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Antimicrobial Activity of Various Extracts of *Taraxacum officinale*

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Abstract

The antimicrobial property of *Taraxacum officinale* plant extracts have been carried out by Agar Well Diffusion method. Five types of microbial strains *viz*. (*Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus aureus and Pseudomonas aeruginosa*) have been used for the estimation of antimicrobial effect of *Taraxacum officinale*. The DCM, ethyl acetate, methanol and water extracts of stem, root and flower of *Taraxacum officinale* gave the varying values of IZD, on their application against the microorganisms with the safe conclusion on the fact that the solvents could extract the different bio-organics varying in number and antimicrobial potential(s). The concentration increase of the extracts resulted in the increase of IZD values resulting in the increase in the antimicrobial activities of the extracts. Among all the plant extracts, the methanolic extracts were found to bear the highest antimicrobial potential against the all bacterial strains, followed by the Ethyl acetate extracts of the plant. The DCM extracts were found to possess the antimicrobial potential in between ethyl acetate extracts and the water extracts. The water extracts were found to have little influence upon the growth of micro-organisms. Among the plant parts observed, roots were observed to be more effective in inhibiting the growth of micro-organisms followed by flower extracts. The stem extracts have a little effect upon the growth of micro-organisms.

Keywords: Antimicrobial; *Taraxacum officinale;* IZD; *Streptococcus mutans; Streptococcus pyogenes; Streptococcus pneumonia; Streptococcus aureus; Pseudomonas aeruginosa*

Introduction

Plants are of most important sources of medicines since times immoral. Large numbers of drugs are being isolated and extracted from plants. The medicinal plants are the sources of secondary metabolites and essential oils of therapeutic importance. The important advantages against the therapeutic use of medicinal plants in various ailments and disorders are their safety besides economical, effective and their easy availability. *T. officinale*, commonly called Dandelion, is an herbaceous perennial herb belonging to family Asteraceae (Compositae). It grows in the intemperate regions of the world, and is found mostly in lawns, on roadsides, on disturbed banks and shores of water ways and other areas with moist soils. Dandelion (*Taraxacum sp.*) has been used in many herbal medical systems, as has been mentioned particularly in Asia, Europe, and North America. The root being primarily considered as gastrointestinal remedy, supporting digestion and liver function, while the leaf is used as a source of diuretic drug and bitter digestive stimulant. Wolbis et al. [1] carried out an analysis in which they mentioned and isolated various polyphenolic compounds from *T. officinale* plant extracts thereby showing that the plant is rich in various antioxidants and can have a direct effect of these phytochemicals with health of an organism.

Cinnamic acid, coumarins and flavonoids and other phytochemicals bearing important medicinal and therapeutic importance have been isolated from different tissue of *T. officinale* plant by various analytical methods by Williams et al. [2].

Budzianwski [3] has isolated a large number of Coumarins and caffeyl tartaric acid from the leaves of *T. officinale*. Also a good number of artifactual methyl esters have been recognized and extracted from the leaves of *T. officinale* by usual analytical techniques.

Dandelion (*T. officinale*) flowers extracts have a potential to suppress the reactive oxygenated compounds like Nitric oxide so prevents lipid oxidation as per the results mentioned by Kitts and Hu [4].

Bevin et al. [5] carried out the anti-diuretic effect of *T. officinale* plant. *T. officinale* ethanolic extract shows good response as a diuretic in humans. The plant *T. officinale* was collected in the month of May in which the plant was found to be full grown. The concerned plant is being consumed in the Kashmir valley from times immoral as source of vegetable and for the lactating mothers as a source of minerals especially calcium. Taking these above mentioned factors into mind the study was carried out to know the microbial properties of the plant.

Experimental

Chemicals and reagents

All the chemicals and reagents used were of analytical grade. The reference plant was collected from the North region of Kashmir (J&K) district Kupwara.

Materials and Methods

Muller-Hilton agar, Gentamicin was bought from Hi-Media (Mumbai India). Hydrochloric acid, sodium acetate, dimethyl sulphoxide, and glacial acetic acid were purchased from S.D. Fine-chemicals limited (Mumbai, India). All the bacterial strains of MTCC grade were sourced from IMTECH Chandigarh, India.

