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Editorial

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY AND BRINE SHRIMP LETHALITY BIOASSAY OF DIFFERENT EXTRACTS OF *ALYSICARPUS VAGINALIS* VAR. *NUMMULARIFOLIUS* (DC.) MIQ. (FAMILY: FABACEAE)

SUSAN KURIAN*, V. S. JOSEKUMAR

Department of Zoology, Mar Ivanios College (Autonomous), Thiruvananthapuram 695015, Kerala, India
Email: susankurianmic@gmail.com

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ABSTRACT

Objective: To evaluate the antimicrobial activity and cytotoxicity of hexane, ethyl acetate and methanol extracts of *Alysicarpus vaginalis* var. *nummularifolius* (DC) along with phytochemical analysis.

Methods: The crude extract of hexane (AVH), ethyl acetate (AVE) and methanol (AVM) of *Alysicarpus vaginalis* var. *nummularifolius* (DC) were prepared and analysed for phytochemical constituents using standard methods. The cytotoxicity activity of the plant extracts was predicted using brine shrimp lethality assay (BSLA). The antimicrobial activity and the minimal inhibitory concentration (MIC) of the plant extracts were examined against 5 bacterial and 2 fungal strains using agar well diffusion method, and two fold serial dilution method, respectively.

Results: The phytochemical screening studies showed a higher concentration of saponins, alkaloids, flavonoids, phenols, quinones and terpenoids in AVM than the other two extracts. The LC_{50} value of AVH and AVE were found to be 900.05 µg/ml and 754.35 µg/ml respectively using BSLA while that of AVM was>1000 ug/ml. All the extracts of the plant showed antimicrobial activity against most of the test organisms. The MIC values of AVM were lower than AVE for all the microbial strains except for *Pseudomonas aeroginosa* where AVE (107.87µg/ml) exhibited higher value than AVM (51µg/ml).

Conclusion: The present study concluded AVM with a high presence of phytochemicals. The AVE and AVM were found to possess promising antimicrobial activity when compared with the standards. The AVM exhibited lesser toxicity when compared with AVH and AVE.

Keywords: *Alysicarpus vaginalis* var. *nummularifolius* (DC), Phytochemical analysis, BSLA, Antimicrobial, Cytotoxicity, Minimum Inhibitory Concentration

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INTRODUCTION

Plants are the indispensable storehouse of many chemical metabolites. The medicinal value of plants have assumed a more important dimension in the past two decades owing largely to the discovery that extracts from plants contain many minerals, primary metabolites and secondary metabolites with antioxidant potential [1]. Chemical metabolites present in green plants are grouped into primary and secondary metabolites (phytochemicals). Phyto-chemicals are good sources of medicinal drugs for instance that with antimicrobial effects are used for the treatment of microbial infections [2, 13].

Alysicarpus vaginalis var. *nummularifolius* (DC.) Miq. Family: Fabaceae (Leguminosae) which is commonly known as alyce clover, one leaved clover (Malayalam: Nilaorila). It is widely used for various diseases related with kidney, diuretics, leprosy and pulmonary problems [3]. Ethnobotanical research on folklore medicine identified the plant is used for jaundice treatment along with goat milk [4]. Antioxidant and antiproliferative activity were reported with ethanolic extract of *A. vaginalis* [5]. Hepatoprotective activity was attributed to an ethanolic extract of *A. vaginalis* in experimental rats with nitrobenzene-induced hepatic damage [6]. Different varieties of *A. vaginalis* have been identified.

Many bioactive compounds are isolated from plant resources, however, the status of them as medicinal drugs required thorough screening [7, 8]. Though many medicinal properties of *Alysicarpus vaginalis* var. *nummularifolius* (DC.) are reported, a systematic investigation to identify cytotoxicity of this plant resource viz., hexane, ethyl acetate and methanolic extracts is not yet done. Brine shrimp lethality assay (BSLA) is an *in vitro* toxicity assay which is useful for assessing the toxicity of bioactive compounds from plant extracts. This plant could be a valuable resource with many pharmacological activities especially as an antimicrobial agent. Phytochemical analysis along with antimicrobial screening of various extracts of this plant is suggested to assess its potential as a medicinal herb. Cytotoxicity screening is also essentially important to identify it for human consumption.

