RESEARCH ARTICLE JPAR 2021

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Assessment of Phytochemical evaluation and Antibacterial activity of *Mirabilis jalapa:* An *in vitro* design

Ceela swetha, Chandragiri Bhavani Shankar, Dabbi Sravanthi, Dandaboina Pooja, Vasudha Bakshi, Narender Boggula*

School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Hyderabad, Telangana, India.

Received: 30.07.2020 Revised: 14.08.2021 Accepted: 22.08.2021 Published: 31.08.2021

ABSTRACT: Back ground: Plants are making use of completely as raw drugs for many formulations in traditional systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, an authentic pharmacognostic study is required for each raw drug. Usually, the drugs are collected by traditional practitioners who have inherited Ayurveda or other herbal practices. **Aim:** The aim of project work is to assess the phytochemical screening, *in-vitro* antibacterial activity of *Mirabilis jalapa* leaves using ethanol as a solvent. **Method:** The coarse powder form of leaves of M. Jalapa was extracted by Soxhlation. The antibacterial activity was assessed by cup plate and disc diffusion method. Streptomycin was used as standard drug. **Results:** Phytochemical results indicated that *M. jalapa* possesses Alkaloids, Terpenes, Carbohydrates, Proteins and Amino acids in. Results reveals that the ethanolic extract of *M. jalapa* leaves was significantly effective against gram-positive, and gram-negative bacteria. The zone of inhibition was measured to be the positive result for the given samples by these two methods. **Conclusion:** It could be concluded that the leaves of M. Jalap significantly showing antibacterial activity. A detailed study needs to be carried out to isolate bioactive compounds that show antibacterial activity.

Corresponding author*
Mr. Narender Boggula
Asst. Professor,
School of Pharmacy, Anurag University,
Venkatapur, Ghatkesar, Medchal,
Hyderabad, Telangana-500088, India.
Tel: +91 966655 2211
Mail ID: narender.b987@gmail.com

Keywords: Cardiovascular Disease, Cross sectional observational study, Antiplatelet, Myocardial Infarction, Ischemic Heart Disease, Prescribing patterns.

INTRODUCTION:

The Indian subcontinent is enriched by a variety of flora-both aromatic and medicinal plants. This is used in a wide diversity of climatic conditions in India ranging from deserts to swamp lands. Numerous types of herbs have been well recognized from the Himalayan to Kanyakumari. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine [1,2]. The traditional system of medicine is so engrained in our culture that, even now, 75 % of the Indian population depend on this

indigenous system for relief [3]. The plant products which have been in use for such a long time be scientifically supported for their efficacy [4]. Throughout the world, about 35,000 to 70,000 species of plants have been used as medicinal, nutraceuticals and cosmeceuticals purposes. In India, about 1,000 plant species are used [5,6].

Antibiotics are one of our most important weapons in fighting bacterial infections. Many commonly used antibiotics have become less and less effective against certain illnesses due to toxic reactions and drug resistance. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [7].

Fig 1. *Mirabilis jalapa* flowering plant.

Fig 2. *Mirabilis jalapa* leaves.

Mirabilis jalapa, the marvel of Peru or four o'clock flower, is the most commonly grown ornamental species of *Mirabilis* plant. *Mirabilis* in Latin means wonderful and Jalapa is the state capital of Veracruz. *M. jalapa* was cultivated by the Aztecs for medicinal and ornamental purposes. The flowers usually open from late afternoon or at dusk. Flowers then produce a strong, sweet-smelling fragrance throughout the night, and then close for good in the morning (Fig 1 and 2) [7,8]. The aim of the present project work is to assess the antibacterial activity of *M. jalapa* leaves.

MATERIALS AND METHODS:

Ethanol was procured from Merck, India. All other chemicals and reagents used in this study were of analytical grade and procured from an authorized dealer.

Collection of plant:

The plant material was collected from surrounding areas in Uppal, Hyderabad, Telangana, India. The fresh leaves were collected and washed with tap water and latter with deionized water and dried under shade. The plant material was regularly checked for fungal growth or rotting. After the plant material was dried it was powdered with the help of an electric blender and sieved through size 80 sieve to obtain a uniform fine particle size. This plant material was stored in airtight containers at 4 °C for future usage [9].

