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Metabolic Profiling and Antibacterial Assessment of *Calotropis procera* extract against *Streptococcus* group B

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Abstract

Calotropis procera is shrub *of Asclepiadaceae* family and is a source of antimicrobial metabolites against a greater range of bacteria including *Streptococcus* Group B (SGB). In the present study, different metabolic compounds were extracted from *C. procera* using 75% methanol. The extract of *C. procera* was assessed against different bacteria of the SGB family by estimating the zone of inhibition. Moreover, minimal inhibitory concentration (MIC) of different concentrations e.g., 0.001, 0.01, 0.1, 1.0 and 10 mg/mL were determined through zone of inhibition. Gas chromatography mass spectrometry (GC-MS) analysis of plant extract revealed 24 compounds. The most important compounds of the GC-MS array were including undecane, terephthalic acid, Cyclohexane, dimethyl-propane-thiosulfinate, Fluorobenzoic acid Octadecenoic acid and others. The antibacterial activities of the plant extracts were might be because of their compound which had been reported previously as well as an antimicrobial compound. The molecular characterization of different bacteria of SGB used in the study revealed the bacteria were consisted of antibiotic resistant genes against.

Key words: *Calotropis Procera*, antimicrobial activity, methanolic extracts, *Streptococcus* group B (SGB)

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1. INTRODUCTION

Calotropis procera belongs to the family of Apocynaceae that is native to South Asia, North Africa, Indochina, Equatorial Africa and West Asia [1-2]. The Procera is an animal bush, green in color and containing a very bitter and poisonous milky sap [3]. They grow as sterile weeds and enjoy abundant fruits and fruits every year [4].*C. procera* exhibits drought tolerance and can survive in extreme conditions such as extreme weather and lower availability [5-6]. *C. procera* is annual bush, having some important medicinal values [4]. *C. procera* is a rich source of biological active chemical groups including, cardenolides, tannins, steroids, glycosides, terpenoids, phenols, alkaloids flavonoids[7]. Therefore, the plant is widely used in traditional Arabic medicine and in the Indian pharmacy to treat various diseases such as spleen, accumulations and abdominal and liver problems [8]. The plant contains several classes of secondary metabolites, including alkaloids, triterpenic sprites and flavonoids, which have demonstrated various biological activities, including very potent antibacterial activities against a wide range of pathogenic bacteria, including streptococci [9].

Plant extracts are natural sources of antimicrobial agents, considered nutritionally safe and easy to dissolve [10]. Many researchers have demonstrated the antimicrobial activity of plant extracts against bacteria [1,3]. In addition, the invalidation of *C. procera* showed antibacterial activity against *S. aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Group B streptococcus (GBS) is one of the main pathogenic groups that affect mammals, reptiles and amphibians. GBS has also caused mastitis in cattle and meningitis in infants [11]. GBS infection is an important cause of neonatal disease and death around the word [9]. S. agalactiae is one of the most important types of GBS that adapts rapidly to asymptomatic colonization of adult humans [9]. It is usually found in the digestive system and the urogenital system, but it is also the main cause of invasion of bacterial diseases in a newborn baby [9,12].

This study provides facts about the antibacterial activities of *C. proceed* using pathogenic bacterial of GBS [9]. Many drugs are being produced by different plants and microbe which have very strong antibacterial activities [13]. However, different reports in the last decade, showed that the pathogenic bacteria has developed resistance against these drugs, which has been a worldwide concern [14]. The genes responsible for resistance against the antibacterial drugs can be transmitted from one to another bacterial species and hence obtain resistance against the drugs used as therapeutic agents. Biologically active plant extracts are a new concept and have been recently reported. The main objective of our work is to study the antibacterial activity in the *C. procera* plant.

2. MATERIALS AND METHODS

2.1 Samples collection

Calotropis procera plants was collected from desert near to Jeddah and brought to the laboratory of the Department of Biological Sciences, King Abdulaziz University Saudi Arabia. The sample was put in shed under pressure and left to dry, then crushed, grinded by mortar and pestle. The crushed *C. procera* was extracted using Hexane, then 70% methanol was used to extract the metabolites from *C. procera*.

