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Survey of medicinal weeds in paddy field ecosystem with special emphasis on *Ammannia baccifera* Linn.–A potent weed

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Abstract

Background: Since the dawn of civilization, humans have utilized herbs for their medicinal value which are used as a traditional medicine that has stood up to the test of time and for the production of many novel prototypes.

Objectives: The study aims to the exploration of medicinal weeds in the agroecosystem of Savandapur village in Gobichettipalayam, Erode District, Tamil Nadu and to investigate the phytochemical compounds and antimicrobial activity of the selected study plant.

Materials and Methods: The documentation of medicinal weeds was carried out with the help of traditional local healers in the paddy fields of Savandapur village. Pharmacognostical studies of selected study plant were followed by standard procedures and antibacterial assay has been done by well diffusion method.

Results: A total of 22 species of common weeds distributed across 21 genera and 13 families were identified. Among these, due to its high therapeutic value, *Ammannia baccifera* L. is evaluated for its potentiality. The aerial plant part contains the phytoconstituents such as carbohydrate, alkaloid, flavonoid, phenol, tannin, glycoside, terpenoid, saponin, coumarin and fixed oils. T.S of stem shows the calcium crystals and oil globules. The petroleum ether and methanolic extracts were active against the tested microorganisms *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* (gram-negative) and also aqueous extract has no antibacterial activity. The methanolic extract has a high degree of potentiality which is more relevant to the antibiotic Kanamycin.

Conclusion: The investigation reveals the importance of weeds in the paddy field ecosystem. Further, the evaluation of the antibacterial properties of these extracts and isolation of the compounds responsible for the antibacterial activity is required.

Keywords: agroecosystem, medicinal weeds, pharmacognostical studies, antibacterial

Introduction

Nature consistently remains as a brilliant imprint to represent the extraordinary and marvel interactions with living and non-living things. The biotic and abiotic components of nature are the most part reliant on one another. The plants are fundamental to man in his life and it has provided an entire storehouse of remedies to heal all ailments. The historical backdrop of herbal medicines is as old as human civilization. The previous reports revealed that the plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. India is endowed with wealthy biodiversity where medicinal plant diversity is also notably spreading over the country. It is a mega bio-diversified country among 12 others on this planet. It has a forest area of 23.81 % of the country's topographical area [1]. Numerous plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as boundless folk medicines, while others gained a reputation in the form of finished products collectively called phytomedicines [2]. Together, the medicinal plants represent a rich source of antimicrobial agents [3]. Agriculture is the major occupation of many parts of the world. Paddy is the most significant crop of Tamil Nadu and people need an intake of rice for their diet. Rice fields are the wetlands to grow various medicinal herbs. Weeds are a significant requirement for crop creation, yet they might be viewed as a basic part of the agro environment and a large number of them are useful as well [4]. With the emphasis on the above background, the present study has been initiated to deal with the survey of medicinal weeds in the paddy field ecosystem and screening their antibacterial activity. In connection with this, the common weeds are surveyed and information about the usage was collected from the local traditional healers of the village of Savandapur in Gobichettipalayam, Erode District. From the detailed survey of medicinal weeds in paddy fields, *Ammannia baccifera* of the Lythraceae family were selected for further study. The studied plant possesses traditional values and some of the scientific investigations of its biological activities are found in previous literature. Yet the study plant should be studied in detail for their antibacterial screening property and impart their importance to society.

Materials and Methods

Collection, identification and documentation of Medicinal Weeds

The present study was conducted in Savandapur Village of Gobichettipalayam, Erode District, Tamil Nadu. The region has vast agricultural fields with rich vegetation around the area (Map 1 and Plate 1). The survey was conducted during November and December Months - 2020. During the

survey, some of the medicinally potent and common weeds in the rice field were collected. The medicinally important weeds in the study area which are widely used by local people to treat various ailments were documented. Some of the major weeds present in the paddy field were photographed. The oral discussion was concentrated to get the knowledge about the vernacular names, flowering, Fruiting, medicinal and economic usage of weed plants in the study area. The collected medicinal plants were identified with the help of the existing Floras [5, 6].

