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# Geographic and intraspecific variability of mesquitol amounts in *Prosopis juliflora* trees from Kenya

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## Abstract

Several studies have shown that the heartwood of *Prosopis juliflora* contains high amounts of a naturally rare flavan-3-ol compound identified as 2-(3,4-dihydroxyphenyl)chromane-3,7,8-triol otherwise known as (–)-mesquitol (**1**). It is known to possess strong antioxidant properties, which may be of valuable interest for further valorization. However, no data exists so far showing the variations of its abundance depending on the different geographic habitats of the tree, the age and the different parts of the tree stem. The variability of flavan-3-ols depending on the geographical area and intra-specific variability within *P. juliflora* trees from Kenya was addressed in this substantive study. The study was done using wood extracts from three different counties in Kenya (Baringo, Garissa and Turkana Counties). Wood samples were separated into two categories of ages; small trees, aged less than 4 years and the big trees, aged more than 8 years. Each sample was divided into five different parts, which included the bark, sapwood, knot wood, heartwood and the pith. Serial extractions were done by the Dionex accelerated solvent extractor using four solvents in increasing polarity (dichloromethane, acetone, toluene: ethanol (2:1 v/v) and finally water). Gas chromatographic analysis coupled to mass spectrometry was employed to identify the different compounds present in the extracts. LC–MS/MS method was thereafter developed and used to confirm the identity and quantify the amounts of mesquitol present. Two other flavan-3-ols identified and quantified included catechin (**2**) and 4'-*O*-methylgallicocatechin (**3**). A systematic study on the mass spectra and the observed fragmentations of the flavonoids showed that mesquitol compound is the most abundant compound in *P. juliflora* with high amounts being found in the heartwood and pith of the acetonic extract (47–72%). Mesquitol abundance was also found to vary depending on the age of the tree and on the geographical areas.

## 1 Introduction

*Prosopis juliflora* is a shrub, or sometimes a tree, known to be highly adapted to dry lands and therefore introduced in Kenya to curb desertification (Pasicznik et al. 2001). *P. juliflora* has recently generated a lot of interest from scientists due to the presence of the mesquitol compound that

has been found to have antioxidant properties (Lakshmi et al. 2015; Sirmah et al. 2011; Azam et al. 2011). It is also known for its anti-termite and anti-fungal properties (Sirmah et al. 2008). However, over the years, the *P. juliflora* species has become a major threat to many livelihoods mainly due to its uncontrolled spread into crop fields, grazing areas, wetlands and lakeshore areas (Muturi 2012; Shackleton et al. 2014). Muturi (2012) suggested that the invasion was spontaneous and independent of the habitat variation. A survey done in local communities around Lake Baringo in Kenya showed that 85–90% of the respondents favored complete eradication of the invasive *Prosopis* species (Mwangi and Swallow 2008). Efforts to eradicate the plant completely have failed mostly because of its deep penetrating taproots, which are able to reach water tables at a depth of more than 50 m (Raven et al. 2005). Cutting down the *Prosopis* trees has also proved to be futile according to studies done in Eritrea by Bokrezion (2008). This author reported that the

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*Prosopis* plants re-grew in a week's time after they had been cut down. The uncontrolled spread of *P. juliflora* into arable land makes it fail its intended purpose of controlling desertification and hence being a nuisance. This study aimed at harnessing the plant as raw material in order to consider potential valorization by studying the geographic and intraspecific variability of mesquitol distribution and especially its abundance in different parts of the tree from varied counties in Kenya. Mesquitol exhibits antioxidant properties (Sirmah et al. 2011) leading to several potential applications in food industry, cosmetics or as starting material for innovative materials or smart molecules. This study proves as a timely research having availed valuable data that will address the plant's transformation from a weed to a raw material of importance.

A study done by Sirmah (2009) showed the presence of two other 3-flavanols catechin (2) and 4'-*O*-methylgalocatechin (3). The quantifications of these compounds were not done, but this study availed information on the presence and the variability of catechin and 4'-*O*-methylepigallocatechin present in the *P. juliflora* (Fig. 1).

## 2 Materials and methods

### 2.1 Reagents and plant samples

Formic acid, methanol, ethyl acetate and acetonitrile used were of HPLC grade and purchased from Merck Company, Germany. Dichloromethane, acetone, toluene and ethanol were of analytical grade and purchased from Merck Company, Germany. The water used for extraction was deionized in the laboratory using the Purelab option-Q Elga water deionizer. The plant samples were collected from their natural habitats in Baringo County (latitude 0°28'0" N, longitude 35°58'0" E), Garissa County (latitude 0°27'09" S, longitude 39°38'45" E) and Turkana County (latitude 03°09' N, longitude 35°21' E) in Kenya. The samples were stems from both the big trees, aged more than 8 years, and the small trees, aged less than 4 years.

