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Antioxidant and antiparasitic activities of *Schima wallichii* (DC.) Korth. from Mizoram, IndiaP.B. Lalthanpuui and K. Lalchhandama[◆]

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Abstract

Schima wallichii (DC.) Korth. is a well-known medicinal plant in the Mizo traditional medicine of Mizoram, India, and is used for the remedy of several parasitic infections including those of intestinal worms, blood flukes, and ectoparasites, among other medicinal applications. To study the medicinal properties of the plant, the bark extracts were prepared using solvents of having different polarities that included petroleum ether, chloroform and methanol. The antioxidant property was evaluated based on the total phenol content, total flavonoid content, total antioxidant content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity assay, hydroxyl radical-scavenging assay, and ferric reducing antioxidant power (FRAP) assays. The overall antioxidant activity was highest in the methanol extract, followed by chloroform extract, and lastly by petroleum ether extract. The antiparasitic activity was assessed using an intestinal roundworm, *Ascaridia galli*, and all extracts showed effectiveness. Scanning electron microscopy revealed structural damages throughout the body of the parasite. The findings thus support the traditional claim of the Mizo people and endorse further investigations.

1. Introduction

Plants are nature's gift to humankind for nutritional sources as well as for medicinal applications, and many plant products and botanical-derived compounds have been used in the therapeutic arsenal of different diseases. Considering their wide roles in the clinical management of various health conditions, medicinal plants are fittingly appreciated as one of the foundations of modern medicine (Süntar, 2020). Even the so-called synthetic pharmaceuticals, in fact 80% of them, are derived or developed from phytochemicals (Fitzgerald *et al.*, 2020). Despite the surge in scientific investigations of plants with technological advances, there is still a serious impediment in pinpointing the exact medicinal sources and the underpinning bioactive compounds (Warrier, 2021; Waris *et al.*, 2022). It is therefore, imperative that systematic analyses and experimentations are carried out bringing into focus the traditional applications of individual plant (Howes *et al.*, 2020).

Among the types of helminth infections, roundworm infection (nematodiasis) is the leading cause of morbidity and disability, especially affecting children in different parts of the world. Around 1.5 billion people are estimated to have the infection, of which over 1 million people developed a disability (Fauziah *et al.*, 2022), and more than 0.1 million die every year (Tinkler, 2020). Despite the lack of statistics on veterinary subjects, different roundworms are responsible for poor economic productivity, and the condition is exacerbated by the ever-increasing anthelmintic resistance in almost all parasite species (Fissiha and Kinde, 2021). There is a limited

number of antiparasitic medications in both clinical and veterinary managements of helminthiasis to combat health issues (Morales and Geary, 2020). The available drugs are synthetic compounds having restricted activity and adverse effects so that the quest for new anthelmintic drugs has been emphasised in botanical arenas (Van Der Kaaij *et al.*, 2022; Ahmed *et al.*, 2023).

Schima wallichii (DC.) Korth. is an evergreen tree in the family Theaceae and is native to Nepal, eastern India, China, Taiwan, and Indonesia (Lalhmingshlu and Jagetia, 2018; Widiyarti and Fitrianiingsih, 2019). Its bark extract has been documented to have anti-inflammatory, analgesic (Dewanjee *et al.*, 2009, 2011), antibacterial, antifungal (Choi *et al.*, 2011; Dewanjee *et al.*, 2008) and free-radical removing activities (Widiyarti and Fitrianiingsih, 2019). Kaempferol 3 O rhamnoside isolated from the leaves was shown to have anticancer property by inhibiting MCF-7 breast cancer cell (Diantini *et al.*, 2012), and antimalarial activity against chloroquine resistant *Plasmodium falciparum* (Barliana *et al.*, 2014). In India, its bark is traditionally used as an antipyretic, antiseptic, and wound healing agent (Barma *et al.*, 2015). In the Mizo traditional medicine, its bark and leaves are commonly used as an antiparasitic agent for intestinal, blood and skin infections (Lalhmingshlu and Jagetia, 2018). Based on the known traditional values of the plant, its antioxidant and potential antiparasitic activities were evaluated.

