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Comparative toxicological studies of *Heracleum maximum* W. Bartram and *Psoralea corylifolia* L. seed extracts on brine shrimp (*Artemia salina*) and wistar rats

Preeti Dhar*◆, Igor Gembitsky, Renato Carvalho*, John Hoffmann*, Brett Pinsky*, Jordan Greenough*, Jamie Woych*, Maureen Morrow**, Steven Jury***, Prashant Kumar Rai**** and Geeta Watal****

* *Department of Chemistry, 1 Hawk Drive, State University of New York, New Paltz, New York 12561, USA*

** *Department of Biology, 1 Hawk Drive, State University of New York, New Paltz, New York 12561, USA*

*** *Sciences Department, 278 Whites Bridge Road, Standish, Saint Joseph's College of Maine, 04084, USA*

**** *Alternative Therapeutics Unit, Drug Discovery and Development Division, Medicinal Research Lab, Department of Chemistry, University of Allahabad, 211002, India*

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Abstract

This study sought to elucidate the reason for the traditional use of *Psoralea corylifolia* (PC) over *Heracleum maximum* (HM) for treating vitiligo, though seeds from both plants are rich in a class of phototoxic compounds called furanocoumarins, that are effective in treating vitiligo. Toxicity studies were carried out by LD_{50} experiments on wistar male and female rats, and the physiochemical parameters associated with toxicity were evaluated. The toxicity results were correlated to the elemental profile obtained for the two plants using laser-induced breakdown spectroscopy (LIBS). The phototoxic/toxic properties of the ethanolic extracts of the seeds of the two plants were compared using brine shrimp bioassay. The PC extract showed lower toxicity levels under long-wave UV light (365 nm) than the HM extract, where 100% mortality was observed at the lowest concentration tested (30 µg/ml). Under dark conditions, the LD_{50} for HM (63.93 ± 6.63 µg/ml) was also lower than that of PC, where only 53.7% mortality was observed at the highest concentration tested (237.1 µg/ml),further suggesting that HM seeds are more toxic than PC seeds. The findings indicate that HM seeds have not been used in traditional medicine for skin ailments because of relatively higher levels of animal toxicity and hence the potential for adverse effects.

1. Introduction

Plant-based drugs have been used for ages, worldwide, to relieve various ailments. The knowledge regarding medicinal plants is usually passed on from generation to generation before these plants become a subject of scientific investigation. From that perspective, characteristics such as the chemical composition of the natural source, the mechanism (s) of action, toxicology, and bioactivity (Tang *et al*., 2023; Usmani*et al*., 2023; Zeng *et al*., 2023) become crucial in developing new commercial drugs. Accordingly, several drug candidates are originally from natural sources (such as medicinal plants) and are undergoing further development (Harvey, 2008).

Psoralea corylifolia L. (also called *Cullun corylifolium*) (Family: Fabaceae), a medicinal plant native to the Indian subcontinent and China, has long been used in traditional medicine (Rangari and Agrawal, 1992). *Psoralea* originates from the Greek word 'Psoraleos', meaning 'itch' (Chopra *et al.,* 2013). In India, PC is called "Kushtanashini", meaning leprosy destroyer (Khushboo *et al*., 2010). PC seeds are rich in compounds called furanocoumarins, which exhibit a broad range of pharmacological activities. In India, PC is used internally to treat impotence, kidney, stomach, gynecological, and heart problems (Yin *et al*., 2004; Dastur, 1962; Chopra *et al*., 1956). Externally, it is used to cure skin disorders (Chakravarti *et al.,* 1948; Latha *et al*., 2000), including vitiligo. Figure 1 shows the furanocoumarins found in PC and HM.

The paste of PC seeds is used topically on affected skin areas to treat vitiligo, wherein,white patches appear on the skin due to the absence of the skin pigment called melanin. Deficiency of melanin results mainly from the destruction of the melanocytes (Chao *et al*., 2018). The furanocoumarin, psoralen, stimulates melanin synthesis (Ren *et al*., 2020). Melanin biosynthesis occurs in melanocytes and is catalyzed by melanocyte-specific proteins such as tyrosinase, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2) (Fang *et al.,* 2001). Several possible believed causes of vitiligo include genetics, autoimmune, and stress-related factors (Halder and Chappell, 2009). The exact cause of melanocyte loss in vitiligous skin is unknown, though several theories have been proposed (Lucia *et al*., 2019; Alikhan*et al*., 2011).Furanocoumarins are phototoxic in the presence of light of wavelengths 320-400 nm (long UV) and can cause the skin to blister and brown (Dall'Acqua *et al*., 1974). The biological activity of furanocoumarins is attributed to their ability to react with the thymine residues of DNA in the presence of long UV light. However, the mechanism by which these compounds stimulate the production of melanin in vitiligous skin is not known (Cardona *et al*., 2005).

Corresponding author: Dr. Preeti Dhar

Department of Chemistry, 1 Hawk Drive, State University of New York, New Paltz, New York 12561, USA

E-mail: dharp@newpaltz.edu

Tel.: +0018452573797

Heracleum maximum W. Bartram (Family: Apiaceae), a plant whose stem and root are edible, grows in North America (Peterson, 1977). HM has previously been known as *H. lanatum* Michx. and *H. Sphondylium* var. *montanum* and has been used in traditional medicine for treating a variety of infectious diseases (Webster *et al*., 2006). HM is also reported to be rich in furanocoumarins (Camm *et al*., 1976). Even though blistering and browning of skin due to the phototoxicity of HM is well known (Camm *et al*., 1976), and is like PC, there is no recorded use of the seeds of this plant for treating vitiligo in folk medicine.

