INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 6, p. 75-80, 2014

RESEARCH PAPER OPEN ACCESS

Antifungal, antioxidant and antibacterial activities of *Calotropis procera*

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Key words: *Calotropis Procera*, latex, antimicrobial, antioxidant, antifungal.

http://dx.doi.org/10.12692/ijb/5.6.75-80 Article published on September 17, 2014

Abstract

Calotropis procera is a species of flowering plant. Traditionally, the plant has been used as an antipyretic and analgesic agent. The dried leaves used as an expectorant, and as anti-inflammatory agent for the treatment of paralysis and rheumatic pains. In the present study, the milky part of *Calotropis procera* was studied in local area (Township, Surrani and Bakka khel) of district Bannu KPK, Pakistan in order to investigate its anti-oxidant, antifungal, antibacterial properties. We reported that the milk of *Calotropis procera* contain high amount of Barium (1579 ppm) and Potassium (109 ppm) in the plant located in township. The *Calotropis procera* latex posses antibacterial activity and inhibited the growth of *salmonella* (0 %), *Shigella* (17.5%), *E. coli* (18.1%). Furthermore it also inhibited the growth of *Aspergillus Niger* (75%), *Aspergillus fumigates* (12.5%), *and Aspergillus flavus* (37.5%). It also showed small amount of anti-oxidant activity in dose dependent manner.

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Introduction

Plants contain a wide variety of free radical scavenging molecules and hence are rich in antioxidant activities (Hertzog *et al.* 1992). Antioxidants are effective free radical scavengers which tend to retard or prevent the oxidation of other molecules by capturing free radicals (Breton, 2008). Plant compounds have various biological activities such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic, antibacterial, antifungal, antiviral antimutagenic and antiallergic activities (Ikken *et al.*,1999., Noguchi *et al.*,1999; Mishra *et al.*, 2009). These biological activities may be associated with their antioxidant activity (Chung *et al.*,1998). Antioxidant compounds help delay and inhibit lipid oxidation, and they play an important role in the maintenance of health and prevention of several diseases. The best way to help prevent diseases is consumption of an optimal diet containing natural antioxidants. When these constituents are added to foods they tend to minimize rancidity, retard the formation of toxic oxidation products, help maintain the nutritional quality and increase shelf life (Fukumoto and Mazza, 2000). The consumption of food such as fruit, vegetables, red wines and juices helps protect the body from being afflicted with diseases such as cancer and coronary heart disease. This protection is due to the capacity of antioxidants in the plant foods to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids.

Aromatic and medicinal plants are known to fabricate certain bioactive molecules which respond to other organisms in the environment, inhibiting bacterial or fungal growth (Khan *et al.*, 2010). The substances that can inhibit microbes and have little or no toxicity to host cells are considered candidates for rising new antimicrobial drugs. Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Scientific experiments since the late 19th century have documented the antioxidant properties of some spices, herbs, and their components. Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms (Khan *et al.*, 2009).

The current investigation was aimed to assess the antibacterial, antifungal and antioxidant potential of *Calotropis Procera*.

Material and method

Collection of plant

*Calotropis procer*a was collected locally from Bannu Township, Khyber Pukhtun Khwah Pakistan. It was accurately identified by taxonomist, dept of Botanical Sciences UST, Bannu. Then Plant stem was thoroughly washed and latex was collected in Falcon tube.

Preparation of Plant Extract

Calotropis procera latex was dried in Oven at 60 C°. 5 gm of dried Calotropis procera latex was dissolved in 5 ml dimethylsulphoxide (DMSO). Then sub solutions of 200 mg/1ml were prepared from stock. Antifungal drug TERBINOFINE (Saffron Pharma) was used as a standard. Same procedure was adapted for the preparation of Terbinofine sub solutions. DMSO was used as a negative control and 1 ml was directly used from the DMSO flask.

Antifungal assay

First SDA media was prepared by taking 3.5 g of SDA and dissolving in 50 ml distill water in conical flask, whose mouth was sealed with cotton plug and aluminum foil. Now the media, test tubes (12), wire loop etc were completely sterilized by autoclaviour. Then media was poured in Test tubes and Slants were prepared in Laminar flow cabinet. Test tubes were labeled as N_1 for *Niger* inoculant with plant extract, N_2 for duplicate as another *Niger* inoculant with plant extract and N_3 for *Niger* inoculant with Anti fungal. Similar steps were carried out for *Flavus* as F_1, F_2 and F_3 representing plant extract, Duplicate and Antifungal respectively. Similarly A_1, A_2, A_3 for fumigates. In 3 test tubes DMSO was poured with media to check their growth, as DN, DF, and DA representing *Niger*, *Flavus* and *Fumigates* respectively. Now all the test tubes containing media

in slant form were measured by Ruler, as total media was 8 cm.

