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ISOLATION, PHYTOCHEMICAL INVESTIGATION AND IN-VITRO ANTIBACTERIAL ACTIVITY OF ETANOLIC EXTRACT USING FABIANA IMBRICATA L.

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Abstract

Fabiana imbricata is a medicinal plant with therapeutic potential, belongs to the family Solanaceae also known as, Made mustard and is known for its anti-bacterial activity. I have been selected groups were divided into positive control, negative control, test groupsT1(20µg/ml), T2(40µg/ml), T3(60µg/ml), T4(80µg/ml) and T5(100µg/ml),standard group ciprofloxacin 2mg/ml and also prepared the stock solutions and the same way prepared the cultured medium by using the formula from the cultured medium taken and prepared sub cultured medium by using microorganisms. The result of antibacterial activity suggested that effects ofFabiana imbricata plant extract were tested on phytochemical, results showed the presence of alkaloids, saponins, Phenolic compounds flavonoids, tannins as chemical constituents may have anti-bacterial activity, which is not evaluated till now.Among that result, shows alkaloids and flavonoids were most effective compounds for the antibacterial activity in higher plants. Apart from that saponins show the most responsible and effectively compound for antibacterial activity of Fabiana imbricata. In this study, two solvents were taken ethanolic whole plant extracts from Fabiana imbricata have been tested for Anti-bacterial activity.Fabiana Imbricata was a traditional medicine. We found strong anti-bacterial activity specifically in the ethanol extracts of Fabiana Imbricata. Plant phenolic compounds have been found to possess potent anti- bacterial activity. These flavonoids have been found to possess anti-bacterial properties in various studies. Strong presence of tannins in all the solvent extracts may explain its potent bioactivities are known to possess potent an anti- bacterial properties. The Saponins have already shown as anti- bacterial activity.

Keywords: Fabiana imbricata, Ciprofloxacin,Anti-antibacterial activity, Escherichia coli, Bacillus Subtilis.

Introduction

Fabiana imbricata is a medicinal plant with therapeutic potential, belongs to the family Solanaceae also known as, Made mustard and is known for its anti-bacterial activity. Recently, many biological activities of Fabiana imbricata all the plant have been reported, including traditionally, said to be medicinal because it can relieve in irritation and inflammation of the bladder, especially if due to mechanical causes, gravel, uric acid, phosphoric or calcareous deposits it is a good remedy. May be thought of in cystitis, gonorrhea, recent cases of simple renal hyperaemia [1-5]. From that, I have been taken Fabiana imbricata plant (Made mustard) is one of most effective and potential anti-bacterial activity, particularly in its whole plant. I have been selected groups were divided into positive control, negative control, test groups T1 (20µg/ml), T2(40µg/ml), T3(60µg/ml), T4(80µg/ml), and T5(100µg/ml), standard group ciprofloxacin 2mg/ml and also prepared the

stock solutions and the same way prepared the cultured medium by using the formula from the cultured medium taken and prepared sub cultured medium by using microorganisms. The result of antibacterial activity suggested that effects of Fabiana imbricata plant extract were tested on phytochemical, results showed the presence of alkaloids, saponins, Phenolic compounds flavonoids, tannins as chemical constituents may have anti-bacterial activity which is not evaluated till now. I have been selected groups were divided into positive control, negative control, test groups T1(20µg/ml), T2(40µg/ml), T3(60µg/ml), T4(80µg/ml)and T5(100µg/ml),standard group ciprofloxacin 2mg/ml and also prepared the stock solutions and the same way prepared the cultured medium by using the formula from the cultured medium taken and prepared sub cultured medium by using microorganisms [6-9]. As a result showed that the zone of inhibition was observed in leaf, stem and root 100 (µg/ml) 1.1mm (Gram (+) B.S) 2mm (Gram (-) E.coli), 40 (µg/ml) 1.1mm (Gram (+) B.S) 0.0mm (Gram (-) E.coli), zone of inhibition not form in the root.Among that result, shows alkaloids and flavonoids were most effective compounds for the antibacterial activity in higher plants. Apart from that, saponins show the most responsible and effectively compound for antibacterial activity of Fabiana imbricata. In this study, two solvent were taken ethanolic whole plant extracts from Fabiana imbricata have been tested for Anti-bacterial activity [10-14]. The antibacterial activity of the extract was examined against Gram-Positive and Gram-Negative bacteria by measuring the zone of inhibition. Results of our findings confirmed the use of Fabiana Imbricata.Fabiana Imbricata was a traditional medicine. We found strong anti-bacterial activities specifically in the ethanol extracts of Fabiana Imbricata [15-19]. High TPC values found in the ethanol extract imply the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been found to possess potent anti- bacterial activity.These flavonoids have been found to possess anti bacterial properties in various studies. Strong presence of tannins in all the solvent extracts may explain its potent bioactivities are known to possess potent an anti- bacterial properties. The Saponins have already shown as anti-bacterial activity [20-22].

