Avicenna Journal of Phytomedicine
Received: Mar 31, 2011; Accepted: April 24, 2011
Vol. 1, No. 1, Summer 2011, 24-28

Phototoxicity activity of *Psoralea drupacea* L. using *Atremia salina* bioassay system

Mohammad Ramezani[1,*], Hossein Hosseinzadeh[2], M. Moradi[3], E.Taghiabadi[3]

Abstract

Objective: Phototoxicity is a kind of dermatitis that is activated by exposure to ultraviolet light following the administration of some drugs or natural products. *Artemia salina (A. salina)* (brine shrimp) has been effectively applied for toxicity testing and is perfect for biological screening of many chemicals for simultaneous evaluation of toxicity and phototoxicity. The objective of this study was to investigate the phototoxic activitiy of the methanolic extract and chloroform and CH_3OH/H_2O_2 fraction of *Psoralea drupacea (P. drupacea).*
Materials and methods: The phototoxic effect of the methanolic extract, chloroform and CH_3OH/H_2O_2 fractions of *P. drupacea* was evaluated using *A. salina* bioassay system. Different concentrations of methanolic extract and fractions of *P. drupacea* were added to the plate of one-day old larvae followed by exposure to UV radiation at 366 nm in three different exposure times (0, 4 and 20 h). Mortality was determined 24h after the start of the irradiation.
Results: The value of LC_{50} of *P. drupacea* methanolic extract and methoxalen as positive control were 0.64 and 3.5×10^{-4} mg/ml, respectively. *P. drupacea* methanolic extract and chloroform fraction demonstrated phototoxic activity after 4 h radiation.
Conclusion: The result showed that *P. drupacea* methanolic extract and chloroform fraction have phototoxicity in *A. salina* bioassay system and their toxic effect is related to phototoxic constituents such as psoralen.

Keywords: *Artemia salina*, *Psoralea drupacea*, Phototoxicity, Toxicity

1- Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I. R. Iran
2- Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I. R. Iran
3- Department of Pharmacodynamy and Toxicology, School of Pharmacy, Mashhad University of Medical sciences, Mashhad, I. R. Iran
***Corresponding author:** Tel: +98 5118823252; Fax: +98 5118823251
E-mail: Ramezanim@mums.ac.ir

Introduction

Psoralea drupacea Bunge (*Papilionaceae*) is one of *Psoralea* species which is found in northeast of Iran. Many members of the *Psoralea* species contain medicinally-active components including isoflavones (Shinde et al.,, 2010), terpenoid, drupanin and drupenin (Backhouse et al., 2001; Chena et al., 2007), angelicin (Jakobs and Piette 1994), bergapten, iso-bergapten, xanthotoxin, umbelliferone and sphondin (Nikonov and Artamonova 1993), daidzein (Shinde et al., 2010), bakuchiol (Backhouse et al., 2001), plicatin B (Mitscher et al., 1996) and furocoumarins such as psoralen, isopsoralen and psoralidin which are the most extensive secondary metabolites in these plants (Backhouse et al., 2001; Chena et al., 2007; Xiao et al., 2010). Many members of *Psoralea* species are used to treat aging symptoms, seasonal affective disorders (by inhibiting of hepatic melatonin metabolism), depression (Chena et al., 2007), osteoporosis (Xina et al., 2010), wounds, stomach ailments, fever (Backhouse et al., 2001), asthma, cough, nephritis, vitiligo, and calvities (Liu et al., 2004). Recent pharmacological studies on *P. drupacea* have shown antidepressant properties (Chena et al., 2007), glycosidase inhibitory activity (Oh et al., 2010) antioxidant effect (Xiao et al., 2010), antiviral and antitumor activities (Liu et al., 2004), estrogenic activities (Lim et al., 2011), antimicrobial effects, anti-inflammatory and antipyretic activities (Backhouse et al., 2001; Khatune et al., 2004). Furocoumarins such as psoralens are naturally occurring phototoxic constituents found in several plants including the species of *Psoralea* (Liu et al., 2004). These phototoxic agents can cross-links pyrimidine-base pairs in DNA that is the basis for their biological effect leading to some activities, such as inhibition of DNA replication (Walter et al., 1982). Furthermore, furocoumarins can induce production of singlet oxygen (oxygen radicals) which can react with proteins rendering them inactive (Frederiksent and Nielsen 1989). Furocoumarins can be harmful and induce eczema and dermatologic reaction which result from exposure to ultraviolet (UV) light followed by touching the plant material (Ojala et al., 1999; Chobot et al., 2006). With careful monitoring, some of the phototoxic compounds have been used as chemotherapeutic agents (PUVA) in the treatment of different skin disorder such as recalcitrant palmoplantar pustolosis, lichen planus, cutaneous mastocytosis, alopecia areata, repigmentation in vitiligo, mycosis fungoides and especially psoriasis (Frederiksent et al., 1989).

