

# Invasive *Acacia nilotica* A Problematic Weed is a Source of Potent Methyl Gallate

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**Abstract:** *Acacia nilotica* the invasive weed is a rich source of highly potent Methyl gallate an alkyl ester of gallic acid the alleochemical of most bark of the plants. Methyl gallate was isolated and purified through an open column chromatography. Analytical thin layer chromatography (TLC) was used to monitor the separation of the compound. The structural details of the isolated expected compound methyl gallate was determined by spectral analysis FTIR, UV, NMR and ES-MS.

**Keywords:** *Acacia nilotica*, methyl gallate, Isolation and spectral analysis.

## 1. Introduction

Prickly acacia a serious weed of national significance, widely, freely and extensively available in the drier regions of India and is indigenous to plains of Maharashtra and Andhra Pradesh, Egypt, South Africa, Asia that is Arabia and eastwards, Burma, Srilanka and widely grown in Australia. Though, known for its ecological impact contributes extensively to its pharmacological attributes, as the plant is the biosynthetic laboratory yielding therapeutic important phytoconstituents that are used in polyherbal formulations and is found to be effective against multitude of health problems due to the presence of principal phytoconstituents including gallic acid, m-digallic acid, catechin, chlorogenic acid, gallolylated flavan-3,4 diol, rabadandiol, betulin, octacosanol,  $\beta$ -sitosterol all contributing to the phenolic nature of the compounds.

Tremendous pharmaceutical properties opens the pathway for a scientist to explore into highly potent compounds and to suggest a source for extracting the metabolites, like, *Acacia nilotica*, (1;2) a widely grown problem weed in many countries which will be highly economic and will account for the useful consumption of these problematic weed.

One such compound of therapeutic importance is methyl gallate, a hydrolysable tannin known for antitumor activity (3) and antibacterial activity (4). The compound that can be panacea for the sufferers which can be extracted from a weed on a commercial scale for nutraceuticals and pharmaceutical purpose. The ever increasing cost of prescription it can provide an active ingredient to curtail the cost of medicine, known for increasing the bioavailability of orally administered pharmaceutical compounds can therefore, of great significance.

For this purpose bark of *Acacia nilotica* was exploited rich in phenolics, glycoside, flavanoids, tannins, alkaloids (5 and 6). Bark ecology suggest that bark of *Acacia nilotica* to be of magnificent medicinal potential being rich in active ingredients contributes to its invasive nature.

## 2. Method and Material

### Collection of plant material:

The plant material that is the powdered bark of *Acacia nilotica* was collected from Shree Baidyanath Ayurved Bhawan Private Limited, Allahabad .Uttar Pradesh, India.

### Extract Preparation

The 1 kg air dried powdered bark of *Acacia nilotica* , was extracted in soxhlet extractor (continuous extraction) the methanolic extract was prepared by successive extraction. The extraction was monitored continuously and at each stage, the completion of extraction was confirmed by colour . The solvent taken from the thimble and evaporated to check the absence of residue. The extract was then filtered through Whatman no.1 filter paper and concentrated by distillation. The concentrated extract (solid content about 50%) was taken in procelein dishes and evaporated in a boiling water bath to evaporate the solvent.

Methanolic extract was concentrated under reduced pressure to obtain a dark viscous mass(150 g).It was adsorbed on silica gel(60-120mesh) to form a slurry, air dried and chromatographed over silica gel( 250g) column ( 100cm) packed in n-hexane and eluted with n Hexane-Chloroform gradient system from 100:0 to 0:100 followed by chloroform-methanol gradient system. Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having similar  $R_f$  values were combined and crystallized. The isolated compounds were recrystallized to get the pure compounds .The melting point of the recrystallized compounds were taken using melting point apparatus and the tannins were determined by thin layer chromatography ,according to Sharma *et.al* (1998) .The following solvent system was used:

- i) Chloroform-methanol-acetic acid (90:10:1)
- ii) Petroleum ether(60-80°C) – ethyl acid and formic acid (40:60:1)
- iii) Chloroform-ethyl acetate-acetic acid (50:50:1)

