Pharmacogn. Res., 2025; 17(3):896-908.
https://www.phcogres.com

Original Article

Phytochemical Insights and Bioactive Potentials of *Alstonia scholaris*, a Variety from Mizoram, India: Studies of the Antioxidant and Anti-Infective Properties

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ABSTRACT

Background: A distinct variety of *Alstonia scholaris* in Mizoram, Northeast India, is a medicinal plant applied in the remedy of various ailments, including diarrhoea, dysentery, gastrointestinal infections, malaria and wounds. **Objectives:** To evaluate *A. scholaris* bark for its secondary metabolite components, antioxidant, antibacterial, antifungal and anthelmintic properties. **Materials and Methods:** The chloroform extract of *A. scholaris* was chemically assessed for its phytocompounds. Antioxidant content was evaluated using phenol, flavonoid and total antioxidant estimations. Antioxidant reaction was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl)-scavenging and ferric-reducing activities. Antimicrobial susceptibility assays were performed against bacteria and fungi. Anthelmintic activity was examined on a parasitic tapeworm of chicken. **Results:** Carbohydrates, reducing sugars, phytosterols and saponins were detected as the main bioactive secondary metabolites. The plant extract possesses appreciable amounts of antioxidants, with the total phenolic content of 11.42±0.08 mg GAE/g (gallic acid equivalent), the total flavonoid content of 57.87±3.53 mg QCE/g (quercetin equivalent) and the total antioxidant capacity of 7.22±0.01 mg AAE/g (ascorbic acid equivalent). Free radical-scavenging estimation against DPPH (2,2-diphenyl-1-picrylhydrazyl) indicated a half-maximal Inhibitory Concentration (IC_{50}) value of 28.96 µg/mL. The Ferric-Reducing Antioxidant Power (FRAP) assay showed a linear concentration-dependent antioxidation reaction similar to the standard ascorbic acid. It effectively inhibited Gram-positive bacteria (*Bacillus cereus, Enterococcus faecalis* and two strains of *Staphylococcus aureus*), as well as Gram-negative species (*Escherichia coli* and *Salmonella typhi*). A notable observation was its effectiveness against an antibiotic-resistant *S. aureus*. The antifungal efficacy against three pathogenic species was in the order *Fusarium keratoplasticum*<*Aspergillus fumigatus*<*Candida albicans*. It was highly effective against an intestinal tapeworm, causing structural damages in all vital parts of the parasite body as revealed by histology and scanning electron microscopy. **Conclusion:** The findings indicate that *A. scholaris* bark contains important bioactive compounds that are health benefit, antimicrobial and anthelmintic in nature. This is the first report on the broad-spectrum anti-infective potential of *A. scholaris* and provides an evident-based therapeutic value of the plant.

Keywords: Anthelmintic, Antifungal, Antibacterial, Antioxidant, Medicinal plant.

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Received: 21-02-2025;
Revised: 08-04-2025;
Accepted: 11-06-2025.

INTRODUCTION

Humanity has relied on natural remedies for centuries to address a range of diseases from common ailments such as colds, allergies, digestive disorders, and dental pain to the most severe and complex medical conditions such as immune diseases, blood disorders and cancers. A variety of synthetic drugs had been developed for the different diseases but with critical drawbacks including adverse effects, drug resistance and non-target toxicity which lead to complications in disease managements.[1,2] For their safety application and often low-cost availability, herbal medicines have been a widely used medical therapy practice in different parts of the world, and especially triggered by the increasing failures of synthetic medication, they continue to gain prominence.[3] This growing preference for natural treatments signifies a global shift from synthetic drugs towards a phenomenon of "return to nature" approach in healthcare and disease prevention.[4] In

DOI: 10.5530/pres.20252145

fact, medicinal plants, sourced from various traditional systems, form the basis of many modern pharmaceuticals.[5,6] As a matter of fact, many botanical compounds became some of the main prescription drugs or lead molecules for the developments of novel medications for different clinical conditions.[7-9]

In India alone, approximately 20,000 species of medicinal plants have been documented, with over 500 traditional communities utilizing around 800 of these species to treat various diseases.[10] These plant-based remedies have not only sustained traditional healthcare systems but have also been harnessed by national and international pharmaceutical companies for treating a wide range of ailments.[11-13] One of the primary properties and uses of medicinal plants is as antioxidants, agents that protect cells from free radical-induced damage, by involving in cellular protective activities for combating oxidative stress, thereby preventing and ameliorating cellular malfunctions. By inhibiting or delaying the oxidation of vital molecules, antioxidants reduce the risk of detrimental diseases associated with free radicals.[14] Plants are normally rich in natural antioxidants, and thus provide the major source of exogenous antioxidation system that scavenge detrimental free radicals produced by cellular activities, establishing protection against oxidation-related diseases.[15,16] Consequently, research into traditionally used herbs for antimicrobial and other therapeutic applications remains crucial, particularly for addressing infections caused by microbial pathogens that are increasing due to the growing cases of antimicrobial resistance.[17,18]

Among the Mizo people of Mizoram, northeast India, the traditional knowledge of medicinal plants, rituals, and cultural practices is deeply ingrained in the indigenous ethnic communities. The traditional practices were passed down by folk literature through generations, representing a vital repository of ethnobotanical heritage.[19] One such genus, *Alstonia* (family Apocynaceae) is among popular medicinal plants in several Indian cultures and commonly referred to as "*saptaparni*" or the devil's tree.[20] The species are large, evergreen trees with over 60 species globally, six of which are found in India.[21] In Mizoram, *A. scholaris* (L.) R.Br. is the sole species recorded of this genus and is distributed widely across the region falling under the Indo-Burma biodiversity hot spot.[22-25]

Different parts of the plants had been documented for analgesic, anti-inflammatory,[26] antihypertensive,[27] anti-tussive, anti-asthmatic,[28] antibacterial,[29] and several health-benefit properties.[30-32] However, an exhaustive study into the chemical and biological properties of *A. scholaris* is limited,[21,32] especially the specific application of its bark among the Mizo people of Northeast India. The variety available in Mizoram is unique in that it possesses leaf whorls arranged radially in eight number on each node, for which a local native name "*ṭhuamriat*" (literally eight branched) is given and is not recorded in any Indian or neighbouring Indo-Burma region (Figure 1). In Mizo traditional medicine, the leaves are curative to asthma, dermal lesions, dysentery, fever, hypertension, typhoid, ulcers, and wounds.[22,24,33] Its bark is specifically employed in the treatment of malaria, diarrhoea, heart diseases and hypertension,[34] while its milky sap from the bark is applied to fresh cuts, sores, ringworms, and warts.[35] To understand the pharmacological properties of the variety in Mizoram and the distinctive application in the Mizo traditional medicine, this investigation was designed to explore the fundamental chemical and pharmacological properties. The plant extract was subjected to an elaborate suite of investigations, including phytochemical screening to identify the presence of bioactive components, evaluation of its antioxidant properties, antimicrobial susceptibility assays to evaluate its potential efficacy against pathogenic bacteria and fungi, as well as anthelmintic test to assess its activity against an intestinal helminth parasite. This multi-faceted approach therefore aimed to underscore a comprehensive understanding of the bioactivity profile and therapeutic potential of the plant.

