PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF *FICUS RELIGIOSA* AND *FICUS RACEMOSA* VARIOUS SOLVENT LEAF EXTRACTS ON GRAM POSITIVE AND GRAM NEGATIVE BACTERIA.

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ABSTRACT

The present investigation focused on exploiting the therapeutic potential of *Ficus religiosa* and *Ficus racemosa* against selected gram positive and gram negative human pathogens. Significant botanicals of leaves of *Ficus religiosa* (Chloroform, Ethanol and Methanol) and *Ficus racemosa* (Petroleum Ether, Chloroform and Methanol) were extracted in solvents of increasing polarity using Cold Masceration technique. Antimicrobial activity of *Ficus religiosa* and *Ficus racemosa* leaf extracts was assessed using agar well diffusion method and MIC assay, against gram positive bacteria *Bacillus subtilis, Staphylococcus aureus* and gram negative bacteria *E.coli, Salmonella typhi*. The highest ZOI values were obtained against *S.aureus* at 1mg/100µl (21mm) of chloroform extract in case of *F.religiosa* whereas *F.racemosa* effectively inhibited *S.typhi* at 1mg/100µl (20mm) of methanol extract. Preliminary phytochemical analysis marked the presence of alkaloids, tannins, saponins, flavonoids as the active bio ingredients in *F. religiosa* and *F. racemosa* various solvent extracts. Conclusive evidences on the basis of data generated showed that selected medicinal plant extracts has a potent inhibitory efficiency in treating bacterial infections caused by gram positive and gram negative bacteria in reference.

Keywords: *Ficus religiosa*, *Ficus racemosa*, Antimicrobial activity, Phytochemical, Zone of inhibition, Human pathogens.

INTRODUCTION

Nowadays due to the frequent use of antibiotics for treatment of various bacterial disease, microorganisms have gained resistance against them that has posed serious problem in present scenario. So, to overcome MDR phenomenon prevailing in society, researches are moving towards the field of Naturopathy, use of herbs as a noble drug for the cure of bacterial disease. The use of alternative medical therapy has increased the interest of pharmacological and herbalist over past decades**.** Antimicrobial resistance (AMR or AR) is ability of microbe to resist the effect of medication previously used to treat them. **(Nathan et al. 2004)**

Microbes resistant to multiple antimicrobial are called multidrug resistant (MDR); extensively drug resistant (XDR) or totally drug resistant (TDR) are sometimes called "superbugs" .Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from one another. Plants have been adapting and evolving along with bacteria for billions of years. Whereas bacteria have quickly adapted to single antibiotic, they have been less successful in resisting the complex patterns found always possible to identify a single active compound using multiple strategies combined provide a synergistic effect.

Pathogens such as *Staphylococcus aureus, Salmonella typhi, E.coli* and *Bacillus subtilis* accounts for serious infections in humans i.e., common cause of skin infections including abscesses, respiratory infection such as sinusitis, food poisoning, **(Drobniewski et al. 1993)**, wound infections,**(Don J. Brenner et al., 1989)** urinary tract infections, neonatal meningitis, gastroenteritis and Crohn's disease **(Jaye et al., 1997).**

Genus *Ficus* has 750 species of woody plants, from which *Ficus religiosa* is one of the important and usable species **(Sharma et al., 2016 and Joseph and Raj, 2010).** *Ficus religiosa* known as peepal is one of the oldest tree in Indian literature **(Gautam et al., 2014).** Its existence can be traced back to the Indus valley civilization (300 BC–1700 BC) where it was embarked on the currency of that time **(Pandey and Pandey, 2016).** Its botanical name was derived from two words i.e. 'Ficus' a Latin word for 'fig' and 'Religiosa' refers to 'religion' indicating its importance in Hindu and Buddhist religions. It is very rich in nutrients as well as in phytochemicals and vital components which are present in every part of plant with several medicinal and health properties. The traditional use of this plant is to cure from many diseases and it is also very important ingredient of Ayush herb. Its effectiveness is proved on animal-based studies which prevent and cure disease and therefore proved and strengthened its importance in herbal medicines. The major use of this plant is in food preparation which can help to meet the nutraceuticals and functional foods. It has been known by more than 150 names **(Bhalerao and Sharma et al., 2014).**

