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EXTRACTIVE CONTENTS OF THE JUVENILE STEMWOOD AND BARK OF TEAK

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ABSTRACT

Teak wood is used at the juvenile stage due to short-rotation, therefore, this study aims to describe the extractive content of stem, bark, branch, and twig parts of the wood as value-added chemicals from secondary metabolites. Moreover, the main stems comprise of sapwood, heartwood, and bark while the branch and twig are made of sapwood together with bark. In this study, the sample trees were 6 and 8 years old with three replications from clonal superior teak wood and were extracted using n-hexane, methanol, and hot water as the solvents. The average of *n*-hexane, methanol, hot water, and total extractives ranged from 0.49 to 2.77%, 2.27 to 17.76%, 0.65 to 7.47%, and 5.96 to 25.40%, respectively. Furthermore, the total phenolic content from soluble *n*-hexane and methanol extracts ranged from 162.16 to 295.24 mg GAE/g, while the total soluble polysaccharides ranged from 166.28 to 423.97 mg GluE/g. The results showed that the 8-year-samples had higher values in methanol extractive content (MEC) and lower in hot-water extractive content (HWEC) than the 6-year-old trees. In addition, the bark together with sapwood in branch and twig parts had higher concentrations of MEC and total extractive content (TEC) compared to the main stems. For radial direction, MEC, HWEC, and TEC levels were greater in bark than in other parts. The branch and twig parts also had higher phenolic concentrations compared to the main stem at the base part. Meanwhile, the sapwood at the branch and twig parts have higher total soluble polysaccharide concentration compared to the main stem.

KEYWORDS: *Tectona grandis*, quinones, fast-growing, soluble polysaccharide, bark, teak.

INTRODUCTION

In Indonesia, teak wood is one of the most important timber sources for construction, sawn wood, furniture, and others. Moreover, the use of young trees as wood from the community

forest for multi-purpose products has increased in the past decade. These trees are managed in the community forest as a short rotation for approximately less than 20 years or juvenile stage. To meet the demand of wooden industries, superior teak wood from clonal breeding with a faster growth rate are introduced in the community forests and Perhutani (own-state forest enterprise) plantations.

Harvesting the trees generates large amounts of biomass residues such as leaves, barks, branches, twigs, and stumps which are left in the field or burned for energy production. Although branch and twig of teak wood have been used for furniture parts, its potential application to value-added products has not been adequately studied. Meanwhile, bark wastage is a major problem in the timber industry which contributes a great amount of biomass as a source of value-added chemicals from secondary metabolites. The extractive composition of teak wood is known, while there is little information about the bark. Moreover, the bark contains phenolic compounds such as stilbenes, lignans, flavonoids, and tannins (Fengel and Wegener 1984, Drozdz and Pyrzynska 2018) that are valuable sources of antioxidants and health care industries.

The necessity of maximizing the use of the remaining scarce resource leads to the complete utilization of trees which has increased the interest in the basic properties of tree parts other than the main stem. Several studies on the chemical analysis of teak wood have focused on the bole wood (Windeisen et al. 2003, Lukmandaru and Takahashi 2008, Niamke et al. 2018) while studies on stem, branch, and twigs are limited. The phenolic compounds from the quinone group of teak wood have attracted much attention among secondary metabolites because they exhibit an array of biological activities and relate to natural durability, dimensional stability, and color (Haupt et al. 2003, Neamatallah et al. 2005, Thulasidas, and Bhat 2007, Niamke et al. 2012, Lukmandaru, and Takahashi 2008, Li et al. 2018). Furthermore, its insoluble polysaccharide or non-structural carbohydrate has also attracted interest due to the association between heartwood formation and natural durability (Niamke et al. 2011).

This study aims to describe the extractive content of stem, bark, and twig parts of juvenile teak wood which are sources of potential biochemicals. Since different positions and ages of the individuals affect extractive contents in teak wood (Lukmandaru and Takahashi 2009, Rizanti et al. 2018), two ages and tree positions with various parts were included to cover a large variety of the potential sources of different extractive content. The results of this study are expected to provide a basis for future applications.

