

Phytochemical screening, antibacterial and antiplasmodial activities of *Chrozophora obliqua* and *Launaea nudicaulis*

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Abstract: The present study showed the different Phytochemicals screening in *Chrozophora obliqua* and *Launaea nudicaulis* plants and also correlates the antibacterial and antiplasmodial activity. The results of the study show that the crude and all fractions having zeroed or negative effects to phytochemicals such as alkaloids and saponins tests, whereas the Flavonoids were present in crude extract and all fractions. While the Tannins test showed the positive results against the crude extracts, chloroform and ethyle acetate fractions, whereas the Crude extracts were completely active against all the bacterial strains. Further from the results of the study found that the Hexane and crude extract having better results against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Furthermore, from the results of the study noted that the Crude hexane, Chloroform, E. acetate, butanol and H₂O having negative result against *Escherichia coli*. Out of all fractions the methanolic fractions were most remarkable fractions obtained against malarial activity, while the n-hexane showed better result in 0.025 µg/ml drug dose level. Chloroform, E-acetate and n-butanol were found to be negative effects against all drug dose level, while the Chloroquine was taken as positive control for drug dose amount for known chemo factor which do not showed the increase in number of schizonts.

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1. Introduction

The exploration of novel Phytochemicals needs a lot of research. Nature has bestowed the wild flora with valuable chemistry [1]. Medicinal herbs are globally used for various purposes such as food, health care, fuel and medicines. Medicinal plants and their constituents are the potential sources of both traditional as well as allopathic drugs [2]. The Medicinal power of these plants lies in Phytochemical constituents which cause definite Pharmacological actions on the human body [3]. Majority of Phytochemicals have been known to bear valuable therapeutic activities such as insecticides [4], antibacterial, antifungal [5], anticonstipative [6], spasmolytic [7], antiplasmodic activities [8]. The genus *Chrozophora* belongs to family *Euphorbiaceae* with 30 species. The *Chrozophora obliqua* is used in food and traditional medicines for the treatment of infectious diseases, whooping cough, appetite and as a stimulant [9].

Launaea is an important genus of family *Asteraceae* with 59 species. The plant is popularly used in folk medicine for the treatment of fever, swelling, cuts, itches, ulcer, toothache and eczema eruption [10]. The World Health Organization (WHO) currently encourages, recommends and promotes traditional remedies in health care programs as they

are easily available at low cost, comparatively safe and are culturally acceptable [11]. Limited information is available regarding antibacterial and antiplasmodial activity of *Chrozophora obliqua* and *Launaea nudicaulis*. Therefore, present study was carried out to investigate the antibacterial and antiplasmodial activity against various bacterial and plasmodial species. Preliminary phytochemical studies of these extracts are also undertaken to find out bioactive compounds having antibacterial and antiplasmodial activity.

2. Materials And Methods

2.1 Plant Material

The amount of desired plants samples of *Chrozophora obliqua* and *Launaea nudicaulis* were collected from the areas of Pakistan. These plants were recognized by the faculty of Botany Department, Kohat University of Science and Technology, Pakistan.

2.2 Preparation of extracts

The plants samples were appropriately rinsed with distilled water to removed soil, sand and other surface like bacteria and virus etc. and then were placed in shadow for drying at temperature of 25 - 30 °C. The dried plants were grinded individually and then stored in dirt free, dried synthetic bags for additional biological and chemical processes.

The weight of dried plants (*Chrozophora obliqua* and *Launaea nudicaulis*) up to 2 kg was saturated in methanol for 15 days individually and were extracted and then filtered. The methanol filtrate was passed through (Vacuum Rotary Evaporator) VRE at 50 °C to obtain dehydrated extracts of methanol. The extracted methanol were suspended in water and consecutively partitioned with n-hexane, chloroform, ethyl acetate and n-butanol to get n-hexane-soluble, chloroform-soluble, ethyl-acetate-soluble, n-butanol soluble and dihydrogen oxide fractions, correspondingly. The isolated material and successive further solvent fractions were after that subjected to phytochemical study, antibacterial and antiplasmodial biological test.

2.3 Phytochemical screening

The extracts were subjected to preliminary phytochemical screening for possible presence of bioactive antimicrobial compounds by the methods of Harborne [12] and Sofowora [13] for identifying alkaloids, saponins, flavons, terpenes and tannins.