Ficus religiosa is known to be native Indian tree, and thought to be originating mainly in Northern and Eastern India Another name is Bodhi tree, Pippaal tree, Peepul tree, Peepal tree or Ashwattha tree (In India and Nepal).The leaves first appears red pinkishin color and then it turns deep green and grow about 12-18 cm long attached to long flexible stalk which makes them rustle flutter. Bark contain 4% tannins along with alkaloid, mineral and certain vitamins **(Evans et al., 2002).**

Ficus racemosa is species of Moracea family ormulberry family. This tree is native to Australia, Malaysia and Indian subcontinent. Its fruit is staple for Indian macaque. It possessanti-inflammatory, antipyretic, and diuretic properties. The bark and leaves area used as an antingent, haemostatic, anti-inflammatory antiseptic prescribed on diarrhea, dysentery, ulcer, vaginal disorder & helps in reducing blood sugar.

F.religiosa & *Ficus racemosa*are also used in treatment of several health ailments as a home-based remedy either singly or in combination with other herbs. It has been traditionally used in the treatment of heart ailments, nose bleeding, diabetes, constipation, fever, jaundice etc. These are also being used to cure various infections and food poisonings. The bark is used to cure skin diseases, mouth ulcers, diabetes and bone fracture. Leaves are used in conditions like vomiting, gonorrhea etc. These can also be consumed in the form of juice in conditions like asthma, cough, diarrhea, gastric problems. Shoots are used in treating skin problems **(Singh and Jaiswal et al., 2014).** Stem can be used in treatment of urinary disorders and problems of digestive system. The dried powder of fruits has been used in treatment of respiratory problems like asthma **(Panchawat et al., 2012).**

Ayurvedic formulations of Ficus *religiosa* & *Ficus racemosa*are consumed as herbal medicine in system as a treatment for several ailments. Parts of these plants can be consumed in the form of oil, as ointments, capsules, tablets or in raw form. Each formulation has itsown function and can be effective in particular kind of disease. It is also being consumed in the powder form by drying it and grinding in traditional grinders. Powder form is also very effective for some conditions like diabetes mellitus (DM), urinary disorders etc. Powder ofstem bark of *F.religiosa* is considered more effective if taken with honey before or after meal **(Anupama et al., 2014).** Similarly, there are various products or formulations available in market which is known to treat diseases. Many present-day herbal medicines contain parts of *Ficus religiosa* & *Ficus racemosa* as an ingredient. Different parts can be consumed either in raw form (powder, extract) or in form of indigenous medicines (either alone or in combination with other herbs for specific ailments).

In the present study an attempt has been made to screen medicinal activity of selected plant and analysis of significant phytochemicals responsible for effective antimicrobial activity against bacterial diseases.

MATERIALS AND METHODS

Plant material and Extraction

Fresh leaves of *Ficus religiosa* & *Ficus racemosa* were collected from local area (FRI Dehradun Nursery, India). Twenty five grams of leaf powder was taken and soaked in 250ml of organic solvents (methanol, ethanol, chloroform extract, petroleum ether) for extraction using Cold Maceration method **(Akhtar et al., 2014)**. All the extracts were made solvent free and concentrated using rotary evaporator and preserved at 4°C in airtight bottle until further use.

Chemicals and Reagents

Chemicals and reagents used for the study were–Ethanol extract, Methanol extract, Chloroform extract, Petroleum ether, Molisch's reagent, Polyvinyl polypyrolidon, Gallic acid, Sulfuric acid, Aqueous NAOH, Ferrous sulphate and Distilled water. These reagents and chemicals were used in pure state.

Microorganism

Four microorganisms representing Gram positiveand Gram negative were used. Two-gram positive bacteria were *Staphylococcus aureus* and *Bacillus* subtilis and two-gram negative bacteria were *Escherichia coli* and *Salmonella typhi.*

Antimicrobial activity

Antibacterial activities of all the extracts (Ethanol, Methanol, Chloroform & Petroleum ether) of *Ficus religiosa* &*Ficus racemosa* were determined by agar well diffusion method **(Chauhan, Neha et al., 2012).** The extracts were dissolved in DMSO (dimethylsulphoxide) to obtain 0.5mg/100µland 1mg/100µl concentration. Commercial antibiotic (Gentamicin) and DMSO was used as positive and negative control respectively. The test was performed in triplicates and the final results were presented as the mean zone of inhibition.

