***A comparative study of the chemical composition and bioactivity of extracts from leaves of two Pecan nut cultivars grown in South Africa***

Stephanie MacDonald1, Gerhard Oosthuizen1, Gert Marais1 and Maria Cawood1

1 *Department of Plant Sciences, University of the Free State, Bloemfontein, 9300, South Africa*

**ABSTRACT**

The South African pecan nut industry is growing at an exponential rate, and the demand for more resistant and higher yielding cultivars remain a concern. Several fungal diseases associated with pecan nuts cripple this industry due to substantial yield losses including anthracnose, black blotch and die-back, brown and white rot and scab. In our study on two cultivars, grown on the same rootstock in the Limpopo province, South Africa, it was observed that the Ukulinga cultivar had increased resistance towards pecan scab compared to the Wichita cultivar. Our aim was thus to determine if certain compounds present in Ukulinga contribute to resistance. Different chromatographic techniques were used to determine the chemical composition of polar and non-polar leaf extracts. The main constituents of the non-polar hexane extract were identified by GC-MS as nerolidol and linolenic acid. Some of the components present in the polar extracts were identified through thin layer and high pressure liquid chromatography as phenolic compounds, such as rutin and saponins. Furthermore, peroxidase enzyme activity was significantly higher in Ukulinga, indicating an induced defence response. Polar extracts of Ukulinga was also found to have an inhibitory effect on the mycelial growth of *Alternaria tenuissima*, *Trichothecium roseum,* and *Cladosporium cladosporioides*. The results are an indication of the potential of compounds present in Ukulinga to contribute to the resistance against fungal diseases.

**Keywords:** Pecan nut, fungal diseases, resistance, chromatography, secondary metabolites.

**1. INTRODUCTION**

The pecan nut (*Carya illinoinensis*), indigenous to the North American continent, has developed into an important agricultural crop. This health-promoting and high prestige value crop became one of the few native U.S crops that is commercially cultivated elsewhere in the world, i.e. Mexico and South Africa (Wood et al., 1990). The first pecan trees were imported and planted in the KwaZulu Natal province, South Africa, around the turn of the 19/20th century (Pecan nuts, 2012). However, no one envisioned the significant impact the pecan nut industry would have on the South African economy. Pecan nut production gradually shifted West as the industry matured and some even believe that the Northern Cape province could become the global pecan nut production centre (Pecan nuts, 2012). Pecan nut production in South Africa experienced a five-fold increase over the past decade with up to 90% of pecan nuts being exported (SAPPA, 2019). However, several fungal diseases cripple this industry due to substantial and erratic yield losses (SAPPA, 2019A). Therefore, to ensure the exponential growth of this industry, more resistant and higher yielding cultivars are required. The disease resistance towards pecan scab observed in the Ukulinga cultivar raised several questions for this rationale. Questions arose due to the susceptibility of the Wichita cultivar grown on the same rootstock. Our aim was thus to determine if certain compounds (secondary metabolites) present in the Ukulinga cultivar contribute to pecan scab resistance.

**2. MATERIALS AND METHODS**

Dried macerated leaf material (5 g) were successively extracted using methanol/water (70:30), hexane and dichloromethane (DCM) as solvents [1:20 (w/v)] to obtain crude extracts. Thin layer chromatography (TLC) studies were conducted as an initial screening process and separated compounds identified based on their Rf values, a developing system used for separation and the color reaction of spots when detection spray reagents were applied (Barbetti et al., 1986; Jork et al., 1990; Wagner and Bladt, 1996). The hexane extracts were further analyzed by gas chromatography-mass spectrometry (GC-MS) and compounds identified with the use of the National Institute of Standard and Technology (NIST) version 5.0 library. Compounds in the polar extracts were separated through high pressure liquid chromatography (HPLC) and some identified by comparing Rt values to known standards (Vidović et al., 2015). The agar dilution method described by Rios et al. (1988), with slight modification, was used for determining the inhibition of mycelial radial growth of *Alternaria tenuissima*, *Trichothecium roseum* and *Cladosporium cladosporioides* by the extracts. The dried methanol/water extracts were dissolved in 500 µl 1% DMSO and amended in the agar to yield a final concentration of 1 g l-1. Working in a laminar flow cabinet, the medium was poured into 90mm sterile plastic Petri dishes, to a thickness of 2–3 mm, and allowed to set. The center of each test plate was subsequently inoculated with a 5-mm size plug of 7–10 d-old cultures, for each of the pathogens separately. A plate containing only the basal medium with 1% DMSO served as a control. Plates were incubated for 14 d at 25 °C in a growth cabinet. Radial mycelial growth was determined after 14 d by calculating the mean of two perpendicular colony diameters for each replicate. Peroxidase activity was measured according to a modified method of Zieslin & BenZaken (1991), using a Varian Cary 100 Bio spectrophotometer with Kinetics program. Change in absorbance was measured at 470 nm for 180 s at 30 °C. The statistical analysis of experimental data was performed by using the NCSS (2007) statistical software package.

**3. RESULTS AND DISCUSSION**

Higher concentrations of compounds were found in both the methanol/water and hexane extracts of Ukulinga. Nerolidol (5.96%) and linolenic acid (6.78%) were the two major compounds identified in the hexane extract of Ukulinga. It has been found that nerolidol plays an active role in the defence system of plants, conferring antioxidant, antibacterial, antifungal and antiparasitic properties to plants (Chan et al., 2016).

