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Phytochemical Screening, Antioxidant and Antibacterial Activity of Indigenous Medicinal Plant against Human Bacterial Pathogens

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Abstract: *Wrightia tinctoria* plant is mostly used by the Tamil Nadu people as a traditional medicine to get relief from pain and inflammation. Latex (plant milk) is directly used to cure infections. In this study, the chemical analysis of aqueous extracts of *Wrightia tinctoria* showed presence of Alkaloids, Phenol, Flavanoids, Saponins, Terpenoids, Quinones, Coumarins and Glycoside and absence of amino acids, carbohydrates, tannins, Phlobatanins and oxalates. The methanol extract from *W. tinctoria* showed presence of Alkaloids, Phenol, Flavanoids, Tannins, Terpenoids, Coumarin, Glycoside and absence of amino acids, carbohydrates, saponins, Phlobatanins, quinones and oxalates. Ethanol extract contained phenol, flavonoids, tannins, saponins, terpenoids, oxalates, coumarins and glycosides while alkaloids, amino acids, carbohydrates, phlobatanins and quinone were absent. The chloroform extract contained alkaloids, carbohydrates, phenol, flavonoids, tannins, saponins, terpenoids, coumarins and glycosides, while amino acids, phlobatanins, quinones, and oxalates were absent. The antibacterial activity of chloroform extracts showed the inhibition area against *Streptococcus aureus* (8 mm) and *Escherichia coli* (3 mm). For methanolic extract 9 mm of inhibitions were recorded in both *S. aureus* and *E. coli*. The antibacterial activity of ethanolic extract shows the inhibition area against *S. aureus* (9 mm) and *E. coli* (10 mm) and there was no aqueous extract activity for both tested bacteria. The antioxidant capacity of *W. tinctoria* extracts was high for methanol extract followed by ethanol, chloroform and aqueous. The results of the study indicated that *W. tinctoria* plant contained active compounds that have antibacterial activity so that it can be used as natural antibacterial agent.

Keywords: Antimicrobial activity, Anti-oxidant activity, Alkaloids, Phenol, Flavanoids, Saponins, Terpenoids, Quinones, Coumarins Phytochemistry, *Wrightia tinctoria*

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Introduction

India contributes eight per cent of the total global biotic resources with its richness. Availability of medicinally important plants are exploited due to urbanization, they are in endemism for past few

years, in the various agro-climatic zones of the country. Approximately 8,000 plant species are considered medicinal and are used by the inhabitants of villages, especially tribal communities in their medicinal systems, such as Ayurveda, Siddha and Unani. Since the beginning of human civilization, people used medicinal plants for their health management. The medicinal plants containing active ingredients can be used in drug development. Besides, these medicinal plants play an important role in developing human cultures around the globe. The present scenario suggests that many developing nations mostly depend on traditional practitioners and herbal plants for their primary health management. The development and commercialization of bio-industries based on medicinal plants in developing countries depend on available facilities and information related to upstream and downstream for biological treatment, plant extraction and purification. *W. tinctoria* (pala indigo), which belongs to Apocynaceae family, is distributed in Tamil Nadu, Rajasthan and Madhya Pradesh States of India. *W. Tinctoria* is a tree widely used by the Tamil Nadu peoples for therapeutic purpose such as asthma, psoriasis, dermatitis, anti-inflammatory, anti-dandruff and also used as chemotherapy drug against skin cancer. In this study, antibacterial and anti-oxidant potential of *W. tinctoria* were evaluated. The phytochemical contents of the extracts were also analysed. The aim of the present research work was to evaluate the biological activities of the extracts of *W. tinctoria* species against bacteria, and to evaluate the phytochemical contents and establish the antioxidant potentials.

Materials and Methods

Collection of W. tinctoria:

W. tinctoria leaf was collected from Yelagiri Hills by hand picking method. The plant was identified and validated by Dr. N.P.M Mohamed Tariq, Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi, India. After identification, *W. tinctoria* was processed for extraction.

Preparation of Stock solution:

W. tinctoria leaves were collected from the tree and then cleaned with distilled water to remove dust particles, dried in shadow at room temperature and powdered using a mechanical pulverizer. The powder was subjected to extraction using various solvents such as aqueous, ethanol, methanol and chloroform. 5 g of powder added to 100 ml solvent (aqueous, ethanol, methanol and chloroform) and stirred occasionally in an orbital shaker (Mangesh Kumar *et al.*, 2016). The mixture was filtered on the second day and the solvent was evaporated at room temperature for 18-24 h to obtain a solid mass and stored in refrigerator for further use.

