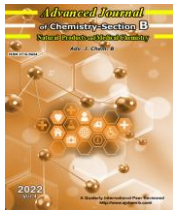
Advanced Journal of Chemistry-Section B
Natural Products and Medical Chemistry

Journal homepage: http://www.ajchem-b.com/



Original Research Article

Isolation, Characterization and Antimalarial Evaluation of Methyl-3,12-dihydroxycholan-24-oate from Bovine Bile

Blessing B. John*, James D. Habila, Adebayo O. Oyewale, AbdulRazaq Tukur
Chemistry Department, Faculty of Physical Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria

ARTICLE INFO

Article history
Submitted: 2022-01-27
Revised: 2022-03-18
Accepted: 2022-05-23
Available online: 2022-05-29
Manuscript ID: AJCB-2201-1107
DOI: 10.22034/ajcb.2022.326645.1107

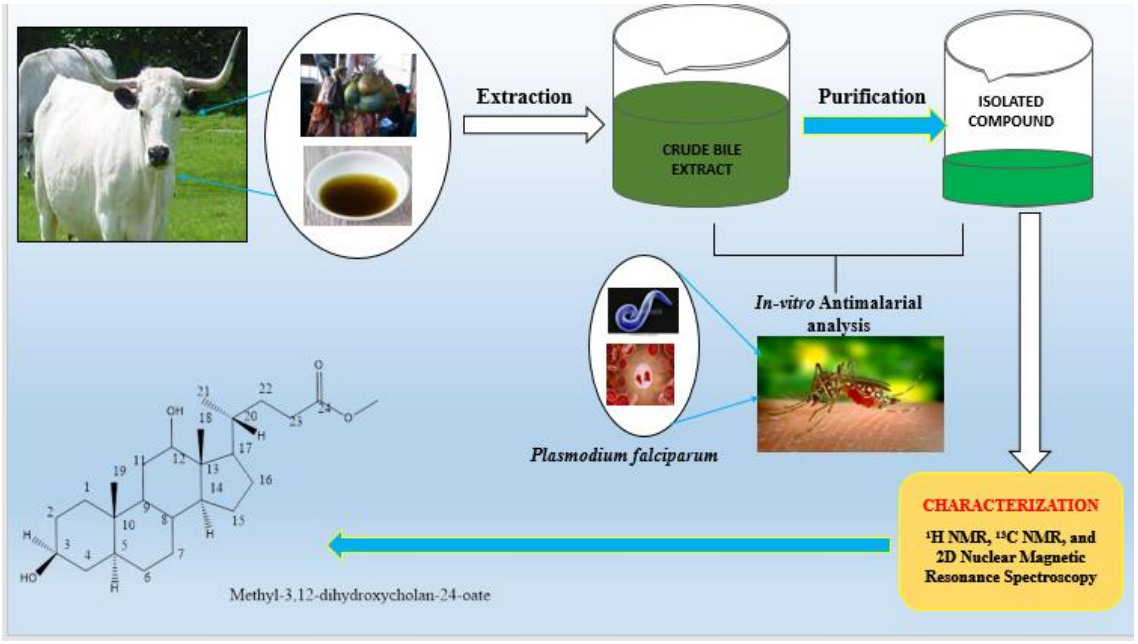
KEYWORDS
Bovine Bile
Isolation
Spectroscopy
Malaria

ABSTRACT

Bile, often known as gall, is a dark-green fluid produced by the liver of most vertebrates. It is largely made up of steroidal detergent-like compounds and membrane lipids including un-esterified cholesterol and mixed phosphatidylcholines. A good number of animal bile has been utilized ethno-medicinally for ages. Animal bile has been administered to cure liver, skin disorders, malaria, diabetes, cancer, and heart disease. The extraction was achieved by solvent extraction in chloroform, where 40% aqueous methanol was added to about 4 liters of the bile sample, mixed thoroughly and acidified with 2M H_2SO_4 solution to a pH of about 3.0 - 4.0. After several agitations, the mixture was allowed to stand for 24 hours, and then extracted with chloroform. The crude chloroform extract was purified using standard purification techniques of column chromatography (CC) and thin layer chromatography (TLC). The structure of the compound was characterized using, - (^{1}H NMR), ^{13}C NMR, and DEPT 135 spectroscopic techniques in order to propose the structure of the compound as (Methyl-3,12-dihydroxycholan-24-oate). The in-vitro antimalarial assay of the crude extract and the isolated compound was carried out on Plasmodium falciparum. The results of the antimalarial activity of the crude bile extract and isolated compound revealed IC50 values of 16.16 µg/mL and 32.09 µg/mL, respectively which indicates the moderate antimalarial activity compared to chloroquine standard control (0.029 µg/mL). The results of the investigation revealed that the bovine bile extract, contain bioactive chemical substances which could be good, therapeutic agents against malaria..

