Effect of extraction techniques and evaluation of antimicrobial activity of *Argemone Mexicana* leaves and roots extracts in different solvents

Abstract:

To estimate effect of extraction techniques and antimicrobial activity of different solvent extract of *Argemone mexicana* leaves and roots. *Argemone mexicana* leaves and roots used in this study was collected. Roots and leaves were shade dried , cut into small pieces of 2-3 inches and grounded in to powdered form using mixer grinder. Leaves and roots extracts were prepared using Soxhlet extraction and Microwave-assisted extraction techniques in solvents named acetone, methanol, aqueous. The antimicrobial activity of the roots and leaves extracts were evaluated against Gram +ve bacteria (*Xanthomonas campesteris*, *Bacillus cereus*, *Staphylococcus aureus)* and fungal species (*Fusarium oxysporum*, *Macrophomina phaseolina* and *Candida albicans)* and their zones of inhibition in mm are measured by Agar well diffusion method. Soxhlet extraction technique gave better extract yield (g/100g) 12.19 and 8.54 while microwave-assisted extraction gave 8.88 and 6.94 for leaves and roots respectively. The methanoilc root and leaves extracts exhibited higher antimicrobial activity followed by acetone and aqueous extracts. The result of the investigation showed that extraction techniques significantly affected extraction yield and antimicrobial activity. Soxhlet extraction is better one extraction method and methanolic extract of leaves was found to be good antimicrobial followed by acetone and aqueous.

Keywords: *Argemone mexicana*, Extraction, Roots, Leaves, Antimicrobial.

Introduction

Plants have long been known as approaching sources of special classes of chemical compounds, known as phytochemicals, having diverse biological and curative activities, which are effective in controlling or treating a variety of diseases. Plant-based traditional medicine system continues to play a vital role in the health care system with about 60 % of the world inhabitants relying mainly on traditional medicines for their primary health care (Khan and Bhadauria 2017). Modern knowledge on medicinal plant research still contains at least 25 % drugs and many others, which are synthetic analogues, built on prototype com- pounds isolated from medicinal plants. The ongoing growing recognition of medicinal plants is due to escalating faith in herbal medicine (S. Dutta *et al*., 2014). The medicinal plant products, which are derived from plant parts such as stem, bark, leaves, fruits, roots and seeds have been part of phytomedicine that produce a definite physiological action on human body. Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in human body (Albuquerque *et al*., 2007). These plant parts possess anti-venom property as well as antimicrobial property (Makhija and Khamar, 2010; Minu *et al*., 2012). The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (D. Chaudhuri *et al*., 2012). *Argemone mexicana* Linn is an exotic weed has wide spread distribution in many tropical and sub-tropical regions in India. *Argemone mexicana* have many biologically important compounds, so it can be recommended as a plant of pharmaceutical importance (Khan *et al*.,2019). This plant grows widely in all dried parts of subtropical India i.e. in Haryana, Madhya Pradesh, Uttar Pradesh, Punjab plains and North-Western part of India like Gujarat and Rajasthan. *Argemone mexicana* possess a wide-range of biological activities, such as antibacterial (Rahman *et al* ., 2011; Rahman M. *et al*., 2006 ; Sahu *et al* ., 2012) , antifungal (Kushtwar *et al*., 2017; Singh *et al* ., 2009; More N. *et al*., 2016; Andleeb S. *et al*., 2020). Plant crude extracts were proved to be higher in antimicrobial activity as a synergistic effect than purified individual constituents. Plants screened for antimicrobial activities have provided modern medicine with abundance of drugs and treatments against various ailments (Mahesh *et al*., 2008) . Plants produce a host of antimicrobial agents, including a wide variety of natural defense compounds, such as phenolics, terpenoids, alkaloids, polyacetylenes and polypeptides. However, with the advent of modern antibiotic drugs mainly of bacterial, fungal and synthetic sources, many of these natural plant derived antibiotic compounds have been left unexplored. In the work herein, a comprehensive evaluation for effect of extraction techniques and evaluation

Comment [LFH1]: Suggest author revise the objective of the study

Comment [LFH2]: Unit of the result?

Comment [LFH3]: Further define special classes

Comment [LFH4]: Suggest author revise the statement and the justification of the study

Comment [LFH5]: Suggest author rephrase the term

Comment [LFH6]: Define Linn

Comment [LFH7]: Probably not necessary in this context

Comment [LFH8]: Same goes to this

Comment [LFH9]: Rephrase the statement. There is only 2 biological activities according to the the statement

of antimicrobial activity of *Argemone Mexicana* leaves, roots extracts in different solvents that had not previously been assessed in the literature in Haryana.

