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Phytochemical and pharmacological evaluation of aerial parts of *Euphorbia pulcherrima L*

[1]*Abdur Rauf and [2]Naveed Muhammad

[1]Institute of Chemical Sciences, University of Peshawar, Peshawar -25120, KPK, Pakistan.
[2]Department of Pharmacy University of Peshawar, Peshawar, Pakistan.

*Corresponding author E-mail: mashaljcs@yahoo.com. Tel: +923469488944

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In the present research work various solvent fractions of *Euphorbia pulcherrima* were subjected to phytochemical and pharmacological activities. The phytochemical screening of E. *pulcherrima* revealed the presence of various classes of chemical constituents like alkaloids, steroids, terpenoids, saponins, glycosides, reducing sugar and amino acid. A significant DPPH free radical scavenging activity was observed by the ethyl acetate fraction and methanol extract having percent effect 91.31% and 90.22% respectively. The ethyl acetate fraction and methanol extract of the *E*. *pulcherrima* also showed moderate antibacterial property especially against *K*. *pneumonia*, *S. epidermidis B. stearothermophihus* and *S. Typhimuriun*. The crude extract and its various solvent fraction exhibited significant phytotoxic effect. Regarding the analgesic effect the crude methanolic extract demonstrated significant effect at tested dose of 100 and 150 mg/kg. The current studies, suggested that *E. pulcherrima* contain potential secondary metabolite which are responsible for these pharmacological activities.

Key words: *Euphorbia pulcherrima*, phytochemical, antioxidant, antibacterial and phytotoxicity, analgesic.

INTRODUCTION

The genus Euphorbia is the largest genus of medicinal plants widely distributed in most part of the china and Pakistan (Bani et al., 2007). Different species of *Euphorbia* are used as a folk medicine for the treatment of various ailments such as skin diseases, intestinal parasites and warts cures.

It has been reported that *Euphorbia* possesses antiarthritis, anticancer (Valente et al., 2003; Luo et al., 2006), anticonvulsant, antidiabetic, anti-eczema, anti-eczema, anti-inflammatory, antimicrobial, antispasmodic, antitumor, antitussive properties hormonal and myelopoiesis properties (Ferreira et al., 2006: Eberle et al., 199: Lin et al., 1983). *E. pulcherrima* is also known as Christ flower. It has a common herbs use as concoction against ailments such as resembling typhoid and gastro-enteritis in Kano Nigeria. There is a little literature on the active secondary metabolite.

The aim of the present study was to identify varies class of chemical constituents responsible for the traditional claim that plant is used in chemotherapy (Yakubu et al., 2011).

MATERIAL AND METHODS

Collection of the plant material

Euphorbia pulcherrima was collected from Razagram, Toormang, Dir, Khyber Pakhtunkhwa Pakistan in month of December 2011. The plant was identified by Ghulam Jelani Department of Botany University of Peshawar Pakistan. Voucher specimen (voucher No. U-808) has been deposited in the herbarium of the Department of Botany, University of Department of Botany University of Peshawar Pakistan.

Animals

Healthy male albino Wistar rats of 150 to 250 g body weight were used for this study. The animals were housed in polypropylene cages and maintained under standard conditions (12/12 h light and dark cycles, at 25±30^{o}C and 35 to 40% humidity). Standard palletized

feed and tap water were provided ad libitum. The experimental protocols were approved by the ethical committee of the Pharmacy Department, University of Peshawar, Peshawar, Pakistan.

Extraction and fractionation

Shade dried plant of *Euphorbia pulcherrima* was filled in the flask and extracted successively with methanol solvent in soxhlet extractor for 48h as discuss earlier (Uddin and Rauf., 2012a). The solvent extract was concentrated under reduce pressure at 40C0 using rotavapor, and suspended in water and successively partitioned with n-hexane, chloroform, ethyl acetate and methanolic fraction.

Animals

BALB/c mice of either sex weighing 18 to 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 profiling

The chemical tests were performed on the crude methanolic extract and its n-hexane, chloroform and ethyl acetate fraction of *E. pulcherrima* using standard procedure (Deepa et al., 2011; Savithramma et al., 2011; Uddin et al., 2011a; Uddin et al., 2011b, Rauf et al., 2012b), to recognize the bioactive secondary metabolite.

Pharmacological screening

Antioxidant assay

The hydrogen atom or electron donation abilities of the corresponding extract/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 according to the standard procedure (Rauf et al., 2012; Uddin et al., 2012c; Blois., 1958).