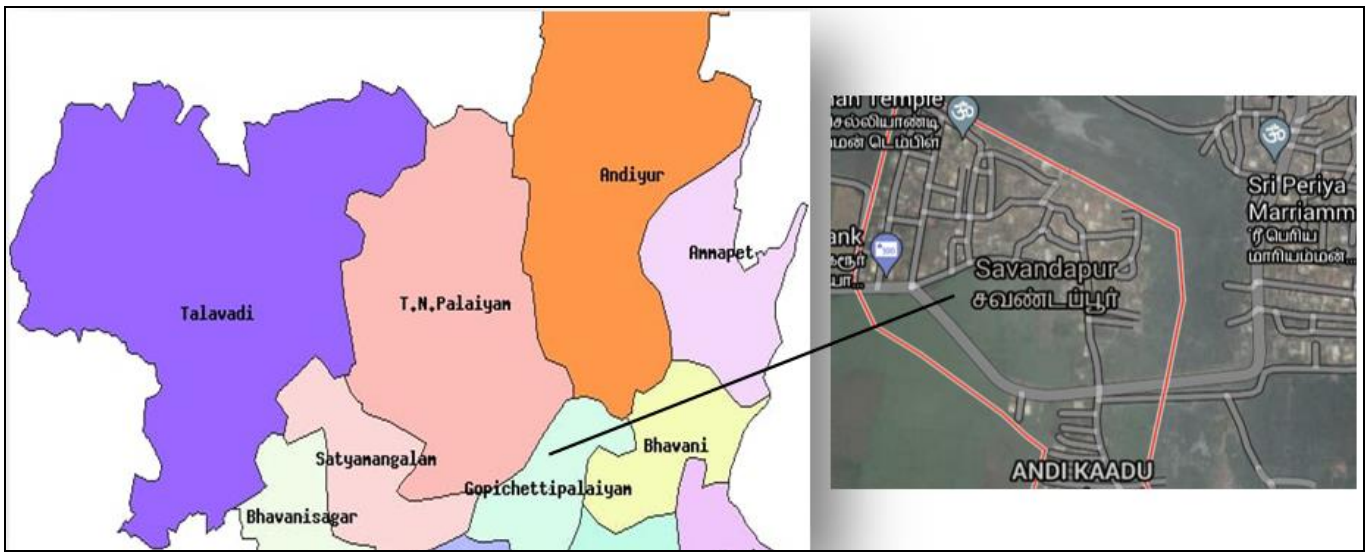


Fig 1: Showing the Location of the Study Area

Plate 1: Paddy fields in Savandapur-Study area

Collection, Preparation of Herbarium and Authentication of Study plant

Fresh aerial plant parts of *Ammannia baccifera* Linn. was collected with the help of traditional local healers inhabited in the study area. The plant parts were collected in the flowering and fruiting season from the natural habitat during December. The collected plant specimen was pressed properly following the method of [7]. A dried specimen was poisoned with 0.1 % $HgCl_2$ dissolved in absolute alcohol and mounted with glue on a standard herbarium sheet (42 x 28 cm). The herbarium was deposited in the Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode. Photographs were also taken to supplement the herbarium. The identity of the type specimen was authenticated by Scientist E and Head, Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu, under the Voucher specimen number - BSI/SRC/5/23/2021/Tech-163.

Pharmacognostical Studies

External Macroscopic Studies

The macroscopic observation of *Ammannia baccifera* was examined as per the methodology of [8, 9].

Microscopical Evaluation

The stem of *Ammannia baccifera* was smoothly cut, safely removed from the plant and finally fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70 % Ethyl alcohol - 90 ml). Thin, free-hand transverse sections were made with the help of a sharp razor blade and cleared with chloral hydrate solution. For each sample section, one drop of water was placed on a clean slide. The specimen section was mounted on it and then stained with a drop of Toluidine blue [10] and Fast green solution. The suitable thin sections were stained, mounted in glycerin and covered with the help of a coverslip [11, 12, 13]. The images were carefully observed under an OLYMPUS (Model AX 70 TRF) light microscope

at (40x and 100x) magnifications. The photomicrographs were taken with the use of a digital camera (Nikon lab photo 2 microscopic unit).

