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RESEARCH ARTICLE

EVALUATION OF TOTAL PROTEIN CONTENT AND PHYTOCHEMICAL ANALYSIS WITH THEIR ANTI MICROBIAL ACTIVITY OF *MIRABILIS JALAPA* LINN AGAINST HUMAN PATHOGENS

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ABSTRACT

Mirabilis jalapa is a medicinal plant which belongs to the family Nyctaginaceae, commonly called the four o'clock flowering plant or marvel of peru, which is the most commonly grown ornamental plant and is native to tropical South America, and introduced in North America, mainly in southern, eastern and western states. However there are some disagreements about where it came from originally: Mexico, Chile and India. It was officially botanically recorded in the year 1753, although it has been long distributed as ornamental plant through the tropics of the world it is a popular garden ornamental in southern climate zones, where it grows rapidly and bears flowers that open in late afternoon, hence the common name. It is been used as folklore remedies around the world. The present study was carried out to explore the protein content, phytochemical constituents and antimicrobial properties of *Mirabilis jalapa* against eight both gram negative and positive test organisms in comparison with standard antibiotics streptomycin. The result reveal that the leaf extract of *Mirabilis jalapa* has high amount of protein content and also has very good anti-microbial activity against both gram negative and gram positive organism when compared to standard antibiotics streptomycin. The ethanol extract has shown significant activity against all the organisms but rather more towards *Shigella dysenteriae*, followed by *Escherichia coli, Lacidophilus, klebsiella pneumonia, Pseudomonas* and *Staphylococcus aureus.* Similarly, petroleum ether, chloroform, and aqueous extract of plant also showed good inhibition against *Vibro cholera* followed by *Pseudomonas*, *Staphylococcus aureus.* This indicates that the leaf extract of *Mirabilis jalapa* has potential antimicrobial activity with concentration dependent and can fight against these organisms and could be a good substitute to the contemporary medicines.

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INTRODUCTION

Plants are used as a medicine since age old for several thousands of years. Medicinal plants are the most important natural source of life saving drugs for the majority of the world's population. They have been the subject of man's curiosity since time immemorial. Almost all civilization has a history of medicinal plants and their applications. The search for components with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infections caused by multi drug antibiotic resistant strains of microorganisms, which adds urgency to search for new infection fighting strategies (Pavithra *et al*., 2015). Many plant families represent reservoir of effective chemotherapeutics and can provide valuable sources of natural antimicrobials (Umamaheswari *et al*., 2008). The antimicrobial activity test is an essential technique in many disciplines of science. It is used in pathology, to determine resistance of microbial strains to antimicrobials and in ethno-pharmacology research; it is used to determine the efficacy of novel antimicrobials against microorganisms, essentially those of medicinal importance (Edeoga *et al*., 2005). Plants provide a valuable material base for the discovery and development of new drugs with natural

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origin without many side effects, besides having enormous therapeutic potential of healing many infections and diseases (Marjorie *et al*., 1999) moreover plants are extensively studied for variety of bio-actives, one such are proteins, proteins are the building blocks of life. Every cell in the human body contains protein. The basic structure of protein is a chain of amino acids. Protein foods are broken down into parts called amino acids during digestion. The human body needs a number of amino acids in large enough amounts to maintain good health. The dietary Reference Intake (DRI) for protein is 0.8 to 1.0 grams of protein per kilogram of body weight. The present study aims to evaluate the protein content, potential phytochemical and antimicrobial activity of *Mirabilis jalapa*.

Mirabilis jalapa is a medicinal plant and its taxonomy is as follows;
Kingdom: Plantae
Order: Caryophyllales
Family: Nyctaginaceae
Genus: Mirabilis
Species: Mirabilis *Jalapa*

Mirabilis jalapa Linn is a long-lived (perennial) herb which grows up to 2 metres height, with a tuberous root. Its leaves are egg-shaped in outline with broad end at base (ovate), oblong, or triangular, measuring to 9 cm long; the leaf tip is acute, base cordate. The leaf stalk (petiole) is 4 cm long. Flowers of *M. jalapa* occur in groups of 3-7; flower stalks more or less absent; flowers are fragrant and open in the afternoon; flowers are tubular, white, pink or red in colour, up to 6.5 long by 3.5 wide with 5-6 stamens. The fruit is a small, one-seeded capsule (anthocarp).