Briefly, a 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample solutions in methanol (containing 20 to 100µg) 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:

% DPPH = Control absorbance – extract absorbance X 100 / control absorbance

Antibacterial assay

The antibacterial activity was done using modified agar well diffusion method as earlier discuss (Uddin et al., 2011d; Uddin et al., 2011e). The Muller-hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37oC 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^{o}C for 24 hours and the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

Phytotoxicity activity

In this the present investigation, the crude extracts and various fractions of *Euphorbia pulcherrima* was tested against Lemna minor (Ahn et al., 1995). In this bioassay, three conical flasks were inoculated with a sufficient stock solution of (20 mg/ml) to achieve a final concentration of 500, 50, and 5µg/ml, respectively.

Each conical flask was then added a 20 ml medium 10 plants each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor. The whole flasks were kept in growth cabinet for incubation up to seven days. After this growth, regulation in percentage was determined with reference to the negative control.

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 writing were counted after 10 min of acetic acid administration (Muhammad et al., 2013).

Table 1. Phytochemical profile of different solvent extracted fractions of *E. pulcherrima*

Constituents	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	+	+	+
Steroids	+	+	-	+
Terpenoids	-	+	+	+
Flavonoids	-	+	+	+
Anthraquinone	-	-	-	-
Tannins	-	-	-	-
Phlobatanins	-	-	+	+
Saponins	-	+	-	+
Glycoside	-	+	-	+
Reducing sugars	-	+	+	+
Cardiac glycoside	-	-	-	-
Comurrine	-	-	-	-
Beta cyanin	-	-	+	+
Protein/amino acid	-	+	+	+

Keywords: – = Absent, + = Present, Frt = Fraction

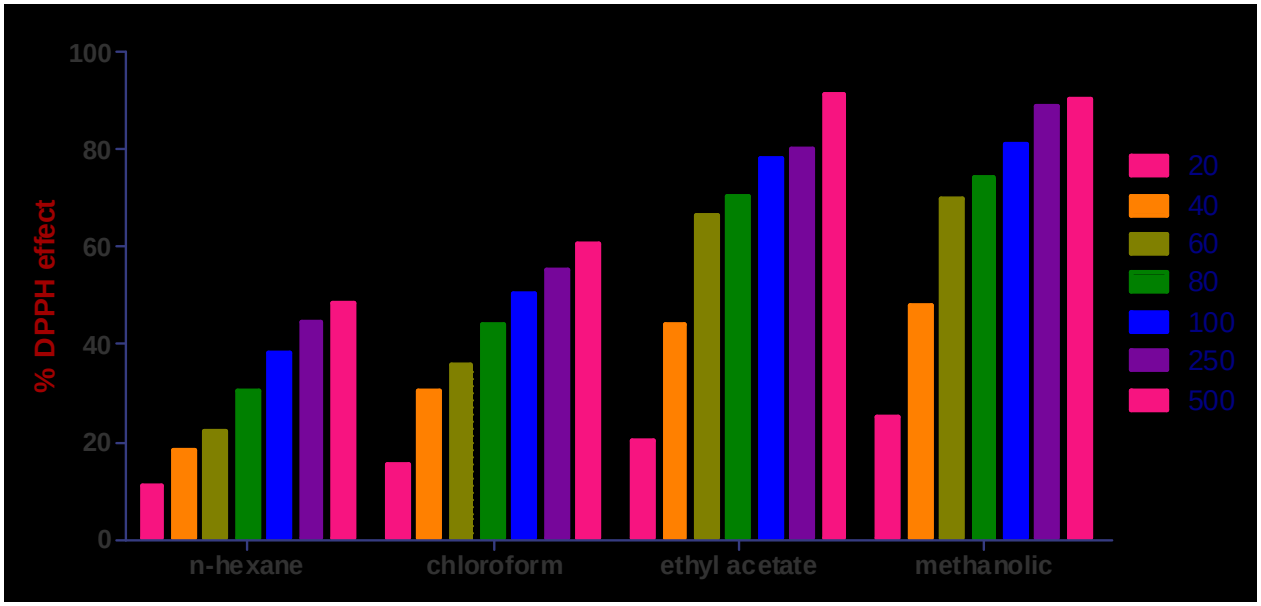

Figure 1. DPPH radical scavenging activity of various solvent extracts of *E. pulcherrima*

RESULTS

Phytochemical screening

The results of phytochemical profile of *Euphorbia pulcherrima* is presented in Table 1. The n-hexane fraction exhibited the presence of steroids while rest of phytochemical was not detected. The chloroform fraction showed the accumulation of alkaloids, steroids, terpenoids, saponins, glycosides, reducing sugar and amino acid. When ethyl acetate was tested for various phytochemical tests, it confirmed the presence of alkaloids, terpenoids, flavoniods, phlobatanins, reducing sugar, beta cyanin and amino acids. Except Anthraquinone, tannins, Comurrine and cardiac glycosides all tested chemical were confirmed in methanolic fraction.

