VOLUME 9 (SPECIAL ISSUE 1) 2023

ISSN 2454 – 3055

Guest Editor: Dr. S. Mohanasundaram
Assistant Guest Editor: Dr. S.S.Syed Abuthahir

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9 (Special Issue 1), 99-105 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Antibiotic and Antioxidant Effects of *Asparagus racemosus*: *In Vitro* Approach

Kumar Anil[1*], Sampatrao Tambe Satish[2], Babasaheb Jige Sandipan[3], Rout Sandeep[4], Prusty Ajay Kumar[5] and Cherian Pinkie[6]

[1]Department of Botany, DDU Gorakhpur University Gorakhpur 273009, Uttar Pradesh, India
[2]Department of Botany, MGV'S Loknete Vyankatrao Hariy Art's and Commerce College, Nashik, Maharashtra, India
[3]Department of Botany, Sant Ramdas College, Ghansawangi, Jalna Maharashtra, India
[4]Faculty of Agriculture, Sri Sri University, Cuttack Odisha 754006, India
[5]Department of Agricultural Extension Education, M S Swaminathan School of Agriculture, Centurion University of Technology and Management, R. Sitapur, Paralakhemundi, Gajapati, Odisha761211, India
[6]Department of Botany, St Joseph's College for Women, Alappuzha, Kerala, India

**Corresponding Author*

Received: 14th January, 2023; **Accepted:** 5th March, 2023; **Published online:** 13th April, 2023

https://doi.org/10.33745/ijzi.2023.v09ispl1.015

Abstract: In this study, the phytochemical composition of *Asparagus racemosus* was investigated both qualitatively and quantitatively. Aqueous, ethanolic, and hexane extracts of *Asparagus racemosus* were tested for antibacterial activity against a variety of bacteria, including *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, and *Enterobacter aerogenes* and were found to be effective at concentrations ranging from 400 µg to 500 µg. The antioxidant activity of these extracts was also studied in comparison to α-tocopherol, which served as a control. *Asparagus racemosus* contains alkaloids, flavonoids, saponins, and tannins. In comparison to a standard reference of 500 µg/ml α-tocopherol, which demonstrated 48.16 per cent antioxidant activity, ethanol extract demonstrated 41.55 per cent antioxidant activity.

Keywords: *Asparagus racemosus* , Phytochemicals, Antibacterial, Antioxidant, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Enterobacter aerogenes*

Citation: Kumar Anil, Sampatrao Tambe Satish, Babasaheb Jige Sandipan, Rout Sandeep, Prusty Ajay Kumar and Cherian Pinkie: Antibiotic and antioxidant effects of *Asparagus racemosus*: *In vitro* approach. Intern. J. Zool. Invest. 9(Special Issue 1): 99-105, 2023.

https://doi.org/10.33745/ijzi.2023.v09ispl1.015

Introduction

Drug resistance has resulted from the indiscriminate use of commercial antimicrobial medicines (Service, 1995). Plants can aid in the treatment of infectious diseases, and various plant extracts have been proven to exhibit antibacterial properties *in vitro* (Sofowora, 1983).

Antibiotic-resistant bacteria are becoming more common as a result of increased antibiotic use, which may render current antimicrobial treatments ineffective in addressing diverse bacterial diseases (Tanaka *et al*., 2006). Herbal therapy is combined with other treatment modalities, namely traditional and folk medicine. It is critical to conduct a scientific evaluation of the potential use of folk medicine for infectious disorders caused by microorganisms. In mild situations of infectious illnesses, medicinal herbs may be used as an alternative treatment. They could potentially be a starting point of new, powerful medications to which disease strains are not resistant. Recently, there has been an increase in the demand for and use of plant-derived pharmaceuticals and nutritional supplements. Ethnopharmacologists, botanists, microbiologists, and natural product chemists are looking for biological chemicals that could be utilised to treat infectious diseases in the medicinal flora. *Asparagus racemosus* is a woody climber that can reach a height of 1-2 m. Like pine needles, the leaves are tiny and uniform. The inflorescence is made up of small white blooms in short spikes. The roots are clumped together and finger-like. In India, Asia, Australia, and Africa, the Liliaceae family plant is available at shaded-low elevations and tropical regions (Thakur *et al*., 1989). The root of *A. racemosus* has several medical characteristics, according to the ancient Ayurvedic literature, and is notably recommended in impending abortion and as a galactogogue. This plant leaf is used as an antiseptic by some rural South Indians, particularly tribals (Gurudeva and Yoganarasimhan, 2009).

