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Phytochemical Screening and Antimicrobial Activities of Purslane Leaf and Stem Extracts

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Abstract

In spite of the fact that pharmaceutical companies have annually produced a huge quantity of antibiotics, emerging bacterial resistance is a main concern for the medical field. Ethno pharmacological studies show that plants and their products are a good source of biologically active antibacterial agents. Characterization of the phytochemical and antimicrobial activity from leaves and steam of purslane and appraisal of its viability is the main purpose of this study. In this work, the phytochemical and antimicrobial activity from leaves and steam of purslane, were determined. The methanol extracted samples from leaf and stem of purslane were made at three concentration levels. Antimicrobial activities were determined using disc diffusion method. Methanol extracts of leaf and stem revealed the presence of tannins, saponins, steroids, terpenoids, and alkaloids as the active phytoconstituents of purslane. The strongest antibacterial activity with maximum zone of inhibition (16.8mm) was recorded with methanol leaf extract against *S. aureus* at 200 mg/mL of concentration. Minimum inhibitory concentration and minimum bactericidal concentration values of 10 mg/mL and 20mg/mL respectively against *S. aureus*; and 100 mg/ml and 125mg/ml respectively against *Aspergillus niger*. These results will facilitate detailed phytochemical and antimicrobial activity purslane further elucidation of its biochemical function.

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Introduction

Portulaca oleracea L, commonly known as purslane is annual herb that grown all over the world and used as a salad and vegetable in many countries, Elkhay *et al.*, (2008). Purslane has been identified as the richest vegetable source of alpha-linolenic acid, an essential omega-3 fatty acid, Simopoulos and Salem (1986). The deficiency of dietary sources of omega-3 fatty acids has resulted in a growing level of interest to introduce purslane as a new cultivated vegetable, Yazici *et al.*, (2007). Alpha-linolenic is an omega-3 fatty acid which plays an important role in human growth and development and in preventing diseases. It also reported to contain other chemical constituents, including urea, calcium, iron, phosphorous, manganese, copper and fatty acids, especially omega-3-acids whose concentration in purslaneis the highest found in leafy vegetables, Simopoulos *et al.*, (1992). Both fresh and dried samples are used in medicinal plants studies. In most cases, dried samples are preferred considering the time needed for experimental design. Purslane is highly perishable in the

fresh state; it has the shortest shelf life among fruits and vegetables due to its high metabolic reactions, which lead to loss quality. Fresh hydro alcoholic purslane extract exhibited the highest radical scavenging potential in 1, 1- diphenyl-2-picrylhydrazine test, whereas dried hydroalcoholic purslane extract showed the highest α-glucosidase inhibitory potential. The active principles isolated from plants appear to be one of the important alternatives, when compared to many sub-standard orthodox synthetic medicines, because of their less or no side effects and better bioavailability, Scazzocchio *et al.*, (2001). In many areas research work on potential use of purslane, as diet in modern society, is hardly found in spite of some traditional use of this plant. The current study was intended to investigate the antimicrobial activity of leaves and steam of purslane and compare the chemical composition, antioxidant and hypoglycaemic properties of dried and fresh purslane leaves and stem. Therefore, analyzing of the phytochemical activity of purslane will provide valuable information to increase its importance on dietary and medical purpose.

Materials and Methods

Study design

The samples were extracted from leaf and stem of purslane at three concentration levels using methanol. Test organisms (2 bacteria and 2 fungi) were used as factorial design in three replications. A complete randomized design was used to determine the antimicrobial activities using disc diffusion method. The least concentration of extract showing antimicrobial activity was selected for further determination of the minimum inhibitory concentration, minimum bactericidal and minimum fungicidal concentrations.

Collection and extract preparation

Leaf and stem of purslane were collected from Dire Dawa district in Ethiopia. The authenticity of the plant material was confirmed at the Herbarium of Haramaya University. The fresh samples were manually washed with distilled water and residual moisture evaporated at room temperature. The leaf and stem samples were cut into pieces then dried in oven at 70 ºC for 72 h. The sample was grinded to fine powder using grinder for 2 min, using the standard methods by, AOAC (2000). Determination of moisture on dry basis was carried out and methanol was used as extraction solvent at boiling range of 40-60 ºC.

