Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN:1673-064X
E-Publication: Online Open Access
DOI 10.17605/OSF.IO/D4ZYU

ANTI-MICROBIAL AND DIURETIC ACTIVITY OF *MIRABILIS JALAPA (LINN)*

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Abstract

Herbs are often used as raw materials in traditional medicine for a variety of formulations. An accurate Pharmacognostical investigation is required to verify the efficacy of raw materials and to detect adulteration of these materials. Traditional practitioners who have inherited Ayurveda or other herbal knowledge and practices are usually the ones who collect the potential materials for certain cause and effect. The aim of the study is to determine phytochemical and pharmacological evaluation of *Mirabilis jalapa* leaf extracts on diuretic and antimicrobial activity by using *in vitro* methods. A cup plate device and the disc diffusion method were used to determine the antibacterial activity. Streptomycin was used as a reference standard. The glycosides, flavonoids, tannins, and saponins were found in the extracts during preliminary phytochemical screening. The aqueous and ethanolic extracts showed effectiveness against both gram positive and negative bacteria. Zone of inhibition is considered as a positive outcome for the given extracts. The phytochemical analyses revealed that the *Mirabilis jalapa* extracts contain various types of alkaloids, terpenes, carbohydrates, proteins, and amino acids in different amounts. The aim of the study is to identify plant extracts with diuretic and antibacterial activities that are free of any negative effects. More research is needed to isolate and characterize the new moiety for its antibacterial and diuretic action in the fight against diverse bacterial infections and pathological conditions.

Key words: anti-bacterial activity, diuretic activity, *Mirabilis jalapa,* phytochemicals, zone of inhibition

Introduction

The herbs and plant products are mainly treated as a traditional source for overcoming various diseases. A varied flora thrives on the Indian subcontinent including fragrant and medicinal plants. In India, it is grown in a variety of climates, from deserts to swamps. From the Himalayan peaks to Kanyakumari's seashore, botanists have determined and described various types of herbs. In Indigenous medical system, this unique flora has historically been employed to produce a variety of remedies [1].

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The Rigveda written between 4500 to 1600 BCE in India, contains the earliest reference of the application of Brihat Samhita and since 1600 BCE the amount of literature on the subject is boundless. The traditional system of medicine is so engrained in our culture that, even now > 75% of population depends on traditional and indigenous medicine [2] medicinal herbs. A detailed account of the world's first symposium on medicinal plants is given the first chapter of, [3]. The increase in

population over decade depend on traditional system of medicine for their well-being and hygiene, so the products derived from herbs are being used since long time and are substantially, scientifically supported [4].

Globally more than 35,000 plant species are identified as a potential medicine in Ayurvedic system. The Chinese system of medicine uses most of its plant resources for their population benefit in a significant manner. The International trade Centre reported an increase in the trade exchange relation and witnessed an upward trend from Jan 2018 onwards. The total raw materials derived from medicinal plants were averaged to US$ 1.28 billion during the financial year 1999 and it is expected to increase 2 to 3 times by 2030.

The Peruvian marvel, also known as the four o'clock flower, has been the most widely planted ornamental plant and appears in a range of colors [5]. *Mirabilis* means splendid in Latin, and Jalapa is the capital city of Veracruz, México. The Aztecs cultivated *Mirabilis jalapa* for medicinal and decorative uses. The notion that the flower blossom in the mid afternoon or at dusk gives it one of its popular names (4 p.m. to 8 p.m.) belonging to family Nyctaginaceae [6]. Flowers then produce a strong, sweet-smelling fragrance throughout the night, and then close for good in the morning. New flowers open the following day. It arrived in Europe in 1525. Today, it is common in many tropical regions and is also valued in Europe as a (not hardy) ornamental plant [7].

The aim of this project work is to assess the phytochemical screening, *in-vitro* anti-bacterial activity of *Mirabilis jalapa* leaves using ethanol as a solvent.

