EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *Cissampelos pareira* L.

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ABSTRACT: In present study antimicrobial and antioxidant activities of leaf and stem ethanolic extracts of *Cissampelos pareira* were evaluated. Stem extract revealed good result as compared to leaf extract. The stem ethanolic extract exhibited good zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa* with 13±0.33 mm and 14±0.58 mm diameter whereas minimum zone of inhibition was observed by leaf ethanolic extract against *Bacillus subtilis* and *Staphylococcus aureus* with 7±0.58 mm and 7±0.88 mm, respectively. The available antibiotics such as tetracycline, cefoperazone and erythromycin were used to compare all these results. The fungal strains i.e. *Aspergillus oryzae* and *Aspergillus niger* were used and significant results were recorded for stem ethanolic extract as compared with antifungal standard discs such as terbinafine and fungivin. The MIC assay was used for further analysis of leaf and stem extracts that inhibited bacterial and fungal growth. The antioxidant effect was evaluated by using 1,1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging assay, total phenolic content and total flavonoid content. The stem extract of plant displayed good antioxidant potential as compared to the leaf extract. The % DPPH free radical scavenging activity was maximum of the stem extract (1.03±0.19 µg/ml). The maximum result of antioxidant activity was observed by total phenolic content i.e. 0.77±0.09 µg/ml at 1000 concentration. The ethanolic extract of stem had exhibited most elevated level of flavonoid content i.e. 0.70±0.06 µg/ml as compared to the leaf extract of *C. pareira.*

Keywords: *Cissampelos pareira*, Antimicrobial, Antioxidant, Minimum Inhibitory Concentration.

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INTRODUCTION

Approximately 250,000-500,000 plant species have been discovered among which nearly 2000 plant species have medicinal importance and therefore used in different forms for medicinal purpose (Amer *et al.*, 2013). The plants have been used for the welfare of human beings such as food, flavoring agents, drugs, lubricants, additives and binders. The significant biologically active compounds derived from plants are proteins, steroids, terpenoids, alkaloids, flavonoids, glucosides, saponins, tannins, resins and essential oils that produce a significant physiological change on human body (Basha *et al*., 2011). According to the (WHO), 80 percent people of the world depends on herbs for folk medicines (Narayana and Thammanna, 1987).

The unnecessary use of antibiotics causes harmful effects on host are; hypersensitivity, allergic reactions and suppression in the immune system (Khan and Latif, 2014). It is needed to develop alternative antibacterial drugs from medicinal plants for the treatment of infectious diseases (Siddiqui *et al*., 2015). Plants produce various compounds which have potent resistance against antibiotics against pathogens (Ajaib *et al.*, 2016a).

Antioxidants contain free radicals and properties of breaking chain reactions. The most effective part of their defense mechanism is to remove and reduce the action of free radicals which cause stress of oxidation (Pourmorad *et al.*, 2006). Antioxidants play vital role in body by reducing the number of free radicals, lower the energy levels and reduce oxidative damage to various biological molecules and prevent diseases caused by oxidative stress like diabetes mellitus, ageing, atherosclerosis, Alzheimer's diseases and cancer. Antioxidants are reported to play a central role in preventing oxidative stress (Ali *et al*., 2008; Mazhar *et al.*, 2015). Since the ancient times, all over the world traditional people depended upon medicinal plants having less toxicity and cost effectiveness due to their biological properties, against various viral infections and ailments, caused by oxidative stress, (Ajaib *et al.*, 2017).

Cissampelos pareira is perennial climbing shrub, 2 to 5 m high commonly known as "Patha" in Ayurveda. It belongs to family Menispermaceae and has significant importance for the treatment of urinary problems, fever and skin infection (Jain *et al.*, 2015).

Menispermaceae is a family of mostly dioecious climbing plants. Most members of this family are tropical, but a few are found in temperate regions (Kim *et al.,* 2004). A number of species in this family are used as medicinal plants in local herbal treatment as well as proven antimicrobial and antioxidant potential such as *Cocculus laurifolius.*

MATERIALS AND METHODS

Plant Material: Stem and leaf material of *C. pareira* plant were collected in May 2017 from District Bhimber, Azad Jammu and Kashmir. The collected plant material was identified and got authenticated from Herbarium Department of Botany, MUST with a voucher number MUST.BOT.5370.

Maceration of plant materials: The collected parts of selected plant i.e. stem and leaves were shade dried at room temperature. Maceration was carried out by soaking of powder in ethanol and then evaporating on a rotary evaporator below 40ºC and extract was obtained respectively.

Antimicrobial activity

Zone of Inhibition: The antimicrobial activity of *C. pareira* was evaluated by using the Agar Well Diffusion method. The antimicrobial activity of *C. pareira* includes antibacterial and antifungal activity. For analysis of antimicrobial activity, two Gram negative bacteria (*Staphylococcus aureus and Escherichia coli),* two Gram positive bacteria (*Pseudomonas aeruginosa* and *Bacillus subtilis*) and two fungal strains *(Aspergillus niger* and *Aspergillus oryzae)* were employed following Cruick-Cruick-Shank *et al*., (1975). The zone of inhibition was determined by employing Agar Well Diffusion technique following Jorgensen and Turnidge (2007).

