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Tectona Grandis: Phytochemical Investigation

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ABSTRACT

The present study reports the phytochemical investigation of the stem bark of *Tectona grandis.* Nine phytoconstituents have been reported namely Eicosanyl Eicosanoate, β-Sitosterol, Ursolic acid, Lupeol, Betulinic acid, Betulin, Betulinaldehyde, Bis (2-ethylhexyl) phthalate and Tectol. Eicosanyl eicosanoate and Bis (2-ethylhexyl) phthalate have been reported for the first time from stem bark of *T. grandis*.

Keywords: *Tectona grandis*, Verbenaceae, Bis (2-ethylhexyl) phthalate, Tectol.

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INTRODUCTION

Tectona grandis, commonly known as Indian Teak is an erect, large deciduous tree having height up to 35 meters; bark having brown or grey colour and shallow longitudinal furrows, belonging to family Verbenaceae[1]. The tree is indigenous to the peninsular India and Madhya Pradesh, extending to parts of Rajasthan, southern Uttar Pradesh, Orissa, Assam, Bihar, Maharashtra, and Southern India[2]. It is known as Saka in Sanskrit, Sagwan in Hindi and Sailo in Konkani[2]. Extensive literature survey revealed that many phytoconstituents like alkaloids[3], glycosides[4], saponins[5], steroids[6], carbohydrates[7], proteins[8] and flavonoids[9] have been reported in *T. grandis.*It was also learnt that no substantial work on the stem bark of *T. grandis* was carried out in terms of phytochemical investigation. Hence an effort was made to investigate the phytoconstituents from the ethanolic extract of the stem bark of *T. grandis*.

MATERIALS AND METHODS

All the melting points were recorded in Bio Technics India, Model No. BT2-38 melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using KBr pellet method on Bruker α-T Spectrophotometer at S. E. T.'s College of Pharmacy, Dharwad and National Facility for Clinical Trials, ISISM, Chennai. ^{1}HNMR spectra of the compounds were taken on Bruker 500 MHz PMR Spectrophotometer using $CDCl_3$ and DMSO and Mass spectra were recorded using LC-MS Shimadzu LC 2020 at National Facility for Clinical Trials, ISISM, Chennai and also using ESI-MS Expression CMS Advion at SynZeal Research Laboratory, Ahmedabad. TLC was carried out using Aluchrosep Silica Gel 60/UV254 from S. D. Fine Chemicals Pvt. Ltd, Mumbai. Column Chromatography was carried out using glass column with stopcock, 30 x 600 mm from Merck Specialities Pvt. Ltd, Mumbai packed with Silica Gel (200-400 mesh) from Molychem, Mumbai. All the Chemicals and Reagents used were obtained in high purity either from S. D. Fine Chemicals Pvt. Ltd, Molychem and ChemportPvt. Ltd, Mumbai.

Collection and authentication:

The stem bark of *T. grandis* were collected from Camurlim, Bardez-Goa during the month of

October, 2012. It was authenticated by Prof. G. I. Hukkeri, Associate Professor, Dhempe College of Arts and Science, Miramar-Goa.[10]

Preparation of ethanolic extract:

The stem bark of *T. grandis* was collected, washed and dried in shade. The dried stem bark was powdered (1 Kg)and exhaustively extracted by maceration with Ethanol (95%) for 3 days. After 3 days, the ethanolic layer was decanted off. This process was repeated thrice. The solvent from the total extract was distilled off using Rotary Vaccum Evaporator (Superfit) and the concentrate was evaporated to dryness (30 g).[11]

Preliminary Phytochemical Screening

Preliminary Phytochemical studies were performed for testing the different phytoconstituents present in the ethanolic extract with reference to standard procedures[12, 13]. The tests were performed and the results are tabulated in Table 1.

Table 1: Result of Qualitative tests for Phytoconstituents.

