Selected Wood Properties of Prunus Africana (Hook) Grown in Kenya as Possible Reasons for its High Natural Durability

Odabrana svojstva drva Prunus africana iz Kenije i mogući razlozi njegove velike prirodne trajnosti

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ABSTRACT • Studies were carried out on the influence of Prunus africana heartwood extractives on the growth of selected wood decay fungi. Also, wood chemical and mineral content, dimensional stability and anatomical features of P. africana were studied. Heartwood extractives were tested in 100 ppm and 500 ppm concentrations on white, brown rot, and blue stain fungi and growth inhibition was determined as a factor of time. Dimensional stability was determined by computing the swelling coefficient after the blocks were saturated with moisture. Klason lignin, Künschner cellulose, extractive and ash contents were determined by standard procedures. Infrared analyses were performed using Perkin Elmer FTIR spectrometer. Microscopic examination was performed using an environmental scanning electron microscope. The results showed that the wood is dimensionally stable, and contains 12.7 % extractives, 37.6 % cellulose and 30.4 % lignin. Extractives deposited in vessels are highly soluble in dichloromethane and mainly composed of terpenes. Extractives were able to inhibit the growth of white rot fungi Coriolus versicolor; brown rot fungi Poria placenta and blue stain fungi Aureobasidium pullulans at different concentrations tested and could explain the high durability of Prunus africana wood species.

Key words: Prunus africana, heartwood, extractives, fungi, inhibition

SAŽETAK • Cilj rada bio je istražiti utjecaj ekstraktivnih tvari u drvu Prunus africana na pojavu i razvoj odredenih vrsta gljiva koje uzrokuju trulež. Također, analiziran je kemijski i mineralni sastav drva P. africana, njegova dimenzijska stabilnost i anatomski obilježja. Ekstraktivi iz drva srži testirani su u koncentraciji 100 ppm i 500 ppm na gljive bijele truleži, smeđe truleži i plavila, a inhibicija rasta određena je kao faktor vremena. Dimenzijska je stabilnost određena izračunavanjem koeficijenta bubrenja nakon što su uzori natopljeni vodom do zasićenja. Klason lignin, Kürschner celuloza, ekstraktivi i sastav pepela određeni su standardnim postupcima.

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**INTRODUCTION**

**1. UVOD**

Prunus africana (Hook) occurs widely in tropical Africa. In Kenya, the plant is widely found in natural indigenous forests of Central and Rift Valley provinces. Wood of P. africana is widely used in Kenya for the construction of bridges and railway sleepers due to its high natural durability (Mburu, 2007). The heartwood is dark brown in color and described as resistant to termites and fungi (Mburu, 2007). Depletion of this wood species has raised concern in the Forestry Sector due to illegal exploitation evident in some parts of the country (Hitimana, 2000). In spite of the logging ban by the Kenyan government, farmers are encouraged to domesticate P. africana, a high value tree species, to ensure its continued existence. Traditionally, water extractives of P. africana bark are used orally to treat benign enlargement of the prostate gland in man (Bombardelli and Morazzoni, 1997; Stewart et al., 2003; Catalano et al., 1984; Breza et al., 1998). Pentacyclic triterpenes (oleanolic and ursolic acids) are believed to inhibit the activity of glycosyl-transferase, an enzyme involved in the inflammation process (Dufour et al., 1984; Bassi et al., 1987; Barlet et al., 1990; Bombardelli and Morazzoni, 1997).

There is no reported data on the influence of P. africana extractives on specific wood destroying fungi by taking time of inhibition as a factor. Similarly, other factors that influence durability of wood such as chemical composition and anatomical features have not been fully investigated. This study aimed at providing important technological information on P. africana wood and testing growth inhibition of aggressive white rot fungi, Coriolus versicolor, brown rot fungi Polia placent and coloration fungi Aureobasidium pullulans by heartwood extractives.

**2. MATERIALS AND METHODS**

**2.1. Soxhlet extraction**

Mature P. africana trees growing in Kimondi, Kapsabet forest, Kenya were sampled, felled and wood sawn into blocks measuring 5 mm × 20 mm × 25 mm. Heartwood and sapwood blocks, air-dried to approximately 18 % MC, were separately ground to fine powder, passed through a 115-mesh sieve and dried at 60 °C to constant weight and 12 % MC before extraction. Drying at 60 °C instead of 103 °C has been shown to minimize extractive degradation (Neya et al., 2004).