The current study intends to qualitatively assess *Asparagus racemosus* in order to investigate the phytochemicals and antibacterial activity of *Asparagus racemosus* extracts against enteric pathogens.

Materials and Methods

Plant Extract Preparation:

Asparagus racemosus leaf for this study was procured from Thirukkalukundram village, Kanchipuram District, Tamil Nadu, India,. A voucher specimen was kept in our departmental laboratory after scientists at the University of Madras' Guindy campus in Chennai identified the plant. Before shade drying at room temperature for 15–20 days, the plant sample was refluxed in running tap water for 1-2 h. Aqueous, ethanolic, and hexane extracts of *Asparagus racemosus* were produced using soxhlet apparatus for 24 h (Hoffman *et al*., 2004). With $CaCl_2$, the extract was distilled and concentrated *in vacuo*. Antifungal, antibacterial, and antioxidant properties of lyophilized aqueous fractions were examined.

Chemicals and Microorganisms:

IMTECH, Chandigarh, India, provided *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Enterobacter aerogenes*, and *Shigella flexineri.* Himedia, Merck, and S.D. Fine-Chemicals, Mumbai, supplied the solvent and other chemicals utilised in this work.

Phytochemical Screening:

The methodologies used for phytochemical screening were adapted from Oloyed (2005). The purpose of this research was to identify the biologically active compounds that determine the flavour, colour, and other aspects of leaves.

Test for alkaloids: The ground sample was pounded separately. 0.2 g was steamed with 5 ml of 2% hydrochloric acid for 5 min. After cooling, the filtrate was divided evenly between three test tubes labeled A, B, and C. 1 ml of filtrate received two drops of each reagent. Dragendroff's reagent precipitated red. Mayer's reagent precipitated a creamy white alkaloid (Harborne, 1973; Trease and Evans, 1989).

Test for flavonoids: 0.5 g of macerated *Asparagus racemosus* was boiled in water with 10 ml of ethyl acetate for 1 min. The combination was filtered and tested again. 4 ml of the filtrate was combined with 1 ml of a 1 per cent aluminium chloride solution. Flavonoids were confirmed by

the development of a yellow colour in 1 ml of mild ammonia solution (Harborne, 1973).

Test for saponins: 0.1 g was boiled in 5 ml of distilled water for 5 min. The hot mixture was filtered and used for the next trials (Trease and Evans, 1989). 2 drops of olive oil were added to 1 ml of filtrates, stirred, and an emulsion formed. Distilled water was added to 1 ml of filtrate. Before testing for stable froth, the liquid was vigorously stirred (Trease and Evans, 1989).

Test for tannins: 2 g of pulverised material was heated for 5 min with 5 ml of 45 per cent ethanol. The mixture was cooled after filtering. 1 ml of filtrate was mixed with 3 drops of lead sub acetate solution. Gelatinous tannin precipitates were notid. 1 ml of filtrate and 0.5 ml of bromine water were combined. Tannins were found as pale brown precipitates (Trease and Evans, 1989).

Test for glycosides: 2 g of the sample and 30 ml of distilled water were boiled for 5 min on a water bath before filtration and use. 5 ml of filtrate and 0.2 ml of Fehling solutions A and B were combined until alkaline and heated in a water bath for 2 min. A light blue precipitate indicated the absence of glycosides (Oloyed, 2005).

Quantitative Phytochemical Constituent Analysis:

Estimation of alkaloids: 0.5 g of the dissolved substance in 1:1 ethanol:H_2SO_4. For 5 min, 1 ml of filtrate was combined with 5 ml of 60% tetraoxosulphate. 5 ml of 0.5 per cent formaldehyde was added and let to stand for 3 h. The absorbance was read at 565 nm (Harborne, 1976). 0.5 g of the material was dissolved in a 1:1 ethanol-H_2SO_4 combination. After 5 min, 1 ml of filtrate was mixed with 5 ml of 60% tetraoxosulphate (VI). After 3 h, 5 ml of 0.5% formaldehyde was added. Absorbance was read at 565 nm (Harborne, 1976).

