.*, 2017; Yagoub, 2008). The antibacterial activity may be due to the presence of alkaloids (inhibiting cell division) in the extracts. Methanolic extract inhibition of *E. coli*, *P. aeruginosa* and *S. aureus* was reported but the concentration was very high (Djeussi *et al.*, 2013). Present study proved that ethyl acetate and chloroform showed best results than water and methanolic extracts. Our study was in accord once with Tane *et al.* (2005) who recorded that Flavonoids, alkaloids and diterpenoids of plant compounds had antibacterial activity. A micro-plate method also prove that methanol's extracts have complete bacterial growth inhibition less than 1mg/ml, which contained grifolic acid (Langfield *et al.*, 2004). The activity of organic solvents extracts is far better than aqueous extracts (De Boer *et al.*, 2005). The best activity against gram-positive was also reported earlier while contradictory to other published data which reported good activity of extract against Gram-negative (Awodele *et al.*, 2013). Previous study also proves the importance of our study being active against both Gram-positive and Gram-negative. The broad spectrum activity was previously reported ranging from 390-6250 µg/ml. The good activity of the extracts as compare to positive control of present study is also reported in literature (Aziz *et al.*, 2017; Pendota *et al.*, 2015). The EC₅₀/IC₅₀ was calculated for the first time for gentamicin against *P. aeruginosa* (Soothill *et al.*, 1992). The IC₅₀ was calculated for 50% growth inhibition of *Bacillus megaterium* of plasma protein (Anderson *et al.*, 2001).

For antifungal activity 4 mg/ml concentrations of all (56) fraction were used. Four parts (branches, flowers, leaves and roots) were used and all the parts (except branches) were observed having antifungal activity. Ethyl acetate > n-hexane-ethyl acetate (1:1) combination > chloroform > acetone was the order of the fraction with increasing rate growth inhibition, supported for chloroform extract earlier (Ertas *et al.*, 2005). The fungicidal compounds were studied in ethyl acetate fraction of *Toddalia asiatica* (L.) a conventional medicinal plant (Duraipandiyani *et al.*, 2009). The maximum inhibitory activity for methanol was reported for lantana leaves and flowers which supported by our studies that the maximum *A. flavus* growth inhibition of methanol fraction was (35%) of *I. tinctoria* (leaves) (Bokhari, 2009). Medicinal plants play important role for controlling of many important bacterial and fungal diseases (Habiba *et al.*, 2016; Khan & Shinwari, 2016; Qasim *et al.*, 2016; Hussain *et al.*, 2015; Jan *et al.*, 2015; Shinwari *et al.*, 2013).

