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Phytochemical analysis and antibacterial properties of *Calotropis procera* against bacterial phytopathogens

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ABSTRACT

Calotropis procera, is known as crown flower or giant milkweed and belongs to the family *Asclepiadacea.* It has been traditionally used for various medicinal purposes. In the present study, the phytochemical analysis, antioxidant property, and antimicrobial activity of *C. procera* were evaluated. Methanol was used as a solvent for the extract preparation using soxhlet extraction. The extracts were subjected to the analysis of the different secondary bioactive metabolites. Furthermore, the antimicrobial activities of these extracts were determined against various pathogens. The qualitative analysis of plant extracts of leaves stems, and roots showed the presence of phenolic compounds, alkaloids, carbohydrates, glycosidic, protein, phytosterols, steroid, saponin, and flavonoid compounds. The leaf extract of *C. procera* plants inhibited 95% 2,2-diphenyl-1-picrylhydrazyl (DDPH) activity at 0.8 mg/mL Methanol extract of *C. procera* showed the maximum antibacterial and antifungal activities against the tested plant pathogen of bacterial and fungal strains. This research explored the various phytochemicals, including carbohydrates, glycosides, alkaloids, steroid, saponins, amino acids, phenols, and flavonoids, present in the stem, leave, root, and flower of the indigenous *C. procera* plant. The study gave a systematic base for the isolation of the novel bioactive phytochemicals with the antioxidant and antibacterial activities from the *Calotropis* species of Balochistan.

Keywords: Phytochemical analysis, antioxidant, antibacterial, *Calotropis procera*.

INTRODUCTION: The *C. procera* is a medicinal plant that belongs to the family *Asclepiadacea*. It is broadly circulated in two species the *C. procera* and *Calotropis gigantia*. These species are known throughout the world, but the more popular is *C. procera* which has a purple flower, and the species *C. gigantia* has a whitish flower. The color difference is the main source to distinguish the two species. Therefore, it is hard to identify the species if the plant has no flower. It is feasible to identify the species by pH test of the' milky latex of the plant (Verma, 2014). The *C.procera* is a salt-tolerant and drought-resistant weed. Rarely do sheep and goats eat the leaves, but cattle and other livestock do not eat it because it is somewhat toxic. The plant is mostly found in sandy soils in areas of minimum rainfall. The plant *C. procera* is a model plant for monitoring sulfur dioxide emissions in the air. The plant has its toxic properties that comprise iridocyclites and dermatitis which act as a poison and have lethal effects (Bobbarala and Vadlapudi, 2009). The plants produce secondary metabolites known as phyto-constituents which have the ability to perform the biological function for us and other living organisms against different diseases and traditionally has been used as medicine in various tribes of the world for curing of various ailments (Mahajan and Badgujar, 2008). The warmed leaves are used as a poultice and also used for the treatment of migraines. *Calotropis procera* latex is used traditionally for the treatment of poison, skin infection, ulcer, colic piles abdominal gland, liver enlargement of the spleen. The latex showed mild toxic effects on the kidney, heart, and liver (Magalhães *et al.*, 2010). The plant roots are used to cure rheumatism, asthma, Eczema, Cough, and Leprosy (Sen and Behra, 2007). The *C. procera* is not suggested for self-medication because it contained poisonous substances (Baloch *et al.*, 2017). *Calotropis procera* contained various compounds in its different parts including root, flower, latex, and leaves. The Plant is known to have a broad range of pharmacological activities such as anticancer, insecticidal, anthelmintic, anti-inflammatory, antimicrobial, larvicidal, antidiarrheal, and acaricidal activities with other useful properties ((Murti *et al.*, 2010; Bader *et al.*, 2021). The occurrence of glycosides, flavonoids, and organic carbonates in the leaf extracts of *Calotropis procera* has been also reported. Moreover, anthocyanins, alkaloids cardenolides, proteolytic enzymes, triterpenoids and cardioactive glycosides are also identified. Numerous bioactive molecules with various biological activities have been found in the plant *C. procera* (Rani *et al.*, 2019; Gyawali *et al.*, 2020). Phenol, 2, 4 bis (1, 1-dimethylethyl) ester with antioxidant and antiviral activity against spot syndrome virus have been found.