Hexane, acetone, dichloromethane, water and a mixture of toluene/ethanol at the ratio of 2:1 (v/v) were used successively for soxhlet extraction of 50 g sample each for 15 hours at a rate of about 10-12 cycles per hour and replicated three times.

**2.2. Fungal growth inhibition**

**2.2.1. Sprečavanje rasta gljiva**

Mycelium was grown in 9 cm diameter Petri dishes filled with 20 ml of malt-agar sterilized medium, prepared by mixing 20 g of malt and 40 g of agar in one liter of distilled water containing 100 ppm or 500 ppm of heartwood extract only. Plates were inoculated by placing a small portion of a malt- agar freshly grown fungal colony of C. versicolor, P. placenta or A. pullulans at the centre of each petri dish and cultures were maintained at 22 °C and 70 % relative humidity. Growth was evaluated by measuring the mean of two perpendicular diameters of the colony every two days. Inhibition was computed using equation (1), when the diameter of control culture filled the petri-dish:

\[
\text{Growth inhibition} \% = 100 \times \left(1 - \frac{d_1}{d_2}\right) \tag{1}
\]

Where \(d_1\) is the diameter of the control culture and \(d_2\) the diameter of the extract culture.

**2.3. Determination of dimensional stability**

**2.3.1. Određivanje dimenzijske stabilnosti**

Twenty four specimens of P. africana were cut into regular blocks of 60 mm × 20 mm × 20 mm \((l, r, t)\) with the surfaces smoothened using hand planer, dried to constant weight and their linear dimensions measured to the nearest 0.01 mm using veneer callipers for the determination of initial volume \(V_i\). The blocks were put in desiccators containing saturated copper sulphate solution and internal relative humidity greater than 80 %. The weights of the blocks were measured every two days until stabilization. The dimensions were measured along the initial axes and used to compute the swollen volume \(V_s\) and swelling coefficient \(S\) using the formula:

\[
S(\%) = \frac{V_s - V_i}{V_i} \times 100 \tag{2}
\]
3 RESULTS
3.1 Amount of wood extractives and their effect on fungal growth

3.1. Količina ekstraktivnih tvari u drvu i njihov utjecaj na razvoj gljiva

Table 1 shows the amount of extractives in the heartwood and sapwood of *P. africana* through series extraction on the same batch of wood powder with different solvents of increasing polarity in the listed order.

Heartwood hexane extraction recorded the lowest percentage of 0.3 % extract followed by dichloromethane 0.4 %. Extract content of acetone, toluene/ethanol and water was above 3 %. Water leads with the highest extract content of more than 5 %. The naturally durable heartwood (Mburu, 2007) contained higher amount of extractives than the perishable sapwood, and it was therefore used for evaluation of durability. Figure 1 shows that heartwood extractives inhibited fungal growth and that the effect depended on the type of extract used and the concentration levels. Total growth inhibition was observed for the three tested fungi at concentration levels of 500 ppm dichloromethane extract. Toluene/ethanol and acetone extracts showed partial inhibition for the three tested fungi, compared to that of the control. The period of mycelia growth inhibition increased with extract concentration for all the three test fungi. During this period, activity of the fungus was detected by formation of a colored area around the fungal inoculate with coloration increasing with the concentration of the extractive. Dichloromethane heartwood extracts showed higher growth inhibition to the three fungi than toluene/ethanol and acetone heartwood extractives even at low concentrations. *A. pullulans*, which is a coloration fungi, showed the least resistance against all extractives, compared to *C. versicolor* and *P. placenta* white rot and brown rot fungi respectively.

3.2 Chemical composition

3.2. Kemijski sastav

The results of chemical analysis of wood showed that heartwood contains a relatively high lignin content, and low hemicellulosates and cellulose contents. Lignin has a complex, non-repetitive three-dimensional structure, which makes it resistant to attack by numerous micro-organisms. The chemical composition of *P. africana* heart wood in percentage was lignin 30.4, cellulose 37.6, hemicelluloses 18.5, extractives 12.7 and ash 0.8.

FTIR spectrum for *P. africana* dichloromethane heartwood extractives is presented in Figure 2. Transmittance (T%) Signals at 1690 cm⁻¹ and 2600 cm⁻¹ (broad absorption) are characteristic of carbonyl and hydroxyl groups respectively of carboxylic acid function. Hydroxyl groups of sugar unit appear at 3400 cm⁻¹, while strong absorption at 2860 cm⁻¹ is characteristic of C-H vibrations present in aliphatic structure of terpenes (Catalano et al., 1984).