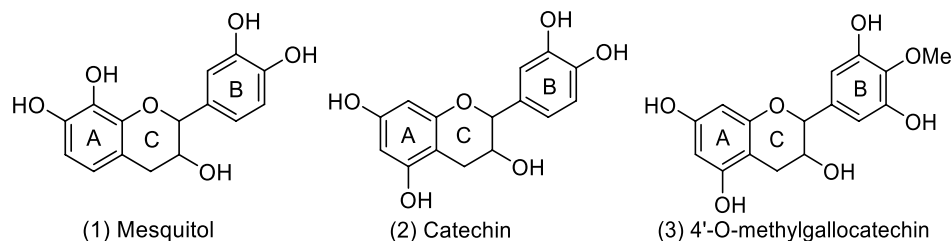
### 2.2 Sample preparation and extraction

Each sample was divided into five different plant parts: the bark, sapwood, knot wood, heartwood and the pith. They were air dried, then dried at 103 °C until weight stabilization and then ground separately into fine powders using a laboratory vibrating cup mill at 1200 rpm for 1 min and passed through a 115-mesh sieve. An ASE Dionex extraction method was developed, where serial extractions of the different samples were done using various organic solvents (dichloromethane, acetone, toluene/ethanol (2/1, v/v) and water) in the order of their increasing polarity. The extractions were performed on a 34 mL cell size on 8 g of sample powder at 100 °C under 3 static cycles of 5 min each. The extractions were done in triplicate and after each extraction, the organic solvents were evaporated under vacuum using a rotary evaporator. The water samples were dried using a lyophilizer. The percentage yields of extractives were thereafter calculated.

### 2.3 GC–MS analysis

GC–MS analysis was performed on a Clarus 500 GC gas chromatograph (Perkin Elmer Inc., USA) coupled to a Clarus 500 MS quadrupole mass spectrometer (Perkin Elmer Inc., USA). Gas chromatography was carried out on a 5% diphenyl/95% dimethyl polysiloxane fused-silica capillary column (Elite-5 ms, 60 m × 0.25 mm, 0.25 mm film thickness, Perkin Elmer Inc, USA). The gas chromatograph was equipped with an electronically controlled split/split less injection port. The injection (injection volume of 1 µL) was performed at 250 °C in the split mode (split flow of 20 mL/min). Helium was used as carrier gas, with a constant flow of 1.2 mL/min. The oven temperature program was 200 °C constant for 4 min, 200–330 °C at a rate of 5 °C/min and then constant for 330 °C. Ionization was achieved in the electron impact mode (ionization energy of 70 eV). The source and transfer line temperatures were 250 °C and 330 °C, respectively. Detection was carried out in scan mode: m/z 35 to m/z 700 a.m.u. The samples were silylated using BSTFA/1% TMCS before aspiration into the GC–MS.

**Fig. 1** Main flavanols identified in *Prosopis juliflora*



## 2.4 LC–MS/MS analysis

Liquid chromatography–mass spectrometry (LC–MS) analyses of samples were carried out using a Shimadzu (Noisiel, France) LC-20A ultra-HPLC (UHPLC) system equipped with an autosampler and interfaced to a PDA UV detector SPD-20A, followed by an LC–MS 8030 triple-quadrupole mass spectrometer. The separation was performed at a flow rate of 0.4 mL/min on a Luna C18 analytical column (inner diameter, 150 mm by 3 mm; Phenomenex, Le Pecq, France) using a 10-min gradient as follows: starting from 2% of acetonitrile (containing 0.1% formic acid) in water (0.1% formic acid solution), acetonitrile proportion was increased linearly to 20% in 3 min, then to 80% in 6.25 min. Initial conditions were then reached in 0.25 min. The injection volume was 1  $\mu$ L. UV–visible spectra were recorded between 190 and 800 nm. Positive and negative ion electrospray mass spectrometric analyses were carried out at a unit resolution between 100 and 2000 m/z at a scan speed of 15,000 U/s. The heat block and desolvation line temperatures were 400 °C and 250 °C, respectively. Nitrogen was used as a drying (15 L/min) and nebulizing (3 L/min) gas. The ion spray voltage was  $\pm$ 4500 V. Data was acquired and analyzed using LabSolutions software, version 5.42 SP4, from Shimadzu. Identification was achieved by comparison of experimental retention times and UV–MS spectra to bibliographic data and standard compounds.

## 2.5 Statistical analysis

Tukey's test analysis was used to compare the data of the amounts of mesquitol of both the small and big trees in the three geographic regions and in the different plant parts. The correlation of the mesquitol quantities between the small trees and the big trees was also computed using ANOVA. The analyses were conducted using R statistical software.

## 3 Results and discussion

### 3.1 Percentage yields of extractives of *P. juliflora*

The percentage yields of extractives according to the different parts of the trees and of the different counties are shown in Table 1. The values of the percentage yields were reported as the mean value of the three replicates of extraction.