2.2 Extraction of metabolites from Plant

All plant parts were isolated, washed in running faucet water for quite a while in order to expel dust particles, dried in outside at room temperature which are then ground to fine powder. The dry plant sample was crushed using mortar and pestle into a fine powder. The *C. procera* extract was carried out using polar solvents such as different percent of n-hexane and MeOH to extract the hydrophilic compounds. The metabolites from the plants were extracted using the solvent-solvent extract method. The extraction was carried out according to the procedure of Ullah et al[15] using 70% MeOH. The samples were then dried, and the dry residues were suspended in 100% MeOH.

2.3 Identification of metabolites in C. procera extract

The dried extract was suspended in 100% MeOH, filtered through 0.45 µm filter, then 2 µL was injected into GC-MS to quantify the metabolite profiles present in the extract of *C. procera* [15]. The career gas was helium (He) and 2 µL sample was injected in the GC injector [16]. The column was temperature was set for 2 min at 60°C and then increased up to 160°C at a rate of 5°C /min for 5 min. In the electron ionization system, the ionization energy to detect the ions was 70 eV in electron impact mode. The spectrum of detected compounds in extract were compared with library present in NIST library.

2.4 Assessment of antimicrobial activity

The antimicrobial activity was determined as described by Ullah et al [15]. The *Streptococcus agalactiae* was used to determine the antibacterial activity of methanolic extract of *C. procera*. An aliquot of 10 µL of the culture was then spread on LB agar plate. Wells were cut off from the agar surface (6 mm diameter) and 30 µL of test samples added in each well and ampicillin (100 µg/mL) was used as positive control. The plates were incubated at 37 ± 2°C for 48 ± 2 h and clear circles known as zones of inhibition were measured and the experiment was triplicated.

2.4 Minimum Inhibitory Concentration of extract

Different concentrations of extract of *C. procera* i.e., 0.001, 0.01, 0.1, 1.0 and 10 mg/mL were prepared. The different concentrations were dropped to culture of the test organisms and incubated at 37°C for 24 h. The minimal inhibitory concentration (MIC) of the compound was determined according to Ullah et al [16] by adding different concentrations of a compound in 5 mL LB media. The respective bacterial species were inoculated and inoculated on a shaker (200 ± 20 rpm) at 37°C and the growth determined at an optical density (OD) 600 nm using a spectrophotometer.

3. RESULTS AND DISCUSSIONS

3.1 GC-MS profiling of C. procera extract

The *C. procera* has greater medicinal value because it has a rich profile of secondary metabolites. The bioactive metabolites are involved in different physiological function including infection, communication and self-defense and reproduction. In the present study, we have identified bioactive metabolites in culture extract of *C. procera* through GC-MS analysis. The major biologically active compounds were including terephthalic acid, propyl tridec-2-yn-1-yl ester, 2-fluorobenzoic acid, 2-methylpropyl este, 2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate, N-Nitrosodimethylamine, Oxalic acid, dineopentyl ester and many other and summarized in Table 1. The corresponding compounds were observed due to the molecular weight, retention time and concentration. GC-MS of the methanol extract of *C. procera* showed 24 peaks, which indicate the presence of 24 compounds (Fig. 1). The spectra of 24 metabolites were compared with the standard compounds present in the NIST library. The compounds detected are presented in Table 1.

The major biologically active compounds were included as terephthalic acid, propyl tridec-2-yn-1-yl ester, 2-Fluorobenzoic acid, 2-methylpropyl este, 2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate, N-Nitrosodimethylamine, Oxalic acid, dineopentyl ester and many other. These compounds were biologically active as antifungal, anti-influenza, antimicrobial, and antioxidants [17,18]. All these compounds are important in the formulation of different medicines. Terephthalic acid is used as an antioxidant, anti-inflammatory, possess hypolipidemic properties and is also used as an antimicrobial agent[15]. The 2-Fluorobenzoic acid has antioxidant activity, and anticancer activity[19]. The terephthalic acid has been reported as strong antimicrobial agent against different pathogenic bacteria. In addition, its antioxidant properties and anti-cancerous activities has also been reported.

