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Research

Antioxidant Effects of Four Heartwood Extractives on Midgut Enzyme Activity in *Heterotermes indicola* (Blattodea: Rhinotermitidae)

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Abstract

Heterotermes indicola (Wasmann) (Blattodea: Rhinotermitidae) is a species of subterranean termite that is a destructive pest of wood and wood products in Pakistan. This study evaluated the antioxidant and antienzyme potential of heartwood extractives against *H. indicola*. Heartwood extractives of four durable wood species, *Tectona grandis* (L.f), *Dalbergia sissoo* (Roxb.), *Cedrus deodara* (Roxb.), and *Pinus roxburghii* (Sarg.) were removed from wood shavings via soxhlet extraction with an ethanol:toluene solvent system. The antioxidant potential of the extractive compounds was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test. Results showed maximum antioxidant activity for extractives of *D. sissoo*. *D. sissoo* had the lowest IC_{50} (the concentration where 50% inhibition of the DPPH radical is obtained) at 28.83 μg/ml among the heartwood extractives evaluated. This antioxidant activity, however, was not concentration dependent as was observed in the other heartwood extractives tested. At the maximum test concentration, *T. grandis* showed the highest percent inhibition at 89.7%, but this inhibition was lower compared to the positive control antioxidant compounds butylated hydroxytoluene and quercetin. When termites were fed filter paper treated with IC_{50s} of the extractives and control compounds, glutathione *S*-transferase activity in the guts of *H. indicola* workers was significantly reduced by *T. grandis* and *D. sissoo* extractives. Similarly, esterase activity was reduced more by *P. roxburghii* extractives compared to control antioxidant treatments and other tested extractives. However, none of the extractives examined significantly reduced the activity of catalase enzymes in *H. indicola* compared to treatments with the antioxidant control compounds.

Key words: gut enzymes, *Heterotermes indicola*, heartwood extractives, antioxidant, termite

Subterranean termites are serious pests of wood and wood products causing significant economic damage in areas they occur. In Pakistan, they have become an increasing object of public interest due to increased urbanization and use of wood for household structures, particularly after devastating earthquakes in the region in 2005 resulted in building code changes. Of the 50 termite species identified in Pakistan, 13 are considered pests of wood in service in rural and urban areas. These species cause significant economic damage and are the focus of ongoing research to mitigate their destructive habits (Manzoor and Mir 2010). Of these economically important species, termites of the genus *Heterotermes* account for a significant proportion of the damage to wood attributed to subterranean termites in this region. This genus occurs in the warm neotropics, Indian subcontinent and Australia. *Heterotermes indicola* (Wasmann) (Blattodea: Rhinotermitidae) is a destructive subterranean termite pest in Pakistan, where it causes significant damage to wooden structures. Much of its ability to cause such destruction is that it is active year round (Saljoqi et al. 2012, Misbah ul haq et al. 2015).

Several synthetic termite control chemicals have been withdrawn from the commercial markets in recent years because of toxicological and environmental concerns (Little et al. 2010). In an era of increasing sensitivity to recalcitrant chemicals in the environment, there is a push for chemical manufacturers to replace more toxic wood preservatives currently in use with biodegradable environmentally friendly alternatives. One approach has been the examination of botanical biocides, some of which can occur in the heartwood of naturally durable wood species. Bark and heartwood of many plant species sequester strong antioxidant compounds, many of which are polyphenolic in nature (Chang et al.1999). Studies have shown that heartwood phenolic compounds include flavonoids, stilbenes, tannins, and lignans. Such compounds are active agents protecting

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plants and wood from termite attack due to their toxic properties. Plant and heartwood compounds that are toxic to termites have been shown to have free radical scavenging (antioxidant) properties (Doi et al. 2001, Sroka and Cisowski 2003, Morimoto et al. 2006, Ragon et al. 2008, Little et al. 2010). Insect antioxidant enzyme systems include superoxide dismutase, catalase (CAT), esterase, glutathione transferase, and glutathione reductase. Plant antioxidant compounds can act as pro-oxidants, effectively reducing the insect antioxidant enzyme system (Lukasik 2007). This reduction in the antioxidant system creates the presence of more reactive oxygen species (ROS) that are cytotoxic and lead to the formation of lesions in the insect gut lumen which can ultimately lead to death (Barbehenn 2002).

