World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Antimicrobial and antioxidant activity of different extracts and polysaccharides isolated from leaves of *Joannesia Princeps Vell.*

Deepak Kumar[1*], Raju Chandra[2] and Tikam[1]

[1*]Department of Pharmaceutical Chemistry, Dolphin PG institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India
[2]Department of Chemistry, Dolphin PG institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, India

Received: 19-12-2014 / Revised: 27-12-2014 / Accepted: 29-12-2014

ABSTRACT

Plants have been used for thousands of years to flavour and conserve food, to treat health disorders and to prevent diseases including epidemics. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases and numerous disorders. The aim of this study was to evaluate the phytochemical contents, antimicrobial & antioxidant activities of *Joannesia princeps* leaves extracts and isolated polysaccharides. Different extracts were prepared using soxhlet assembly and after depigmentation of leaves, leaves were undergoing for the isolation of polysaccharides. All four extracts were tested for the presence of phytoconstituents and all four extract and isolated polysaccharides were also evaluated for antimicrobial and antioxidant effects. From antimicrobial activity, methanol extract showed maximum inhibition zone at a concentration of 200 mg/ml. The concentration of 500 µg/ml of methanol extract showed maximum antioxidant activity 83.69 % in comparison to other extract. CWP and HWP polysaccharides showed a significant antimicrobial and antioxidant effects. Methanol and acetone were richest in presence of phytoconstituents.

Keywords: *Joannesia princeps Vell.*, Antimicrobial, Antioxidant, Polysaccharides, DPPH

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now days, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants[1].

Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Currently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents or through the search of natural sources for as yet undiscovered antimicrobial agents. Herbal medications in particular have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option [2].

Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species. The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules

***Corresponding Author Address:** Dr. Deepak Kumar, Assistant Professor, Department of Pharmaceutical Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun (UK) India**;** E-mail: deepsingh2304@gmail.com*

commonly called "free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating [3].

Water-soluble polysaccharides, long-chain polymers with high molecular weight, are generally present in cell walls of higher plants. In recent years, there has been a growing interest in polysaccharides obtained from higher plants that may have wide diversity of chemical structures and biological activities [4]. The polysaccharides extracted from higher plants are widely used in diverse fields in the food, pharmaceutical and many other industries [5]. The polysaccharides from plants have proved to be one of the most promising groups of antioxidant compounds [6].

Many plants have been reported for their antimicrobial and antioxidant properties like *Spilanthes acmella* [7], *Aegle marmelos*[8], *Bombax ceiba* [8], *Lantana camara* [8], *Trapa natans* [9], *Aloe barbadensis*[10], *Sida cordata* [11], *Rhododendron arboreum* [11], *Hedychium spicatum* [12], *Syzygium cumini* [13], *Emblica officinalis* [14], *Mangifera indica* [14], *Desmodium heterocarpon* [15] *etc*. Till date there is not much literature available on biological activities of leaves of *Joannesia princeps.* Thus the objective of the present study was to evaluate the antimicrobial and antioxidant activity of different extract and polysaccharides isolated from leaves of *Joannesia princeps Vell.*

MATERIAL AND METHODS

Experimental work was carried out under following headings on the leaves of *Joannesia princeps Vell* for their antimicrobial & antioxidant activity.

Collection and identification of leaves of Joannesia princeps: Leaves of *Joannesia princeps* were collected from locality of Dehradun. Plant material was authenticated by Kumar Ambrish (Scientist C), in Botanical Survey of India, Northern regional centre, Dehradun (BSI). Authenticated specimen no is- Acc. No. 114848.

Extraction of leaves of Joannesia princeps in different solvents (Non-polar to Polar): The collected plant material was washed with water to removed other undesirable material and dried under shade. The air-dried leaves (100gm.) of *Joannesia princeps* were crushed. The crushed leaves extracted with different solvents of increasing polarity viz. Petroleum ether, Chloroform, Acetone and Methanol by hot percolation method using Soxhlet Apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure.

Phytochemical Analysis: The extracts of leaves of *Joannesia princeps* undergo various qualitative phytochemical tests. They showed their presence and absence in the different solvent systems. The different extracts of leaves of *Joannesia princeps* were tested for various phytoconstituents viz., alkaloid, carbohydrate, sterols and Terpenoids, Phenolic compound and tanins, Protein and amino acid, saponin etc.

Isolation of Polysaccharide from depigmented leaves

Cold water polysaccharides (C W P): 50 g depigment leaves (after extraction) is subjected to soaked with 1 litre distilled water and keep over night at 10˚C. The material was filtered through muslin cloth and the filtrate was again filtered vacuum using a Buchner funnel and centrifuged at 8000 rpm for 30 minute to remove water insoluble matters. The supernatant was concentrated up to 1/3 of its volume on a rota-evaporator under reduced pressure and was treated with IPA (Isopropyl alcohol-filtrate 3:1 v/v), the precipitate was washed with 80% and 100% isopropyl alcohol-water and isopropyl alcohol respectively and dried in vacuum desiccators.