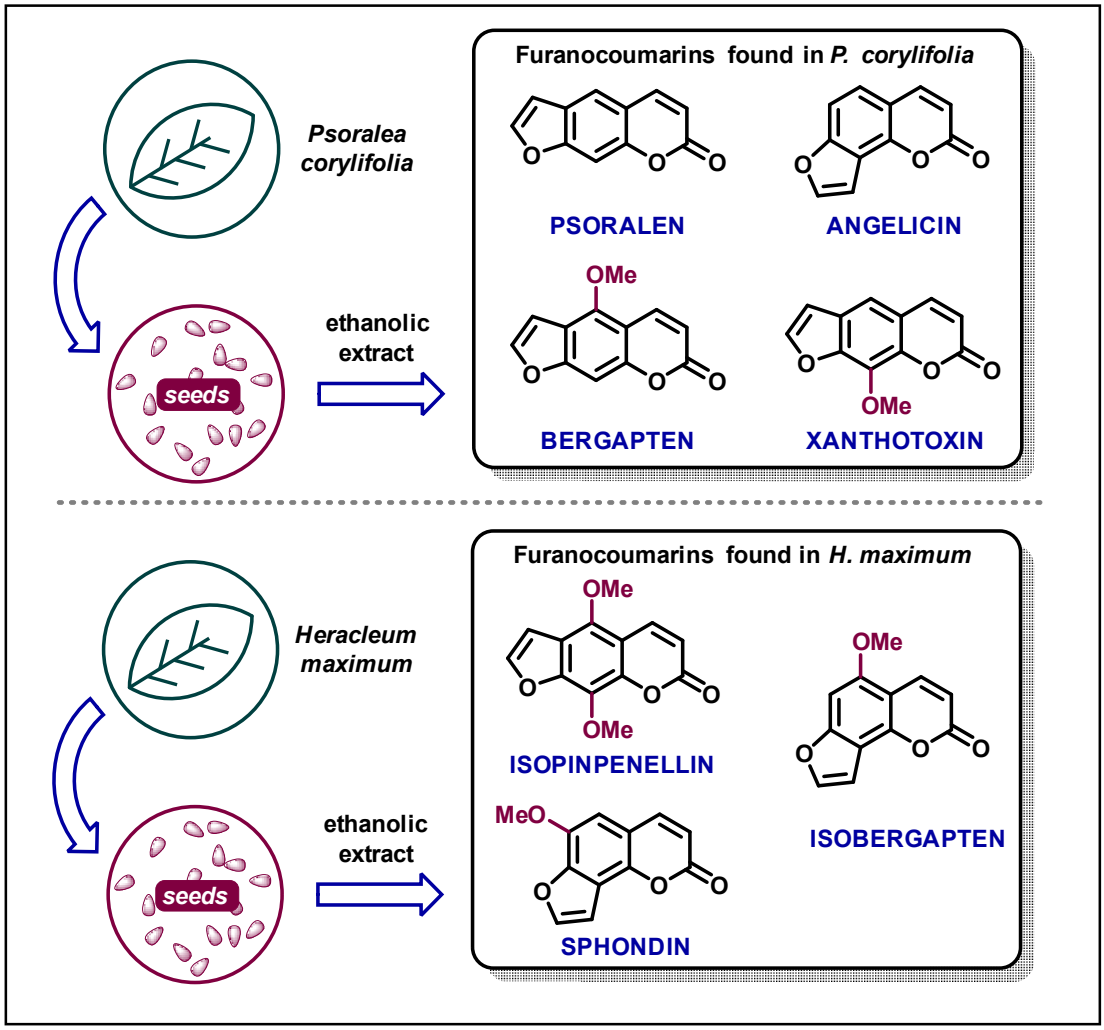

Figure 1: Furanocoumarins found in *P. corylifolia* and *H. maximum*.

In this research, we sought to understand why these two plants growing in different parts of the world and having the same class of compounds (furanocoumarins) were used for different medicinal purposes by the Native Indian and American Indian cultures and whether HM could be considered a potential candidate for treating vitiligo.

PC and HM plant extracts were screened for toxicity/phototoxicity using a standard brine shrimp bioassay (Persoone and Wells, 1987; Libralato *et al*., 2016). The dose-response curve and the median lethal dose (LD_{50}) of the extracts leading to mortality were determined. PC and HM extracts were also used to evaluate acute and sub chronic toxicity in albino wistar rats, a standard mammalian ecotoxicology model organism (Weber *et al*., 2011; Chellan *et al*., 2008). Thin-layer chromatography was used to evaluate both plants' crude seed extracts and qualitatively estimate the various furanocoumarins present. LIBS analysis was carried out to compare the elemental profile of both the plants (Rai *et al*., 2014) and see their role in melanogenesis.

2. Materials and Methods

2.1 Materials

The seeds of PC were obtained from an herbal drug store in Lucknow, India and authenticated by Dr. Kanak Sahai, Botanist at the National Botanical Research Institute (NBRI) Lucknow, India. A sample was deposited in the NBRI herbarium under registration # 1Aa on August 22, 2003. HM seeds were purchased from the Prairie Moon Nursery, Inc., in Winona, MN, USA (lot number EW595B) in 2006. San Francisco Bay Brand Brine Shrimp Eggs were used (Aquatic Ecosystems, Florida). Bergapten, xanthotoxin, psoralen, angelicin, isobergapten, sphondin, isopinpenellin, and sea salts were purchased from Sigma Aldrich. All solvents used were ACS grade and purchased

from Sigma-Aldrich or Fisher Scientific. Albino wistar rats were housed in an air conditioned room and had free access to water and a pellet diet (Pashu Aahar Kendra, Varanasi, India). All experimental procedures were in accordance with the internationally accepted guidelines for the care and use of laboratory animals. All animal procedures used were in strict accordance with the Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines, under the Animal Welfare Division, Govt. of India, India. Experimental protocols for all the studies conducted on rats were approved on 01/10/2004 by the Institutional Ethical Committee (83 a/a/04/CPCSE) of Allahabad University, India.

