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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *MONSTERA DELICIOSA* LIEBM. STEM EXTRACTS

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ABSTRACT

The present study was carried out to investigate the preliminary phytochemical screening, total phenolic, tannin and flavonoid contents, in vitro antibacterial and antioxidant activities of different solvent extracts of *Monstera deliciosa* stem. Phytochemical screening was carried out using standard protocols. The antibacterial activity was carried out by agar well diffusion method and the antioxidant activity was performed by DPPH (2, 2-diphenyl-1-picryl-hydrazyl) method. The phytochemical screening study of *Monstera deliciosa* stem extracts revealed the presence of important phytochemicals namely Tannins, Steroids, Flavonoids, Alkaloids and Saponins. Hexane extract exhibited antibacterial activity against both Gram positive and Gram negative microorganisms tested. Ethyl acetate extract exhibited larger zone of inhibition on *Serratia marcescens* than the standard Streptomycin. Hexane, Chloroform, Ethyl acetate and Methanol extracts at 100µg/ml and 200µg/ml concentrations exhibited more free radical scavenging activity than the standard Ascorbic acid, whereas hexane and chloroform extracts at 300µg/ml concentrations showed more free radical scavenging than the standard Ascorbic acid. The results of this study are suggesting the medicinal importance of this plant due to the presence of various phytochemicals.

KEYWORDS: *Monstera deliciosa*, Phytochemical screening, Antibacterial activity, Antioxidant activity.

INTRODUCTION

Medicinal plants are the richest bio-source of drugs for traditional system of medicine, food supplements, chemical entities for synthetic drugs and many more therapeutic products. Various medicinal plants have been used worldwide in daily life to treat diseases all over the world.[1] In fact, plants produce a diverse range of bioactive molecules, thus making them a rich source of different types of medicine. Higher plants, as source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times.[2] Medicinal plants are useful for healing as well as curing of human diseases because of the presence of phytochemical constituents as natural bioactive compounds. Development of multi drug resistance in microorganisms due to indiscriminate use of antibiotics, infectious diseases are becoming the major cause of morbidity and mortality. Long-term solutions must acknowledge this and nurses and other health care professionals must take a proactive part in finding alternative solutions,[3] therefore scientists now started focusing on plant medicines which possess relatively lower incidence of side effects, decreased toxicity and increased activity compared to that of chemically synthesized drugs which cause chronic diseases on long term use. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), that may generate in the human body, can cause oxidative damages associated with many diseases such as Atherosclerosis, Coronary heart diseases, Parkinson's disease, Diabetes and Cancer. Too high levels of free radicals and low amount of antioxidant levels lead to a condition of oxidative stress, chronic injury and depletion of immune system. The synthetic antioxidants like Butylated hyroxytoulene (BHT), Butylated hydroxylanisole (BHA) and Tert-butylhydroquinone (TBHQ) using in Food industry currently may be linked to the development of liver disease and cancer. Hence, demand for antioxidants containing less synthetic additives is driving research efforts to seek out alternative sources of natural antioxidants mainly from plant source.[4] Polyphenols are the natural antioxidant compounds found in variety of fruits, vegetables and other plant parts.[5] These compounds are attracting a great deal of attention due to increasing evidence suggesting that they may prevent chronic conditions such as cancer, atherosclerosis and neurological diseases.[6] *Monstera deliciosa* also called as fruit salad plant belongs to Araceae family native to Mexico. Common names include Fruit Salad plant (in reference to its edible leaves and fruits), ceriman and Swiss cheese plant. In the present study, an attempt was made to evaluate the phytochemical screening, antibacterial activity and antioxidant activity and also to determine the total phenolic, tannin and flavonoid contents of *Monstera deliciosa* stem extracts of different solvents.

MATERIALS AND METHODS

Chemicals and reagents

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was obtained from sigma-Aldrich and all the solvents from Merck. The remaining chemicals which were used in the present study were of analytical grade.

Preparation of the plant extracts

Monstera deliciosa plant was collected from the Herbal garden of Acharya Nagarjuna University, Guntur, Andhra Pradesh. The stem part of the plant was thoroughly washed, cut into small pieces and air dried in shade. The dried plant material was ground into a coarse powder by means of electrical grinder. The powdered plant stem material was extracted in different solvents viz., Hexane, Chloroform, Ethyl acetate and Methanol in that order of their polarity. The extracts thus obtained were concentrated separately in rotary evaporator and the crude extracts were preserved in sterile air tight container for further analysis.

