Title :

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

Abstract:

Aim - The main objective of the study is to estimate the effect of extraction techniques and antimicrobial activity of different solvent extract of *A. mexicana* leaves and roots.

Study Design - The research is experimental in nature.

Place and Duration of Study: Department of Chemistry and Microbiology at CCSHAU, Hisar, between late 2020 and January2022.

Methodology - The leaves and roots of *A. mexicana* were harvested for this study. Shade dried roots and leaves were cut into small pieces of 2-3 inches and processed into powder using a mixer grinder. Soxhlet extraction and microwave-assisted extraction procedures were used to extract leaves and roots in solvents such as acetone, methanol, and water. 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.

Results - Soxhlet extraction technique gave better extract yield 12.19g/100g and 8.54 g/100g while microwave-assisted extraction gave 8.88 g/100g and 6.94 g/100g for leaves and roots respectively. The methanoilc root and leaves extracts exhibited higher antimicrobial activity followed by acetone and water extracts.

Conclusion - The result of the investigation showed that extraction techniques considerably affected extraction yield and antimicrobial activity. Soxhlet extraction is better one extraction method among these two and methanolic extract of leaves was found to be good antimicrobial followed by acetone and aqueous.

Keywords: *Argemone mexicana*, Soxhlet Extraction, Microwave- Assisted Extraction, Roots, Leaves, Antimicrobial.

Introduction

Plants have long been recognized as potential sources of diverse classes of chemical compounds, known as phytochemicals, having diverse biological and therapeutic activities, which are effective in controlling or treating various diseases. Medicine based on the plant system continues to play a significant part in the health-care system. Plant-based medications are used by over 60% of the world's population for primary health care. Because therapeutic plants are becoming more widely recognised, there is a growing belief in herbal medicine. The medicinal plants are long-lasting acknowledgment owing to growing trust in herbal medicine (S. Dutta *et*

al., 2014). The medicinal plant's parts (stem, bark, leaves, fruits, roots, and seeds) have been used in phytomedicine and have a specific physiological function on the human body. According to Chaudhuri *et al*. (2012) the medicinal plants had natural bioactive elements such as alkaloids, tannins, flavonoids, and phenolic compounds.Medicinal plants also contain massive amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein and other carotenoids, which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (A. Bind *et al*., 2014). In various places, *A. Mexicana* also known as prickly poppy or satyanashi, is utilised as a medicinal plant. *A. Mexicana* possess biological activities such as antibacterial as reported by Bhattacharjee *et al*. (2006); Rahman, *et al*. (2011); Rahman, M. *et al*. (2006) ; Sahu *et al*. (2012) as well as antifungal reported by Kushtwar *et al*.(2017); Santosh *et al*.(2009); More, N. *et al*. (2016); Andleeb, S. *et al*. (2020). Strong medicinal potential of *A. mexicana* has been analyzed by several researchers as well as scholars groups to determine its main secondary metabolites, that includes phenolics, flavonoids, tannins, terpenoids (such as glycosides) , N-containing compounds (such as alkaloids), as well as saponins and steroids (Ibrahim and Ibrahim, 2009). In our study, we were used two different methods for extraction that is soxhlet (traditional) and microwave assisted (noval) extraction for root, leaves extract preparation in three different solvent namely methanol, acetone and water. Extraction techniques and nature of the solvent greatly affected the yield, phenolic contents as well as antioxidant activity as reported by Bushra *et al*. (2009) ; Tushar *et al*. (2017). Many of these natural plant-derived antibiotic compounds have been left undiscovered since the introduction of current antibiotic medications mostly derived from bacterial, fungal, and synthetic sources. A detailed evaluation of the effect of extraction procedures and antibacterial activity of A. mexicana leaves, roots extracts in different solvents that had not before been examined in the literature in Haryana was carried out in this study.

Materials and methods

Plant materials – In late March 2021, the entire plant A. mexicana was obtained from roadside and unoccupied plots in Haryana's districts of Bhiwani and Hisar. The entire plant was brought to the lab. Roots and leaves were separated, then washed with double distilled water after being cleansed with running tap water for 2-3 times. After that, it was shade dried for thirty days. Roots and leaves were dried before being cut into small pieces of 2-3 inches and powdered in a grinder. The powdered form was sieved, and leaf and root samples were stored separately in airtight containers.

Chemicals and Reagents

The solvents were used for extraction and evaluation of antimicrobial activity of analytical grade from hi- media chemicals limited. The solutions used in assay were prepared fresh for experimental procedures.

Extraction

Extracts were made with acetone, methanol, and water, and then two extractions were performed (soxhlet and microwave- assisted extraction). Antimicrobial activity was assessed using the extraction method with the highest extract yield.

