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Anticancer and Antibacterial Activity of *Calotropis procera* Leaf Extract

Hassan Swed Alzahrani[1*], Mohamed Mutwakil[1], Jamal Sabir[1], Kulvinder S. Saini[1], Walied M. Alarif[2] and Mohamed R. Rizgallah[3]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.
[2]Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, P.O. Box 80207, Jeddah 21589, Saudi Arabia.
[3]Faculty of Education, UMM Al-Qura University, P.O. Box 715, Makkah 21955, Saudi Arabia.

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ABSTRACT

In this study, we investigated the anticancer and antimicrobial potential of Calotropis procera, a medicinally important plant found in Asia. Leaves of C. procera were extracted with methanol and characterization was performed using FTIR and UV-VIS spectrophotometry. The extract was investigated for its anticancer activity against MCF7 breast cancer cell line by performing MTT assay as well as for antibacterial activity against methicillin resistant Staphylococcus aureus (MRSA) by performing disc diffusion test. Methanolic fraction of C. procera proved effective against the MCF7 cell line and inhibited the growth of MRSA significantly. Our results reveal the importance of the metabolic fraction of leaves of C. procera in inhibiting the growth of the MCF7 cell line and its potential as an effective antimicrobial agent.
KEYWORDS: C. procera, FTIR, UV-VIS, MRSA.

INTRODUCTION

Breast cancer is the most lethal form of cancer in women worldwide [1]. It has been ranked second just behind cervical and lung cancer. Almost 30 % of breast cancer incidences are reported to be due to genetic aberrations in interleukin-18 [2,3], p53, BRCA1 and BRCA2 genes [4]. Nevertheless, toxic chemicals in the environment such as polycyclic aromatic hydrocarbons (PAHs), obesity [5] also play an important role in increased incidences [6]. However, recent advances in detection techniques and improved treatment regimens have kept the mortality rate constant over decades. Still, there is a lack of efficient treatments for advance stage, metastatic breast cancer. This necessitates the development of new therapies for advanced stage disease [7].
Last few decades saw an increase in the discovery of anti-microbial natural products against multiple infections. Additionally, studies are being carried out to discover natural compounds effective against multidrug resistant organisms. This is due to the ineffectiveness of current available compounds against these multidrug resistant organisms. Increased demand for natural products further increases the importance of natural compounds. The risks posed by multidrug resistant pathogens could be overcome by isolation and identification of natural compounds having a broad spectrum of effectiveness against numerous pathogenic bacteria. In this regard *Calotropis procera*, a traditional medicinal plant has also been investigated to identify compounds having antibacterial activity against multidrug resistant microbes [8].
Various compounds derived from medicinal plants have documented anti-cancer properties with almost negligible side effects [9-11]. Apart from that, epidemiological studies also indicate that utilization of natural products [12], rich in anti-oxidants could help ameliorate or in some cases, prevent cancer progression [13]. *Calotropis procera* belonging to the family Asclepidaceae is a known medicinal plant [14] used traditionally against various diseases [15]. Extracts obtained from leaves and bark from plants are used against dermatological and bronchial infections [16]. The aqueous solution obtained from the bark of *Calotropis procera* showed effectiveness against bronchial irritation in animal models [17]. Additionally extracts from aerial parts have commonly been used for treating joint pain, fever, muscular spasm and constipation in Saudi Arabia. Ethanolic extract of *Calotropis procera* has been studied for its antipyretic, analgesic, antibacterial, anti-inflammatory, purgative [18], and as relaxant [19]. Fractions obtained using various solvents such as n-hexane, 1-butanol, ethyl acetate, chloroform and water have substantial anti-inflammatory activity [20]. The root bark is effective against dysentery and diarrhea [21]. Additionally the ethanolic extract of roots has been

***Corresponding Author:** Hassan Swed Alzahrani, Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

investigated for antifertility in albino rats [22]. Latex present in *C. procera* has traditionally been used for treating dermal infections, poison, ulcer, spleen enlargement, worms and inflammation [23]. Moreover, latex shows mild toxicity towards the heart and liver [24]. Protiens such as laticifer [25], and osmotin present in latex also exhibit anti-fungal [26], antimycoplasmal [27], anti-inflammatory [23], insecticidal [28], larvicidal [29], antioxidant [30], and anticancer [31] activities. In this preliminary study, we investigated the anticancer and antibacterial potential of *C. procera* leaf extract against MCF7, breast cancer cell line and Methicillin resistant *Staphylococcus aureus* (MRSA) respectively.