MATERIALS AND METHODS

Sample identification

The plant parts of *A. scholaris* were collected from a forest surrounding Lungdai village in Kolasib district, Mizoram, India (23°52' N 92°44' E). The herbarium specimens of the leaves and flowers were validated for species identification at the Eastern Regional Centre of Botanical Survey of India, Shillong, India (*vide*. BSI/ERC/Tech/2023-24/102-17-05-23). A voucher herbarium with catalogue code PUC-A-23-01 was deposited in the botanical collection of Pachhunga University College.

Plant extraction

The bark samples of *A. scholaris* were carefully washed with distilled water to remove surface impurities and subsequently dried in a closed but well-ventilated environment for two months. This ensured the complete natural dehydration of the materials crucial for the subsequent extraction process. Once fully dried, the bark was mechanically ground using a grinder to produce a coarse powder. The powder was then accurately weighed, and batches of 450 g were utilized for the extraction in a 5-L Soxhlet apparatus. Using a medium polar solvent, chloroform (polarity index 4.1), hot extract was run for 72 hr to maximise the yield of extracts. The chloroform was removed and recovered by vacuum evaporation of the crude extract in a rotary evaporator Büchi Rotavapor® R-100 (Flawil, Switzerland).

Phytochemical analysis

A series of qualitative chemical analyses was conducted on *A. scholaris* bark extract based on standard biochemical protocols.[36] The chemical detection assays included alkaloids by Dragendorff's, Hager's, Mayer's and Wagner's tests; anthraquinones by ammonium hydroxide and Borntrager's tests; carbohydrates by

Barfoed's, Benedict's test, Fehling's and Molisch's tests; flavonoids by lead acetate, Shinoda's and zinc hydrochloride reduction tests; glycosides by Keller-Kiliani, Legal's and Liebermann tests; gums by alcohol test; phytosterols by Liebermann-Burchard and Salkowski tests; protein and amino acid by biuret and ninhydrin tests; reducing sugars by Benedict's and Molisch's tests; saponins by foam test; and tannins by ferric chloride, lead acetate and potassium dichromate tests.

Total phenolic content

A quantitative test for the total phenolic content was performed using Singleton's Folin-Ciocalteu assay with necessary standardizations.[37] A stock solution (100 mg/mL) was prepared for *A. scholaris* bark extract. Varying concentrations (like 10, 20, 40, 60, 80, and 100 µg/mL) of a standard antioxidant, gallic acid were prepared. Folin-Ciocalteu Reagent (FCR) in 5 mL was mixed with the samples to allow chemical reaction at room temperature for 3 min. For the test sample, 200 µL of the stock extract was processed similarly. Then, 4 mL of 0.7 M sodium carbonate solution was added to all the samples and agitated for an hour. Absorbance of the solutions was read at the wavelength of 765 nm in a UV-vis spectrophotometer (Labtronics LT39, Haryana, India). The absorbance was adjusted to that of a blank concentration consisting of a mixture of 1 mL methanol, 5 mL FCR, and 4 mL sodium carbonate. A calibration curve was generated for gallic acid from which the total phenol concentration was determined, expressed as milligram of gallic acid equivalent per gram (mg GAE/g) of the dry weight of the plant extract. Each assay was repeated three times.

Total flavonoid content

A modified approach technique of Zhishen *et al.*,[38] using aluminium reduction test was followed for the determination of total flavonoid content. A reference antioxidant flavonoid, quercetin was prepared in 10, 20, 40, 60, 80, and 100 µg/mL. From the stock solution of *A. scholaris* bark extract, 1 mL was taken for the test. 2 mL of distilled water was added to all the samples and kept for 5 min. Then, 3 mL of 5% sodium nitrite and 0.3 mL of 10 % aluminium chloride were added and mixed well. After 6 min, 2 mL of sodium hydroxide was added to the mixture. The final volume was made to 10 mL with distilled water. After an hour, the absorbances all the samples were detected at 510 nm. The total amount of flavonoid content was extrapolated from the standard curve of quercetin and given as milligram of quercetin equivalent per gram (mg QCE/g) of the dry weight of the plant extract.

Total antioxidant content

The total antioxidant content was analyzed by phosphomolybdate reaction following the standard protocol of Prieto *et al.*, with a small modification.[39] Ascorbic acid was used as a reference antioxidant and was prepared in 10, 20, 40, 60, 80, and 100 µg/mL. 3 mL of a reagent mixture (4 mM ammonium molybdate, 0.6 M sulphuric acid, and 28 mM sodium phosphate) was added to 100 µL of each ascorbic acid sample and 100 µL of *A. scholaris* bark extract. The mixtures were allowed to undergo reactions at 95°C for 90 min. After cooling them down, the absorbances were measured at 695 nm. The total antioxidant content was calculated from the standard curve of ascorbic acid and expressed as milligram of ascorbic acid equivalent per gram (mg AAE/g) of the dry weight of the plant extract.

DPPH radical-scavenging activity

Progressive antioxidant degradation of a free radical, DPPH (2,2-diphenyl-1-picrylhydrazyl) developed by Blois was used to determine the free radical-scavenging potential.[40] Concentrations of 10, 20, 40, 60, 80, and 100 µg/mL of both *A. scholaris* bark extract as well as a standard antioxidant compound, Butylated Hydroxytoluene (BHT), were prepared. 0.5 mL of freshly prepared DPPH was added to 3 mL of all the solutions. The samples were adequately mixed and then incubated for 30 min at 37°C. The solutions were cooled down, the Optical Densities (ODs) were taken at 517 nm. Then, the amount of DPPH radicals scavenged was determined by the formula:

$$\text{Free radicals scavenged (\%)} = \frac{\text{OD of control} - \text{OD of treatment}}{\text{OD of control}} \times 100$$

The half-maximal Inhibitory Concentration (IC_{50}) values were determined in GraphPad Prism version 10.4.1 (Dotmatics, Boston, Massachusetts, USA). The log doses were calculated from three concentrations, 1, 0.5, and 0.25 mg/mL of BHT and the plant extract.

