



BILE CHEMICAL SUBSTANCE FROM NORTHERN NIGERIAN RED GOAT INHIBIT MICROBES



J. D. Habila¹, J. I. Achika^{2*}, A. J. Habila³ and M. Abubakar¹

¹Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

²Department of Chemistry, Federal University Lokoja, Kogi State, Nigeria

³Department of Science Laboratory Technology, Nigerian Institute of Leather & Science Technology, Samaru, Zaria, Nigeria

*Corresponding author: achijonile@yahoo.com

Received: February 14, 2020 Accepted: May 28, 2020

Abstract: This aim of this research was to evaluate the antimicrobial activity of bile chemical substance from Northern Nigerian red goat (*Capra hircus*). The antimicrobial activity of the bile was done using standard methods on some selected microorganisms; *Escherichia coli*, *staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans*. The result showed significant antimicrobial activity against the microorganisms with zone of inhibition ranging from 12 – 38 mm and Minimum inhibitory concentration (MIC) values ranging from 6.25 – 50 mg/ml. The MBC and MFC values against the selected microorganism range between 25 and 100 mg/ml. The study shows that the goat bile extract had significant antimicrobial activity and compare well with the standard drugs (Ciprofloxacin and Terbinefin) having the zone of inhibition in the range of 20 – 50 mm. Goat bile therefore has potentials to explore in the search for antibacterial or antifungal agent from nature.

Keywords: Bile chemical substance, *Capra hircus*, antimicrobial activity

Introduction

The usage of animal biles in China for the treatment of a wide number of disorders in human beings enjoys a three millennial history (Wang *et al.*, 2014). Bile is a yellow, orange, or slightly green aqueous fluid that is the “exocrine” secretion of the liver. It forms first in bile canaliculi enclosed between parenchymal cells of the liver and flows continuously into ever enlarging ducts to exit the liver *via* two hepatic ducts (Chen, 1987). Bile is composed principally of a mixture of dissimilar molecular lipid species; bile acids, the principal catabolic products of cholesterol, a sterol found in the biles of all vertebrates and invertebrates; Bile pigments (bilirubin, biliverdin or both) the final products of heme catabolism. Phospholipids and cholesterol are responsible for the detergent-like properties of bile salts. In addition, bile contains small amounts of proteins, especially mucin glycoproteins and a wide variety of mineral salts (Tan, 1999). The known naturally-occurring bile acid species run into many hundreds and are typified by the most evolutionary advanced species in human (Hofmann *et al.*, 2010; Une and Hoshita, 1994).

Goat bile was believed to be effective in treating optic atrophy, including acute hemorrhagic conjunctivitis, marginal suppurative blepharitis, and epiphora (non-emotional tearing). Goat bile was also used to treat temporary blindness following a life-threatening illness (which was most likely smallpox), and eye injury from foreign bodies (Tao, 1986). This bile was believed to be effective in ameliorating various infectious skin diseases, chancre in children (most likely impetigo), and constipation. This bile was also prescribed as a facial lotion for minimizing chloasma in pregnant women (Jiangsu, 1977). Also, when goat bile was decocted thrice with ox bile and wine the resulting liquor was believed to be effective in reversing any olive discoloration of the skin from itchy dermatitis, which was most likely secondary to tinea versicolor (Li, 1957). Bile was also reported to contain a wide variety of antioxidants, the most powerful ones being bilirubin, glutathione, vitamin E, and melatonin (*N*-acetyl-5-methoxytryptamine) (Tan, 1999). This study aimed to report the anti-microbial properties of Goat bile extracts against some selected microorganisms.

Materials and Methods

Bile collection

One litter of goat bile was collected from Kakuri Abattoir in Kaduna State, Nigeria, in the month of March, 2018. Methanol was added to the bile for preservation the bile until needed for further studies.

Extraction of goat bile

The bile was partitioned with chloroform and separated using a separating funnel, the chloroform extract was concentrated *in vacuo* using a *rota vapor* at 40°C and finally air dried to remove all residual chloroform.

Antimicrobial profile (susceptibility test)

The antimicrobial screening was carried out using the agar diffusion method as described by (NCCLS, 2000). Overnight culture of the various bacteria in blood agar were sterilized at 121°C for 15 min. The sterilized media were then seeded with 0.1 mL standard inoculum of the test microorganism. The inoculum was spread evenly over the surface of the seeded media by the use of a sterile swab. By the use of a standard cork-borer of 6 mm in diameter, a well was cut at the center of each seeded medium and 0.1 mL solution of the compounds (10 mg/mL of each) was then introduced into each well on the medium. The inoculated plates were incubated at 37°C for 24 h, after which the plates were observed for the zone of inhibition of growth of microorganisms. The zone was measured with a transparent ruler and the result recorded in millimeters.