Materials and methods

Plant materials - *Argemone Mexicana* roots and leaves were procured from the roadsides, dry region in Haryana in the end of the March 2021. Roots and leaves were brought to lab, cleaned using running tap water 2-3 times and then finally washed with distilled water followed by shade dried. After drying, roots and leaves were cut into small pieces of 2-3 inches and were ground in to powdered form using mixer grinder.

Comment [LFH10]: Suggest author explain the sampling in details.

Chemicals and Reagents

HPLC grade solvents were used for extraction and evaluation of antimicrobial activity. All the solutions were prepared fresh and utilized on the same day of the assay and used for experimental procedures.

Extraction

For evaluation of antimicrobial activity extracts were prepared using different solvents named acetone, methanol and aqueous followed by two extraction methods.

Soxhlet Extraction

Four gram of powdered samples of *Argemone mexicana* roots and leaves were placed in a filter paper (Whatman No. 1) thimble in a classical Soxhlet apparatus fitted with a 250 mL round bottom flask. The acetone and methanol solvent were added up to one and a half siphons that are approximately 150 mL. After the completion of first extraction step of 5 h, residue in thimble was again extracted twice (each extraction time 2 and 1 h, respectively) with suitable amount of acetone and methanol solvent. Filtrates of acetone and methanol solvent from three extraction steps were pooled and their volumes were noted. But in the case of aqueous as a solvent it takes longer to extract through the siphon mechanism, requiring more time to complete the 7-8 cycles. longer. After extraction, the volume of each filtered solvent was measured. These extracts were filtered and used for evaluation of antimicrobial activity.

Comment [LFH11]: Which method are you referring from? AOAC? ISO? Manuscript?

Microwave-assisted extraction

Extraction was carried out using a microwave oven (IFB, model : 2301) with output of 800W and operating frequency 2450 MHz. Eight gram of powdered samples of *Argemone mexicana* roots and leaves were placed in a 250 ml conical flask with solvents 100 ml each acetone, methanol and aqueous. Flasks were left overnight. Samples containing flasks were irradiated for 10 seconds in microwave oven at 40 per cent power and not allowed to boil. Then cooled to room temperature and irradiation step was repeated up to 12 times to complete the process. After extraction, the volume of each filtered solvent was measured and used for evaluation of antimicrobial activity.

Comment [LFH12]: Same comments shown above.

Comment [LFH13]: Why requires at least 12 times of irraditation to complete the study?

Evaluation of Antimicrobial Activity

The antimicrobial activity of the roots and leaves extracts were evaluated by Agar well diffusion method described by Bayer *et al*., (1966). Eighteen to 24 hrs single colonies on agar plates were used to prepare the bacterial suspension with the turbidity of 0.5 McFarland (equal to 1.5×10^{8} colony-forming units (CFU)/ml). Turbidity of the bacterial suspension were measured at 600 nm. Agar plates were inoculated with 100 µl of the test microorganisms and were spreaded uniformly with the help of spreader, then allowed to dry for 5 minutes. Mueller hinton agar plates and Potato dextrose agar were inoculated with bacterial strain and fungal strain respectively under aseptic conditions and wells (diameter=6mm) were filled with 50 µl of the test samples and incubated at 37°C for 24 hours for bacteria and 72 hours for fungi. After the incubation period, the diameter of the growth inhibition zones was measured in mm. Zone around each well was measured after 24 h for bacteria and 72 h for fungi. All the experiments were performed in triplicate ~~to reduce error~~. Cycloheximide was used as standard for fungi and for bacteria tetracycline was used. After incubation, zone of inhibition was measured in mm. The antimicrobial activity of root and leaves extracts obtained was tested against Gram +ve bacteria *Xanthomonas campesteris*, *Bacillus cereus*, *Staphylococcus aureus* and fungal species *Fusarium oxysporum*, *Macrophomina phaseolina* and *Candida albicans* and their zones of inhibition in mm are measured.

Comment [LFH14]: Suggest author provide specific details on cultivation of cultures. Bacteria and mold exhibited different value of McFarland reading. How did author perform in order to justify 10^8 CFU concentration for McFarland ?

