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Isolation of an elastase inhibitor, (+)-gossypol from the bark of *Thespesia populnea* plant

T. Annamalai, G. Venkateswara Rao* and T. Mukhopadhyay

M/s. Cavin Kare Research Centre, 12, Poonamalle Road, Ekkattuthngal, Chennai, Tamilnadu, India

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ABSTRACT

*Bioassay guided purification of methanolic extract of the bark of the plant, Thespesia populnea yielded a naphthalene derivative, (+)-gossypol (**1**). The structure of compound, (+)-gossypol has been established based on its physical and spectral data (UV, IR, ^{1}H & ^{13}C NMR and Mass). The compound has been shown moderate elastase inhibition activity when compared with control compound, ursolic acid.*

Key words: *Thespesia populnea*, bark, (+)-gossypol, elastase inhibition

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INTRODUCTION

The plant, *Thespesia populnea* belongs to Malvacea family and distributed in the tropics of the world. It commonly known as false rose wood or Indian Tulip tree. The tree is largely being cultivated for ornament and shade, and it blooms throughout the year in the tropics. Various parts of the tree, bark, leaves, flowers and fruits are reported to be useful in cutaneous affections, such as scabies, psoriasis, ringwarm, guineaworm and eczema. The decoction made from the bark is given internally in the diseases of skin and fruits are being used as antidote for poisoning. The bark, roots and fruits are stated to be used in dysentery, cholera and haemorrhoids. The young buds, leaves and flowers are eaten either raw, cooked or fried in butter. The extracts of the leaves are active against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*.[1] The ethanolic extract of the fruits were reported activity against Ranikhet disease virus and also anticancer against Lewis lung-carcinoma in the mice.[2] The chemical examination of the different parts of the tree yielded variety of the compounds, like steroids and its glycosides,[2] terpenoids, [3] flavonoids and their glycosides,[4] triglycerides,[5] fatty acids,[6] and sesquiterpene derivatives.[7]

Based on our interest and continuous search on bioactive secondary metabolites from medicinal plants for personal care / cosmetic applications,[8-17] we have under taken the bark of *Thespesia populnea*, for chemical examination. In this article, we wish to report the isolation and structure elucidation of biologically active compound, (+)-gossypol from the bark. The structure of the compound has been established based physical and spectral data and comparison with literature data. The present paper describes the isolation and structure elucidation of (+)-gossypol and its elastase inhibition studies.

MATERIALS AND METHODS

General procedures

Melting point was reported uncorrected. IR spectrum was recorded on a Shimadzu Prestige 21 FT IR. Optical rotation was measured on Autopol IV, serial no. 80305. UV spectrum was recorded on Shimadzu UV spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX 400 with TMS as an internal standard. Mass spectrum was recorded on Jeol SX 102/DA 600 mass spectrometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh). Purity of the samples was checked by TLC on pre-coated aluminum sheets, silica gel 60 F_{254} (20 X 20 cm, 0.2mm thickness, Merck) and compounds were detected under UV light (254 & 366 nm) and spraying with 5% sulphuric acid in methanol followed by heating the plates at 110^{o}C for 5 min. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz.

Plant material

The dried bark of *Thespesia populnea* Soland. Ex. Correa (203 g) was collected from Chengalpattu, Kanchepuram district, Tamil Nadu in August 2007 and identified by Dr. P. Santhan, Toxanamist, M/s. Durva Herbal Centre, Chennai, Tamil Nadu, India. A voucher specimen of the species was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation procedure

The dried bark of *T. populnea* Soland. Ex. Correa (203 g) was coarsely powdered, subjected for an extraction with methanol (6.0 L) by using soxhlet apparatus. The solvent was distilled off by rotary evaporator under reduced pressure at ~40^{o}C to get 21.08 g crude methanolic extract. The methanolic extract was showed good elastase inhibition activity (78.7%@48.48 µg/ml) and it was suspended in methanol : water (1:4) and fractionated with chloroform, ethyl acetate and n-butanol to get corresponding fractions, 7.43g, 2.84g and 2.93g respectively. All three fractions were submitted for biological activity. The chloroform and ethyl acetate fractions were shown more or less same activity and their TLC was checked and found to be similar. The n-butanol fraction has shown very poor activity when compared with other two fractions.

Again fresh lot of dried bark (500g) was extracted directly with ethyl acetate through soxhlet apparatus to get 28.06g. The extract has been submitted for elastase inhibition activity and showed 87.9%@ 46.0µg/ml. The extract was adsorbed on the silica gel and performed vacuum liquid chromatography (VLC) by using each two 2L of mixture of hexane: chloro-form (1:2), chloroform and mixture of chloroform : MeOH (85:15) to get corresponding fractions, 20.58g (Fr.1), 6.28 g (Fr.2) and 1.22g (Fr.3) respectively. All fractions were submitted for biological activity along with original crude extract and Fr.1 found to be more potent than other fractions. The fraction 1 was further purified by another column of silica gel and eluted with mixture of hexane: ethyl acetate (9:1, 8:2, 7:3 and 1:1) to get four sub-fractions, 2.97g (TP01A), 1.54 g (TP01B), 2.1g (TP01C) and 0.76 g (TP01D). Again all these fractions were submitted for biological studies and fraction TP01B was found to be potent. This fraction showed solid nature and was crystallized with chloroform and methanol to get pale yellow colored crystals, 1.12g (**1**). The compound has been submitted for elastase inhibition activity and showed moderate inhibition (53%@48 µg/ml).