A. salina (brine shrimp) has been effectively used for toxicity testing; therefore it seemed to be applicable for the initial biological screening of many samples for simultaneous detection of both toxicity and phototoxicity (Ojala et al., 1999). Phototoxicity is a kind of dermatologic reaction that is activated by exposure to ultraviolet light following the administration of different types of drugs or natural products (Magne et al., 2010). Hence, it can be helpful to develop a rapid, simple and inexpensive procedure to test it.

As *P. drupacea* contains some phototoxic constituents such as psoralen, in this study the phototoxicity of the methanolic extract and chloroform fraction of *P drupacea* using *A. salina* bioassay system was evaluated.

Material and method

Plant

Aerial parts of *P. drupacea* were collected from northen region of Mashhad, in July and identified by M.R. Joharchi. A voucher specimen was deposited at the Herbarium of School of Pharmacy, Mashhad University of Medical Sciences, Iran.

Preparation of Extract

The aerial parts of *P. drupacea* were cleaned, dried in shadow and powdered by mechanical grinder. Then, the aerial parts powder (100 g) was defatted with petroleum ether using the Soxhlet apparatus for a period of 3 days. For the methanolic extract, powder was subsequently macerated in methanol, three times and for 3 days and the mixture was subsequently filtered and concentrated under reduced pressure at 40 °C. The residue was suspended in saline solution and sonicated for 20 min. The concentrated extract was then fractionated with an equal volume of chloroform or CH_3OH/H_2O, three times, to give two fractions containing nonpolar and polar compounds, respectively.

Artemia assay

The experiment is based on the modification of methods developed by Ojala et al., (Ojala et al., 1999). Briefly, the dried brine shrimp *(A. salina,* local market) were placed in saline solution (artificial seawater, g/L) included NaCl 27 g, $MgCl_2.6H_2O$ 4 g, KCl 0.5 g, $CaCl_2.2H_2O$ 1 g, $MgSO_4.7H_2O$ 2.39 g and $NaHCO_3$ 0.1 g in distilled water, pH 8 to 9 and were illuminated with lamp (1000-4000 lux) for 1 hour. Then, eggs were incubated for 24 hours at 25°C in dark. The hatched larvae were used as such in phototoxicity bioassay method [9].Fifty microliters of one-day old larvae of *A. salina* was pipetted into 24-well plates (5–15 larvae per well). Different concentrations (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/ml) of methanolic extract of *P. drupacea* and chloroform or CH_3OH/H_2O fractions at concentration of 1 mg/ml were added to the plate. Methoxalen and saline solution were considered as a positive and negative control, respectively. In each case, three replicates of each concentration were assayed. 15 minutes after adding all solutions and suspensions into the plates, the larvae were exposed to UV radiation at 366 nm (DESAGA, Germany) at a distance of 30 cm at 25-30 ˚C. The plates were covered to prevent the solution from evaporation. Three different radiation times (0, 4 and 20 h) were used in each test. The control set of larvae were kept in dark. Mortality was determined 24h after the start of the irradiation.

Statistical analyses

The LC_{50} values and 95% confidence intervals (CI) were calculated by using Litchfield and Wilcoxin Π program from PHARM/PCS Version 4.2 software. The analyses were performed by GraphPad Prism.

