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Full Length Research Paper

Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms

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Preliminary phytochemical screening of the stem bark extracts of *Vitellaria paradoxa* revealed the presence of carbohydrates, alkaloids, saponins, tannins and cardiac glycosides. Ethanol, acetone and aqueous extracts of the plant inhibited the growth of pathogenic *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Shigella dysenterie* and *Salmonella typhi* with varying degrees of activity with the ethanol extracts demonstrating the highest activity against all the test organisms. The activity of the extracts increased as the pH was adjusted towards alkalinity, but no significant increase was observed with increase in temperature. The MBC and MIC values ranged between 6.5-200 mg/ml and to some extent comparable to those of the conventional antibiotic chloramphenicol (6.5 mg/ml). There is scientific basis for the use of this plant as a traditional medicine and can therefore be used to source new antibiotic substances for the treatment of various enteric infections.

Key words: antibiotic, enteric infections, extracts, MBC, MIC, pathogenic, phytochemical screening, *Vitellaria paradoxa*.

INTRODUCTION

Enteric bacteria are a huge heterogeneous group of gram-negative rod-shaped bacteria inhabiting the intestinal tract of humans and animals. This group of organisms causes various intestinal and extra-intestinal diseases though some are only commensals. Strains causing infections harbor numerous virulence factors encoded on plasmids, bacteriophages or the bacterial chromosomes within pathogenicity islands (Nester et al., 1998). Antimicrobial resistance among enteric pathogens is becoming a matter of serious concern. This is because antimicrobial resistance leads to therapeutic failures of empirical therapy (Parekh and Chanda, 2007), thus, the need for search of novel antibiotics to which these groups of organisms are yet to develop resistance. It is against this background that we thought it was of interest to study the efficacy of the *Vitellaria paradoxa* leaves and stem back against some pathogenic enteric bacteria.

V. paradoxa (formaly *Butryspermum paradoxum*), (Sapotaceae) is an immensely popular tree with many applications in folkloric medicine. It is commonly called Shea butter (English), Kareje (Fulfulde, Nigeria), Kadanya (Hausa, Nigeria) Koita (Gbagi, Nigeria), Mmameng (Cham, Nigeria). The tree grows naturally in the wild of the dry savanna belt of West Africa, from Senegal in the West to Sudan in the East and onto the foothills of the Ethiopian mountains. *V. paradoxa* is considered a sacred tree by many communities and ethnic groups and plays important roles in religious and cultural ceremonies. It is also believed to have some spiritual protective powers (Pretarious and Watt, 2001; Agbahungba and Depommier, 1989). Different parts of the plant including leaves, roots, seeds, fruit and stem bark have been used in the treatment of enteric infections such as diarrhea, dysentery, helminthes and other gastrointestinal tract infections, skin diseases and wound infections (Soladoye et al., 1989). The bark is used to suppress cough and also to treat leprosy (Ferry et al., 1974). It is rich in oil and together with the oil palm serve as sources of edible oil for many households in many parts of the Sahel Africa, particularly Northern Nigeria (Ndukwe et al., 2007). Fat

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extracts from the kernel of the plant is used extensively in cosmetics and chocolate industries (Nester et al., 1998; Parekh and Chanda, 2007; Pretarious and Watt, 2001; Agbahungba and Depommier, 1989; Soladoye et al., 1989; Dalziel, 1937; Ferry et al., 1974; Ndukwe et al., 2007; Ndukwe et al., 2007). There are however, few reports in the scientific literature concerning the antimicrobial properties of this plant. The work was therefore designed to investigate the antibacterial activity of stem bark extracts of *V. paradoxa* and to determine the phytocostituents present in the plant extracts.

MATERIALS AND METHOD

Plant materials

The stembark of *V. paradoxa* were collected from Sangere village of Girei, Girei Local Government Area, Adamawa State, Nigeria. The taxonomic identity of the plant was confirmed by Mr Bristone Basiri of the Department of Biological Sciences, School of Pure and Applied Sciences, Federal University of Technology, Yola. The plant parts were air-dried under shade to constant weight for 7-14 days and the dried materials were reduced to powdered form using a pestle and mortar and then micronized using an electric blender (Kenwood). The powders were stored in airtight bottles until required.

