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In vitro antimicrobial studies on various extracts and fractions of *Baptisia tinctoria* roots

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Abstract
In well-established homeopathic arrangement of medication, roots of plant *Baptisia tinctoria* has been utilized broadly to treat the typhoid, however the detailed examination for antimicrobial action has never been done on this medicinally active plant. So the current work was intended to assess the antimicrobial action on various strains. The unrefined or uncharacterized extracts and fraction of bioactive unrefined extract were readied using standard protocols in the order of increasing polarity. In present investigations disc diffusion method was utilized to examine the antimicrobial activity on different gram positive and gram negative bacterial strains and fungal strains. The methanol extract and ethyl acetate fraction has most extreme antimicrobial activity while the remaining test samples did not show any inhibitory action of different bacterial as well as fungal strains. As the preliminary phytochemical screening suggested the presence of phenolic as well as flavonoids as major classes of compounds in methanol extract and ethyl acetate fraction, so finally it can be concluded that these compounds may be responsible for the antimicrobial profile of plant.

Keywords: bacterial strain, *Baptisia tinctoria*, disc diffusion method, fungal strain

Introduction
In homeopathic arrangement of medication, the plant *Baptisia tinctoria* has been utilized generally to cure the typhoid, mental issues, pain, inflammation and various urinary issues. Phytochemically the plant contains flavonoids, phenols and alkaloids as major classes of compounds. The different phytochemicals were scientifically separated from the different parts of plant such as alkaloids, phenols, flavonoids, coumarins, triterpenes and polysaccharides till date. The plant has been scientifically reported various pharmacological exercises such as CNS related activities and immune system stimulatory properties (Richa *et al* 2017; Sujata *et al* 2017) [3, 5].
Despite the huge phytochemical and biological profile of the plant, yet the plant has never been subjected to investigate antimicrobial profile to till date. Therefore, the plant was exposed to assess the antimicrobial action of different uncharacterized extracts and fraction of bioactive extract of plant root against various bacterial and fungal strains using well established procedures available in reference books.

Materials and Methods
Plant Materials
The dried root part of *Baptisia tinctoria* Linn was gathered from K.R. Indo German American company situated in Kurukshetra, Haryana, India. Dr. Sunita Garg, Head of raw material Herbarium and Museum, NISCAIR, New Delhi. India verified the personality of the plant (Ref. No. NISCAIR/RHMD/Consult/2019/3507-08 dated 14/10/2019.

Chemical and Instrumental Material
The different reagents of LR/AR grade utilized in present research work were benefited from S.D. School of Pharmacy Barnala. The reagents were purchased from Ranbaxy authorized firms. The different test samples were concentrated by utilizing rotating vacuum evaporator (Perfit, Ambala) to acquired solvent free test samples. The microbial strains were obtained from CSIR-Institute of Microbial Technology, Chandigarh, India. The different extracts and fraction were set up according to standard technique announced in writing available online scientifically (Richa *et al* 2017) [3]. The antimicrobial activity was investigated using disc diffusion method (Sahoo *et al* 2010) [4].

Antibacterial Activity Investigations
Media
Mueller Hinton stock gelled by the expansion of 2% agar (bacteriological grade)

Ingredients
Casein enzymatic Hydrolysate: 17.5 g/Lt.
Beef Infusion: 300 g/Lt.
Soluble starch: 105 g/Lt.
Final PH at 25^0C: 7.4$\pm$0.2

Method of Preparation
The above written raw materials were blended well in with refined water by unbreakable mixing utilizing warmth and pH were changed between 7.2 to 7.6 by utilizing weaken salt or weaken acids.

Sterilization
Test tube containing Muller-Hinton agar 15-20 ml and packed with non-retentive cotton were kept in Autoclave at a temperature of 121°C and pressing factor 15 psi for under 15 minutes.

Organisms Used
The different strains of bacteria used in present studies such as *Staphylococcus aureus* MTCC 7443, *staphylococcus epidermis* MTCC 1133, *Pseudomonas aeruginosa* MTCC 2449, *Escherichia Coli* MTCC 1235 were gathered from Department of Biotechnology, S.D. School of Pharmacy Barnala. The strains were exposed to gram's staining strategy and biochemical responses to confirm the equality and character of the life forms. The chose strains were preserved by sub-refined occasionally on agar inclines and put away under frozen temperature. For examination new 24 hours stock culture were utilized after standardization.

