CHEMICAL STUDY OF ANTI-TERMITE EXTRACT OF SAW DUST OF HAGENIA ABYSSINICA

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CHEMICAL STUDY OF ANTI-TERMITE EXTRACT OF SAW DUST OF HAGENIA ABYSSINICA

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## Abbreviations and symbol

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<thead>
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<th>Description</th>
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<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas liquid chromatography</td>
</tr>
<tr>
<td>HMBC</td>
<td>Hetronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>HSQC</td>
<td>Hetronuclear Single Quantum Correlation</td>
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<tr>
<td>IR</td>
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<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetra methyl silane</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra Violet</td>
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<tr>
<td>Δ (delta)</td>
<td>the symbol used to indicate chemical shift value</td>
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Abstract

The monotypic genus *Hagenia*, which belongs to the family Rosacea, commonly known as Kosso is the most widely used taenicide against tapeworm. The flowers of this plant have been studied before, whereas no previous chemical work has been reported from the wood of *H. abyssinica*.

*C. macrostachyus* stakes treated with successive extracts of *H. abyssinica* saw dust with EtOAC and MeOH, and direct extract of *H. abyssinica* saw dust with ethanol were damaged at different levels. However, *C. macrostachyus* stakes treated with diethyl ether extract of *H. abyssinica* female flower were not damaged at all.

Phytochemical investigation from the saw dust of *H. abyssinica* resulted in the isolation of one compound partially characterized on the basis of spectroscopic data. Search in the chemical literature indicated that the isolated compound has not been reported before from the genus *Hagenia*. 
1. Introduction

1.1 The family Rosaceae

Rosaceae, in the order Rosales, is a large family containing more than 100 genera and 2,000 species of herbs, shrubs and trees of economic value, both for food (e.g., fruit trees including plums, apples, pears, loquats, blackberries and strawberries) and as ornamentals (e.g., flowers of the genus *Rosa*) (Hedberg, 1989). This family is represented on all continents except Antarctica, but the majority of species are found in Europe, Asia and North America.

1.2 The genus *Hagenia*

Taxonomy

*Hagenia abyssinica* (Bruce) J. F. Gmel. is commonly known as Kosso in Ethiopia. Taxonomically it has also been treated as *Brayera anthelmintica* Kunth, *Bankesia abyssinica* Bruce, *Hagenia anthelmintica* (Kunth) Eggeling, and *Hagenia abyssinica* (Bruce) Gmelin var. *viridifolia* Hauman (Jansen, 1981). The name Kosso is used in several ways in Ethiopia. It can indicate the tree (*Hagenia*), the female inflorescence (the medicine), the parasite, or in general other taenicidal medicine.

Ecology and distribution

In Ethiopia, *H. abyssinica* was once abundant in the semi-humid mountain woodlands with the altitudinal range of between 2,450 and 3,250 m (Hedberg, 1989). In forest depressions, it can descend to 2,000 m. Friis (1992) gives a wider altitudinal range of 1,850 to 3,700 m. The species occurs naturally within the undifferentiated Afromontane (mixed
Podocarpus forest) and dry, single-dominant Afromontane forest (Juniperus forest) or forest dominated by Hagenia (Friis, 1992). It is often found in association with tree species such as Schefflera abyssinica (A. Rich.), S. volkensii (Harms) Harms, as well as with the mountain bamboo, Arundinaria alpina. The tree is so dominant in the mountains of tropical Africa; it is characterized as a typical example of Afromontane endemism (Friis, 1992).

Outside Ethiopia, H. abyssinica is reported from the mountainous regions of Kenya, Tanzania, Malawi, Zimbabwe, Zambia, Zaire, Burundi, Rwanda, Uganda and Sudan (Jansen, 1981).

**Botanic description**

*H. abyssinica* is a dioecious tree with distinct male and female trees, both of which are endowed with colourful flowers. The tree attains heights of up to 20 m, with short trunk and thick branches; branchlets covered in silky brown hairs and ringed with leaf scars. The bark, which can sometimes be very thick in older specimens, is usually brownish in colour, and readily peels in strips. Hairs are quite abundant on young branchlets, petioles and leaves. The leaves are composed of several leaflets, ranging in number from 11 to 13. They measure up to 40 cm in length (Negash, 1995) (See Fig. 1).

![Fig. 1 Picture showing the leaves of H. abyssinica (photo by prof. Ermias D.)](image-url)
The flowers are greenish, or white, turning reddish with maturity in female flowers. The individual flowers of the female tree are small and inconspicuous. However on aggregate, they form rather a bright inflorescence that appears exceptional to other wind-pollinated inflorescences/flowers which, usually, are dull in colour. The reddish female inflorescence is bulkier than the dull-cream inflorescence of the male. Apparently, the larger size of the female inflorescence is meant for enhancing its receptive capability for the wind–borne pollen grains of the male inflorescence (Negash, 1995).

Fruit small, dry, winged, asymmetric, single seeded, brown syncarp with a single more or less ovoid carpel and fragile pericarp. The wood is dark red, hard and useful for furniture but attacked by borers (Bekele, et al. 1993). *Hagenia* is a monospecific genus confined to Africa and is most closely related to the monospecific genus *Leucosidea*. The specific name means ‘from Ethiopia’.

### 1.3 Ethnobotany of *H. abyssinica*

Plants have been used as a source of medicine in Ethiopia from time immemorial to treat different ailments. Due to its long history, traditional medicine has in fact become an integral part of the culture (Pankhurst, 1965). It is not unusual for people living in the countryside to treat some common ailments using plants available around them (e.g. *H. abyssinica* to expel tapeworm).