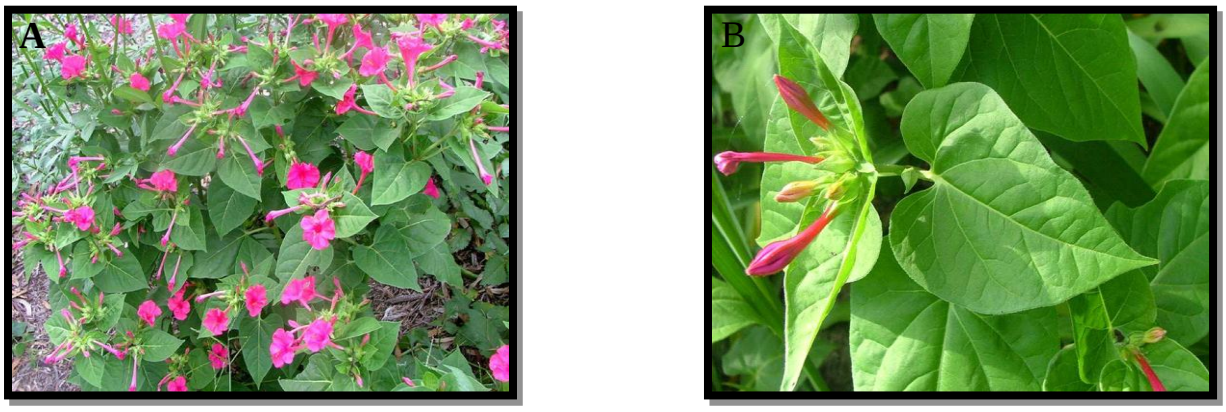

Fig. 1. “(a)” *Mirabilis jalapa* L. “(b)” Leaves of *Mirabilis jalapa* L.

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Materials and methods

Collection of plant material

The plant material was procured in the Ghatkesar neighborhood of Hyderabad, Telangana, India. Fresh leaves were picked and cleaned with tap water, then deionized water, before being dried in the shade. The plant raw material was thoroughly monitored for any fungal growth or rotten smell and the material was dried and made to powder form by using electric blender for obtaining a uniform size of 80 meshes. The final product was retained in airtight containers at 4 ^{0}C. The extracts were tested for their anti-microbial and diuretic activity [8], [9].

Preparation of plant extract

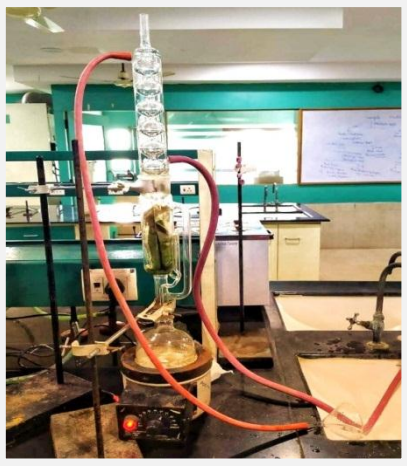
Extraction of plant

Alcoholic extraction

The dried powdered (250gm) was extracted with alcohol (600ml and 70%). The powder was wrapped in filter paper and placed in Soxhlet apparatus with solvent. The extract so obtained was concentrated by solvent recovery, dried completely using hot air oven (40^{0}C) and the obtained extract was kept in a desiccator to remove moisture and stored, for the experiment the extract was dissolved in suitable vehicle [10], [11].

Aqueous extraction

The dried powder (250gm) was soaked for 3 days in hot boiling water (1L) at room temperature followed by filtration using muslin cloth and Whatman filter paper. The process was repeated for 3 times. The filtrate was evaporated under reduced pressure (760 mm Hg) using a rotary evaporator [12].



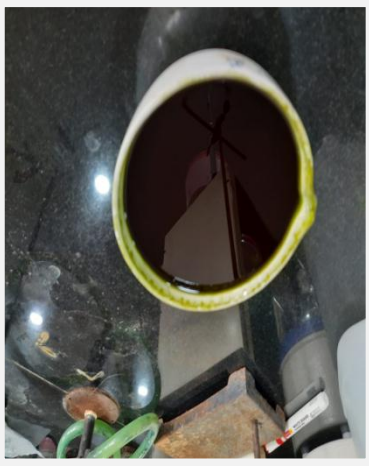


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Fig. 2. Typical Extraction Apparatus Setup for extracting both Aqueous and ethanolic leaf extract of *Mirabilis jalapa* by using soxhlation method.

Experimental design

Lipschitz Method

A total of 36 Wistar albino rats of body weight 130-150 gm. were used and rats were allocated randomly into 6 equal groups. The animal studies were conducted as per the guidelines of CPCSEA and approval from Institutional Animal Ethics Committee wide (Ref. No. I/IAEC/AGI/024/2020WR ♂or ♀), Hyderabad, Telangana, India. Each group contains 6 rats. The test animals were fasted overnight (12-14hr) but they had free access to fresh water and deprived of food and water for 15 hr prior the experiment. The test drug was given for 5 consecutive days [13], [14].

Group I Received normal saline (0.9%NaCl solution, 10ml/kg p.o.)

Group II Received Furosemide (Standard Drug 20mg/kg p.o.)

Group III and **IV** Received Aqueous extract of *Mirabilis jalapa (*200 and 400mg/kg p.o.)

Group V and **VI** Received Alcoholic extract of *Mirabilis jalapa (*200 and 400mg/kg p.o.)