OBJECTIVES: The current study was designed to analyze the phytochemical composition of different parts of the local *C. procera* plant of Kech, Balochistan. Furthermore, the antimicrobial applications of the extract of different parts of *C. procera* plant will be analyzed against different bacterial phytopathogens.

MATERIALS AND METHODS: Collection and preparations of plant extract: Healthy and mature plant *C. procera* was collected from Turbat. Plant parts including leaves,flowers, and stems were thoroughly washed with tap water. The washed plant parts were shade dry for ten days and then ground in a mechanical grinder. The powder of the plant parts which was later sieved through mesh size 80 got the powder of uniform size. The powder was used for extraction by the soxhlet apparatus.

Preliminary phytochemicals screening: It refers to the extraction, screening, and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids; tannin, antioxidants, and phenolic compounds. The crude methanol extracts were obtained which were used for the phytochemical screening method followed by literature (Evans, 2009).

Determination of antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay: The antioxidant properties of various extracts of *C. procera* extracts were determined by measuring the DPPH scavenging activities. The reaction mixture of 100μL of DPPH and 100μL of the diluted test sample was prepared and incubated at 37°C for 30 minutes. The absorbance was measured at 515nm using UV Visible Spectrophotometer. Percent inhibition of DPPH radical scavenging activity was calculated as follows;

$$Percent\ inhibition = [(A0 - A1)/A0] \times 100$$

Determination of antibacterial activity: Antibacterial assay was performed via the ager-well diffusion method (Jack *et al.*, 1995). The experiments were performed by the formation of four 6 mm diameter bored in Mueller-Hinton agar and 20µL of DMSO dissolved extracts were added in each sterilized condition with the incubation of tested bacterial strains at pH 7.4 and 37ºC for 24 hours. Ciprofloxacin (20 lg/mL), gentamicin (20 lg/mL) and nystatin (20 lg/mL) were used as positive controls.

RESULTS AND DISCUSSION: The useful usage of herbal medicine from the old days to now cannot be denied. Herbal medicine has important for various therapeutic uses. Traditionally *C. procera* is used to treatment of various diseases such as the leaf is used to cure joint problem and reduction of swelling. *C. procera* parts are used for different treatment purposes (Meena *et al.*, 2011)

Phytochemical constituents: The phytochemical constituent of *C. procera* was analyzed and showed existence of glycoside, tannin, alkaloid, saponins, and flavonoids. The existence of different consistent is an indication that the plant may be a great potential for bioactivity and suggest that it is very important for medicinally drug discovery. As it was indicated from the previous tests, stems, leaves, fruit, flowers, and roots of *C. procera* gave positive response for the presence of sterols, triterpenes, cardiac glycosides, and saponins (table 1).

Experiment	Methanolic extract of Calotropis procera leaves	Methanolic extract of Calotropis procera Stem	Methanolic extract of Calotropis procera Root	Methanolic extract of Calotropis procera Flower
Alkaloids				
Mayer's reagent test	+++ve	-ve	+++ve	-ve
Wagner's reagent test	+++ve	-ve	+++ve	-ve
Hager's reagent test	+++ve	-ve	+++ve	-ve
Carbohydrates				
Molish's test	+ve	-ve	+ve	+ve
Fehling's test	+ve	-ve	+ve	+ve
Benedict's test	+ve	-ve	+ve	+ve
Glycoside				
Borntrager test	+ve	+ve	-ve	+ve
Legal's test	+ve	+ve	-ve	+ve
Saponin				
Foam Test	-ve	+ve	-ve	+ve
Phytosterols and steroid				
Salwonski Test	+ve	-ve	+ve	+ve
LibbermanBurchard's test	+ve	-ve	+ve	+ve
Flavonoids				
Alkaline reagent test	+ve	+ve	-ve	+ve
Shinoda test	+ve	+ve	-ve	+ve
Lead acetate test	+ve	+ve	-ve	+ve
Tannin and phenolic compound				
Ferric chloride test	+ve	+ve	+ve	+ve
Lead Acetate test	+ve	+ve	+ve	+ve
Dilute Iodine solution	+ve	+ve	+ve	+ve
Test for proteins and amino acid				
Ninhydrin test	+ve	+ve	-ve	+ve
Biuret test	+ve	+ve	-ve	+ve

