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STANDARDIZATION AND ANTIBACTERIAL ACTIVITY OF *SPHAERANTHUS AMARANTHOIDES* LINN.

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ABSTRACT

The present work is aimed at exploring the physic-chemical parameter, Preliminary phytochemical analysis, TLC Photo documentation, HPTLC fingerprint, Heavy metal analysis of Lead, Cadmium, Mercury and Arsenic, and antibacterial activity of ethanolic extract of whole plant of *Sphaeranthus amaranthoides* Linn. (Fam: Asteraceae). The physicochemical screening was carried out and loss on drying of the sample ,total ash content, acid-insoluble ash, water& alcohol soluble extractive values of the sample were estimated. The preliminary phytochemical screening was performed and the presence of alkaloids, aminoacids, flavonoids, glycosides, phenol, steroid, tannin, triterpenoid and saponin were observed. HPTLC fingerprinting showed 9 peaks at 254 nm, the TLC photo documentation showed 7 visible spots under 254nm, 9 spots under 366nm and 9 spots after derivatization. Heavy metals such as Cadmium, Mercury and Arsenic were not detected and lead content was within the permissible limit. The antibacterial activity of methanolic extract of whole plant of *Sphaeranthus amaranthoides* Linn.was carried out against gram positive and gram negative bacteria. The antibacterial activity studied is expressed in terms of zone of inhibition measured in mm. A significant growth inhibition was shown by many of the organisms tested indicating the profound potency of the plant drug. Among the tested organism *Bacillus subtilus* was found to be the most sensitive organism followed by *Escherichia coli, Pseudomonas aeruginosa and Klebsiella Pneumonia.*, with maximum zone of diameter ranging from 32mm to 9 mm.

KEYWORDS: *Sphaeranthus amaranthoides* Linn., Standardization, HPTLC, Heavy

metals antibacterial activity.

INTRODUCTION

In Siddha System of Medicine, *Sphaeranthus amaranthoides* Linn. (family Asteraceae) is one of the kaya kalpa drugs. Kalpa drugs meant for rejuvenation therapy eradicates the infective organisms from our body and prevents further by developing an immune response. It also prevents the destruction and aging of the soft parts, muscles, nerves, bones, bone marrow, blood cells etc. It is commonly known as Sivakarantai in Tamil. *Sphaeranthus amaranthoides* is an erect fragrant herb with woody stem found in South India. Particularly in Tanjore, Tirunelveli and also found in Southern Mysore and Travancore.[1] The plant is used in the treatment of eczema, blood disorders, stomach worms, filarial and fever. It also removes kapha, vata and piles.[2] Protective role of this plant extract on dermal wounds in wistar rats was studied.[3] Whole plant is reported to posses analgesic, anti inflammatory, anti microbial, cytotoxicity, antidiarrhoeal and antioxidant activities.[3-8] This leaf juice is used in the purification of Mercury.[9]

MATERIALS AND METHODS

The fresh plant specimens for the proposed study was collected from Mettur, Salem Dt., Tamilnadu and it was authenticated by Dr.Sorna Subramaniyan, Research Officer (Botany),SMPG, Mettur Dam.The whole plant of *Sphaeranthus amaranthoides* Linn.were cleaned and shade dried at room temperature. The dried material was pulverized mechanically into coarse powder (sieve No. 80). The coarse powders were used for the various studies. Coarse powdered was extracted with ethanol by cold percolation method (48 hrs). The extract was filtered through Whatman No.1 filter paper, concentrated on a water bath and finally dried in vaccuum.

Physico-chemical parameters

Physico-chemical parameters were carried out as per WHO guidelines.[10]

Estimation of Heavy Metals

Heavy Metals like Lead, Cadmium, Mercury and Arsenic were estimated as per the procedure given in WHO.[11]

TLC/HPTLC finger printing studies

The HPTLC finger printing of the ethanol extract of whole plant of *Sphaeranthus*

amaranthoids L. was carried out by adopting standard procedure. The plates developed in different solvent systems as given in TLC studies were scanned using CAMAG TLC scanner with Cats 4.05 version software using Deuterium lamp at UV 254 nm. Finally the plates were dipped in vanillin-sulphuric acid. Allowed to dry and then heated in a hot-air oven at 105ºC till the coloured spots of the separated compounds appeared.[11,12]

Preliminary phytochemical studies

Preliminary phytochemical analysis was carried out as per the procedure.[13,14]

Antimicrobial Activity

Collection of microorganism

To evaluate the microbial studies, the cultures were procured from various laboratories in and around Chennai. The organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogens*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Proteus vulgaris*, and *Klebsiella pneumoniae*. All the organisms were confirmed using specific biochemical tests.[15]

Inoculum Preparation

A uniform suspension of the organisms listed above were prepared in 6ml of saline, and compared with the McFarland's standards.[16] Each microbial suspension was diluted with the saline to a density visually equivalent to the Barium sulphate standard, 0.5 McFarland's unit. The plates were inoculated within 15 minutes of the preparation of the suspension to avoid changes in the density of the cultures.