Preliminary phytochemical screening

Test for flavonoids

a) Ferric chloride test

Two ml of the test solution was boiled with distilled water and filtered. Then, few drops of 10% ferric chloride solution were added to the 2 ml of filtrate. A greenish blue or violet coloration indicates the presence of a phenolic hydroxyl group.

b) Shinoda's test

Five grams of each extract was dissolved in ethanol, warmed and then filtered. Small pieces of magnesium chips were then added to the filtrate followed by few drops of conc. HCl. The pink, orange, or red to purple coloration indicates the presence of Flavonoids.

c) Sodium hydroxide test

Extract of 0.2 gm was dissolved in water and filtered. To this, 2 ml of the 10% aqueous sodium hydroxide was added to produce yellow coloration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was the indication for the presence of Flavonoids.

d) Lead acetate test

Extract of 0.5 gm was dissolved in water and filtered. To the 5 ml of each filtrate, 3 ml of lead acetate solution was added. Appearance of a buff-coloured precipitate indicates the presence of Flavonoids.

Test for alkaloids

Five grams of crude powder was stirred with 1% aqueous HCl on water bath and then filtered. To the 1 ml filtrate, few drops of Dragendroff's reagent were added. Orange Red precipitate was taken as positive. To another 1 ml filtrate, few drops of Mayer's reagent were added and appearance of buff- coloured precipitate will be taken as presence of alkaloids. Test for soluble starch Crude extract of 0.2 gm was boiled in 1 ml of 5% KOH, cooled and acidified with H_2SO_4. Yellow coloration indicates the presence of soluble starch.

Test for Saponins

Crude powder of 0.5 g was shaken with water in a test tube and it warmed in a water bath. The persistent froth indicates the presence of saponins.

Test for terpenoids

Five grams of crude extract was dissolved in ethanol. To this, 1 ml of acetic acid was added followed by conc. H_2SO_4. A change in colour from pink to violet confirms the presence of terpenoids.

Test for steroids

a) Salkowskii test

In 2 ml of chloroform, 0.2 g of extract was dissolved and added the conc. H_2SO_4. The development of reddish brown colour at inter phase indicates the presence of steroids.

b) Keller-Killiani test

To 0.5 ml of test solution, 2 ml of 3.5% $FeCl_3$, small amount of glacial acetic acid and 2 ml of conc. H_2SO_4 were added carefully. Appearance of reddish brown ring at inter phase is a positive indication for the presence of steroids.

c) Liebermann-Burchard test

To 0.2 g of each extract, 2 ml of acetic acid was added and the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or

bluish-green indicates the presence of a steroidal ring (i.e. Aglycone portion of cardiac glycoside).

Test for carbohydrates

a) Molisch's test

Two ml of Molisch's reagent was added to the extract dissolved in distilled water and 1 ml of conc. H_2SO_4 was dispensed along the walls of the test tube. The mixture was allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a dull violet colour at theinter phase of the two layers indicates the positive test for carbohydrates.

b) Fehling's test (for free reducing sugars)

The crude extracts were treated with 5.0 ml of Fehling's solution (A & B) and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of free reducing sugars.

c) Fehling's test (for Combined Reducing Sugars)

Extract of 0.5 g was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and then heated on a water bath for 2 minutes. Appearance of a reddish brown precipitate of cuprous oxide indicates the presence of combined reducing sugars. d) Barfoed's test (for monosaccharide) In distilled water, 0.5 g of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

d) Barfoed's test (for monosaccharide)

In distilled water, 0.5 g of the extract was dissolved and filtered. To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and then heated on a water bath for 2 minutes. Reddish precipitate of cuprous oxide formation is the positive test for the presence of monosaccharide.

Test for tannins

Crude extract of 0.5 g was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

a) Borntrager's Test

Extract of 0.2 g was shaken with 10 ml of benzene and then filtered. To the filtrate, 5 ml of 10% ammonia solution was added and then shaken the tube well. Appearance of pink, red or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.

b) Phlonatanins test

To 0.2g of extract, 1% HCl solution was added. Formation of red precipitate indicates the presence of tannins.

In vitro antioxidant assay

2, 2-diphenyl-1-picryl hydrazyl (DPPH) Free radical scavenging activity

The DPPH free radical scavenging activity of the different extracts was measured according to the method of Chew *et al.*[7] The crude extracts in different concentrations viz., 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml were prepared in DMSO. One ml of each concentration was mixed with 4 ml of the 0.004% (w/v) solution of DPPH prepared in methanol. The reaction mixture was kept for incubation in dark for 30 minutes. Methanol was used as control and Ascorbic acid was used as standard antioxidant. The absorbance was measured at 517 nm.

