A DISSERTATION ON

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *BERBERIS ARISTATA* AGAINST PATHOGENIC BACTERIA

SUBMITTED TO THE DEPARTMENT OF BIOSCIECES,

INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILLMENT

FOR THE

DEGREE OF MASTER OF SCIENCE

IN MICROBIOLOGY

By

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UNDER THE SUPERVISION OF

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DECLARATION

I hereby declare that the present work on **Antioxidant and antimicrobial activity of *Berberis aristata* against pathogenic bacteria** is a record of original work done by me under guidance of Dr. Mohammad Hayatul Islam, Assistant Professor, Department of Biosciences, Integral University, Lucknow during Feb, 2022 to June, 2022. I also declare not part of ther thesis has previously been submitted to my Institution or any examining body for acquiring any diploma or degree.

Place: **Shabeena Khatoon**

Date:

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TO WHOM IT MAY CONCERN

Ther is to certify that Ms. Shabeena khatoon , a student of M.Sc. Microbiology (II Year, IV semester), Integral University has completed her Four months dissertation work entitled **"Antioxidant and antimicrobial activity of *Berberis aristata* against pathogenic bacteria"** successfully. she has completed ther work from Department of Biosciences, Integral University, under the guidance of **Dr. Mohammad Hayatul Islam**

The dissertation was a compulsory part of her M.sc degree. I wish her good luck and bright future.

(Dr. Mir Snober Shabnam)

Head,

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CERTIFICATE OF ORIGINAL WORK

Ther is to certify that the study conducted by Ms. Shabeena Khatoon during the Four months training from February –June 2022 reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and script of the thesis has been written by candidate herself. The thesis entitled is **"Antioxidant and antimicrobial activity of *Berberis aristata* against pathogenic bacteria"** Therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology , Department of Biosciences, Integral University, Lucknow (U.P.).

(Supervisor)

Dr. Mohammad Hayatul Islam

Assistant Professor

Department of Biosciences

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ACKNOWLEDGEMENT

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I would like to express my special thanks to **Dr. Snober S. Mir (Head, Department of Biosciences)** for given me an opportunity to join the department laboratory and providing all the necessary facilities ever since I started my work.

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My thanks also go to all of my friends who were instrumental in my academic success.

My appreciation would be inadequate if I did not include my parents, with whose blessing I was able to successfully finish my aim. There are no words to convey how I feel about them. I discreetly express my gratitude to them.

Shabeena Khatoon

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1. INTRODUCTION

Berberis aristata commonly known as “Daruhaldhi and chitra” is spinous shrub native to northern Himalaya region. The plant is widely distributed from Himalayas to Srilanka, Bhutan, and hilly areas of Nepal in Himalaya region. It is found in Himachal Pradesh. It grows at the height of 2000-3000m especially in Kumaon and Chammba region of Himachal Pradesh. It is also found in Nilgiris hills in South India.The shrub *B. aristata* belongs to Berberidaceae family and commonly known as tree turmeric, Daruhaldi (in Hindi), Daruharidra (in Sanskrit), Indian barberry (in English) and Zarishk (in Unani). The genus *Berberis* comprises approximately 450-500 species of deciduous evergreen shrubs and is found in the temperate and sub-tropical regions of Asia, Europe, and America [1]. *B. aristata* is used in ayurvedic medicines from very long time. The plant is used traditionally in inflammation, wound healing, skin disease, menohrrhagia, diarrhea, jaundice and infection of eyes. A very valuable ayurvedic preparation ‘Rashut’ is prepared by this plant. *B.aristata* is a spinous shrub with height ranging from 2 to 3 metres. Wood of the plant is hard and bark is yellow to brown in colour in outer appearance whereas from inside it is of deep yellow colour. Roots of berberis aristate are also yellow in colour [2].

Classification:

Class -Magnoliopsida

Subclass-Magnoliidae

Order- Ranunculales

Family- Berberidaceae

Genus- *Berberis*

Species-*aristata*

Leaves

The leaves are arranged in tufts from five to eight and are about 4.9 cm long and 1.8 cm wide. Leaves are simple, spiny, lanceolate, toothed, leathery in texture, sessile, acuminate, verticillate, intense green on the dorsal surface and light green on the ventral surface and show reticulate pinnate venation.

Flowers

The flowers are yellow in colour, hermaphrodite, actinomorphic and form a racemose inflorescence, with 11-16 flowers per cluster, arranged along a central stem. The calyx whorl of the flower is yellow in colour, polysepalous in condition, 6 in number out of which 3 sepals are large and 3 sepals are small, 4-5mm long caducous calyx Corolla has six petals in total which are yellow in colour. The petals are free (polypetalous), imbricate with 2-3 bracts. The male reproductive structure, androecium, is polyandrous and contains six stamens, 5–6 mm long. The female reproductive structure, the gynoesium, is 4-5 mm long and is composed of a short style and a broad stigma. Ovary is simple, club shaped with 1-12 ovules that are erect and in sub basal arrangement.

A **B** **C**

Figure 1. A) Plant B) Leaves and flowers, C) Fruits of *B. aristata*

Fruits

Fruits are globose to ovoid, usually covered with bloom as in plums. Fruits are 7 mm long, 4 mm in diameter, weighing 227 mg, 237 microlitres in volume. Fruit colour is aconite violet. Seeds are 2 to 5 in number, varying in colour from yellow to pink, each weighing 25 mg each.

