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RESEARCH ARTICLE

Antibacterial Activity of Leaf and Bark Extracts of *Crataeva Tapia* L.

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ABSTRACT

Antibiotics are commonly used to treat most microbial infections. But their irrational use, resistance of organism towards them and their side-effects have put their significance in jeopardy. Medicinal plants are known to cure microbial diseases since ancient times and exhibit minimum side effects. In the present study an attempt has been made to study antibacterial activity of *Crataeva tapia* L. (leaf and bark extracts) against *Escherichia coli* 2184, *Proteus mirabilis* 2241, *Bacillus subtilis* 2063 and *Staphylococcus aureus* 2079 procured from National Collection of Industrial Microorganisms (NCIM, Pune). Petroleum ether, ethanol and aqueous extracts of leaf and bark were used to evaluate the antibacterial activity of *Crataeva tapia* L. Antibacterial activity was established by determining Minimum Inhibitory Concentration (MIC) of leaf and bark extracts followed by agar well diffusion method. For agar well diffusion method, Ciprofloxacin (Ciplox), a broad spectrum antibiotic and sterile Nutrient Broth served as positive and negative control respectively. The results obtained from the present study revealed potential use of the plant for developing antibacterial compounds against tested bacteria.

KEYWORDS

Crataeva tapia L., *Staphylococcus aureus* 2079, *Bacillus subtilis* 2063, *Escherichia coli* 2184, *Proteus mirabilis* 2241, Antibacterial, MIC, Agar Diffusion

INTRODUCTION

Traditional medicine is in practice for many centuries by a substantial proportion of the population. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases[1]. According to World Health Organisation, medicinal plants would be the best source for obtaining a variety of drugs[2].The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infections[3-5].

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Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines[6]. In pharmaceutical field medicinal plants are mostly used for the wide range of substances present in plants which have been used to treat chronic as well as infectious diseases[7].

Widespread use of antibiotics is thought to have spurred evolutionary changes in bacteria that allow them to survive treatment with the powerful drugs[8]. Antibiotic resistance is accelerated by the overuse of antibiotics which has driven selection of mutations in bacteria that bring about drug resistance [9]. In the past decade, the numbers of bacterial isolates which are

antibiotic resistant have increased considerably. As a consequence, some agents are no longer useful for the treatment of infections. It is also of concern that an increasing number of bacteria species are becoming resistant to more than one antibiotic[10].

Crataeva tapia L. is a small much branched tree (Family: Capparaceae). The plant has been reported to possess several medicinal properties which include anti-inflammatory[11,] anti-oxidant[12], anti-arthritic[13,] anti-fertility[14], anti-mycotic[15], anti- diabetic[16], anti-microbial[17], anti-diarrhoeal[18], wound healing[19], anti-helminthic[20], urolithic property[21], nephrolithic property[22], hepatoprotective and cardioprotective activity[23]. In Northeast of Brazil, the fruits are used as tonic and febrifuge[24]. Therefore, in the present work an attempt has been made to study antibacterial activity of *Crataeva tapia* L. (leaf and bark extracts) against *Escherichia coli* 2184, *Proteus mirabilis* 2241, *Bacillus subtilis* 2063 and *Staphylococcus aureus* 2079.

MATERIALS AND METHOD

Solvents (Petroleum ether and ethanol) used for the present study were from SD fine Chemicals Ltd. (AR grade). Nutrient Broth and Nutrient Agar were from HiMedia and Ciprofloxacin was procured from Cipla (Ciplox).

Collection of Plant material

The flowering twig of *Crataeva tapia* L. was collected from Kalyan M.S., India. Herbarium was prepared and authenticated from Blatter herbarium, St. Xavier's College, Mumbai, M.S., India. Antibacterial activity of *Crataeva tapia* L. (leaf and bark extracts) was determined against different bacterial strains. Fresh leaves and bark were collected, washed under running tap water and dried in an oven at 40 ± 2^0C for one week. After drying, both the plant parts were ground separately to fine powder and stored till further use.

Preparation of Extracts

The plant extracts were prepared using petroleum ether, ethanol and water as solvents. 10 gms of the plant powder (leaf and bark) was refluxed for 6 hours in 100 ml of petroleum ether, ethanol and water at 60^0C, 70^0C and 80^0C respectively. The extract was filtered using vacuum filtration assembly. It was evaporated to dryness using a vacuum rotary evaporator. The extracts were stored in refrigerator in sterile amber colored bottles for further antibacterial study.

Test Microorganisms

Bacterial strains used for the antibacterial assay were, Gram positive bacteria *Staphylococcus aureus* 2079 and *Bacillus subtilis* 2063and Gram negative bacteria *Escherichia coli* 2184, *Proteus mirabilis* 2241 procured from National Collection of Industrial Microorganisms (NCIM), Pune, M.S., India. All the bacterial cultures were maintained on Nutrient agar slants at 4^0C in refrigerator and were used as parent cultures. Fresh bacterial cultures were obtained by subculturing the organisms from parent cultures on sterile Nutrient agar slants under aseptic conditions and were incubated for 24 hours at 37^0C.

