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PHYTOCHEMICAL SCREENING AND SUSCEPTIBILITY OF BACTERIA PATHOGENS TO EXTRACTS OF *Evolvulus alsinoides*

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ABSTRACT

The *in vitro* antibacterial activity of ethanolic and aqueous extract of the whole plant (leaves and twigs) of *Evolvulus alsinoides* was investigated on gram-positive and gram-negative bacteria by agar well diffusion technique. The results indicate that glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil were better extracted in ethanol than water. The ethanolic extract of the plant had MIC values ranging from 16mg/ml to 512.5mg/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 512.5mg/ml to >1025mg/ml. *Salmonella typhi, Micrococcus luteus* and *Staphylococcus aureus* were not inhibited by the water extract.

Key words: *Evolvulus alsinoides*, Phytochemical Screening, Antibacterial, Activity.

INTRODUCTION

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 antimicrobial drugs especially from natural sources (Girish & Satish, 2008).

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 & Singh (2005) reported its use in the treatment of neurodegenerative diseases, asthma and amnesia. Pre-clinical research has justified its ancient claim as 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 *Evolvulus 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 stomaachic. 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 of *Evolvulus alsinoides* 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 *Evolvulus alsinoides* on some clinical microbial isolates.

MATERIALS AND METHODS

Plant Sample Collection and Preparation: The whole plant of *Evolvulus alsinoides* used for the investigation was obtained from a private botanical garden in Sokoto, Nigeria. The plant leaves and twigs were air-dried for two weeks. They were pulverized into powder with a mechanical blender and sieved with 0.50mm mesh. The powdered samples were stored in clean brown bottles at room temperature (28 $\pm$ 2^{O}C) before extraction.

Preparation of Aqueous and Ethanol Extract: Ninety (90) grams of the powdered plant leaves and twigs was dispensed in 900ml distilled water in a 1L conical flask. The mixture was stirred vigorously intermitently with a magnetic stirrer and then allowed to stand for 48 hours. It was stirred again and filtered through a Whatman filter paper No.1 into a conical flask. The filtrate was evaporated at 40 ^{O}C on a water bath to obtain the solid crude extract.

The same procedure was followed for ethanol extraction except that the crude solid extract was obtained by concentrating the filtrate on a rotary evaporator. All extracts obtained were stored in a refrigerator until they were required for analysis.

Phytochemical Analysis: The extracts of *Evolvulus alsinoides* were tested for alkanoids, tannins, glycosides, steroids, flavonoids, saponins, volatile oil and resins using standard procedures (Trease & Evans, 1996).

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 sub cultured in nutrient agar plates and stored in nutrient agar slants at 4^{O}C until they were required for activity assay.

Antibacterial activity assay: The agar well diffusion technique was used for the determination of antibacterial activity**.** (Cheesebrough, 2000).The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24hours at 37^{O}C, a loop of inoculum was transferred into 5ml of nutrient broth, incubated for 2hours at 37^{O}C. This served as fresh suspension inoculum. Wells of 5mm 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 50µl of aqueous extract or ethanol extract of the whole plant (*Evovulus alsinoides*) were placed in the wells made in inoculated plates. Controls were set up with 50µl of sterile distilled water or ethanol. The plates were incubated at 37^{O}C for 24hours and zones of inhibition if any around the well were evaluated in millimeters (mm) (Girish & Satish, 2008).

Determination of minimum inhibitory concentrations (MICS): Determination of the minimum inhibitory concentration (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) to obtain 1025, 512.5, 256, 128,64,32 & 8mg/ml.

Equal volume of extract and Muller Hinton broth (2ml) was dispensed into sterilized test tubes. 0.1ml of standardized inoculum (1.25×10^7 cfu/ml) was added to each of the test tubes which were incubated aerobically at 37 ^{0}C for 24 hours each. 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 minimum inhibitory concentration (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 37^{0}C for 24hours. The lowest concentration of the extract that yielded no growth was recorded as the minimum bactericidal concentration (MBC).

RESULTS

Phytochemical properties of *Evolvulus alsinoides:* The different phytochemical constituents present in the whole plant extract of *Evolvulus 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. The phytochemicals present in the ethanol extract were alkaloids, glycosides, saponins, tannins and flavonoids. Amongst the phytochemicals assayed for, only glycosoides and alkaloids where found in the water extract (Table 1).

TABLE 1. PHYTOCHEMICAL CHARACTERISTICS OF THE WHOLE EXTRACTS OF *E. alsinoides*

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

+ = present , - = Absent.

ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Evolvulus alsinoides:* The results of the antibacterial activity of the aqueous and ethanolic extracts of *Evolvulus alsinoides* against the test organisms are shown in Tables 2, 3, and 4. The zone of inhibition of the growth of the isolates was a function of the relative antibacterial potency of the extracts. Thus zones of inhibition decreased as the concentration of the extracts decreased (Tables 2 and 3). At a concentration of 1025mg/ml, the highest zone of clearance was obtained from ethanol extract against *Klebsiella pnuemoniae* with a diameter of 38mm. This was followed by *Pseudomonas aeruginosa* (33mm), *Salmonella typhi* (30mm) and *Escherichia coli* (26mm) respectively. The lowest zone of inhibition at this concentration was 8mm against *Staphylococcus aureus*

TABLE 2. THE ANTIBACTERIAL ACTIVITIES OF THE ETHANOL EXTRACT OF *E. alsinoides*

Conc. (Mg/ml)	Zone of Inhibition(mm) of bacterial Isolates						
	B. cereus	*S. aureus*	*M. luteus*	*K. pneumonia*	*P. aeruginosa*	*E. coli*	*S. typhi*
1025	12	08	14	38	33	26	30
512.5	08	05	10	34	28	23	24
256	00	00	04	30	20	18	21
128	00	00	00	26	15	12	16
64	00	00	00	24	00	08	10
32	00	00	00	10	00	00	08
16	00	00	00	00	00	00	06
08	00	00	00	00	00	00	00
04	00	00	00	00	00	00	00

TABLE 3. THE ANTIBACTERIAL ACTIVITIES OF WATER EXTRACT OF *E. alsinoides*

Conc. (Mg/ml)	Zone of Inhibition (mm) of Bacterial Isolates						
	B. cereus	*S. Aureus*	*M. luteus*	*K. pneumonia*	*P. aeruginosa*	*E. coli*	*S. typhi*
1025	08	00	00	24	06	12	00
512.5	00	00	00	18	04	08	00
256	00	00	00	15	00	06	00
128	00	00	00	12	00	04	00
64	00	00	00	00	00	02	00
32	00	00	00	00	00	00	00
16	00	00	00	00	00	00	00
08	00	00	00	00	00	00	00
04	00	00	00	00	00	00	00

TABLE 4. THE MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS OF ETHANOL AND WATER EXTRACTS OF *E. alsinoides* ON BACTERIAL ISOLATES.

ISOLATES	ETHANOL EXTRACT		WATER EXTRACT	
	MIC	MBC	MIC	MBC
B. cereus	512.5	>1025	1025	>1025
S. aureus	512.5	>1025	Nil	Nil
M. leutus	256	256	Nil	Nil
K. pneumoniae	32	64	128	512
P. aeruginosa	128	128	512	1025
E. coli	64	256	64	512
S. typhi	16	32	Nil	Nil

Higher growth inhibition was obtained with the ethanol extract compared with aqueous extracts. In Table 3, the antibacterial activity of the aqueous extract of *Evolvulus alsinoides* revealed the highest zone of inhibition of 24mm against *klebsiella pnuemoniae* compared to 38mm of ethanolic extract at the same concentration (1025mg/ml). *Staphylococcus aureus, Micrococcus leutus,* and *Salmonells typhi* were not inhibited with the water extract and thus showed no zone of inhibition. The lowest zone of clearance in this experiment was 2mm against *Escherichia coli* using water extract at a concentration of 64mg/ml.

Table 4 shows the results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Evolvulus alsinoides* on the test bacteria. The ethanolic extract of the plant had MIC values ranging from 16mg/ml to 512.5mg/ml. The least MIC was 16mg/ml against *Salmonella typhi* while *Bacillus cereus* and *Staphylococcus aureus* showed the highest MIC of 512.5mg/ml. The MBC values of the ethanol extract ranged between 32mg/ml to >1025mg/ml. The MIC and MBC values of the aqueous extract ranged between 0-1025mg/ml and 0 to >1025mg/ml respectively.In the water extract *Escherichia coli* showed the least MIC of 64mg/ml and the highest was 1025mg/ml against *Bacillus cereus.*

DISCUSSION

The preliminary phytochemical screening carried out showed *Evolvulus 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 therapeutic activity. Singh & 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 & Shailendra, 2006). The presence of glycosides and alkaloids in *Evolvulus alsinoides* may be associated with their use by traditional medicine practitioners in healthcare systems in the treatment of cough, fever, cold and venereal diseases. The results of this research highlight 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 & Singh, 2000; Natarajan *et al*., 2005).

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. These suggest their higher susceptibility to the extract of *Evolvulus alsinoide*s On the basis of the results obtained in this investigation it was concluded that ethanol extract of *Evolvulus alsinoides* had *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* may also find treatment with the extracts of this medicinal plant.The results obtained in this study justify the use of *Evolvulus alsinoides* by traditional medical practitioners.

CONCLUSION

The results from this work showed that the antimicrobial activity of *Evolvulus alsinoides* can inhibit both gram-positive gram negative organisms. Ffurther work should concentrate on the extraction and characterization of the bioactive compounds of this plant.

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