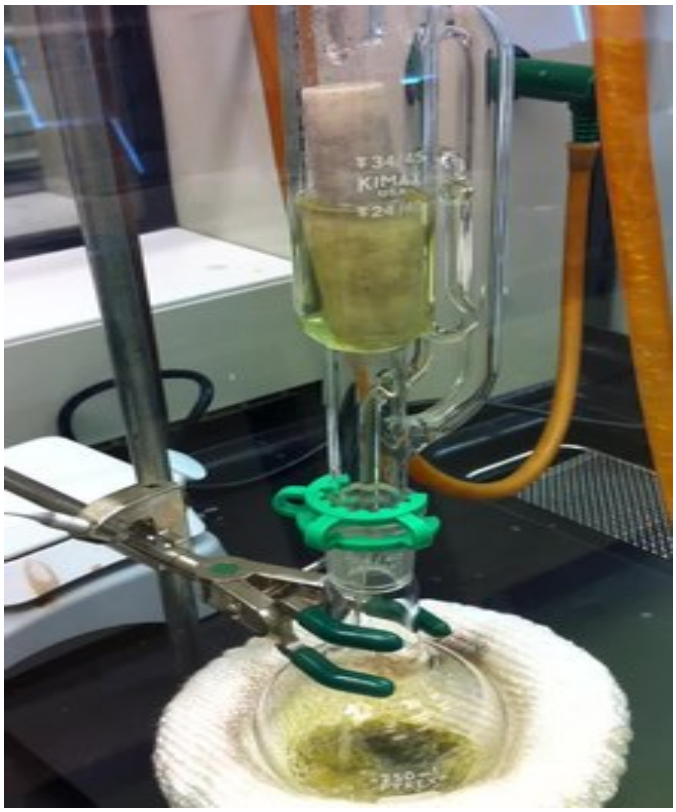

Fig 3. Extraction of leaves by Soxhlation.

Preparation of extract:

Accurately weighed 250 g of the sieved coarse powder was extracted with ethanol in a Soxhlet extractor (Borosil, India) for 72 h as presented in Fig 3. The extract thus obtained was concentrated under reduced pressure to yield crude plant extract. The extract was stored at 4 °C in amber coloured glass stopper vials for further use [10,11].

Qualitative Phytochemical Evaluation:

The ethanolic leaves extract of *M. jalapa* was screened for the detection of phytochemicals that are Alkaloids, Carbohydrates, Glycolides, Terpenes, Flavonoids, Saponins, Sterols, Tannins, Resins, Proteins, Quinones, Fats, Volatile oils, Fixed oils and Amino acids, as per the standard procedure mentioned in the Ayurvedic Pharmacopoeia [12,13].

***In-vitro* antibacterial activity screening [14-17]:**

***Cup plate method*:**

The stock solution of the antibiotic was prepared at solution strength of 1000 µg/ml. the stock solution was diluted suitably. The known concentration of the standard and the test antibiotic solution was prepared. The Muller-Hinton agar medium was sterilized in an autoclave at 121 °C at 15 lbs pressure for 15 min. About 1 ml suspension of standard test organism was added to Muller Hinton medium and mixed thoroughly while maintaining temperature at 50 °C. The above mixture was poured into petridish to form a layer of about 3 mm thickness. The medium allowed solidifying. At the centre of the medium, a hole was made with sharp tool such as cork borer. The cup was marked as per dilutions and each cup was added with the respective dilutions of the antibiotic. The plate was kept carefully in the refrigerator for diffusion of anti-biotic for 20 min. The condensed water was wipped carefully from the lid of the petridish, with the sterile cotton plugs and the petridish was incubated at 37 °C for 24 h.

***Disc diffusion method*:**

Select a pure culture plate of one of the organisms to be tested. Aseptically emulsify a colony from the plate in the sterile saline solution. Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution. Repeat until the turbidity of the saline solution visually matches that of the standard turbidity. Take a sterile swab and dip it into the broth culture of organism. Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab. Take a sterile Mueller-Hinton agar (MHA) plate or a nutrient agar (NA) plate. Use the swab with the test organism to streak an MHA plate or a NA plate for a lawn of growth. After the streaking is complete, allow the plate to dry for 5 min. Antibiotic discs can be placed on the surface of the agar using sterilized forceps. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop. Carefully invert the inoculated plates and incubate for 24 h at 37 ^{0}C. After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone. Compare the measurement obtained from the individual antibiotics to the standard table to determine whether the tested bacterial species is sensitive or resistant to the tested anti-biotic.

