IJBPAS, November, 2019, 8(11): 2163-2170

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF *NANNORRHOPS RITCHIANA* AGAINST RESISTANT DENTAL BACTERIAL STRAINS

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Received 8th March 2019; Revised 11th April 2019; Accepted 10th May 2019; Available online 1st Nov. 2019

https://doi.org/10.31032/IJBPAS/2019/8.11.4862

ABSTRACT

Different properties like anti-hypertensive, anti-microbial, anti-sickling, and anti-schistosomal (molluscicidal) have been found in medical plants. Medical plants have bioactive compounds which treat various ailments caused by microorganisms. Culture media was prepared in a conical flask, where 0.84 g of Muller Hinton Broth and 0.4 g of agar technical was added and the volume was made up to 1000 ml. Two bacterial strains of *Escherichia coli* and *Pseudomonas aeruginosa* were used. Disc diffusion method was designed to check over the activeness of *Nannorrhops ritchiana*. The minimum inhibitory concentration of the ethanolic extract of *Nannorrhops ritchiana* was found to be 100 mg, 200 mg, 300 mg, 400mg, 600 mg against *Escherichia coli* and 600 mg against *Pseudomonas aeruginosa at* different concentrations of leaf extracts ranging from 100 to 600 mg, *Escherichia coli* showed no Zone of inhibition except at 500 mg. On the other hand, *Pseudomonas aeruginosa* showed Zone of inhibition in concentrations ranging from 100 to 500 mg except at 600 mg no Zone of inhibition was seen. In conclusion, the plant showed minor activity against *Escherichia coli* but showed potent activity

against *Pseudomonas aeruginosa*; and this study concluded that *Nannorrhops ritchiana* can be used as an antibacterial agent.

Keywords: *Escherichia coli*, Pseudomonas aeruginosa, antibacterial activity, *Nannorrhops ritchiana*, Muller Hinton Broth

INTRODUCTION

Different properties like anti-hypertensive, anti-microbial, anti-sickling, and anti-schistosomal (molluscicidal) have been found in medical plants [1]. Different ways have been used which contain resistance of antibiotic and administration of disease evaluated from higher plants. In comparison to expensive artificially made drugs which have contrary effects, it is believed and scientifically proved that traditional medicines are found to be safer [2]. The traditional healing systems all around the world uses plant based antimicrobial that shows a greater untrapped and further research of plant microbials is needed. According to World Health Organization (2001), 80% of African and Asian populations depend on traditional medicine basically plant biodiversity for primary health care because plant derived medicines are relatively cheaper and safer in comparison to the synthetic alternatives. Medical plants have bioactive compounds which treat various ailments caused by microorganisms. These compounds may have been evolved in plants as self-defense against pests and pathogens that help plants to establish them in their environment [3]. *Nannorrhops ritchiana* belonging to the family Arecaceae. It is an exclusive species of genus Nannorrhops. The local name of *Nannorrhops ritchiana* is Mazari palm. In different parts of the world such as Afghanistan, Iran and Pakistan this plant is highly observed. In different locations of Balochistan, this plant is extensively dispersed especially in Khuzdar and Harnai at an altitude about 16000 meters. It is an adaptable palm that has the tendency to survive in harsh weather positions [4]. It is an affable, feathered and shrubby palm. The leaves of Mazari palm are blue-grey costapalamate in shape. The young leaves of Mazari palm have an astringent taste and are widely used as a medication for treatment against diarrhea and dysentery. They are also used as a laxative in veterinary practice. The root extract of *Nannorrhops ritchiana*, Arecaceae contains alkaloids, phenols, polyphenol saponins, tannins, antraquinones and sterols [5].

MATERIALS AND METHODS

The plant sample (Leaves) of *Nannorrhops ritchiana* was collected from the local market of Lahore. The plant was identified by Prof. Dr Ijaz Rasood of Department of Botany from Agriculture University of Faisalabad. Extraction procedure was used. 200 g of the plant in powder form was dissolved in 600 ml ethanol and was left for 24 hours. It was then filtered; where the filtrate was collected and then was kept in an electric water bath at temperature between 50-60^{o}C and it took 3 to 4 days. The plant extract was then kept in a lyophilizer at -50^{o}C temperature and reduced pressure, where the dried form of plant extract was obtained and then were tested against the two bacterial strains used.The standard concentrations of 100mg, 200mg, 300mg, 400mg, 500mg, and 600mg were prepared and then dissolved in 1 ml DMSO for 1 day.