Ferric-reducing antioxidant power (FRAP) assay

A modified approach of Oyaizu's potassium ferricyanide reduction assay was followed for determining the ferric-reducing antioxidant activity.[41] Ascending concentrations, *viz.* 10, 20, 40, 60, 80, and 100 µg/mL were prepared for ascorbic acid, as reference antioxidant, and *A. scholaris* bark extract. 1 mL of all the test samples were added to 2.5 mL of Phosphate Buffer (6.6 pH) and 1% of potassium ferricyanide. Chemical reaction was allowed in an incubator at 50°C for 30 min 30. After adding 2.5 mL of 10% trichloroacetic acid, they were centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant solutions were diluted with 2.5 mL of deionized water and mixed thoroughly. The samples were mixed with 0.5 mL of 0.1% ferric chloride and left for reaction for 10 min. A blank sample was made from a mixture of equivalent amounts of distilled water, phosphate buffer and potassium ferricyanide. ODs were read at 700 nm and recorded after adjustment with the blank sample reading.

Antibacterial susceptibility assay

A standard agar-well diffusion technique was employed to evaluate the antibacterial potential.[42] *A. scholaris* bark extract was prepared at three multiple concentrations, *viz.* 50, 100 and 200 mg/mL. Ciprofloxacin (0.5 µL) and dimethyl sulfoxide

(DMSO, 10 µL) were employed as the positive and negative controls respectively. Antibacterial susceptibility was tested against three Gram-positive species such as *Bacillus cereus* (ATCC 13061), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 700698); and two Gram-negative bacteria such as *Escherichia coli* (ATCC 10536) and *Salmonella typhi* (ATCC 51812). Culture media for each bacteria and test consisted of 30 mL sterilized nutrient agar in Petri dishes. 6 mm wells created using a cork borer on the solidified agar. The bacterial inoculums at 40 µL were uniformly spread onto the agar surface using a sterile spreader. Subsequently, ciprofloxacin, DMSO and 50 µL of the each of extract concentration were carefully dispensed into individual wells. The Petri dishes were sealed and incubated at 24°C for 24 hr to allow for microbial growth.

Antifungal toximetric assay

The antifungal potential of *A. scholaris* extract was assessed with the poison plate technique of Grover and Moore,[43] modified with potato-dextrose agar culture method, the most sensitive antifungal culturing for the most common fungi.[44] Sub-cultures of three clinically important fungi, namely *Aspergillus fumigatus* (ATCC 204305), *Candida albicans* (ATCC 26790) and *Fusarium keratoplasticum* (ATCC 36031) were supplied by HiMedia Laboratories Private Limited, Mumbai, India, from the American Type Culture Collection (Manassas, Virginia, USA). The fungal specimens were streaked evenly using disinfected cotton patches over the sterilised agar media in Petri dishes. They were allowed to grow at 27±2°C for a week in an aseptic microbiological chamber (Igene IG-95I, New Delhi, India). Serial dilutions, *viz.* 10, 5, 2.5 and 1.25 mg/mL, of the plant extract were made by mixing with 20 mL molten potato-dextrose agar (~50°C). A control medium was maintained containing only the growth medium. Using a sterile cork borer, 6 mm discs of the fully-grown fungi were harvested and inoculated at the centre of the culture plates containing the plant extract-treated and control media, which were then cooled down at room temperature to solidify. After hermetic sealing the culture plates with parafilm, the specimens were grown at 27±2°C for a week. The growth zones formed radially around the inoculum were measured at two opposite circumference every 24 hr. The degree of inhibitory action was determined from the equation:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth of control} - \text{growth of treated}}{\text{Growth of control}} \times 100$$

Helminth survival assay

Intestinal tapeworm, *Taenia echinobothrida* Mégnin, 1880 (family Davaineidae; class Cestoda; phylum Platyhelminthes) were recovered from freshly sacrificed fowl, *Gallus gallus* Linnaeus, 1758. Based on the standardized helminth survival assay,[45] the tapeworms were maintained at 37±1°C in 0.9% neutral Phosphate-Buffered Saline (PBS) mixed with 1% Dimethyl Sulfoxide (DMSO) in a microbiological chamber. Increasing concentrations of *A. scholaris* extract, such as 5, 10 and 20 mg/mL, were prepared in PBS + DMSO, and treated with the tapeworms. A batch of untreated worms remained as negative control. Positive control was set up with worms treated with albendazole.

Histology and light microscopy

Tapeworms treated with *A. scholaris* bark extract were processed for histology for observation under light microscopy. The tapeworms were washed in PBS and fixed in Bouin solution for 24 hr under ambient environment. After repeated washing in distilled water, they were dehydrated through varying concentrations of ethanol, from 30 to 100%. They were dealcoholized in ascending concentrations of xylene up to pure xylene. They were then paraffinized into solid cubes and cut at 4-5 µM thickness using auto-microtome (MRM-ST, Medimeas, Haryana, India). The sections were dehydrated again in ethanol, double stained with eosin and haematoxylin, mounted on glass slides, and observed under an image analyzer (Nikon Eclipse, Tokyo, Japan).

Scanning electron microscopy

Tapeworms treated with *A. scholaris* extract were first washed with PBS solution and fixed in 10% neutral-buffered formaldehyde at 4°C for 4 hr. Complete dehydration of the tissues was achieved through different grades of acetone. For solidifying, the samples were immersed tetramethylsilane for 10 min and then the solvent was allowed to evaporate at 25°C in an air-drying chamber. The dried specimens were coated with gold in MC1000 ion sputter coater (Hitachi, Ltd., Tokyo, Japan), and electron images were taken with TM4000Plus II scanning electron microscope (Hitachi, Ltd., Tokyo, Japan).

Statistical analysis

Duration of survival were expressed as means±standard deviations of the means or standard error of the means. The mean difference of treatment against control was analyzed using Student's *t*-test. The differences of the means between different treatments were analyzed using analysis of variance and Tukey's multiple comparison test. The mean differences were considered significantly different at p value less than 0.05. The final data were generated from Graphpad Prism.

RESULTS

Phytocompounds

Qualitative chemical detection of the chloroform extract of *A. scholaris* bark encompassing assays for eleven phytochemical groups was performed and each assay consisted of multiple chemical detections as presented in Table 1. Out of the secondary metabolite group tested only three compound groups were detected, namely carbohydrates (including reducing sugars), phytosterols and saponins. This indicates that the specific

bioactive compounds of the plant were restricted to carbohydrate and fatty acid compounds.

Total phenolic content

The standard graph of gallic acid used for estimating the total phenolic content of *A. scholaris* extract is presented in Figure 1A. The quantification based on the linear regression graph yielded the value of phenol as 11.42±0.08 mg GAE/g.

Total flavonoid content

The total flavonoid present in the *A. scholaris* extract was quantified from the calibration curve of standard quercetin (Figure 1B). The analysis revealed that the flavonoid concentration was 57.87±3.58 mg QCE/g.