Antibacterial Assay

Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits growth of the tested organism. When growth occurs in all dilutions containing the antimicrobial agent, the MIC is recorded as greater than the highest concentration. The MIC is recorded as less than or equal to the lowest concentration, when no growth occurs in any of the concentrations tested[25]. The lowest concentration which did not permit any visible microbial growth when compared with negative control was recorded as MIC value.

A. Preparation of Inoculum

24 hour old bacterial culture was suspended in sterile saline and O.D. was set as 0.1 at 660 nm for all organisms tested.

B. Scheme for MIC Determination

Leaf and bark extracts were prepared in petroleum ether, ethanol and water. MIC of the plant extracts was determined in triplicates

according to the procedure described in Table 1. Stock solutions of leaf and bark extracts (petroleum ether, ethanol and aqueous) were prepared as 500 mg/ml in sterile Nutrient Broth (HiMedia). The stock solutions were diluted to prepare a range of 10-150 mg/ml (petroleum ether) and 10-100 mg/ml (ethanol and aqueous) concentration of the plant extracts using sterile Nutrient broth (diluent) to make up the final volume as 5.0 ml in pre-sterilized test tubes. 0.1 ml of respective bacterial culture was inoculated in the tubes. Positive control consisted of 5.0 ml of sterile Nutrient Broth inoculated with each of the bacterial strain whereas negative control consisted of 5.0 ml of sterile Nutrient Broth. All the tubes were incubated at 37^0C for 24 hrs.

Agar Well Diffusion Method

A. Preparation of Inoculum

24 hour old bacterial culture was suspended in sterile saline and O.D. was set as 0.1 at 660 nm for all organisms tested.

B. Positive and Negative Controls

Ciprofloxacin (Ciplox), a broad spectrum antibiotic and sterile Nutrient Broth served as positive and negative control respectively. Standard Ciprofloxacin (2 mg/ml) was serially diluted in sterile distilled water to get a final concentration of 5 and 0.1µg/ml.

Table 1: Scheme for MIC determination

No.	Concentrations of plant extract (mg/ml)	Stock solution (500mg/ml)	Diluent (sterile NB) (ml)	Culture suspension(ml)
1.	10	0.02	4.98	0.1
2.	20	0.04	4.96	
3.	30	0.06	4.94	
4.	40	0.08	4.92	
5.	50	0.1	4.9	
6.	60	0.12	4.88	
7.	70	0.14	4.86	
8.	80	0.16	4.84	
9.	90	0.18	4.82	
10.	100	0.2	4.8	
11.	110	0.22	4.78	
12.	120	0.24	4.76	
13.	130	0.26	4.74	
14.	140	0.28	4.72	
15.	150	0.3	4.70	

C. Preparation of Media

20 ml of the molten Nutrient Agar (HiMedia) was prepared in tubes and autoclaved at 15 psi at 121^0C for 20 minutes. 1 ml of the culture was seeded to 20 ml of sterile molten Nutrient agar (about 40^0C), mixed thoroughly, poured in pre-sterilized petri plates under aseptic conditions and was allowed to solidify at room temperature.

Wells were made using sterile 8 mm diameter cork borer (HiMedia). Four equidistant wells were prepared (for positive control, negative control, leaf and bark extracts). Once MIC is determined, thrice of its concentration was added in wells. 100 µl of the positive control, negative control and plant extracts (leaf, bark) were added to the wells using micropipette. These plates were kept for prediffusion and incubated at 37^0C for 24 hours.

The antibacterial activity was assayed by measuring the diameter of inhibition zone formed around the wells after incubation.

The zone of inhibition was measured using HiMedia Antibiotic Zone Scale and the results were tabulated. The whole experiment was performed in triplicates. A separate control plate was prepared in triplicates to check for visible growth.

RESULTS AND DISCUSSION

Minimum Inhibitory Concentrations (MIC's) are considered as the 'gold standard' for determining the susceptibility of organisms to antimicrobials. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate.

The MIC is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism after overnight incubation (this period is extended for organisms such as anaerobes, which require prolonged incubation for growth)[26].

Petroleum ether, ethanolic and aqueous extracts (leaf and bark) of *Crataeva tapia* L. were screened to check for antibacterial activities. The plant extracts were effective against all the test organisms. Petroleum ether, ethanolic and aqueous leaf extracts exhibited MIC as 60-70, 20-30 and 30-40 mg/ml whereas bark extracts exhibited MIC as 80-90, 40-50 and 60-70 mg/ml against *Staphylococcus aureus* 2079 (Table2, 3 and 4). Petroleum ether, ethanolic and aqueous leaf extracts exhibited MIC as 90-100, 50-60 and 60-70 mg/ml whereas bark extracts exhibited MIC as 80-90, 30-40 and 40-50 mg/ml against *Bacillus subtilis* 2063 (Table 2 and 3**)**.

Petroleum ether, ethanolic and aqueous leaf extracts exhibited MIC as 70-80, 30-40 and 50-60 mg/ml whereas bark extracts exhibited MIC as 40-50, 10-20 and 20-30 mg/ml against *Escherichia coli* 2184 (Table2, 3). Petroleum ether, ethanolic and aqueous leaf extracts exhibited MIC as 80-90, 40-50 and 50-60 mg/ml whereas bark extracts exhibited MIC as 70-80, 30-40 and 40-50 mg/ml against *Proteus mirabilis* 2241 (Table 2 and 3).