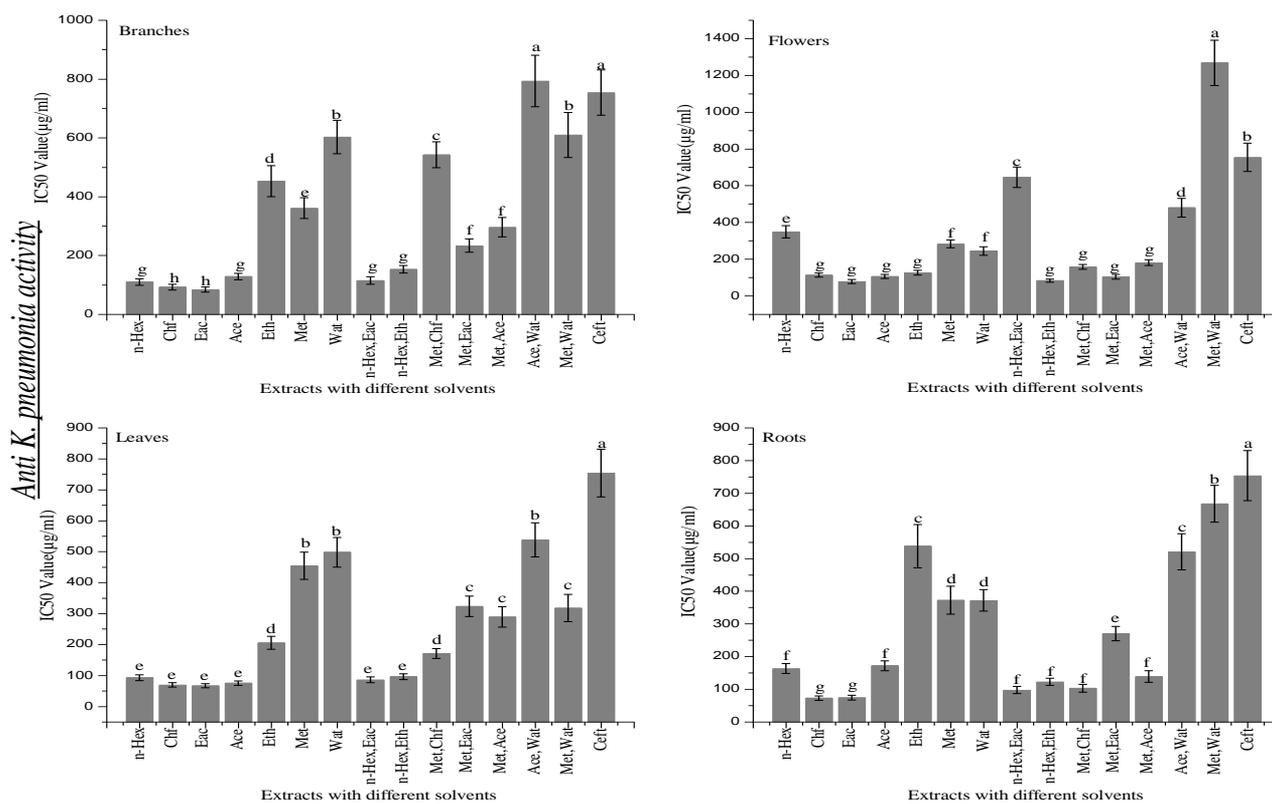


Fig. 5. Anti *Klebsiella pneumoniae* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