In general, amounts of extractives were similar to those found in previous studies (Sirmah et al. 2009, 2011). Extractives were present in high amounts in the acetonic and the water extracts with the water extracts dominating in the bark and sapwood of the samples. For the bark, the water extracts gave the highest yields compared to all other solvents apart from the samples from Turkana County, where acetone gave a higher yield. There was a considerable difference in the

**Table 1** Percentage yields of extractives of *P. juliflora* samples

Plant part	Solvent	Baringo county		Turkana county		Garissa county	
		Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	Dichloromethane	1.86 $\pm$ 0.18	1.72 $\pm$ 0.92	1.63 $\pm$ 0.00	1.42 $\pm$ 0.51	1.27 $\pm$ 0.09	1.21 $\pm$ 0.26
	Acetone	6.58 $\pm$ 0.73	6.59 $\pm$ 0.64	4.46 $\pm$ 2.13	6.50 $\pm$ 0.57	5.31 $\pm$ 1.39	5.11 $\pm$ 0.77
	Toluene:ethanol	1.92 $\pm$ 0.19	0.99 $\pm$ 0.23	1.63 $\pm$ 0.66	1.13 $\pm$ 0.25	1.28 $\pm$ 0.54	1.44 $\pm$ 0.27
	Water	8.28 $\pm$ 0.48	6.99 $\pm$ 1.00	3.13 $\pm$ 0.78	3.13 $\pm$ 0.90	13.21 $\pm$ 1.90	16.42 $\pm$ 2.22
Sapwood	Dichloromethane	0.63 $\pm$ 0.23	0.52 $\pm$ 0.11	0.56 $\pm$ 0.06	0.46 $\pm$ 0.07	0.57 $\pm$ 0.18	0.70 $\pm$ 0.07
	Acetone	1.30 $\pm$ 0.18	1.42 $\pm$ 0.02	2.07 $\pm$ 6.64	1.38 $\pm$ 0.22	1.97 $\pm$ 0.18	1.61 $\pm$ 0.13
	Toluene:ethanol	0.65 $\pm$ 0.06	0.40 $\pm$ 0.09	1.05 $\pm$ 0.53	0.38 $\pm$ 0.00	0.66 $\pm$ 0.15	0.74 $\pm$ 0.11
	Water	4.78 $\pm$ 0.56	4.21 $\pm$ 0.43	7.44 $\pm$ 0.38	3.13 $\pm$ 0.25	5.05 $\pm$ 1.09	4.92 $\pm$ 1.49
Knot wood	Dichloromethane	1.09 $\pm$ 0.24	0.99 $\pm$ 0.04	1.07 $\pm$ 0.12	0.71 $\pm$ 0.19	1.70 $\pm$ 1.00	1.00 $\pm$ 0.00
	Acetone	9.91 $\pm$ 1.96	12.62 $\pm$ 0.58	8.17 $\pm$ 1.71	4.79 $\pm$ 0.19	17.73 $\pm$ 0.72	8.67 $\pm$ 1.21
	Toluene: Ethanol	0.75 $\pm$ 0.05	0.68 $\pm$ 0.10	1.05 $\pm$ 0.30	0.54 $\pm$ 0.14	0.94 $\pm$ 0.31	0.58 $\pm$ 0.07
	Water	7.08 $\pm$ 0.10	7.44 $\pm$ 0.80	7.13 $\pm$ 1.07	4.63 $\pm$ 1.09	8.52 $\pm$ 1.65	7.67 $\pm$ 1.38
Heartwood	Dichloromethane	2.60 $\pm$ 1.51	2.25 $\pm$ 1.02	1.08 $\pm$ 0.26	0.75 $\pm$ 0.13	1.48 $\pm$ 0.78	1.38 $\pm$ 0.13
	Acetone	8.73 $\pm$ 0.54	16.42 $\pm$ 1.41	6.58 $\pm$ 0.38	9.46 $\pm$ 0.69	14.80 $\pm$ 0.39	8.92 $\pm$ 0.36
	Toluene:ethanol	2.13 $\pm$ 0.62	1.88 $\pm$ 1.56	0.88 $\pm$ 0.38	0.58 $\pm$ 0.07	0.85 $\pm$ 0.09	0.63 $\pm$ 0.25
	Water	5.10 $\pm$ 0.62	7.47 $\pm$ 1.42	7.67 $\pm$ 1.28	4.46 $\pm$ 0.51	6.18 $\pm$ 0.65	5.83 $\pm$ 0.38
Pith	Dichloromethane	1.77 $\pm$ 0.03	1.09 $\pm$ 0.28	1.00 $\pm$ 0.25	0.79 $\pm$ 0.07	1.48 $\pm$ 0.46	1.13 $\pm$ 0.13
	Acetone	12.17 $\pm$ 0.83	17.92 $\pm$ 2.33	6.54 $\pm$ 0.92	5.96 $\pm$ 0.83	16.57 $\pm$ 0.74	8.13 $\pm$ 1.32
	Toluene:ethanol	1.87 $\pm$ 0.78	0.87 $\pm$ 0.03	0.88 $\pm$ 0.33	0.54 $\pm$ 0.07	1.05 $\pm$ 0.30	0.58 $\pm$ 0.47
	Water	5.48 $\pm$ 1.12	7.34 $\pm$ 1.39	8.21 $\pm$ 2.06	4.33 $\pm$ 2.06	7.81 $\pm$ 0.58	6.83 $\pm$ 0.47