The termite digestive tract, especially the midgut, is important not only for cellulose digestion but also for the detoxification of plant compounds such as allelochemicals, entomopathogens, and other xenobiotic chemicals. Termite gut cells secrete laccases, glutathione S-transferases (GSTs), CATs, esterases, and cytochrome P450s (Tartar et al. 2009). Of these, many are involved in the degradation or metabolism of plant chemicals and insecticides. GSTs (EC 2.5.1.18), a super family of detoxifying enzymes, are phase II metabolizing isozymes that play a fundamental part in the protection of living cells against injury by the toxic compounds. Several xenobiotic compounds such as pesticides, plant allelochemicals, drugs, organic pollutants, and other toxins are detoxified by these enzymes in insects (Hayes et al. 2005). Hydroxyl radicals and singlet oxygen are directly scavenged by these enzymes, so these also play protective roles against oxidative stress (Bamidele et al. 2017). They are mostly secreted by midgut tissues and fat body of the insects. Guts of several termite species, e.g., *Coptotermes curvignathus* (Holmgren) (Blattodea: Rhinotermitidae), *Mastotermes darwiniensis* Froggatt (Blattodea: Mastotermitidae), *Reticulitermes virginicus* (Banks) (Blattodea: Rhinotermitidae), *Reticulitermes flavipes* (Kollar) (Blattodea: Rhinotermitidae), *Coptotermes acinaciformis* (Froggatt) (Blattodea: Rhinotermitidae), and *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae) contain cytochrome P450s monooxygenases, carboxylesterase, GST, and *N*-acetyltransferase which are involved in all three biotransformation phases of xenobiotic and detoxification metabolism (Valles et al. 1998, Tartar et al. 2009, Raychoudhury et al. 2013, Charles at al. 2014, Tramontina et al. 2017) .

Esterases (EC 3.1) are hydrolytic enzymes that break ester bonds in several biomolecules. They are also directly involved in xenobiotic, acetylcholine, lipid, and JH metabolism (Wheeler et al. 2010). These enzymes also play a role in insect resistance to insecticides and allelochemicals and are part of the insect antioxidant system (Singh et al. 2001, Lehane and Billingsley 2012). In the termite gut, these are phenolic acid esterases and are potentially important in the cleavage of polyphenolic lignin and lignin monomers from hemicellulose in the fore- and midgut after ingestion of cellulosic material (Scharf and Tartar 2008, Wheeler et al. 2010). However, previous studies indicate that some esterases can be produced by some of the protozoa species (*Pseudotrichonympha grassii* Koidzumi) which are present in hindgut of *Coptotermes formosanus* (Shiraki) (Blattodea: Rhinotermitidae), a lower termite (Wang and Grace 2000).

Catalases (CATs) (EC 1.11.1.6) are secreted in the termite gut lumen to catalyze the breakdown of hydrogen peroxide to oxygen and water, thereby protecting from the activity of ROS, which can cause cellular oxidative damage (Engel and Moran 2013).