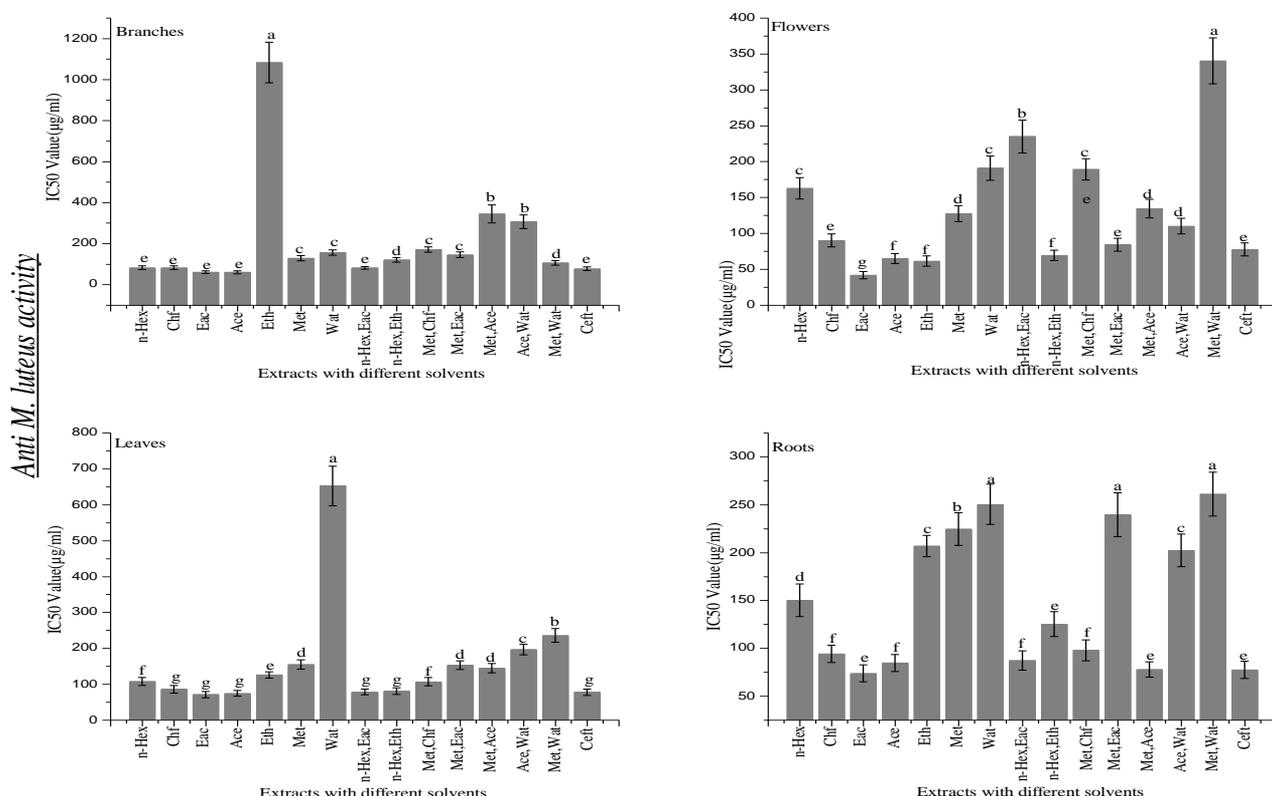


Fig. 6. Anti-*Micrococcus luteus* activity of 14 fractions with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

Table 1. % inhibition of fungal growth by 14 fractions each from branches, flowers, leaves and roots. % Fungal growth inhibition using 4000µg/ml concentration of different extracts from *Isatis tinctoria* L.