A. Alkaloids: Dilute 70 % Hydrochloric acid solutions were mixed up with extracts, fractions and filtered. The solution of Hager's reagent was inserted to the filtrate. Yellow color precipitate shows alkaloids existence [14].

B. Saponins: Extracts, fractions were individually heated with 10 mL of dihydrogen oxide (distilled) for about 15 minutes and mixed materials were then cleaned and permitted to cool. Then 3 mL of filtrates were collected and mixed with 15 mL sterilized water and vibrated dynamically for 4 minutes. Froth formation showed the occurrence of saponins in the filtrate [15].

C. Tannins: Extracted material and fraction were heated individually with 20 mL of H₂O (sterilized) for 10 minutes in a water bath and then cleaned. Then 2 mL of the filtrates were combined with 10 mL dihydrogen oxide and 4-5 drops of 20 percent Ferric tri-chloride solution were inserted. A blue color or brown-green precipitate shows tannins occurrence [16].

D. Flavonoids: Extracted material and fraction were mixed up to 15 mL of ethyl acetate. Solution was heated for 5 minutes and then cleaned. Mix 1 mL of dilute NH₃ solution to 5 mL filtrate. Yellowish color precipitate shows flavonoids occurrence [17].

E. Terpenes: Mix solution 0.5 mg of extract and fraction individually to 6 mL dihydrogen oxide and then mixed with 5-6 ethyl acetate drops. Occurrence of bright green color shows terpenes [17].

3. Antibacterial activity

Extracts and various fractions of *Chrozophora obliqua* and *Launaea nudicaulis* were studied for antibacterial assay using gram negative, gram positive

bacteria. Three bacterial strains *E. coli*, *Staphylococcus aureus* and *Salmonella typhi* were used by Agar Well Diffusion Susceptibility Method [18]. In Dimethyl Sulfoxide (DMSO) solutions the extracts and fractions were prepared at the same amount of 3 µg/µL and DMSO was used as a solvent, because it cannot demonstrate any type of activity against bacteria. During the investigation, 28 g/L nutrient agar was used and in the solution media, petri plates and borer were cleaned for 20 minutes at very elevated pressure and at the temperature of 130 °C in autoclave. Agar media were poured in Petri dishes for laminar flow. The 6 mm of sterile discs were dug in bacterial media by means of plastic borer. Every well were given a named special. The cultured bacterial consequent to 10⁴ to 10⁶ (colony forming unit) were speckled on the solid media surface. The Solutions of methanolic extracts and different fractions of same amount 2 µg/µL were prepared. The stock solution of each 40 mL was further added into the applicable wells. The zones of inhibition were calculated once a day for incubation at 40 °C in the incubator. Amoxicillin standard of 5 µg/µL were used for positive control and DMSO was used as a negative control. The inhibition zones of methanolic extracts and other soluble fractions were matched with given drugs as inhibition zones i.e. amoxicillin. The quantity of bacterial growth in each well was calculated.

3. Antiplasmodial activity of *Chrozophora obliqua*

3.1 Antiplasmodial bioassay
The modified method of Kerhoro and Adam (1974) was followed. 100 µL of blood sample were used for the patient of malaria in 8 wells of micro titer plates and were subjected equally amount for the media culture, Roswell Park Memorial Institute-1640. 200 (IU) penicillin and 15 µL kanamycin were subjected into micro titer plate well for the protection of bacterial contamination. Next the micro titer plates were shaken continuously to obtain same phase and standardized suspension in every well. 20 µL drugs for each dose level i.e. 0.025 µg/mL and 0.050 µg/mL were transferred into apprehensive well and chloroquine were used for positive control. The drug amount of famous chemo factor was placed in well number [7]. The negative control in well 8 was kept at the end of the test. The micro titer plates were placed in incubator at 40 °C for the next five days. The duration of incubation, every day different research slides of each wells were made for micro titer plate. Every day all slides are noticeable for detection. Each research Slides were dehydrated and set with CH₃OH. When the slides were dried, every slide was marked with the Giemsa solution (methylene blue) and examined with the help of microscope at 10 x, 40 x and 100 x specification. In this way the growth of schizont were detected and recorded.