The DPPH scavenging activity (%) was calculated by using the following formula

DPPH scavenging activity (%) = $[(A_0 – As) / A_0] \times 100$,

Where,

A_0 -- absorbance of the control.

As -- absorbance of the sample.

Antibacterial screening

Microorganisms used

The antibacterial activity of solvent extracts was determined by using both Gram positive and Gram negative bacteria. Three Gram positive bacteria including *Staphylococcus aureus* MTCC 737, *Bacillus megaterium* MTCC 428 and *Streptococcus mutans* MTCC 497. Three Gram negative bacteria which include *Escherichia coli* MTCC 1687, *Serratia marescens* MTCC 7298 and *Salmonella enterica* MTCC 3858.

Antibacterial screening by agar well diffusion method

Antibacterial activity was determined by agar well diffusion method 12. Bacterial suspensions of different bacteria were prepared by using 24 hours old bacterial cultures. After solidification of agar medium, 6mm diameter wells were punched in agar medium with a sterile cork borer. Streptomycin standard antibiotic was used as positive control in the concentration of 10µg/ml DMSO. A minute quantity of sterile agar suspension was added to the well. The sample of 100 µl, prepared by dissolving 100mg of sample in 1 ml of DMSO, was added to each well. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 370 C for 24 hrs. After incubation, the diameter of the inhibition zone was measured. For each sample and bacterial species, triplicates were maintained.

RESULTS

Phytochemical screening

The phytochemical screening study of *M. deliciosa* stem extracts showed the presence or absence of various phytochemicals in different solvent extracts (Table-1). Tannins, Free anthraquinones, Steroids and Carbohydrates were found to be present in all the four solvent extracts. Flavonoids were present in all the solvent extracts except the Ethyl acetate extract. Alkaloids, Saponins, Monosaccharides, Free reducing sugars and combined reducing sugars were present only in Methanol extract. Cardiac glycosides were present in all solvent extracts except hexane. However, Terpenoids and Soluble starch were found to be absent in all solvent extracts.

Table 1: **Phytochemical analysis of *Monstera deliciosa* stem extracts in different solvents.**

S.No	Phytochemicals	H	C	E	M
1	Carbohydrates	+	+	+	+
2	Monosaccharides	-	-	-	+
3	Free reducing sugar	-	-	-	++
4	Combined reducing sugars	-	-	-	++
5	Tannins	+	+	+	+
6	Free anthraquinones	++	+	+	+
7	Steroids	+	+	++	++
8	Cardiac glycosides	-	+	+	+
9	Terpenoids	-	-	-	-
10	Saponins	-	-	-	+
11	Flavonoids	+	+	-	++
12	Soluble starch	-	-	-	-
13	Alkaloids	-	-	-	+

H-Hexane, **C**- Chloroform, **E**-Ethyl acetate, **M**-Methanol.

Antibacterial activity

The different solvent extracts of *M. deliciosa* showed varied antibacterial activity against six test organisms which include, three Gram positive bacteria and three Gram negative bacteria (Figures-1&2). Hexane extract exhibited the activity on all the tested bacteria, with highest activity against *Bacillus megaterium* and least against *Salmonella enterica*. The chloroform, ethyl acetate and methanol extracts did not show any antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica*, respectively. Hexane extract against *Bacillus megaterium* and ethyl acetate extract against *Serratia marcescens* showed high antibacterial activity by causing larger inhibition zones than that of positive control antibiotic namely streptomycin. Of the four solvent extracts, hexane and ethyl acetate extracts showed relatively higher activity against the tested Gram positive bacteria. On the whole, the four crude extracts of the *M. deliciosa* stem in different solvents showed a mixed result exhibiting more or equal or less antibacterial activity when compared to that of streptomycin against the tested bacteria.

