Radical Scavenging Activities of a Novel Flavonoid (−)-Mesquitol Isolated from Prosopis juliflora

Article · September 2018

CITATION
1

READS
131

1 author:

Peter Sirmah
Prof. University of Kabianga
45 PUBLICATIONS **331** CITATIONS

SEE PROFILE

International Journal of Forestry and Wood Science
Vol.5(1), pp. 048-053, September, 2018. © www.premierpublishers.org. ISSN: 2167-0465
IJFWS

Research Article

Radical Scavenging Activities of a Novel Flavonoid (-)-Mesquitol Isolated from *Prosopis juliflora* Heartwood

Peter Kipkosgei Sirmah
Department of Agroforestry and Rural Development, University of Kabianga, P.O. BOX 2030, Kericho, Kenya
Email: sirmahkipkosgei110@hotmail.com; Tel:+254725873652

Radical scavenging activities of a novel flavonoid (-)-mesquitol isolated from *Prosopis Juliflora* heartwood was estimated by measuring methyl linoleate oxidation inhibition in a closed borosilicate glass reactor containing ((2, 2'-azobis (2-methylpropionitrile)) (AIBN) in 1-butanol as initiator and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) disappearance in UV Perkin Elmer spectrophotometer respectively. (-)-mesquitol like (+)-catechin was able to slow down oxidation of methyl linoleate induced by AIBN as well as inhibit the activities of DPPH radical. IC_{50} values for (-)-mesquitol were observed at approximately 23µg/ml while 50µg/ml, inhibited more than ninety percent of the DPPH radical. The IC_{50} value of well-known antioxidant compounds, (+)-catechin and butylated hydroxytoluene (BHT), used as reference controls in this study were approximately 29µg/ml and 268µg/ml respectively. Increasing (-)-mesquitol concentration from 0.009g/l to 0.09g/l decreased oxygen uptake from 70% to 20% respectively after 3hrs. (-)-mesquitol from *P. juliflora* heartwood could therefore be of valuable interest as potential source of antioxidant from renewable source.

Keywords: *Prosopis Juliflora,* heartwood, radical scavenging, Flavonoid, (-)-mesquitol, catechin

INTRODUCTION

Extractives are low molecular weight compounds present in plants in more or less important quantity, alongside cellulose, lignin and hemicelluloses (Chang *et al.,* 2001). Many extractives have specific biological activities such as taxane diterpene (taxol), has strong antitumor activity (Toshiaki, 2001). Similarly, other group of extractives such as aryltetralin lignin (podophyllotoxin) alkaloid have both toxic (gastrointestinal toxicity) as well as beneficial effects (antitumor) on human health (Harborne and Williams, 2000; Toshiaki, 2001). Biological and pharmacological studies strongly suggest that plant extractives used as additives in foods and beverages give various health benefits (Harborne and Williams, 2000).

Indeed, more than 4000 different types of flavonoids have been isolated from different plants (Neacsu *et al.,* 2007). The flavonoid constitutes a large potential resource of natural antioxidants and radical scavengers for the food and pharmaceutical industries and for use as technical antioxidants (Neacsu *et al*., 2007). Antifungal, antibacterial and antitermitic properties of some flavonoids such as 3,4,7,8-tetrahydroxyflavanone and 4,7,8-trihydroxyflavanone are closely associated with their radical scavenging properties (Khairullina *et al*., 2006; Mihara *et al.,* 2005). Flavanones 3′,4′-dihydroxy-5-methoxy-6-methylflavanone, 7-O-β-D-glucopyranoside and 7,4′-dimethoxy-6,8-dimethylflavanone 5-0-β-D-galactopyranoside (Malhotra and Misra, 1983) and flavonoids (leucodelphinidin-3-O-α-L-rhamnopyranoside and leucodelphinidin-3-O-β-D-glucopyranosyl-(1-4)-O-α-rhamnopyranoside isolated from the roots of *P. juliflora* (Shukla *et al*. (1980) have important radical scavenging properties.