The heartwood of naturally durable woods, a rich source of bioactive chemicals and antioxidants, have various pharmacological and insect growth-reducing activities (Omar et al. 2000, Belt et al. 2017). Antioxidant flavonoids, found in some heartwoods, have been shown to be inhibitors of several enzymatic pathways, including catabolic glycohydrolases, which hydrolyze cellulose and starch (Tadera et al. 2006). Previous studies show that esterases such as acetylcholinesterase (AChE), a neurotransmitter that catalyzes the breakdown of acetylcholine and other choline esters, was inhibited by a naturally occurring flavonoid, kaempferol (Thors et al. 2008, Priya 2012). Its stimulating effects were found to correlate with the toxicity of tetrahydronootkatone, a derivative compound isolated from *Chamaecyparis nootkatensis* (D. Don) (Ibrahim et al. 2007). Özkan et al. 2015, showed that olive wood extracts had high antioxidant activities and proved to be good inhibitors of AChE and butyrylcholinesterase, a plasma esterase. Digestive enzymes in the guts of termites, particularly cellulases, have been the target of several synthetic and natural inhibitors (Zhu et al. 2005). Plant extracts can also have significant effects on semiochemicals, hormones, and lignocellulose processing in the termite gut where esterases can play a major role (Nisar et al. 2015). Similarly, juniper wood extractives where shown to inhibit the activity of amylase and glucosidase (Ozkan et al. 2015). Plant flavonoids have also been identified as inhibitors of GSTs in *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) (Yu and Abo Elghar 2000). Tang et al. (2007) showed a reduction in GST activities in *Odontotermes formosanus* (Shiraki) (Blattodea: Termitidae) and *Reticulitermes chinensis* (Snyder) (Blattodea: Rhinotermitidae) after exposure to quercetin and tannic acids, which ultimately reduced the detoxification mechanism in these termites. Wheeler et al. 1993 reported that quercetin, ellagic acid, juglone, catecholamines, and quinones were successful antioxidants and insect GST inhibitors.

Many extractives present in naturally durable wood are not only toxic to termites but are also a rich source of antioxidants/radical scavengers (Gao et al. 2007, Huang et al. 2009, Lamounier et al. 2012, Kadir and Hale 2017), which may act synergistically to affect termite mortality. These free radical scavengers serve as synergists by interfering with the GST-mediated detoxification mechanism and act as alternative substrate for the antioxidant enzymes. They can also reduce the activities of antioxidant enzyme systems by acting as competitive and noncompetitive inhibitors toward the substrate of GST and esterases (Das et al. 1984). Thus, the balance between ROS and antioxidants is always optimal as both extremes, oxidative and antioxidative stress, can be damaging to an organism (Poljsak 2011). Reduction in the antioxidant system of termites due to antioxidant stress ultimately leads to death of termites due interference with physiological processes. In earlier work, we showed that extractives from durable wood species used in Pakistan were toxic to termite gut protozoa (Hassan et al. 2017). In further efforts to identify potential botanical insecticides for wood preservation in Pakistan, the current study was designed to examine the antioxidant potential and physiological impacts of four naturally durable heartwood extractives on esterase, GST and CAT activity in the guts of *H. indicola*.

Materials and Methods

Preparation of Heartwood Extractives

Heartwood shavings of *Tectona grandis*, *Dalbergia sissoo*, *Cedrus deodara*, and *Pinus roxburghii* were air dried and extracted separately using 300-ml mixture of ethanol:toluene (2:1) as solvent system according to ASTM D1105-96 (ASTM 2014) to obtain crude extractives per Kirker et al. (2013). Shavings (12–15 g) were added to soxhlets with a small amount of cotton under and above the shavings to prevent clogging of the reflux tubes. The 300 ml of solvent was heated in a heating mantle at medium heat and run for six hours after the first reflux for each extraction. The resulting aliquot was placed in a tared round-bottom flask and vaporized using a rotary

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evaporator (BUCHI, R-114). After the flask had cooled to room temperature, it was reweighed and the resulting residue was rehydrated with the ethanol:toluene solvent mixture to produce a stock solution of 10 mg/ml, based on the dry weight of the extractive residue. Solutions of extractives of the four wood species were stored in darkness at 4°C in tightly capped air tight bottles and further sealed with a plastic paraffin film (Parafilm, Bemis, Inc, Oshkosh, WI).

