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# Antioxidant Activity of Oxochromen derivative Isolated From the Stem Bark of *T. populnea*

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## ABSTRACT

A novel antioxidant oxochromen derivative was isolated and characterized from *Thespesia populnea* stem bark (Linn.) with the evaluation of its *in vitro* antioxidant activity. The isolated compound was characterized by FTIR, $^{1}$H NMR, $^{13}$C NMR, and mass spectral technique. The spectral data revealed the structure [(2S,3R,4S,5S,6R)-2-(5,7-dihydroxy-2-methyl-4-oxochromen-6-yl)-4,5-dihydroxy-6-(hydroxyl methyl) oxan-3-yl] 3,4,5-trihydroxybenzoate. Antioxidant activity of the isolated compound was evaluated by DPPH scavenging activity, ABTS scavenging activity and Ferric reducing /antioxidant power assay. Results show that isolated compound from the acetone extract fraction of *T. populnea* stem bark exhibit better antioxidant activity at lower concentration in all the methods and at higher concentrations they act as prooxidants. In conclusion the present results suggested that the isolated compound could be a potential antioxidant agent for preparing functional foods and nutraceuticals applied in food and pharmaceutical industries.

**Keywords:** *Thespesia populnea*, Antioxidant, DPPH, ABTS, FRAP

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# INTRODUCTION

Rancidity and deterioration of food which affect the nutritional quality, colour, flavour, texture and safety of foods on storage occurs due to oxidation[1,2]. Oxidation of biological membranes, genotoxicity and tocopherol inhibition are associated with consumption of oxidised lipids[3]. These unfavourable effects of lipid oxidation can be overcome by adding chemical agents known as antioxidants. Various antioxidants are used to prevent the effects of excessive oxidations by encountering the reactive oxygen species. Butylated hydroxytoluene is frequently used synthetic antioxidant but more toxicity is associated with it[4]. Tertiary butylhydroquinone and butylated hydroxyanisole have been banned and removed from the safety usage in food[5]. Hence search for safer antioxidants have been initiated[6,7]. Many countries traditionally use plants to increase shelf life of food[8,9].

*Thespesia populnea* (L.) Soland. is a large tree belongs to the family of Malvaceae, found in coastal forests and tropical regions of India. It is commonly known as 'Indian tulip tree' or 'Portia tree'. In Ayurveda system of medicine, the fruits are used for the control of diabetes. Gossypol was found to be the major component of *T. populnea* produce anti-fertility effects in rats as well as in human beings[10,1112,13]. The fruits contain thespesin and β-sitosterol. The flower part contains gossypetin and kaempferol. Four naturally occurring quinones viz thespone, thespesone, mansonone-D, and mansonone-H have been extracted from heart wood of the plant[14]. The infusion of stem bark of *T. populnea* is used in the management of diarrhoea by traditional medicine practitioners in Tamil Nadu.

The aim of the present study was to evaluate the possible antioxidant activity of isolated compound from the acetone extract fraction of *T. populnea* stem bark.

# MATERIALS AND METHOD

**Collection and processing of plant part**

The stem bark of *T. populnea* (Linn.) was collected from Mysore and was authenticated by Dr.M.S. Sudarshana, Department of studies in Botany, University of Mysore, Mysore (Specimen Number BOT-0004).

**Extraction and isolation of the fraction**

The collected stem bark of *T. populnea* was shade dried, powdered and sieved in mesh 40. The powdered material was extracted with 5 L of 70% acetone at 60$^{o}$ C for 2 hrs. Extraction was repeated twice with 5 L of acetone. The acetone extracts were combined and evaporated under reduced pressure.