Preparation of methanol extract

Sixty grams of the powdered plant material was soaked in 400 ml of 97% methanol in a conical flask sealed with aluminium foil and allowed to stand for 72 h. The crude extract was filtered using what man filter paper to obtain a solution. The resulting alcoholic filtrate was concentrated using freeze dryer. After solvent evaporation, the remaining crude extract was kept in air tight bottle in a refrigerator until use at 4^{o}C, Taura *et al.*, (2014). Different concentrations of 100 mg/ml, 150mg/ml and 200mg/ml) of the extracts were prepared from their respective stocks in 20 mL of sterile distilled water (w/v dilution) respectively as per, Alabi *et al.*, (2012), and kept in closed well labeled containers for antimicrobial testing.

Phytochemical composition analysis

Qualitative analysis of major secondary metabolites including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids of thepurslane leaf and stem was carried out on dried and powdered plant specimens using standard procedures by Savithramma *et al.,* (2011). Each 1 mL of solid extract was placed into separate test tubes and mixed with 10 mL of distilled water. The mixture was boiled in a water bath for 10 min. Hence, 1mL aqueous hydrochloric acid was added to each mixture and shaken to develop red precipitate that indicates the presence of phlobatannins, Ajayi *et al.,* (2011). The presence of saponins was determined by adding 1mLof the extract sample from leaf and stem of purslane into 5mLof distilled water. The mixture was shaken vigorously to observe for the appearance of stable persistent froth on warming, as preliminary evidence for the existence of saponins, Abba *et al.,* (2009). Foam test was conducted by adding 1 mL of filtrate 10 mL distilled water and shaken vigorously for 2 min. Formation of froth confirmed the presence of saponin in the filtrate. Alkaline reagent test was conducted through 1mL of concentrated extract was added in to test tube, then 4 drops of NaOH solution was added and mixer was heated in water bath for 10 min. Test for steroids was done using 1 mL of chloroform and 10 drops of acetic acid placed in test tube. The change of red color through blue to green serves as an indicator for the presence of steroids, Edeoga *et al.,* (2005). Test for terpenoids 3 ml of each extract was mixed with 1 mL of chloroform in separate test tubes, and 1mL of concentrated H_2SO_4 was carefully added and shaken gently to form a layer. Formation of a reddish brown coloration at the interface

was considered as a positive indicator for the presence of terpenoids.

Media preparation and inoculum

Nutrient agar, potato dextrose agar, and Muller Hinton agar were used for sub-culturing of bacterial test organism, fungal test organism, and determination of antimicrobial activities, respectively. These media were prepared and sterilized using an autoclave according to the manufacturers' instructions. The spores of the test fungi were harvested by washing the surface of the fungal colony using 5mL of sterile saline solution (0.85%). The resulting suspension was used as inoculum for the test pathogen in the antimicrobial susceptibility test.

Disk diffusion method

The disk diffusion method is performed using Mueller-Hinton Agar. In this study, discs of 6 mm diameter were prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then impregnated with a volume up to 200 µL of the antimicrobial agent solution at desired concentration to be inoculated with test pathogens.

Measuring zones of inhibition

After 24 hours of incubation at 37 °C the diameters of the zone of inhibition around each disc was measured to the nearest millimeter along two axes 90° to each other using a transparent calibrated ruler (mm) and the means of the two readings were recorded, Biswas *et al.,* (2013). All tests were carried out in triplicate and the mean of zones of inhibition and Standard deviation of Mean were calculated to each microbe.

