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Anti-Bacterial activity of total flavonoids of *Portulaca oleracea* L.

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Abstract

Flavanoids are uniquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers and wine. For centuries, preparations canting these compounds as the principal, physiologically active constituents have been isolated and used to treat human diseases. *Portuleca olerace L* is an important medicinal plant that has been used in traditional medicine. The present study was aimed to isolate the total flovanoids and to investigate an anti-bacterial activity of total flavonids extracted from aerial part of *Portulaca olerace L*. we have used five standard pathogenic bacterial strains like *Pseudomonas aeruginosa, Salmonella typhimurium, Proteus mirabilis, Klebsiella pneumonia and Enterobacter aerogenes,* among all the bacterial strains *Salmonella typhimurium* (14.33±0.2886) and *Proteus mirabilis* (17.16±0.0281) have shown maximum zone of inhibition for total flovanoids and remaining bacterial strains have shown moderate zone of inhibition when compared with control (20.66±0.2881). In case of bio-assay method *Salmonella typhimurium* was shown more sensitive by low turbidity of OD value (0.187) indicating most significant result. The Minimum inhibitory Concentration (MIC) of the total flavonoids isolated from *Portuleca olerace* L was tested at the concentration ranging from undiluted sample to 10mg/ml. the minimum inhibition concentration (MIC) for the total flavonoids for all tested bacterial strains was >10mg/ml.

Experimental results supports that these flavonoids have antibacterial properties which helps in the developing antibacterial agents in the form of drugs for the therapy of infectious diseases caused by these bacterial pathogens.

Keywords Flavonoids, *Portuleca oleracea* L, Antibacterial, Medicinal plant

Introduction

Medicinal plants are the source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grown in different parts of the country. India is rich in all 3 levels of Biodiversity, namely species diversity, Genetic diversity and habitant diversity. Thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient time [1]. India can be virtually called as the Herbarium of the World and also called Botanical Garden of the World [2]. The medicinal plants have been used since ancient times, evidence of using these nature resources (herbal remedies) in Iran goes back to the history itself there are lots of scientific documents in this area, Ibn-sina (Avicenna, 980-1037) has wrote many books on a wide range of topics but he is perhaps most famous for his laws of medicines which contains sections on the formulation of medicine, general medicine and other subjects that discuss the herbal medicines in details [3]. In recent years, antibiotic resistance has become a global concern and this problem is more important in developing country because the infectious diseases are still at important to cause of morbidity and mortality among humans. Plants rudely synthesize substances for their defense against insects, herbivores and microorganisms [4]. Nowadays, multiple drug resistance is developed due to the indiscriminate use of drugs which are commonly used in the treatment of infectious diseases [5, 6]. In addition to this problem, antibiotics are sometimes associated with adverse effect on the host including hypersensitivity, immune-supression and allergic reaction [7]. The plants may produce secondary antimicrobial metabolites as part of their normal growth and development program or in response to stresses [8]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity [9]. Furthermore, the active components of herbal remedies have an advantage of being combined with many other substances that appear to be inactive. However, these complementary compounds give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [10]. Because of side effect and resistance that the pathogenic microorganism developed against antibiotics, recently much attention has been paid to extraction of biologically active compounds isolated from plant species used in herbal medicine. Plant based antimicrobials represent a vast update

source of medicine and further explanation is that plant anti-microbial needs to occur. Anti-bacterial plant origin has enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. *Portulaca oleracea L* is an annual prostrate or spreading, succulent, branched herb of the Postulacacea family. A very common well of cultivated and undisturbed land [11]. Recent studies on *Portuleca olerace L* extracts have showed muscle relaxant activity [12], reduction in locomotors activity, increase in onset time of pentylenetrazole-induced convulsion [13], analgesic and ant-inflammatory effects [14]. It is used in Iranian folk medicine as a diuretic, vermifuge, anti-scorbutic, anti-tussive, analgesic and in gastro-esophageal reflux [15]. In traditional medicine, this plant is utilized as anti-vomiting, anti-bleeding, anti-hepatitis and in treatment of gastric mucosal diseases [16]; in some Middle East countries, it is considered beneficial for small tumors and inflammation, urinary disorders, liver obstruction and ulcers of mouth and stomach [17]. The aim of this investigation was to determine antibacterial activity of total flavonoids extract from *Portulaca oleracea L* against some Pathogenic Bacterial strains.

