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Full Length Research Paper

In vitro antimicrobial activity of some phytochemical fractions of *Euphorbia pulcherima* L. (Poinsettia)

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The antimicrobial activities of aqueous and ethanolic extracts of flower, leaf, stem and whole plant of *Euphorbia pulcherima* were evaluated against bacteria and fungi by agar disc diffusion and macro broth dilution techniques. This was with the aim of substantiating the ethno medicinal use of the plant as anti-typhoid. The bacterial isolates showed sensitivity at discs concentrations of 1000 µg/disc to 1000 µg/disc. High sensitivity was shown by *Salmonella typhi* and *Escherichia coli* 0157:H7 on aqueous and ethanolic extracts as well as whole plant, but least on flower extract. The fungal isolates showed sensitivity at disc potencies of 2000 µg to 10,000 µg. High sensitivity was shown by *Aspergillus niger* to aqueous extract of leaves. Low sensitivity was shown by *C. albicans*. The minimum inhibitory concentration (MIC) for ethanol extract of leaves and the whole plant was 0.10, 100 and 10 mg/ml on *S. typhi*, *S. paratyphi* and *E. coli* 0157:H7 respectively. The values for flower ethanolic and aqueous extracts where 100 and > 1000 mg/ml respectively. Leaves and stem extracts contain active compounds against the test organisms. But the ethanolic extracts of flower were specifically antifungal on *A. niger* and *Trichophyton tonsurans*. The minimum bactericidal concentration (MBC) test showed that the active fractions acted by bacteriostatic effect. The chemotherapeutic potential of the plant may be due to the presence of tannins, resins, steroids, glycoside, alkaloids, reducing sugars and sapoinins. Further biotechnological study on the pharmacological values of the plant is thus recommended.

Key words: *Euphorbia pulcherima*, phytochemicals, antimicrobial, antitrichophyton, bacteriostatic.

INTRODUCTION

The increasing technological skills in chemical, pharmacological and microbiological researches nowadays, has paved the way for efficient screening of higher plants for active compounds. Many of the photochemicals have unequivocally been established to be efficious in the chemoteraphy of infectious diseases (West et al., 2004; Mukhtar and Tukur, 2000). As the result of the fact that the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens, the world health organization is progressively approving the treatment of many infectious diseases with herbal remedies. However, such plant products must be those with established high potency, safely, efficacy, reliability and availability (Iwu et al., 1999). This is also by recollecting the fact that, throughout the history, pure compounds, natural products, either as pure compounds or as standardized plant extracts; provided unlimited opportunities for drug leads.

To keep out potential invaders, plant produces a wide range of selective antimicrobial compounds either in a constitutive or inducible manner (Cuilel, 1994). Among these compounds several low molecular weight protein or peptides with antimicrobial activity have been isolated in recent years (Colombo and Bosisio, 1996). Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents.

Therefore researchers are turning attention to folk medicine, looking for new leads to develop better drugs against microbial infections especially from plant sources (Benkebila, 2004). *Euphorbia pulcherima L.* (Poinsettia)

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also known as “Christ flower” was among the common herbs put to use as concoction against ailments resembling typhoid and gastro-enteritis by the people living around Kano Nigeria (verbal communication). However, there is scantly literature on the active components of the plant supported with recently published microbiological findings. It was for this purpose that the present study was carried out to substantiate the traditional claim that the plant would be of value in chemotherapy.

MATERIALS AND METHODS

Collection and identification of the plant materials

E. pulcherima (Poinsettia) was collected from the Botanical Garden of the Department of Biological Sciences, Bayero University, Kano Nigeria and identified at the herbarium of the same Department with the help of keys from Encyclopedia of flowering plant (Novak, 1966).

Extraction of plant material

The leaves, stem, inflorescence and whole plant were separately collected, washed gently and thoroughly with distilled water and dried at room temperature. This was finely powdered using a clean mortar and pestle and sieved to get a fine powder. 100 g of each it was soaked in 100 ml sterile distilled water or 1000 ml of 95% ethanol for 2 weeks with constant shaking at regular intervals. The solution was then filtered and the solvent were evaporated using Rotor evaporator (R 110) at 40°C and extracts were stored in a refrigerator at 4°C until needed for further analysis (Adoum et al., 1997).

Determination of physical properties of the plant fractions

The colour of fractions was visually assessed immediately after the removal of the solvent by rotary evaporation process. Texture was felt manually with the help of glass rod and feeling of the particulate nature of the resultant fraction in between the fingers (Adoum et al., 1997). The pH was tested using Jean way pH meter, while the conductivity was measured using conductivity meter (Mukhtar and Huda, 2005).