The Ethiopian taenicide Kosso (*H. abyssinica*) was known in Ethiopia for many centuries. The drug was reported to the rest of the world in the early seventeenth century by the Portuguese Jesuit Almeida and described in detail in the eighteenth century by the Scottish traveler
James Bruce (1790), who visited Ethiopia from 1768-1773. His Italian companion, L. Balugani, who died in Gondar in 1770, provided an artistic illustration. Bruce was hopeful that this plant would be developed as a drug to combat the worm infestation widely prevalent in Europe at the time. Additional attributes and observations were provided by the physician Brayera and the botanist Kunth in 1822 and the traveler Johnston in 1844 (Abegaz et al., 1999).

The Czech traveler Prutky was, however, the first writer to describe the manner in which the Kosso medicine was prepared and to mention the custom of taking Kosso every month or so. This was adopted because the drug in many instances failed to expel the head of the worm and the worm grew again regaining its original form and size in a few weeks (Pankhurst and Pearson, 1972).

The female flowers of Kosso have long been used to expel tapeworm, a very common infestation among Ethiopians due to the age-long practice of eating raw beef by a large sector of the population. Only female flowers of the Kosso are said to be active as anthelmintics, and they are called ‘red Kosso’. According to Lemordant (1972, in Jansen, 1981), the male flowers cause much more vomiting and are called by the Ethiopians: donkey’s Kosso, dog Kosso or hyena-Kosso. Abegaz and Dagne (1978), however, showed that both male and female flowers have similar toxicity effect to earthworms. On the other hand Yohannes and Dagne (1983) showed the absence of kosotoxin and protokosin in the male flowers from a comparative study of the female and male flowers. As a result, if kosotoxin is the one, which is responsible for poisoning the common human tapeworm, and if this same substance was shown to be absent from the male flowers of the species then this may explain the reason why the male flower is not traditionally used as a taenicide. Abate and
Dagne (1995) also demonstrated that kosotoxin is lethal to brine shrimp (*Artemia salina*) with an IC$_{50}$ of 30-40 $\mu$g/ml.

The female flowers of Kosso are readily available in markets throughout the year. In the main market in Addis Ababa, Kosso flowers are sold at about US$5/Kg (Dagne and Abate, 1995). The traditional healer G. Abate (1989) claims that the flowers are also useful in treating high blood pressure and diabetes.

The Kosso medicine can be prepared by soaking the crushed flowers overnight in water, local beer (tella), or mead (tej) before the filtered solution is drunk in the morning to expel *Taenia saginata* the common beef tapeworm. It is normally taken on empty stomach, and food abstention is continued until the huge segments of the worm are expelled in the feces. When Kosso was administered in a paste form to human volunteers, the effective dose was determined to be in the range 10 to 15 g, and the worm expulsion time to be in the rage 10 to 11 h (Fullas, 2001).

It is, however, important to note that the traditional medical practice varies from region to region, and, owing to the introduction of anti-tapeworm tablets, the practice is now becoming more and more obsolete, especially in the bigger towns and cities of Ethiopia (Negash, 1995).

The action of an anthelmintic agent may involve: paralysis of the parasite, or its dislocation from normal habitat in the host, or it may affect the surface membrane in such a way that it becomes easy for the host defence mechanism to launch its attack. Further more, some anthelmintic agents may inhibit respiration and thus block glucose uptake by the parasite (Dawit et al., 2003).
Despite widespread use as anthelmintic, Kosso was found toxic to mice in high concentrations. However, Kosso was not hepatotoxic in normal, starved and paracetamol-fed mice. On the other hand, Kosso was fatal to mice, fatality being maximum during periods of starvation (Tsega et al., 1978). While assuming that hepatitis in Ethiopia is primarily related to a virus, clinical observations of hepatitis-like presentations after ingesting Kosso raise the possibility of other causative agents (Tsega 1977). However, Kosso may have been taken coincidentally during the prodromal phase of hepatitis to relieve symptoms.

Rokos (1969) noted that Kosso appears to cause in certain individuals under condition still unknown, a simple or total optic atrophy which is usually bilateral. Low et al. (1985) observed defects in visual behavior in chicken treated with Kosso flowers.

In Ethiopian highlands, parents are said to have used Kosso as a means of disciplining their children. The punishment involved making the children inhale the smoke from a burning Kosso, which made them sneeze and cough. Despite the medicinal importance of this plant, the profession of selling Kosso was looked down upon traditionally (Fullas, 2001). Young leaflets of *H. abyssinica* are so spongy that, in some localities, they are used as a mattress for a mother who has just given birth to a new baby (Negash, 1995).

The species has been playing a number of important roles in the early, as well as in the present economic welfare of the people. For example (owing to its characteristic branching habit), the tree has been used in traditional apiculture. Peasants use the branches of the tree for keeping and stabilizing their beehives on them. Thousand years ago, the wood of *H. abyssinica* was mainly used for fuel, for house construction, as well as for fencing. It is easy to split, but it has to dry while the tree is still
standing, either through a natural death or through girdling. According to some unconfirmed reports, honey obtained from the nectar of *H. abyssinica* may sometimes purge tapeworm (Negash, 1995).

**Ethnobotany of *H. abyssinica* in other African Countries**

In E. Africa the roots of *H. abyssinica* are cooked with meat and the soup drunk against general illness and against malaria, besides its use as an anthelmintic (Jansen, 1981). The dried female flower heads and the bark infusion serve as a reputed, powerful remedy for intestinal parasites, especially against cestodes. It is also claimed that the bark cures diarrhea and stomach ache in humans; however it is also reputed to cause abortions. *H. abyssinica* has been used as an anthelmintic in ruminants by farmers and pastoralists in Kenya. Hauman (1952, in Jansen, 1981) reported that the medicinal use of Kosso as anthelmintic is unknown in Zaire, although the plant is common. In Zaire, however, the wood is used for fences and for the preparation of pans in which the milk is curdled. Kobert thought that the stiff hairs found in Kosso might play a part in producing the anthelmintic action.