Each rat of each group was placed into a separate metabolic cage, with wire mesh floor provided with a conical-shaped bottom underneath designed to collect urine (5hr and 24hr) in a replaced without faecal contamination and estimate the electrolytes (Sodium, Potassium and Chloride) by using flame photometry and also calculate diuretic index as well as Lipschitz value by using following formulas [15], [16].

- Diuretic index = Mean urine volume of test / Mean urine volume of control.
- Lipschitz value = Mean urine volume of test / Mean urine volume of standard.

Statistical analysis

All the values were expressed mean±SEM six animals in each group. The difference in means was statistically assessed using one-way ANOVA followed by Dennett's test. The $p<0.05$ was considered as significant [17].

Qualitative Phytochemical Evaluation

The phytochemical evaluation test was conducted on all the extracts for determining various chemical constituents using standard procedures [18].

In-vitro anti-bacterial activity screening

Cup plate method:

Prepare stock solution of the antibiotic as 1000µg/1ml (1mg/1ml). Prepare dilutions of the antibiotic of known concentration of the standard and the test antibiotic to be

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examined. Sterilize the Muller-Hinton agar medium in an autoclave at 121 ^{0}C at 15lbs pressure for 15 min. To Muller Hinton medium, add 1ml suspension of standard test organism and mix well [19]. Maintain the temperature at 50^{0}C. A layer of roughly 3mm thickness is formed by pouring the prepared mixture into a petridish. Allow time for the medium to harden. Now use a sharp tool, such as a cork borer, to cut the reservoirs/cup. Using a knife or sharp forceps, remove the cylindrical plugs. Mark the cups according to the dilutions and pour the anti-biotic dilutions into each cup. Refrigerate the plate for 20 minutes to allow the anti-biotic to diffuse. Wipe condensed water carefully from the lid of the Petridish, with the sterile cotton plugs [20], [21]. Incubate the petridish at 37 ^{0}C for 18-24 h.

Disc diffusion method:

Pick a pure culture plate from one of the species being investigated. Aseptically emulsify a colony off the plate in the sterile saline solution. By vigorously mixing the saline solution, ensure that there is no apparent solid substance from the colony in it. Continue till the turbidity of the saline solution and the standard turbidity are almost identical. Dip a sterile cotton swab into the broth culture of the organism. Squeeze the swab lightly against the inside of the tube to remove any excess fluid. Take a sterile Mueller-Hinton agar (MHA) plate or a nutritional agar (NA) plate. Use the swab containing the test organism to streak an MHA plate or a NA plate for a lawn of growth. When you're done streaking, let the plate dry for 5 minutes. Put antibiotic discs on the agar's surface with sterile forceps. Press gently the discs on to agar's surface with flame-sterilized forceps or an inoculation loop. Carefully invert the contaminated plates and incubate at 37°C for 24 hours. With a metric ruler, measure the diameter of the zone of inhibition for each antibiotic used after incubation [22], [23]. Compare the measurements from the various antibiotics to the reference table to determine the sensitivity zone. Match the findings obtained from the various medications to the standard table to determine if the studied bacterial species is responsive or tolerant to the tested antibiotic.

Results and discussion

The extracts were subjected to extraction and the percent yields were calculated on the dry basis and the results were depicted in Table: 1.

Table 1: Plant used for screening of Diuretic activity and yield of extractions

Plant Species	Plant family	Part used	Type of extraction	% yield / 250g of dry plant
Mirabilis jalapa	Nyctaginaceae	Leaves	Aqueous	37.8
			Alcoholic	41.6

Phytochemical investigation

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Qualitative chemical analyses were performed on the ethanolic and aqueous extracts of *Mirabilis jalapa* leaves to identify chemical constituents such as alkaloids, terpenoids, glycosides, tannins, and flavonoids. The results were shown in Table 2.

Table 2: Preliminary phytoconstituents screening of *Mirabilis jalapa* L. leaves extract of Aqueous and ethanol using standard qualitative methods

S.No	Constituents	Aqueous Extract	Ethanol 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	Glycosides	+	+

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18	Resins	+	+

'+' = Present, '-' = Absent

In-vitro anti-bacterial activity

At three different doses, an anti-bacterial assay of ethanolic extract of dried leaves of *Mirabilis jalapa* showed dose-dependent anti-bacterial and diuretic efficacy against the tested microorganisms [24], [25]. The extract's potential sensitivity was determined against all of the microorganisms tested, and the inhibition zone was recorded and reported in the tables and figures below. The antibacterial assay was carried out using the cup plate method and the disc diffusion method, with the Zone of Inhibition observed at various concentrations [26].