Preparation of plates and Inoculation of microbial cultures

The required quantities of the Muller Hinton agar were prepared. The pH of medium was adjusted to 7.2. Each plate was poured with 20ml of the media and was allowed to solidify. The tubes containing 0.5 McFarland's unit equivalent microbial cultures were dipped with sterile cotton swabs, and excess of the fluid was removed by gently rotating the swabs against the sides of the test tube. The dipped swabs were swabbed over the Muller Hinton agar plates covering the entire surface of the plate by rotating the plates in all the directions. After solidification wells of 6 mm diameter were punched in agar plates. Plates were then allowed to set for few minutes.

Drug concentration

500mg of plant extract was accurately weighed and dissolved in 2.5ml of DMSO solvent[17] to make the stock solution containing 0.2mg/ml concentration. A series of dilutions were made from the stock solution to obtain 100µg/µl, 50µg/µl, 25µg/µl, 12.5µg/µl, 6.25µg/µl, 3.1µg/µl, 1.5µg/µl, 0.7 µg/µl and 0.3µg/µl ml for determination of MIC.

Antibacterial assay and Determination of MIC

Antibacterial activity was assayed in duplicates by agar well diffusion method[18] using the above mentioned test organisms. The well was loaded with 80µl of the drug (0.2mg/ml conc.). The commercially available drug Ampicillin (10mcg/disc) was used as control.The plates were incubated at 37°C for 24 hrs. The diameter of the clearing zones were measured in mm using the calipers. The precise assessment of the effectiveness of the extract against the susceptible bacteria was achieved by determining the MIC with varying concentration ranging from 1.5µg/ml to 100 µg/µl by agar well diffusion method. The plates were incubated at 37^0C for 24 hrs and were observed for the MIC, which was read as the lowest concentration of the drug required to completely inhibit the growth of the organism.

RESULTS & DISCUSSION

The physico-chemical data of the whole plant (80mesh) are given in Table 1. The whole plant of *Sphaeranthus amaranthoides* contain 9.46 % moisture content. The ethanolic extract of whole plant of *Sphaeranthus amaranthoides* Linn. contains 2.71 % amount of silicates as shown by the acid insoluble ash, but contains considerable amounts of inorganic materials as shown by the higher ash value (11.19 %). The alcohol soluble extractive value 4.33% shows the polar constitutes of the plant and water soluble extractive values 16.62 % showed the polar and inorganic constituents of the plant.

Table. 1. Physico-chemical data

S. No.	Parameters	Result
1.	Loss on drying at 105^oC	9.26 %; 9.48 %; 9.64 %
2.	**Ash values** a. Total Ash b. Acid Insoluble Ash	 11.09 %; 11.13 %; 11.37 % 2.52 %; 2.72 %; 2.91 %

3.	**Extract Values** a. Alcohol b. Water	 4.28 %; 4.32 %; 4.40 % 16.48 %; 16.64 %; 16.76 %

The amount of various heavy metals found in the plant material are given in Table:2. The heavy metal contents of cadmium, mercury and arsenic were not detected and lead content was found to be within the permissible limits.

Table. 2: Heavy Metals Analysis

S. No.	Name of the Element	Results	Permissible Limit
1	Lead	0.0097 ppm	10 ppm (WHO)
2	Cadmium	Not detected	0.3 ppm (WHO)
3	Mercury	Not detected	1 ppm (API, 2008)
4	Arsenic	Not detected	3 ppm (API, 2008)

Preliminary phytochemical tests

The preliminary phytochemical tests of ethanol extract carried out are tabulated in Table: 3. Ethanol extract showed the presence of alkaloid, amino acid, flavonoid, glycoside/sugar, phenol, steroid, tannin, triterpenoid and saponin.