Fungal strains	<i>Aspergillus niger</i>				<i>Aspergillus fumigatus</i>				<i>Aspergillus flavus</i>				<i>Mucor mycosis</i>				
	Branches (Mean ± SD)	Flowers (Mean ± SD)	Leaves (Mean ± SD)	Roots (Mean ± SD)	Branches (Mean ± SD)	Flowers (Mean ± SD)	Leaves (Mean ± SD)	Roots (Mean ± SD)	Branches (Mean ± SD)	Flowers (Mean ± SD)	Leaves (Mean ± SD)	Roots (Mean ± SD)	Branches (Mean ± SD)	Flowers (Mean ± SD)	Leaves (Mean ± SD)	Roots (Mean ± SD)	
Extracts with different solvents	n-Hex	-	38 ± 3 ^d	-	-	38 ± 3 ^d	-	38 ± 3 ^d	-	-	63 ± 7 ^a	32 ± 4 ^d	-	-	29 ± 3 ^e	12 ± 2 ^f	
	Chf	-	40 ± 5	43 ± 3 ^c	41 ± 4 ^c	-	38 ± 3 ^d	23 ± 3 ^c	33 ± 4 ^d	12 ± 1 ^f	55 ± 6 ^a	60 ± 7 ^a	44 ± 5 ^c	11 ± 3 ^f	25 ± 3 ^e	26 ± 3 ^e	23 ± 3 ^e
	Eac	-	60 ± 5 ^a	45 ± 6 ^c	36 ± 3 ^d	-	58 ± 4 ^b	65 ± 7 ^a	32 ± 4 ^d	32 ± 4 ^d	68 ± 6 ^a	61 ± 6 ^a	39 ± 5 ^d	17 ± 3 ^f	28 ± 2 ^e	35 ± 3 ^d	10 ± 2 ^f
	Ace	-	40 ± 4 ^c	61 ± 6 ^a	27 ± 4 ^e	10 ± 2 ^f	40 ± 5 ^c	52 ± 5 ^b	29 ± 3 ^e	-	46 ± 5 ^c	37 ± 5 ^d	49 ± 4 ^c	10 ± 2 ^f	19 ± 3 ^f	37 ± 5 ^d	23 ± 3 ^e
	Eth	-	27 ± 4	28 ± 4 ^e	-	-	14 ± 1 ^f	20 ± 5 ^e	16 ± 2 ^f	-	45 ± 5 ^c	37 ± 5 ^d	25 ± 3 ^e	9 ± 2 ^f	20 ± 2 ^e	20 ± 3 ^e	9 ± 1 ^f
	Met	14 ± 2 ^f	10 ± 2 ^f	12 ± 2 ^f	23 ± 3 ^e	-	14 ± 2 ^f	21 ± 3 ^e	13 ± 2 ^f	19 ± 3 ^f	10 ± 1 ^f	35 ± 5 ^d	29 ± 4 ^e	15 ± 2 ^f	16 ± 2 ^f	23 ± 2 ^e	16 ± 1 ^f
	Wat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	n-Hex, Eac	-	52 ± 5 ^b	26 ± 2 ^e	42 ± 3 ^c	-	51 ± 5 ^b	29 ± 4 ^e	-	-	55 ± 7 ^b	47 ± 4 ^e	51 ± 6 ^b	-	33 ± 2 ^d	27 ± 4 ^e	-
	n-Hex, Eth	-	22 ± 3 ^e	23 ± 3 ^c	38 ± 5 ^d	13 ± 2 ^f	34 ± 5 ^d	26 ± 4 ^e	-	-	59 ± 6 ^b	22 ± 3 ^e	29 ± 3 ^e	-	22 ± 3 ^e	11 ± 2 ^f	19 ± 3 ^f
	Met, Chf	-	10 ± 1 ^f	-	36 ± 5 ^d	-	13 ± 2 ^f	28 ± 3 ^e	33 ± 4 ^d	-	34 ± 5 ^d	-	48 ± 4 ^c	-	20 ± 4 ^e	14 ± 2 ^f	28 ± 3 ^e
	Met, Eac	-	16 ± 2 ^f	20 ± 3 ^c	21 ± 3 ^e	-	29 ± 2 ^e	21 ± 2 ^e	30 ± 4 ^d	-	34 ± 3 ^d	47 ± 4 ^e	37 ± 5 ^d	-	19 ± 3 ^f	13 ± 3 ^f	24 ± 3 ^e
	Met, Ace	-	28 ± 3 ^e	26 ± 3 ^c	-	-	29 ± 3 ^e	25 ± 2 ^e	32 ± 4 ^d	22 ± 2 ^e	40 ± 3 ^c	41 ± 6 ^c	46 ± 6 ^c	13 ± 1 ^f	17 ± 3 ^f	9 ± 2 ^f	24 ± 3 ^e
	Acc, Wat	-	-	19 ± 2 ^f	31 ± 3 ^d	-	-	-	12 ± 2 ^f	-	11 ± 2 ^f	-	16 ± 2 ^f	-	-	23 ± 3 ^e	19 ± 3 ^f
	Met, Wat	-	17 ± 2 ^f	-	12 ± 2 ^f	-	-	-	-	-	-	16 ± 1 ^f	19 ± 3 ^f	-	-	10 ± 2 ^f	10 ± 3 ^f

(±) indicate standard deviation, (-) indicate no activity, (f) subscript containing have significantly (p<0.05) minimum activity which increase with alphabetical order, and (a) obtaining values have significantly (p<0.05) maximum activity. Values (µg/ml) with similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. Light green (branches), yellow (flowers), dark green (leaves), and dark yellow color (roots)