Effect of Heartwood Extractives on DPPH Radical-Scavenging Activity

An assay method employed by Lu et al. (2014) was used with a slight modification for the determination of DPPH (2,2-diphenyl-2-picrylhydrazyl) scavenging activity/antioxidant properties of each extractive. The modification used 2.5 µmol instead of 50 µM as described in the assay method cited. Extractives were dissolved in methanol to produce a series of concentrations ranging from 25 to 800 µg/ml. One hundred microliters of methanolic DPPH (Sigma-Aldrich) solution was added to an equal amount of each extractive concentration. This solution was added in 200-µl aliquots to each well of a 96-well microtiter plate. Methanol was used as a negative control in this test. The well-plate was shaken by hand for two minutes and incubated for 20 min at 37°C in darkness. The resulting yellow-colored solutions were measured spectrophotometrically at 517 nm using a BioTek Power Wave HT microplate spectrophotometer linked to a computer with Gen5 software. This test was repeated thrice and the percentage of radical scavenging activity was calculated by using the formula cited by Lu et al. (2014).

Concentrations of butylated hydroxytoluene (BHT) and quercetin as positive controls were prepared as mentioned above. The IC_{50} values were calculated by using Graph pad Prism 6 software, where IC_{50} represents the concentration where 50% inhibition of the DPPH radical is obtained.

Enzyme Activity of the Gut Contents of *H. indicola* Workers Exposed to the Values of IC_{50} of Heartwood Extractives and Control Antioxidant Compounds

Sets of filter paper were treated with the values of IC_{50} of the four heartwood extractives and control compounds at 42.63, 8.17, 167.97, 28.83, 508.69, and 335.24 µg/ml for BHT, quercetin, *T. grandis*, *D. sissoo*, *C. deodara*, and *P. roxburghii*, respectively. Ethanol:toluene (2:1) was used as solvent to prepare above-mentioned concentrations of compounds and extractives. About 200-µl solution of each concentration was applied to each filter paper and allowed to air dry. Each treatment was replicated three times along with a control treatment (only ethanol:toluene treated). Jars containing 20 g of sand, moistened with 3.6 ml of deionized water to produce a moisture content of 18% based on the weight of sand, were used. Each jar received a total of 50 termites with treated filter papers, which were maintained in an incubator at 27 ± 2°C and 75 ± 1% RH for 7 d. After 7 d, termites were briefly rinsed in 80% ethanol and the termite guts were then dissected in a saline solution. The dissected guts were stored at –20°C until used for the enzyme assay.

Protocol for Esterase Activity

Procedure outlined by Nisar et al. (2015) was used for the determination of esterase activity. Ten termite worker guts per replicate were homogenized in 0.1 M phosphate buffer with a yellow line IKA DI 25 Basic Homogenizer at 15,000 rpm for 20 s. Glass wool was used to filter the homogenate, which was used as the enzyme test solution. An aliquot of uncentrifuged homogenate was incubated at 25°C with α-naphthyl acetate (0.25 mM final concentration) in a total volume of 3.0 ml of 0.1 M phosphate buffer (pH 7.0). After 30 min, 0.5 ml of Fast blue B salt (*tetra*-azotized *o*-dianisidine/$ZnCl_2$, 1%) + sodium dodecyl sulfate (5%) was added to the incubated mixture. A red color immediately developed that quickly changed into a fairly stable blue color, which was measured at 605 nm on a spectrophotometer (CECIL, CE2041, 2000 series). The quantity of naphthols produced was determined from a standard curve of naphthol. The α-naphthol standard curve was obtained by plotting concentrations (3–30 µmol) of α-naphthol against absorbance at 605 nm. Specific activity of esterase was, thus, represented as µmole naphthol produced per minute per milligram of protein.

Protocol for GST Activity

The guts of 10 termites were homogenized in 0.1 M sodium phosphate, pH 7.4, and centrifuged at 3,000 *g* for 10 min at 4°C, storing the supernatant as enzyme source at –80°C. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma-Aldrich) following the technique described by Habig et al. (1974). Each well of a spectrophotometer plate contained 300 µl of reaction mixture: 213 µl of 0.1 M sodium phosphate buffer (pH 6.5), 8 µl of synergist, 70 µl of enzyme and incubated for 10 min at 30°C, 6 µl of 50 mM CDNB. Then, 3 µl of 100 mM GSH (Sigma-Aldrich) was added to start the reaction. The change in absorbance at 340 nm was recorded for 6 min with a spectrophotometer.