* Corresponding author: Blessing B. John
✉ E-mail: johnbapinah22@gmail.com

GRAPHICAL ABSTRACT



1. Introduction

Bile is a natural product formed in the liver and stored in the gall bladder [6]. It is a yellow, slightly green aqueous fluid, secreted by the liver of animals [16]. Bile contains a wide range of principal components- bile acids and bile salts, antioxidants including bilirubin, glutathione, vitamin E, and melatonin (N-acetyl-5-methoxytryptamine) [7]. Bile has been reported to have several therapeutic applications, some of which include improving liver function, dissolving of gallstones, inhibiting bacterial and viral multiplication, as well as anti-diabetic, antipyretic, anticancer, and anti-allergic effects [16].

The bile is produced as a byproduct of cholesterol catabolism [16]. In addition to its well-known enabling hepatobiliary secretion of endogenous metabolites and xenobiotics and intestine absorption of lipophilic nutrients, bile regulates glucose and lipid metabolism in the enterohepatic system, as well as energy expenditure in the peripheral tissues [6].

Malaria is a widespread infectious disease spread by the Anopheles mosquito [8], that has affected people since its inception [2], resulting in about 228 million cases of fevers and approximately 500,000 fatalities yearly [18]. The scientific and medical communities have been researching malaria prevention and therapy for hundreds of years and overtime, many antimalarial medications have rapidly lost their potency as a result of continued usage and increased resistance of the parasite [4]. The pregnant women and children (under 5 years) are more susceptible to malarial infections, accounting for a larger chunk of the susceptible population due to their lower immune system, thereby constituting about 92% of total prevalence and mortality rate [1]. The use of chloroquine and other antimalarial drugs to prevent and treat *Plasmodium falciparum* malaria has led to a widespread occurrence of chloroquine-resistant strains [12]. We report for the first time on the isolation, characterization of the bioactive components of Bovine bile, and evaluation of its anti-malarial potential **(Fig.1)**.

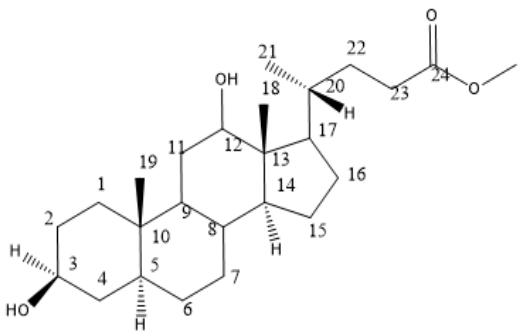


Fig. 1: In-vitro Antimalarial Analysis of bovine bile

2- Experimental

All chemicals and reagents used in the work were of analytical grade purchased from commercially available sources. The thin-layer chromatography was carried out using pre-coated silica gel 60 (F254) that was obtained from MERCK (Germany). Spots on the TLC plates were visualized under the UV light (254 nm and 366 nm), by spraying with 10% H_2SO_4 in 90% MeOH followed by heating at 100 °C. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Avance III 400 MHz spectrometer at room temperature, using TMS as a reference. Chemical shift values (δ) were reported in parts per million (ppm) relative to TMS and the coupling constants were given in Hz. The solvent used for these measurements was deuterated DMSO.

2.1- Sample collection

Fresh sample of bovine bile was collected from the abattoir in Zaria, Kaduna State, Nigeria, between the months of July-August, 2020. The collected bile was stored in 40 % aqueous methanol.

2.2- Extraction

The extraction was achieved according to the method as described by Harborne (1973) with some modifications. Briefly, four liters of the fresh bile were measured, acidified with 2 M H_2SO_4, and extracted with chloroform. After 24 hours, the aqueous layer was partitioned from the organic layer, and the extract was concentrated to one-third of its original volume using a rotary evaporator (42 °C) and dried at room temperature to afford the crude bile chloroform extract (20.4 g).

