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Research Article

MICROWAVE-MEDIATED EXTRACTION OF *LANTANA CAMARA* (L) WITH COW URINE

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ABSTRACT

Objective: There are approximately 150 species of perennial blooming plants in the Verbenaceae family, which includes *Lantana camara* (L). The instantaneous preparation of the cow urine extract in the microwave, followed by the partition process of making different solvent crude extracts, and subsequent investigation of the biological efficacy against bacterial strains *Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus,* and *Escherichia coli* by disk diffusion method, constitutes the main goals. Additionally, thin-layer chromatography (TLC), IR, and phytochemical studies will be used to analyze the contents of the phytochemicals.

Methods: The development of a plant profile comprises authentication, collecting, synthesis of a cow urine extract, and biological analysis. Phytochemical, TLC, and IR spectral methods were used to validate the presence of phytoconstituents in the solvent crude extracts.

Results: It is shown that microwave method improves the yield when compared to cold percolation method and the maximum zone of inhibition shown by the crude extract is compared to standard and control. Among the three extracts, chloroform extract displayed promising inhibitory action against four bacterial strains. Further IR spectra absorption peaks provide evidence of the kind of functional groups present.

Conclusion: The current novel research demonstrates that an efficient way for producing bioactive extract in short time and with a higher yield is due to the combination of microwave irradiation with cow urine. *In vitro* screening of crude extracts of the leaf of the plant shows promising activity against bacterial strains and thus suggests its application in drug discovery research.

Keywords: *Lantana camara* (L), Cow urine, Microwave, Partition, Antimicrobial activity, TLC, IR.

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INTRODUCTION

The products of plants, in particular fruits, vegetables, and leaves, include essential constituents including flavonoids, coumarins, phenolics, tannins, lignin, carotenoids, anthocyanins, antioxidants, and other worthwhile phytochemicals [1-3]. Natural products represent a formidable reservoir of potentially useful leads for new medicines in drug discovery research and these are called as secondary metabolites which produced by various living organisms [4-8]. The plant name *Lantana camara* (L) is obtained from Latin "lento" which means "to bend," it is a terrestrial weed, belongs to Verbenaceae family with 600 varieties, and the genus comprises 2500 species [9-11]. It originates from south and central parts of America, which is considered among the top 10 worst invasive species by IUCN and it is naturalized in approximately 80 tropical and sub-tropical countries also severely affects the native composition of terrestrial ecosystems [12]. It was instigated in India around 1809, later conquered most of the disturbed forest, pasture, and it has been standing as one of the most rudimentary medicinal weeds in the world [13]. It is frequently employed as herbal remedy, and in some places, as mulch and fuel [14]. There has been a significant amount of research done, particularly in India, on the chemical components of *L. camara* (L) [15-18]. Flowers have been utilized for children's chest problems, roots are used to cure toothaches, leaf crude extract and oil is exhibited anti-proliferative, antimicrobial, fungicidal, insecticidal, and nematocidal activities and used as an antiseptic for scars [19,20]. In the opinion of Bashir *et al.*, *L. camara* (L) shoot extract demonstrated quite significant antioxidant activity [21]. The fruits can be used to cure rheumatism, tumors, pocks, and fistulas. *L. camara* (L) essential oil demonstrated a wide range of antibacterial, antimicrobial, and antifungal properties [22]. The chemical constituents of *L. camara* (L) that has been successfully isolated and structurally characterized are triterpenes, including as lantadenes A, B, C, and D, alkaloids, flavonoids, saponins, tannins, germacrene A, B and D and chief compounds are valencene and γ-gurjunene [23]. Even many investigators have effectively documented the presence of beneficial phytochemicals in *L. camara* (L), however, it is anticipated that this plant will include numerous active chemical components [24].