Total antioxidant content

The total antioxidant composition of *A. scholaris* extract was quantitatively determined from the standard curve of ascorbic acid (Figure 1C). Based on dose-responsive calibration, the antioxidant level of the plant extract was determined to be 7.22±0.01 mg AAE/g.

DPPH scavenging activity

The molecular scavenging activity evaluated using DPPH radical demonstrated that *A. scholaris* bark extract possesses appreciable oxidant-scavenging capabilities in concentration-dependent manner. The increasing activity corresponding to the concentration was in similar pattern to that of BHT. However, the scavenging efficacy of the extract was lower at all tested concentrations than the pure antioxidant compound.

Further, the IC_{50}, defined as the concentration necessary to scavenge 50% of free radicals in a given sample, was estimated from the dose-response curve (Figure 1D). The IC_{50} value of *A. scholaris* bark extract against DPPH radicals was determined as 28.96 µg/mL, while that of BHT as 5.73 µg/mL, indicating lower effectiveness of the plant extract than the pure compound.

FRAP

The ferric-reducing activity of the plant extract was evaluated against that of ascorbic acid. Reaction with potassium ferricyanide indicated concentration-dependent response to ferric ion degradation, signifying an ability to transfer electrons for converting Ferric Ions (Fe^{+3}) to Ferrous Ions (Fe^{+3}), thereby reducing the highly oxidant form of iron. The standard ascorbic acid consistently showed relatively higher ferric-reducing efficacy to the plant extract (Figure 1E).

Antibacterial activity

The susceptibility of different bacteria against *A. scholaris* bark extract in comparison with ciprofloxacin is summarized in Table 2. Antibacterial sensitivity was observed for the plant extract in all the bacterial sample, including antibiotic-resistant strain. The plant extract exerted highest activity against *S. aureus* (strain 1) showing inhibition zones of 5.3, 7.49 and 8.65 mm against the concentrations of 50, 100 and 200 mg/mL respectively. The antibacterial susceptibility was followed by *S. typhi, B. cereus, E. coli* and then lastly by *S. aureus* 2. An interesting observation was that *S. aureus* 2 was totally resistant to ciprofloxacin with no signs of inhibition. However, the plant extract effectively inhibited the bacterial growth at 100 and 200 mg/mL, producing inhibition zones of 1.47 and 2.25 mm respectively.

A statistical comparison of the efficacy of different concentrations of the chloroform extract of *A. scholaris* bark with that of standard antibiotic showed significant difference between the tested groups ($p<0.05$) as shown in Figure 2. Ciprofloxacin at 20 µg/mL was comparatively more potent than the plant extract even at all concentrations tested.

Antifungal activity

The effect of *A. scholaris* bark extract on the rate of proliferation was examined against *A. fumigatus, C. albicans* and *F. keratoplasticum* as shown in Table 3. The three fungi steadily proliferated for the seven days as indicated by the increasing size of growth zones around the sample wells. The control, i.e., 0 treatment, showed maximum growth (largest growth zones) in all species. The relative rate of growth was in the order *C. albicans* < *F. keratoplasticum* < *A. fumigatus*. The plant extract was effective in inhibiting growth of the three fungi and highest significant inhibition was seen on *C. albicans* in comparison to the respective controls. The lowest concentration, i.e., 25 mg/mL, was mostly ineffective upon *A. fumigatus* and *F. keratoplasticum*. Experimental group comparison of the efficacy between the varying concentrations of the plant extract and between the different fungi each day for one week is depicted in Figure 3.

Anthelmintic activity

The efficacy of *A. scholaris* bark extract against a parasitic tapeworm, *T. echinobothrida* is presented in Table 4. The extract displayed significant effectiveness ($p<0.05$) at all concentrations tested and exhibited a fine concentration-dependent lethal action. The reference drug, albendazole also indicated similar pattern of activity with higher potency. The extract was equally effective ($p>0.05$) as the drug at higher concentrations, but less so at the lowest concentration, i.e., at 5 mg/mL.

Histological analysis of tapeworms treated with the plant extract using light microscopy showed several anthelmintic effects (Figure 4A). Tissue damages were evident on the outermost layer, the tegument, that showed several depressions and swellings all around the margin. The subtegumental layer and longitudinal muscles appeared intact. However, the parenchymatous tissues surrounding the sub-tegument and the longitudinal muscle layers were massively degenerated, appearing as fuzzy and