Table 2: MIC of leaf extracts (petroleum ether, ethanol, aqueous) of *Crataeva tapia* L. against bacterial strains tested

No.	Bacterial strains	Petroleum ether (mg/ml)	Ethanol (mg/ml)	Aqueous (mg/ml)
1.	*Staphylococcus aureus* 2079	60-70	20-30	30-40
2.	*Bacillus subtilis* 2063	90-100	50-60	60-70
3.	*Escherichia coli* 2184	70-80	30-40	50-60
4.	*Proteus mirabilis* 2241	80-90	40-50	50-60

Values are average of three determinants

Table 3: MIC of bark extracts (petroleum ether, ethanol, aqueous) of *Crataeva tapia* L. against bacterial strains tested

No.	Bacterial strains	Petroleum ether (mg/ml)	Ethanol (mg/ml)	Aqueous (mg/ml)
1.	*Staphylococcus aureus* 2079	80-90	40-50	60-70
2.	*Bacillus subtilis* 2063	80-90	30-40	40-50
3.	*Escherichia coli* 2184	40-50	10-20	20-30
4.	*Proteus mirabilis* 2241	70-80	30-40	40-50

Values are average of three determinants

Table 4: Antibacterial activity positive and negative control against bacterial strains by agar well diffusion method

No.	Bacterial strains	Positive Control Standard Ciprofloxacin		Negative Control
		Concentrations (µg)	Zone of Inhibition (mm)	Zone of Inhibition (mm)
1.	*Staphylococcus aureus* 2079	5	29 ± 0.58	0.00
2.	*Bacillus subtilis* 2063	5	26 ± 0.00	0.00
3.	*Escherichia coli* 2184	0.1	23 ± 0.58	0.00
4.	*Proteus mirabilis* 2241	5	22 ± 0.58	0.00

Values are Mean ± S.D. of three values of determinants
Zone of inhibition (mm) includes diameter of well (8 mm)

Table 5: Antibacterial activity of leaf extracts (petroleum ether, ethanol, aqueous) *Crataeva tapia* L. against bacterial strains by agar well diffusion method

No.	Bacterial strains	Zone of Inhibition (mm)		
		Petroleum ether	Ethanol	Aqueous
1.	*Staphylococcus aureus* 2079	20±0.58	23 ±0.00	19 ±0.58
2.	*Bacillus subtilis* 2063	23±0.58	21 ±0.58	19 ±0.58
3.	*Escherichia coli* 2184	17±0.58	25±0.58	23±0.58
4.	*Proteus mirabilis* 2241	22±0.58	19 ±0.58	18 ±0.58

Values are Mean ± S.D. of three values of determinants
Zone of inhibition (mm) includes diameter of well (8 mm)

Petroleum ether, ethanolic and aqueous leaf extracts showed lowest MIC against *Staphylococcus aureus* 2079 (70, 30 and 40 mg/ml) whereas bark extracts revealed lowest MIC against *Escherichia coli* 2184(50, 20 and 30 mg/ml) (Table 2 and 3).

Agar well diffusion assay involve the application of antibiotic solutions of different concentrations to cups, wells or paper discs, placed on the surface of or punched into agar plates seeded with the test bacterial strain. Antibiotic diffusion from these sources into the agarose medium leads to inhibition of bacterial growth in the vicinity of the source and to the formation of clear "zones" without bacterial lawn. The diameter of these zones increases with antibiotic concentration. The assays are usually carried out using multiple discs on the same Petri dish to eliminate differential effects from growth time and temperature. Care is required during preparation for the assay, as agar homogeneity and thickness, as well as other factors can affect zone size and shape[27].

Results of antibacterial activity tabulated in Table 3, 4 and 5 clearly showed that petroleum ether, ethanolic and aqueous extracts have shown antibacterial activity against all microorganisms tested at various concentrations.

In agar well diffusion method, the negative control (Nutrient broth) showed no activity against the test microorganisms whereas positive control (Ciprofloxacin) gave 29, 26, 23 and 22 mm zone of inhibition against *Staphylococcus aureus* 2079, *Bacillus subtilis* 2063, *Escherichia coli* 2184 and *Proteus mirabilis* 2241, *Staphylococcus aureus* 2079 and *Bacillus subtilis* 2063 respectively as shown in Table 4, 5 and 6. The antibiotic was tested at 5µg against *Staphylococcus aureus* 2079, *Bacillus subtilis* 2063 and *Proteus mirabilis* 2241 whereas 0.1 µg against *Escherichia coli* 2184 (Table 4, Fig. 1, 2 and 3).

Petroleum ether leaf extract showed maximum zone of inhibition against *Bacillus subtilis* 2063 (23 mm) followed by *Proteus mirabilis* 2241 (22 mm), *Staphylococcus aureus* 2079 (20 mm) and *Escherichia coli* 2184 (17 mm), whereas petroleum ether bark extract showed maximum zone of inhibition against *Bacillus subtilis* 2063 (24 mm)followed by *Proteus mirabilis* 2241 (23 mm), *Staphylococcus aureus* 2079 (19 mm) and *Escherichia coli* 2184 (16 mm) as shown in Table 5, 6and Figure 1, 4 and 5.