Broth dilution MIC tests (NCCLS, 2000)

This test was done to check the zone of inhibition which was minimum. This process is named as macro broth dilution assay. Muller- Hinton broth diluents was taken and 2-fold serial dilutions of all extracts were prepared in the well on the basis of result obtained from agar well diffusion method. Gentamicin and DMSO are positive and negative control respectively 20µl of test culture at concentration (5 x 10^5 cfu/ml) was inoculated and plates were incubated for 24h at 37ºC. Plate with minimum growth was taken and concentration is noted as minimum inhibitory concentration. Another value minimum bacterial count was calculated by spreading 20µl of MIC test broth on a new plate incubating for 18- 24h at 37^0C. Dilution of plates showing no single bacterial growth was taken as MBC concentration. Triplicates were used to perform. Test and mean MIC and MBC value were calculated and noted.

Phytochemical analysis

Phenolic,saponins, flavonoid, ascorbic acid,tannins and alkaloids provide antibacterial properties to themedicinal plant against S. *aureus, S.typhi, E. coli* & *Bacillus* **(Negi et al., 2012).** Phenolic, saponins, flavonoids, ascorbic acid were detected by quantitative test and tannins and alkaloids by Qualitative tests.

Qualitative Analysis

Phytochemical analysis was done in accordance to **Sharma et al., 2013.**

Test for Glycosides

1ml of plant extract was taken and few drops of Sulphuric acid were added, the mixture was allowed to stand for some time, formation of reddish precipitate confirmed presence of glycosides.

Test for Carbohydrate (Molisch's test)

1ml of extract was taken and 2ml of Molisch's reagent was added. Now to this mixture 2ml conc. Sulfuric acid was added along the side of test tube. Presence of carbohydrates was confirmed by formation of reddish violet ring at the junction of two liquids.

Test for Flavonoid (Aqueous test)

1ml plant extract was taken, 1ml of aqueousNAOH was added. Presence of flavonoids was confirmed by the formation of yellowish colour.

Test for Saponins (Aqueous test)

1ml extract was taken and 5ml water was added to it, shaken well in test tube shaker. Presence of saponin was confirmed by lather formation.

Test for Tannins (Ferric chloride test)

1ml plant extract was taken and 1ml of ferric chloride was added to it. Presence of tannins was confirmed by the formation of greenish black colour.

Test for alkaloids (Dragondroff's reagent)

1ml of plant extract was taken to which 5-6 drops of Dragondorff's reagent were added.Presence of alkaloid was confirmed by formation of creamish/ brownish red/ orange precipitates.

RESULT AND DISCUSSION

Extract preparation

Leaf extracts of *Ficus religiosa* & *Ficus racemosa* were prepared by cold maceration technique. The extracts were prepared, their yield color and state were tabulated in table 1. Maximum yield was found in Ethanol extract followed by methanol, chloroform and petroleum ether extract.

Table 1- Yield and physical properties of *Ficus religiosa* & *Ficus racemosa* leaf extract

S.NO.	Solvent used	*F.religiosa*			*F.racemosa*		
		Yield	**Colour**	**State**	**Yield**	**Colour**	**State**
1	Methanol	1.29	Light green	Viscous	1.4	Blackish green	Viscous
2	Ethanol/ Petroleum ether(in *racemosa*)	1.20	Dark green	Solid	1.1	Blackish green	Viscous
3	Chloroform	0.98	Dark green	Viscous	1.00	Yellowish green	Liquid