2.2 Extraction of plant material

Seeds of PC and HM were pulverized and run through a Soxhlet extractor in a sequential manner using the following solvents (in order of polarity): hexane, ether, ethyl acetate, and ethanol. The seeds were also extracted with either ethanol or water using the Soxhlet extractor. The extractions were run until the solvent was clear. The solvent was then removed using a Labconco rotary evaporator, with a Brinkmann Model B169 vacuum aspirator and an Alcatel 2008A high vacuum pump.

2.3 Furanocoumarin screening

Preliminary qualitative furanocoumarin analysis was carried out using thin-layer chromatography on silica plates developed with a mixture of ethyl acetate/hexane (30%-50%) for optimal resolution of spots. A 5% methanolic sodium hydroxide solution was sprayed on half of the TLC plate, causing any spots which contain coumarins to fluoresce differently under long-wave UV light due to the lactone ring opening of coumarins (Stahl, 1969). Authentic samples of various furanocoumarins were purchased from Sigma Aldrich.

2.4 Toxicity studies with wistar rats

LD_{50} for both the plant extracts was determined on wistar rats using a single dose of 2.5 g and 3.75 g. Single doses of 2.5 g and 3.75 g are 10 and 15 times, respectively, of the amount that is determined to be the most effective dose (MED). MED was identified as 250 mg per kg body weight (b. wt.) through a previous study (Dhar *et al.,* 2013). Five groups consisting of six rats each (3 females and 3 males), weighing 180-200 g were used for the study. Group I rats were orally administered a single dose of 2.5 g of the PC extract, group II rats were administered 2.5 g of HM extract, group III rats were administered 3.75 g of PC extract, group IV rats were administered 3.75 g of HM extract and group V, a control group was fed the normal pellet diet. All the rats at all times had access to water and the pellet diet. After administering the extracts, the rats were observed for their gross behavior, neurologic, autonomic, and toxic effects continuously. Food consumption, feces and urine were examined 2 h after the extract administration and then subsequently at 6 h intervals for 24 h.

2.5 Phototoxicity bioassay using brine shrimp

The brine shrimp bioassay as previously described (Ojala, 1999) was used with modifications as described below.

2.5.1 Hatching shrimp

Brine shrimp cysts (1 g) were soaked for an hour in fresh water with light aeration. The eggs were filtered and transferred to a litre of 2% saline solution (which was prepared using sea salts and distilled deionized water (wt./v), filtered, and brought to pH 7.5-8 using sodium bicarbonate), temperature was maintained between 28-30ºC, and illumination with a 40 W bulb provided direct continuous light. Hatching occurred over 24 h after which aeration was stopped and the bottom of the beaker was illuminated to allow the nauplii (phototactic) to separate from the unhatched cysts and nauplii were transferred using a Pasteur pipet to fresh saline solution. Newly hatched nauplii which were less than 5h post-hatch were used for all bioassays.

2.5.2 *Artemia salina* (brine shrimp) bioassay

Stock solutions of all the PC extracts (15 µg/µl) and HM (1µg/µl) seed extracts were prepared in DMSO. Tissue culture plates (untreated falcon, 96 well, 350 µl) were used for the bioassay. Two identical sets of 96 well plates having wells of 350 µl capacity were prepared and nauplii (5-15) were gently pipetted into each well. One well plate was exposed to long-wave UV light (365 nm) and the other was placed in the dark. Mortality was assessed after 24 h and death was determined if an individual brine shrimp did not exhibit any motion of appendages over the course of 10 seconds. The total number of nauplii per well was counted when they died of starvation. Wells (n=16) at each freshly made extract concentration (30, 60, 119 and 237 µg/ml, respectively) were tested. Table 1 for example, shows how the 96 well plates were set up for HM extracts when the seeds were extracted with solvents of increasing polarity.

Table 1: Set up of the light and dark well plates for HM extracts when they were sequentially extracted with solvents of increasing polarity

Experimental samples						Positive control	Negative control
Extract volume (µl)	0	2	4	6	8	0	0
DMSO volume (µl)	0	8	6	4	2	0	10
Saline volume (µl)	350	340	340	340	340	340	340
Bergapten volume (µl)	0	0	0	0	0	10	0
Total volume (µl)	350	350	350	350	350	350	350
Concentration of extract (µg/ml)	0	5.7	11.4	17.1	22.8	0	0

A negative control consisted of saline (340 µl) with DMSO (10 µl) and the positive control consisted of a known furanocoumarin, bergapten (5-methoxy-psoralen) at a concentration of 30 µg/ml. DMSO concentration in each well was 2.86% (10 µl). An additional brine shrimp bioassay with varying amounts of DMSO in saline (so that the total volume of both the DMSO and saline was 350 µl,) was performed previously to determine the effect of DMSO on brine shrimp mortality (Figure 2). Several studies have tested the effects

of various solvents on brine shrimp mortality rates (Barahona-Gomariz *et al.,* 1994; Banti *et al*., 2021). Results show that 10 µl of DMSO mixed with 340 µl of saline had very little effect on the brine shrimp mortality, but was necessary for keeping the extract in solution. Lower amounts of DMSO precipitated the extract. No lethality of brine shrimp was observed in the negative control.