Determination of minimum inhibitory concentration (MIC) of the compounds

Minimum inhibitory concentration of the compounds was carried out on the test organisms using the broth dilution method as described by Vollekova *et al.*, 2001. In this method, 10 mL nutrient broth was dispersed into test tubes and sterilized at 121°C for 10 min and allowed to cool. McFarland’s turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared, 10 mL was dispensed into sterile test-tube and the test microbes inoculated and incubated at 37°C for 6 h. Dilution of the test microbes in the normal saline was made until the turbidity matched that of the McFarland scale by visual comparison. At this point the test microbes had a concentration of about 1.5×10^8 cfu/mL. Two-fold serial dilutions of the compounds in the sterile broth was made to obtain concentrations of 100, 50, 25 and 12.5 mg/mL. The initial concentration was obtained by dissolving 0.2 g of the compound in 10 mL of the sterile

broth. Having obtained the different concentration of the compounds in the serial broth, 0.1 mL of the test microbes in the normal saline was then inoculated into the different concentrations of the compounds. Incubation was made at 37°C for 24 h after which each test-tube was observed for turbidity (growth). The lowest concentration of the compound in the broth which shows no turbidity was recorded as the minimum inhibitory concentration.

Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller-Hinton agar was prepared, sterilized and poured into sterile petri. The plates were allowed to cool and solidify. The content of the MIC in the serial dilution were then sub-cultured onto the prepared medium. Incubation was made at 37°C for 24 h after which each plate was observed for colony growth. The MBC/MFC were the plates with lowest concentration of the compound without colony growth (NCCLS, 2000).

Results and Discussion

The zone of inhibition (Fig. 1) of the goat bile against *S. aureus* was 28 mm at 100 mg/ml. The goat bile was also active against *S. typhi* and *E. coli* with a zone of inhibition of 35 and 29 mm, respectively at 100 mg/ml. The growth of *C. albican* was also inhibited with a zone of 25 mm at 100 mg/ml (Fig. 1). The MIC of the bile against *S.aureus* and *S. typhi* was 6.25 mg/ml and 50 mg/ml against *P. aeruginosa* and *E.coli*. The MBC was 25, 50 and 100 mg/ml against *S. typhi*, *S. aureus* and *E. coli*. The MFC of the bile against *C. albicans* was 100 mg/ml (Fig. 2).

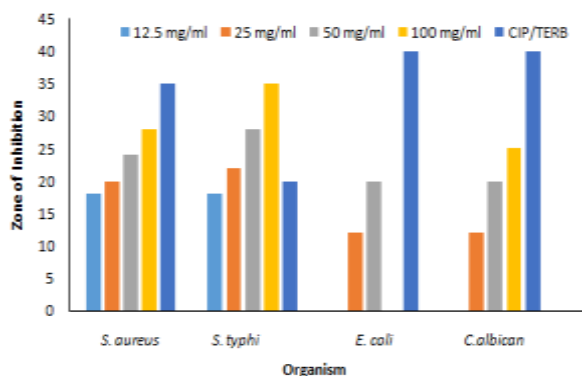


Fig. 1: Results of the determination of zone of inhibition in (mm)

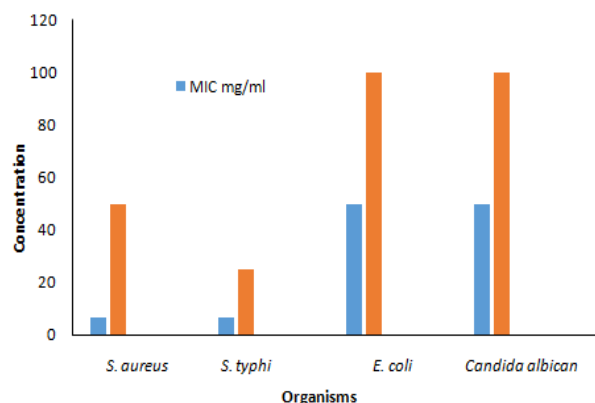


Fig. 2: Minimum inhibition concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of goat bile extract (mg/ml)

The bile extracts had broad spectrum activity in that it inhibited growth of both gram positive and gram negative bacteria. The inhibition zones increased on increasing the concentration of the extract in the discs showing a concentration dependent activity and also varied with the species of bacteria tested. Although the concentrations of the extract were in the range of 100 times more than the standard antibiotic (ciprofloxacin and terbinafin), they showed marked anti-bacterial activity as evidenced by their zones of inhibition.

