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Characterization and Quantification of Phenolic Compounds in the Extract of Teak (*Tectona grandis* Lin. f) Fruits

NATALYA KRISHNAMBIKA AND K. SUDHAKARA

College of Forestry, Kerala Agricultural University, Thrissur 680 656
sudhakarakudlu@yahoo.com

ABSTRACT The present study was conducted at College of Forestry, Kerala Agricultural University, Thrissur, to investigate the presence of major phenolic compounds and to estimate the total phenolic content occurring in the pericarp of fresh and one year old teak fruits obtained from Nilambur Forest Division. Characterisation of the phenolic compounds was done using Thin Layer Chromatography and the quantification of the total phenolic content was done using colorimetry. It was found that phenolic compounds occurring in the teak fruits are basically hydroxybenzoic acids. These include syringic acid with R_f value of 0.79 (in the mesocarp and the endocarp irrespective of the age of the fruits), gentisic acid with an R_f value of 0.46 (in the endocarp of one year old fruit), *p*-hydroxybenzoic acid with an R_f value of 0.57 (in the endocarp of fresh fruit), vanillic acid with an R_f value of 0.87 (in the mesocarp of one year old fruit) and protocatechuic acid with an R_f value of 0.19 (in the mesocarp of fresh fruit). The amount of total phenolic content in the mesocarp of the fruit was relatively higher than those found in the endocarp irrespective of the age of the fruit lot. The total phenolic content with respect to ageing of fruits proved statistically non-significant. The bioassay studies using the leachate of teak fruits are contradictory; this may be due to fact that the physiological effect of hydroxybenzoic acids upon seed dormancy is yet to be verified.

Key words: Phenolic compounds, Teak fruits

Teak (*Tectona grandis* Linn. F) is a significant and premier timber species of the tropics due to its unparalleled durability and versatility. Huge requirement of teak wood makes it imperative to establish large scale plantations to achieve maximum productivity. In tropical countries seed is the most economical tool for artificial regeneration of forest trees. Teak is no exception to this. However, teak cultivation is hampered by poor fruit/seed germination. Once germination has started, it proceeds easily and briskly.

Various reasons for the cause of dormancy have been put forth by different workers. Joshi and Kelkar [1] have shown that seeds in the quadrilocular fruit of teak is not uniformly developed i.e., on an average, one seed is fully developed and three remain undeveloped. The X-ray radiography studies conducted by Kamra [2] revealed that about 40 per cent of the fruits contain only one developed seed and about 16 per cent, two such seeds. The studies also showed that fruits lacking a fully developed seed may vary from 20-45 per cent in the sample which considerably affects the germination. This emptiness is one of the major causes of poor germination percentage [3]. Gupta and Pattanath [4] attributed that germination inhibitor in the mesocarp are responsible for slow and low germination of teak seeds. The present study has been attempted to characterize and estimate the major phenols present in the pericarp of teak fruit, with special reference to the mesocarp and endocarp of old and fresh fruits.

MATERIALS AND METHODS

Seed source

Two fruit lots were obtained from 1945 Teak Seed Production Area, Cherupuzha of Karulai Range,

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Nilambur Forest Division. The fruit lots were collected between January-February of 2004 and 2005 respectively.

The fruits collected during the year 2004 were cleaned and stored in a gunny bag in room under ambient conditions for one year.

Separation of layers of the pericarp

Since teak fruit is a drupe,enclosed within a persistent calyx, three distinct layers are present, namely the flaky exocarp with trichomes, the felty mesocarp and the hard endocarp. Hand pounding using mortar and pestle aided the separation of the pericarp. To improve the efficiency of separation, quartz sand was added in the proportion of one part of quartz for two parts of fruits. Afterwards winnowing and sieving was done to separate the quartz sand from the test sample. The mesocarp and the endocarp were ground to fine dust using Wiley Mill. Sample was kept in airtight-labeled polythene bags and stored in refrigeration at a temperature of ≃ 5°C until required for analysis during March-April 2005.

Quantitative estimation of phenols

The total phenol estimation was carried out with the Folin-Ciocalteau reagent. The principle is that the phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex, Molybdenum blue. The preparation of standard Catechol and that of the test material was in accordance with the prescriptions [5].

