Phenolic and Flavonoid Compounds from Leaves and Branches of *Schotia brachypetala* for the Development of Biofungicide for Wood Protection

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GRAPHICAL ABSTRACT



Phenolic and Flavonoid Compounds from Leaves and Branches of *Schotia brachypetala* for the Development of Biofungicide for Wood Protection

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The extracts of Schotia brachypetala were tested against the molecularly identified fungi Alternaria alternata, Botrytis cinerea, and Fusarium oxysporum, which cause early blight of tomatoes, gray mold of cucumber immature fruits, and Fusarium wilt, respectively. Leaves and branches of S. brachypetala were extracted using acetone and bio-assayed for their antifungal activity at 2%, 4%, and 6% when applied to white mulberry wood samples. Using high-performance liquid chromatography analysis, the most abundant compounds in leaf extract were kaempferol (37900 µg/g extract) and gallic acid (7480 µg/g extract), and in branch extract were gallic acid (3120 µg/g extract) and chlorogenic acid (1320 µg/g extract). By increasing the extract concentration to 6%, the percentage inhibition of fungal mycelial was significantly increased compared to the positive (Cure-M) and negative control samples. This study indicates that extracts from leaves and branches of S. brachypetala can be effective as bio-based agents in wood protection and that they can prevent the growth of pathogenic fungi.

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Keywords: Schotia brachypetala; Leaf extract; Branch extract; Antifungal activity; HPLC; Phenolic compounds; Flavonoid compounds; Gray mold; Fusarium wilt; Early blight

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INTRODUCTION

Schotia brachypetala Sond. is a deciduous tree that grows to a medium to large size and belongs to the Caesalpinaceae family. It is native to southern Africa (Coates Palgrave 1997). *Schotia brachypetala* is one of four species and three hybrids of *Schotia* in southern Africa (Arnold and Wet 1993). Roasted seeds of all species are edible (Watt and Breyer-Brandwijk 1962). The plant, especially the bark and root, are used in traditional medicine and to treat diarrhea and dysentery (Gelfand 1985; Hutchings *et al.* 1996; Coates Palgrave 1997; McGaw *et al.* 2002a).

In an initial screening, the antibacterial activity of *S. brachypetala* leaves was found to be higher than that of the roots (McGaw *et al.* 2002a); thus, activity-directed fractionation from the leaf extract was carried out once more to specify the most bioactive compounds. The bark and roots of *S. brachypetala* are used for nervous conditions (Van

Wyk and Gericke 2000). Acetylcholinesterase (AChE) inhibition was seen in the extracts from the roots and bark of *S. brachypetala* (Adewusi *et al.* 2011). The promising antityrosinase and antibacterial activities of *S. brachypetala* root bark extract, together with its low to moderate cytotoxicity against HaCat cells (a human epidermal keratinocyte line), were reported (Lall *et al.* 2019).

The dried leaves ethanol extract contained what are thought to be the active fractions: methyl-5,11,14,17-eicosatetraenoate and 9,12,15-octadecatrienoic (linolenic acid), both of which showed antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (McGaw *et al.* 2002b). Furthermore, at 0 and 24 h of bacterial development, the acetone extracts of *S. brachypetala* reduced the formation of biofilms from *Enterococcus faecalis* by 113% and 135%, respectively (Khunoana *et al.* 2019).

Several plant extracts have been used to manage fungal diseases that infect plants. *Leucaena leucocephala* (Lam.) is one such extract that is used to combat *Rhizoctonia solani*, *Fusarium solani*, and *Alternaria solani* (Elbanoby *et al.* 2024). *Eucalyptus camaldulensis* and *Origanum majorana* essential oils were tested against *Fusarium oxysporum* (Shakam *et al.* 2022). This study focused on three common plant pathogenic fungi, namely *Alternaria alternata*, *Fusarium oxysporum*, and *Botrytis cinerea*. One of the most prevalent fungi, *B. cinerea*, can cause gray mold in greenhouses and cause significant fruit losses in cucumber plants (Kumar *et al.* 2016; Ziedan *et al.* 2022). The tomato (*Solanum lycopersicum* L.) is particularly susceptible to several fungi that can cause significant reductions in tomato yield, such as fusarium wilt (*F. oxysporum*) (Sharma 2023). One of the pathogens that most drastically reduces agricultural productivity is *Alternaria alternata*, which causes tomato early blight disease and harms crops all over the world (Sharma *et al.* 2021).

