http://dx.doi.org/10.4314/bajopas.v6i2.14

Bayero Journal of Pure and Applied Sciences, 6(2): 65 - 68
Received: May 2013
Accepted: September 2013
ISSN 2006 – 6996

ANTIBACTERIAL ACTIVITY OF *EUPHORBIA HIRTA AGAINST STREPTOCOCCUS PNEUMONIAE, KLEBSIELLA PNEUMONIAE* AND *PROTEUS VULGARIS*

***Kuta[1], F.A., Damisa[1], D., Adamu, A., Nwoha[1], E. and Bello, I. M.[2]**
Department of Microbiology, Federal University of Technology, Minna.
Department of Biological Sciences, Federal University of Technology, Minna
*Correspondence author

ABSTRACT
This investigation was conducted to determine the in-vitro effect of aqueous, ethanol and methanol crude extracts of Euphorbia hirta at concentrations ranging from 10mg/ml – 100mg/ml against three pathogenic bacteria (Streptococcus pneumoniae, Klebsiella pneumoniae and Proteus vulgaris) using cup plate method. The extracts showed appreciable inhibitory effect (6-11mm and 2-20mm) on Streptococcus pneumoniae and Proteus vulgaris when compared to the positive control (penicillin). Klebsiella pneumoniae was resistant to all the extracts. The minimum inhibitory concentration (MIC) was between 60mg/ml – 80mg/ml on Streptococcus pneumoniae and 60mg/ml-100mg/ml on Proteus vulgaris. The minimum bactericidal concentration (MBC) was between 90-100mg/ml on Streptococcus pneumoniae and 100mg/ml on Proteus vulgaris. Phytochemical analysis revealed the presence of tannins, flavonoids, cardiac glycosides and volatile oils. The result from this preliminary study suggests that the plant contains active compounds that could be used for the development of drug for the treatment of ailments associated with the test organisms. More work needs to be done on the separation and purification of active compounds in the plant extract in order to determine the role of each compound in the crude extracts.
Keywords: Antibacterial activity, Euphorbia hirta, Crude extracts, Bacterial isolates.

INTRODUCTION

Euphorbia hirta belongs to the family Euphorbiaceae. It is a small annual herb common in tropical countries (Sofowora, 1986). The plant can grow to a height of 40cm. The stem is slender and cylindrical, with many branches from the base to the top and often reddish in colour, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lancelet and are usually greenish or reddish underneath measuring about 5cm long, blotched with purple in the middle and toothed at the edge. The flowers of the plant appear very small and cluster around the axial of the leaves. The stem and leaves produce white or milky juice when cut. Capsules are broadly ovoid, hairy, three-angled and about 1.5cm (Lind and Tallanttire, 1971).

The plant is abundant in refuse dump site. It prefers light sandy to medium loamy soil that is well drained. The plant prefers acid, neutral and alkaline soils. It cannot grow on the shade and requires dry or moist soil. It is not tolerant to frost. The ripe seed is released explosively from the seed capsules. Germination of the seed usually takes place within 2-3 weeks at 20^{o}C (Huxley, 1992).

Euphorbia hirta commonly known as asthma weed has traditionally been used in Asia to treat bronchitic asthma, laryngeal spasm and various other lung complaints though in modern herbalism, it is used more in the treatment of intestinal amoebic dysentery. The aerial parts of the plant are harvested at the flowering stage, during the summer and air dried for later use. The stem taken internally is famed as a treatment for asthma, bronchitis and various other lung complaints. The herb relaxes the bronchioles but apparently depresses the heart and general respiration (Kokwaro, 1993). It is usually used in combination with other anti-asthma herbs such as *Grindelia camporum* and *Lobelia inflate* (Kokwaro, 1993). The whole plant is decocted and used in the treatment of athletes foot, dysentery, enteritis and skin conditions. It has been used in the treatment of syphilis (Duke, 1985). The sap from *Euphorbia hirta* is used to suppress warts (Duke, 1985).

In Nigeria, crude extracts of the plant are used as ear drops and in the treatment of boils, sores and promoting wound healing (Anon, 2005). This study was aimed at determining the antibacterial effect of *Euphorbia hirta* on some pathogenic organisms (*S. pneumoniae, Proteus vulgaris, Klebsiella pneumoniae*).

MATERIALS AND METHODS

Plant Collection and Identification

The whole plant was collected from the premises of Federal University of Technology, Minna and was identified by a botanist in the Department of Crop production, school of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State.

Preparation of Plant Material

The fresh whole plant was ground into particles using electrical blender after thorough washing. This was done to enhance the permeability of the extracting solvents into the cells, thus facilitating the release of active ingredients (Iyabo, 1991).