Stem

Stem has rhytidome with cork consisting of 3 to 45 rectangular and square, yellow coloured, thin-walled cells that are arranged radially. Phloem fibers are arranged tangentially into rows having 1 to 4 rows of cells, the fibers are short, spindle-shaped, thick walled with wide lumen these cells contain the yellowish-brown content. The walls of phloem fibers are lignified. The secondary phloem rays are obliquely arranged having parenchyma cells that are elongated. These rays for the half portion rhytidoma. Secondary xylem consists of xylem vessels, tracheids, xylem fibers and transverse by multi seriate xylem rays. Xylem vessels are numerous ranging from small to medium in size, distributed either in groups or individually throughout the xylem region. They are usually arranged radially, walls are thick and filled with lignin. Xylem rays are straight and the cells are rectangular, some of the cells have brown content [3, 4].

B. aristata, The berberine alkaloid can be found in the roots, rhizomes, and stem bark of the plants. Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and chlamydia. In China, berberine is an overthe-counter drug for the treatment of bacterial diarrhea. In 1988, the hypoglycemic effect of berberine was firstly reported when berberine was prescribed to treat diarrhea in diabetic patients. Moreover, several clinical and preclinical studies demonstrate ameliorative effect of berberine against several disorders including metabolic, neurological and cardiological problems. This review provides a summary regarding the pharmacokinetic and pharmacodynamic features of berberine, with a focus on the different mechanisms underlying its multispectrum activity.However, numerous literatures had been published by various authors exploring the phytochemical and pharmaceutical aspects along with traditional uses yet there is no much more literature concerning so far the importance of Berberine, which is important constituent of this species

Ayurveda is a traditional system of medicine using a wide range of modalities to create health and well-being. The primary aim of Ayurveda health care is to restore the physical, mental and emotional balance in patients, thereby improving health, preventing disease and treating any current illness. The number of patients seeking

alternate and herbal therapy is growing exponentially. Herbal medicines are now in great demand in the developing world for primary healthcare not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries for primary healthcare2. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phyto-chemically. Therefore, it seems necessary to evaluate the herbs properly. Berberis aristata DC.

(Berberidaceae) is one of the herbs mentioned in all ancient scriptures of Ayurveda, Charaka andSusruta have mentioned it's different properties along with various used for the treatment of numerous illnesses3. The genus Berberisrepresents the around 12 genera and 600 species worldwide and about 77 species have been reported fromIndia4. In Indian Himalayan ecosystem most of the species have reported from Nilgiri hills at an altitude of 1,000–3,000 mASL5. Among the various species of Berberis genus Berberi aristata DC is one of the most important species due to its wide medicinal properties and its occurrence has reported from sub-tropical areas (1800-3000 m ASL) of the mountain state of Uttarakhand and Himachal Pradesh6. It is used invarious crude drug formulations and in different ayurvedic and homeopathic medicines since ancient times.

Potential secondary metabolites/bioactive compounds of *B. aristata*

Different plant extracts, essentials oils and their bioactive secondary metabolites have been extensively used for the treatment and management of distinct metabolic diseases due to their safety and less or no toxicity [5–7]. A recent study demonstrated that *B.aristata* constitutes phytochemicals of different classes such as alkaloids, flavonoids, saponins, steroids, coumarins, glycosides, tri-terpenoids, polyphenols, tannins, reducing sugars, metal ions and, among these, the alkaloids are the most abundant phytoconstituents from *B. aristata* stem [1,8].

OBJECTIVES

I. **Solvent based extraction and phytochemical screening of *B. aristata stem*.**

II. **Evaluation of antioxidant potential of *B. aristata* stem extracts.**

III. **Antibacterial activity of *B. aristata* extracts against pathogenic bacteria.**

2. REVIEW AND LITERATURE

2.1. Pharmacological effects of *B. aristata* in targeting various diseases

B. aristata is well known for their pharmacological activities such as; anticancer, hepatoprotective, anti-diabetic, anti-inflammatory and neuroprotective activity. various *in vitro* and *in vivo* studies are the agreement of its medicinal propreties.

2.1.1 Antioxidant effects

Till date, various *in-vitro* methods have been used for the determination of antioxidant properties of different medicinal plants and their secondary metabolites *i.e*., DPPH, FRAP, OH radical and ABTS assay along with other *in-vivo* biomarkers of oxidative homeostasis *i.e*., CAT, SOD, GST, GPx, and PON-1 [9-14].Gaccheand Dhole assessed the antioxidant potential of ethanolic extract of *B. aristata* and reported that it exerts potent *in-vitro* DPPH free radical scavenging activity with an IC_{50} value of 150 µg/ml [15]. Further, they also analyzed the hydroxyl radical scavenging activity of *B. aristata* ethanolic extract and demonstrated that it does not show hydroxyl radical quenching activity [15].

Recently, berberine has been shown to exhibit potent antioxidant activities and suppressed the mitochondrial ROS generation in spiral ganglion cells via targeting sNMDAR1/Nox3 pathway [16]. .In addition, berberine significantly alleviated the oxidative stress in the lenses of diabetic rats via modulation of SOD, CAT, and GPx activities and the level of thiobarbituric acid reactive substances (TBARS) [17]. Berberine also protected against lead (Pb)-mediated oxidative imbalance and hepatotoxicity in rats by increasing SOD, CAT, and GPx activities along with GSH content, a non-enzymatic antioxidant. These beneficial effects of berberine against Pb-induced oxidative stress, hepatic necrosis and inflammatory response were comparable to the effects of reference standard silymarin [18].