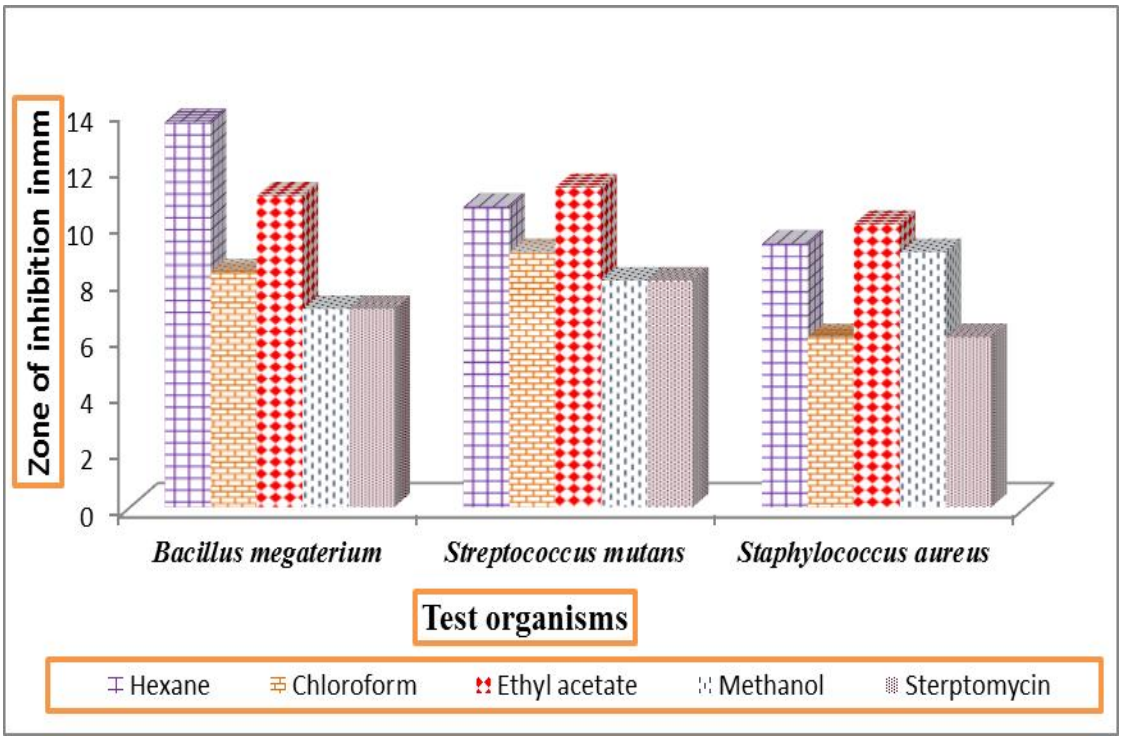

Figure-1: Antibacterial activity of different solvent extracts of *M. deliciosa* Stem on Gram positive bacteria.

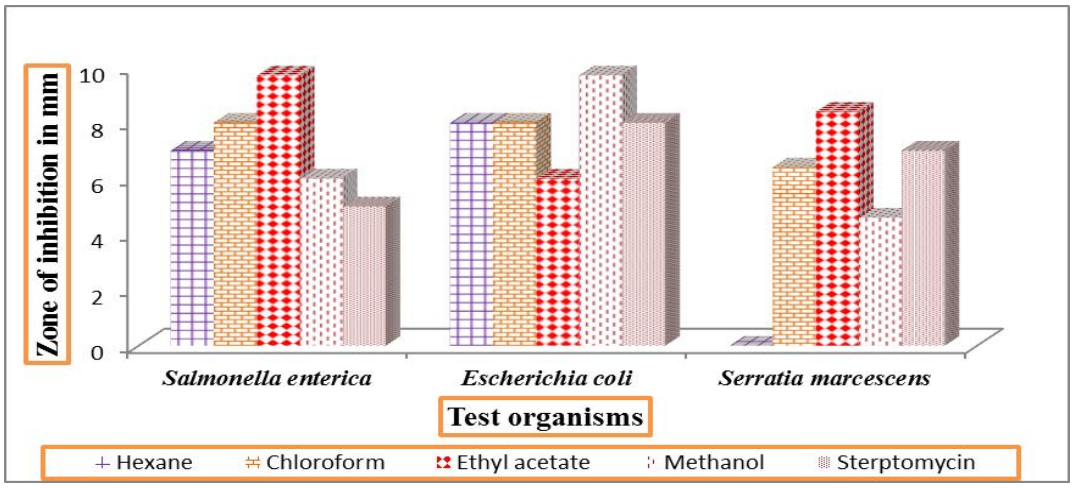

Figure-2: Antibacterial activity of different solvent extracts of *M. deliciosa* stem on Gram negative bacteria.