A great diversity in extractive composition and distribution is found throughout wood species. Individual extractive compounds are often found in specific tissues of individual trees. The amount of extractives in the wood from the temperate zone ranges from 5 to 10% (Toshiaki, 2001) but higher amounts of up to 17.5% have been reported in *Prosopis africana* and other tropical wood species (Gérardin *et al*., 2004). The amount however can vary from season to season even in the same tissue or are restricted in certain wood species (Taylor *et al.,* 2006). Many

phenolic compounds are accumulated in heartwood, whereas they are found only in trace amounts in the corresponding sapwood (Toshiaki, 2001). Extractives compounds in wood, bark, and leave range from simple products such as vanillin to polymeric condensed tannins (Taylor *et al.,* 2006). Such features provide the basis of chemotaxonomy of woody plants (Toshiaki, 2001).

In our previous studies on *Prosopis juliflora*, we isolated a novel flavonoid (-)-mesquitol from the heartwood and 3, 5, 7, 3′, 5′-penta-O-acetyl-4′-O-methylgallocate from the bark extractives (Sirmah, 2009a; Sirmah *et al.,* 2009b; Sirmah *et al*., 2011; Odero *et al.,* 2017). The flavonoid (-)-mesquitol is able to significantly inhibit the growth of brown and white rot fungi hence confer natural resistance to this species (Sirmah *et al.,* 2009b). It has however been hypothesized that flavanoids protect heartwood against fungal colonization by a dual mechanism involving fungicidal and antioxidant activities (Toshiaki, 2001).

There is a growing interest in development and use of phytochemicals from natural origin as antioxidant for food, and pharmaceutical industries. This will be possible if radical scavenging activities of such phytochemicals are well understood. This study therefore examined the radical scavenging activities of the flavonoid (-)-mesquitol with a view to understanding its potential uses as a product from renewable source.

MATERIALS AND METHODS

Preparation of (-)-mesquitol

(-)-mesquitol was prepared as described by Sirmah*,* 2009a and briefly as follows: Mature *P. juliflora* wood was randomly sampled and cut in Baringo (latitude 0°, 20' N, longitude 35°, 57'E), Kenya. The heartwood was excised and grounded to fine powder using a vibrating hammer mill to pass through a 115-mesh sieve anddried at 60°C to constant weights before extraction using acetone in accelerated solvent extractor (Dionex ASE 200). Extraction was performed in 33 mL cell size on 10gm of *P. juliflora* heartwood powder at 100°C under a pressure of 100 bars (3 static cycles of 5 minutes each). This was replicated three times. After each extraction, the solvent was evaporated under reduced pressure and the crude extract dried under vacuum in a desiccator over P_2O_5 to dryness and stored at -20°C awaiting its use.

Radical-Scavenging Activity by DPPH Free Radical

Scavenging actions of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical by (-)-mesquitol was measured as follows: Dry powder of (-)-mesquitol and reference controls ((+)-catechin and BHT (Butylated hydroxytoluene) were separately prepared to concentrations of 10^{-4} M, 4.10^{-5} M and 2.10^{-5} M respectively. The reaction mixture contained 100µM of 0.09g/l DPPH solution and 10, 20, and 50*µ*g/ml of test samples in butanol. DPPH free radical and the different (-)-mesquitol concentrations were separately and rapidly mixed in the kinetic accessory SFA- II (Hi-Tech Scientific, Salisbury, United Kingdom). Injection was carried out in UV Perkin Elmer spectrophotometer (Lambda 16). Reduction of the DPPH free radical was measured by reading the absorbance at 520nm exactly 20 min after adding each of the test samples and evaluating IC_{50} value which corresponds to the antioxidant concentration scavenging 50% of DPPH.

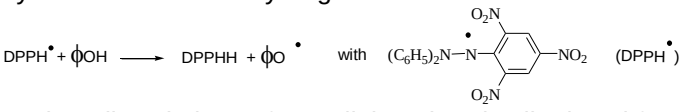
DPPH inhibition ratio was expressed as a percentage after being calculated from the following equation:

$$\% \text{ inhibition} = 100 \times (a_c - a_e / a_c)$$

where a_c is the absorbance of control and a_e the absorbance of (-)-mesquitol test extracts. All experiments were repeated two times.

Evaluating IC_{50} value of (-)-mesquitol

The radical scavenging activity of (-)-mesquitol was investigated more precisely by measuring IC_{50} value, which corresponds to the concentration scavenging 50% of DPPH. For comparison purposes, IC_{50} values were also determined for (+)-catechin and BHT. This experiment was intended to corroborate the previous study since DPPH is a stable radical, dark violet in color. Its color is bleached by its reaction with a hydrogen donor.



