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PRELIMINARY PHYTOCHEMICAL SCREENING AND SUSCEPTIBILITY OF BACTERIA PATHOGENS TO WHOLE EXTRACT OF *EVOLVULUS ALSINOIDES* (L.)

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Abstract

Context: Plant based antimicrobial represent a vast untapped source for medicines and further exploration of plant antimicrobial neeto occur. *Evolvulus alsinoides* (L) (Convolvulaceae) is a perennial herb is used in traditional medicine in East Asia, India, Africa and Philippines to cure fever, cough, cold, venereal diseases, azoospermia, adenitis and dementia.

Objective: The objective of this research was to evaluate the antimicrobial activity of the extracts of *E. alsinoides* on some clinical microbial isolates.

Materials and Methods: The ed thanolic and aqueous extracts of the whole plant (leaves and twigs) were analysed for alkanoids, tannins, glycosides, steroids, flavonoids, saponins, volatile oil and resins. The determination of antibacterial activity was done using the agar well diffusion technique. Pure cultures of pathogenic bacteria such as *Bacillus cereus, Staphylococcus aureus, Micrococcus leutus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* were used for antibacterial activity assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results: The ethanolic extract of the plant had MIC values ranging from 16 mg/ml to 512.5 mg/ml. The least MIC was 16mg^{-ml} against *Salmonella typhi* while *Bacillus cereus* and *Staphylococcus aureus* showed the highest MIC of 512.5 mg^{-ml}. In the aqueous extract the MIC ranged between 512.5 to >1025 mg/ml. *Salmonella typhi, Micrococcus luteus* and *Staphylococcus aureus* were not inhibited by the water extract. Phytochemical result showed ethanol to be a better solvent for the extraction of the bioactive agents in this plant which include: glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil.

Conclusion: In this study the gram-negative organisms had the lowest MICs and MBCs. This suggests their higher susceptibility to the extract of this plant. On the basis of the result obtained in this investigation it can be concluded that ethanol extract of *Evolvulus alsinoides* had significant *in vitro* broad spectrum antimicrobial activity.

Keywords: *Evolvulus alsinoides*, Phytochemical screening, Antibacterial activty.

Introduction

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being (Iwu *et al*. 1999). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity. Many plants have tropisms to specific organs or systems in the body. Phytomedicines usually have multiple effects on the body, their actions act beyond the symptomatic treatment of diseases. Plant based antimicrobial represent a vast untapped source for medicines and further exploration of plant antimicrobial need to occur. Human infections particularly those involving microorganisms cause serious infections in tropical and subtropical countries of the world. In recent years, the problem of multiple drug resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken not only to understand the genetic mechanisms of resistance but to develop new drugs especially natural ones (Girish and Satish 2008).

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Evolvulus alsinoides (L) is a perennial herb belonging to the family *Convolvulaceae* with a small woody and branched root stock (Austin 2008). This plant is used in traditional medicine in East Asia, India, Africa and Philippines to cure fever, cough, cold, venereal diseases, azoospermia, adenitis and dementia. It has a known nootropic and anti-inflammatory activity (Singh 2008). Goyal and Singh (2005) reported its use in the treatment of neurodegenerative diseases, asthma and amnesia. Preclinical research has justified its ancient claim as a brain tonic (Singh 2008). Several other uses reported for this plant include its ability to boost memory and improve intellect (Sethiya *et al.* 2009), immuno-modulatory, adaptogenic as well as anti-oxidant properties (Siripurapu *et al.* 2005).

Singh (2008) reported that *E. alsinoides* is used in the Philippines to cure certain bowel irregularities and as a vermifuge and febrifuge. Infusion of roots, stalks and leaves are all used in Nigeria as stomaachics. In addition, the plant is sold in Ghana and Northern Nigeria principally as a charm worn as a girdle or circlet on the arm to procure love or favour (Burkill 1985). Bussman *et al.* (2006) reported that in Kenya (Kwale Province) sores are treated by application of the powdered leaves and in Tanganyinka (Lake province), the powdered leaves are put onto enlarged gland in the neck. The objective of this research was to evaluate the antimicrobial activity of the extracts of *E. alsinoides* on some clinical microbial isolates.

Materials and Methods

Plant sample collection and preparation: The whole plant of *E. alsinoides* used for the investigation was obtained from a private botanical garden in Sokoto, Nigeria. The plant parts; leaves and twigs were air-dried for two weeks. They were ground into powder with a mechanical blender and sieved with a mesh of size 0.5 mm. The powdered samples obtained were thereafter stored in clean brown bottles at room temperature (28 $\pm$ 2ºC) until needed for use.

Preparation of aqueous and ethanol extract: Ninety (90) grams of the powdered whole plant (leaves and twigs) was dispensed in 900 ml of distilled water in an one litre capacity conical flask. The mixture was stirred vigorously intermittently with a magnetic stirrer and then allowed to stand for 48 h. It was stirred again and filtered through a Whatman filter paper lined funnel into a conical flask. The filtrate was evaporated at 40ºC with a water bath to obtain the solid crude extract. The same procedure was carried out for ethanol extraction except that the crude solid extract was obtained by concentrating the filtrate with a rotary evaporator. All extracts obtained were stored in a refrigerator until required for use.

