Validation of a green extraction method based on Ultrasound Assisted Aqueous Extraction from Fresh Plants of *Argemone mexicana*

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Abstract

Argemone mexicana is a well known medicinal plant abundantly grown in arable and non-arable land all over Bangladesh. The whole plant of *A. mexicana* was selected for the validation of a green extraction method conducted by ultrasound treatment. The proposed aqueous Ultrasound Assisted Extraction (UAE) from fresh plants of *A. mexicana* was compared with the conventional methanol and decoction extraction method. Higher extraction yield was observed in the UAE method with a maximum number of phytochemicals almost similar to the methanol extract. Both UAE and methanol extracts showed moderate antimicrobial sensitivity against *Staphylococcus aureus* and *Salmonella typhi* which was much higher than the extract obtained from the decoction method. Insignificant difference was observed in aqueous UAE crude extracts obtained both from fresh and dried plants give an option to avoid the time consuming drying stages of plant materials before extraction. Ultrasound mediated extraction successfully reduces the overall extraction time and cost as well as it allows aqueous solvent instead of hazardous organic solvent. Recent study indicates the suitability of the method both for laboratory and industrial setup.

Key words: *Argemone mexicana*, Prickly poppy, Green extraction, Ultrasound, *Staphylococcus aureus, Salmonella typhi*

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Introduction:

Argemone mexicana L. is locally known as “Shiail Kata” and a very common annual herbaceous weed extremely grow in the cultivated and abandoned land in all parts of Bangladesh[1]. The plant is belongs to the family Papaveraceae[2] and commonly known as prickly poppy[3]. It contains 30-32 species, all with prickly stems, leaves and capsules[4] and widely distributed in many tropical and subtropical regions and has naturalized in the United States, Ethiopia, India and Bangladesh[3,5]. *A. mexicana* is a prickly, glabrous, branching herb with eye-catching yellow flowers. It is a popular medicinal plant and widely used in folk medicine to alleviate several ailments especially for its analgesic, antibacterial, antimalarial, antispasmodic, sedative and narcotic effects[6]. Several studies specifically identified its antioxidant [7], antihelmentic [8], anti-inflammatory[8], anti-bacterial[8], anti diabetic[9] activities.

The plant is also useful for the treatment of warts, cold sores, cutaneous infections, skin diseases, itches, dropsy, jaundice[10] and even sometime as an antidote to snake poisoning[11,12].
In the present study, the whole plant of *A. mexicana* was used to prepare crude extract by using an optimized green extraction method known as "Aqueous Ultrasound Assisted Extraction (UAE) from Fresh Plants" as per the process proposed by Sadat et al.[13]. The basic principle of the UAE method is to utilize ultrasonic waves on the vegetal material that breaks the cells and releases the cells' contents into the extraction medium[14]. This method is capable of reduceing the use of extraction solvents, processing time, and therefore, energy consumption enhancing during the extraction of the desired biocomponents [15-18].

Botanical nomenclature [19]
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida Dicotyledons
Subclass: Magnoliidae
Order: Papaverales
Family: Papavaraceae
Genus: Argemone
Species: *A. mexicana*

Figure 1: Washing stage of freshly collected *Argemone mexicana* plants

Materials and Methods

Study protocol

Ultrasound was applied on the fresh plants of *Argemone mexicana* for facilatating the extraction of phytochemicals in water and subsequently compared to the conventional decoction and methanol cold extraction method (Table-1). Both fresh and shade dried plant materials were used in the present study[13, 20-22]. Extraction yield (Eq. 1), presence of common phytochemicals (Table-2) and antimicrobial sensitivity were the parameters set for the comparison.

Collection of Plant Material

The whole plants of *Argemone mexicana* was collected from Botanical Pesticide Garden of the Institute of Environmental Science (IES) of Rajshahi University (RU), Bangladesh and duly identified by the professional taxonomist of the Department of Botany, RU and a voucher specimen was deposited at the herbarium of the institute.