water extracts from Garissa County, which gave very high yields of extractives compared to the rest of the counties.

The sapwood had generally low amounts of extractives with the water solvent showing a higher percentage compared to the rest of the solvents. Water is known to extract polar compounds like sugars and gums constituted of polysaccharides. This was further confirmed by the GC–MS analyses, where the presence of sugars dominated in the sapwood.

The higher yields of extractives from the knot wood, heartwood and pith were obtained with acetone in all the trees from the different counties. These high yield values may be explained by the presence of high amounts of flavonoids being extracted by the acetone solvent as seen in the GC–MS.

Compared to the rest of the solvents used for extraction, toluene: ethanol solvent gave lower percentage yields of extractives in all the regions (0.4–2.1%) indicating that most of the organic solvent soluble extractives have already been extracted before by dichloromethane and acetone.

### 3.2 Identification of the extractives

#### 3.2.1 GC–MS analysis

GC–MS analyses were performed on all the extractives obtained from the four solvents used. According to the NIST library, the dichloromethane extracts showed the presence of mostly fatty acids, sterols, terpenes, alcohols and sugars. The acetone extracts, toluene/ethanol extracts and water

extracts were mostly composed of sugars and flavonoids as previously reported by Sirmah et al. (2011). Of the three flavan-3-ols (mesquitol, catechin and 4'-*O*-methylgallo catechin), catechin was observed first with a retention time of 20.61 min, followed by mesquitol with a retention time of 20.82 min and finally 4'-*O*-methylgallo catechin with a retention time of 21.60 min. This pattern was repeated in all the samples, which contained the flavan-3-ols. The mass spectrum of the penta-TMS derivative of mesquitol was  $m/z$  (%): 650 ( $M^+$ ), 383, 369 (100), 355, 267, 179. The mass spectrum for catechin was similar to that of mesquitol, since it has a similar fragmentation pattern. This is because it only differs from mesquitol by the position of the hydroxyl OH at the A-ring. The MS spectrum of the penta-TMS derivative of 4'-*O*-methylgallo catechin was  $m/z$  (%): 680 ( $M^+$ ), 398, 368, 297, 267, 73 (100), which corresponds to the data reported by Sirmah (2009) on the presence of 4'-*O*-methylgallo catechin in *P. juliflora*. The percentages of the three main flavan-3-ols relative to TIC in the acetonic extracts are listed in Table 2.

The 4'-*O*-methylgallo catechin was found in higher percentages in the bark compared to the other plant parts. It was also found more in the small trees compared to the big trees.

#### 3.2.2 LC–MS analysis

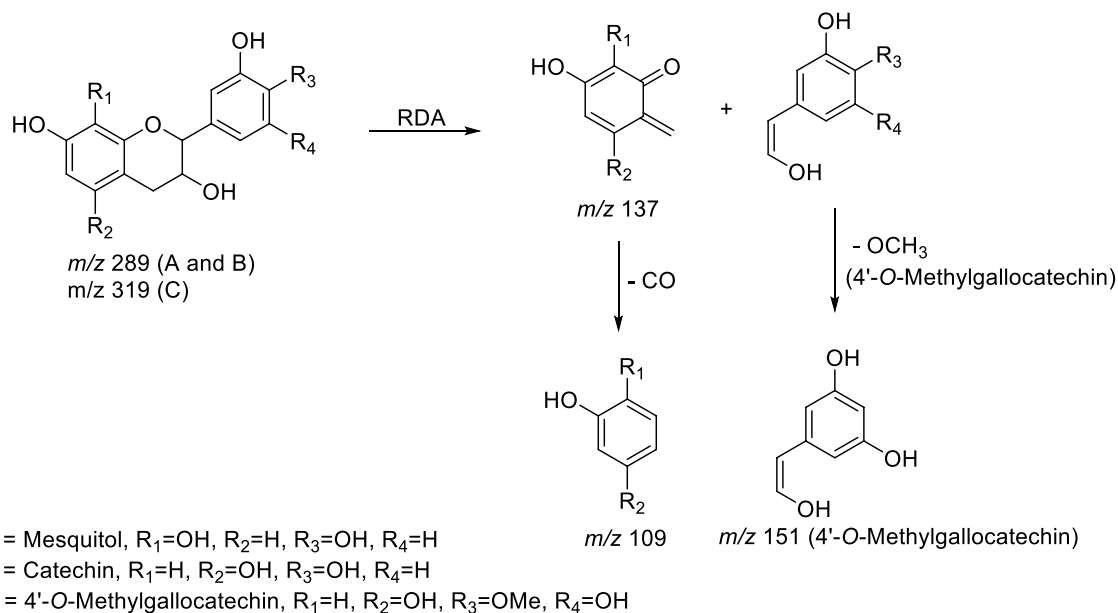
LC–MS/MS analyses of the acetonic extracts were undertaken in order to additionally confirm the identity and quantify the three flavan-3-ols. The quantification of the flavan-3-ols was done in the negative ion mode.