RESULTS AND DISCUSSION

Phytochemical investigation:

The ethanolic extract of *M. jalapa* leaves were subjected to qualitative chemical tests whore result is illustrated in Table 1. The leaves of M. jalapa contains the phytochemicals that are Terpenoids, Saponins, Steroids, Flavonoids, Carbohydrates, Alkaloids, Tannins, Proteins, Anthraquinones and Terpenoids.

Table 1. Preliminary phytochemical screening of *M. jalapa* leaves.

Sl. No.	Phytochemicals	Ethanolic extract
1	Terpenoids	+
2	Saponins	+
3	Steroids	+
4	Phenols	-
5	Flavonoids	+
6	Coumarins	-
7	Carbohydrates	+
8	Alkaloids	+
9	Quinones	-
10	Tannins	+
11	Proteins	+
12	Oils & fats	-
13	Anthraquinones	+
14	Anthocyanins	-
15	Amino acids	-
16	Volatile oils	-
17	Terpenoids	+

(+) indicates present, (-) indicates absent.

***In-vitro* antibacterial activity:**

Antibacterial assay of the ethanolic extract of dried leaves of *M. jalapa* exhibited dose dependent antibacterial activity against the tested micro-organisms at three different concentrations. The potential sensitivity of the extract was obtained against all the tested micro-organisms and the zone of inhibition was recorded and presented in the following tables and figures given below (Table 2 and 3, Fig 4 and 5).

Table 2. Zone of inhibition shown by the standard drug (Streptomycin) by cup plate method.

Sl. No.	Name of microorganism	Conc. (µg/ml)		
		10	20	30
		ZOI (cm)		
1	*E. coli*	2.5	2	2.7
2	*Klebsiella pneumonia*	0.5	1.5	1.5
3	*Staphylococcus aureus*	1.5	1.3	1.7
4	*Bacillus subtilis*	1.6	1.5	1.8

ZOI - Zone of Inhibition.

Table 3. Zone of inhibition shown by the ethanolic extract of *M. jalapa* leaves by cup plate method.

Sl. No.	Name of microorganism	Concentration (µg/1ml)		
		10	20	30
		ZOI (cm)		
1	*E. coli*	1.4	2.5	2.6
2	*Klebsiella pneumonia*	1.4	1.6	1
3	*Staphylococcus aureus*	1.2	1.4	1.6
4	*Bacillus subtilis*	2	2.2	2.5

ZOI - Zone of Inhibition.

The antibacterial assay was done by cup plate and disc diffusion method with the observations of Zone of Inhibition (ZOI) at different concentrations (Given in Table 4 and 5, Fig 6).

Plants are important source of pharmacophore which will function as new chemotherapeutic agents. The first step to develop a chemotherapeutic agent from plants would be the assay of *in-vitro* antibacterial activity. The extracts thus found active will help to identify the active compounds responsible for the activities from the plant. In recent years multi drug resistance is seen in pathogenic bacteria which has revived interest in the search of new antibacterial agents from natural sources. In fact, gram negative bacteria *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the anti-biotics. But the extract shows a good activity against bacterial strains. The antibacterial agents from natural sources also eliminate the side effects of synthetic or semi synthetic antibacterial agents. The antibacterial activity of the plant extract was variable with various organisms.

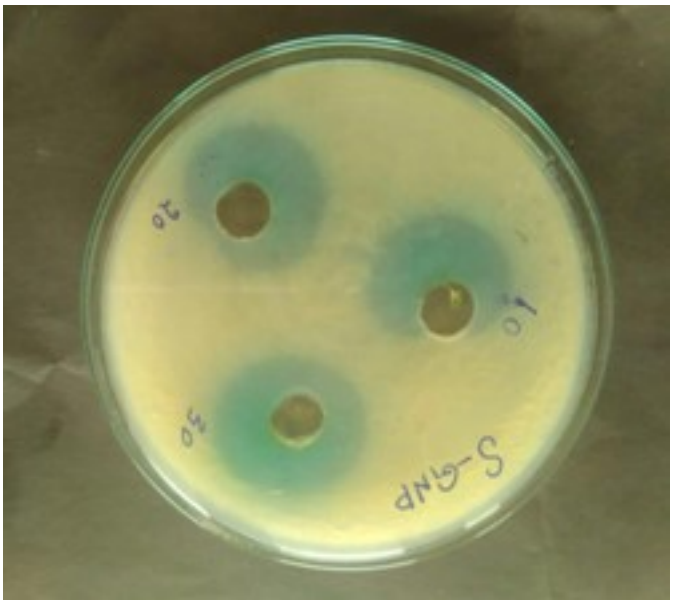

Fig 4. Zone of inhibition shown by the standard drug (Streptomycin).