Comment [LFH15]: Provide the details of the spectrophotometer used in this study.

Comment [LFH16]: Suggest author explain this methodology.

Comment [LFH17]: What is the standardization CFU/mL spreaded on the agar plate? What kind of the soft agar used to achieve the spreading?

Comment [LFH18]: What is the concentration of both standard antimicrobial?

Comment [LFH19]: Suggest author justify the selection of the cultures for the study.

Comment [LFH20]: There's redundant of the statements found in this session. Suggest rephrase the sentences.

Results and Discussion

Extract Yield:

Extract yield of *Argemone mexicana* roots, leaves prepared by soxhlet extraction and microwave-assisted extraction technique was given in Table 1. Among *Argemone mexicana* roots and leaves extracts prepared by two extraction technique, yield (g/100g) of extracts prepared by soxhlet extraction technique was highest than microwave-assisted extraction technique for the solvents aqueous followed by methanol and acetone. The results are in agreement with other researchers (Kanhiya Mahour *et al.*, 2011; Datkhile Kailas, *et al*., 2020). Among plant parts , extract yield of leaves was highest. Extraction yield is a measure of solvent and extraction method efficiency. Soxhlet extraction gave higher yield and results are in agreement with other researchers. Literature results showed that supercritical carbon dioxide(SC-CO_2) extraction give the lowest extraction yield as compared to soxhlet extraction for *Quercus infectoria galls* (Hasmida *et al*., 2014). Soxhlet extraction was found to be 1.8 and 3 times higher than ultrasound extraction and maceration extraction but slightly (1.2 times) higher than microwave extraction in aerial parts of *Potentilla atrosanguinea Lodd*. Reported by kalia *et al*., (2008).

Evaluation of Antimicrobial Activity

Extract obtained from soxhlet extraction technique suitable to better yield was used to evaluate antimicrobial activity. Among different solvent extracts , methanol extract showed good activity against bacterial culture *Bacillus sp.* and *Staphylococcus aureus as well as* antifungal activity against *Candida albicans*, Fusarium oxysporum and *Macrophomina phaseolina* as in Table 2,3 & Fig.1. Among leaves and roots extracts, leaves extracts showed better activity for antibacterial as well as antifungal in methanol.The antibacterial activity in terms of inhibition zone against *Bacillus sp., Xanthomonas campestris* and *Staphylococcus aureus* was observed. The antifungal activity in terms of inhibition zone against *Candida albicans, Macrophomina phaseolina* and *Fusarium oxysporum* was observed. But the antifungal activity against *Fusarium oxysporum* was found to be nil in all leaves extracts.

Abdulkarim *et al.*, (2016) evaluated ethanol leaf extract that exhibit more potent antibacterial activity than methanol leaf extract in *Argemone mexicana.* Chloroform extract of seeds of *Argemone Mexicana* exhibited antibacterial activity with minimum inhibitory concentrations (MIC) of 2.0 -5.0 mg/ml, against both Gram-positive and Gram-negative bacteria reported by Singh *et al*., (2009;). Bhatacharjee *et al*., (2006) studied that methanol extracts of the leaves and seeds of the *A. mexicana* showed greater antibacterial activity than the corresponding water extracts. Methanol leaves extracts of *Argemone mexicana* at 100µl concentration showed better activity against two Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*) and 4 fungi (*Aspergillus niger*, *Fusarium moniliforme*, *Candida albicans* and *Mucor plumbeus)* as reported by Shyam Prasad and Dhanapal, (2010). Nuclear magnetic resonance analysis of the root and leaf methanol fractions showed two main antibacterial compounds, chelerythrine and berberine and data highlight the importance of plants as an invaluable pharmaceutical resource at a time when antimicrobial and anticancer drug discovery had reported by Orozco *et al*., (2021).

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Research Content : The research content is original and has not been published.

Ethical Approval : Not Applicable

Data from other sources: Not applicable

Consent to publish : All the authors agree to publish the paper in Journal of Environmental Biology.

COMPETING INTERESTS DISCLAIMER:

Comment [LFH21]: Why is Soxhlet extraction is better yield than other extraction technique? Possible author justify more how is the phenomenon happening.

Comment [LFH22]: Suggest author cite their findings.

Comment [LFH23]: Suggest author rephrase the term used as this study involves fungal inhibition activity.