Hot Water polysaccharides (H W P): 50 g depigment leaves (after extraction) is subjected to soaked with 1 litre distilled Hot water and keep over one day at 70˚C. The material was filtered through muslin cloth and the filtrate was again filtered vacuum using a Buchner funnel and centrifuged at 8000 rpm for 30 minute to remove water insoluble matters. The supernatant was concentrated up to 1/3 of its volume on a rota-evaporator under reduced pressure and was treated with isopropyl alcohol (isopropyl alcohol-filtrate 3:1, v/v), the precipitate was washed with 80% and 100% isopropyl alcohol-water and isopropyl alcohol respectively and dried in vacuum desiccators.

Test for Polysaccharides

***Iodine test*:** Iodine test use to test for the prince of polysaccharides. Iodine dissolve in aqueous

solution potassium Iodine reacts with the polysaccharide producing a purple black colour.

In-vitro antimicrobial and antioxidant activity of different extracts and Polysaccharides isolated from leaves of Joannesia princeps

Antimicrobial activity [16]***:*** The antimicrobial activity of the leaves of *Joannesia princeps Vell.*was carried out. The leaves extract were screened for anti-bacterial and antifungal activities at a dose of 200 mg/ml.

Anti-bacterial activity of leaves extract: Antibacterial activity was studied against the micro-organism and the bacterial cultures used in the study were: 1. Escherichia coli 2. Bacillus cerus 3. Pseudomonas 4. Proteus These bacteria were provided by Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun checked for purity by convention biochemical method. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 370 c for about 18-24 hours and then stored at 40 c as stock for anti-bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37˚C overnight. To test anti-bacterial activity, the well diffusion method used.

Antifungal activity of leaves extract: In this study, the antifungal activity was against the micro-organism and the fungi culture used for this study were: 1. Aspergillus Niger. These cultures were obtained from the standard cultures maintained in the Dolphin (PG) Institute Of Biomedical & Natural Sciences, Manduwala, Dehradun . These cultures were maintained on sabouraud dextrose agar (SDA) at first being incubated at 30˚C for about 32-48 hours and then stored at 40˚C at stock cultures for further anti-fungal activities. Fresh cultures into sabouraud Dextrose Broth and incubated at 30˚C for 48 hours. To test anti-fungal activity, the well diffusion method was used.

Antioxidant Activity[17]: DPPH was prepared in dark because of its highly oxidisable properties. Weigh accurately 20 mg DPPH and dissolved in their respective solvent. Generally Methanol and for some cases Ethanol is used as a solvent for DPPH. Ascorbic acid is an strong antioxidant agent. It is taken as standard. Standard solution of ascorbic acid is prepared. viz. 50 µg/ml, 100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500 µg/ml. Different concentration of the test sample *Joannesia princeps* extract and isolated polysaccharides which is to be examined for anti-oxidant activity is prepared. Viz. 50µg/ml, 100µg/ml, 200µg/ml, 300 µg/ml, 400µg/ml and 500 µg/ml. 3 ml of different concentration of test sample *Joannesia princeps Vell.* Extract, isolated polysaccharides and standard (ascorbic acid) were mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour. When procedure is done than absorbance is taken with the help of UV Spectrophotometer at 517 nm. We calculate the % activity of individual concentration of individual extract from the following formula:-

$$\% \text{ Activity} = \frac{\text{Abs.of control} - \text{Abs. of individual concentration}}{\text{Abs.of control}} \times 100$$

RESULTS AND DISCUSSION

The yield (in w/w) of leaves extracts in different solvents system viz. petroleum ether 2.36gm, chloroform 5.61gm, acetone 3.41gm, methanol 3.72gm, Hot water polysaccharides 3.10gm, and Cold water polysaccharides 2.10gm. Different extracts of leaves of *Joannesia princeps* undergoes various qualitative phytochemical tests. Phytochemical studies revealed that methanol extract was the richest extract among all the extracts. It showed the presence of carbohydrate, alkaloid, Phenolic compounds, saponin and Protein and amino acids. Acetone extract showed the presence of carbohydrate, alkaloid and Phenolic compounds. Petroleum ether showed only presence of steroids. Isolated hot water and cold water polysaccharides showed the distinct purple black colour for Iodine test for test of polysaccharide.