Methanolic solutions of 2, 2-diphenyl-1-picrylhydrazyl free radical and 10, 20, and 50*µ*g/ml of (-)-mesquitol extractives in butan-1-ol were therefore separately and rapidly mixed and the absorbance measured at 517 nm (a wavelength at which only 2, 2-diphenyl-1-picrylhydrazyl absorbs). Radical scavenging activity of (-)-mesquitol extracts was then evaluated according to the remaining DPPH concentration.

Radical Scavenging Activity by Methyl Linoleate Oxidation Inhibition

The inhibition of oxygen uptake by (-)-mesquitol with methyl linoleate (LH) as the substrate was evaluated. L° free radicals are generated by the initiator AIBN (2, 2'-azobis (2-methylpropionitrile)), and oxidation of methyl linoleate is a chain reaction propagated by the processes:

$$L° + O_2 \leftrightarrows LOO°$$
$$LOO° + LH \rightarrow LOOH + L°$$

In the presence of (-)-mesquitol as an inhibitor having labile hydrogen called AH, chain carriers easily abstract an H atom from AH (called a chain-breaking antioxidant), giving a non reactive free radical A°:

$$LOO° + AH \rightarrow LOOH + A°$$

therefore inhibiting chain propagation.

Oxidation of methyl linoleate (2mL of a 0.4M solution in 1-butanol) was therefore performed in a closed borosilicate glass reactor containing 1mL of a 9×10^{3}M solution of AIBN in 1-butanol as initiator. The double shell reactor was thermostated at 60°C by an external heating bath. Oxygen (150 Torr) was bubbled by a gas-tight oscillating pump. A small condenser was inserted on the reactor in the gas circulation to ensure condensation of the solvent. Oxygen uptake (torr) was monitored continuously with a pressure transducer (Viatron model 104) in the presence or not of 1mL of 0.9, 0.05 and 0.009g/l of (-)-mesquitol extracts in butan-1-ol to evaluate radical scavenging activities. Each experiment was repeated thrice. The volumes of the liquid and the gas phases were, respectively, 4 and 100 mL.

RESULTS AND DISCUSSION

Antioxidant Properties of (-)-Mesquitol Extractives According to Methyl Linoleate Oxidation Inhibition

Antioxidant properties of the (-)-mesquitol extractives, estimated using methyl linoleate oxidation inhibition induced by AIBN, are presented in Figure 1, 2 and 3.

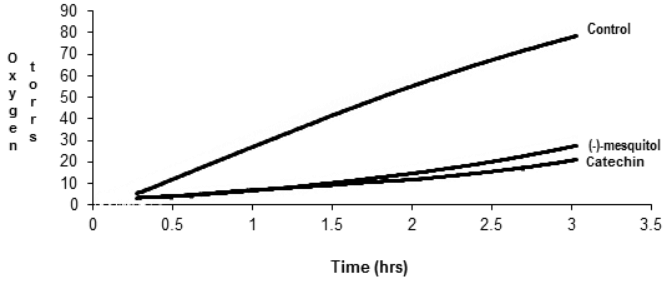


Figure 1: Antioxidant properties of (-)-mesquitol and catechin at 0.09g/l estimated using methyl linoleate oxidation inhibition

Generally (-)-mesquitol extractives presented more or less similar antioxidant properties in comparison with commercially available catechin suggesting its high ability to quench free radicals. Indeed, correlation of phenolic contents in plants to their antioxidant activities has been reported (Wang *et al*., 2004; Haupt *et al*., 2003). Such plant extracts have been widely utilized in pharmaceutical industry to treat various human ailments (Nithya *et al.,* 2018). Antioxidant properties of green tea catechins, methylgallocatechins and epigallocatechins and their application in food and pharmaceutical industry has been well described in literature (Valcic *et al*., 2000; Sang *et al*., 2003; Bors, 1990). These results suggest possible utilization of (-)-mesquitol like catechin in food and pharmaceutical industry.

To further evaluate potential of (-)-mesquitol as antioxidant, additional experiments was performed with BHT a widely used synthetic antioxidant and catechin (Figure 2).