After spraying the TLC’s with the different detection reagents, phenolic compounds, terpenes, and saponins were identified in the polar extracts. Ukulinga contained higher concentrations of these compounds. Further separation on HPLC showed the major difference in phenolic compounds between the two cultivars were the concentration of flavonoid glycosides and more specific, rutin. The presence of rutin in other plant species was found to confer antifungal activity, antimicrobial activity, the inhibitory activity of RNA viruses and can even be used as a priming agent for plant resistance to bacterial pathogens (Orhan et al., 2010; Girardi et al., 2014; Yang et al. 2016).

The Ukulinga polar extract significantly inhibited the mycelial growth of all three pathogens (p<0.05) by 45%, 22 % and 25% for *A. tenuissima, T. roseum,* and *C. cladosporioides* respectively. The inhibitory effects of the Ukulinga extract could be ascribed to the high concentrations of phenolic compounds like rutin and the presence of saponins.

Peroxidase is a key enzyme in the defence response of plants, mediating reactions like lignification, crosslinking of glycoproteins, signaling cascades, etc. (Asada, 1992). Peroxidase activity has thus been linked to various plant defence responses and is now used as a general indicator of induced plant defence response (Hammond-Kosack and Jones, 1996). Peroxidase in Ukulinga was found to be significantly higher than that of Witchita, indicating an induced defence response.

**4. CONCLUSIONS**

Chromatographic techniques identified major compounds that can contribute to resistance in pecan nuts including phenolic compounds like rutin in the polar extracts, and nerolidol and linolenic acid in the non-polar extract. The Ukulinga cultivar displayed a significantly higher peroxidase enzyme activity indicative of an induced defence response. The polar and non-polar extracts of the Ukulinga cultivar contained antifungal and anti-oxidant compounds that could confer disease tolerance and resistance like phenolic compounds, saponins, nerolidol and linolenic acid. This study found a link between the disease resistance in the Ukulinga cultivar and increased production of secondary metabolites.

**ACKNOWLEDGMENTS**

The University of the Free State (UFS) and the South African Pecan nut Producers Association (SAPPA) for financial support.

**REFERENCES**

Asada, K., 1992. Ascorbate peroxidase–a hydrogen peroxide‐scavenging enzyme in plants. *Physiologia Plantarum*, *85*(2), pp.235-241.

Barbetti, P., Grandolini, G., Fardella, G., & Chiappini, I. (1986). Indole alkaloids from *Quassia amara*. Planta Medica, 53, 289-290.

Chan, W.K., Tan, L., Chan, K.G., Lee, L.H. and Goh, B.H., 2016. Nerolidol: a sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules*, *21*(5), p.529.

Girardi, F.A., Tonial, F., Chini, S.O., Sobottka, A.M., Scheffer-Basso, S.M. and Bertol, C.D., 2014. Phytochemical profile and antimicrobial properties of Lotus spp. (Fabaceae). *Anais da Academia Brasileira de Ciências*, *86*(3), pp.1295-1302.

Hammond-Kosack, K.E. and Jones, J.D., 1996. Resistance gene-dependent plant defense responses. *The Plant Cell*, *8*(10), p.1773.

Jork, H., Funk, W., Fischer, W., & Wimmer, H. (1990). Thin layer chromatography- reagents and detection methods- Physical and chemical detection methods: Fundamentals, Reagents. Volume 1. Wiley Publishers New York.

Orhan, D.D., Özçelik, B., Özgen, S. and Ergun, F., 2010. Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological research*, *165*(6), pp.496-504.

Pecan nuts. 2012. Pecan History. [ONLINE] Available at: http://www.pecannut.co.za/history/. [Accessed 5 April 2019].

Rios, J.L., Recio, M.C. and Villar, A., 1988. Screening methods for natural products with antimicrobial activity: a review of the literature. *Journal of ethnopharmacology*, *23*(2-3), pp.127-149.

SAPPA. 2019. Industry statistics – SAPPA. [ONLINE] Available at: https://www.sappa.za.org/industry-statistics/. [Accessed 5 April 2019].

SAPPA. 2019A. Research – SAPPA. [ONLINE] Available at: https://www.sappa.za.org/industry- research/. [Accessed 5 April 2019].

VIDOVIĆ, M., Morina, F., MILIĆ, S., Zechmann, B., Albert, A., Winkler, J.B. and VELJOVIĆ JOVANOVIĆ, S.O.N.J.A., 2015. Ultraviolet‐B component of sunlight stimulates photosynthesis and flavonoid accumulation in variegated P lectranthus coleoides leaves depending on background light. *Plant, cell & environment*, *38*(5), pp.968-979.

Wagner, H., & Bladt, S. (1996). Plant drug analysis. A thin layer chromatogrphy atlas. 2nd edition. Springer-Verlag, Berlin.

Wood, B.W., Payne, J.A. and Grauke, L.J., 1990. The rise of the US pecan industry. *HortScience*, *25*(6), pp.594-723.

Yang, W., Xu, X., Li, Y., Wang, Y., Li, M., Wang, Y., Ding, X. and Chu, Z., 2016. Rutin-mediated priming of plant resistance to three bacterial pathogens initiating the early SA signal pathway. *PloS one*, *11*(1), p.e0146910.

Zieslin, N. and Ben-Zaken, R., 1991. Peroxidases, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers [flower quality, peduncle bending, vascular system]. *Plant Physiology and Biochemistry (France)*.

**AUTHOR DETAILS**

Stephanie Elizabeth MacDonald

Department of Plant Sciences

University of the Free State

Nelson Mandela road

Bloemfontein

Free State

9300

macdonaldstephanie06@gmail.com

Tel: +27-725058123