Phytochemical analysis: The extracts of were analysed for alkanoids, tannins, glycosides, steroids, flavonoids, saponins, volatile oil and resins using standard procedures.

To 1 ml of the extract was added 2 ml of acetic acid and then cooled in an ice bath at 4°C. To that mixture 1 ml of concentrated tetraoxosulphate acid (H_2SO_4) was added drop wise. The formation of an oil layer on top of solution indicated the presence of glycosides (Odebiyi and Sofowora 1978). To 3 ml of the extract was added 1ml of 1% HCl. This resulting mixture was then treated with few drops of Meyer's reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids (Ogukwe *et al*. 2004). Five drops of olive oil was added to 2 ml of the plant extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins (Trease and Evans 1996). Two drops of 5% $FeCl_3$ was added to 1 ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins (Trease and Evans 1996). To 1 ml of the extract was added 3 drops of ammonica solution (NH_3^+) followed by 0.5 ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids (Odebiyi and Sofowora 1978). To 1 ml of the plant extract was added 1ml of concentrated tetraoxosulphate acid (H_2SO_4). A red colouration confirmed the presence of steroids (Trease and Evans 1996). To 5 ml of the extract was added 5 ml of copper acetate solution. The mixture was shaken

vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins (Elmahmood and Doughari 2008).

Source of test microorganisms: Pure cultures of pathogenic bacteria such as *Bacillus cereus, Staphylococcus aureus, Micrococcus leutus, Klebsiella Pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. They were gram stained and subjected to biochemical tests to confirm their identity (Cheesbrough 2000). The organisms were subcultured in nutrient agar plates and stored in nutrient agar slants at 4°C until needed for use.

Antibacterial activity assay: The determination of antibacterial activity was done using the agar well diffusion technique (Cheesebrough 2000). The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24 h at 37°C, a loop of inoculum was transferred into 5 ml of nutrient broth, incubated for 2 h at 37°C. This served as fresh suspension inoculum. Wells (5 mm diameter) were made in sterile nutrient agar plate using a sterile cork borer (flame sterilized) and inoculum containing 10^7 CFU/ml of test bacteria were spread on solid plates with the aid of sterile swab moistened with the bacterial suspension. Then 50 µl of aqueous extract or ethanol extract of the whole plant (*E. alsinoides*) were placed in the wells made in inoculated plates. Controls were set up with 50 µl of sterile distilled water or ethanol. The plates were incubated at 37°C for 24 h and zones of inhibition if any around the well were evaluated in millimeters (Girish and Satish 2008).

Determination of minimum inhibitory concentrations (MIC): Determination of the MIC of the extracts was carried out using the tube-dilution technique described by Cheesebrough (2000). A double fold serial dilution was made using Muller Hinton broth (MHB).The following concentrations used were 1025, 512.5, 256, 128, 64, 32, 16 and 8 mg/ml. Equal volume of extract and MHB (2 ml) was dispensed into sterilized test tubes. A quantity (0.1 ml) of standardized inoculum (1.25×10^7 cfu/ml) was added to each of the test tubes which were incubated aerobically at 37ºC for each 24 h. A tube containing broth and inoculum without extract served as organism control. The tube with broth and extract without inoculum served as extract control. The lowest concentration of the extracts which inhibited microbial growth (no turbidity) was recorded as the MIC.

Determination of minimum bactericidal concentration (MBC): Sterile Muller Hinton agar plates were inoculated with samples from each of the test tubes that showed no visible growth from the MIC test. The plates were then incubated at 37ºC for 24 h. The lowest concentration of the extract yielding no growth was recorded as the MBC.

Results

Phytochemical properties: The different phytochemical constituents present in the whole plant extract of *E. alsinoides* is shown in Table 1. It was observed that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity. In the ethanol extract, the phytochemicals present include alkaloids, glycosides, saponins, tannins and flavonoids. Of the phytochemicals assayed for, only two: glycosoides and alkaloids where found in the water extract.

Antibacterial activity: The results of the antibacterial activity of the aqueous and ethanolic extracts of *E. alsinoides* against the test organisms are shown in Tables 2 and 3. The zone of inhibition of the growth of the isolates was found to be a function of the relative antibacterial potency of the extracts. Thus zones of inhibition decreased as the concentration of the extracts decreased (Table 2). At a concentration of 1025 mg/ml the highest zone of clearance was obtained from ethanol extract against *K. pnuemoniae* with a diameter of 38 mm. This was followed by *P. aeruginosa* (33 mm), *Sa. typhi* (30 mm) and *E. coli* (26 mm) respectively. The lowest zone of inhibition at this concentration was 8 mm against *St. aureus.*

Higher growth inhibition was obtained with the ethanol extract compared with aqueous extracts. In Table 2 the antibacterial activity of the aqueous extract of *E. alsinoides* revealed the highest zone of inhibition to be 24 mm against *K. pnuemoniae* compared to 38 mm of ethanolic extract at the same concentration (1025 mg/ml). The bacteria *St. aureus, M. leutus,* and *Sa. typhi* were not inhibited with the water extract and thus showed no zone of inhibition. The lowest zone of clearance in this experiment was 2 mm against *E. coli* using water extract at a concentration of 64 mg^{-ml}.