Physico-chemical Studies

Organoleptic characters of plant powder and the extract

The organoleptic evaluation of aerial plant powder and the extracts was followed as per the methodology of [8].

Qualitative Phytochemical analysis

Soxhlet Extraction and their extractive yield Percentage

The air-dried and powdered aerial plant powder of *Ammannia baccifera* was filled in the thimble and extracted successively with petroleum ether (60-80°C) and methanol (64.7 °C) (20 g / 200 ml) using a Soxhlet extractor for 6 - 8 hours. Finally, the material was macerated using hot water (99.98°C) with occasional stirring for 5 hours and the water extract was filtered. The percentage of yields was calculated as well as colour and consistency of the extracts were also observed. The prepared extracts were subjected to further phytochemical analysis and antibacterial studies.

Phytochemical screening Procedure

Phytochemical screening of different successive solvent extracts was followed as per the methods adopted by [14, 15, 16].

Antibacterial Studies

Bacterial Strains used

In-vitro well diffusion method antibacterial activity was examined for the aerial plant part extracts (petroleum ether and methanol) of the species, *Ammannia baccifera* against two bacterial strains which include the Gram-positive strain *Staphylococcus aureus* (MTCC 3160*)* and Gram-negative strain *Pseudomonas aeruginosa* (MTCC 424) which was obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Agar well Diffusion Method

The 28.0 g of Nutrient agar media was weighed and mixed in 1000 ml of distilled water. Liquid nutrient agar media and the Petri plates were sterilized by autoclaving at 121◦ C for about 30 minutes at 15 lbs pressure. Then the media was cooled to 45 - 50◦C, mixed well and poured into sterile petriplates for further use (HIMEDIA – M001-500G). Under aseptic conditions in the laminar air flow chamber, about 20 ml of the agar medium was dispensed into each petriplate to yield a uniform depth of 4 mm. After solidification of the media, 24 hrs cultures of microorganisms were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with the loading of 50 µl and 100 µl concentration of plant extract sample to the distinct well with 50 µl of sterile distilled water as negative control and Kanamycin (30 mcg/disc) as a positive control. The sample plates were then incubated at 37◦C for 24 hours to observe the zone of inhibition [17, 18].

Results and Discussion

Survey of Medicinal Weeds

Study Area

Savandapur is a Panchayat of Gobichettipalayam Taluk in Erode District of Tamil Nadu, India. The total geographical area of the village is 736.17 hectares. It has a population of about 5500. The male and female populations are 2800 and 2700. Savandapur lies between 11°52’ N and 77 °51” E and the altitude is 193 m. The temperature and climate are moderate.

Enumeration of Medicinal Weeds

In the present investigation, about 22 species of common weeds distributed across 21 genera and 13 families, in the study area which is widely used by people to treat various ailments were documented (Table 1).

Table 1: Surveyed List of Medicinally important Weeds and their uses in the Paddy Field Ecosystem – Savandapur, Gobichettipalayam