MATERIALS AND METHODS

Samples preparation

The trees were 6 and 8 years old (14 to 18 cm DBH) from a clonal superior teak wood (Jati Unggul Nusantara) in Gunungkidul Regency, while three trees were sawn from each age for replication. Meanwhile, the discs were sawn (5 cm in thickness) at the base (0.5 m above the ground), top (4.5 m above the ground), branch, and twigs (Fig. 1) for each tree. The branch (diameter of 2.5 to 5.5 cm) was defined as the first ramification from main the stem and twig (diameter of 1.0 to 2.2 cm) was the second. Also, the proportion of the heartwood at the base and top part were 26 to 56% and 9 to 32%, respectively. For radial direction, the discs from

the trunk were divided into sapwood, heartwood, and bark, while the branches and twigs were divided into sapwood and bark parts. Each part was converted into a wood meal in 40 to 60 mesh size.

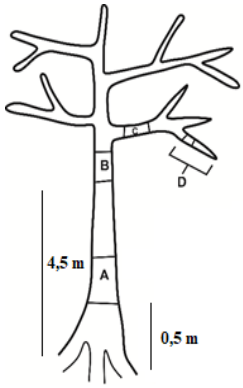

Fig. 1: The scheme of teak trees sampling, A - base stem, B - top stem, C - branch, D - twig.

Extraction

The wood meal (5 g) was extracted using n-hexane, methanol, and hot water. Meanwhile, the extraction with *n*-hexane and methanol solvents was carried out in a soxhlet apparatus for 6 hours. The solvent was later evaporated using a rotary evaporator and the extract was dried in an oven for 1 hour (100°C). Furthermore, the hot-water extraction was conducted in a water bath by reflux for 3 hours (100°C) and the extract was obtained after filtering and evaporating the water. This extractive content was measured using an oven-dry wood meal, while the total extractive content (TEC) was calculated by determining the sum of all extractive contents.

Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Singleton et al. 1999) with slight modifications. The extracts (*n*-hexane and methanol) were dissolved in methanol (1 $mg \cdot ml^{-1}$) and 2.5 ml diluted (10 times) Folin-Ciocalteu reagent (Merck, Germany) was added. This mixture was maintained for 2 min and 2 ml Na_2CO_3 (7.5%) was added. After 30 min at room temperature, the mixture was placed in the equipment and was followed by the sample absorbance which reads 765 nm (Spectrophotometer UV-Vis Optima Nano 3000 SP). The results were expressed as gallic acid equivalents based on dry extract (mg GAE/g). Furthermore, the calibration curve was determined using gallic acid at 0.125, 0.0625, 0.03125, 0.015625, and 0.0078125 $mg \cdot ml^{-1}$ concentrations ($y = 0.1064x - 0.0056$, $R^2 = 0.9954$). The TPC was calculated as the mean ± standard deviation of three replications for each sample. The combined total phenolic content (CTPC) was calculated as the sum of all TPC contents.

Total soluble polysaccharide

The total soluble polysaccharides (TSP) contents were determined using the phenol-sulfuric acid method (DuBois et al. 1956). The amount of 1 ml of hot-water extract (1 $mg \cdot ml^{-1}$ in water) was mixed with 1 ml of phenol (5%) and 5 ml of concentrated sulfuric acid (98%). This mixture was allowed to stand for 20 min at room temperature, while the sample's absorbance was measured at 490 nm wavelength. Furthermore, the TSP value was calculated from the

calibration curve using glucose at 0.250, 0.125, 0.0625, 0.03125, and 0.015625 $mg \cdot ml^{-1}$ concentration ($y = 0.1264x - 0.0076$, $R^2 = 0.9939$). The results were expressed as glucose equivalent based on dry extract (mg GluE/g) from the three replications.

Statistical analysis

Statistical analysis was carried out with Microsoft excel 2010 on Windows and the results were expressed as means ± standard deviation.

RESULTS AND DISCUSSION

Extractive content

The extractive content of samples using three different solvents is shown in Figs. 1-4. The average of n-*h*exane, methanol, hot water, and total extractives ranged from 0.49 to 2.77%, 2.27 to 17.76%, 0.65 to 7.47%, and 5.96 to 25.40%, respectively. Similarly, the number of extractives is lower than the TEC of the teak from the forest in India (Thulasidas and Bhat 2007), farmlands, and Perhutani plantations except for the sapwood parts (Lukmandaru and Takahashi 2008). Furthermore, the TEC levels were higher compared to the teak wood from 10 and 40-year-old trees in Indonesia (Rizanti et al. 2018). The average total extractives of teak bark (50 to 60 years) were 10.7% which corresponds to polar extractives with water-soluble extract as the major fraction (Baptista et al. 2013). That result was lower compared to this experiment at the same part (12-24%).