Phytochemical Screening:

Alkaloids:

Wagner's test:

To 1 ml of extract added 1 ml of Wagner's reagent and observed the colour changes.

Amino Acid:

Biuret test:

To 1 ml of the extract few drops of 2% $CuSO_4$, 1 ml of ethanol and 3-5 pellets of NaOH were added. The appearance of colour reactions was recorded.

Carbohydrate:

Benedict's test:

To 1 ml of extract, 1 ml of Benedict's reagent was added and heated in a boiling water bath for 2-3 min. The reaction was observed and the results were recorded.

Barfoed's test:

To 1 ml of the extract added 1 ml of Barfoed's reagent and heated in a boiling water bath for 2-3 min. The reaction was observed and the result was noted.

Phenol:

$FeCl_3$ test:

To 1 ml of the extract, added 1 ml of 5% ferric chloride. The action was observed and the result was noted.

Flavonoids:

Alkaline reagent test:

To 1 ml of extract added 1 ml of 10% NaOH and observed fluorescence was noted.

Ammonia test:

To 1 ml of extract, 2 ml of 10% of ammonia solution and 1 ml of concentrated sulphuric acid were added and appearance of colour was recorded.

Tannins:

$FeCl_3$ test:

To 2 ml of the extract, 2 ml of 5% ferric chloride was added. The appearance of colour was observed and noted.

Saponin:

Foam test:

To 2 ml of the extract, 2 ml of the Dis.H_2O was added and shaken vigorously. The formation of stable foam was observed and recorded.

Terpenoids:

Salkowskis test:

To 1 ml of extract, added 2 ml of chloroform and 3 ml of H_2SO_4. The appearance of colour was observed and noted.

Phlobatanins:

1% Hydrochloric Acid test:

To 2 ml of the extract, 2 ml of the 1% HCl was added and heated in boiling water bath and the presence of colour was observed and noted.

Quinones:

Hydrochloric acid test:

To 1 ml of the extract, 1 ml of the conc. HCl was added. The appearance of colour was observed and noted.

Coumarin:

Sodium hydroxide test:

To 1 ml of the extract, 1 ml of 10% sodium hydroxide was added. The appearance of colour was observed and noted.

Oxalate:

Glacial acetic acid test:

To 3 ml of extract, 1 ml of glacial acetic acid was added. The appearance of colour was observed and noted.

Glycoside:

Keller-Killiani's test:

To 2 ml of the extract, 2 ml of the glacial acetic acid and few drops of the 5% $FeCl_3$ and conc.H_2SO_4 were added. The appearance of colour was observed and noted.

Antioxidant Activity:

The antioxidant activity was performed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. A solution of 0.135 mM DPPH was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. Different concentrations such as 50 µg/ml,100 µg/ml,150 µg/ml, 200 µg/ml and 250 µg/ml of each extract were prepared as stock solution. About 2 ml of each concentration of the extracts were taken and added 2 ml of DPPH solution. The control was prepared with 2 ml DPPH alone without extracts and Ascorbic acid is used as standard solution. Then all test tubes were incubated in a dark room for 30 minutes at room temperature. After the incubation, absorbance was measured at 517 nm by UV-Vis spectrophotometer. Methanol blank was used to set zero in the UV spectrophotometer. The antioxidant activity in inhibition ratio was calculated using the following formula:

$$\text{Inhibition percentage} = \frac{[\text{Control O.D} - \text{Sample O.D}]}{\text{Control O.D.}} \times 100$$

Antibacterial Activity:

The well diffusion method was used to test antimicrobial sensitivity to find the presence of antibacterial activity of the selected extracts. *Streptococcus aureus* and *Escherichia coli* were used for the antibacterial activity. These bacteria were collected from Department of Microbiology, Shanmuga Industries Arts and Sciences College, Thiruvannamalai, India. Bacteria were cultured

Table 1: The phytochemical analysis of various organic extract of *Wrightia tinctoria*