2.3- Isolation and purification

2.3.1 Packing the column

With the aid of a long glass rod, a clean glass wool was used to block the opening of the column. Silica gel (300 g) was mixed with n-hexane (900 cm^3) in a beaker and stirred to make slurry. This was poured into the column with the aid of a funnel and continued washing with n-hexane.

2.3.2 Loading the column

About (5 g) was subjected to column chromatography using 100 g of 60:200 mesh silica gel and n-hexane/chloroform (3:7) solvent system. The column was eluted using a gradient solvent system with n-hexane and ethyl acetate in the following order: 100% n-hexane; -95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 40:60, 30:70, 20:80, and 10:90. Subsequently, 100% ethyl acetate was used to wash the column.

2.4- Anti-malarial assay

2.4.1 Plasmodium parasite culture

Plasmodium parasites were cultivated in type A^+ erythrocytes and cultured in RPMI-1640 (Roswell Park Memorial Institute) enriched with 0.5 mL FBS (Foetal Bovine Serum). Incubation was carried out at 37 °C under anaerobic condition for 48 hours. The culture was routinely monitored every 24 hours by smearing on strips and staining with Geimsa. Parasitaemia was calculated as a percentage based on the viable parasitic forms observed. Parasite quantification was conducted by optical microscopy. Chloroquine was used as the standard positive control (0.029 µg/mL) [14]. The percentage parasitemia after incubation was calculated as described by [10].

The percentage inhibition per concentration was calculated using the formula:

$$Percentage\ parasitemia\ (PP\%) = \frac{Ei}{40{,}000} x\ 100 \qquad [1]$$

[Ei is the number of infected erythrocytes]

$$\%\ Inhibition = \frac{\%\ Parasitaemia\ in\ control\ wells - \%\ Parasitaemia\ of\ test\ wells}{\%\ Parasitaemia\ of\ the\ control} x\ 100 \qquad [2]$$

The IC_{50} values were determined graphically on dose response curves. A plot of concentration versus percent inhibition was plotted on Microsoft excel for the crude extract and the isolated compound. The slope was calculated, which equaled to the IC_{50} values. The antimalarial activity was analyzed in accordance to the norm of antimalarial activity in [9]. According to this norm, highly active, promisingly active, good activity, moderate activity, marginal potency, and poor or inactive has IC_{50} values of: ≤ 5 µg/mL, 5.1–10 µg/mL, 10.1–20 µg/mL, 20.1–40 µg/mL, 40.1–70 µg/mL, and 70.1–4100 µg/mL, respectively.

2.5-Spectroscopic analysis

The isolated compound was subjected to ^{1}H-NMR, ^{13}C-NMR, and DEPT 135 spectroscopic analysis to elucidate its structure.

3- Results and discussion

The compound was isolated as a green colored semi-solid substance (10 mg) and subjected for spectral analysis (**Table 1**).

Table 1. Physical characteristics of the isolated compound (A)

Gradient solvent system	% yield	R_f Value	Color
Hexane: ethyl acetate (70:30)	10 mg	0.36	Green

Table 2. Comparison of experimental values of compound A with literature values

Position	^{13}C-NMR Experimental	^{13}C-NMR Literature	^{1}H-NMR Experimental	^{1}H-NMR Literature	Nature Of Carbon
C-1	33.87	36.70	-	-	CH_2
C-2	30.68	30.78	-	-	CH_2
C-3	72.03	72.08	3.61 (m)	3.63	CH
C-4	36.24	36.70	-	-	CH_2
C-5	42.28	42.38	1.27(m)	-	CH
C-6	27.33	27.41	-	-	CH_2
C-7	26.33	26.63	2.10 (dd)	-	CH_2
C-8	35.40	36.08	-	-	CH
C-9	36.62	40.40	-	-	CH
C-10	34.32	34.79	-	-	C
C-11	28.87	21.04	-	-	CH_2
C-12	73.37	40.40	-	-	CH
C-13	46.70	42.96	-	-	C
C-14	48.47	56.72	-	-	CH
C-15	23.84	24.41	-	-	CH_2
C-16	27.64	28.38	-	-	CH_2
C-17	47.53	56.20	1.20 (m)	-	CH
C-18	12.94	12.24	0.67 (s)	0.63	CH3
C-19	23.35	23.57	0.90 (s)	0.91	CH3
C-20	36.2	38.61	-	-	CH
C-21	35.30	35.58	0.89(d)	0.99	CH3
C-22	31.10	31.29	-	-	CH_2
C-23	31.28	31.23	-	-	CH_2
C-24	174.92	174.95	-	-	C
C-25	51.70	51.63	3.61(s)	3.65	OCH_3