Now all the test tubes were kept in Incubator at 37 C° for 24 hour.

Antibacterial assay

For antibacterial assay, agar well diffusion assay method was used, described by Holder and Boyce (1994).

Preparation of media (nutrient broth and nutrient agar)

Nutrient broth medium was prepared by dissolving 1.5 g of nutrient broth and 3 g of agar in 125 ml of distilled water; pH was adjusted 7.0 and the solution was mixed well by using magnetic stirrer and then media was sterilized by autoclaving at 121 C for 15 mins.

Preparation of seeded agar plates

Nutrient medium was prepared by dissolving 1.5 g Nutrient Broth and 3 g of agar in 125 ml of distilled water; pH was adjusted to 7.0 and the medium was autoclaved. Petri plates were prepared by pouring 25 ml of nutrient agar and allowing it to solidify. 3 wells were made per plate using a sterile cork borer under aseptic conditions.

Using a micropipette, 120 µl of test solution (from sub solution in epindroff) was poured in respective wells. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of clear zones of inhibition was measured with a ruler. And so antibacterial activity of C. *procera* latex was determined against three bacterial strains by comparing zone of inhibition.

Antioxidant or DPPH (1, 2-diphenyl-2-picrylhydrazyl) radicals Scavenging assay

The DPPH assay was performed according to the standard procedure of Gyamfi *et al.* (1999) with slight modifications. The fresh stock solution was prepared by dissolving 3mg DPPH into 100 ml of methanol and then stored at 20°C. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 (±0.02) at 517 nm using the spectrophotometer. A 900µl aliquot of this solution was mixed with 100µl of the plant extract at varying concentrations (50, 100, 150, 200, 250 µg/ml in respective solvent). The solution in the test tubes were shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was noted at 517 nm. The EC50 of scavenging activity was calculated from the percentage of DPPH radical scavenged with the equation:

$$\text{Scavenging effect (\%)} = \left[\frac{(\text{control absorbance} - \text{sample absorbance})}{(\text{control absorbance})} \times 100\right]$$

IC50 value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid standard was applied as positive reference. Each extract was assayed in duplicate.

Results

Antifungal Assay

The antifungal activity was determined by measuring the diameter of growth zone recorded. DMSO extracts of *Calotropis procera* latex were found to have antifungal activity. As *Calotropis procera* latex shows potent antifungal activity against *Aspergillus niger* showing diameter of growth zone viz. 2 cm while *Calotropis procera* latex showed similar antifungal activity against *Aspergillus Flavus* showing diameter of growth zone viz. 7 cm and *Calotropis procera* latex showed similar antifungal activity against *Aspergillus Fumigates* showing diameter of growth zone viz. 6 cm. The present study thus *Calotropis procera* latex is effective against fungal infections caused by *Aspergillus Niger*, and somewhat against *Aspergillus fumigates*, and *Aspergillus flavus*. The results are illustrated in given Figure No 1.

Antibacterial assay

For assay first juvenile culture was prepared in test tubes (10ml) which shows maximum activity. Juvenile culture activity was checked by turbidity method. Petri plates were prepared by pouring 25 ml of nutrient agar and allowing it to solidify. Now

Bacterial cultures from those juvenile cultures were inoculated on petriplates in zigzag manner. 3 wells were made per plate using a sterile cork borer under aseptic conditions. Using a micropipette, 120 µl of test solution (from sub solution in epindroff) was poured in respective wells. *Calotropis procera* latex was tested against different bacterial cultures to determine and investigate their antibacterial potential. We observed that the crude DMSO extract of *Calotropis procera* showed significant antibacterial activities against all the tested bacterial strains. Maximum activity was conferred against *Eschershia Coli* (4mm) and *Shigella* (3.5mm) while minimum was observed against *Salmonella* (0 mm) which mean inhibition zone diameter.

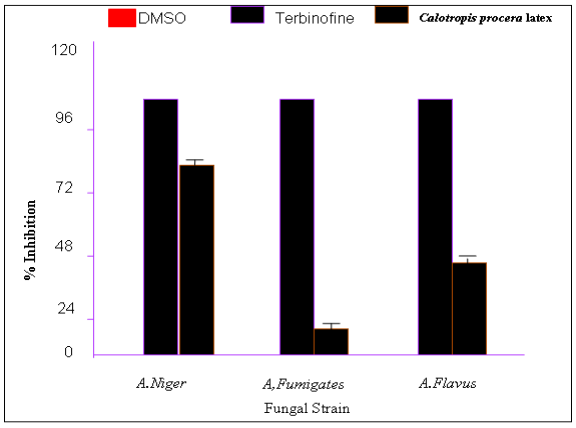

Fig. 1. Inhibition of fungal stain by *Calotropis Procera* Latex.