Working Conditions
The entire work was done under aseptic conditions by utilizing horizontal laminar air stream machine. The supplement agar plate was utilized for air testing and presented to aseptic climate inside the hood prior to starting the workout hatched for checking bacterial development. The absence of bacteria life forms affirms the aseptic working conditions.

Preparation of Inoculum
The examination the inoculum was arranged newly in Mueller Hinton stock from the saved frozen inclination culture was incubated at 37ºC for 24 hr and utilized after standardization. The different concentrates and part of *B. tinctoria* root part are utilized as test tests, dimethyl sulphoxide is utilized as vehicle and Ciprofloxacin (10 µg/circle) is utilized as standard antibacterial medication.

Antibacterial Screening by Kirby-Bauer Method
Thickness of 5-6 mm Mueller Hinton agar plates were arranged aseptically and permitted to set in upset position and dried at 37ºC before inoculation. The living organisms were infused in the plates soaked. Sterile swab in inoculum. The swab was altogether turned against the sides of the culture cylinders to eliminate abundance of inoculums. The swab was streaked multiple times over the outside of medium and each occasion the plates are pivoted at a point of 60º. The sterile circle containing test medication, standard and vehicle were set on the outside of the Muller Hinton agar plates and kept in a cooler for one hour to work with uniform appropriation of the medication. Three arrangements of plates were arranged and afterward hatched for 18-24 hr at 37ºC. Zones of hindrance for each test sample were estimated in diameter and compared with standard. Each test sample was tried for antibacterial activity against gram positive and gram negative microorganisms.

Antifungal Activity Investigations
Media
In the examination of antifungal action the media utilized was potato dextrose agar media.

Ingredients
Mycological peptone: 10 gm/Lt
Dextrose: 40 gm/Lt
Agar: 15 gm/Lt
Final pH at 250C: 5.4±0.2
Distilled water to make: 1000ml

Preparation
65 gm of Potato dextrose agar was suspended in 1000 ml. of refined water and boiled to disintegrate the medium totally.

Sterilization
In test tubes 15 to 20 gram of potato dextrose agar was moved and fixed with non-permeable cotton plug and autoclaved at the pressing factor of 15 Psi at 121ºC temperature for 15 minutes.

Organism Used
Candida albicans MTCC 1637 and *Aspergillus niger* MTCC 1235 were obtained from Department of Biotechnology, S.D. School of Pharmacy, Barnala.

Working Conditions
The entire work was done under aseptic conditions by utilizing horizontal laminar wind stream machine. The supplement agar plate was utilized for air testing and presented to aseptic climate inside the hood prior to starting the workout hatched for checking fungal development. The absence of fungal life forms affirms the aseptic working conditions.

Preparation of Inoculum
The inoculum was arranged newly in potato Dextrose stock and hatched at 25ºC for 24-48 h and sanitized. The different concentrates and part of *B. tinctoria* root part are utilized as test tests, dimethyl sulphoxide is utilized as vehicle and Fluconazole (25µg/circle) is utilized as standard antibacterial medication.

Antifungal Screening
Thickness of 5-6 mm Potato dextrose agar plates were arranged aseptically and permitted to solidify in inverted position and dried at 37ºC before inoculation. The life forms were infused in the plates soaked. Sterile swab in inoculum. The swab was thoroughly turned against the sides of the cylinders to eliminate overabundance of inoculums. The swab was streaked multiple times over the outside of medium and each occasion the plates are pivoted at a point of 60º. The sterile circle containing test medication, standard and vehicle were put on the outside of the Potato dextrose agar plates and kept in a fridge for one hour to work with uniform dispersion of the medication. Three arrangements of plates were arranged and afterward brooded for 18-24 hr at 37ºC. Zones of restraint for each test sample were estimated in the form of diameter and compared with the standard. Each test sample of *B. tinctoria* roots were tried for antifungal activity against *Candida albicans* MTCC 1637 and *Aspergillus niger* MTCC 1235 (Manoj *et al* 2009) [2].