- iv) Toluene – acetonitrile- formic acid (70:30:1)
- v) Petroleum ether(60-80°C) – methanol-acetic acid (90:10:1)

The chromatograms were developed at room temperature (about 20°C), air dried and the spots were detected as follows:

- i. Ferric chloride reagent was prepared by dissolving 1g anhydrous ferric chloride in 100ml. Blue spots appeared confirmed the presence of gallic acid and its esters.
- ii. Vanillin-sulphuric acid reagent was prepared by dissolving 0.5g vanillin in 100ml sulphuric acid (40:10). The plates were observed after spraying. Methyl gallate was confirmed with similar Rf as the markers.

Further, Gallic acid ester-methyl gallate was confirmed by spectral analysis, recrystallized samples were sent to SAIF, Panjab University for UV, FTIR, NMR  $^1\text{H}$  and  $^{13}\text{C}$  and Mass Spectrometry.

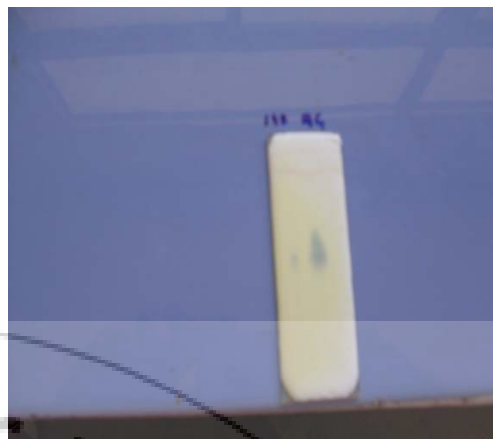
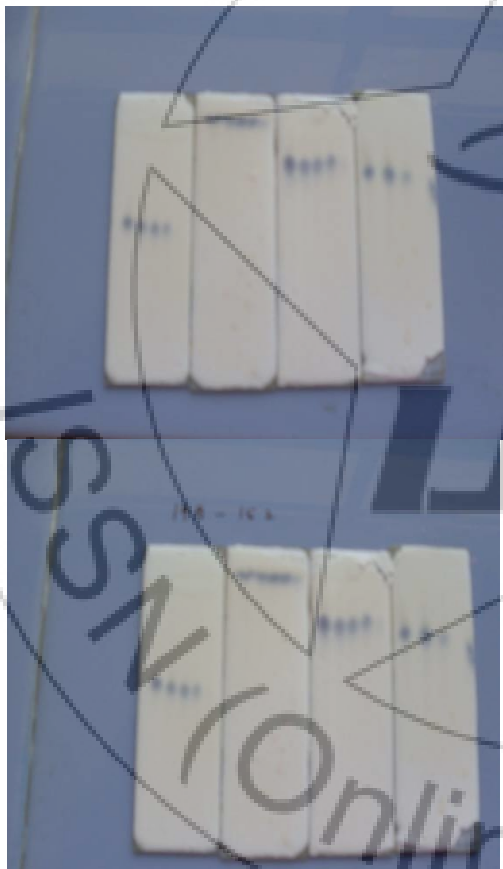
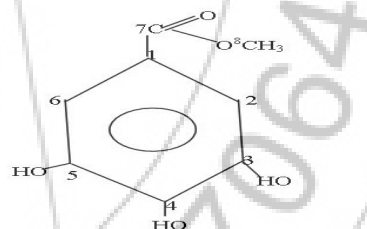


Figure 1: The chromatograms of the elute having similar spots as the marker methyl gallate

### 3. Result and Discussion

The isolated methyl gallate of the methanolic extract of bark of *Acacia nilotica* was obtained at 95:5 (chloroform:methanol gradient) was recrystallized in chloroform buff white crystals (1.6g) were obtained with melting point 188-189° C and about ten microliters of the fraction was spotted on the silica gel precoated TLC plates and developed in chloroform-methanol-acetic acid (90:10:1), Toluene-acetonitrile-formic acid (70:30:1) and petroleum ether-methanol-acetic acid (90:10:1) according to 7. The chromatograms (fig.1) were developed at room temperature in ferric chloride reagent, and blue spots obtained for this fraction of tannins was similar to the marker – methyl gallate.