Ethanolic leaf extract showed maximum zone of inhibition against *Escherichia coli* 2184 (25 mm), followed by *Staphylococcus aureus* 2079 (23 mm), *Bacillus subtilis* 2063 (21 mm) and *Proteus mirabilis* 2241 (19 mm), whereas ethanolic bark extract showed maximum zone of inhibition against *Escherichia coli* 2184 (22 mm) followed by *Bacillus subtilis* 2063 (20 mm), *Proteus mirabilis* 2241 (17 mm) and *Staphylococcus aureus* 2079 (16 mm) as shown in Table 4,5 and Figure 2, 5 and 6.

Aqueous leaf extract showed maximum zone of inhibition against *Escherichia coli* 2184 (23 mm) followed by *Staphylococcus aureus* 2079 and *Bacillus subtilis* 2063 (19 mm) and *Proteus mirabilis* 2241 (18 mm), whereas aqueous bark extract showed maximum zone of inhibition against *Escherichia coli* 2184 (19 mm) followed by *Bacillus subtilis* 2063 and *Staphylococcus aureus* 2079 (17 mm) and *Proteus mirabilis* 2241 (16 mm) as shown in Table 5, 6 and Figure 3, 4 and 5.

Amongst the different extracts (petroleum ether, ethanolic and aqueous) of leaf and bark, bark extracts were found to have better antibacterial activity against *Escherichia coli* 2184, *Proteus mirabilis* 2241and *Bacillus subtilis* 2063 whereas leaf extracts against *Staphylococcus aureus* 2079 (Table 2, 3). Ethanolic extracts were found to have better antibacterial activity than petroleum ether and aqueous extracts (Table 2, 3).

Lowest MIC of 30 and 20 mg/ml was observed for ethanolic extracts of leaf and bark against *Staphylococcus aureus* 2079 and *Escherichia coli* 2184 respectively (Table 2, 3). Antibacterial activity was found to be least in petroleum ether extracts of leaf with MIC of 100 mg/ml against *Bacillus subtilis* 2063 and bark

Table 6: Antibacterial activity of bark extracts (petroleum ether, ethanol, aqueous) *Crataeva tapia* L. against bacterial strains by agar well diffusion method

No.	Bacterial strains	Zone of Inhibition (mm)		
		Petroleum ether	**Ethanol**	**Aqueous**
1.	*Staphylococcus aureus* 2079	19±0.58	16 ±0.00	17 ±0.58
2.	*Bacillus subtilis* 2063	24±0.58	20 ±1.00	17 ±0.58
3.	*Escherichia coli* 2184	16±0.58	22±0.58	19±0.58
4.	*Proteus mirabilis* 2241	23±0.58	17 ±0.58	16 ±0.00

Values are Mean ± S.D. of three values of determinants
Zone of inhibition (mm) includes diameter of well (8 mm)

against *Bacillus subtilis* 2063 and *Staphylococcus aureus* 2079 with MIC of 90 mg/ml (Table 2, 3).In agar well diffusion method, petroleum ether leaf and bark extracts showed maximum zone of inhibition of 23 mm and 24 mm against *Bacillus subtilis* 2063 (Table 5, 6). Ethanolic and aqueous extracts (leaf and bark) showed maximum zone of inhibition against *Escherichia coli* 2184. Zone of inhibition of ethanolic leaf and bark extracts were 25 mm and 22 mm whereas for aqueous leaf and bark extracts was 23 mm and 19 mm against *Escherichia coli*2184 (Table 5, 6).