MATERIALS AND METHODS

Extraction

Leaves of *Calotropis procera* obtained from the outskirts of Jeddah region, Saudi Arabia were a shade dried and ground. Ground leaves were then subjected to methanolic extraction using soxhlet apparatus.

UV-VIS and FTIR Spectroscopic analysis

Total extract obtained was analyzed using UV-VIS and FTIR spectrophotometer for proximal analysis. The Sample was diluted 1:10 in DMSO and scanned at 200-1100 nm for UV-VIS analysis. Afterwards, to observe peaks and their corresponding functional groups FTIR analysis was performed on the same samples using the ATR technique of the Thermo scientific ATR-FTIR instrument.

Cell Culture

Breast cancer cell line, MCF7 obtained from King Fahd Medical Research Center, King Abdualaziz University, Jeddah, Saudi Arabia, were cultured in DMEM (high) with 10 % FBS and 1 % PS at 37^{o}C.

Cytotoxicity assay

To perform MTT assay 5000 cells/ well were cultured in 96 well plates and incubated overnight. Latter, 5, 10 and 25 µl/ml of *C. procera* total extract was added to each well and incubated for 48h. Subsequently, the media were removed and 100µl of fresh media was added. Cytotoxicity assay was performed according to manufacturer's instructions. Briefly, each well was incubated with 10µl of MTT solution for 4h. Latter 50 µl of DMSO was added and the plates were further incubated for 10 min, afterwards absorbance was checked at 540nm using a microplate reader.

Antibacterial assay

The Disc diffusion method was performed to evaluate the antibacterial activity of *Calotropis procera* extract. Methicillin resistant *Staphylococcus aureus* (MRSA) obtained from the East Jeddah Hospital was grown in Muller hinton agar medium for 24 h. After 24 h, 50 µl of *Calotropis procera* extract was added and pictures were obtained at 0, 24 and 48 h of treatment to evaluate the antibacterial activity of *Calotropis procera* extract. Vancomycin and oxacilline were used as positive and negative controls respectively.

Statistical analysis

Data obtained was represented as mean ± standard error. One-way ANOVA was used to measure significance with $p < 0.05$ considered significant.

RESULTS

FTIR and UV-VIS spectra of *Calotropis porcera* total extract

The FTIR spectrum of the crude extract of *Calotropis porcera*, as shown in figure 1. Since a large number of organic compounds are usually contained in a crude extract, the functional groups present in any compound may contribute to the peaks observed in the FTIR spectrum. In the present case, the peaks present at 3400, 1651, 900 and 650 cm-1 correspond to the –OH, aromatic ring stretching symmetric, aromatic out-of- or siccoring deformation of $–CH_2$, and alkyl halide, respectively. Whlile figure 2 shows the UV-VIS spectrum of *Calitropis procera* extract. In the UV region, a cut-off of the signals was observed which might be due to the usage of glass UV-cuvette of high concentration of the extract. A single absorption peak at 350nm was the indication of the presence of a conjugated alkene in the sample.

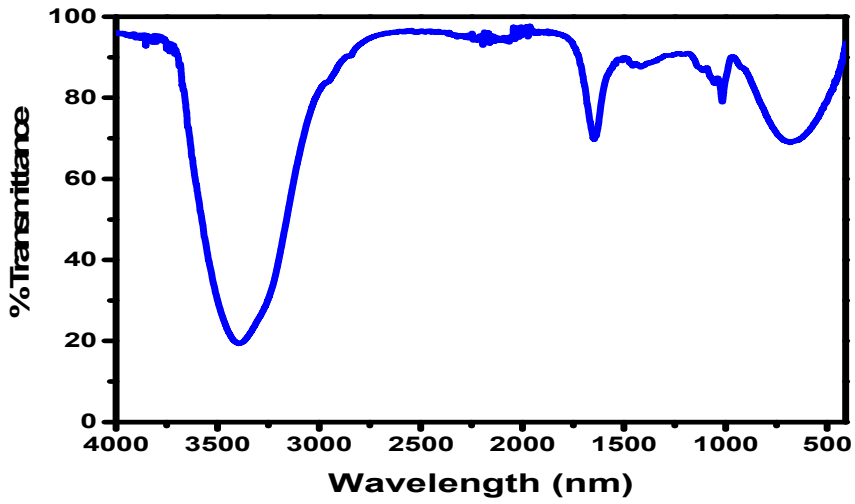

Figure 1: FTIR spectra of *Calotropis procera* total extract.