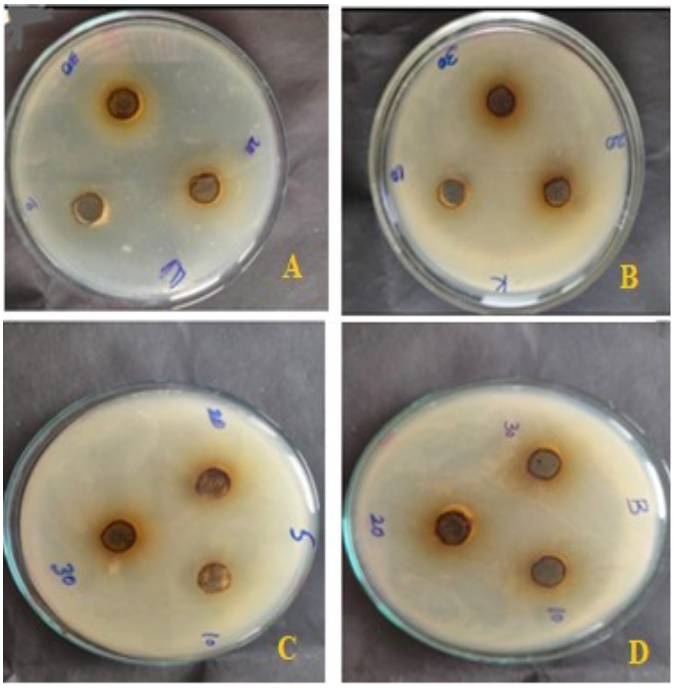

Fig 5. Zone of inhibition shown by the ethanolic extract of *M. jalapa* leaves A) *Escherichia coli* B) *Klebsiella pneumonia* C) *Staphylococcus aureus* D) *Bacillus subtilis,* by cup plate method.

Table 4. Zone of inhibition shown by the standard drug (streptomycin) by Disc diffusion method.

Sl. No.	Name of microorganism	Concentration (µg/1ml)		
		10	20	30
		ZOI (cm)		
1	*E. coli*	2.5	2	2.7
2	*Klebsiella pneumonia*	0.5	1.5	1.5
3	*Staphylococcus aureus*	1.5	1.3	1.7
4	*Bacillus subtilis*	1.6	1.5	1.8

Table 5. Zone of inhibition shown by the ethanolic extract of *M. jalapa* leaves by Disc diffusion method.

Sl. No.	Name of microorganism	Concentration (µg/1ml)		
		10	20	30
		ZOI (cm)		
1	*E. coli*	1.5	1.2	1.38
2	*Klebsiella pneumonia*	1.38	1.3	1.5
3	*Staphylococcus aureus*	1.5	1.6	1.8
4	*Bacillus subtilis*	1.7	1.5	1.5

Results reveal that ethanolic extract of *Mirabilis jalapa* leaves were significantly effective against Gram-positive, Gram-negative bacteria. The antibacterial activity of the given samples is tested using cup-plate (or) cylinder plate method and disc diffusion method.

The samples exhibited positive test results are as following for the ethanolic leaf extract of *Mirabilis jalapa*. Thus, further work can be carried out on the isolation. In addition, these results confirmed the evidence in previous studies, which reported that ethanol is a better solvent for more consistent extraction of antibacterial substances from medicinal plants compared to other solvents.

The antibacterial activity of the samples is assessed using the different concentration of the sample i.e., low, intermediate and high. The present investigation reveals that the zone of inhibition is found in the sample mentioned above in the tabular column i.e., *Mirabilis jalapa* ethanolic leaf extract. The standard drug streptomycin is found to be very effective anti-microbial agent. Here it is found that the standard drug show antibacterial activity on both Gram+ve and –ve bacteria and it is found that the zone of inhibition increased as the concentration of the sample increased.