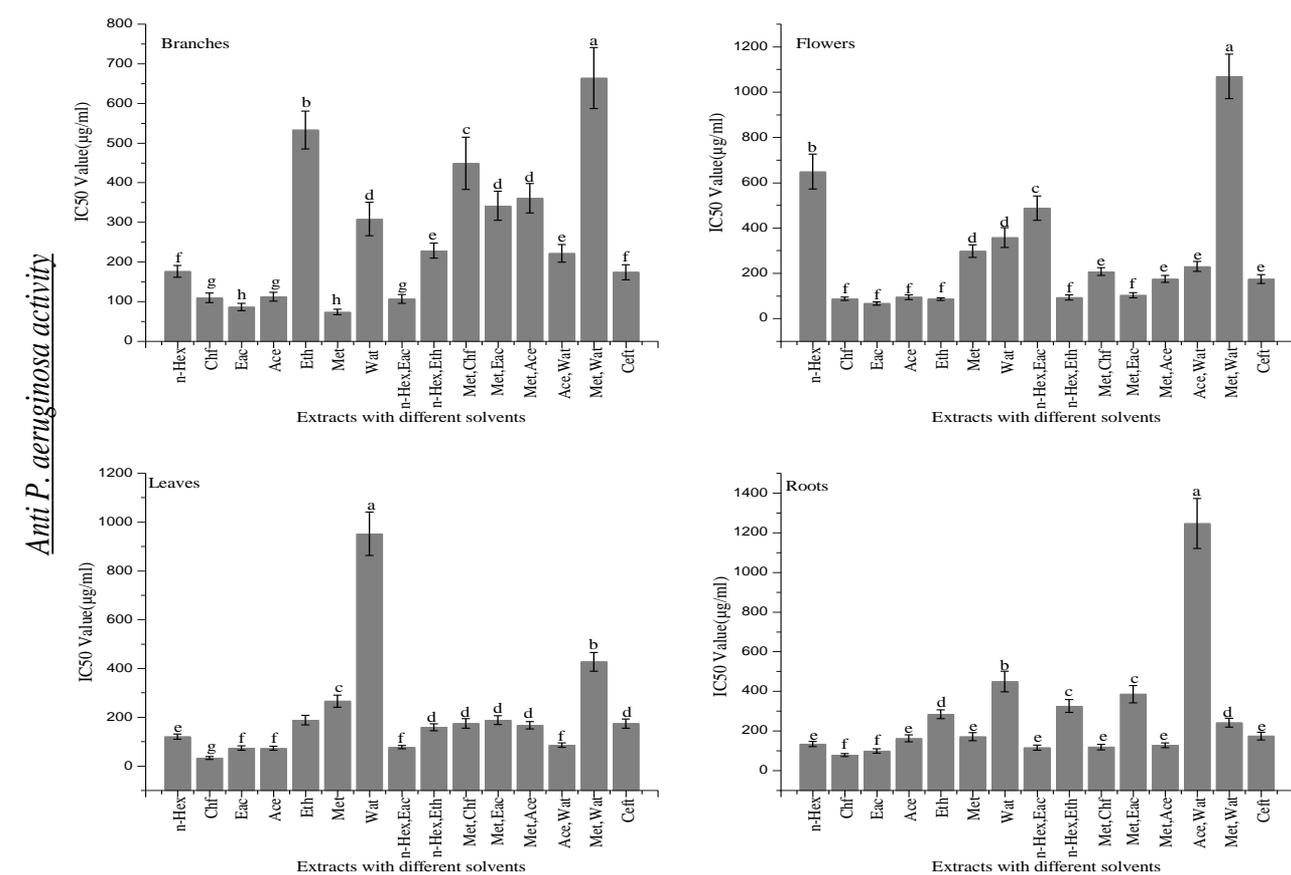


Fig. 7. Anti-*Pseudomonas aeruginosa* activity of 14 fraction with respective solvents from each branches, flowers, leaves and roots. Graphs indicate IC₅₀ values µg/ml bars with standard deviation bar having similar subscripts letters indicate no significant (p<0.05) difference, while different subscripts letters shows significant (p<0.05) difference among different extracts. The highest inhibitory effects are shown with last alphabet in alphabetical order. The minimum inhibitory fractions with maximum IC₅₀ values are nominated with (a).

Conclusion

Isatis tinctoria L. is one of the important medicinal plant shows great potential to control many lethal diseases. Our results showed that this plant species has the strong potentials to control the growth and other activity of many lethal bacterial and fungal species such as *Klebsiella pneumonia*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mucor mycosis*, *Aspergillus nigar*, etc. However the susceptibility and tolerance response vary with type of microbe, type of plant parts used and with type of different extracts. The *M. mycosis* strain showed more resistant to all plant parts as compared to other tested organisms. The present study reported toxic activities of this important plant species against many lethal microbes and serve as model to test other plant species against these pathogens.

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(Received for publication 1 September 2016)