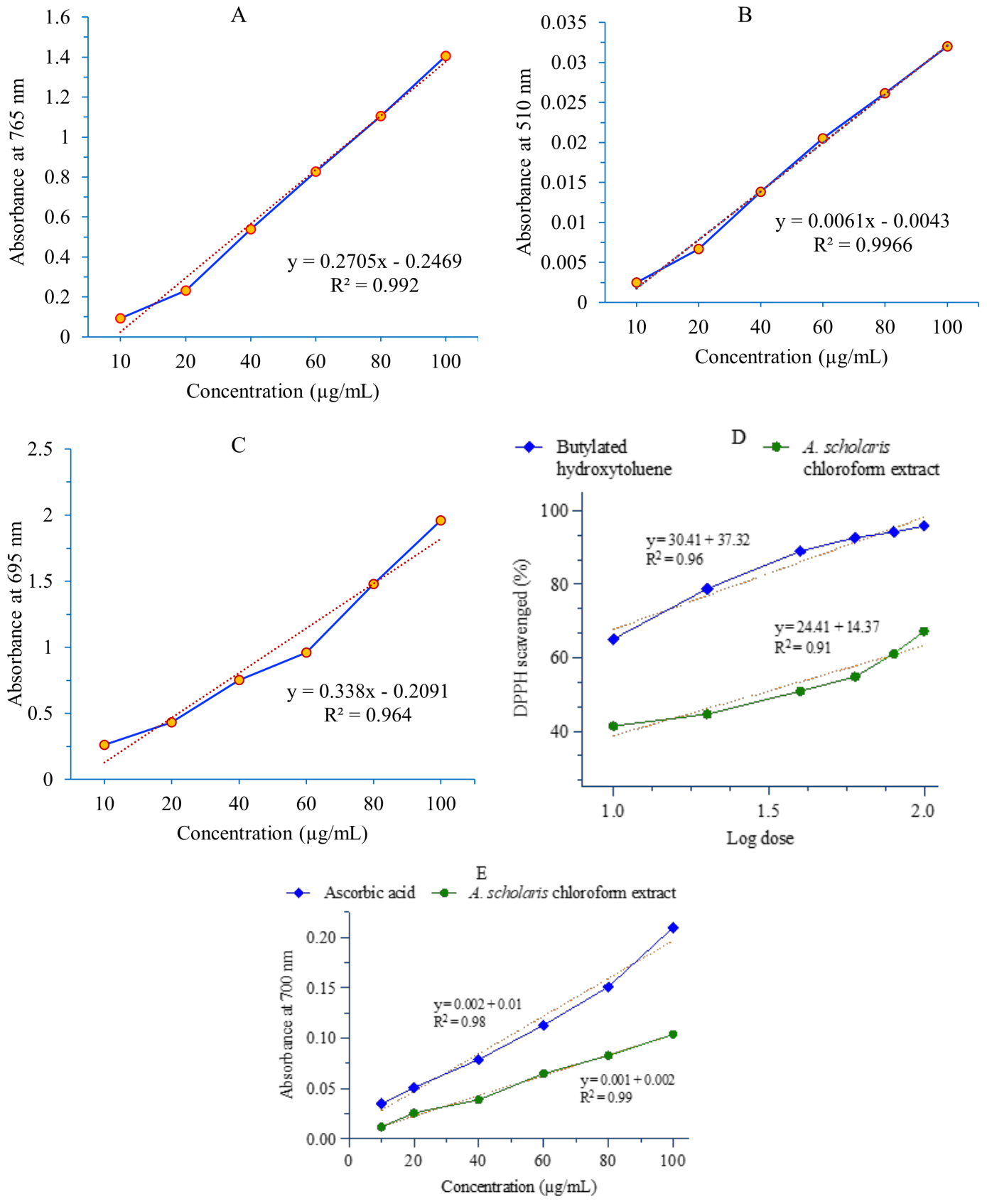

Figure 1: Standard graphs for quantifying the antioxidant property of the chloroform extract of *A. scholaris* bark. (A) Gallic acid for the total phenol. (B) Quercetin for the total flavonoid. (C) Ascorbic acid for total antioxidants. (D) DPPH scavenging reaction in log dose. (E) Ferric reducing reaction. The dotted line represents the linear graph.

diffused strands. The circular muscle was also degenerated and almost totally disappeared, only represented by faint traces of red fibrils. The parenchyma is expected to be completely smooth in normal condition. Although the egg capsules did not show structural damages, they were highly compressed and clamped together at the centre of the body. The lateral excretory canals, which should be normally circular in shape, were distended into irregular shapes. There was also formation of an aberrant vacuole near one of the lateral canals.

Scanning electron micrographs revealed the major anthelmintic effects on the body of *T. echinobothrida*. Structural disruption and organ damages were evident throughout the body. The head-like anterior region, known as the scolex, was found to be entirely disfigured (Figure 4B). The vital organs such as the rostellum at the apical end and the lateral suckers were all visibly destroyed. The rostellum was lost in the tegumental folds. The suckers showed varying degrees of deformation, with one completely bereft of any spine (Figure 4C), while one was still having circular rows of spines around the rim but with several spines disentangled and about to fall off (Figure 4D). Tegumental destruction extended throughout the body segments (Figure 4E). The series of immature body segments exhibited convolutions and folds due to collapse of the tegument (Figure 4F). The mature segments formed sharper folds that were seen as cracks and grooves (Figure 4G). Tegumental depressions are quite deep and the hairy microtriches were disintegrating (Figure 4H).

DISCUSSION

Plants serve as vital reservoirs of diverse secondary metabolites, which are established to play vital roles in the physiology and adaptive stress of plants, and are exploited as medicinal compounds for the treatment and prevention of several diseases.[46] A history of drug development and exploration of medicinal plants have contributed to the production of a substantial proportion of modern pharmaceuticals from these herbal secondary metabolites and their associated compounds.[47,48] Many of them developed and substantiated to be used as prescription drugs for various types of diseases.[7-9] Secondary metabolites, which are essential for the survival of plants, exhibit a wide range of pharmacological activities, from analgesic, antiallergic, antibacterial, anticancer, anti-diuretic, anthelmintic, antifungal, antimalarial, anti-inflammatory, antioxidant, to antiviral properties.[49,50] Our phytochemical tests revealed the occurrence of reducing sugars, carbohydrates, saponins and phytosterols in the chloroform extract of *A. scholaris* bark, highlighting its distinct phytochemical profile and potential implications for its biological activity and therapeutic applications.

Oxidative stress is a major physiological dilemma in animals including humans as it arises from a disproportion between the accumulation of Reactive Oxygen Species (ROS) and the cells' ability to eliminate the free radicals, thereby initiating a cascade of reactions that are deleterious to cellular lipids, proteins, and DNA.[51] Plants have historically been recognized as some of the best natural antioxidants with relative effectiveness, safety and sustainability, particularly phenolic compounds such as anthocyanins, flavonoids, phenolic acids, stilbenes, and tannins that are demonstrably powerful free radical scavengers.[52] Our findings on *A. scholaris* bark extract emphasise the plant's high antioxidant value, linking it to its beneficial source as nutraceutical phytocompounds. For instance, flavonoid content, which contributes to pharmacologically relevant bioactive properties and the phenolic composition, a key factor in antioxidation

Table 1: Qualitative phytochemical analysis of the chloroform extract of *A. scholaris* bark.

Phytocompounds	Name of test	Extract indication
Alkaloid	1. Dragendorff's test	-
	2. Hager's test	-
	3. Mayer's test	-
	4. Wagner's test	-
Anthraquinone	1. Ammonium hydroxide test	-
	2. Borntrager's test	-
Carbohydrate	1. Barfoed's test	+
	2. Benedict's test	-
	3. Fehling's test	+
	4. Molisch's test	+
Flavonoid	1. Lead acetate test	-
	2. Shinoda test	-
	3. Zinc hydrochloride reduction	-
Glycoside	1. Keller-Kiliani test	-
	2. Legal's test	-
	3. Liebermann's test	-
Gum	1. Alcohol test	-
Phytosterol	1. Liebermann-Burchard test	+
	2. Salkowski reaction	+
Protein and amino acid	1. Biuret test	-
	2. Ninhydrin test	-
Reducing sugar	1. Benedict's test	+
	2. Molisch's test	+
Saponin	1. Foam test	+
Tannin	1. Ferric chloride test	-
	2. Lead acetate test	-
	3. Potassium dichromate test	-

+ = presence; - = absence.

reactions, could bely the plants medicinal values. The plants ability to effectively neutralise ROS is chemically demonstrated through DPPH and FRAP assays, suggesting its potential role in mitigating oxidative stress. Such attributes are integral to its potential therapeutic and health-promoting applications.