Extraction Procedure

Healthy plants of *Argemone mexicana* were collected from the field before sun rise and immediately washed by the running tap water and distilled water. After shade drying of the surface water, the plants were devided into five parts (Table 1). As per Toma *et al.*,[23] the optimum material-solvent ratio is 1:5 for effective ultrasound extraction which is also frequently followed in many conventional extraction methods[24-26]. Extraction from Part A & B of the fresh plants were conducted immediately by using UAE and decoction method. Whereas "Part-C, D and E" of fresh plants were first allowed for week-long drying and powder form were used for UAE, decoction and methanol cold extraction. Dissolved phytochemicals

were seperated from the debris by using five layers of polyester cloths and dried at 60^{oC} in a conventional water bath. The dried crude extracts were then stored in an air tight bottle with identical labels and preserved in a cold chamber for further use. The efficiency of the extraction process was mesured by comparing yield (Equation 1)[27] and the presence of common phytochemicals (Table 2). The efficacy of the crude extracts were compaed by using antimicrobial sensitivity study. All process were repeated three times for calculating significance level. SPSS 16.0 was used for all types of statistical calculation.

Table 1: Extraction procedure of *Argemone mexicana* whole plants

Method	Plant parts	Treatment	Method	Ref.
A	Fresh Plants*	Ultrasound	Juice were placed in the ultrasonic bath (Power Sonic 405) for 30 minutes ultrasonic treatments at 40^{oC} bath temperature.	[13, 20-21]
B	Fresh Plants*	Decoction	Juice were allowed to boil for 5 minutes before extraction	[28-29]
C	Dried Plants**	Ultrasound	Fine powder was mixed with distiled water (1:5 ratio) and treated in ultrasonic bath for 30 minutes at 40^{oC} bath temperature	[13, 20-21]
D	Dried Plants **	Decoction	Fine powder was mixed with distil water (1:5 ratio) and allowed to boil for 5 minutes before extraction	[28-29]
E	Dried Plants **	Methanol cold extraction	Fine powder was mixed with methanol (1:5 ratio) and allowed for cold extraction up to 72 hours with intermittent shaking as per standard method	[24-26]

* Juice of fresh plants (100 gm) was prepared by conventional blender by adding distilled water q.s. to 500 ml.
** Powder was prepared and mixed with respective solvents as per ratio 1:5.

$$\% Yield = \frac{(W2\ x\ 100)}{W1} \text{ ---------- } (Eq.1)$$

Where, W1: weight of the plant materials for extraction,
W2: weight of the crude extract after drying

Table 2: Phytochemical Screening Test of *Argemone mexicana*

Phytochemicals	Qualitative test	Pharmacological importance
Alkaloids	Mother solution + 2% of H_2SO_4 + Heat + few drops Dragendoff's reagent → Orange red precipitate (This is known as **Dragendoff's test)**[30-31]	Anaesthetics, CNS stimulants, narcotics, poisons due to their potent biological activities[4, 32]
	Mother solution + 2% of HCl + Heat + few drops Mayer's reagent → turbidity or yellow precipitation (This is known as **Mayer's test)**[30, 33]	
Anthraquinones	Mother solution + benzene or chloroform + 10% (v/v) ammonia solution → pinkish or color change[31, 34]	Anthraquinones provide anticancer, anti-inflammatory, diuretic, antiarthritic, antifungal, antibacterial, and antimalarial activities[35].
Flavonoids	Mother solution + dilute ammonia Solution + Conc. H_2SO_4 → yellow coloration that disappear on standing[34]	Its antioxidant property provides protection against diseases like cancer, ageing, atherosclerosis, inflammation[5, 32]
	Mother solution + few drops of 1% aluminium solution → yellow coloration[34]	

