Evaluation of the antimicrobial and cytotoxic effect of *Dendranthemaindicum* (L Desmoul) leave extract

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Abstract

Hexane, chloroform, ethyl acetate and ethanol extracts of *Dendranthema indicum* leaf were studied for their cytotoxic effect and antimicrobial activities against seven human pathogenic bacteria *Shigella dysenteriae, Salmonella typhi, klebsiella pneumonia, Staphylococcus aureus, proteus mirabilis, Escericia coli and pseudomonas aeriginosa,*) using disc diffusion method. All the extracts exhibited moderate to good antibacterial activity against all the pathogens tested *except proteus mirabilis* and *Salmonella typhi*, The chloroform extract exhibited the largest zone of inhibition (20 mm) against *Staphylococcus aureus.*The ethyl acetate extracts exhibited the highest lethality on brine shrimp larvae at LC 50 values 211.6. (LC 50) were determined at 95% confidence intervals by analyzing the data on a computer loaded with "Finney Programme."

Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used as medicine in different countries and are a source of many potent and powerful drugs (Chandra, 2013), For centuries, many plant compounds have an outstanding role in medicine. Their pharmacological and economical values have lost nothing to its importance until date. They are either used directly or after they have been subjected to certain chemical modification processes. These plants which are medicinal in nature however contain bio active compounds (Sasidharan *et al.*, 2010) that over the years have been exploited in ayurvedic medicines for the treatment of various ailments. Indeed, indigenous plants play an important role in the treatment of many diseases and 80% of the people worldwide are estimated to use herbal remedies. However, few data are available on their safety (Nguta *et al* ., 2010). The prevalence of bioactive components such as tannins, terpenoids, flavonoids, alkaloids, steroids etc. underscores the

needs for continuous search for bioactive and active principles from plant, but some of these active ingredients become obsolete because of drug resistance (Ojiako, 2014).

The brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24 h (Krishnaraju *et al.*, 2006). The commercial availability of inexpensive brine shrimp eggs, the low cost and ease of performing the assay make brine shrimp lethality assay, a very useful bench-top method (McLaughlin *et al* 1991). A number of studies have demonstrated the use of the brine shrimp assay to screen plant extracts (Sleet and Brendel 1983 ,Harwing and Scott 1971 and Nguta *et al* 2011). It has been demonstrated that activity against Artemia salina Leach (Artemiidae) larva correlates well with cytotoxic activity (Meyer *et al* 1982), as well as other pharmacological activities (Solis *et al* 1993).

Chrysanthellum indicum also known as "Dunkufe" among the Hausas (Kano) is a faintly aromatic branching annual herb which belongs to the family of composite (Dalziel and Hatchinson 1976). The leaves are depurative while the flowers are apereint and bitter. The Hausas (Kano) use the plant in the treatment of fever and it is used among the Chinese to treat eye ailments . In conjunction with black pepper, it is used in the treatment of gonorrhoea (Carvalho, 2005)., the flowers are also used in the treatment of scrofula, deep rooted boils, inflammation of the throat eyes and cervix, eczema and itchiness of the skin. An essential oil obtained from the plant contains chryssanthenone, which is active on the brain centre affected by parkinson's disease (van and Heuvelink 2006).

In the current study, results of biological screening of the methanol,ethyl acetate, chloroform and hexane extracts of leaves of *D. indicum* for lethality towards Artemia salina larvae and its antimicrobial activity against pathogenic bacteria are presented.

Experimental

Plant material

The plant material was collected fresh from the field in federal college of education Kano, Nigeria. Taxonomical identification was done at the Herbarium of the Biological Science Department Bayero University Kano and at herbarium unit of Biological Science Department Ahmadu Bello University Zaria and and its voucher specimen with number 676 deposited there.. The plant was air-dried under shade, segregated and pulverized by mechanical pounding using wooden mortar and pestle. The pulverized plant material was stored away from moisture until needed.

Extraction of plant materials

The pulverized leaves of Dendranthema indicum (500 g) was carefully weighed and macerated with 95% ethanol for one weeks. The extract was decanted, filtered and labelled. The process was repeated three times for exhaustive extraction. The three sets of extracts were combined on confirmation by TLC. The combined ethanol extract was partitioned with hexane, chloroform and ethyl acetate. The extracts were concentrated in vacuum at 40˚C using rotary evaporator and later subjected to air drying to give dried crude extracts.