Determination of Minimum Inhibitory Concentration (MIC): antimicrobial potential of plant parts was evaluated by using Broth-dilution method following Murray *et al.,* (1999).

Determination of antioxidant activity: Investigation of antioxidant activity included following parameters: Total Phenolic Contents (TPC)**,** Total Flavonoid Contents (TFC)**,** DPPH radical scavenging action.

DPPH radical scavenging action: The DPPH free radical scavenging activity of ethanolic extracts of parts (bark and leaves) of *C. pareira* was examined by using the method of Hassan *et al*., (2016).

Total Phenolic Content: Estimation of Total Phenolic Content of *C. pareira* was carried out following the procedure of Yang *et al*., (2005).

Total Flavonoid Contents: The Total Flavonoid Content of *Cissampelos pareira* was determined by employing the method of Dewanto *et al*., (2002).

RESULTS AND DISCUSSION

The investigation of antimicrobial activity of leaf and stem extract of *C. pareira* was evaluated against antibacterial and antifungal strains. Different antibiotics and antimycotics were used for the assessment of result in antimicrobial activity. Both extracts of stem and leaves of *C. pareira* showed activities against all sample bacteria but the ethanolic extract of stem of *C. pareira* exhibited maximum zone of inhibition. Maximum zone of inhibition is shown by ehtanolic stem extract against *P. aeruginosa*, i.e. 14±0.58 mm and minimum 7±0.58 of leaf extract against *B. subtilis* (Table-1).

In antifungal activity, the ethanolic extract of stem showed maximum activity against *A. oryazae* with zone of inhibition 16±0.57 mm and against *A. niger* with 16±0.577 mm zone of inhibition. In case of antimycotics, zone of inhibition formed by Fungivin and Terbinafine against *A. niger* were 19±2.08 mm and 18±0.05 mm respectively (Table-2).

Antioxidant activity: The percent DPPH free radical scavenging potential of ethanolic leaf and stem extract of *C. pareira* was evaluated and results obtained were compared with BHT as a standard. The ethanolic extract of stem of *C. pareira* showed most elevated antioxidant potential 1.03±0.19 µg/ml to neutralize DPPH radicals while minimum capacity was observed for ethanolic extract of leaves i.e. 0.95±0.17 µg/ml (Table-4).

Minimum inhibitory concentration (MIC): The MIC values were recorded at various concentrations (Table-3).

The total flavonoids content of *C. pareira* was measured maximum activity reported by ethanolic extract of stem of *C. pareira.* The flavonoid content increased with increase in concentration of extract (Table 5). The maximum potential showed by ethanolic extract of stem 0.70±0.06 µg/ml at 1000 concentration while the ethanolic extract of leaves showed minimum potential 0.66±0.07 µg/ml.

The total phenolic contents of leaf and stem extracts of *C. pareira* were measured by using folin-ciocalteu reagent. The Gallic acid is used as a standard to compare the results in this assay. The ethanolic extract of stem had macerated more phenolic content 0.77±0.09 µg/ml and attribute to the total antioxidant activity while the phenolic constituent was present in lower concentration in the ethanolic extract of leaves 0.60±0.06 µg/ml as shown in (Table 6).

Table-1: Inhibition Zone produced by extracts of leaf and stem of *Cissampelos* ***pareira*** **and antibiotics against bacterial strains (mm).**

Plant parts	Zone of Inhibition (mm)			
	Escherichia coli	***Bacillus subtilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Leaf	11±0.88	7±0.58	7±0.88	10±1.14
Stem	13±0.33	12±0.88	9±0.57	14±0.58
Antibiotics				
Tetracycline	10±0.58	11±0.88	7±0.57	12±0.33
Erythromycin	11±1.86	8±0.88	4±0.57	8±0.06
Cefoperazone	-	3±0.2	2±0.57	-

Table-2: Inhibition Zone produced by ethanolic extracts of leaf and stem of *Cissampelos* ***pareira*** **and antimycotic against fungal strains (mm).**

Plant parts	Zone of Inhibition (mm)	
	Aspergillus niger	***Aspergillus oryazae***
Leaf	11±0.577	16±0.57
Stem	16±0.577	17±1.45
Antifungal standard disc		
Fungivin	19±2.08	18±0.05
Terbinafine	14±0.66	13±0.88

Table-3: MIC values (mg/mL) exhibited by leaf and stem of ***C. pareira*** **against bacterial and fungal strains.**

Plant parts	***Escherichia coli***		***Bacillus subtilis***		***Staphylococcus aureus***		***Pseudomonas aeruginosa***	
	Conc. mg/mL	MIC	Conc. mg/mL	MIC	Conc. mg/mL	MIC	Conc. mg/mL	MIC
Leaf	1.0	1.27±0.18	1.0	1.11±0.01	0.9	1.26±0.02	0.9	1.36±0.10
Stem	0.9	1.07±1.05	1.0	1.07±0.02	1.0	0.34±0.01	1.0	1.12±0.03

Plant parts	***Aspergillus niger***		***Aspergillus oryzae***	
	Conc. mg/mL	**MIC**	**Conc.** mg/mL	**MIC**
Stem	1.0	1.12±0.06	0.9	1.16±0.05
Leaf	0.9	0.53±0.04	1.0	0.39±0.06

Table-4: Free Radical Scavenging of leaf and stem of ***C. pareira*** **by DPPH assay**.

