INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 186-195, 2015

RESEARCH PAPER OPEN ACCESS

Antimicrobial activities, essential element analysis and preliminary phytochemical analysis of ethanolic extract of *Mirabilis jalapa*

Syed M. Salman[1*], Iltaf Ud Din[1], Ghausia Lutfullah[2], Durr e Shahwar[3], Zarbad shah[5], Abdul Waheed Kamran[4], Saleem Nawaz[1], Shaukat Ali[1].

[1]*Department Of Chemistry, Islamia College University, Peshawar, KPK, Pakistan*

[2]*Center of Biotechnology, University of Peshawar, KPK Pakistan*

[3]*Departments of Zoology, Islamia College University Peshawar, KPK Pakistan*

[4]*Department of Chemistry, University of Malakand, Chakdara, Dir, KPK, Pakistan*

[5] *Department of Chemistry, Bacha Khan University Charsadda, KPK, Pakistan*

Key words: *Mirabilis Jalapa*, phytochemical analysis, essential elements analysis, antimicrobial activities, AAS.

http://dx.doi.org/10.12692/ijb/7.4.186-195 Article published on October 25, 2015

Abstract

The main aims of this study was to identify all the bio active compounds present in the *Mirabilis jalapa* plant (leaves and stem) qualitatively by using four different solvent system (water, n-hexane, ethanol, and ethyl acetate). The qualitative study show the presence of phytochemicals like, carbohydrates, flavonoids, alkaloids, steroids, tannin, protein etc. but some compounds like, Cumarin,, saponin etc. were not identified. The determination of trace amount of the essential elements in the leaves and stem of Mirabilis jalapa plant was also the part of this research, the essential elemental analysis shows the presence of elements like, Fe, Mn, Pb, Cr, Zn, Cu, in different concentrations in both the leaves and stem of the plant. The antimicrobial activities of ethanolic extract of *Mirabilis Jalapa* were investigated against four strains of bacteria*, Salmonella typhi, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginos*a. The plants extract show high sensitivity against *Pseudomonas aeruginosa* and least sensitivity against *Staphylococcus aureus*. The percent zone inhibition was also determined by comparing the zone inhibition of the extract with standards. Ethanolic leaves extract was also used to determine the sensitivity against fungi. *Penicellium* and *Rhizopus* strains were taken and the extract shows high sensitivity against *Penicellium* than *Rhizopus*. Moreover, the current research work will help to introduce new and cost effective antibiotic resources and will help to solve the problem of resistance develop by microbes against allopathic antibiotics.

* **Corresponding Author:** Dr. Syed Muhammad Salman ✉ salmanchemist80@yahoo.com

Introduction

Nature is always being more beneficial to mankind, providing tools for the treatment of different diseases. Even Ancient civilization depended on plants extracts for the treatment of various diseases. Plant materials are the main important resource for curing of many illnesses, also for the treatment of infectious problems and used for the production of new drugs and also for the formulation of therapeutic agents. (Habila *et al.*, 2011). The natural products that are responsible for their medicinal importance are derived from different parts of the plant like flowers, leaves, bark, fruits and roots. Due to the medicinal values plants play very important role for the sustaining of lives of both individuals and communities. There are many bioactive substances present in different parts of the plants that show definite physiological behavior on the human body. Those bioactive compounds are alkaloids, tannins, flavonoids and phenolic compounds etc. (Hill *et al.*, 1952).

Phytochemicals are can be categorized into two main classes, primary and secondary phytochemicals. Primary phytochemicals include carbohydrates, proteins, chlorophyll, and flavonoids, alkaloids, terpenoids and phenolic compounds are constituents of secondary compounds. (Krishnaiah *et al.*, 2007; Edeoga *et al.*, 2005). All these compounds are very important due to their antimicrobial activities. Most of the phytochemicals have shown valuable therapeutic activities such as insecticidal (Kambu *et al.*, 1982). Antifungal, antibacterial, anticonstipative, spasmolytic, antiplasmodial and antioxidants activities etc.(Kambu *et al.*,1982; Lemos *et al.*, 1990; Ferdous *et al.*, 1992; Sontos *et al.*, 1998; Benoitvical *et al.*, 2001; Vardar-unlu *et al.*, 2003). Terpenoids are very much active and act as anti-cancer, anti-inflammatory, anti-malarial, reduce the cholesterol synthesis, and is also show anti-bacterial and anti-fungal activities. (Mahato *et al.*, 1997). Another important compound is alkaloid that is used as anesthetic agents during operation. (Hérouart *et al.*, 1988) .There is hundreds of other bio-active compounds present in plants are helpful for the treatment of diabetic diseases and also used in the lowering of glucose level in the blood. (Marles *et al.*, 1995). Some evidences also come forward that fruits and vegetable rich in phytochemicals like alkaloids, tannins flavonoids etc. can decrease the cardiovascular disorders and also reduce the risk of cancer. (Javanmardi *et al.*, 2003).