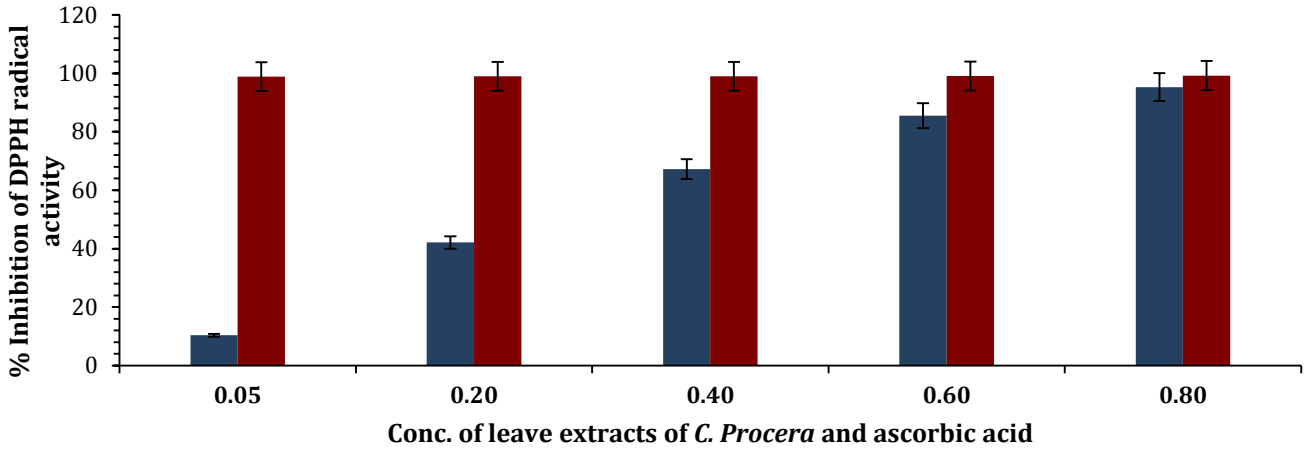
Table 1: Phytochemical analysis of leave, stem, root and flower in methanol.

On the other hand, flavonoid glycosides were detected only in leaves and flowers while tannins were found in the leaves, fruit, and flowers. A low quantity of alkaloids was found in stems and roots. Anthraquinones (combined) were only detected in the flowers. The presence of cardiac glycosides in high quantity in the different organs of *C. procera* prompts the author to study it quantitatively in the future.

The DPPH free radical scavenging activity: The antioxidant due to its scavenging properties of free can be useful for various diseases like neurodegenerative diseases, cancer, etc. The DPPH free radical scavenging method was used for the determination of the antioxidant activity of methanolic extracts of leaves of *C.*

procera. The potential of DPPH radical scavenging properties was measured by determining the reduction of the violet color strength at 517 nm. DPPH being a stable free radical can accept an electron or hydrogen to form a stable diamagnetic molecule. The leave extract showed the inhibition of DPPH and maximum percent inhibition of free radical scavenging activity was observed at 0.8 mg/ml of *C. procera* leave extracts (figure 1). The results indicated that *C. procera* leaves have the ability to donate electrons or hydrogen for the inhibition of free radicals. The percent inhibition of leave extracts is due to the presence of polyphenol and flavonoids compounds because most of these compounds are responsible for the antioxidant properties of medicinal plants (Larson *et al.*, 1988).