Table.3. Preliminary phytochemical analysis

S.No	Test	Ethanol extract
1.	Alkaloid	+
2.	Amino acid	+
3.	Coumarin	-
4.	Flavonoid	+
5.	Glycoside/Sugar	+
6.	Phenol	+
7.	Quinone	-
8.	Steroid	+
9.	Tannin	+
10.	Triterpenoid	+
11.	Saponin	+
12.	Furanoid	-
13.	Carboxylic acid	-
14.	Lignan	-

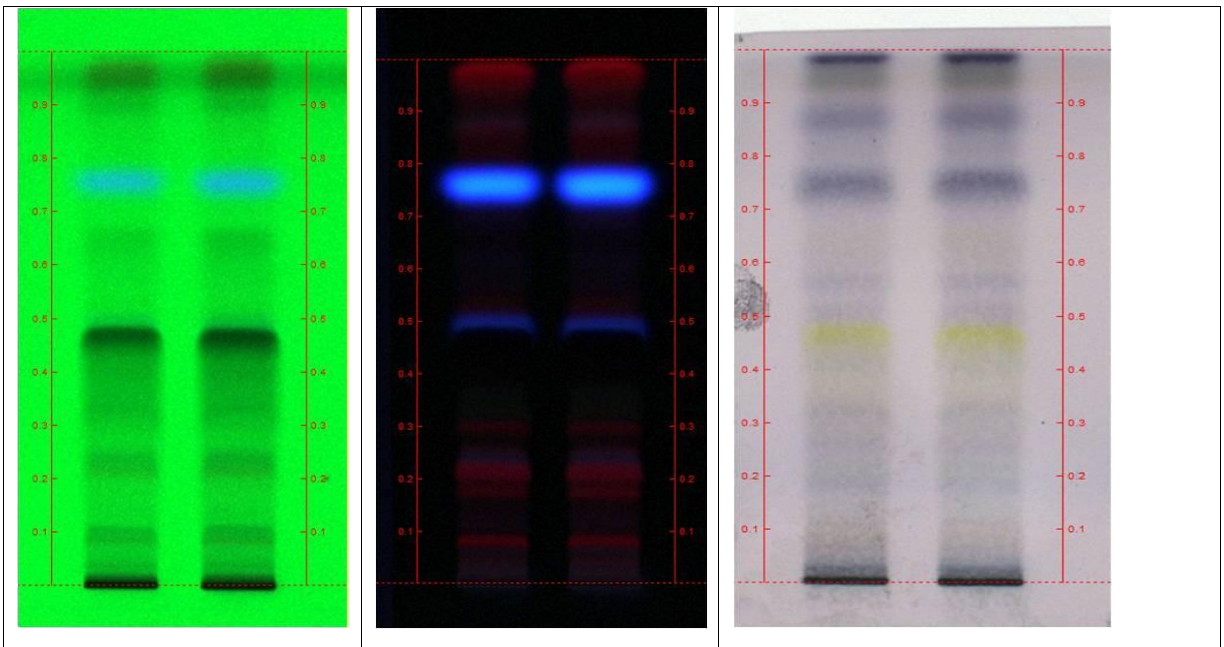
HPTLC finger print

The Rf values of various spots of ethanol extract in the TLC profile are given in Table:4. and spots were observed at 254 nm and 366 nm respectively. The spots were visualised

by spraying with vanillin-sulphuric acid reagent. The chromatograms of the ethanol extract are shown in Fig:1. HPTLC finger print profile at UV 254 nm is presented in Fig:2. Rf values and colour of spots are shown in Table:4 & 5.

Table:4. Rf. Values and colour of spot at UV 254 and 366 nm and after derivatization.

Solvent System	UV-254 nm	UV-366 nm	Visible light (Vanillin – Sulphuric acid Derivatization)
Toluene: Ethyl acetate (1:1)	0.89 Green	0.87 Red	0.95 Grey
	0.75 Blue	0.76 Fluorescent blue	0.87 Violet
	0.64 Green	0.53 Red	0.75 Violet
	0.47 Green	0.49 Violet	0.56 Grey
	0.33 Green	0.30 Red	0.51 Grey
	0.23Green	0.24 Violet	0.47 Yellow
	0.10 Green	0.21 Red	0.32 Grey
		0.17 Red	0.26 Violet
		0.10 Red	0.18 Grey



UV-254 nm	UV-366 nm	Visible light (Derivatization with Vanillin-Sulphuric acid)

Fig:1.TLC of the ethanol extract of *Sphaeranthus amaranthoides*

It showed 9 peaks of which one peak Rf. 0.55 was major peak and others were moderately smaller peaks.