Literature*: Methyl 3α-hydroxycholan-24-oate reported by - Do Nascimento *et al.* (2015)

3.1 Spectroscopic characterization

From the ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of compound A **(Fig. 2)** revealed the presence of protons signals at δ (ppm) 0.67 (s, 3H, 18-H), 0.90 (s, 3H, 19-H), 0.96 (d, 3H, 21-H, J= 6 Hz), 1.27 (m, 1H, 5-H), 2.10 (dd, 2H, 7-H, J = 78 Hz), 3.61 (s, 1H, 3-H), and 3.97 (s, 1H, 12-H); ^{13}C (DMSO-d_6, 600MH_Z): δ (ppm) 174.92 (C-24), 73.38 (C-12), 72.03 (C-3), 51.70 (C-25), 48.48 (C-14), 47.53 (C-17), 46.70 (C-13), 42.28 (C-5), 36.62 (C-9), 36.24 (C-4), 35.41 (C-8), 35.30 (C-20), 34.32 (C-10), 33.87 (C-1), 31.28 (C-23), 31.10 (C-22), 30.68 (C-2), 28.87 (C-11), 27.65 (C-16), 27.33 (C-6), 26.33 (C-7), 23.84 (C-15), 23.36 (C-19), 35.30 (C-21), and 12.95 (C-18).

The ^{1}H NMR spectrum of compound A **(Fig. 2)** revealed overlapping methyl, methylene, and methine proton signals in regions between 0.6 and 2.5 ppm (typical of steroidal nucleus). Peaks for protons H-18 and H-19 were both singlets and H-21 methyl peak was a doublet distinctively observed between 0.6 and 1.0 ppm (0.67, 0.90, and 0.96, respectively). Protons H-3, H-7, and H-12 were seen further downfield away from the other signals at δ 3.61, 2.10, and 3.97 respectively. A pair of ^{1}H single is seen downfield at δ 3.61 (H-3) and δ 3.97 (H-12) was indicative of steroid protons. The ^{13}C-NMR spectrum revealed a total of twenty-five carbon signals **(Fig. 3**). Three methyl carbons were seen at [δ: 12.9 (C-18), 23.4 (C-19), and 35.3 (C-21), 10 methylene carbons at [δ: 33.9 (C-1), 30.7 (C-2), 36.2 (C-4), 27.3 (C-6), 26.3 (C-7), 28.9 (C-11), 23.8(C-15), 27.6 (C-16), 31.1 (C-22), and 31.3 (C-23), 8 methine at [δ: 72.0 (C-3), 42.3 (C-5), 35.4 (C-8), 36.6 (C-9), 73.4 (C-12), 48.5 (C-14), 47.5 (C-17) and 35.3 (C-20), and 3 quaternary carbon resonances at [δ: 34.3 (C-10), 46.7 (C-13), and 174.9 (C-24) were further assigned with the aid of the Distortionless Enhancement by Polarization Transfer (DEPT 135) spectrum (**Fig 4)**.

The structure of the isolated compound was successfully characterized and confirmed using some spectroscopic and spectrometric techniques such as: FT-IR, GC-MS, and NMR. The IR spectrum **(Fig. 5)** demonstrated a broad band at 3380 cm^{-1} due to an -OH stretching, 2929- 2862 cm^{-1} corresponds to C-H stretching and 1729 cm^{-1} which is due to a C=O stretching of an ester. All the bands detected in the FTIR spectra were in agreement with the values reported in the literature for esters (-OH, 3518 cm^{-1}, C-H, 2932 cm^{-1}, C-H, 2861 cm^{-1}, and C=O, 1712 cm^{-1}) [3]. The mass spectrum was derived from an Agilent technology GC-MS 7890A coupled with MSD 5975C, with GC ALS as injection source and a column size 30 m×320 µm×0.25 µm. The sample was dissolved in methanol with an injection volume (2 µL). From **Fig. 6**, the result interpretation obtained from the GC-MS spectrum revealed m/z values of -41.1, 74.1, 87.1, 115.1, 143.1, 171.1, 214.2, 253.0, 281.0, 331.1, and 406.1. The GC-MS of the compound indicated the molecular ion peak at 406.1 which correlates with the calculated relative molecular mass of 406.

The results of the spectroscopic analysis, were in agreement with the known compound methyl 3α-hydroxycholan-24-oate reported by [3] as seen in **Table 2**.

