Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **04 Year 2023** Page **1009:1013***

Characterization Studies of Medicinal Plants & I'ts Biological Evaluation Towards Anti-Bacterial Study

Rajakumari K[1], Parthiban Brindha Devi[1*], S Ivo Romauld`[1], Karthika J[1], Mirunaalini Kannan[1]

[1]*Department of Bio-Engineering, School of Engineering, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai-600117, Tamil Nadu*

**Corresponding author's E-mail: pbrindhadevi@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 04 Dec 2023

Abstract

The traditional system of medicine proves to be one of the best sources for treating illness and infections. Murraya koenigii (curry leaves) and Asparagus racemosus (shatavari) are the two medicinal plant species that have numerous medicinal properties like antimicrobial, anticancer, anti-inflammatory, etc. In this study, the Ultrasound extraction and the Soxhlet mode of extraction were carried out using two different solvents such as Hexane and Ethyl acetate. Analysis and Retention factor were was carried out thin layer chromatography. The purification was performed using column chromatographic method and the various phytoconstituents such as tannins, terpenoids, carbohydrates, proteins, steroids, flavonoids, phlobatannins were analysed for the extracted samples. Retention factors have been calculated for both the extracted samples and the samples were further processed for sterility test in order to check for any contamination. The extracted samples were characterized using GC-MS analysis. The anti-bacterial evaluation was performed. The highest zone of inhibition found in 1000µg/ml concentration for Pseudomonas aeruginosa and 500µg/ml concentration for Staphylococcus aureus. The minimum inhibitory concentration for Pseudomonas aeruginosa is predicted as 250µg/ml concentration and for Staphylococcus aureus as 500µg/ml concentration. The rate of kill was higher in 500µg/ml concentration for both bacterial samples. The crystal violet assay was performed and observed the cell viability. Further the In-silico analysis was performed towards the GC analysis compounds and their structure activity relationship were studied towards the protein.

Keywords: *Murraya koenigii, Asparagus racemosus, characterization studies, Anti-bacterial evaluation, Structure activity Relationship.*

1. Introduction

In recent the prevalence of diseases has been increasing in our environment. These diseases are growing more resistant to the current modern and traditional medicines and treatments. Traditionally plants are used to treat multiple diseases and injuries exclusively over the world. They are massively available renewable source that persists to exist despite harsh environments and natural disasters. New technologies and methods are present in the modern world to prevent plant species from annihilation such as international seed banks, synthetic seed cultivation, green house plantation, plant sanctuaries, etc. These methods help to store existent plant seeds and the plant itself so that they can be cultivated in the future when they are nearing destruction or when their need is of vital importance. In India, many outmoded siddha books are available on medicinally available plants and their uses [1].

Plants serve as the primary source for many of the modern medicines for example cocaine drug which is used as local anaesthetic is from the plant source *Erythroxylum coca* (Coca plant). Nowadays, greater interest has been shown for herbal drug synthesis for traditional medicines have caused a surge in the scientific study of medicinal plants [2,3].

The medicinal plants are found over many regions under various conditions. Such plants are also consumed daily by men as a source of food. Two of such food products are *M. koenigii* and *A. racemosus* which possess vital medicinal values. *M. koengii* is commonly known as curry leaves. It's naturally found in India, Sri Lanka and other south Asian countries. Traditionally they are used for treating

various ailments. The plant *A. racemosus*is known as Shatamuli which is used as the traditional medicine in Bangladesh. These plants are found in India, Sri Lanka, Himalayas and Nepal. *A. racemosus* treats various disorders such as tumours, inflammation, cardiac related problems, bronchitis and other infectious diseases [4,5].

Figure 1. *Murraya koenigii*

Figure 2. *Asparagus racemosus*

2. Materials And Methods

Sample Collection:

Fresh curry leaves and the *A. racemosus* were collected from Thanjavur district in the village Valuthur, Tamilnadu. The samples were washed and dried thoroughly to form dry combined mixtures. The dry leaves were coarse grinded and stored in an airtight container [6,7].

Bioactive Component Analysis

The combined plant compounds were analysed for different solvent polarity ranges from polar to non-polar. Among the various solvents like Hexane, Chloroform, Ethyl acetate and ethanol, hexane and ethyl acetate have extracted the metabolites better via ultrasonication method. The retention factors were calculated and the samples extracted were analysed using thin layer chromatography method [8]. Further the extracted samples were partially purified using column chromatographic method with different polarity ranges of solvents. The purified samples were analysed for its presence of various phytochemicals using the standard protocol [9].