S. No.	Phytochemical Tests		Aqueous	Methanol	Ethanol	Chloroform
1.	Alkaloids	**Wagners test**	+	+	-	+
2.	Amino Acid	**Biuret test.**	-	-	-	-
3.	Carbohydrate	**Benedicts test.**	-	-	-	+
		Barfoed's test.	-	-	-	+
4.	Phenol	**$FeCl_3$ test.**	+	+	+	+
5.	Flavanoids	**Alkaline reagent test**	+	+	+	+
6.	Tannins	**$FeCl_3$ test.**	-	+	+	+
7.	Saponin	**Foam test.**	+	-	+	+
8.	Terpenoids	**Salkowskis test.**	+	+	+	+
9.	Phlobatanins	**1% HCL test**	-	-	-	-
10.	Quinones	**Hydrochloric Acid test**	+	-	-	-
11.	Coumarin	**Sodium hydroxide test**	+	+	+	+
12.	Oxalate	**Glacial acetic acid test**	-	-	+	-
13.	Glycoside	**Killarkillanis test**	+	+	+	+

overnight at 37°C in nutrient broth. Muller Hinton Agar (Hi-media) plates were prepared. Different concentrations such as 100 mg/l, 200 mg/l, 300 mg/l and 400 mg/l of each extract were prepared on stock solution. Then the plates were seeded with 0.2 ml of chosen bacterial suspension and spread with L-Rod to obtain an even culture across all plates. Next, wells of 8 mm in diameter were drilled in the agar medium with well cutter. A total of six well with sufficient space between each well were made. Ampicillin was used as a positive control and related solvents (in which the sample was dissolved) were used as a negative control. Wells were poured with positive control, negative control, four different concentrations of plant extracts. Then all plates were kept for incubation at 37°C for 12-18 h. After incubation, the zone of inhibition was measured and results were tabulated.

Results

Phytochemical Screening:

Phytochemical analysis was conducted in all the solvent extracts. A total of 13 phytochemicals were screened. The phytochemicals such as phenol, Flavonoids, Terpenoids, Coumarin and Glycoside were present in all the extracts whereas Amino acids acid Phlabotanins were absent in all the extracts. Phytochemical analysis was performed using standard protocols. The aqueous extracts, ethanol, methanol, and chloroform from *W. Tinctoria* were tested for the presence of phytochemicals. The alkaloids were absent in ethanol extract, tannins were absent in aqueous extract, saponins were absent in methanol extract but these three phytochemicals were present in all other extracts. Carbohydrates were present in chloroform extract only, quinones were present in aqueous extract and oxalate was present in ethanol extract. Plant amino acids and Phlobatanins phytochemicals were absent in all solvent extracts of *W. tinctoria* (Table 1; Fig. 1).

Antioxidant activity:

The antioxidant activity of the aqueous, ethanol, methanol and chloroform extracts of *W. tinctoria* was performed by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method and was compared with standard ascorbic acid. Antioxidant activity is very important in counteracting the deleterious role of free radical in food biological system. The DPPH solution was deep purple in colour with absorption at 517 nm