Root rot disease, primarily caused by *Fusarium* spp. species, is a severe problem for the commercially important mulberry (Nguyen *et al.* 2019), which is grown for its nutritious leaves that are needed to generate the most valuable silkworm cocoons. According to Beevi and Qadri (2010), this disease diminishes acreage and produces significant damage. Fig fruit disease is caused by *A. alternata* (Anwaar *et al.* 2022). More than 200 plant species are susceptible to gray mold disease, which is caused by the fungus *B. cinerea*. According to AbuQamar *et al.* (2016), this fungus is one of the primary pathogenic fungi that can infect mulberries. These pathogens can be identified through Internal Transcribed Spacer (ITS) ribosomal RNA (rRNA) sequencing, molecular identification has emerged as one of the most significant and sophisticated techniques for fungus identification (Abd-Elkader *et al.* 2021; Ziedan *et al.* 2022).

Synthetic fungicides, which are the cornerstone of fungal disease control schemes, are the most effective way to minimize fruit fungus deterioration or stains. These days, growing worries about wood safety and environmental issues have led to the development of creative and eco-friendly control techniques, specifically involving natural extracts (Singh and Singh 2012; Broda 2020; Kirker *et al.* 2024; Taha *et al.* 2024), and their use for nanomaterials as antifungal agents (Borges *et al.* 2018; Salem 2021; Khadiran *et al.* 2023; Elshaer *et al.* 2024). Along with their protective qualities, the extract's phenolic components helped slow down the aging process of the wood by lowering light-induced and other-caused deterioration (Alonso-Hurtado *et al.* 2024). The plant extracts were investigated for their effects on anatomical features and specific gravity of wood species (Atılgan *et al.* 2013; Atılgan 2023).

Mulberry wood's ease of burnishing and varnishing and its flexibility and elastic properties when steam-cooked make it a valuable material for the sporting equipment

sector. Mulberry wood produces most hockey sticks, tennis, and badminton rackets. Mulberry planks can also be used to make wood accessories, furniture, and beautiful veneers. Paper is made in China and Europe from the very fibrous stem bark of the white mulberry (Lochynska and Oleszak 2011). In Beni-Suef, Egypt, it is a year-round wild plant that grows in abundance (Hussein *et al.* 2010). According to reports, the wood is long-lasting (Se Golpayegani *et al.* 2010; Se Golpayegani 2011). The methanol extract from *M. alba* heartwood showed good inhibition against the growth of *Aspergillus niger* at a concentration of 32 μ g/mL (Mansour *et al.* 2015a).

The authors' ongoing work assesses the antifungal effect of extracts from *S. brachypetala* leaves and branches against the growth of three common mold fungi: *Alternaria alternata, Botrytis cinerea,* and *Fusarium oxysporum.* This was done as part of the ongoing study using natural products to develop an eco-friendly wood bio-preservative against mold fungi. Therefore, in the present work, the commonly used local wood species white mulberry was used to study the effect of extracts from leaves and branches of *Schotia brachypetala* as bio-fungicides. Furthermore, a high-performance liquid chromatography (HPLC) approach was used to assess the phenolic and flavonoid components present in the extracts.

EXPERIMENTAL

Materials

The biomass parts (leaves and branches) of Schotia brachypetala Sond. were collected from Antoniadis Garden, Alexandria, Egypt. The plant was identified at the Timber Trees Research Department, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt, by the coauthor Nashwa H. Mohamed and vouchered with number Z0013. Leaves and branches were first air-dried at room temperature and then ground to powder. The particles that were passed through a 0.4-mm sieve (40-mesh) and retained on a 0.27-mm sieve (60-mesh) were used for the extraction. Acetone solvent was used for the extraction, where about 100 g of each powdered material were soaked in 200 mL of acetone solvent (99.99%, technical grade-Merck) for three days, separately in a stoppered conical flask (500 mL). After this period, the extracted biochemical compounds were filtered with a cotton plug and Whatman filter paper No. 1 to afford the acetone extracts. The extracts were concentrated in a vacuum using a rotary evaporator. The extract percentage was calculated as follows: Extract percent = [extract amount (g)/air-dry leaf or branch sample amount (g)] \times 100. The percentages of extracts were 1.23% and 2.5% from branches and leaves, respectively. The concentrated extracts were poured into Petri dishes and air-dried before further analysis (Elbanoby et al. 2024). The plant extracts were stored in a refrigerator at 4°C in airtight glass vials (Eldesouky et al. 2024).