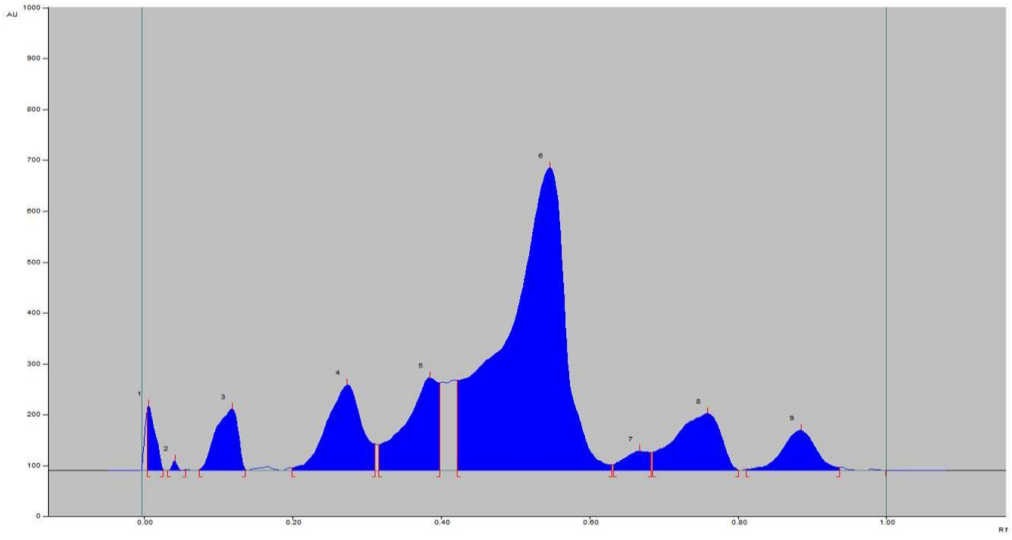


Fig:2.HPTLC finger print profile of Ethanol extract

Table:5. HPTLC finger print profile and Rf Values of ethanol extract at UV 254 nm:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	121.9 AU	0.01 Rf	127.2 AU	8.84 %	0.03 Rf	1.6 AU	980.6 AU	1.73 %
2	0.03 Rf	0.0 AU	0.04 Rf	19.0 AU	1.32 %	0.06 Rf	1.0 AU	89.3 AU	0.16 %
3	0.07 Rf	0.7 AU	0.12 Rf	120.3 AU	8.36 %	0.14 Rf	1.4 AU	2572.1 AU	4.55 %
4	0.20 Rf	4.5 AU	0.27 Rf	167.7 AU	11.66 %	0.31 Rf	52.4 AU	5629.1 AU	9.95 %
5	0.32 Rf	50.1 AU	0.39 Rf	182.0 AU	12.65 %	0.40 Rf	72.2 AU	5832.0 AU	10.31 %
6	0.42 Rf	176.5 AU	0.55 Rf	594.5 AU	41.31 %	0.63 Rf	10.8 AU	33102.2 AU	58.50 %
7	0.63 Rf	11.2 AU	0.67 Rf	37.7 AU	2.62 %	0.68 Rf	35.6 AU	945.5 AU	1.67 %
8	0.69 Rf	35.6 AU	0.76 Rf	111.5 AU	7.75 %	0.80 Rf	0.5 AU	4903.9 AU	8.67 %
9	0.81 Rf	2.2 AU	0.89 Rf	79.0 AU	5.49 %	0.94 Rf	5.8 AU	2534.6 AU	4.48 %