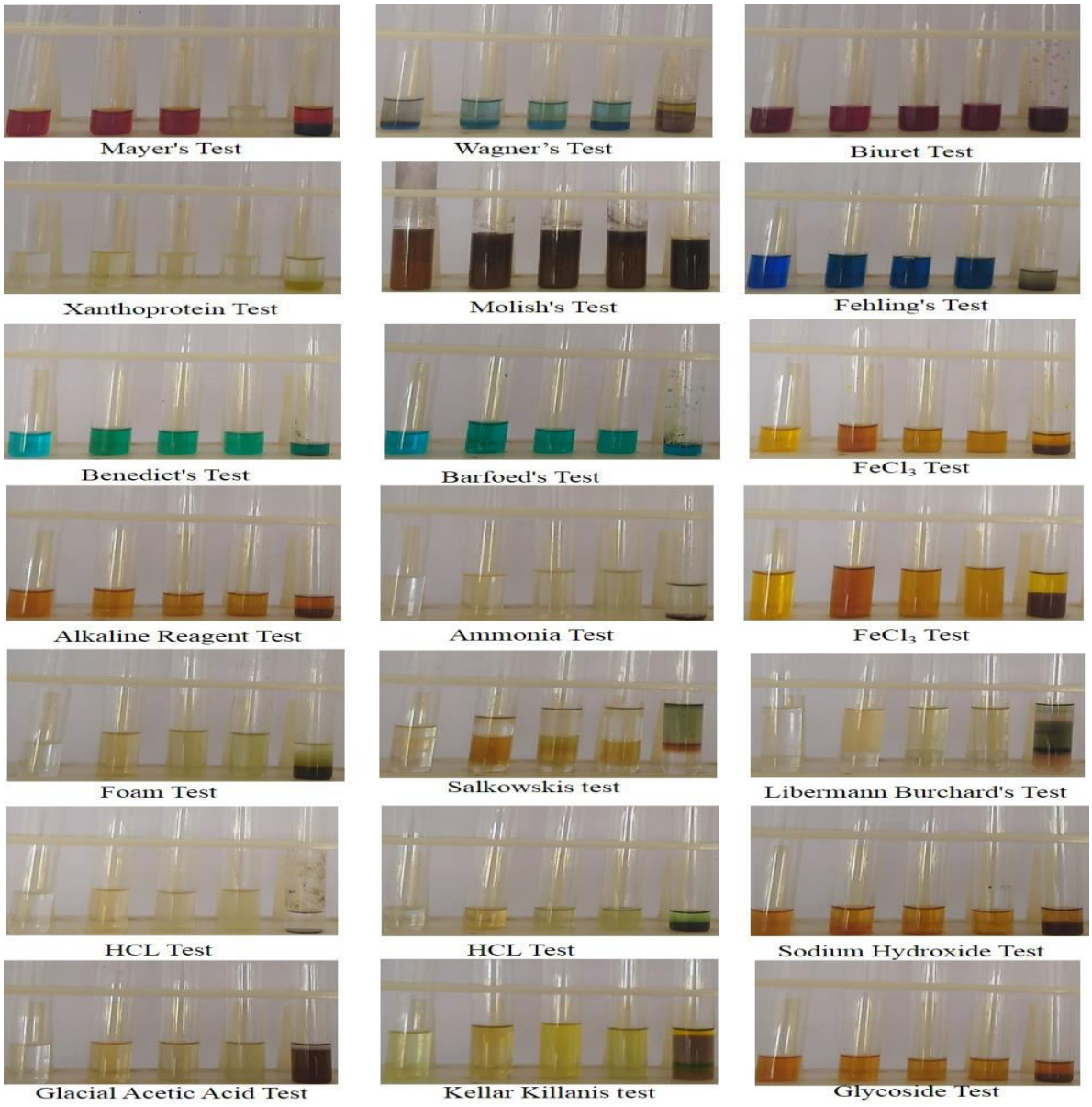
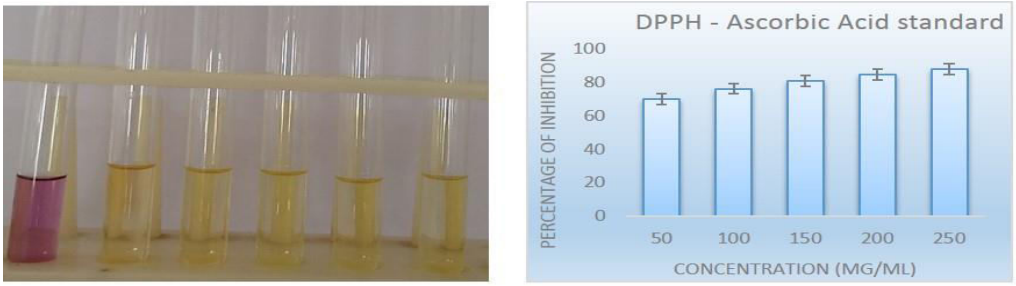