Compound 1 (**(+)-Gossypol**): Pale yellow color crystals, mp:186-188^{o}C, $[\alpha]_D^{26}$ +0.177(c 0.05, $CHCl_3$); UV ($CHCl_3$) nm: 238, 290, 379; IR (nujol) cm^{-1}: 2929, 1658, 1454, 1058, 889; ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.55 (12H, s), 2.15 (6H, s), 3.90 (2H, t, J=6.3Hz), 6.04 (2H, s), 6.39 (2H, d, J=9.0Hz), 7.77 (2H, s), 11.1 (2H, s), 15.03 (2H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.2, 20.3, 27.9, 111.8, 114.7, 115.9, 118.1, 129.7, 133.6, 134.1, 143.4, 150.5, 156.0, 199.3, EIMS (m/z) : 518 (M^{+}, 0.4%), 500 (8%), 482 (100%), 467 (65%), 439 (17%).

RESULTS AND DISCUSSION

The compound **1** was isolated as pale yellow colored crystals from chloroform methanol. Its molecular formula was fixed as $C_{30}H_{30}O_8$ based on its mass spectrum. Its UV spectrum showed three bands at 238, 290 and 379nm indicating conjugation in the molecule. The IR spectrum showed the presence of hydroxyl group at 3500cm^{-1}, an aldehyde group at 1730cm^{-1} and an aromatic group at 1620cm^{-1}. The ^{1}H NMR spectrum clearly showed the presence of an isopropyl group ie., two methyls at δ1.55 as singlet and methine proton at δ3.90 as triplet (J=7.0Hz), an aromatic methyl group at δ 2.14 as singlet, one aromatic proton at δ 7.77 (s) and an aldehyde proton at δ 11.11 as singlet. Further, the spectrum also showed three D_20 exchangeable protons at δ 6.04, 6.39 and 15.03 each as singlet and belongs to phenolic hydroxyl groups. The carbon spectrum showed a total of fourteen carbons. Out of 14

carbons, three belongs to aliphatic (δ 20.2, 20.3, 27.9), ten aromatic carbons at δ111.8, 114.7, 115.9, 118.1, 129.7, 133.6, 134.1, 143.4, 150.5, 156.0 and an aldehydic carbon at δ199.3. Its mass spectrum showed molecular ion peak at 518. By revealing the literature search, the compound spectral data is well corroborated with literature compound and it has been identified as (+)-gossypol which was reported from the different parts of the same plant [7, 18]. Total synthesis has been reported by using Ullmann coupling method.[19] The compound has been reported for anti-fertility[20] and cytotoxic activities.[21]

Elastase inhibition activity: The elastase inhibition activity of crude extract, different fractions, isolated compound and ursolic acid (control) were studies in cell free system. The assay method is most reliable and reported in the literature.[22] Fresh solution of 300 µl (0.6 mg) of succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide (the enzyme substrate), 1200 µl of buffer and varying amounts of the elastase inhibitor under testing are incubated at 37^{o}C for 20 minutes. The hydrolysis is measured by the spectrophotometric measurement of the release of p-nitroaniline at a wavelength of 410 nm. In this method, the methanolic extract, EA fraction and isolated compound were tested and the results documented in table.1.

Table1: Elastase inhibition study results

Extract/Fraction/Compound	Inhibition (µg/ml)
Ursolic acid	IC_{50}= 13.1
Methanolic extract	78.7%@ 48.8
$CHCl_3$ fraction	79.6%@ 43.2
Ethyl acetate fraction	87.9%@ 46.0
n-Butanol fraction	43.2% @ 43.2
Ethyl acetate extract	87.9% @ 46.0
Fr.1	92.5% @ 39.4
Compound **1**	53.0% @48.0 or IC_{50}= 42.5

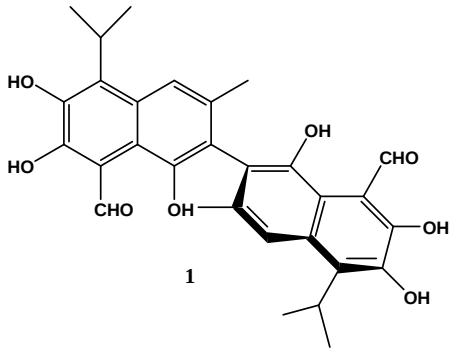

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Figure 1: Compound from *Thespesia populnea*

CONCLUSION

The present bioassay guided isolation of the bark of the plant, *Thespesia populnea* yielded only one active compound, (+)-gossypol (**1**). Its structure has been confirmed based on physical and chemical data. The crude extracts, solvent fractions and active fraction have showed superior elastase inhibition activity, where as the compound showed moderate inhibition activity. This is the first report on elastase inhibition.

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