The antibacterial activity was taken on the basis of diameter of zone of inhibition, which was measured after 24 hours of incubation.

The results of antibacterial assay are in the Figure No 2:

Antioxidant assay

Our result found that *Calotropis Procera* methanolic extract (CPME) was effective at reducing the stable radical DPPH, indicating that CPME is active in DPPH radical scavenging. CPME showed significant scavenging effects with increasing concentrations in the range of 50 to 500 µg/ml. Ascorbic acid was used as a reference one with scavenging DPPH free radical in increasing concentration in the range of 50 to 1000 µg/ml. The % scavenging of both are shown in the Figure No 3.

Discussion

Our results showed antifungal potential of the *Calotropis Procera* latex and its various fractions against three fungal species: *Aspergillus fumigates, Aspergillus flavis* and *Aspergillus Nigar* with different potencies. These fungi are major cause of liver, lungs, mouth, blood and skin infections (Pelczar *et al;* 2006 Willey *et al;* 2008). The DMSO fraction was found most potent against fungus *Aspergillus Niger,* followed by the plant DMSO extract in case of *Aspergillus flavus*. The DMSO fraction was found devoid of any effect against *Aspergillus fumigates* fungal strains.

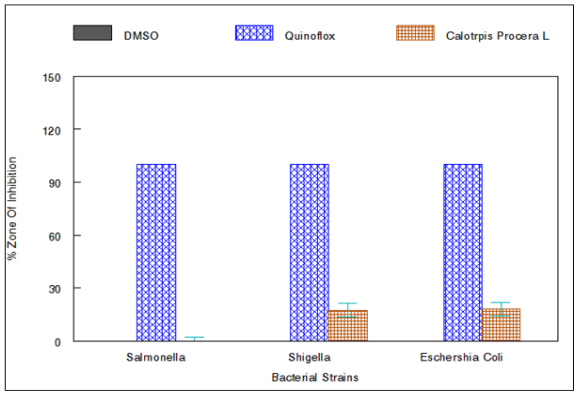

Fig. 2. Zone of inhibition of *Salmonella, Eschershia Coli and, Shigella* in the presence of *Calotropis Procera* Latex.

Calotropis Procera latex from stem samples displayed bactericidal effect against the pathogenic bacteria. Skin, ear, soft tissues, respiratory and urinary tracts are the common bacterial infections caused by these species (Brooks *et al;* 2010) DMSO fraction of *Calotropis Procera* latex was found most effective in inhibiting the *Escherichia coli*, while the the growth of *Shigella* was also inhibited somewhat. *Calotropis Procera* latex DMSO fraction showed no effect against *Salmonella T.*. Radicals (often referred to as free radicals) are atoms, molecules or ions with at least one unpaired electron in the outermost shell, and are capable of independent existence. Free radicals are highly reactive due to the presence of unpaired electron(s). Oxidative stress is defined as an imbalance between radical-generating and radical scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. These state results in an excess of free

radicals, which can react with cellular lipids, proteins, and nucleic acids, leading to local injury and eventual organ dysfunction. Humans have evolved with antioxidant systems to protect against free radicals. Total antioxidant capacity (TAC) of tested substance indicates its ability to prevention of oxidation reactions in exactly defined conditions. Thus, methods to evaluate free radicals scavenging activity of plant extract have been employed by measuring the extracts ability to stabilize a synthetic free radical in a polar organic solvent such as methanol at room temperature over a period of time.

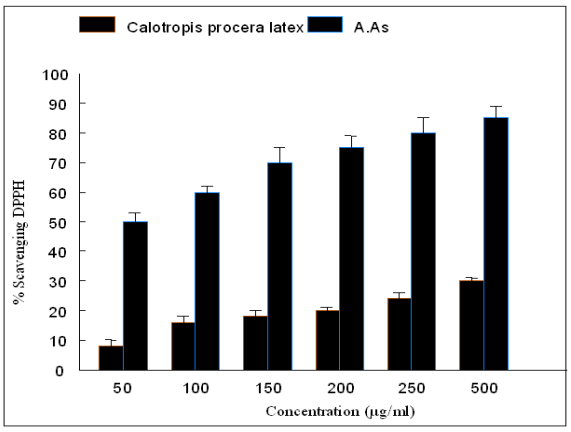

Fig. 3. %Scavenging of *Calotropis procera* methanolic extract (CPME) VS Ascorbic.

The aim of this research was to investigate the antioxidant activity of methanolic extract of *Calotropis Procera* latex from their ability to scavenge synthetic DPPH free radicals. Our research data discovered that *Calotropis Procera* latex methanolic extract (CPME) possesses potent DPPH scavenging ability at levels comparable to ascorbic acid.

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