The bacterial species employed were *Escherichia coli*and *Pseudomonas aeruginosa*, and Amikacin (30µg) was an antibiotic that was contrary used as a positive control. Culture media was prepared in a conical flask, where 0.84 g of Muller Hinton Broth and 0.4 g of agar technical was added and then dissolved in 1000 ml distilled water. Disc diffusion method was used.The bacterial cultures of *Escherichia coli* and *Pseudomonas aeruginosa* (50µl) were adjusted to 0.5 McFarland standard solution, and that was then used to evenly spread the petri plates that contained Muller Hinton and that was done evenly by the use of a sterile cotton swap. The plates were then dried for 15 minutes. After solidification of the petri plates, the filter paper discs were placed on media then plant extract (5µl) on each disc of all six concentrations were placed on discs with the help of micro pipette. Then the plates were left for 24 hours to observe the plants antibacterial activity against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

RESULTS

The minimum inhibitory concentration of the ethanolic extract of Nannorrhops ritchiana was found to be 100 mg, 200 mg, 300 mg, 400mg, 600 mg against *Escherichia coli* and 600 mg against *Pseudomonas aeruginosa*. At different concentrations of leaf extracts, varying from 100-600 mg; *Escherichia coli* showed no Zone of inhibition except 500 mg. On the other hand, *Pseudomonas aeruginosa* showed Zone of inhibition in concentrations ranging from 100 to 500 mg except at 600 mg where no zone of inhibition was observed. Plant showed minor activity against *Escherichia coli*, but showed potent activity against *Pseudomonas aeruginosa*.

Table 1: Leaf Extracts of *Nannorrhops ritchiana* Showing Antibacterial Activity

Sr. No	Name of bacteria	Concentration of leaf extract (mg)	Zone of inhibition (mm)
1.	*Escherichia coli*	100	0
		200	0
		300	0
		400	0
		500	8
		600	0
2.	*Pseudomonas aeruginosa*	100	5
		200	6
		300	7
		400	8
		500	7
		600	0

