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RESEARCH ARTICLE

PHYTOCHEMICALS ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF GENETIC VARIANTS OF MIRABILIS JALAPA

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ABSTRACT

Mirabilis jalapa Linn. is widely used as a traditional medicine in many parts of the world for the treatment of various diseases. The methanol, acetone, chloroform and diethyl ether extracts from different varieties of *M. jalapa* were screened for phytochemical analysis and antibacterial activities. Phytochemical analysis showed the presence of Alkaloids and glycosides were detected only in the methanolic extracts of all varieties, but were not detected in the other solvent extracts, viz. acetone, chloroform and diethyl ether. Flavonoids and terpenoids were detected in all the four solvent extracts of each variety. Among the four different solvents used for the extraction, the methanol extracts followed by acetone extracts of all variety showed maximum inhibitory activity than the diethyl ether and chloroform extracts. However the methanolic leaf extract of the white flowered variety showed highest antibacterial activity against all organisms at 500 mg/ml concentration. Best activity was observed against *S. aureus* NCIM 5021 followed by *B. subtilis* NCIM 2063, *P. aeruginosa* NCIM 5029 and *E. coli* NCIM 2931. Then second best antibacterial activity was shown by 500 mg/ml concentration of methanolic leaf extracts of pink flowered variety, followed by yellow and orange flowered variety, respectively.

INTRODUCTION

Due to worldwide increasing demand in the field of herbal medicines, it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs. There is a need for the application of this knowledge in authentication, detailed study and practical utilization of crude drugs. The use of traditional medicines and medicinal plants in most countries, as a normative basis for the maintenance of good health has been widely observed (Trivedi, 2006). It has been observed that the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effect. These natural compounds form the basis of modern drugs which we use today (Edeoga *et al*., 2005; Akinmo-laudn *et al*., 2007; Rout *et al*., 2009).

One of such plant which has been used in traditional medicine is *Mirabilis jalapa*, belonging to the family Nyctaginaceae and commonly known as "Four O' clock plant". It is a popular ornamental plant grown worldwide for the beauty of its flowers which can be red, white, yellow, orange or multicoloured and their sweet fragrance. It is extensively used for treatment of dysentery, diarrhoea, conjunctivitis, edema, inflammation, swellings, muscular pain, swelling and abdominal colic, also used as a laxative by people from different countries (Holdsworth, 1992; Comerford, 1996; Encarnaci'on *et al*., 1998; M'arquez *et al*., 1999).

Several constituents have been isolated from the root and aerial parts of this plant including some rotenoids, terpenoids, steroids, phenolic compounds, D-glucoside, ursolic acid, mirabalisoic acid, trigonellin, alanine, alphaamyrins, arabinose, beta amyrins, campesterol, daucosterol and dopamine (Stanic *et al*., 1988; Siddiqui *et al*., 1990; Ali *et al*., 2001; Yang *et al*., 2001; Yi-Fen *et al*., 2002; Wei *et al*., 2003) and Mirabilis Antiviral Protein (Vivanco *et al*., 1999).

The plant has been extensively studied for a variety of bioactive principles and screened for different pharmacological activities. Irrespective of the flower colour, extracts of *M. jalapa* have been reported to possess various bioactive properties including antibacterial, antifungal (Chakraborthy, 2009), antiviral (Vivanco *et al.* 1999), protein synthesis inhibition (Kataoka *et al*., 1991), antinociceptive (Walker *et al*., 2013) anti-inflammatory (Nath *et al*., 2010), anti-allergic and anti-asthmatic (Maxia *et al*., 2010), antidiabetic (Sankar *et al*., 2011), carminative (expel gas), purgative, stomachic tonic (stimulating digestion) and vermifuge properties (expels worms) (Dimayuga *et al*., 1998).

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In order to scientifically apprise some of the ethnomedical uses of the plant, the present study intends to evaluate the bioactive chemical constituents and antibacterial properties of *M. jalapa* commonly used in herbal medicine in India.