Phytochemical screening

Test for reducing sugars

1 ml of the ethanol extract was diluted to 3.0 ml of distilled water followed by addition of Fehling’s solution (A+B) and the mixture warmed to 40°C in water bath. Brick red precipitates at the button of the test tube indicated the presence of reducing sugars (Brain and Turner, 1975).

Test for tannins

2 ml of the ethanol extract was diluted to 3.0 ml with distilled water in a test tube, two drops of 5% ferric chlorides solution was added. A dark green or blue coloration indicates the presence of tannins (Cuilel, 1994).

Test for steroid glycosides

2 ml of the ethanol extracts was evaporated to dryness. The residue was dissolved in 2.0 ml acetic acid and was added by the side of the test tube. A brownish ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer indicates the presence of steroids (Cuilel, 1994).

Test for resins

The method reported by Tanaka et al. (2002) was used. 2 g of the ethanolic extract was dissolved in 10 ml of acetic anhydride. One drop of concentrated sulphuric acid was added. Appearance of purple colour, which rapidly changed to violet, indicated the presence of resins.

Test for flavonoids

2 g of the ethanol extract was dissolved in 50% methanol by heating. Magnesium metal and 5 to 6 drops of concentrated hydrochloric acid were added. Appearance of red colour indicated the presence of flavonoids.

Test for alkaloids

1.0 ml of the ethanol extract in 2 separate test tubes, 2 to 3 drops of Dragendoff’s and Meyer’s reagents were separately added. An orange red precipitate/turbidity with Dragendoff’s or white precipitate with Meyers reagent denoted the presence of alkaloids (Cuilel, 1994).

Test for saponins

500 mg of the powder was taken in a test tube. 5 ml of water was added and vigorously shaken. A persistent froth that lasted for at least 15 min indicated the presence of saponin.

Test organisms

Clinical isolates of bacteria namely *Salmonella typhi* and *Salmonella paratyphi* as well as fungal isolates namely: *Aspergillus niger, Candida albicans* and *Trichophyton tonsurans* were obtained from Pathology Department of Aminu Kano Teaching Hospital and Murtala Muhammad Teaching Hospital, while the *Escherichia coli* 0157:H7 were obtained from the stock cultures of the Microbiology Laboratory, Bayero University, Kano, Nigeria. All the isolates were maintained under appropriate environmental conditions on their respective media at 4 to 8°C until required.

Culture media

Muller Hinton Agar (Biotech), Sabroud dextrose agar (Lab M.) and nutrients broth media (Lab M) were prepared according to the manufactures guide. These were used as growth media in the test.

Sensitivity disc preparation

Whatman No. 1 filter paper disc of 6.0 mm diameter were punched and sterilized by autoclaving. The extracts of the plant were impregnated to arrive at a potency of 100, 1000, 2000, 5000 and 10,000 µg/disc using dimethyl sulphuroxide as diluents.

Table 1. Some physical characteristics of different extracts of *Euphorbia pulcherima*.

Extract	Colour	Texture	Solvent	pH	Conductivity (uscm)
Leaf	Dark green	Solid	Water	5.92	2.45
Leaf aqueous	Dark green	Solid	Water	5.68	4.23
Stem aqueous	Dark green	Solid	Water	5.12	2.1
Whole plant aqueous	Dark green	Solid	Water	5.62	2.84
Inflorscence ethanol	Green	Gummy	DMSO	6.43	0.07
Leaf ethanolic	Dark green	Gummy	DMSO	6.11	0.11
Stem ethanol	Green	Gummy	DMSO	7.07	0.10
Whole plant ethanol	Green black	Gummy	DMSO	6.29	0.15

DMSO = Dimethyl sulfuroxides.

Table 2. Some phytochemical agents isolated from various parts of ethanolic extract of *Euphorbia pulcherima*.

Part of plant	Tannins	Resins	Steroid glycoside	Alkaloids	Reducing sugar	Saponin
Whole plant	+	-	+	-	+	+
Stem	+	+	-	+	+	+
Leaves	+	-	-	+	+	+
Flower	-	+	+	+	+	+

Standardization of inoculums

An overnight broth culture of the test organisms was used to prepare bacterial inoculums and for fungi, the colonies were taken from the sabrouds dextrose agar culture of the organisms. Inoculums preparation of about 3.2 x 10^6 cfu/ml population density was arrived at by appropriate dilutions of the culture in 0.85% NaCl (w/v) to match with the standard turbidity of 1% barium sulphate suspension (Mukhtar and Tukur, 2000).