Methods

Isolation of fungal pathogens

The pathogens of early blight and fusarium wilt of tomato and gray mold of cucumber fruits were isolated. To meet the development requirements of the organisms, the fungal pathogen was isolated from tissues using potato dextrose agar medium (PDA media) and incubated at 26 ± 1 °C for a week (Klug *et al.* 2024).

Molecular characterization of genomic DNA

Genomic DNA was extracted from three pure isolates of *Botrytis* spp., *Fusarium* spp., and *Alternaria* spp. grown on PDA, and their genomic DNA was extracted using the rapid micro preparation technique (Shakam *et al.* 2022). Using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGA TATGC-3'), the ITS DNA region of these isolates was amplified by polymerase chain reaction (PCR). The conserved ribosomal internal transcribed spacer (ITS) region was used to identify fungal cultures (Moore *et al.* 2011; Mohamed *et al.* 2021; Salem *et al.* 2021), and three isolates' amplified ITS1-5.8s and ITS2 regions (500 to 700 bp) of selected *Botrytis* spp., *Fusarium* spp., and *Alternaria* spp. isolates were sent for sequencing (Macrogen, Scientific Services Company, Korea) (Kumar *et al.* 2016). Using the BLAST search on the National Centre for Biotechnology Information (NCBI), the sequences were compared to those in GenBank (http://www.ncbi.nlm.nih.gov).

Antifungal activity

First, the acetone extracts of S. brachypetala leaves and branches were prepared in concentrations of 2%, 4%, and 6% by dissolving the respective amounts (g) in dimethyl sulfoxide (10% DMSO) in stock solutions of 100 mL. Air-dried wood blocks of 2 (L) \times 2 (R) \times 0.7 cm³ from white mulberry (*Morus alba* L.) with a moisture content range from 8.12 to 11.2% were autoclaved at 121 °C for 20 min and left to cool (Abo Elgat et al. 2021). A culture of Alternaria spp., Botrytis spp., and Fusarium spp., each aged seven days, was cultivated on PDA medium. The PDA medium was added to the Petri dishes and left to settle in the incubator. The concentrations of acetone extracts (2%, 4%, and 6%) were applied to wood samples in conditions of surface sterilization using Laminar Flow Sterile Cabinets. Each wood sample was given 150 µL of the concentrated acetone extracts and positive and negative samples. After covering the PDA medium with all the treated wood blocks, each fungus was immediately inoculated onto a Petri dish using a disc that measured 5 mm in diameter. The dishes were then cultured for 10 days at 25 ± 1 °C. Every treatment was assessed three times. The samples were treated as a negative control sample with 10% DMSO and as a positive control with the commercial fungicide Cure-M 72% WP (Mancozeb 64%+ Metalaxyl 8%). The percentage of mycelial growth inhibition was measured with the following formula (Ashmawy et al. 2020; Shakam et al. 2022); MGI = $[(A_c - A_t) / A_c] \times 100$; where MGI is the mycelial Growth reduction (%) and A_c and A_t are average diameters of the fungal colony of the control and treatment, respectively.

HPLC conditions

The HPLC analysis of the acetone extract was carried out using an Agilent 1260 series device. The separation was performed using a Zorbax Eclipse Plus C8 column (4.6 mm \times 250 mm, id, 5 µm film thickness). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 mL/min. A mobile phase linear gradient program was implemented with a step size of 1 min and durations of 5, 8, 12, 15, 16, and 20 min, using (A) concentrations of 82, 80, 60, 60, 82, 82, and 82%, respectively. The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µL for each of the sample solutions (redissolved in acetone). The column temperature was maintained at 40 °C (Hamzah *et al.* 2024). Standard HPLC-grade phenolic and flavonoid compounds were used (Fig. 1), including gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, pyrocatechol, rutin, ellagic acid, *p*-coumaric acid, vanillin, ferulic acid, naringenin, rosmarinic acid, daidzein, quercetin,

cinnamic acid, kaempferol, and hesperetin. The identification of compounds was confirmed by comparing their retention time with the standard one. All chemical standards (high-performance liquid chromatography (HPLC grade) were from Sigma–Aldrich (St. Louis, MO, USA).