**Table 2** Percentages of the three main flavan-3-ols identified by GC–MS in acetonic extracts from different plant parts of *P. juliflora* of the three counties. (The threshold relative area for all the samples was set at 1.0)

Plant part	Flavan-3-ol	Baringo county		Turkana county		Garissa county	
		Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	Mesquitol	6	23	0	1	19	0
	Catechin	0	0	0	0	0	0
	4'- <i>O</i> -methylgallo catechin	45	32	71	14	30	29
Sapwood	Mesquitol	6	0	0	1	8	16
	Catechin	1	0	0	0	0	0
	4'- <i>O</i> -methylgallo catechin	0	4	1	0	0	0
Knot wood	Mesquitol	48	57	22	63	63	81
	Catechin	8	15	0	5	2	2
	4'- <i>O</i> -methylgallo catechin	11	0	19	0	15	12
Heartwood	Mesquitol	74	74	43	68	42	70
	Catechin	7	14	0	6	3	9
	4'- <i>O</i> -methylgallo catechin	17	8	40	6	11	7
Pith	Mesquitol	70	78	45	68	62	74
	Catechin	10	22	3	4	5	7
	4'- <i>O</i> -methylgallo catechin	15	0	30	8	25	11

Identification was achieved by comparison of experimental retention times and UV–MS spectra to bibliographic data and by the use of the standard compounds of mesquitol and catechin. All the three flavan-3-ols showed strong UV absorption ( $\lambda_{\max}$ ) at 278 nm. This is in agreement with the fact that the aromaticity of phenol groups of organic compounds absorbs strongly near 280 nm (Kalsi 2004). Multiple reaction monitoring (MRM) was done on the precursor ions of the three flavan-3-ols and the consequent product ions obtained are explained in Fig. 2.

Catechin is firstly eluted with a retention time of 3.3 min (MS, 289 [M–H]; MS/MS [M–H]<sup>–</sup>, 151, 137, 109) followed by 4'-O-methylgallo catechin, which was eluted at a retention time of 3.4 min (MS, 319 [M–H]; MS/MS [M–H]<sup>–</sup>, 166, 151, 137, 109). Mesquitol was lastly observed at a retention time of 3.7 min (MS, 289 [M–H]; MS/MS [M–H]<sup>–</sup>, 151, 137, 109).



**Fig. 2** Fragmentation pathway of the flavan-3-ols proposed on the basis of the ESI–MS/MS spectra in the negative mode (RDA retro diels–alder reaction)

**Table 3** Percentage of mesquitol in acetone extracts identified by LC–MS in the different parts of *P. juliflora* of the three counties

Plant part	Baringo County		Turkana County		Garissa County	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	0	0	0	3	19	0
Sapwood	33	22	18	19	44	43
Knot wood	62	58	31	61	55	57
Heartwood	61	69	47	63	67	72
Pith	63	57	51	59	58	65

### 3.3 Quantification

Having confirmed the presence of the three flavan-3-ols in the different samples, their quantifications were done in the negative ion mode.

#### 3.3.1 Mesquitol quantification

A systematic study of the percentages of mesquitol present in *P. juliflora* showed that it is the most abundant compound in most parts of the stem with high amounts being found in the knot wood, heartwood and pith. These results corroborated those reported by Sirmah et al. (2008) and Odero et al. (2017). Mesquitol percentage in the acetonic extract ranged from 31 to 72% in the knot wood, heartwood and pith. Small amounts of mesquitol were also detected in the bark and in the sapwood. Percentages of free mesquitol in acetonic extracts from the various parts and regions are listed in Table 3.

Percentages of mesquitol were however lower than those measured by GC–MS suggesting that mesquitol may exist in different forms, especially as glycoside derivative.

The knot wood, heartwood and the pith of the small trees from Turkana County showed lower percentages of mesquitol in the acetonic extract compared to the other counties.