Glycosides	Mother solution + 3 ml of glacial acetic acid + 1 drop of 5% ferric chloride Solution + 0.5 ml of Conc. H_2SO_4 →Brown or blue ring of the interface[33-34]	Glycosides play numerous important roles in living organisms, including antioxidant, antiinflammatory, antihypertensive, and antidiabetic activities[36]
Saponins	Mother solution + equal volume water + vigorous shaken→ foam stable more than 10 minutes[33]	Saponins provide hypolipidemic and anticancer activity and are also used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc.[5, 37-38]
Steroids / Terpenoids	Mother solution + 2ml Chloroform + Carefully added 3 ml Conc. H_2SO_4 to form a layer → reddish brown colour of the interface (This is known as Salkowski test)[34]	steroids possess cardiotonic activity, also insecticidal and antimicrobial properties[5, 32]
Tannins	Mother solution + 1% $FeCl_3$ solution → dark green colour[39-40]	These are used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhoea[5, 38]

Antimicrobial Study

Disc diffusion method[41-44] was used for antimicrobial sensitivity study on *Staphylococcus aureus* (Gram +ve bacteria) and *Salmonella typhi* (Gram -ve bacteria). Microorganisms were collected from the Microbiology Lab, Department of Biochemistry and Molecular Biology, Rajshahi University, Bangladesh. The filter paper discs impregnated with the 400µg/disc of extracts were placed on the surface of the inoculated nutrient agar media with the aid of sterilized pair of forceps. After allowing 30 minutes of pre-diffusion, the petridish was then placed in an incubator for 24 hours at 37^{0C}. The degree of sensitivity of the organisms to the extracts was determined by measuring the diameter of visible zones of inhibition to the nearest millimetre. The procedure was repeated thred times for each batch and the average result was counted for statistical analysis.

Results and Discussion

High extraction yields were observed in both cases of fresh (27.83±1.09%) and dried (24.54± 1.19%) plants of *A. mexicana* treated by ultrasounds. Insignificant difference ($p>0.05$) observed in the above two methods giving space to avoid the time consuming drying stages from the extraction method. Conventional extraction methods like decoction from fresh plants (yield, 8.7± 0.47%), decoction from dried plants (yield, 9.81± 0.51%) and methanol cold extraction from dried plants (yield, 15.41± 0.44%) were observed significantly ($p<0.05$) lower than the ultrasound method of extraction. On the basis of above results the ultrasound assisted extraction was proved better than the conventional decoction and cold extraction method.

It was observed that *Argemone mexicana* extract was rich in different phytochemicals (Table 4). Qualitative phytochemical analysis indicated the presence of alkaloid, anthraquinone, flavonoids, glycoside, saponin, steroid/ terpenoid and tannin in the crude extracts obtained from fresh and dried plants by ultrasound treatment and methanol extracts. Similarly extracts obtained from decoction were rich of anthraquinones, flavonoid, saponin and steroid truly matched with the work of Veni and Puspanathan [45].

Ultrasound treated extracts from fresh and dried plants and methanol extracts of *Argemone mexicana* were found promising antimicrobial activities on *Staphylococcus aureus* (Gram +ve) and *Salmonella typhi* (Gram –ve) presented in the Chart-1. Statistically insignificant

antimicrobial differences were observed on the microorganisms indicating the same efficacy of the applied extracts obtained both from the ultrasound treated and methanol extracts (Table-5). Extracts obtained by decoction method from fresh and dried plants showed comparatively poor sensitivity.