2. Materials and Methods

2.1. Plant specimen and extracts

Schima wallichii (DC.) Korth. was collected from Pachhunga University College campus in Aizawl, Mizoram, India, located at 23.7233° N, 92.7271° E (Figure 1). The fresh leaves and flowers were used for preparing a herbarium, which is catalogued as PUC-S-18-01 at Pachhunga University College. The barks of *S. wallichii* were peeled off, cleansed in demineralised water, chopped into fine pieces and kept in the shade to dry for four weeks. The dried samples

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(400 g) were packed into the Soxhlet apparatus for extraction using solvents of decreasing polarity, such as methanol > chloroform > petroleum ether. Extraction was run for 72 h in each solvent. A

vacuum rotatory evaporator, Buchi Rotavapor® R-100, was used to concentrate the extracts. The final extracts were refrigerated at 4°C for chemical and biological assays.



Figure 1: *S. wallichii* at Pachhunga University College, adjacent the Central Library.

2.2 Total phenolic content

The total phenolic content was estimated after the method of Singleton and Rossi (1965). 1 ml of the plant extracts (from a stock of 100 mg/ml) and standard gallic acid of increasing concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were mixed with 5 ml of Folin-Ciocalteu reagent (FCR). The reaction was allowed to run for 3 min. 4 ml of sodium carbonate was added to each and then left undisturbed at room temperature for 1 h. A blank concentration was made from 1 ml of methanol, 5 ml of FCR, and 4 ml of sodium carbonate. The sample absorbances were measured at 765 nm wavelength in a UV-Vis spectrophotometer. A standard curve for gallic acid was generated. The total phenolic content was calculated as gallic acid equivalent in milligrams per gram (GAE mg/g) of dry weight of the plant sample. The experiment was done in triplicate.

2.3 Total flavonoid content

The total flavonoid content was determined by the aluminium chloride assay of Zhishen *et al.* (1999). The plant extracts (1 ml) and quercetin at varying concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were prepared. 2 ml of distilled water was added to all the samples. After letting them remain for 5 min, 3 ml of 5% sodium nitrite with 0.3 ml of 10% aluminium chloride was added. The reaction was allowed for 6 min, after which 2 ml of 1 M sodium hydroxide was added. All samples were made to 10 ml by adding distilled water. After 1 h, the absorbances were measured at 510 nm. From the quercetin standard graph, the total flavonoid concentration was calculated and presented as quercetin equivalent in milligrams per gram (QE mg/g) of the dry weight of the sample.

2.4 Total antioxidant content

The method of Prieto *et al.* (1999) was used to evaluate the total antioxidant activity. The plant extracts (1 ml) and increasing concentrations of ascorbic acid (at 10, 20, 40, 60, 80, and 100 µg/ml) were mixed with 3 ml of a reagent. A reagent solution was prepared from a mixture of 4 mM ammonium molybdate, 0.6 M sulphuric acid, and 28 mM sodium phosphate. They were incubated for 90

min at 95°C. After allowing them to cool down to room temperature, the absorbances were taken at 695 nm. From the ascorbic acid calibration curve, the total flavonoid amount was worked out as ascorbic acid equivalent in milligrams per gram (AAE mg/g) of the dry weight of the sample.

2.5 DPPH free radical-scavenging activity assay

The antioxidant activity was assessed using the method developed by Blois (1958). The plant extract and standard butylated hydroxytoluene (BHT) were prepared at concentrations such as 10, 20, 40, 60, 80, and 100 µg/ml. 3 ml of all the samples were added to 0.5 ml of 1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl). The control sample was made by mixing 3 ml of methanol with 1 ml of DPPH. The samples were incubated and allowed to react for 30 min at 37°C. Absorbances were recorded at 517 nm. The following formula was then used to translate absorbance into antioxidant activity:

$$\text{Free radical-scavenging activity (\%)} = \frac{AC - AS}{AC} \times 100$$

where, AC represents absorbance of the control sample, while AS is of the sample extract or standard.