The concentrated acetone extract was portioned between n-hexane and methanol (1:1). The methanol part was evaporated under reduced pressure to obtain a semi solid extract. This extract was poured into cold diethyl ether to precipitate the crude mixture. This was repeated several times and the precipitate was collected by filtration. The filtered crude mixture (400g) was subjected to column chromatography (Silica gel, 60-120 mesh) and eluted with n-hexane (100%), n-hexane : ethyl acetate mixture (70:30), ethyl acetate : methanol mixture (60:40), methanol(100%).

All the eluted fractions were monitored by thin layer chromatography using pre coated aluminum plates. Three fractions were obtained. The major fraction (350 g) was taken for further study and other two fractions were not considered as they were negligible amount. The major fraction was subjected for repeated HPLC on a reverse phase C-18 semi preparative column using Acetonitrile: water (7:3) as mobile phase with flow rate of 10 ml/min. The eluted peak of fraction was collected and concentrated to dry mass. The isolated compound was recrystallized to get pure compound (1.5 g).

**Phytochemical investigation**

The isolated compound of *T.populnea* was subjected to preliminary qualitative investigations[15].

**Spectral analysis**

Nuclear magnetic resonance (1H NMR and 13C NMR) spectra were recorded on Bruker Avance 300 NMR spectrometer (300 MHz) at 300 K, in DMSO $d_6$ solution. Chemical shifts were reported as d (ppm) relative to tetramethylsilane (TMS) as internal standard. Infrared (IR) spectra were recorded as KBr pellet on Perkin Elmer FT-IR spectrometer. The wave number is given in $cm^{-1}$. Mass spectrometry (GC-MS) was recorded on Thermo GC-Trace Ultra Ver: 5.0

**DPPH scavenging activity**

DPPH scavenging activity were carried out according to the method of Banerjee et al 2005[16].Varying concentrations of AFTP 1, 2, 4, 8 and 10 µg/ml were added to 100 µg/ml of 0.1 mM DPPH solution. Equal volume of methanol and DPPH (100 µl) was used as control. Ascorbic acid treated as standard. The resulting solution was incubated for 20 min in dark and the absorbance was recorded at 490 nm using ELISA micro plate reader. The capacity to scavenge DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (1-As/Ac) \times 100$$

Where, As and Ac absorbance of the AFTP and control respectively. The experiment was performed in triplicates and the antioxidant activity was reported as 50% inhibitory concentration ($IC_{50}$) obtained from the graph of % radical scavenging against concentration of AFTP/ standard.

**ABTS scavenging activity**

50 µl of various concentrations of the AFTP 1, 2, 4, 8 and 10 µg/ml, 170 µl of phosphate buffer and 30 µl of ABTS solutions were added. An equal volume of methanol, phosphate buffer and ABTS solution as in the above step were used in control. The standard used was ascorbic acid. The resulting solution was incubated for 10 min in dark place.

The absorbance was recorded at 630 nm using ELISA micro plate reader (AK and Gulcin 2008)[17]. The capacity to scavenge ABTS cation was calculated using the following equation:

ABTS scavenging activity (%) = (1-As/Ac) x 100

Where, As and Ac absorbance of the AFTP and control respectively. The experiment was performed in triplicates and the antioxidant activity was reported as $IC_{50}$ obtained from the graph of % radical scavenging against concentration of AFTP/standard.

**Ferric reducing /antioxidant power (FRAP) assay**

300 µl FRAP working reagent was mixed with 490 µl double-distilled water and AFTP (1, 2, 4, 8 and 10 µg/ml ) were warmed to 37°C in a water-bath. The reagent blank reading was recorded at 593 nm using 10 µl of methanol and distilled water. The difference in absorbance between the AFTP and the blank reading was calculated and the data were expressed as mM ferric reduced to ferrous form / L (Benzie and strain 1996)[18].

% reduction of ferric ion = [(A test - A blank) / A blank] x 100

## RESULTS AND DISCUSSION

**Phytochemical investigation**

It was found that the isolated compound contains alkaloids, flavanoids, tannins and carbohydrates. It has been reported that phytoconstituents such as phenolics, flavonoids and essential oils are good source of antioxidants[19,20].