		Absorbance at different concentrations (µg/ml)				
Plant parts	Fraction	60	125	250	500	1000
Leaf	Ethanol	0.43±0.11	0.56±0.13	0.66±0.13	0.78±0.13	0.95±0.17
Stem	Ethanol	0.46±0.21	0.62±0.18	0.75±0.21	0.89±0.17	1.03±0.19
BHT		0.44±0.10	0.53±0.10	0.61±0.11	0.74±0.10	0.99±0.11

Table-5: Total Flavonoids Content in the leaf and stem of ***C. pareira.***

Plant parts	Absorbance at different concentrations (µg/ml)					
	Fraction	60	125	250	500	1000
Leaf	Ethanol	0.27±0.06	0.36±0.07	0.44±0.08	0.55±0.07	0.66±0.07
Stem	Ethanol	0.27±0.09	0.37±0.09	0.47±0.09	0.60±0.06	0.70±0.06
Ascorbic acid		0.27±0.05	0.34±0.05	0.40±0.06	0.55±0.05	0.64±0.05

Table-6: Total Phenolic Content in the leaf and stem of *C. pareira.*

Plant parts	Fraction Ethanol	Absorbance at different concentrations (µg/mL)				
		60	125	250	500	1000
Leaf		0.24±0.03	0.33±0.04	0.39±0.06	0.48±0.06	0.60±0.06
Stem	Ethanol	0.27±0.09	0.37±0.09	0.47±0.09	0.63±0.09	0.77±0.09
Gallic acid		0.28±0.04	0.35±0.04	0.44±0.05	0.54±0.05	0.75±0.06

The present study was conducted to evaluate the antimicrobial and antioxidant activity of ethanolic extract of leaf and stem of *C. pareira*. The antimicrobial activity was evaluated by calculating the inhibition zone and MIC value produced by ethanolic extract of leaf and stem of *C. pareira* against bacterial and fungal strains. Both parts i.e. stem and leaf of *C. pareira* exposed activity against all the bacteria used in the study. The maximum zone of inhibition was observed by stem extract as compared to the leaf extract. The stem extract showed good result against *E. coli* and *P. aeruginosa*, i.e. 13±0.33mm and 14±0.58mm respectively whereas minimum zone of inhibition was observed by leaf ethanolic extract, i.e. *B. subtilis* and *S. aureus* with 7±0.58 mm and 7±0.88 mm respectively. Okwulehie *et al*. (2013) reported the similar results while working on antimicrobial activity of ethanolic extract of four indigenous plants from south eastern Nigeria against same bacterial strains. The leaf and stem extract of *C. pareira* showed activity against *A. niger* and *A. oryzae.* The maximum zone of inhibition was formed by *A. oryzae* with 17±1.45mm in stem ethanolic extract while leaf extract showed minimum zone of inhibition against *A. niger* with diameter 11±0.577 mm. Erturk (2006) reported same result while working on the antimicrobial and antifungal activity of whole ethanolic extract of eleven spice plants.

Considerably good MIC value, i.e. 0.34±0.01 mg/mL of stem ethanolic extract was observed against *S. aureus.* The same results were obtained from the work of Ajaib *et al*., (2011) on *Sauromatum venosum* Ait. The antioxidant activity was evaluated by using DPPH radical scavenging activity, Total phenolic contents and Total flavonoids contents. Several methods were used for analysis of the antioxidant compounds. The result obtained by DPPH radical scavenging activity had recognized that the ethanolic extract of stem showed maximum radical scavenging potential 1.03±0.19 µg/mL at 1000 µg/mL concentration. BHT was used as a standard to compare the results. This agreed with those results that were documented by Aziz-ur-rehman *et al*. (2011) while reporting the antioxidant potential of *Artemisia incisa.*

The maximum value of total flavonoids contents was determined in stem extract of *C. pareira* 0.70±0.06 µg/mL when compared with the extract of leaves and the result obtained from the plant under investigation were nearly close to those findings reported by Muhammad and Saeed (2011) during the biological screening of whole plant of *Viola betonicifolia*. The calculation of total flavonoids contents was based upon the aluminum chloride complex that it forms with flavonoids.

Phenolic compounds have significant importance because of their antioxidant, anti-inflammatory and anticancerous functions (Sacchetti *et al*., 2005) and phenolic compounds were present in both edible and non-edible plant components. The ethanolic extract of stem exhibit maximum potential of phenolic contents that was found to be 0.70±0.06µg/mL. These results were documented to the findings of Ajaib *et al*. (2016b) while working on the antimicrobial and antioxidant activity of different parts of *Ficus natalensis.*

Conclusion: It was concluded that the antimicrobial and antioxidant potential was exhibited by the both leaf and stem ethanolic extracts of *C. pareira*

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