Susceptibility testing

Agar diffusion method was employed (Mukhtar and Tukur, 2000; Mukhtar and Okafor, 2002). The growth of media (Saborauds dextrose and Mueller Hinton (agar plates) were dried in a drier for about 10 min to remove excess surface moisture. The plates were aseptically inoculated with the test of organisms by streaking method. With the aid of sterile pair of forceps impregnated paper disc containing the extract of *E. pulcherima* parts at different concentration were arranged radically and pressed firmly to the inoculated agar surface to ensure even contact. Each disc was sufficiently spaced-out and kept at least 15 mm from the edge of the plate to prevent overlapping of zones. This process was repeated for the replicate plates and the plate were allowed pre-diffusion time of 15 min. Control test were done on the same plate by placing a standard antibiotic disc of ciprofloxacin (30 µg) for bacteria and fluconazole (25 µg), ketaconazole (40 µg) for fungi and disc containing only dimethyl sulphur oxide (DMSO) on the inoculated plate. Plates were incubated 35°C aerobically for 18 h for bacteria and 24 to 48 h for fungi. Diameters of zone of inhibition were measured using meter rule and recorded in milliliter.

Determination of minimum inhibitory concentration (MIC) of the extracts

Macrobroth dilution technique was adopted in which various drug concentrations (1.0, 100, 1000, 2000, 5000 and 10,000 mg/ml) were prepared in DMSO and sterile distilled water. A series of 9 ml nutrient broth was arranged in a rack and inoculated with 3 loops full of the test organism followed by the addition of 1.0 ml of specific concentration of the test agent or the control drugs as the case may be. The cultures were incubated aerobically at 37°C for 18 to 24 h. Uninoculated tube without any drug and another with only drug were also incubated as control.

Determination of minimum bactericidal concentration of the plant fractions (MBC)

Subcultures on nutrient agar by pour plate method were prepared from the MIC test culture tubes without growth using nutrient agar by pour plate techniques. This was incubated at 35°C for 18 h (Mukhtar and Tukur, 2000).

RESULTS AND DISCUSSION

Table 1 shows the physical parameters of aqueous and ethanolic extract of *E. pulcherima* (flower, leaf, stem and whole plant). The colour of the leaf stem and whole plant was dark green while that of the flower was simply greenish. Texture was powdery (dry) for the aqueous extracts and water soluble gummy (sticky) for all ethanolic fractions being soluble in dimethylsulphoside (DMSO). The pH range between 5.0 and 12.0 for stem aqueous extract to 5.92 for inflorescence aqueous extract, with conductivity ranging from 2.1 to 4.23 µscm respectively. Table 2 shows the phytochemical compounds present in *E. pulcherrima* the whole plant indicated presence of tannins which was absent in the flower. Resins were only detected in the stems and

Table 3. Antimicrobial activities of various extracts of *Euphorbia pulcherima*.

		Zone of inhibition on test organisms (mm)					
Fraction	**Extract potency (µg)**	***S. typhi***	***S. paratyphi***	***E. coli 0157:H7***	***C. albicans***	***Aspergillus niger***	***Trichophyton***
Ethanol leaf	5000	10.0	9.0	8.0	8.0	0.00	10.00
	10000	12.0	13	12.0	14.0	8.00	14.00
Ethanol leaf	5000	10.00	12.00	8.00	0.00	0.00	0.00
	10000	14.00	13.00	9.00	0.00	0.00	0.00
Whole plant ethanol	5000	8.00	16.00	8.00	8.00	0.00	8.00
	10000	9.00	17.00	9.00	9.00	10.00	11.00
Aqueous leaf	5000	8.00	0.00	13.00	12.00	14.00	0.00
	10000	10.00	0.000	7.00	0.00	0.00	0.00
Stem aqueous	5000	8.00	0.00	8.00	0.00	0.00	0.00
Whole plant aqueous	2.000	9.00	0.00	8.00	0.00	0.00	0.00
Inflorescence aqueous	2000	00	00	0.00	0.00	10.00	0.00
	5000	00	00	0.00	0.00	10.00	10.00 etoh
Ciprofloxacin control	10.000	00	00	00	00	12.00	13.00 etoh
	30.00	21	14	22	NA	NA	NA
Ketpnazole control	35	NA	NA	NA	NA	NA	NA
Fluconazole	25	NA	NA	NA	NA	NA	NA

NA –not applicable as control, *aqueous flower extract, *etoh-ethanolic flower extract.