S.No.	Botanical Name	Family	Vernacular Name	Ailments Treated
1.	*Acalypha indica* L.	Euphorbiaceae	Kuppaimeni	Asthma and Skin infections
2.	*Achyranthes aspera* L.	Amaranthaceae	Naayuruvi	Kidney stone and Haemorrhoids
3.	*Aerva lanata* Juss.	Amaranthaceae	Sirukanpoolai	Cough, Headache and Asthma
4.	*Amaranthus viridis* L.	Amaranthaceae	Kuppaikeerai	Dysentery, Urinary disorder and Diabetes
5.	*Ammannia baccifera* L.	Lythraceae	Kalluruvi	Skin disease, Typhoid, Ulcer, Stomach disorder and Snake bite
6.	*Cardiospermum halicacabum* L.	Sapindaceae	Modakathan	Inflammation, Ear ache and Urinary infection
7.	*Centella asiatica* Urb.	Apiaceae	Vallarai	Ulcer and Wound healing
8.	*Chloris barbata* Sw.	Poaceae	Korai pillu	Diabetes and Skin diseases
9.	*Cleome viscosa* L.	Capparidaceae	Vaelaikeerai	Fever and Wound healing
10.	*Commelina benghalensis* L.	Commelinaceae	Kaanakuzhai	Stomach disorders and Burns
11.	*Cynodon dactylon* Pers.	Poaceae	Arugampul	Skin disease and Piles
12.	*Euphorbia hirta* L.	Euphorbiaceae	Amman pacharsi	Lung infections and Digestive
13.	*Leucas aspera* Spr.	Lamiaceae	Thumbai	Wounds and Fever
14.	*Mimosa pudica* L.	Fabaceae	Thottalsurungi	Haemorrhoids and Jaundice
15.	*Phyllanthus niruri* L.	Euphorbiaceae	Keelanelli	Jaundice and Stomach pain
16.	*Physalis minima* L.	Solanaceae	Sodakkuthakkali	Diabetes and Inflammation
17.	*Ricinus communis* L.	Euphorbiaceae	Amanaku	Arthritis and Constipation
18.	*Solanum nigrum* L.	Solanaceae	Manathakkali	Ulcer and Skin infection
19.	*Solanum trilobatum* L.	Solanaceae	Thoodhuvalai	Cold, Cough and Asthma
20.	*Tephrosia purpurea* Pers.	Fabaceae	Kolunji	Ulcer and Asthma
21.	*Trichodesma indicum* R. Br.	Boraginaceae	Kallutaitumapi	Inflammation and Fever
22.	*Tridax procumbens* L.	Asteraceae	Vettukayapoondu	Wound healing and Skin disease

Some of the major weeds present in the paddy field were photographed (Plate 2). Out of 22 plants, 19 were Dicotyledons and 3 were Monocotyledons. Among Dicotyledons, 6 were of Polypetalae, 6 were of Gamopetalae and 7 were under Monochlamydeae.

Among the collected weeds in the survey area, some of the plants have high medicinal value such as, *Euphorbia hirta* is used to cure lung infection and digestive disorders, *Acalypha indica* is used to cure asthma and skin infections, *Phyllanthus niruri* is used to cure jaundice and stomach pain, *Solanum trilobatum* is used to cure a cold, cough and asthma. Among these, the potent weed *Ammannia baccifera* was used predominantly by the local community to treat various ailments and also has various traditional therapeutic uses such as; it is used to cure typhoid, renal calculi, skin infections, ulcer, stomach disorders and snake bite. Due to the high therapeutic value, *Ammannia baccifera* L. – A potent weed is subjected to pharmacognostical and pharmacological studies.

Pharmacognostical studies of Selected Plant

Macroscopical Studies

The aerial plant parts of *Ammannia baccifera* are green in colour and when matured the stem and leaves turn into reddish or purplish colour. Leaves are simple, dorsiventral, opposite decussate, narrow, lanceolate, entire, margin, acute tip, often cordate at base, 1-3 cm long and 0.5 cm wide, exstipulate, glabrous, midrib red on lower surface and green on the upper surface. The inflorescence is axillary clusters, sessile or pedunculate dichasia. Flowers are bisexual, small, red, or purple, 4 merous and 1.2 mm long. The calyx is campanulate, epicalyx, obscure, petals alternate when present alternate the calyx lobe and sometimes 0. Stamens 4 or 8, connate at the base and filaments 0.5 mm long. Style erect, stigma capitate, ovary ovate and sessile. It has been observed that the petals were absent, flowers are bisexual, axillary in clusters, fruit capsule and globose in structure and seeds are black [19].

Microscopical Studies

T.S. of Stem Anatomy

T.S. of *Ammannia baccifera* stem shows quadrangular with four protuberances with well-differentiated epidermis, cortex, vascular bundle and pith (Plate 3). Epidermis is made up of rectangular shaped parenchyma cells and is compactly arranged, followed by 2-3 layers of hypodermis which are made up of parenchymatous cells without any intercellular spaces. Cortex is made up of loosely arranged parenchyma cells with numerous crystals of calcium oxalate and oil globules are present. The endodermis is single-layered and made up of somewhat barrel-shaped parenchyma cells. Vascular bundles are radially arranged, protoxylem is facing towards the pith and metaxylem is towards the periphery, medullary rays are biseriate to multiseriate and phloem is located above the xylem. The central pith is composed of parenchyma cells. The quadrangular protuberances, the presence of oil globules and calcium oxalate crystals are seen in the cortex region which is following the results of [20].