Soxhlet Extraction

The extract was prepared according to the M.D.Luque *et al*. (1998) technique. *A. mexicana* roots and leaves powdered samples were packed in a whatman no.1filter paper to make a thimble that fit in a traditional soxhlet apparatus 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. Extractions completed in three steps that consisted of first extraction step of 5 hours, residue in thimble was again extracted twice completed in 2 hours and then repeated third time; extraction completed in 1 hour. Suitable amount of acetone and methanol solvent was added in the siphon to make up a volume of 150mL. Filtrates of acetone and methanol solvent from three extraction steps were taken and their volumes were noted. But in the case of water as a solvent it takes longer to extract through the siphon mechanism, here extraction completed in 8 cycles rather than 3 cycles in case of acetone and methanol. After extraction, the volume of each filtered solvent (acetone, methanol, water) was measured. All these extracts were filtered and further concentrated on rotator evaporator (Buchii-R300) for evaluation of antimicrobial activity. Root and leaves extracts stored in a refrigerator at 4°C. Extract yield of *A. mexicana* roots, leaves prepared by soxhlet extraction was calculated using formulae

$$\text{Extract yield (g/100g)} = \frac{(W1 \times 100)}{(W)}$$

W1 is the weight of the extract residue
W is the weight of the extract

Microwave-assisted extraction

The main benefits of using microwave assisted extraction is that it reduces the amount of solvent used, waste generated, solvent release into the environment, and human exposure. (M. letellier *etal*., 1999). In that case a microwave oven (IFB, model : 2301) with output of 800W and operating frequency 2450 MHz was used for extraction. Eight gram of each powdered samples of *A. mexicana* roots and leaves were placed in a 250 ml conical flask. Then 100 ml each acetone, methanol and aqueous was added to these flasks. Flasks were left overnight. Flask samples were microwaved for 10 seconds at 40% power in a microwave oven, but not allowed to boil. After cooling to room temperature, the extraction procedure was repeated up to 12 times (in order to complete the extraction) with the irradiation stage. After extraction, the volume of each filtered solvent was measured and further concentrated on rotator evaporator (Buchii-R300) for evaluation of antimicrobial activity. Root and leaves extracts stored in a refrigerator at 4°C. Extract yield of *A. mexicana* roots, leaves prepared by microwave-assisted extraction technique was calculated using formulae

$$\text{Extract yield (g/100g)} = \frac{(W1 \times 100)}{(W)}$$

W1 is the weight of the extract residue
W is the weight of the extract

Evaluation of Antimicrobial Activity

The antibacterial activity of the root and leaf extracts were determined using Bayer *et al*. (1966), Agar's well diffusion method. Single colonies on agar plates were grown for 18 to 24 hours to produce a bacterial solution with a turbidity of 0.5 McFarland (equivalent to 1.5×10^8 colony-forming units (CFU)/ml). At 600 nm, the turbidity of the bacterial suspension was measured. Agar plates were inoculated with 100 µl of the test microorganisms and spread evenly using a spreader before being allowed to dry for 5 minutes. Under aseptic circumstances, Mueller hinton agar plates and Potato dextrose agar were inoculated with bacterial and fungus strains, respectively, and 50 µl of the test samples were poured into wells (diameter=6mm) and incubated at 37°C for 24 hours for bacteria and 72 hours for fungi. The diameter of the growth inhibition zones was determined in millimetres after the incubation time. After 24 hours for bacteria and 72 hours for fungi, the zone around each well was measured. To reduce error, each experiment was repeated three times. For fungus, cycloheximide was employed as a standard, while tetracycline was utilised for bacteria. The zone of inhibition was measured in millimetres after incubation. 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.

Statistical analysis

Triplicates of each sample were used for statistical analysis, and the results were reported as mean, standard error (S.E.). One-way and two-way analysis of variances (ANOVA) were employed in Online Statistical Analysis (OPSTAT) to examine if there were any significant differences between the sample means.

Results and Discussion

Extract Yield:

Extract yield of *A. mexicana* roots, leaves prepared by soxhlet extraction and microwave-assisted extraction technique was given in Table 1. The yield of extracts in g/100g prepared by soxhlet extraction technique was higher than microwave-assisted extraction technique for the solvents aqueous, methanol, and acetone among *A. mexicana* roots and leaves extracts prepared by two extraction techniques. In case of Soxhlet extraction the analyte is concentrated from the matrix as a whole or isolated from specific interfering compounds using a mixture of percolation and maceration procedures while in case of microwave assisted extraction involves heating solvents containing samples, which allows analytes from a sample matrix to be partitioned into the solvent. 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. For *Quercus infectoria* galls, literature results showed that supercritical carbon dioxide (SC-CO2) extraction yielded the lowest extraction yield when compared to soxhlet extraction (Hasmida *et al*., 2014). In aerial portions of *Potentilla atrosanguinea* Lodd, soxhlet extraction was shown to be 1.8 and 3 times greater than ultrasound and maceration extraction respectively, but only slightly (1.2 times) higher than microwave extraction according to Kalpana *et al*. (2008).