MATERIAL AND METHODS

Collection of plant material

The plants of *Mirabilis jalapa* L. of different flower colours (pink, white, yellow, orange) were collected from Maharashtra, India. The plants were maintained in the greenhouse of R.J. College, Mumbai. The leaves of plants were collected in the month of October and used for further analysis.

Preparation of the plant extract

Mirabilis jalapa leaves were thoroughly washed with water to remove dirt, dust and shade dried on a clean filter paper. Once the leaves were completely dried, they were ground into fine powder using mechanical grinder, and kept in air tight container until use (Muthumani *et al.*, 2009). Extraction was performed using the Soxhlet apparatus. Hundred grams of leaves powder was packed into a thimble made up of Whatman filter paper No. 1. The packed thimble was placed into the main extraction unit of the Soxhlet apparatus. All the joints were sealed with petroleum jelly. The temperature of the heating mantle was adjusted to the boiling point of the solvent. Different solvents like methanol, acetone, chloroform, diethyl ether, with decreasing polarity were used. The extraction was carried out for 2 hrs. The extracts thus obtained were concentrated to a gummy material in incubator (Muthumani *et al.*, 2009).

Activated charcoal treatment

1 g of extract was treated with 0.5 g of activated charcoal slurry in the respective solvents and incubated at room temperature for 45 mins. The treated extracts were then filtered through a Whatman filter paper No. 1 and concentrated in incubator at 40°C. The extracts were then kept in sterile bottles at 10°C until further use for phytochemical analysis (Abeysinghe and Weeraddana, 2011).

Sample preparation and Analysis of phytochemicals by HPTLC

HPTLC precoated, silica gel G 60 F25 (Merck, Germany) plates were used for the application of samples. Charcoal treated extracts were dissolved in respective solvents, to prepare 10% of each sample. Samples were applied on precoated plate with the help of Linomat 5 applicator. Phytochemical screening for alkaloids, glycosides, flavonoids and terpenoids were done according to Reich and Schibli (2006).

Preparation of samples for antibacterial analysis

For each extract 500 mg/ml stock solution was prepared by dissolving dried extract residues in respective solvents. From stock solution required concentrations, i.e. 100 mg/ml, 300 mg/ml and 500 mg/ml, were prepared for assay. Solution of streptomycin (10 µg/ml) in sterile distilled water was prepared as positive control. Pure solvents were used as negative control.

Microorganisms used

Four bacterial species were employed as test organisms. These included: Gram negative bacteria: *Pseudomonas aeruginosa* NCIM 5029, *Escherichia coli* NCIM 2931; Gram positive bacteria: *Staphylococcus aureus*, NCIM 5021, *Bacillus subtilis* NCIM 2063, these were obtained as fresh pure cultures from the National Collection of Industrial Microorganisms, Pune, India.

Preparation of bacterial suspension

A loop full of *Pseudomonas aeruginosa* NCIM 5029, *Staphylococcus aureus*, NCIM 5021, *Bacillus subtilis* NCIM 2063 cultures were inoculated in sterile Nutrient broth and *Escherichia coli* NCIM 2931 in sterile Luria broth and incubated for 6 to 8 hrs. at 37 °C. The turbidity of cultures were adjusted to 0.5 McFarland's standard by diluting with sterile saline obtain a bacterial suspension of 10^8 CFU/ml before use (Ezhilarasu and Prabakaran, 2013).