Results and Discussion
The percentage yield of petroleum ether, chloroform, methanol and water extracts was found to be 0.65, 2.80, 14.89 and 5.98 % w/w while the percentage yield of ethyl acetate fraction was found to be 26.80 % w/w in relation to methanol extract. The various unrefined or uncharacterized extracts plant roots were prepared systematically in the increasing order of polarity such as petroleum ether, chloroform, methanol and water using standard protocols and ethyl acetate fraction of methanol extract was also prepared systematically. The various prepared extracts and fraction was subjected to antimicrobial investigations again gram +ve living organisms (*Staphylococcus aureus* and *Staphylococcus epidermides*), gram -ve living organisms (*Pseudomonas aeruginosa* and *Escherichia coli*) and fungal

strains (*Candida albicans* and *Aspergillus Niger*). The zone of inhibition in mm was determined by cup plate technique. The higher diameter of zone of hindrance indicates the higher antimicrobial action of the test samples. The movement was controlled by cup plate strategy to decide zone of hindrance in mm.

In antibacterial investigation, the ethyl acetate fraction of methanol extract exhibited higher antibacterial action against gram - ve organisms (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram +ve life organisms (*Staphylococcus aureus* and *Staphylococcus epidermides*) at the different dose tested than the other crude unrefined extracts. The all test samples showed antibacterial profile in the dose dependent manner. The scope of the zone of hindrance of ethyl acetate extract was found to be 7.01 to 22.79 mm. The petroleum ether and water extracts (close antibacterial profile) of plant roots showed slightly higher activity than chloroform and methanol extracts (close antibacterial profile) in case of Gram positive organism *Staphylococcus epidermides* (Table 1 and Fig 1). The petroleum ether, chloroform, methanol and water extracts of plant roots showed marginally close antibacterial profile in case of Gram negative organism *Pseudomonas aeruginosa* (Table 2 and Fig 2). The chloroform and methanol extracts (close antibacterial profile) of plant roots showed maximum activity than petroleum ether and water extracts (close antibacterial profile) in case of Gram negative organism *Escherichia coli* (Table 3 and Fig 3). The methanol extract of plant roots showed maximum activity than petroleum ether, chloroform and water extracts (close antibacterial profile) in case of Gram positive organism *Staphylococcus aureus* (Table 4 and Fig 4).

In antifungal investigation, the ethyl acetate fraction followed by methanol extract showed huge antifungal action against *Candida albicans* (Table 5 and Fig 5) and *Aspergillus niger* (Table 6 and Fig 6) at the dosages tested. The scope of the zone of restraint of ethyl acetate fraction was found to be 10.54 to 13.22 mm. Other extracts for example pet ether, chloroform, and water were exhibit mild antifungal action against *Candida albicans* and *Aspergillus niger* at the dosages appeared.

From above examinations, obviously plant is dynamic against Gram -ve life organism, Gram -ve life organism and fungal strains.

Table 1: Antibacterial activity of various extracts / fraction of *B. tinctoria* roots against Gram positive organism *Staphylococcus epidermides*

Tested Concentration/ Dose(mcg)	Zone of Inhibition (mm)				
	Petroleum either extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	7.18	6.63	6.63	7.18	7.01
2000	7.73	6.74	6.74	7.73	8.12
4000	7.80	6.83	6.83	7.80	9.64
8000	7.93	6.94	6.91	7.93	10.22
10 (Ciprofloxacin)	17.22	17.22	17.22	17.22	17.22

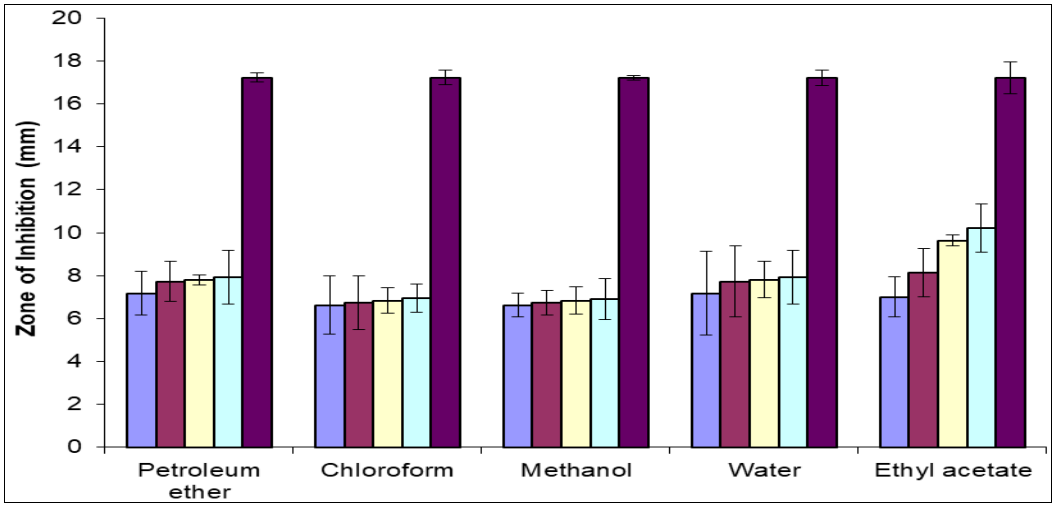

Fig 1: Graphical presentation of antibacterial activity of various extracts / fraction of *B. tinctoria* roots against Gram positive organism *Staphylococcus epidermides*