Evaluation of Antimicrobial Activity

Antimicrobial activity was assessed using extract derived from a soxhlet extraction procedure that yielded a higher yield. As shown in Table 2,3 and Fig.1, methanol extract has good antibacterial action against *Bacillus sp*. and *Staphylococcus aureus* as well as antifungal activity against *Candida albicans, Fusarium oxysporum*, and *Macrophomina phaseolina*. 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. In *A. mexicana*, Abdulkarim *et al*. (2016) found that ethanol leaf extract had higher antibacterial activity than methanol leaf extract. Singh *et al*. (2009) found that chloroform extract of *A. Mexicana* seeds has antibacterial activity with minimum inhibitory concentrations (MIC) of 2.0 -5.0 mg/ml against Gram-positive and Gram-negative bacteria. According to Bhatacharjee *et al*. (2006), methanol extracts of the leaves and seeds of *A. mexicana* had higher antibacterial activity than water extracts. According to Shyam Prasad and Dhanapal.(2010), methanol leaves extracts of *A. mexicana* at 100 µl concentration showed better activity against two Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris*, *Salmonella typhi*), and four fungi (*Aspergillus niger, Fusarium moniliforme, Candida albicans* and *Mucor plumbeus*). The antibacterial compounds chelerythrine and berberine were discovered by nuclear magnetic resonance analysis of the root and leaf methanol fractions, and the findings highlighted the importance of plants as a valuable pharmaceutical resource at a time when antimicrobial and anticancer drug discovery was reported by Orozco *et al*. (2021).

Conclusion

A. mexicana is a Papaveraceae plant with antibacterial and antifungal properties against a wide range of microorganisms. Water extract had the highest extraction yield for soxhlet extraction (12.19 g/100g) and microwave assisted extraction (8.54 g/100g) among the different solvents (water, methanol and acetone). For both antifungal and antibacterial action, methanol extract was found to be the most effective, followed by aqueous, and finally acetone. The results of this investigation revealed that the extraction technique and solvents had a substantial impact on the extract yield and antimicrobial activity of *A. Mexicana* leaf and root extracts. *A. Mexicana* leaf and root extracts can thus be considered effective antimicrobials.

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Conflict of Interest : The authors claim to have no competing interests.

Competing Interests: Authors have declared that no competing interests exist.

Author's Contribution : Neha Goel, Monika Panghal,and Indu Rani : Plant part collection, roots , leaves extract preparation, Sachin Kumari: manuscript preparation; Meena Sindhu : Antimicrobial activity ; Sushila Singh : Help in planning and execution.

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

Plant and Location	Plant Parts Extraction Technique	Leaves **(g/100g)**			Roots **(g/100g)**		
		Water	Methanol	Acetone	Water	Methanol	Acetone
A. mexicana and Hisar	Soxhlet	12.19 ± 0.05	11.48 ± 0.05	9.00±0.02	8.54±0.03	7.65±0.02	5.63±0.01
	Microwave	8.88 ± 0.02	7.46 ± 0.05	7.31±0.04	6.94±0.03	4.84±0.01	4.83±0.02
	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

(SE -standard error, CD- critical difference)

Table 2. Antimicrobial activity of roots extracts of *A. 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
Water	12	08	12	28	28	18
Acetone	11	08	14	31	20	17
Tetracycline	21	16	18	-	-	-
Cycloheximide	--	--	--	13	15	10

Table 3. Antimicrobial activity of leaves extracts of *A.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
Water	11	11	11	9	-	11
Acetone	13	12	14	34	-	28
Tetracycline	21	16	18	-	-	-
Cycloheximide	--	--	--	13	15	10

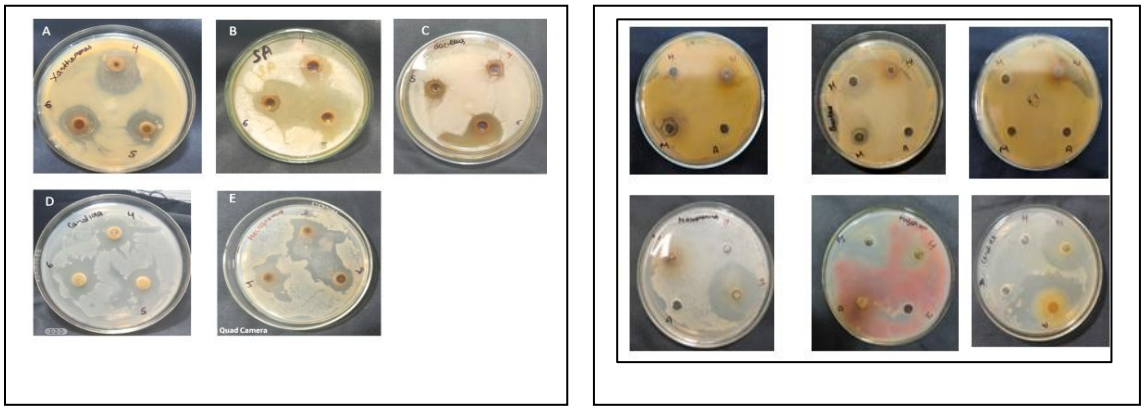

Figure1. Antimicrobial activity of leaves and roots extracts of *A. mexicana* against tested microorganism