2.6 Hydroxyl radical-scavenging assay

Hydroxyl radical (HO•)-scavenging assay was performed according to the protocol of Halliwell and Gutteridge (1989). Different concentrations (10, 20, 40, 60, 80, and 100 µg/ml) were prepared for the plant extracts and ascorbic acid by dissolving in distilled water. To each concentration, 0.1 ml of 1 mM EDTA, 0.01 ml of 10 mM FeCl₃, 0.1 ml of 10 mM H₂O₂ and 0.36 ml of 10 mM deoxyribose were added. Further, 0.33 ml of phosphate buffer (pH 7.4) and 0.1 ml of 0.1 mM ascorbic acid were added and then incubated for an hour at 37°C. 1 ml of each incubated solution was mixed with 1 ml of trichloroacetic acid and 1 ml of 0.5% thiobarbituric acid. The solutions were then heated to 80°C for 10 min to develop pink colour. After cooling at room temperature, the absorbance was taken at 532 nm.

The results were calculated as inhibition in percentage of the deoxyribose using the following formula:

$$\% \text{ of HO}\cdot \text{ scavenging} = \frac{AC - AS}{AC} \times 100$$

2.7 Ferric reducing antioxidant power (FRAP) assay

A modified method by Oyaizu (1986) was used to assess the reducing (antioxidant) power. Ascorbic acid, a standard antioxidant, and the plant extract were prepared in different concentrations, such as 10, 20, 40, 60, 80, and 100 µg/ml. To 1 ml of all the samples, 2.5 ml of phosphate buffer (6.6 pH) and 2.5 ml of 10% potassium ferricyanide were added. The solutions were centrifuged for 10 min at 3000 rpm. The supernatant portion (2.5 ml) was collected from each sample and diluted with 2.5 ml of distilled water. After mixing with 0.5 ml of 0.1% ferric chloride, the reaction was allowed to undergo. 1 ml of distilled water, 2.5 ml of phosphate buffer, and 2.5 ml of potassium ferricyanide were mixed to make the blank concentration. After 10 min, absorbance was taken at 700 nm against the blank concentration.

2.8 Antiparasitic test

The antiparasitic activity was tested on a roundworm, *Ascaridia galli*, following the method of Lachhandama *et al.* (2009). Live parasites were collected from the intestines of local fowls, *Gallus gallus domesticus*. They were exposed to the plant extracts and standard drug, albendazole, at the concentration of 20 mg/ml in a microbiological incubator maintained at $37 \pm 1^\circ\text{C}$. 0.9% neutral phosphate-buffered saline supplemented with 1% dimethylsulfoxide was used to prepare the extract, albendazole as the positive control, and the negative control samples. The antiparasitic activity was assessed in terms of survival in the culture media. The data were analysed by Student's *t*-test, with the level of significance considered at a *p* value less than 0.05.

2.9 Scanning electron microscopy

The roundworms treated with the plant extract were processed for scanning electron microscopy following the method of Lalthanpui and Lachhandama (2020). They were fixed in 10% neutral-buffered formaldehyde at 4°C for 4 h. Acetone was used for dehydration. After treating with tetramethylsilane, they were dried in an air-drying chamber at 25°C . After sputter coating with gold in JFC-1100 (JEOL Ltd., Tokyo, Japan), they were observed under a JSM-6360 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an electron accelerating voltage of 20 kV.

3. Results

3.1 Total phenolic content

The total phenolic content of the methanol (MES), chloroform (CES) and petroleum ether (PES) extracts of *S. wallichii* bark was estimated based on Folin-Ciocalteu reaction. Gallic acid as a standard compound indicated concentration-dependent antioxidant activity as shown in Figure 2. From the gallic acid calibration curve, the amount of phenols

was found to be 15.71 GAE mg/g of the dry weight of the sample for MES, 3.47 for CES, and 0.47 for PES.