Characterization studies

The purified samples were characterized using Gas Chromatography to determine the presence of volatile compounds in the mixture. Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique that can be used to identify and determine the compounds present in a plant sample. GC-MS plays a major role in the phytochemical screening and chemotaxonomic studies of medicinal plants containing physiologically active components. Conversely, peak regions are connected with the pertinent chemical's concentration. When complex samples are separated by GC-MS, numerous distinct peaks are produced; each peak yields a distinct mass spectrum that is utilised to identify the molecule.

Target analytes and unknown substances could be found and analysed using large, commercialised mass spectra collections.

Antibacterial evaluation for *Pseudomonas aeruginosa* and *Staphylococcus aureus*:

Zone of Inhibition using Agar well diffusion method

The dried sample of combined extracts of *Murraya Koenigii* and *Asparagus racemosus* was taken in the ratio of 1:1 and dissolved in 2ml of (5%) DMSO with 1mg/ml concentration. Serial dilution was done for three different concentrations namely Muller Hinton agar medium was prepared for the study. Serial dilution was done for three different concentrations from 1mg/ml to 250µg/ml and control was kept as ampicillin. Muller Hilton agar prepared, autoclaved and poured into Petri plates and get solidified under UV cabinet. Overnight grown culture *Pseudomonas aeruginosa* and *Staphylococcus aureus* was poured into the plates separately and spread using L-rod. The wells were punched in each agar plates using well puncher, 200µl of different concentrations samples and control with ampicillin was poured into the different well punched agar plates. Then the plates were kept at incubator for 24 hours. After incubation, the zone was observed and measured in mm [10].

Rate of Kill

To measure the percentage level of the plant sample inhibiting the bacterial growth at different time intervals, the initial concentration of combined plant samples was kept as 1mg/ml and it is serially diluted till 62.5µg/ml with 5% DMSO and similarly for the standard ampicillin. Both the bacterial samples were serially diluted at four different concentrations separately [10]. The serially diluted samples were kept for overnight incubation. The growth was observed periodically at different time intervals and the OD was measured at 595nm and the percentage of inhibition were calculated using the formula,

$$\%\text{Inhibition} = \frac{100\text{x [Control (OD)- Sample (OD)]}}{\text{[Control (OD)]}}$$

Crystal Violet Assay

The alteration in membrane permeability was predicted using crystal violet assay. Overnight grown culture poured in four different concentrations and it is centrifuged using cooling centrifuge for the collection of pellets. The cells washed twice with phosphate buffer saline (pH 7.4). The plant sample is serially diluted into two different concentrations 1000µg/ml, 500µg/ml and control was kept as ampicillin. Then the diluted sample is added to the cell suspension and incubated at 37ºC for 30 minutes. The cells were harvested at 9300rpm for 5 minutes. The cells were resuspended with Phosphate buffer saline containing 10µg/ml of crystalviolet. The suspensions are then incubated at 37ºC for 10 minutes. The cell suspension was then centrifuged using cooling centrifuge in 9500 rpm for 15 minutes. OD of the supernatant was measured using UV- spectrophotometer at 595nm. The percentage is calculated using the formula [13].

$$\text{Percentage of cell viability} = \frac{\text{(OD) value of sample}}{\text{(OD) value of crystal violet assay}} \text{x100}$$

3. Results and Discussion

Combined activities of *Murraya koenigii* and *Asparagus racemosus*

In this study, Ultrasonic modes of extractions were carried out to extract the phytoconstituents of combined plant species. The Anti-bacterial evaluation of *Murraya koenigii* and *Asparagus racemosus* against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The combined species ultrasonicated extract were analysed by thin layer chromatography which gave different bands visualised under UV and the samples were partially purified using Column chromatographic method.

Phytochemical Analysis & Sterility Test

Phytochemical tests are carried out with the purified dried mixtures. From these analyses, tannins, flavonoids, glycosides, terpenoids, steroids, carbohydrates, proteins were confirmed for their presence. Further the sterility test was performed for the partially purified samples to detect anti-microbial growth for the period of 24hrs. After incubation the samples were check for any microbial growth. There is no growth observed in the samples which implies the samples are sterile free for further analysis.

Characterisation Studies Using GC-MS

The dry mixtures were given for GC-MS evaluation and observed the biological compounds present in the plant samples. The volatile compounds eluted are mentioned in the Table- 1

Table 1- Biological compounds observed from the plant extract

S.NO	BIOLOGICAL COMPOUNDS	MOLECULAR FORMULA
1.	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]	$C_{15}H_{24}$
2.	Trans-z-alpha-bisabolene epoxide	$C_{15}H_{24}O$
3.	Caryophyllene oxide	$C_{15}H_{24}O$
4.	1,3,6,10-dodecate tetrane 3,7,11-trimethyl-(Z,E)	$C_{15}H_{24}$
5.	1H-cyclopro p[E]azulene, decahydro-1,1,7- trimethyl -4methylene, [1AR	$C_{15}H_{24}$
6.	Aromadendrene	$C_{15}H_{24}$
7.	1 butanol, 4-butoxy-	$C_8H_{18}O_2$
8.	Sulphurous acid, pentadecyl 2-propylester	$C_{15}H_{32}O_3S$
9.	Hexatriacontane	$C_{36}H_{74}$

Anti-Bacterial Evaluation with *Pseudomonas aeruginosa* and *Staphylococcus aureus*

The anti-bacterial activity of the plant samples against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were analysed initially by agar dilution method and agar well diffusion method later carried out by Minimum Inhibitory Concentration (MIC), rate of kill & Crystal violet assay.