Material and Method

Plant material

The healthy aerial part of the plant of *Portuleca oleracea L* was collected from around Gulbarga university campus during the month of June 2011. The plant material was identified and authenticated from the Department of Botany Gulbarga University Gulbarga Karnataka (India), voucher specimen has deposited in herbarium of the same Department.

Chemicals

Methanol, ethanol, ethyl acetate, Petroleum ether Diethyl ether, H_2SO_4 , Chloroform, HCl, KOH, hexane, silica Gel 60-120 mesh, Tween 80 Phosphate buffer saline, FCR Reagent, all the chemical, solvents and reagents were analytical grade and were obtained from Hi media.

Extraction of flavonoids

Before extraction, *Portuleca oleracea L* was crushed into powder by versatile plant pulverizer. The powder of the sample was degreased by soxhlet extractor with petroleum ether until the color of the elute become colorless. The same powder sample was accurately weighed and placed in soxhlet extractor by adding 80 ml of ethanol: water (70:30) solvent, followed by the extraction for 5 hrs, and then extract solution was concentrated. The extract was centrifuged at 11000 rpm for 30 min; supernatant was taken for further use.

Qualitative test for flavonoids

The presence of flavonoids were further conformed by specific test for flavonoids like shinoda test, lead acetate test, sodium hydroxide test, Sulphuric acid test, aqueous test. These are the specific test for detection of flavonoids.

Solubility determination of flavonoids

The solubility test of total flavonoids was performed in various solvents and gradient solvents to determine dissolving capacity of flavonoids in different solvents because the solubility of compounds in organic solvent will helps in further spectral analysis and also gives more information about chemical nature of that particular compound. Dried flavonoid sample of known quantity was added to 1ml of solvent taken in watch glass and mixed properly. The complete dissolving and crystal formation of sample indicate soluble and insoluble, respectively.

Antimicrobial susceptibility test

Microorganisms

The bacterial strains employed in the current study were procured from Institute of Microbial Technology (IMTECH), Chandigarh (India) which includes *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumonia* (MTCC 109), *Salmonella typhimurium* (MTCC 98), *Proteus mirabilis* (MTCC 425), and *Enterobacter aerones* (MTCC 111). These species were originally isolated from clinical samples and identified by standard biochemical reactions.

Media

Nutrient broth (Hi Media M002) contain peptic digest of animal tissue (5g/L), yeast extract (1.50g/L), Beef extract (1.5g/L) was used for the growth of bacterial cultures. Antibiotic assay media No:11 (Hi Media MM004) congaing peptic digest of animal tissue (6g/L), casein enzyme hydrolyte (4g/L), Yeast extract (1.50g/L), Dextrose (1.00g/L). Agar (15.00g/L) was used for anti-bacterial activity.

Agar well diffusion Method

The antimicrobial activity of flavonoids was determined by using agar well diffusion technique. For this 25 ml of sterile Muller-Hinton agar No.2 (Hi Media), was poured in sterile autoclaved Petri plates, before pouring 100µl activated bacterial culture was added, and then allowed to stand for solidification completely. The well was prepared with the help of sterile 6mm diameter cork-borer. Than 100µl of prepared flavonoid extract (60mg/ml) solution were poured in to the wells. Then the plates were sealed with plasticine and transferred to refrigerator to diffuse out of 30 min. the plates were then incubated at 37 ºC for 24 hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well, were recorded. 0.01mg/ml streptomycin was used as positive control. Inoculums turbidity was maintained constant throughout the

experiment to 0.8 OD at 660nm. Level of turbidity is equivalent to approximately $1X10^8$ CFU/ml.