As plants have many antimicrobial activities and important medicinal values, but all plants have not been screened yet, so the investigation on new and already exploited plants is still in progress for their pharmacological values. (Palaksha *et al.*, 2013). The effectiveness shown by plants against pathogens is mostly due to the secondary phytochemicals. (Khushad *et al.*, 2003).

The plants also had shown antispasmodic and anti-nociceptive properties against different pathogens. It is also found that the plant also possess many bioactive compounds like flavonoids, triterpenes, alkaloids, steroids and amino acids based proteins called antiviral proteins. Phytochemical screening also exposed the components like alanine, alpha-amyrins, arabinose, beta-amyrins, campesterol, C-methyle abronisoflavone, stigmasterol, tartaric acid, trigolline present in the plant extract. Ribosome-inactivating protein (RIP) also extracted from *Mirabilis Jalapa* have antiviral activity, phenolic compounds show antifungal activity. (Yang *et al* ., 2001). The roots of the plants in paste form are used to treat scabies and muscular swelling .But the juice of roots is used to treat diarrhea, stomach or gastric problems and fever. The root powder is mixed with corn flour and baked then use for the treatment of menstrual problems. (Manandhar., 2002).

Mirabilis jalapa (family:Nyctaginaceae), is a herbaceous climbing plant with opposite leaves, tuberous roots and stem, large flowers, and planted as an ornamental plants throughout the world. The different parts of plants are used traditionally throughout the world of the treatment of varieties of diseases like dysentery, conjunctivitis, diarrhea, edema, inflammation, swelling, muscular pain and abdominal colic. (Daniel *et al.*, 2006; Holdsworth *et*

al., 1992). The extract of this plant have also much effective properties against viruses, bacteria and fungi. (Oladunmoye *et al.*, 2007).

Therefore the aim of the current research work is to explore the essential qualitative and quantitative phytochemicals present in the selected plant *Mirabilis Jalapa,* and to determine their antimicrobial activities against different bacterial and fungal strains as well as to analyze various essential elements present in the selected plant.

Materials and methods

Crude extracts preparation

The leaves and stem were washed thoroughly, dried and made powder. 5g of both powder taken in 150ml of four different solvents [water, ethanol, n-hexane, and ethyl acetate] and kept for two days then filtered with filter paper and the extracts were then used for further qualitative tests.

Qualitative Analysis of Phytochemicals

Flavonoids

The extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

Tannins

0.5 ml of extract was added to 1 ml of distilled water and when the mixture gives blue or green color on the addition of 2 drops of ferric chlorides confirmed the presence of tannin. (Tyler ., 1994).

Alkaloids

Hager's solution (saturated picric acid solution) was added to the test solution formation of yellow precipitate show the presence of alkaloids. (Satheesh *et al.*, 2012**).**

Terpenoids

5ml of each extract was added with 2ml of methanol followed by the addition of conc. H_2SO_4 carefully till to formation of layer. The appearance of reddish brown color confirmed the presence of terpenoids.

Saponins

Foam test. The extract was mixes with distilled water and shaken vigorously for some time and appearance of colloidal solution is positive test for saponins.

Carbohydrates

Molish test. 2-3 mL of each extract was taken and two drops of alcoholic alpha-naphthol solution were added and shacked followed by the addition of conc. H_2SO_4 from sides of test tube. Violet ring is formed at the junction of two liquids.

Cardiac glycosides

5ml of each extracts were mixed with 2ml glacial acetic acid having one drop of ferric chloride solution, this is under layered with conc. H_2SO_4. A brown ring of interface confirmed the presence of de-oxy sugar. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may appear just slowly through thin layer.