Based on the assignment and correlation with a similar compound, the proposed structure for the isolated compound A is as follow:

Table 3. Percentage inhibitory concentration of the crude extract and fractions of bile acid on plasmodium

Samples	Percentage inhibitory concentrations					
	100	**50**	**25**	**12.5**	**6.25**	**IC_{50}**
Fraction A	75.78	59.09	47.22	34.01	17.65	32.09
Extract K	87.38	82.51	64.06	52.03	51.55	16.16

A: hexane/ethyl acetate fraction of bile acid (7:3), K: chloroform extract of bile acid

Based on the criteria of the IC_{50} of antimalarial activity (in-vitro) reported by [9], the results (**Table 3**) showed that the crude bovine bile extract (extract K), had the highest antimalarial activity (good activity) as recorded by the IC_{50} of 16.16 µg/m as compared to the isolated compound. The isolated compound showed moderate antimalarial activity of 32.09 µg/mL. The observed results indicated that the concentration of the extract has a linear relationship with the inhibition percentage; for the isolated compound (A); 6.25 mg/mL (17.61%), 12.5 mg/mL (34.01%), 25 mg/mL (47.22%), 50 mg/mL (59.09%), and 100 mg/mL (75.78%). The crude extract (K) also followed a similar pattern. The results of this investigation clearly demonstrated the potential of bovine bile as an antimalarial agent.