**Identification active compound**

M.F ($C_{23}H_{22}O_{13}$) Mol Wt 506.6 IR (υmax, $cm^{-1}$, KBr): 3364.60 (-OH str), 1711.70 (ester C=O str), 1614.80, 1533.11 (Ar C=C str), 1452.26 (C-H bend), 1332.04 (acyl C-O), 1204.80(Ar C-O), 1023.76 (Ar alkoxy C-O), 871.92, 751.18 (Ar-$sp^2$ C-H bend). $H^1$ NMR ($CD_3OD$, 300 MHz ) δ: 10.03-9.27 (6H, s,m, phenol -OH), 7.39-6.76 (8H, s, Ar-C-H), 6.16, 5.58 (2H, s, Ar-CH), 4.48 (3H, t,, 3.80, ali -CH),3.45 (OH, m, ali –OH),1.98 (3H, m, $CH_3$); $^{13}C$ NMR ($CD_3OD$, 300 MHz ) δ: 165.36 (Ester-COO), 145.97-138.26 (Ar-C-OH), 109.56-108.99 (C-C), 40.56-38.90 (pyran -CH), 21.08, 14.21 ($sp^3$ -C) ppm. FAB-MS (*m/z*): 506.7 (M+) ($C_{23}H_{22}O_{13}$),491.2 ($C_{23}H_{23}O_{12}$), 427.9 ($C_{21}H_{19}O_{10}$), 383.08 ($C_{17}H_{19}O_{10}$), 362.7 ($C_{17}H_{19}O_9$), 334.4 ($C_{16}H_{17}O_8$), 293.06($C_{14}H_{13}O_7$), 261.3 ($C_{14}H_{13}O_5$),194.1 ($C_{10}H_{10}O_4$), 135.1($C_9H_{10}O$), 94.2 ($C_6H_6O$)

Based on the above structural data, the tentative structure of the [(2S,3R,4S,5S,6R)-2-(5,7-dihydroxy-2-methyl-4-oxochromen-6-yl)-4, 5-dihydroxy-6-(hydroxyl methyl) oxan-3-yl] 3,4,5-trihydroxybenzoate as shown in Figure 1.

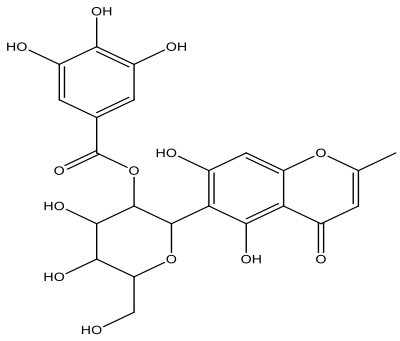


**Figure 1: Structure of the [(2S,3R,4S,5S,6R)-2-(5,7-dihydroxy-2-methyl-4-oxochromen-6-yl)-4, 5-dihydroxy-6-(hydroxyl methyl) oxan-3-yl] 3,4,5-trihydroxybenzoate.**

**DPPH scavenging activity**

1, 1-diphenyl-2-picryl hydrazyl radical (DPPH), is a stable free radical having a purple colour**.** Antioxidants, interacts with DPPH, either by transferring electrons or hydrogen atoms to and converts DPPH into a stable diamagnetic molecule, and thus neutralizing free radical character. In the radical form, this molecule has an absorbance at 517 nm which decreases after the acceptance of an electron or hydrogen radical from an antioxidant compound, and the colour of the reaction mixture changes from purple to yellow (Banerjee et al 2005)[18].

The %DPPH radical scavenging activity of AFTP was presented in Table 1. AFTP exhibited a maximum DPPH scavenging activity of 88.24±1.53 at 10µg/ml whereas for standard Ascorbic acid (AscA) was found to be 94.85±1.13 at 10µg/ml by inhibiting hydrogen atom transfers process.