inflorescence. Steroids glycosides were observed in whole plant and inflorscence. However, alkaloid was common to stem, leaves and flower. Reducing sugar and saponins were deducted from all the parts of the plant. Table 3 shows the result of antimicrobial activities of aqueous sand ethanolic extract *E. pulcherima* on some bacterial and fungal clinical isolates. The minimum inhibitory concentration and the minimum bactericidal concentrations of the fractions are presented on Tables 4 and 5 respectively. High inhibitory effect was exhibited against *S. typhi* and *E. coli* 0157:H7 by aqueous and ethanolic extract at disc potencies of 5000 to 10000 µg, with zones of inhibition ranging from 8.0 to 14.0 mm. Additionally, the whole plant extracts were active at 2000 to 5000 µg, with 9.0 to 10.0 mmzone of inhibition diameters respectively. Inflorescence extract did not show any observable antibacterial effect. The fungal isolates especially *A. niger* and *T. tonsurans* showed sensitivity even to inflorscence extract at discs potencies of 2000 and 5000 ug of sensitivity respectively. *C. albicans* was apparently insensitive. The minimum inhibitory concentration (MIC) for ethanolic extract of leaves and the whole plant was0.10, 100 and 10.0 mg/ml on *S. typhi*, *S. paratyphi* and *E. coli*

Table 4. Minimum inhibitory concentration (MIC) of various extract of *Euphobia pulcherima* on pathogenic bacteria.

Fractious	*Salmonella typhi* (MIC; mg/ml)	*Salmonella paratyphi* A (MIC;mg/ml)	*E. coli* 0157:H7 (MIC;mg/ml)
Flower ethanolic	100	100	100
Leaves ethanolic	0.10	100	10.0
Whole plant ethanolic	0.10	100	10.0
Stem ethanol	10.0	1.0	10.0
Flower aqueous	>1000	100	>1000
Leaves aqueous	1.00	100	10.0
Whole plant aqueous	1.00	1.0	10.0
Stem aqueous	10.0	1.0	10.0

Table 5. Minimum bacterial concentration (MBC; mg/ml) and extracts *Euphorbia pulcherima*.

Fractious	*Salmonella typhi*	*S. paratyphi* A	*E. coli* 0157:H7
Flower ethanolic	>100	>100	>100
Leaves ethanolic	>0.10	>100	>10.0
Whole plant ethanolic	>0.10	>100	>10.0
Stem ethanol	>10.0	>1.00	>1000
Flower aqueous	1000	>100	>100
Leaves aqueous	>1.00	>100	>100
Whole plant aqueous	>1.00	>1.0	>10.0
Stem aqueous	>10.0	>1.0	>10.0

> = Values greater than the MICS (Table 3) showed bactericidal concentrations were very high indicating that the inhibitory effect of the plant was perhaps due to bacteriostatic mechanism rather than "cidal action.

0157:H7 respectively. The values were however higher for inflorescence ethanolic and aqueous extracts with 100 and > 1000 mg/ml respectively (Table 4). Leaves and stem extract contain active compounds against the test organism (Tanaka et al., 2002) while all the fractions of the flower were active against *A. niger. T. tonsurans* was the only fungus shown to be sensitive to the ethanolic extract of the inflorscence. There was no observable effect on *C. albicans*. Considering the result of MIC the ethanolic extracts of whole plant and leaves was found to be the most potent on *S. typhi,* in which is minimum inhibitory concentration was found at concentration of 0.1 mg/ml. This can be considered as a scientific basis (Rojas et al., 2003) that reflects the idea of traditional healer for using this plant for curing of typhoid like fever even though this is an *in vitro* study. Among the fungi isolates high sensitivity was shown by *A. niger* to aqueous extract of leaves, while all extract excepting the ethanol extract of the inflorescence shows little activity on *T. tonsurans* and *C. albicans.* The inflorscence may possess greater value as antifungal and by considering a report on such infections (Mukhtar and Huda, 2005) the minimum bactericidal concentration was not ascertained since bacteria were re-grown from the initial MIC tubes, hence the minimum bactericidal concentration were very high, indicating that the inhibitory effect of the extract of *E. pulcherima* was perhaps due to bacteriostatic mechanism rather than "cidal" action. The minimum bactericidal concentration (MBC) test showed that the active fractions *Salmonellosis* and the emerged *E. coli* 0157:H7 gastroenteritis and dermatophytic fungi tested in this investigation may be due to the presence of tannins, resins, steroids, glycosides, alkaloids, reducing sugars and saponins. Further biotechnological study on the pharmacological values of the plant is thus recommended.

Conclusion

The present study showed that various extracts of parts of *E. pulcherima* (Poinsettia) had antimicrobial activity on *S. typhi, S. paratyphi A, E. coli* 0157:H7, *C. albicans T. tonsurans* and *A. niger*. Hence this provides a scientific basis that reflects the idea of traditional healers for using this plant for curing of typhoid like fever as well as some dermatophytic and perhaps systemic fungal infections.

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