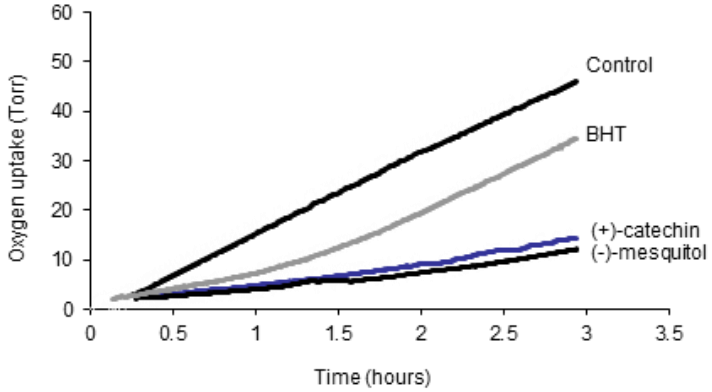


Figure 2: Antioxidant properties of (-)-mesquitol, BHT and catechin (0.09g/l) estimated using methyl linoleate oxidation inhibition

(-)-mesquitol extractives presented similar antioxidant properties as (+)-catechin. Howe ever in both cases, the two flavanols present higher antioxidant properties compared to butylated hydroxytoluene (BHT) chosen as reference antioxidant that is widely used as food preservative and medicine to treat genital herpes and acquired immunodeficiency syndrome (AIDS) (Nithya *et al.,* 2018. Antioxidant properties of (-)-mesquitol (M) and (+)-catechin (C) at concentarations of 0.9, 0.05 and 0.009g/l was evaluated further and results are presented in Figure 3.

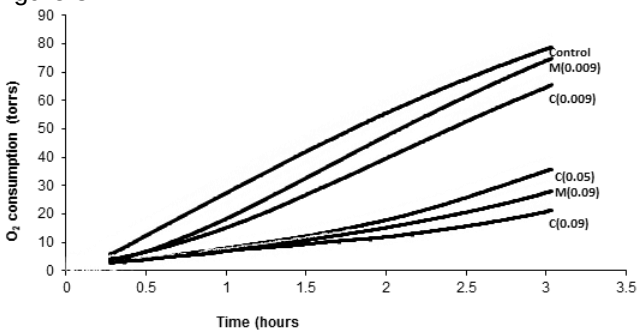


Figure 3: Antioxidant properties of (-)-mesquitol (M) and (+)-catechin (C) at different concentrations(g/l) estimated using methyl linoleate oxidation inhibition

Independent of the concentration level tested (-)-mesquitol and (+)-catechin gave important antioxidant properties which increase with increasing concentration. For a given concentration, (+)-catechin seems to be slightly more effective than (-)-mesquitol.

Antioxidant Properties According to DPPH Assay

To corroborate the previous properties, free radical scavenging activities of (-)-mesquitol extracted from *P. juliflora* heartwood by different solvents (dichloromethane, toluene/ethanol, water and acetone) were assessed by DPPH assay and its antioxidant activity evaluated according to the remaining DPPH concentration (Figure 4).

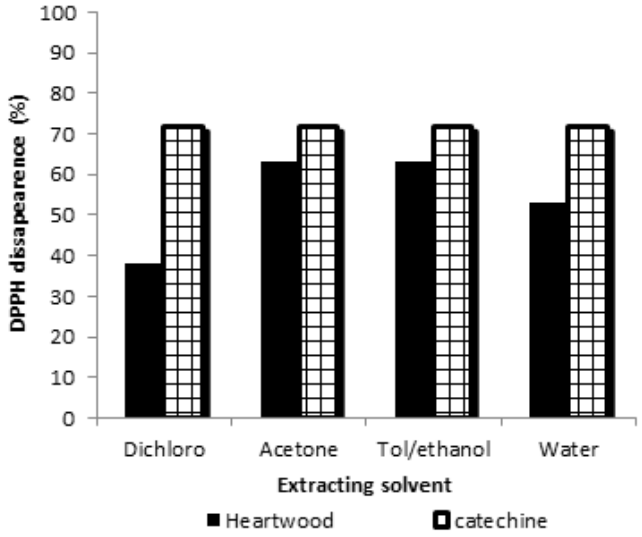
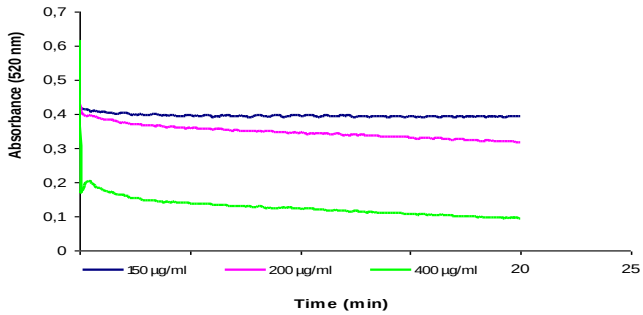


