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Isolation of phenolic compounds from the methanolic extract of *Tectona grandis*

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ABSTRACT

Phenolic compounds like phenolic acids, flavonoids and tannins are important plant metabolites that are important for many pharmacological activities. We had earlier demonstrated the wound healing activity, analgesic and anti-inflammatory of the methanolic extract. The extract was investigated to determine the nature of the phytoconstituents responsible for these activities and this led to the isolation of gallic acid, ellagic acid, rutin and quercetin from the methanolic extract of the leaves of *Tectona grandis*. Phenolic compounds gallic acid, ellagic acid (phenolic acids), rutin and quercetin (flavonoids) were isolated and identified by their melting points, chemical tests, IR, NMR and mass spectra. The isolated compounds were qualified by comparison with the R_f values of that of the standards. Presence of these four phenolic compounds in the methanolic extract of the leaves of *Tectona grandis* may be an important contributing factor for these activities.

Key words: *Tectona grandis*, isolation, gallic acid, ellagic, rutin and quercetin.

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INTRODUCTION

The plant *Tectona grandis* is native to India and Myanmar and is found in the monsoon vegetation forest. It is commonly known as teak. It is a large deciduous tree with a height up to 35 meters, leaves simple, opposite, broadly elliptical or acute or acuminate, with minute glandular dots; the Flowers are white in color and small with a pleasant smell [1]. The various phytoconstituents reported are tectoquinone, 5-hydroxylapacol, tectol, betulinic aldehyde, betulinic acid, squalin, lapachol [2-5]. The survey has also revealed that the plant is used in the treatment of urinary discharge, bronchitis, common cold and headache, as a laxative, sedative, diuretic and in scabies [1]. We have earlier reported that the methanolic extract of the leaves showed a significant wound healing, analgesic and anti inflammatory activity [6, 7]. The extract was investigated to determine the nature of the phytoconstituents responsible for these activities. Literature survey has revealed that plant metabolites like phenolic compounds (simple phenols, phenolic acids, Flavonoids, tannins etc), and sterols play an important role in many of the activities like wound healing, analgesic, anti inflammatory and anti microbial activity [7,8]. This paper reports the isolation and identification of phenolic compounds gallic acid, ellagic acid, rutin and quercetin.

MATERIALS AND METHODS

Plant material

The frontal leaves of *Tectona grandis* were collected from the rural areas of Bangalore in the month of October 2006. The plant was identified and authenticated by the Regional Research Institute, Bangalore where the specimen voucher (RRCBI Acc no 12474) has been deposited for future reference. The material was shade dried, pulverized and preserved in air tight containers.

Preparation of the extracts

The methanolic extract of dried powder (1 kg) of the leaves was prepared by using Soxhlet apparatus. The extract was then concentrated and dried to give dark brown mass. The yield of the extract was 6.6 %. The extract was then subjected to preliminary phytochemical analysis [9] and majority of the constituents were found to be polar in nature.

Isolation and identification

The methanolic extract was subjected to column chromatography on silica gel using solvents of increasing polarities starting from petroleum ether, chloroform, ethyl acetate and methanol in different ratios to yield several sub fractions (160 fractions). Fractions 16-20 (50 % chloroform in petroleum ether) were mixed due to their similar TLC pattern this fraction was coded as TG1 (*Tectona grandis*). The solvent system Toluene: Ethyl acetate: formic acid: acetic acid in the ratio 1.4:2.2:0.1:1.1 showed an R_f value of 0.52 for the major spot which was compared to that of the standard gallic acid. This fraction TG 1 was eluted with chloroform and petroleum ether in different ratios to get fractions of 20ml. Fraction 6 showed a single spot. This fraction was collected dried and was recrystallized using methanol to get 0.48mg of TG1 which was identified as gallic acid. TG2 was isolated from fractions 94-99% (20% methanol in ethyl acetate). The solvent system ethyl acetate: butanol: formic acid in the ratio 2.5:1.5:0.5 showed an R_f value of 0.5 for the major spot which was comparable to standard rutin. The fractions TG 2 was eluted using the ethyl acetate and methanol in different ratios. Fractions of 20ml each were collected. The compound started to elute at 7.5% methanol in ethyl acetate. This was then allowed to stand overnight. Yellowish color crystals were obtained. This was further purified and recrystallized by dissolving the compound in hot water. The crystals were then filtered, collected and dried to yield 0.54 mg of TG2 identified as rutin. TG 3 was isolated from fractions 89-93 (10 % methanol in ethyl acetate) and subjected to TLC using the solvent system butanol: acetic acid: water in the ratio 5:3:5 which showed a single spot of the targeted compound at an R_f value of 0.8. The isolated TG 3 was identified by comparing the R_f value with that of standard quercetin. The column was then eluted using methanol in ethyl acetate in different ratios and fractions of 10 ml were