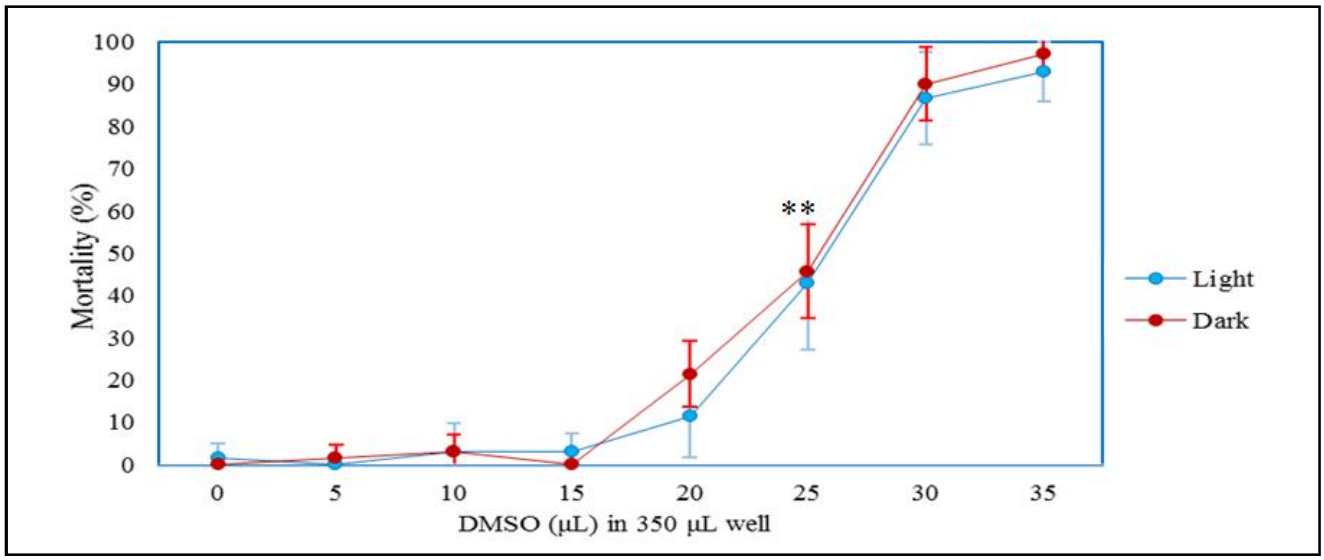

Figure 2: Brine shrimp mortality at 24 h with varying amounts of DMSO mixed with saline in a 350 µl well. Light refers to UV light (365 nm). **p<0.01 as compared with control.

Earlier, due to the length of time necessary to observe mortality rates in each well, some brine shrimps were exposed to the experimental conditions for a longer period than others before mortality rates were recorded. To overcome this shortcoming, a webcam (Logitech Fusion © webcam) was attached to a dissecting microscope and each well was recorded for 10 seconds. Reviewing these recordings significantly cut down on the discrepancy in exposure time from the first and last brine shrimp wells observed.

2.5.3 Data analysis

The brine shrimp mortality vs. extract concentrations were plotted and compared using Probit analysis (MacStatPlus, www.analyst.com). LD_{50} (mean ± SEM) were determined only for HM under dark conditions as mortality and survivorship were within the range of concentrations tested. However, given the very high toxicity of HM under UV light (estimated LD_{50}<30 µg/ml) and the very high survivorship of brine shrimp in the PC extract, under dark conditions (estimated LD_{50}>150 µg/ml), we do not present LD_{50} values for these treatments given the range of concentrations tested.

2.6 LIBS-based screening of HM and PC

LIBS was used to detect trace elements in HM and PC seed extracts and the intensity ratios of trace elements present were estimated. HM and PC seed extracts were lyophilized and dissolved in distilled water (1g/10 ml) and LIBS analysis was carried out. The LIBS spectra were recorded at a repetition rate of 2 Hz and 175-mJ-laser energy covering the wavelengths from 200-290 and 390-430 nm.

3. Statistical analysis

The mean and standard deviation were calculated for each set of data. Statistically significant differences were determined using the student's t-test (p<0.05).

4. Results

4.1 Extraction of plant materials

Powdered seeds of PC and HM were extracted with ethanol and concentrated to give the corresponding crude ethanolic extracts (CEE).The powdered seeds of both plants were also sequentially extracted with solvents of increasing polarity and the extracts were concentrated and weighed (Table 2). Finally, the seeds were extracted with water to give aqueous extracts of PC and HM.

Table 2: Amounts of extract obtained on extracting 20 g of PC and HM seeds with solvents of increasing polarity

	PC (g)	HM (g)
Hexanes	8.17	4.44
Ether	2.56	0.15
Ethyl acetate	2.96	0.14
Ethanol	1.56	0.23

4.2 Furanocoumarin screening

A four spotter TLC plate was taken, the crude extract was spotted in the first and third space and an authentic sample of the furanocoumarin was spotted in the second and fourth place. Thin layer chromatography (TLC) was done on silica plates, using authentic samples of furanocoumarins known to be present in the two plants (Figure 1). TLC was run in hexane: ethyl acetate solvent system (7:3 ratio) and visualized under UV light (365 nm). After visualization, half the TLC plate was sprayed with methanolic NaOH solution and viewed again under the UV light. Methanolic NaOH opens the lactone ring of the coumarins and allows them to fluoresce differently. Comparing the R_f values, nature, color and intensity of the spots with the authentic samples, the HM extract was determined to have isobergapten, sphondin and isopinpenellin. Similarly, PC extract was found to have psoralen, bergapten, xanthotoxin and angelicin.

