

Original Research Article

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HPTLC Finger Print Profile and Evaluation of *in vitro* Antimicrobial Activity of *Mirabilis jalapa* L. Flowers

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ABSTRACT

The present study has been designed to carry out the phytochemical screening, HPTLC finger print profile and *in vitro* antimicrobial activity of aqueous flowers extract of *Mirabilis jalapa*. Preliminary phytochemical screening confirmed the presence of alkaloids, anthraquinones, glycosides, flavonoids, tannins, terpenes etc., In HPTLC analysis, quercetin bands were identified in sample chromatogram (5 and 10 μ l) which was confirmed by the chromatogram obtained from the standard quercetin and by comparing retention factors of quercetin (Rf - 0.51) value is from sample and standard solution. *In vitro* antimicrobial activity of plant extract (50, 100 and 150 μ g/ml) was also performed by using disc diffusion method against the pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *E.coli*, pathogenic fungi such as *Candida albicans* and *Trichoderma viride*. 150 μ g of aqueous flowers extract was active against both the bacterial and fungal species. Standard antibiotics gentamicin, amphotericin-B were also performed for antibacterial and antifungal activity respectively. The results of our study indicated that flavonoid quercetin is present in the plant extract which may responsible for potent antimicrobial activity against pathogenic microorganisms.

Keywords

Antimicrobial, flowers extract, HPTLC, *Mirabilis jalapa*, phytochemical, quercetin.

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Introduction

Human beings have used plants for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries together with their side effects and lack of healthcare facilities (Griggs *et al.*,

2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al.*, 1999; Idu *et al.*, 2007; Mann *et al.*, 2008; Ammara *et al.*, 2009).

In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for

hundreds of years (Ali *et al.*, 1998; Barbour *et al.*, 2004; Yasunaka *et al.*, 2005). Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Ibrahim, 1997; Koshy *et al.*, 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with drugs and this has resulted in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics (Okemi *et al.*, 2003) has led to the search new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages (Bouamama *et al.*, 2006). Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno medicinal uses (Arora *et al.*, 2007). The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases. In recent times, plants are being extensively explored for harboring medicinal properties. Keeping in view the above states, the present study deal with the phytochemical investigation, development of HPTLC finger printing and *in vitro* antimicrobial activity of aqueous flowers extract of *Mirabilis jalapa* L.

Mirabilis jalapa (Family: Nyctainaceae) is a small glabrous herb grows up to 0.5m in height, with succulent stems. Flowers are tubular, cluster, funnel-shaped, simple or double, fragrant, white, yellow, pink or purple. An individual flower opens for one

night in the early evening, the exact time depending on temperature and relative humidity, and closes the next morning. An individual plant produces between 25 and 75 flowers in one flowering season. The seeds are olive, brown or black in color. The root system consists of a fairly thickened and tuberous up to 1mm high, stem swollen at nodes (Muthumani *et al.*, 2009).

Phytoconstituents

Roots, seeds and leaves are the reported plant part used from ancient times. The major phytoconstituents found in *Mirabilis jalapa* are the rotenoids (mirabijalones A- D boeravinones C and F), an isoquinoline derivate as well as terpenoids, steroids, phenolic compounds, alanine, alpha-amyrine, arabinose, beta-amyrins, oleanolic acid, trophan, stigmasterols, betasistosterol, beta-D -glycoside, urolic acid, mirablisoic acid, mirabalisol, trigonellin and antiviral protein, C-methylabron isoflavone, tartaric acid, betanin, brassicasterol, betalanic acid. *Mirabilis jalapa* is rich in many active compounds of which, particular interest to researchers is a group of amino acid proteins, called *Mirabilis* antiviral protein.

Traditional Uses

Mirabilis jalapa L is known as a very bioactive plant and used in various diseases as folklore medicine. The leaves are used as traditional folk medicine in the south of Brazil to treat inflammatory and painful diseases and as a laxative. In traditional medicine, *Mirabilis jalapa* is widely used as antidiarrhetic, antiparasitic, carminative, digestive stimulant, tonic, vermifuge, wound healer. Seeds are used as cathartic. This plant contains bio active components like trigonellin as a purgative and rotenoids used as an antispasmodic. In Ayurveda, this plant used for boils, inflammations, constipation,

diabetes, urinary disorders. Dried flowers used as a snuff for headaches, fungal infection and root decoction is used to wash wounds, treat skin afflictions as leprosy (Kiran kumar *et al.*, 2010). It is also used a remedy for the kidney stones and gall bladder (Vardhana, 2008).