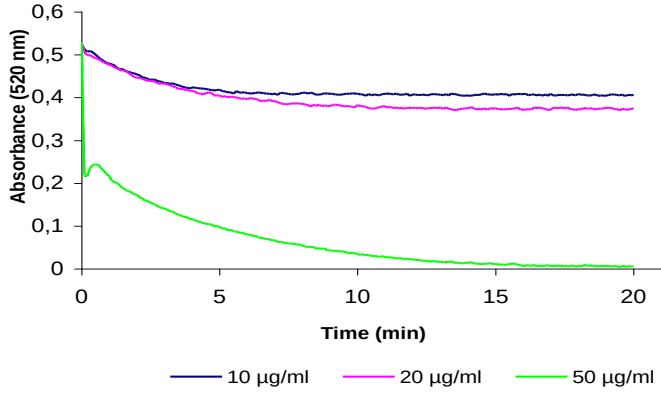
Figure 4: DPPH inhibition by (-)-mesquitol extracted from *P. juliflora* heartwood by different solvents

As shown in Figure 4 the heartwood of *P. juliflora* is rich in (-)-mesquitol and like (+)-catechin exhibited significant inhibitory activity (≥ 60%) against the DPPH radical, irrespective of extracting solvent.

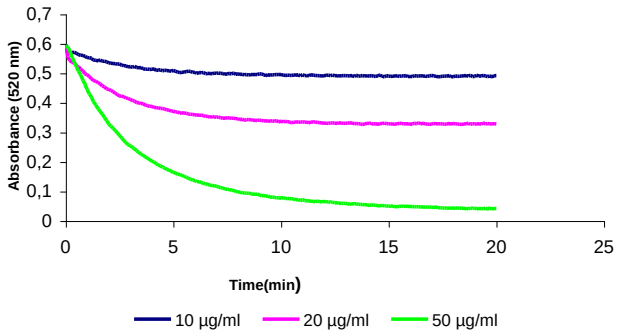
The antioxidant activity of (-)-mesquitol was investigated more precisely by measuring IC_{50} value, which corresponds to the antioxidant concentration scavenging 50% of DPPH. For comparison purposes, IC_{50} values were also determined for (+)-catechin and BHT. Results are presented in Figure 5(a-c).



a. DPPH treated with different concentrations of BHT



b. DPPH treated with different concentrations of (+)-catechin



c. DPPH treated with different concentrations of (-)-mesquitol

Figure 5 (a-c). Absorption of 100µM DPPH treated with different concentrations of antioxidants flavanols

The inhibitory concentration at 50% DPPH (IC_{50}) was determined for (-)-mesquitol, BHT and (+)-catechin by reporting the remaining DPPH concentration as a function of antioxidant concentration. Figure 6 shows the typical curve obtained for (-)-mesquitol allowing determination of IC_{50}.

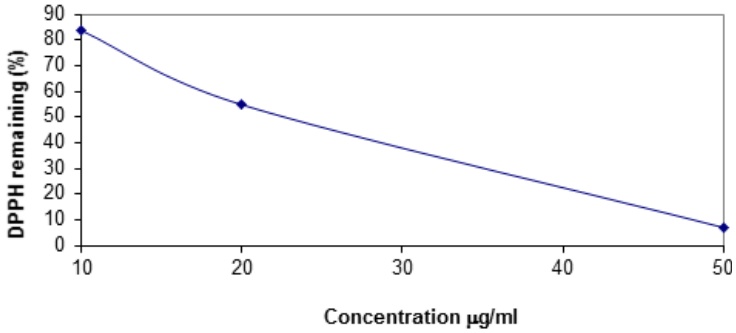


Figure 6: Determination of IC_{50} of (-)-mesquitol

IC_{50} values for (-)-mesquitol from *P. juliflora* heartwood were observed at approximately 23µg/ml. At 50µg/ml, the extract inhibited more than 90% of the DPPH radical. The IC_{50} value of (+)-catechin and BHT, used as a reference in this study, is approximately 29µg/ml and 268µg/ml respectively. The IC_{50} values of well-known antioxidant compounds reported in literature are presented in Table 1.