2.1.2. Anti-inflammatory effects

Administration of *B. aristata* extract markedly suppressed the protein expression of inflammatory markers i.e., interleukin-1β (IL-1 β), IL-6, TNF-R1 and cyclooxygenase-1 (COX-1) that are reckoned to trigger the inflammatory cascades, whereas, the expression of anti-inflammatory IL-10 was stimulated in peritoneal macrophages [19].

Further, *B. aristata* extract supplementation resulted in down-regulation of IL-1 β), IL-6, TNF-R1, NF-κB and vascular endothelial growth factor (VEGF) expression in an adjuvant-induced arthritis (AIA) model. In addition, the HO-1/Nrf-2 ratio was also improved and the protein level of matrix metalloproteinases (MMP)-3 and 9, known to degrade the extra cellular matrix, was also suppressed after *B. aristata* extract treatment [19].

Similarly, the treatment with berbamine also regulated the MAPK (JNK and ERK1/2) pathways and reduced the activation of NF-κB in LPS-stimulated macrophages [20]. Moreover, incubation with berbamine evidently combated the LPS or CH_3COOH-induced IL-1β and IL-6 mRNA expression and p65 and STAT3 phosphorylation in RAW264.7macrophage cells and protected C57BL/6J mice against alcoholic liver disease [21]. Two sitosterol's namely β-sitosteryl-3β-glucopyranoside-6′-O-palmitate and 3-O-(6′-O-linoleoyl-β-D-glucosyl)-β-sitosterol isolated from *Berberis spp*. exhibited strong anti-inflammatory effects via inhibiting *in-vitro* activity of COX-1 and COX-2. In conclusion, *B. aristata* and its secondary metabolitesexhibit promising anti-inflammatory effects and may be further evaluated for their therapeutic potential against various inflammatory diseases including atherosclerosis, diabetes and cancer.

2.1.3. Antidiabetic effects

In this regard, *B. aristata* has been reported to positively affect glycemic control, significantly decreasing HbA1c, basal insulin, homeostatic model assessment of insulin resistance[22].A randomized and placebo control clinical trial on 102 patients observed that treatment with *B. aristata* extract in combination with *Silybum marianum*improves the glycemic control in diabetic dyslipidemic subjects via increasing the insulin C-peptide [23].Another clinical trial with same combination showed the protective effects on T1DM as well as reduced the insulin doses required before meals [24].The level of fasting blood glucose (FBG), PPG, HbA1c, TC, TGRLs and LDL-C were also reduced after the administration of *B. aristata/S. marianum* extracts [24]. Another interventional study concluded that *B. aristata* increases the level of insulin through its antioxidant mechanisms and ability to repair damaged pancreatic β-cells in T1DM and T2DM cases [25].

2.1.4. Anticancer effects

A recent study showed that the treatment with methanolic extract of berberis exerted significant anticancer effects in human osteosarcoma cells (HOS) via induction of ROS generation, enhanced apoptosis, nuclear fragmentation, autophagy, and caspase-3 activity, as well as diminished cell viability (either alone or in combination) [26].

Another study with stem plus bark extract of *B. aristata* evident its anti-tumorigenesis potential in Ehrlich ascites carcinoma (EAC)-bearing mice and it also increased the survival rate and declined the body weight due to the decreased tumor cell proliferation after receiving intraperitoneal injection of 100 mg/Kg B.W. and 6.5 mg/Kg B.W. of aqueous and ethanolic extracts, respectively [27]. Apart from the anticancer effects of *B. aristata* extracts, its bioactive ingredients like berberine, oxyberberine, berbamine and palmatine also showed remarkable antitumorigenic and antiproliferative efficacy in different *in-vitro* and animal model studies. In this context, berberine has been extensively investigated for its beneficial effects against the management of different cancers [28-30].

2.1.5. Antimicrobial effects

Extract of *B. aristata* showed potent bactericidal effects against a serious of microbes with maximum antibacterial efficacy against *Klebsiella pneumoniae* having a zone of inhibition (ZOI) of 25 mm, however, *K. pneumoniae* and some other bacteria showed a marked resistance against *B. aristata* extract. The same extract exerted strong antimicrobial effects against *Candida albicans* (ZOI: around 23 mm) [31]. Berberine, a major constituent of *B.aristata*, showed potent inhibitory effects against various methicillin-resistant *Staphylococcus aureus* (MRSA)strains with minimum inhibitory concentrations (MICs) between 32 and 128 µg/mL and extended the antibacterial efficacy of commercially available beta-lactam antibiotics*i.e.*, ampicillin and oxacillin [32,33].

3. MATERIALS AND METHODS

3.1 Chemicals

Chemicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), ascorbic acid, and dimethyl sulfoxide (DMSO), were purchased from Himedia Laboratories, Mumbai, India. Methanol (MeOH) was obtained from Merck, India. All chemicals were of analytical grade.

3.2 Plant material and its extracts preparation

Berberis aristata stem was obtained locally from Botanical garden, NBRI, Lucknow. The formal identifcation of the plant material was carried out in NBRI, Lucknow. Plant stem was shed dried and made in coarse powder, avoiding sun dried due to the signature modification of the biochemicals. The dried powder (25 g) of the plants was extracted using nonpolar, partially polar, and polar solvents successively with the required amount of each solvent (n-hexane, ethyl acetate, dicholoromethane, methanol and aqueous) and water solvents in Soxhlet apparatus until it turned colour less. The solvent was removed, filtered, and dried at room temperature, and residues were scratched out and stored at −20◦C for future use [34].