Materials and Methods

Collection of Plant Materials

The mature flowers of *Mirabilis jalapa* were collected from Koothanallur at Thiruvarur District in Tamil Nadu. Then the plant flowers were identified, authenticated and the specimen was deposited in the Department of Biochemistry, S.T.E.T. Women's College, Mannargudi, Thiruvarur District, Tamil Nadu.. The plant material was cleaned, and then they were shade dried. The dried plant materials were coarsely powdered using a mechanical grinder. The powder was stored in air light resistant container for further analysis.

Preparation of Plant Extract

100g of the flower powder was macerated using 500 ml of distilled water for 8-10 hours. The extract was filtered through what man No. 1 filter paper to remove all extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure. The dried extract was used for further analysis.

Preliminary Phytochemical Screening

The flowers extract was subjected to preliminary phytochemical investigations to determine the different phytoconstituents using standard procedures (Harborne, 1984).

HPTLC Finger Print Profile

About 1mg of the extract of the *Mirabilis jalapa* L. was taken and dissolved in respective solvent and the volume was up to 1ml in a standard flask (1000 µg/ml). This solution was used as test solution for HPTLC analysis. Silica gel 60 F254 and HPTLC aluminum sheets were used as adsorbent (stationary phase). 5 and 10 µL of the above test solution and 2 µL of a standard solution (quercetin) were loaded on HPTLC aluminum sheets as different tracks in the form of 6 mm wide bands by using a CAMAG semi-automatic Linom at 5 spotters at a distance of 12mm. Nitrogen gas was also supplied for simultaneous drying of bands.

The samples-loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with mobile phase toluene: ethyl acetate: formic acid (5:4:1). The developed plates were then dried and scanned using a TLC scanner 3 with Wincats software under 364 nm. All plates were visualized directly after drying and a fingerprint profile was photo documented using a CAMAG Reporter- 3 under 254 nm and 366 nm in UV and visible light. The peak table, peak display and peak densitogram were recorded (Srivastava, 2011; Sethi, 2013).

Antimicrobial Activity

Selection of Microorganism

Totally six pathogenic microorganism such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Bacillus subtilis* and *Aspergillus niger*, *Trichoderma viride* were selected for antimicrobial activity. The pure microbial culture were collected from Centre for Life Science Research, Thanjavur, and inoculated in the tube using inoculation needles or loops. The tubes were

incubated at 37°C for 24 hours.

Screening of Antimicrobial Activity by Agar Well Diffusion Method

The antibacterial and antifungal activities of flowers extract of *Mirabilis jalapa* was tested against the selected bacterial and fungal strains. Nutrient agar medium (for bacteria) and potato dextrose agar medium (for fungi) were prepared by sterilizing them with the use of autoclave at 121°C and 15 lbs pressure for 15 minutes; petriplates were also sterilized using autoclave. After sterilization, about 25 ml the cooled medium was poured into petriplates and allowed to solidify in a laminar air flow chamber. After solidification, the test bacterial and fungal cultures were spread by using a sterile cotton swab over the plate by spread plate technique. Well of 5mm size made in the agar plates with the help of sterile cork borer, each well were loaded with different concentration (50, 100, 150 µl) of aqueous flowers extract of *Mirabilis jalapa* using micropipette and allowed to diffuse at room temperature for 2 hours. The antibiotics gentamicin and amphotericin B were used as standard for antibacterial and antifungal activity respectively. All the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the antibacterial and antifungal activities. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (Bauer *et al.*, 1966; Perez *et al.*, 1990)

Statistical Analysis

Values of antimicrobial activities were expressed as mean value of triplicate ± S.D

Results and Discussion

In the present study, phytochemical

screening, HPTLC finger printing analysis and *in vitro* antimicrobial activity were carried out in aqueous flowers extract of *Mirabilis jalapa*. The obtained results were recorded and tabulated.

Preliminary Phytochemical Screening

Table 1 shows the phytoconstituents of aqueous extract of *Mirabilis jalapa* flowers. Results revealed the presence of alkaloids, anthroquinones, glycosides, flavonoids, steroids, tannins, terpenes and absence of carbohydrate, saponins, and proteins.

HPTLC Finger Printing Analysis

HPTLC finger prints of aqueous flowers extract (5µl and 10µl) of *Mirabilis jalapa* were done by using selected solvent system toluene: ethyl acetate: formic acid (5:4:1), visualized under UV 254 nm before derivatization. The peaks obtained were analyzed and the R_f value was compared to the standard. The presence of a specific peak for quercetin at R_f 0.51 was recorded and considered as a positive result for quercetin (fig. 2, 3 & table 2, 3,4).