Characterization of phenols in mesocarp and endocarp of teak fruits

TLC plates were prepared by coating 300 µm thick layer of silica gel G of SRL brand on glass plates of 20 cm x 20 cm size. The test samples were prepared as per the recommendations [6]. Spotting on TLC plates was done in circular shapes with the help of capillary tubes. About 5 µl volume was spotted per sample. For the purpose of quantifying the phenols, the elution was done in a solvent mixture of acetic acid: chloroform [7] in the ratio of 1:20.

To detect the phenols on the TLC plates, 2N Folin-Ciocalteau reagent spray was used. The sprayed plate was kept at 110°C for 3-5 minutes in a chromatographic oven to develop coloured spots. The developed plate was scanned by naked eye immediately before the colour of the phenol faded due to cooling.

Estimation of total phenolic content

The phenolic content in five different concentrations from 0.2 ml - 1.0 ml increasing by 0.2 ml was recorded for four samples viz., fresh and old endocarp and mesocarp of six replication each.

Characterization of the phenols from TLC plates

The R_f values as well as the colour of phenolic spots were recorded. Colour was identified by purple and blue-violet colours.

Statistical analysis

All experiments had six replications. Adopting completely randomized design the analysis of variance was done by NESTED ANOVA.

RESULTS

Characterization of phenolic compounds

By using standard TLC technique, characterization of the phenolic compounds was performed. The colour of the compound eluted, mean, standard deviation and the coefficient of variation of the R_f value are shown in Table 1. The results show that a characteristic phenolic acid, 'syringic acid' is present in both the endocarp as well as in the mesocarp of the fruit (of both the old and the fresh fruits) with a R_f value of 0.79. The other compounds that have been characterized include the gentisic acid with a R_f value of 0.46 in the old endocarp; *p*-hydroxybenzoic acid with a Rf value of 0.57 in the fresh endocarp; vanilic acid with a R_f value of 0.87 in the old mesocarp and protocatechuic acid with a R_f value of 0.19 in the fresh mesocarp.

Total phenolic content in the different layers of the pericarp

Results pertaining to the total phenolic content of the various components of the pericarp of the fresh

and the old fruit lot in five different concentration are presented in Table 2. The results obtained after conducting NESTED ANOVA concerning the above parameters are presented in Table 3. The results show that phenolic content in the old and fresh fruit lot were statistically at par. However, the phenolic content between the mesocarp and endocarp within the old and fresh fruit lot were significantly different ($P \geq 0.01$). On an average, phenolic content in the mesocarp was 11 per cent more than in the endocarp. The total phenolic content increased significantly ($P \geq 0.01$) with increase in the concentration of the test sample. At 1 ml concentration, it was 5.833 mg compared to 1.023 mg at 0.2 ml concentration of the test sample. Rest of the treatments were non significant.

DISCUSSION

The germination percentage of teak fruit is highly variable and in some cases total failure of germination indicates seed dormancy. According to Pattanath [8], cited by Willan [9] there is no evidence of physical dormancy in *T. grandis*. Teak fruit takes up sufficient water for germination within 24 hrs of immersion of the fruit [10]. This uptake relates to the anatomical structure of the endocarp. There are numerous, paired, quite broad pits penetrating the highly thickened cell wall of the endocarp, hence the high permeability. Fairlamb and Davidson [11] observed the presence of water soluble inhibitors in the pericarp. They found that aqueous extract obtained by soaking teak fruits for four days and used to moisten filter paper inhibited germination of cress seeds. The present study reveals that the total phenolic content in the mesocarp (irrespective of the age of the fruits) was higher when compared with the content in the endocarp. Perhaps this could explain the higher germination percentage in the mesocarp removal studies of teak. Termite aided mesocarp removal of teak fruits is an efficient pre-sowing technique [12]. The term phenolic compound embraces a wide range of plant substances, which possess in common an aromatic ring and one or more hydroxyl substitutes. Phenolic substances tend to be water-soluble since they most frequently occur combined with sugars, as glycosides. At present, conventional methods like alternate wetting and drying at twelve-hour interval for

Table 1. Colour and R_f value of the components of the pericarp of fresh and old fruits

Pericarp component	Colour	Eluted compound I				Colour	Eluted compound II			
		R_f value					R_f value			
		Mean	S.d	CV%	Compound		Mean	S.d	CV%	Compound
OM	Purple	0.873	0.006	0.74	vanillic acid	Pale blue	0.793	0.02	3.71	Syringic acid
OE	Brown	0.460	0.010	2.17	Gentisic acid	Pale blue	0.790	0.03	4.20	Syringic acid
FM	Purple	0.194	0.002	1.27	Protocatechuic acid	Pale blue	0.791	0.04	5.61	Syringic acid
FE	Brown	0.570	0.022	3.99	*p* - hydroxybenzoic acid	Pale blue	0.790	0.06	7.59	Syringic acid