Antibacterial assay by agar well diffusion method

Antibacterial activity was determined against four pathogens by agar well diffusion method (Spooner and Sykes, 1972). Petri plates containing 20 ml of solidified sterile Mueller-Hinton agar media were inoculated with 100 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Each plate containing 5 wells of 8 mm were cut using a cork borer on the surface of the inoculated agar. Different concentrations of Soxhlet extracts, viz. 100, 300, 500 mg/ml were made using respective solvent as diluting agent. 0.1 ml of each of the leaf extracts were aseptically dispensed into three of the wells using a 1.0 ml sterile pipette. Respective solvents of 0.1 ml were introduced into middle well to serve as control. Streptomycin (10 µg/ml) used as the reference antibacterial agent was introduced into fourth well. The plates were incubated in refrigerator for 30 min. in order to allow pre-diffusion of the extracts in the agar wells (Esimone *et al.*, 1998). The antibacterial assay plates were incubated at 37 °C for 24 hrs. After incubation, the plates were observed for zones of inhibition around the well. The observed zones of inhibition were measured and recorded in millimeters (mm). The degree of antimicrobial activity was evaluated using the values obtained from the readings of the zone of inhibition on each of the agar plates. The antibacterial activity of each flower colour extracts of *M. jalapa* were compared with streptomycin, which was taken as a standard (Ullah *et al.*, 2010).

Statistical analysis of data

All reported values are means of triplicates (n = 3). Statistical analysis of the data was performed using the IBM SPSS version 19 software. Comparison between antimicrobial activity was performed using Duncan's Multiple Range Test.

Means that are assigned the same letter(s) are not significantly different from each other at $p < 0.05$.

RESULTS

The preliminary phytochemical screening of *M. jalapa* extract showed the presence of bioactive components like alkaloids, terpenoids, glycosides and flavonoids (Table 1). Alkaloids, glycosides were only detected in methanol extracts of leaf of all varieties. While terpenoids and flavonoids were methanol, acetone, chloroform, diethyl ether extracts of leaves of all varieties.

Gram negative bacteria (*Pseudomonas aeruginosa* NCIM 5029, *Escherichia coli* NCIM 2931) and Gram positive bacteria (*Staphylococcus aureus* NCIM 5021, *Bacillus subtilis* NCIM 2063). The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. The values for zones of inhibition (mean ±SD) have been reported in the Tables 2 - 5.

Among the different solvent extracts studied, methanol showed high degree of inhibition followed by acetone, diethyl ether and chloroform extracts (Fig 1 – 4).

Table 1 Phytochemical screening results of various extracts of leaf of *M. jalapa* varieties.

Sample	Alkaloids	Terpenoids	Glycosides	Flavonoids
Methanol extract of pink leaves	+	+	+	+
Acetone extract of pink leaves	-	+	-	+
Chloroform extract of pink leaves	-	+	-	+
Diethyl ether extracts of pink leaves	-	+	-	+
Methanol extract of white leaves	+	+	+	+
Acetone extract of white leaves	-	+	-	+
Chloroform extract of white leaves	-	+	-	+
Diethyl ether extracts of white leaves	-	+	-	+
Methanol extract of yellow leaves	+	+	+	+
Acetone extract of yellow leaves	-	+	-	+
Chloroform extract of yellow leaves	-	+	-	+
Diethyl ether extracts of yellow leaves	-	+	-	+
Methanol extract of orange leaves	+	+	+	+
Acetone extract of orange leaves	-	+	-	+
Chloroform extract of orange leaves	-	+	-	+
Diethyl ether extracts of orange leaves	-	+	-	+

Key: - '+' Detected and '-'Not detected

Table 2 Antimicrobial activity of leaf extracts of pink flowered *M. jalapa* against test microorganisms

Standard/ Solvent Extracts	Conc.	Zone of Inhibition (mm) Mean ±SD			
		P. aeruginosa NCIM 5029	*E. coli* NCIM 2931	*S. aureus* NCIM 5021	*B. subtilis* NCIM 2063
Streptomycin	10 µg/ml	30.00±0.00	28.00±0.00	33.00±0.00	32.00±0.00
Methanol	100 mg/ml	14.67±0.47	13.00±0.00	16.67±0.47	15.67±0.94
	300 mg/ml	22.33±0.47	20.67±0.47	26.00±0.00	23.67±0.47
	500 mg/ml	25.00±0.00	24.00±0.00	27.67±0.47	27.33±0.47
Acetone	100 mg/ml	13.67±0.47	12.33±0.47	15.67±0.00	15.33±0.47
	300 mg/ml	21.67±0.47	21.33±0.00	24.33±0.00	23.00±0.00
	500 mg/ml	23.00±0.00	22.00±0.47	26.33±0.00	25.67±0.00
Chloroform	100 mg/ml	11.33±0.00	11.00±0.47	13.67±0.47	12.33±0.47
	300 mg/ml	17.33±0.00	16.33±0.47	19.33±0.47	18.33±0.00
	500 mg/ml	20.00±0.47	19.33±0.47	23.00±0.47	22.00±0.00
Diethyl ether	100 mg/ml	12.33±0.47	11.33±0.47	15.00±0.00	13.00±0.00
	300 mg/ml	19.33±0.47	18.33±0.47	21.00±0.00	20.00±0.00
	500 mg/ml	20.67±0.47	20.00±0.00	23.67±0.47	23.00±0.00