3.3 Phytochemical Screening

Qualitative chemical tests were carried out to identify the phytochemicals present in various extracts of *B. aristata* using standard procedure [34].

3.4 DPPH Radical Scavenging activity

The DPPH radical scavenging capacity of the plant was determined by the method of Brand-Williams *et al. (*1995). Briefly the free radical scavenging activity based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. DPPH molecule determines with the occurrence of a purple colour. DPPH solution (132mM) was prepared in methanol in a dark reagent bottle. 100µl of the Iridin (dissolved in 5% CH_3OH) and reference standard drug, ascorbic acid (dissolved in water) (Concentration ranging from 0 to 1000µg/ml) was added to 2ml of DPPH solution and the reaction mixture was incubated for 15 minutes at 27ºC in a water bath and absorbance was measured at 517 nm. The reduced form of DPPH was generated, accompanied by the disappearance of the violet colour. Ascorbic acid

was used as a reference standard. Percent (%) scavenging of DPPH free radical was measured using the following equation [9].

% DPPH scavenging= (Abs. of Control-Abs. of sample)/Abs. of control x100

Further, IC50 [9].

3.5 Superoxide radical scavenging assay

This activity was measured by the reduction of NBT according to a previously reported method [5]. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations (0–20 µg/ml) of sample solution. After incubation for 5 min at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated.

3.6 Antibacterial activity of selected extracts of *B. aristata* against pathogenic bacteria

3.6.1 Inoculum preparation

The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10-15 colony forming units per milliliter ($cfuml^{-1}$) [35].

3.6.2 Agar well diffusion assay

The agar well diffusion method was used to test the anti-microbial effect of *B.aristata* extracts. All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar. For test, three doses of each extract (25, 50, 75 µl/well). Piperacillin (100Mcg), Ofloxacin (5 Mcg), Amoxyclav (30 Mcg) Kanamycin (30 Mcg), Nalidixic (30Mcg), Amikacin (30 Mcg), Methicillin (5 Mcg) and Cefoxitin (30Mcg) were used as positive standard antibiotics.

Diluted microbial suspension was inoculated on nutrient agar plates using sterile non-absorbent cotton swab. The inoculums were allowed to dry for 5 min and incubate at 37°C for 24 h. After incubation at 37°C for 24 h, all plates were examined for any zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The anti-microbial effect was recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

4. RESULTS AND DISCUSSION

4.1 Phytochemical screening of B. aristata extracts

The sequentially extracted fractions were analysed for the presence or absence of different phytochemicals using standard protocols. The results have been reported in Table 1.

Table 1: Phytochemical profiling of sequentially extracted *B. aristata* extracts

S. No.	Phytochemical groups	*B. aristata* stem extracts				
		n-Hex	EtOAc	DCM	MeOH	Aqu.
1.	Tannins	+	+	+	+++	++
2.	Flavonoids	+	+	++	+++++	++++
3.	Quinones	++	+++	+++++	++++	+
4.	Terpenoids	+++++	+	++	+++	++++
5.	Cardiac glycosides	-	-	-	-	-
6.	Coumarins	-	-	+	++++	+++
7.	Steroids	+++	++++	+++++	-	+

*(n-hexane, ethyl acetate, dicholoromethane, methanol and aqueous)

Phytochemical analysis showed presence of secondary metabolites was higher in methanol and aqueous extracts of *B. aristata* including flavonoids, quinones, terpenoids, tannins etc.

4.2 Antioxidant activity of *B. aristata* extracts

4.2.1 DPPH free radicals scavenging activity of *B. aristata* extracts

The sequentially extracted fractions (0-200 µg/ml) were analysed for the DPPH free radical scavenging activity using standard protocols. All five extracts showed significant DPPH scavenging activity when compared to standard drug, ascorbic acid. The results have been reported in Figure 1.