MATERIALS AND METHODS

Collection of plant materials

Alysicarpus vaginalis var. *nummularifolius* (DC.) Miq was collected from Nalanchira, Thiruvananthapuram, Kerala, India. It is a common creeping herb found in open ground and waste land; widely distributed in India, Sri Lanka, Pakistan, Africa and Australia. The leaves are obovate–oblong, obtuse at both ends and having a diameter of 1-2.5 cm. Its flowers are pink in dense racemes. They flower and fruit during the month of September to January. The plant specimen was authenticated by Dr. G. Valsaladevi, Curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala. The voucher specimen has been deposited in the Herbarium of Department of Botany, the University of Kerala with voucher no KUBH-5928 for future reference.

Chemicals and reagents

Hexane, ethyl acetate, methanol, dimethylsulfoxide (DMSO), hydrochloric acid, Dragendorff reagent, Mayer's reagent, Wagner's reagent, Benedict's reagent, sulphuric acid, ferric chloride, sodium hydroxide, lead acetate, ninhydrin, nitric acid, α-naphthol, chloroform, glacial acetic acid, potassium dichromate, nutrient agar medium, Potato dextrose agar were purchased from Himedia Chemical: India. All the chemicals and solvents used were of standard analytical grades.

Preparation of the plant extracts

The entire fresh plant materials were collected washed and shade dried. The dried plant materials were grinded to a fine powder using an electric grinder. The dried and powdered plant material was successively extracted with hexane (AVH), ethyl acetate (AVE) and methanol (AVM) in the order of their increasing polarity in soxhlet apparatus until it became colourless according to the standard methods [9]. Each extract was concentrated by using rotary vacuum evaporator (Superfit, ROTAVAP PBU-6) and stored in the refrigerator for further analysis.

The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material using the formula:

%Yield = (Dry weight of the extract/Dry weight of leaf sample) x 100. The colour and consistency of the extracts were also noted.

Phytochemical screening

The plant extracts were analysed for the presence of alkaloids, saponin, flavonoids, phenol, carbohydrates, proteins and amino acids, cardiac glycosides, steroids, anthraquinone and terpenoids using the standard methods [9-11].

Cytotoxicity study: brine shrimp lethality assay (BSLA)

BSLA was used to predict the cytotoxicity activity of hexane, ethyl acetate and methanol extract of the plant. The cysts of brine shrimp were obtained as a gift from Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram. The cysts were hatched in filtered and well-aerated sea water under illumination. After 24 h the nauplii (larva) hatched, and the phototropic nauplii were collected by pipette. The extracts were dissolved in DMSO and diluted with sea water. An alternative dilution method was adopted in the preparation of the different dilutions of the plant extract and the final concentrations were1000, 100, 10 and 1 (µg/ml). Three replicates in each concentration were done. Potassium dichromate and DMSO of the same concentrations were used as positive and negative controls respectively.

The solutions were added to the pre-marked vials containing 10 brine shrimp nauplii in 5 ml simulated seawater. Dry yeast suspensions were added as food to each vial and maintained under illumination. After 24 h the survived nauplii in each vial was counted using a magnifying glass. The percentage of mortality of brine shrimp nauplii was calculated for each concentration and compared with positive and negative controls [12]. Since the mortality was observed only in 1000 µg/ml concentration of AVH and AVE, the further assay was conducted using varying concentrations between 100 and 1000µg/ml for calculating LC_{50}. Using the probit analysis method described by Finny [13], LC_{50} and 95% confidence intervals were determined from the 24 h.

Microbial strains

Five bacterial strains [*Staphyllococcus aureus* (ATCC 25923), *Sreptococcus mutans* (MTCC 890), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883) and *Pseudomonas aeroginosa* (ATCC 27853)] and two fungi [*Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404)] were used. American type culture collection (ATCC) strains were purchased from Himedia while *S. mutans* was obtained from Institute of Microbial Technology (MTECH), Chandigarh.

Antibacterial activity

The antimicrobial activity of the different extracts of the plant was assayed by agar-well diffusion method as described in NCCLS, 1993 [14]. Petri plates containing 20 ml nutrient agar medium was seeded with bacterial strains. Wells of approximately 10 mm was bored using a well cutter. Plant extracts were prepared in DMSO (stock: 1 mg/ml DMSO). The plant extracts of 25, 50, and 100 µl concentrations were added. Streptomycin (20 µl) and DMSO (100 µl) were used as positive and negative controls respectively. The plates were then incubated at 37 °C for 24 h. The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact with the test organisms in the freshly seeded plate. The diameter of the zone of inhibitions was measured in millimetres after 24 h.

