In Vitro Biological Activity of Prunella Vulgaris from Kashmir, Himalayas

Asif Ahmad Bhat[1] Kumari Manisha[2]
[1,2]Department of Agriculture and Botany Science
[1,2]SBBSU University, Jalandhar, India

Abstract— The most popular medicinal plant Prunella Vulgaris L, (Lamiacae) is a perennial widely distributed in temperate zone and tropical mountains of Europe and Asia. Due to its medicinal and industrial importance, the demand for P Vulgaris has increased drastically in recent years, in the present study methonalic extract was obtained by soxhlet extraction method (Soxhlet extractor), The MIC, extract was accumulated by broth dilution, The Total phenolics in methonalic extract was determined by using Folin-Ciocalteau reagent. The DPPH assay was performed by using the method of Braca etal (2011). The plant extract showed Zone of Inhibition aganist five bacterial strains namely Escherichia coli, Klebsiella pnemoniae, Staphylococcus aureus, Shigella dysentery, Salmonella typhi and three Fungal strains namely Penicillium notatum, Aspergilllus niger, Accremonium alternatum respectively. Minimum Inhibitor concentration (MIC) against all tested microorganisms showed antimicrobial and anti-fungal activities .The present investigation recognized plant extract of P vulgaris as a promising antibacterial, antifungal and antioxidant agent. However further investigations are needed to understand the mechanistic basis of this effect of this extract and its chemical composition.

Keywords: Prunella vulgaris, MIC, Soxhelt extractor, DPPH, antifungal, antimicrobial

I. INTRODUCTION

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize ecofriendly and natural or plant-based products for the prevention and cure of different human diseases.P. vulgaris L. (Labiatae), a rediscovered herb belonging to the mint family also known as self heal, was very popular in European, Asian and Chinese medicine and was used against fever, wounds and throat infections. The epithet of the species “vulgaris” is from the Latin adjective “vulgar” meaning “common” as the plant is widespread. Prunella vulgaris is widely distributed in Asia, Europe and Iran. It is traditionally used for eye pain, inflammation, headache, dizziness, sore throat and wound healing. P. vulgaris is known as selfheal; contains several active components, including oleanolic acid, betulinic acid, ursolic acid, flavonoids and rosmarinic acid. Some pharmacological activities such as the immune modulatory effect, antioxidant activity and anti hyperglycemic action were confirmed. In spite of its traditional uses as an antiseptic agent for treatment of wounds and sore throat, there are a few literatures on its antimicrobial activity. The P. vulgaris were used in oral preparations for control of gingivitis. In China, the aqueous extract from fruit spikes were used in typical dose of 9–15 gm per day for different ailments. The aim of this research was to evaluate the total phenolic and total flavonoid contents of P. vulgaris methanol, ethanol, aqueous extracts and their antimicrobial activities.

Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate in vitro antibacterial and antifungal activity of extracts from Prunella vulgaris against the most common microbial pathogens.

II. MATERIAL AND METHODS

A. Plant Material Collection

The plant Prunella vulgaris was collected from higher reaches of Naranag in the month of July and was identified and authenticated courtesy Centre of Plant Taxonomy, Department of Botany,University of Kashmir. The voucher specimen has been retained in the herbarium of Taxonomy, Department of Botany, University of Kashmir for future reference under herbarium number:(KASH-612). The aerial sample was grinded and subjectedto extraction in methanol by using soxhlet extractor apparatus at the temperature range of 55-65 ^{0}C.At the end of hot extraction, the extract was filtered. The filtrate was concentrated on a hot water bath and the solvent was recovered using distillation unit. The extract was then kept in desiccator to remove any moisture if present and finally stored at 4^{o}C for further use.

B. Collection of Bacterial and Fungal Strains

Three specific human pathogenic bacterial strains, Escherichia coli, Klebsiellapneumoniaeand Staphylococcus aureus were obtained from Department of Microbiology, SKIMS and two bacterial strains Shigella dysenteryand Salmonella typhi and two fungal strains Penicillium notatum and Aspergillus niger were obtained from the Microbiology Laboratory, Centre of Research and Development (CORD), University of Kashmir.