Antimicrobial Activity

Antimicrobial activity was performed in aqueous flowers extract of *Mirabilis jalapa* against pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *E.coli* and pathogenic fungi such as *Candida albicans*, and *Trichoderma viride* by disc diffusion method.

Antibacterial Activity

The anti bacterial activities of plant extract against tested bacterial pathogen were represented in table 5. Three different concentrations (50, 100, and 150 µg/ml) of plant extracts were selected, all are found to

be active against both gram positive and gram negative bacterial strains. Maximum zone of inhibition was observed in 150µg of plant extract against *Pseudomonas aeruginosa* (56 mm) followed by *Bacillus subtilis* (36 mm), *E.coli* (28 mm) and *Staphylococcus aureus* (27 mm). Moderate results were obtained in 100µg of plant extract against *Pseudomonas aeruginosa* (42.5 mm) followed by *Bacillus subtilis* (27 mm), *Staphylococcus aureus* (26 mm) and *E.coli* (25 mm) and least activity was observed in 50 µg of plant extract against *Pseudomonas aeruginosa* (42.4 mm) followed by *Bacillus subtilis* (26 mm) and *E.coli* (25 mm) and *Staphylococcus aureus* (26 mm). Antibacterial activity of standard antibiotic gentamicin was also performed and the zone of inhibition were 63mm for *E.coli*, 68.8 mm for *P. aeruginosa*, 46.5mm for *B. subtilis* and 58.2 mm for *S.aureus*. When compared with antibiotic, plant extract exhibited moderate antibacterial activity against all tested pathogenic bacteria (fig.7).

Antifungal Activity

In the present investigation, antifungal activities of three different concentration of (50, 100, 150 µg) flower extract of *Mirabilis jalapa* have been evaluated *in vitro* against fungal strains *Trichoderma viride* and *Candida albicans* (table 5, fig 8). Maximum zone of inhibition was observed in 150 µg of plant extract against *Candida albicans* (33.5 mm) followed by *Trichoderma viride* (25.5 mm). Moderated results were obtained in 100 µg of plant extract in the order of *Candida albicans* and *Trichoderma viride* and their zone of inhibition in mm were 32.5 and 27 respectively. Similarly, least result of 25.5 and 33.5 mm was observed in 50µg of plant extract against *Candida albicans* and *Trichoderma viride*. Standard antibiotic amphotericin-B was also performed for its

antifungal activity and the inhibitory zone were 30mm and 37.3mm for *Trichoderma viride* and *Candida albicans* respectively. When compared with standard, plant extract exhibited moderate antifungal activity against all tested pathogenic fungi.

In the recent years, research on medicinal plants has attracted a lot of attention globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Phytochemicals are chemical compounds synthesized during the various metabolic processes. Various phytochemicals possess a variety of pharmacological activities. These chemicals are often called secondary metabolites. Some of these are found to have *in vitro* antimicrobial properties (Dahanukar *et al.*, 2000; Cowan, 1999) and serve as plant defence mechanisms against pathogenic organisms. These compounds are classified as phenols, quinines, flavonoids, tannins, alkaloids, glycosides and polysaccharides.

In the present study, result of preliminary phytochemical analysis revealed the presence of alkaloids, anthroquinones, glycosides, flavonoids, steroids, tannins, terpenes and absence of carbohydrate, saponins, and proteins. Flavonoids like quercetin and catechin are becoming the subject of medical research (Gupta *et al.*, 2012). Flavonoids interfere with specific intracellular or surface enzymes and many bacterial virulence factors such as toxins, enzymes and signal receptors (Cushnie, 2011).

HPTLC, High Performance Thin Layer Chromatography, is the most recent evolution of planar chromatography and has been specifically tailored for analysis of

natural products (Pharmacopée Française, 1965; Reich and Schibli, 2007). It is one of the modern sophisticated techniques that can be used for evaluating the potency, authenticity, quality and purity of crude drugs (Mamatha, 2011). It offers better resolution, and estimation of active constituents can be done with reasonable accuracy in a shorter time (Syed *et al.*, 2013).

In the present study, HPTLC finger printing profile of plant extract was performed at the wave length of 254nm in TLC scanner. The result gave a sharp and well -defined band with $R_f = 0.51$ for quercetin. This showed the presence of the bioactive compound flavonoid. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the

reference standard solution (Sachin Rakesh *et al.*, 2009). Quercetin is a flavonol, one of the most abundant natural flavonoid, being extensively found in many plants. It is of great interest because of its pharmacological function (Walid *et al.*, 2015).