Bio-assay Method

Muller Hinton broth media was prepared and distributed in several test tubes, each with 5ml of broth. These tubes were autoclaved and cooled. To these test tubes containing 5ml of medium 100µl activated bacterial culture was added, the 100µl of flavonoid extract (60mg/ml) solution was added. 0.01mg/ml streptomycin was added to one tube and served as positive control. All the test tubes were then incubated at 37 ^oC for 24 hrs. Subsequently after 24 hrs 0.1ml of formaldehyde solution was added to each inoculated tube to arrest the bacterial growth. Then the O.D of each tube was measured at 660nm and compared with the positive control. Triplicate test tubes were prepared for each treatment and the average OD was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined through the broth dilution method. Bacteria were grown in Muller Hinton broth for 6 hrs. After this, 20µl of 106 cells/ml were inoculated in tubes with Muller Hinton broth supplemented with 4 different concentrations (60, 40, 20, and 10 µg/ml) of the flavanoids. After 24 hrs incubation at 37 o C, the MIC of each sample was measured through optical density in the spectrophotometer at 660nm, though the non-inoculated Muller Hinton broth [18]. All determinations were performed in duplicate [19, 20].

Results

The qualitative determination of flavonoids

The qualitative test for flavonoids were performed and all the test were given positive by formation yellow colored precipitation and shinoda test has given positive by formation of pink color. The result of all the test were tabulated in the table-1

Table 1. Showing results of qualitative test for flavonoids

Sl. No.	Test	Observations
1.	Shinoda test	Positive
2.	Lead acetate test	Positive
3.	Sodium hydroxide test	Positive
4.	Salphuric acid test	Positive
5.	Aqueous test	Positive

Solubility determination of flavonoids

The solubility test of total flavonoids was performed in various solvents and gradient solvents to determine dissolving capacity of flavonoids in different solvents because the solubility of compounds in organic solvent will helps in further spectral analysis and also gives more information about chemical nature of that particular compound. The results of solubility of isolated compound in various solvents were shown in the table-2.

Table 2. Showing solubility tests of isolated flavonoid compound.

Sl. No.	Solvent	Solubility
1.	Methanol	Partially soluble
2.	Acetonitryle	Insoluble
3.	Glacial acetic acid	Partially soluble
4.	Water	Partially soluble
5.	Petroleum ether	Insoluble
6.	Chloroform	Insoluble
7.	Hexane	Insoluble
8.	Ethyl acetate	Insoluble
9.	Benzene	Insoluble
10.	Methanol: Water (7:3)	Soluble
11.	Methanol: Acetonitryal (7:3)	Partially soluble
12.	Acetonitryl: Water (7:3)	Soluble

Antibacterial susceptibility test

Agar well diffusion Method

The antibacterial activity of total flavonoids extract of *Portuleca olerae* L against pathogenic bacteria was assayed. The results from Agar well diffusion method show that among all the bacterial strains *Salmonella typhimurium* (14.33±0.2886) and *Proteus mirabilis* (17.16±0.0281) have show maximum zone of inhibition for total flovanoids and remaining bacterial strains have shown moderate zone of inhibition when compared with control (20.66±0.2881), (Table 3).

Bio-assay Method

Antimicrobial bio-assay is also another standard method of assaying antimicrobial activity of total flavonoids extracted From *Portuleca olerace* L. In addition to agar diffusion technique, here the flavonoid extracts was further subjected to antimicrobial bio-assay against five bacterial strains. In this method *Salmonella typhimurium* (14mm) was shown more sensitive by low turbidity of OD value indicate most significant result, but all other bacterial strains were shown moderate results, (Table 4).

