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Antibacterial Activity of *Trianthema portulacastrum* Linn. Against Human Pathogens

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Abstract : *Trianthema portulacastrum* Linn.is a species of flowering plant that belongs to the family Aizoaceae (Ficoideae). It is a commonly used medicinal plant in India. The present investigation was carried out to assess the antibacterial property of the ethanolic and aqueous extracts of *T. portulacastrum* Linn. on the common human pathogens *viz. Escherichia coli*, *Vibrio harveyi, Staphylococcus aureus* and *Bacillus cereus* by Kirby-Bauer disc diffusion assay. The antibacterial activity of the biosynthesized silver nanoparticles was also carried out. The preliminary phytochemical screening revealed the presence of alkaloids, tannins, phenols, glycosides, saponins, flavonoids, protein, carbohydrate and terpenoids in both aqueous and ethanolic extracts. The nanoparticles biosynthesized using the plant extract was spherical in shape. Ethanolic extract showed superior activity against all the four strains of bacteria when compared to the aqueous extract. Antibacterial activity of nanoparticle solution was less when compared to the ethanolic extract. The most susceptible bacterium was found to be *Bacillus cereus* and the most resistant bacterium was found to be *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was observed at a concentration value of 40µg for ethanolic extract, 80µg for aqueous extract and 40µg for nanoparticle solution.

Keywords: *Trianthema portulacastrum*, phytochemical screening, silver nanoparticle, antibacterial activity, MIC.

Introduction

Plant resources have remained an integral part of human society throughout the history. Almost all plants have its own medicinal properties that encouraged early man to utilize them for various kinds of diseases. Plants have been used for various medicinal purposes since many centuries in many parts of the world. In India, Ayurvedic, Siddha and Unani System of medicines utilize plants as medicinal agents. According to the World health organization (WHO) report an average of about 55% of the world population relies on the traditional system of medicines, mainly on plant source for their health care (Ekta *et al*., 2016;Samiriti *et al*., 2019). Hence, one such plant is *Trianthema portulacastrum* Linn. which is well known for its medicinal properties.

T. portulacastrum Linn.is a species of flowering plant that belongs to the family Aizoaceae. It is a weed species commonly known as horse pulsane, back pig weed or carpet weed. It is a prostrate herb distributed in sub-tropical regions of the world, and also found abundantly in India (Divya *et al*., 2017). *T. portulacastrum* Linn. has been recognized in different systems of traditional medicines (Ayurvedic, Siddha and Unani System) for the treatment of diseases and aliments of human beings. The plant is bitter and used as analgesic, stomachic, laxative and serves as alterative cure for bronchitis, heart disease, anaemia and inflammation. The root applied to the eye to treat corneal ulcers, itching, dimness of sight and night blindness. A decoction of the herb is used as a vermifuge and is useful in rheumatitis (Shyam *et al*., 2009). The plant has a remarkable protection against the hepatotoxicity and hepatocarcinogenesis induced by the various chemical methods (Ekta *et al*., 2016, Anum *et al*., 2015). The present study focuses on the preliminary phytochemistry, antibacterial activity and silver nano particle assay of *T. portulacastrum* Linn. Plants synthesize phytochemicals as a means of self-defence but these phytochemicals play a significant role in the human body for the treatment against various diseases (Kavitha *et al*., 2013). Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism and many components of plants products have been shown to be specially targeted against resistant pathogenic bacteria (Selvakumar and Prashanth, 2017). A variety of phytoconstituents present in plants are active against a wide range of microorganisms. The present study was carried out to find out whether the plant possesses antibacterial activity. The minimum inhibitory concentration was also found out. The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium or bacteria. The MIC is determined by preparing solutions of the crude extract at increasing concentrations, and incubating the solutions with separate batches of cultured bacteria, and measuring the results using agar dilution or broth micro dilution (Andrews, 2001). Nowadays plant extracts act as reducing as well as capping agents for the synthesis of nano particles which is more advantageous than chemical and microbial synthesis. Among the metal nano particles silver has been consumed largely due to their antimicrobial and pharmaceutical applications. The small sized nano particles have large surface area to improve the antimicrobial activity. Currently, silver can also be utilized either in the textile industry by incorporating it in to the fibre or employed in filtration membranes of water purification systems (Vanaja *et al., 2013*; Abhishek, 2015).

Materials and Methods

Collection and identification of the plant material

Whole plant of *Trianthema portulacastrum* Linn. were collected from Atlantis railway gate, Panampilly Nagar, Ernakulam, Kerala (India). The plant specimen was identified using standard authentic taxonomic literature (Gamble, 1921).