MATERIALS AND METHODS

Plant Material

The plant material *Mirabilis jalapa* Linn (Figure 1) used for this study was collected from Nizamabad district, Telangana State, India. The plant specimen was authentically identified with the help of report of documentation of folk knowledge on medicinal plants (Ghatapanandi *et al*., 2011).

Fig. 1. Photograph of *Mirabilis Jalapa* Linn

Extraction of Protein from leaves of *Mirabilis jalapa* Linn

For the isolation of total protein from *Mirabilis jalapa* Linn plants, 100 mg of leaf material was taken, cleaned with double distilled water and placed in pestle and motor, to this protein extraction buffer was added and homogenized and then transferred to a 1.5 ml eppendroff tube, then the samples was centrifuged (13,000 rpm) for 15 min at 4°C, and separated from cellular debris and the supernatant was taken as test sample.

Table 1. Total protein extraction buffer

S.No	Chemical	Quantity
1	Glycerol (10%)	1ml
2	Tris-HCl (1,5 M)pH = 8,0	1.4ml
3	ß-mercaptoethanol	0.5ml
4	SDS (10%)	2ml
5	Water	10ml

Quantitative evaluation of protein content in leaves of *Mirabilis jalapa* Linn

The total proteins extracted from leaves of ***Mirabilis jalapa*** Linn was quantitatively estimated using Biuret method and compared against calibration curve of standard protein BSA (bovine serum albumin). Protein estimation using Biuret method lies on the principle that the –CO-NH- bond (peptide) in polypeptide chain reacts with copper sulphate (present in the biuret reagent) in an alkaline medium to give a purple colour which can be measured at 540 nm.

Reagents required for estimation of protein by biuret method;

1. Prepration of 0.2N NaOH (250ml): 2g of NaOH is taken and made up to 250ml with distilled water.

2. Preparation of Biuret Reagent: Dissolve 0.3 g of copper sulphate (CuSO4.5H2O) and 4.5g of sodium potassium tartarate, 2.5g of potassium iodide, dissolved in 100ml of 0.2N NaOH.

3. Preparation of Working Standard Protein: 0.5g of BSA was taken, dissolved and made up the volume to 100ml in volumetric flask using 0.2N NaOH.

3. Procedure

- A series of seven clean dry autoclaved test tubes were taken and marked B,S1,S2,S3,S4,S5 and T1 and T2 with the help of marker, to this working standard protein was pipetted out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml respectively (leaving test tube marked "B" as Blank without adding any sample, expect reagent and water) and to the test tube marked T1 and T2 - 0.5ml and 1ml of test sample(protein extract of *Mirabilis jalapa* Linn) was added
- To all these test tubes 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 1.5, 1.0 ml of distilled water was added serially. Subsequently 6ml of Biuret reagent was added to all these test tubes.
- All the test tubes were heated for 10min, cooled and the optical density /absorbency was recorded at 540 nm against

blank. Then a standard calibration curve was plotted by taking concentration of protein along X-axis and absorbance at 540 nm along Y-axis. Then from this standard calibration curve, then amount of total protein present in extract of ***Mirabilis jalapa*** Linn was calculated.

Extraction of Secondary Metabolites from *Mirabilis jalapa* Linn

The leaves of *Mirabilis jalapa* Linn was shade dried and grinded to a fine powder and further used for extraction of secondary metabolites using various solvents such as petroleum ether, chloroform, ethanol (95 %) and distilled water successively through cold extraction.