Antibacterial activity

The antibacterial assay was performed by agar well diffusion method for all the extract of leaves i.e. Petroleum ether, Chloroform, Ethanol Chloroform &Methanol, against four microorganisms investigated, two gram positive bacteria were *Bacillus subtilis & Staphylococcus aureus* while remaining two were gram negative bacteria i.e *E.coli* & *Salmonella typhi.* Microbial growth was determined by measuring ZOI. In case of *Ficus religiosa* , it was concluded that for 1mg/100µl dose of Methanolic extract the most susceptible was *S.aureus* followed by *S.typhi, B.subtilis* &*E.coli* with ZOI 20mm,14mm, 8mm and 7mm respectively whereas for 0.5mg/100µl dose the maximum ZOI was observed for *S.aureus* followed by *S.typhi, E.coli* & *B. subtilis* i.e. 9mm, 6mm, 4mm & 3mm respectively. In case of Chloroform extract for 1mg/100µl dose, the maximum susceptibility was shown by *S. aureus* followed by *S. typhi, B. subtilis* & *E.coli* with the ZOI 21mm, 18mm, 11mm & 10mm respectively whereas for 0.5mg/100µl dose the maximum ZOI was of *S. aureus* followed by *S.typhi,, E.coli* & *B. subtilis* i.e. 10mm, 7mm & 5mm for both *B.subtilis* and *E.coli*. It was observed that in case of Ethanol extract for 1mg/100µl dose the maximum ZOI was of *B.subtilis* followed by *S.typhi, S.aureus* & *E.coli* i.e. 12mm, 10mm, 8mm & 6mm respectively whereas in case 0.5mg/100µl dose the most susceptible bacteria was *B.subtilis* followed by *S.typhi & S.aureus* with ZOI 5mm,4mm and 2mm respectively while in case of *E.coli*, no zone was observed.

While for *Ficus racemosa*, it was concluded that for 1mg/100µl dose of Methanolic extract the most susceptible was *S.typhi* followed by *S.aureus, E.coli & B.subtilis* with ZOI 20mm, 13mm (for both *E.coli* &*S.aureus*), 9mm respectively whereas for 0.5mg/100µl dose the maximum ZOI was observed for *S.typhi* followed by *S.aureus* &*B.subtilis* i.e. 8mm, 6mm & 3mm respectively while no zone of inhibition was formed by *E.coli*.In case of Chloroform extract for 1mg/100µl dose, the maximum susceptibility was shown by *S.typhi* followed by *E.coli, S.typhi* & *B.subtilis* with the ZOI 19mm, 11mm, 8mm &6mm respectively whereas for 0.5mg/100µl dose the maximum ZOI was of *S.typhi* followed by *E.coli, S.aureus* & *B.subtilis* i.e. 7mm, 5mm, 3mm & 2mm respectively. It was also observed that in case of Petroleum ether extract for 1mg/100µl dose the maximum ZOI was of *S.typhi* followed by *S.aureus, E.coli* & *B.subtilis*i.e. 13mm, 12mm, 8mm & 4mm respectively whereas in case 0.5mg/100µl dose the most susceptible bacteria was *S.typhi* followed by *S.aureus, E.coli* &*B.subtilis* with ZOI 5mm,5mm, 4mm and 2mm respectively.

Table 2- Antibacterial activity of *Ficus religiosa* leaves for various extract

S. No.	Microbial culture	Concentration of Various Extracts											
		F.religiosa						*F.racemosa*					
		Methanol extract		Chloroform extract		Ethanol extract		Methanol extract		Chloroform extract		Petroleum ether extract	
		0.5mg/ 100µl	1mg/ 100µl	0.5mg/ 100µl	1mg/ 100µl	0.5mg/ 100µl	1mg/ 100µl	0.5mg/ 100µl	1mg/ 100µl	0.5mg/ 100µl	1mg/ 100µl	0.5mg/ 100µl	1mg/ 100µl
1	*B. subtilis*	3mm	8 mm	5 mm	11mm	5 mm	12 mm	3mm	9mm	2mm	6mm	1.5mm	4mm
2	*E. coli*	4 mm	7 mm	5 mm	10mm	No zone	10 mm	No zone	13mm	5mm	11mm	4mm	8mm
3	*S. aureus*	9 mm	20 mm	10 mm	21mm	2mm	8 mm	6mm	13mm	8mm	3mm	5mm	12mm
4	*S. typhi*	6 mm	14 mm	7 mm	18mm	4 mm	6 mm	8mm	20mm	2mm	6mm	5mm	13mm