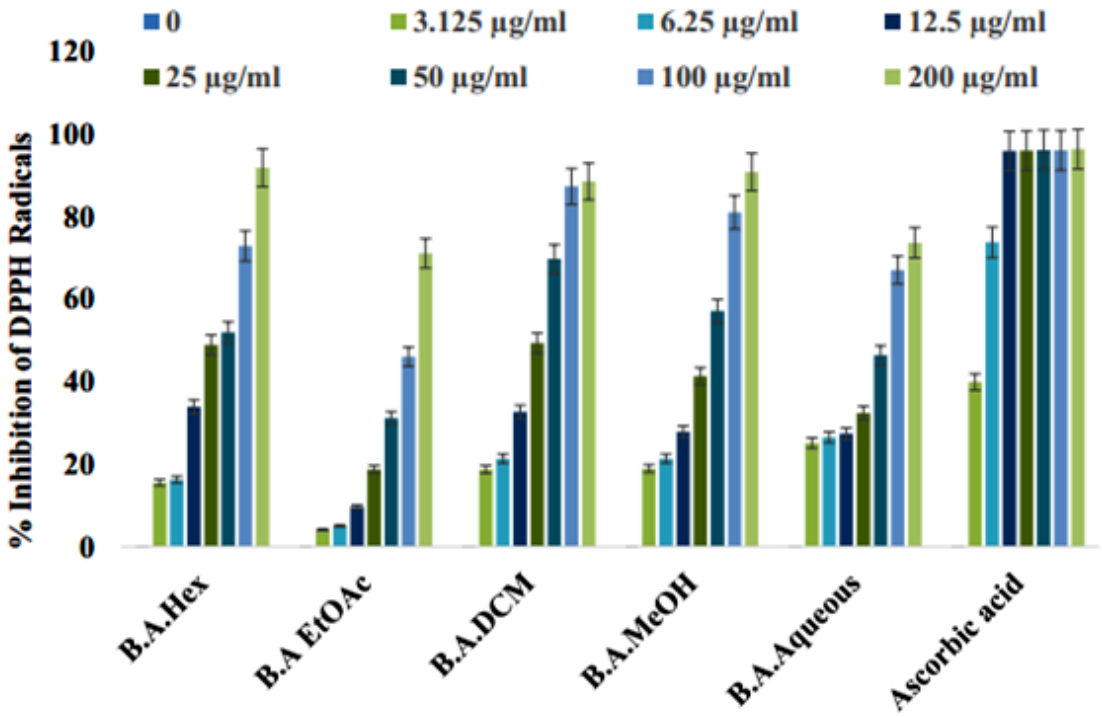

Figure 2: DPPH free radicals scavenging activity of *B. aristata* extracts.

4.2.2 Super oxide radical (O2.-) scavenging activity of *B. aristata* extracts

The sequentially extracted fractions (0-200 µg/ml) were analysed for the super oxide radical scavenging activity using standard protocols. All five extracts showed significant super oxide radical scavenging activity when compared to standard drug, ascorbic acid. The results have been reported in Figure 2.

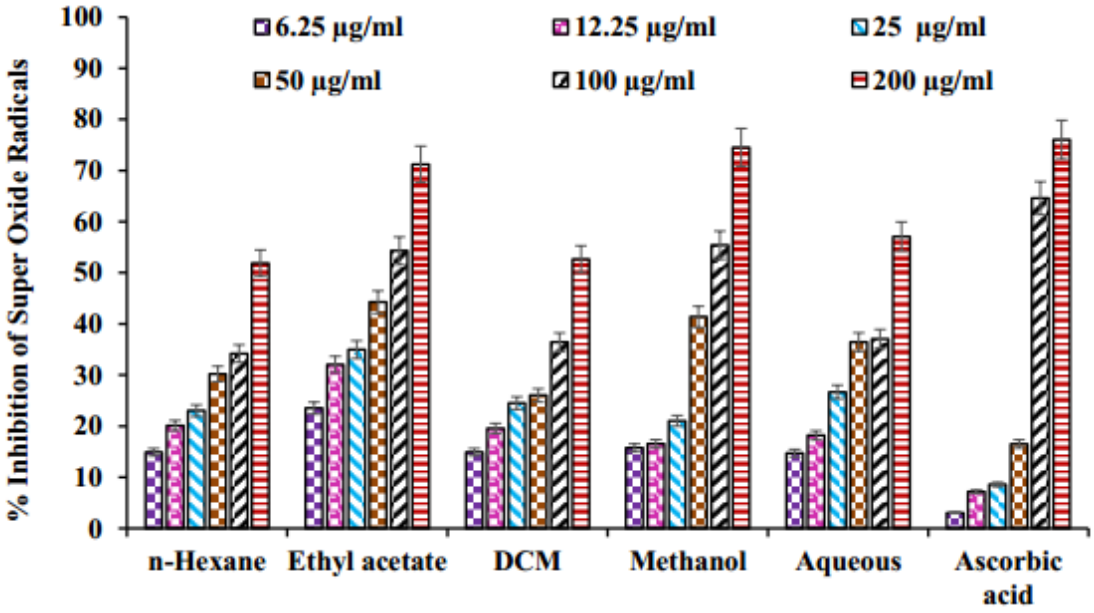

Figure 3: Superoxide radicals scavenging activity of *B. aristata* extracts.

4.3 Antibacterial activity of *B. aristata* extracts

Antibacterial activity of *B. aristata* extracts was evaluated against *E. coli, Bacillus subtilis and Klebsiella pneumoniae* strains. The antibacterial potential of all the extracts against tested bacterial strains has given very good results. The results indicated that different doses of extracts showed different degrees of growth inhibition depending Table 2.

Table 2. Antibacterial activity (Zone of inhibition) of *B. aristata* extracts and commercially available antibiotics against pathogenic bacteria.

Bacteria	*B.aristata Extracts*						*Antibiotics*							
	Aqueous (µg/ml)			*Methanol (µg/ml)*			*PC*	*OF*	*AC*	*KN*	*ND*	*AC*	*MC*	*CF*
	25	**50**	**75**	**25**	**50**	**75**								
E. coli	*18*	*19*	*23*	*13*	*17*	*20*	*14*	*25*	*R*	*12*	*R*	*20*	*R*	*R*
Bacillus subtilis	*R*	*R*	*R*	*R*	*R*	*R*	*20*	*31*	*R*	*9*	*22*	*25*	*R*	*R*
Klebsiella pneumoniae	*R*	*12*	*15*	*12*	*14*	*20*	*R*	*23*	*R*	*19*	*R*	*16*	*R*	*R*

*PC-Piperacillin (100Mcg), OF-Ofloxacin (5 Mcg), AC-Amoxyclav (30 Mcg), KN-Kanamycin (30 Mcg), ND-Nalidixic (30Mcg), AC-Amikacin (30 Mcg), MC-Methicillin (5 Mcg) and CF-Cefoxitin (30Mcg).