The non-polar solvent dissolved quinone compounds in teak wood (Windeisen et al. 2003, Lukmandaru and Takahashi 2009). Meanwhile, there was no consistent trend for *n*-hexane extractive content (NHEC) and TEC levels between the 6 and 8 years old samples. It is assumed that TEC between these years is in a narrow range compared to other earlier studies on teak wood (Haupt et a. 2003, Lukmandaru and Takahashi 2008, Rizanti et al. 2018). Generally, the 8-year-old sample had higher values in methanol extractive content (MEC) and lower in hot-water extractive content (HWEC) than the 6-year-old. Based on extract weight (Fig. 5), the differences between 6 and 8-year-old samples were the percentage of MEC and HWEC fractions, especially in bark and in sapwood at the base part. Their MEC values were not greater than 50% and had a higher portion of HWEC when compared with the 8-year-old samples and vice versa. Furthermore, phenolic compounds such as tannins, flavonoids, and stilbenes are soluble in methanol (Sjöström 1993), while the bark contains excess polyphenols (Drozdz and Pyrzynska 2018). The higher MEC levels in the stem indicated a heartwood formation which is characterized by polyphenols increase in heartwood and decrease sugars in sapwood (Hillis 1987, Niamke et al. 2011).

A previous work showed that the variation among different parts of the tree indicated that the stem base had the highest extractive concentration (Caron et al. 2013). Meanwhile, some systematic differences were observed in line with the vertical direction or stem part. The results showed that sapwood at the main stem had higher levels than sapwood at branch and twigs for NHEC, while the base part had a higher level of NHEC than other parts. When compared to the bark in the main stems, the branch and twig showed higher concentrations in MEC, HWEC, and

TEC. Furthermore, TEC in the sapwood of branch and twig has also higher values than the sapwood in the main stem. This is due to a large number of living cells related to the cambial part and is active in the metabolism processes of the stem (Yeh et al. 2006). Moreover, the amount of extractive is relatively high in certain parts such as bark, heartwood, root, wounded tissue, branches, and twigs (Hillis 1987).

NHEC in sapwood tends to decrease in values than other parts in the radial direction. Meanwhile, heartwood contained higher amounts than sapwood in main stems for NHEC, MEC, and TEC. The huge differences in MEC between sapwood and heartwood were observed at the base part of 6-year-old samples. Furthermore, the bark had higher amounts than other parts in MEC, HWEC, and TEC. Based on extract weight, MEC was the major fraction in all parts except bark and sapwood in the main stem of the 6-year-old trees. These comparatively high values were due to the presence of parenchyma cells in the bark as storage tissues (Sjöström 1993).

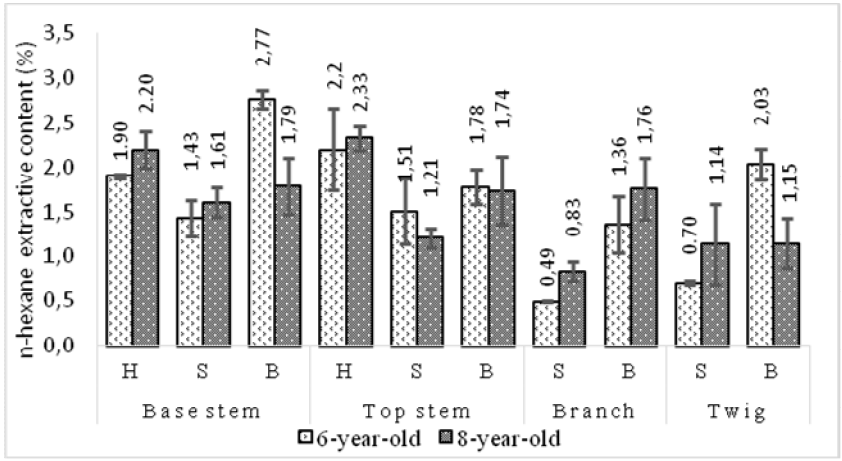

Fig. 1: The hexane extractive content (% based on oven-dry wood) from T. grandis (means of three trees) with error bar standard deviation. H- heartwood, S- sapwood and B-bark.