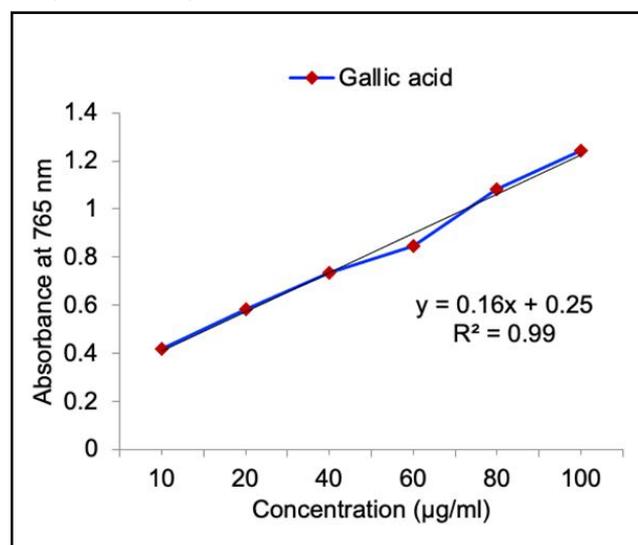


Figure 2: Standard calibration curve for gallic acid from Folin-Ciocalteu reaction.

3.2 Total flavonoid content

From the linear graph of quercetin as given in Figure 3, the flavonoid concentration was determined as 97.79 QE mg/g of the dry extract for MES, 9.56 for CES, and 3.68 for PES.

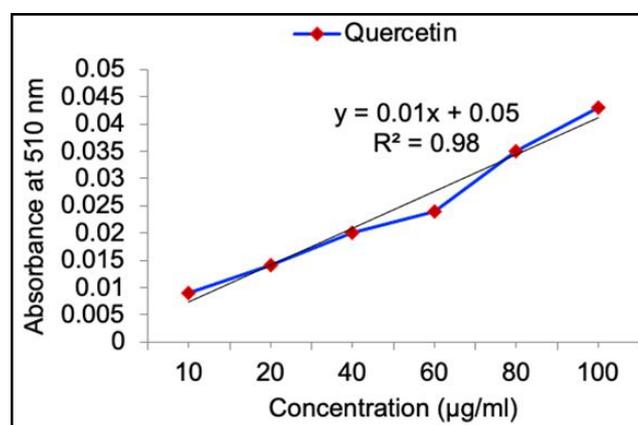


Figure 3: Standard calibration curve for quercetin from aluminium chloride reaction.

3.3 Total antioxidant content

From the ascorbic acid calibration curve as given in Figure 4, the total antioxidant content was calculated as 188.20 AAE mg/g of the dry extract for MES, 35.53 for CES, and 7.14 for PES.

3.4 DPPH free radical-scavenging assay

S. wallichii bark extracts showed clear concentration-dependent antioxidation reaction, as indicated in Figure 5. MES was the most active with an activity comparable to that of the standard BHT. From the log dose, the half-maximal inhibitory concentration (IC_{50}) value was determined as 5.60 µg/ml for BHT, 21.56 µg/ml for MES, 155.03 µg/ml for CES, and 52.38 µg/ml for PES.

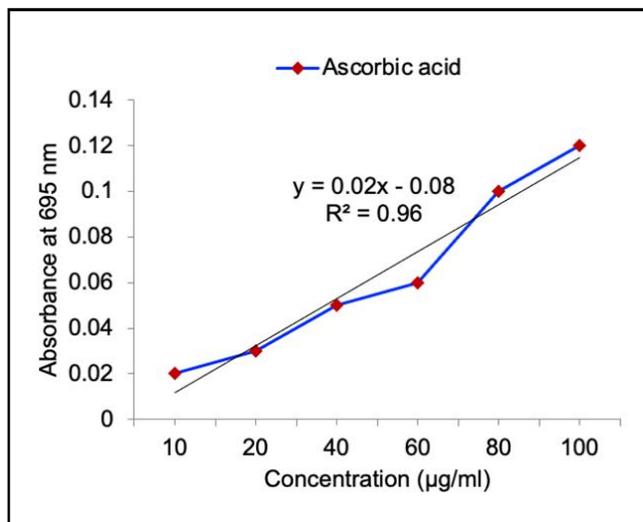


Figure 4: Standard calibration curve for ascorbic acid from ammonium molybdate reaction.