From the Anova test, the amounts of mesquitol present in the acetonic extracts of small trees are significantly different ( $p=0.002$ ) in the three counties and are also significantly different ( $p=0.000$ ) in the plant parts. The amounts of mesquitol present in the acetonic extracts of big trees are significantly different ( $p=0.001$ ) in the three counties and are also significantly different ( $p=0.000$ ) in the plant parts. There is a very strong positive correlation ( $r=0.91$ ) between the amounts of mesquitol present in the acetonic extracts between small trees and big trees from the three counties.

Table 3 shows that mesquitol is either absent or present in the bark of *P. juliflora*. However, after a systematic study of the LC chromatograms of the barks, it was revealed for the first time that the mesquitol or an isomer of this latter one is present in the barks in glycosylated form. This compound

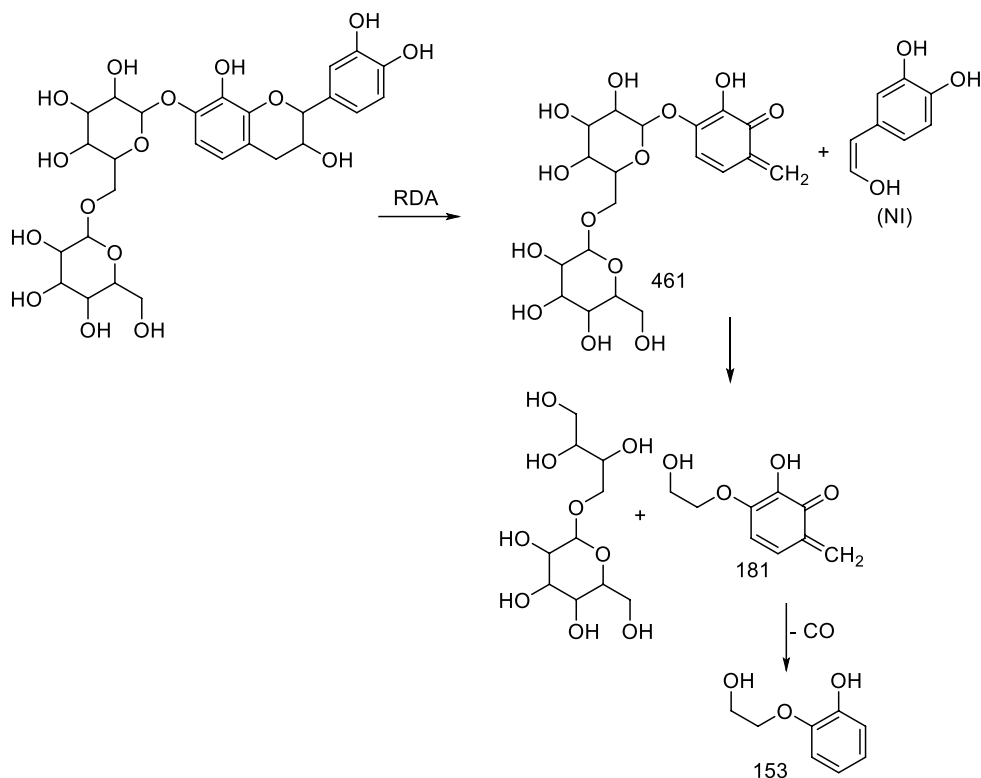
with a retention time of 5.7 min was identified by its MS/MS ions as shown in Fig. 3.

C-glycosidic bonds are more stable than O-glycosidic bonds; the fragmentation pathway of O-glycosylated flavonoids begins with the cleavage of glycosidic bonds and removal of the sugar moieties. In C-glycosylated flavonoid, complete removal of the sugar moiety is not observed but rather elimination of fragments of the sugar ring (Ramesh 2017). From the fragmentation pattern (Fig. 3), the mesquitol diglycoside is a C-glycosylated flavonoid since the sugar moiety fragments are missing. C-glycosylated flavonoids are more stable than O-glycosylated flavonoids. The percentage of glycosylated flavonoid corresponding to a molecular mass of  $290 \text{ g mol}^{-1}$  in the acetonic extracts from the barks of the *P. juliflora* is shown in Table 4.

The estimation of the total amounts of mesquitol present in *P. juliflora* was thereafter done and is shown in Table 5.