Protocol for CAT Activity

CAT activity was measured as described by Aebi (1984). Ten termite guts from each treatment were homogenized in 67 mM potassium phosphate buffer (pH 7) for 5 min at 0°C. Homogenates were filtered through two layers of cheesecloth and centrifuged at 3,000 *g* for 15 min. A 0.5 ml aliquot of the termite gut homogenate was added to 0.5 ml of 30 mM H_2O_2 and the disappearance of hydrogen peroxide was measured at 240 nm during 3 min at 30 s intervals. CAT activity was expressed as µmol decomposed H_2O_2 per minute per mg protein. Protein concentration of the sample (10 µl) was determined by the method described by Bradford (1976).

Statistical Analysis

Differences in enzyme activity in the different treatments were determined by ANOVA and means were separated by Tukey's HSD test at 5% level of significance.

Results

Effect of Heartwood Extractives on DPPH Radical-Scavenging Activity

The radical-scavenging ability and IC_{50}s for the extractives/compounds tested are presented in Fig. 1. The minimum IC_{50} was produced by quercetin at 8.17 µg/ml. Of the tested extractives, *D. sissoo* showed the maximum percent inhibition with an IC_{50} of 28.83 µg/ml. The IC_{50} value of *D. sissoo* heartwood extractives was between that of the two control antioxidant compounds, BHT and quercetin. The percent inhibition of *D. sissoo* was not concentration dependent within the range evaluated while the percent inhibition of the other heartwood extractives was somewhat linearly dependent on concentration (Fig. 1). Of the heartwood extractives, *T. grandis* showed the highest percent inhibition at 89.7%, but this inhibition was to some extent lower compared to the BHT and quercetin at the maximum concentration tested.

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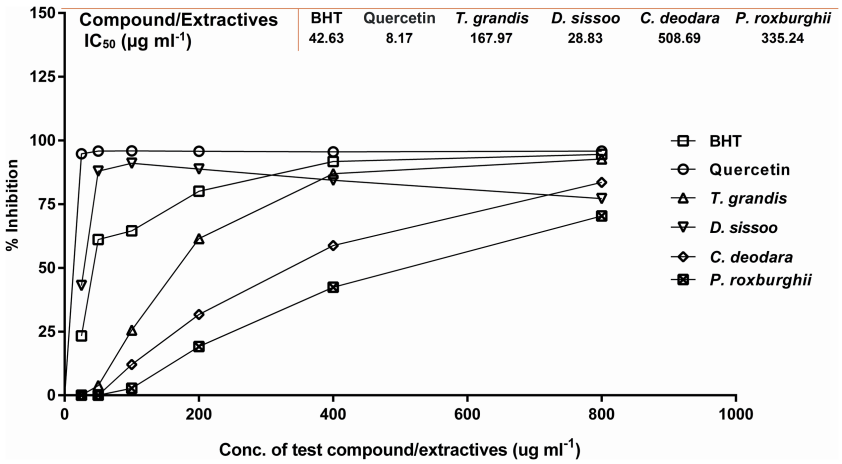

Fig. 1. Effect of heartwood extractives on DPPH radical scavenging activity (% inhibition); Conc. = concentration.

Table 1. Mean GST activity in the gut of *H. indicola* exposed to IC_{50} concentrations of BHT, Quercetin, and four heartwood extractives

Compound/extractives	IC50 (µg/ml)	GSTs activity	Grouping
Control—ethanol:toluene	—	77.60 ± 1.07	a
BHT	42.63	23.96 ± 0.73	e
Quercetin	8.17	12.83 ± 0.72	f
T. grandis	167.97	29.33 ± 1.17	de
D. sissoo	28.83	30.76 ± 0.54	d
C. deodara	508.69	54.27 ± 1.39	b
P. roxburghii	335.24	44.50 ± 1.80	c
	F= 365.73	$P < 0.05$	

Means (n = 3) sharing same letters in a column are not significantly different from each other at $P > 0.05$.