Fig. 1. HPLC chromatograms of the standard phenolic and flavonoid compounds

Statistical Analysis

The inhibition of fungal growth measured from the treated wood samples with the acetone extracts from *S. brachypetala* leaves and branches at 2%, 4%, and 6% was statistically analyzed. The statistical method was performed using analysis of variance (ANOVA) and SAS software (SAS Institute, Release 8.02, Cary, NC, USA), and the means were compared against the control treatments using Duncan's Multiple Range Test at the 0.05 level of probability.

RESULTS AND DISCUSSION

Phytochemical Compounds by HPLC

Table 1 shows the polyphenolic compounds identified in the leaves and branches of *S. brachypetala* extracts (in the solid form). The total identified phenolic and flavonoid compounds were 10500 μ g/g extract and 43200 μ g/g extract, respectively from the acetone extract of leaves. In the branch acetone extract, the total identified phenolic and flavonoid compounds were 6370 μ g/g extract and 2130 μ g/g extract, respectively.

The most abundant phenolic compounds in leaf acetone extract (Fig. 2) were gallic acid (7480 μ g/g extract), pyrocatechol (907 μ g/g extract), methyl gallate (548 μ g/g extract), chlorogenic acid (541 μ g/g extract), and rosmarinic acid (456 μ g/g extract). The concentrated flavonoid compounds in the leaf acetone extract were kaempferol (37900

 μ g/g extract), rutin (1710 μ g/g extract), naringenin (1660 μ g/g extract), and catechin (1550 μ g/g extract).

The most abundant phenolic compounds in branch extract (Fig. 3) were gallic acid (3120 μ g/g extract), chlorogenic acid (1320 μ g/g extract), vanillin (633 μ g/g extract), rosmarinic acid (589 μ g/g extract), syringic acid (403 μ g/g extract), and ellagic acid (174 μ g/g extract). The abundant flavonoid compounds in the acetone extract from branches were catechin (1030 μ g/g extract), naringenin (647 μ g/g extract), and quercetin (325 μ g/g extract). The acetone extracts from the leaves and branches of *S. brachypetala* contained a total of 53700 and 8500 μ g/g extracts of recognized phenolic and flavonoid components. These findings imply that phenolic chemicals found in naturally occurring antifungal drugs may be useful in the management of plant pathogens.

Compounds		Leaves Branches							
		Conc.			Conc.	Conc.			
	Area	(µg/mL	ovtract)	Area	(µg/mL	(µg/g			
	(mAU*s)	extract)	extract)	(mAU*s)	extract)	extract)			
Phenolic Compounds									
Gallic acid	1681.73	149.69	7484.43	700.79	62.38	3118.80			
Chlorogenic									
acid	79.83	10.81	540.54	194.55	26.35	1317.26			
Ferulic acid	39.79	2.39	119.63	8.76	0.53	26.33			
Methyl gallate	211.35	10.97	548.26	ND	ND	ND			
Caffeic acid	27.66	2.22	110.93	11.46	0.92	45.96			
Syringic acid	14.62	1.12	55.76	105.55	8.05	402.60			
Pyrocatechol	123.57	18.15	907.41	ND	ND	ND			
Ellagic acid	22.39	2.04	102.24	38.02	3.47	173.64			
p-Coumaric									
acid	11.87	0.44	21.83	24.94	0.92	45.85			
Vanillin	57.47	2.17	108.31	335.65	12.65	632.51			
Cinnamic acid	33.63	0.63	31.30	19.49	0.36	18.14			
Rosmarinic									
acid	82.88	9.13	456.48	106.93	11.78	588.99			
Total phenolic									
compounds		209.76	10487.12		127.41	6370.08			
Flavonoid Compounds									
Catechin	136.02	30.94	1547.20	90.39	20.56	1028.15			
Rutin	203.56	34.12	1705.86	10.18	1.71	85.29			
Naringenin	351.01	33.19	1659.37	136.78	12.93	646.61			
Daidzein	54.00	3.14	156.79	ND	ND	ND			
Quercetin	8.09	1.08	53.76	48.90	6.50	324.87			
Kaempferol	11095.20	758.37	37918.60	1.43	0.10	4.89			
Hesperetin	80.53	4.13	206.42	15.05	0.77	38.57			
Total		864.97	43248		42.57	2128.38			
flavonoid									
compounds									
Total									
concentration		1074.73	53735.12		169.98	8498.46			