Table 3 Antimicrobial activity of leaf extracts of white flowered *M. jalapa* against test organisms

Standard/ Solvent Extracts	Conc.	Zone of Inhibition (mm) Mean ±SD			
		P. aeruginosa NCIM 5029	*E. coli* NCIM 2931	*S. aureus* NCIM 5021	*B. subtilis* NCIM 2063
Streptomycin	10 µg/ml	30.00±0.00	28.00±0.00	33.00±0.00	32.00±0.00
Methanol	100 mg/ml	16.33±0.47	14.33±0.47	20.00±0.00	18.00±0.00
	300 mg/ml	24.33±0.47	23.00±0.00	28.00±0.00	27.00±0.00
	500 mg/ml	26.00±0.00	24.67±0.47	30.00±0.00	29.00±0.00
Acetone	100 mg/ml	15.00±0.00	13.00±0.00	17.67±0.47	16.33±0.47
	300 mg/ml	23.33±0.47	22.00±0.00	25.00±0.00	24.00±0.00
	500 mg/ml	24.33±0.47	23.00±0.82	27.67±0.47	26.67±0.47
Chloroform	100 mg/ml	12.00±0.00	11.33±0.47	14.67±0.47	13.00±0.00
	300 mg/ml	20.00±0.00	19.00±0.00	22.67±0.47	21.00±0.00
	500 mg/ml	21.00±0.00	20.00±0.00	25.00±0.00	24.00±0.00
Diethyl ether	100 mg/ml	13.00±0.00	12.33±0.47	15.67±0.47	14.33±0.47
	300 mg/ml	21.33±0.47	19.67±0.47	23.00±0.00	21.67±0.47
	500 mg/ml	22.67±0.47	21.33±0.47	26.33±0.47	25.33±0.47

The sixteen different crude extracts of methanol, acetone, chloroform and diethyl ether of four varieties, i.e. pink, white, yellow and orange of *M. jalapa* leaves were tested against

It was observed that at 500 mg/ml concentration, methanolic extracts of all *M. jalapa* varieties showed effective antimicrobial activity against all four test organisms.

Table 4 Antimicrobial activity of leaf extracts of yellow flowered *M. jalapa* against test organisms

Standard/ Solvent Extracts	Conc.	Zone of Inhibition (mm) Mean ±SD			
		P. aeruginosa NCIM 5029	*E. coli* NCIM 2931	*S. aureus* NCIM 5021	*B. subtilis* NCIM 2063
Streptomycin	10 µg/ml	30.00±0.00	28.00±0.00	33.00±0.00	32.00±0.00
Methanol	100 mg/ml	13.00±0.00	12.67±0.47	16.33±0.47	15.33±0.47
	300 mg/ml	20.00±0.00	18.33±0.47	24.67±0.47	22.00±0.00
	500 mg/ml	24.33±0.47	22.33±0.47	26.67±0.47	26.00±0.00
Acetone	100 mg/ml	12.67±0.47	12.33±0.47	14.67±0.47	14.00±0.00
	300 mg/ml	21.00±0.00	20.00±0.00	23.33±0.47	21.33±0.47
	500 mg/ml	22.33±0.47	21.33±0.47	25.00±0.00	24.00±0.00
Chloroform	100 mg/ml	11.33±0.47	11.00±0.00	12.00±0.00	11.67±0.47
	300 mg/ml	16.00±0.00	15.33±0.47	18.33±0.47	17.00±0.00
	500 mg/ml	19.00±0.00	18.33±0.47	21.67±0.47	20.00±0.00
Diethyl ether	100 mg/ml	11.33±0.47	11.00±0.00	14.00±0.00	12.33±0.47
	300 mg/ml	17.67±0.47	17.00±0.00	20.00±0.00	19.67±0.47
	500 mg/ml	20.67±0.47	19.33±0.47	23.00±0.00	22.00±0.00