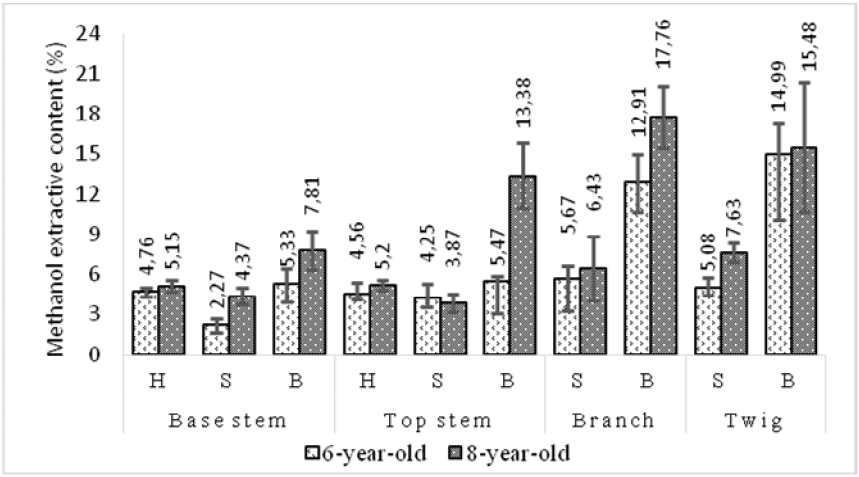

Fig. 2: The methanol extractive content (% based on oven-dry wood) from T. grandis (means of three trees) with error bar standard deviation. H- heartwood, S- sapwood and B-bark.

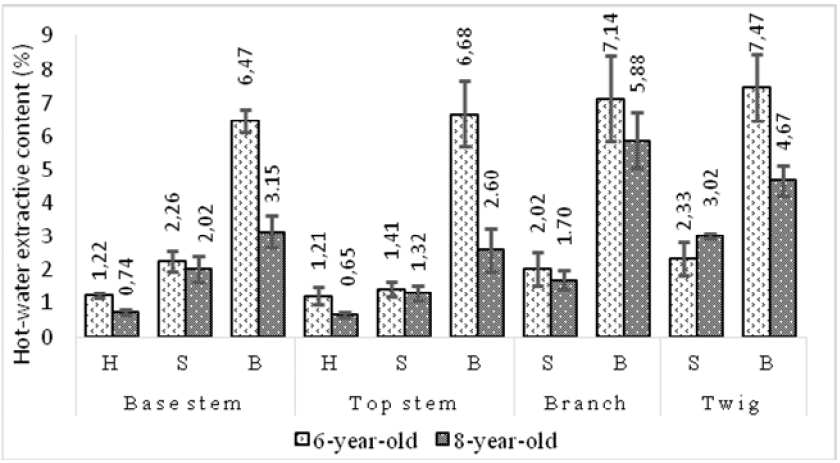

Fig. 3: The hot-water extractive content (% based on oven-dry wood) from T. grandis (means of three trees) with error bar standard deviation. H- heartwood, S- sapwood and B-bark.

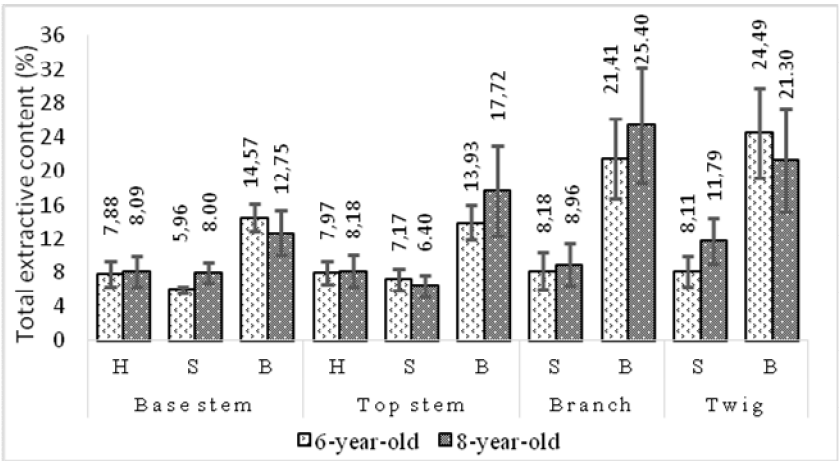

Fig. 4: The total extractive content (% based on oven-dry wood) from T. grandis (means of three trees) with error bar standard deviation. H- heartwood, S- sapwood and B-bark.