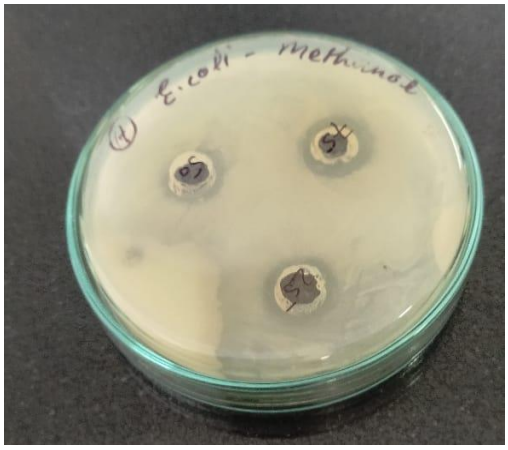

Figure 1 E. coli Methanol

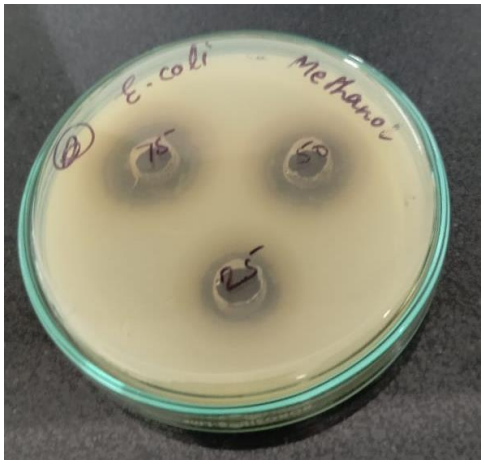

Figure 2 E. coli Methanol

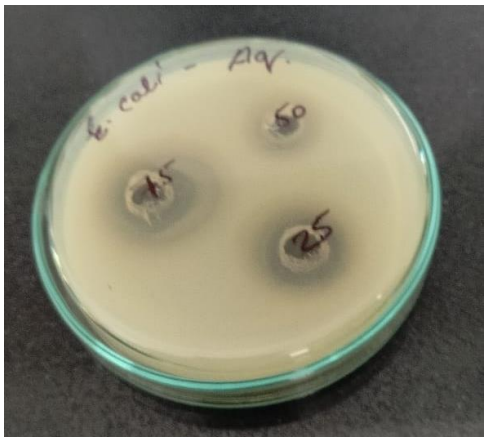

Figure 3 E. coli Aqueous

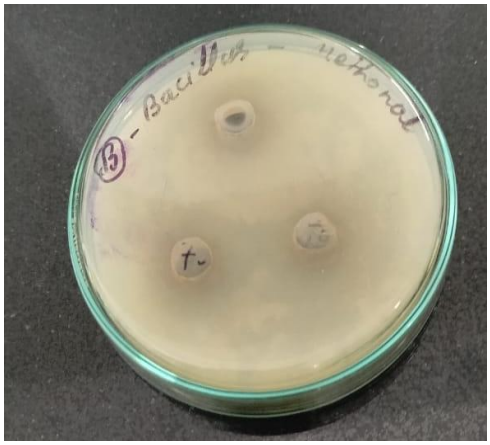

Figure 4 Bacillus subtilis Methanol

Figure 5 Bacillus subtilis Methanol

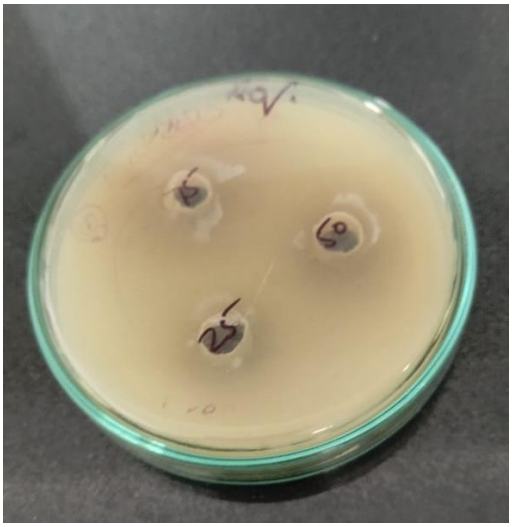

Figure 6 Bacillus subtilis Aqueous

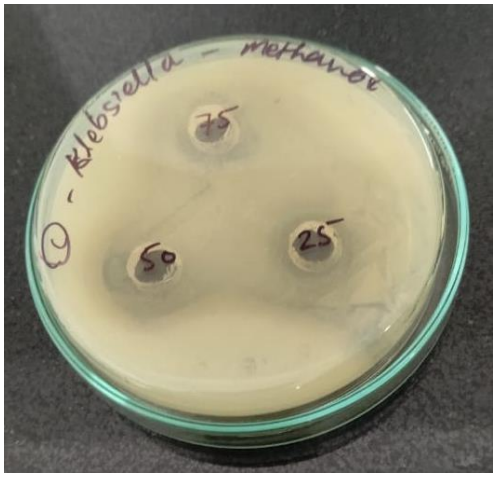

Figure 7 Klebsiella pneumoniae Methanol

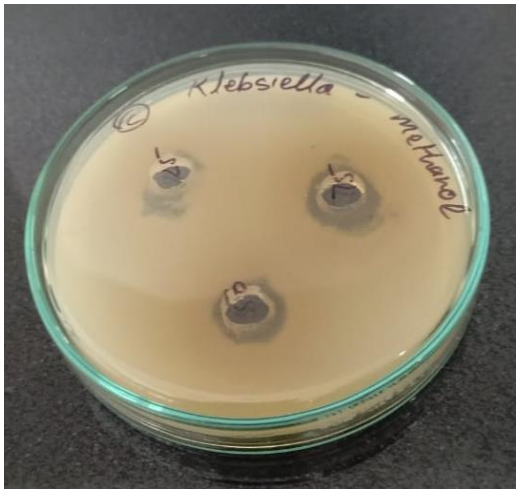

Figure 8 Klebsiella pneumoniae Methanol

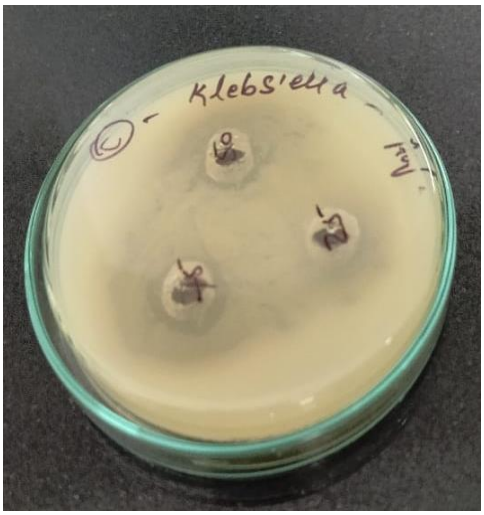