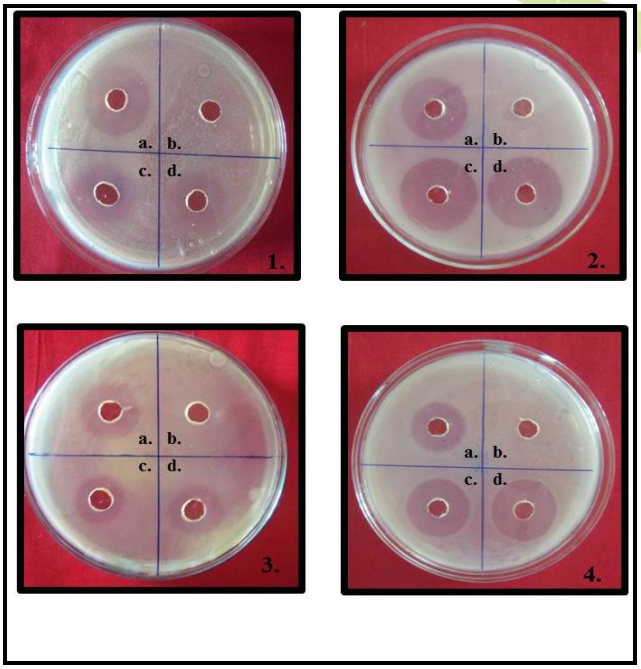

Figure 1

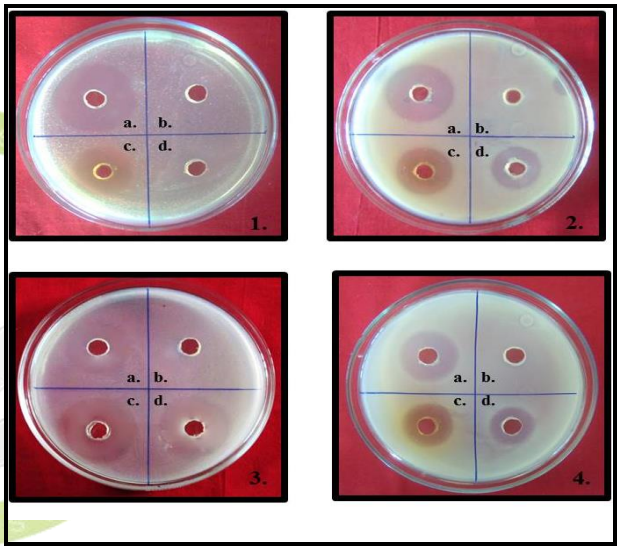

Figure 2

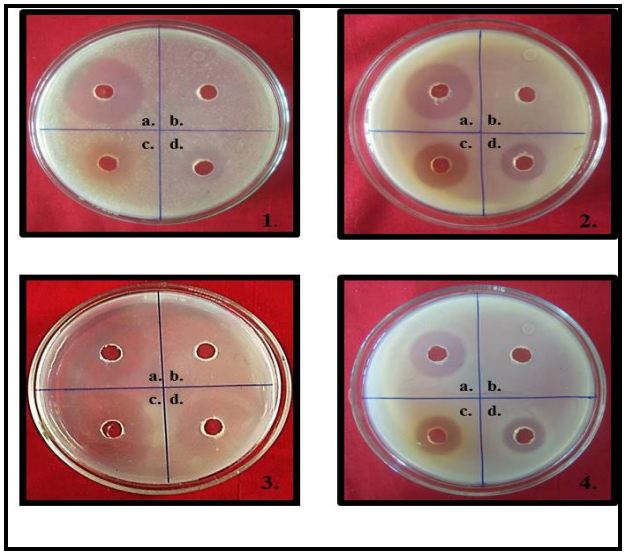

Figure 3

Antibacterial activity of leaf and bark petroleum ether, ethanolic and aqueous extracts of *Crataeva tapia* L.

1. *Staphylococcus aureus* 2079
2. *Bacillus subtilis* 2063
3. *Escherichia coli* 2184
4. *Proteus mirabilis* 2241

Where,

a. Positive Control

b. Negative Control

c. Leaf Extract

d. Bark Extract

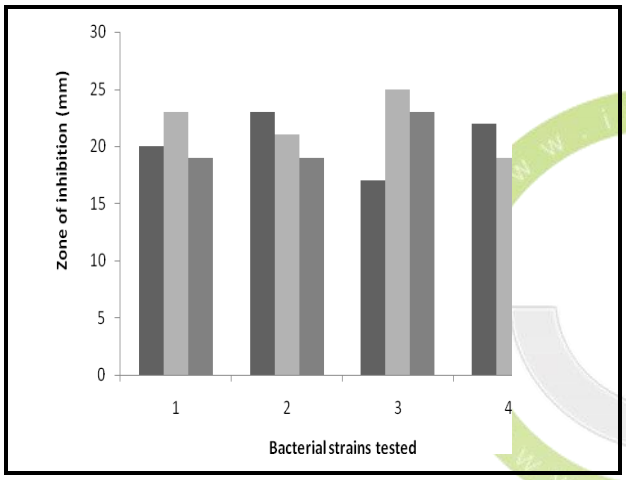

Figure 4: Effect of petroleum ether, ethanolic, aqueous leaf extracts of *Crataeva tapia* L. on bacterial strains tested

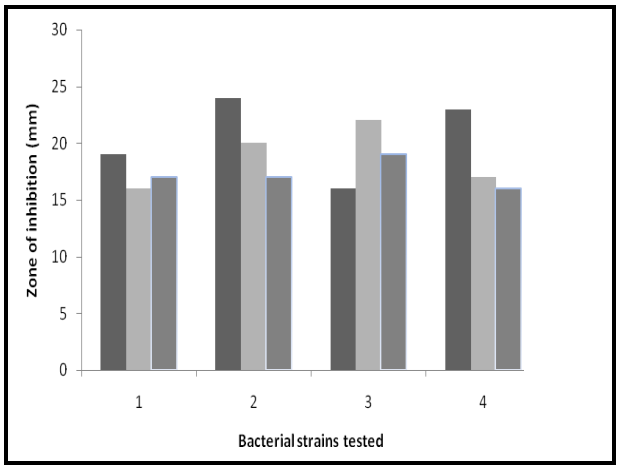

Figure 5: Effect of petroleum ether, ethanolic, aqueous bark extracts of *Crataeva tapia* L. on bacterial strains tested

Key	
1 = *Staphylococcus aureus* 2079	2 = *Bacillus subtilis* 2063
3 = *Escherichia coli* 2184	4 = *Proteus mirabilis* 2241
Petroleum ether extract	Ethanolic extract
Aqueous extract	

The present data is in agreement with previous results obtained on antibacterial activity of *Crataeva tapia* L. Parvin*et al*. (2012) studied antibacterial activity of chloroform extract of stem bark of *Crataeva nurvula* against two Gram positive bacteria (*Bacillus cereus* and *Bacillus megaterium*) and four Gram negative bacteria (*Escherichia coli*, *Shigelladysenteriae*, *Shigellasonnei* and *Shigellaboydii*). The bark extract showed highest activity against *Shigelladysenteriae* and lowest against *Bacillus cereus*. *Bacillus megaterium* showed no sensitivity to the test material[28].