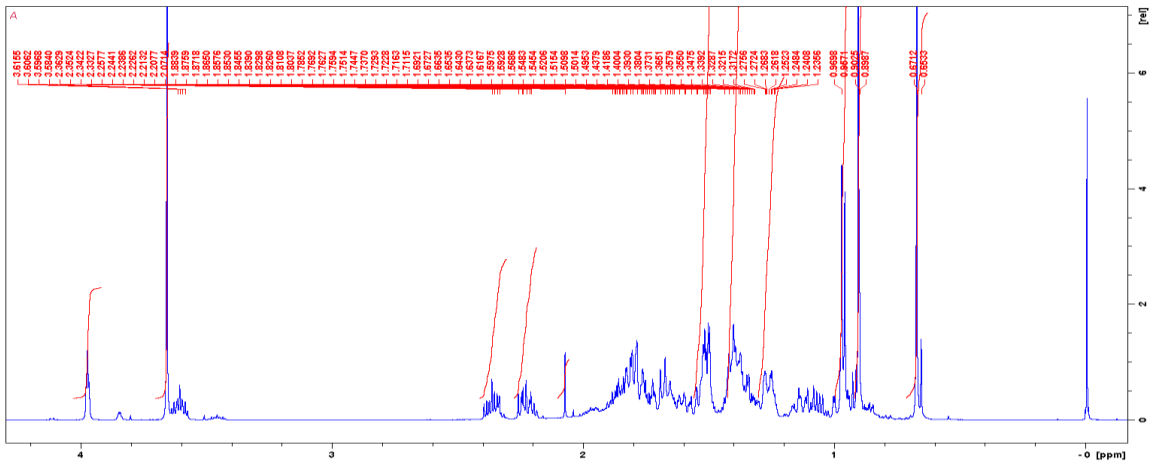


Figure 2. ^{1}H NMR spectrum of compound A

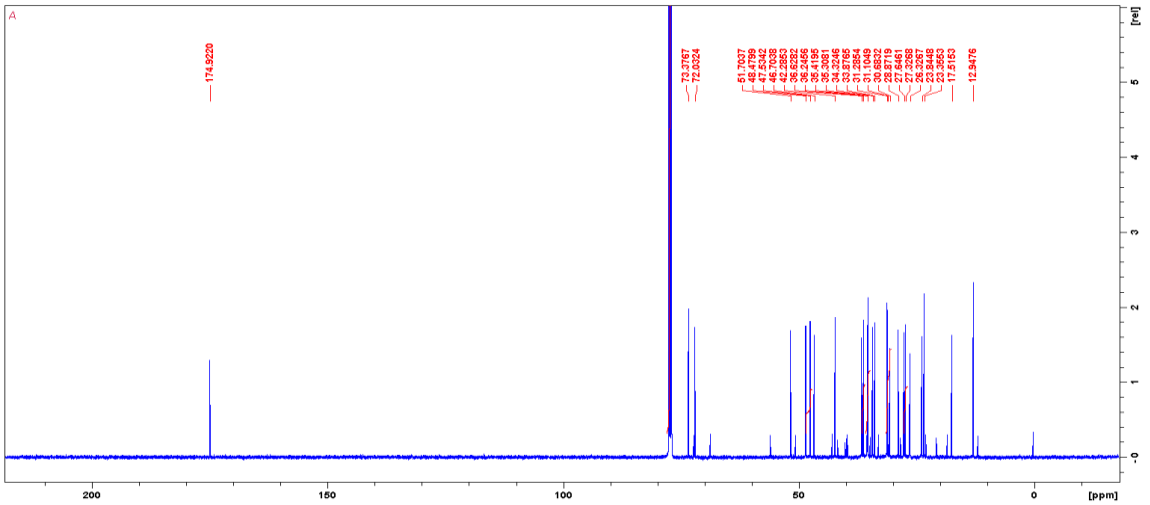


Figure 3. ^{13}C NMR spectrum of compound A

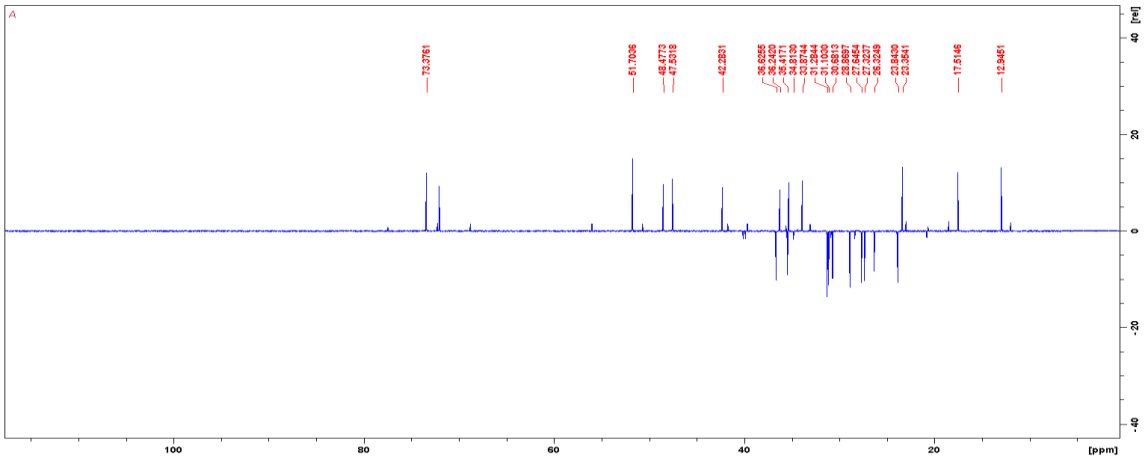


Figure 4. DEPT 135 Spectrum of Compound A

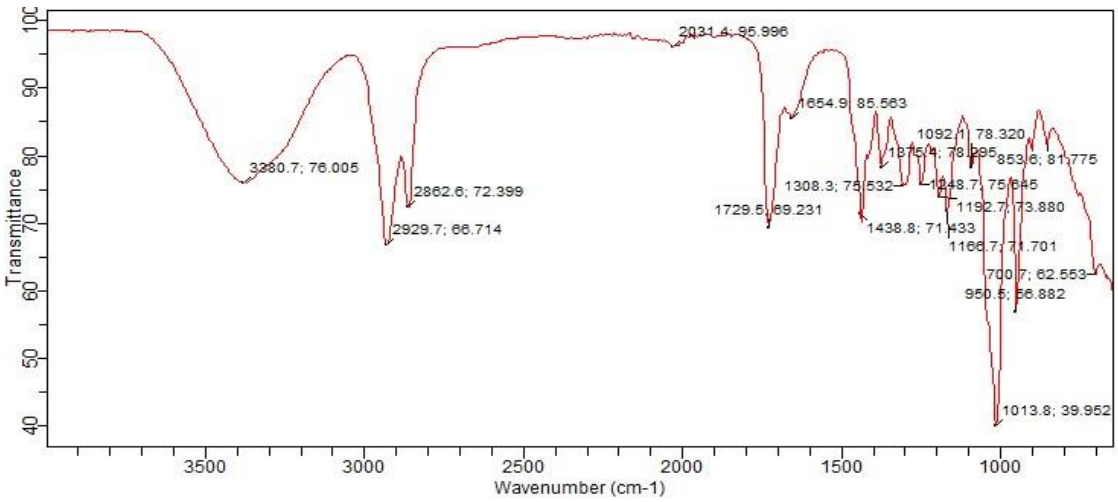


Figure 5. FT-IR Spectrum of Compound A

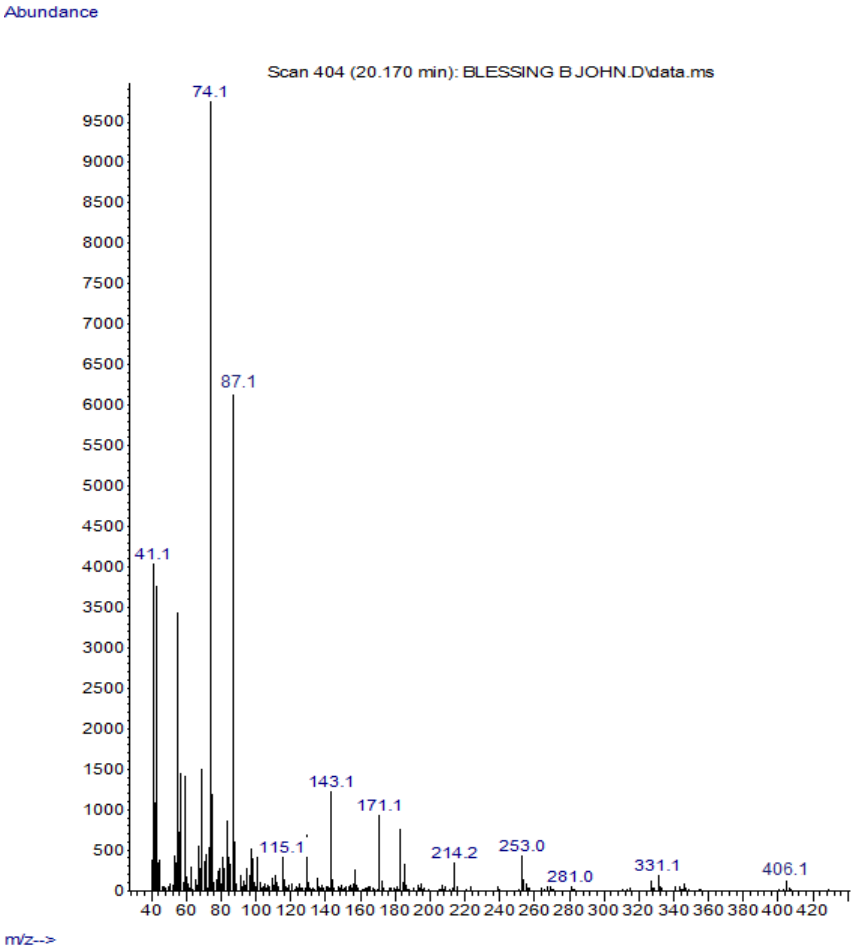


Figure 6. GC-MS Spectrum of Compound A

Conclusion

The isolated compound was characterized with FT-IR, GC-MS, and NMR spectroscopic techniques and identified to be Methyl-3,12-dihydroxycholan-24-oate. The results of the in vitro antimalarial screening of the isolated compound and the crude bile extract revealed that bile possesses a reasonably good activity against malaria, clearly indicating the potential of bovine bile extract as an antimalarial agent relative to the standard drug used. This justifies the applications of bovine bile in ethno medicine. With the rate of increasing resistance of the malaria parasite to the existing drugs and the continuous efforts of researchers to find ways of combating this disease, we hope that this research will lead to further discoveries in the fight against malaria in the near future. It can also serve as a precursor for the synthesis of other therapeutic medications.

Acknowledgements

The authors would like to express their sincere gratitude to the staff and laboratory technologists of Chemistry Department, Ahmadu Bello University Zaria, Nigeria, for giving the resources and support that allowed this study to be completed.

References

[1] A. Abdulkadir, I. A. Umar, S. Ibrahim, E. Onyike, A. Y. Kabiru, Evaluation of antimalarial potential of cysteine protease inhibitors from *calotropis procera* linn and *moringa oleifera* lam on *plasmodium berghei* (nk 65) infected mice, *Journal of Advances in Medicinal and Pharmaceutical Sciences*, 6(3) (2016) 1-13.

[2] C. Foundation, Destructive effects of malaria in Africa. *Carter Foundation*., (2005) 23–45.