Cow urine is a unique liquid discharge and potent, and numerous research reports have used it to extract phytochemicals despite having significant pharmacological significance and having great esthetic and medicinal values [25,26]. The main constituents are water: 95%, followed by urea: 2.5%, and the rest is a mixture of different minerals, salts, hormones, and enzymes: 2.5%. Cow urine exhibits antimicrobial and germicidal characteristics owing to the presence of urea, creatinine, aurum hydroxide, carbolic acid, phenols, and calcium and manganese salts [27,28]. It has an excellent polarity index value that is sufficient to extract a wide spectrum of phytochemicals from the medicinal plant and its pH value ranges from 7.3 to 8.7 and varies depending on the type of cow [29-32].

Microwave-assisted extraction (MAE) is one of the most widely recognized extraction techniques that are being investigated right now in natural product chemistry [33-35]. By the exploitation of microwave energy, solvents are concentrated with the plant metabolites, and it is proved to be safe for most specimens because to its durability and convenience of use [36,37]. While the application of MAE is still in the stages of development, research into the practical application of microwaves for the commercial production of phytochemicals is ongoing.

Encouraged by the findings of earlier studies on *L. camara* (L), we decided to conduct the current investigation of microwave-assisted extraction of leaves of *L. camara* (L) using cow urine [38].

METHODS

The solvents (hexane, chloroform, ethyl acetate, DMSO, petroleum ether, and methanol) used for the current study are procured from reputed

companies such as Thermo-Fisher and SD fine and distilled before use. The cow urine samples were purchased from Patanjali Pharmaceuticals (under standard managemental practices) near Mhow Naka, Indore. Perkin-Elmer FTIR spectrometer was used to record the IR spectra of chloroform crude extract.

Modification of commercial microwave oven
The commercial microwave oven has been tailored to facilitate the continuous extraction of phytoconstituents and to facilitate the assembly of a cold-water circulation system for the condenser, which is helpful for the condensation of cow urine.

Authentication and collection of plant material
Botanical identity was done with the help of a botanist Dr. Anand Krishna, Department of Life Science, SVIS, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore who recognized, validated, and certified the *L. camara* (L) leaves voucher specimen that was deposited in the Herbarium.

Collection of plant material
To get rid of dust and debris, the *L. camara* (L) leaves were gathered, cleaned under running water, and rinsed with distilled water. After that, the leaves were separated, shade dried, and ground into powder with the help of a mechanical grinder. When needed, the fine powder was made from the dried powder by passing it through a mesh screen, then it was stored in an airtight container.

Specifications of a microwave oven
The domestic microwave oven was modified for the current research and the specifications are 2450 MHz frequency and the power output was 900 W.

Preparation of cow urine extract and solvent partition
50 gm of dried, pulverized leaves of *L. camara* (L) are prepared into crude extracts using 300 mL of cow urine distillate as a solvent medium (1:6 ratio) and kept in a rotary shaker equipment for 2 h to aid in effective mixing and the mixture was repeatedly exposed to microwave radiation for 90 min, followed by a 2-min rest with regular 15-min breaks. The microwave oven is equipped with cool water circulation system which is designed to help the cow urine vapors to condense and after 90 min of irradiation, the extract solution in the flask was filtered through the muslin cloth. The filtrate thus obtained was once more filtered using Whatman filter paper No. 1 to prepare the solvent crude extract as well as extraction of phytoconstituents. The L. camara (L) powder, microwave reactor, and appropriately dried chloroform crude extract are highlighted in Fig. 1. The solvent crude extracts prepared as cow urine extract solution is taken for sequential solvent extraction from non-polar (hexane) to medium polar (ethyl acetate) solvents [39]. The cow urine extract solution is taken in 1000 mL conical flask equipped with mechanical overhead stirrer, 200 mL of hexane solvent is added, and then, the mixture was agitated for 1 h. Later, hexane layer was collected in a conical flask with the help of separating funnel and this process was repeated for 2 times to make the extraction completely. Cow urine extraction and flow chart of solvent partition of L. camara (L) leaves in detail are presented in Fig. 2. The solvent is removed under vacuum and the extract is completely dried with high vacuum. The % yield of the different solvent crude extracts has been determined using the microwave – cow urine method and the cold percolation method and the results are shown in Table 1 at results and discussion section. It is evident that microwave radiation has a beneficial effect on the yield percentage of the relevant crude extract when compared to the conventional cold percolation procedure. The same experimental procedure was followed to prepare the other solvent crude extracts and weight of *L. camara* (L) leaf powder taken for microwave-cow urine method and the cold percolation method is 50 g.