Phytochemical of Screening of *Mirabilis jalapa* Linn

The leaves of *Mirabilis jalapa* Linn was shade dried, powdered and were qualitatively tested for presence of various phytochemicals using various standard tests such as test for flavanoids (Peach and Tracey 1959), test for steroids (Gibbs 1974), test for tritepenoids, test for tannins (Trease and Evens 1980), test for glycosides (Kokate *et al*., 1997), test for Saponins (Gibbs 1974), test for Alkaloids (Meyers test, Wagner's test, Dragendroffs test). These tests were based on visual observation of colour modification and precipitation upon the addition of specific reagents.

Antimicrobial Studies on *Mirabilis jalapa* Linn

The successive extracts such as petroleum ether extract, chloroform extract, ethanol extract and aqueous extract of *Mirabilis jalapa* Linn, was concentrated by distilling the solvent through evaporation, air dried and then dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 1mg/ml. Antimicrobial screening was done using agar diffusion method (Bauer *et al*., 1966).

Nutrient agar plates were prepared and the bacterial samples of 0.1ml were spread uniformly on to the plates, wells were made with help of crock borer and 0.1ml of crude drug extract dissolved in DMSO was loaded as bioassay for studying the antimicrobial activity of *Mirabilis jalapa* Linn against few test organisms *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeroginosa, Klebsiella pneumoniae, Bacillus cereus, Lactobacillus acidophilus, Enterococcus faecalis, Salmonella typhi* and *Shigella dysenteriae*

RESULTS AND DISCUSSION

Evaluation of Protein content in leaves of *Mirabilis jalapa* Linn:

In present study efforts were made to determine protein content in *Mirabilis jalapa* Linn. Proteins are made up of amino acids, and they are the "building blocks" of life. Our skin, muscles, tendons, cartilage, even hair and nails, are all because of protein. Protein helps to form enzymes, hormones, antibodies and new tissues. It replaces old cells with shiny new ones, and it transports important nutrients in and out of those cells. The human body can manufacture all but nine of the 22 amino acids that make up proteins. These nine amino acids are known as "essential" amino acids, and therefore must be derived from what we eat. In our present study it was evaluated that *Mirabilis jalapa* Linn is protein rich which is evident from our present study that 0.1g of leafs of *Mirabilis jalapa* Linn contain 5800 micrograms or 58mg of protein. But there are limited evidences that the leaves of *Mirabilis jalapa* Linn are used for cooking (Tanaka, 1976; Facciola, 1990; Manandhar, 2002). There are few reports that it can be used as an emergency food, only eaten when all else fails (Kunkel, 1984). Research in the edible nature of *Mirabilis jalapa* leaves is further necessary since it has high amount of protein content.