Determination of minimum inhibitory concentration

The methanol extracts of stem and leaf of significant antimicrobial activity were selected and determined by broth dilution method. The extract solution at 100mg/ml was serially diluted in a two-fold dilution to get 150mg/ml, 200mg/ml, and 50mg/ml, and 25mg/ml concentrations. Two mL of nutrient broth and potato dextrose broth for bacteria and fungi were added into all test tubes and 0. 1 ml of the prepared concentration of each extract was mixed with the nutrient broth and potato dextrose. Standardized inoculums of 0.1 ml of the respective test pathogens were dispensed into the test tubes containing the suspensions of the broth and the extract. All test tubes were properly corked and incubated at 37°C for 24 h for bacteria and 27°C for 72 h for fungi. Minimal bactericidal and minimum fungicidal concentration were also done according to Riffel *et al.*, (2002). The tests were performed in triplicate. Dilutions of amoxicillin served as positive control.

Results and Discussion

The phytochemical constituents of purslane are presented in Table 1. Phytochemical screening of methanolic extracts of leaf and stem revealed the presence of tannins, saponins, steroids, terpenoids, and alkaloids as the active phytoconstituents of purslane. Flavonoids were found to be absent in methanolic extract of leaf but present in methanolic extract of stem. Phlobatannin has been found absent in both stem and leaf examined. Agar well diffusion assay revealed that the crude extracts of purslane leaf and stem were found to exhibit a considerable antibacterial activity against test bacteria and fungi Table 2. Susceptibility of the two tested bacteria and fungi to the extracts didn't show significance difference between leaf and stem extracts. Extracts with colony growth inhibitory effect at the highest dose showed a mean zone of inhibition ranged from 15.5 to 16.8mm. Amoxycillin showed a significant superiority ($p<0.05$) in the zone of inhibition as compared to the test extracts Table 2. For most of the test extracts, the highest concentration (200mg/mL) exhibited a significantly higher ($P<0.05$) zone of inhibition as compared to the respective lowest concentration (100mg/mL). The strongest antibacterial activity with maximum zone of inhibition (16.8mm) was recorded with methanolic leaf extract *against S. aureus* at 200mg/ml of concentration. The results of minimum inhibitory concentration and pathogenic tests are presented in Table 3. All the tested extract exhibited antimicrobial activity against the test pathogens. The extracts presented minimum inhibitory concentration and minimum bactericidal concentration values ranging from 10 to 20.5 mg/ml and 20 to 50mg/ml respectively against *E. coli* and *S. aureus*. The leaf extract exhibited strong minimum inhibitory concentration and minimum bactericidal concentration values of 10 mg/ml and 20mg/ml respectively against *S. aureus*. The relatively weak minimum inhibitory concentration and minimum bactericidal concentration values of 20.5 and 50mg/ml respectively of methanolic extract of stem of purslane were observed for *S. aureus.* The methanolic extracts of purslane presented minimum inhibitory concentration and minimum fungal concentration values ranging from 50 to 150 mg/ml and 100 to 200 mg/ml respectively against *A. niger* and *A.*

versicolor. The methanol extracts of purslane leaf exhibited strong minimum inhibitory concentration and minimum fungal concentration values of 100 mg/ml and 125mg/ml respectively against *A. niger*. The relatively weak minimum inhibitory concentration and minimum bactericidal concentration values of 150 and 200mg/ml respectively of methanolic extract of stem of purslane were observed for *A. versicolor*.

Table.1 Phytochemical screening of crude extracts

Phytoconstituents	Leaf extract		Stem extract	
	Aqueous	Methanol (70%)	Aqueous	Methanol (70%)
Tannins	+	+	-	-
Phlobatannins	-	-	+	-
Saponins	+	+	+	+
Flavonoids	+	-	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	+	+	+	+

+: detected; -: not detectable.

Table.1 Antimicrobial activity of the crude extracts pathogenic bacteria, and fungi.