Phytochemical analysis (qualitative analysis)
In accordance with the previously described procedure, a phytochemical evaluation of solvent crude extracts of *L. camara* (L) leaves was carried out to identify the presence of following phytochemicals saponins, tannins, flavonoids, steroids, alkaloids, terpenoids, glycosides, quinones, coumarins, and phenols [40,41]. The results are displayed in Table 2 of results and discussion section.

Table 1: Comparison of cold percolation and microwave method-crude extracts (% Yield)

No	Solvent	Microwave method		Cold percolation method (Maceration)	
		Extract (Wt) g	% Yield	Extract (Wt) g	% Yield
1	Hexane	1.64 g	3.3%	0.46 g	0.9%
2	Chloroform	2.68 g	5.4%	0.38 g	0.7%
3	Ethyl acetate	1.2 g	2.4%	0.92 g	1.8%

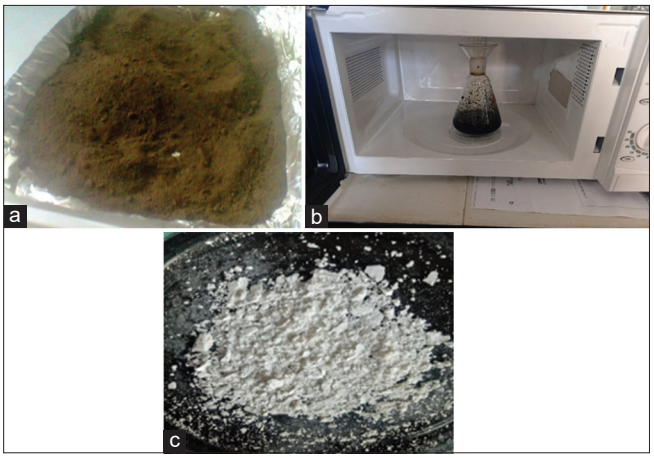

Fig. 1: (a) *Lantana camara* (L) powder, (b) Microwave extraction, (c) Chloroform crude extract

RESULTS AND DISCUSSION

The purpose of the investigation was to determine the % yield of various solvent crude extracts, which is illustrated in Table 1 and further discussed in the context of the results and objective of the study. It is confirmed that microwave method is superior to the conventional cold percolation procedure.

The results of phytochemical analysis of different crude extracts are displayed in Table 2.

The antibacterial attributes of cow urine extract and solvent crude extracts can be examined with making use of four bacterial strains, namely *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* by means of the disk diffusion method, and their antibacterial efficacy can be determined through growth inhibition zones, which is expressed in mm [42]. The test organism was inoculated in nutrient broth and incubated overnight at 37°C. The *L. camara* (L) solvent crude extracts were prepared in 100 ppm concentrations with DMSO. Four petri plates of agar media were prepared and the solution of crude extracts in DMSO concentration of 100 ppm is prepared. The plates are incubated for 24 h at 37°C after that observed for the formation of zone of inhibition, which measure the antibacterial efficacy of extracts, and the data are shown in Table 3. Fig. 3 demonstrated the antibacterial activity of solvent crude extracts of L. camara (L). The research findings highlighted that chloroform extract had the most profound antibacterial effect against the four bacterial strains, *P. aeruginosa* - 23 mm, *S. typhimurium* - 21 mm, *S. aureus* - 22 mm, and *E. coli* - 20 mm. The results of this study agreed with literature values, with inhibition in the range of 18–24 mm and it confirms that chloroform crude extract is a source of bioactive phytoconstituents.