collected. Fraction 9 and 10 of the second column of TG3 showed single spot which was sensitive at UV 254. These fractions were collected mixed concentrated and left overnight to obtain 0.32 mg pure TG3 which was identified as quercetin. TG4 was isolated from fractions 94-99 (20 % methanol in ethyl acetate) of the main column. This was then subjected to TLC using the solvent system ethyl acetate: toluene; formic acid in the ratio 2.2:1.1:1.1. The TLC showed a sharp single spot which was sensitive at UV 254 and after spraying with ferric chloride reagent showed a greenish blue spot. The targeted spot showed at an R_f value of 0.6. A second column was prepared as described earlier and the fraction TG4 was eluted using different ratios of the solvents methanol and ethyl acetate i.e. 10%, 12%, 14%, 16%, and 20%. Fractions of 20ml were collected. The compound started eluting at 14% i.e. fraction 9 of the second column for TG 4. This fraction was collected, recrystallized and to get of TG4 which was identified as ellagic acid.

RESULTS AND DISCUSSION

Literature survey has revealed that plant metabolites like phenolic compounds (simple phenols, phenolic acids, Flavonoids, tannins etc), and sterols play an important role in many of the activities like wound healing, analgesic, anti inflammatory, anti oxidant and anti microbial activity. The structure and the presence of free hydroxyl groups in the various phenolic compounds make them an important class of compounds. The chemical activities of these phenolic compounds in terms of their reducing properties as electron or hydrogen donating is important for their anti oxidant activity [10, 11, 12]. The extract possesses significant wound healing, analgesic and anti inflammatory activity [6, 7] which could be due to the nature of the phytoconstituents present in the plants like phenolic compounds, tannins sterols etc. This resulted in the isolation of gallic acid, ellagic acid, rutin and quercetin.

The structures of the isolated compounds were established by chemical test, melting point, IR, NMR, Mass spectroscopy. All the four compounds gave a bluish green color with ferric chloride suggesting that they are phenolic compounds. TG 2 and TG 3 were positive for the magnesium ribbon test suggesting them to be flavonoids.

TG1 was obtained as a pale buff powder with a melting point of 250-253^o. Mass spectra of TG1 gave a base peak at 170 m/z (C₇H₆O₅). IR (Kbr cm⁻¹): 3367 cm⁻¹ (OH stretch), 3064 cm⁻¹ (C-H stretch), 2654-2907 cm⁻¹ (C-H aliphatic), 1702 cm⁻¹ (C=O), 1618 (OH), 1541 (C=C), 1246 (C-O), C-C (1026). ¹H NMR : (400MHZ, CDCl₃): 3.37-3.86 (dr, 4H, OH), δ 6.96 (s, 2H, Ar-H), δ 9.21 (s, 1H, COOH). ¹³C : (100MHZ, CDCl₃): δ 109.042, 120.75, 178.29, 145.65 (Ar -C), 167.84 (COOH).

TG 2 was obtained as yellow powder with a melting point of 210-213^o. Analysis by mass spectroscopy gave molecular mass at 610 m/z. The IR, NMR, melting point and the chemical test of TG2 can be correlated to the available literature data of the flavonoid rutin [14,15]. (Kbr cm⁻¹): 3423 cm⁻¹ (OH stretch) 2938 cm⁻¹, 2909 cm⁻¹ (C-H stretch), 1457 cm⁻¹ (C-H bend), 1656 cm⁻¹ (C=O), 1505 cm⁻¹ (C=C) ¹H NMR : (400MHZ, CDCl₃): 12.6 (s, 1H, CHO), 7.57 (s, 5H, ArH), 6.87 (d, 1H, Ar-H), 6.4 (s, 1H, ArH), 6.2 (s, 1H, Ar-H). ¹³C : (100MHZ, CDCl₃) : 144.8 (C2), 133.41 (C3), 177.48 (C4), 161.33 (C5), 98.53 (C6), 156.73 (C7), 93.69 (C8), 100.84 (C9), 156.55 (C10), 121.70 (C1¹), 116.70 (C2¹), 144.84 (C3¹), 148.5 (C4¹), 115.31 (C5¹), 116.36 (C6¹), 110.132 (C1¹¹), 74.18 (C2¹¹), 76.57 (C3¹¹), 68.33 (C4¹¹), 76.01 (C5¹¹), 67.09 (C6¹¹), 101.0 (C1¹¹¹), 70.52 (C2¹¹¹), 70.48 (C3¹¹¹), 70.67 (C5¹¹¹), 68.33 (C5¹¹¹), 17.72 C-CH₃. (C¹¹ and 3¹¹¹ – sugars).