Cup plate method:

Table 3: Determination of Minimum inhibitory concentration of standard and Test extract (*Mirabilis jalapa*) using respective microorganisms.

S.No.	Organism	Zone of Inhibition (cm)					
		10 (µg/ml)		20 (µg/ml)		30 (µg/ml)	
		S	T	S	T	S	T
1	*E. coli*	2.5±0.21	1.6±0.02	2.2±0.06	1.6±0.02	2.7±0.08	2.8±0.06
2	*K. pneumonia*	0.5±0.04	1.3±0.01	1.5±0.02	1.3±0.01	1.5±0.05	1.1±0.04
3	*S. aureus*	1.5±0.31	1.3±0.05	1.3±0.24	1.3±0.05	1.7±0.31	1.8±0.03
4	*B.subtilis*	1.6±0.24	2.4±0.02	1.5±0.27	2.4±0.02	1.8±0.23	2.4±0.03

S=Standard T=Test

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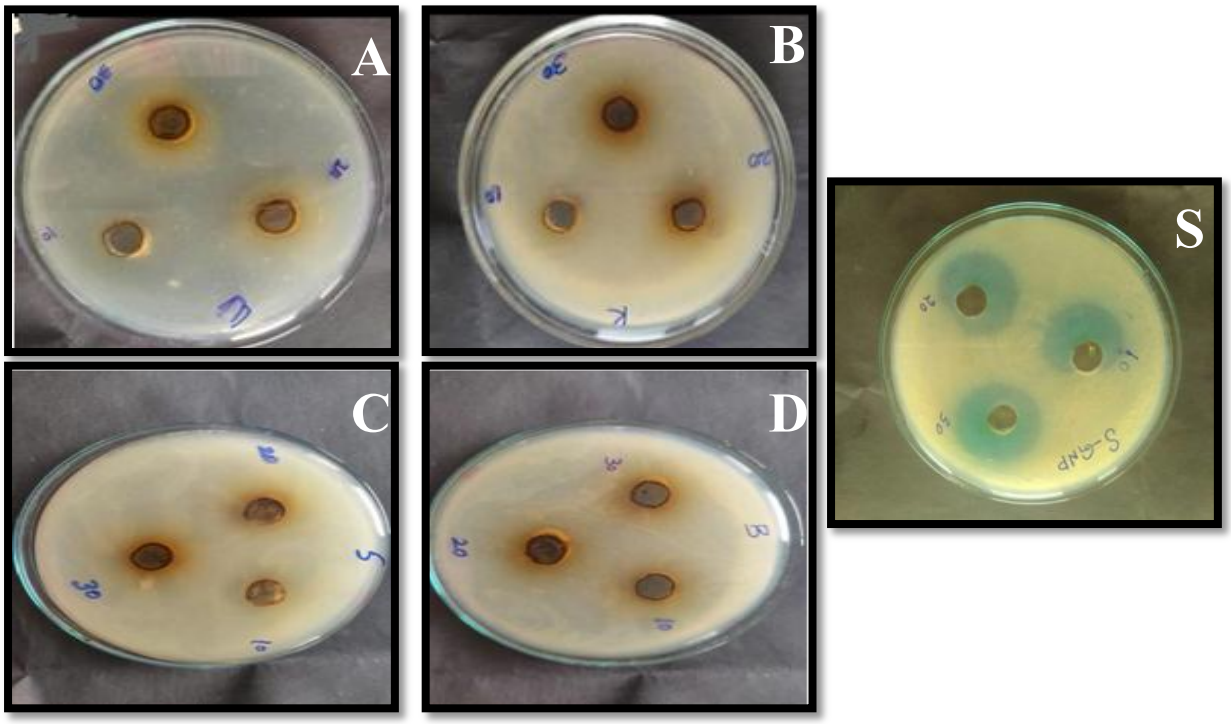

Fig. 3. Anti-Microbial Activity of *Mirabilis jalapa* ethanolic extract using Cup plate method. A) *Escherichia coli* B) *Klebsiella pneumonia* C) *Staphylococcus aureus* D) *Bacillus subtilis and S)* Streptomycin

Disc diffusion method:

Table 4: Determination of Minimum inhibitory concentration of standard and Test extract (*Mirabilis jalapa*) using respective microorganisms.