Brine Shrimp Lethality Test (BST):

The extracts were evaluated for lethality on brine shimp larvae as described by Adoum ,2009. Solution of instant sea salt was made by dissolving the salt (2.86 g) in distilled water (75ml), Artenia salina (heach) eggs (50mg) were added in a hatching chamber. The hatching chamber was kept under an inflorescent bulb for 48 hrs for the eggs to hatch into shrimp larvae. Each test extract (20 mg) was separately dissolved in methanol (2 ml) from which 500µl, 50µl, and 5µl of each solution was transferred into vials corresponding to 1000, 100 and 10µg/ml respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control containing 500µl of solvent (methanol) were allowed to evaporate to dryness in about 48 hrs at room temperature. The instant ocean sea salt solution (4.5 ml) was added to each vial and 10 larvae of Artenia salina (taking 48 – 72 hrs) after initiation of hatching were added to each vial, the final volume of the solution in each vial was adjusted to 5ml with the salt solution, immediately after adding shrimps 24 hrs later, the number of surviving shrimps at each dosage was counted and recorded, LC50 values were determined with 95% confidence interval before analyzing the data on a kintech AT-compatible computer loaded with a “Finney Programme.”

Antimicrobial studies

The antimicrobial activities of the extracts and standard drug (Ciprofloxacin) were determined using microbial strains obtained from the Department Microbiology, Bayero university Kano, Nigeria .The test microorganisms used are *Shigella dysenteriae*, *Salmonella typhi*, klebsiella pneumonia, *Staphylococcus aureus, ,proteus mirabilis, Escericia coli and pseudomonas auriginosa*,. The disc diffusion method of Anonymous (1996) and Mahesh and Satish (2008) was used to determine the antibacterial activity of the test extracts. . The micro-organisms were grown overnight at 37°C in Mueller-Hinton Broth (Oxoid, England) at pH 7.4. Different concentration of the extracts (1000 µg / ml) was prepared by reconstituting with Dimethylsulphoxide (DMSO). The test microorganisms were seeded into respective medium

by spread plate method. 10 µl with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extract were placed on test organism-seeded plates, Cyprofloxacine 5 mg/mL used as positive control and DMSO used as negative control. The antibacterial assay plates were incubated at 37^0 C for 24h. The diameter of the inhibition zones were measured in milimeters (mm).

Results and Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Recently there has been considerable interest in the use of plant material as an alternative method to control pathogenic microorganism (Aqil et. al., 2005) and many components of plants products have been reported to be specially targeted against resistant pathogenic bacteria (Nostro et. al., 2006). The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover,; high cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India (Gopalakrishna Sarala et. al., 2010).Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. Many reports are available on the antiviral, antibacterial antifungal, anthelmintic, antimolluscal and anti inflammatory properties of plants (Mahesh and Satish, 2008). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use.The present work was a done to explore the cytotoxic effect and antimicrobial property of *D. indicum* .Extracts obtained from *D. indicum* leaves were found to be cytotoxic except hexane extract .The ethyl acetate extract was the most cytotoxic followed by ethanol and then chloroform ,these extracts have exhibited high lethality on brine shrimps at LC50 values 211.6, 217.4 and 327.1 µg/ml, respectively (Table 1). This result validates the popular uses of the plant in the treatment of ailments such as fever , eye ailments, and gonorrhoea. The result of the antimicrobial activity of the extracts is shown in Table 2. The extracts exhibited appreciable activity against five out of the seven tested bacterial . The results were compared with standard antibiotic drug. The extracts exhibited good antibacterial against all the pathogens tested except *Pr.mirabilis and S. typhi.* Chloroform extract exhibited the highest zone of inhibition (20 mm) against *S.aureus and* the hexane extract exhibited the lowest zone of inhibition (7 mm) against *K. pneumonia.*

The plant based products have been effectively proven for their utilization as source for antimicrobial compounds. For instance, ethanol extracts of *D indicum* exhibited inhibitory

activity against *S.aureus, Escericia coli and pseudomonas aeriginosa,* (Wang et al 2013),the highest activity was recorded against *S.aureus.*. The findings in this work is also in agreement with that of Liu et al.,(2005) who reported the antimicrobial activity of the Extracts of Forsythia suspensa and in there work the inhibition activity by the extract *Dendranthema indicum,*of *D. indicum* was stronger than by the extract of *F. suspensa* . The broad spectrum exhibited by the extracts as shown in the antimicrobial results lend credence to the traditional uses of the plant in folk medicine.

Table 1: Brine Shrimp Lethality Test (BST):

Extract	LC_{50}
Hexane	> 1000
Chloroform	327.1
Ethyl acetate	211.6
Ethanol	217.4

Table 2: Zones of Inhibition (mm) of extracts and standard drug

Test organism	HE	CL	EA	ET	Ciprofloxacin
S. aureus	10	20	16	13	37
Sh .dysenteriae	10	18	12	14	39
S. typhi	-	-	-	-	41
K. pneumoniae	7	10	14	12-	40
E.coli	9	18	14	17	32
Pr.. mirabilis	-	-	-	-	-
Ps. aeruginosa	8	10	16	18	-

Key: HE = Hexane extract, CL= chloroform extracts, EA = Ethyl acetate extracts, ET = ethanol extracts, - =No activity

Conclusion

The extracts of *D. indicum* were found to be active on most of the test microorganisms. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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