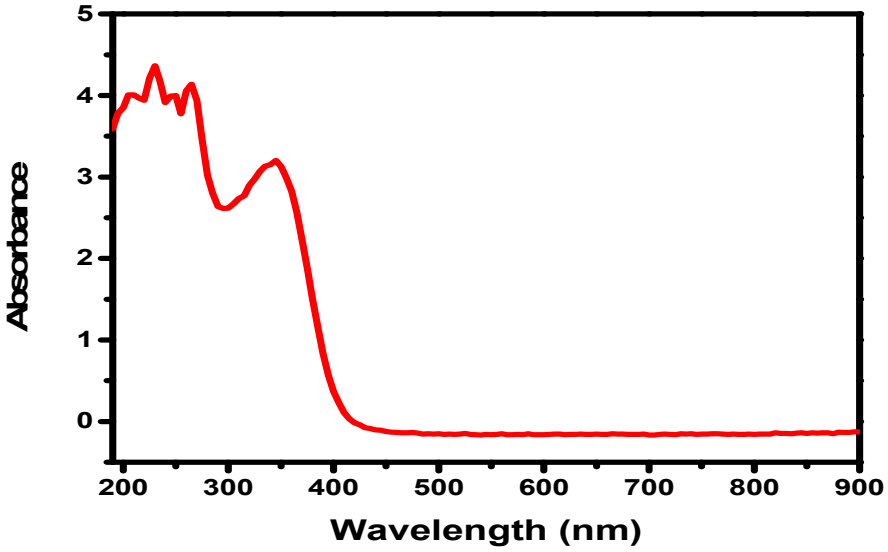

Figure 2: UV-VIS spectra of *Calitropis procera* total extract.

Cytotoxicity

MTT assay was performed to evaluate the cytotoxicity of methanolic extract of *C. procera* total extract towards MCF7 breast cancer cell line. Three different concentrations of mathanolic extract were used, 5, 10 and 25 µl. MTT assay was performed to examine cytotoxicity of C. procera total extract after 48 h of treatment as shown in figure 3. Total extract of *C. procera* was able to significantly reduce cell viability ($p < 0.05$) by more than 70 %.

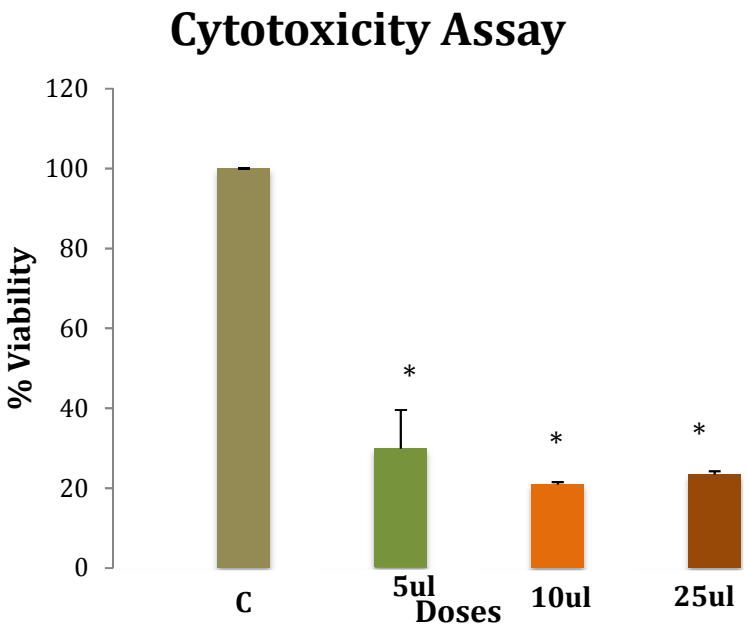

Figure 3: % viability: MCF7 cell line was cultured in 96 well plate at a density of 5x10^3 cells/well. Plates were incubated at 37^{o}C for 48 h in a CO_2 incubator with 0, 5, 10 and 25 µl of *Calotropis procera* total extract. * denotes significance, p <0.05.

Antimicrobial activity

The Disc diffusion method was employed to explore the antimicrobial activity of methanolic leaf extract of *C. procera* against drug resistant *Staphylococcus aureus*. Total extract proved to be an efficient antibacterial agent (Figure 4). The zone of inhibition observed was 18 mm for MRSA as shown figure 5.