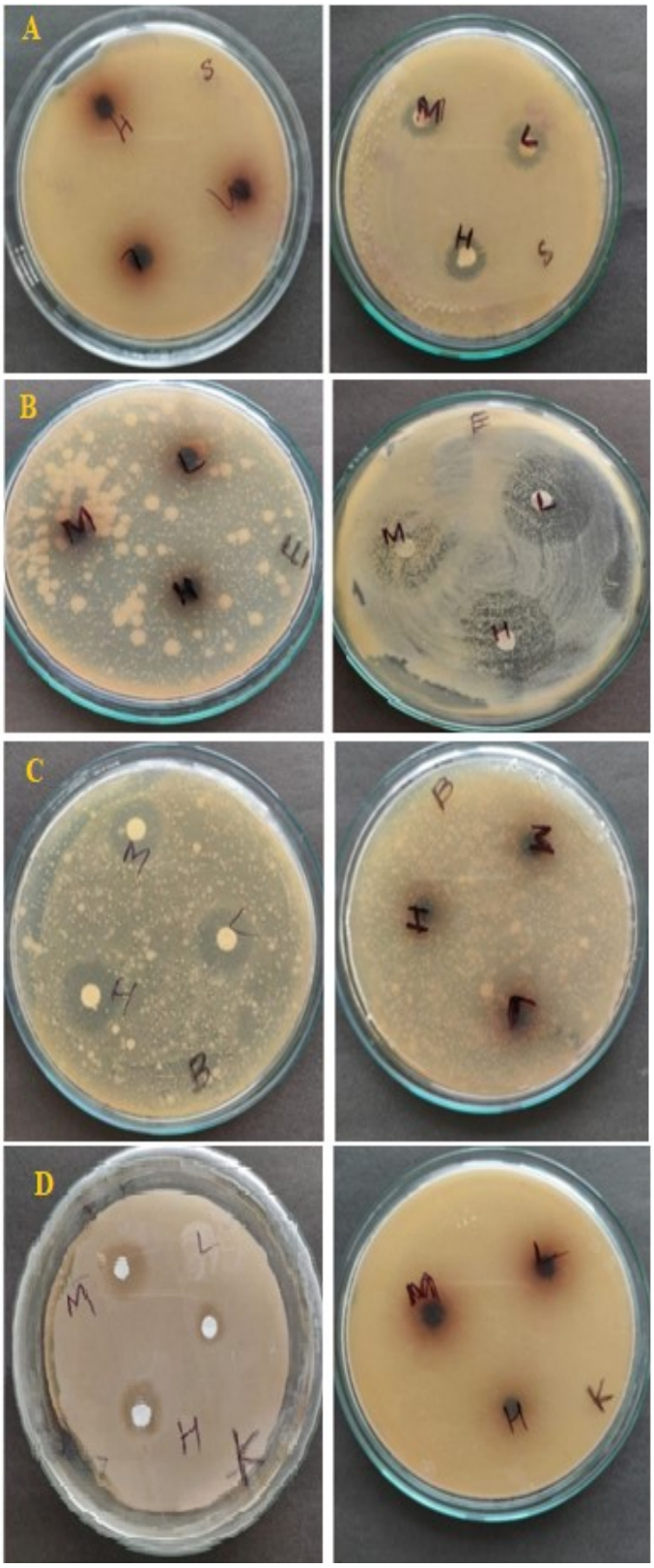

Fig 6. Zone of inhibition shown by the ethanolic extract of *M. jalapa* leaves A) *Escherichia coli* B) *Klebsiella pneumonia* C) *Staphylococcus aureus* D) *Bacillus subtilis,* by Disc diffusion method.

CONCLUSION:

Mirabilis jalapa has been medicinally used as a therapeutic agent for a variety of diseases. Moreover, numerous research works have proven its uses beyond the medicinal ones in experimental animals. Alkaloids and flavonoids which were isolated from this plant may be responsible for its pharmacological activities. Therefore, the cultivation, collection, and further pharmacological exploration of *Mirabilis jalapa* are essential.

The findings of this study support the view, that the ethanolic extracts of plants are promising sources of potential antibacterial and may be efficient as preventive agents in some diseases and can be considered as a natural herbal source in pharmaceutical industry. Further detailed studies on isolation of phytoconstituents of the plant extracts are essential to characterize them as biological antibacterial agents. This knowledge about the medicinal plant's usage can also be extended to other fields like field of pharmacology. In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind.

ACKNOWLEDGEMENT:

We express our indebtedness and sense of gratitude the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Hyderabad, Telangana, India, for providing the necessary facilities, constant encouragement, praiseworthy inspiration and support.