Table 2. Mean Esterase activity in the gut of *H. indicola* exposed to IC_{50} concentrations of BHT, quercetin, and four heartwood extractives

Compounds/extractives	IC50 (µg/ml)	Esterases activity	Grouping
Control—ethanol:toluene	—	41.03 ± 0.89	a
BHT	42.63	25.03 ± 3.24	bc
Quercetin	8.17	0.22 ± 0.01	e
T. grandis	167.97	27.70 ± 3.96	b
D. sissoo	28.83	16.26 ± 0.63	cd
C. deodara	508.69	32.60 ± 0.70	ab
P. roxburghii	335.24	10.70 ± 1.67	d
	F = 43.82	$P < 0.05$	

Means sharing same letters in a column are not significantly different from each other at $P > 0.05$.

Enzyme Activity in *H. indicola* Worker Gut Material Exposed to IC_{50} Concentrations of BHT, Quercetin, and Heartwood Extractives

Tables 1–3 show the activity of the gut enzymes tested in *H. indicola* fed on filter paper treated with IC_{50s} of the antioxidant compounds and heartwood extractives.

The results revealed lowest GSTs activity at 12.83 µmol/min mg/protein in termites exposed to quercetin. This was significantly lower compared to all tested extractives and BHT ($P < 0.05$). BHT reduced significantly more GSTs compared to all extractives except *T. grandis*. Extractives from *D. sissoo* and *T. grandis* did not significantly reduce the activity of GSTs but were significantly different compared to the other extractives and quercetin (Table 1). A maximum GST activity of 54.27 µmole/min mg/protein was observed in *H. indicola* workers fed on filter paper treated with *C. deodara* extractives, but it was significantly lower compared to the ethanol:-toluene control treatment.

Evaluation of esterase (ESTs) activity in the gut of *H. indicola* workers fed on filter paper treated with the different concentrations of heartwood extractives and control compounds (BHT, quercetin) is presented in Table 2. The compound quercetin resulted in significantly lower activity of esterase activity, 0.22 µmol/min mg/protein in the workers of termite as compared to all treatments. Termites fed on extractives of *P. roxburghii* showed significantly lower EST activity at 10 µmol/min mg/protein compared to all other treatments except *D. sissoo*. However, *D. sissoo* extractives performed similarly to BHT. Additionally, extractives of *T. grandis* and *C. deodara* performed similarly to each other but significantly different from all other treatments including control except BHT. This was contrary to GST activity, where *D. sissoo* and *T. grandis* showed minimal activity.

Table 3 gives the results for CAT activity in workers of termite (*H. indicola*) after feeding on filter paper treated with heartwood extractives of four species and control compounds. The results revealed that there was no substantial decrease in CAT activity in *H. indicola*-fed extractives of *C. deodara*, *P. roxburghii*, or *D. sissoo*. Compared to the control, the maximum decrease in the activity of CAT was detected in termites after feeding on quercetin and *T. grandis* treated filter papers at 9.23 and 11.00 µmol H_2O_2/min mg/protein, respectively.

Discussion

In our study, *D. sissoo* extractives were observed to have maximum antioxidant activity with the lowest IC_{50} among the evaluated heartwoods. The synthetic antioxidant control, BHT, showed higher IC_{50} at 42.63 µg/ml compared to *D. sissoo* extractives. Bark from this tree has been previously reported to have a high potential to

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Table 3. Mean CAT activity in the gut of *H. indicola* exposed to IC_{50} concentrations of BHT, quercetin, and four heartwood extractives

Compound/extractives	IC50 (µg/ml)	CATs activity	Grouping
Control—ethanol:toluene	—	16.41 ± 0.35	a
BHT	42.63	15.07 ± 0.43	ab
Quercetin	8.17	9.23 ± 1.30	c
T. grandis	167.97	11.00 ± 0.52	bc
D. sissoo	28.83	12.48 ± 0.74	abc
C. deodara	508.69	15.36 ± 0.48	a
P. roxburghii	335.24	16.53 ± 1.58	a
	$F = 10.24$	$P < 0.05$	