Table 1: IC_{50} values for different phytochemical extracts

No	Phytochemical	Plant source	IC_{50}(µg/ml)	Reference
1	Betulin	Birch bark	6.2	Nakurte *et al*., 2017
2	Crude extract	*P. Juliflora* bark	37	Siahpoosh and Mehrpeyma, 2014
3	Crude extract	*H. Perforatum*	21	Fathi and Ebrahimzadeh, 2013
4	Hexadecanoic acid	*S. Xanthocarpum*	197	Nithya *et al*., 2018
5	Ascorbic acid	Reference control	239	Nithya *et al*., 2018
6	Tannic acid	*Q.Faginea*	2.6	Miranda *et al*., 2017
7	Trolox	Reference control	3.8	Miranda *et al*., 2017

Lower IC_{50} values of plant phytochemicals implies that low amount will give high effect in fighting oxidative damage, therefore high preference for use in pharmaceutical and cosmetic industry (Nakurte *et al.,* 2017) through quenching free radical elements, chelating metals and suppressing enzyme activities (Nithya *et al.,* 2018). Indeed free radical

scavenging is one of the known mechanisms whereby antioxidants inhibit lipid peroxidation or stop the enzymatic activities of micro-organisms (Pongtip *et al*., 2007). DPPH assay is used extensively for screening antioxidants from natural products such as fruit, vegetable juices and wood extractives (Raza and John, 2007).

In general, this study shows that *P. juliflora* extractives have important antioxidant activity to prevent the fungal deterioration of natural products. Indeed (-)-mesquitol in comparison with existing antioxidants, such as BHT, catechin, probucol and alpha-tocopherol, shows better antioxidant activity, which can be useful in controlling and managing inflammatory diseases such as cancer and diabetes (Madhusudana *et al.,* 2004).

CONCLUSION

Antioxidant properties, estimated using methyl linoleate oxidation inhibition test showed that (-)-mesquitol like (+)-catechin is able to slow down oxidation of methyl linoleate induced by AIBN as well as inhibit the activities of DPPH radical. In both cases, the flavanols presented higher antioxidant properties compared to BHT chosen as reference antioxidant. These results suggest that (-)-mesquitol from *P. juliflora* heartwood could be of valuable interest as potential source of antioxidants for the development of new antimicrobial drugs. This study however recommends the evaluation of the occurrence and abundance of (-)-mesquitol within *P. juliflora* trees under different ecological zones.

ACKNOWLEDGEMENTS

This work was partially supported by a grant from the French Government through its Embassy in Nairobi Kenya. I am grateful to Lorraine University staff in the Wood Fibre Science section for allowing me use their facilities during my stay.

REFERENCES

Bors W, Heller W, Michel C, Saran M (1990). Flavonoïds as antioxidants: determination of radical- scavenging efficiencies. Methods in Enzymology*,* 186, 343-355.

Chang S, Cheng S, Wang S (*2001)*. Antitermitic activity of essential oils and components from Taiwania (*Taiwania cryptomerioides*). Journal of Chemical Ecology, 27(4), 717- 724.

Fathi H, Ebrahimzadeh MA (2013). Antioxidant and free radical scavenging activities of *Hypericum Perforatum* L. International Journal Forest, Soil and Erosion, 3(2) ;68-72.

Gérardin P, Neya B, Dumarcay S, Petrissans M, Serraj M, Huber F (2004). Contribution of gums to natural durability of *Prosopis africana* heartwood. Holzforschung, 58, 39–44.

Harborne JB, Williams CA (2000). Advances in flavonoïd research since 1999. Phytochemistry, 55, 481-504.

Haupt M, Leithoff H, Meier D, Puls J., Richter HG, Faix O (2003). Heartwood extractives and natural durability of plantation grown teakwood (*Tectona grandis* L.) - a case study. Holz Roh- Werkst, 61 (6), 473-474.

Khairullina GM, Garifullina GG, Gerchikov A, Ostroukhova LA and Babkin VA (2006). Quantitative antioxidant activity of the ethyl acetate extract of *Larix sibirica* bark and its individual components. Chemistry of Natural Compounds*,* 42 (2), 160-163.