Table 2: Antibacterial activity of various extracts/ fraction of *B. tinctoria* roots against Gram negative organism *Pseudomonas aeruginosa*

Tested Concentration/Dose(mcg)	Zone of Inhibition (mm)				
	Petroleum either extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	10.63	10.64	9.45	9.66	11.10
2000	11.00	9.90	9.84	16.76	14.52
4000	10.67	11.36	8.56	9.64	16.90
8000	11.13	11.60	9.50	10.20	17.38
10 (Ciprofloxacin)	28.18	28.18	28.18	28.18	28.18

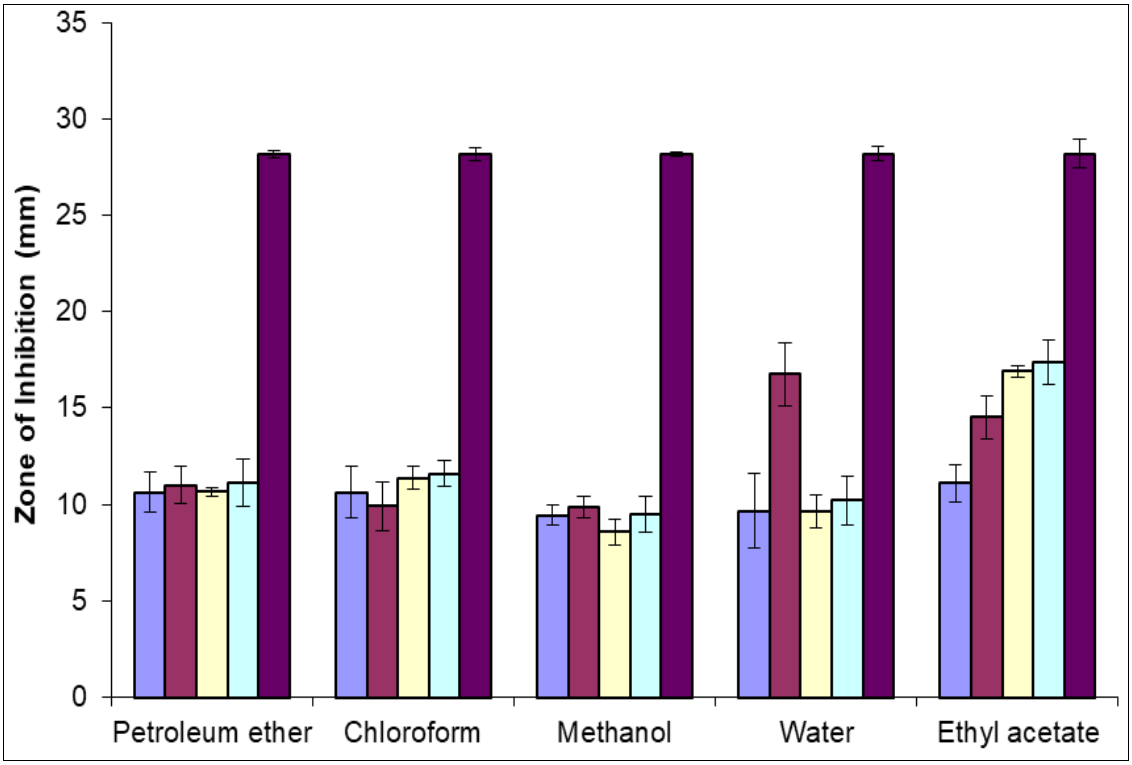

Fig 2: Graphical presentation of antibacterial activity of various extracts/fraction of *B. tinctoria* roots against Gram negative organism *Pseudomonas aeruginosa*

Table 3: Antibacterial activity of various extracts / fraction of *B. tinctoria* roots against Gram negative organism *Escherichia coli*