Antifungal activity

The potato dextrose agar plates were prepared and inoculated with a fungal culture. Wells of approximately 10 mm was bored using a well cutter and samples of different concentration was added. The zone of inhibition was measured in millimetres after overnight incubation and compared with that of standard antimycotic (Clotrimazole) (10 µl) which was used as positive control and DMSO (10%) as the negative control.

The percentage of inhibition (% I) was calculated by using the following formula:

$$\text{Inhibition (\%)} = (\text{Zone of Inhibition of extract} / \text{Zone of inhibition of the positive control}) \times 100.$$

The diameter of the test sample of 100 µl concentration of various plant extracts was taken for calculating the inhibition %.

Minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) of the ethyl acetate and methanol extracts was determined by using two-fold serial dilution methods in 96-well plates against *S. mutans, E. coli, P. aeroginosa, K. pneumoniae*, *C. albican* and *A. niger*. Samples were dissolved in DMSO to a final concentration of 1 mg/ml and added in increasing concentration such as 6.25, 12.5, 25, 50, 100 µg/ml respectively. Solvent control was prepared with DMSO (10%), and blank control was prepared from virgin media. The plates were prepared in triplicates and incubated overnight at 37 °C. Growth was observed by visual inspection and by measuring the optical density (OD) at 620 nm using microplate reader (ERBA, Germany, Model: ELISCAN FT_3, 1x96 wells). The OD was measured immediately after the visual reading. The growth inhibition for the test wells at each plant extract dilution was determined by the formula:

$$\text{Percent inhibition} = (\text{OD of control} - \text{OD of test}) / (\text{OD of control}) \times 100\%$$

Spectrophotometric MICs were calculated based on the density of the growth control and the lowest drug concentrations that resulted in a 50 % reduction in growth was compared with that of the drug-free growth control [15].

Statistical analysis

Experimental results were expressed in mean±standard error mean (SEM) of the triplicates. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package (version 16). P value<0.01 was considered as significant at 1% level.

RESULTS

The present investigation shows the phytochemical analysis, antimicrobial activity and cytotoxicity assay of the different extract of the plant *Alysicarpus vaginalis* var. *nummularifolius* (DC). The yield % of the successive extraction of hexane, ethyl acetate and methanol were 1.3%, 4.28% and 5.12% respectively. They were a light green solid powder (AVH), brown waxy (AVE) and greenish brown waxy (AVM) in appearance.

Phytochemical analysis

Various phytochemical analyses with the plant extracts showed the presence of alkaloids, saponin, flavonoids, phenol, carbohydrates, proteins and amino acids, cardiac glycosides, steroids, anthraquinone and terpenoids (table 1). The phytochemical test of the crude hexane (AVH) revealed the presence of tannin, flavonoids, steroids and terpenoids while ethyl acetate (AVE) and methanol (AVM) extract showed the presence of proteins, amino acids, cardiac glycosides, alkaloids, tannins, flavonoids, steroids, quinines and terpenoids. The methanol extract (AVM) showed the higher concentration of saponins, alkaloids, flavonoids, phenols, quinones and terpenoids than the other two extracts.

Table 1: Phytochemical screening of hexane (AVH), Ethyl acetate (AVE) and Methanol (AVM) extract of *Alysicarpus vaginalis* var. *nummularifolius* (DC.)

S. No.	Phytochemical constituent	Test performed	AVH	AVE	AVM
1	Saponin	1. Froth Test	_	_	++
		2. Foam Test	_	_	++
2	Carbohydrate	1. Molisch's Test	+	+	+
		2. Benedict's Test	_	_	_
		3. Fehling's Test	_	_	_
3	Protein and Amino acid	1. Xanthoprotec Test	_	+	+
		2. Ninhydrin Test	_	_	+
4	Cardiac glycosides	Keller Kelliani's Test	++	++	++
5	Alkaloids	1. Wagner's Test	_	_	+
		2. Mayer's Test	_	+	++
		3. Dragondroff Test	+	_	++
6	Tannin	Braymer's Test	+	+	++
7	Flavanoids	1. Alkaline reagent Test	+	++	+
		2. Lead acetate	+	_	++
8	Phenol	Ferric chloride Test	+	+	++
9	Steroids	Salkawski's Test	++	+	++
10	Anthraquinone	Borntrager's Test	_	+	_
11	Quinones	Hydrochloric Acid	+	+	++
12	Terpenoids	Salkawski's Test	++	+	++