C. Determination of total phenolic content:

The total phenolics in methanolic extract of Prunella vulgaris was determined by using Folin- Ciocalteau reagent. One hundred mg of extract was dissolved in 200 ml of methanolic/water (60:40, v/v, 0.3% HCl) and filtered through Whatman’s filter paper. To 100µl filtrate, 100 µl of 50% Folin- Ciocalteau reagent, and 2 ml of 2.5% sodium carbonate were added and mixed completely. After two hours incubation at room temperature, the absorbance of the solution at 750nm was measured with spectrophotometer. Quantitation was based on the standard curve of gallic acid (50 mg%), which was dissolved in methanol/water (60:40, v/v, 0.3% HCl) (Kyoung et al., 2005).

D. DPPH radical scavenging activity:

The DPPH assay was performed by using the method of Braca et al. (2001). Various concentrations of plant extracts (100-1000 µg/ml) were added to 1ml of the 20mg%

All rights reserved by www.ijsrd.com

methanol solution of DPPH, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 30 minutes in dark, and the absorbance was taken at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. α-tocopherol was taken as positive control. The percentage inhibition was calculated by using the formula.

Percent inhibition = $\frac{Ac-As}{Ac} \times 100$

Where Ac is the absorbance of the control and As is absorbance in the presence of plant extracts and known standards.

III. Results and Discussion

A. *Anti-microbial activity of Prunella vulgaris*

The methanolic extract of Prunella vulgariswas tested for antimicrobial activity against a set of microbial strains including both gram positive and gram negative bacterial strains and fungal strains. The plant extract showed anti-microbial activity which was visible as the zones of inhibition formed in the different cultures of Gram positive and Gram negative bacteria as well as in case of fungal cultures. Five bacterial strains Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,Shigellaand Salmonellatyphi and three fungal strains Penicillium notatum, Acremonium alternatum and Aspergillus niger were tested for their susceptibility to Prunella vulgaris extract.

B. *Antibacterial activity*

The methanolic extract of Prunella vulgaris extract showed a high antimicrobial activity against the pathogenic bacteria like Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Shigella and Salmonella typhi. The maximum activity was seen for Klebsiella pneumoniae with inhibition zone diameter of 10.66 ± 1.15 mm and least Inhibition zone 8.00±2.00mm was observed in Salmnonella as is depicted in Table-1 and clearly shown in Figure-1.

C. *Antifungal activity*

In case of antifungal activity, the methanolic extract of Prunella vulgaris was found to be effective against the inhibition of growth of three fungal strains. The maximum inhibition zone diameter of 8.00 ± 2.00 mm was observed for Acremoniumalternatumfollowed by 17.33 ± 1.15 mm for Aspergillusnigerand 16.33 ± 1.15 mm for Penicilliumnotatumas can be seen in Table-2 and clearly depicted in Figure-2.

D. *Minimum inhibitory concentration*

The MICs of methanolic extract of Prunella vulgarisagainst eight microorganisms were tested and the results are tabulated in Table-3. It was observed thatall tested bacterial (gram positive as well as gram negative) and fungal species were susceptible to methanolic extract of Prunella vulgaris and exhibited the MICs ranging from 2000 to 4000 µg/ml.

S.no	Bacterial strain	Zone of inhibition (mm)		
		Methanolic extract (50 mg/ml)	Positive control (30µg/ml)	Negative control (DMSO)
1.	Escherichia coli	8.66 ± 1.52	25.66 ± 4.04	--
2	Staphylococcus aureus	10.33 ± 1.52	30.66 ± 4.04	--
3.	Klebsiellapneumoniae	10.66 ± 1.15	30.00 ± 5.00	--
4.	Shigella dysentery	8.00 ± 1.00	18.66 ± 0.55	--
5.	Salmonella typhi	8.00 ± 2.00	17.33 ± 1.15	--

Table 1: In vitro antibacterial activity of methanolic extract of Prunella vulgarisagainst different bacterial cultures as represented by the diameter of zone of inhibition (mm).
*Chloramphenicol was used as positive control at the concentration of 30µg/ml.