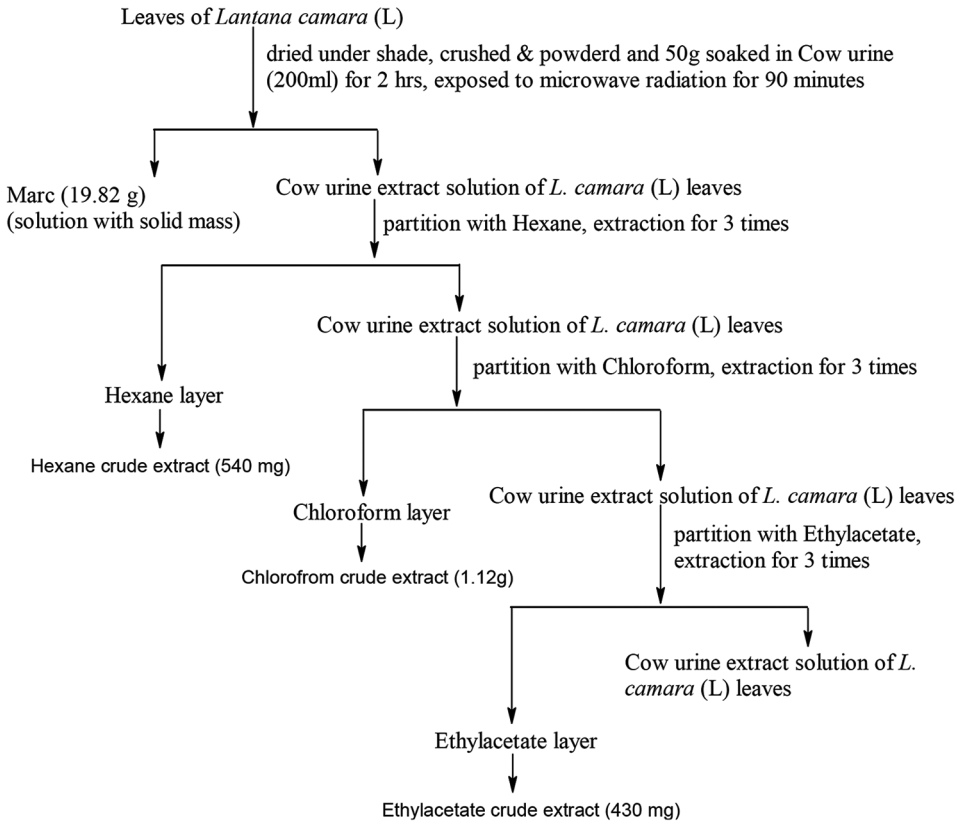

Fig. 2: Flow chart of cow urine extraction and solvent partition of *Lantana camara* (L) leaves

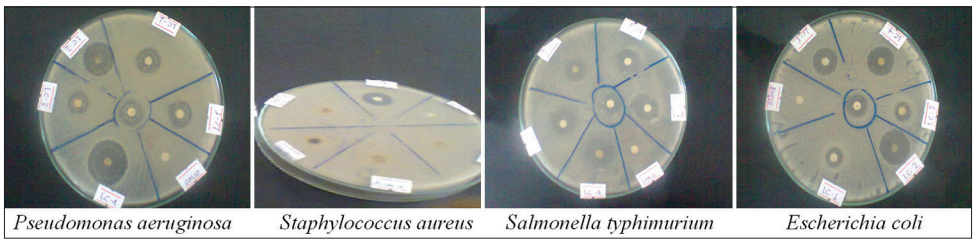

Fig. 3: Antibacterial activity of solvent crude extracts of *Lantana camara* (L) cow urine extract

Table 2: Phytochemical constituents of different solvent crude extracts

No	Phytochemicals (type/class)	Cow urine extract	Hexane extract	Chloroform extract	Ethyl acetate extract
1	Alkaloids	+++	--	++	++
2	Flavonoids	+	--	+	+
3	Glycosides	+++	--	+	++
4	Terpenoids	+++	++	+++	+++
5	Steroids	+++	+	++	+
7	Coumarins	+++	+	+	+