Estimation of flavonoids: Acid hydrolysis spectrophotometry was used to measure the test sample's flavonoid concentration. For 30 min, 5 ml of dilute HCl was heated with 0.5 g of processed plant material. 1 ml filtrate, 5 ml ethyl acetate, and 5 ml 1 per cent NH_3 were mixed. Three absorbance scans were performed from 420 nm to 520 nm (Harborne, 1976).

Estimation of saponins: 20 ml of 1N HCl was heated with 0.5 g of the material for 4 h. The ether layer was dried by adding 50 ml of petroleum ether to the filtrate after cooling. The residue was acetone-ethanoled. Each tube got 0.4 ml and received 6 ml of ferrous sulphate and 2 ml of saturated H_2SO_4. After 10 min absorbance was read at 490 nm (Oloyed, 2005).

Estimation of tannins: After agitating 5 g of pulverized material in a test tube with 3 ml of methanol for 1 min, it was put into a Buchner funnel with the suction on. After cleaning the tube with 3 ml of methanol, the contents were dumped into the funnel. After mixing 50 ml of water, the filter was tested. Aqueous extractions employed 5 ml of water for extraction and washing and 50 ml for the filtrate. To 5 ml of extract, 3 ml of 0.1 ml $FeCl_3$ in 0.1 NH_4Cl was added, followed by 3 ml of 0.008 ml $K_2Fe(CN)_6$. Absorbance was read at 720 nm (Onwuka, 2005).

Antibacterial Effect:

Asparagus racemosus was tested for antibacterial activity using agar well diffusion (Chung *et al*., 1990). Fresh Muller Hinton agar media filled the petri dishes. A sterile cotton swab with bacterial culture dispersed it over the media (mid log phase). Agar wells were made with a sterile cork borer (10 mm diameter). The plates were incubated at 37°C for 24 h after adding the test component. Aseptically, the experiment was done five times. The organism's sensitivity was assessed by the zone of inhibition diameter. Repeating each experiment five times yielded the mean value. The control study utilised streptomycin and chloramphenicol (Shobana *et al*., 2009).

Antioxidant Effect:

Aqueous, ethanolic, and hexane extracts of *Asparagus racemosus* were tested for antioxidant activity using ferric thiocynate (Mistuda *et al*., 1996). Different concentrations (100, 200, 300,

400, and 500 µg/ml) were obtained by diluting each extract in 99.5 per cent ethanol. A screw cap vial with a 2 ml sample in 99.5 per cent ethanol, 2.052 ml of 2.51 per cent linoleic acid in ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0), and 1.948 ml of water combination was baked at 60 °C in a dark oven. 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocynate were added to this sample solution. After adding 0.1 ml of 2 x 10^{-2} M ferrous chloride in 3.5 per cent hydrochloric acid, the red color's absorbance was measured at 500 nm in 3 min (Mokbel and Hashinaga, 2005). The control received solvent, whereas the sample received the same amount of α-tocopherol (reference component) as the standard (Yildirim *et al*., 2001). The % inhibition of lipid peroxidation was calculated by using the equation:

$$\% \text{ Inhibition} = 1 - (A1/A2) \times 100$$

Where, A1 - test sample's absorbance; A2- reaction of absorbance control.

Results and Discussion

Phytochemical Analysis:

Phytochemical analysis is extremely useful for identifying therapeutic plants' active biological components. Wet and dry samples were assessed quantitatively and qualitatively. *Asparagus racemosus* contains alkaloids, flavonoids, saponins, and tannins (Table 1). This proves its nutritional and therapeutic benefits (Oloyed, 2005). Some of the vegetable species' components, such tannins, are hazardous to humans and farm animals (Odebiyi and Sofowora, 1979). Because they reduce feed palatability and digestibility, some of these active chemicals have antinutritional effects (Odebiyi and Sofowora, 1979).

Table 2 shows phytochemical values (bioactive compounds). The dried sample contained more bioactive compounds than the wet sample. Non-volatile bioactive chemicals have large dry weights. Table 2 shows that the vegetable treats cardiovascular disease and oxidative stress due to its high flavonoid concentrations (56.26 2.18 and 47.43 2.29) as natural antioxidants. Antioxidants protect cells from singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrile. Oxidative stress, caused by a lack of antioxidants, damages cells (Burlon and Ingold, 1984). Oxidative stress causes cancer, ageing, atherosclerosis, inflammation, ischemia injury, and neurological diseases including Parkinson's and Alzheimer's (Palozza, 1998). Flavonoids boost the body's antioxidant defences when combined with antioxidant vitamins and enzymes. Epidemiological studies show that flavonoids and carotenoids reduce coronary heart disease and heart attack risk (Donald and Cristobal, 2006).