Phlobotannins

In a test tube take 10 mL of extract of each plant sample and boiled it with 1 %HCl. If the sample of plant carries phlobotannins, a deposition of a red precipitate will occur and indicates the presence of phlobotannins.

Amino acid

Ninhydrin test

3mL of samples extract and 3 drops of 5% Ninhydrin solution was heated in boiling water bath for 10 min. Purplish or blue color appears confirmed the presence of amino acids.

Coumarins

3ml of 10% NH_4OH was added to 2ml of aqueous extract formation of yellow coloration indicates the presence of Cumarin.

Steroids

Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of ethanolic extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; we added 0.5 ml of the filtrate

chloroform. Treated with the reagent of Libermann Burchardt, the appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Quantitative Analysis of phytochemicals

Determination of total Phenols

To determine the total phenols, 5g of leaves in powder form was taken in 250ml titration flask and 100ml of n-hexane was added twice for 4 hours each, then the solution was filtered and the filtrate was discarded for fat free sample preparation. Then, 50ml diethyl ether was added twice and heated for 15 minutes each, then cooled up to room temperature and was filtered in separating funnel. 50ml of 10% NaOH was added twice and shook well each time to separate the aqueous layer from the organic layer. Then it was washed three times with 25ml distilled water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 50ml dichloromethane (DCM) twice for acidification of aqueous layer in the separating funnel. At last the organic layer was collected, dried and weight for total phenol contents.

Determination of total Alkaloids.

(Harborne method) (Harborne., 1973) To determine the alkaloids from plants leaves, 5 g of grinded leaves were taken into 250ml beaker and 200ml of 10% acetic acid in ethanol was added to the sample and the beaker was covered with aluminum foil and left for 4 hours. This was then filtered and the extract was collected and made concentrated on the water bath till the volume remains one quarter of the original volume. Then concentrated ammonium hydroxide was added drop by drop until the precipitation is completed. The whole solution was left to settle. The precipitate was then collected dried and weight. The collected dried precipitate is the total alkaloids contents in the leaves of *Mirabilis Jalapa* plants.

Essential elemental Analysis

3 g of dried powder form of *Mirabilis Jalapa* leaves were taken in crucible and ignited in open air. The ignited leaves were then made ash at 550-600 °C for at least 3 hours. The ashes were then transferring into 250ml Pyrex beaker. Then 10ml of concentrated Nitric acid along 3ml of 60% per chloric acid to the ashes of leaves, the mixture was left for 24hrs covered with watch glass. After that the mixture left at sand bath for 80°C for 6 hrs, till the digestive materials converted into white powder. Then 10 ml of deionized water was added to the powder and filtered the sample and diluted the extract up to 100ml. The trace elements present in the leaves of *Mirabilis Jalapa* were determined by Flame Atomic Absorption Spectrophotometer. (Mohammed *et al*., 2007).

Analysis of Antimicrobial Activities

There are two types of media used in bacterial activity, Nutrient Agar media and Nutrient Broth media.

Media preparation

1.3 g /100ml of Nutrient Broth media and 2.8 g/100ml of Nutrient Agar media were prepared in distilled water and then poured in to conical flasks 20ml/flask. Nutrient broth media was taken in test tubes about 7-8 ml/test tubes. Now all the test tubes, media and flasks were closed with cotton plugs and then placed in autoclave machine for steam sterilization for 1 hour at 1.5 psi pressure and 121 °C. After sterilization the nutrient agar media was taken in Laminar Flow hood machine for microbe free environment and the media was poured into pre-sterilized petri plates, sterile environment should be maintained during the pouring of media. The media was then allowed to solidify for an hour, the solidified media on the petri plates were then kept in incubator for 24 hrs. After 24 hours the plates were examined and the contaminated plates were made side and the clear and un-contaminated plates were collected and used for further fungi and bacteria culturing. The nutrient broth media in flask was used for shaking and incubation of microorganisms, and media in test tubes were used for microbial culture standardization.

Microbial Strains used

There were four bacterial strains used, three were gram negative and one was gram positive strain. They are *Salmonella typhi, Staphylococcus aureus, Escheichia coli, Pseudomonas aeruginosa*. And two

fungal strains *Penicillium notatum, Rhizopus stolonifer* were also used for antifungal activities.