The various analytical methods have been applied for the phytochemical analysis of *T. officinale* and are mentioned with a reference cited accordingly.

Preparation of Plant Samples

The separated and segregated plant parts (*viz.* Stem, Roots and

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Flowers) were collected. The shade dried dirt free plant parts were powdered and stored in the air tight container in the dark until further use (Figures 1-5).

Preparation of Plant Extracts

The thimble filled with about 55 g of each plant sample (Stem, Root and Flower) powder was subjected to Soxhlet extraction method with the successive use of solvents of increasing polarity. Tables 1-3 represent the polarity index of the solvents used for the extraction procedure. The solvents were evaporated to dryness in the rotary evaporator to obtain the solvent free extracts. The plant extracts so obtained were lyophilized at a definite temperature. The extracts powder was stored in airtight bottles at 40°C till further experimentation as carried out by Krishnamoorthy et al. [6]. The physical properties, percentage yield of the extracts are mentioned in the Table 1.

Microorganisms and Culture Conditions

The lyophilized cultures of *Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus aureus and Pseudomonas aeruginosa* were procured from IMTECH, Chandigarh Punjab, India. Bacterial strains along with their MTTC number, used in this study are listed in Table 4.

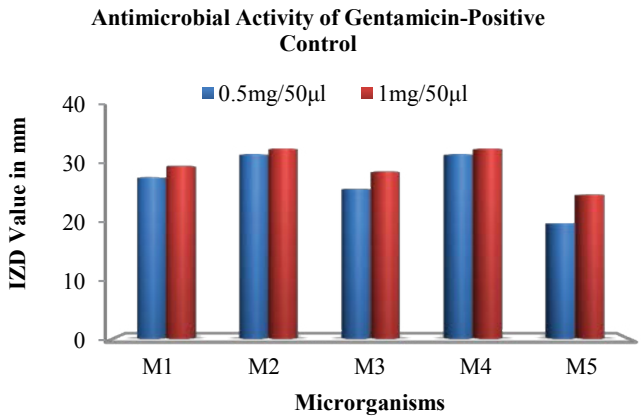

Figure 1: Demonstration of IZD value (mm) of Gentamicin against various microbes.

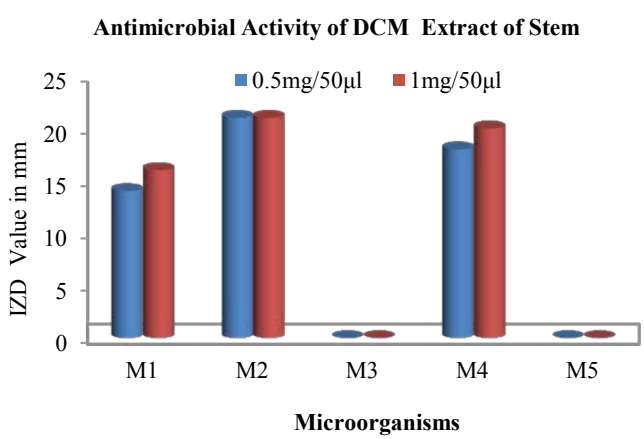

Figure 2: Demonstration of IZD value (mm) of dichloromethane extract of *T. officinale* stem against various microbes.

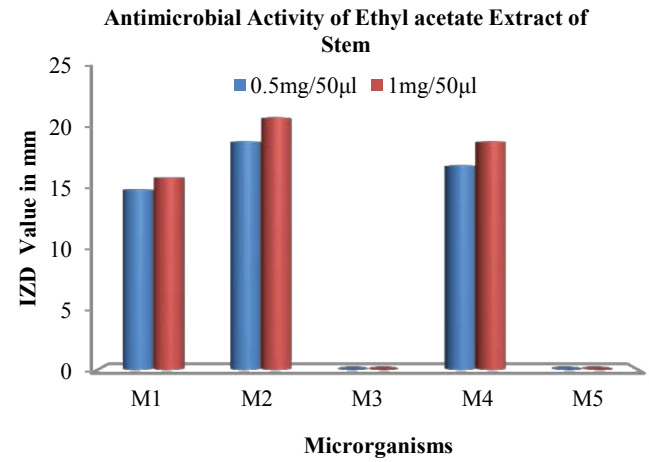

Figure 3: Demonstration of IZD value (mm) of ethyl acetate extract of stem of *T. officinale* against various microbes.