Extraction and determination of phytoconstituents

Ten grammes (10.0 g) of the powdered plant material was soaked in 100 ml each of distilled water, 95% ethanol and acetone in separate 500 ml sterile conical flasks at room temperature with uniform shaking in a shaker water bath for 48 h. The content was then filtered with a muslin cloth and then Whatman No. 1 filter paper. The filtrates were evaporated to dryness and then packed in separate clean dry bottles and stored at room temperature until required. The extracts were screened for the presence of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones and cardiac glycosides as described by Trease and Evans, (1996) and Parekh and Chanda (2007).

Test Organisms

Eschericia coli, Klebsiella pneumonia, *Proteus mirabilis, Shigella dysenterie* and *Salmonella typhi* were collected in peptone water with the help of the laboratory staff from the Microbiology Laboratory of Specialist Hospital (750 bed capacity) Yola, Nigeria. Preliminary identification of the test organisms were carried out in the hospital laboratory. Biochemical tests to confirm the identity of the organisms were carried out at the Microbiology Laboratory, Department of Microbiology, Federal University of Technology, Yola, Nigeria (Cheesbrough, 2002). The organisms were stored on Nutrient agar slants in a refrigerator (2-8^{o}C). Purity of the cultures was checked at regular intervals as described by Acheampong et al. (1988).

Determination of antimicrobial activity of extracts

The agar well dilution method as described by Lino and Deogracious (2006) was used for this purpose. Standardized inoculum (0.5 McFarland turbidity standard equivalent to 5 x 10^{8} cfu/ml) NCCLS, 1990) of each test bacterium was spread onto sterile nutrient agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (6.0 mm diameter) was used to bore wells in the agar plates. The extracts were prepared to achieve different concentrations of 10, 20, 30, 40, and 50 mg/ml by redissolving in 20% dimethylsulphoxide (DMSO). Subsequently, 0.5 ml of each concentration of the extracts was introduced in triplicate wells earlier bored in the nutrient agar plate cultures. Chloramphenicol (10 mg/ml) (Joabez Corporation) was used as a positive control and 20% nutrient agar as negative control. The plates were then incubated at 37^{o}C for 24 h. Antimicrobial activity of the extracts was determined after incubation period by measurement of zones of inhibition produced against the test bacteria.

Effect of temperature and pH on activity

This was carried out as described by Emeruwa (1982). Briefly 2.0 g of the sample powder was dissolved in 5 ml of distilled water in a test tube and the contents filtered. 1 ml each of the filtrate was pipetted into two separate test tubes. To the first test tube few drops of 1N HCl was added dropwisely until pH 3 was attained, and to the second test tube few drops of 1N NaOH was also added dropwislely until pH 10 was attained. All the extracts were allowed to soak for 30 min after which they were neutralized (pH 7) once again using the appropriate solvents. Another test tube containing extracts only (untreated) was left for the same period (30 min) to serve as control. Antimicrobial activity of both the treated and untreated extracts were then determined as earlier described.

For the effect of temperature on antimicrobial activity, 2.0 g each of powdered plant extracts was dissolved in 6 ml of distilled water and filtered. 2 ml of the filtrate was pipetted into two different test tubes. Each of the test tubes were then treated at 4^{o}C (in the refrigerator) 60^{o}C and 100^{o}C for 30 min respectively using a shaker water bath. Another test tube containing the extracts was left at room temperature (untreated) for 30 min (control). After heat treatment, antimicrobial susceptibility of both the treated and untreated extracts was carried out against the test bacteria as earlier described.

Determination of minimum inhibitory concentration MIC) and minimum bactericidal concentration (MBC)

The MIC of the extracts against the test organisms was determined using the broth dilution method (Sahm and Washington, 1990). Briefly, 1.0 ml of the extract solution at concentration of 200 mg/ml was added to 1ml of nutrient broth to obtain extract concentrations of 200, 100, 50, 25, 12.5 and 6.25 mg/ml in different test tubes. 1 ml of an 18 h culture adjusted to 0.5 McFarland turbidity standard (1.0 x 10^{8} cfu/ml) was inoculated in each test tube. It was then mixed thoroughly on a vortex mixer. The tubes were incubated at 37^{o}C for 24 h. The tube with the lowest dilution with no detectable growth was considered the MIC.

