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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 phyto-chemicals, while a combination of column and preparative thin layer chromatography lead to the compound (F_A) a light yellow crystal. The compound F_A showed significant inhibition on the tested organisms, E.coli (IC_{50} = 0.81) *S. typhi* (IC_{50}= 0.84) *S. aureus* (IC_{50}= 0.66) *K. Pneumonia* (IC_{50}= 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 gnus 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 diethylether 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% H_2SO_4, followed by heating in an oven. The ^1H (100MHz) and ^{13}C NMR (400MHz) spectra were run in a Brucker AV3 spectrometer using $CDCl_3$ 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,

Borno State, Nigeria. Specimen voucher number 8012B was allocated to the plant material and deposited for reference. The sample was air-dried and pulverized using a wooden pestle and mortar. The pulverized plant material was then stored in an air-tight polythene bag ready for analysis. The solvents used were of general purpose grade.

2.3 Preliminary Phytochemical Screening
The crude ethanol extracts of the stem bark of *Ficus sycomarus* was subjected to preliminary phytochemical screening of secondary metabolites using standard methods (Sofowora 1993 [7]; El-olemmy *et al.*, 1994 [7]; Trease and Evans 2002) [8]; Abulude 2007 [9]; Hatil *et al*. 2015 [10])

2.4 Sample extraction and Isolation
One thousand five hundred grams (1.5kg) of the pulverized sample material was extracted with 96% ethanol using soxhlet extractor. The crude extract was concentrated over a water-bath at 100ºC and then exposed to air at 25 ^{0}C to dryness. The dry extract was weighed, labeled and stored in a desiccator, subject to further analysis. 100 g of the pulverized plant material was fractionated by open column chromatography with silica gel 60 (70-120mesh). The elution started with *n*-hexane to ethyl acetate (7:3) ratio with 10% increment in polarity using ethyl acetate until a final collection with EtOAC and *n*-hexane (7:3). 300ml was collected for each increment made. Fraction A eluted at 30% ethylacetate (7:3) and showed single spot on TLC, further purification on Sephadex LH-20(CH_3Cl-MeOH) gave the compound F_A (196mg). Subsequent fractions with increment gave compound with two or more spot on TLC. Thus, they were pooled into F_B-F_D based on number of spots on TLC.

2.5 Susceptibility Assay
The zone of inhibition of F_A-F_D against test organisms were determined by disc diffusion test according to Eucast (2016) [11]. The agar plates inoculated with test organisms were used in these assays. Wells of 6mm diameter and 4mm deep were punched on the agar with the aid of a sterile cork borer. Each of the plates was allowed to dry, and then incubated at 37 ^{0}C for 24hrs. Antibacterial activity was evaluated by measuring the diameters of zones of growth inhibition in triplicates and results were presented as Mean ±SEM.

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 Flavonoid, sterols and Phenolic acid e.t.c (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* (Poumale 2008) [15].

A total of 11 fractions were pooled together on the basis of their R_f values after several eluate from column chromatogram were obtained from 100g of plant extract. Subsequent Pool on the basis of R_f values gave four fractions which were designated F_A-F_D. Fraction F_A alone (196mg) gave 1 spots (R_f = value: 0.78 with benzene:hexane, 1:1)on TLC. It was mounted on Sephadex LH-20 for further purification. F_A was partially soluble in hexane and insoluble in ethanol and acetone with a melting point of 190-196 ^{0}C. The proton (^{1}H NMR) of the compound indicated the presence of eight angular methyl protons in the region δ 0.88 to 1.24 ppm; methylene 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 ^{13}C 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 C-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].

Table 1: ^{1}H NMR (δ ppm), ^{13}C NMR (δ ppm) and carbon type for the isolated compound from *Ficus sycomorus* stem bark and the literature

S/N	^{1}H*	^{13}C*	^{1}H**	^{13}C**	Carbon type
1		38.80α		38.55α	CH_2
2		27.00α		27.01α	CH_2
3	4.5 (dd, 1H)	78.00α	4.48α(dd, H)	77.30α	CH
4		38.00α		38.12α	C
5		55.12α		55.23α	CH
6		18.34α		18.30α	CH_2
7		33.66α		32.67α	CH_2
8		40.02α		40.09α	C
9		47.54α		47.64α	CH
10		37.00α		37.15α	C
11		23.30α		23.46α	CH_2
12	5.12(α)(t,1H)	122.54α	5.10(α) (t, 1H)	121.74α	CH
13		143.52α		145.24α	C
14		41.54α		41.64α	C
15		28.34α		28.50α	CH_2
16		26.25α		26.22α	CH_2
17		32.54α		32.56α	C
18		47.22α		47.30α	CH
19		46.80α		46.86α	CH