Sr. No.	Tests	Inference
1.	Alkaloids	+
2.	Glycosides	+
3.	Carbohydrates	+
4.	Flavonoids	+
5.	Steroids	+
6.	Triterpenoids	+
7.	Tannins	+
8.	Resins	+
9.	Proteins	-
10.	Saponins	+

+ Present, - Absent

Isolation of Phytoconstituents

The ethanol soluble fraction (15 g) was mixed with Silica gel (2 g). The sample was then loaded onto a column packed with 200 g of silica gel (200-400 mesh, Molychem) prepared in petroleum ether (60-80^{0}C). The column was eluted with different solvents like petroleum ether (60-80^{O}C)100%, followed by petroleum ether: chloroform graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40, 50:50), chloroform 100%, followed by chloroform: ethyl acetate graded mixtures (95:5, 90:10, 80:20, 70:30, 60:40, 50:50), ethyl acetate 100% and finally ethyl acetate: methanol graded mixture (99:1,98:2, 97:3, 96:4, 95:5). The elutions were monitored by TLC (Silica gel G; visualization by UV at 254nm; 366 nm and Vanillin-Sulphuric acid spaying reagent heated at 110^{0}C). Each time 10 ml were collected and identical elutes (TLC monitored) were combined and concentrated to 5 ml and kept aside.[11]

Elutions carried out with Petroleum Ether: Chloroform (70:30) resulted in a single spot on TLC (Petroleum Ether: Chloroform, 70:30). After evaporation of the solvent a brown solid resulted, which was designated as Compound 1 (50 mg). Elutions carried out with Petroleum Ether: Chloroform (50:50) resulted in a single spot on TLC (Petroleum Ether: Chloroform, 50:50). After evaporation of the solvent bright yellow crystalline powder resulted, which was designated as Compound 2 (100 mg). Elutions carried out with Chloroform (100%) resulted in a single spot on TLC (Chloroform, 100%). After evaporation of the solvent yellowish white crystalline powder resulted, which was designated as Compound 3 (250 mg). Elutions carried out with Chloroform: Ethyl Acetate (95:5) resulted in a single spot on TLC (Chloroform: Ethyl Acetate, 95:5). After evaporation of the solvent white powder resulted, which was designated as Compound 4 (180 mg). Elutions carried out with Chloroform: Ethyl Acetate (80:20) resulted in a single spot on TLC (Chloroform: Ethyl Acetate, 80:20). After evaporation of the solvent pale yellow powder resulted, which was designated as Compound 5 (500 mg). Elutions carried out with Chloroform: Ethyl Acetate (70:30) resulted in a single spot on TLC (Chloroform: Ethyl Acetate, 70:30). After evaporation of the solvent white powder resulted, which was designated as Compound 6 (75 mg). Elutions carried out with Ethyl Acetate: Methanol (99:1) resulted in a single spot on TLC (Ethyl Acetate: Methanol, 99:1). After evaporation of the solvent pale yellow crystals resulted, which was designated as Compound 7 (175 mg). Elutions carried out with Ethyl Acetate: Methanol (96:4) resulted in a single spot on TLC (Ethyl Acetate: Methanol, 96:4). After evaporation of the solvent yellow oily liquid resulted, which was designated as Compound 8 (80 mg). Elutions carried out with Ethyl Acetate: Methanol (95:5) resulted in a single spot on TLC (Ethyl Acetate: Methanol, 95:5). After evaporation of the solvent brown solid resulted, which was designated as Compound 9 (200 mg). Elutions carried out with other graded mixtures resulted in brown resinous mass which were not processed further.[11]

RESULTS AND DISCUSSIONS

Phytochemical screening of the ethanolic extract of *T. grandis* led to the presence of phytoconstituents like alkaloids, glycosides, carbohydrates, flavonoids, steroids, triterpenoids, tannins, resins and saponins.

The chemical investigation led to the isolation of nine compounds from the ethanolic extract of stem bark of *T. grandis*. The isolated compounds are Eicosanyl Eicosanoate, β-Sitosterol, Ursolic acid, Lupeol, Betulinic acid, Betulin, Betulinaldehyde, Bis (2-ethylhexyl) phthalate and Tectol.