Preparation of plant extract

The whole plants collected were washed under running tap water to get rid of dust particles, cut into small pieces, shade dried and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work. The aqueous and ethanolic extracts were prepared by weighing 20gm of each of the powdered samples and mixed thoroughly with 200ml of each solvent. They were allowed to soak in the solvent for 48 hours at room temperature. The extracts were then filtered through Whatman no. 1 filter paper. The ethanolic extract obtained was air dried and later in a water bath. The aqueous extract obtained was evaporated at 50°C in hot air oven. The extracts were then dissolved in known amount of distilled water for further studies.

Preliminary phytochemical screening

The plant extracts were tested for the presence of various phytochemicals by using standard methods (Thulasi and Krishnakumar, 2018).

Silver nanoparticle assay

Silver Nano particle assay was carried out in order to find out whether the plant biosynthesized silver nano particle or not. The powdered samples were used for the assay (Nisha *et al*., 2017).

Preparation of silver nitrate solution for nanoparticle synthesis

2 mM silver nitrate solution was prepared by adding 0.0339g of silver nitrate in 100 ml of double distilled water. The solution was mixed thoroughly and stored in brown coloured bottle in order to prevent auto-oxidation of silver.

Preparation of the plant extract for nanoparticle synthesis

25 gm of the powdered sample was taken in 250 ml beaker and boiled along with 100 ml distilled water. After 10 minutes of boiling solution was cooled to room temperature and filtered using Whatman's no. 1 filter paper. The collected extract was used for the synthesis of silver nano particles.

Synthesis of silver nano particles

10 ml of extract was added to 90 ml of 2 Mm aqueous silver nitrate solution (1:9 ratio) and mixed thoroughly by manual shaking. The beaker was then placed under sun light for reduction into silver nitrate nano particle. After 10 minutes colour changes were noted. This indicates the preliminary confirmation for the formation of plant mediated silver nano particle

Purification of silver nano particles

After 5 hours, grey nano particles started to settle at the bottom. The solution was centrifuged at 8000 rpm for 15 minutes, supernatant was discarded and the pellet containing nanoparticles were taken out on a petri plate and kept in hot air oven to dry at 50° C for 4-5 hours. The silver nanoparticles were then taken out on a glass slide and observed under 40 X resolution of the microscope (Biolinkz M2000 series) and photographs were taken.

Antibacterial Assay

Kirby- Bauer disc diffusion method was performed for the antibacterial assay (Woldeyes, 2012). Four different bacterial strains were used in the present study which belongs to gram-positive (*Staphylococcus aureus, Bacillus cereus*) and gram-negative categories (*Escherichia coli, Vibrio harveyi*). *Bacillus cereus* is a gram positive, rod shaped, facultatively anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are gram positive, facultatively anaerobic, round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are gram negative, facultative anaerobic, rod shaped, coli form bacterium commonly found in the lower intestine of warm-blooded organism which cause food poisoning in their hosts. *Vibrio harveyi* are gram negative, bioluminescent, marine, rod shaped bacterium responsible for Luminous Vibriosis.

The pure cultures maintained in slants were collected from Microbiology laboratory, Post Graduate and Research Department of Botany, Maharaja's College, Ernakulam, Kerala, India for the study. Each strain was separately inoculated into 5 ml nutrient broth and was incubated at 37° C for 24 hours. Filter paper discs (Whatman filter paper No.1) were prepared using paper punch and sterilized. Lawn cultures of the test organisms were made on nutrient agar plates using a sterile cotton swab under aseptic conditions. The filter paper discs were loaded with plant extracts (aqueous and ethanolic) and nano particle solution using a micropipette under aseptic conditions. Discs impregnated with Amoxycillin served as positive control (standard) and the filter paper disc soaked in solvents were used as negative control. The discs were placed on the surface of nutrient agar with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar plate. The prepared plates were incubated at 37 °C for 24 hours. Inhibition zones were measured after incubation period.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the ethanolic extract as well as the biologically synthesized nano particles were carried out. Minimum inhibitory concentration (MIC) was determined by making decreasing concentrations (100 µg, 80 µg, 60 µg 40 µg, 20µg) of the stock extracts (ethanolic and nano particle) into nutrient broth using a pipette. Suspension of the bacterium was added to the test tubes containing the different concentrations of the extract aseptically and incubated to allow growth of the bacteria at 37° C for 24 hours. The test tube was examined for growth or turbidity in the samples (Ogundipe *et al*., 2008).