20		31.14α		31.15α	CH
21		34.82α		34.83α	CH_2
22		37.22α		37.25α	CH_2
23	0.99α (s,3H)	28.40α	0.99 (s, 3H)	28.12α	CH_3
24	0.88 α (s, 3H)	15.61α	0.82α (s, 3H)	15.64α	CH_3
25	0.96 α (s, 3H)	15.52α	0.96 α (s, 3H)	15.52α	CH_3
26	1.02α (s, 3H)	15.95α	1.01α (s, 3H)	16.80α	CH_3
27	1.16α (s,3H)	26.00α	1.11α(s, 3H)	26.04α	CH_3
28	0.84α (s, 3H)	27.34α	0.84α (s, 3H)	27.53α	CH_3
29	0.88α (s,3H)	33.22α	0.86α (s, 3H)	33.45α	CH_3
30	0.88α (s,3H)	23.70α	0.86α (s, 3H)	23.79α	CH_3
1^1		171.40		175.10	COO
2^1	2.02 (s, 3H)	21.70	2.01 (s, 3H)	21.65	CH_3C00

*(Saeed& Sabir, 2003; Sissay and Abeba, 2005) [16, 17] ** (The isolated compound)

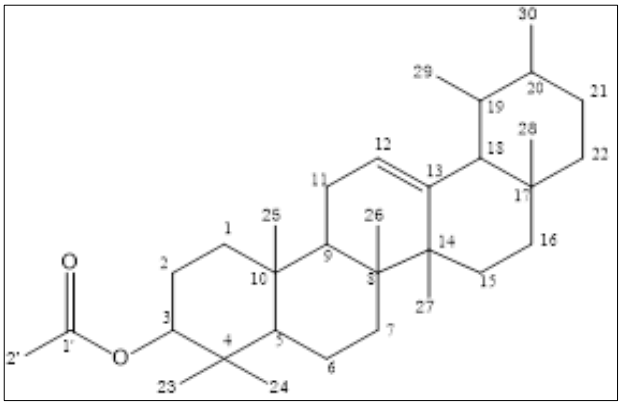

Fig 1: Chemical structure (α-amyrin acetate) isolated from the ethanol extract of stem-bark of *Ficus sycomorus*.

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).

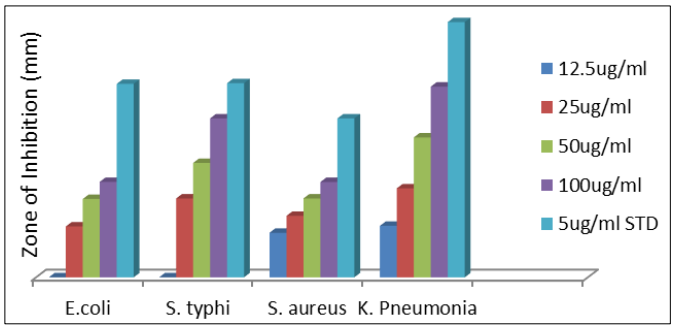

Fig 2: *In vitro* Susceptibility Test for F_A

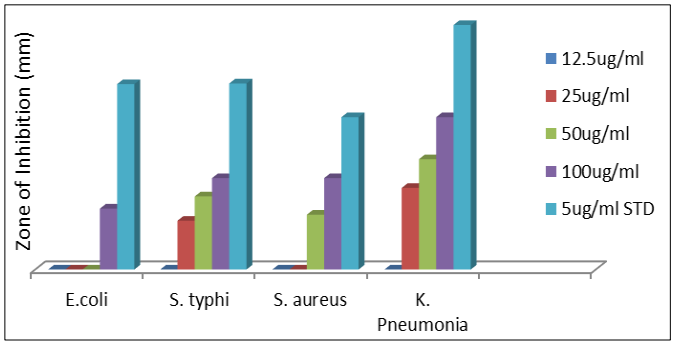

Fig 3: *In vitro* Susceptibility Test for F_B

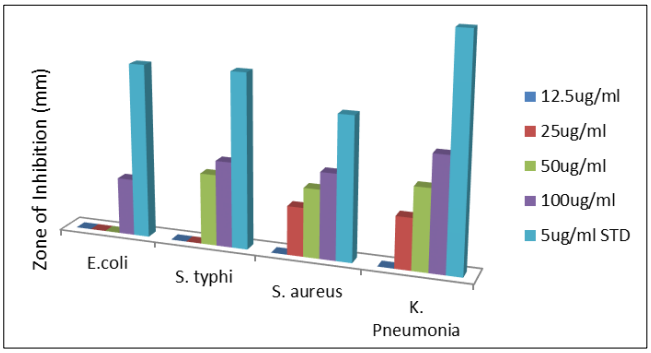

Fig 4: *In vitro* Susceptibility Test for F_C

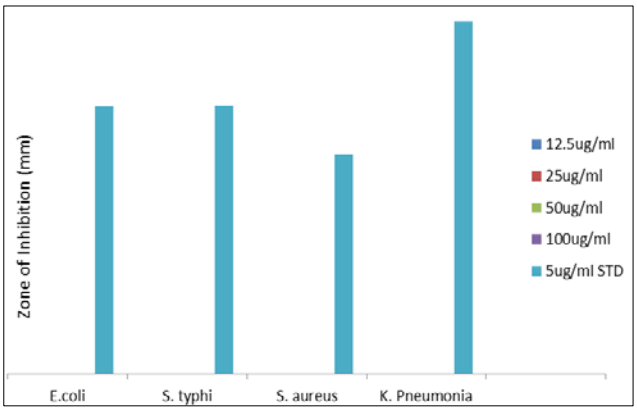

Fig 5: *In vitro* Susceptibility Test for F_D

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