**1.4 The chemistry of *Hagenia abyssinica***

Investigations of Kosso were made by numerous workers from 1839 onwards, but the early literature was in a very confused state; a useful summary of the early work was given by Kondakow (1899, in Hems and Todd, 1937). The first definite crystalline product from Kosso appears to have been the kosin, m.p. 142 °C, prepared by Messers. E. Merck. The extraction process involved treatment of the flowers with hot milk of lime and subsequent extraction with alcohol. Leichsenring (1894, in Hems and Todd, 1937) isolated from an ethereal extract of Kosso, protokosin, a
colourless crystalline compound with no vermicidal properties. Interestingly, Leichsenring also managed to isolate an amorphous and a highly toxic substance known as kosotoxin. Lobeck (1901, in Hems and Todd, 1937), using a modified form of the old alkaline extraction process, isolated kosin together with small quantities of protokosin and a third substance, kosidin, having very similar properties. His analytical data for kosin and protokison agreed with those of Leichsenring but he was able to show the so-called kosin was really a mixture of two isomers, $\alpha$- and $\beta$-kosin.

Kosotoxin and kosin are anthelmintic against cestodes. It is reported that these compounds have been used as a taenicide in veterinary medicine (Dawit et al., 2003). According to Abegaz and Dagne (1978), kosins exhibit comparable potency as the marketed drugs dichlorophene and niclosamide.

The kosins are presumably located in the typical glandular hairs occurring on the epidermis (Lounasmaa et al.1973, 1974). Bernard et al. (1974, in Jansen, 1981) analyzed a sample of Kosso and reported the following composition: moisture 9%, ash 6.4%, sodium 0.02%, potassium 1.22%, calcium 0.82%, acids 49.5 meq. /100g. They identified six organic acids: fumaric acid (3% of total acidity), $\alpha$-ketoglutaric acid (3%), succinic acid (3%), citric acid (19%), glycolic acid (3%), malic acid (59%). The following amino acids were identified (in total ca 18.2% of the sample, values are mg/g in dried flower sample): aspartic acid 26.2, threonine 9.2, serine 9.9, glutaminic acid 26.1, proline 14.4, glycocol 9.9, alanine 10.4, valine 10.9, cystine 0.0, methionine 0.4, iso-leucine 10.5, leucine 15.2, tyrosine 5.8, phenylalanine 14.2, histidine 6.1, arginine 12.4. Except for the higher proline content, these values are in accordance with comparable findings in plant tissues.
Structure determination of kosins

Hems and Todd (1937) isolated from commercial Kosso a substance having the recorded properties of protokosin together with some kosotoxin. However none of the reported kosidin was found. They proposed structure (1) for protokosin. Later Birch and Todd (1952) on the basis of studies of reduction products and spectral analysis revised the structure of protokosin to (2). Again Riedl and Orth and Riedl (in Lounasmaa et al., 1973) on the basis of synthetic work rejected formula (2) for protokosin and proposed a new tricyclic structure (3). All of these workers made significant contributions but not arrived at the correct chemical structures of the active constituents until 1974.

![Chemical Structures]
Lounasmaa et al. (1973) reinvestigated the structurally related kosins in *H. abyssinica* by preparing ether extracts and crude kosins (phloroglucinol mixtures) from Kosso. These workers isolated four phloroglucinol derivatives (kosins), and referred to them as K1, K2, K3, and K4. For the four kosins isolated, the structure (4) (K1) and partial structures (5), (6) and (7) (K2-4, respectively) were proposed. Due to the very small amounts of isolated pure kosins, the analytical data were insufficient for elucidating all structural details.

![Chemical structures](image)

Mixture of

![Chemical structures](image)

R = -CH(CH₃)₂, -CH₂CH(CH₃)₂, -CH(CH₃)CH₂CH₃
The acyl side chains of the four kosins were cleaved by strong alkali and the acids formed were identified by GLC. Isobutyric acid was the main component, accompanied by isovaleric acid and 2-methylbutyric acid. In previous investigations, only isobutyric acid was found. The methylene bridges between the different ring systems were cleaved by alkali and the products obtained were mixtures of closely related homologous pseudo-aspidinols (acyl-3-methylphloroglucinol-2-methyl ethers) slightly differing from pseudo-aspidinol B (8a) in their acyl side-chains. The composition of the acids obtained from the kosins indicated that it could be a mixture of the pseudo-aspidinols isobutyryl (IB) (8b), isovaleryl (IV) (8c), and 2-methylbutyryl (2-MeB) (8d).

![Chemical Structures](image)

8a R = -CH₂CH₂CH₃
8b R = -CH(CH₃)₂
8c R = -CH₂CH(CH₃)₂
8d R = -CH(CH₃)CH₂CH₃

The existence of pseudo-aspidinol isobutyryl (8b) (isobutyryl-3-methylphloroglucinol-2-methyl ether) unit was previously postulated in the molecule of protokosin by Orth and Riedl. The phloroglucinols obtained after alkaline cleavage of K1 (4) are shown in Scheme 1. In addition to pseudo-aspidinol iB (8b) (IV (8c), 2-MeB (8d)) two more breakdown products were identified as phloroglucinol iB (9a) (IV (9b), 2-MeB (9c)) and methyl phloroglucinol iB (10a) (IV (10b), 2-MeB (10c)) through comparison with authentic samples.
The MS of K1 presents four molecular peaks at m/e 710, 696, 682 and 668 corresponding to C_{39}H_{50}O_{12}, C_{38}H_{48}O_{12}, C_{37}H_{46}O_{12} and C_{36}H_{44}O_{12}, respectively. These peaks, as well as the peaks at m/e 653, 639 and 625, which can be assigned to the cleavages of C_{4}H_{9}- and C_{3}H_{7}- side chain units from the molecular ions, are in good agreement with the results of alkaline cleavages and confirm that K1 is a mixture of side chain homologues. The general fragmentation of K1 supports the proposed structure (4).