Table 5 Antimicrobial activity of leaf extracts of orange flowered *M. jalapa* against test organisms

Standard/ Solvent Extracts	Conc.	Zone of Inhibition (mm) Mean ±SD			
		P. aeruginosa NCIM 5029	*E. coli* NCIM 2931	*S. aureus* NCIM 5021	*B. subtilis* NCIM 2063
Streptomycin	10 µg/ml	30.00±0.00	28.00±0.00	33.00±0.00	32.00±0.00
Methanol	100 mg/ml	13.33±0.47	13.00±0.00	15.33±0.47	15.00±0.00
	300 mg/ml	19.33 ±0.47	18.00±0.00	22.33±0.47	20.33±0.47
	500 mg/ml	23.33±0.47	21.67±0.47	25.00±0.00	24.33±0.47
Acetone	100 mg/ml	12.00±0.00	12.00±0.00	15.33±0.47	13.33±0.47
	300 mg/ml	20.00±0.00	19.00±0.00	21.67±0.47	20.67±0.47
	500 mg/ml	21.00±0.00	20.67±0.47	23.67±0.47	22.67±0.47
Chloroform	100 mg/ml	11.00±0.00	10.67±0.47	11.67±0.47	11.33±0.47
	300 mg/ml	15.33±0.47	14.00±0.00	17.67±0.47	16.00±0.00
	500 mg/ml	18.00±0.00	17.33±0.47	21.33±0.47	19.33±0.47
Diethyl ether	100 mg/ml	11.00±0.00	10.67±0.47	12.67±0.47	12.00±0.00
	300 mg/ml	16.00±0.00	16.33±0.47	19.00±0.00	18.00±0.00
	500 mg/ml	19.33±0.47	18.33±0.47	22.00±0.00	21.00±0.00

The acetone extracts of all variety showed less activity than methanol extract, but showed more activity than chloroform and diethyl ether extracts. While the minimum inhibitory concentration (MIC) of the all extracts were 100 mg/ml.

However, the methanol extract of white flowered plant leaves showed good antibacterial activity against four test organisms than other three colours i.e. pink, yellow and orange. From the results it has been revealed that irrespective of flower colour, highest growth of inhibition were recorded against *Staphylococcus aureus* NCIM 5021 in methanol extracts of all varieties (25.00 to 30.00 mm) followed by *Bacillus subtilis* NCIM 2063 (24.33 to 29.00 mm), *Pseudomonas aeruginosa* NCIM 5029 (23.33 to 26.00 mm), *Escherichia coli* NCIM 2931 (21.67 to 24.67 mm).

Similarly, in acetone extracts, the maximum zone of inhibition were observed against *Staphylococcus aureus* NCIM 5021 (23.67 to 27.67 mm) followed *Bacillus subtilis* NCIM 2063 (22.67 to 26.67 mm), *Pseudomonas aeruginosa* NCIM 5029 (21.00 to 24.33 mm), *Escherichia coli* NCIM 2931 (20.67 to 23.00 mm).