Table 1. Phenolic and Flavonoid Compounds Identified in the Extracts of Schotia

 Brachypetala Leaves and Branches

mAU: milli-Absorbance Units



Fig. 2. HPLC chromatograms of the phenolic and flavonoid compounds from leaf extract of *Schotia brachypetala*



Fig. 3. HPLC chromatograms of the phenolic and flavonoid compounds from branch extract of *Schotia brachypetala*

Molecular Characterization of Genomic DNA

Alternaria spp., *Botrytis* spp., and *Fusarium* spp. isolates were identified through ITS ribosomal RNA (rRNA) sequencing. The ITS sequences were submitted to GenBank. The sequence data alongside BLAST tool search showed the identities to be *Botrytis cinerea* (accession no. PP758475), *Fusarium oxysporum* (accession no. PP737874), and

Alternaria alternata (accession no. PP737870). The DNA sequence obtained for each fungal isolate revealed 100% similarity with the isolates listed in GenBank.

Antifungal Activity of Wood Treated with Leaf and Branch Extracts

The antifungal activity of wood treated with acetone extracts from *S. brachypetala* leaves and branches was visually apparent (Fig. 4) against the growth of three mold species: *A. alternata*, *B. cinerea*, and *F. oxysporum* compared with the positive control. Furthermore, the three molds' fungal mycelia growth was seen on the 10% DMSO-treated wood.

The wood blocks that were exposed to Cure-M 72% positive controls exhibited the highest percentage of inhibition against the growth of all three molds. The treated wood with acetone extracts showed distinct inhibitory zones. After treating wood blocks, the extracts and their concentrations demonstrated a notable suppression of the growth of *A*. *alternata*, *B. cinerea*, and *F. oxysporum* fungi. Furthermore, fungal linear growth inhibition increased with an increase in the concentration of acetone extract.

Note that each wood sample was treated with 150 μ L of the concentrated extract before being positioned over the substrate that has been inoculated with fungi. As a result, the inhibition of fungal growth may be caused by the leaching or spreading of dissolved extracts. As shown in Fig. 4, the three fungi grew over the media, but neither growth nor inhibition was observed over the treated wood with 2% extract, suggesting that the extract is protecting the wood from mold growth when compared to the negative control.





Fig. 4. Antifungal bioassay of wood treated with acetone extracts from leaves and branches of *Schotia brachypetala* against the growth of *Alternaria alternata*, *Botrytis cinerea*, *and Fusarium oxysporum.* Positive control Cure-M 72% (a, d, g), negative control (b, e, h), and the morphological characterization of fungi (c, f, i)

Table 2 illustrates how wood samples treated with the acetone extracts of *S*. *brachypetala* leaves and branches at varying concentrations (2%, 4%, and 6%) inhibited the growth of *A*. *alternata*, *B*. *cinerea*, and *F*. *oxysporum* to varying degrees in comparison to the control treatment (wood samples treated with 10% DMSO). When compared to the

control, the acetone extracts of the leaves and branches at 4% and 6% levels showed the highest percentage of inhibition (100%) of mycelial growth of *A. alternata*. Additionally, the extract from leaves and branches at 6% demonstrated the highest percentage of inhibition (100%) of *B. cinerea* and *F. oxysporum* mycelial development. In contrast, the percentage suppression of fungal mycelia of *F. oxysporum*, *B. cinerea*, and *A. alternata* increased significantly with increasing concentration.