Fig.1- Represents antibacterial activity of *F. religiosa* extracts against various bacterial cultures at concentration 0.5mg /100µl

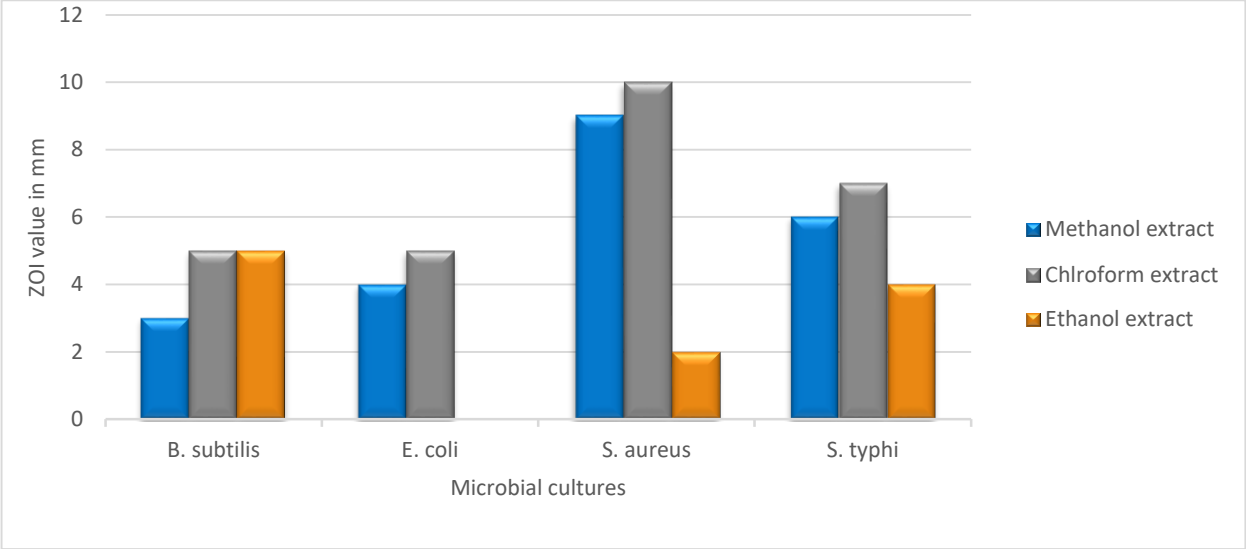


Fig.2- Represents antibacterial activity of *F. religiosa* extracts against various bacterial cultures at concentration 1mg /100µl

