

Phytochemical and Pharmacological Studies of the Whole Plant of *Calotropis procera*

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Abstract: Phytochemical and biological screening is necessary for the isolation of new compound which lead to the discovery of drug. In the present work the crude MeOH extract and milky latex of *Calotropis procera* were tested for phytochemical test which indicated the presence of various class of bioactive secondary metabolite such as terpenoids, flavonoids, saponins, steroids and cardiac glycosides. *C. procera* also exhibited good antibacterial, antioxidant and analgesic effect.

Key words: *Calotropis procera* • Phytochemical • Antibacterial • Antioxidant • Analgesic

INTRODUCTION

Calotropis procera belong to family Asclepiadaceae which is commonly known as Giant Swallow wort, Milkweed. It is native to North Africa, Tropical Africa, Western Asia, South Asia and Indochina. *C. procera* is use as a folk medicine in traditional system for the treatment of various diseases. *C. procera* various parts are used for the treatment of various diseases including liver dysfunctions [1]. It has been reported in literature that the title plant possess potential anthelmintic, analgesic, anticancer, anticoagulant, anti-inflammatory, antimicrobial, purgative and antipyretic properties and is also used in the treatment of leprosy, leucoderma, liver and abdomen [2]. *C. procera* flowers causes temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodes in naturally infected sheep [3]. The latex of *C. procera* plants has important indigenous medicinal uses because of its purgative, antisyphilitic and antidontalgic action [4]. *Calotropis procera* possesses potent antioxidant and anti-hyperglycemic effects against alloxan-induced diabetes in rats. It decreases blood glucose and increase hepatic glycogen content and prevents body weight loss, increases hepatic levels of the endogenous antioxidants, viz. superoxide dismutase, catalase and glutathione and

brings down the levels of thiobarbituric acid-reactive substances [5]. Dry latex of *Calotropis procera* has potential anti-cancer properties due to its differentiable targets and non-interference with regular pathway of apoptosis [6]. The current work demonstrated the chemical constituents, antibacterial, antioxidant and analgesic properties of *Calotropis procera*.

MATERIALS AND METHODS

Plant Collection and Identification: *Calotropis procera* was collected from the garden of Institute of Chemical Sciences. The plant was identified by Ghulam Jelani Department of Botany University of Peshawar Pakistan.

Extraction and Fractionation: Shade dried plant of *Calotropis procera* was filled in the flask and extracted successively with methanol solvent in soxhlet extractor for 48h. The solvent extract was concentrated under reduce pressure at 40°C using rotavapor, according to standard protocol [7-9].

Animals: BALB/c mice of either sex weighing 18-25 g were used as experimental animals. The animals were bred in the animal house, PCSIR of Peshawar. The animals were

maintained in clean and hygienic conditions with optimum room temperature. Clean and properly dried food was given to the animals and water *ad libetum*. Animals were divided into different groups comprising of six mice in each.

Phytochemical Screening: The chemical tests were performed on the crude extract and latex of *Calotropis procera* are using standard procedure [10-12] to recognize the bioactive secondary metabolite.

Antioxidant Activity: The antioxidant activity was performed by DPPH radical scavenging assay according to standard protocol as earlier discusses [13- 16]. The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) Experiments were carried out in triplicate. Briefly, a 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample (extracts/fractions) solutions in methanol (containing 10-100ug) and control (without sample). The solution was stand for 30 min, in dark the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows.

$$\% \text{ DPPH} = (\text{OD control} - \text{OD sample}) \times 100 / \text{OD control}$$

where, OD control is the absorbance of the blank sample and OD sample is the absorbance of samples or standard sample.

Antibacterial Activity: The antibacterial activity was done using modified agar well diffusion method according to standard protocol [17, 18]. The muller-hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 hours. The broth culture (0.6 ml) of the test organism was placed in a sterile Petri-dish to which 20 ml of the sterile molten MHA was added. Holes were bored in to the medium using 0.2ml of the extract. Streptomycin was the standard antimicrobial agent at concentration of 2 mg /ml. Inoculation was done for 1 hour to make possible the diffusion of the antimicrobial agent into the medium.

Incubation was done at 37°C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

Analgesic Activity

Acetic Acid Induced Writhing Test: Animals were divided in various groups. The group I was injected with normal saline (10 ml/kg, i.p.), group II was injected with diclofenac sodium (10 mg/kg, i.p.) and rest of groups were treated with crude methanolic extract (50, 100 and 150 mg/kg). After 30 min of the above treatment animals were injected 1% acetic acid (10 ml/kg, i.p) and then the number of abdominal writhing were counted after 10 min of acetic acid administration [19].

RESULTS

The preliminary phytochemical screening test of *Calotropis procera* are listed in Table 1. The antioxidant, antibacterial and analgesic effect of this plant are given in Figure 1, 2 and 3 respectively.