To obtain the MBC, two loopfuls of broth culture were taken from the tubes that showed no growth in the above test tubes used for the MIC determination and were inoculated on to agar plates. The plates were incubated at 37^{o}C for 24 h and observed for possible growth. Concentrations that did not show any growth after incubation were regarded as the MBC.

RESULTS

The result of phytochemical screening of the stem bark of *V. paradoxa* showed the presence of saponins, alkaloids phenolics, tannins, cardiac glycosides and carbohydrates (Table 1). Antimicrobial susceptibility of the extracts (50 mg/ml) against the test organisms showed that in all the three solvents used, the ethanol extracts demonstrat

Table 1. Phytochemical Screening of crude stem bark extracts of *Vitellaria paradoxa*

Phytoconstituents	Ethanol extracts	Acetone extracts	Water extracts
Saponins	+	+	-
Carbohydrates	+	-	-
Alkaloids	+	+	+
Phenolics	-	-	+
Tannins	+	+	-
Cardiac glycoside	+	-	+
Anthraquinones	-	+	-

+ = present; - = absent

Table 2. Antimicrobial activity of extracts of *Vitellaria paradoxa* (ambient temperature)

Organisms	Aqueous extracts	Ethanol extracts	Acetone extracts	Chloramphenicol
Klebsiella pneumoniae	5.0	8.0	7.0	18.0
Proteus mirabilis	5.0	7.0	8.0	23.0
Shigella dysenterie	5.0	7.0	7.0	23.0
Eschericia coli	4.0	6.0	7.0	18.0
Salmonella typhi	-	8.0	8.0	23.0

= no measureable zone of inhibition

Table 3. Effect of pH on antimicrobial activity of *Vitellaria paradoxa* stem bark extracts (50 mg/ml)

Organisms	Acetone extracts		Water extracts		Ethanol extracts		*Ethanol Extracts	*Acetone extracts
	pH 3	pH 10	pH 3	pH 10	pH 3	pH 10		
Klebsiella pneumoniae	27.0	25.0	18.0	18.0	27.0	25.0	27.0	27.0
Proteus mirabilis	28.0	25.0	16.0	20.0	26.0	22.0	26.0	28.0
Shigella dysentriae	26.0	24.0	18.0	14.0	27.0	24.0	27.0	26.0
Escherichia coli	27.0	24.0	12.0	8.0	26.0	18.0	26.0	27.0
Salmonella typhi	28.0	25.0	10.0	10.0	27.0	20.0	27.0	28.0

*Untreated extract pH 4.5, **Untreated pH 4.2.

the highest activity followed by the acetone extracts. Water extracts demonstrated the least activity against all the test bacteria. For the effect of the extracts against the test bacteria, ethanol extracts demonstrated the highest activity (8.0 mm zone diameter of inhibition) against *S. typhi* and *K. pneumonia* and 7.0 mm (zone diameter of inhibition) against *P. mirabilis* and *S. dysenterie,* while the least activity (6.0 mm zone diameter of inhibition) was demonstrated against *E. coli* at 50 mg/ml. For the acetone extracts, the highest activity (8.0 mm zone diameter of inhibition) was demonstrated against *P. mirabilis* and *S. typhi* and 7.0 mm (zone diameter of inhibition) each against *K. pneumoniae*, *S. dysentrie* and *Es. coli.* For the aqueous extracts the most effective concentration (50 mg/ml) demonstrated the activity of 5.0 mm (zone diameter of inhibition) and 4.0 mm (zone diameter of inhibition) each against *K. pneumoniae*, *P. mirabilis* and *S. dysenterie.* The lowest activity (4.0 mm zone diameter of inhibition) was demonstrated against *E. coli.* There was no activity against *S. typhi* (Table 2).