Tested Concentration/ Dose(mcg)	Zone of Inhibition (mm)				
	Petroleum either extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	7.21	14.31	12.77	8.21	19.35
2000	8.20	13.36	15.34	9.20	19.87
4000	9.15	15.68	15.67	9.64	21.70
8000	11.54	16.16	15.87	10.80	22.79
10 (Ciprofloxacin)	29.71	29.71	29.71	29.71	29.71

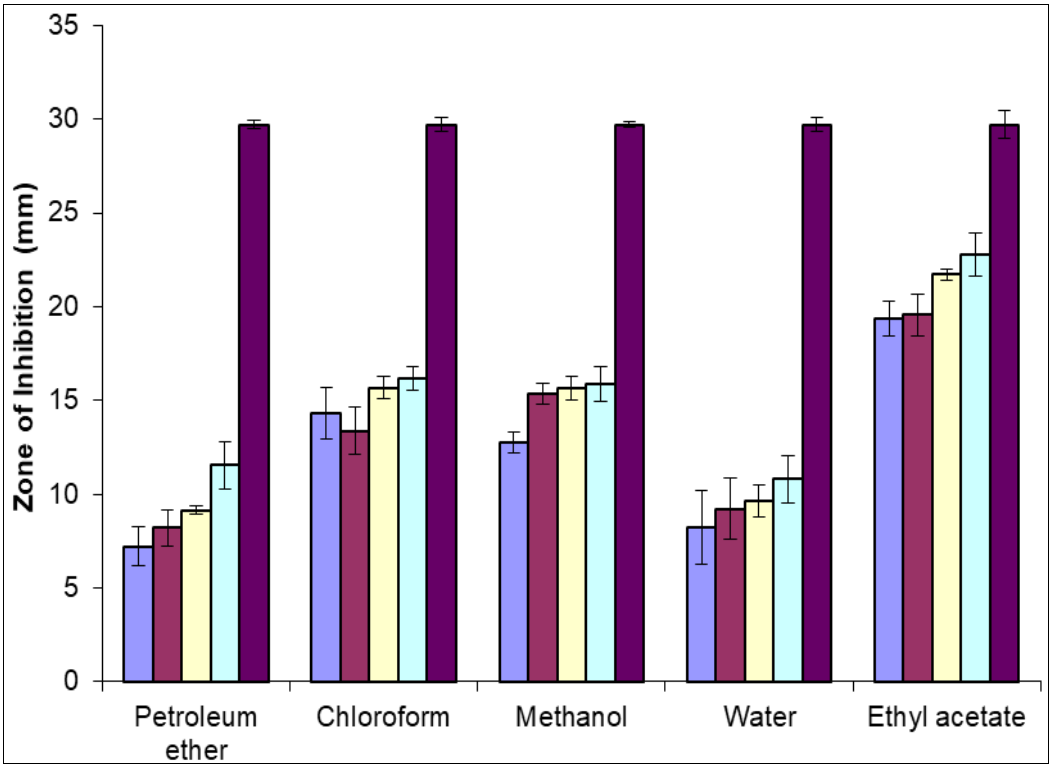

Fig 3: Graphical presentation of antibacterial activity of various extracts/fraction of *B. tinctoria* roots against Gram negative organism *Escherichia coli*

Table 4: Antifungal activity of various extracts / fractions of *B. tinctoria* roots against Gram positive organism *Staphylococcus aureus*

Tested Concentration/ Dose (mcg)	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	7.24	8.48	10.15	7.17	11.94
2000	7.50	8.98	10.78	8.22	12.05
4000	8.10	9.45	11.05	8.70	11.10
8000	8.25	9.78	11.90	8.98	13.38
10 (Ciprofloxacin)	17.48	17.48	17.48	17.48	17.48