#### Spectral Data



#### UV-vis

$\text{C}_6\text{H}_6$   $\lambda_{\text{max}}$  nm 202-284,

IR (KBR)  $\nu_{\text{max}}$  in  $\bar{\nu}$   $\text{cm}^{-1}$

3410 (OH str)  $\text{cm}^{-1}$

2955 (C-H Str)  $\text{cm}^{-1}$

1676 (C=O)  $\text{cm}^{-1}$

1613 (C=C benzene ring)  $\text{cm}^{-1}$

$^1\text{H}$  NMR (DMSO 400 MHz) in  $\delta$

3.7318 (3H, s,  $\text{OCH}_3$ ), 6.935 (2H, s, H-2, H-6) and 8.9 (brs Ph-OH)

$^{13}\text{C}$  NMR (DMSO 400MHz) in  $\delta$

51.26 ( $\text{OCH}_3$ ), 108.62 (C-2, C-6), 119.49 (C-1), 138.09 (C-4), 145.6 (C-3, C-5) and 166.31 ( $\text{CO}_2\text{CH}_3$ )

MS: m/z (rel int.), 185(50), 153.0(100), 125(25) and 107(10).

The compound methyl gallate was isolated as buff needle like crystals. The molecular formula was determined to be  $\text{C}_8\text{H}_8\text{O}_5$  from the MS and NMR data. The FTIR spectrum

confirmed a carbon-carbon double bond ( $\bar{\nu}$  1613  $\text{cm}^{-1}$ ), and C-H stretching ( $\bar{\nu}$  2955.00  $\text{cm}^{-1}$ ) indicating the aromatic nature of the compound, ( $\bar{\nu}$  1676  $\text{cm}^{-1}$ ) absorption was because of the C=O stretching and revealed the presence of OH ( $\bar{\nu}$  3410.00  $\text{cm}^{-1}$  broad). Close examination of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum showed a symmetrical molecule with two aromatic protons,  $\delta$  6.935 (2H, s, H-2, H-6) which were symmetrical to each other. Therefore, both protons were giving the same signal at  $\delta\text{H}$  6.935, which was further confirmed by two symmetrical aromatic carbon signals at  $\delta\text{C}$  108.62 (C-2, C-6). In the higher field region, there is another signal from aliphatic methoxy protons at  $\delta$  H 3.7318 (3H, s,  $\text{OCH}_3$ ) and based on the integration of the signal, the presence of these protons was in agreement with the number of protons in the methoxy substituent supported by a ester carbonyl resonance at  $\delta$  C 51.26. The  $^{13}\text{C}$  NMR spectrum of the isolated compound also exhibited resonances for C=O signal comprising  $\delta\text{C}$  at 166.31 indicating the presence of carbonyl ester, three hydroxyl  $\delta\text{C}$  145.6 (C-3, C-5) that were symmetrical to each other and  $\delta\text{C}$  138.09 (C-4), one quaternary aromatic carbon signals at  $\delta\text{C}$  119.49 (C-1). Therefore, in all 8 Carbons and 5 protons attached to carbon were observed in the  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra and eight signals appeared in the  $^{13}\text{C}$  NMR spectrum (C x 5, CH x 2,  $\text{CH}_3$  x 1) which were inconsistent with NMR data provided by **8** and **9** in one of their literature.

The structure of methyl gallate revealed the presence of methyl ester of 3,4,5-trihydroxybenzoate a six-membered ring and the side-chain was determined from the MS. The fragment ions were observed at  $m/z$  153 due to the loss -  $\text{OCH}_3$  from  $m/z$  185 [ $\text{M}^+$ ] and  $m/z$  125 due to the loss of - C=O from ion ( $m/z$  153), the presence of ester group ( $\text{COOCH}_3$ ) was disclosed. Besides that the fragment ions were observed at  $m/z$  107 due to the loss  $\text{H}_2\text{O}$ . This reveals the presence of three hydroxyls on the benzene ring. Therefore, the compound was confirmed as methyl gallate.