Means sharing same letters in a column are not significantly different from each other at $P > 0.05$.

scavenge DPPH radicals (Roy et al. 2011). *T. grandis* at the highest concentration showed maximum percent inhibition at 89.7%, but this was not statistically significant different than BHT or quercetin, unlike previous findings where ethyl acetate extraction of *T. grandis* wood showed more antioxidant potential than quercetin and trolox (Krishna and Jayakumaran 2010).

The use of heartwood extractives as termiticides has been previously tested and practiced. In previous laboratory studies, tested extractives showed very strong insecticidal activities against termites (Kirker et al. 2013, Mankowski et al. 2016, Hassan et al. 2017). Currently, we are investigating the effects of nondurable pine and cottonwood treated with the heartwood extractives used in this study on termites in field tests. Results after 1 yr for heartwood extractive treated stakes and blocks exposed in Pakistan and the United States show that untreated control nondurable pine or cottonwood were severely damaged by termites in ongoing field tests. Extractive plus linseed oil combinations appear to add some protection to the treated nondurable wood species (Hassan et al. 2017b). Several studies have shown that it may not be solely toxic activity of the heartwood extractives that gives resistance to heartwood against termites, but that the toxicity and antioxidant properties of heartwood extractives act in synergistic manner to affect termite mortality (Little et al. 2010, Ragon et al. 2008). Heartwood extractives tested here appear to be inhibitors of some of the gut enzymes in *H. indicola* similar to other plant compounds previously examined (Yu 1982). The majority of active compounds identified in heartwoods in this study are classified as polyphenols having powerful antioxidants. *D. sissoo* extractives showed higher antioxidant activity compared to the other tested extractives. Gas chromatography–mass spectrometry results (Hassan et al. 2017) confirmed that these extractives contain large amounts of resveratrol, a phenol, which has been noted to have biocidal and antioxidant functions (Sambangi and Rani 2016). Resveratrol is a potential pro-oxidant which can inhibit the antioxidant systems in the insect gut or can generate ROS in the digestives tract of insects. These ROS can lead to fatal oxidative damage to the cells of the midgut (Baxter et al. 1998, Sambangi and Rani 2016). In earlier work, we showed that *R. flavipes* and *H. indicola* exposed to extractives from *T. grandis*, *D. sissoo*, *C. deodara*, *P. roxburghii*, and *Morus alba* (L.) incurred increased mortality with increased concentration of extractives when termite were exposed to extractive treated nondurable southern pine (Hassan et al. 2016, Mankowski et al. 2016). Extractives from these woods were also shown to rapidly lower protozoan numbers in the hindgut of exposed termites, and this correlated with termite mortality. (Hassan et al. 2017a).

The inhibition of enzymes has been observed to correlate with the contents of phenols and tannins in the extracts of plants (Owen and Johns 1999). Two neurotransmitter enzymes, glutamic acid decarboxylase, and monoamine oxidase were inhibited by extracts of different plant species (Milestone et al. 2012, Waye et al. 2014). How plant allelochemicals act on the enzymes, is generally not known yet, although some seem to disrupt the digestive system and process by targeting insect gut digestive enzymes, such as proteases and α-amylases (Duffey and Stout 1996). For example, ingestion of azadirachtin by *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) and *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Crambidae) caused a decrease in gut enzyme activities (Nathan et al. 2005a,b). Phenolic compounds present in the extractives can act as enzyme and metabolism inhibitors as these binds to proteins and thus act as nutritional protein precipitating agents and ultimately reduce the protein digestibility in insects (Torres et al. 2003). It has also been suggested that extractive compounds bind to the active enzyme site inhibiting the detoxification role of the enzyme (Kolawole et al. 2009).