Treatment	Concentration	Fungal Inhibition Percentage (%)				
		Alternaria	Botrytis cinerea	Fusarium		
		alternata		oxysporum		
Control	10% DMSO	0.00D	0.00F	0.00F		
	(Mancozeb					
Cure-M 72% WP	64%+ Metalaxyl	100 ± 0.00A	100 ± 0.00A	100 ± 0.00A		
	8%)					
	6%	100 ± 0.00A	100 ± 0.00A	100 ± 0.00A		
Leaves extract	4%	100 ± 0.00A	77.43 ± 1.68B	77.43 ± 0.63D		
	2%	85.56 ± 1.15C	48.90 ± 1.10E	72.56 ± 0.63E		
Branchae extract	6%	100 ± 0.00A	100 ± 0.00A	100 ± 0.00A		
branches extract	4%	100 ± 0.00A	62.70 ± 1.40C	87.80 ± 1.10B		
	2%	93.30 ± 1.10B	55.56 ± 2.25D	85.20 ± 0.69C		

Table 2. Inhibition Percentages of the Antifungal Activity of Extracts from Schotia

 brachypetala Leaves and Branches

WP: Wettable powder; Means with the same letter are not significantly different according to Duncan's Multiple Range Test at 0.05 level of probability

This study showed that the acetone extracts from *S. brachypetala* leaves and branches had a promising antifungal activity against the growth of *A. alternata*, *B. cinerea*, and *F. oxysporum* when applied to *Morus alba* wood samples. These bioactivities could be related to the presence of several phenolic and flavonoid compounds in the extract. The identified compounds gallic acid, chlorogenic acid, ferulic acid, methyl gallate, caffeic acid, syringic acid, pyrocatechol, ellagic acid, *p*-coumaric acid, vanillin, cinnamic acid, rosmarinic acid, catechin, rutin, naringenin, daidzein, quercetin, kaempferol, and hesperetin were present in different concentrations in the acetone extract from leaves and branches.

Quercetin was found in concentrations of 53.8 and 325 μ g/g extract from leaves and branches, respectively. Quercetin and its derivatives were identified and isolated from leaf, aerial parts, and stalk extracts of *S. brachypetala* (Hassaan *et al.* 2014; Du *et al.* 2014; Thakur *et al.* 2019). Daidzein was found in a concentration of 157 in the leaf extract but not detected in the branch extract, while another previous study showed the presence of daidzein in the aqueous alcohol from the stalk extract (Hassaan *et al.* 2014). Additionally, the aqueous alcohol stalk extract of *S. brachypetala* identified the following polyphenolic compounds: gallic acid, and ellagic acid (Hassaan *et al.* 2014).

Previous studies have demonstrated the bioactivity of various *S. brachypetala* extracts. For example, methyl-5,11,14,17-eicosatetraenoate and linolenic acid that were extracted from *S. brachypetala* showed some antibacterial action against two Grampositive bacteria and less activity against two Gram-negative species (McGaw *et al.* 2002b). The total phenols, flavonoids, and proanthocyanidin contents of *S. brachypetala* root extracted by methylene chloride:methanol(1:1) were 304 (mg tannic acid/g of dry plant material), 4.24 (mg quercetin/g of dry plant material), and 19.6 (mg catechin/g of dry plant material), respectively (Adewusi *et al.* 2011). The yield of acetone extract from *S.*

brachypetala was 1.2%, and the content of total phenols was 4.67 mg/mL gallic acid equivalents (Würger *et al.* 2014). Part of the activity *of S. brachypetala* may be attributed to astringent tannins in the bark (Bruneton 1993). Tannins are also thought to be present in the wood dust and root (Watt and Breyer-Brandwijk 1962). The extract from the tree's heartwood showed the presence of small amounts of *trans*-3,3,4,5-tetrahydroxystilbene, catechin, epicatechin, and trace amounts of *cis*-3,3,4,5,5-pentahydroxystilbene (Drewes 1971; Siegfried and Ian 1974). Epicatechin, and catechin isolated from the bark of *S. latifolia* were effective in inhibiting the growth of some bacteria (Masika *et al.* 2004).