Fig.1:Fabiana imbricata plants (Made mustard)

Escherichia coli, commonly abbreviated E. coli, it is a gram-negative rod-shaped bacteria that is commonly found in the lower intestine of warm blooded organisms (endotherm). Most E. coli strains are harmless and are occasionally responsible for product recalls due to food contamination [4]. The harmless strains are part of normal flora of the gut, and can benefit their hosts by producing vitamin K and by preventing the establishment of pathogenic bacteria within the intestine. E. coli and related bacteria constitute about 0.1% of gut flora, and fecal-oral transmission is the major route through which pathogenic strains of the bacteria cause disease cells are able to survive outside the body for a limited time, environmental samples for fecal contamination. There is, however, a growing body of research that has examined environmentally persistent E. coli, which can survive for extended periods outside of the host.

Bacillus subtilis is a Gram-positive bacteria bacterium, rod-shaped and catalase positive. It was originally named Vibrio subtilis by Christian Gottfried Ehrenberg, and renamed Bacillus subtilis by Ferdinand Cohn in 1872 (subtilis being the Latin for "fine"). B. subtilis cells are typically rod-shaped, and are about 4-10 micrometers (um) long and 0.25-1.0 um in diameter, with a cell volume of about 4.6 fl at stationary phase.

Fig.2: Escherichia coli are gram-negative bacteria.

As with other members of genus Bacillus, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation. B. subtilis is a facultative anaerobe conditions. B. subtilis is heavily flagellated, which gives it the ability to move quickly in liquids. B. subtilis has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially for sporulation, which is simplified example of cellular differentiation. The number of spores found in human gut was too high to be attributed solely to consumption through food contamination7. B. subtilis appears in normal honey bee gut flora in some bee habitats.

Fig.3: Bacillus subtilis is a Gram-positive bacterium

Materials and Methods

Materials

Fabiana imbricata plants (Made mustard) were procured from local market, Potassium dihydrogenorthophosphate, Sodium hydroxide, dihydrogen phosphate, Sodium chloride and ethanol was procured from S.D. Fine chemicals, Mumbai.Ciproflaxin drug was obtained as a gift sample from Aurobindo Pharm Ltd., Hyderabad. Clinical isolates of Staphylococcus sps., Escherichia coli, were obtained from Acharya Nagarjuna University, Guntur. All the other chemicals were procured of Hindu College of pharmacy Guntur.

Methods

Fabiana imbricata plants (Made mustard) of good quality were purchased from local market andMade mustardwhole plantwere purchased were cleaned and washed and air dried for 7 days, then pulverized using mortar and pestle under aseptic conditions and ground to powder using a blender core powder. Powdered whole plant (leaf, stem, and root) materials were then each one weighed (175 gm) and kept in airtight containers until further usage.

Extraction of leaf, stem and root

Ethanolic extract

10 g of leaf stem and root powder were weighed and transferred into soxhlet apparatus and the leaf stem and rootpowder was extracted with ethanol at 35°c for 3-4 cycles. The extract was collected and the ethanol was evaporated after extraction by using rotary evaporator connected to a vacuum pump. The final extract in semi solid form was dried by placing in

desiccators. A rotary evaporator, yielding the extracted compound and their percentage yield is calculated respectively and used for further extracted crude drug phytochemical evaluation studies.