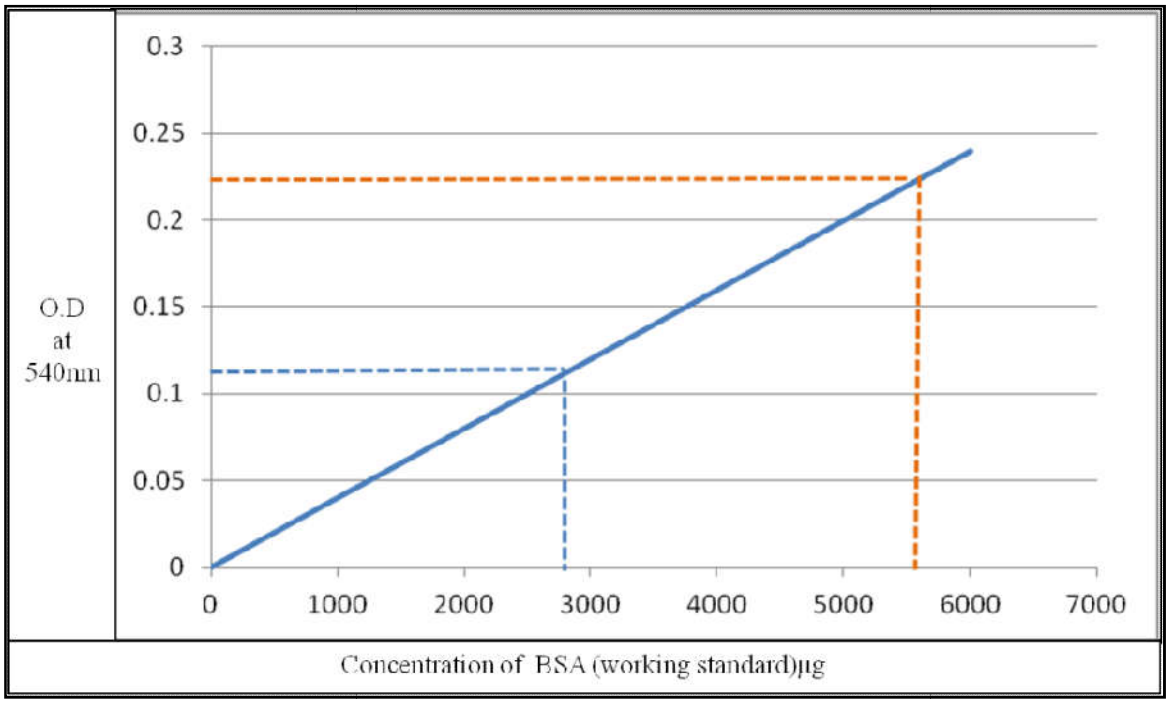

Figure 2. Quantitative estimation of total protein content in leaves of *Mirabilis jalapa* Linn using Biuret method

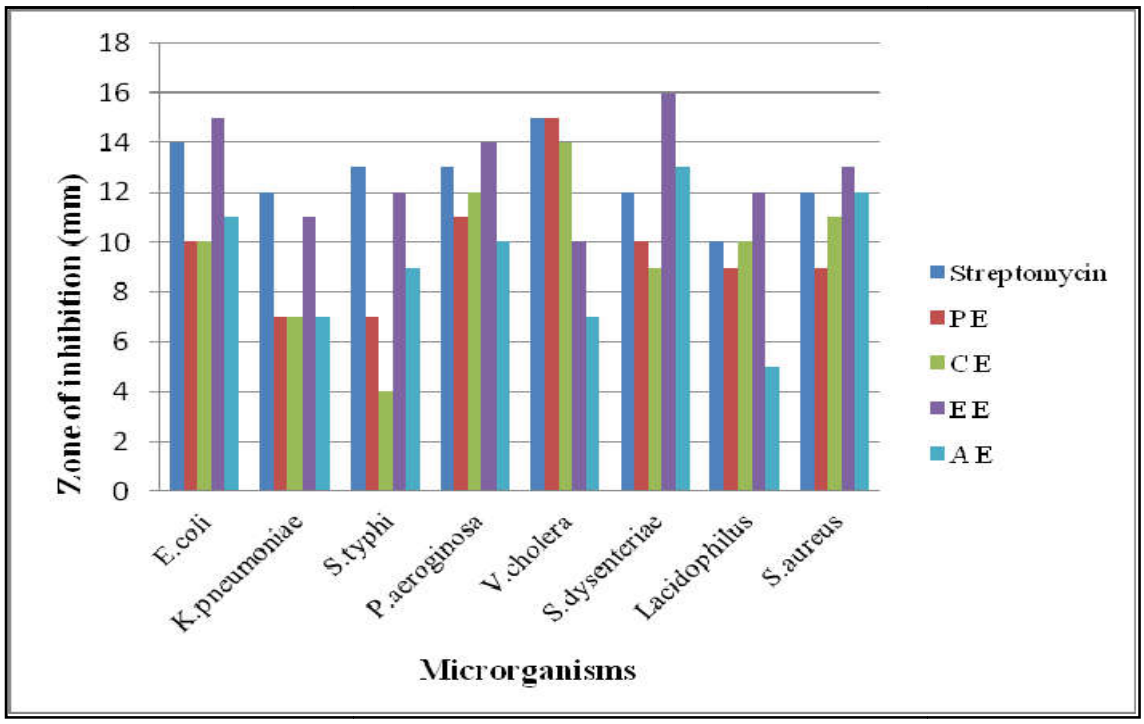

Figure 3. Anti-Microbial Activity of Various Extracts of *Mirabilis jalapa* Linn leaves on different microorganisms and their Comparative Study with Antibiotic Streptomycin as Control