The diethyl ether extracts exhibited maximum zone of inhibition against *Staphylococcus aureus* NCIM 5021 (22.00 to 26.33 mm) followed *Bacillus subtilis* NCIM 2063 (21.00 to 25.33 mm), *Pseudomonas aeruginosa* NCIM 5029 (19.33 to 22.67 mm) and *Escherichia coli* NCIM 2931 (18.33 to 21.33 mm) (Fig. 1 – 4)

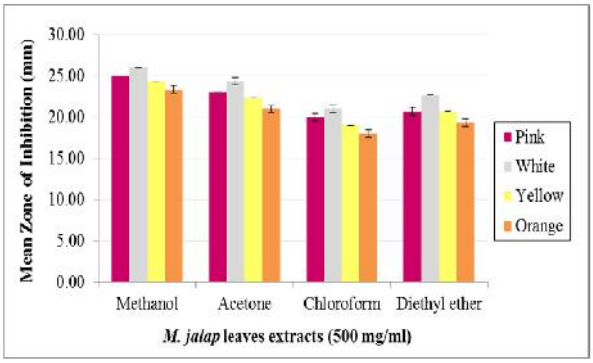

Figure 1 *Antimicrobial activity of different* M. jalapa *variety leaves extracts against* Pseudomonas aeruginosa *NCIM 5029*

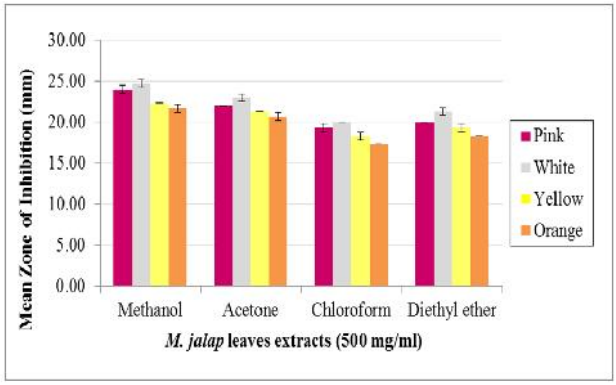

Figure 2 Antimicrobial activity of different *M. jalapa* variety leaves extracts against *Escherichia coli* NCIM 2931

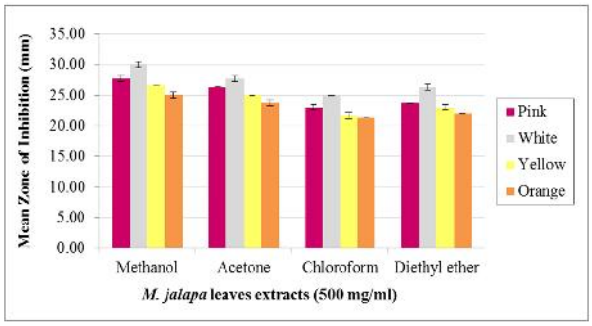

Figure 3 Antimicrobial activity of different *M. jalapa* variety leaves extracts against *Staphylococcus aureus* NCIM 5021

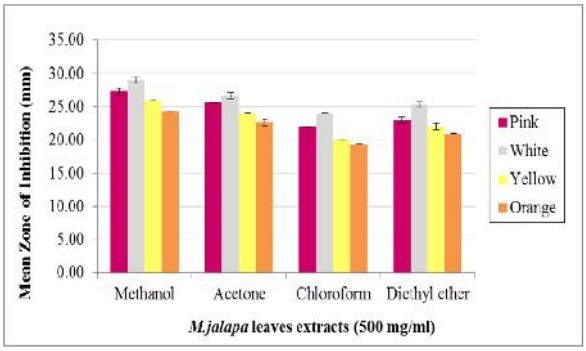

Figure 4 Antimicrobial activity of different *M. jalapa* variety leaves extracts against *Bacillus subtilis* NCIM 2063

Since the methanol extracts of all varieties leaves of a concentration of 500 mg/ml had showed effective antimicrobial activity against four of the strains tested than that of the other three solvent extracts. Therefore the methanol extracts with concentration of 500 mg/ml of all varieties were used for statistical analysis of the data which had been performed using the software SPSS version 19. Comparison between four varieties methanol extracts against four test organisms has been performed using Duncan's Multiple Range Test. Those are assigned the same letter(s) (a,b,c,d), which are not significantly different from each other at $p < 0.05$. The result for the same are shown in Fig. 5 – 8).