Compound 1 (Eicosanyl eicosanoate): m.p.: 68°C; IR (KBr): 2933.39 cm^{-1} (C-H str. in CH_3), 2865.54 cm^{-1} (C-H str. in CH_2), 1727.82 cm^{-1} (ester C=O), 1458.14 cm^{-1} (C-H deformation in CH_3), 1128.05 cm^{-1} (C-O str.). ^{1}HNMR ($CDCl_3$): δ 0.881 – δ 0.998 (m, 6H, 2 X CH_3), δ 1.256 – δ 1.698 (m, 70H, 35 X CH_2), δ 2.273 (t, 2H, C-21), δ 4.096 (t, 2H, C-19).The ESI-MS spectra showed a molecular ion peak at 592.6, corresponding to molecular formula $C_{40}H_{80}O_2$. Further fragment ion at m/z 313.1 and 279.3 suggested the acid and alcohol moiety of C-20 chain length.[14]

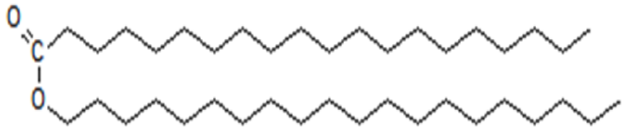

Figure 1: Eicosanyl Eicosanoate

Compound 2 (β-Sitosterol): m.p.: 142°C; IR (KBr): 3469.98 cm^{-1} (br, OH), 2940.74 cm^{-1} (C-H str in CH_3), 2868.80 cm^{-1} (C-H str. in CH_2), 1687.99 cm^{-1} (C = C str.), 1377.91 cm^{-1} (C-H deformation in geminal dimethyl), 1035.68 cm^{-1} (C-O str. of secondary alcohol). ^{1}HNMR (DMSO): δ 0.660 (s, 3H, C-18), δ 0.774 (s, 3H, C-26), δ 0.879 (s, 3H, C-29), δ 0.939 (s, 3H, C-21), δ 1.006 (s, 3H, C-27), δ 1.084 (s, 3H, C-19), δ 1.106 – δ 1.545 (m, 22H, 11 X CH_2), δ 1.553 – δ 2.135 (m, 7H, methine protons), δ 2.510 (m, 1H, OH), δ 2.962 (m, 1H, C-3), δ 4.697 (d, 1H, vinylic protons). In the LC-MS spectrum, characteristic fragment ions occurred at m/z 391.30, 368.25, 331.20 and 301.78. The last two ions were known to be diagnostic for sterols having Δ^5-unsaturation. Other important fragments were observed at m/z 273.10 and 255.31 indicating loss of [M-side chain] and [M^+side chain-H_2O]. It exhibited the molecular ion peak at m/z 414.25 $[M]^+$ corresponding to molecular formula $C_{29}H_{50}O$.[15, 16]

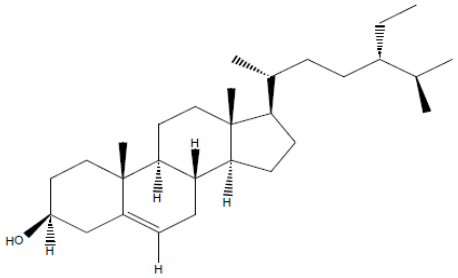

Figure 2: β-Sitosterol

Compound 3 (Ursolic acid): m.p.: 286°C; IR (KBr): 3468.31 cm^{-1} (br, OH), 2940.62 cm^{-1} (C-H str. in CH_3), 2868.93 cm^{-1} (C-H str. in CH_2), 1688.00 cm^{-1} (C = O str. of COOH), 1644.33 cm^{-1} (C = C str.), 1454.74 cm^{-1} (C-H deformation in CH_3), 1377.67 cm^{-1}(C-H deformation in geminal

dimethyl). ^{1}HNMR (DMSO): δ 0.684 – δ 1.049 (m, 21H, 7 X CH_3), δ 1.220 – δ 2.014 (m, 18H, 9 X CH_2), δ 2.036 – δ 2.792 (m, 4H, methine protons), δ 2.317 (d, 1H, C-18), δ 2.762 (m, 1H, OH), δ 3.289 (d, 1H, C-3), δ 4.500 (s, 1H, vinylic proton), δ 11.841 (s, 1H, COOH). In the LC-MS spectrum, the molecular fragment at m/z 456.25 [M^+] corresponds to molecular formula $C_{30}H_{48}O_3$. The spectrum also showed other peaks at m/z 248.1 and 203.1 due to Retro-Diels Alder fragmentation, typical for Δ^{12}oleanene and ursine triterpenes[17].