Antibacterial Activity:

As indicated in Tables 3, 4, and 5, the aqueous, ethanolic, and hexane extracts of *Asparagus racemosus* displayed growth inhibitory effectiveness only at higher dosages ranging from 400 µg to 500 µg. *E. aerogens and P. mirabilis* were more sensitive to aqueous extracts of *Asparagus racemosus* than ethanolic and hexane extracts. *S. typi* showed modest resistance to all extracts when compared to others (Tables 3, 4, 5). Bacterial strains were found to be less vulnerable to *Asparagus racemosus* ethanolic and hexane extracts, even at higher concentrations than aqueous extract.

Antioxidant Activity:

Table 6 shows ferric thiocynate antioxidant activity of *Asparagus racemosus* aqueous, ethanolic, and hexane extracts (FTC). The FTC method calculated peroxide production, which interacts with ferrous chloride ($FeCl_2$) to generate reddish ferric chloride ($FeCl_3$). As extract antioxidant activity improves, peroxide concentration decreases. Aqueous, ethanolic, and hexane extracts (100, 200, 300, 400, and 500 µg/ml) showed concentration-dependent antioxidant activity. Ethanol extract demonstrated 41.55 per cent antioxidant activity and α-tocopherol 48.16 per cent at 500 µg/ml.

Table 1: Qualitative analysis of different extracts of *Asparagus racemosus* for phytochemicals

S. No.	Name of the phytochemical	Different extracts of *Asparagus racemosus*		
		Aqueous extract	Ethanolic extract	Hexane extract
1.	Alkaloids	+ve	+ve	+ve
2.	Flavonoids	+ve	+ve	+ve
3.	Saponins	+ve	+ve	+ve
4.	Tannins	+ve	+ve	+ve
5.	Glycosides	-ve	-ve	-ve

+ve – present; -ve – absent

Table 2: Quantitative analysis of *Asparagus racemosus* for Phytochemicals

S. No.	Name of the phytochemical	*Asparagus racemosus*	
		Dry sample	Wet sample
1.	Alkaloids (mg/100g)	31.11 ± 2.32	26.18 ± 1.19
2.	Flavonoids (mg/100g)	56.26 ± 2.18	47.43 ± 2.29
3.	Saponins (mg/100g)	03.11 ± 0.14	01.37 ± 0.11
4.	Tannins (mg/100g)	01.91 ± 0.29	01.39 ± 0.28

Table 3: Antibacterial activity of aqueous extract of *Asparagus racemosus*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Asparagus racemosus	100	NI	NI	NI	NI
	200	NI	NI	NI	NI
	300	08.2	12.6	NI	09.1
	400	11.4	16.1	10.6	12.3
	500	16.5	19.3	14.2	16.6
Streptomycin	100	21.2	18.2	18.4	19.2
Chloramphenicol	100	20.8	18.6	19.1	18.6

NI – No inhibition

Table 4: Antibacterial activity of ethanolic extract of *Asparagus racemosus*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Asparagus racemosus	100	NI	NI	NI	NI
	200	NI	NI	NI	NI
	300	NI	08.2	10.1	10.1
	400	11.6	10.7	10.2	12.4
	500	13.3	14.2	14.3	16.3
Streptomycin	100	19.8	17.1	18.2	19.1
Chloramphenicol	100	20.1	19.6	19.8	20.7

NI – No inhibition

Table 5: Antibacterial activity of hexane extract of *Asparagus racemosus*

Aqueous extract	Concentration in µg	Mean diameter of the zone of inhibition (mm)			
		E. coli	***P. mirabilis***	***S. typhi***	***E. aerogenes***
Asparagus racemosus	100	NI	NI	NI	NI
	200		NI	NI	NI
	300	11.5	10.4	10.8	10.3
	400	13.1	12.8	12.3	11.9
	500	16.2	14.7	15.7	14.2
Streptomycin	100	19.9	20.1	18 .9	21.4
Chloramphenicol	100	19.1	19.9	20 .1	20.1

NI – No inhibition

Table 6: Antioxidant activity of aqueous, ethanolic and hexane extracts of *Asparagus racemosus*

S. No.	Extract	% of inhibition of lipid peroxidation				
		100 µg/ml	**200 µg/ml**	**300 µg/ml**	**400 µg/ml**	**500 µg/ml**
1.	Water	09.11	14.22	19.41	25.21	29.95
2.	Ethanol	14.37	19.93	26.21	32.17	41.55
3.	Hexane	10.11	18.12	23.13	29.34	36.55
4.	α-Tocopherol	19.13	26.41	31.15	37.11	48.16

Asparagus racemosus extracts inhibited lipid peroxidation. Increased extract concentration showed minimal antioxidant action.

