



Effect on Physicochemical Characters and Bioactivity of *Argemone mexicana* in Different Plant Extraction Methods

Kanhiya Mahour*, Ashok Kumar and V.S. Vihan

*Medicine Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, P.O. Farah (Mathura)-281122, U.P. India.

Abstract: A study was conducted to find out the variation in physicochemical characters and bioactivity in plant extract prepared by two different methods, in order to decide a preferential method. Methanolic extract of *Argemone mexicana* plant extract was prepared by Soxhlet extraction method and reflux method. Bioactivity of plant extract was ascertained by a brine shrimp lethality test. The percentage yield of methanolic extract was 18% and 42% in soxhlet and reflux extraction methods respectively. In the first method, extract was greenish brown and sticky, but blackish and nonsticky in later method. Soxhlet method showed solubility in ethanol, chloroform, methanol and acetone whereas the reflux method extract did not show solubility in methanol. In both the method, plant extract was positive for alkaloids and saponins tested with standard methods. Nauplii of brine shrimp were hatched in artificial seawater with routine technique and collected in a test tube. The methanolic extract at concentrations of 250, 500 and 750µg/ml was tested on quantified numbers of larvae, in comparison to negative and positive control to test bioactivity. Mortalities were noted after 24 hrs post treatment and calculated as LC₅₀. The LC₅₀ was lower (118.23µg/ml) in Soxhlet extraction method, as compared to the reflux method (192.69µg/ml). The above results indicated that soxhlet extraction is superior to the reflux method to obtain better bioactivity.

Keywords: *Argemone mexicana*, Soxhlet assembly, Brine shrimp, Multiwell plate.

1. Introduction

The importance of medicinal plants in traditional health systems in solving the healthcare problems is gaining increasing attention worldwide due to their safe nature and easy availability. In drug development, various practices are adopted for the preparation of herbal extract with an objective to preserve or maintain bioactivity in plants. It is also evident that physical, chemical and biological property is affected to a degree in different extraction procedure. Therefore, it is desirable to test commonly used extraction methods with respect to variation in biological property, in order to decide the best and most suitable method. In this

paper, two commonly adopted extraction procedure such as Soxhlet and reflux method have been compared.

2. Materials and Methods

The plant material (Leaves) of *Argemone mexicana* was collected from Farah, Mathura district in the month of January. *Argemone mexicana* (Papaveraceae) commonly known as 'Shailkanta'. An annual weed with prickly leaves, bright yellow flowers and brist capsules, containing many black mustard like seeds. It is an American plant now naturalized in India as a troublesome weed¹. The plant is used externally for

*Corresponding author:
E-mail: kris_mathura@yahoo.com; Contact no: +91-9412404655.

skin and venereal diseases. It acts as an antiseptic, antibacterial and antifungal agent², while leaves useful in cough, wounds, ulcers and skin diseases³. The plant's leaves were authenticated with related taxonomy in literature. Crude extract of *Argemone mexicana* was prepared by two different methods such as the Soxhlet method and reflux method with their physical and chemical profile, while the biological activity of these two different extracted methods was screened by BSLT.

2.1 Preparation of plant extract

The collected plant material was shade dried and grind to a coarse powder. The coarse powder (100gm) of the shade dried leaves of the *Argemone mexicana* was divided into two parts. One part exhaustively extracted using methanol in Soxhlet extractor and other in reflux extracted assembly for a period of 22 hrs, as per standard methods. Prepared liquid extracts were concentrated by vacuum rotary evaporator (Heidolph, Japan) to a syrupy consistency after which it was evaporated to dryness.

2.2 Analysis of active constituents

Qualitative analysis of active constituents was done by standard methods⁴ to find out the constituents like alkaloids, glycosides, carbohydrates, protein, fixed oils or fats, tannins, flavonoids, saponins and triterpenoids.

Percentage yield was also noted in both extraction methods.

2.3 Collection of brine shrimp

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of biological activity and has also been suggested for screening pharmacological activities in plant extract (Carballo *et al.*, 2002)⁵. The eggs of brine shrimp, *Artemia salina*, are readily available at pet shops as a food for tropical fish, and they remain viable for years in the dry state. Brine shrimp eggs were hatched as per (Micheal *et al.*, 1956)⁶ filled with artificial seawater which was prepared with a commercial salt mixture in double distilled water. After 48 hrs, the phototrophic nauplii were collected by pipette in a test tube. The test was conducted in a multiwell plate in filtered (0.45µm pore diameter) and sterilized seawater (final volume 5ml). The extract was tested at 750, 500 and 250µg/ml of extract concentration, by keeping potassium dichromate as positive control and seawater and Tween-20 as negative control. The entire test was performed in a temperature-controlled room at 28°C, under a continuous light regime. The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC₅₀ values were obtained by Finney⁷.

Table-1. Effect on solubility, physical characters, active constituents and bioactivity of plant extract in different extraction methods.

Extraction method	Solubility									
	Distilled water	Ethanol	Methanol	Chloroform	Acetone	Petroleum ether				
Soxhlet	-	+	+	+	+	-				
Reflux	-	+	-	+	+	-				
	Physical characters									
	Colour	Odour	Nature	Consistency						
Soxhlet	Greenish brown	Not agreeable	Sticky	Semi-solid						
Reflux	Blackish green	Peculiar	Non sticky	Solid						
	Active constituents									
	Alkaloids	Glycosides	Carbohydrates	Protein	Fixed Oils	Tannins	Flavonoids	Saponins	Triterpenoids	% yields
Soxhlet	+	-	-	-	-	-	-	+	-	18%
Reflux	+	-	-	-	-	-	-	+	-	42%
	Bioactivity									
	Experiment types	LC50 (µg/ml)	Upper confidential limit	Lower confidential limit						
Soxhlet	Test	118.23	569.11	694.18						
Reflux	Test	192.69	311.65	7.03						
-	Positive control	0.00	-	-						
-	Negative control	0.00	-	-						

3. Results and Discussion

Solubility of extract was tested in organic solvents revealed that an extract prepared by the Soxhlet method was soluble in ethanol, methanol, chloroform and acetone while insoluble in distilled water and petroleum ether. On the other hand, extract prepared by reflux method was soluble in ethanol, chloroform and acetone, insoluble in distilled water, methanol and petroleum ether. Physical characteristics such as colour, odour, consistency and percentage yield in Soxhlet method found greenish brown, sticky, semisolid, 18% yield with not agreeable odour while, extract prepared by reflux method was blackish green, non-sticky, solid, 42% yield with peculiar odour. The plant extracts were tested for chemical analysis such as alkaloids, flavonoids, tannins, protein, amino acids, saponins, fixed oils and carbohydrates. Both the extracts were found to be positive for alkaloids and saponins only. The percentage yield was varied significantly in two methods, which was 18% and 42% of the extract in Soxhlet and reflux method.

The bioactivity of both plant extracts were tested against brine shrimp nauplii. The calculated LC₅₀ was lower 118.23µg/ml in the extract by Soxhlet extraction method and 192.69µg/ml in reflux extraction method. It indicated that Soxhlet extraction is superior in respect to maintaining bioactivity, as compared to reflux

method, whereas percentage yield is poorer in Soxhlet than reflux method.

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