(OM = One year old mesocarp, OE = One year old endocarp, FM = Fresh mesocarp, FE = Fresh endocarp)

Table 2. Total phenolic content of the various components of the fresh and old teak fruit lot in five different concentrations

Pericarp component	Concentration of the test sample													
	0.2ml			0.4ml			0.6ml			0.8ml			1.0ml	
	Mean	Sd	Cv%	Mean	Sd	Cv%	Mean	Sd	Cv%	Mean	Sd	Cv%	Mean	Sd	Cv%
OM	0.278	0.001	0.559	0.559	6.6E-08	0	0.836	0.002	3.44	1.115	0.003	3.447	1.37	1.8E-08	3.44
OE	0.218	0	0	0.436	0	0	0.654	0	0	0.87	0	0	1.09	1.88E-08	1.73E-08
FM	0.289	0	0	0.582	0	0	0.873	1.33E-08	1.53E-08	1.16	0	0	1.45	1.88E-08	1.29E-08
FE	0.238	0	0	0.476	0	0	0.71	0	0	0.953	0	0	1.923	0	0

(OM = One year old mesocarp, OE = One year old endocarp, FM = Fresh mesocarp, FE =Tresh endocarp)

Table 3. NESTED ANOVA

Source	df	MSS	F value
Between old and fresh fruits	1	0.11857954	0.16
Between mesocarp and endocarp (within old and fresh fruits)	2	0.73946625	32507.1**
Between concentrations	4	9.69082021	426011**
Concentration x old and fresh fruits	4	9.69082021	0.03
Concentration x mesocarp and endocarp (within old and fresh)	8	0.00000028	0.02
Error	100	0.00002275	

seven consecutive days is being followed as a pre-treatment for teak by the Kerala Forest Department. This is based upon the belief that the chemical, which may be present in the pericarp, will be washed off and soften the seed coat. The percentage of germination by this method ranges from 30-50 per cent. The free phenols and the phenolic acids are best considered together, since they are usually identified together during plant analysis. There has been universal occurrence of *p*-hyroxybenzoic acid, protocatechuic acid, vanillic acid and ortho-protocatechuic acid among the angiosperms. A large number of studies have been carried out on phenols of the seeds. List of inhibitory compounds and seed parts in which they occur are given [13, 14]. Kannan *et al.* [15] showed the presence of coumarin like substance with R_f value of 0.22 in the seed coat of *Albizia odoratissima*. Phartyal *et al.* [16] reported the presence of inhibitors in different parts of *Acer caesium* samara. The inhibitors in *A. caesium* appeared most likely to be non-polar terpenes and acids (petroleum ether soluble of polar compounds as Phenolics, which are water-soluble). Plant extracts from *Portulaca oleraccea* L. contained ferulic acid, o-coumaric, *p*-hydroxy-benzoic acid, vanillic acid, gentisic acid, caffeic acid, cinnamic acid and p-coumaric acid which inhibited the germination of 12 crop seeds [17]. *p*-hydroxybenzoic acids is the major phenolic germination inhibitor in papaya seeds [18]. Phenolic substances like protocatechuic and *p*-hydroxybenzoic acid, catechol and *p*-hydroxy-acetophenone found in the humus are responsible for the inhibition of natural regeneration of spruce [19]. The present study revealed that the phenolic content is concentration dependent. Perhaps, this

could probably explain the relatively higher responses both stimulatory [20] and inhibitory [11] in the laboratory conditions than in field conditions, where the treatment differences are nullified due to the external environmental conditions [21]. A major complication with chemical dormancy is that most seeds from which germination inhibitors have been isolated have also been shown to exhibit physiological dormancy [21]. Further, there is no evidence whether the chemicals present in the seed would still prevent germination even though the physiological dormancy is broken. Chemical dormancy should be used to describe only a condition where seeds lack physiological dormancy.

Though the major phenolic compounds in the mesocarp and the endocarp of the teak fruits have been identified by the present study, the influence of these compounds on dormancy have to be studied. Most of the literature available on bioassay studies are contradictory [20, 11] hence there is wider scope for further research into the effect of the phenolic compounds on dormancy, using bioassay as a tool.

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