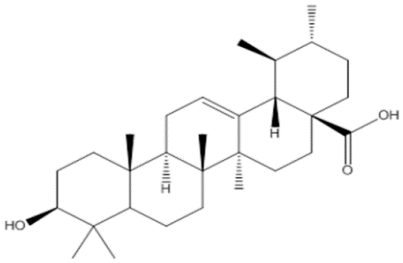

Figure 3: Ursolic acid

Compound 4 (Lupeol): m.p.: 213ºC; IR (KBr): 3469.20 cm^{-1} (br, OH), 2936.33 cm^{-1} (C-H str. in CH_3), 2866.84 cm^{-1} (C-H str. in CH_2), 1643.37 cm^{-1} (C = C str.), 1454.62 cm^{-1} (C-H deformation in CH_3), 1036.12 cm^{-1} (C-O str. in secondary alcohol), 749.46 cm^{-1} (str. in isopropyl alcohol). ^{1}HNMR (DMSO): δ 0.769 (s, 3H, C-24), δ 0.814 (s, 3H, C-28), δ 0.848 (s, 3H, C-25), δ 0.936 (s, 3H, C-23), δ 0.972 (s, 3H, C-27), δ 0.992 (s, 3H, C-26), δ 1.651 (s, 3H, C-30), δ 1.781 – δ 2.254 (m, 20H, 10 X CH_2), δ 2.248 (m, 1H, C-19), δ 2.964 (m, 1H, C-3), δ 2.926 – δ 2.995 (m, 4H, methine protons), δ 4.279 (d, 1H, OH), δ 4.692, δ 4.696 (d, 2H, C-29). In the LC-MS spectrum, the fragments occurring at m/z 385.4 [M^+-41], 218.02 [M^+-$C_{14}H_{24}O$] and 207.2 [M^+-$C_{16}H_{27}$] were characteristic to lupane series. The other fragment ion at m/z 411.10 [M^+-CH_3] is a characteristic feature of pentacyclictriterpene with an isopropyl group. It exhibited a molecular ion peak at m/z 426.30 [M]$^+$ corresponding to molecular formula $C_{30}H_{50}O$[18].

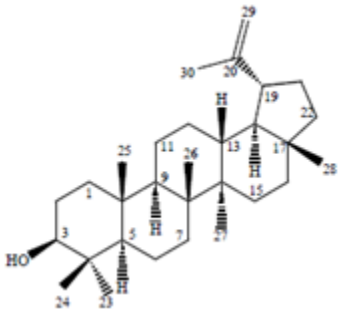

Figure 4: Lupeol

Compound 5 (Betulinic acid): m.p.: 297ºC; IR (KBr): 3469.56 cm^{-1} (br, OH), 2928.73 cm^{-1} (C-H str. in CH_3), 2861.48 cm^{-1} (C-H str. in CH_2), 1687.13 cm^{-1} (vibration of carboxylic acid),

1643.24 cm^{-1} (C = C vibration), 1455.79 cm^{-1} (symmetric vibration of CH_2 of cyclopentane), 1377.56 cm^{-1} (CH_3 symmetric and asymmetric str.), 1076.64 cm^{-1} and 1035.02 cm^{-1} (C-O str. of alcohol). ^{1}HNMR (DMSO): δ 0.704 (s, 3H, C-24), δ 0.895 (s, 3H, C-25), δ 0.924 (s, 3H, C-23), δ 0.983 (s, 3H, C-27), δ 1.018 (s, 3H, C-26), δ 1.698 (s, 3H, C-30), δ 1.845 - 2.278 (m, 20H, 10 X CH_2), δ 2.303 (d, 4H, methine protons), δ 2.558 (t, 1H, C-19), δ 3.022 (m, 1H, C-3), δ 4.076 (d, 1H, OH), δ 4.744 (d, 2H, C-29 a, b), δ 12.115 (s, 1H, COOH). The LC-MS spectrum displayed an $[M]^+$ peak at m/z 456.25 corresponding to molecular formula $C_{30}H_{48}O_3$, together with fragments at m/z 441.15 $[M^+-15]$ and 438.10 $[M^+-18]$[18, 19].