Phytochemical screening

Tab.1: Phytochemical analysis of solvent extracts of Fabiana imbricata

S.No	TEST	ETHANOL	LEAF	STEM	ROOT
1	alkaloids	Ethanol	+++	++	+
2	phenols	Ethanol	+	+++	++
3	tannins	Ethanol	_	++	+
4	flavonoids	Ethanol	+++	+	++
5	saponins	Ethanol	++++	+	_

The result of antibacterial activity indicate that effects of Fabiana imbricata plant extract were tested on phytochemical, results showed the presence of alkaloids, flavonoids, corticoids, saponins and tannins in the extracts of Fabiana imbricata . Among that result showed alkaloids and flavonoids were most effective compounds for the antibacterial activity in higher plants. Apart from that, saponins show the most effectively compound for antibacterial activity of Fabiana imbricata.

Anti-bacterial activity

The antibacterial activity ofFabiana imbricata was carried out against the Staphylococcus aureus and Escherichia coli. The antibacterial activity of the compounds can be assessed by Bore plate method.

Procedure of bore plate

The antibacterial activities are performed by bore plate method. The fresh culture of bacteria are obtained by inoculating bacteria nutrient broth media and incubated at 37 ± 2° C for 18 - 24 hours. This culture mixed with nutrient agar media and poured into Petri dishes by following aseptic techniques. After solidification of the media, the plates were placed in a refrigerator for 2 hours. After two hours of cold incubation, four bore are made at equal distance by using bore (5 mm diameter).Dip these solvent in to different concentrations. Water was used as a control. After introduction of standard drugs and plant extract the plates were placed in a refrigerator for 2hrs for proper dipping of drug into the media. After 2hrs the plate were placed in an incubator and maintained at 370±20C for 18-24 hours. After the incubation period, over mean the Petri plates were observed for zone of inhibition by using vernier scale. The results evaluated by comparing the zone of inhibition shown by the plant extract with standard drugs. The results are the mean value of zone of inhibition measured in millimeter of two sets. The results are tabulated.

Tab.2: Nutrient agar composition

S.No	Ingredients	Quantity
1	Beef extract	3g
2	Peptone	5g
3	Sodium chloride	5g
4	Agar	20g
5	Distilled water	1000ml.

Preparation of culture medium

- All the Ingredients were weighed as for required quantity the transferred into sterial conical flask then added to 1000ml distilled water then after conical flask kept in autoclave for 15min.

Preparation of sub culture

- Boiling test tubes should be taken.
- Then after 5ml culture medium transferred into boiling test tube
- Then sterilized 15min boiling test tube for 370c.
- Boiling test tubes kept in cross position.
- Before doing work we neatly clean the area of laminar air flow chamber with sprit.
- Then arranged the loop, burner, and sensitive micro organism gram +, gram -, like B.C (+), E.C (-),
- Nearby Laminar air flow chamber.

- With the help of loop sensitive microorganism gram +, gram -, like B.C (+), E.C (-), Are transferred into boiling test tubes.
- Then after labeled.
- Kept in incubator and observed for one day.

Anti bacterial activity against Bacillus subtilis

Concentrations : 20µg/ml 40µg/ml, 60 µg/ml80 µg/ml100 µg/ml

Control : Water

Standard : ciprofloxacin

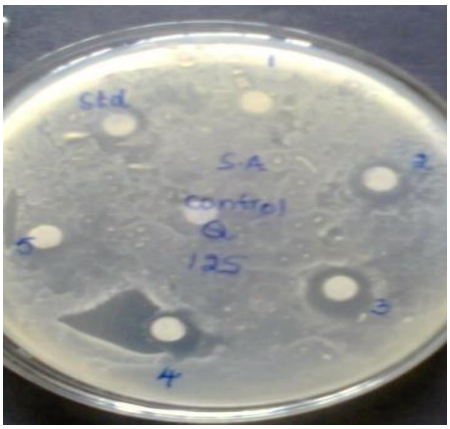

Fig. 4: Bacillus subtilis

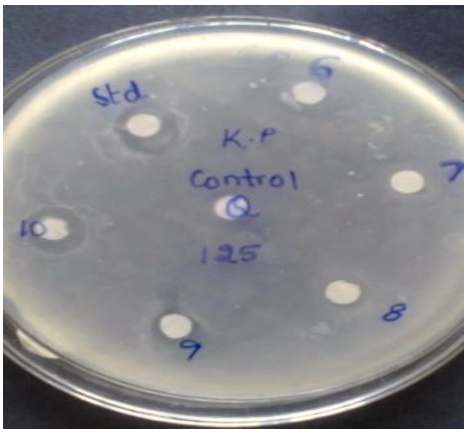

Fig.5: Escherichia coli

Anti-bacterial activity against Escherichia coli

Concentrations : 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, and 100µg/ml

Control: Water.