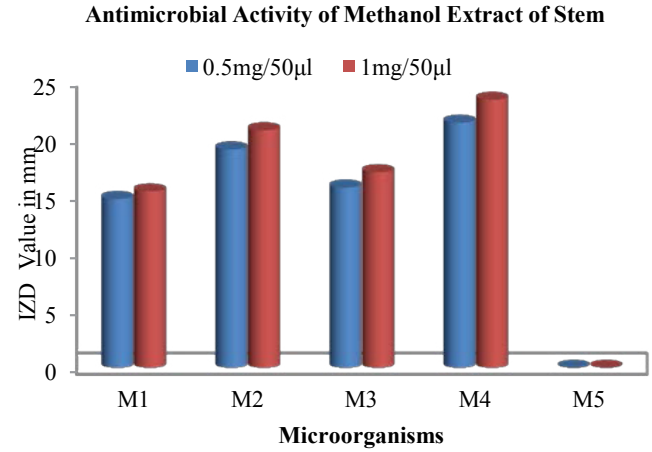

Figure 4: Demonstration of IZD value (mm) of methanol extract of *T. officinale* stem against various microbes.

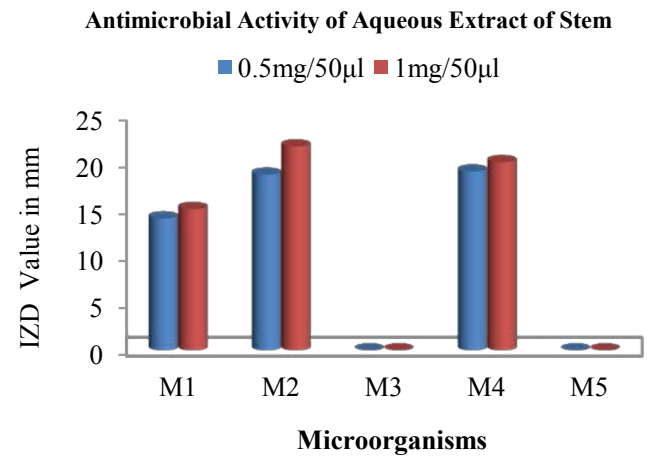

Figure 5: Demonstration of IZD value (mm) of aqueous extract of *T. officinale* stem against various microbes.

Solvent	Plant part	Physical properties	Yield obtained g/500 ml	Percentage yield (W/W)
DCM	Stem	Greenish Orange-Solid	5.5	10%
	Root	Chocolate Brawn-Powder	7.5	13.63%
	Flower	Yellowish Green Powder	6.4	11.63%
Ethyl acetate	Stem	Blackish Green Powder	3.7	6.7%
	Root	Brawn Powder	4.8	8.7%
	Flower	Yellow Brawn Powder	5.7	10.36%
Methanol	Stem	Honey Brown Powder	9.5	17.27%
	Root	Honey Brown Powder	6.9	12.54%
	Flower	Brown Powder	9.8	17.81%
Water	Stem	Honey Brown Powder	14.7	26.72%
	Root	Honey Brown Powder	11.7	21.27%
	Flower	Blackish Brown Powder	15.4	28%

Table 1: Represents the physical properties, yield obtained and percentage yield of the stem, root and flower of *T. officinale*.

Microorganisms	MTCC Number
Streptococcus mutans **(M1)**	MTCC/497
Streptococcus pyogenes **(M2)**	MTCC/655
Streptococcus pneumonia **(M3)**	MTCC/442
Streptococcus aureus **(M4)**	MTCC/96
Pseudomonas aeruginosa **(M5)**	MTCC/1036

Table 2: Represents the microorganisms used for the study along with their MTCC numbers.