From antimicrobial studies methanol and acetone extracts showed antibacterial activity against all bacterial culture at a concentration of 200 mg/ml. Methanol extract showed inhibition zone 20 mm against E.coli, 14 mm against Bacillus, 16 mm against proteus, 18 mm against Pseudomonas. Acetone extract showed inhibition zone 18 mm against E.coli, 16 mm against Bacillus, 18 mm against proteus, 12 mm against Pseudomonas. Chloroform extract showed inhibition zone 12 mm against E.coli, 14 mm against Bacillus, 18 mm against proteus, 14 mm against Pseudomonas. Pet.Ether extract showed inhibition zone 14 mm against E.coli, 16 mm against Bacillus, 14 mm against proteus, 10 mm against Pseudomonas. Standard drug Chloramphenicol 20 mm against E.coli, 17 mm against Bacillus, 27 mm against Pseudomonas and 20 mm against proteus. Hot water polysaccharides extract showed inhibition zone 16 mm against Bacillus, 22 mm against Pseudomonas, 20 mm against Proteus and 20 mm against E. coli. Cold water polysaccharides showed inhibition zone 22 mm against Bacillus, 20 mm against Pseudomonas, 18 mm against Proteus and 21 mm against E. coli.

All extracts and polysaccharides was subjected to antifungal activity against fungal culture Aspergillus Niger at a concentration of 200 mg/ml. Acetone extracts showed maximum inhibition zone against Aspergillus Niger in comparison to other extracts. Acetone extract showed inhibition zone 25 mm against Aspergillus Niger. Methanol extract showed inhibition zone 22 mm against Aspergillus Niger. Chloroforam extract showed inhibition zone 7 mm against Aspergillus Niger. Pet.ether extract showed inhibition zone 5 mm against Aspergillus Niger. Standard drug chloramphenicol showed inhibition zone 12 mm against Aspergillus Niger. HWP showed inhibition zone 20 mm and CWP showed inhibition zone 21 mm against Aspergillus niger. Results are shown in table 1-2. Microbial antibiotic resistance are increasing day by day so more studies in this field are necessary for the identification of natural antimicrobial compounds and the future development of these compounds through structure/activity studies provides a promising avenue of research for novel antimicrobials.

Table 1 antibacterial activity of different extracts and isolated polysaccharides.

Test Organism	Inhibition zone in mm						Standard drug
	Pet. ether	Chloroform	Acetone	Methanol	HWP	CWP	Chloramphenicol
E. Coli	14	12	18	20	20	21	20
Bacillus cerus	16	14	16	14	16	22	17
Pseudomonas	10	14	12	18	22	20	27
Proteus	14	18	18	16	20	18	20

Table 2 antifungal activity of different extracts and isolated polysaccharides.

Test Organism	Inhibition zone in mm						Standard drug
	Pet. ether	Chloroform	Acetone	Methanol	HWP	CWP	Chloramphenicol
Aspergillusniger	05	07	25	22	20	21	12

From antioxidant studies methanol extract of *Joannesia princeps* leaves showed maximum antioxidant activity in comparison to all extracts. The concentration of 500µg/ml of methanol extract showed 83.69% antioxidant activity in comparison to all extract and standard drug Ascorbic acid. 500µg/ml concentration of methanol extract showed higher activity than acetone (79.77%) and chloroform extract (49.91%). Petroleum ether showed 61.66% antioxidant activity. The concentration of 500µg/ml of hot water polysaccharides showed 49.09% antioxidant activity as well as cold water polysaccharides showed 50.44 % antioxidant activity. Ascorbic acid is used as a standard drug comparision to all extracts. Results are shown in table 3-4.

Table 3 absorbance of different extracts and isolated polysaccharides

S. No.	**Concentration (µg/ml)**	**Absorbance of extracts at 517 nm**				**Polysaccharides**		**Standard drug**
		Pet. ether	Chloroform	Acetone	Methanol	HWP	CWP	Ascorbic acid
1	Control	1.620	1.11	3.09	0.742	1.11	1.13	1.299
2	50	1.050	1.01	0.967	0.556	0.826	0.938	0.041
3	100	0.874	0.830	0.897	0.284	0.742	0.856	0.039
4	200	0.769	0.704	0.804	0.208	0.683	0.652	0.036
5	300	0.633	0.664	0.776	0.196	0.665	0.612	0.037
6	400	0.625	0.661	0.654	0.124	0.570	0.566	0.036
7	500	0.621	0.556	0.625	0.121	0.565	0.560	0.034

Table 4: percent antioxidant activity of different extracts and isolated polysaccharides.