Results

Phototoxic activity of the methanolic extract, chloroform and CH_3OH/H_2O_2 fraction of *P. drupacea* was studied by *A. salina* bioassay test. In the absence of radiation (0 h) *P. drupacea* methanolic extract did not induce phototoxicity in any concentrations while methoxalen demonstrated phototoxicity at the highest concentration. *P. drupacea* methanolic extract at the highest concentration and methoxalen induced 100% mortality in *A. salina* after 4 h radiation. Also 100% mortality was observed at all concentrations of extract, positive and negative controls after 20 h radiation. The extracts did not show any toxicity in *A. salina* in the absence of UV radiation after 20 h. The LC_{50} value of *P. drupacea* methanolic extract was 0.64 mg/ml, (95% CI, 0.4965 to 0.8246), after 4 h of radiation. The LC_{50} value of methoxalen was 3.5×10^{-4} mg/ml, (95% CI, 1.75×10^{-4} to 7.03×10^{-4}), after 4 h radiation. The result of phototoxicity tests of *P. drupacea* methanolic extract is shown in Figure 1. The result of phototoxicity tests of methoxalen is shown in Figure 2. The results of chloroform fraction

phototoxicity were similar to that of methanolic extract of *P. drupacea* but no mortality was observed in CH_3OH/H_2O fraction at 4 h radiation (the results of neither fractions were shown).

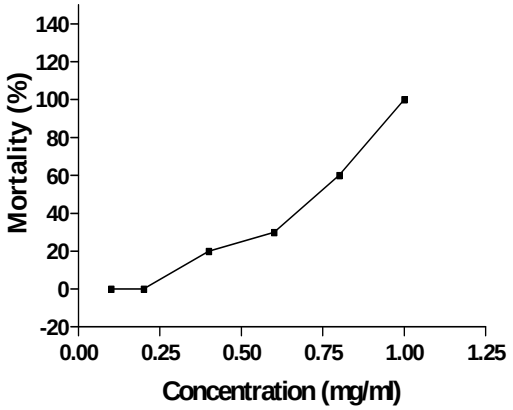

Figure 1. Effect of the *P. drupacea* methanolic extract in conjunction with UV 366 nm on *Artemia salina* at 4 h after radiation. Mean value of all measurements is 3.

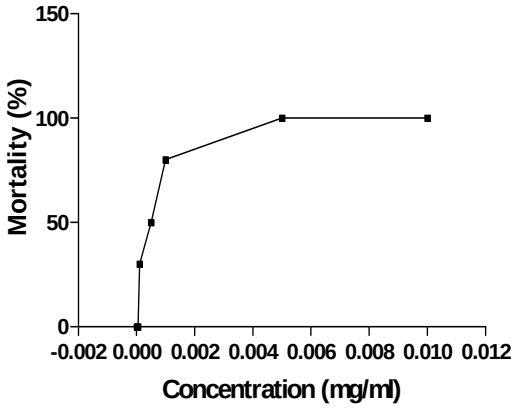

Figure 2. Effect of methoxalen in conjunction with UV 366 nm on *Artemia salina* at 4 h after radiation. Mean value of all measurements is 3.

Discussion

To evaluate phototoxicity of many chemicals, the application of *A. salina* is a beneficial method and it is a fast, simple and inexpensive technique. Considering the results of the other phototoxicity tests, for this study three different radiation times (0, 4 and 20 h) was employed. To show any toxicity, 0 h was chosen and for evaluating the extract phototoxicity, 4 h was used while 20 h exposure time was for making sure if there was phototoxicity at all (Ojala et al., 1999). In the absence of radiation (0 h) methoxalen exhibited phototoxicity at the highest concentration tested which was in agreement with other studies (Ojala et al., 1999). Results of 4 h radiation time showed 100% mortality at the highest concentration of methanolic extract and methoxalen, so 20 h radiation was not considered. The results of this study showed that *P. drupacea* methanolic extract and chloroform fraction induced phototoxic activity in *A. salina* after 4 h radiation. *P. drupacea* is a rich source of furanocoumarins which is a kind of nonpolar phototoxic constituents and phototoxicity of *P. drupacea* is related to phototoxic constituents that are present and accumulate in nonpolar chloroform fraction (Frederiksent et al., 1989; Liu et al., 2004). According to the data, it is concluded that the methanolic extract of *P. drupacea* and its chloroform fraction have phototoxicity activity using *A. salina* bioassay system.