Preparation of Reference Antibiotic-Gentamicin

One ml of Gentamicin has 40,000 units. In order to obtain the concentration of Gentamicin 0.5 mg/50 ul and 1 mg/50 ul, 62.5 ul and 125 ul of antibiotic was made to a final volume of 500 ul, by adding 437.5 ul and 375 ul of DMSO. The concentration of antibiotic was used for the antimicrobial study. Gentamicin was used as a positive control.

Preparation of Extract Concentration

0.5 mg/50 uL and 1 mg/50 uL concentrations of all the extracts in reference were used for the antimicrobial susceptibility study. The concentrations were prepared by mixing 5 mg of the extract in 1ml of DMSO (Stock concentration) having a concentration of 1 mg/50 uL. Further 0.5 mg/10 uL concentration was obtained by reconstituting 50 uL of the extract from the stock with 50 ul of the extract from the stock with 50 ul of DMSO (Figures 5-10).

Disc-Diffusion Method

The MHA plates were prepared and fresh inoculum was spread over the surface of the media. The sterile filter paper discs of size 6 mm were dipped into the extract solutions (T1-T12) of concentration (0.5 mg/50 ul, 1 mg/50 ul). Then the disc was placed over the center of medium surface and plates were inoculated at 37°C for 18-24 h. Zone of inhibition (ZOI) was observed and calculated by subtracting the size of the disc with the total ZOI observed.

Susceptibility Test by Agar Well Diffusion Method

Antimicrobial susceptibility tests were performed by a modified agar-well diffusion method NCCLS. 20 ul volume of the standard suspension of test bacterial strain having inoculum size of 5×10^5 CFU/ml was spread evenly on MHA plates using a sterile glass rod spreader and the plates were allowed to dry at room temperature in LAF (Laminar Air Flow Chamber). Subsequently 9 mm diameter wells were bored in the agar plates and a 100 ul volume of various extract concentration (T1-T12) and Gentamicin of 0.5 mg/50 ul and 1 mg/50 ul of each extracts reconstituted in DMSO was transferred into wells.

S. No.	Micro-organisms	Zone of Inhibition (mm) Gentamicin	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	28.00 ± 0.00	30.00 ± 0.50
2	M2	32.00 ± 0.90	33.00 ± 0.90
3	M3	26.00 ± 0.50	29.00 ± 0.95
4	M4	32.00 ± 0.00	33.00 ± 0.00
5	M5	20.00 ± 0.00	25.00 ± 0.00

Table 3: Antimicrobial activity (IZD value) of Gentamicin (positive control), tested against different microorganism at 0.5 mg/50 ul and 1 mg/50 ul concentration.

S. No.	Micro-organisms	Zone of Inhibition (mm) T1 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	14.00 ± 0.47	16.00 ± 0.47
2	M2	21.00 ± 0.14	21.00 ± 0.15
3	M3	Nil	Nil
4	M4	18.00 ± 0.00	20.00 ± 0.50
5	M5	Nil	Nil

Table 4: Antimicrobial activity (IZD value) of dichloromethane extract of stem of *T. officinale* against different microorganisms.

The plates were kept at room temperature for 2 h to allow diffusion of the extract into the media, and then the plates were incubated at 37°C for 24 h. Gentamicin antibiotic was used as a reference antibiotic (positive control) against all he bacterial strains.

Inhibition zone was observed after 24 h and zone of inhibition was recorded for all the extracts, and Gentamicin. Inhibition zone diameter (IZD) was measured to the nearest millimeter (mm) by reducing the IZD value with diameter of the well.

$$\text{Total Zone of Inhibition} = \text{Inhibition Zone Diameter} - \text{Diameter of the well}$$

DMSO was used as a negative control. The tests were performed in triplicates for the microorganism evaluated and the final results were presented as the Mean Inhibition Zone Diameter (IZD) (Tables 5-9).