Preparation of stock solutions and standards

5g of pulverized dried leaves of *Mirabilis Jalapa* plants were dissolved in 200ml of ethanol and the beaker was closed with aluminum foil and kept for 24 hours. Then the solution was filtered and the residue was discarded and filtrate was taken on water bath and the solvent was evaporated and extract was collected. The extract was then dissolved in DMSO (240mg/ml) and then used for further activity against the given strains of bacteria. And Azithromycin, Cefoxitin, streptomycin, and Ciprofloxacin were used as a positive control or standard against the four strains of bacteria.

Inoculation of culture

The original culture of microorganisms was taken into the solidified freshly prepared media in petri plates and was refreshed. The refresh microbes' culture was then inoculated into nutrient broth media and then kept in incubator for uniform growth.

Disc diffusion susceptibility method

A standard method was followed for the determination of antibacterial activity of *Mirabilis Jalapa* leaves extract against four strains of bacteria. The required amount of Nutrient Agar (2.8 g/100ml) and Nutrient Broth (1.3 g/100ml) were dissolved in distilled water. 8 ml nutrient broth was taken in test tubes for refreshment of microbial culture. All the apparatus (test tubes, conical flasks, petri plates, yellow tips etc.), normal saline and media were made sterilized in autoclave at 1.5 psi pressure for one hour at 121 °C. After sterilization the nutrient agar media was poured into sterilized petri plates inside laminar flow hood, the media was then left for solidification. Then the nutrient agar petri plates were placed in incubator for one day to avoid contamination.

The bacterial strains were made refreshed by the help of nutrient broth media in test tubes. The uncontaminated petri plates were taken into laminar flow hood. Small amount (0.2 micro liters) of each bacterial strain were streaked from refreshed nutrient broth media by the help of sterilized fore cep and spread on the petri plates containing nutrient agar with glass spreader, the plates were placed in refrigerator for 15 minutes for absorption. Then 6mm of diameter of wells were made by the help of sterile cork borer on the nutrient plates. 100 micro liter of leaf extract was poured into each well by the help of yellow tip micropipette. The standards were also placed on their respective wells. The plates were then placed in incubator at 37°C for 24 hours. After 24 hours the activities of the ethanol extract was determined by calculating the %zone inhibition of the extract against the microbial strains by comparing with that of control groups used.

Results and discussion

In the current study phytochemical investigation of *Mirabilis jalapa* plant was performed in four different solvents, because the bioactive compounds present in the plant are different from each other on the basis of their structure and solubility in different nature of solvents. Phytochemical analysis on *Mirabilis Jalapa* by (Oladunmoye *et al.*, 2007) showed the presence of important chemical compounds, e.g. carbohydrates, flavonoids, alkaloids, protein, steroids etc. but the result showed that the important phytochemical saponin is absent in any of the solvent extract. But the current result showed the presence of saponin in all the solvents extract. Anthraquinone glycoside is an important phytochemical present in many species of plant. The qualitative analysis done on the leaves of *Mirabilis jalapa* plant reveled that anthraquinone glycosides can't be extracted from this medicinal plant by using any solvents, but according to our study the test for anthraquinone glycosides is positive only for ethanol extract. The leaves of the selected plants show the presence of phytochemicals like, alkaloids, flavonoids, tannins and saponins, phenolic compounds, protein, cardiac glycosides in the majority of the solvents, but bioactive compounds like, steroids, emodin, starch, phlobotannins, fats, Cumarin etc. are absent in most of the solvents. Table 1.

Table 1. Qualitative phytochemical test of *Mirabilis Jalapa* leaves.

Sl. No.	Phytochemicals	Water	Ethanol	n-hexane	Ethyl acetate
1	Carbohydrate	+	+	+	+
2	Flavonoids	+	+	+	+
3	Tannin	+	+	+	_
4	Terpenoids	+	+	_	_
5	Alkaloids	+	+	+	+
6	Steroids	_	+	_	_
7	Saponin		+	+	+
8	Phlobotannins	_	_	+	_
9	Cardiac glycosides	+	+	+	_
10	Starch	_	_	_	_
11	Protein	+	+	+	+
12	Phenolic compounds	+	_	+	_
13	Amino acids	+	_	_	+
14	Fats	_	_	_	_
15	Cumarin	_	_	_	_
16	Emodin	+	_	_	_
17	Anthraquinone glycosides	_	+	_	+

Note. + Means present and - means absent.