Determination of MIC

The Minimum Inhibitory Concentration (MIC) of the total flavonoids isolated from *Portuleca olerace* L was tested at the concentration ranging from undiluted sample to 10mg/ml. the minimum inhibition concentration (MIC) for the total flavonoids for all tested bacterial strains was >10mg/ml.

Table 3: Showing Results of antibacterial effect of flavonoids isolated from *Portuleca olerace L* by Agar well Diffusion Method.

Sl. No.	Pathogenic Bacteria	MTCC No.	Zone of inhibition in mm	
			Streptomycin (0.01mg/ml)	Flavonoids (60mg/ml)
1.	*Pseudomonas aeruginosa*	MTCC424	15.33±0.7637	10.33±0.7637
2.	*Klebsiella pneumonia*	MTCC109	20.33±0.5773	09.66±0.5773
3.	*Salmonella typhimurium*	MTCC98	24.33±0.5275	14.33±0.2886**
4.	*Proteus mirabilis*	MTCC425	20.66±0.2881	17.16±0.0281***
5.	*Enterobacter aerones*	MTCC111	20.66±0.2881	11.033±0.2133

Table 4: Showing Results of antibacterial effect of flavonoids isolated from *Portuleca olerace L* by Bio- assay Method.

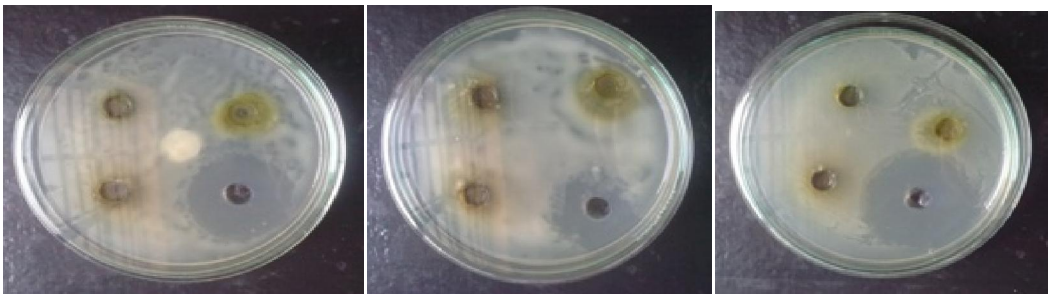
Sl. No.	Pathogenic Bacteria	MTCC No.	Optical Density at 660nm	
			Streptomycin (0.01mg/ml)	Flavonoids (60mg/ml)
1.	*Pseudomonas aeruginosa*	MTCC424	0.756	0.314
2.	*Klebsiella pneumonia*	MTCC109	0.943	0.234
3.	*Salmonella typhimurium*	MTCC98	0.518	0.187
4.	*Proteus mirabilis*	MTCC425	0.755	0.352
5.	*Enterobacter aerones*	MTCC111	0.711	0.315

Table 5: Showing Results of Minimu Inhibition Concentration (MIC) of total flavonoids isolated from *Portuleca olerace L* by Bio- assay Method.

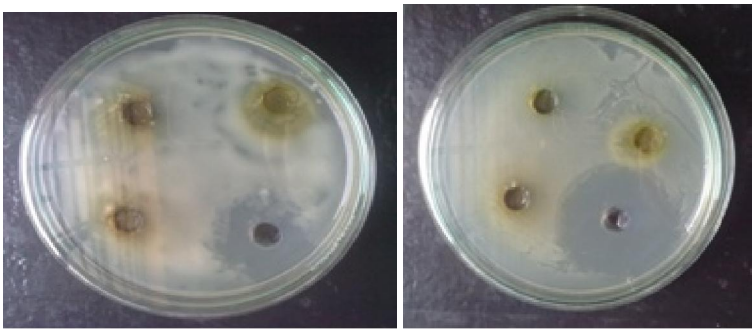
Sl. No.	Pathogenic Bacteria	MTCC No.	Flavonoids concentration (mg/ml)				
			Streptomycin (0.01mg/ml)	60	40	20	10
1.	*P. aeruginosa*	MTCC424	-	-	-	-	+
2.	*K. pneumonia*	MTCC109	-	-	-	-	+
3.	*S typhimurium*	MTCC98	-	-	-	-	+
4.	*P. mirabilis*	MTCC425	-	-	-	-	+
5.	*E. aerones*	MTCC111	-	-	-	-	+

Note: +; Indicates Presence of Growth, -; Absence of Growth.