In our study, activity of the tested gut enzymes esterase and GST were significantly reduced after feeding on filter paper treated with heartwood extractives or the synthetic antioxidants, BHT and quercetin. Total phenolic content present in the plant extracts has been interrelated with its antioxidant activity via assessment of phenols on enzyme inhibition (Skerget et al. 2005, Coruh et al. 2007a,b, Kolawole et al. 2009). GST serves a crucial role in the defense system of insects. Thus, reduction in the detoxification mechanism of this enzyme may lead to higher mortality in termites exposed to extractives that inhibit this enzyme system. Our results agree with Tang et al. (2007) who observed a decrease in GSTs activities in *R. chinensis* (Snyder) and *O. formosanus* (Shiraki) after treatment with two antioxidants (quercetin and tannic acids). Similar results were observed by Wang et al. (2014) where reduction of this enzyme (GST) in the gut of the Colorado potato beetle by cone and bark extract of pine was noted. This inhibition was positively correlated to phenolic contents of the tested extracts. Reduction of GST activity has also been reported in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Callosobrochus maculatus* (F.) (Coleoptera: Chrysomelidae) by different plant extracts (Kolawole et al. 2009, Rizwan-ul-haq et al. 2010, Kolawole and Kolawole 2014). Esterase, a detoxifying enzyme, play very important role in hydrolyzing of the esteric bond of synthetic chemicals (Zibaee and Bandani 2010). Our findings agree with Bouayad et al. 2013 who showed a reduction in esterase from the extracts of 10 plant species in the guts of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Similarly, esterase reduction was observed in *Eurygaster integriceps* (Puton) (Hemiptera: Scutelleridae) after topical application of two medicinal plants extracts (Zibaee and Bandani 2010). In our study, CAT activity was not significantly reduced by the control antioxidant compounds or tested heartwood extractives. This is contrary to the findings of Kaur et al. (2014) where a reduction in CAT was observed in *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) after feeding on a diet mixed with plant extracts. Reduction in enzymes activities can be due to synergistic effect of plant chemicals on antioxidant enzyme system of termites. These plant chemicals act as inhibitors of enzymes which increases the concentration of ROS in termite gut which ultimately leads to death of insect.

Gut symbionts of some insects, particularly termites, contribute to the survival of the insect by the production of detoxifying enzymes (GST, EST) and thus augment the hosts' ability to detoxify poisons (Shen and Dowd 1992). Previous studies showed an increased number of bacteria in the insect gut may produce detoxifying enzymes such as esterases, which also aid in depolymerization of lignin in wood-feeding termites (Scharf and Tartar 2008, Ramya et al. 2016). Esterases present in the gut of insects have previously been identified

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from symbiotic gut bacterial and fungal communities of higher termites. McSweeney et al. (1999) identified esterase activity from a bacterial gut symbiont (*Clostridium xylanolyticum*) of *Tumilitermes pastinator* (Holmgren) (Blattodea: Termitidae), a grass-feeding higher termite. *Clostridium* bacteria have also been identified from the gut of *R. flavipes* (Kollar) (Fisher et al. 2007). Symbiotic protozoans in the guts of lower termites (Rhinotermitidae) such as *Heterotermes* are a rich reservoir of novel Fe hydrogenase, *endo*-β-glucanase, and other enzymes (Inoue et al. 2007, Cairo et al. 2011). In earlier work we performed (Hassan et al. 2017), we found that extractives of the four heartwoods tested here drastically reduced the number of gut protozoa in *H. indicola*. Reduction in the activity of tested enzymes may be due to gut protozoan depletion in response to compounds in the extractives as some studies shows that esterases are produced by some of the protozoa species (*P. grassii*) of lower termites. However, we are currently investigating whether or not the origin (self-produced or symbiotic or both) of the three enzymes tested here are produced by the termites themselves or from their symbiotic protozoa.

Conclusion

Heartwood extractives of tested species have potential to reduce the activity of GST and esterase present in the gut of *H. indicola*. This reduction appears to increase termite mortality due to antioxidant properties of theextractives.

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