REFERENCES:

1. Kokate CK, Purohit AP, Gokhale SB. Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. In: Text book of Pharmacognosy. 7th ed. New Delhi: Nirali Prakashan; 2001.
2. Irena S, Phyllis GW, Abdullah G, Yehudit T, Sara S. Phytoplasma infection in the four o'clock flower (*Mirabilis jalapa*). Bulletin of Insec, 2007; 60(2): 281-282.
3. Pramanick DD, Mondal M, Maiti GG. Pollen morphological studies on some members of the family Nyctaginaceae in India. Asian J Plant Sci Res, 2015; 5(2): 72-76.
4. Boggula N, Reddy S, Reddy N, Alla TS, Farhana A, Battineni J, *et al*. Phytochemical evaluation and *in vitro* antibacterial activity of dried seeds of *Abrus precatorius.* Int J Pharm Sci Rev Res, 2017: 44(1): 101-107.
5. Vyshali V, Nettem LJ, Bakshi V, Ismail S, Manoj M. Formulation, evaluation and comparative studies on antibacterial activity of zinc oxide and copper oxide nanoparticles. J Global Trends Pharm Sci, 2018; 9(3): 5848-5860.
6. Bakshi V, Jyotsna NL, Ahmed MSA, Choudary M. Formulation and Evaluation of Antimicrobial Activity of Topical Niosomal Gel of Erythromycin. Am J Pharmacy Health Sci, 2018; 6(6): 27-38.
7. Towers GH, Lopez A, Hudson JB. Antiviral and antimicrobial activities of medicinal plants. J Ethnopharmacol, 2001; 77: 189-196.
8. Eneji SM, Inuwa HM, Ibrahim S, Ibrahim AB, Abdulfattah A. *In-vitro* assessment of bioactive components of *Mirabilis jalapa* ethanolic extract on clinical isolates of *Salmonella typhi* and *Bacillus cereus*. Afr J Biotechnol, 2011; 10(7): 16006-16011.
9. Jyothi B, Akshmi SM, Anitha K. Protective effect of *Mirabilis jalapa* leaves on anti-tubercular drugs induced hepatotoxicity. Asian J Plant Sci Res, 2013; 6(3): 221-224.
10. Sayeed M, Patchigalla JRP, Oggu R, Srinivas RRP, Pajjuru SN, Bakshi V, Boggula N. Antibacterial and Phytochemical Screening of *Trachyspermum ammi* - An *in-vitro* Approach. Int Res J Pharmacy Med Sci, 2018; 1(4): 40-45.
11. Ikawati Z, Sudjadi, Widyanengsih E, Dyah P, Sismindari. Induction of apoptosis by protein fraction isolated from the leaves of on HeLa and Raji cell-line. Orien Pharm and Exp Med, 2003; 3(3): 151-156.
12. Thilagavathy P, Saratha R. *Mirabilis jalapa* flowers extract as corrosion inhibitor for the mild steel corrosion in 1M HCl. IOSR J Appl Chem, 2015; 8(1): 30-35.
13. Vanker PS, Dhara B. Preparation of gold nano particles from *Mirabilis jalapa* flowers. Ind J Biochem Biophys, 2010; 47: 157-160.
14. Peng S, Qixing Z, Zhang C, Zhening Z. Phytoremediation of petroleum contaminated soils by *Mirabilis jalapa* in a greenhouse plot experiment. J Hazard Mat, 2009; 168: 1490-1496.
15. Ingale SA, Somwanshi NR, Gaykar BM. Phytochemical analysis of *Cyathocline purpuria* (Don) O. Ktze. in different solvent system-A

specified medicinal plant. Indo Global J Pharm Sci, 2016; 9(2): 98-102.
16. Boggula N, Bojjala N, Mandula T, Priyanka SM, Chinnala KM. Phytochemical investigation and *in-vitro* antibacterial activity of dried leaves of *Aerva lanata*. Indo Am J Pharm Sci, 2016; 3(6): 637-643.
17. George S, Ashika M, Swetha C, Cyriac JM, Kavya Manoj M, Thomas B. Effect of extraction methods on the phytoconstituents of different parts of *Murraya koenigii* (L.) Spreng. – The Aromatic spice plant. J Pharm Adv Res, 2020; 3(2): 787-792.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Swetha C, Shankar CB, Sravanthi D, Pooja D, Bakshi V, Boggula N*. Assessment of Phytochemical evaluation and Antibacterial activity of *Mirabilis jalapa:* An *in vitro* design. J Pharm Adv Res, 2021; 4(8): 1353-1359.