3.2 Assessment of antibacterial activity of plant extract

The disc diffusion method is widely employed to check the susceptibility of chemical components of extracts against selected bacterial strains and is a reliable method too. The results of bacterial inhibition by extract of *C. procera* are shown in figure 1. The antibacterial activity of the extract revealed that the extract was consisted of metabolites, responsible for bactericidal activities (Fig. 1). The zone inhibition against tested bacteria in the plats indicated efficacy of the extract of the *C. procera*. The differences in the antibacterial activity of extract might be due to the phytochemical components present in the extract[20-21]. Medicinally important plants have a significant role in traditional and commercial medicine due to the chemical contents present in the plant extract. Previous studies showed that the extract of medicinal plants are rich with chemical constituents which have very prominent role against the pathogenic bacteria [15, 19-20].

Table 1. The list of detected secondary metabolites through GC-MS from the extract of *C. procera*

Peak#	Ret.Time	Area	Height	A/H	Name
1	5.803	12758	4089	3.12	Indolizine, 2-(4-methylphenyl)-
2	6.218	350144	81439	4.3	Undecane
3	7.725	20200	8522	2.37	Butane, 2,2-dimethyl-
4	10.588	79249	47874	1.66	Cyclohexane, 1-ethenyl-1-methyl-
5	11.417	2266	2141	1.06	2,2-Dimethyl-propyl 2,2-dimethyl-propane-thiosulfinate
6	11.459	7953	3920	2.03	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-
7	11.889	42530	14819	2.87	(SR)- or (RS)-4-methyl-2,3-pentanediol
8	11.949	22734	7784	2.92	Phosphoric acid, dipentyl octyl ester
9	12.082	9201	4906	1.88	Terephthalic acid, propyl tridec-2-yn-1-yl ester
10	12.45	12715	7839	1.62	Decane, 1-iodo-
11	13.164	13257	8227	1.61	Hexane, 3,3-dimethyl-
12	13.989	10681	6728	1.59	RS-2,3-hexanediol
13	14.295	8119	4075	1.99	2-Fluorobenzoic acid, 2-methylpropyl ester
14	14.355	19744	6881	2.87	Octadecane, 1-iodo
15	14.508	29774	7665	3.88	Nonane, 1-iodo
16	14.648	9313	2190	4.25	N-Nitrosodimethylamine
17	15.002	3705	3597	1.03	Oxalic acid, dineopentyl ester
18	15.467	3472	2154	1.61	Phosphorus P4
19	15.708	11343	2221	5.11	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-
20	15.81	12953	5068	2.56	RS-2,3-hexanediol
21	15.94	15727	11412	1.38	R(-)3,7-Dimethyl-1,6-octadiene
22	16.367	4293	3188	1.35	3-Benzyloxy-butyric acid, t-butyl ester
23	16.644	32296	19038	1.7	9-Octadecenoic acid (Z)-, methyl ester
24	16.849	1578718	880715	1.79	Hexadecanoic acid, methyl ester