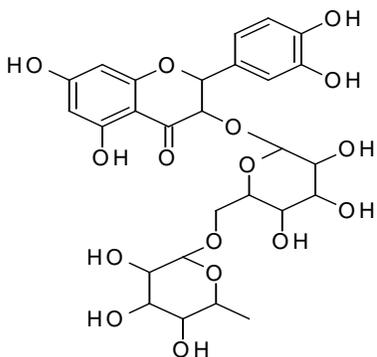
TG3 was obtained as a brownish powder and the melting point was found to be 317-319^o. Analysis by mass spectroscopy gave base molecular peak at 302 m/z. The IR, NMR, melting point and the chemical test of TG3 suggests that the isolated compound is flavonoid quercetin [14,15]. (IR (Kbr cm⁻¹): 3419 cm⁻¹ (OH stretch) cm⁻¹, 1666 cm⁻¹ (C=O), 1610 cm⁻¹ (OH), 1516 cm⁻¹ (C=C), 1240 (C-O), 1163 cm⁻¹ (C=C). ¹H NMR : (400MHZ, CDCl₃): δ 12.5, 10.9, 10.8, 9.6, 9.3 (s-5OH), 7.6 (d 1H-2¹), 7.4 (d 2H-5¹ and 6¹), 6.8 (d 1H, H8), 6.2 (d 1H, H6) ¹³C: (100MHZ, CDCl₃): 145.40 (C2), 136.07 (C3), 176.18 (C4), 164.22 (C5), 98.53 (C6), 161.07 (C7), 93.69 (C8), 156.49 (C9), 101.36 (C10), 121.95 (C1¹), 115.43 (C2¹), 145.40 (C3¹), 147.51 (C4¹), 115.43 (C5¹), 120.32 (C6¹).

TG4 was obtained as a brownish powder and the melting point was found to be 317⁰. Analysis by mass spectroscopy gave molecular mass at 301 m/z. The compound gave a bluish green color with ferric chloride suggesting the compound to be a phenolic compound. The IR, NMR and melting point is similar to the reported literature for ellagic acid. Therefore confirming that the isolated phenolic compound to be ellagic acid. IR (Kbr cm⁻¹): 3556 cm⁻¹ (OH stretch) cm⁻¹, 1699 cm⁻¹ (C=O), 1618 cm⁻¹ (OH), 1508 cm⁻¹ (C=C), 1192 (C-O). ¹H NMR :(400MHZ, CDCl₃):7.5(s, 2H, aromatic H), 2.7-4.4(4H, 4OH)¹³C: (100MHZ,CDCl₃): 136.30(C2),140.07(C3andC10) 148.03 (C4andC11),112.21(C5 and C12),110.07(C6 and C13),159.00(C7 and C14).

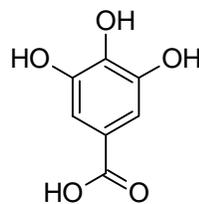
CONCLUSION

The current study resulted in isolation of four phenolic compounds (TG1, 3, 2 and 4) i.e. Gallic acid and ellagic acid (phenolic acids), rutin and quercetin (flavonoids) from the methanolic extract of *Tectona grandis*. The presence of these constituents may be one of the contributing factors responsible for the activities by virtue of their different properties like anti oxidant, anti inflammatory, analgesic and antimicrobial activities.

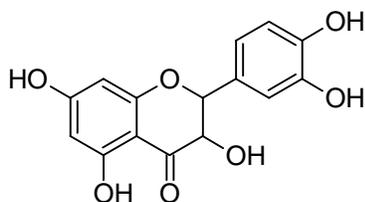
Structures of the isolated compounds TG1-TG4



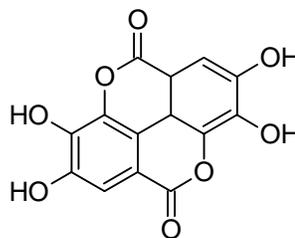
TG2-rutin



TG1-gallic acid



TG3-quercetin



TG4-ellagic acid



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