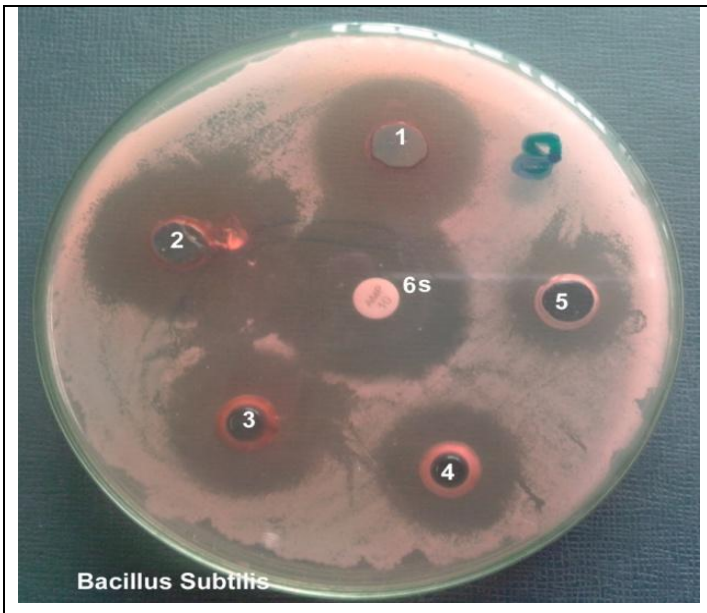
Antibacterial activity

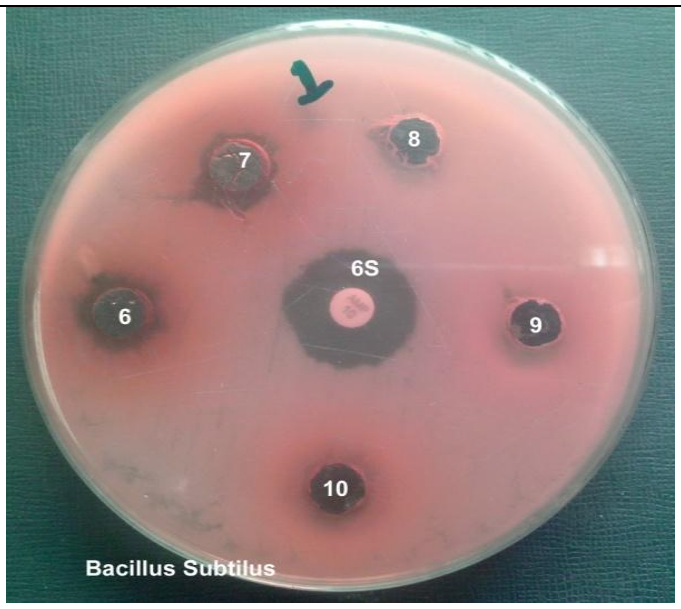
The antibacterial activity of the ethanol extract was studied against gram positive and gram negative bacteria. The antibacterial activity studied is expressed in terms of zone of inhibition measured in mm. A significant growth inhibition was shown by many of the organisms tested indicating the profound potency of the plant drug. Among the tested organism *Bacillus subtilus* was found to be the most sensitive organism followed by *Escherichia coli*, *Pseudomonas aeruginosa and Klebsiella pneumonia*, with maximum zone of diameter ranging from 32mm to 9mm. The organisms *Staphylococcus*

aureus, *Enterobacter aerogens*, *Proteus spp.*, and *Salmonella typhimurium* did not exhibit any activity. The result observed with the minimal inhibitory concentration is depicted in Table : 6.

Table :6. Antibacterial activity of the whole plant of *Sphaeranthus amaranthoides* Linn.

S. No	Organism	Zone diameter in mm										
		200 µg/µl	100 µg/µl	50 µg/µl	25 µg/µl	12.5 µg/µl	6.2 µg/µl	3.1 µg/µl	1.5 µg/µl	0.7 µg/µl	0.3 µg/µl	Standard Amp
1	***Proteus vulgaris***	-	-	-	-	-	-	-	-	-	-	+
2	***Enterobacter aerogens***	-	-	-	-	-	-	-	-	-	-	-
3	***Escherichia coli***	**29**	**28**	**27**	**20**	**19**	**18**	**15**	**13**	-	-	+
4	***Pseudomonas aeruginosa***	**13**	**12**	**11**	**10**	-	-	-	-	-	-	+
5	***Klebsiella pneumonia***	**15**	**13**	**12**	**11**	**9**	-	-	-	-	-	-
6	***Salmonella typhimurium***	-	-	-	-	-	-	-	-	-	-	-
7	***Staphylococcus aureus***	-	-	-	-	-	-	-	-	-	-	+
8	***Bacillus subtilis***	**32**	**30**	**28**	**25**	**18**	**15**	**13**	-	-	-	+