Standard: Ciprofloxacin.

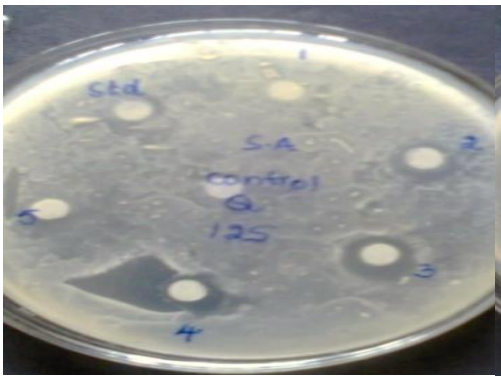

Fig. 6: Escherichia coli

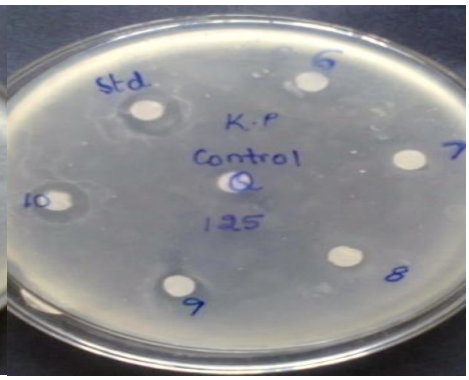

Fig. 7: Bacillus subtilis

Results

Tab.3: In-vitro anti-bacterial activity of ELEFI (bore plate method)

S.No	Concentration of ELEFI (µg/ml)	Zone of inhibition in mm	
		Gram (+) B.S	Gram(-)E.coli
1	Control	+	+
2	20(µg/ml)	-	-
3	40(µg/ml)	-	-

4	60(µg/ml)	-	-
5	80(µg/ml)	-	-
6	100(µg/ml)	1.1mm	2mm
7	Ciprofloxacin(2µg/ml)	5mm	5mm

Tab.4: In-vitro anti-bacterial activity of ESEFI (Bore plate method)

S.No	Concentration of ESEFI (µg/ml)	Zone of inhibition in mm	
		Gram (+) B.S	Gram(-)E.coli
1	Control	+	+
2	20(µg/ml)	-	-
3	40(µg/ml)	1.1mm	-
4	60(µg/ml)	1.5mm	1mm
5	80(µg/ml)	2mm	1.5mm
6	100(µg/ml)	3mm	2mm
7	Ciprofloxacin(2µg/ml)	5mm	5mm

Tab.5: In-vitro anti-bacterial activity of EREFI (Bore plate method)

S.No	Concentration of EREFI (µg/ml)	Zone of inhibition in mm	
		Gram (+) B.S	Gram(-)E.coli
1	Control	+	+
2	20(µg/ml)	-	-
3	40(µg/ml)	-	-
4	60(µg/ml)	-	-
5	80(µg/ml)	-	-
6	100(µg/ml)	-	-
7	Ciprofloxacin(2µg/ml)	5mm	5mm