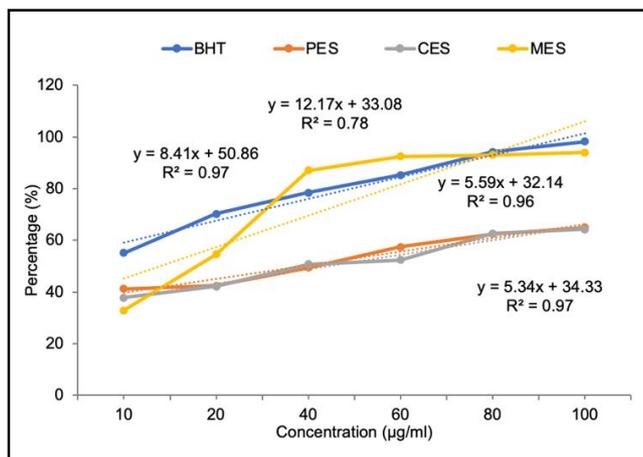


Figure 5: DPPH-scavenging activity of butylated hydroxytoluene (BHT), petroleum ether (PES), chloroform (CES) and methanol (MES) extracts of *S. wallichii*.

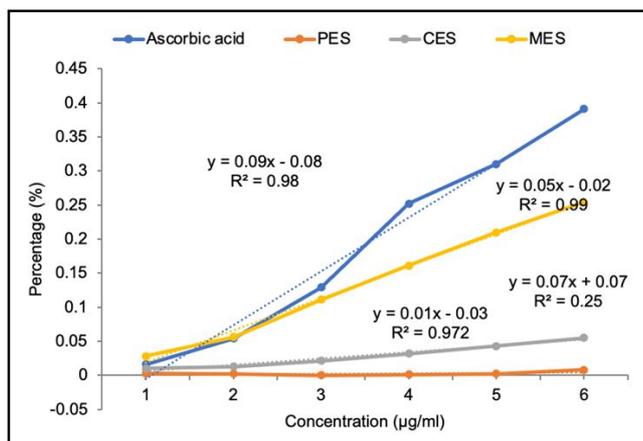


Figure 6: Hydroxyl radical (HO•)-scavenging activity of ascorbic acid, petroleum ether (PES), chloroform (CES) and methanol (MES) extracts of *S. wallichii*.

3.5 Hydroxyl radical-scavenging assay

Hydroxyl radical (HO•)-scavenging activity of *S. wallichii* extract is shown in Figure 6. MES was almost as potent as the ascorbic acid, while PES failed to indicate appreciable activity. The IC₅₀ value was 4.13 µg/ml for ascorbic acid, 10.83 µg/ml for MES, and 33.81 µg/ml for CES.

3.6 FRAP assay

The potassium ferricyanide reducing power of *S. wallichii* extracts is shown in Figure 7. The plant extracts and standard ascorbic acid both showed increasing antioxidant activity corresponding to increased concentration of the samples. However, the plant extract was less effective than ascorbic acid at each concentration tested.

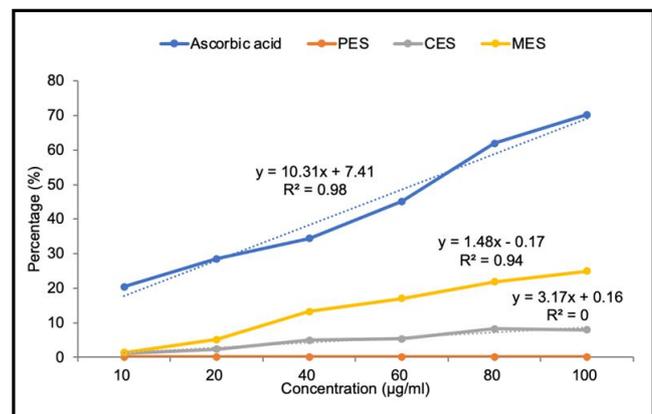


Figure 7: Ferric-scavenging activity of ascorbic acid, petroleum ether (PES), chloroform (CES) and methanol (MES) extracts of *S. wallichii*.