Agar diffusion method

The zone of inhibition is the effective response of the antimicrobial agent from *Murraya Koenigii* and *Asparagus racemosus* against the bacterial strains *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The zone of inhibition observed in the plates spread with *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the maximum zone is developed at 1000 µg/ml when compared with control drug Ampicillin. This study has proved that the compounds are having good effects towards the pathogenic bacteria.

Name of the Organism	Concentration of Plant samples (µg/ml)			Control (ampicillin) (µg/ml)		
	1000	500	250	1000	500	250
P. aeruginosa	25mm	14mm	8mm	20mm	15mm	10mm
S. aureus	30mm	18mm	10mm	25mm	20mm	9mm

Rate of Kill

The rate of kill was performed to find the effectiveness and the time taken for the bactericidal activity of the plant sample against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The percentage inhibition was calculated by using the OD values measured at 595nm. The rate of kill of the bacterial strains was given below in the tables. The rate of kill of *P. aeruginosa* was observed at 75% at 1000µg/ml concentration in 16th hour shows that at minimum time period the metabolites in the extracts were able to kill the organisms.

Table 2. Percentage inhibition of growth (*Pseudomonas aeruginosa*) by plant samples at different time intervals.

Concentration of Plant samples (µg/ml)	4th hour % of Inhibition	8th hour % of Inhibition	16th hour % of Inhibition	24th hour % of Inhibition
1000	25	55.7	75	100
500	15	32.6	80	100
250	21.1	65.0	90	100
125	18.2	32.0	15	100
Control	16	15	17	76.47

Table 3. Percentage inhibition of growth (*Staphylococcus aureus*) by plant samples at different time intervals.

Concentration of Plant samples (µg/ml)	4th hour % of Inhibition	8th hour % of Inhibition	16th hour % of Inhibition	24th hour % of Inhibition
1000	28	56.7	72	100
500	18	33.6	82	100
250	23.2	67.0	92	100
125	19.2	34.0	55	100
Control	17	16	20	76.47

Crystal Violet Assay

To find the cell viability the crystal violet assay was performed. The crystal violet assay was performed for *P. aeruginosa* and *Staphylococcus aureus* and the values are summarised in the table 4. The percentage of cell inhibition occurs in 1000µg/ml concentration is 11.34% and 500µg/ml is 2.77% with the control of 4.86%. At 1000µg/ml concentration there was an effective inhibition. For *Staphylococcus aureus* the inhibition occurred at 1000µg/ml concentration with 10.34%. The result implies that both the bacteria's have been effectively killed by the extracted plant metabolites.

Table 4. Crystal violet assay for cell viability - *P. aeruginosa* and *S. aureus*

P. aeruginosa		*S. aureus*	
Concentration of Plant samples (µg/ml)	Percentage of Cell viability	Concentration of Plant samples (µg/ml)	Percentage of Cell viability
1000	11.34%	1000	10.34%
500	2.77%	500	5.77%
Control (ampicillin)	4.86%	Control (ampicillin)	5.86%

4. Conclusion

In the present generation, the interest in the study of natural herbal products and their formulation is growing rapidly, especially while extracting a bioactive compound and qualitative analysis. Hence, we carried out our work on the varying extraction methods for the selected plant materials. Among the above methods done, ultrasonication method gave the finest result and by using various solvents like hexane, ethyl acetate, petroleum ether, ethanol used for extraction of bioactive compounds from leaf extract of *Murraya Koenigii* and *Asparagus racemosus*, the solvent ethyl acetate was more efficient. Based on the R_f value the sample has extracted well in polar solvent. The sterility test has proven that the sample employed has been free from bacterial contamination. Through GC-MS characterization the bioactive compounds of the mixture have known. Based on the test carried out, the anti-bacterial property of the mixture was efficient.

Acknowledgement

The author's thanks Vels Institute of Science Technology and Advanced Studies for providing the facilities to execute the project work effectively.

Conflict of interest

The authors declare that there are no conflicts of interest.

Financial support

No funding sources available.

Ethics statement

No animals were involved in the study.

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