**Table 1: Free radical scavenging activity of AFTP by DPPH method**

| **Concentration (µg/ml)** | **Percentage radical scavenging Activity** | |
|---|---|---|
| | **AFTP** | **AscA** |
| 1 | 34.53±9.13 | 35.26±1.66 |
| 2 | 81.59±1.82 | 35.65±1.80 |
| 4 | 89.71±0.36 | 92.64±0.99 |
| 8 | 90.42±0.88 | 93.72±0.88 |
| 10 | 88.25±1.53 | 94.85±1.13 |
| $IC_{50}$ | 1.15± 0.24 | 1.64±0.045 |

**ABTS scavenging activity**

This method is based on a decolouration reaction. The reaction is initiated by adding ABTS (2, 2-azino bis (3-ethyl benzthiazoline-6-sulphonic acid) and potassium per sulphate to produce ABTS radical. Incubation of ABTS with potassium per sulphate results in the production of radical cation

$ABTS^{\bullet+}$. This species is blue-green in colour and can be detected at 734 nm. Antioxidants or radical scavenger in the sample cause suppression of this colour production to a degree that is proportional to their concentration. The generation of ABTS radical is allowed to proceed until a stable colour of ABTS radical obtained. Antioxidants which have scavenging activity, therefore, decolourize a mixture of ABTS radical, thus giving an index of their antioxidant capacity (Ak and Gulcin 2008)[19]. $ABTS^{\bullet+}$ are more reactive than DPPH radicals and unlike the reactions with $ABTS^{\bullet+}$ radicals involve an electron-transfer process.

$ABTS^{2-}$ radicals are more reactive than DPPH radical (generated by lipid auto oxidation) and involve electron transfer process. AFTP was showing scavenging activity in a dose dependent manner up to 8 µg/ml and then start to show pro oxidant activity with increase in concentration (Table 2).

**Table 2: Free radical scavenging activity of AFTP by ABTS method**

| Concentration (µg/ml) | Percentage radical scavenging Activity | |
|---|---|---|
| | AFTP | AscA |
| 1 | -8.59± 3.72 | 2.41± 4.29 |
| 2 | 10.94± 2.31 | 6.78± 1.81 |
| 4 | 55.11± 2.09 | 15.03±4.21 |
| 8 | 65.34± 2.73 | 23.27±8.19 |
| 10 | 57.12 ±2.73 | 44.36±11.4 |
| $IC_{50}$ | 6.96±1.34 | 12.69±2.13 |

**Ferric reducing/antioxidant (FRAP) power Assay**

At low pH, when a ferric-tripyridyltriazine ($Fe^{III}$-TPTZ) complex is reduced to the ferrous ($Fe^{II}$) form which gives an intense blue colour has an absorption maximum at 593 nm (Benzie and Strain 1996)[20].

FRAP assay is more sensitive and widely used antioxidant assay. Table 3 presents the antioxidant activity of AFTP. Extract is having ability to reduce $Fe^{3+}$ to $Fe^{2+}$ at concentration of 3.42 µg/ml.

**Table 3: Free radical scavenging activity of AFTP by FRAP method**

| Concentration (µg/ml) | Percentage radical scavenging Activity | |
|---|---|---|
| | AFTP | AscA |
| 1 | -9.93±4.84 | 16.75±5.76 |
| 2 | 54.44±4.93 | 21.10±6.51 |
| 4 | 112.23±12.56 | 52.90±12.11 |
| 8 | 138.22±10.57 | 131.24±15.04 |
| 10 | - | - |
| $IC_{50}$ | 3.42±0.34 | 2.50± 0.02 |

## CONCLUSION

The *in vitro* antioxidant of MFATP has been performed by different methods such as DPPH assay, ABTS and FRAP assay. Results show that these extract exhibit better antioxidant activity at lower concentration in all the methods and at higher concentrations they act as prooxidants.

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