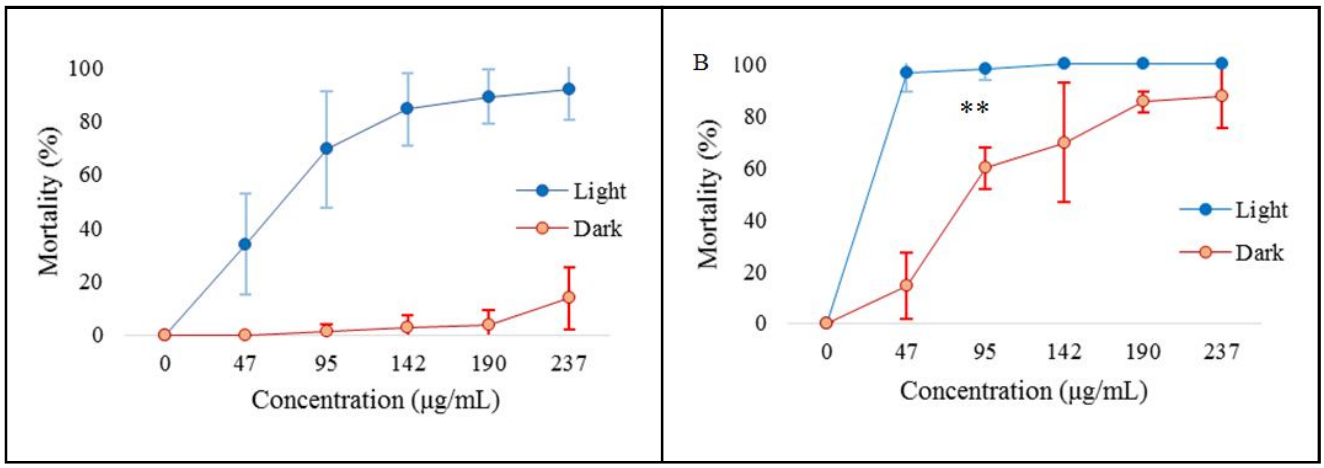

Figure 3: Brine shrimp mortality assay at 24 h for crude ethanol extract A. PC and B. HM. ** p<0.01 as compared with control.

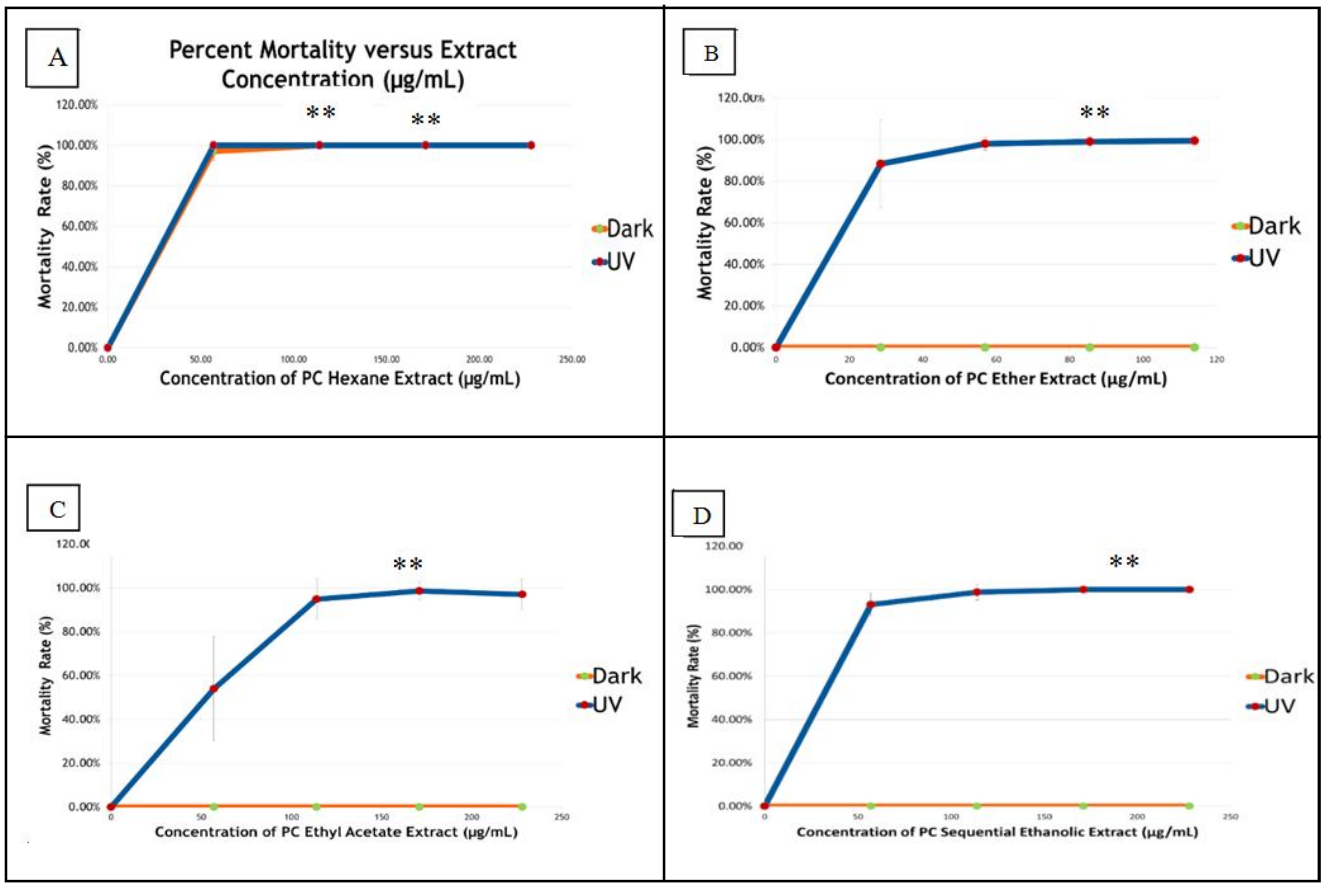

Figure 4: Brine shrimp mortality assay at 24 h for the sequential extracts of PC. A. Hexane, B. Ether, C. Ethyl acetate, D.Ethanol

4.3 Brine shrimp bioassays

Brine shrimp bioassays with crude ethanol (Figure 3), sequentially used solvents in the order of increasing polarity-hexane, ether, ethyl acetate and ethanol (Figure 4 for PC and Figure 5 for HM) and aqueous extracts of PC and HM (Figure 6) are given below.