Table 2.The results of preliminary screening of secondary metabolites

S No	TESTS	P.E EXTRACT	C.E EXTRACT	E.E EXTRACT	A.E EXTRACT
1	Test for Flavonoids				
	a.Flavonoid test	+	+	+	+
	b.Lead acetate test	+	+	+	+
2	Test for Steroids				
	a.Acetic anhydride	-	-	+	-
	b.Chloroform	-	-	+	-
3	Test for Tripenoids	+	+	+	+
	a.Salkowiski test	+	+	+	+
	b.Libermann Burchards test				
4	Test for Tannins				
	a.Ferric chloride test	+	+	+	+
	b.Gelatin test	+	+	+	+
5	Test for Glycosides				
	Kellar killani's test	-	-	+	-
6	Saponins				
	Foam test	-	-	+	+
7	Test for Alkaloids				
	a.Mayers test	+	+	+	+
	b.Dragendroffs test	+	+	+	+
	c.Wagner test	+	+	+	+

P.E= Petroleum, ether; C.E= chloroform, ethanol; E.E= Ethanol extract; A.E= Aqueous extract; '-'= negative; '+'= Positive

Preliminary Screening of Secondary Metabolites and Antimicrobial Activity of *Mirabilis jalapa Linn*

Preliminary phytochemical screening showed the presence of flavonoid, steroids, tripenoids, tannins, glycosides, saponins and alkaloids.

Antimicrobial Studies on *Mirabilis jalapa* Linn

Antimicrobial activity of various solvent extracts of *M. jalapa* leaves have been evaluated *in vitro* against eight test organisms like gram negative *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeroginosa, Vibrio cholera, Shigella dysenteriae* and grampositive bacteria like *Lacidophilus* and *Staphylococcus aureus.* The range of antimicrobial activity are represented graphically in Figure 3, this clearly depicts that all the extracts of *Mirabilis jalapa* Linn leaves shows antimicrobial activity against all the test organisms. The ethanol extract has evidently shown significant activity against all the organisms but rather more towards *Shigella dysenteriae* (16 mm) followed by *Escherichia coli,* (15 mm), *Lacidophilus* (12 mm), *klebsiella pneumonia*

(11 mm), *Pseudomonas* (14 mm) and *Staphylococcus aureus* (13 mm). Similarly, petroleum ether, chloroform, and aqueous extract of plant also showed good inhibition against *Vibrio cholera* (15, 14 and 7mm) followed by *Pseudomonas aeroginosa* (11, 12 and10mm) *Staphylococcus aureus* (9, 11, and 12 mm) against standard antibiotic streptomycin inhibition ranging from 10-15mm. This is due to the presence of major secondary metabolites like steroids, flavonoids, saponins, terpenoids, tannins, alkaloids and glycosides in ethanolic extract. The antimicrobial activity of petroleum, chloroform, and aqueous extracts is due to the presence of secondary metabolites like, flavonoids, terpenoids, tannins and alkaloids.

Conclusion

Change in the life style and habits of human beings are leading to various new diseases, of which some are found to be multidrug resistant. These types of diseases are better treated using the extracts of medicinal plants which are rich in secondary metabolites and are source of manufacturing potential drugs. This secondary metabolite includes Flavonoids, Glycosides, Alkaloids, Saponins, Terpenoids, Tannins and Steroids etc. Hence, in this regard we anticipated to find out the *invitro* antimicrobial activity of *Mirabilis jalapa* Linn, which has confirmed to inhibit the growth of few gram positive and gram negative test organisms, in which all the extracts of *Mirabilis jalapa* Linn showed antimicrobial activity from which the ethanolic extract and petroleum ether showed major significance. Based on the results obtained it is evident that the medicinal plant *Mirabilis jalapa* Linn has antimicrobial activity against various microorganisms used in this study and can be used as a potential drug. As the leaves are rich in source of protein, they may be consumed, but only as an emergency food.

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