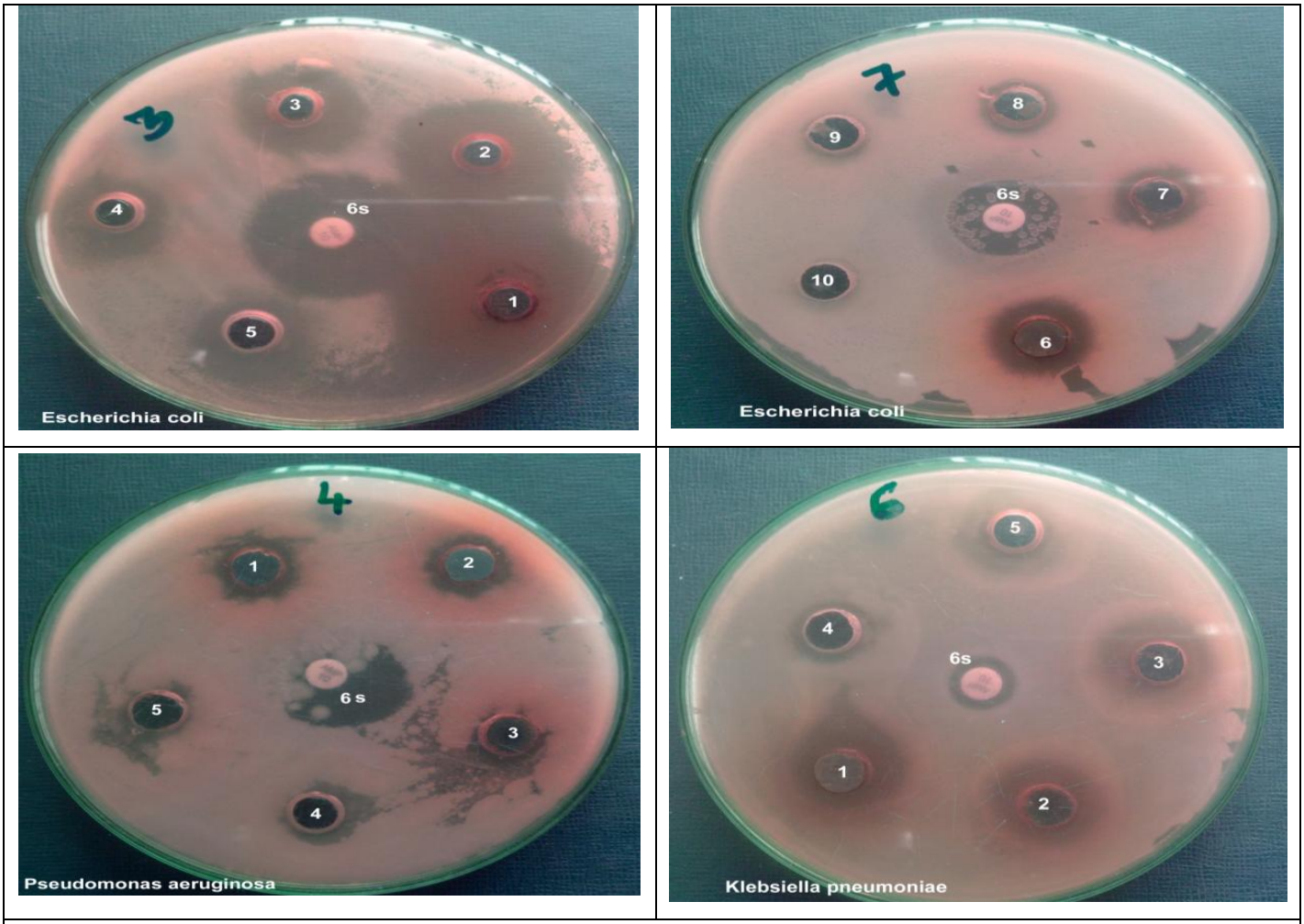


Fig:3. Antibacterial activity of the ethanol extract of the whole plant of the *S. amaranthoides L.*

CONCLUSION

Physicochemical standards such as loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractives were evaluated in the ethanolic extract of whole plant of *Sphaeranthus amaranthoides* Linn.

Preliminary phytochemical analysis of the ethanol extract showed the presence of alkaloid, amino acid, flavonoid, glycoside/sugar, phenol, steroid, tannin, triterpenoid and saponin.

TLC Photodocumentation and HPTLC finger printing was also developed for the ethanolic extract of whole plant of *Sphaeranthus amaranthoides* Linn. The HPTLC finger print profile at UV 254 nm showed 9 peaks of which peak at R_f 0.55 was major and others were moderately smaller peaks. Heavy metals such as lead, mercury, cadmium were not detected and arsenic was found to be below the permissible level.

The ethanol extract of whole plant of *Sphaeranthus amaranthoides* Linn. has a great potential as antibacterial agent against tested pathogenic bacteria viz. *Bacillus subtilus ,Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia.*

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