Figure 9 Klebsiella pneumoniae Aqueous

Figure 10 E.coli Amikacin & Nalidixic (Ab)

Figure 11 E.coli Methicillin & Cefoxitin (Ab)

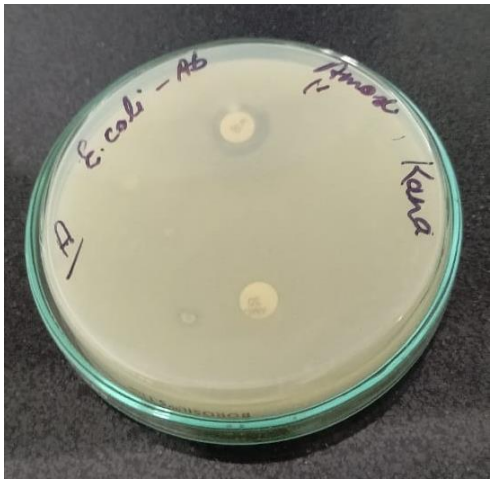

Figure 12 E.coli Amoxyclav & Kanamycin (Ab)

Figure 13 E.coli Piperacillin & Ofloxacin (Ab)

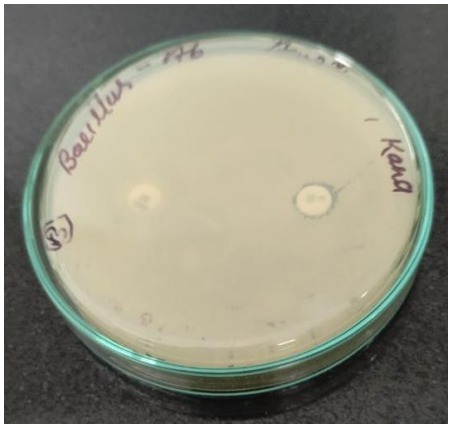

Figure 14 Bacillus Amoxyclav & Kanamycin Ab)

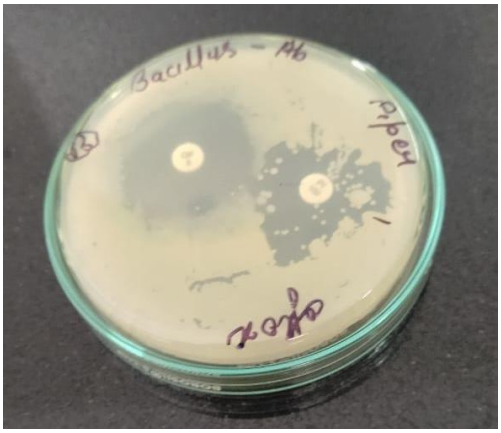

Figure 15 Bacillus Piperacillin & Ofloxacin (Ab)

Figure 16 Bacillus Cefoxitin & Methicillin (Ab)

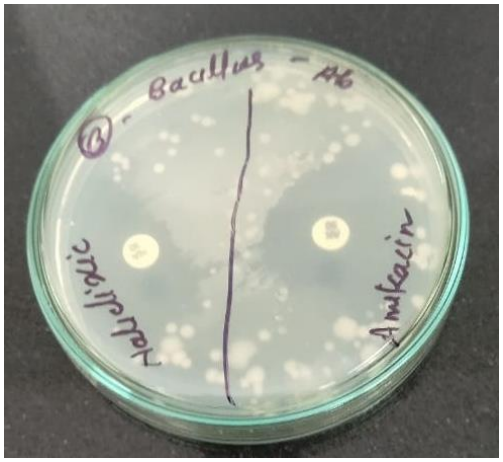

Figure 17 Bacillus Nalidixic & Amikacin (Ab)