As observed in Fig. 5 the methanol extract of white flowered *M. jalapa* leaves showed significantly higher antimicrobial activity against *Pseudomonas aeruginosa* NCIM 5029 than other varieties leaves. While methanol extracts of yellow and pink flowered *M. jalapa* leaves showed similar significant antimicrobial activity and higher than methanol extract of orange flowered variety leaves.

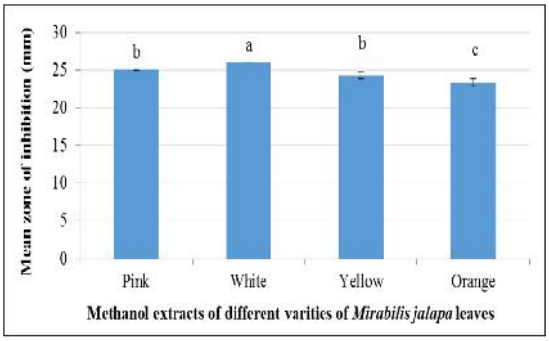

Figure 5 Antimicrobial activity of methanol extracts of different varieties of *M. jalapa* against *Pseudomonas aeruginosa* NCIM 5029.

In Fig. 6 it is observed that methanol extracts of pink and white flowered *M. jalapa* leaves had significantly similar and more antimicrobial activity than yellow and orange flowered *M. jalapa* leaves extracts against *Escherichia coli* NCIM 2931 as test organism. The yellow and orange flowered *M. jalapa* leaves had similar significant effect on *Escherichia coli* NCIM 2931.

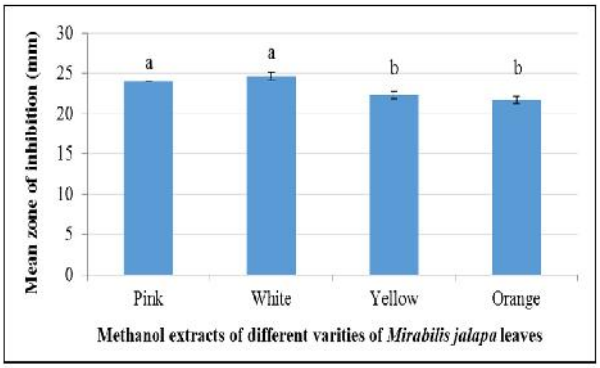

Figure 6 Antimicrobial activity of methanol extracts of different varieties of *M. jalapa* against *Escherichia coli* NCIM 2931.

As seen in Fig. 7 and 8**,** the methanol extract of white flowered *M. jalapa* leaves showed significantly higher antimicrobial activity against *Staphylococcus aureus* NCIM 5021 and *Bacillus subtilis* NCIM 2063 as a test organisms than other methanol extracts. While pink flowered *M. jalapa* showed significantly higher antimicrobial activity against *Staphylococcus aureus* NCIM 5021 and *Bacillus subtilis* NCIM 2063 as a test organisms than methanol extract of yellow flowered *M. jalapa* followed by methanol extract of orange flowered *M. jalapa*.

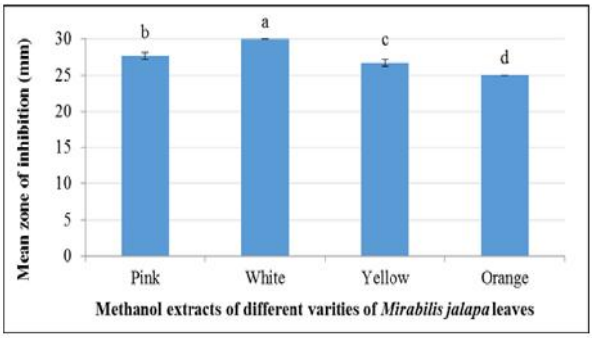

Figure 7 *Antimicrobial activity of methanol extracts of different varieties of* M. jalapa *against* Staphylococcus aureus *NCIM 5021.*