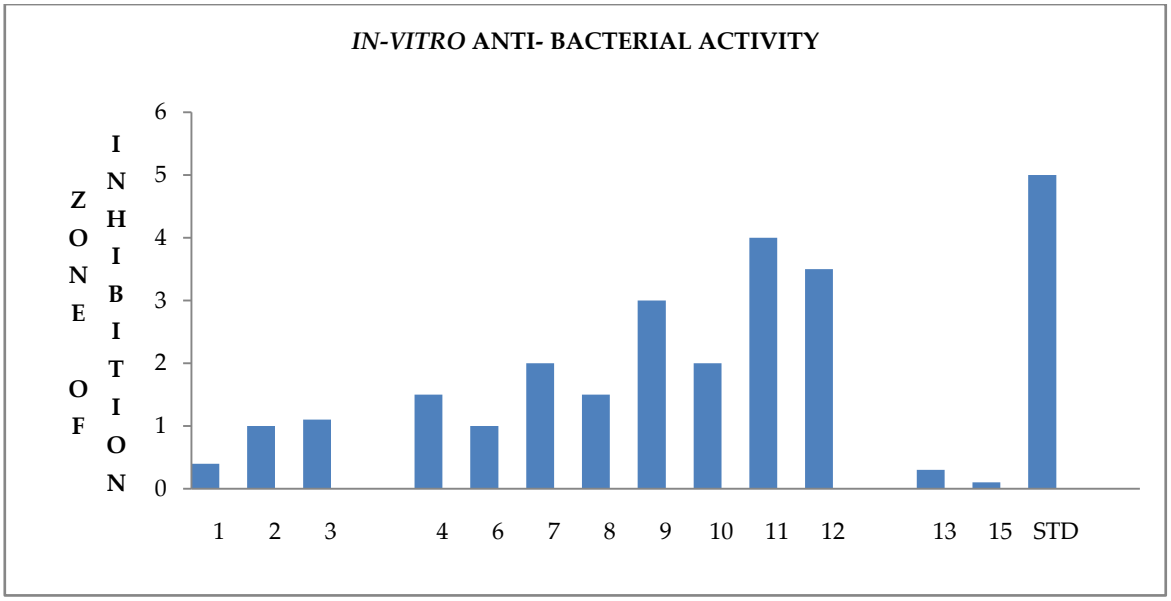

Fig.8: In-Vitro Anti-bacterial Activity

Discussion

In-vitro Anti-bacterial Activity

In recent years, the search for phytochemical possessing anti- bacterial properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging Results of our findings confirmed the use of Fabiana Imbricata. As traditional medicine. We found strong anti-bacterial activities specifically in the ethanol extracts of Fabiana Imbricata. High TPC values found in ethanol extract imply the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been found to possess potent anti- bacterial activity. These flavonoids have been found to possess anti-bacterial properties in various studies. Strong presence of tannins in all the solvent extracts may explain its potent bioactivities are known to possess potent an anti- bacterial properties. The Saponins have already shown as anti-bacterial activity.

Conclusion

Fabiana imbricata was a medicinal plant with therapeutic potential, belongs to the family Solanaceae.Fabiana imbricata plant (Made mustard) is one of most effective and potential anti-bacterial activity, particularly in its whole plant. I have been selected groups were divided into positive control, negative control, test groupsT1(20µg/ml), T2(40µg/ml), T3(60µg/ml), T4(80µg/ml),and T5(100µg/ml),standard group ciprofloxacin 2mg/ml and also prepared the stock solutions and the same way prepared the cultured medium by using the formula from the cultured medium taken and prepared sub cultured medium by using microorganisms. Among that result, shows alkaloids and flavonoids were most effective compounds for the antibacterial activity in higher plants. Apart from that saponins show the most responsible and effectively compound for antibacterial activity of Fabiana imbricata. In this study, two solvent were taken ethanolic whole plant extracts from Fabiana imbricata have been tested for Anti-bacterial activity. The antibacterial activity of the extract was examined against Gram-Positive and Gram-Negative bacteria by measuring the zone of inhibition. We found strong anti bacterial activities specifically in the ethanol extracts of Fabiana Imbricata. High TPC values found in the ethanol extract imply the role of phenolic compounds in contributing these activities. Plant phenolic compounds have been found to possess potent anti- bacterial activity. These flavonoids have been found to possess anti bacterial properties in various studies. Strong presence of tannins in all the solvent extracts may explain its potent bioactivities are known to possess potent an anti- bacterial properties. The Saponins have already shown as anti- bacterial activity.

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Conflict of Interest
All authors are declared that no Conflict of Interest.

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Not Applicable

Ethical Statement
Not Applicable

Author Contribution
All authors contributed equally

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