Antioxidant effect

The antioxidant effect of various solvent fractions *E. pulcherrima* of is presented in Figure 1. The maximum (90.22%) free radical scavenging effect was observed with methanolic extract at higher concentration (500 µg/ml), while the effect was weak (24.94%) at 20 µg/ml.

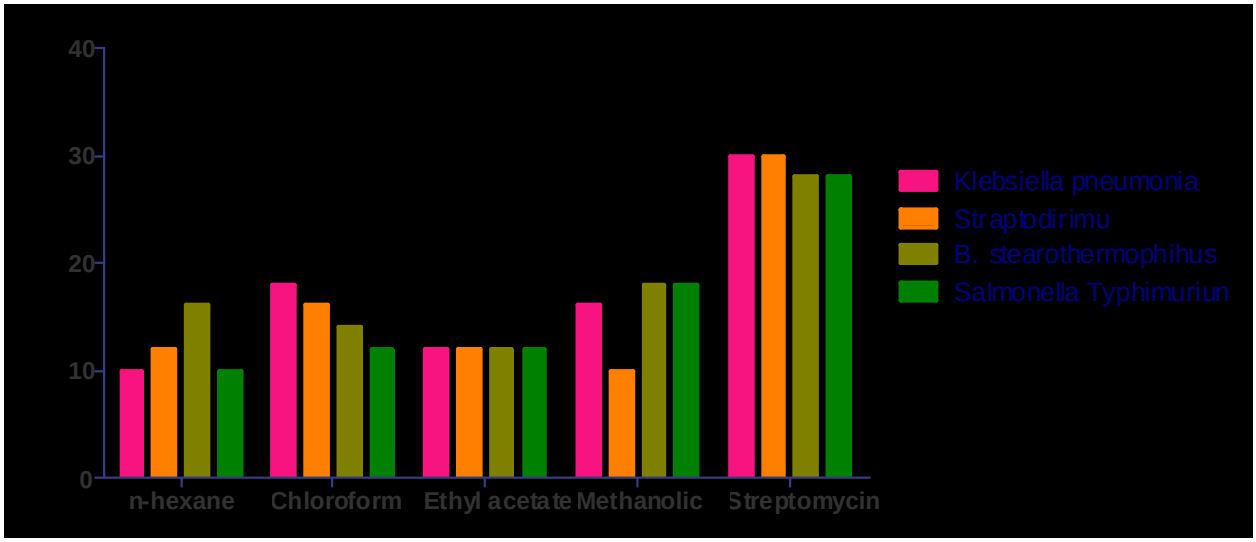

Figure 2. Antibacterial effect of various solvent extracts of *E. pulcherrima*

the antioxidant effect of ethyl acetate was higher (91.31%, at 500 µg/ml) but the effect at lowest concentration (20 µg/ml) was 20.41%. the free radical scavenging effect of chloroform and *n*-hexane fraction was moderate (60.55 and 48.41%) at tested concentration of 500 µg/ml.

Antibacterial effect

The antibacterial effect of various solvent fraction of *E. pulcherrima* is presented in Figure 2. Among the tested bacteria *Escherichia coli* and *Staphylococcus aureus* exhibited complete resistance against all the tested solvent fractions. The *n*-hexane fraction exhibited 10, 12, 16 and 10 mm zone of inhibition against *Klebsiella pneumonia*, *S. epidermidis, B. stearothermophihus* and *Salmonella Typhimuriun* respectively. When chloroform was tested against these bacterial strains, it caused 18, 16, 14 and 12 mm zone on inhibition respectively. Ethyl acetate fraction demonstrated moderate antibacterial effect (12 mm) against all tested bacterial and methanolic fraction was responsible for 16, 10, 18 and 18 mm zone of inhibition against *Klebsiella pneumonia, S. epidermidis, B. stearothermophihus* and *Salmonella Typhimuriun* respectively.

Phytotoxic effect

Figure 3 presents the phytotoxic profile of various solvent fractions of *E. pulcherrima*. The maximum effect was observed with methanolic extract and showed 50, 70 and 80% phytotoxicity at the tested concentration of 10, 100 and 1000 µg/ml respectively. In case of ethyl acetate fraction the percent growth regulation was 40, 70 and 80 at the same tested concentrations. The percent growth regulation effect with chloroform fraction was also significant. A comparatively moderate phytotoxic effect was demonstrated by n-hexane fraction.