Observation and Results

Antimicrobial activity of stems of *T. officinale*

Comparative study of antimicrobial activity of extracts in reference: Solvent free extracts when subjected to antimicrobial potential revealed that the growth of microbes was significantly inhibited both by polar as well non-polar extracts of the plant. As the results show, M1 was effectively inhibited by T5 and T11 extracts (DCM extract of Root and methanol extract of Flower respectively). M2 was comparatively inhibited significantly by T5 (DCM extract of Root) extract whereas T6

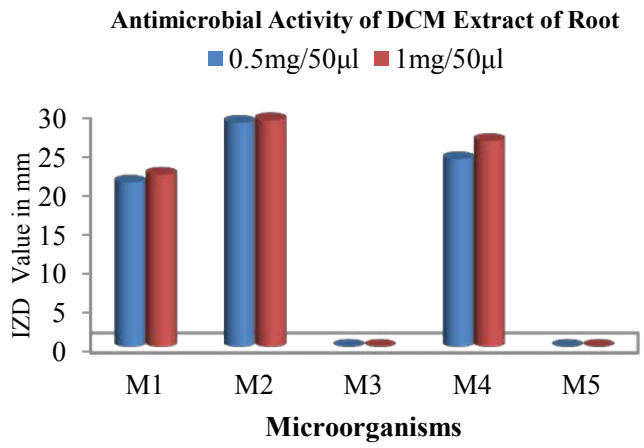

Figure 6: Demonstration of IZD value (mm) of dichloromethane extract of *T. officinale* root against various microbes.

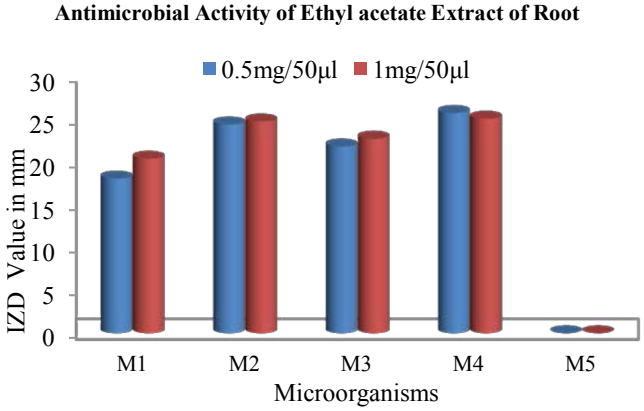

Figure 7: Demonstration of IZD value (mm) of ethyl acetate extract of *T. officinale* root against various microbes.

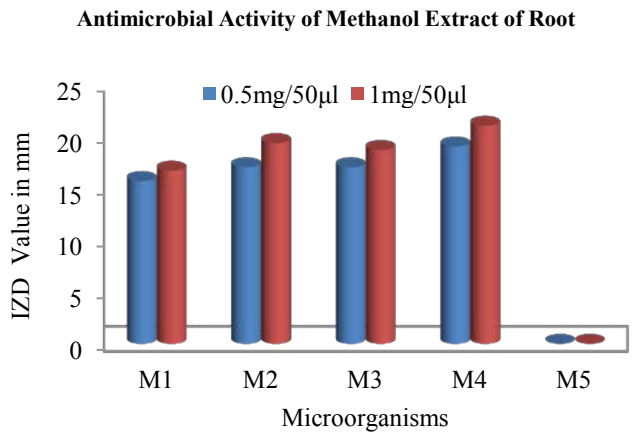

Figure 8: Demonstration of IZD value (mm) of methanol extract of *T. officinale* root against various microbes.

Ethyl acetate extract of root was found effective against inhibiting the growth of M3 microbes. M4 was the second highly susceptible microbe next to M2. M4 revealed its susceptibility to T5 extract of root and T6 (Ethyl acetate) extract of roots have also the same results.

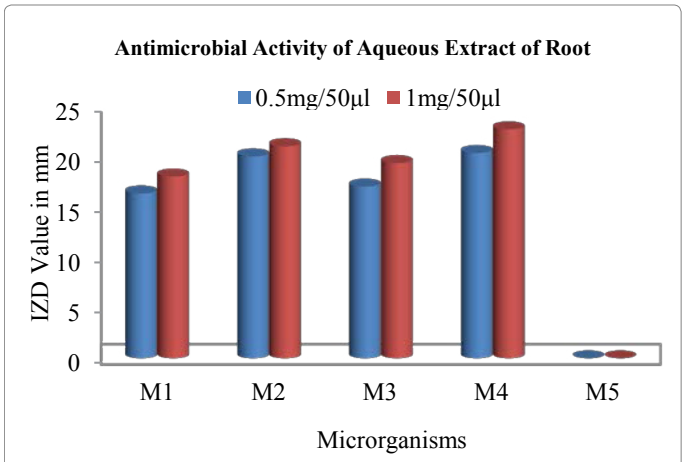

Figure 9: Demonstration of IZD value (mm) of methanol extract of *T. officinale* root against various microbes.