S. No.	Test Fungi	Zone of inhibition (mm)	
	Methanolic extract (50 mg/ml)	Positive control (30µg/ml)	Negative control (DMSO)
Penicillium notatum	16.33 ± 1.15	19.00 ± 1.00	--
Aspergillus niger	17.33 ± 1.15	20.67 ± 1.52	--
Accremonium alternatum	18.00 ± 2.00	21.33 ± 1.15	--

Table 2: In vitro antifungal activity of methanolic extract of Prunella vulgaris against different fungal cultures as represented by the diameter of zone of inhibition (mm).

S. No	Microorganism	Methanolic extract (µg/ml)
	Bacterial Strains	
1.	Escherichia coli	3000
2.	Staphylococcus aureus	4000
3.	Klebsiella pneumoniae	3000
4.	Salmonella typhi	4000
5.	Shigella dysentery	2000
	Fungal Strains	
1.	Penicillium notatum	3000
2.	Aspergillus niger	4000
3.	Accremonium alternatum	3000

Table 3: The MICs of methanolic extract of Prunella vulgaris against different microorganisms

IV. Conclusion

This study supports the argument that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant activities showed by the plant provide a scientific validation for the traditional use of this plant. Further work on isolation and identification of active compounds and its efficacy needs to be done.

References

[1] Adamkova H, Vičar J, Palasova J, Ulrichova J, Šimanek W. Macleya cordata and Prunella vulgaris in oral hygiene products- their efficacy in the control of gingivitis. Biomed Papers 2004; 148(1):103-105.

All rights reserved by *www.ijsrd.com*

[2] Askun T, Tumen G, Satil F, Ates M. Characterization of the phenolic composition and antimicrobial activities of Turkish medicinal plants. Pharm. Biol. 2009; 47(7):563-571.
[3] Baba M, Snoeck R, Pauwels R, De-Clercq E. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus and human immunodeficiency virus. Antimicrob Agents Chemother 1988; 32(11):1742-1745.
[4] Cheung LM, Cheung PCK, Vincent ECO. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 2003; 81(2):249-255.
[5] Determination of substances characteristic of green and black tea. Part 1: Content of total polyphenols in tea. Colorimetric method using Folin-Ciocalteu reagent. 2005.
[6] Fang X, Yu MM, Yuen WH, Zee SY, Chang RC. Immune modulatory effects of Prunella vulgaris L on monocytes/macrophages. Int J Mol Med 2005; 16(6):1109-1116.
[7] Han EH, Choi JH, Hwang YP, Park HJ, Choi CY, Chung YC, Seo JK, Jeong HG. Immuno stimulatory activity of aqueous extract isolated from Prunella vulgaris. Food Chem Toxicol 2009; 47(1):62-69.
[8] Hu SY. Herbal teas and populace health care in tropical China. Am J Chin Med 1997; 25(1):103- 34.
[9] Lamaison JL, Petitjean-Freyet C, Carnat A. Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid. Pharm Acta Helv 1991; 66(7):185-188. extract. Phytother Res 2003; 17(9):1082-1087
[10] Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of Mentha pulegium L. essential oil. J Ethnopharmacol 2008; 119(2): 325-327.
[11] Osakabe N, Yasuda A, Natsume M, Sanbongi C, Kato Y, Osawa T, Yoshikawa T. Rosmarinic acid a major polyphenolic component of Perilla frutescens, reduces lipopolysaccharide (LPS)- induced liver injury in D-galactosamine sensitizied mice. Free Radic Biol Med 2002; 33(6):798- 806.

All rights reserved by *www.ijsrd.com*