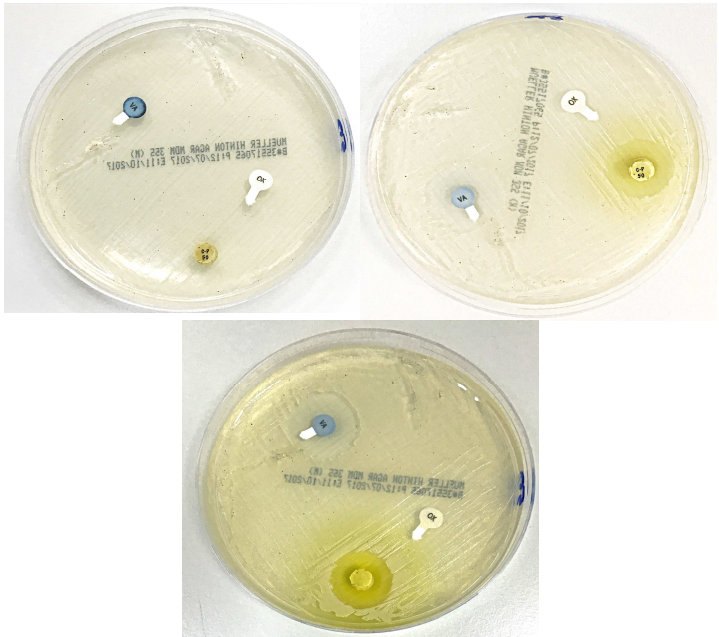

Figure 4: Methicillin resistant *Staphylococcus aureus* (MRSA) at 0, 24 and 48 h after treatment with 50 µl of *C. procera* total extract and (vancomycin) positive and (oxacilline) negative control.

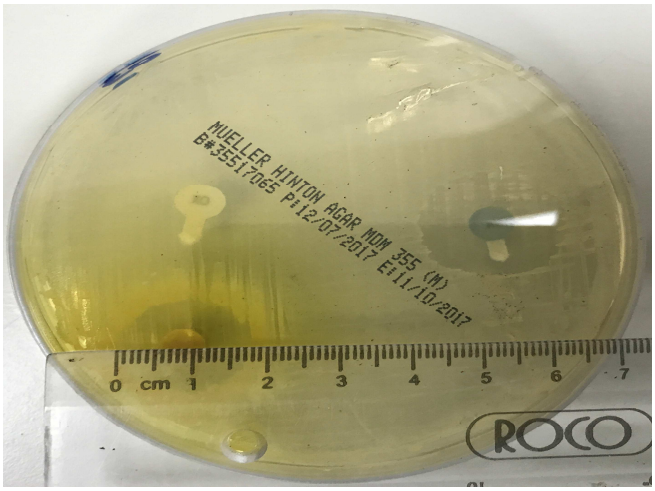

Figure 5: Zone of inhibition measurement for Staphylococcus aureus MRSA after 48 h of treatment with 50 µl of *C. procera* total extract. Zone of inhibition with diameter of 1.8 cm (18 mm) was observed after 48 h of treatment.

DISCUSSION

Pharmacologically plant extracts are of great importance in anticancer research [24,32]. Numerous studies on extracts from *C. procera* have been carried out in recent years [33] documenting the anticancer potential of different part of this medicinally important plant [34]. Cardiotonic steroid isolated from the methanolic extract of *C. procera* root bark displays anticancer potential comparable to taxol by inducing apoptosis [35]. The latex from *C. procera* induced DNA fragmentation in leukemia cells [34]. In this study, we investigated the anticancer activity of methanolic leaf extract of *C. procera* by perfomring MTT assay. The percentage of cell viability after 48 h of incubation with total extract was less than 30 %.

Drug resisting microbes are of immense concern globally [36]. The fact that new cases are emerging and the increase in demand for natural products with antimicrobial activity is pushing the scientific community to identify natural compounds isolated from medicinally important plants [8]. Total extract of *C. procera* proved significant in restricting the growth of MRSA as observed in our disc diffusion test.

CONCLUSION

Recently many researchers have studied the pharmacological importance of medicinal plants. This is partly due to the fact that these plants have been used for centuries as remedies against different aliments in the traditional medicinal system. Additionally, extracts from these plants have little side effects, making them good candidates for drug development. Our preliminary data substantiate the importance of the methanolic leaf extract of *C. procera* as both anticancer and antimicrobial agent. It effectively inhibited the growth of MRSA, which is a potential threat to humans due to its resistance to numerous antibiotics. Moreover, its efficacy against MCF7 cell line is commendable. Further, *in vivo* studies are required to shed light on the mode of action of this medicinally important plant and isolation of individual compounds would help in developing treatments with fewer side effects.

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Declaration: The manuscript is part of my PhD thesis.

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