Antimicrobial activity can be regarded as a very important study, particularly at this specific period of human history due to bacterial resistance that is constantly imposing new scientific challenges. In spite of great development in drug therapy, infectious diseases remain as the most common reasons for mortality in many developing countries. Resistance to antimicrobial agents has become an increasingly important and pressing global problem. According to the WHO, plants are a source of compounds that have the ability to combat disease.

Table.1 Preliminary phytochemical screening of *Mirabilis jalapa* flowers

S.No.	Phytochemicals	Results
1.	Alkaloids	+
2.	Anthraquinones	+
3.	Carbohydrates	-
4.	Glycosides	+
5.	Flavonoids	+
6.	Saponins	-
7.	Steroids	+
8.	Tannins	+
9.	Terpenes	+
10.	Protein	-

+ - indicate presence: - indicate absent

Table.2 HPTLC finger print profile for standard (2 µl)

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.45	6.4	0.51	468.1	100.00	0.55	0.1	9671.8	100.00	Quercetin

Table.3 HPTLC finger print profile for sample (5 µl)

peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.03	0.1	0.07	98.4	49.39	0.11	0.1	2169.1	43.94	Unknown
2	0.14	0.1	0.21	50.9	25.54	0.24	3.7	1533.5	31.07	Unknown
3	0.24	3.8	0.27	15.8	7.91	0.32	0.1	445.3	9.02	Unknown
4	0.40	5.5	0.43	11.1	5.56	0.44	10.0	232.5	4.71	Unknown
5	0.47	11.3	0.50	23.1	11.60	0.53	0.1	555.9	11.26	Quercetin

Table.4 HPTLC finger print profile for sample (10 µl)

peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.03	4.2	0.07	123.0	34.87	0.10	0.4	3343.6	34.12	unknown
2	0.14	0.2	0.21	91.7	26.00	0.24	12.5	2975.5	30.36	unknown
3	0.24	12.9	0.27	32.1	9.11	0.33	0.0	1046.8	10.68	unknown
4	0.39	3.1	0.45	17.4	4.92	0.43	16.5	627.5	6.40	unknown
5	0.47	16.5	0.50	27.3	7.73	0.51	25.7	655.3	6.69	Quercetin
6	0.51	25.9	0.53	38.0	10.77	0.57	1.8	930.5	9.49	unknown
7	0.96	0.2	0.98	23.3	6.60	0.99	17.2	221.2	2.26	unknown

Table.5 Antibacterial activity of aqueous extract of *Mirabilis jalapa* L. flowers

S. No.	Bacterial strains	Zone of Inhibition (mm in diameter)			
		Plant extract (µg/ml)			Standard antibiotic
		50 µg/ml	100 µg/ml	150 µg/ml	
1	<i>Escherichia coli</i>	25.5±0.5	25±0.28	28±0.76	63±0.36
2	<i>Pseudomonas aeruginosa</i>	42.5±1.04	42.5±1.25	56±0.76	68.8±0.11
3	<i>Bacillus subtilis</i>	26±1.60	27±2.17	36±0.86	46.5±0.5
4	<i>Staphylococcus aureus</i>	24±1.32	26±2.46	27±1.32	58.2±97.4

All values are expressed as mean ± SEM for three determinations.

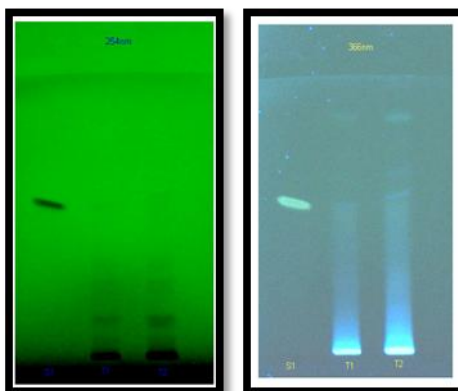
Table.6 Antifungal activity of aqueous extract of *Mirabilis Jalapa* L.flowers

S. No.	Fungal strains	Zone of Inhibition (mm in diameter)			
		Plant extract (µg/ml)			Standard antibiotic
		50 µg/ml	100µg/ml	150 µg/ml	
1	<i>Trichoderma viride</i>	19.5±0.5	27±1.32	25.5±0.86	30±0.25
2	<i>Candida albicans</i>	26.5±0.76	32.5±1.60	33.5±1.04	37.3±0.40

All values are expressed as mean ± SEM for three determinations.