Plant-derived bioactive compounds have drawn considerable pharmacological interest for their antimicrobial potentials so that their experimental validations are pivotal in the ongoing pursue for novel antibiotics and antifungal agents.[53,54] The emergence of antibiotic-resistant microorganisms underscores the urgency to identify alternative antimicrobial sources, with millions of people dying and estimated to die in the near future purely because of drug resistance in the pathogenic microbes.[55] Phytocompounds with their safe and ready availability offer a promising avenue for exploration into lead molecules.[56,57]

Table 2: Inhibitory action of *A. scholaris* bark extract and ciprofloxacin against different bacteria determined from the zones of inhibition (mm). Values in means±standard deviations of the means (*n*=3).

Bacteria	*A. scholaris* bark extract			Ciprofloxacin
	50 mg/mL	100 mg/mL	200 mg/mL	20 µg/mL
Bacillus cereus	4.05±0.76	3.80±0.60	3.77±1.01	31.79±1.15
Staphylococcus aureus 1	5.34±0.72	7.49±0.75	8.65±0.46	23.30±0.98
Staphylococcus aureus 2	-	1.47±0.48	2.25±0.68	-
Escherichia coli	4.05±0.63	3.07±0.51	2.72±0.51	32.61±1.12
Salmonella typhi	4.47±0.41	4.87±0.70	5.05±0.71	26.03±0.87

Table 3: Fungal growth and inhibition measured in growth zones (mm) from progression of proliferation during seven days. Values expressed in means±standard deviations of the means (*n*=3).

Duration	Growth zone (mm)				
	0 (control)	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL
Aspergillus fumigatus					
Day 1	06.50±0.47	05.90±0.12†	05.09±0.24*	05.09±0.45*	03.64±0.53*
Day 2	19.59±0.90	17.78±0.40†	17.15±0.24*	15.75±0.41*	14.61±0.52*
Day 3	28.90±0.86	26.18±1.34*	26.71±0.73*	24.75±0.64*	23.82±0.74*
Day 4	46.35±2.03	42.71±0.59*	38.78±0.63*	37.62±0.48*	34.76±0.57*
Day 5	51.46±0.65	50.28±0.71*	48.26±0.89†	44.96±0.71*	43.71±0.56*
Day 6	68.04±0.51	67.38±0.23†	64.70±0.43*	61.61±0.53*	59.71±0.61*
Day 7	82.30±1.11	80.66±0.49†	78.12±0. 44*	75.06±0.68*	71.41±1.18*
Candida albicans					
Day 1	06.47±0.50	05.52±1.25*	05.03±0.46*	04.16±0.98*	02.42±0.42*
Day 2	10.36±0.54	09.26±1.07*	08.28±0.92*	07.69±0.62*	05.97±0.66*
Day 3	12.02±1.34	09.69±0.26*	09.02±0.31*	08.27±0.14*	06.45±1.23*
Day 4	14.02±0.44	13.18±1.74†	11.41±0.51*	11.01±0.79*	08.93±0.30*
Day 5	15.36±1.55	14.79±0.328	13.03±1.97*	12.80±1.10*	10.61±1.95*
Day 6	24.7±0.55	23.56±0.51*	21.73±0.44†	18.53±0.48*	15.23±0.39*
Day 7	26.64±0.49	24.22±0.44*	22.64±0.50*	20.15±0.45*	18.13±0.30*
Fusarium keratoplasticum					
Day 1	04.50±0.52	04.18±0.70†	03.58±0.69†	03.24±0.28*	02.96±0.40*
Day 2	10.91±0.16	09.68±0.25*	08.98±0.49	06.69±0.42*	06.21±0.24*
Day 3	23.19±0.83	22.94±0.49†	21.94±0.19†	20.25±0.27*	18.37±0.66*
Day 4	37.51±0.64	36.92±0.87†	33.82±0.63*	31.26±0.66*	28.99±1.64*
Day 5	47.94±0.40	47.43±0.37†	42.43±0.45*	40.66±1.15*	36.82±0.63*
Day 6	63.66±0.52	55.87±0.33*	53.17±0.47*	50.17±0.42*	46.87±1.15*
Day 7	66.15±0.98	63.58±0.49*	61.60±0.48*	58.65±0.54*	56.15±0.66*

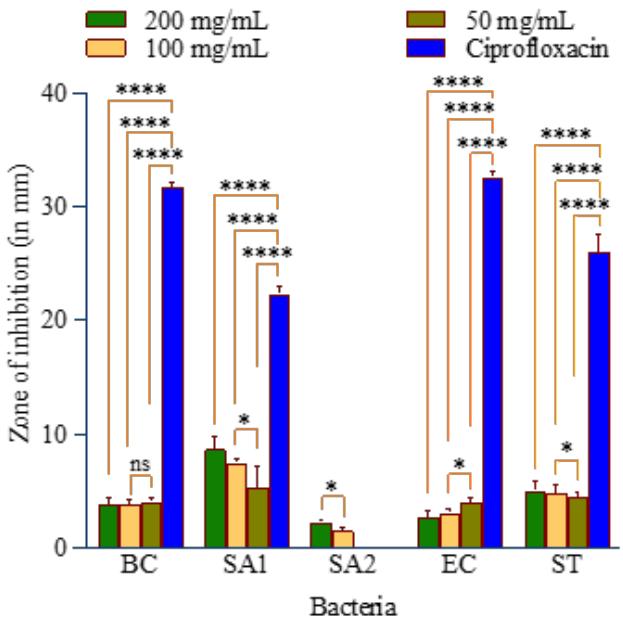

Figure 2: Comparative antibacterial activity of A. scholaris bark extract and ciprofloxacin against *Bacillus cereus* (BC), *Staphylococcus aureus* 1 (SA1), *Staphylococcus aureus* 2 (SA2), *Escherichia coli* (EC), and *Salmonella typhi* (ST). Values are in means±standard error of means (*n*=3); ****$p \leq 0.0001$; *$p < 0.05$, ns=not significant.