++= Highly present; += Moderately present; -= Absent

Brine shrimp lethality assay (BSLA) for cytotoxicity

The brine shrimp lethality assay of the three plant extracts was found to be concentration dependent (table 2). The LC_{50} value of AVH and AVE found to be 900.05µg/ml and 754.35 µg/ml respectively. Meyer *et al.* [16] suggested that crude plant extract is considered toxic (active) if it has an LC_{50} value of less than 1000 µg/ml while non-toxic (inactive) if it is greater than 1000 µg/ml. The methanolic plant extract of *Alysicarpus vaginalis* var. *nummularifolius* (DC.) showed no lethality up to 1000µg/ml. Hence its LC_{50} value is insignificant. While Potassium dichromate which was used as standard showed LC_{50} value as 24.8µg/ml.

Table 2: Brine shrimp lethality assay of different extracts of *Alysicarpus vaginalis* var. *nummularifolius* (DC.) after 24 h

Plant extracts	LC_{50}(µg/ml)	95% Confidence interval
Potassium Dichromate	24.8	14-37
AVH	900.05	849.80-965.36
AVE	754.35	724.93-786.51
AVM	>1000	

Table 3: Antimicrobial activity of *Alysicarpus vaginalis* var. *nummularifolius* (DC.) extracts using disc diffusion assay

Microbial strains	Diameter of inhibition zone (mm)			
Staphyllococcus aureus	**25 µl**	**50 µl**	**100 µl**	**Streptomycin (20 µl)**
Hexane	12.1±0.05	13.2±0.05	21.23±0.03	41±0.14
Ethyl acetate	11.23±0.06	14.3±0.05	16.23±0.08	40±0.07
Methanol	12.3±0.05	19.33±0.18	22.26±0.08	44±0.06
Sreptococcus mutans				
Hexane	10.3±0.05	12.33±0.12	15.3±0.05	49.3±0.00
Ethyl acetate	11.23±0.03	13.3±0.05	16.23±0.08	40.3±0.05
Methanol	12.3±0.05	12.16±0.03	15.33±0.08	44.5±0.05
Escherichia coli				
Hexane	Nil	13±0.04	14.16±0.16	39.2±0.15
Ethyl acetate	10.1±0.05	12.03±0.03	14.1±0.1	35.23±0.18
Methanol	10.03±0.03	11.10±0.1	12.16±0.08	38.13±0.13
Klebsiella pneumonia				
Hexane	12.1±0.05	13.2±0.05	21.23±0.03	41±0.03
Ethyl acetate	11.23±0.06	12.3±0.06	16.23±0.08	40.03±0.03
Methanol	12.3±0.05	11.3±0.18	22.26±0.08	44.13±0.13
Pseudomonas aeroginosa				
Hexane	12.13±0.03	16.4±0.04	20.3±0.17	40.5±0.28
Ethyle acetate	10.03±0.03	14.3±0.05	20.23±0.23	40.16±0.08
Methanol	10.23±0.23	12.2±0.21	16.33±0.18	44.0±0.05
Candida albicans				**Clotrimazole (20 µl)**
Hexane	Nil	10.1±0.05	10.7±0.05	29.96±0.08
Ethyl acetate	12.2±0.05	13.16±0.12	19±0.05	31.93±0.06
Methanol	12.3±0.06	16±0.5	21±0.05	35.03±0.08
Aspergillus niger				
Hexane	10±0.00	11.13±0.03	13.13±0.13	30.9±0.05
Ethyl acetate	11.2±0.05	14.23±0.03	26±0.15	29.93±0.08
Methanol	10.2±0.05	11.46±0.27	12.2±0.20	30.36±0.31

Values are mean inhibition zone (mm)±SE of three replicates

According to Moshi *et al.* [17], the brine shrimp results were interpreted as follows: LC_{50}<1.0 µg/ml–highly toxic; LC_{50}–1.0-10.0 µg/ml–toxic; LC_{50} 10.0-30.0 µg/ml–moderately toxic; LC_{50}>30<100 µg/ml as non–toxic. The hexane, ethyl acetate and methanol extract of *A. vaginalis* which is having the LC_{50} value greater than 100 µg/ml can be considered as non-toxic while that of potassium dichromate as moderately toxic. The lethality of the plant extracts to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of these plants.

Antimicrobial activity

Plant extracts are generally rich in antimicrobial compounds. The *in vitro* antimicrobial activity of the different extracts of *Alysicarpus vaginalis* var. *nummularifolius* (DC.) under different concentration with the standard are shown (table 3). All the extracts of the plant showed antimicrobial activity against most of the test organisms. The percent of inhibition of different extract is shown in the fig. 1.