This structure of methyl gallate makes it very versatile, that is, the molecule of an alkyl gallate consists of three sections, a lipophilic alkyl chain at one end is connected via an ester linkage to the galloyl group bearing the polar hydroxyl groups at the other end and this amphiphilic property makes the cell membrane the target sites for the alkyl gallates **10**. The amphiphilic nature of methyl gallate imparts therapeutic features that makes it pharmacologically and nutraceutically potent substance. The ester linkage present in it as explained by **11** makes it medicinally important along with the hydroxyl group. The study of this compound gives the leads to further explore the alkyl gallate which are considered as important ingredients in plants as they possess the alkyl chains which might play important role in enhancing the biological activities and therefore can be used in biological activity tests. The in depth study is required to explore into the efficacy of these natural products.

## References

[1] Wassel, G.M.; Abd-El-Wahab, S.M.; Aboutabl, E.A.; Ammar, N.M.; Afifi, M.S. (1990). Study of phenolic constituents and tannins isolated from *Acacia nilotica*

(L.) Willd and *Acacia farnesiana* (L.) Willd growing in Egypt. *Herba Hung* 29 : 43-49

- [2] Khalid, S.; Yagi, S.; Khristova, H. and Duddeck, H. (1989). (+)-Catechin-5-galloyl Ester as a Novel Natural Polyphenol from the Bark of *Acacia nilotica* of Sudanese Origin. *Planta Medica*, 55(6): 556-558.
- [3] Lee, T. H.; Chiou, J. L.; Lee, C. K. and Kou, Y. H. (2005). Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. roxburghiana. *Journal of the Chinese Chemical Society*. 52:833-841
- [4] Choi, J.G.; Kang, O.H. and Lee, Y.S. (2009). Antibacterial activity of methyl gallate isolated from *Galla Rhois* or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. *Molecules* 14(5) :1773-80
- [5] Banso, A. (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. *Journal of Medicinal Plants Research*. 3(2):82-85
- [6] Siddiqui, M.B. and Husain, W. (2009). Traditional treatment of diarrhea and dysentery through herbal drug in rural India. *Fitoterapia* 62:325-529.
- [7] Sharma, O.P.; Bhat, T.K. and Singh, B. (1998). Thin-layer chromatography of gallic acid, methyl gallate, pyrogallol, phloroglucinol, catechol, resorcinol, hydroquinone, catechin, epicatechin, cinnamic acid, p-coumaric acid, ferulic acid and tannic acid. *Journal of Chromatography A* 822 : 167-171
- [8] Haddock E.A.; Gupta R.K.; Al-Shafi S.M.K.; Haslam E. and Magnolato D. (1982). The metabolism of gallic acid and hexahydroxydiphenic acid in plants 1. Introduction -naturally- occurring galloyl esters. *Journal of Chemical Society Perkin Trans*, 1:2515-2524.5.
- [9] Lee, H.; Lee, H.; Kwon, Y.; Lee, J.; Kim, J.; Shin, M.; Kim, S. and Bae, H. (2010). Methyl Gallate Exhibits Potent Antitumor Activities by Inhibiting Tumor Infiltration of  $\text{CD4}^+\text{CD25}^+$  Regulatory T Cells. *Journal of immunology* 185(11):6698-6705
- [10] Fan, W.W.; Yuan, G.Q.; Li, Q.Q. and Lin, W. (2014). Antibacterial mechanisms of methyl gallate against *Ralstonia solanacearum*. *Australasian Plant Pathology*. 43(1) 4.
- [11] Nam, S. (2001). "Ester bond-containing tea polyphenols potentially inhibit proteasome activity in vitro and in vivo," *Journal of Biological Chemistry*, 276:13322