In 1952 Birch and Todd suggested the formulae (11) and (12) for α- and β-kosin, respectively. On the bases of extensive synthetic work, Orth and Riedl later rejected the proposed structures. As their synthetic 5,5’-methylene-bis-(3-methyl-phloroisobutyrophenone-2-methyl ether) [13; R = -CH(CH_{3})_{2}] proved to be identical with the α-kosin of Birch and Todd, the structure of α-kosin was settled. Moreover, Orth and Riedl showed that β-kosin was not identical either with compound (12) or any other
synthetical dimethyl ether of 5,5’-methylene-bis-(3-methylphloroisobutyrophenone) containing the two methoxyl groups in different rings. Therefore they proposed for β-kosin the tentative formula (14), where the two methoxyl groups are in the same ring.

\[
\begin{align*}
\text{α-kosin 11} & \\
\text{β-kosin 12} & \\
\text{α-kosin 13} & \\
\text{β-kosin 14} & \\
R = \text{-CH(CH}_3\text{)}_2
\end{align*}
\]

Lounasmaa et al. (1974) prepared kosin with slightly varying melting points (α-kosin, 148-150 °C; β-kosin, 120 °C) and crystalline forms by column chromatography after treatment of crude kosin, kosotoxin or protokosin with alkali. These kosin were resistant against further breakdown with alkali and detected by TLC in the reaction mixture even after 24 h heating in 15% NaOH on a water bath. The sole decomposition products observed consisted of pseudo-aspidinols iB, iV and 2-MeB (15a, b, and c) (Scheme 2). 3-methyl-phloroisobutyrophenone-2,6-dimethyl ether (16) or other isomeric dimethyl ethers were not detected, which could be expected to appear among the decomposition products of 14 or its isomer. On the other hand, some methyl-phloroglucinol-2-methyl ether (17), resulting from the reductive alkaline cleavage of the acyl side chains of 15a, b and c was recognized by TLC (Scheme 2). The same
ether (17) has previously been isolated by Lobeck after strong reductive alkaline cleavage of his α-kosin.

R = -CH(CH$_3$)$_2$(a), -CH$_2$CH(CH$_3$)$_2$(b), -CH(CH$_3$)CH$_2$CH$_3$(c)

Scheme 2. Reductive alkaline cleavage of kosin (13)

In the light of these facts, it seems very probable that α- and β-kosin simply differ in their side chains, which consist of iB, iV and 2-MeB groups in different ratios.

The mass spectra of different kosin fractions show molecular peaks at m/e 488, 474 and 460 corresponding to C$_{27}$H$_{36}$O$_8$, C$_{26}$H$_{34}$O$_8$ and C$_{25}$H$_{32}$O$_8$, respectively. The peaks at m/e 431 and 417 support the assumption that kosin fractions are mixtures of C$_4$H$_9$- and C$_3$H$_7$-side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern is in agreement with the proposed structure (13). The relative intensities of the molecular peaks are in good agreement with the conclusion that α- and β-kosin differ only in their side chains. Thus the fraction melting at 148-150 °C shows a relatively
strong molecular peak at m/e 460 and weak molecular peaks at m/e 474 and 488 supporting the conclusion that α-kosin contains mainly isobutyryl side chains. On the other hand, the relative intensities of the molecular peaks at m/e 474 and 488 increase as the melting points of the different kosin fractions decrease, approaching the indicated melting point of β-kosin (120 °C). Such changes point to the growing contribution of iV and 2-MeB side chains in the fractions.

Lounasmaa et al. (1974) also proposed the now accepted structures (18) and (19), as a result of extensive spectral analysis and examinations of degradative products, for kosotoxin and protokosin respectively.

![kosotoxin structure](image)

kosotoxin 18

![protokosin structure](image)

protokosin 19

\[ R = \text{-CH(CH}_3\text{)}_2, \text{-CH}_2\text{CH(CH}_3\text{)}_2, \text{-CH(CH}_3\text{)CH}_2\text{CH}_3 \]

The first step in the decomposition of kosotoxin (18) and protokosin (19) (Scheme 3 and 4) was the cleavage of the methylene bridges, resulting in
large amounts of pseudo-aspidinol iB, iV and 2-MeB (20a, b, c). Although expected, no 3-methyl-isobutyrylfilicinic acid (21a), 1,3-dimethyl-5-isobutyrylphloroglucinol (22), or 3-methyl-pseudo-aspidinol iB (23a) or its iV or 2-MeB homologues were detected by TLC. Neither were 3,3-dimethylisobutyrylfilicinic acid (24a) or its iV and 2-MeB homologues observed in the case of protokosin (19).

The second step in the decomposition of both kosotoxin (18) and protokosin (19) (Scheme 3 and 4) lead to compounds of unknown structure. These compounds were decomposed by a much more drastic variant of the reductive alkaline cleavage and large quantities of methylphloroglucinol-2-monomethyl ether (25) and trace amounts of 3-methylfilicinic acid (26) were detected in the reaction mixture. The former was previously found by Lobek after reductive alkaline cleavage of kosotoxin and kosin. However, the key degradation product expected from protokosin, 3,3-dimethylfilicinic acid (27) could not be detected with certainty.
The mass spectrum of kosotoxin shows molecular peaks at m/e 488, 474 and 460 corresponding to C_{27}H_{36}O_{8}, C_{26}H_{34}O_{8} and C_{25}H_{32}O_{8} respectively. The peaks at m/e 431 and 417 support the assumption that kosotoxin is a mixture of C_{4}H_{9}- and C_{3}H_{7}- side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern supports the proposed structure (18).