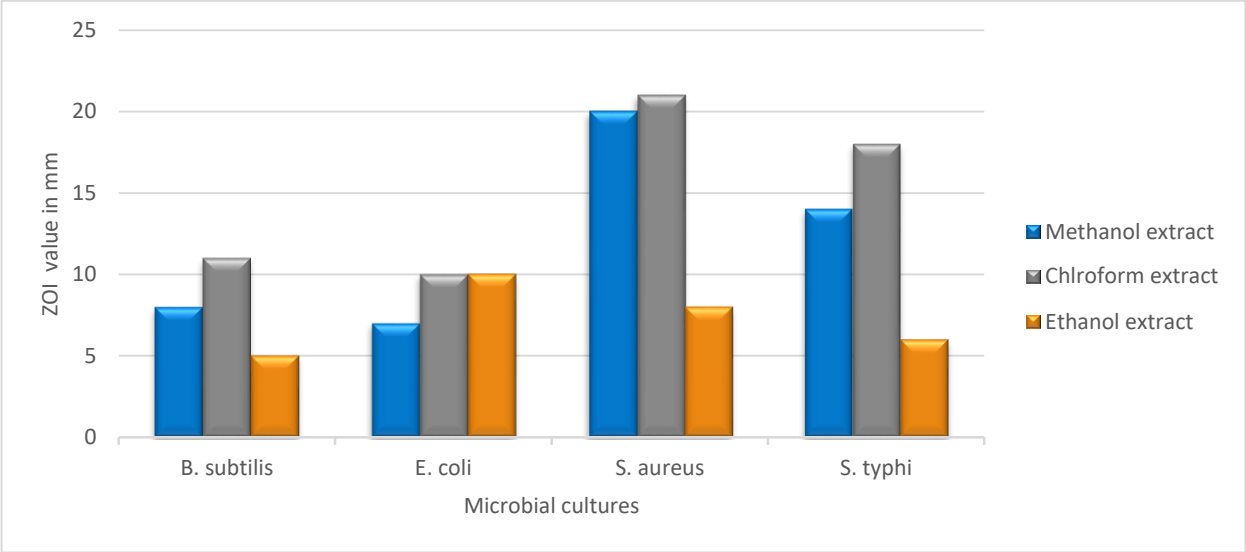


Fig.3- Represents antibacterial activity of *F. racemosa* extracts against various bacterial cultures at concentration 0.5mg /100µl

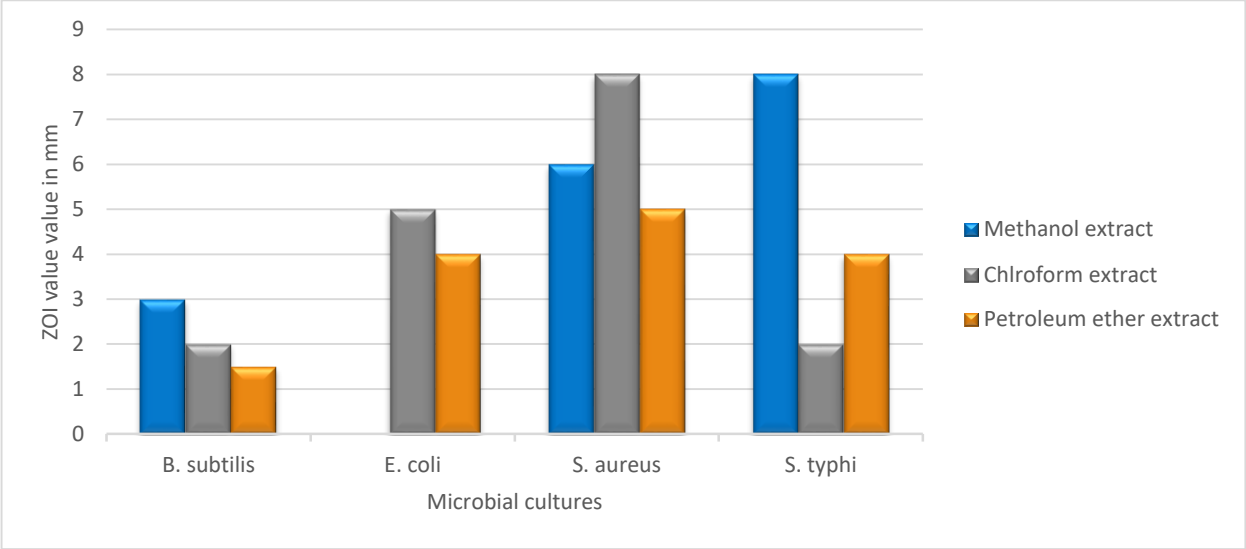
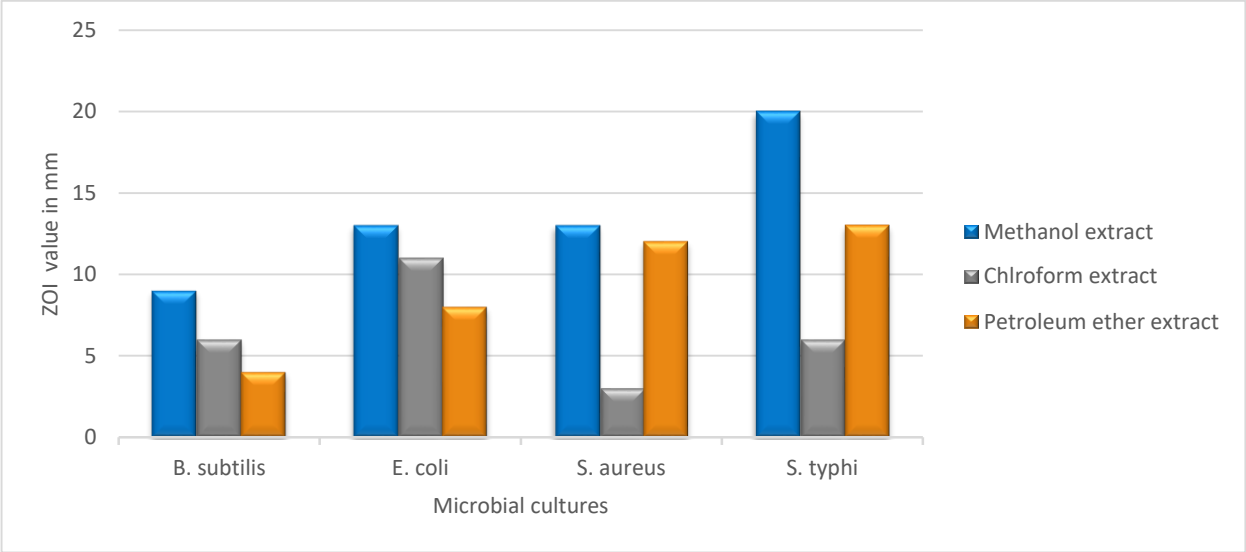