Biological Screening: It is well recommended fact that the pharmacological properties of a plant is directly attributed the presence of various chemical constituents. Therefore during scrutinizing the plants materials for their pharmacological activities the determination of their chemical composition preliminary is vary essential. The present research work proved that our tested plant is a rich source of various chemical constituents like steroids, terpenoids, saponins and glycosides. These chemicals constituents performing various pharmacological actions [12]. The production of free radicals in the living body is responsible for a large number of disorders. Antioxidants are responsible for protection or minimizing the production of these free

Table 1: Phytochemical screening of crude extract and latex of *Calotropis procera*

Chemical constituents	MeOH	Latex
Alkaloids	-	-
Terpenoids	+	+
Flavonoids	+	+
Anthraquinones	-	-
Tannins	+	-
Phlobatanins	+	-
Saponins	+	+
Glycosides	+	+
Reducing sugars	-	-
Steroids	+	+
Cardiac glycosides	+	+

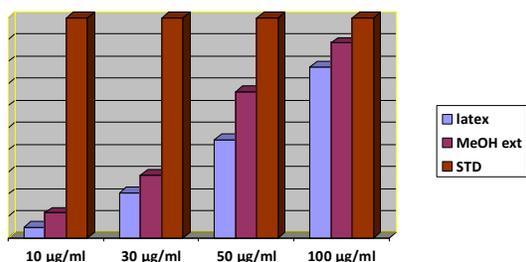


Fig. 1: Antioxidant activity of crude extract and latex extracted from the *Calotropis procera*

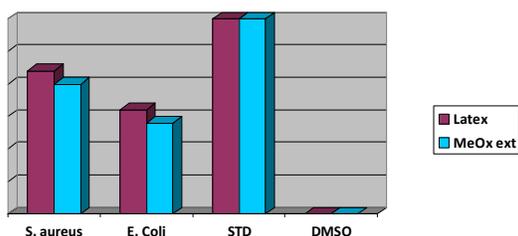


Fig. 2: Antibacterial activity of crude extract and latex extracted from the *Calotropis procera*

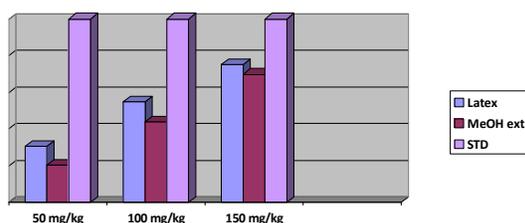


Fig. 3: Analgesic activity of crude extract and latex isolated from the *Calotropis procera*

radicals. The plants are rich source of this antioxidant agent therefore we tested our selected plant for antioxidant potential [13]. Searching new antimicrobial agent is a big challenge for the scientist of the present modern era and plants the biggest source of these agents. Screening of plants for their antimicrobial properties with the hope of finding safe and effective antimicrobial agents is very essential. The growth regulation of weeds is very important for better crop production. A large number of synthetic compounds are available as weedicidal but due to their environmental pollution and adverse effect on human body there use is restricted. To find the safe, effective and environmental friendly weedicidal we tested *Calotropis procera*. To some of the plant extract are also use as analgesic to give relive 10 µg/ml from pain. The analgesic activity of the crude extract and the medicine prepared may be due to the presence of various class of compound present in the crude extract and medicine prepared from the plant [19].

The crude extract exhibited 10, 30, 68, 88.5% radical scavenging properties at 10, 30, 50 and 100 µg/ml which were followed by latex which exhibited 4, 10, 22, 77% antiradical activity at 100 µg/ml.

The crude methanolic extract and Milky latex extracted from *Calotropis procera* were also seen for antibacterial properties the crude MeOH extract exhibit good antibacterial activity with zone of inhibition ranging from 12-20mm while latex was found more active which showed activity with inhibition zone ranging from 15-22 mm at the tested concentration 22 mg/ml.

Analgesic Effect: The methanolic extract as well as medicine exhibited dose dependent analgesic properties. Regarding the acetic acid induced writhing test the crude methanolic extract and tested medicine significantly attenuated the induced writhing. The percent effect of the crude was 20.4, 44 and 70% at the tested doses of 50, 100 and 150 mg/kg respectively, while the effect of latex was 30.6, 55 and 75% at the tested dose of 50, 100 and 150 mg/kg, respectively. The percent effect of diclofenac sodium was 82.34% was nearest to our tested compound. The crude methanolic extract demonstrated a dose and time dependent effect.

CONCLUSION

The current research work strongly support the use of *Calotropis procera* in the management of various infection, painful conditions, as well as a good antioxidant. The medicinal properties of our tested extract and the medicine prepared are directly attributed to the presence various phytoconstituents in crude extract as well as in medicine. Both the extract and medicine prepared proved central analgesic and antioxidant. Our current research suggest that future research is needed to isolated these compounds responsible for the activates of the *C. procera*.

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