S.No.	Organism	Zone of Inhibition (cm)					
		10 (µg/ml)		20 (µg/ml)		30 (µg/ml)	
		S	T	S	T	S	T
1	*E. coli*	2.4±0.03	1.4±0.01	2.6±0.02	1.3±0.04	3.1±0.01	1.5±0.03
2	*K. pneumonia*	0.6±0.03	1.5±0.02	1.8±0.02	1.2±0.03	1.9±0.04	1.6±0.01
3	*S. aureus*	1.6±0.01	1.4±0.03	1.4±0.02	1.5±0.02	1.8±0.03	1.9±0.01
4	*B.subtilis*	1.7±0.02	1.8±0.02	1.6±0.01	1.6±0.01	1.9±0.02	1.7±0.02

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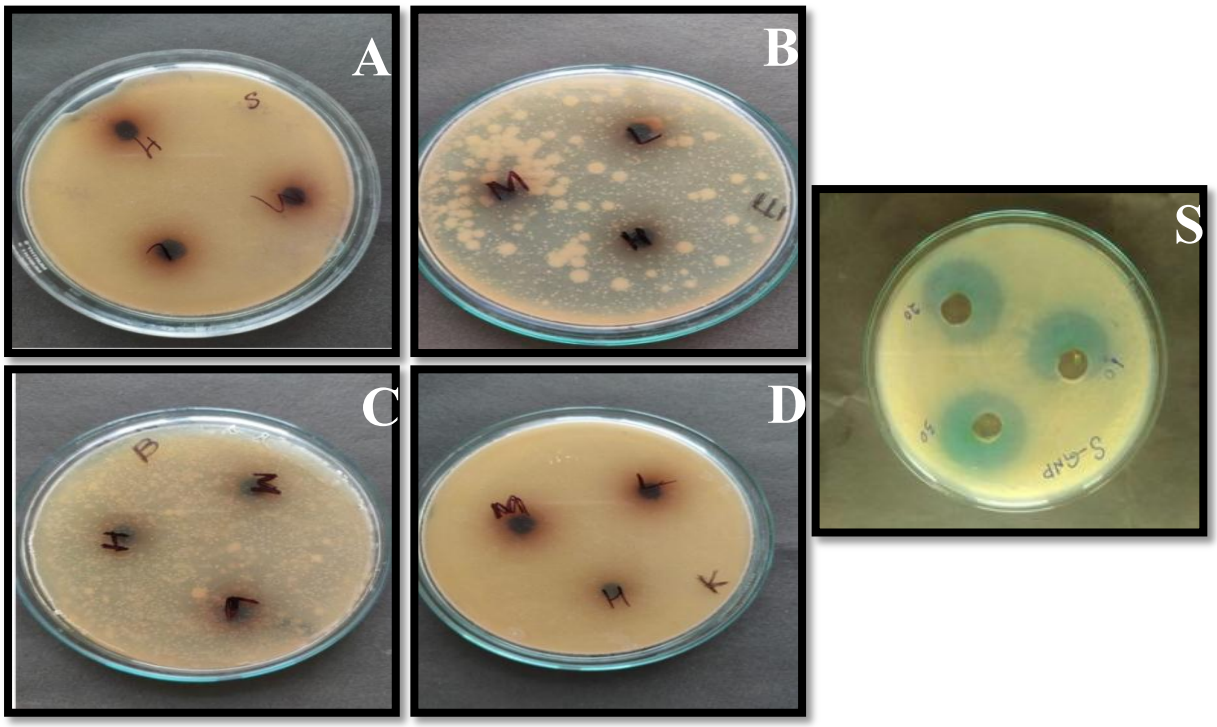

Fig. 4. Anti-Microbial Activity of *Mirabiis jalapa* ethanolic extract using Cup plate method. A) *Escherichia coli* B) *Klebsiella pneumonia* C) *Staphylococcus aureus* D) *Bacillus subtilis and S)* Streptomycin

Diuretic Activity:

Table 5: Effect of Aqueous and Alcoholic extracts of *Mirabilis jalapa* on urine volume at 5hr and 24hr interval and pH.

Treated Groups	5hrs Urine volume (mL)	24hrs Urine volume (mL)	pH (at 24hrs)
Control 0.9% NaCl10ml/kg	1.43±0.10	1.55±0.20	5.89±0.23
Furosemide 20mg/kg	3.95±0.26***	7.91±0.38***	7.42±0.27
Aqueous Extract 200mg/kg	2.75±0.05*	4.88±0.02*	6.89±0.16
Aqueous Extract 400mg/kg	3.69±0.12**	5.37±0.20**	7.11±0.28
Alcoholic Extract 200mg/kg	3.25±0.14**	5.14±0.41**	7.18±0.19
Alcoholic Extract 400mg/kg	3.81±0.02***	6.32±0.02***	7.27±0.24

Values are expressed as mean ± SEM of six rats per group, significant at P<0.05*,

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P<0.01**and P<0.001*** with respect to control group by using one- way ANOVA followed by Dunnett's test.