Fig. 1: Phytochemical analysis of *W. tinctoria.*

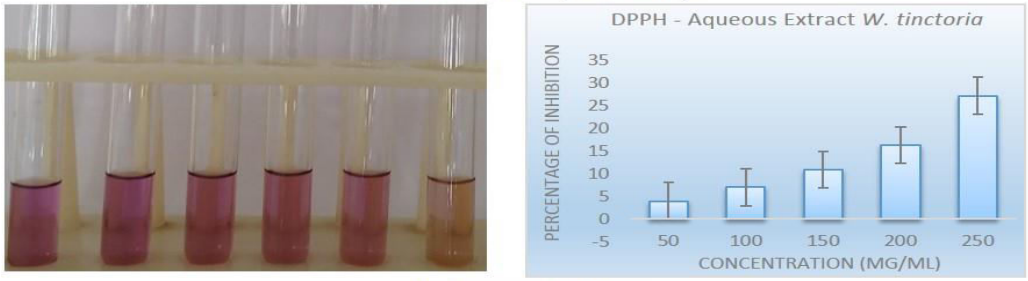
which disappeared in the presence of radical scavengers in the reactive system. Evidence gathered in the past year points to the implication of free radicals and other oxidizing substances as the main cause of oxidative stress that leads to a variety of diseases and disorders. This led to an increasing interest in natural products having antioxidant properties. Plants have been considered as richer sources of antioxidants. In this study the antioxidant activity was high in the methanol extract followed by ethanol, chloroform and aqueous extract. The O.D value of the tested samples was used to calculate the Percentage of Inhibition and Standard Error. Then data of inhibition percentage and standard error were expressed and a graphical representation as presented in Table 2 and Figure 2.

Antibacterial Activity:

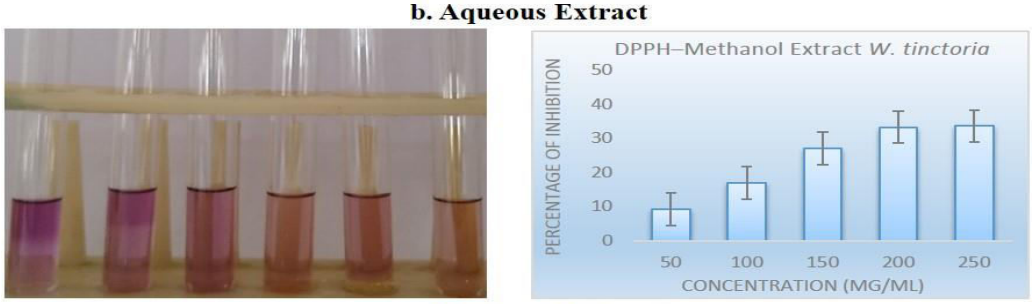
The well diffusion method was used to test antibacterial susceptibility to assess the presence of antibacterial activities of different solvent



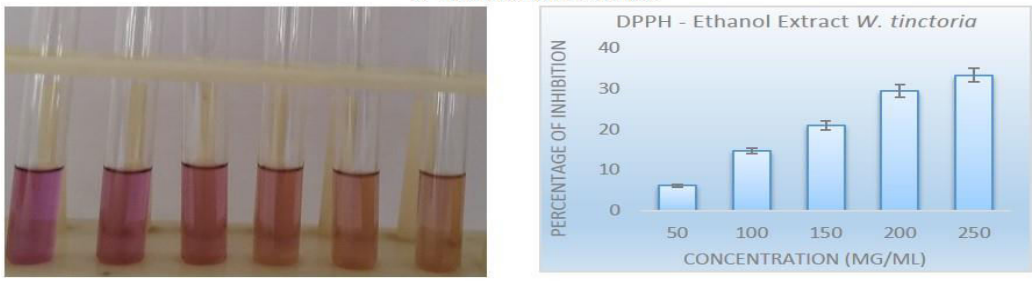
a. Ascorbic Acid (Standard)