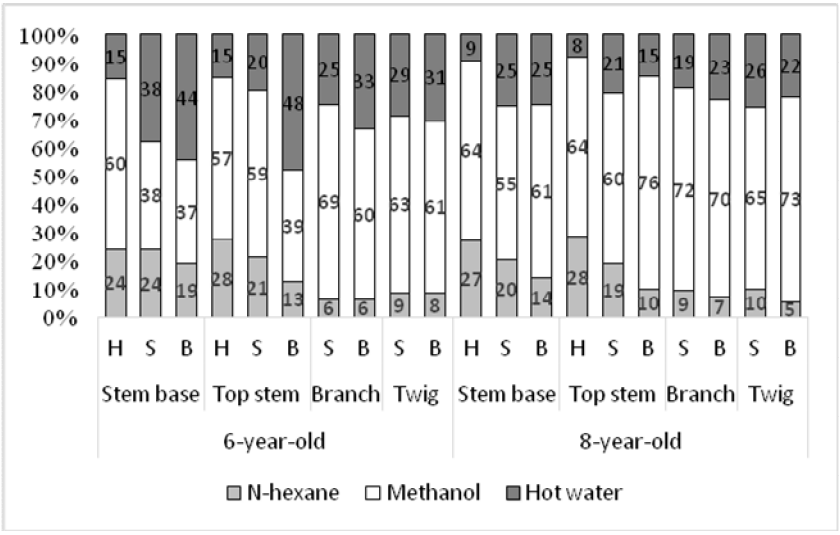

Fig. 5: The composition of extractive based on extract weight (n-hexane, methanol, and hot water) from T. grandis (means of three trees). H- heartwood, S- sapwood and B-bark.

Total phenolic contents

The total phenolic contents were measured from *n*-hexane and methanol soluble extracts. Meanwhile, the methanol total phenolic contents (MTPC) (ranged from 149.68 to 261.90 mg GAE/g) had higher values compared to the *n*-hexane total phenolic contents (HTPC) (ranged from 10.87 to 47.51 mg GAE/g). This low level of HPTC was due to the presence of more non-phenolic components by less polar solvent. The largest amount of HTPC in all radial parts, age groups, and part of the tree were in the sapwood branch of the 8-year-old trees (47.51 mg GAE/g) and the lowest was in the bark of the top stem of 6-year-old. (10.87 mg GAE/g) (Tab. 2). Furthermore, the HTPC of the bark showed higher levels in 8-year-old samples than 6-year-old. However, the reverse pattern was observed in the sapwood at the main stem parts, while it gives higher values of HTPC than its bark at the branch and twigs parts.

The combined total phenolic content (CTPC) ranged from 162.16 to 295.24 mg GAE/g (Tab. 2). These results were higher than a previous study conducted by Villagomez (2005), which showed that the same parts of pines ranged from 1.8 to 98.2 mg GAE/g. However, these values were considerably lower in the same parts compared to previous studies on the bark of *Swietenia macrophylla* (Masendra et al. 2021) and stem as well as the bark of *Eucalyptus pellita* (Arisandi et al. 2019).

The phenolic content in plants changes gradually in the tissues and is distinguished based on growth rate as due to tree age (Lattanzio et al. 2008). Meanwhile, the MTPC and CTPC had a similar pattern due to the high proportion of MPTC. This showed that the tissues of the base part have lower concentrations in MPTC and CPTC compared to the branch and twig parts. Furthermore, there was no systematic pattern in tree age and radial direction for MTPC and CTPC values. A previous study showed that the heartwood contains more phenolic compounds than sapwood (Hillis 1987, Fengel and Wegener 1984). This is caused by the wood region which has a smaller dimension of juvenile wood than the mature (Zobel and Buijtenen 1989). Meanwhile, the positive trend of CTPC levels with tree age (Ayton et al. 2007, Aregay et al. 2021) was not clearly shown in this study. The increase in phenolic compounds in older trees is affected by mineral nutrient imbalance and water stress due to a reduction in xylem and wounded tissue efficiency (Aregay et al. 2021).