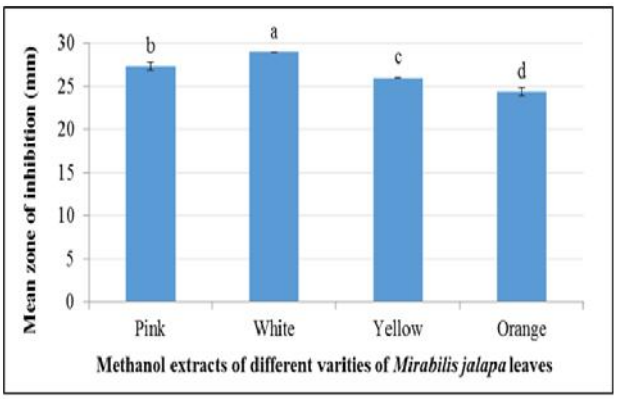

Figure 8 *Antimicrobial activity of methanol extracts of different varieties of* M. jalapa *against* Bacillus subtilis *NCIM 2063*

DISCUSSION

The search for antimicrobials from natural sources had received much attention, and efforts had been put into identifying natural compounds that can act as suitable replacements for synthetic antimicrobial agents. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines for controlling the growth of microorganisms (Ahmad and Beg, 2001). The medicinal plants are rich in these secondary metabolites and among the vast array of bioactive compounds alkaloids, flavonoids, glycosides and terpenoids are of high interest. These compounds have a significant therapeutic application against human pathogens, including bacteria, fungi and viruses (Sharma, 2011).

From the several methods available for separating plant constituents, the chromatographic procedure is the most commonly used techniques for general application (Kokate *et al.*, 2006). The present HPTLC studies confirmed the presence of active metabolites alkaloids, terpenoids, flavonoids and glycosides in *M. jalapa*. In this present investigation, only methanol extracts of all varieties showed the presence of alkaloids and glycosides, which in is contradictory to previous reports of Kumar *et al.* (2010), who observed the presence of alkaloids in acetone and chloroform, Muthumani *et al.* (2009), who observed the presence of glycosides in chloroform extracts and by Selvakumar *et al.* (2012), who detected alkaloids and glycosides in acetone extract. Therefore it can be concluded that the other extracts may contain trace amount of alkaloids and glycosides which could not detected at the concentrations used in the present study.

In the present study we investigated the presence of terpenoids and flavonoids in methanol, acetone, chloroform and diethyl ether extracts of all varieties, which is in accordance with the results obtained by Chakraborthy (2009) and Ezhilarasu and Prabakaran (2013).

Antibacterial activity of various solvent extracts of *M. jalapa* (flower colour variants) has been evaluated *in vitro* against four test organisms by agar well method. Antibacterial activity was found to be concentration dependent for all different samples tested. Among the four different solvents used for the extraction, the methanol extracts followed by acetone extracts of all variety showed maximum inhibitory activity than the diethyl ether and chloroform extracts. Similar results on antibacterial activities of *M. jalapa* were also reported by other studies (Chakraborthy, 2009; Ullah *et al.*, 2011). The effectiveness of the extracts in inhibiting the growth of the test organisms improved with increasing concentration. The zones of inhibition at 500 mg/ml concentration were higher than 100 mg/ml concentration. From all the results, it can be inferred that the activity of the extract is concentration-dependent. This is in agreement with an earlier report that an increase in the concentration of an antimicrobial agent might result in an increase in its effectiveness (Aspen, 2000). However, the methanolic extract of white flowered plant leaves showed good antibacterial activity against all tested microbial strains followed by pink then yellow and orange coloured plant leaves, which is similar to the result obtained by Ullah *et al.* (2011).

The results of this study revealed that these extracts contained compounds which were able to inhibit the growth of the selected bacteria. *M. jalapa* has a wide variety of secondary metabolites, such as alkaloids, terpenoids, and flavonoids which have antibacterial properties (Adebajo *et al.*, 1983). According to the phytochemical screening of active compounds, it was observed that the plant contains alkaloids, flavonoids, glycosides and terpenoids. As noted earlier, these secondary metabolites may have antimicrobial activity against the tested bacterial species.

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