Gowsalya and Saravanababu, (2013) studied antibacterial activity of chloroform, ethanol and hexane extracts of *Crateavareligiosa* bark against three pathogenic bacterial species *Enterococcus faecalis*, *Esherichiacoli* and *Staphylococcus*. The ethanol extract of bark was effective than chloroform and hexane extract[29].

Ethanolic extract of roots of *Crataeva nurvula* (10, 20 and 50 mg/ml) showed anti-bacterial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa*. The extract showed inhibition of the test bacteria in a concentration dependent manner. Among these, *Staphylococcus aureus* was found to be more susceptible followed by *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa*. Extract exhibited significant antibacterial activity at highest concentration of 50 mg/ml [20].

Chandra and Gupta (2001), isolated bacterial strains *Escherichia coli*, *Klebsiella*sp. *Pseudomonas* sp., from patients urine suffering from Urinary Tract Infection (UTI). Antibacterial activity of methanolic bark extract

of *Crataeva nurvula* was tested against these bacterial strains. The maximum growth inhibition of 35.3, 28.4 and 18.6 mm was recorded by the crude bark extract against *E. coli* followed by *Pseudomonas* and *Klebsiella*sp. respectively[30].

Methanolic extract of *Crataeva nurvula* bark was tested against *Bacillus subtilis* ATCC 6051, *Proteus vulgaris* ATCC 6380, *Salmonella typhimurium* ATCC 23564, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* K-12 and *Staphylococcus aureus.* Methanolic extract (200 mg/ml) showed maximum potency against *Bacillus subtilis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Zone of Inhibition: 10-19 mm), but showed moderate activity against *Escherichia coli* K-12 and *Proteus vulgaris*[31].

Patil and Gaikwad (2012), found methanolic extract of apical bark more effective than middle and mature bark in inhibiting growth of bacterial species like *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiellap-neumoniae, Salmonella typhi, Proteus mirabilis* and *Micrococcus*[32].

Similar results were observed in antimicrobial assay of different plants such as *Aloe vera* which was tested against human pathogenic bacteria such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* using four different solvents *viz.* Ethyl acetate, ethanol, hexane, petroleum ether. Out of the four solvents used, ethanol exhibited significant activity against *B. subtilis, S. aureus* and moderate activity against *K. pneumonia* and *P. aeruginosa*[33].

Igbinosa *et al*. (2009) studied the antimicrobial activity of stem bark of *Jatrophacurcas* against *Escherichia coli, Streptococcus faecalis, Staphylococcus epidermidis, Shigella-dysenteriae, Micrococcus kristinae, Klebsiella pneumoniae, Bacillus cereus, Bacillus subtilis, Proteus vulgaris and Serratia marcescens* using ethanol, methanol and aqueous extracts. The extracts exhibited antimicrobial activities with zone of inhibition ranging from 5 to 12, 8 to 20 and 2 to 8 mm for ethanol, methanol and aqueous extracts respectively against all the tested organisms[34].

Petroleum ether, chloroform, ethanol and aqueous extracts of aerial parts (leaves, stem and fruits) of *Momordicacymbalaria* were tested for antimicrobial activity against clinically isolated bacteria, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. The ethanol and aqueous extracts of plant have shown significant activity against tested organisms[35].

Arivuselvan *et al*. (2011) investigated antibacterial activity of leaves and bark extracts of *Ceriopstagal* and *Pemphisacidula* using acetone, methanol, ethanol and aqueous extract against human pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio parahaemolyticus, Staphylococcus aureus* and *Vibrio cholera*. The bark extract inhibited the growth of all tested pathogens than the leaf extracts in all the solvents. *Pemphisacidula* possessed higher antibacterial potency than *Ceriopstagal* and the highest activity was recorded in methanol extract of bark against *S. aureus* (17.2 ± 0.1 mm)[36].