Concentration (µg/ml)	**% antioxidant acitivity**				**Polysaccharides**		**Standard drug**
	Pet. ether	Chloroform	Acetone	Methanol	HWP	CWP	Ascorbic acid
Control	-	-	-	-	-	-	-
50	35.18	9.0	68.70	25.00	25.58	16.99	96.82
100	46.04	25.22	70.97	61.72	33.15	24.24	96.97
200	50.53	36.56	73.98	71.96	38.46	42.30	97.20
300	60.92	40.18	74.88	73.58	40.09	45.84	97.13
400	61.42	40.45	78.83	83.28	48.64	49.91	97.20
500	61.66	49.91	79.77	83.69	49.09	50.44	97.36

However, the chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like alkaloids, phenolic compounds, tannins, reducing sugars and proteins present in the extract may be responsible for such activity. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. Polysaccharides are a structurally diverse group of biological macromolecules of widespread occurence in nature.

They are composed of repetitive structural features that are polymers of monosaccharides residues joined to each other by glycosidic linkage. The effect of polysaccharides isolated from plant parts are studied by very large number of researchers in the world. Furher studies needed for the mechanism of action of polysaccharides.

CONCLUSION

Present study showed that the *Joannesia princeps Vell* leaves extracts and isolated polysaccharides possessed significant in vitro antimicrobial and antioxidant property. From the above study it is concluded that the methanol extracts showed the maximum antimicrobial activity and antioxidant activity in comparison to other extracts. So further study needed for the isolation of active constituents which may be useful for therapeutic purpose.

Conflict of interest: The authors do not have any conflict of interests regarding the content of this research paper.

Acknowledgement: Author's are thankfull to Chairman and Principal of Dolphin PG Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand for providing necessary facilities for completion of this work.

REFERENCES

1. Eloff JN. Which extract should be used for the screening and isolation of antimicrobial components from plants. J Ethano 1998; 60(1):1-8. http://dx.doi.org/10.1016/S0378-8741(97)00123-2
2. Cock IE. Antimicrobial Activity of *Aloe barbadensis* Miller Leaf Gel Components. The Internet Journal of Microbiology 2007; 4:2.
3. Percival M. Antioxidant, Clinical Nutritional Insight; 1998; 1-4
4. Kardosova A, Machova E. Antioxidant activity of medicinal plant polysaccharides. Fitoterapia 2006; 77:367-373. http://dx.doi.org/10.1016/j.fitote.2006.05.001
5. Ridley BL et al. Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 2001; 57(6):929-967 http://dx.doi.org/10.1016/S0031-9422(01)00113-3
6. Ng TB et al. A gallic acid derivative and polysaccharides with antioxidative activity from rose (*Rosa rugosa*) flowers. Journal of Pharmacy and Pharmacology 2004; 56 (4):537-545 http://dx.doi.org/10.1211/0022357022944
7. Arora S et al. Phytochemical and antimicrobial studies on the leaves of *Spilanthes acmella.* J. Chem. Pharm. Res. 2011; 3(5):145-150.
8. Dabur R et al. Antimicrobial activity of some indian medicinal plants. Afr. J. Trad. CAM 2007; 4 (3): 313 – 318.
9. Kumar D et al. Evaluation of invitro anti-inflammatory, antimicrobial and antioxidant effects of *Trapa natans* (linn.) Leaves extract. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3 (2): 1697-1710.
10. Nejatzadeh-Barandozi F. Antibacterial activities and antioxidant capacity of Aloe vera. Organic and Medicinal Chemistry Letters 2013; 3:5. http://dx.doi.org/10.1186/2191-2858-3-5
11. Kumar D et al. Membrane stabilizing activity and antioxidant effect of *Sida cordata (Burm F.) Bioss and Rhododendron arboreum Sm.* leaves. Int J Pharm Sci Res 2013; 5(1): 165-70. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(1).165-70
12. Sarvani T, Paarakh PM. Antioxidant activity of Hedychium spicatum Buch.-Ham. Rhizome. Indian Journal of Natural Products and Resources 2012; 3(3):354-358.
13. Kumar D et al. Pharmacognostical Standardisation and antimicrobial activity of leaves of Syzygium cumini (Linn.) from various region of North India, Int. Res. J. Pharm. 2014; 5 (2): 62-65. http://dx.doi.org/10.7897/2230-8407.050112
14. Gupta VK, Sharma SK. Plants as natural antioxidants. Natural Product Radiance 2006; 5(4): 326-334.
15. Arora S et al. Antimicrobial studies of leaf extracts from *Desmodium heterocarpon* (L.) DC. Medicinal Plants 2014; 6(3): 206-208. http://dx.doi.org/10.5958/0975-6892.2014.00011.2
16. Barry A Lea, and Fibiger. The Antimicrobial Susceptibility Test. Principle and Practices. 1976; 180.
17. Williams WB et al. Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm.-Wiss. u.-Technol. 1995; 28: 25-30.