The stem of the plant was also analyzed for the essential bio compounds in the same solvents, it show the presence of carbohydrate, cardiac glycosides, alkaloids, saponin, flavonoids, tannin, protein, phenolic compounds and amino acids, and show negative result for, fats, steroids, Emodin, phlobotannins, and starch. The current result shows that leaves and stem of the *Mirabilis jalapa* plants contain the most medicinally important bio active compounds, they can be extracted quantitatively, purified and can be applied for drug formulation against different pathogens. Tabel 2.

Table 2. Qualitative phytochemical test of *Mirabilis Jalapa* stem.

Sl. No.	Phytochemicals	Water	Ethanol	n-hexane	Ethyl acetate
1	Carbohydrate	+	+	+	+
2	Flavonoids	+	+	+	+
3	Tannin	+	+	+	_
4	Terpenoids	_	+	_	+
5	Alkaloids	+	+	_	+
6	Steroids	_	+	_	_
7	Saponin	+	+	+	+
8	Phlobotannins	_	_	+	_
9	Cardiac glycosides	+	_	_	+
10	Starch	_	_	_	_
11	Protein	+	+	+	_
12	Phenolic compounds	+	+	+	+
13	Amino acids	+	+	_	_
14	Fats	_	_	_	_
15	Emodin	+	_	_	_

Note. + Means present and - means absent.

Essential elemental analysis was also performed for both of the leaves and stem of the plants, that shows the presence of Fe, Mn, Cr, Cu, Zn, and Pb.in both leaves and stem Fe is present in large quantity than other elements as shown in Table 3 and 4. These elements are very important for the survival of the plant, their growth and metabolism.

Table 3. Essential elements in *Mirabilis Jalapa* leaves.

Sl. No.	Metals contents in leaves	Concentration mg/kg
1	Mn	0.42
2	Fe	5.02
3	Zn	1.19
4	Pb	0.04
5	Cr	0.14
6	Cu	0.067

Table 4. Essential elements in *Mirabilis Jalapa* stem.

Sl. No.	Metal contents	Concentration mg/kg
1	Pb	0.13
2	Zn	1.74
3	Cu	0.58
4	Cr	0.13
5	Mn	0.72
6	Fe	4.88
7	Cd	Nil

Quantitative study was also performed for the extraction of total phenol contents and alkaloids from the leaves of the plant and the two important compounds were extracted in small quantity of 0.054mg/kg and 0.034mg/kg respectively. Table 5.

Table 5. Quantitative phytochemical test of *Mirabilis Jalapa* leaves.

Sl. No.	Phytochemicals	Extracted (mg/kg)	Crude sample(grams)	% extracted
1	Alkaloids	0.054	5	1.08
2	Phenols	0.032	5	0.64

Table 6. Antifungal activities of ethanolic extract of *Mirabilis Jalapa* leaves.

Fungal strains	Standard used	Zone inhibition of standard (mm)	Zone inhibition of extract (mm)	% zone inhibition
Rhizopus oligosporus	Media without plant extract.	70±0.21	30±0.11	42.85
Penicillium marneffei	״״	60±0.12	35±0.13	58.33

In the current study ethanol extract of leaves of *Mirabilis Jalapa* plant at concentration of 240mg/ml was used against four strains of bacteria and two strains of fungi. The plant extract showed much high sensitivity against all the strains of bacteria and fungi. Table 6 and Fig 2.

The results shows that the leaves extract is much sensitive against gram negative bacteria as *E.coli* and *Pseudomonas aeruginosa* showing maximum zone inhibition for these bacteria, and moderate sensitivity against gram positive bacteria like *Staphylococcus aureus* Table 7 and Fig 1. The activity of the plant extract against microbes is due to the presence of the bio-active compounds especially alkaloids, flavonoids, tannin and saponins. The ethanol plant extract is also active against some fungal strains as it is much effective against *penicellium* and *rhizopus*. So from the current study it is confirmed that the extract of *Mirabilis jalapa* plant is much effective against the common diseases causing bacteria like *S.typhi* which is responsible for causing pathogenic diseases like typhoid fever; and *E.coli* which is responsible for causing infection to the urinary tract, diarrhea. Thus Mirabilis Jalapa plant extract can be used against these pathogens.

Table 7. Antibacterial activities of ethanolic extract of *Mirabilis Jalapa* leaves.