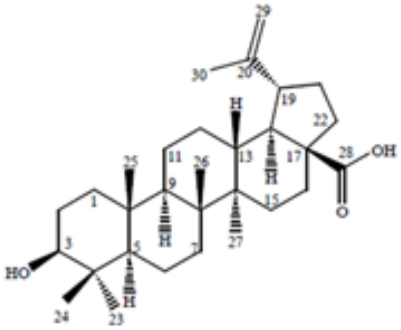

Figure 5: Betulinic acid

Compound 6 (Betulin): m.p.: 250°C; IR (KBr): 3424.73 cm^{-1} (br, OH), 2930.72 cm^{-1} (C-H str. in CH_3), 2863.73 cm^{-1} (C-H str. in CH_2), 1687.30 cm^{-1} and 1602.07 cm^{-1} (C=C str.), 1454.74 cm^{-1} (C-H deformation in CH_3), 1278.58 cm^{-1} (C-O str.), 883.20 cm^{-1} (olefinic C-H deformation). ^{1}HNMR ($CDCl_3$): δ 0.754 (s, 3H, C-24), δ 0.824 (s, 3H, C-25), δ 0.935 (s, 3H, C-23), δ 0.965 (s, 3H, C-26), δ 0.976 (s, 3H, C-27), δ 1.690 (s, 3H, C-30), δ 1.943 – δ 2.214 (m, 20H, 10 X CH_2), δ 2.249 – δ 2.281 (m, 4H, methine protons), δ 2.262 (m, 1H, C-19), δ 2.984 – δ 3.027 (m, 1H, C-3), δ 3.195 (d, 1H, C-28), δ 4.096 (d, 1H, C-28'), δ 4.221 (m, 2H, OH), δ 4.609 (t, 1H, C-29), δ 4.741 (d, 1H, C-29'). In the ESI-MS spectrum, the fragment ion at m/z 411.4 $[M^+-CH_2OH]$ is a characteristic feature of pentacyclictriterpene with an isopropyl group. It exhibited a molecular ion peak at m/z 442.4 $[M]^+$ corresponding to molecular formula $C_{30}H_{50}O_2$.[20, 21]

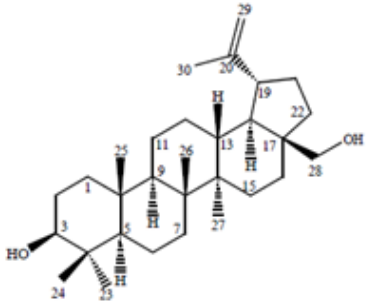

Figure 6: Betulin

Compound 7 (Betulinaldehyde): m.p.:194°C; IR (KBr): 3439.31 cm^{-1} (OH str.), 2937.09 cm^{-1} (C-H str. in CH_3), 2867.81 cm^{-1} (C-H str. in CH_2), 1727.67 cm^{-1} (C = O str.), 1640.15 cm^{-1} (C=C str.), 1455.22 cm^{-1} (C-H deformation in CH_3), 883.44 cm^{-1} (olefinic C-H deformation). ^{1}HNMR ($CDCl_3$): δ 0.754 (s, 3H, C-24), δ 0.824 (s, 3H, C-25), δ 0.937 (s, 3H, C-27), δ 0.965 (s, 3H, C-23), δ 0.975 (s, 3H, C-26), δ 1.690 (s, 3H, C-30), δ 1.943 – δ 2.230 (m, 20H, 10 X CH_2), δ 2.247 – δ 2.286 (m, 4H, methine protons), δ 2.974 – δ 3.018 (m, 1H, C-19), δ 3.027 (s, 1H, OH), δ 3.172 – δ 3.204 (m, 1H, C-3), δ 4.608 (m, 1H, C-29b), δ 4.741 (d, 1H, C-29a), δ 9.669 (s, 1H, C-28). The ESI-MS spectrum exhibited a $[M]^+$ peak at m/z 440.4 corresponding to molecular formula $C_{30}H_{48}O_2$, together with fragments at m/z 425.4 [M^+-15] and 422.4 [M^+-18], corresponding to a lupeol type triterpenoid[18, 19].