Madhusudana R, Jagadeeshwar R, Ashok K, Jhillu S, Kondapuram VR (2004). Antioxidant from natural source. US Patent 20040116716, 5 pp.

Malhotra S, Misra K (1983). New Flavanones from *Prosopis juliflora* roots**.** India Planta Medica, 47(1), 46-48.

Miranda I, Sousa V, Ferreira J, Pereira H (2017). Chemical characterization and extractives composition of heartwood and sapwood from *Quercus faginea*. Plos One, 12(6); e0179268.

Nakurte I, Stankus K, Virsis I, Paze A, Rizhikovs J (2017). Characterization of antioxidant activity and total phenolic compound content of birch outer bark extractives using micro plate. Environment, Technology, Resources, 1: 197-201.

Neacsu M, Eklund PC, Sjoholm RE, Pietarinen SP, Ahotupa MO, Holmbom BR, Willfor SM (2007). Antioxidant flavonoïds from knotwood of Jack pine and European Aspen. Holz als Roh Werkst, 65, 1-6.

Nithya M, Ragavendran C, Natarajan D(2018). Antibacterial and free radical scavenging activities of a medicinal plant Solanum xantocarpum. International journal of food properties, 21(1), 328-342.

Odero MP, Munyendo WL, Kiprop, AB (2017). Quantitative analysis of the flavonoidmesquitol in the medicinal plant *Prosopis juliflora* with seasonal variations in Marigat Baringo County, Kenya. Science Journal of analytical chemistry, 5(6): 107- 112.

Pongtip S, Charlotte U, Carlsen B, Mogens LA, Wandee G, Leif HS (2007). Antioxidative effects of leaves from Azadirachta species of different provenience. Food Chemistry, 104, 1539–1549.

Raza H and John A (2007). In vitro protection of reactive oxygen species induced degradation of lipids, proteins and 2- deoxyribose by tea catechins. Food and Chemical Toxicology, 45(10), 1814-1820.

Sang S, Tian S, Wang H, Stark RE, Rosen RT, Yang CS, Ho CT (2003). Chemical studies of the antioxidant mechanism of tea catechins: Radical reaction products of epicatechin with peroxyl radicals. Journal of Bioorganic and Medicinal Chemistry Letters, 11, 3371-3378.

Shukla R, Trivedi KK, Misra K (1980). New leucoanthocyanins from *Prosopis juliflora* bark. India Planta Medica (Suppl.), 48-51.

Siahpoosh A, Mehrpeyma M (2014). Antioxidant effects of albizia lebbek and prosopis juliflora barks. International Journal of Biosciences 5(9):273-284.

Sirmah P (2009a). Towards valorisation of Prosopis Juliflora as an alternative to declining wood resource in kenya. Ph.D Thesis, Nancy I University, France.

Sirmah P, Dumarcay S, Masson E, Gerardin P (2009b). Unusual amount of (-)-mesquitol from the heartwood of *Prosopis juliflora.* Natural product research 23(2): 183-189.

Sirmah P, Mburu F, Iaych K, Durmaçay S, Gérardin P (2011). Potential antioxidant compounds from different parts of *Prosopis juliflora*. Journal of Tropical Forest Science 23 (2): 187- 195.

Taylor A, Gartner BL, Morrell JJ, Tsunoda K (2006). Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi. J. Wood Sci., 52:147-153.

Toshiaki U (2001). Chemistry of Extractives. In: "Wood and cellulosic chemistry". Ed Marcel Dekker, Inc. New York, pp 213-241.

Valcic S, Burr JA, Timmermann BN, Liebler DC (2000). Antioxidant chemistry of green tea catechins. New oxidation products of (-)-epigallocatechin gallate and (-)-epigallocatechin from their reactions with peroxylradicals. Chemical Research in Toxicology, 13(9), 801-810.

Wang S, Wu J, Cheng S, Lo C, Chang H, Shyur L, Chang S (2004). Antioxidant activity of extracts from *Calocedrus formosana* leaf, bark and heartwood. Journal of Wood Science, 50, 422-426.

Accepted 23 August 2018

Citation: Sirmah PK (2018). Radical Scavenging Activities of a Novel Flavonoid (-)-Mesquitol Isolated from P*rosopis juliflora* Heartwood. Int. J. Forestry Wood Sci. 5(1): 048-053.