Aqueous Extraction
Thirty (30) grams of the grounded plant material was weighed into 300ml of distilled water in a 500ml conical flask. The mixture in the conical flask was covered with foil paper, shaken in every 30 minutes for 6 hours and was allowed to stand for 72 hours (Akujobi *et al*., 2004). The mixture was filtered using sterile muslin cloth. The filtrate was evaporated to dryness in a steam bath. A sticky dark semi solid substance was obtained. It was weighed and stored in sterile universal sampling bottles and kept in the refrigerator at 4^{o}C for further analysis (Chika *et al*., 2007).

Ethanol Extraction
Thirty (30) grams of the grounded plant material was weighed into 300ml ethanol in a 500ml conical flask. The mixture was shaken at 30 minutes interval, allowed to stand for 72 hours. It was filtered and evaporated to dryness. A sticky dark semi-solid substance was obtained. This was weighed and transferred into universal sampling bottles and were stored in the refrigerator at 4^{o}C for further analysis (Sofowora, 1986).

Methanol Extraction
Thirty (30) grams of the grounded plant material was weighed into 300ml of methanol in a 500ml conical flask. The mixture was shaken, allowed to stand for 72 hours. The mixture was filtered and evaporated to dryness. A sticky dark semi-solid substance was obtained. It was weighed and stored in a sterile sampling bottle and kept in the refrigerator at 4^{o}C for further analysis (Sofowora, 1986).

Preparation of Crude Extracts for Antibacterial Assay
Half of a gram, 0.4g, 0.3g, 0.2g, 0.1g of the crude extracts was dissolved in 5ml of distilled water separately to obtain the following concentrations;100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 10mg/ml. The concentrations were stored at 15^{o}C in sample bottles for further analysis (Kuta *et al.,* 2012)

Source of Test Organisms
Streptococcus pneumoniae, *Klebsiella pneumoniae* and *Proteus vulgaris* were obtained from the bacteriology laboratory of the Federal College of Veterinary and Medical Laboratory Technology, Vom, Jos.

Confirmation of Identity of the Bacteria Isolates
The bacteria isolates were identified using the API system and characterized by examining the colonial morphology through gram's reaction, followed by biochemical test such as motility, indole, catalase , citrate utilisation, sugar fermentation (Kuta *et al.,* 2012).

Standardization of inoculums
The procedure described by Macfarland was adopted (Akujobi *et al*., 2004).

Evaluation of the Antibacterial Activity of the Extracts
Cup plate method was used for the study. Twenty millilitre (20ml) of nutrient agar was dispensed into sterile Petri dishes and allowed to set. The plates containing the media were kept in the oven for 30 minutes at 40^{o}C. Wire loop was used to smear the surface of the agar in the plates with standardized inoculum. A cork borer with a diameter (5mm) was used to bore 5 wells on the media in the plates. Different concentrations (5ml) of the plant extract were introduced into the wells on the media. The plates were incubated for 24-48 hours and the results were recorded (Kuta *et al.,* 2012).

Determination of Minimal Inhibitory Concentration (MIC)
Two millilitre nutrient broth was sterilized in five test tubes and was allowed to cool to 45^{o}C then 0.5ml of different concentrations (10-100mg/ml) of the crude extract was introduced into the test tubes containing the broth. 10^{6} cfu/ml of the organisms, 0.1ml was also added in the test tubes containing the broth and was incubated at 37^{o}C for 24 hours.

Determination of Minimum Bactericidal Concentration (MBC) of the Extracts
A loopful of broth from the test tubes used for minimum inhibitory concentrations (MIC), particularly the test tubes that showed no visible growth and was sub-cultured onto fresh nutrient agar plates, the plates were incubated at 37^{o}C for 24 hours (Kuta *et al* ., 2012).

Phytochemical screening
Screening for phytocompounds such as tannins, cardiac glycosides, flavonoids and volatile oil was conducted and result recorded.

RESULTS
All the concentrations had activity on the test organisms ranging from 2mm-16mm, except *Klebsiella pneumonia* (Table 1). The minimum inhibitory concentration (MIC) of the aqueous, ethanol and methanol crude extracts of *Euphorbia hirta* against *S. pneumoniae, K. pneumoniae* and *Proteus vulgaris* ranged from 60mg/ml-100mg/ml (Table 2). The minimum bactericidal concentration (MBC) of the aqueous, ethanol and methanol crude extracts of *Euphorbia hirta* on *S. pneumoniae, K. pneumoniae* and *Proteus vulgaris* ranged from 100mg/ml-160mg/ml. The Phytochemical components of the crude extracts of *Euphorbia hirta* include; Tannins, Cardiac glycosides, flavonoids and volatile oil.