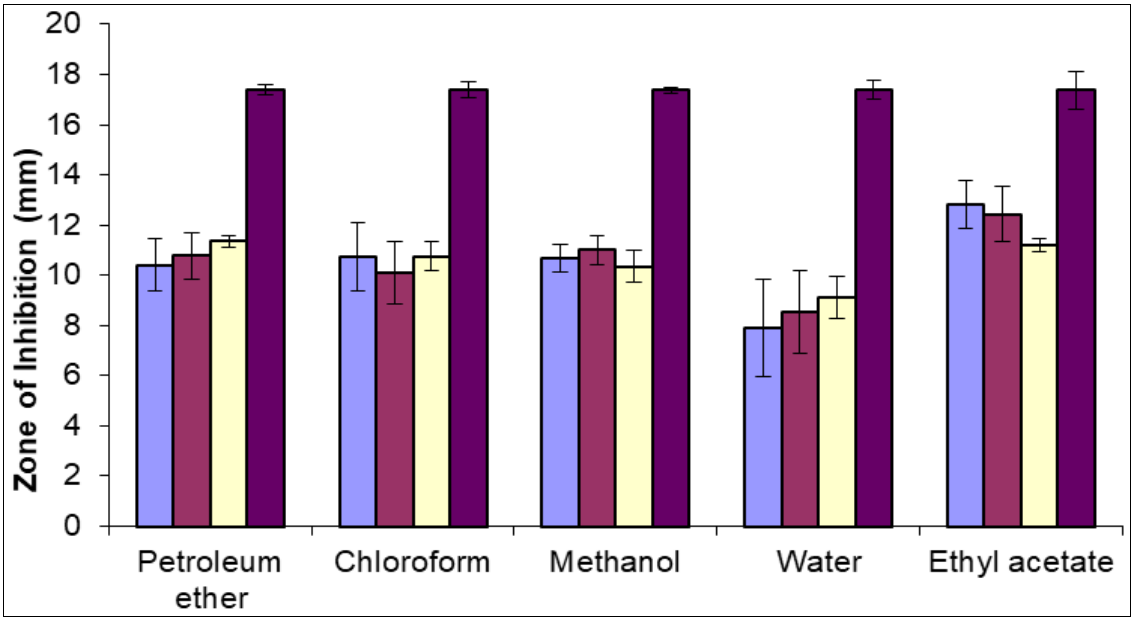

Fig 4: Graphical presentation of antifungal activity of various extracts / fractions of *B. tinctoria* roots against Gram positive organism *Staphylococcus aureus*

Table 5: Antibacterial activity of various extracts / fraction of *B. tinctoria* roots against fungal organism *Candida albicans*

Tested Concentration/ Dose(mcg)	Zone of Inhibition (mm)				
	Petroleum either extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	8.91	8.44	9.52	6.78	11.48
2000	9.33	8.78	9.68	10.04	12.11
4000	8.13	9.79	9.81	8.52	12.86
8000	8.87	10.27	10.29	9.26	13.22
10 (Fluconazole)	12.04	12.04	12.04	12.04	12.04

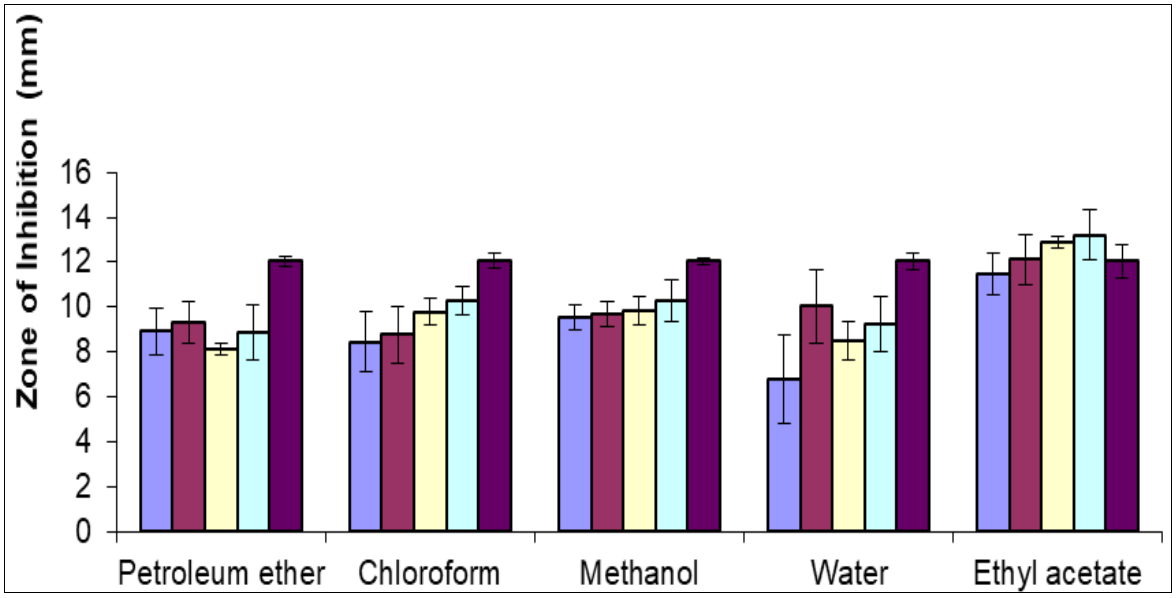

Fig 5: Graphical presentation of antibacterial activity of various extracts / fraction of *B. tinctoria* roots against fungal organism *Candida albicans*