Ali and Dixit (2012) evaluated antibacterial activity of flavonoids, Orientin and Vicenin, isolated from leaves of *Ocimum sanctum* against *Staphylococcus aureus, Staphylococcus cohni, Escherichia coli, Proteus* and *Klebsiella pneumoniae* at different concentrations (50, 100, 200, 400 mg/ml) separately and in combination. Orientin was found active against *Staphylococcus aureus, Staphylococcus cohni* and *Klebsiella-pneumonia*e in all concentrations whereas Vicenin was effective only against *Escherichia coli* and *Proteus* respectively. Maximum zone of inhibition in Orientin (18.04, 17.13 and 16.11 mm) was observed at concentration of 400 mg/ml against *Staphylococcus aureus, Staphylococcus cohni* and *Klebsiellae-pneumonia*e respectively. Vicenin gave maximum inhibition zone of 18.84 and 17.16 mm against *Escherichia coli* and *Proteus.* The combination of these flavonoids

was found to be most active against all bacterial strains. The highest zone of inhibition (20.12, 20.75, 20.95, 19.55 and 20.1 mm) was observed at 400 mg/ml against *Escherichia coli*, *Proteus*, *Staphylococcus aureus, Staphylococcus cohni* and *Klebsiella pneumoniae* respectively[37].

Plants represent a rich source of antimicrobial agents, potent drugs and are used medicinally in many different countries. A wide range of medicinal plant parts are used for the extraction of agents having variety of medicinal properties. The different plant parts used include root, stem, flower, fruit, twig exudates and modified plant organs[38]. The antibacterial activity of *Crataeva tapia* leaf and bark extracts may be attributed to the presence of phytochemicals.

CONCLUSION

It can be concluded from the above results that *Crataeva tapia* L. leaf and bark extracts (petroleum ether, ethanol, aqueous) possess antibacterial activity against *Staphylococcus aureus* 2079, *Bacillus subtilis* 2063, *Escherichia coli* 2184 and *Proteus mirabilis* 2241. Antibacterial activity of these leaf and bark may be attributed to the phytoconstituents present in petroleum ether, ethanolic and aqueous extracts.

The antibacterial activity of petroleum ether, ethanolic and aqueous extracts of *Crataeva tapia* (leaf and bark) were comparable to the standard antibiotic (Ciprofloxacin).The inhibitory effect of extracts against bacterial strains can introduce the plant as a potential candidate for drug development for treatment of ailments caused by these pathogens. However, it is necessary to isolate the secondary metabolites from the extracts studied in order to test specific antibacterial activity. Further research work is necessary for separation, purification and characterization of biologically active compounds.

REFERENCES

1. Mothana, R. A., Linclequist, V. (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. *Journal of Ethnopharmacology*, 96, 1-2, 177-181.
2. Santos, P. R.V., Oliveria, A .C. X., Tomassini, T. C. B. (1995). Controls microbiological products fitoterapices. *Rev. Farm Bioquim.*, 31, 35-38.
3. Reddy, P. S., Jamilk, M. P. (2006). Antimicrobial activity of isolates from *Piper longum* and *Taxusbaccata*. *Pharmaceutical Biology*, 39, 236-238.
4. Atefl, D. A., ErdoUrul, O. T. (2003). Antimicrobial activities of various medicinal and commercial plant extracts. *Turk Biol*., 27, 157-162.
5. Edro U. O. T. (2002). Antibacterial activities of some plant extracts used in folk medicine. *Pharmaceutical Biology*, 40, 269-273.
6. Sukanya, S. L., Sudisha J, Hariprasad, P., Niranjana S. R., Prakash, H. S., & Fathima S. K. (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8(23), 6677- 6682.
7. Okigbo R. N., Anuagasi C. L., Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plants Research*, 3(2), 86-95.
8. Anonymous. National Institute of Allergy and Infectious Diseases, NIAID (2004). The problem of antibiotic resistance, http://www.niaid.nih.gov/.
9. Anonymous. World Health Organization, (WHO) (2000). Report on infectious disease: Overcoming antimicrobial resistance. Available at URL: http://www.who.int/infectious-disease report/index Html Accessed September 23.
10. Piddock, L. J. V. (2006). Multidrug-resistance efflux pumps-not just for resistance. *Nature Review Microbiology*, 4, 629-36.
11. Tripathy, S., Asha, M., Pradhan, D. (2010). Acute and Chronic anti-inflammatory evaluation of *Crataeva religiosa* in rats. *International Journal of Pharmacy & Technology*, 2(4), 1270-1279.

12. Kumari, A. Kakkar. P. (2008). Screening of Antioxidant potential of selected barks of Indian medicinal plants by multiple *in vitro* assays. *Biomedical and Environmental Science*, 21, 24-29.

13. Bani, S., Kaul, A., Ahmad, S. F., Suri, K. A., Gupta, B. D, Satti N. K., & Qazi G. N. (2006). Suppresion of T lymphocyte activity by lupeol isolated from *Crataevareligiosa*. *Phytotherapy Research*, 20(4), 279-87.

14. Bhaskar V. H., Profulla, K. M., Balakrishnan B. R. Balakrishnan, N., Sangameswaram, B. (2009). Evaluation of the anti-fertility activity of stem bark of Crataevanurvulabuch-hum. *African Journal of Biotechnology*, 8(22), 6453-6456.

15. Sahoo, S., Mishra S. K., Panda P. K., Tripathy, S., Mishra, S. R., Ellaiah, P., Dash, S. K. (2008). Antimycotic potential of *Crateavareligiosa* Hook and Forst against some selected fungal pathogens. *ActaPoloniacPharmaceutica*, 65(2), 245-247.