The known furanocoumarin, bergapten, proved to be very phototoxic under UV light (365 nm), and non-toxic in the dark and was used as a positive control. The brine shrimp bioassays shown in Figures 3 were conducted with crude ethanol extracts. PC extract exhibited lower levels of toxicity under long-wave UV, compared to HM extract where100% mortality was observed at the lowest concentration tested (30 µg/ml). Under dark conditions, the LD_{50} for HM (63.93 ± 6.63 µg/ml) was also lower than that of PC, where only 53.7% mortality was observed at the highest concentration tested (237.1 µg/ml), therefore LD_{50} for PC was>237.1 ug/ml.

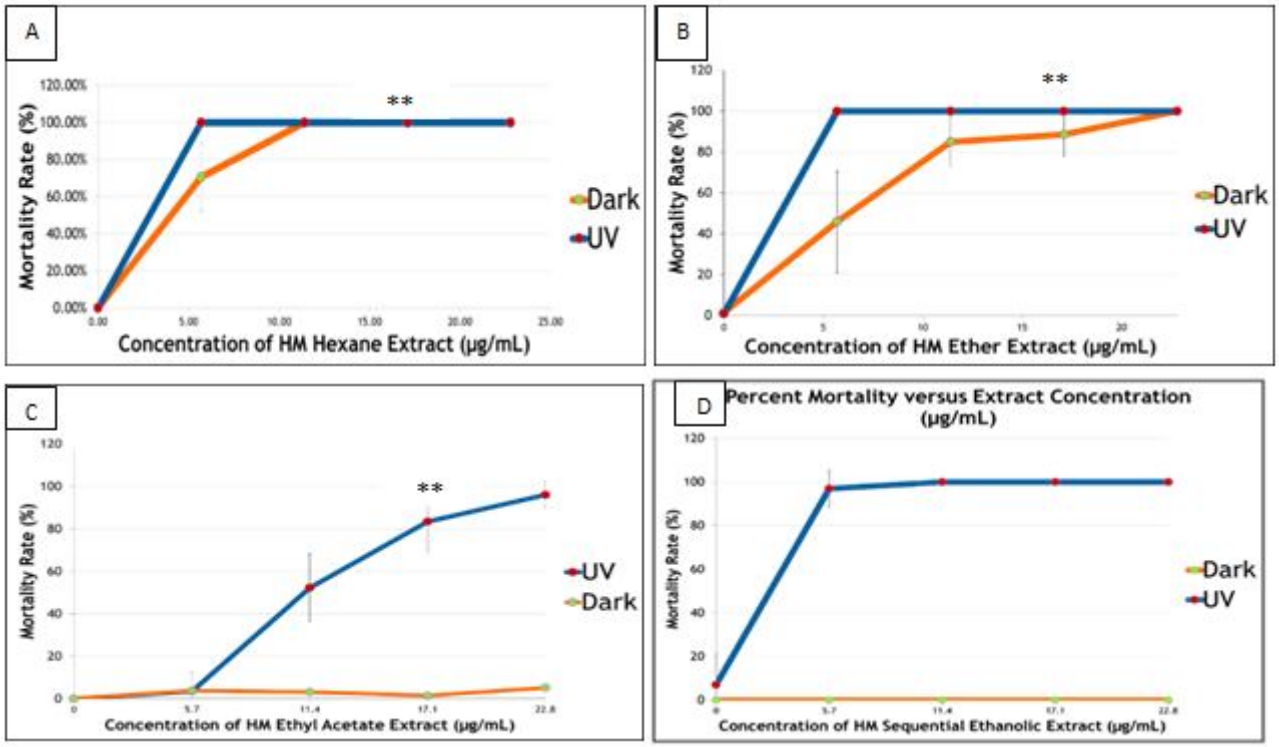

Figure 5: Brine shrimp mortality assay at 24 h for the sequential extracts of HM. A. Hexane, B. Ether, C. Ethyl acetate, D. Ethanol $p<0.01$ as compared with control.