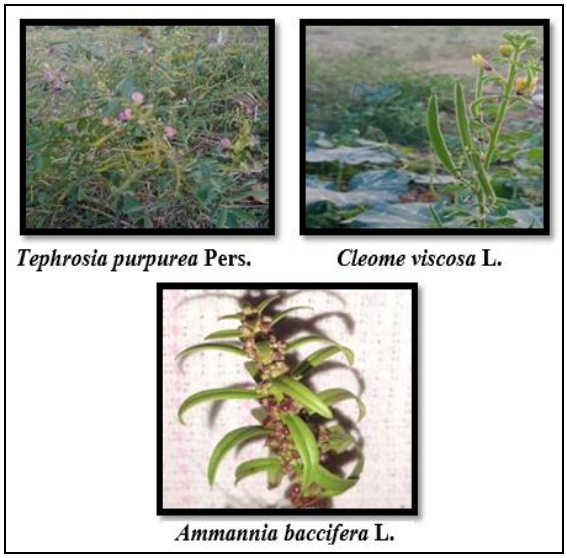
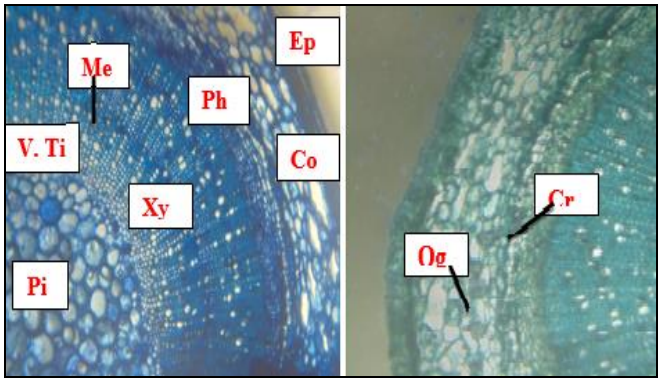


Plate 2: Major surveyed Medicinal Weeds in Paddy Field Ecosystem-Savandapur



Ep: Epidermis; Co: Cortex; Ph: Phloem; Xy: Xylem; Pi: Pith; V. Ti: Vascular tissues; Og: Oil globules; Cr: Calcium oxalate crystals; Me: Medullary rays.

Plate 3: T.S of Stem - A Portion Enlarged

Organoleptic Characters of Plant Powder

The plant powder showed a characteristic odour and pungent taste. Upon drying and powdering the colour of the powder changed from dark green to brown. The organoleptic characters such as colour, consistency and odour were noted in the petroleum ether, methanol and aqueous successive solvent extracts of *Ammannia baccifera*.

Percentage yield and Qualitative Phytochemical Screening

The percentage yield was maximum in methanol extract (8.6 %) followed by petroleum ether extract (7.3 %) and the aqueous extract showed a minimum yield percentage (6.0 %).

The results of the preliminary phytochemical screening of *A. baccifera* powder with different solvents showed the presence of various phytochemicals (Table 2).

Table 2: Qualitative Phytochemical screening of different extracts of *A. baccifera* aerial plant powder

S.No.	Constituents	Petroleum ether	Methanol	Water
1.	Test for Protein			
	Biuret Test	-	-	-
2.	Test for Carbohydrate			
	Barfoed's Test	-	+	+

3.	Test for Aminoacid			
	Ninhydrin Test	-	-	-
4.	Test for Alkaloids			
	Wagner's Test	-	+	-
5.	Test for Flavonoid			
	Ammonium Hydroxide Test	+	+	-
6.	Test for Phenol			
	Lead Acetate Test	+	+	-
7.	Test for Tannin			
	Ferric Chloride Test	+	+	+
8.	Test for Glycosides			
	Borntrager's Test	+	+	+
9.	Test for Terpenoids	+	+	-
10.	Test for Saponin			
	Foam Test	-	-	+
11.	Test for Coumarin	-	+	-
12.	Test for Quinine	-	-	-
13.	Test for Anthroquinone	-	-	-
14.	Test for Fixed oil	+	-	-

Note: '+', '-' symbol indicates the presence/absence of phytoconstituents

The petroleum ether extract revealed the presence of flavonoid, phenol, tannin, glycoside, terpenoid and fixed oils. The methanol extract revealed the presence of carbohydrate, alkaloid, flavonoid, phenol, tannin, glycoside, terpenoid and coumarin. This finding is somewhat correlated with the work of [21], who reported the presence of alkaloids, flavonoids, steroids, tannin and saponin in the methanolic extract. The aqueous extract revealed the presence of carbohydrate, tannin, glycoside and saponin which is similar to the work of [22].