The effect of pH and temperature on antimicrobial activity of the plant extracts are shown in Table 3 and 4. Results showed that adjustment of pH towards alkalinity had slight diminishing effect on the activity of the extracts, and increased temperature generally enhanced the activity. For instance, at pH 3, the ethanol extracts (50 mg/ml) demonstrated the activity of 27.0 mm against *K. pneumonia*, *S. dysenterie,* and *S. typhi*, and 26.0 mm (zone diameter of inhibition) against *P. mirabilis* and *E. coli.* But at pH 10, the activities reduced to 25 (*K. pneumonia*), 24 (*S. dysenterie*), 22 (*P. mirabilis*), 20 (*S. typhi*) and 18 mm (*E. coli*) zone diameters of inhibition (Table 3).

For the effect of temperature, at 4 and 30^{o}C (untreated), the activity of the extracts was not affected with the ethanol extracts demonstrating the activity of 9.0

Table 4. Effect of temperature (°C) on antimicrobial activity of the extracts (50 mg/ml) of *Vitellaria paradoxa*

Organisms	Acetone extracts			Water extracts			Ethanol extracts			*Ethanol Extracts	*Acetone extracts
	4	60	100	4	60	100	4	60	100		
Klebsiella pneumoniae	7.0	10.0	12.0	7.0	7.0	10.0	8.0	10.0	12.0	8.0	7.0
Proteus mirabilis	8.0	10.0	12.0	8.0	7.0	13.0	9.0	10.0	12.0	9.0	8.0
Shigella dysentriae	8.0	10.0	12.0	7.0	7.0	10.0	9.0	10.0	10.0	9.0	8.0
Escherichia coli	8.0	9.0	12.0	8.0	10.0	13.0	9.0	9.0	10.0	9.0	8.0
Salmonella typhi	8.0	9.0	12.0	8.0	9.0	13.0	8.0	10.0	10.0	8.0	8.0

*Untreated extract (ambient temperature 30°C)

Table 5. Minimum inhibitory concentration (MIC) values of the stem bark extract of *Vitellaria paradoxa*

Organisms	MIC (mg/ml)																	
	Aqueous						Ethanol						Acetone					
	200	100	50	25	12.5	6.5	200	100	50	25	12.5	6.5	200	100	50	25	12.5	6.5
Escherichia coli	+	+	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	+
Klebsiella pneumoniae	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+
Proteus mirabilis	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+
Shigella dysentriae	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	+	-	+
Salmonella typhi	+	+	-	-	+	+	+	-	-	-	-	+	+	-	-	+	+	+
Chloramphenicol	-	-	-	-		-												

+ = presence of bacterial growth; - + = absence of bacterial growth

Table 6. Minimum bactericidal concentration (MBC) values of the stem bark extract of *Vitellaria paradoxa*

Organisms	MBC (mg/ml)																	
	Aqueous						Ethanol						Acetone					
	200	100	50	25	12.5	6.5	200	100	50	25	12.5	6.5	200	100	50	25	12.5	6.5
Escherichia coli	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	+	+	+
Klebsiella pneumoniae	+	+	+	-	+	+	+	-	-	-	+	+	-	-	-	+	+	+
Proteus mirabilis	+	+	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+
Shigella dysentriae	+	+	-	-	+	+	-	-	-	-	+	-	-	-	-	+	+	+
Salmonella typhi	+	+	-	-	+	+	+	-	-	-	+	-	+	-	-	-	+	+
Chloramphenicol	-	-	-	-	+	-												

+ = presence of bacterial growth; - + = absence of bacterial growth.

mm (zone diameter of inhibition) each against *E. coli, S. dysenterie* and*, P. mirabilis*; and 8.0 mm (zone diameter of inhibition) against *K. pneumonia,* but as the temperature was raised to 60°C there was no significant change in activity (10.0 mm zone diameter of inhibition each) against all the test organisms. A similar trend was observed with the extracts of all the other solvents (Table 4).

The MIC and MBC values of the extracts against the test organisms ranged between 25-200 mg/ml for the water extracts and acetone extracts and 6.5 - 200 mg/ml for the ethanol extracts (Table 5 and 6). These values were to some extend comparable to those of chloramphenicol even though the antibiotic is in a pure state.