[3] P.G.G. Do Nascimento, T.L.G. Lemos, M.C.S. Almerida, J.M.O. De Souza, A.M.C. Bizerra, G.M.P. Santiago, J.G.M. Da Costa, H.D.M. Countinho, Lithocholic acids and derivatives: antibacterial activity. *Steroid,* (2015) STE 7816.

[4] B. Gitta, N. Kilian, Diagnosis of Malaria Parasites Plasmodium spp. In endemic areas: Current strategies for an ancient disease. *Bioassays Journal*, 42 (2019) 1900138.

[5] J.D. Habila, N. Habila, I.G. Ndukwe, J.O. Amupitan, A.J. Nok, F.O. Shode, 4-(ethyl-pophenylglycinylamino)-7-chloroquinoline: Synthesis and in vivo antimalarial evaluation. *Journal of Pharmacognosy and Phytotherapy*, 3 (2011) 160-163

[6] R.L. Harris, G.M. Goodwin, The therapeutic potential of psychedelic drugs: past, present and future. *Neuropsychopharmacology,* 42 (2017) 2105-21113.

[7] J. Kandrac, S. Keveresan, J.K. Gu, M. Mikov. Isolation and determination of bile acids. *European journal of drug metabolism and pharmacokinetics*, 31 (2006) 157-77.

[8] R.E Lagerberg, Malaria in pregnancy: a literature review. *J midwifery women's Health*, 53 (2008) 209–15

[9] C.A.N. Nadia, W.J. Pone, K.N. Kaushi, M.D. Krishnan, M. Garima, N.T. Arlette, M. Shubbi, M. Mbida, D. Sahal, In Vitro Antiplasmodial activity and cytotoxicity of extracts and fractions of *Bidens pilosa*. *Asian Journal of Biomedical and Pharmaceutical Sciences*, 7 (2017).

[10] M.N. Ngemenya, T.M. Akam, J.N. Yong, P.Tane, S.N.Y. Fanso Free, K. Berzins, P.K. Titanji, Antiplasmodial activities of some products from Turreanthus africanus (Meliaceae). *African Journal of Health Sciences*, 13 (2006)

[11] U.E. Odoh, P.F. Uzor, C.L. Eze, T.C. Akunne, C.M. Onyegbulam, P.O. Osadede, Medicinal plants used by the people of Nsukka local government area south-eastern Nigeria for the treatment of malaria: An ethnobotanical survey. *Journal of Ethnopharmacology,* 218 (2018) 1-15.

[12] N.N.N.A. Rahman, T. Furuta, K. Takane, M.A. Mohd, Antimalarial activity of extracts of Malaysian medicinal plants. *Journal of ethnopharmacology*, 64 (1999) 249-254.

[13] B. Staels, V.A. Fronseca, Bile acids and metabolic regulations: mechanisms and clinical responses to bile acid sequestration. *Diabetes journal*, 32 (2009)

[14] W. Trager, J. Jensen, Human malaria parasites in continuous culture. *Science*, 193 (1976) 673-675.

[15] B. Wahlin, M. Wahlgren, H. Perlmann, K. Berzins, A. Björkman, M.E. Patarroyo, P. Perlmann, Falciparum antigen efficiently inhibit merozoites invasion. *Proceedings of the National Academy of Science, USA*., 18 (1984) 7912-7916.

[16] D.Q.H. Wang, M.C. Carey, Therapeutic uses of animal biles in traditional Chinese medicine: An ethnopharmacological, biophysical chemical and medicinal review. *World Journal of Gastroenterology*, 20 (2014) 9952–9975.

[17] World Health Organization. World malaria report. License: cc By NC-SA 3.0 IGO, Geneva: https://www.who.int/publications-detail/world-malaria-report, 2018 (accessed 11 August 2020).

[18] World Health Organization. World malaria report. License: cc By NC-SA 3.0 IGO, Geneva: https://www.who.int/publications-detail/world-malaria-report. 2019 (accessed 11 August 2020)

[19] World Health Organization. World malaria report. 2020 (accessed 15 April 2021)

HOW TO CITE THIS ARTICLE

Blessing B. John*, James D. Habila, Adebayo O. Oyewale, AbdulRazaq Tukur. Isolation, Characterization and Antimalarial Evaluation of Methyl-3,12-dihydroxycholan-24-oate from Bovine Bile, Ad. J. Chem. B, 4 (2022) 135-143

DOI: 10.22034/ajcb.2022.326645.1107

URL: http://www.ajchem-b.com/article_150591.html