+ + +Heavily present; ++Slightly present; + Present; – Absent

Table 3: Antibacterial activities of different solvent crude extracts

Bacterial strain	Conc. Mg/mL	Zone of inhibition in (mm)				
		Hexane extract	Chloroform extract	Ethyl acetate extract	Gentamicin	DMSO
Escherichia coli	100	9	20	15	22	NI
Staphylococcus aureus	100	10	22	16	22	NI
Salmonella typhimurium	100	12	21	14	23	NI
Pseudomonas aeruginosa	100	12	23	15	24	NI

NI: No inhibition

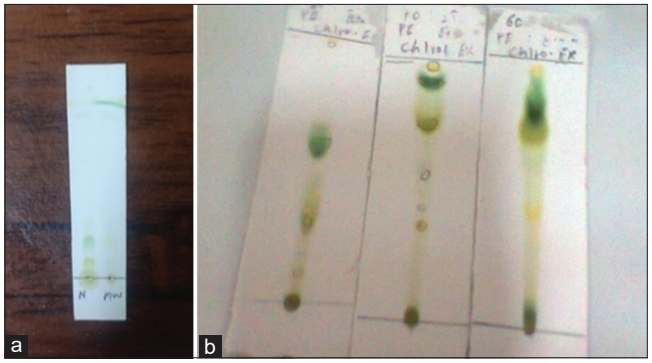

Fig. 4: TLC of cow urine extract and chloroform extract of *Lantana camara* (L) leaves (a) TLC of cow urine extract (b) TLC-chloroform extract in different mobile phase

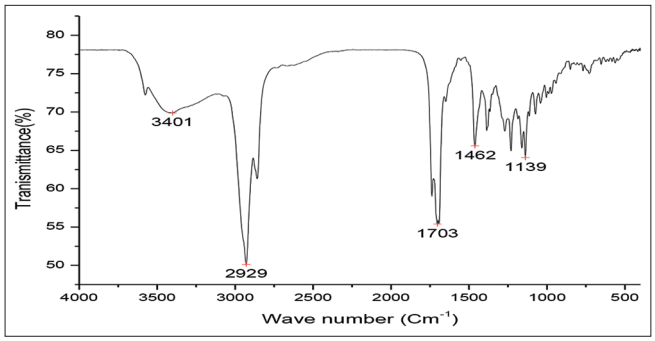

Fig. 5: IR spectra of chloroform extract of *Lantana camara* (L) leaves

Thin-layer chromatography (TLC) analysis

Encouraged by the results of antibacterial studies, TLC experiment is performed on cow urine and chloroform crude extracts with the following mobile phase, (a) 20% Ethyl acetate + Pet. Ether, (b) 40% Ethyl acetate + Hexane, (c) 60% Ethyl acetate + Hexane. The TLC of chloroform crude extract in different mobile phase is presented in Fig. 4 It reveals that both cow urine and chloroform extracts contain essential bioactive phytochemicals.

Analysis of IR spectra of chloroform crude extract

The IR absorption peak values disclosed that may be the presence of following groups in the compounds of chloroform crude extract. The presence of -OH group is confirmed by the appearance of peak at 3401 cm^{-1}, peak at 2929 cm^{-1} indicates the C-H stretching of -CH group, 1703 cm^{-1} confirms the presence of carbonyl group, and the presence of aromatic ring is confirmed by the absorption peaks at 1462 and 1139 cm^{-1}. IR spectra of L. camara (L) chloroform crude extract is displayed in Fig. 5.

CONCLUSION

Plants can synthesize a vast array of distinctive bioactive compounds, which will be valuable for therapeutic purposes. In this manuscript, it is highlighted that the combination of cow urine and microwave irradiation influences the yield of the crude extract. The antibacterial studies, TLC, and phytochemical analysis revealed that the chloroform crude extract contains bioactive phytochemicals, column chromatography isolation, and characterization of the lead bioactive molecule will be published in the due course of time.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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