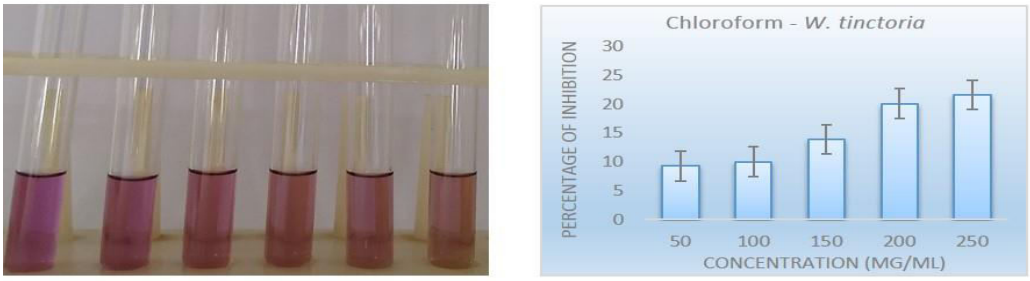
b. Aqueous Extract



c. Methanol Extract



d. Ethanol Extract



e. Chloroform Extract

Fig. 2: Antioxidant Activity of various solvent extracts of *W. tinctoria.*

Table 2: Optical Density of *W. tinctoria* extracts at 517 nm

Concentration	Ascorbic Acid	Aqueous	Ethanol	Methanol	Chloroform
50 µg / ml	0.13	0.43	0.40	0.39	0.42
100 µg / ml	0.10	0.40	0.37	0.36	0.39
150 µg / ml	0.08	0.38	0.34	0.31	0.37
200 µg / ml	0.07	0.36	0.30	0.29	0.34
250 µg / ml	0.05	0.31	0.29	0.25	0.34

Table 3: Antibacterial activity

Microorganism	Solvents	*P.C	*N.C	Zone of Inhibition (in mm)			
				100 µg	200 µg	300 µg	400 µg
Streptococcus aureus	Aqueous	10	-	-	-	-	-
	Ethanol	10	-	5	7	8	9
	Methanol	10	-	3	6	8	9
	Chloroform	10	-	5	6	7	8
E. coli	Aqueous	15	-	-	-	-	-
	Ethanol	15	-	6	8	9	10
	Methanol	15	-	5	6	7	9
	Chloroform	15	-	-	1	2	3

***PC**=Positive control (Ampicillin); ***NC**=Negative control

extracts of *W. tinctoria*. The higher concentration of aqueous, ethanol, methanol and chloroform extract of *W. tinctoria* showed activity against the tested pathogens by the following order from the highest zone of inhibition: *S. aureus* (9 mm), *E. coli* (10 mm) by ethanol extract, *S. aureus* (9 mm), *E. coli* (9 mm) by methanol extract, *S. aureus* (8 mm), *E. coli* (3 mm) by chloroform extract and there is no activity by the aqueous extract of *W. tinctoria* (Table 3, Figs. 3a, b).

Discussion

In tnis study terpenoids were present in all tested plant extract which have also benn reported by Khyade and Vaikos (2011). Vedhanarayanan *et al.* (2013) reported the presence of tannins in ethanol extract and flavonoids, phenol and steroids in methanolic extract of leaves of *W. tinctoria*. Subhashini *et al.* (2014) found that methanol extracts from *W. tinctoria* root contained carbohydrates, proteins, tannins, and steroids in Chloroform extract. Similar to the present study, absence of flavonoids, saponins, glycosides, and alkaloids were observed by Ramesh *et al.* (2014). The inhibition percentage was observed by Sharifi-Rad *et al.* (2015). The methanolic leaves extract of *W. tinctoria* had the highest total antioxidant activity and the aqueous extract had the lowest antioxidant activity as observed by Sharifi-Rad *et al.* (2015). The antibacterial activity results could be matched with results of many researchers. Ranjani *et al*. (2012) found that methanolic and ethanolic extracts of *W. tinctoria* leaves have strong bacterial inhibitory activity

Fig. 3a: Antibacterial activity of various solvent extract of *W. tinctoria* against *Escherichia coli* and Fig. 3b: Antibacterial activity of various solvent extract of *W. tinctoria* against *Streptococcus aureus*. (a) 100 mg/ml, (b) 200 mg/ml, (c) 300 mg/ml, and (d) 400 mg/ml.

against *Staphylococcus* and *Bacillus* species. Ethanol extract of *W. tinctoria* flower also showed the potential activity against both gram positive and gram negative bacteria (Shruthi *et al.*, 2010). The antibacterial activities of *W. tinctoria* in petroleum ether, chloroform, acetone and methanol extracts were qualitatively assessed by the presence or absence of inhibition zones and zone diameter was observed by Khyade and Vaikos (2011). The dried leaf extracts of *W. tinctoria* possess both antibacterial and antifungal activity. Chloroform and ethanol extract showed maximum zone of inhibition while methanol extract exhibited moderate activity against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Khyade and Vaikos, 2011). Sravanthi Maddila and Hemalatha (2017) reported that all three crude leaf extracts of *W. tinctoria* exhibited significant *in vitro* antibacterial activity against all the tested bacterial strains at a concentration of 500 µg/50 µl, whereas in our study, maximum zone of inhibition was observed in ethanol and Methanol extracts against *Streptococcus aureus* and *E. coli* at a concentration of 400 µg.

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