Fig 1: Showing results of antibacterial activity by agar well diffusion method.



S typhimurium *K. pneumonia* *S typhimurium*



P. mirabilis *E. aerones*

Discussion

Nowadays multiple drug resistance developed due to the indiscriminate use of drugs commonly used in the treatment of infectious diseases treatment. Unfortunately, bacteria have genetic ability to transmit and acquire resistance to drugs and chemicals [21]. Behind the increasing prevalence of antibiotic resistance among pathogenic bacteria, undesirable side effects of some synthetic antibiotic add urgency to the search for new infection-fighting stratage, as well. Scientist and pharmaceutical industries considered medicinal plants as a good choice, because these natural resources have ordinary fewer side effects, are costless and effective against broad spectrum of antibiotic resistance bacteria. In many parts of world, extracts of medicinal plants used for thiere antibacterial, anti-fungal and anti-viral properties [22]. *Portuleca lerace* L was among the most important plant extensively used in traditional medicine in India. Based on the results of anti bacterial studies shown in Table 3 and 4 of agar well diffusion and Bioassay method respectively. The favonoid extract had antibacterial activity more significant on *S typhimurium,* and *P. mirabilis* at 60mg/ml of concentration of flavonoid extracts of *Portuleca olerace* L When compared to control group. This may be due to the chemical nature and number of total favonoids available in the total flavoniod extract, cell membrane permeability and other factors. In general, composition of the inhibition zones diameter showed that flavonoid extract of *Portuleca olerace* L was more effective against both gram positive and gram negative bacterial strains. This difference may be due to several possible reason such as permeability barrier provided by the presence of cell wall with multi layer structure in gram negative bacteria or the membrane accumulation mechanism or presence of enzymes in periplasmic space which are able to break down foreign molecules introduced from outside [23]. The flavonoid extract of *Portuleca Olerace L* have shown a very less activity on the other bacterial strains like *Pseudomonas aeruginosa, Klebsiella pneumonia and Enterobacter aerogenes.* The MIC (Table-5) for total flavonoid extract of *Portuleca olerace L* was 10mg/ml, it is reported that for bacterial antimicrobials, the MIC was often near or aquilial values [24]. Based on above result it can be concluded that flavonid extract of *Portuleca olerace* L have bactericidal effect on *S typhimurium,* and *P. mirabilis.* Extracted flavonids of *Portuleca olerace* L were unable to show bactericidal activity against *Pseudomonas aeruginosa, Klebsiella pneumonia and Enterobacter aerogenes,* even at 200mg/ml (MIC>200mg/ml)., this result may caoused by high bactriostic effect of *Portuleca olerace L* flavonoid extracts, we can call these extracts as bacteriostatic which can inhibit bacterial growth but generally do not kill them [25]. Gram – negative bacteria were resistance to the flavonoids and can be attributed in part of the great complicity of the double membrane containing cell envelop in contrast to the single membrane structure of gram-positive bacteria [26].

Conclusion

In conclusion the results of present study suggests that, the total flavanoids extracts of *Portuleca olerace L* have promising antibacterial activity on pathogenic bacterial strains. The *Salmonella typhimurium* and *Proteus mirabilis* have shown more significant sensitivity. So it indicates that these flavanoids can be used to prepare antibacterial drugs to cure the diseases caused by these bacterial strains. Flavanoids of *Portuleca olerace L* may serve as potential source of industrial drug useful in chemotherapy of some bacterial infections.

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