Figure 8: Scanning electron microscopic image of *Ascaridia galli* treated with *S. wallichii* bark extract. The portion shows the anterior end with massive shrinkage of the cuticle and constriction of the head parts.

3.7 Antiparasitic test

The model parasite, *A. galli*, responded well to the drug, albendazole, and the different extracts of *S. wallichii*, as given in Table 1. The prescription dosage of albendazole, i.e., 20 mg/ml was consistently

used for all the treatments. Relative (normalised with respect to the survival of the control group) survival values indicate that the roundworms died at 86.64 h in MES, at 72.65 h in CES, and at 77.92 h in PES.

Table 1: Antiparasitic activity of albendazole and *S. wallichii* bark extract on *Ascaridia galli*

Treatment media	Dose (mg/ml)	Normalised survival time (hour) in mean \pm SD	<i>t</i> value	<i>t</i> critical value
Negative control	0	100.00 \pm 2.00	NA	NA
Albendazole	20	001.78 \pm 0.44*	149.72	2.45
Methanol extract	20	086.64 \pm 1.81*	013.74	2.23
Chloroform extract	20	072.65 \pm 1.69*	019.83	2.26
Petroleum ether extract	20	077.92 \pm 2.25*	029.27	2.22

*Significantly different at $p < 0.05$ against negative control at $n = 6$; NA = not applicable.

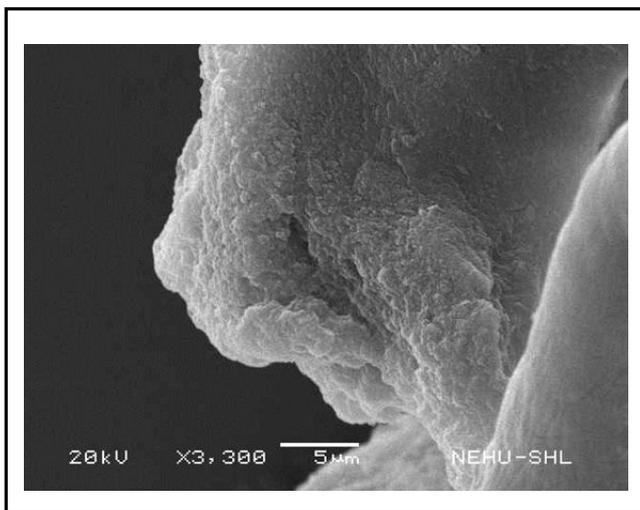


Figure 9: The mouthpart of *A. galli* treated with *S. wallichii* bark extract indicating distortion of the smooth lip and disappearance of the denticles.

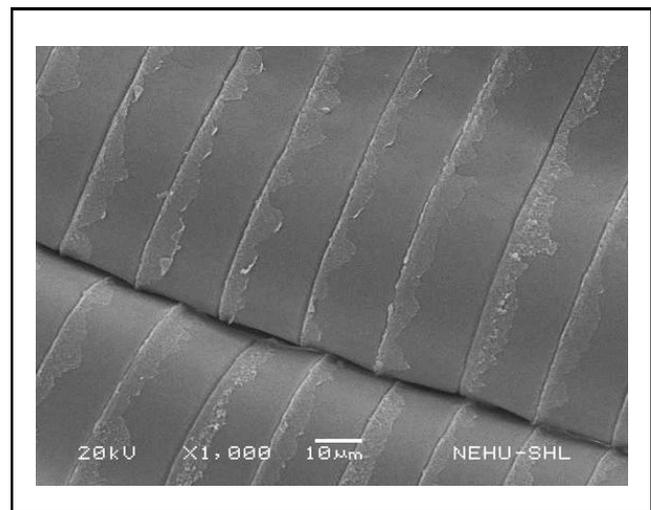


Figure 11: The main body of *A. galli* treated with *S. wallichii* bark extract showing a magnified portion of the cuticle. The transverse lines called annulations are surrounded by scars and eroded portions.