The highest sensitivity was recorded for *Salmonella typhi* at inhibition zone inhibition of 35 mm with a concentration of 100 mg/ml, MIC of 6.25 µg/mL and MBC of 25 mg/ml. This antibacterial activity could be due to the fact that the bile contains chemical substances which act by complexing proteins and disrupting microbial membranes (Samy and Gopalakrishnakone, 2008). These chemicals can cross the cell membranes, penetrate up to the cytoplasm and interact with intracellular targets critical for antibacterial activity (Trombetta *et al.*, 2005). They are also used to alleviate epilepsy, to relieve cold, influenza, cough and acute bronchial disease (Victor *et al.*, 2005), and this could offer a justification relative to why the bile is used in managing infectious diseases.

Therefore, the concentration of the active components in the extract could be much lower than the standard antibiotic used. It is important to note that, if the active components were isolated and purified, they would probably show higher antibacterial activity than those observed in this study. Over the past two decades it has been discovered that bile acids are potent regulatory molecules and do so via activation of specific nuclear receptors, a G-coupled protein receptor and several cell signaling pathways within the gastrointestinal tract and liver (Lefebvre *et al.*, 2009; Chiang, 2003; Kuipers *et al.*, 2007).

In conclusion, goat bile had significant antimicrobial activity against the selected microorganism and its use in the treatment of infectious disease is justified.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

References

Chen SP 1987. Research on Calculus bovis. *ZhongyaoTongbao*, 12: 59–61.

Chiang JY 2003. Bile acid regulation of hepatic physiology: III. Bile acids and nuclear receptors. *Am. J. Physiol. Gastrointest Liver Physiol.*, 284: G349–G356.

Hofmann AF, Hagey LR & Krasowski MD 2010. Bile salts of vertebrates: structural variation and possible evolutionary significance. *J. Lipid Res.*, 51: 226–246.

Jiangsu New Medical College 1977. Great Dictionary of Chinese Materia Medica. Shanghai: People’s Publishing House of Shanghai.

Kuipers F, Stroeve JH, Caron S & Staels B 2007. Bile acids, farnesoid X receptor, atherosclerosis and metabolic control. *Curr. Opin. Lipidol.*, 18: 289–297.

Lefebvre P, Cariou B, Lien F, Kuipers F & Staels B 2009. Role of bile acids and bile acid receptors in metabolic regulation. *Physiol. Rev.*, 89: 147–191.

Li SZ 1957. Compendium of Materia Medica (Ben Cao Gang Mu) Beijing: People’s Health Publishing House (1596) reprinted 1957.

NCCLS 2002. Performance standard for antimicrobial susceptibility testing, 12th informational supplements. NCCLS document M100-512. Wayne PA: National Committee for Chemical Laboratory Standards.

- Samy RP & Gopalakrishnakone P 2008. Review: Therapeutic potential of plants as antimicrobials for drug discovery. *J. Evidence-Based Complimentary and Alternative Med.*, 10: 1093.
- Tan D, Manchester LC, Reiter RJ, Qi W, Hanes MA & Farley NJ 1999. High physiological levels of melatonin in the bile of mammals. *Life Sci.*, 65: 2523–2529.
- Tao HJ 1986. Records of Famous Physicians (Ming Yi Bie Lu) Beijing: People's Publishing House, (c. 510 CE), reprinted 1986.
- Trombetta D, Castelli F, Sarpietro M, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G & Bisignano G 2005. Mechanisms of anti-bacterial action of three monoterpenes. *J. Antimicrob. Agents and Chemotherapy*, 49: 2474-2478.
- Une M & Hoshita T 1994. Natural occurrence and chemical synthesis of bile alcohols, higher bile acids, and short side chain bile acids. *Hiroshima J. Med. Sci.*, 43: 37–67.
- Victor J, Siebert S, Hoare D & Wyk BV 2005. Sekhukhuneland Grasslands: A Treasure House of Biodiversity. *FAO, Rome*.
- Vollekova A, Košťálová D & Sochorova R 2001. Isoquinoline alkaloids from Mahonia aquifolium stem bark are active against *Malassezia* spp. *Folia Microbiologica*, 46(2): 107-111.
- Wang HQ & Carey MC 2014. Therapeutic uses of animal biles in traditional Chinese medicine: An ethnopharmacological, biophysical chemical and medicinal review, *World J. Gastroenterol.*, 20(29): 9952–9975.