Table 6: Antifungal activity of various extracts / fraction of *B. tinctoria* roots against fungal organism *Aspergillus niger*

Tested Concentration/Dose (mcg)	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract	Ethyl acetate fraction
1000	6.32	6.00	8.45	6.54	10.54
2000	6.48	6.03	9.53	6.62	11.45
4000	6.72	6.59	9.67	6.83	11.85
8000	6.84	9.53	10.84	6.98	11.27
10 (Ciprofloxacin)	10.34	10.34	10.34	10.34	10.34

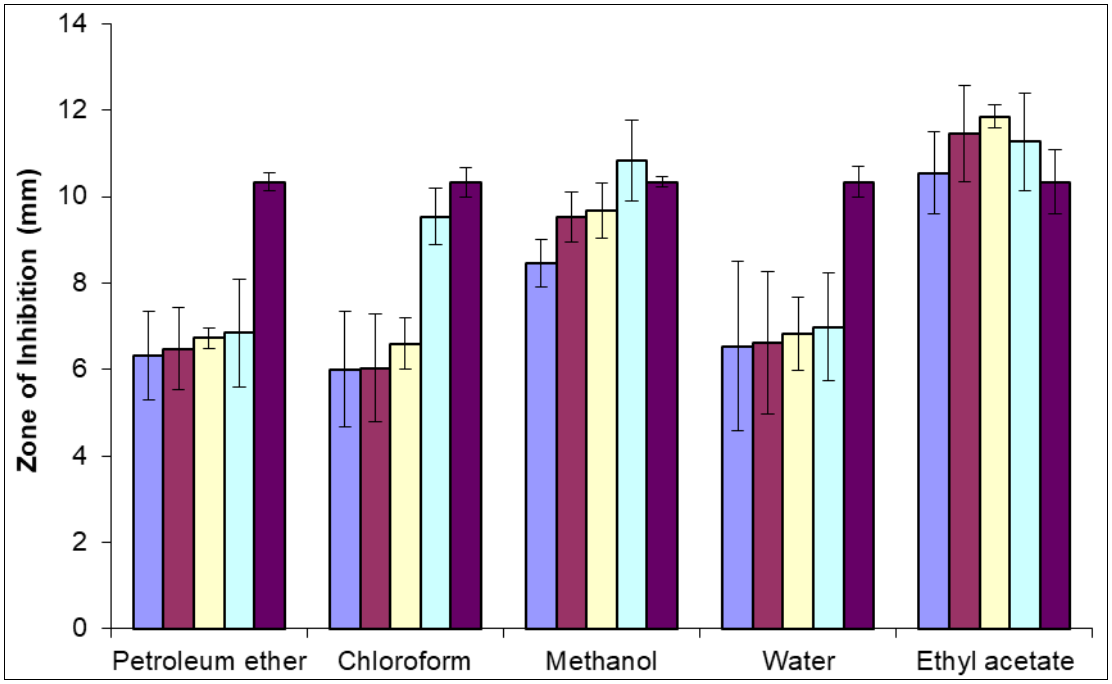

Fig 6: Graphical presentation of antifungal activity of various extracts/fraction of *B. tinctoria* roots against fungal organism *Aspergillus Niger*

Conclusion

The exhaustive review of literature suggested that most of the pharmacological profile of the plant is due to the presence of its phenolic and flavonoidal compounds. The research paper available online suggested that phenolic and flavonoidal compounds are present in methanol and its ethyl acetate fraction (Richa *et al* 2017; Sujata *et al* 2017) [3, 5]. Further, the comprehensive study of writing proposed that polyphenols are answerable for the inhibition/harm of the tried microorganism (Chibane et al 2017; Xie et al 2017) [1, 6]. In consent to these confirmations, at last, it was discovered that polyphenols are greater part answerable for antimicrobial profile of *B. tinctoria*. Further, the column chromatography investigations of ethyl acetate fraction of plant are under progress to separate polyphenols liable for the antimicrobial exercises of plant.

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