Scheme 3. Reductive alkaline cleavage of kosotoxin 18
The mass spectrum of protokosin presents molecular peaks at m/e 738, 724, 710 and 696 corresponding to C_{41}H_{54}O_{12}, C_{40}H_{52}O_{12}, C_{39}H_{50}O_{12} and C_{38}H_{48}O_{12} respectively. The peaks at m/e 681, 667 and 653 support the assumption that protokosin is mixture of C_{4}H_{9}- and C_{3}H_{7}- side chain homologues and confirm the results of the alkaline cleavages. The general fragmentation pattern supports the proposed structure (19).
1.5 Protection of *H. abyssinica* wood against termites attack

Forests in Ethiopia have been indispensable sources of timber, most common and widely used renewable material in all parts of the country for construction, forest industries and wood-based energy sectors. Wood is the material produced in the stems and branches of trees and other woody plants, having a base diameter of $\geq 10$ cm and a length of $\geq 1.5$ m. Wood is a multi-purpose, biological and renewable product from nature, which has to be protected and rationally utilized (Willeitner and Leise, 1992 in Desalegn et al (2003)). Almost all houses in rural areas of the country and in the majority of urban areas are still made of wood as the major construction material.

Though mostly overlooked, sever degradation of wood, wooden structures and commodities, both in service and during storage, occur due to biodeteriorating agents (mainly termites, fungi, beetles and marine borers) and problems of timber seasoning are among the major causes of forest destruction and degradation of wood in Ethiopia. Specifically, these factors usually lead to low quality of timber, low recovery rate at sawmills as well as during further processing and manufacturing of products, and short service life of timber based constructions, that trigger frequent and excessive mining of the last remaining forests and re-building constructions (Desalegn et al (2003)).

Termites are the most important wood pests in all tropical and subtropical regions of the world, although they are important for nutrient recycling in forest ecosystems by converting dead trees into organic matter. They cause more damage to homes than all other natural disasters combined (Anonymous, 1997 in Desalegn et al (2003)). The potential damage of termites on forests and forest products was estimated in several countries. The worldwide cost of treatments to
eradicate termite infestations has been estimated at approximately USD 1.92 billion (Eaton and Hele, 1993 in Desalegn et al (2003)).

The termites and fungi groups found in Ethiopia are diverse and less known. It has been estimated that the damage caused by termites in Ethiopia is commonly over 20% and rarely exceeding 50%. In some cases, the termite damage has led to total crop loses. However, there are only few wood preservation studies made so far on a few timbers, and only for short periods (< five years) to evaluate natural durability of timbers.

Desalegn et al. (2003) investigated on 32 timber species to select naturally durable and treatable species for construction purposes. As a result they reported ten naturally durable timber species, which resisted termites and fungal attack for more than 10 years. Among these *H. abyssinica* showed 5% and 45% resistance/survival rate against termites and fungal attack, respectively in 13 years exposure. However the best naturally durable timber species, *J. procera*, showed 65% and 55% resistance/survival rate against termites and fungal attack, respectively in 13 years exposure. The workers found no naturally very durable timber species during their work. The more durable timber species owe their resistance mainly to their extractives, which serve as natural preservatives. Preference and resistance of plants to termites rely on many factors such as: hardness, lignin content or chemical constituents of wood. Many researchers have examined the effect of wood extracts on the behavior of termites.

Taking the above report into consideration, Gedeon Yohannes (2006) conducted an experiment on the resistant plants and demonstrated that *J. procera* stakes were 100% resistant to termites attack in one month.
exposure. However, *H. abyssinica* and *C. macrostachyus* stakes were 82% and 17% resistant to termites attack, respectively in one month exposure. Similarly *C. macrostachyus* stakes treated with CHCl₃, acetone and ethanol extracts of *H. abyssinica* and *J. procera* saw dust showed different resistance to termites attack after a month (see Table 1).

Table 1. Mean % resistance of *C. macrostachyus* (control), *H. abyssinica*, *J. procera* and *C. macrostachyus* stakes treated with acetone, CHCl₃ and ethanol extracts of *H. abyssinica* and *J. procera* saw dust against termites’ attack.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean % resistance after a month</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. macrostachyus</em> (control)</td>
<td>17</td>
</tr>
<tr>
<td><em>H. abyssinica</em></td>
<td>82</td>
</tr>
<tr>
<td><em>J. procera</em></td>
<td>100</td>
</tr>
<tr>
<td><em>H. abyssinica</em> (acetone)</td>
<td>61</td>
</tr>
<tr>
<td><em>J. procera</em> (acetone)</td>
<td>54</td>
</tr>
<tr>
<td><em>H. abyssinica</em> (CHCl₃)</td>
<td>48</td>
</tr>
<tr>
<td><em>J. procera</em> (CHCl₃)</td>
<td>52</td>
</tr>
<tr>
<td><em>H. abyssinica</em> (ethanol)</td>
<td>75</td>
</tr>
<tr>
<td><em>J. procera</em> (ethanol)</td>
<td>58</td>
</tr>
</tbody>
</table>

He also showed that acetone, ethanol and water extracts of *H. abyssinica* and *J. procera* fine saw dust showed no mortality to worker termites at 10%, 15%, and 20% extraction and at 1, 2 and 3 ml application. However, he found that aqueous extract of *H. abyssinica* female flower and *Milletia ferruginea* seed powder killed termites at three levels of extraction (10%, 15% and 20% W/V) and at 3 ml level of application after 24 h.
1.6 Objective of the study

It is reported that *H. abyssinica* showed 5% resistance against termites attack and classified as durable timber species. The more durable timber species owe their resistance mainly to their extractives, which serve as natural preservatives. The ethanol extract of *H. abyssinica* saw dust applied on *C. macrostachyus* stakes was found to resist (75%) termites’ attack by Gedion Yohannes, Biology Department, Addis Ababa University. The main objective of this project was to undergo chemical investigation to isolate and characterize the anti termite component/s from the ethanol extract of *H. abyssinica* saw dust. To our knowledge the wood of *H. abyssinica* has not yet been subjected to phytochemical study.
2. Results and discussion

The ground *H. abyssinica* saw dust was successively extracted with EtOAC and MeOH at room temperature, filtered and concentrated using rota vapor to give yellowish and red extracts respectively. TLC of the EtOAC extract using Hexane/Toluene/EtOAC (1:1:1) solvent system revealed presence of six spots when sprayed by 1% vanillin in conc. H$_2$SO$_4$ (see Fig. 2). The spot with the highest $R_f$ value was labeled as HAW1 and the lowest as HAW7. The EtOAC extract was subjected to flash column chromatography and fractions were monitored by TLC. Those fractions that had similar retention factor were combined and purified by precipitation.