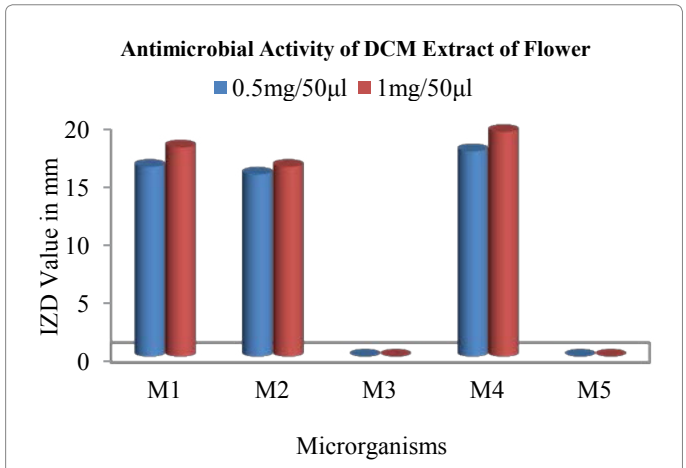

Figure 10: Demonstration of IZD value (mm) of dichloromethane extract of *T. officinale* flower against various microbes.

S. No.	Micro-organisms	Zone of Inhibition (mm) T2 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	15.00 ± 0.90	16.00 ± 0.51
2	M2	19.00 ± 0.90	21.00 ± 0.50
3	M3	Nil	Nil
4	M4	17.00 ± 0.00	19.00 ± 0.50
5	M5	Nil	Nil

Table 5: Antimicrobial activity (IZD value) of ethyl acetate extract of stem of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T3 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	14.67 ± 0.47	15.33 ± 0.47
2	M2	19.00 ± 0.82	20.67 ± 0.47
3	M3	15.67 ± 0.82	17.00 ± 0.47
4	M4	21.33 ± 0.14	23.33 ± 0.82
5	M5	Nil	Nil

Table 6: Antimicrobial activity (IZD value) of methanol extract of stem of *T. officinale* against different microorganisms.

All the extracts of *T. officinale* were not found effective against *P. aeruginosa*, which was found resistant all through the study. Among all the extracts of individual plant, root extracts were found to posses' high antimicrobial potential as compared to other parts of the plant.

The positive control, which is a pure form of antibiotic (Gentamicin), bacteria, showed high susceptibility giving highest zone of inhibition in comparison to extracts used for the study**.** DMSO gave no susceptibility results, as all the microbes in study were found resistant to this solvent.

S. No.	Micro-organisms	Zone of Inhibition (mm) T4 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	14.00 ± 0.00	15.00 ± 0.00
2	M2	18.67 ± 0.82	21.67 ± 0.47
3	M3	Nil	Nil
4	M4	19.00 ± 0.00	20.00 ± 0.00
5	M5	Nil	Nil

Table 7: Antimicrobial activity (IZD value) of aqueous extract of stem of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T5 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	21.00 ± 0.00	22.00 ± 0.94
2	M2	28.67 ± 0.47	29.00 ± 0.00
3	M3	Nil	Nil
4	M4	24.00 ± 0.00	26.33 ± 0.82
5	M5	Nil	Nil

Table 8: Antimicrobial activity (IZD value) of dichloromethane extract of root of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T6 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	18.00 ± 0.67	20.33 ± 0.94
2	M2	24.33 ± 0.47	24.67 ± 0.82
3	M3	21.75 ± 0.82	22.67 ± 0.00
4	M4	25.00 ± 0.47	25.79 ± 0.94
5	M5	Nil	Nil

Table 9: Antimicrobial activity (IZD value) of ethyl acetate extract of root of *T. officinale* against different microorganisms.