Antibacterial assay

The antibacterial activity of petroleum ether, methanolic and aqueous extracts of *A. baccifera* was investigated by using the agar well diffusion method and results were tabulated in Table 3 and Fig. 1.

Table 3: Antibacterial activity by petroleum ether, methanol and aqueous extracts of *Ammannia baccifera* plant against two bacterial pathogens

Microorganisms	Extracts	Concentration (µg/ml)	Zone of inhibition (mm)
Staphylococcus aureus (Gram positive)	Petroleum ether Extract	50 µL	15
		100 µL	20
	Kanamycin	30 (mcg/disc)	22
	Control	50 µL	-
	Methanol Extract	50 µL	18
		100 µL	25
	Kanamycin	30 (mcg/disc)	23
	Control	50 µL	-
	Aqueous Extract	50 µL	-
		100 µL	-
	Kanamycin	30 (mcg/disc)	22
	Control	50 µL	-
Pseudomonas aeruginosa (Gram negative)	Petroleum ether Extract	50 µL	17
		100 µL	20
	Kanamycin	30 (mcg/disc)	22
	Control	50 µL	-
	Methanol Extract	50 µL	20
		100 µL	25
	Kanamycin	30 (mcg/disc)	23
	Control	50 µL	-
	Aqueous Extract	50 µL	-
		100 µL	-
	Kanamycin	30 (mcg/disc)	22
	Control	50 µL	-

Results of the present investigation indicated that two dilutions (50 µL and 100 µL) of petroleum ether and methanolic extracts produced inhibitory effects against the tested microorganisms and aqueous extract has no activity against microorganisms, *Staphylococcus aureus* (gram-positive) and *Pseudomonas aeruginosa* (gram-negative) (Plate 4).

The maximum zone of inhibition was observed by methanolic extract (100 µl) against the gram-positive bacterium *Staphylococcus aureus* (25 mm) and gram-negative bacterium *Pseudomonas aeruginosa* (25 mm), while the petroleum ether extract (100 µl) showed a moderate zone of inhibition (20 mm) for the above bacterial strains tested. Similarly, the antibacterial potential of *Ammannia baccifera* was screened using disc diffusion and well diffusion method against five bacterial strains. The results indicated that the methanolic extract was active against all the five strains used *viz*, *Bacillus cereus* (15 mm), *Staphylococcus aureus* (13 mm), *Klebsiella pneumoniae* (11 mm), *Escherichia coli* (15 mm) and *Pseudomonas pseudoalcaligenes* (25 mm). At the same time, the aqueous extracts were inactive [23].