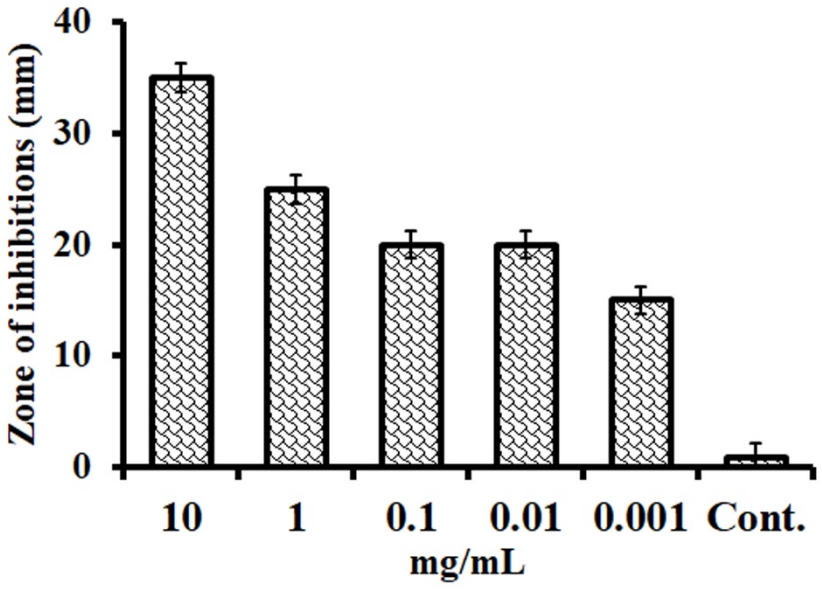

Fig. 1. Antibacterial activity of the extract of *C. procera* against pathogenic bacteria. Different concentrations were applied against the bacteria

The plant extracts have a wider range of secondary metabolites which have very vital activities against bacteria and fungi. Previous reports showed that these secondary metabolites are rich with flavonoids, unsaturated fatty acid, alkaloids, linolenic, anticancer compounds, anti-inflammatory and many other valuable compounds[11, 22]. In addition, other characteristics of the secondary metabolites have been reported including repellent characteristic, attraction of beneficial organisms and some phytoprotectants respond to environmental changes[10].

3.3 Assessment of MIC and MBC of plants extract

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extract against pathogenic were determined using disc diffusion method. The effect of the plant extracts was shown in Figure 2, revealed that that bacterial strain was susceptible to the extract. The MICs ranged up to 40 mg/mL and MBCs ranged up to 70 mg/mL (Fig. 2).

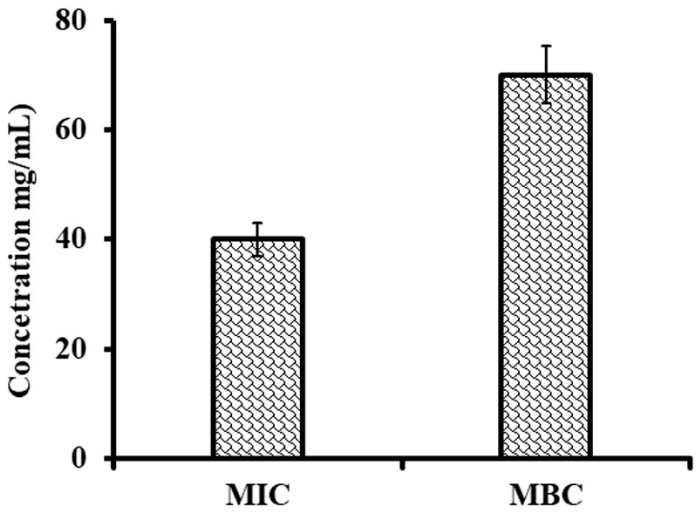

Fig. 2. Minimal inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) of the extract of *C. procera* against pathogenic bacteria.

Plant extracts were found to be ineffective against a wider range of bacteria pathogenic and nonpathogenic bacteria [20]. In addition, previous findings also revealed that a higher concentration of plant extract were effective against pathogenic fungi and bacteria of causing food spoilage [21]. Many researchers have studied the efficacy of plant extracts and their active compounds as antimicrobial agents to control bacterial growth and food degradation [19].

4. CONCLUSIONS

The GC-MS profile of the C. procera showed a great variety of secondary metabolites which have been successfully used as antimicrobial compound. However, these compounds have been profiled from C. procera in the present study and applied against a pathogenic bacterium. The results revealed that the extract of C. procera could be a better and alternative source of antibacterial compound. The extract can be used as antibacterial agent against the pathogenic bacterial strain which have developed resistance against some conventional antibacterial drugs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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