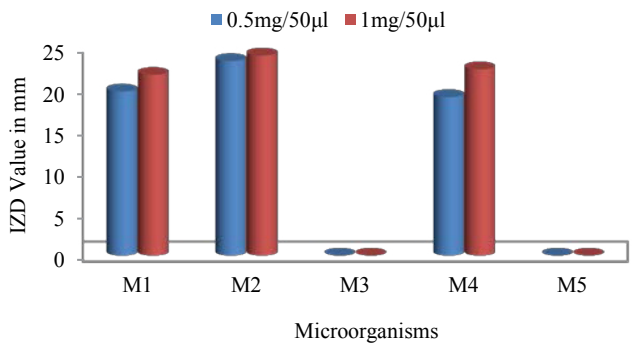

Figure 11: Demonstration of IZD value (mm) of ethyl acetate extract of *T. officinale* flower against various microbes.

Discussion

The antimicrobial property of *T. officinale* plant extracts have been carried out by Agar Well Diffusion method. Five types of microbial strains *viz.* (*Streptococcus mutans, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus aureus, and Pseudomonas aeruginosa*) have been used in order to know about the antimicrobial effect of *T. officinale*. The DCM, ethyl acetate, methanol and water extracts of stem, root and flower of *T. officinale* gave the varying values of IZD on their application against the microorganisms with the safe conclusion on the fact that the solvents could extract the different bio-organics varying in number and antimicrobial potential(s). The concentration increase of the extracts resulted in the increase of IZD values resulting in the increase in the antimicrobial activities of the extracts (Figures 10-13).

Among all the plant extracts, the root extract (T5) was found to be more effective in suppressing the growth of the all microorganisms except *Pseudomonas aeruginosa* which was found resistant against all the extracts of the plant at all concentrations. After root extracts the flower extracts were found to be more effective in inhibiting the growth of microorganisms followed by stem extracts. The most susceptible microorganism was found *Streptococcus mutans,*

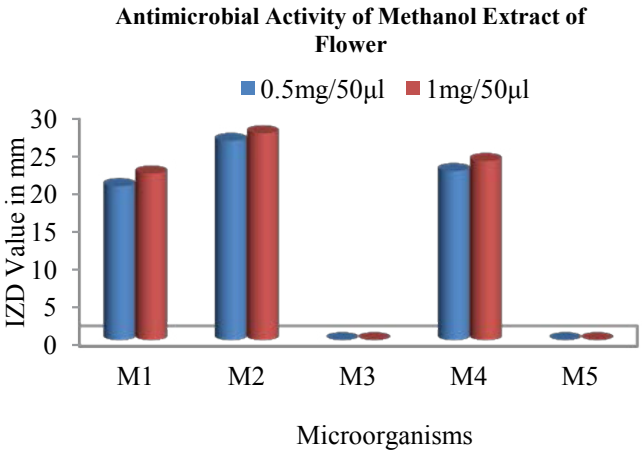

Figure 12: Demonstration of IZD value (mm) of methanol extract of *T. officinale* flower against various microbes.

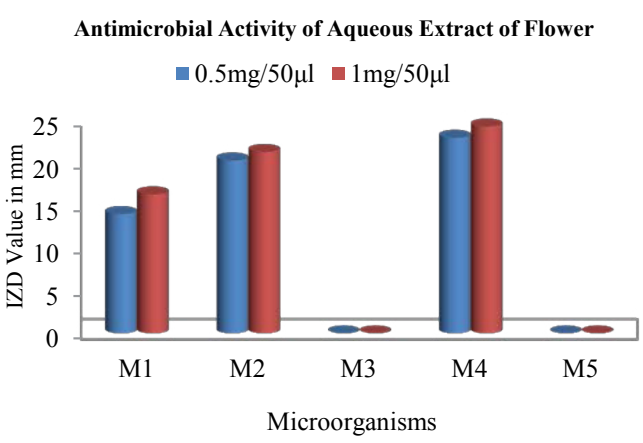

Figure 13: Demonstration of IZD value (mm) of aqueous extract of *T. officinale* flower against various microbes.