The ethanolic extract of the plant had MIC values ranging from 16 to 512.5 mg/ml. The least MIC was 16 mg/ml against *Sa. typhi* while *B. cereus* and *St. aureus* showed the highest MIC of 512.5 mg/ml. The MBC values of the ethanol extract ranged between 32 to >1025 mg/ml. The MIC and MBC values of the aqueous extract ranged between 0-1025 mg/ml and 0 to >1025 mg/ml respectively. In the water extract *E. coli* showed the lowest MIC of 64 mg/ml and the highest was 1025 mg/ml against *B. cereus*.

Table 1. Phytochemical characteristics of the whole extracts of *Evolvulus alsinoides*

Phytochemical constituent	Ethanol Extract	Water Extract
Glycosides	+	+
Alkaloids	+	+
Saponins	+	-
Steroids	-	-
Tannins	+	-
Flavonoids	+	-
Resins	-	-
Volatile oil	+	-

Table 2. The antibacterial activities of bacterial isolates of the ethanol (water) extract of *Evolvulus alsinoides*

Conc. (mg/ml)	Zone of inhibition (mm)						
	B. cereus	*St. aureus*	*M. luteus*	*K. pneumonia*	*P. aeruginosa*	*E. coli*	*Sa. typhi*
1025	12 (8)	8 (0)	14 (0)	38 (24)	33 (6)	26 (12)	30 (0)
512	8 (0)	5 (0)	10 (0)	34 (18)	28 (4)	23 (8)	24 (0)
256	0 (0)	0 (0)	4 (0)	30 (15)	20 (0)	18 (6)	21 (0)
128	0 (0)	0 (0)	0 (0)	26 (12)	15 (0)	12 (4)	16 (0)
64	0 (0)	0 (0)	0 (0)	24 (0)	0 (0)	8 (2)	10 (0)
32	0 (0)	0 (0)	0 (0)	10 (0)	0 (0)	0 (0)	8 (0)
16	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (0)

Table 3. The Minimum Inhibitory and Bactericidal Concentrations of both Ethanol and Water extracts of *Evolvulus alsinoides* on bacterial isolates

Isolates	Ethanol Extract		Water Extract	
	MIC	MBC	MIC	MBC
Bacillus cereus	512.5	>1025	1025	>1025
Staphylococcus aureus	512.5	>1025	Nil	Nil
Micrococcus leutus	256	256	Nil	Nil
Klebsiella pneumonia	32	64	128	512
Pseudomonas aeruginosa	128	128	512	1025
Escherichia coli	64	256	64	512
Salmonella typhi	16	32	Nil	Nil

Discussion

The preliminary phytochemical screening carried out showed *E. alsinoides* contain some secondary metabolites such as glycosides, alkaloids, saponins, volatile oil, flavonoids and tannins. In general secondary metabolites present in plants have been reported by Rabe (2000) to be responsible for their therapeutic activity. Singh and Bhat (2003) reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants.

Plant essential or volatile oils and their individual components have been used in traditional systems of medicines for a variety of bacterial infections for centuries. Furthermore, it has been demonstrated that antibacterial properties of these oils can be attributed to their hydrocarbon and terpene constituents (Amit and Shailendra 2006). The presence of glycosides and alkaloids in *E. alsinoides* may be attributed to their use by traditional medicine practitioners in healthcare systems in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases. The results of this research highlights the fact that the organic solvent (ethanol) extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted more or only through the organic solvent medium.

This observation agrees with the report of other investigators of medicinal plants that organic solvents are more suitable for extraction of phytochemicals. (Singh and Singh 2000, Natarajan *et al*. 2005).

Conclusion

Microorganisms vary widely in their degree of susceptibility to anti-microbial agents. A high MIC value indicates low activity and *vice versa*. In this study the gram negative organisms had the lowest MICs and MBCs. This suggests their higher susceptibility to the extract of this plant. On the basis of the result obtained in this investigation we conclude that ethanol extract of *Evolvulus alsinoides* had significant *in vitro* broad spectrum antimicrobial activity. Thus extracts from the plant can be used to control infections caused by *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus.* Opportunistic infections such as bronchopneumonia, bacterial endocarditis and meningitis caused by *Micrococcus* spp. and *Pseudomonas aeruginosa* will also find treatment with the extracts of this medicinal plant. The results obtained in this study justify the use of this plant by traditional medical practitioners.

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