In this work it was possible to isolate from the EtOAC extract one compound exhibiting $R_f$ value of 0.8 HAW2. The characterization of this compound was based on IR, $^1$H, $^{13}$C and 2D NMR spectra. Details about the extraction and isolation of this compound are given in the Experimental Section.

![Fig. 2 analytical thin layer chromatogram of HAW2 (A) and crude extract (B)](image_url)
2.1 Characterization of compound HAW2

HAW2 was obtained as a white powdery solid, soluble in CHCl₃ with m.p. 254-257 °C, not UV active (254 nm), red coloration with 1% vanillin-H₂SO₄ reagent and precipitated from hexane. Based on the MS spectrum (Appendix 1), which exhibited a molecular ion peak (EIMS) at m/z 496, and ¹³C NMR spectrum the molecular formula of HAW2 was established as C₃₂H₄₈O₄. In the IR (KBr) spectrum (Appendix 2) absorption bands at 1735 and 1246 cm⁻¹ showed the presence of ester functional group which are due to C=O and C-O stretching vibrations respectively. Absorption at 2934 cm⁻¹ is due to C-H stretching vibrations of aliphatic CH₃ and CH₂.

The ¹H NMR spectrum (Appendix 3) of HAW2 in CDCl₃ suggested the presence of eight methyl signals at δ 0.74 (3H, s), δ 0.88 (6H, d), δ 0.96 (3H, s), δ 0.98 (3H, s), δ 1.23 (3H, s), δ 1.26 (3H, s), and δ 2.07 (3H, s). The spectrum also showed one singlet at δ 5.35 integrating for one vinylic proton and again one triplet at δ 4.53 corresponding to a proton attached to a carbon bearing an electronegative atom such as oxygen. The chemical shift of one of the methyls at δ 2.07 (3H, s) was indicative of its attachment to a carbonyl carbon.

The ¹³C NMR spectrum (Appendix 4), analyzed with the aid of DEPT-135 (Appendix 5), showed nine quaternary carbons, which were attributable to two carbonyls (both ester), one oxygenated carbon, five aliphatic carbons and one vinylic carbon. In addition, six methines, nine methylenes and eight methyl carbons were observed. Out of the six methine carbons one was in the olefinic region based on the results of 2D ¹³C-¹H correlation spectroscopy (HSQC) and this suggests the presence of a trisubstituted double bond.
In the HMBC spectrum (Appendix 8), pertinent correlations were observed between the ester carbonyl carbon at $\delta$ 171.1 with the methyl protons at $\delta$ 2.07 and the oxygenated methine proton H-3, the oxygenated methine carbon at $\delta$ 81.0 (C-3) with the methyl protons at $\delta$ 2.07, H-24, H-25 and the methine proton H-5, the methine carbon at $\delta$ 55.2 with the methyl protons H-24, H-25 and H-26. These observations together with HSQC (Appendix 7) correlation of H-3 at $\delta$ 4.53 with C-3 at $\delta$ 81.0, the methine proton H-5 at 0.88 with C-5 at $\delta$ 55.17 and the methyl protons H-26 at 0.96 with C-26 at $\delta$ 15.3 led to partial skeleton (I).

The HMBC spectrum also showed correlations between the methine carbon at $\delta$ 47.1 with the vinylic proton H-12 and the methyl protons H-27, the vinylic quaternary carbon at $\delta$ 137.9 with the methyl protons H-28 and the vinylic proton H-12, the vinylic carbon at $\delta$ 129.3 (C-12) with the methylene protons H-11, the aliphatic quaternary carbons at $\delta$ 40.0 (C-8) and $\delta$ 47.8 (C-14) with the methyl protons H-28 and H-27 respectively and the methyl carbon at $\delta$ 17.1 (C-27) with the methylene protons H-7. These observations together with HSQC correlations give an indication for partial skeleton (II).

Partial skeleton III is based on methine proton H-17 which showed correlations with carbonyl carbon at $\delta$ 184.7 (C-20), oxygenated quaternary carbon at $\delta$ 73.1 (C-8), methine carbon at $\delta$ 41.1 (C-16) and aliphatic quaternary carbon at $\delta$ 41.0 (C-18). In addition, correlations were observed between the aliphatic quaternary carbon at $\delta$ 41.0 (C-18) with the methyl protons H-30, oxygenated quaternary carbon at $\delta$ 73.1 (C-19) and methine carbon at $\delta$ 52.7 (C-17) with the methyl protons H-29.
Further correlations of the methine proton H-9 with the aliphatic quaternary carbon at $\delta$ 36.9 (C-10) and the methine carbon at $\delta$ 47.09 with the methyl protons H-26 joined partial skeletons I and II to form partial structure (IV). Moreover, correlations of the methine proton H-17 with the vinylic quaternary carbon at $\delta$ 137.91 and the vinylic proton H-12 with the aliphatic quaternary carbon at $\delta$ 41.00 (C-18) linked partial skeletons III and IV to form partial structure (V). Although no definite evidence was obtained for the connectivity between the methylene carbons, probably due to complex nature of the peak observed for the methylene protons, joining the partial structure (V) to form methylene carbons resulted in the proposed structure (VI). The suggested partial skeletons are indicated in Figure 3.

Fig. 3. Suggested partial skeletons for HAW2 together with selected HMBC correlations (arrows are from proton to carbon).
Fig. 3. Suggested partial skeletons for HAW2 together with selected HMBC correlations (arrows are from proton to carbon).
Besides to the observed correlations, a portion of (partial skeleton IV in which the methylenes are joined) the proposed structure was found to agree with literature data (Sheng-Xiang, 1993).