References

Backhouse CN, Delporte CL, Negrete RE, Erazo S, Zuniga A, Pinto A ,Cassels BK (2001). Active constituents isolated from *Psoralea glandulosa* L. with antiinflammatory and antipyretic activities. J Ethnopharmacol, 78:27–31.

Chena Y, Wangb HD, Xiaa X, Kungc HF, Pana Y , Kong LD (2007). Behavioral and biochemical studies of total furocoumarins from seeds of *Psoralea corylifolia* in the chronic mild stress model of depression in mice. Phytomed, 14:523–529.

Chobot V, Vytlačilová J, Kubicová L, Opletal L, Jahodář L, Laakso I ,Vuorela P (2006). Phototoxic activity of a thiophene polyacetylene from Leuzea carthamoides. Fitoter, 77:194–198.

Frederiksent S ,Nielsen PE (1989). Lysosomes: a possible target for psoralen photodamage. J Photochem Photobiol, 3:437 - 441.

Jakobs A ,Piette J (1994). Photobiological activity of sulphur and selenium analogues of psoralen. J Photochem Photobiol B: Biol, 22:9-15.

Khatune NA, Islam ME, Haque ME, Khondkara P ,Rahman MM (2004). Antibacterial compounds from the seeds of *Psoralea corylifolia*. Fitoter, 75:228–230.

Lim SH, Ha TY, Ahn J , Kim S (2011). Estrogenic activities of *Psoralea corylifolia* L. seed extracts and main constituents. PhytomedArticle in Press, Corrected Proof

Liu r, Li A, Sun A ,Kong L (2004). Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. J Chromatogr A, 1057:225–228.

Magne N, Chargari C, Auberdiac P, Moncharmont C, Merrouche Y ,Spano JP (2010). Ultraviolet recall dermatitis reaction with sorafenib. Invest New Drug, 9476-5.

Mitscher LA, Telikepalli H, McGhee E , Shankel DM (1996). Natural antimutagenic agents. Mutat Res, 350:143-152.

Nikonov GK, Artamonova NA (1993). Phenolic components of the unsaponifiable fraction of the lipids of *Psorlea drupaceae*. Chem Nat Comp, 29:251-252.

Oh KY, Lee JH, Curtis-Long MJ, Cho JK, Kim JY, Lee WS , Park KH (2010). Glycosidase inhibitory phenolic compounds from the seed of *Psoralea corylifolia.* Food Chem, 121:940-945.

Ojala T, Vuorela P, kiviranta J, Vuorela H ,Hiltunen R (1999). A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. Planta Med, 65:715-718.

Shinde AN, Malpathak N, Fulzel DP (2010). Determination of isoflavone content and antioxidant activity in *Psoralea corylifolia* L. callus cultures Purchase. Food Chem, 118:128-132.

Shinde AN, Malpathak N ,Fulzele DP (2010). Determination of isoflavone content and antioxidant activity in *Psoralea corylifolia* L. callus cultures. Food Chem, 118:128–132.

Walter JF, Gange RW, Mendelson IR ,San Diego C (1982). Psoralen-containing sunscreen induces phototoxicity and epidermal ornithine decarboxylase activity. Am Acad Dermatol, 6:1022-1027.

Xiao G, Li G, Chen L, Zhang Z, Yin JJ, Wu T, Cheng Z, Wei X , Wang Z (2010). Isolation of antioxidants from *Psoralea corylifolia* fruits using high-speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J Chromatogr A, 1217:5470-5476

Xin D, Wang H, Yang J, Su YF, Fan GW, Wang YF, Zhu Y ,Gao XM (2010). Phytoestrogens from *Psoralea corylifolia* reveal estrogen receptor-subtype selectivity. Phytomed, 17:126-131.