Fig.4- Represents antibacterial activity of *F. racemosa* extracts against various bacterial cultures at concentration 1mg /100µl



MIC

MIC is the lowest concentration of the test sample or drug at which it shows the highest inhibitory activity against microorganisms. The extracts showing high efficacy against microorganisms were subjected to minimum inhibitory concentration (MIC) assay by two folds serial dilution method (1:1) **(Florey et al.,1989 and Drummond et al., 2000).**From the data obtained it was observed that the MIC value ranged from 0.25 – 0.03125mg/ml.In case of *F.religiosa* it was observed that Methanol extract showed lowest MIC value for *E.coli* &*S.typhi* i.e. 0.125 (Table 3). In case of Chloroform extract & Ethanol extract the MIC value for all the four bacteria were observed to be same i.e. 0.25 (Table 4 and 5)while when *F.racemosa* was observed the lowest MIC value for both Methanol extract& Chloroform extract werefound to be of *S.aureus* i.e. 0.0125& 0.03125 respectively (Table 6 and 7). In case of Petroleum ether extract the lowest MIC value was 0.03125 for *B.subtilis*(Table 8).

Table 3: The MIC index value of methanol extract of *F.religiosa* against different pathogens

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MBC extract	MIC index (control)	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.0625	0.5	2	2
E. coli	0.5 – 0.0156	0.0156	0.0312	0.03125	0.5	2	2
S. aureus	0.5 -0.0156	0.0156	0.0312	0.0125	0.5	2	2
S. typhi	0.5 – 0.0156	0.0156	0.0312	0.03125	0.5	2	2

Table 4: The MIC index value of chloroform extract of *F.religiosa* against different pathogens

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MBC extract	MIC index (control)	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.25	0.5	2	2
E. coli	0.5 - 0.0156	0.0156	0.0312	0.25	0.5	2	2
S. aureus	0.5 - 0.0156	0.0156	0.0312	0.25	0.5	2	2
S. typhi	0.5 - 0.0156	0.0156	0.0312	0.25	0.5	2	2

Table 5: The MIC index value of ethanol extract of *F.religiosa* against different pathogen

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MBC extract	MIC index (control)	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.625	0.5	2	2
E. coli	0.5 – 0.0156	0.0156	0.0312	0.125	0.5	2	2
S. aureus	0.5 -0.0156	0.0156	0.0312	0.25	0.5	2	2
S. typhi	0.5 – 0.0156	0.0156	0.0312	0.125	0.5	2	2

Table 6: The MIC index value of methanol extract of *F.racemosa* against different pathogens

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MIC index (control)	MBC extract	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.25	2	0.5	2
E.coli	0.5 - 0.0156	0.0156	0.0312	0.25	2	0.5	2
S. aureus	0.5 - 0.0156	0.0156	0.0312	0.25	2	0.5	2
S.typhi	0.5 - 0.0156	0.0156	0.0312	0.25	2	0.5	2