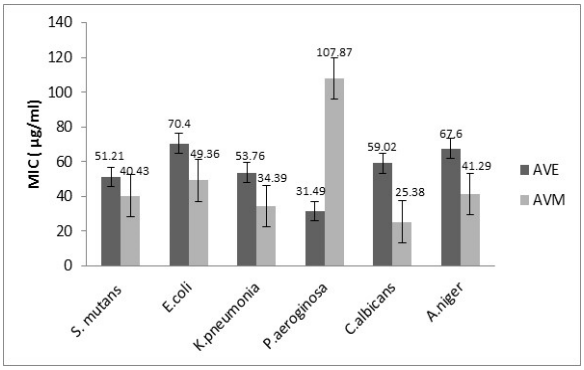

Fig. 1: Minimum inhibitory concentration (MIC) of ethyl acetate (AVE) and methanol (AVM) extract of *A. vaginalis* var. *nummularifolius (DC.)* against microbial organisms expressed as mean±SEM (standard error mean; n=3)

Table 4: Statistical analysis of antimicrobial activity of hexane (AVH), ethyl acetate (AVE) and methanol (AVM) extracts (100 µl) of *A. vaginalis* var. *nummularifolius* (DC.) with respect to various microbial strains

Microbial strains	Diameter of inhibition zone(mm)				
	AVH	AVE	AVM	F	P
Staphyllococcus aureus(SA)	21.23±0.03[a]	16.23±0.08[b]	22.26±0.08[c]	1874.067	0.000**
Sreptococcus mutans(SM)	15.3±0.05[a]	16.23±0.08[b]	15.33±0.08[a]	44.529	0.000**
Escherichia coli(EC)	14.16±0.16[a]	14.1±0.1[a]	12.16±0.08[b]	84.976	0.000**
Pseudomonas aeroginosa(PA)	20.3±0.17[a]	20.23±0.23[a]	16.33±0.18[b]	131.231	0.000**
Candida albicans(CA)	10.7±0.05[a]	19±0.05[b]	21±0.05[c]	894.9	0.000**
Aspergillus niger(AN)	13.13±0.13[a]	26.00±0.15[b]	12.2±0.20[c]	2199.836	0.000**

Values are mean±SEM (standard error mean) (n=3). ANOVA followed by Duncan's Test. Mean followed by a common letter are not significantly different at the 1% level (p<0.01) by Duncan's test

ANOVA showed that there exist significant mean differences in antimicrobial activities among various extracts at 100 µl (P>0.01) (table 4). Duncan's test showed that the inhibition zone of the hexane (AVH) and ethyl acetate (AVE) extracts against *S. aureus* were more or less same (P>0.05). Methanol extract (AVM) showed highest inhibition zone against *S. aureus.* (22.26±0.08) *K. pneumonia* (21.23±0.03) and *C. albicans* (21+0.05) when compared to hexane (AVH) and ethyl acetate (AVE) extracts. Inhibition zone of the extract AVH (15.3±0.05) and AVM (15.33±0.08) against *S. mutans* were more or less the same (p>0.05). AVH and AVE are having a similar reaction and higher zone of inhibition against *E. coli* and *P. aeroginosa*. AVE is having the highest zone of inhibition (26.00±0.05) against *A. niger.*

The MIC of the AVE and AVM against *S. mutans, E. coli, P. aeroginosa, K. pneumoniae*, *C. albican* and *A. niger* is shown in the fig. 1. The MIC values of AVE was higher than AVM for all the microbial strains except for *P. aeroginosa* were AVM exhibited higher activity than AVE. The AVE showed the lowest MIC against *P. aeroginosa* i.e. 31.49µg/ml and higher value for *S. mutan* (51.21µg/ml), *E. coli* (70.4µg/ml), *K. pneumonia* (53.76µg/ml), *C. albicans* (59.02µg/ml) and *A. niger* (67.6µg/ml). AVM showed higher MIC value for *P. aeroginosa* (107.87µg/ml) while lowest against *C. albica* ns (25.38 µg/ml).