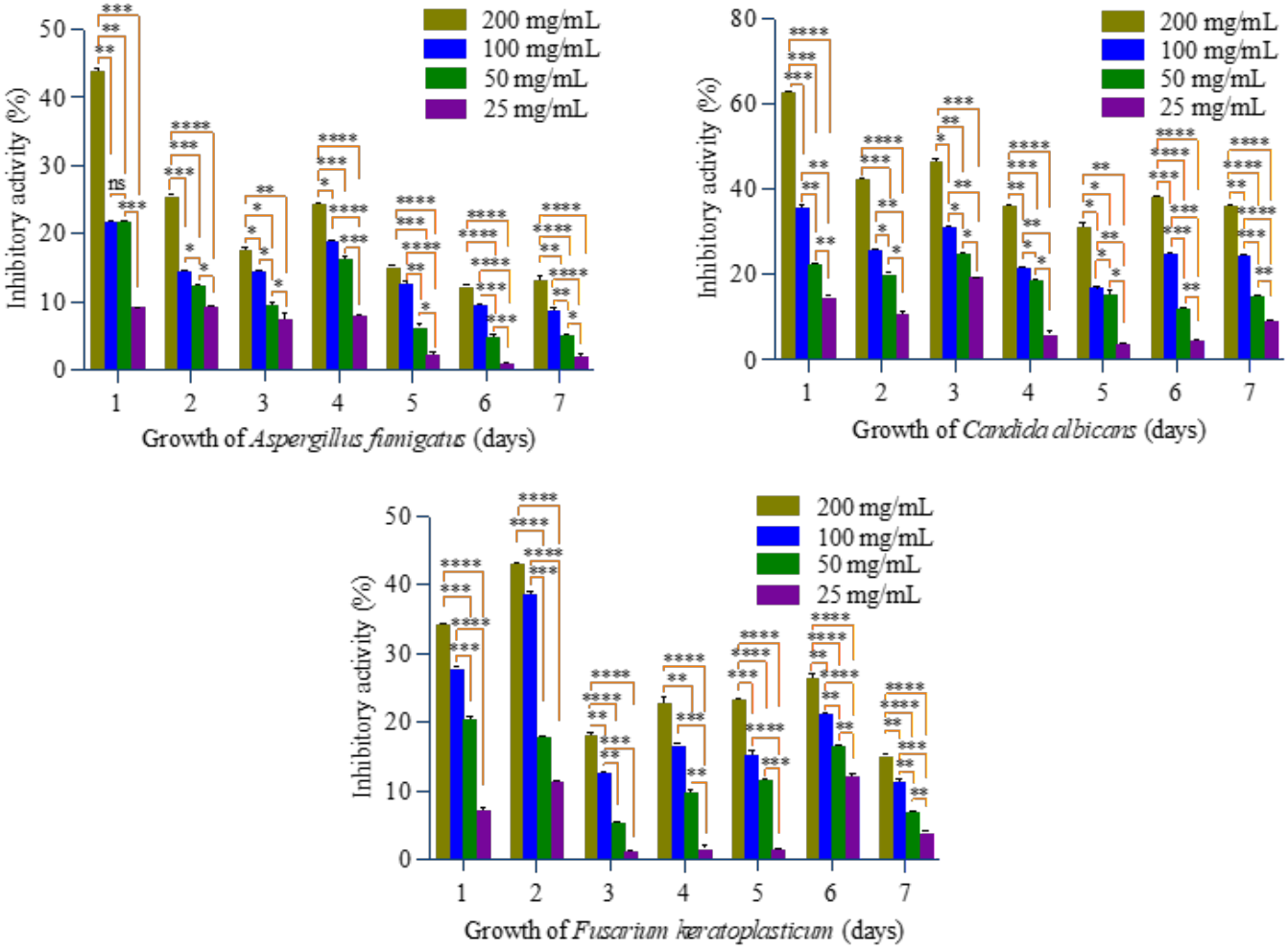

Figure 3: Comparison of the growth pattern of fungal species, *Aspergillus fumigatus, Candida albicans* and *Fusarium keratoplasticum* after treatments with different concentrations of *A. scholaris* bark extract. Values are in means±standard error of means (*n*=3); ****$p < 0.0001$; ***$p < 0.002$; **$p < 0.001$; *$p < 0.05$, ns=not significant.