Table 1: Result of activity of the crude aqueous, ethanolic and methanolic extract of *Euphorbia hirta against S. pneumoniae, K. pneumoniae and Proteus vulgaris*

Test organism	Conc. of extract (mg/ml)	Aqueous extract	Ethanolic extract	Methanolic extract	Penicillin (control)
Streptococcus pneumoniae			Zone of inhibitions in mm		
	100	-	11	10	28
	80	-	10	9	26
	60	10	8	9	26
	40	10	8	8	24
	10	9	7	6	18
Klebsiella pneumoniae	100	-	-	-	31
	80	-	-	-	26
	60	-	-	-	25
	40	-	-	-	23
	10	-	-	-	20
Proteus vulgaris	100	9	16	15	20
	80	7	16	12	18
	60	-	11	12	16
	40	-	11	10	16
	10	-	2	-	06

Key: mg/ml - concentration of the crude extract and penicillin (control).

Table 2: Minimum Inhibitory Concentrations (MIC) of the crude (aqueous, ethanol and methanol) extracts on the test organisms

Test Organism	Aqueous extract (mg/ml)	Ethanol extract (mg/ml)	Methanol extract (mg/ml)
S. pneumoniae	80	60	60
Proteus vulgaris	100	60	80

Key: mg/ml - concentration of the extract.

Table 3: Minimum bactericidal Concentration (MBC) of the crude (aqueous, ethanol and methanol) extracts on the test organisms

Test Organism	Aqueous extract (mg/ml)	Ethanol extract (mg/ml)	Methanol extract (mg/ml)
S. pneumoniae	140	120	120
Proteus vulgaris	160	100	140

Table 4: Phytochemical component of *Euphorbia hirta* crude (aqueous, ethanol and methanol) extracts

Phytochemical component	Detected
Tannins	+
Cardia glycosides	+
Flavonoids	+
Volatile oil	+

Key: + present

DISCUSSION

In this study, the results obtained from the antibacterial investigation of the crude (aqueous, ethanol and methanol) extracts of *Euphorbia hirta* revealed that the extracts had activity against *Streptococcus pneumoniae,* and *Proteus vulgaris* except *Klebsiella pneumoniae*. Although the activity of the crude extracts of the *Euphorbia hirta* cannot be compared to that of synthetic antibiotic (penicillin) (Table 1). It will not be out of point to say that the crude extracts had appreciable activity on the test organisms. The resistance by *Klebsiella pneumonia* against the crude extracts of *Euphorbia hirta* as observed in this study is in conformity with the earlier report by Afolabi *et al.(* 2007). Reason to explain the resistance exhibited by *Klebsiella pneumoniae* in this study could be due to the polysaccharide based capsule surrounding the cell of the organism.

Streptococcus Pneumonia and *Proteus vulgaris* were more susceptible to ethanol and methanol crude extracts compared to aqueous extract (Table 1). This could be attributed to the extraction capability of the two solvents (ethanol and methanol). Previous reports by Junaid *et al*. (2005) revealed that tannins and polyphenols are more soluble in alcohol and are active against cell wall synthesis , it could be the reason for the inhibitory or cidal effects of the alcoholic crude extracts (Table 1). The outcome of this study agreed with the report by Sofowora (1986) who identified alcohol as the best extraction solvent for antimicrobial investigations.

The inability of the aqueous extract to have significant activity on the test organisms (Table 1) could be explained by the fact that when ground plant tissues are in aqueous medium, phenolases and hydrolases are released by the tissues. These enzymes have effect on the active components of the plant extracts. Akpata (1977) reported same in his study. Therefore, this may have been the reason why the aqueous crude extract was not active against the test organisms.

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained in this study (Table 2 and 3) showed that the MIC values were low compared to values for MBC. This could be attributed to low concentration of active compound. Similar result has been reported by Lansing *et al*. (2005) who asserted that variation in the values of MIC and MBC could occur due to low amount of active compounds contained in the crude extract.

The Phytochemical components identified in the crude extracts (Table 4), showed that extracts contain Tannins, cardiac glycosides, flavonoids and volatile oil. This has corroborated the previous reports. Several studies (Jones *et al*., 1997 and Chika *et al*., 2007) reported that Tannins, flavonoids, glycosides and essential oil have irreversible activity which could lead to inhibition of cell wall or cell membranes synthesis. The presence of the above mentioned phytochemical components could be used to justify the effect of the crude extracts on the test organisms in this study. The result of this study suggest that the crude extract of *Euphorbia hirta* contains active compounds that could be used for the treatment of ailments associated with the test organisms. However, further investigation should be conducted on the extracts to ascertain the chemistry of action of the active compounds and their toxicity.

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