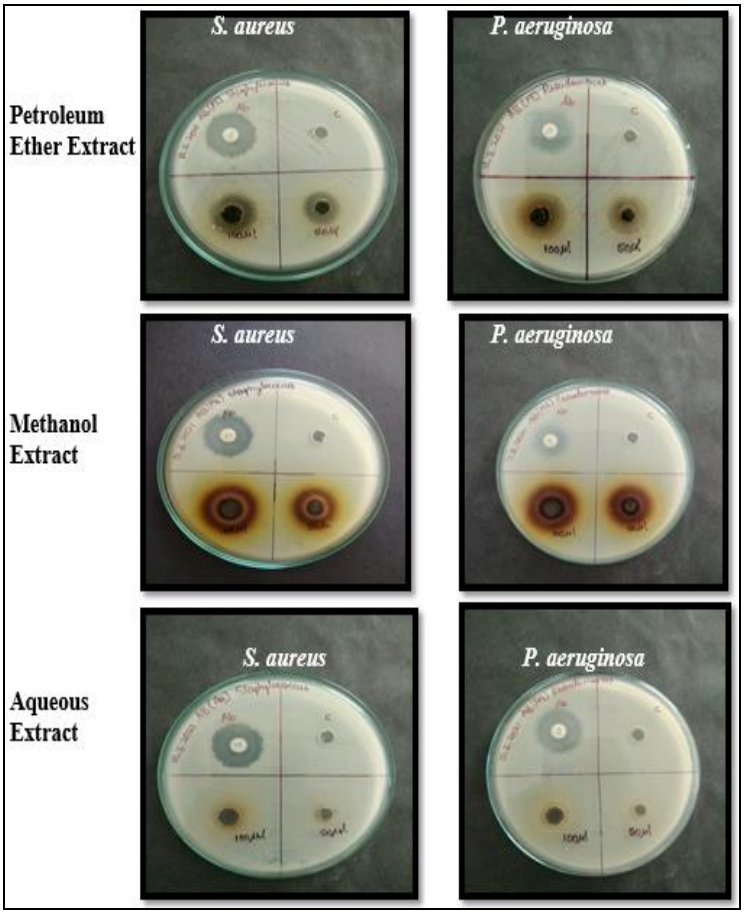
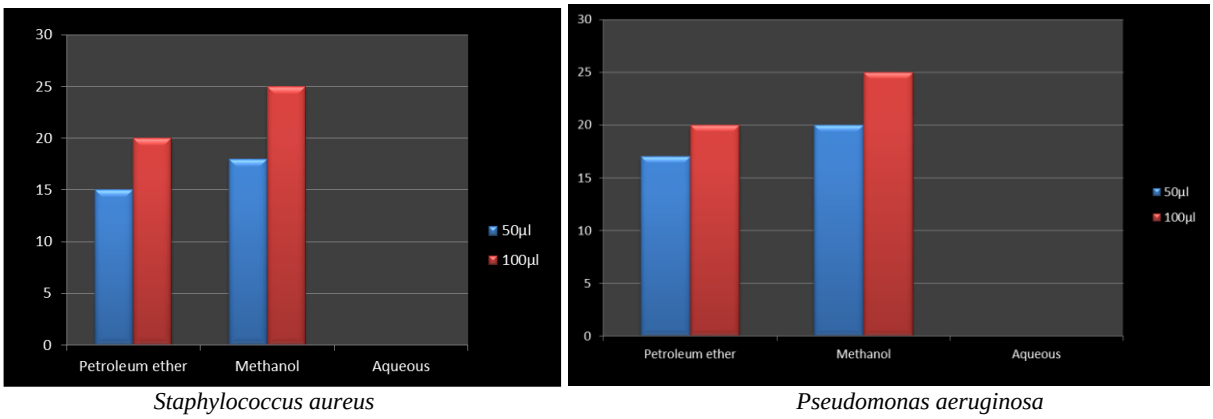


Plate 4: Antibacterial activity of *Ammannia baccifera* by Agar well Diffusion Method against two Bacterial Pathogens



Staphylococcus aureus *Pseudomonas aeruginosa*

Fig 1: Antibacterial activity of *Ammannia baccifera* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Conclusion

In the present study, the information about the medicinally important weeds is well documented with the help of traditional healers inhabited in the study area and predominantly used potent weed *Ammannia baccifera* has been subjected to further studies. The aerial study plant part contains the phytoconstituents such as carbohydrate, alkaloid, flavonoid, phenol, tannin, glycoside, terpenoid, saponin, coumarin and fixed oils. The petroleum ether and methanolic extracts were active against the tested microorganisms and the aqueous extract has no activity. The maximum zone of inhibition was observed by methanolic extract (100 µl) against the gram-positive bacterium *Staphylococcus aureus* (25 mm) and gram-negative

bacterium *Pseudomonas aeruginosa* (25 mm). It is understood that the methanolic whole aerial plant part extract of *A. baccifera* showed a broad spectrum of antibacterial activity in comparison to other extracts. This information might be useful to the future study exploration of the *A. baccifera* plant. Also, this study aims to enlighten the importance of medicinal weeds in agricultural lands. The proposal for future study is that better understanding and finding more pharmacological activities of the *Ammannia baccifera* plant for the betterment of society.

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