Conclusion

Certain bacterial strains have been shown to be extremely resistant to *Asparagus racemosus* ethanolic and hexane extracts, even at concentrations higher than the aqueous extract. Extracts of *Asparagus racemosus* in both water and hexane have been shown to exhibit anti-lipid peroxidation properties. The extract's antioxidant activity was found to increase with increasing concentration.

References

Burlon GW and Ingold KU. (1984) B-Carotene, an unusual type of lipid antioxidant. J Sci. 224: 573.

Chung KT, Thomasson WR and Wu-Yuan CD. (1990) Growth inhibition of selected food-borne bacteria, particularly *Listeria monocytogenes*, by plant extracts. J Appl Bacteriol. 69: 498-503.

Donald RB and Cristobal M. (2006) Antioxidant activities of flavonoids, J Agric. 52: 125-757.

Gurudeva MR and Yoganarasimhan SN. (2009) Bibliography of medicinal plants of India (Pharmacognosy and Pharmacology), Divyachandra Prakashana, Bangalore, pp. 1024.

Harborne JB. (1973) Textbook of phytochemical methods, 1st Ed., Champraan and Hall Ltd. London. pp. 110-113.

Harborne JB. (1976) Phytochemical methods, 2nd Ed., Chapman and Hall Ltd, London, pp: 52-55.

Hoffman BR, Delas Atlas H, Blanco K, Wiederhold N, Lewis RE and Williams L. (2004) Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. J Pharm Biol. 1(42): 13-17.

Mistuda H, Yuasumoto K and Iwami K. (1996). Antioxidation action of Indole compounds during the autoxidation of linoliec acid. Nihon Eiyo Shokuryo GakkaiShi 19: 210-214.

Mokbel ML and Hashinaga F. (2005) Antibacterial and antioxidant activities of Banana (*Musa* AAA *cv. Cavendish*) fruits peel. Am J Biochem Biotechnol. 1(3): 126-132.

Odebiyi OO and Sofowora EA. (1979) Phytochemical screening of Nigerian Medicinal plants 2nd OAU/STRC Inter-African Symposium on traditional Phamacopoeia and African Medicinal, pp: 216-220.

Oloyed OI. (2005) Chemical profile of unripe pulp of *Carica pagaya.* Pak J Nutr. 4: 379-381.

Onwuka, GI. (2005) Food analysis and instrumentation theory and paractice, 1st Ed., Naphthali Prints, Lagos, pp. 114-169.

Palozza P. (1998) Pro-oxidant actions of carotenoids in biologic systems. Nutr Revolution 56: 257-256.

Service RF. (1995) Antibiotics that resist resistance. Science 270: 724-727.

Shobana S, Vidhya VG and Ramya M. (2009) Antibacterial activity of garlic varieties (*Ophioscordon* and *Sativum*) on enteric pathogens. Curr Res J Biol Sci. 1(3): 123-126.

Sofowora EA. (1983) Proceedings of the 5th International Symposium on medicinal plants. University of Ile-Ife, Nigeria, pp. 7.

Tanaka JCA, da Silva CC, de Oliveira AJB, Nakamura C and Dias Filho BP. (2006) Antibacterial activity of iodole alkaloids from *Aspidosperma ramifloram*. Br J Med Biol Res. 39(3): 387-391.

Thakur RS, Puri HS and Husain A. (1989) Major Medicinal Plants of India. Central Institute of Medicinal and Aromatic Plants, Lucknow.

Trease GE and Evans WC. (1989) Pharmacognosy. 2nd Ed., Braille Tiridel and Macmillan Publishers.

Yildirim A, Oktay M and Bilaloglu V. (2001) The antioxidant activity of the leaves of *Cydonia vulganis*. Tr J Med Sci. 31: 23-27.