Streptococcus pyogenes and *Streptococcus aureus* bacterial strains in which their growth was inhibited by all the extracts of the concerned plant even at lower concentrations. The investigation against these three microorganisms gets also supported by the study carried out by Rex et al. [7], in which they mentioned that most of the plant extracts have a good response towards the inhibition of the concerned microbes. Polar as well non polar solvent extracts of dandelion have more inhibiting effect against the *Streptococcus aureus* bacteria. Ionescu et al. [8] mentioned that polar compounds have more potential to inhibit microbial growth than non-polar compounds. So the results obtained in the concerned study get fully agreed in which it has been found that polar extracts of the plant possess higher antimicrobial effect than the non-polar solvent extracts. Chi et al. [9] carried out a study on *Streptococcus mutans* by various plant extracts, but the results obtained were found less than the results shown by *T. officinale* plant extracts, so it could be concluded that *T. officinale* plant parts bear a good antimicrobial effect. Among all the bacterial strains, *Streptococcus pyogenes* bacteria was found to be the most susceptible bacteria by all the plant extracts (T1-T12) followed by *Streptococcus aureus. Pseudomonas aeruginosa* bacteria was found resistant to all the plant extracts, in which no any sort of inhibition by any plant extract was noticed, and the results obtained gets supported by the investigation carried out by Oseni and Yussif [10]. Among all the plant extracts, the methanolic extracts (T3, T7, T11) (Tables 8-15) were found to bear the highest antimicrobial potential against the all bacterial strains, followed by the Ethyl acetate extracts (T2, T6, T10) of the plant.

S. No.	Micro-organisms	Zone of Inhibition (mm) T7 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	15.67 ± 0.47	16.67 ± 0.94
2	M2	17.00 ± 0.47	19.33 ± 0.00
3	M3	17.00 ± 0.82	18.67 ± 0.47
4	M4	19.00 ± 0.00	21.00 ± 0.94
5	M5	Nil	Nil

Table 10: Antimicrobial activity (IZD value) of methanol extract of root of T. officinale against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T8 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	16.33 ± 0.47	18.00 ± 0.94
2	M2	20.00 ± 0.47	21.00 ± 0.00
3	M3	17.00 ± 0.82	19.33 ± 0.00
4	M4	20.33 ± 0.82	22.67 ± 0.47
5	M5	Nil	Nil

Table 11: Antimicrobial activity (IZD value) of aqueous extract of root of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T9 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	16.33 ± 0.82	18.00 ± 0.00
2	M2	15.67 ± 0.47	16.33 ± 0.92
3	M3	Nil	Nil
4	M4	17.67 ± 0.82	19.33 ± 0.82
5	M5	Nil	Nil

Table 12: Antimicrobial activity (IZD value) of dichloromethane extract of flower of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T10 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	19.67 ± 0.82	21.67 ± 0.00
2	M2	23.33 ± 0.47	24.00 ± 0.92
3	M3	Nil	Nil
4	M4	19.00 ± 0.47	22.33 ± 0.47
5	M5	Nil	Nil

Table 13: Antimicrobial activity (IZD value) of ethyl acetate extract of flower of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T11 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	20.33 ± 0.47	22.00 ± 0.47
2	M2	26.33 ± 0.47	27.33 ± 0.82
3	M3	Nil	Nil
4	M4	22.33 ± 0.82	23.67 ± 0.82
5	M5	Nil	Nil

Table 14: Antimicrobial activity (IZD value) of methanol extract of flower of *T. officinale* against different microorganisms.

S. No.	Micro-organisms	Zone of Inhibition (mm) T12 Extract	
		0.5 mg/50 ul	1 mg/50 ul
1	M1	14.00 ± 0.00	16.33 ± 0.00
2	M2	20.33 ± 0.94	21.33 ± 0.82
3	M3	Nil	Nil
4	M4	23.00 ± 0.82	24.33 ± 0.82
5	M5	Nil	Nil

Table 15: Antimicrobial activity (IZD value) of aqueous extract of flower of *T. officinale* against different microorganisms.

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