Pathogens	plant	Methanol (70%) extract			Amoxicillin
	organ	100mg/ml	150mg/ml	200mg/ml	(100mg/ml)
E, coli	Leaf	13.05±0.07aD	14.75±0.35aC	16.4±0.57aB	18.5±0.71aA
E. coli	Steam	12.5±0.71aC	14.9±0.57aB	15.25±0.35aB	18.85±0.21aA
S. aureus	Leaf	13.4±0.56aC	15.85±0.21aB	16.8±0.28aB	19.25±0.35aA
S. aureus	Stem	13.8±0.28aD	14.75±0.35aC	15.46±0.64aB	18.4±0.14aA
A. niger	Leaf	10.2±0.28aD	11.25±0.35aC	12.9±0.14aB	15.15±0.21aA
A. niger	Stem	8.85±0.21aD	10.25±0.35aC	12.75±0.35aB	14.2±0.28aA
A. versicolor	Leaf	9.3±0.28aC	10.75±0.36aC	13.35±0.21aB	14.8±0.28aA
A. versicolor	Stem	8.7±0.28aD	10.3±0.42aC	12.8±0.43aB	14.35±0.21aA

Table.2 Minimum inhibitory concentration (mg/ml) of pathogens methanolic extract

Microbes	Plant organ	MIC (mg/ml)	MBC/MFC (mg/ml)
E. coli	Leaf	18.5	30
E. coli	Steam	30	50
S. aureus	Leaf	10	20
S. aureus	Stem	20.5	50
A. niger	Leaf	50	100
A. niger	Stem	100	125
A. versicolor	Leaf	100	100
A. versicolor	Stem	150	200

Findings from the current in vitro study revealed that methanolic extracts from purslane plants exhibit antimicrobial activity against all the experimented microbes. The high percentage content of saponin may be indicative of the plant's major use as an anthelminthic and antichloristic, Maurice (1993). The presence of tannin in the plant shows its potential as an antiviral, antibacterial and anti-parasitic, Kolodziejand and Kiderlen(2005). The presence of flavonoids is indicative of its potential use as an anti-allergic, anti-inflammatory, anti-oxidative, antimicrobial anti-diarrhea and anticancer, Cazarolli *et al.,* (2008). Ketokonazole used as positive control showed a significant superiority ($p<0.05$) in the zone of inhibition as compared to the test extracts Table 2. For most of the test extracts, the highest concentration (200mg/mL) exhibited a significantly higher ($P<0.05$) zone of inhibition as compared to the respective lowest concentration (100mg/mL). The strongest antifungal activity with maximum zone of inhibition (13.35mm) was recorded with methanolic leaf extract against *A. versicolor* at 200mg/ml of concentration. Minimum inhibition concentration assay was employed to evaluate the effectiveness of the extracts to inhibit the growth of the tested bacteria. The plant extracts with high activity against a particular organism usually give low minimum inhibition concentration value while the extracts with low activity give high minimum inhibition concentration value, Fabry*et al.,* (1998) and extracts with minimum inhibition concentration s below 8 mg/mL are classified as possessing potent antimicrobial activity. The antimicrobial activity of the extract could also be attributed to the terpenoids which were also present during the phytochemical screening of the plant. In consonance with this general assertion, in the present study, the minimum inhibition concentration value of the extracts agreed with their corresponding antibacterial activities.

In conclusion, the present study was planned to investigate the phytochemical and antimicrobial activity of methanol extract of leaves and steam of purslane against some human pathogenic bacteria and plant pathogenic fungi. A complete randomized design was used to determine the antimicrobial activities using disc diffusion method. Phytochemical screening of methanolic extracts of leaf and stem revealed the presence of tannins, saponins, steroids, terpenoids, and alkaloids as the active phytoconstituents of purslane and potency for use in producing pharmaceutical bioactive compounds for therapeutic drugs.

Competing interests

The authors declare they have no competing interests.

Authors' contributions

MD, ZY outset and designed experiments. MD, ZY and KA performed experiments. MD and ZY contributed reagents and materials. MD&KA performed all the experiment and data analyses and wrote the manuscript. All authors discussed and revised the manuscript. All authors commented on the manuscript before submission. All authors read and approved the final manuscript.

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