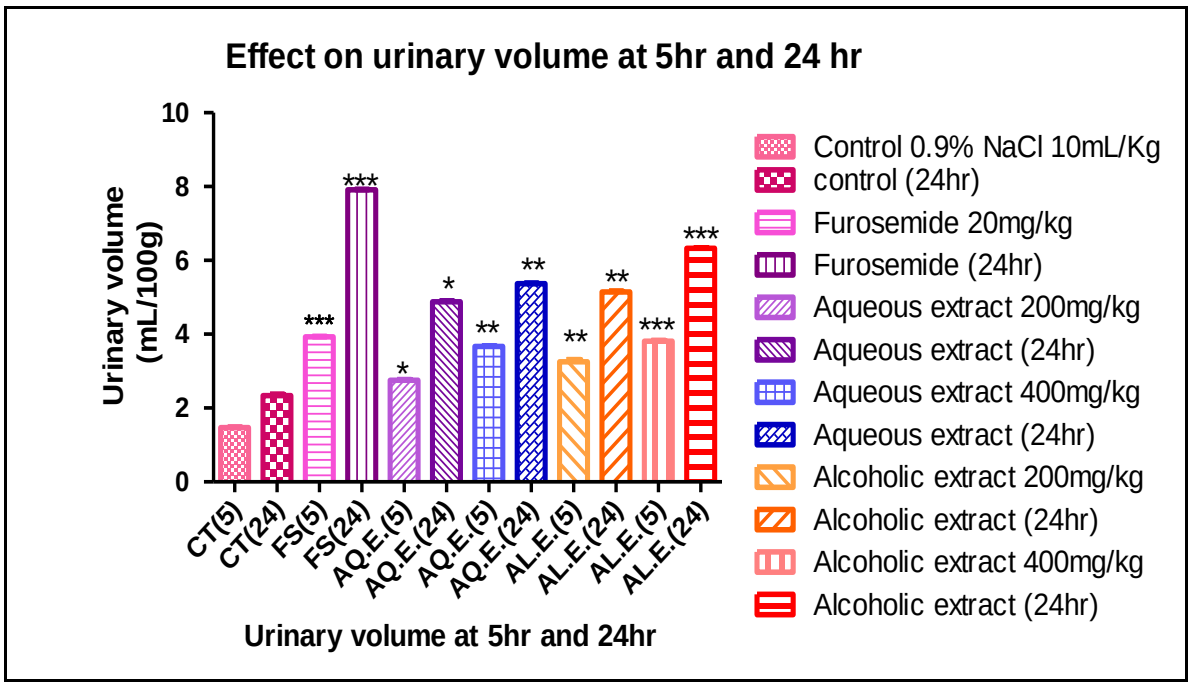

Fig. 5. Effect of Aqueous and Alcoholic extracts of *Mirabilis jalapa* on urinary volume at 5hr and 24hr

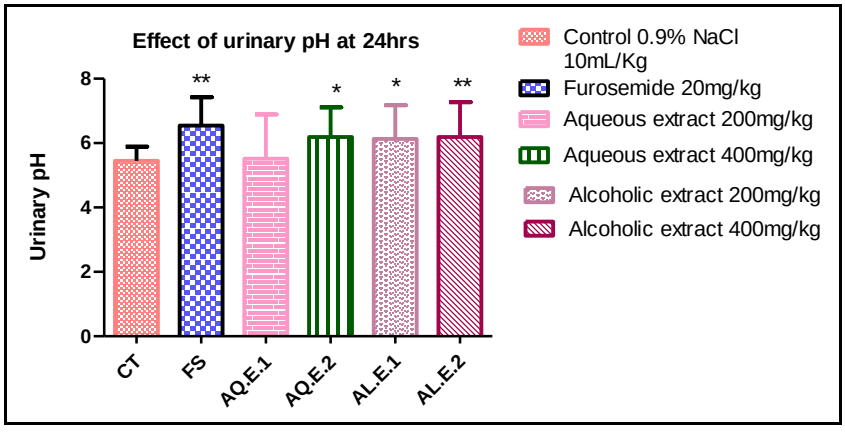

Fig. 6. Effect of Aqueous and Alcoholic extracts of *Mirabilis jalapa* on urinary pH.