DISCUSSION

Preliminary phytochemical investigations of the stem bark and leaves of *V. paradoxa* revealed the presence of some phytochemical compounds. Phytoconstituents (alkaloids) has earlier been reported in different plants including the stem bark of *Setigera* and *Nauclea latifolia* (El-kheir and Salim, 1980; Tona et al., 1998). These secondary metabolites are linked to antimicrobial activity of the plant material. Drugs contained in medicinal plants are called active principles and these active principles are divided into a number of groups. Carbohydrates present in plants are mostly in the form of pentoses, sucrose and soluble sugars which are intermediate plant constituents

and whose pharmacognostic significance stem from the fact that they combine with a numerous variety of compounds to form glycosides. The presence of glycoside moieties like saponins, anthracene and cardiac glycolsides, some of which are known to structurally resemble sex hormones (oestrogens, gestrogens and androgens) are known to protect against gastric infections caused by enteric pathogens thus justifying the use of this plant in traditional medicine practice. All the extracts from the different solvents demonstrated antimicrobial activity with the ethanol extracts demonstrating the highest activity. Variation in activity among different extracting solvents has earlier been reported (Falodun et al., 2006). Differences in polarity among various solvents have been reported to be accountable for the differences in solubility of plant active principles, hence variation in degree of activity. The water extracts however, demonstrated the least activity against all the test bacteria. When plant materials are grounded in water or the plant cells are damaged, some phenolases and hydrolases are often released and these enzymes might have modulatory effect on the activity of the active compounds in the extract or there may incomplete extraction of the active principles thus explaining the low activity. Traditionally, however crude plant extracts are prepared with water as infusions, decoctions and poultices, therefore it is very unlikely that the herbalist is able to extract all these compounds, which are responsible for the activity observed in acetone and ethanol extracts. Generally, acetone and ethanol showed broader and greater spectra of activity against the tested organisms.

Results also showed that activity of all the extracts were concentration dependent. Similar results have been reported by several researchers. Highest activity was demonstrated by the standard antibiotic chloramphenicol (control). This is because the antibiotic is in pure state and has refined processes that have established it as a standard antibiotic (Prescott et al., 2002).

The organisms used for the purpose of this investigation are associated with various forms of infections; *S. dysentriae* (dysentery), *K. pneumoniae* (pneumonia), *P. mirabilis* (wound infections), *E. coli* (gastrointestinal tract infections) and *S. typhi* (typhoid fever) (Prescott et al. 2002). Results of this investigation therefore have shown that *V. paradoxa* is a potential source of antibiotic substances for drug development for use against this group of enteric organisms. In earlier works however, *K. pneumoniae* was observed to be resistant to the aqueous, methanol and acetone bark extracts of eight plants used for traditional medicine in Paraguay. In this investigation however, ethanol and acetone bark extracts inhibited the growth of *K. pneumoniae* significantly. The ethanol extracts of the stembark was previously found to inhibit the growth of *E. coli* and *P. mirabilis* (Legrand et al., 1988).

Acid and alkaline treatment was carried out in order to slightly simulate the stomach and duodenal conditions and also to predict the condition under which the drug would be more effective, if it is to be formulated for com-mercial purposes and since the plant is also used in the treatment of constipation and diarrhea traditionally. As the temperature of the extracts was increased, their activity also gradually increased. It is assumed that the increase in temperature increases the activity of the bioactive components of the extracts probably due to increased solubility. This explains why traditionally, the extracts are still effective in remedying the ailment they are being used to against; besides the extracts are taken while still warm ensuring effectivity and consequently their continued usage in traditional medicine.

Demonstration of low MIC and MBC values by especially the ethanol extracts is an indication that the phytoconstituents of the plant have therapeutic properties and therefore justifies its traditional medicinal uses.

Conclusion

Results from this study showed the therapeutic activity of *V. paradoxa* against some selected members of the enterobacteriaceae. The plant can therefore be used to manage enteric infections like diarrheal diseases. Toxicological studies, purification and identification of the plant active principles should be embarked upon in addition to investigating its activity on a wider range of bacteria and fungi.

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