Meanwhile, the high value of CTPC is due to the function of the bark for protecting from fungal attack and resistance against pathogens (Withouck et al. 2019, Sakai 2001). Furthermore, there were no striking differences in the CTPC levels of the barks and woods. This finding is different from other species such as *Picea abies* (Neiva et al. 2018) and Belgian apple (Withouck et al. 2019). It is noticed that the highest values were measured in the bark at the twig part while the lowest was in the sapwood at the base part. This shows that phenolic compounds are transported from source cells to other tissues and are usually accumulated in sub-epidermal cells of leaves and shoots (Lattanzio et al. 2008).

Phenolic compounds are the most common secondary metabolites in plants that are known to have some important roles. The quinones from teak wood have been detected and related to their natural durability in sapwood and heartwood regions (Windeisen et al. 2003, Lukmandaru and Takahashi 2009, Niamke et al. 2011, 2012). Therefore, the phenolics in sapwood including in the branch and twig parts might be the precursor for toxic components in the heartwood.

Although antibacterial activity has been observed from a teak bark quinone (Neamatallah et al. 2005), the phenolics in the bark are less investigated. The high content of phenolics in some parts of teak wood from juvenile age stems showed the potential of raw material for bioactive compounds extraction which are used in commercial applications.

Tab 2: Total phenolic content (mg GAE/g) measurement for n-hexane and methanol soluble extracts of Tectona grandis.

No	Stem part	n-hexane	Methanol	Combination
1	6BH	31.72 (7.89)	188.84 (42.82)	220.56 (78.56)
2	6TH	31.04 (10.82)	184.54 (18.14)	215.58 (68.83)
3	6BS	26.27 (8.05)	163.92 (6.98)	190.19 (84.07)
4	6TS	38.66 (2.88)	200.85 (14.84)	239.51 (76.75)
5	6BBa	21.31 (1.04)	189.45 (16.26)	210.76 (81.1)
6	6TBa	10.87 (1.81)	193.33 (5.18)	204.2 (91.23)
7	6BrS	33.99 (4.87)	211.77 (47.21)	245.76 (88.89)
8	6BrBa	21.39 (3.84)	208.46 (9.77)	229.85 (93.54)
9	6TwS	46.75 (1.41)	237.17 (24.59)	283.92 (95.21)
10	6TwBa	25.86 (8.04)	261.38 (47.43)	287.24 (117.76)
11	8BH	28.2 (10.92)	183.54 (21.75)	211.74 (77.67)
12	8TH	44.92 (1.05)	213.62 (8.53)	258.54 (68.6)
13	8BS	12.48 (13.16)	149.68 (50.05)	162.16 (91.65)
14	8TS	11.93 (19.10)	181.37 (29.37)	193.3 (84.35)
15	8BBa	23.93 (2.64)	207.14 (30.89)	231.07 (84.72)
16	8TBa	31.45 (14.07)	208.94 (40.40)	240.39 (88.75)
17	8BrS	47.51 (5.68)	245.73 (35.54)	293.24 (99.11)
18	8BrBa	23.23 (12.58)	254.85 (50.75)	278.08 (115.81)
19	8TwS	45.08 (9.54)	214.99 (12.06)	260.07 (84.96)
20	8TwBa	33.34 (7.90)	261.90 (7.53)	295.24 (114.28)

Remark: B- base stem, T- top stem, Br- branch, Tw- twig, H- heartwood, S- sapwood, Ba- bark, 6- six-year-old, 8- eight-year-old. Mean of three trees with standard deviation in parentheses.

Total soluble polysaccharide

The sugar contents in wood serve as a nutrient that provides energy for metabolic processes, osmoregulation, and is the source of the C-skeleton (Niamke et al. 2018, Kampe and Magel 2013). Meanwhile, the most important reserved carbon compounds are non-structural carbohydrates (NSC), which consist of starch and low molecular weight sugar (soluble sugar) such as glucose, fructose, and sucrose (Hoch et al. 2003). In this study, total soluble polysaccharides (TSP) were calculated from the hot-water soluble extract. The highest value was from the sapwood of twig of 8-year-old trees (423.97 mg GluE/g) and the heartwood of the top stem of 6-year-old gave the lowest value (166.28 mg GluE/g sample) (Fig. 6). Compared to previous studies, these results were lower than the spruce bark (Le-Normand et al. 2012).