Sl. No.	Bacterial strains	Antibiotic Used (Standard)	Zone of inhibition of standard (mm)	Zone of inhibition of extract (mm)	% Zone of inhibition
1	*Salmonella typhi*	Azithromycin	22±0.11	13.5±0.22	61.36
2	*Staphylococcus aureus*	Cefoxitin	22±0.54	11.1±0.27	50.4
3	*Escherichia coli*	Streptomycin	22±0.13	15±0.15	68.18
4	*Pseudomonas aeruginosa*	Ciprofloxacin	23. ±0.45	15.5±0.32	67.39

Penicillium can cause a lethal systemic infection (penicilliosis) with fever and anemia. Rhizopus microspores is responsible for the skin and gastrointestinal infections, it can also affect the blood vessels, and causing thrombs and tissue necrosis. Thus *Mirabilis Jalapa* plant has much medicinal importance as the results show the activity of the extract against the disease causing bacteria and fungi. But the most important task is to extract the bio-active compounds quantitatively.

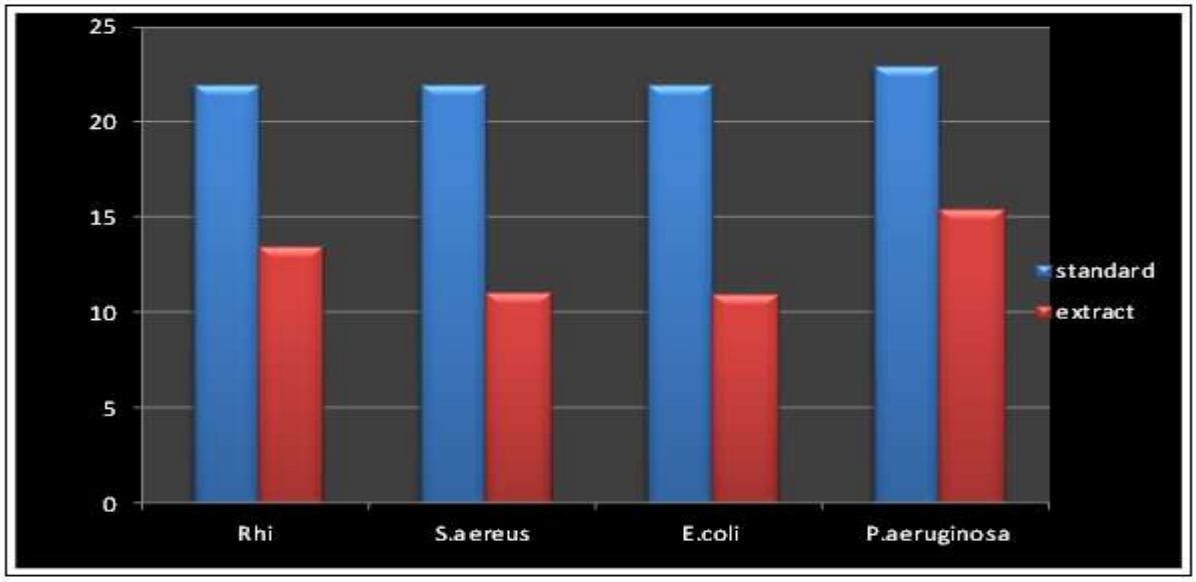

Fig. 1. Antibacterial activity of ethanolic extract of *Mirabilis Jalapa* plant leaves.