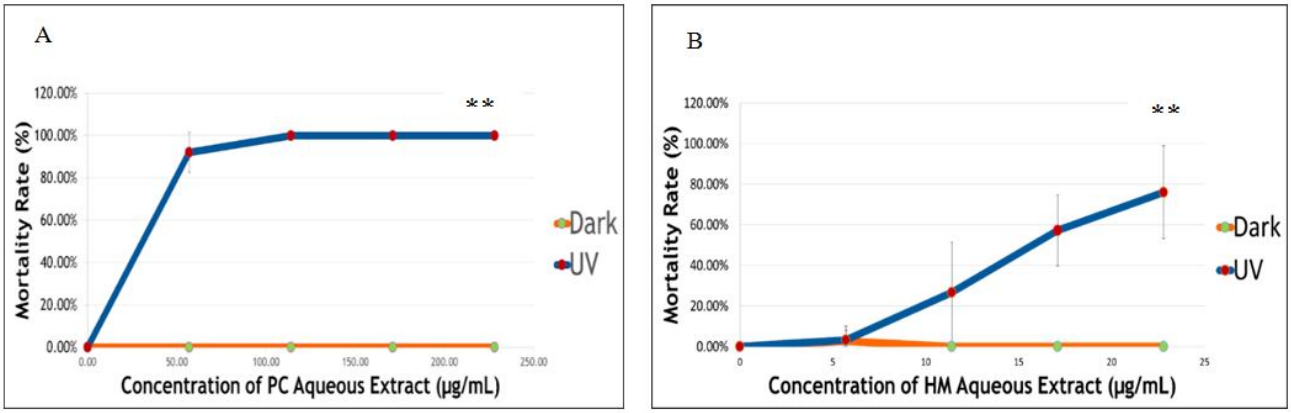

Figure 6: A. Brine shrimp mortality assay in aqueous extract of PC at 24 h. B. Brine shrimp mortality in aqueous extract of HM at 24 h.

** $p<0.01$ as compared with control.

4.4 Toxicity studies with wistar rats

After oral administration of a single dose of 2.5 g (10 times of MED) of PC and HM extracts to group I and II of wistar rats, respectively, and a single dose of 3.75 g (15 times of MED) of PC and HM extracts to groups III and IV of wistar rats, respectively, all the rats were observed for their gross behaviour, neurologic, autonomic, and toxic effects continuously for 24 h. Their food consumption, feces and urine were examined 2 h after the extract administration and then subsequently at 6 h intervals for 24 h. All the rats at all times had access to water and the pellet diet including the control group. No deaths were reported in groups I, II, III and V and the rats appeared normal. However, four out of the six rats died in group IV within 24 h of administering the dose. The surviving two rats in group IV were slower and disoriented on administration of the dose, but eventually recovered and overcame the symptoms.

4.5 Comparative LIBS studies

Comparative study of the LIBS spectra (Figure 7) of both the plants reveals that HM is richer in calcium than PC. Higher concentration of calcium is reflected in its intensity ratio (Tables 3 and 4).

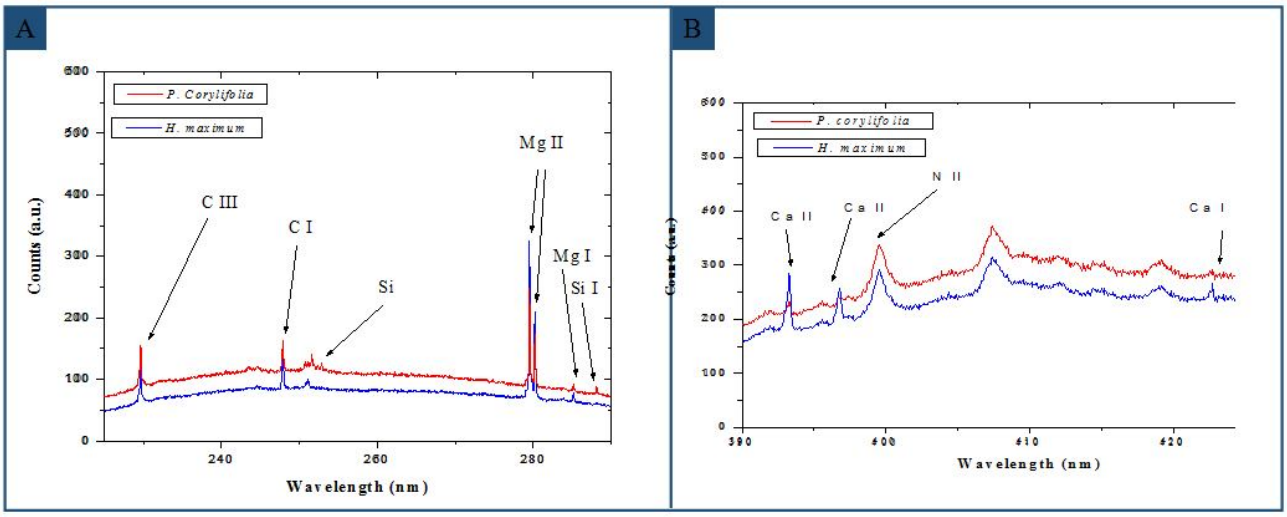

Figure 7: LIBS spectra of *P. corylifolia* and *H. maximum* in spectral ranges 200-290 nm (A) and 390-430 nm (B).

Table 3: Intensity ratio of elements with respect to Carbon (247.856 nm) in *P. corylifolia*

Element (wavelength)	Intensity ratio*
Mg (285.213)	0.14724
Mg (279.078)	0.06626
Mg (279.553)	5.59525
Mg (279.8)	1.62309
Mg (280.271)	4.8671
C (247.856)	1
C (229.689)	1.91963
Ca (393.366)	0.70153
Ca (396.847)	0.16626
Ca (422.673)	0.11718
Si (250.69)	0.11994
Si (251.611)	0.36626
Si (251.92)	2.32684
Si (252.411)	0.14479
Si (252.851)	0.1865
Si (288.158)	0.31902

*Calculated by the ratio between the intensity of emission of the specific element at the mentioned wavelength and the emission of carbon at 247.856 nm. Emissions determined by LIBS.

Table 4: Intensity ratio of elements with respect to Carbon (247.856 nm) in *H. maximum*

Element (wavelength)	Intensity ratio*
Mg (285.213)	0.1401
Mg (279.078)	0.06766
Mg (279.553)	4.70787
Mg (279.8)	1.09846
Mg (280.271)	3.83106
C (247.856)	1
C (229.689)	1.23878
Ca (393.366)	2.20755
Ca (396.847)	0.99631
Ca (422.673)	0.15268

*Calculated by the ratio between the intensity of emission of the specific element at the mentioned wavelength and the emission of carbon at 247.856 nm. Emissions determined by LIBS.