The big trees had slightly higher amounts of mesquitol in the knot wood, heartwood and the pith from both Turkana and Baringo Counties compared to the small trees, which was contrary to the samples from Garissa County, where

**Fig. 3** Fragmentation pathway of mesquitol diglycoside proposed on the basis of the ESI–MS/MS spectra in the negative mode. (Retention time, 5.7 min; UV, 274, 278 nm; MS, 613 [M–H]<sup>−</sup>; MS/MS [M–H]<sup>−</sup>, 461, 181, 153)



**Table 4** Percentage of glycosylated mesquitol in acetone extracts identified by LC–MS in the bark of *P. juliflora* from the three counties

Plant part	Baringo county		Turkana county		Garissa county	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	30	22	24	29	21	28

**Table 5** Estimation of percentages of mesquitol in *P. juliflora* (%)

Plant part	Baringo Small trees	Baringo Big trees	Turkana Small trees	Turkana Big trees	Garissa Small trees	Garissa Big trees
Bark	2.0	1.5	1.1	2.1	2.1	1.4
Sapwood	0.4	0.3	0.4	0.3	0.9	0.7
Knot wood	6.1	7.3	2.5	2.9	9.8	4.9
Heartwood	5.3	11.3	3.1	6.0	9.9	6.4
Pith	7.7	10.2	3.3	3.5	9.6	5.3

the opposite effect was observed. Surprisingly, mesquitol was present in small amounts in the samples from Turkana County. The exact factors that could lead to these differences among the counties are not yet completely understood, but a possible explanation could be the higher altitude of Turkana location compared to Baringo and Garissa Counties. For the small trees, Garissa County had the highest amounts of mesquitol, whereas for the big trees, samples from Baringo County showed the highest amounts of mesquitol in the knot wood, heartwood and pith. Harnessing of mesquitol from these two counties for valorization is therefore profitable.

### 3.3.2 Catechin quantification

Catechin, an isomer of mesquitol that has almost the same chemical and biological properties as mesquitol, was identified in the *P. juliflora* and quantified. It was found in little amounts in some parts of the stem, which included the knot wood, heartwood and the pith. In the sapwood, it was present only in the small trees from Baringo and Garissa Counties. An interesting result was the absence of catechin in the barks.

The percentages of catechin in acetonetic extracts from the various plant parts and counties are presented in Table 6.

In the case of the small trees from Baringo County, catechin was only found in the heartwood (5%) and not in the other parts. The highest percentages of catechin were found in the small trees from Turkana County. Examination of the LC chromatograms of the knot wood, heartwood and pith extracts from the different counties revealed some interesting results concerning the ratio of mesquitol and catechin. Figure 4 shows the different LC chromatograms

for the heartwood of small trees and big trees from the three counties.

The heartwoods like knot woods and piths of the big trees were generally dominated by only one peak corresponding to mesquitol (retention time of 3.7 min). On the other hand, the extractives of small trees were dominated by two peaks for catechin and mesquitol. It was interesting to note that in all the counties, the amounts of catechin were higher in small trees and its amounts decreased significantly in big trees. Braicu et al. (2013) argued that catechin can play two different roles in plants according to its stereochemistry. The (+)-catechin has antioxidant properties, whereas the (–)-catechin induces oxidation and cellular death in root cells of neighboring plants. The absolute configuration of asymmetric carbons present in catechin isomer in the *P. juliflora* is yet to be established.

Ratios of mesquitol and catechin in the knot wood, heartwood and pith for the different counties are shown in Table 7.

The results showed that mesquitol dominated in the big trees. Catechin was present in huge amounts in the small trees from Turkana County and its ratio was half that of mesquitol (2:1) in all the different parts of the stem. Catechin amounts decreased drastically in the big trees.