Scheme. 5 Proposed Mass fragmentation pattern of HAW2
Table 2 Observed $^1$H and $^{13}$C NMR spectral data of HAW2 in CDCl$_3$

<table>
<thead>
<tr>
<th>C</th>
<th>$^1$H</th>
<th>$^{13}$C</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>81.0</td>
<td>37.7</td>
<td>H$^3$→C$^2$, C$^1$</td>
</tr>
<tr>
<td>4</td>
<td>4.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.96</td>
<td>55.2</td>
<td>H$^5$→C$^3$, C$^2$</td>
</tr>
<tr>
<td>6</td>
<td>18.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>32.5</td>
<td></td>
<td>H$^7$→C$^{27}$</td>
</tr>
<tr>
<td>8</td>
<td>40.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>47.1</td>
<td></td>
<td>H$^9$→C$^{10}$</td>
</tr>
<tr>
<td>10</td>
<td>36.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>23.5</td>
<td></td>
<td>H$^{11}$→C$^{12}$, C$^{13}$</td>
</tr>
<tr>
<td>12</td>
<td>5.35</td>
<td>129.3</td>
<td>H$^{12}$→C$^9$, C$^{13}$, C$^{18}$</td>
</tr>
<tr>
<td>13</td>
<td>137.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>47.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>28.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>41.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2.49</td>
<td>52.7</td>
<td>H$^{17}$→C$^{13}$, C$^{16}$, C$^{18}$, C$^{19}$, C$^{20}$</td>
</tr>
<tr>
<td>18</td>
<td>41.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>73.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>184.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>38.0</td>
<td></td>
<td>H$^{21}$→C$^{17}$</td>
</tr>
<tr>
<td>22</td>
<td>26.0</td>
<td></td>
<td>H$^{22}$→C$^{19}$</td>
</tr>
<tr>
<td>23</td>
<td>25.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.88</td>
<td>16.7</td>
<td>H$^{24}$→C$^3$, C$^4$, C$^5$</td>
</tr>
<tr>
<td>25</td>
<td>0.88</td>
<td>28.0</td>
<td>H$^{25}$→C$^3$, C$^4$, C$^5$</td>
</tr>
<tr>
<td>26</td>
<td>0.96</td>
<td>15.3</td>
<td>H$^{26}$→C$^9$, C$^5$</td>
</tr>
<tr>
<td>27</td>
<td>0.74</td>
<td>17.1</td>
<td>H$^{27}$→C$^9$, C$^{14}$</td>
</tr>
<tr>
<td>28</td>
<td>1.26</td>
<td>24.5</td>
<td>H$^{28}$→C$^8$, C$^{13}$</td>
</tr>
<tr>
<td>29</td>
<td>1.23</td>
<td>27.4</td>
<td>H$^{29}$→C$^{17}$, C$^{19}$</td>
</tr>
<tr>
<td>30</td>
<td>0.96</td>
<td>16.2</td>
<td>H$^{30}$→C$^{18}$, C$^{19}$</td>
</tr>
<tr>
<td>COMe</td>
<td>2.07</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td>COMe</td>
<td>171.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2 Antifeedant effects of H. abyssinica and C. macrostachyus stakes

Different resistance levels were observed on the wood stakes of *C. macrostachyus* and *H. abyssinica* in this test. *H. abyssinica* stakes and *C. macrostachyus* stakes treated with diethyl ether extract of *H. abyssinica* female flower were 100% resistant whereas untreated stakes of *C. macrostachyus* were 48% resistant to termites attack after a month. On the other hand *C. macrostachyus* stakes treated with MeOH, EtOAC and ethanol extract of *H. abyssinica* saw dust were 84%, 68% and 70% resistant, respectively against termites’ attack after a month (see Table 3). This result is in agreement with the result obtained by Gedeon Yohannes.

Table 3. Mean % resistance of *C. macrostachyus* stakes treated with EtOAC, MeOH and ethanol extracts of *H. abyssinica* saw dust and diethyl ether extract of *H. abyssinica* female flower against termites attack.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean% resistance after a month</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. macrostachyus</em> (control)</td>
<td>48</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (MeOH)</td>
<td>84</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (ethanol)</td>
<td>70</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (EtOAC)</td>
<td>68</td>
</tr>
<tr>
<td><em>C. macrostachyus</em> (diethyl ether)</td>
<td>100</td>
</tr>
<tr>
<td><em>H. abyssinica</em> (control)</td>
<td>100</td>
</tr>
</tbody>
</table>
Fig. 4 Picture showing how the stakes were attacked by termites. Stake no. 1 (H. abyssinica) and 2 (C. macrostachyus treated with diethyl ether extract of H. abyssinica female flower) were 100% resistant. However stake no. 3, 4, 5, 6 and 7 were attacked by termites to different extents.
3. Experimental

3.1 Instruments and materials
The IR spectrum was recorded with Perkin Elmer BX Infrared spectrometer in the range 4000-400 cm⁻¹.

¹H NMR, ¹³C NMR and 2D NMR spectra were recorded on a Bruker Advance 400 spectrometer at 400 MHZ and 100 MHZ. The chemical shifts are expressed in ppm relative to internal reference: for ¹H NMR spectra, the residual CHCl₃ resonance at δ 7.24, for ¹³C NMR, the center line of the solvent triplet at δ 77.0, downfield of TMS at 0 ppm. For the ¹³C NMR spectra, multiplicities were determined by DEPT-135 experiment.

Melting point was determined on a capillary melting point apparatus. TLC was done on silica gel 60 F₂₅₄ 0.2 mm thick layer on aluminum sheets (Merck). Flash chromatography was performed on silica gel (230-400 mesh, Merck). Components on TLC were visualized by spraying with vanillin-H₂SO₄ and heating. All chromatography solvents were distilled before use.