3.3 Anatomical characteristics and dimensional stability

3.3. Obilježja anatomske građe i dimenzijska stabilnost

Heartwood features of *P. africana* are highlighted in Figure 3 manifesting a radial arrangement of solitary, paired or clustered vessels of up to 5 elements and
Most of the extractives deposited in the heartwood vessels were removed during extraction (Figure 3 (c) and (d)) and those from dichloromethane showed higher inhibition rate against growth of fungi indicating that extractives deposited in the vessels and rays contribute to the reported natural durability of *P. africana*.

Abundant extractive deposits, thick-walled fibers measuring 10 µm and 30 µm, multiseriate rays 3 to 6 cells wide and 15 cells high, and low proportion of parenchyma cells associated with vessels characterize the cellular structure of the wood.

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Figure 1 Inhibition of *C. versicolor*, *P. placenta* and *A. pullulans* growth by *P. africana* heartwood extractives from different solvents

Slika 1. Usporene raste gljiva *C. versicolor*, *P. placenta* i *A. pullulans* uz pomoć ekstraktivnih tvari dobivenih različitim otapalima iz srži drva *P. africana*

Figure 2 FTIR spectrum of *P. africana* dichloromethane heartwood extractives

Slika 2. FTIR spektar ekstraktivnih tvari dobivenih iz srži drva *P. africana* primjenom diklormetana kao otapala
cana wood. Additionally, the relatively low mean swelling coefficient of 4.5% indicates that *P. africana* heartwood is dimensionally stable since it picks less moisture. This partially explains inhibition of fungal growth by heartwood extractives and contribution to the high natural durability of this species even in the outdoor use.

4 DISCUSSION

4.1 Wood extractives and their effect on fungal growth

4.1. Ekstraktivne tvari iz drva i njihov utjecaj na razvoj gljiva

The observed initial inhibition and subsequent fungal proliferation in the second and third week can be associated with detoxification of the agar medium by fungal enzymes (Neya et al., 2004). This indicates that only toluene/ethanol, acetone and water heartwood extracts have inhibitory rather than toxicity properties. *P. africana* recorded high quantities of heartwood extracts at 12.7% and as described in the literature of other tropical species, may be one of the reasons of high natural durability (Neya et al., 2004).

Since dichloromethane extractives inhibited the growth of fungi more than acetone, toluene/ethanol and water extracts, it indicates that extractives deposited in the vessels and rays also contribute to the reported natural durability of *P. africana* wood. The observed dimensional stability is linked to lower uptake of water, which can enhance natural durability of this species even in outdoor use.

4.2 Chemical composition and anatomical characteristics

4.2. Kemijski sastav i anatomska obilježja

Heartwood contains a relatively high lignin content, and low hemicelluloses and cellulose contents. The only organisms capable of mineralizing lignin into...
water and carbon dioxide are white-rot fungi (Anke et al., 2006) due to its complex, non-repetitive three-di-
mensional structure. The high lignin content partly ex-
plains high resistance to the tested fungi species and
hence the high natural durability of P. africana (Mbu-
ru, 2007). The presence of oleanolic and ursolic acid
and associated antifungal activities is consistent with
previous findings on these extractives (Becker et al.,
2005; Deepak and Handa, 2000). Saponins derived
from oleanolic acid are also described to possess anti-
fungal properties against phytopathogenic fungi ex-
plaining the durability of heartwood to fungal degra-
dation (Escalente et al., 2002).

5 CONCLUSION

5. ZAKLJUČAK

The results showed that the wood of P. africana is
dimensionally stable, and contains a high amount of
extractives and lignin, which may partly explain its high
durability in outdoor use. Extractives deposited in wood
vessels and rays are highly soluble in dichloromethane
and also contain terpenes, as indicated by FTIR spectra
measurements. Terpenes, which possess antifungal
properties, may explain the high fungal growth inhibi-
tion of heartwood extractives against C. versicolor, P.
placenta and A. pullulans in a sterile chamber. Different
heartwood extractives were able to inhibit the growth of
all test fungi at different concentrations tested. Inhibi-
tion period before mycelia growth increased with ex-
tact concentration while the rate of growth increased
with time after detoxification of malt-agar by fungi.

The heartwood extracts could be at the origin of the
reported heartwood durability of P. africana.

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