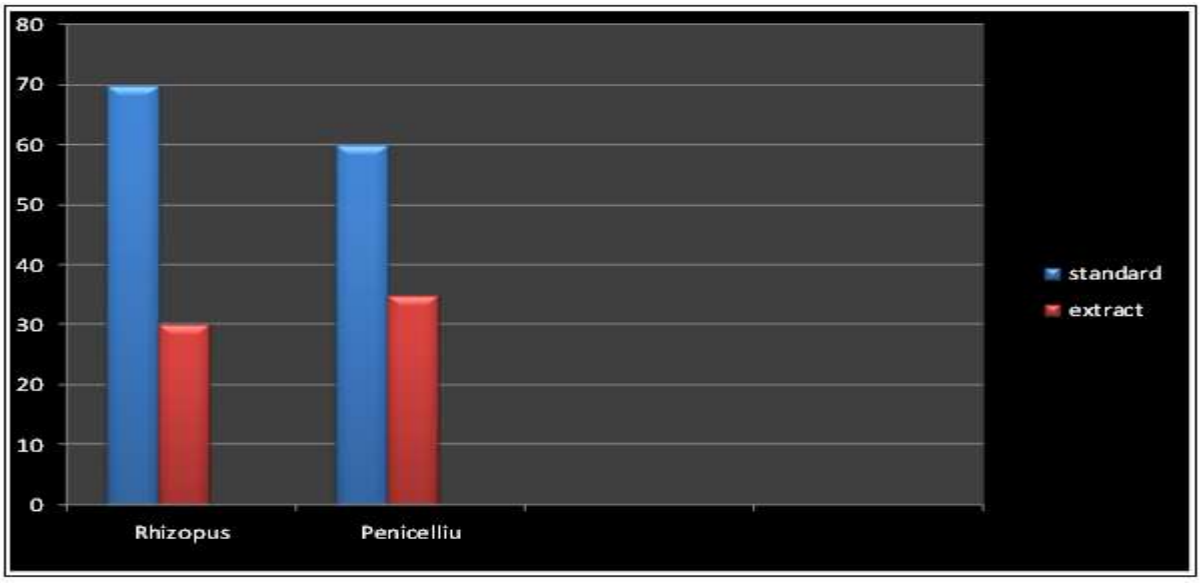

Fig. 2. Antifungal activity of ethanolic extract *Mirabilis Jalapa* plant leaves.

Conclusion

The current research was on the medicinally important plant *Mirabilis Jalapa*. The important bio-active compounds, carbohydrates, flavonoids, alkaloids, tannin, saponin, were qualitatively analyzed through different identification tests. Moreover the anti-inflammatory, anti-bacterial. Anti-fungal, anti-viral and anti-malarial activities of plants are due to the presence of the mentioned secondary metabolites, therefore the ethanolic extract of leaves and stem of *Mirabilis Jalapa* shows amazing activities against some gram positive, gram negative as well as some fungal strains The essential elements, Cu, Cr, Mn, Pb, Zn, Fe, etc. are also analyzed by Flame Atomic Absorption Spectroscopy.

In this research ethanol leaves extract of *Mirabilis Jalapa* plant was treated against four strains of bacteria and two strains of fungi. The result showed that the leaves extract is much sensitive against gram negative bacteria as *E.coli* and *Pseudomonas aeruginosa* showing maximum zone inhibition for these bacteria, and moderate sensitivity against gram positive bacteria like *Staphylococcus aureus*. The activity of the plant extract against microbes is due to the presence of the bio-active compounds especially alkaloids, flavonoids, tannin and saponins. The ethanol plant extract is also active against fungi; it is much effective against *penicellium* and *rhizopus*.

Some standard procedure should be introduced to explore these bio-active components of plants for the beneficial of human health. Extraction in purified form and high concentration is much needed to use for traditional medicines and other drugs formulation. This research will provide a detail evidences and information for the extraction of the essential phytochemicals and their activity against microbes.

Acknowledgment

We are thankful to Higher Education Commission of Pakistan for financial assistance, PCSIR Laboratories Complex Peshawar for providing us essential element analysis facilities and center of biotechnology university of Peshawar for antimicrobial analysis facilities.

References

Benoitvical F, Valentin A, Mallic M, bassierc JM. 2001. Anti-plasmodialactivity of *Colchlospermum planchonii* and *C. tentorium* tubercle essential oils. Journal of Essential Oil Research **13(1),** 65-67. http://dx.doi.org/10.1080/10412905.2001.9699609

Daniel M. 2006. Medicinal plants chemistry and properties. Science Publishers, Enfield, NH, U.S.A.

Edeoga HO, Okwu D, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian Medicinal plants. African Journal of Biotechnology **4(7),** 685-688. http://dx.doi.org/10.5897/AJB2005.000-312

Ferdous AJ, Islam SM, Ahsan M, Hassan CM, Ahmad ZV. 1992. *In vitro* antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug-resistant isolates of *Shigella* spp and isolates of *Vibriocholerae* and *Escherichia coli:* Phytotherapy Research **6(3),** 137-140. http://dx.doi.org/10.1002/ptr.2650060307

Habila JD, Bello IA, Dzikwe AA, Ladan Z, Sabiu M. 2011. Comparative Evaluation of Phytochemicals, Antioxidant and Antimicrobial Activity of Four Medicinal Plants Native to Northern Nigeria. Australian Journal of Basic and Applied Sciences **5(5),** 537.