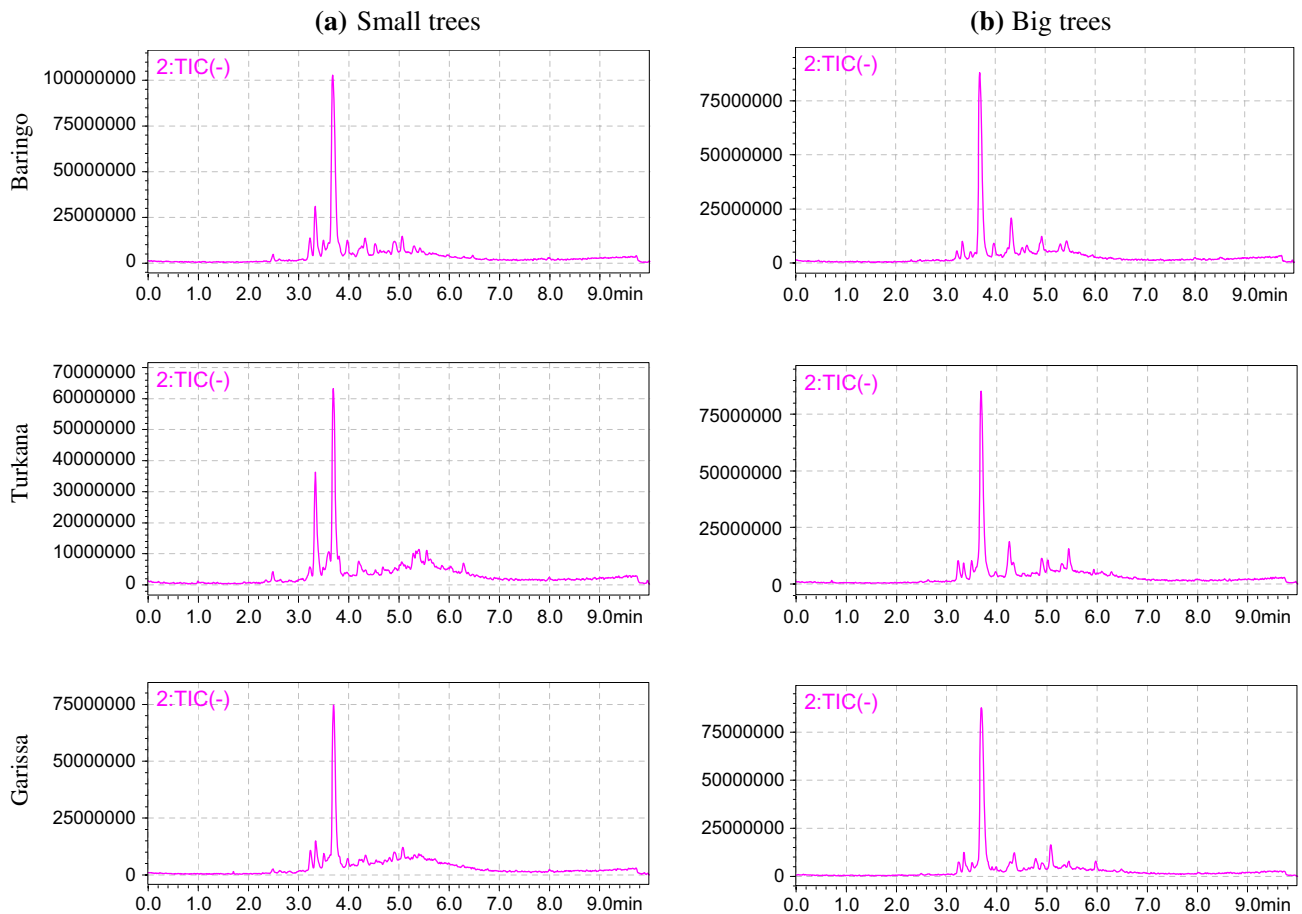
### 3.3.3 4'-O-methylgallo catechin

4'-O-methylgallo catechin was the most abundant free flavonoid that was found in the barks of *P. juliflora* ranging between 18 and 28%. The percentages of 4'-O-methylgallo catechin in acetonetic extracts from different parts of *P. juliflora* and counties found in the bark and sapwood are listed in Table 8.

**Table 6** Percentage of catechin in acetone extracts identified by LC–MS from the various plant parts in the three counties

Plant part	Baringo county		Turkana county		Garissa county	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	0	0	0	0	0	0
Sapwood	8	0	14	0	0	0
Knot wood	11	0	16	5	14	5
Heartwood	13	5	26	3	9	6
Pith	10	0	24	3	15	5





**Fig. 4** LC chromatograms for the heartwood of **a** small trees and **b** big trees from the three counties of Baringo, Turkana and Garissa

**Table 7** Ratios of mesquitol: catechin present in the heartwood of the three different counties

County	Knot wood		Heartwood		Pith	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Baringo	6:1	No catechin	5:1	16:1	6:1	No catechin
Garissa	4:1	11:1	8:1	13:1	4:1	15:1
Turkana	2:1	12:1	2:1	19:1	2:1	22:1

**Table 8** Percentage of 4'-*O*-methylgalocatechin in LC-MS

Plant part	Baringo county		Turkana county		Garissa county	
	Small trees	Big trees	Small trees	Big trees	Small trees	Big trees
Bark	18	23	20	28	26	19
Sapwood	9	13	23	0	0	0

In all cases, 4'-*O*-methylgalocatechin was more abundant in barks of the different trees independently from the counties. Similar to catechin, 4'-*O*-methylgalocatechin was completely absent in the sapwood of Garissa trees. Catechin was probably a precursor of 4'-*O*-methylgalocatechin.

## 4 Conclusion

The study on the variability and viability of mesquitol in *P. juliflora* reveals that mesquitol abundance in *P. juliflora* varies greatly depending on the different geographical habitats of the tree as demonstrated in the data from the three

different counties. The age and the different plant parts of the tree stem also showed variability. The highest amounts of mesquitol were found in the heartwood of the big trees in Baringo County, where mesquitol can be extracted with an unusually high yield of 11% with very high purity. Small trees from Turkana County had the lowest amounts of mesquitol (3%) making it an unlikely site for harnessing of mesquitol. However, it had the highest amounts of catechin and 4'-*O*-methylgallo catechin as compared to the other counties. From the data obtained on the abundance of mesquitol, it is evident that mesquitol is viable for valorization.

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### Compliance with ethical standards

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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