DISCUSSION

The aim of the present investigation is to evaluate the different extracts of *A. vaginalis* var. *nummularifolius* (DC.) for their phytochemical composition, cytotoxicity assay and antimicrobial activity. The detailed literature studies showed that the entire plant of *A. vaginalis* is used in traditional medicine for the treatment of renal calculi [18]; the leaf juice was also used for the improvement of eyesight and ear ache [19]. The root of this plant is widely used for kidneys, diuretics; leprosy and pulmonary troubles [20]. Preliminary phytochemical screening of ethanolic extract of *A. vaginalis* reported the presence of polyphenol [5]. The total phenolic content, total flavonoid content and antioxidant activity of the ethanolic extracts of the plant was evaluated [21]. The free radical scavenging activity and reducing power showed that the plant has significant antioxidant status. The presence of these phytochemicals reveals that the plant will possess various pharmacological activities like anti-inflammatory, anticancer, estrogenic gonadotropic, hepato-protective functions [21].

More than four different varieties of *A. vaginalis* have been identified. Literature studies reveal that no scientific work have been done on this particular variety of *A. vaginalis*. However, present study is the first ever report on qualitative screening, cytotoxicity study and antimicrobial activity of different extracts of *A. vaginalis* var. *nummularifolius* (DC.)Miq.

The percent yield of the successive extraction of hexane, ethyl acetate and methanol showed that the methanol extract (AVM) has the highest yield. Studies reveal the presence of the major phytochemicals are in polar fractions [22, 23]. The preliminary phytochemical analysis of the three extract revealed the presence of alkaloids, flavonoids, tannin, phenol and steroid (table 1). The analysis showed that amongst the three extracts, the polar fraction, methanol (AVM) contain high phenol, flavonoid, alkaloid, saponin, tannin, steroids and terpenoids.

A similar result was reported in the ethanolic extract of *A. vaginalis* revealed the presence of alkaloids, flavonoids, sterols, tannins, polyphenols, and triterpenoids [7]. The HPTLC studies on the petroleum ether extracts of *A. vaginalis* reported the presence of lupeol, beta sitasterol and stigmasterol [24].

All the three extracts were screened for cytotoxicity using brine shrimp lethality assay for LC_{50}. Higher LC_{50} values indicate a lesser toxicity. Of the three extracts, LC_{50} value of methanol (AVM) was>1000. Hence it is considered purely non-toxic considering Mayers *et al.* standards of evaluation [16], while LC_{50} value of AVH and AVE were 900.05µg/ml and 754.35 µg/ml. The LC_{50} value of the three extracts can be considered very less toxic to normal cells. The intrinsic functions of the cells may be disturbed if a compound is acutely toxic [25]. A positive correlation was reported between the

brine shrimp lethality test in mice in medicinal plant research [26]. Hence, the brine shrimp lethality assay of *A. vaginalis* can be considered as an ideal preliminary screening for toxicity *in vivo*. Antimicrobial activity of plant extract was considered to be good if its MIC was less than 100 µg/ml, moderate if MIC was from 100.0 to 500.0 µg/ml and poor over 500.0 µg/ml [27]. Since the MIC of the ethyl acetate and methanol extracts of the plant against all the microbial strains were analysed to be less than 100 µg/ml, the antimicrobial activity of the plant extracts can be considered good quality. The antimicrobial potential of the plant extracts could be due to its ability to bind to the cell wall of the bacteria, thereby inhibiting its synthesis probably because of the flavonoids, alkaloids and tannins present in the plant [28].

The flavonoids, alkaloids, tannins, triterpenes and steroids present through phytochemical screening might be responsible for the pharmacological activities of the plant [29]. Alkaloids are widely used for medicinal purposes and have positive and negative effects to human beings [10, 30]. All these natural phytochemicals possess antioxidant activity [31-33]. The plant extracts showed the LC_{50} value to be considered very less toxic to normal cells and exhibit a high antimicrobial activity. Due to these reasons, this plant can be identified as a good source for medicinal compounds.

CONCLUSION

The present study concluded that hexane, ethyl acetate and methanol extract of *A. vaginalis* var. *nummularifolius* (DC.) showed the presence of various phytochemicals of which methanol extract showed the high presence of alkaloids, phenols, flavonoids, saponins, tannin and terpenoids. Using the brine shrimp lethality assay the plant extracts showed the LC_{50} value to be considered very less toxic to normal cells. The present study revealed significant antimicrobial activity of the plant extracts when compared with standards. Since the minimum inhibitory concentration of the plant extracts was less than 100µg/ml, the plant was considered to have a very good antimicrobial activity. However, further studies are required to throw light on the biological activity of *A. vaginalis* and its bioactive compounds against various diseases.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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