Harborne JB. 1973. Phytochemical methods, London Chapman and Hall, Ltd, UK.

Hérouart D, Sangwan RS, Fliniaux MA, Sangwan-Norreel BS. 1988. Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura anoxia. Planta Med **54,** 14-17. http://dx.doi.org/10.1055/s-2006-962320

Hill AF. 1952. Economic Botany. A textbook of useful

plants and plant products. 2nd edn. McGarw-Hill Book Company Inc, New York.

Holdsworth DK. 1992. A preliminary study of medicinal plants of Easter Island, South Pacific. International Journal of Pharmacognosy **30(1),** 27-32.
http://dx.doi.org/10.3109/13880209209054626

Javanmardi J, Stushnoff C, Locke E, Vivanco JM. 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Journal of Food Chemistry **83(4),** 547-550.
http://dx.doi.org/ 10.1016/S0308-8146(03)00151-1

Kambu K, midi Phenzu, N Coune C, Wauter JN, Angenot L. 1982. Plants Medicine ET Phytotherapie, 34.

Krishnaiah D, Sarbatly R, Bono A. 2007. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnology Molecular Biology Review **2(4),** 097-104.

Kushad M, Masiuymas J, Smith M, Kalt W, Eastman K. 2003. Health Promoting Phytochemicals in Vegetables. Horticultural reviews **28,** 125-185.

Lemos TLG, Matos FJA, Alencar JW, Crareiro AA, Clark AM, Chesnary JD. 1990. Antimicrobial activity of essential oils of Brazilian plants. Phytotherapy Research **4(2),** 82-84.
http://dx.doi.org/10.1002/ptr.2650040210

Mahato SB, Sen S. 1997. Advances in terpenoids research, 1990-1994. Phytochemistry **44(7),** 1185-1236.
http://dx.doi.org/10.1016/S0031-9422(96)00639-5

Manandhar NP. 2002. Plants and People of Nepal Timber Press. Oregon.

Marles RJ, Farnsworth NR. 1995. Antidiabetic plants and their active constituents. Phytomedicine **2(2),** 137-189.
http://dx.doi.org/10.1016/S0944-7113(11)80059-0

Mohammed MT. 2007. Study of Some *Vinca Rosea* (Apocynaceae) Leaves Components and Effect of Its Extract on Different Microorganisms", Al-Mustansirya Journal of Science **18(1),** 28-36.

Oladunmoye MK. 2007. Comparative Evaluation of Antimicrobial Activities of Leaf Extract of *Mirabilis Jalapa*. Trends Medical Research **2(2),** 108-112.

Palaksha MN, Ravishankar K, Girija Sastry V. 2013. Evaluation of in-vitro antibacterial and anthelmintic activities of *Melochia corchorifolia* plant extracts. International journal of Biological and Pharmacy Research **4(8),** 577-581.

Santos FA, Rao VSN, Silveria ER. 1998. Investigations on the antinociceptive effect of *Psidium guajava* leaf essential oil and its major constituents. Phytotherapy Research **12(1),** 24-27.
http://dx.doi.org/10.1002/(SICI)10991573(199802012)12:1<24:AID-PTR181>3.0.CO;2-B.

Satheesh KB, Suchetha KN, Vadisha SB, Sharmila KP, Mahesh PB. 2012. Preliminary phytochemical screening of various extracts of *punica granatum* peel, whole fruit and seeds. Nitte University Journal of Health Science **2(4),** 34-38.

Tyler V. 1994. Phytomedicines in Western Europe: their potential impact on herbal medicine in the United States *Herbalgram* **30,** 24-30.

Vardar-Unlu G, Cadan F, Sokmen A, Deferera, Polissiou M, Sokmen M, Donmez E, Tap Bektas. 2003. Antimicrobial and Antioxidant Activity of the Essential Oil and Methanol Extracts of *Thymus pectinatus* Fisch et Mey. Var. *pectinatus* (Lamiaceae) Journal of Agriculture Food Chemistry **51(1),** 63-67.
http://dx.doi.org/10.1021/jf025753e

Yang SW. 2001. Three new phenolic compounds from a manipulated plant cell culture, *Mirabilis Jalapa*. Journal of Natural Products **64,** 313-317.
http://dx.doi.org/10.1021/np0004092