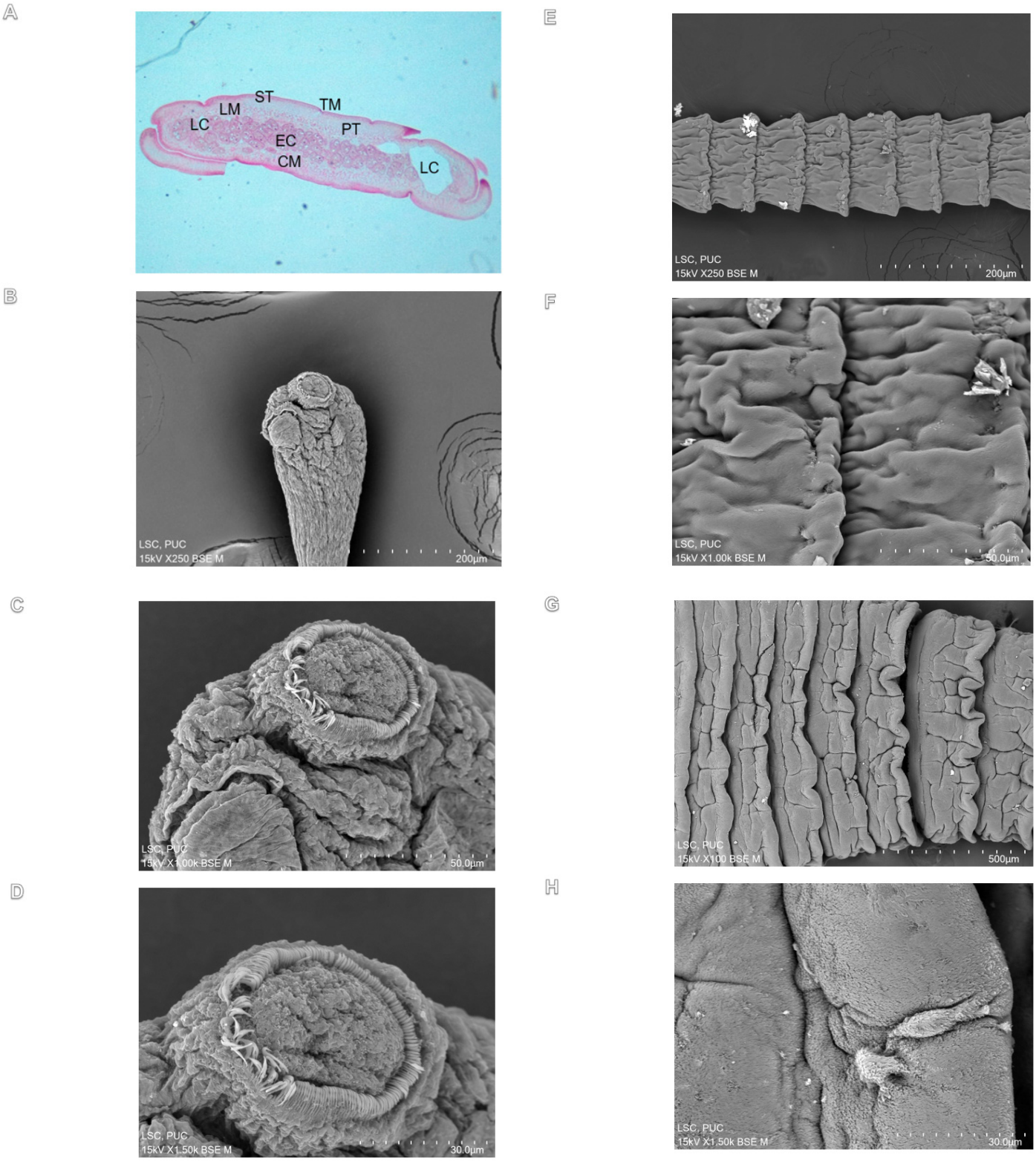

Figure 4: Anthelmintic effects of *A. scholaris* chloroform extract on *T. echinobothrida*. (A) Light micrographs of a histological section of a body segment showing the Tegument (TM), Sub-Tegument (ST), Longitudinal Muscle (LM), Circular Muscle (CM), Parenchymatous Tissue (PT), Lateral Canals (LC) and Egg Capsules (EC). (B) Scanning electron micrograph of the anterior scolex showing two lateral eye-like suckers. (C) The two suckers magnified with an apical rostellum. (D) A focus of the upper sucker. (E) The main body with mature segments. (F) A magnified view of two segments. (G) The egg-containing gravid proglottids. (H) A magnified portion of a gravid proglottid.

Table 4: Susceptibility of the tapeworm, *T. echinobothrida* to *A. scholaris* bark extract and albendazole.

Treatment media	Dose (mg/mL)	Survival value (h)	*t* Stat	*t* Critical
A. scholaris extract	5	32.34±2.81*	20.92	2.13
	10	14.99±1.40*	29.19	2.13
	20	05.48±0.41*	33.65	2.13
Albendazole	5	12.84±5.32*	20.98	2.13
	10	05.10±1.49*	70.26	2.13
	20	01.62±0.60*	82.90	2.13

*Significantly different at $p<0.05$ against negative control ($n=3$). Values are normalised to control and expressed in means±standard deviations.

The key finding of our study was that *A. scholaris* bark extract is an interesting broad-spectrum antimicrobial, not only active against both Gram-negative and Gram-positive bacteria, as well as important pathogenic fungi, but also effective against antibiotic-resistant strain. Among the bacterial species tested, its showed maximum efficacy against *S. aureus*, a Gram-positive species that is a causative pathogen of skin infections (boils and pimples), pneumonia, sepsis, and bone disease (osteomyelitis) and is notorious for its ability to develop resistance against all types of antibiotics.[58] Among the fungi tested, it exhibited highest activity against *C. albicans*, one of the most prevalent pathogenic fungi that is responsible for life-threatening immune disease and inflammation (candidiasis) of different organs of the body; it is also known to acquire antimicrobial resistance to different drugs.[59]

Many drugs and plant products are complicated by their limited activity such as target species specify, further restraining their applications. Even for broad-spectrum medications, there is a need to optimise their effects against different pathogens to circumvent further development of drug resistance.[60] An interesting observation for *A. scholaris* was that it was highly effective against *S. aureus* strain 2, which was totally resistance to ciprofloxacin. Further, anthelmintic effectiveness against an intestinal parasite established the therapeutic value of the plant. This observation is critically noteworthy in itself as anthelmintic resistance is one of the biggest issues in veterinary care and animal industry leading to excessive economic crisis.[61,62] A major turn of pharmaceutical research is looking for anthelmintic compounds from plant sources, many of which are being actively investigated.[63] Some of the phytocompounds are quite established for their potential drug development and have been advocated as alternative or complementary to standard medications.[64] The demonstrated broad-spectrum activity of *A. scholaris* therefore suggested that its bioactive principles could be a potential source of alternative antibiotic and anthelmintic so that the evidence in the present study espouse further chemical studies and pharmacological investigations.

CONCLUSION

Our finding indicate that *A. scholaris* bark contains bioactive compounds that are potentially important for health benefits. We report here for the first time that the plant is uniquely a broad-spectrum acting antimicrobial and anthelmintic agent, substantiating the folk medicine as in the Mizo traditional practice. It was not only active against selected Gram-positive and Gram-negative bacteria, but also specifically active against antibiotic-resistant strain. It was most active against *Staphylococcus aureus* among the bacteria, and *Fusarium keratoplasticum* among the fungi used in the experimental assays. We also demonstrated for the first time that the plant exhibits strong anthelmintic property as indicated by susceptibility test upon an intestinal helminth, *T. echinobothrida.* This is the first documentation of *A. scholaris* as a broad-spectrum antimicrobial and anthelmintic agent. However, the study does not provide information on the specific bioactive compound(s) responsible for such antimicrobial and anthelmintic activities, and thus, opens a vista for further research.

ACKNOWLEDGEMENT

The study was funded by the Department of Biotechnology, Government of India (BT/INF/22/SP41398/2021). Experiments were carried out at the DBT-BUILDER National Laboratory of Pachhunga University College.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***A. scholaris*:** *Alstonia scholaris*; ***A. fumigatus*:** *Aspergillus fumigatus*; **AAE:** Ascorbic acid equivalent; **ANOVA:** Analysis of Variance; **ATCC:** American Type Culture Collection; **BHT:** Butylated hydroxytoluene; **C. albicans:** *Candida albicans*; **DMSO:** Dimethyl sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **FCR:** Folin-Ciocalteu reagent; ***F. keratoplasticum*:** *Fusarium keratoplasticum*; **FRAP:** Ferric-reducing antioxidant power; **GAE:** Gallic acid equivalent; **IC_{50}:** Half-maximal inhibitory concentration; **QCE:** Quercetin equivalent;

PBS: Phosphate-buffered saline; ***T. echinobothrida*:** *Taenia echinobothrida*

SUMMARY

Chemical analysis of the chloroform extract of *A. scholaris* bark showed that the plant contains important secondary metabolites such as carbohydrates, phytosterols, reducing sugars, and saponins. It also contains substantial antioxidant molecules as indicated by the total flavonoid, total phenolic and total antioxidant tests. The antioxidant capacity was demonstrated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and Ferric-Reducing Antioxidant Power Assay (FRAP) assays. An important finding was that the plant extract showed broad-spectrum antimicrobial activity against all Gram-positive and Gram-negative bacteria, as well as pathogenic fungi used in the experiments. It showed highest potency against *Staphylococcus aureus* among the bacteria, and *Fusarium keratoplasticum* among the fungi. Anthelmintic susceptibility assay also indicated high effectiveness against an intestinal parasite, *T. echinobothrida*. This is the first documentation of *A. scholaris* as a broad-spectrum antimicrobial and anthelmintic agent.

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Cite this article: Lalngaihmanawmi, Lalthanpuii PB, Tlau L, Lalawmpuii L, Lalrosangpuii, Lalnundanga, *et al*. Phytochemical Insights and Bioactive Potentials of *Alstonia scholaris*, a Variety from Mizoram, India: Studies of the Antioxidant and Anti-Infective Properties. Pharmacog Res. 2025;17(3):896-908.