Antimicrobial evaluation of Amyrin acetate from the stem bark of *Ficus sycomorus* (Moraceae)

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**Abstract**

In this study, the antimicrobial evaluation of alpha amyrin obtained from the stem bark of *ficus sycomorus* was reported. Standard method was adopted for the screening of phytochemicals, while a combination of column and preparative thin layer chromatography lead to the compound (FA) a light yellow crystal. The compound FA showed significant inhibition on the tested organisms, E.coli (IC₅₀ = 0.81) S. typhi (IC₅₀ = 0.84) S. aureus (IC₅₀ = 0.66) K. Pneumonia (IC₅₀ = 0.06) and was identified based on spectra evidence to contain a mixture of α-amyrin acetate.

**Keywords:** *Ficus sycomorus*, moraceae, triterpenoids, α-amyrin acetate, antimicrobial

1. **Introduction**

*Ficus sycomorus* belongs to moraceae, a family that is reputable for its medicinal values, and consist of about 40 genera and over 1,400 species of trees, vines and herbs, often with milky latex juices (Zerega et al., 2005) [1]. It is commonly known as fig mulberry. The Hausa people of Northern Nigeria call it Farin Baure or Bore. The genus Ficus consist of a variety of phytochemicals which includes phenolics, polyphenols, flavonoids, tannins, anthocyanins, coumarins, volatile components, glycosides, saponins, carotenoids, alkaloids, triterpenoids and vitamins (Nawaz et al., 2019) [2]. *Ficus* species have been used for a long time in herbal medicine. Traditionally, the plant is used for the treatment of sexually transmitted infections, gastrointestinal, respiratory, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers. Adeshina et al. (2010) [3] reported the antibacterial activity of ethanol extract of *F. sycomorus* L. and *F. platyphylla* Del. The antibacterial activity of *F. sycomorus* L. could be related to the presence of bioactive compounds, such as flavonoid (Adeshina et al., 2010) [3], alkaloid, tannin, saponin and steroid (Salem et al., 2013) [4]. Mohammed et al. (2015) [5] reported the antihelmitic potential of the *F. sycomorus*. While Bello et al. (2015) [6] reported that the plant material finds relevance in the management of diabetic conditions and infectious diseases. Literature has reported the isolation of α and β-amyrin acetate, a pentacyclic triterpenoid of the oleanane series from *Ficus* species example include the isolation of α-amyrin acetate, from the diethylfether fraction of the methanol extract of the stem bark of *Ficus kamerunensis*. However, its potential as an antimicrobial agent is being reported for the first time in the stem bark of *F. sycomorus* from literature survey.

2. **Expérimental Procédure**

2.1 **General**

Column chromatography was performed using silica gel (60-120 mesh), whereas TLC was performed on aluminium plates coated with silica gel 60 F254. The spots were visualized by spraying with 10% H₂SO₄, followed by heating in an oven. The ¹H (100MHz) and ¹³C NMR (400MHz) spectra were run in a Bruker AV3 spectrometer using CDCl₃ as solvent and TMS as internal standard. Both 1D and 2D NMR were run at the Strathclyde Institute of Pharmacy and Biological Sciences, University of Strathclyde Glasgow, Scotland.

2.2 **Plant material**

Fresh stem-bark of the medicinal plant *Ficus sycomorus* was collected from it natural habitat at Alau-dam environ in Maiduguri, Borno State, Nigeria. The herbarium specimen was identified by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri,
3. Results and Discussion

The pulverized stem bark of the plant yielded 23.21% of the sample using 96% ethanol as solvent. The crude ethanol extract revealed the presence of Phytochemicals which were previously reported from the plant such as Flavonoids, steroids and Phenolic acid etc (Bello et al. 2013) [12] except for anthraquinones which were not previously reported in F. sycomorus but in other ficus species such as F. thunbergii (Kitagima et al. 1994) [13]; F. Polita (Kuet et. 2011) [14] and F. cordata (Pournale 2008) [15].

A total of 11 fractions were pooled together on the basis of their Rf values after several eluate from column chromatogram were obtained from 100g of plant extract. Subsequent Pool on the basis of Rf values gave four fractions which were designated FA-FD. Fraction FA alone (196mg) gave 1 spots (Rf value: 0.78 with benzene:hexane, 1:1) on TLC. It was mounted on Sephadex LH-20 for further purification. FA was partially soluble in hexane and insoluble in ethanol and acetone with a melting point of 190-196 °C. The proton (1H NMR) of the compound indicated the presence of eight angular methyl protons in the region δ 0.88 to 1.24 ppm; methane protons in the region δ 1.5 to 2.8 ppm; de-shielded methyl proton at δ 2.05 ppm indicate the presence of an acetate moiety and this was confirmed by the presence of carbonyl carbon at δ-171.5. The compound also indicated the presence of two two olefinic protons; at δ 5.15ppm(α) assigned to H-12 (Saeed and Sabir, 2003) [16] and an oxygenated proton at δ 4.48ppm (α) assigned to H-3 thus, suggesting a triterpenoid or steroid acetate, see table1(Sissay and Abeba, 2005) [17]. The 13C NMR spectra indicated the presence of 30 carbon peaks; with a C-C double bond (δ 121.74 ppm (α) at C-12. Oxygenated carbon shift was observed at 77.30(α) for H-3. The forgoing spectral analysis and, comparison with reported data, led us to identify the structure of the isolated compound as a known triterpene, α-amyrin acetate (figure1). The pentacyclic triterpene α-amyrin acetate (12-ursen-3β-yl acetate) Figure 1 is a constituted triterpene, that belong to the group of ursane series though their chemical structure are similar to that of the steroid, and are extremely useful in prevention or treatment of many diseases in experimental animals, particularly those in which oxidative and inflammatory stress plays a key role in pathogenesis (Sporn et al. 2011) [18].

| S/N | 1H ppm | 13C ppm | δH ppm | δC ppm | Carbon type |
|-----|--------|---------|--------|--------|-------------|
| 1   | 38.80α | 38.55α  | CH2    |        |             |
| 2   | 27.00α | 27.01α  | CH2    |        |             |
| 3   | 78.00α | 77.30α  | CH     |        |             |
| 4   | 38.00α | 38.12α  | C      |        |             |
| 5   | 55.12α | 55.23α  | CH     |        |             |
| 6   | 18.34α | 18.30α  | CH2    |        |             |
| 7   | 33.66α | 32.67α  | CH2    |        |             |
| 8   | 40.02α | 40.09α  | C      |        |             |
| 9   | 47.54α | 47.64α  | CH     |        |             |
| 10  | 37.00α | 37.15α  | C      |        |             |
| 11  | 23.30α | 23.46α  | CH2    |        |             |
| 12  | 122.54α| 121.74α | CH     |        |             |
| 13  | 143.52α| 145.24α | C      |        |             |
| 14  | 41.54α | 41.64α  | C      |        |             |
| 15  | 28.34α | 28.50α  | CH2    |        |             |
| 16  | 26.25α | 26.22α  | CH2    |        |             |
| 17  | 32.54α | 32.56α  | C      |        |             |
| 18  | 47.22α | 47.30α  | CH     |        |             |
| 19  | 46.80α | 46.86α  | CH     |        |             |
Compound F_A showed better activity than F_B, F_C with no activity recorded in F_D as determined by agar well diffusion method against some selected organisms (Escheria coli, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia) as shown (Figure 3-6). The IC_50 showed more activity of Compound F_A on Klebsiella pneumonia (IC_50= 0.06) and least on Salmonella typhi (IC_50= 0.84).

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