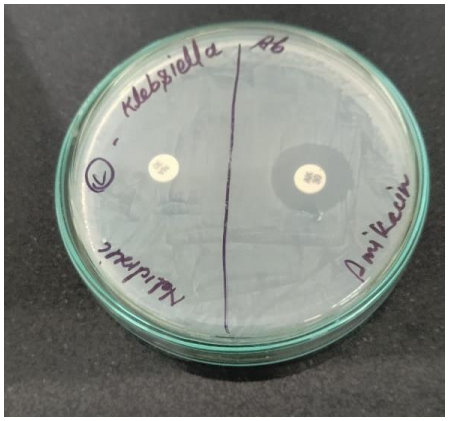

Figure 18 Klebsiella Nalidixic & Amikacin (Ab)

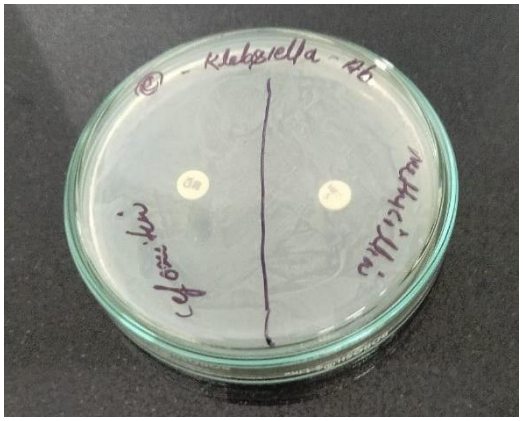

Figure 19 Klebsiella Cefoxitin & Methicillin (Ab)

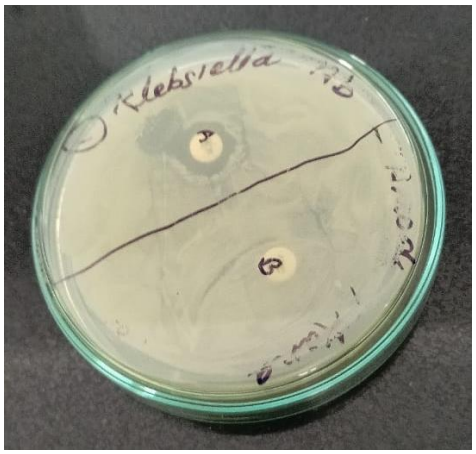

Figure 20 Klebsiella Amoxyclav & Kanamycin (Ab)

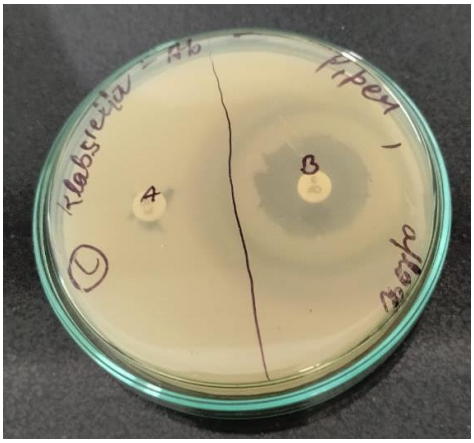

Figure 21 Klebsiella Piperacillin & Ofloxacin (Ab)

Oxidative stress induced by reactive oxygen species (ROS) can cause cell membrane disintegration, protein, lipid, and deoxyribose nucleic acid (DNA) damage which can further initiate or propagate the development of many chronic and degenerative diseases [10]. The uppermost concern in the mind of a sick person is always going to be whether or not full health can ever be achieved again. Sometimes the answer to that question is “yes,” and sometimes it’s “no”; sometimes it’s a very heavily qualified “yes” with lots and lots of imponderables. For some neurological issues, the outlook can be pretty good with treatment and adequate rehabilitation, while for others the prognosis can be grim. Rather than disability dwelling on a potentially unavailable cure, sometimes what’s called for is an adjustment to the patient’s lifestyle to better accommodate whatever is imposed by the affliction [5].

There has been enormous interest in natural antioxidants due to their ability to neutralize the effects of ROS that are not only responsible for alleviating the oxidative stress condition as well as neurological disorders. The growing interest to combat the side effect of the drugs available for diabetes leads to the development of green medicines due to their higher stability, higher antioxidant potential, low cost, and low cytotoxicity. Plants are rich sources of phytochemicals, which possess a variety of biological activities including antioxidant and antidiabetic potential both in vitro and in vivo [19].

In the current study, we evident the great antioxidant potential of *B. aristata* extracts. We found that methanolic and aqueous extracts exerted significant inhibition of DPPH radicals activity. These findings showed that *B. aristata* possess potent antioxidant activity almost comparable to ascorbic acid which is being used as standard for antioxidant assays (Alvi et al., 2016; Hashim et al., 2013). In addition Methanolic and aqueous extracts also exhibited the good superoxide radical scavenging activity. This potent antioxidant potential of *B. aristata* encouraged us to precede further assays (Alvi et al., 2016; Hashim et al., 2013). Phytochemical analysis showed the presence of higher amount of metabolites in methanol and aqueous extracts. Furthermore, these extracts are subjected to test the anti bacterial activity against selected pathogenic bacteria results are presented in table 2.

CONCLUSION

B. aristata is abundant of secondary metabolites, present study showed the presence of various secondary metabolites in different extracts. Methanol and aqueous extracts of *B. aristata* showed significant antioxidant potential in terms of DPPH and superoxide dismutase scavenging activity. Both extracts also showed antibacterial activity against the pathogenic bacteria. Further, a thorough and full-fledged *in vitro* study is needed to explore the role of *B. aristata* in order to establish a better treatment approach to get rid of oxidative stress consequences and infectious disease.

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