Regarding the age, it was shown that the TSP in heartwood at the main stem and bark at the branch of 8-year-old had higher values than 6-year-old samples. These results confirmed the previous studies on *Nothofagus pumilio* (Piper and Fajardo 2011) and *Pinus ponderosa* (Sala and Hoch, 2009). This is due to the difference in trees height which affects the light intensity and causes a low carbohydrate storage capacity from juvenile trees (Niinemets 2010). Furthermore,

the lower TSP of 6-year-old trees was correlated to the harvesting season where the trees were felled in a longer drought period in the same year. During the drought, the teak wood also molts gradually which leads to a decrease in carbon reserves (Lloret et al. 2018).

For vertical direction, the sapwood at the branch and twig had a higher of TSP concentration compared to the main stem. This shows that the location of the twig is adjacent to leaves on which the photosynthesis products are formed and translocated (Rosell et al. 2020). Furthermore, previous results in other species also showed a similar phenomenon (Barbaroux et al. 2003, Sala and Hoch 2009). The high level of TSP in a twig is also correlated to the anatomical change in organs and the cambium aging process which enhances vessel formation (Barbaroux et al. 2003).

A previous study by Niamke et al. (2011, 2018) showed the drastic decrease from sapwood toward heartwood of the teak (5 to 10 years) in NSC content. This trend was observed only from the sapwood to heartwood in 6-year-old samples. Furthermore, the TSP levels increase from the heartwood toward the bark at the main stem and were also observed in previous studies (Rosell et al. 2020, Barbaroux et al. 2003). Moreover, soluble sugars are the dominant constituent in bark (Rosell 2020). The high value of TSP in the bark is related to secondary phloem that translocates photosynthate (Savage et al. 2016) and the hydrolysis process of tannins to generate gallic acids and sugars (Sjöström 1993).

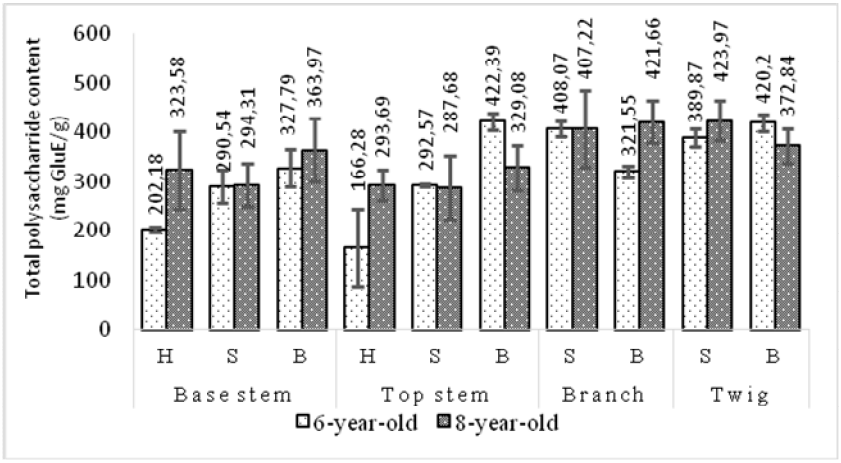

Fig. 6: The total soluble polysaccharide (mg GluE/g sample) from T. grandis (means of three trees) with error bar standard deviation. H- heartwood, S- sapwood and B- bark.

CONCLUSION

The evaluation of the extractive content of the main stems, branches, and twigs of the two different ages of juvenile teak wood showed the variation between the wood and bark. These results showed that the 8-year-old trees were higher in methanol extractive content and lower in hot water than the 6-year-old trees. Furthermore, it was also higher in methanol, hot-water, and total extractive contents in the bark than other parts in a radial direction. The branch and twig parts contained more methanol and total extractive content than the main stem. The phenolics content and polysaccharides concentrations were higher in branch and twig parts at the base.

Therefore, this high extractive and phenolics contents in bark parts attracted interest in exploring valuable extracts and compounds.

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