3.2 Plant material

The wood material of *H. abyssinica* and *C. macrostachyus* used in this study were purchased from the main market in Addis Ababa and Ziway town respectively. The saw dust of *H. abyssinica* wood and stakes of *C. macrostachyus* were prepared at the Workshop of Science faculty Addis Ababa University. The female flowers of *H. abyssinica* used in this study was also purchased from the main market in Addis Ababa from traditional medicine vendors.
3.3 Extraction and isolation

The ground *H. abyssinica* saw dust was successively extracted with EtOAC (2 X 250 ml/100 g) and MeOH (2 x 250 ml/100 g) for a minimum of 24 h extraction at room temperature. After each extraction, the sample was filtered and the solvent removed from the filtrate by rota vapor to yield 3 g of a yellowish powder from the EtOAC extraction and 2 g of a red powder from the MeOH extraction. The EtOAC extract (3 g) was subjected to flash chromatography on silica gel (230-400 mesh, 60 g) using a gradient of EtOAC in petroleum ether to give 13 main fractions. Fractions 7, 8 and 9 eluted with petroleum ether/EtOAC (72:28, 69:31, 65:35) showed similar retention factors. These fractions were combined and purified by precipitation from hexane to yield white powder solid (HAW2) (50mg).

3.4 Coding system

In the coding system H stands for the genus name *Hagenia*, A stands for species name abyssinica, W stands for wood and the number attached to HAW indicate the position of the compound starting from the highest R<sub>f</sub> value to the lowest. Thus, HAW2 stands for the second compound.

**HAW2:** White powder solid, IR (KBr) v<sub>max</sub> 2934, 1735, 1692, 1459, 1370, 1246 cm<sup>-1</sup>; <sup>1</sup>H NMR and <sup>13</sup>C NMR see Table 2.

3.5 Antifeedant test

The test was done at the actual habitat of the insects at Ziway. Stakes of susceptible plant (*C. macrostachyus*) having relatively equal volumes (70 ml) were prepared. The ground *H. abyssinica* saw dust (30 g) was successively extracted with EtOAC and MeOH to give 540 mg from the
EtOAC extraction and 420 mg from the MeOH extraction. Another 30 g of ground *H. abyssinica* saw dust was extracted directly with ethanol to give 1 g of ethanol extract. EtOAC and ethanol extracts were dissolved with CHCl₃ and MeOH mixture (8 ml) (1:1), and applied each on three stakes of *C. macrostachyus*. The same was done with MeOH extract dissolved with MeOH (8 ml). After solvents were evaporated, the stakes of susceptible plant that were treated with the extracts and other untreated stakes of susceptible plant, resistant plant stakes (*H. abyssinica*) as controls were taken to the field and buried around termite mounds. After a month stakes were dug out and brought to the laboratory and their volumes were measured. This was done by pouring water into a measuring cylinder of 500 ml in volume. Then each stake was immersed into the cylinder, the difference in volume of damaged and undamaged stake was measured and the percentage of the plant material eaten was calculated as follows:-

\[
\frac{(\text{volume of undamaged stake} - \text{volume of damaged stake})}{\text{volume of undamaged stake}} \times 100
\]

### 3.6 Toxicity test

Diethyl ether extract of *H. abyssinica* female flower (1 g) was dissolved with 6 ml of CHCl₃ and divided into three parts. Each part was then applied onto the filter paper in the petri dish and the solvent was allowed to evaporate at room temperature for 30 minutes. Then, 1 ml of distilled water was added to each petri dish as a carrier. This makes the extracted active ingredient to adhere to the body of the insects. Finally, 20 termites were added to each petri dish. In addition to the application of the extract, 2 ml of the solvent was used for the experiment as control for comparison. Then mortality was observed 24 h
after application of treatments. Both the toxicity test and antifeedant test were made following the method used by Gedeon Yohannes.

4. Conclusions and recommendations

Conclusions

- Diethyl ether extract of *H. abyssinica* female flowers powder was found toxic to termites. The toxicity of the extract is most likely due to the kosotoxin component of the female flower extract.

- *C. macrostachyus* stakes treated with EtOAC, MeOH and ethanol extracts of *H. abyssinica* saw dust showed varying degrees of susceptibility to termites attack whereas the stakes treated with diethyl ether extract of *H. abyssinica* female flowers were 100% resistant to termites attack.

Recommendations

- In this study *H. abyssinica* stakes and *C. macrostachyus* stakes treated with diethyl ether extract of *H. abyssinica* female flowers were observed to be highly resistant to worker termites' damage followed by *C. macrostachyus* stakes treated with MeOH extract of *H. abyssinica* saw dust. But, because of time constraint the reason for their resistance was not identified. Therefore, it would be better if further study is carried on identifying the protective substance from these extracts and make available for the end users.

- In the absence of other options, people use commercial insecticides to control termite attack. These insecticides are costly and are not easily obtained. Moreover, harmful to non target organisms and to the environment at large. Hence, the use of resistant plants and
natural insecticides in termite infested area should be encouraged and research should be pressed forward to identify and formulate such natural insecticides and use (Yohannes, 2006)

➢ To elucidate the complete and correct structure of HAW2 additional experimental data is recommended.
References


APPENDIX 1. MS spectrum of HAW2

ED-967
Sample01 179 (4.177) Cm (179:187-79:85)

TOF MS EI+
1.51e5
APPENDIX 2. IR spectrum of HAW2
APPENDIX 3. $^1$H NMR spectrum of HAW2
APPENDIX 4. $^{13}$C NMR spectrum of HAW2
APPENDIX 5. DEPT-135 spectrum of HAW2
APPENDIX 6. COSY spectrum of HAW2
APPENDIX 7. HSQC spectrum of HAW2
APPENDIX 8. HMBC spectrum of HAW2