Significant antifungal activity was shown by quercetin-7-O-diglucoside, which was extracted from *Terminalia brownii* stem bark and wood against strains of *Fusarium* and *Aspergillus* (Salih *et al.* 2017). The mechanisms of action of phenolic and flavonoid compounds were found to be the inhibition of cytoplasmic membrane function, nucleic acid synthesis, and energy metabolisms (Cushnie and Lamb 2005). Dihydroquercetin, which was extracted from barley, has been shown to inhibit the growth of *Fusarium* spp. (Mierziak *et al.* 2014). Furthermore, it has been shown that quercetin and its derivatives have effective antifungal properties (Tempesti *et al.* 2012; Alves *et al.* 2014; Céspedes *et al.* 2014). Some antifungal and antibacterial activity was shown by naringenin and its derivatives (Duda-Madej *et al.* 2020; Duda-Madej *et al.* 2022).

Rutin in the present work was found in concentrations of 1710 and 85.3 μ g/mg extracts from leaves and branches, respectively. Concentrations of 10 and 20 mg/mL of methanol extract from *Muscari aucheri* (flower + peduncle) resulted in a 100% poisonous effect against *F. oxysporum* f. sp. *cucumerinum*, *Alternaria solani*, *Verticillium dahliane*, *R. solani*, and *Botrytis cinerea* (Onaran and Başaran 2018), where this extract had rutin content. Strong antimicrobial properties were demonstrated by rutin, naringenin-7-O-b-D-glucopyranoside, and four flavonoid compounds found in *Galium fissurense*, *Viscum album*, and *Cirsium hypoleucum* (Orhan *et al.* 2010).

Furthermore, rutin extracted from *Polygala paniculata* showed possible antifungal action against some pathogens (Johann *et al.* 2011). The fruit of *Phaleria macrocarpa* possesses some flavonoid chemicals, such as rutin, myricetin, and naringin, which are responsible for its antimicrobial properties (Hendra *et al.* 2011). Strong antibacterial capabilities against pathogenic microbes have been demonstrated by gallic acid, caffeic acid, vanillic acid, rutin, and quercetin (Vaquero *et al.* 2007).

The antifungal activity of the acetone extracts from *S. brachypetala* leaves and branches applied to white mulberry (*Morus alba* L.) wood samples showed good antifungal activity against the growth of *A. alternata*, *B. cinerea*, and *F. oxysporum* when compared to the standard fungicide Cure-M 72%. These activities could be related to the presence of phenolic and flavonoid compounds as reported from the literature.

Acacia saligna flower extract showed the presence of benzoic acid, naringenin, ocoumaric acid, quercetin, and kaempferol, and inhibited the growth of *F. culmorum*, *P. chrysogenum*, and *R. solani* when applied to wood samples of *Melia azedarach* at 1, 2, and 3% (Al-Huqail *et al.* 2019). Following a three-month incubation period, the wood surface treated with the methanol extract of *Maclura pomifera* bark did not exhibit any fungal growth from *Alternaria tenuissima* or *Fusarium culmorum* (Mansour *et al.* 2015b). At concentrations of 5%, 10%, and 20%, the treated wood samples of *Acacia saligna* containing the methanolic extract of *Cupressus sempervirens* wood displayed an inhibitory zone against the growth of *T. harzianum* surrounding the treated wood (Mansour and Salem 2015). Several fungi, such as *A. alternata*, were suppressed in their growth to varying degrees on the wood surfaces of *Pinus sylvestris*, *Pinus rigida*, and *Fagus sylvatica* by applying essential oils or extracts of *Pinus rigida* (wood), *Eucalyptus camaldulensis* (leaves), and *Costus speciosus* (leaves) (Salem *et al.* 2016a, 2016b). When applied to wood samples from *Acacia saligna*, *Fagus sylvatica*, *Juglans nigra*, and *Pinus rigida*, extracts from *Schinus terebinthifolius* and *Pinus rigida* and essential oils from *Thymus vulgaris* and *Origanum majorana* were found to have varying degrees of bioactivity against the growth of *Trichoderma harzianum* and *A. niger* (Salem *et al.* 2019a).