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Results

Phytochemical Screening

The present study carried out with *Trianthema portulacastrum* Linn. revealed the presence of various bioactive metabolites which might be responsible for their medicinal attributes. Aqueous extract showed the presence of alkaloids, tannins and phenols, glycosides, saponins, flavonoids, protein, carbohydrates, etc.(Table-1). The ethanolic extract showed the presence of alkaloids, tannins, and phenol, saponins, flavonoids, protein, and carbohydrate. The preliminary phytochemical screening resulted in the identification of many phytoconstituents so they have notable phytochemical properties. The obtained results are shown in the Table-1.

Table-1. Preliminary phytochemical screening of *T. portulacastrum* Linn. extracts

Sl. No.	Phytoconstituents	Test	Aqueous	Ethanol
1.	**Alkaloids**	a. Mayer's test b. Wagner's test c. Hager's test	+ve +ve +ve	- ve +ve +ve
2.	**Tannins and phenols**	a. $FeCl_3$ test b. Lead acetate test	-ve +ve	+ve +ve
3.	**Glycosides**	a. Borntrager's test b.Keller- Killianitest	+ve -ve	-ve +ve
4.	**Saponins**	a. Foam test	+ve	+ve
5.	**Flavonoids**	a. Shinoda test b. Con. H_2SO_4	-ve +ve	+ve -ve
6.	**Protein**	a. Biuret test b. Millon's test	-ve +ve	-ve +ve
7.	**Carbohydrate**	a. Molish test b. Benedict's test	-ve +ve	+ve +ve
8.	**Terpenoids**	a. Salkowski test	+ve	+ve

Silver Nanoparticle Assay

Green synthesis of silver nanoparticle was shown by *Trianthema portulacastrum* Linn. For the extract the colour change observed after adding silver nitrate solution was from pale yellow colour to reddish- brown colour. After 5 hours the nanoparticle started to settle down at the bottom and was centrifuged and washed. The intensity of colour change is representative of the amount of silver nanoparticle synthesized. Under 40x resolution of the microscope, the nanoparticle produced were found to be small and spherical in shape.

Antibacterial Assay

Ethanolic extract, aqueous extract and silver nanoparticle showed antibacterial activity against the strains of bacteria studied. Amoxycillin was used as the standard antibiotic drug. The zone of inhibition in aqueous extract was higher for *Escherichia coli* with an inhibition zone of 7mm and for *Bacillus cereus, Staphylococcus aureus* and *Vibrio harveyi,* the inhibition zones were 6mm. In the case of ethanolic extract, the zone of inhibition was higher for *Bacillus cereus* with 12 mm followed by *Vibrio harveyi* (9mm), *Staphylococcus aureus* (11mm), and *Escherichia coli* (10mm). The zone of inhibition of nanoparticle solution against *Escherichia coli* (8mm) and *Bacillus cereus* (8mm) was maximum, followed by *Vibrio harveyi, Staphylococcus aureus* with 7mm. For Amoxycillin the zone of inhibition was higher in *Bacillus cereus* with 9mm followed by *Vibrio harveyi, Staphylococcus aureus* and *Escherichia coli* with inhibition zone of 8mm, 7mm and 6mm. The zone of inhibition was greater for Amoxycillin in the case of all the four strains of bacteria studied (*Bacillus cereus, Staphylococcus aureus, Escherichia coli, Vibrio harveyi).* The zone of inhibition is least in the case of ethanol. The most resistant strain of bacteria was *Staphylococcus aureus* and the most vulnerable strain was *Bacillus cereus*. Obtained results are given in the following Fig.1 & Table-2. The minimum inhibitory concentration (MIC) was observed at a concentration value of 40µg for ethanolic extract, 80µg for aqueous extract and 40µg for nanoparticle solution.

Fig. 1. Agar plates showing zones of inhibition exhibited by the four types of bacteria