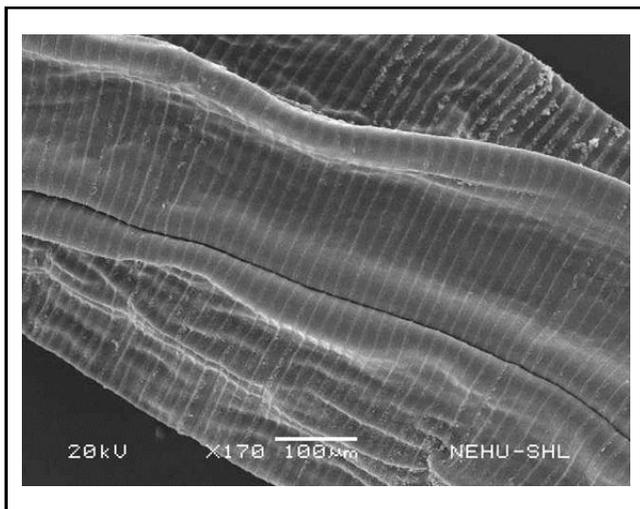


Figure 10: The main body of *A. galli* treated with *S. wallichii* bark extract showing distortion of the general cuticular surface and distortion of the otherwise smooth body covering.

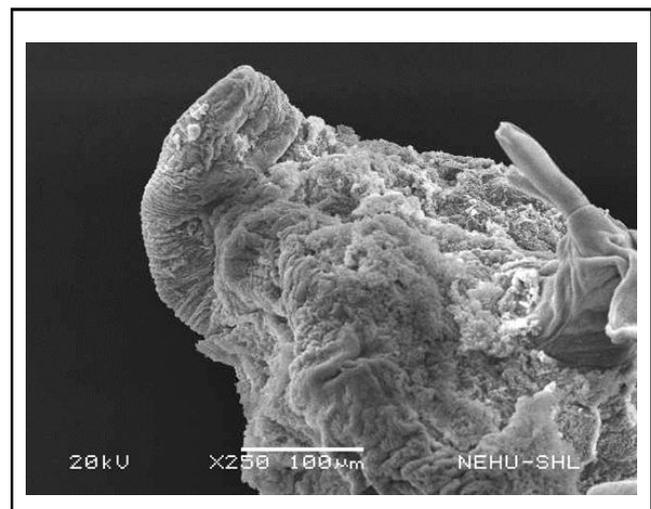


Figure 12: The main body of *A. galli* treated with *S. wallichii* bark extract showing distortion of the general cuticular surface and distortion of the otherwise smooth body covering.

3.8 Scanning electron microscopy

The roundworms treated with 20 mg/ml of MES appeared severely damaged. The head part shown in Figure 8 reveals extensive shrinkage and folding of the cuticle leading to loss of the normal smooth topology. The extent of compression indicates that the underlying anatomical tissues and muscle layers were completely devastated. The mouth region (Figure 9) where a rounded lip and rows of denticles should be present shows only barren and warty surface. The general body is also entirely shrunk (Figure 10), and the transverse rings called annulations are marked with irregular scars and eroded surface (Figure 11). The extreme posterior end (Figure 12) shows complete obliteration of the cuticle along with posterior organs including the anal opening, or cloaca. Two tiny stick-like projections, called spicules, which are the external reproductive organs, are also blunted.