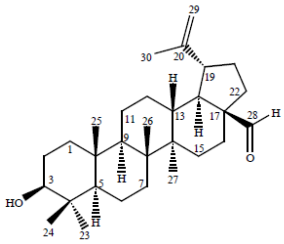

Figure 7: Betulinaldehyde

Compound 8 [Bis (2-ethylhexyl) phthalate]: b.p.: 229°C; IR (KBr): 2925.98 cm^{-1} (C-H str. in CH_3), 2858.58 cm^{-1} (C-H str. in CH_2), 1729.33 cm^{-1} (ester C=O group), 1458.26 cm^{-1} (methylene C-H bending), 1126.56 cm^{-1} (C-O str.), 742.58 cm^{-1} (C-H bending of aromatic ring). ^{1}HNMR ($CDCl_3$): δ 0.880 – δ 0.998 (m, 12H, C-8', C-8", C-10', C-10"), δ 1.130 –δ 1.321 (m, 12H, C-5', C-5", C-6', C-6", C-7', C-7"), δ 1.622 – δ 1.747 (m, 2H, C-4', C-4"), δ 2.016 (m, 4H, C-9', C-9"), δ 4.235 (m, 4H, C-3' C-3"), δ 7.527 (m, 2H, C-3, C-4), δ 7.715 (m, 2H, C-2, C-5). The molecular formula was established as $C_{24}H_{38}O_4$ based on ESI-MS which showed the presence of molecular ion $[M+H]^+$ at m/z 391.4. Other important peaks were detected at m/z 279.3 (protonated hydrolytic monoester, MEHP), 163.1 (phthalic acid + H) and 149.1 (phthalic acid – H_2O + H). The presence of a phthalate was inferred from the peaks at m/z 163.1 and m/z 149.1.[22, 23]

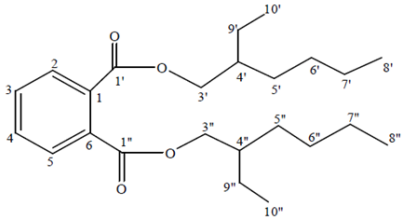

Figure 8: Bis (2-ethylhexyl) phthalate

Compound 9 (Tectol): m.p.: 207°C; IR (KBr): 3385.31 cm^{-1} (br, OH), 2925.53 cm^{-1} (C-H stretching in CH_3), 1687.30 cm^{-1} (C=C str.), 1456.94 cm^{-1} (C-H deformation in CH_3), 1383.56 cm^{-1} (C-H deformation in geminal dimethyl). ^{1}HNMR ($CDCl_3$): δ 1.447 (s, 6H,C-15, C-15'), δ 1.538 (s, 6H, C-14, C-14'), δ 5.010 (d, 2H, C-12, C-12'), δ 5.821 (m, 2H, C-11, C-11'), δ 7.376 (m, 2H, C- 6, C- 6'), δ 7.527 (m, 2H, C-7, C-7'), δ 7.637 (s, 2H, OH), δ 7.709 (m, 2H, C-5, C-5'), δ 8.044 (d, 2H, C-8, C-8'). The ESI-MS spectra showed a molecular ion peak at m/z 451.4 $[M+1]^+$ corresponding to molecular formula $C_{30}H_{26}O_4$. The spectrum also showed other diagnostic peaks at m/z 435.2 and m/z 211.5.[24]

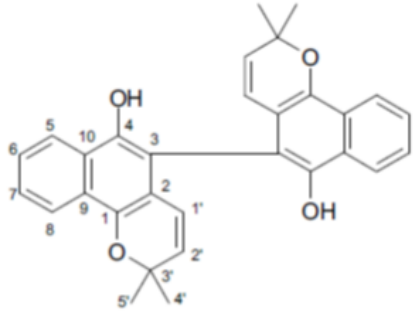

Figure 9: Tectol

CONCLUSION

The chemical investigation led to the isolation of nine compounds from the ethanolic extract of stem bark of *T. grandis*. The isolated compounds are Eicosanyl Eicosanoate, β-Sitosterol, Ursolic acid, Lupeol, Betulinic acid, Betulin, Betulinaldehyde, Bis (2-ethylhexyl) phthalate and Tectol. The constituents isolated and characterized from the stem bark of *T. grandis* can be categorised under fatty acid ester, steroid, triterpenoids, phthalate derivative and chromene flavonoid. Eicosanyl eicosanoate and Bis (2-ethylhexyl) phthalate have been isolated for the first time from the stem bark of *T. grandis*.

AKNOWLEDGEMENT

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