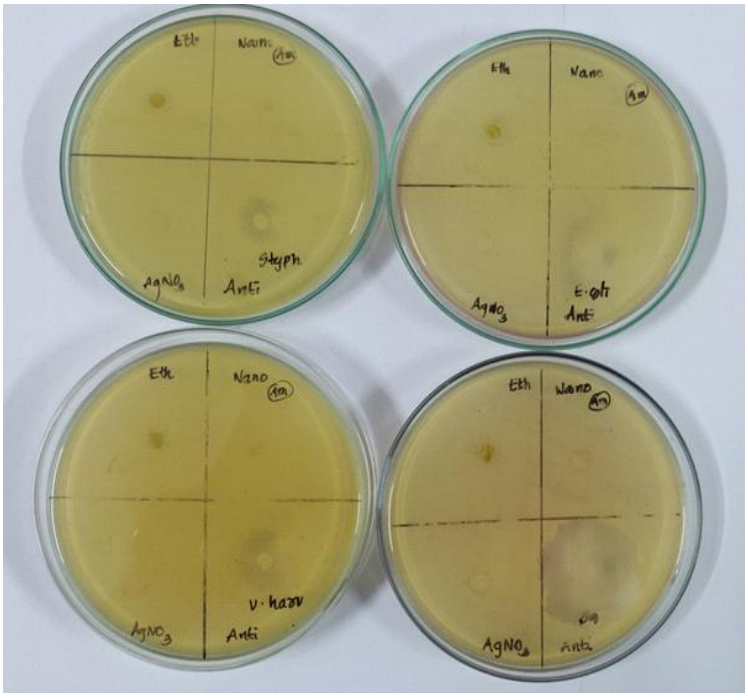

Table- 2. Antibacteriala ctivity of aqueous and ethanolic extract of *T. portulacastrum* Linn.

Sample	Zones of inhibition of different bacteria (in mms)			
	B. cereus	***S.aureus***	***E. coli***	***V.harveyi***
Aqueous	6	6	7	6
Ethanol	12	11	10	9
Nanoparticle solution	8	7	8	7
Amoxycillin	31	14	23	18

Discussion

Trianthema portulacastrum Linn. is traditionally used as a medicinal plant in many parts of the world. A variety of compounds that are of plant origin are found to act against a wide range of microbes. Hence such compounds can be exploited as antimicrobial agents to treat a variety of diseases (Abubacker *et al*., 2013; Tom and Benny, 2016). In the present study, preliminary phytochemical screening of *T. portulacastrum* Linn. was carried out and the result showed that both aqueous and ethanolic extract contain chemical compounds like alkaloids, glycosides, saponins, flavonoids, protein, carbohydrate, terpenoids, tannins and phenols. Earlier workers (Ogundipe *et al*., 2008; Camporese *et al*., 2003; Woldeyes *et al*., 2012; Annam *et al*., 2016) reported that *Gomphrena celosioides* L., *Hamelia patens* Jaq., *Sida rhombifolia* Linn., *Trianthema portulacastrum* Linn. possessed alkaloids which were found to be antimicrobial against bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus aureus, Enterococcus faecalis.*

In the present investigation *T. portulacastrum* Linn. showed the presence of alkaloids. As per previous knowledge alkaloids were found to be antimicrobial against *T. portulacastrum* Linn. also contain tannins and phenols which also act against a variety of strains of bacteria (Woldeyes *et al*., 2012; Ajaykumar *et al.,* 2016). Both aqueous and alcoholic extract showed significant antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. The standard antibiotic Amoxycillin was used to compare the antibacterial activity of *T. portulacastrum* Linn. It was found that alcoholic extract showed 33.84% inhibitory activity while that of aqueous extract was 20.13%. Earlier studies (Samiriti *et al*., 2019; Abubacker *et. al*., 2013) revealed that ethanolic extract of *T. portulacastrum* Linn. showed antibacterial activity. In the present study we studied the antibacterial properties against bacterial stains like *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. *Bacillus cereus* is a gram positive, rod shaped, facultative anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are gram positive, facultative anaerobic, round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are gram negative, facultative anaerobic, rod shaped, coli form bacterium commonly found in the lower intestine of warm-blooded organism which cause food poisoning in their hosts. *Vibrio harveyi* are gram negative, bioluminescent, marine, rod shaped bacterium responsible for Luminous Vibriosis. Hence, the present study revealed that *T. portulacastrum* Linn. is a potential source of antibacterial agents. However, further fractionation and characterisation are required to identify the exact principle.

Silver nanoparticle synthesized with various plants showed antibacterial activity. The green synthesized nanoparticles are usually easy to handle as compared to the plant extract. In the present investigation green synthesized nanoparticles were used as antimicrobial agents. $AgNO_3$ was used as the control (Nisha *et al*., 2012). The percentage of inhibition was found to be more than that of hydro alcoholic extracts. This indicates that green synthesized nanoparticles possessed a promising antibacterial activity.

The present study revealed the antibacterial property associated with *Trianthema portulacastrum* Linn. was significant and hence further investigation is required to ascertain the actual principle behind the antibacterial activity.

Conclusion

The present study has shown that *Trianthema portulacastrum* Linn. possess antibacterial properties. This principle can be used against the pathogens investigated in this experiment. However, further characterisation and screening including toxicological evaluations is needed to ascertain the exact molecule behind the antibacterial property of *Trianthema portulacastrum* Linn.

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