Analgesic effect

The crude methanolic extract was tested at 50, 100 and 150 mg/kg as presented in Table 2. The extract significantly attenuated the induced writhing at dose dependant manner.

DISCUSSION

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 alkaloids, steroids, terpenoids, saponins, glycosides, reducing sugar and amino acid. These chemicals constituents performing various pharmacological actions (Muhammad et al., 2012). The production of free radicals in the living body is responsible for a large number of disorders (Muhammad et al., 20111). Antioxidants are responsible for protection or minimizing the production of these free radicals. The plants are rich source of this antioxidant agent therefore we tested our selected plant for antioxidant potential. Searching new antimicrobial agent is a big challenge for

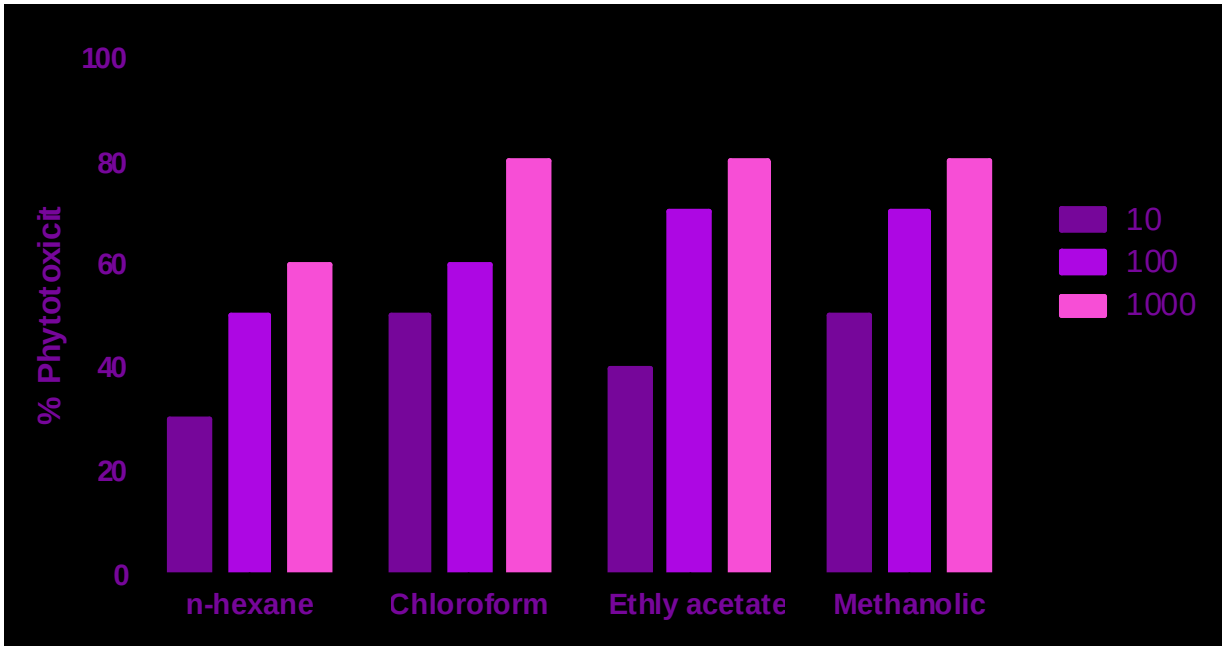

Figure 3: Phytotoxic profile of various solvent extracts of *E. pulcherrima*

Table2. Analgesic profile of Methanolic extract of *Euphorbia pulcherrima*

Diclofenac sodium		**12.34 ± 1.24**
Euphorbia pulcherrima	50	20.34± 3.98
	100	44.34± 2.56

the scientist of the present modern era and plants the biggest source of these agents (Khan et al., 2012). 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 *E. pulcherrima*. Acetic acid induced writing model is also known as chemical induced pain model. This pain paradigm is simple and well established in the present piece of research work our tested plant showed good analgesic effect. The acetic acid induced writhing model is performed for investigation of peripheral pain.

Conclusion

The current studies, suggest that *E. pulcherrima* contain potential secondary metabolite which may be responsible for its use as folk medicine for the treatment of antiarthritis, anticancer, anticonvulsant, antidiabetic, anti-eczema, anti-eczema, anti-inflammatory, antimicrobial and antioxidant. To isolated these bioactive compounds the title plant need extensive studies to be explore.

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