16. Sikarwar, M. S., Patil, M. B. (2010). Antidiabetic activity of *Crataevanurvula* stem bark extracts in alloxan induced diabetic rats. *Journal of Pharmacy & BioAllied Sciences*, 2(1), 18-21.

17. Patil, U. H., Dattatraya, K. G. (2012). Differential bactericidal potential and phytochemical evaluation of *Crataevareligiosa* stem bark. *International Journal of Pharmaceutical Research & Development*, 2 (11), 82-88.

18. Inayathulla, Shariff W. R., Karigar, A. A., Sikarwar, M. S. (2010). Evaluation of anti-diarrhoeal activity of *Crataevanurvula* root bark in experimental animals. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2 (1), 158-161.

19. Asuti, N. (2010). Wound healing property of alcoholic extract of root bark of *Crataevanurvula*. *Journal of Pharmacy Research*, 3(5), 1121-1123.

20. Kamath, R., Shetty, D., Bhat, P., Shabaraya, A. R., Hegde, K. (2011). Evaluation of antibacterial and antihelminthic activity of root extract of *Crataevanurvula*. *Pharmacology online*, 1, 617-622.

21. Agarwal, S., Gupta, S. J., Saxena, A. K., Gupta, N. and Agarwal, S. (2010). Urolithic property of Varuna (*Crataeva nurvula*): An experiment. *Ayu*, 31(3), 361-366.

22. Rajat, M., Walia, A., Gupta, S. (2011). New frontiers on Nephrolithiasis: Pathophysiology and management of kidney stones. *International Journal of Research in Ayurveda & Pharmacy*, 2 (3), 775- 786.

23. Bopana, N., Saxena, S. (2008). *Crataevanurvula*: A valuable medicinal plant. *Journal of Herbs, Spices & Medicinal Plants*, 14(1-2), 107-127.

24. Agra, M. F., Silva, K. N., Basilio I. J. L. D., Freitas, P. F., Barbosa-Filho, J. M. (2008). Survey of medicinal plants used in the region Northeast of Brazil. *Brazilian Journal of Pharmacognosy*, 18(3), 472-502.

25. Weigand, I., Hilpert, K., Hancock, E. W. R. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), 163-175.

26. Andrews, M. J. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48 Suppl. S1, 5-16.

27. Bonev, B., James, H., Judicae, P. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy*, 61, 1295–1301.

28. Parvin, S., Kader, M. A., Rahman, M. A., Wahed, M. I. I., Haque, M. E. (2012). Antibacterial activities and brine shrimp lethality bioassay of the chloroform extract of stem bark of *Crataeva nurvula* Buch Ham. *International Journal of Pharmaceutical Sciences and Research*, 3(3), 830-834.

29. Gowsalya, P., Saravanababu. (2013). Phytochemical and antimicrobial activity of selected microorganism of bark extract of the

plant. *Crataeva religiosa*. *International Journal of Pharmaceutical & Biological Archives*, 1(6), 179-181.

30. Chandra, S., Gupta, C. P. (2001). Antibacterial activity of medicinal plant *Crataeva nurvula* (bark) against bacterial strains causing urinary tract infection. *Asian Journal of Chemistry*, 13(3), 1181-1186.
31. Mathur, A., Purohit, R., Mathur, D., Prasad G. B. K. S., Dua, V. K. (2001). Pharmacological investigation of methanol extract of *Syzigumcuminii* seeds and *Crataeva nurvula* bark on the basis of antimicrobial, antioxidant and anti-inflammatory properties. *Pelagia Research Library*, 2(1), 174-181.
32. Patil, U. H., Dattatraya, K. G. (2012). Differential bactericidal potential and phytochemical evaluation of *Crataeva religiosa* stem bark *International Journal of Pharma. Research & Development*, 2(11), 82-88.
33. Thiruppathi, S., Ramasubramanian, V., Sivakumar, T., Thirumalai, A. V. (2010). Antimicrobial activity of *Aloe vera* (L.) Burm. F. against pathogenic microorganisms. *Journal of Biosciences Research*, 1(4), 251-258.
34. Igbinosa, O. O., Igbinosa, E. O., Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatrophacurcas* (Linn). *African Journal of Pharmacy and Pharmacology*, 3(2), 58-62.
35. Natarajan, A., Silambarasan, D., Govindan, T., & Kathiresan, K. (2011). Antibacterial activity of mangrove leaf and bark extracts against human pathogens. *Advances in Biological Research*, 5(5), 251-254.
36. Sajjan, S., Chetana, S. H., Paarakh, M. P., Vedamurthy, A. B. (2010). Antimicrobial activity of *Momordicacymbalaria* Fenzl aerial parts extracts. *Indian Journal of Natural Products and Resources*, 1(3), 296-300.
37. Huma, A., & Dixit, S. (2012). *In vitro* antimicrobial activity of flavonoids of *Ocimum sanctum* with synergistic effect of their combined form. *Asian Pacific Journal of Tropical Disease*, S396-S398.
38. Chetia, B., & Gogoi, S. (2011). Antibacterial activity of the methanolic extract of stem bark of *Spondiaspinnata*, *Moringaoleifora* and *Alstoniascholaris*. *Asian Journal of Traditional Medicines*, 6(4), 163-167.