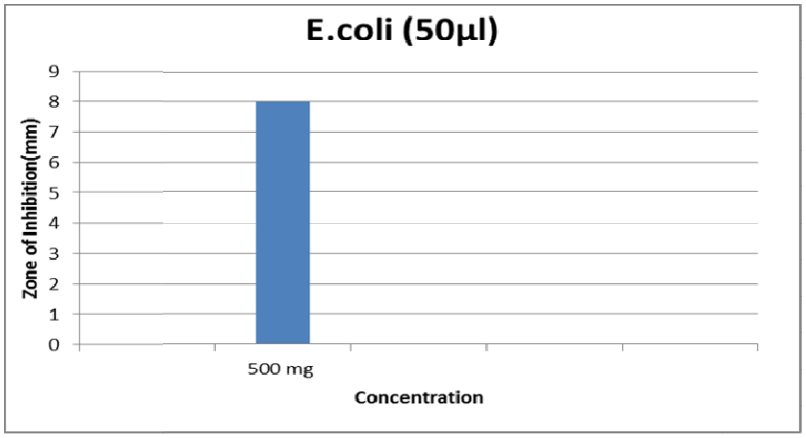

Figure 1.1: *Escherichia coli*

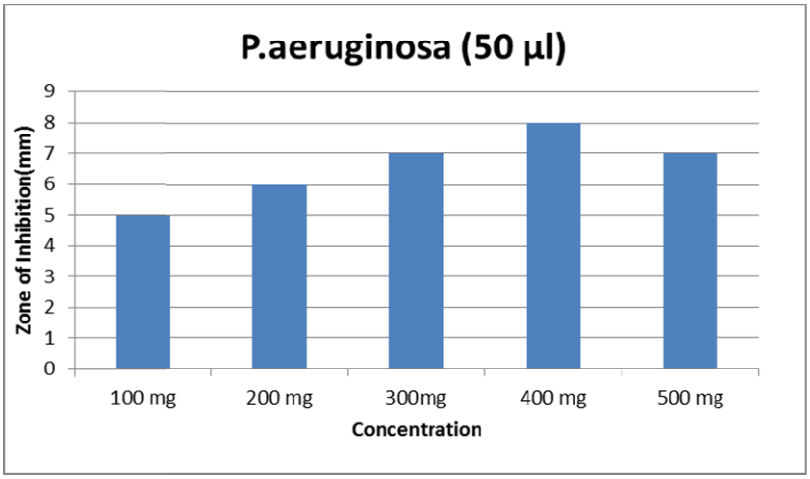

Figure 1.2: *Pseudomonas aeruginosa*

DISCUSSION

The traditional healing systems all around the world uses plant based antimicrobial that shows a greater untrapped and further research of plant microbials is needed. Many commercial drugs that are used in traditional modern medicine are derived from plants that are following ethno-botanical and ethno-medical knowledge [6]. Medical plants have bioactive compounds which treat various ailments caused by microorganisms. These compounds may have been evolved in plants as self-defense against pests and pathogens that help plants to establish them in their environment [7]. Many reports have shown the effectiveness of traditional herbs against microorganisms, and therefore plants are one of the bedrocks for modern medicine to attain new principles. To determine invulnerable, latest and valuable agents that have the tendency to fight against pathogenic bacteria higher plants are being used [8]. Biologically active compounds that are present in the medical plants have always been of a great interest to the scientists that are working in this field. In recent years, this interest to evaluate the plants possessing antibacterial activity for various diseases is growing [9]. Traditional herbs have been tested from extracts that differ from each other. Medicinal plants are very important in sheltering the fundamental health in growing countries and against microorganisms that cause disease and they exhibit new antibacterial, antiviral and antifungal agents. The compounds responsible for the antibacterial activity of this extract (*Nannorrhops ritchiana*) are not revealed. However, fundamental phytochemical tests revealed that the root extracts of Nannorrhops ritchiana contained alkaloids, phenols, polyphenol saponins, tannins, antraquinones and sterols [10].

According to a biological point of view, bacteria has shown to exhibit four superior mechanisms and these are: (1) Change in the target, that causes lessening in the property of the drug (2) formation of an enzyme that alter the prescription (3) by the damage of porin some material cannot be passed out from it (4) discharge of medicines that require energy [11]. Antibiotic resistance is brought about by an alternation in the gene that could be caused by the absence of selective pressure, usually in the company of antibiotics which are not as ordinary as the acquired one. Bacterial resistance brought out by the presence of microbial-ecological pressure, which is expended by the habitation of an antibiotic [12]. Chromosomal alternation is known to be a major cause of bacterium resistance. The relocation of

resistant determinants is travelled by plasmids and transposons and at a faster rate they can deliver wide variety of microbial organisms in comparison to recent agents that can be exhibited to fight against them [13]. Nearly, the genetic transfer of bacteria has the capability to transfer and attain drug resistance thatis used by therapeutic agents. On the basis of the results obtained during the present study, it was observed that out of *Escherichia coli* and *Pseudomonas aeruginosa*; *Pseudomonas aeruginosa* (50µl) was shown to be more susceptible to the plant extracts ranging from 100 to 500 mg; where no zone of inhibition was observed at 600 mg concentration of the plant extract used [14]. Moreover, the maximum Zone of inhibition (8mm) was achieved at 400 mg of the plant extract used. It exhibited maximum antibacterial activity at 400mg of the plant extract used. In contrast, to *E.coli* that showed the presence of antibacterial activity at 50 µl showed maximum Zone of inhibition (8mm) only at 500 mg concentration of the plant extract, where at other concentration of the plant extracts used; no Zone of inhibition was observed [15]. The reason why *E.coli* did not show any Zone of inhibition at any other concentrations of the plant extracts except 500 mg could be due to several reasons that could include the alternation in the method of extraction of medicinal plants used, culture media, temperature, pH, age of the plant or environmental factors, the method of antibacterial study, and lastly the genetic variation of plant.It could also be due to experimental error while performing in the laboratory and also the concentration of the bacteria used was not enough to show a potent antibacterial activity against the plant extracts of varying concentrations [16].

CONCLUSION

This study has shown antibacterial activity of *Nannorrhops ritchiana* against two gram-negative bacterial strains of *Escherichia coli* and *Pseudomonas aeruginosa* that cause diarrhea and urinary tract infections respectively. The plant showed potent antibacterial activity against *Pseudomonas aeruginosa*, but minor activity against *Escherichia coli*. *Nannorrhops ritchiana* is an effective medical plant used to treat diarrhea and dysentery. This study has concluded that *Nannorrhops ritchiana* can be considered and therefore can be used as an antibacterial drug to treat various alignments caused by microorganisms.

ACKNOWLEDGEMENTS

The authors are greatly thankful to the Director IMBB, and the students of LAB-313 for their consistent support and hard

work without whom the current project was not possible.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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