Comment [LFH24]: According to the methodology, methanol and acetone were used in this study. How author make sure that the inhibition activity is solely from the phytochemical extracted from the plant not from the methanol and acetone? Is there any negative control test performed in this study to prove that the result is not affected after the extraction.

Comment [LFH25]: Justify the culture author wish to investigate.

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1. Extract yield (g/100g) of *Argemone Mexicana* roots, leaves prepared by two extraction technique

Plant & Location	Plant Parts Extraction Technique	Leaves			Roots		
		Aqueous	Methanol	Acetone	Aqueous	Methanol	Acetone
Argemone Mexicana & Hisar	Soxhlet	12.19	11.48	9.00	8.54	7.65	5.63
	Microwave	8.88	7.46	7.31	6.94	4.84	4.83
	Mean	10.5	9.47	8.15	7.74	6.24	5.23
	SE(m)	0.05	0.03	0.05	0.03	0.05	0.03
	CD at 5 %	0.16	0.19	0.16	0.12	0.16	0.12

Comment [LFH26]: Suggest author define aqueous (Is it water extraction?)
What is the unit of the study?
Is there any comparison test performed in this study?
There's some abbreviation in this table. Suggest author describe the abbreviation in details.
The first left column (Argemone Mexicana and Hisar) is unclear. Suggest author define Hisar

Suggest author define the mean, SE(m) and CD at 5%, how author calculate the result?
As per stated at the materials and methods, the sample was performed in triplicate. Suggest author include the mean and SEM in each of the result stated at the table.

Table 2. Antimicrobial activity of roots extracts of *Argemone Mexicana*

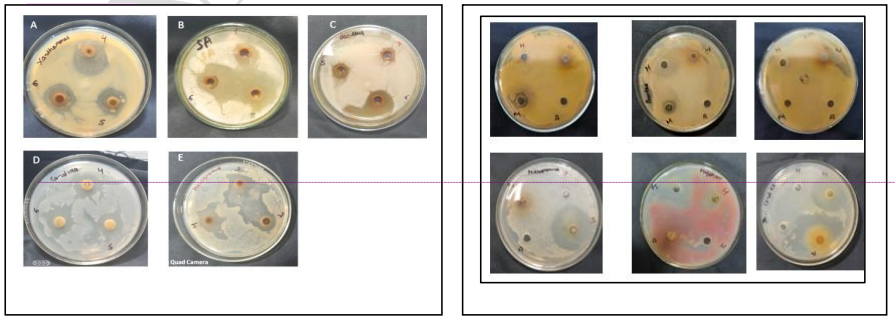
Plant extract	**Antimicrobial activity (mm) roots**					
	Antibacterial activity (mm)			Antifungal activity (mm)		
	Bacillus sp.	*Xanthomonas campestris*	*Staphylococcus aureus*	*Candida albicans*	*Fusarium oxysporum*	*Macrophomin aphaseolina*
Methanol	13	18	18	34	30	32
Aqueous	12	08	12	28	28	18
Acetone	11	08	14	31	20	17
Tetracycline	21	16	18	-	-	-
Cycloheximide	--	--	--	13	15	10

Comment [LFH27]: As per stated at the materials and methods, the sample was performed in triplicate. Suggest author include the mean and SEM in each of the result stated at the table.
Suggest author consider either holo or clearzone shown on the plate.

Table 3. Antimicrobial activity of leaves extracts of *Argemone Mexicana*

Plant extract	**Antimicrobial activity (mm) leaves**					
	Antibacterial activity (mm)			Antifungal activity (mm)		
	Bacillus sp.	*Xanthomonas campestris*	*Staphylococcus aureus*	*Candida albicans*	*Fusarium oxysporum*	*Macrophomina phaseolina*
Methanol	21	16	18	38	-	32
Aqueous	11	11	11	9	-	11
Acetone	13	12	14	34	-	28
Tetracycline	21	16	18	-	-	-
Cycloheximide	--	--	--	13	15	10

Fig. 1. Antimicrobial activity of roots and leaves extracts of *Argemone Mexicana - A) Staphylococcus aureus B) Bacillus sp C) Xanthomonas campestris D) Macrophamina phaseolina E) Fusarium oxysporum F)*



Comment [LFH28]: The inhibition test shown in the Figure 1 are not very satisfactory as the zone shown on the plate is unclear. Some of the agar is not slanted properly. The bacteria load shown on the plate is not well-distributed and not standardized terms of bacteria concentration (the cultured soft agar on the plates did not filled properly)