4. Discussion

Plants are our mainstay diet for nutraceutical requirements and therapeutic necessities. Their cohorts of metabolites are bioactive chemicals that are directly or indirectly vital molecules for normal cellular metabolism and functional activities, especially those that are critical in the development of debilitating diseases (Verma *et al.*, 2022; Rani *et al.*, 2023). Our data indicate that *S. wallachii* contains valuable phytochemicals that may be lead molecules for nutraceutical compounds. Plants have been a major group of sources various therapeutic medications and will remain so in the future investigations for novel drugs (Süntar, 2020; Huang *et al.*, 2022).

Our primary observation that *S. wallachii* possesses antioxidant property and exhibit free radical-scavenging activity is pharmacologically relevant. Free radicals or reactive oxygen species are biochemical products of normal metabolic reactions in our cells, but they become detrimental when they stockpile in the cytoplasm. They begin to wreak havoc by thwarting the integrity of the fundamental cellular constituents such as DNA, RNA, proteins, and lipids (Ali *et al.*, 2020), thereby leading to the actuation of chronic ailments such as cancer, blood disorders, and heart diseases (Hajam *et al.*, 2022). Plants in our diet are the primary source of antioxidants to counterbalance cellular oxidative stress (Nwozo *et al.*, 2023). DPPH and FRAP assays are reliable chemical tests to assess antioxidant activity (Mohammadnezhad *et al.*, 2023). From DPPH and FRAP assays, we noted that *S. wallachii* exhibits powerful antioxidant activity, with the methanol extract being the richest source of the antioxidants. It is known that extraction solvents influence antioxidant levels (Nidhi *et al.*, 2021; Aggarwal *et al.*, 2022)

Roundworms are unique parasites in that their body is protected by a hard but flexible cuticle that acts as a tough exoskeleton maintaining an effective barricade from external factors such as chemicals and digestive enzymes of the host, the property of which is essential for an effective parasitic adaptation (Basyoni and Rizk, 2016). In contrast to other parasites like tapeworms (cestodes) and flukes (trematodes), they have a complete digestive system. Yet, like in all other parasites, any antiparasitic drug acts through the body surface by transcuticular diffusion (Lifschitz *et al.*, 2017). In this way, the principal drug activity is primarily observed in the structural alterations of the cuticle. In different roundworms, the drug effects are noted as shrinkage, cracking, blistering and sometimes erosion of the cuticle (Chaweeborisuit *et al.*, 2016; Shanti *et al.*, 2016; Njom *et al.*, 2021).

A. galli is an excellent roundworm model for antiparasitic assay as it is resilient and easily maintained in culture; moreover, susceptible to cuticular damage upon drug treatments (Lalthanpuii and Lalchandama, 2020; Lalthanpuii *et al.*, 2020). The untreated individuals show three protruding lips and smooth cuticle on the head end, while fine transverse corrugations called annulations are regularly present throughout the body length. The posterior terminal is slantly tapering with an anal opening near the tip of the pointy end (Lalchandama, 2010). We have shown that *Acacia oxyphylla* extract caused loosening and collapse of the lips, shrinkage and irregular wrinkles of the cuticle (Lalchandama *et al.*, 2009); while *Milletia pachycarpa* extract caused general shrinkage, cracks, lacerations and scars on the cuticle (Lalchandama, 2019). These observations are in line with the present findings in which *A. galli* displayed cuticular distortion and damages after treatment with *S. wallachii* extract, thus, vindicating the traditional claim as an antiparasitic agent.

5. Conclusion

S. wallachii bark extracts showed considerable antioxidant properties. All three extracts; namely, methanol, chloroform and petroleum ether extracts contained antioxidant components. The methanol extract had the highest contents of total phenols, flavonoids, and antioxidants. The methanol extract also showed highest activities in scavenging free radicals based on DPPH scavenging, hydroxyl scavenging and potassium ferricyanide reducing assays. The three extracts exerted antiparasitic activity on the roundworm, *A. galli*. Scanning electron microscopic observation showed that the plant extract caused extensive damage to the body of the parasite, characteristic of the antiparasitic effects of drugs.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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