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Table 6: Effect of Aqueous and Alcoholic extracts of *Mirabilis jalapa* on diuretic index and Lipschitz value

Treated Groups	At 5hr after drug administration		At 24hr after drug administration	
	Diuretic Index	Lipschitz Value	Diuretic Index	Lipschitz Value
Control (0.9%NaCl 10mL/kg)	---	---	---	---
Furosemide (20mg/kg, p.o.)	2.67	---	3.38	---
Aqueous extract (200mg/kg p.o.)	1.87	0.69	2.08	0.61
Aqueous extract (400mg/kg p.o.)	2.49	0.93	2.29	0.67
Alcoholic extract (200mg/kg p.o.)	2.21	0.82	2.19	0.64
Alcoholic extract (400mg/kg p.o.)	2.59	0.97	2.85	0.84

- *Diuretic Index = Mean urine volume of test /Mean urine volume of control.*
- *Lipschitz Value = Mean urine volume of test /Mean urine volume of Furosemide.*

Table 7: Effect of Aqueous and Alcoholic extracts of *Mirabilis jalapa*on urinary electrolyte excretion of Wistar albino rats at 24 h urine sample collection.

Group	Treated Groups	Concentration of Na^+(mmol/L)	Concentration of K^+(mmol/L)	Concentration of Cl^- (mmol/L)
I	Control (0.9%NaCl 10mL/kg)	86.7±1.94	59.17±1.07	93.17±1.55
II	Furosemide (20mg/kg, p.o.)	148.92±1.11***	97.01±1.69***	159.06±1.65***
III	Aqueous extract (200mg/kg p.o.)	97.24±1.72	70.42±1.53*	113.67±2.95
IV	Aqueous extract (400mg/kg p.o.)	118.36±1.43**	81.17±1.65**	121.57±1.64**
V	Alcoholic extract (200mg/kg p.o.)	109.12±1.08*	73.67±1.33*	113.85±1.80*
VI	Alcoholic extract (400mg/kg p.o.)	126.18±1.28**	84.33±1.14**	137.74±1.49**

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Values are expressed as mean±SEM of six rats per group, significant at P<0.05, P<0.01**and P<0.001*** with respect to control group byusing one- way ANOVA followed by Dunnett's test.*

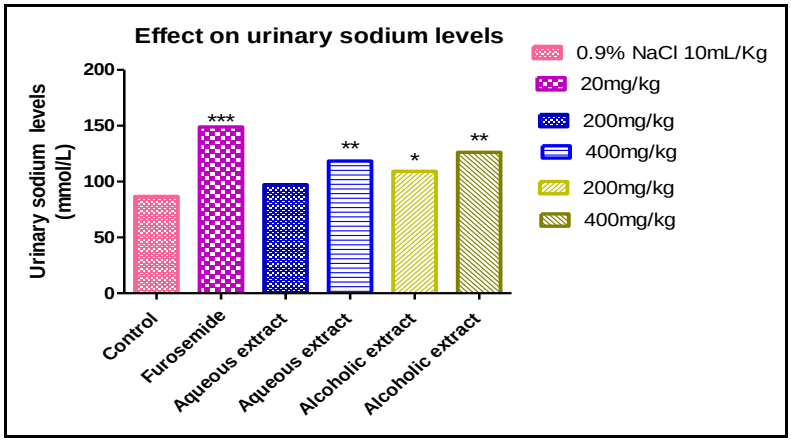

Fig. 7. Effect of aqueous and alcoholic extracts of *Mirabilis jalapa* on urinary sodium levels

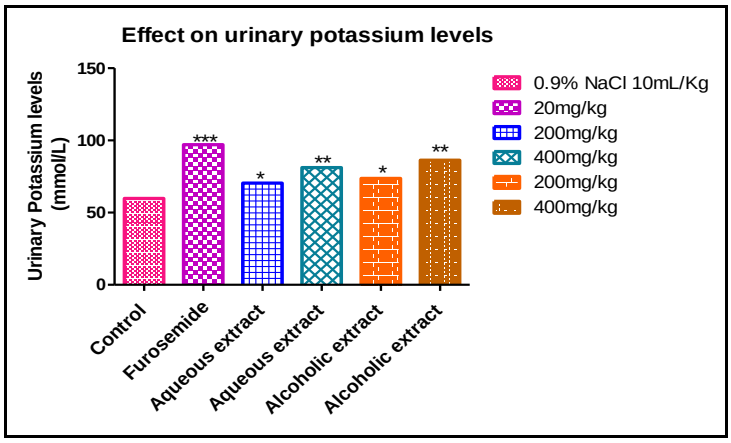

Fig. 8. Effect of aqueous and alcoholic extracts of *Mirabalis jalapa* on urinary potassium levels.

