# BIOACTIVE POTENTIALS OF *VITELLARIA PARADOXA* (GAERTN) STEM BARK EXTRACT ON BACTERIAL ISOLATES ASSOCIATED WITH HUMAN INFECTIONS


**BY**

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## ABSTRACT

This study extracted the crude extract from stem bark of *Vitellaria paradoxa*, investigated the antibacterial potentials and mechanisms of action of potent fractions of the extract on susceptible bacterial isolates. It also assessed the phytochemical constituents and antioxidant properties of the plant. This was with a view to tackling problem of multidrug resistance development by microorganisms.

The stem bark of *V. paradoxa* was harvested from Ijagbo, Kwara State, Nigeria, and oven-dried at 40°C using hot-air oven and ground into fine powder. The powdered sample was cold extracted using methanol and sterile distilled water in ratio 3:2 (v/v). The mixture obtained was concentrated *in vacuo* using a rotary evaporator and then lyophilized. The crude extract collected was screened for antimicrobial activity against selected bacterial isolates associated with human infections. The crude extract was later partitioned into fractions using different organic solvents in the order of their polarity. The antimicrobial potentials of the crude extract and along with the fractions were determined using agar-well diffusion method. The active fractions were further partially purified by combination of thin layer and column chromatography. The antimicrobial activity of the resulting samples was tested against the bacterial strains previously used. The rate of killing, protein, nucleotide and potassium leakages were determined using *Staphylococcus aureus* and *Escherichia coli* as representatives of Gram positive and Gram negative bacteria respectively. The most active fraction of the partially purified butanol fraction was analysed using GC-MS.

The stem bark extract of *V. paradoxa* and various fractions obtained from it exhibited varying degrees of antibacterial activities. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, reducing sugar and cardiac glycosides. The minimum inhibitory concentration of the crude extract ranged between 0.545 mg/mL and 2.187 mg/mL while those of aqueous, butanol and ethylacetate fractions ranged between 0.31 mg/mL and 5.00 mg/mL, 0.31 mg/mL and 2.50 mg/mL and 0.31 mg/mL and 2.50 mg/mL respectively. The time kill assay showed that the percentage of the cells killed increased with increasing concentrations of the fractions, as well as, contact time intervals. Leakages of protein, potassium ions and nucleotides followed the same trend observed for killing rate. *Vitellaria paradoxa* extract exhibited 50% inhibition at 0.008777 mg/mL, whereas ascorbic acid used as standard had $IC_{50}$ of 0.078777 mg/mL. The major active constituent of the purified sample was identified as 14-methylhexadecanoic acid.

The study concluded that *V. paradoxa* stem bark extract which possessed antioxidant properties exhibited appreciable antimicrobial activities against the test pathogens.

**Keywords: Bioactive pontentials/ Vitellara paradox/ Stem bark/ Bacterial isolate / Human infections.**

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Folklore medicine in healthcare delivery

Human infections are caused by several aetiological agents which include bacteria, fungi and viruses. These agents vary in their virulence and degree of resistance to available chemotherapeutic agents. Increase in prevalence of multidrug-resistant (MDR) pathogens due to misuse of antibiotics in clinical practices, has generated to a high degree of concern in health care delivery (Lewis and Stanley, 2012). This has now led to an intensive search for newer and more effective antimicrobial agents to deal with these problems. Such agents are now been sourced for from bioactive components of the medicinal plants (Gary, 2003). Several hundreds of plants worldwide present good sources of therapeutic agents and are used traditionally for different purposes, including treatment of bacterial, fungal, and viral infections (Obafemi *et al*., 2006a). Out of 250,000 flowering plants sampled more than 50,000 are used for medicinal purposes (Anthonio and Roland, 2013). Hence, plant products are seen as alternative solutions in treating human infections in developing countries.

Human infections caused by multidrug-resistant pathogens constitute a serious problem for intensive care patients throughout the world (George, 1993). Occurrence of epidemics due to multi-drug resistant microorganisms and emergence of unknown disease causing microbes even at the moment, pose enormous public health concern (Iwu *et al*., 1999). According to an estimate by the Centre for Disease Control and Prevention (USA), 13,300 patients died of antibiotic-resistant bacterial infections in the US during 1992. An incredible 150% increase in the

occurrence of drug-resistant pneumococci was noted between 1987 and 1994, while a twenty-fold increase in the frequency of hospital-acquired enterococci and resistant to vancomycin was seen between 1989 and 1993 (Saswati and Madhab, 2012). The frequency of methicillin-resistant *Staphylococcus aureus* rose from 2% in 1975 to 32% in 1992. By this time, resistance to virtually all the therapeutically useful antibiotics had been evidenced (Saswati and Madhab, 2012).

Plant-derived bioactive compounds have become of great interest owing to their versatile applications (Baris *et al*., 2006). Medicinal plants are of great importance to the health of individuals and communities and thus showing them to contain certain chemical substances that produce a definite physiological action on the human body. Plants can synthesize many different types of secondary metabolites, which have been subsequently exploited by humans for their beneficial role in a diverse array of applications (Balandrin *et al*., 1985). Alkaloids, tannins, flavonoids, and phenolic compounds are the most important of the bioactive constituents of plants (Acamovic and Brooker, 2005). Screening techniques of biologically active medicinal compounds have been conducted on well-known species of plants used in traditional medicine and these plants have shown antimicrobial activity (Arunkumar and Muthuselvam, 2009).

Many of the indigenous medicinal plants are used as spices and food. They are also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 1999; Okwu, 2001). One of the great advantages of medicinal plants is that they are readily available and have very low side effects (Widkar *et al*., 2008). New drugs of herbal origin discovered through ethnopharmacological studies have shown interesting results (Ricardo *et al*., 2004), for example, plant oils containing terpenes have shown increasing promise *in vivo*,

against multiple drug resistant species of bacteria. There is global resurgence in the use of herbal preparations in some developing countries like Nigeria and now it is being gradually integrated into the primary and secondary health care systems (El-Mahmood and Ameh, 2007). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002).

According to the World Health Organisation (WHO), up to 80 percent of the people still rely mainly on traditional remedies for their ailments (Arunkumar and Muthuselvam, 2009). James (2010) stated