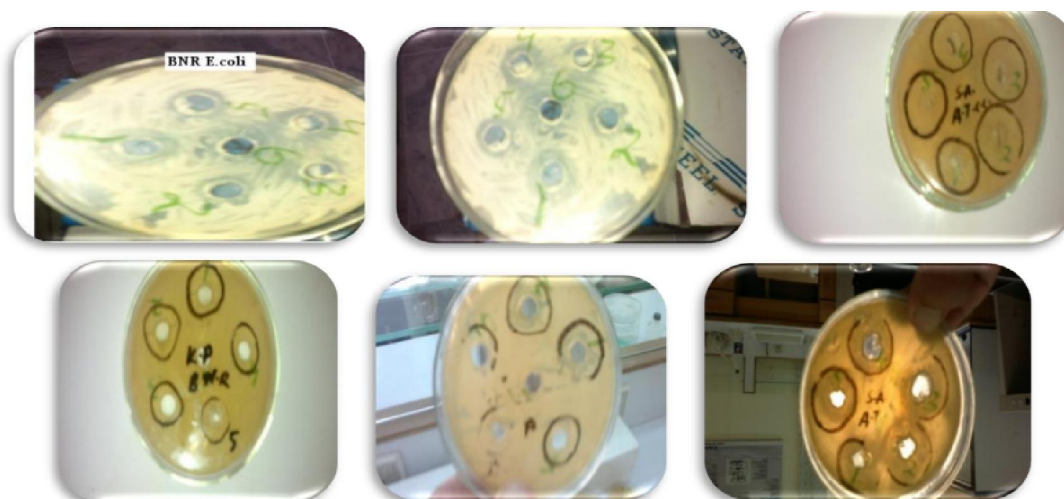


Figure 1. Photographic view of antibacterial activity of *Chrozophora obliqua* and *Launaea nudicaulis*

4. Results And Discussion

4.1 Phytochemical screening of *chrozophora oblique* and *launaea nudicaulis*

The following results showed the detection of various group of phytochemicals in different methanolic extracts and various organic fractions of *chrozophora obliqua* and *launaea nudicaulis* as shown in tables 1. It shows the phytochemical examination of methanolic crude extract and various chemical fractions of *chrozophora obliqua* plant. The methanolic crude extract and all chemical made fractions give zero or negative results to phytochemical like alkaloids and saponins tests. These two phytochemicals were absent in the given tested samples. Flavonoids were present in methanolic crude extract and in all chemical fractions. Terpenes were present in all fractions except n-butanol and aqueous fractions. Tannins were present in methanolic crude extract, chloroform and E. acetate fraction. The table shows the phytochemical examination of methanolic crude extract and the various chemical fractions of

Launaea nudicaulis plant. The methanolic crude extract and all the chemical fractions give zero or negative results to alkaloids and saponins tests and hence were observed absent in the tested samples.

4.2 Antibacterial Activity

The extracts and fractions of *Chrozophora oblique* and *Launaea nudicaulis* plants were same concentrations ration of 2 $\mu\text{g}/\mu\text{L}$ for getting duplicate results carried various inhibition zones. *In vitro* antibacterial activities of crude extract and chemical fractions of *chrozophora obliqua* and *launaea nudicaulis* were subjected, these activities were matched with the given antibiotics (Amoxicillin). In the following antibacterial activity three bacterial strains *E. coli*, *S. aureus* and *S. typhi* was analyzed by AWDSM “Agar Well Diffusion Susceptibility Method”. The inhibition zone for antibacterial activity of *Chrozophora obliqua* is displayed in table 2.

4.3 Antibacterial activity of *Chrozophora oblique* and *launaea nudicaulis*

Table 1. Phytochemical screening of *chrozophora obliqua* and *launaea nudicaulis*

Plants	Extracts	Alkaloids	Flavonoids	Terpenes	Tannins	Saponins
Chrozophora obliqua	Crude	-	+	+	+	-
	n-Hexane	-	+	+	+	-
	Chloroform	-	+	+	+	-
	E-acetate	-	+	+	-	-
	n-Butanol	-	+	-	-	-
	Aqueous	-	-	-	-	-
Launaea nudicaulis	Crude	-	+	+	+	-
	n-Hexane	-	+	+	+	-
	Chloroform	-	+	+	+	-
	E-acetate	-	+	+	-	-
	n-Butanol	-	+	+	-	-
	Aqueous	-	+	+	-	-

Table 2. Antibacterial activity of *Chrozophora obliques* and *Launaea nudicaulis*