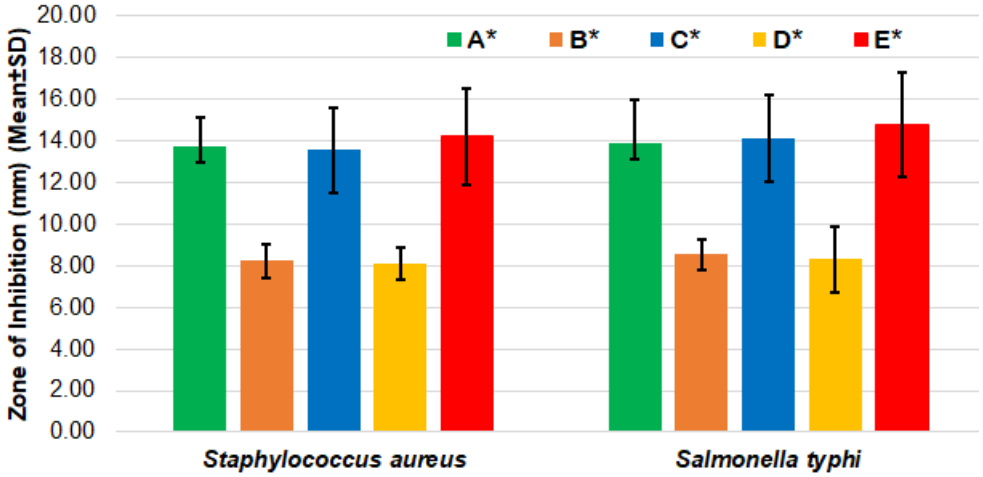

Chart 1: Antimicrobial sensitivity study (Mean±S.D.) of the crude extracts of *A.mexicana* plants. (Here, * method of extraction as per Table-1)

Table 3: Yield variation of different extaction methods

Method of Extraction	Starting materials (gm)	Weight (gm) after drying (Mean±SEM)	p (Weight variation)	Solvent used (ml)	% Yield	p (Yield variation)
A*	100	-		q.s. to 500	27.83±1.09	-
B*	100	-		q.s. to 500	8.7±0.47	0.001^{x}
C*	100	41.90±1.36		5 times to the dry wt	24.54±1.19	0.266^{x}
D*	100	40.57±1.52	0.211^{a}	5 times to the dry wt	9.81±0.51	0.008^{x} 0.005^{y}
E*	100	40.60±1.10	0.615^{a}	5 times to the dry wt	15.41±0.44	0.005^{x} 0.030^{y}

* Method of extraction as per Table-1. Mean calculated by considering successive 3 studyis.
[a] Significance level comparison with "C", where, $p \geq 0.05$, is statistically insignificant.
[x] Significance level comparison with "A", where, $p \geq 0.05$, is statistically insignificant.
[y] Significance level comparison with "C", where, $p \geq 0.05$, is statistically insignificant.

Table 4: Phytochemical screening study of crude extracts of *A. mexicana*

Phytochemical Tests	Crude extract				
	A*	B*	C*	D*	E*
1.Alkaloid,(i) Dragendroffs' test	+	+	+	+	+
(ii) Mayer's test	+	+	+	+	+
2. Anthraquinones	+	-	+	-	+
3. Flavonoid (i): by H_2SO_4	+	-	+	-	+
(ii): by aluminum	+	-	+	-	+
4. Glycoside	+	+	+	+	+
5. Saponin	+	-	+	-	+
6. Steroid/ Terpenoid	+	-	+	-	+

7. Tannin	+	+	+	+	-

* Method of extraction as per Table-1.
Here, (+) indicated presence of compound, and (–) indicated absence of compound

Table 5: Antimicrobial sensitvity study of crude extracts of *A. mexicana*

Method* of extraction	**Zone of inhibition (mm)**			
	Staphylococcus aureus		*Salmonella typhi*	
	Mean ±SEM	p	Mean ±SEM	p
A	13.78±0.46	-	13.89±0.66	-
B	8.22±0.28	0.000[a]	8.56±0.24	0.000[a]
C	13.56±0.69	0.782[a]	14.11±0.66	0.801[a]
D	8.11±0.26	0.000[a]; 0.000[b]	8.33±0.50	0.000[a]; 0.000[b]
E	14.22±0.77	0.609[a]; 0.299[b]	14.78±2.49	0.442[a]; 0.563[b]

* Method of extraction as per Table-1. Mean calculated by considering successive 3 studyis.
[a] Variation compared to method "A", p≤0.05indicate significant variation
[b] Variation compared to method "C", p≤0.05indicate significant variation

Conclusion:

Aqueous ultrasound assisted extraction from fresh plants of *Argemone mexicana* have many points of green extraction without compromising the efficiency and efficacy obtained from conventional extraction methods. The proposed method was proved cost effective and environment friendly which successfully reduced the overall processing time and hazardous organic solvents for extraction procedure. Results obtained from phytochemical and antimicrobial studies indicate the potential medicinal values of the studied plants. Statistical similarity with conventional methanol extraction proved the justification of using the ultrasound on fresh plant's material.

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