Antibacterial activity of *C. procera* methanolic leave extracts: The extract from *Calotropis procera* was investigated for antibacterial activity. The methanol extracts of leaves which showed high phytochemical constitutes were screened for antibacterial activities against phytopathogens including *Xanthomonas Species, Erwinia Species, Serratia species* and *Klebsiella Species* through the agar diffusion method. The extract showed sensitivity against all strains and the highest zone of inhibition was observed against Xanthomonas *Species* (figure 2).



Figures 1: Antioxidant Activity of Leave Extracts of *C. Procera*.

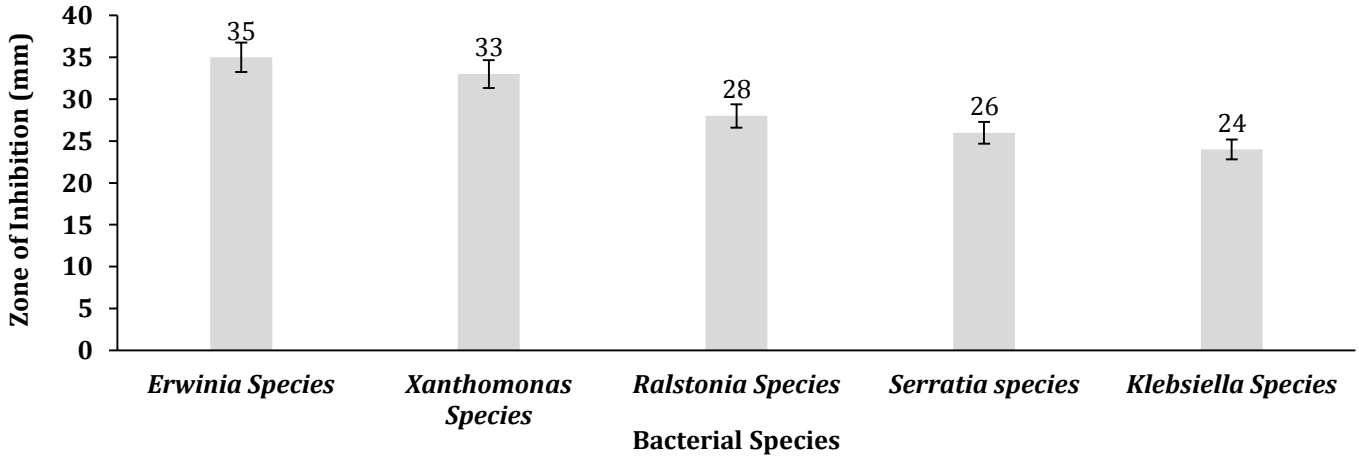


Figure 2: Antibacterial activities of leave methanolic extracts of *C.procera* against bacterial phytopathogens.

A moderate zone of inhibition was seen in *Serratia species and Ralstonia Species*, and the lowest zone of inhibition was observed against *Erwinia Species*, and no inhibition was seen against *Klebsiella Species.* Similar findings were previously reported that methanol extracts of *Calotropis procera* showed the highest antibacterial activities (Nenaah, 2013). The result concluded that the methanol extracts of *Calotropis procera* leave shown antibacterial activity with a diameter range from 33 mm to 12 mm against *Xanthomonas species, Erwinia species, Serratia species, Klebsiella species* using the agar diffusion method.

CONCLUSION: This study has explored the various phytochemicals, including carbohydrate, glycosides, alkaloids, steroids, saponins, amino acids, phenols, and flavonoids, present in the stem, leave, root, and flower of indigenous *Calotropis procera* plant. The Methanolic, extracts showed the presence of various phytochemical compounds. Furthermore, the antimicrobial activities of these extracts were determined against various pathogens. The Methanolic extracts of flowers and leaves of *Calotropis procera* showed promising antibacterial activity. The antimicrobial activity of these extracts definitely will enhance if these biomolecules will be purified. Therefore, further research is needed to obtain more benefit from this plant and change the traditional system of medicine into a scientific and standard medication system of purified biomolecules.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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