Organisms	Inhibition zone (mm)						
	Chrozophora oblique						
	Crude	n-hexane	Chloroform	E. Acetate	n-butanol	Aqueous	Standards
<i>Escherichia Coli</i>	9	7	7	6	-	-	19
<i>Salmonella typhi</i>	14	16	17	10	9	9	21
<i>Staphylococcus Aureus</i>	11	16	17	15	12	7	21
Launaea nudicaulis							
<i>Escherichia Coli</i>	6	6	-	-	-	-	19
<i>Salmonella typhi</i>	12	18	15	12	13	10	21
<i>Staphylococcus Aureus</i>	15	17	12	11	10	9	21

Table 3. Antiplasmodial activity of *chrozophora obliqua*

S. No	Drug	Drug dose level	Drug Concentration (µg/ml)	Isolation of parasites in different fractions (Schizonts)					
				Methanol	n-Hexane	Chloroform	E. Acetate	n-Butanol	Aqueous
1	A	A ₁	0.025	01	02	03	03	04	05
		A ₂	0.050	0	01	02	03	03	04
2	B	B ₁	0.025	0	03	03	04	04	05
		B ₂	0.050	0	03	03	03	04	05
3	C	C ₁	0.025	01	03	02	04	03	04
		C ₂	0.050	0	02	01	03	02	04
4	D	D	Chloroquine	No Growth					
5	E	E	Distilled water media	Hundred percent growth					

Table 4. Antiplasmodial activity of *launaea nudicaulis*

S. No	Drug	Drug dose level	Drug Concentration (µg/ml)	Isolation of parasites in different fractions (Schizont)					
				Methanol	n-Hexane	Chloroform	E.Acetate	n-Butanol	Aqueous
1	A	A ₁	0.025	01	02	03	03	04	05
		A ₂	0.050	0	01	02	03	03	04
2	B	B ₁	0.025	0	03	03	04	04	05
		B ₂	0.050	0	03	03	03	04	05
3	C	C ₁	0.025	01	03	02	04	03	04
		C ₂	0.050	0	02	01	03	02	04
4	D	D	Chloroquine	No growth					
5	E	E	Distilled water media	Hundred percent growth					

Methanolic Crude extract was completely active throughout all bacterial strains. Hexane and the crude extract showed good results against *S.aureus*, *E.coli* and *s typhi*. Chloroform, E. acetate, n- butanol and H_2O soluble fraction show negative result against *E.coli*. crude, n-hexane and chloroform showed maximum inhibition (19mm). n- hexane and chloroform fraction showed good activity against *salmonella typhi* and *staphylococcus aureus*. Methanolic Crude extract and n- hexane were throughout active against *salmonella typhi* and *staphylococcus aureus*. Hexane and crude extract show good activity against *S.aureus*, and *s typhi*. methanolic Crude

hexane Chloroform, E. acetate, butanol and H_2O show negative result against *E.coli*.

4.3 Antiplasmodial activity

4.3.1 Antiplasmodial activity of *Chrozophora oblique* and *launaea nudicaulis*

The table 3 demonstrates the results of antiplasmodial activity. Out of all fractions, the CH_3OH fraction of *Chrozophora obliqua* is the most remarkable fraction obtained against malarial activity. n-hexane revealed good result in A2 drug dose level. Chloroform fraction exhibited good result in C2 drug dose level.. E-acetate and n- butanol showed negative results against all drug dose level (≤ 1 level is

significant). Chloroquine were taken as positive control for drug dose amount for known chemo factor which do not showed the increase in number of schizonts. The table 4 shows the results of antiplasmodial activity of *launaea nudicaulis*. Out of all fractions, the *CH₃OH* fraction of *launaea nudicaulis* are most remarkable fractions obtained against malarial activity. n-hexane showed good result in A2 drug dose level. Chloroform, E-acetate and n-butanol showed negative result against all drug dose level. (≤ 1 level is *significant*). Chloroquine were taken as positive control for drug dose amount for known chemofactor which do not showed the increase in number of schizonts.

5. Conclusion

The results proved that the plants store different classes of phytochemicals with different concentration. These phytochemicals are responsible for different biological activity by inhibiting the reproduction of different microbes and destroy them. The antibacterial and antiplasmodial results of *chrozophora oblique* and *launaea nudiculis* indicate that crude extracts, ethyl acetate, chloroform and n-butanol fraction of all parts were the most active fraction. It is recommended that *chrozophora oblique* and *launaea nudiculis* are important plants from medicinal point of view and can be potent candidates for further *in-vivo* bioassays which would leads to the synthesis of safe herbal drugs with or less side effects of global interest.

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