When *Melia azedarach* wood samples are treated with the methanolic extract of *M. paradisiaca* peels, the polyphenolic compounds ellagic acid, gallic acid, ferulic acid, *o*-coumaric acid, catechol, salicylic acid, cinnamic acid, rutin, myricetin, and naringenin should exhibit strong antifungal activity against *Fusarium culmorum* and *Rhizoctonia solani* (Behiry *et al.* 2019). Additionally, the same wood demonstrated encouraging antifungal properties against three fungi *Fusarium culmorum*, *Rhizoctonia solani*, and *Penicillium chrysogenum* when sprayed with n-hexane from *E. camaldulensis* and *Matricaria chamomilla* (Salem *et al.* 2019b).

When acetone extracts from the inner and outer barks of *Acer saccharum var*. *saccharum* were combined with citric acid and applied *to Leucaena leucocephala* wood, good antifungal activity was observed against the growth of three common molds: *A. niger*, *F. subglutinans*, and *Trichoderma viride*. These were associated with phenolic chemicals, including caffeine and other phenolic acids such as *p*-hydroxybenzoic, gallic, and salicylic acids (Salem *et al.* 2019c).

Wood durability is impacted by extractive leaching during weathering conditions because phenolic compound leaching was less noticeable than that of other extractives. Wetting ability and natural durability are weakened by weathering (Keržič *et al.* 2024). The natural ability of wood to withstand destructive action from organisms that break down wood is known as its natural durability. The natural durability of wood is crucial in predicting the service life of wooden items, which can be limited by the effects of global change, in addition to exposure to the climate, product design, and use conditions. Since wood's moisture content affects the establishment of wood-degrading organisms, the main wood properties that affect natural durability are the amount and composition of heartwood extractives, the xylem's anatomy, the lignin content in the cell wall, nutrient availability, and the presence of moisture-regulating elements (Martín and López 2023).

Blocks of both extracted and unextracted wood from nine different species were tested for endurance in termite bioassays and fungal soil bottles, as well as to look at the impact of extractives. Compared to unextracted controls, extracted blocks were typically far less durable (Kirker *et al.* 2013). Even after being extracted, tali and moabi wood species maintained their exceptional durability. The high presence of extractives in tali is responsible for both its inherent longevity and the high level of fungal inhibition it exhibits (Tchinda *et al.* 2018). The investigated fungi, *Aspergillus flavus*, and *P. chrysogenum*, were able to attack the two different species of wood in different deterioration patterns during the long-term study, depending on how durable the woods of *Ficus sycomorus* and *Tectona grandis* were. The two fungi were unable to grow in *T. grandis* wood, which is likely due to the presence of phenolic chemicals in the wood extractives (Mansour *et al.* 2023).

Leaching of the bio-preservatives (natural extracts) can limit their effectivity; therefore, the search for application possibilities with a fixed formulation or repeated application of these extracts is needed. The study offers important new information about the antifungal characteristics of the extract from *S. brachypetala*. However, the results

might be strengthened by using a larger range of concentrations, a more comprehensive approach to extraction techniques, and the addition of more fungal isolates. Furthermore, investigating certain antifungal pathways and carrying out further evaluations on other species might increase the study's applicability.

CONCLUSIONS

- 1. Phytochemicals were extracted from leaves and branches of *Schotia brachypetala* using acetone as a solvent.
- 2. The high pressure liquid chromatography (HPLC) analysis of the extracts showed the presence of various phenolic and flavonoid-type compounds. The most abundant compounds in leaf extract were kaempferol, gallic acid, rutin, naringenin, catechin, and pyrocatechol, respectively. The most abundant compounds in branch extract were gallic acid, chlorogenic acid, catechin, naringenin, and vanillin, respectively.
- 3. Significant antifungal activity against the phytopathogenic fungi was observed by applying the acetone extracts from *S. brachypetala* leaves and branches against *B. cinerea*, *F. oxysporum*, and *A. alternata* when applied to white mulberry wood samples. Antifungal activity against the phytopathogenic fungi was observed by applying the acetone extracts from *S. brachypetala* leaves and branches against *A. alternata*, *B. cinerea*, and *F. oxysporum*. The inhibition effectiveness at 6% was 100% and was equal to the positive control (Cure-M). The inhibition rate with the remaining concentrations was significant compared to the negative control.
- 4. *S. brachypetala* extracts are a good alternative source of phytochemicals for use as potential antifungal agents.

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