DPPH Free radical scavenging assay

The DPPH Free radical scavenging assay results revealed that the *M. deliciosa* stem extracts were found to have good antioxidant activity (Figure-3). DPPH radical scavenging activity of *M. deliciosa* was increased with an increase in concentration of all the solvent extracts. When compared to the other solvent extracts, chloroform extract showed relatively higher activity at all concentrations. At 100µg/ml and 200µg/ml concentrations, all the solvent extracts exhibited higher activity than ascorbic acid. Chloroform extract at 300µg/ml and 400µg/ml concentrations and hexane extract at 300µg/ml concentration were found to be superior in activity than ascorbic acid. However, the activity of all the extracts at 500µg/ml concentration was found less than ascorbic acid. But on the whole, all the extracts at different concentrations exhibited considerably good antioxidant activity.

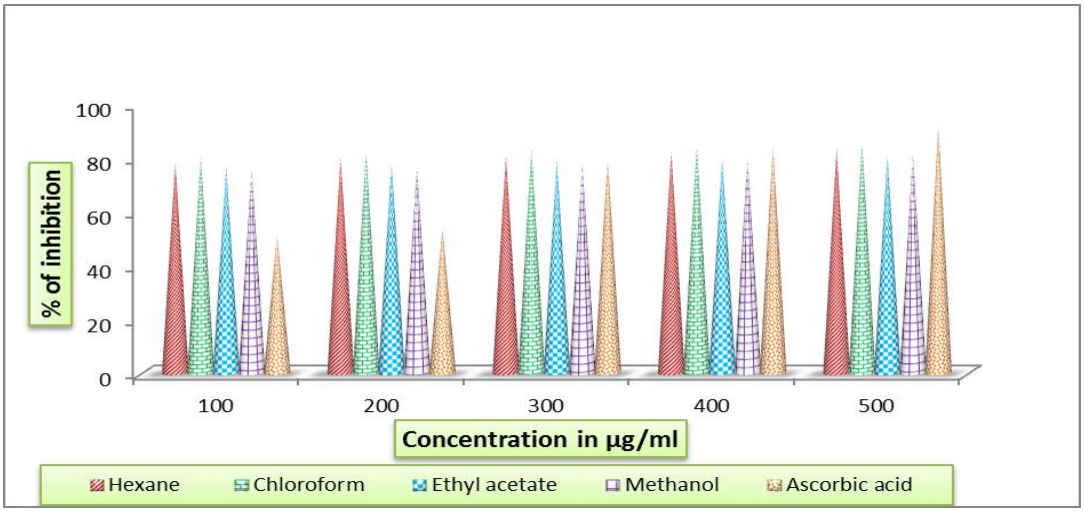

Figure-3: Antioxidant activity of different solvent extracts of *M. deliciosa* stem.

DISCUSSION

In the present investigation, the studies revealed the presence of different phytochemicals, total amounts of the some phytochemicals and potential antibacterial and antioxidant activities. Phytochemicals are either primary compounds (e.g. chlorophyll, proteins and common sugars) or secondary compounds (e.g. terpenoids, alkaloids, phenolics) The medicinal value of plants lies in their phytochemical substances that have a definite physiological action. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids and saponins protect against chronic diseases. Steroids and triterpenoids show the analgesic properties. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. The use of antibiotics has led to an alarming incidence of development of multi drug resistant microorganisms thus demanding the invention of new medicines which work on multi drug resistant microorganisms. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has been reported earlier.[8,9] Lucas *et al*.[10] reported the antibacterial activity of ethanol extract of *Monstera deliciosa* leaf and flower against *Staphylococcus aureus*. Similar antibiotic and antioxidant results were reported with different solvent extracts of a medicinal weed *Chromolaena odorata*.[11] Many plants have been investigated in the past for their activities and the search is gradually increasing in recent times due to rapid generation of ROS. Many plant species have been reported to exhibit antioxidant activity, the majority of active antioxidant compounds are flavonoids, is oflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, β-carotene and α-tocopherol are known to possess antioxidant potential.[12,13,14] In the current study, all the solvent extracts showed a potential antimicrobial activity than the positive control Streptomycin. *Monstera deliciosa* plant stem extracts also contained considerable amounts of Phenolics, Flavanoids and Tannins. Different extracts of this plant also exhibited antioxidant activity higher than that of the standard Ascorbic acid. Thus, this study is suggesting the medicinal importance of this plant.

CONCLUSION

The selected medicinal plant for this study was found to be a good source of the secondary metabolites (Alkaloids, Flavonoids, Terpenoids, Tannins and reducing sugars), anitimicrobial

and antioxidant compounds. Further extensive studies need to be carried out on this plant to identify the bioactive compounds responsible for their antibacterial and antioxidant activities.

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