Fig.1 HPTLC finger print profile

Photo documentation under UV



S1 S2 S3
AT 254 nm

S1 S2 S3
AT 366nm

3D DISPLAY & 254 nm

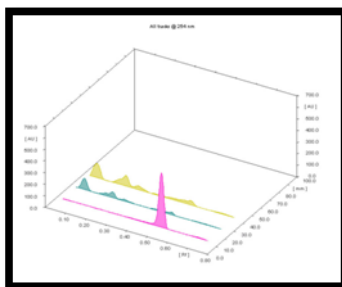


Fig.2 HPTLC Chromatogram for standard

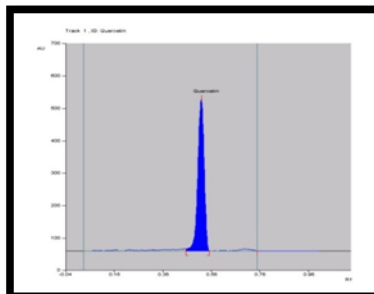


Fig.3 HPTLC Chromatogram for sample (5µl)

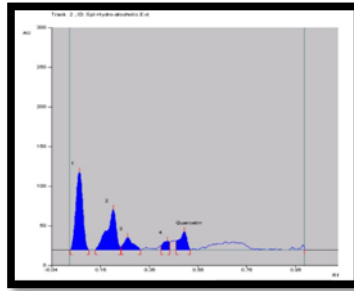


Fig.4 HPTLC Chromatogram for sample (10µl)

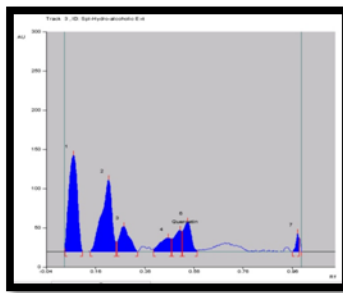


Fig.5

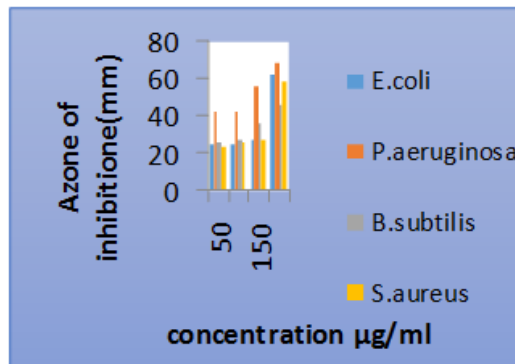
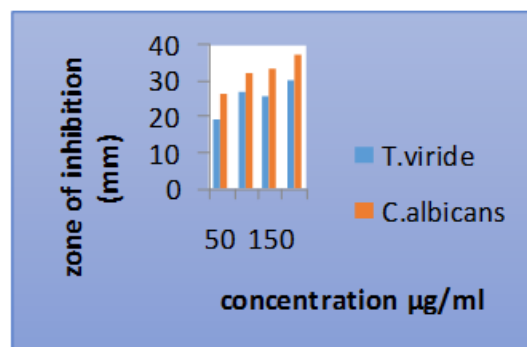


Fig.6



In the present study, antibacterial activity has been performed in the plant extract against bacterial pathogens by disc diffusion method. The maximum zones of inhibition were observed which was ranged between 27 mm and 56 mm diameter at the concentration of 150 µg. Almost all the bacteria were susceptible to plant extract, though in different concentrations which was less than that of standard antibiotic. The result revealed that the aqueous flower extract of *Mirabilis Jalapa* possessed significant inhibitory activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *E.coli*. Antimicrobial activities of the aqueous extract of *Mirabilis jalapa* have been attributed to the presence of the phytoconstituents (Oladunmoye, 2007). Previous study reported that various leaf extracts of *Mirabilis jalapa* have been shown to be significantly effective against gram positive and gram negative bacteria (Muthumani *et al.*, 2009).

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper price (Mann *et al.*, 2008). Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Bennett and Wallsgrove, 1994; Osbourne, 1996).

In the present study, antifungal activity of plant extract was performed against pathogenic fungi *Trichoderma viride* and *Candida albicans* by disc diffusion method. Among the three different concentration of plant extract, 150 µg showed maximum

zone of inhibition ranges between 19.5 to 33.5 mm which was less than that of standard antibiotic amphotericin-B (30 to 37.3mm). The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999).

In conclusion, the result of the present study showed that the aqueous extract of *Mirabilis jalapa* flowers exhibited the greatest antimicrobial activity against gram positive, gram negative and fungal species. The study also reports HPTLC analysis of quercetin from flower extract. The overall study, thus, emphasizes the potency of *Mirabilis jalapa* as a sustainable source of new broad spectrum of antimicrobial products which may be due to the presence of active constituent quercetin. However, further research is required to determine the compound that has contributed to the antimicrobial activity of the extract and its exact mode of action.

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