5. Discussion

Furanocoumarins are a class of naturally occurring compounds widely distributed in the plant kingdom and contain a coumarin(benzo-á-pyrone) fused to a furan ring either in a linear or angular fashion leading to psoralen or angelicin type of furanocoumarins. Preliminary phytochemical analysis of both the extracts showed the presence of furanocoumarins. Furanocoumarins become toxic in the presence of UV light (Bethea *et al*., 1999; Del Rio *et al*., 2014). Quick and cost-effective screening for the presence of phototoxic compounds was done by running HM and PC plant extracts through brine shrimp bioassay, in the presence and absence of UV light, and brine shrimp

mortality rates were observed, as previously described (Ojala, 1999). If, high mortality is observed under 'light' and 'dark' conditions in this bioassay, the extract is classified as toxic; but if high mortality is observed under 'light' conditions while low or no mortality is observed under 'dark' conditions, then the extract is classified as phototoxic.

The seeds of HM and PC were extracted with ethanol (crude ethanol extract) (CEE). High levels of mortality under conditions of UV light and low mortality in the dark (Figure 3A), suggest that the CEE of PC is highly phototoxic and otherwise has little toxicity. In contrast, high levels of mortality in the dark (Figure 3B) suggest that CEE of HM is highly toxic. However, the aqueous extracts of PC and HM (Figure 6) are both phototoxic, but not toxic. These results prompted us to extract the powdered seeds of PC and HM with solvents of increasing polarity in a sequential fashion. Table 2 provides the amounts of various extracts obtained when seeds from both the plants were sequentially extracted with solvents of increasing polarity (hexane, ether, ethyl acetate and ethanol). Figure 4 shows the brine shrimp bioassays done on PC extracts obtained in a sequential fashion. The hexane extract is very toxic as it shows high mortality, both under UV light and dark conditions (Figure 4A). The rest of the sequential extracts from PC (ether (Figure 4B), ethyl acetate (Figure 4C) and sequential ethanol extract (Figure 4D), show no mortality in the dark and high mortality under UV light, indicating that they are all phototoxic but not toxic. Figure 5 shows the brine shrimp bioassay conducted on extracts obtained from HM using solvents of increasing polarity. The hexane extract (Figure 5A) and ether extract (Figure 5B) of HM are toxic as high mortality is observed under both light and dark conditions. The ether extract is less toxic than the hexane extract, as would be expected. The ethyl acetate extract and the sequential ethanol extract obtained from HM were both phototoxic as there was high mortality under UV light, but almost no mortality under dark conditions. We chose to proceed with the CEE of PC and HM for a few reasons. The aqueous extracts of PC and HM were tedious to concentrate as they foamed a lot and, therefore it took a long time to concentrate these extracts. We had a couple of options with the extracts obtained through sequential extractions, but the yield of the extracts (Table 2) were very low. The CEE of PC (Figure 3) was highly phototoxic and had low toxicity and the time and effort required to get a good amount of extract was short. Since, the CEE of PC was chosen, to have a fair comparison, we chose the CEE of HM for further studies. Overall, HM showed a higher toxicity than the PC extract.

Qualitative assessment based on the intensity of the spots of various furanocoumarins and their R_f values (in CEE of PC and HM) against standards purchased, HM seemed to have a higher concentration of furanocoumarins.

The results of LD_{50} experiments carried out on normal healthy wistarrats, further validate the higher toxicity of CEE of HM over CEE of PC. The behavior of the rats treated with PC extract (groups I and III) appeared normal and no toxic effects were observedeven when the doses up to 10 and 15 times the effective dose (250 mg/ kg body weight) (Dhar *et al.,* 2013) were administered. The rats treated with 2.5 g of the HM extract which is ten times the effective dose (group II) appeared to show normal activity but group IV rats that were administered the highest dose of 3.75 g of CEE of HM (which was 15 times the effective dose) showed abnormal activity and four of the six rats died.

Furanocoumarins are known to cause skin pigmentation. It is reported that calcium (Ca^{2+}) plays an important role in melanogenesis (Patil*et al.,* 1993), therefore, we used LIBS for screening the elements present in the two plants to see if a possible correlation could be drawn to explain the melanin-stimulating activity of the two plants. It is known that the action of melanin stimulating hormone (MSH) on dermal melanocytes requires calcium for the transduction of signal and cyclic AMP production (Seldenrijk *et al.,* 1979; D'Mello *et al*., 2016). Calcium can regulate adenylate cyclase activity or inhibit cAMP phosphodiesterase activity leading to increased cAMP levels, and there by help in melanogenesis (Veseley and Hadley, 1971). A comparative study of the LIBS spectra of both plants revealed (Figure 7, Tables 3 and 4) that HM is richer in calcium than PC, and hence might be stimulating melanogenesis *via* the pathway that requires calcium, validating hyperpigmentation caused by HM (Bush and Simon, 2007). Higher concentrations of calcium, reflected in its intensity ratio (Tables 3 and 4) may be responsible for enhancing the melanogenetic properties of HM over PC.

6. Conclusion

The results suggest that the difference in toxicity between the ethanolic extracts of seeds of HM and PC plays an essential role in their respective application as medicinal plants. Based on these findings, it is probable that the lack of use of HM as a traditional folklore medicine in treating pigment disorders like vitiligo is due to its high levels of toxicity. The lower toxicity of the PC may explain why this plant has been used in folklore medicine to treat vitiligo. The sequential ethanol extract of HM is highly phototoxic and has little or no toxicityand can be pursued for melanogenetic studies on human cell lines.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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