Table 7: The MIC index value of chloroform extract of *F.racemosa* against different pathogens

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MIC index (control)	MBC extract	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.125	2	0.5	2
E.coli	0.5 - 0.0156	0.0156	0.0312	0.125	2	0.5	2
S. aureus	0.5 - 0.0156	0.0156	0.0312	0.03125	2	0.5	2
S.typhi	0.5 - 0.0156	0.0156	0.0312	0.0625	2	0.5	2

Table 8: The MIC index value of petroleum ether extract of *F.racemosa* against different pathogens

Organism	Range (mg/ml)	MIC control (mg/ml)	MBC control (mg/ml)	MIC extract (mg/ml)	MBC extract	MIC index (control)	MIC index (extract)
B. subtilis	0.5 - 0.0156	0.0156	0.0312	0.03125	0.5	2	2
E. coli	0.5 – 0.0156	0.0156	0.0312	0.0625	0.5	2	2
S. aureus	0.5 -0.0156	0.0156	0.0312	0.125	0.5	2	2
S. typhi	0.5 – 0.0156	0.0156	0.0312	0.125	0.5	2	2

Qualitative analysis

Phytochemical analysis of *Ficus religiosa*in chloroform extract exhibit the presence of alkaloid, flavonoid, glycosideand tannin. Same as these phytochemicals saponin was present in ethanolic extract. Flavonoid, glycosides and tannin were present in methanolic extract of*Ficus religiosa.*In case of *Ficus racemosa* methanol extract exhibit the presence of glycosides, carbohydrates, flavonoid, tannin, saponins & alkaloid whereas in case of both Chloroform extract &Petroleum ether extract glycosides, saponins, flavonoids & carbohydrates were absent. Tannin is present in Petroleum ether but absent in Chloroform extract whereas alkaloid was present in Chloroform extract but absent in Petroleum ether extract.

Table 9: Presence of phytochemicals in *F.religiosa* &*F.racemosa*

S.NO.	Phytochemicals	Leaves					
		F.religiosa			*F.racemosa*		
		Ethanolic extract	Methanolic extract	Chloroform extract	Methanolic extract	Chloroform extract	Petroleumether extract
1	Alkaloid	+ve	-ve	+ve	+ve	+ve	-ve
2	Flavonoid	+ve	+ve	+ve	+ve	-ve	-ve
3	Glycosides	+ve	+ve	+ve	+ve	-ve	-ve
4	Tannin	+ve	+ve	+ve	+ve	-ve	+ve
5	Saponin	+ve	-ve	-ve	+ve	-ve	-ve
6	Carbohydrates	-ve	-ve	-ve	+ve	-ve	-ve

CONCLUSION

Medicinal plants are important for pharmacological research and drug development. They are used directly as therapeutic agent as well as raw material for synthesis of drug or as modern pharmacologically active compound. A significant number of modern pharmaceuticals are based on or derived from secondary metabolite of medicinal plant. Efficacy of medicinal plants had been in use to cure various human afflictions since ages due to the possession of bioactive ingredients like: alkaloids, terpenes, and polyphenols. From the experimental data it was

concluded that *F. religiosa* showed more effective antibacterial activity towards all the four organisms taken, as compared to that of *F. racemosa*. For *B. subtilis, E. coli, S.aureus* and *S. typhi*, the highest ZOI observed for different extracts of *F. religiosa*. Use of antibiotics has provided immediate relief but has posed a serious threat in the form of MDR. So the usage of medicinal plant various extracts as alternative means of therapy is the best option to treat bacteria, diseases. The present study is extendable to in vivo experiments to determine the specific mode of action of the extracts. There are number of medicinal plants in India which possess medicinal properties in the form of various drug formulation (there are several ingredients-like alkaloids, terpenes, polyphenols present in plants which are responsible for their medicinal property), the use of this plant is to cure various alignment which is safe and effective and used to form new drug which is safe and effective, a gift for industrialized and underdeveloped countries.

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