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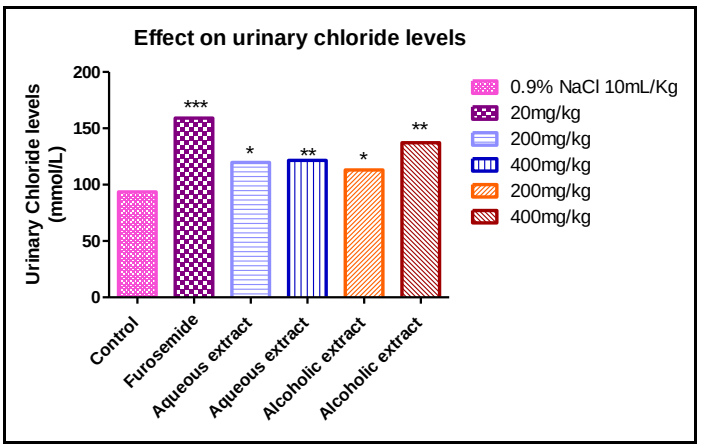

Fig. 9. Effect of aqueous and alcoholic extracts of *Mirabilis jalapa* on urinary chloride levels.

Plants are a valuable source of pharmacophore, which can be used to develop new chemotherapeutic drugs. The evaluation of in vitro antibacterial activity would be the initial stage in developing a chemotherapeutic drug from plants. The active extracts will aid in identifying the active chemicals responsible for the plant's actions. Multidrug resistance has been observed in pathogenic bacteria in recent years, rekindling interest in the hunt for novel antibacterial medicines from natural sources. P. aeruginosa, Gram-negative bacteria, has been reported to have acquired multi-drug resistance to a variety of antibiotics [27]. However, the extracts have shown to be effective against bacterial strains [28]. Natural antibacterial agents also have fewer negative effects than synthetic or semi-synthetic antibacterial agents. With different species, the plant extract's anti-bacterial efficacy was variable.

The extracts of *Mirabilis jalapa* leaves were found to be highly effective against both gram positive and gram negative bacteria. Cup-plate (or) cylinder plate method and disc diffusion method are used to assess the antibacterial activity of the samples. For the supplied samples, the zone of inhibition is deemed a positive outcome. Antibacterial activity is assessed by using microbial strains listed in the preceding tables.

The leaf extracts of *Mirabilis jalapa* contained glycosides, anthraquinones, tannins, carbohydrates and proteins, according to preliminary phytoconstituents screening. As a result, more work on the isolation can be done. Furthermore, these findings complemented prior research that found ethanol to be a better solvent for reliable extraction of antibacterial compounds from medicinal plants than other solvents [29], [30], [31], [32].

Conclusion

Mirabilis jalapa is a medicinal plant that has been used to treat a range of ailments. Furthermore, various research studies in experimental animals have demonstrated its usefulness beyond therapeutic ones. The properties may be due to the presence

ISSN:1673-064X
E-Publication: Online Open Access
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of glycosides, resins and anthraquinones extracted from it. As a result, *Mirabilis jalapa* cultivation, collection, and future pharmacological research are critical.

The concentration of the samples determines their antibacterial activity, which is categorized into 3 categories: low, intermediate, and high. A common antibiotic, streptomycin, has been proved to be an extremely efficient antibacterial agent. The standard drug was discovered to exhibit antibacterial activity against gram +ve and –ve bacteria, and the zone of inhibition grew as the sample concentration grew.

The present study indicates that *Mirabilis jalapa* planthas potential to be used as a diuretic agent. The phytochemical tests revealed the presence of glycosides, resins, proteins and anthraquinones in both aqueous and alcoholic extracts. Both the aqueous and alcoholic extracts showed a dose dependent increase in urine excretion. Relatively alcoholic extract produced significant diuresis than aqueous extract. The study confirms the significant diuretic activity of the alcoholic extract of *Mirabilis jalapa* during the measurement period of the study (24hrs). However, activity guided fractionation is need of further research to separate the potent diuretic phytochemical constituentsand further studies are recommended to know the mechanism of diuretic activity.

The findings of this study illustrates that the ethanolic extracts of plant showed promising results as a potential antibacterial and may be efficient as preventive agents in various diseases and can be considered as a natural source in pharmaceutical industry. Further detailed studies on isolation and characterization of phytoconstituents of the plant extracts are required to prove them as anti-bacterial agents. Research work can be taken up in humans for evaluating multifarious effects will be an added advantage in the scientific community. Furthermore, a rigorous and methodical strategy can be taken in utilizing and finding phytopharmacology in order to discover the plant's maximum potential benefit to the mankind.

Acknowledgments

The authors would like to thank Anurag University chairman and management, for encouraging research and development.

Compliance with ethical standards

All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of interest

I hereby confirm that all authors have approved the manuscript for submission. We declare that we have no conflicts of interest.

ISSN:1673-064X
E-Publication: Online Open Access
DOI 10.17605/OSF.IO/D4ZYU

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