



Research Article

ETHNO BOTANICAL SURVEY OF PLANTS USED BY FOLKLORE PRACTITIONERS OF HAVERI DISTRICT, KARNATAKA, INDIA IN TREATING SEPTIC CONDITIONS WITH SPECIAL REFERENCE TO ACACIA FARNESIANA LINN AND PERGULARIA DAEMIA LINN AND THEIR ANTI BACTERIAL ACTIVITY
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DOI: 10.7897/2230-8407.06668**ABSTRACT**

Ayurveda is time tested, treasured and archaic science. 70% of India's medicinal plants are found in the tropical zone. Mixed vegetation of both wet and dry landscape of Haveri district sources a variety of medicinal plants having various pharmacological actions. Haveri district is also famous for folklore medicines in various ailments. Survey was conducted through a structured questionnaire in 64 villages of selected four taluks of Haveri district having 44 traditional folklore practitioners. Preliminary physico chemical, phytochemical and primary metabolites of aqueous and alcoholic extracts of leaves of both *Acacia farnesiana* Linn and *Pergularia daemia* Linn plants were analyzed. HPTLC of *Acacia farnesiana* Linn and *Pergularia daemia* Linn was conducted. Quantification of phenol group was done using gallic acid. Antibacterial activity of *Acacia farnesiana* Linn and *Pergularia daemia* Linn was conducted on selected bacterial stains.

Keywords: Varada river, ailment**INTRODUCTION**

India is a vast country with a huge tropical variation. The characteristics of the plant vary according to the region and climate of an area. 70% of India's medicinal plants are found in the tropical zone. The proportion of medicinal plants recorded in the dry and moist deciduous tropical forests is higher as compare to those recorded in the tropical evergreen forests. ¹Haveri district is exactly in the centre of Karnataka and it is bounded by Dharwad district on the north, by Gadag on north east, by Bellary on East, by Davangere on south, by Shimoga on southwest and by Uttar Kannada on west and northwest. Currently the botanical survey of India has revealed that Haveri is an under explored area having rich black soil, tropical climate and an average of 691.1 mm of annual rainfall. The district is spread across an area of 4848 Sq.Kms. which is 2.53% of the area of Karnataka state. Varada, Kumadhvati, Dharma and Tungabhadra are the main rivers of Haveri district. Among them the Varada river covers the major part of the district including Haveri, Hangal, Shiggaon and Savanur taluks. ² The bank of varada river is rich in medicinal plants which is yet to be explored and documented. Haveri district is famous for folklore medicines used for jaundice, fractures etc.,. The modern world is realizing the importance of folklore practice and these practitioners do have valuable information about the medicinal plants. Nearly 28% of the plants are estimated to be used in ethno medicine. Hence being need of the hour this survey was carried out during 2010-2011 to explore and document the known and unknown medicinal treasure of traditional knowledge of Haveri district with special reference to bank of Varada river.

MATERIALS AND METHODS

- Study area is located in the centre of Karnataka situated between 14.28 & 14.59 latitudes and 75.07 & 75.38 longitudes.

- Grid method was followed for the selection of villages over the bank of Varada river.
- Format and data sheets for the documentation of folklore practices were prepared along with the consent letter.
- During ethno-botanical exploration of selected villages over the bank of river traditional practitioners were documented for the identification of the plants, local name, parts used, method of preparation of the medicine, mode of administration, duration, and their parameters for the diagnosis of different diseases.
- Audiovisual medias were used during the documentation for the authenticity and rapid assessment method was conducted.
- Assessment of fidelity level, relative popularity level and informant consensus factor among the plants surveyed during folklore documentation was conducted.
- Botanical authentication of 10 samples of *Acacia farnesiana* Linn and 5 samples of *Pergularia daemia* Linn collected from different folklore practitioners was conducted by FRLHT Bangalore, India.
- Preliminary physico chemical, phytochemical and primary metabolites of aqueous and alcoholic extracts of leaves of both plants were analyzed. HPTLC of *Acacia farnesiana* Linn and *Pergularia daemia* Linn was conducted.
- Quantification of phenol group was done using gallic acid.
- An *In-vitro* study to assess the antibacterial susceptibility of *Acacia farnesiana* and *Pergularia daemia* were carried out against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This was done under two phases.
 - * Minimum inhibitory concentration
 - * Zone of inhibition

The present study was carried out to determine minimum inhibitory concentration by pour plate method and to determine zone of inhibition by agar well diffusion method. ^{3,4,7}

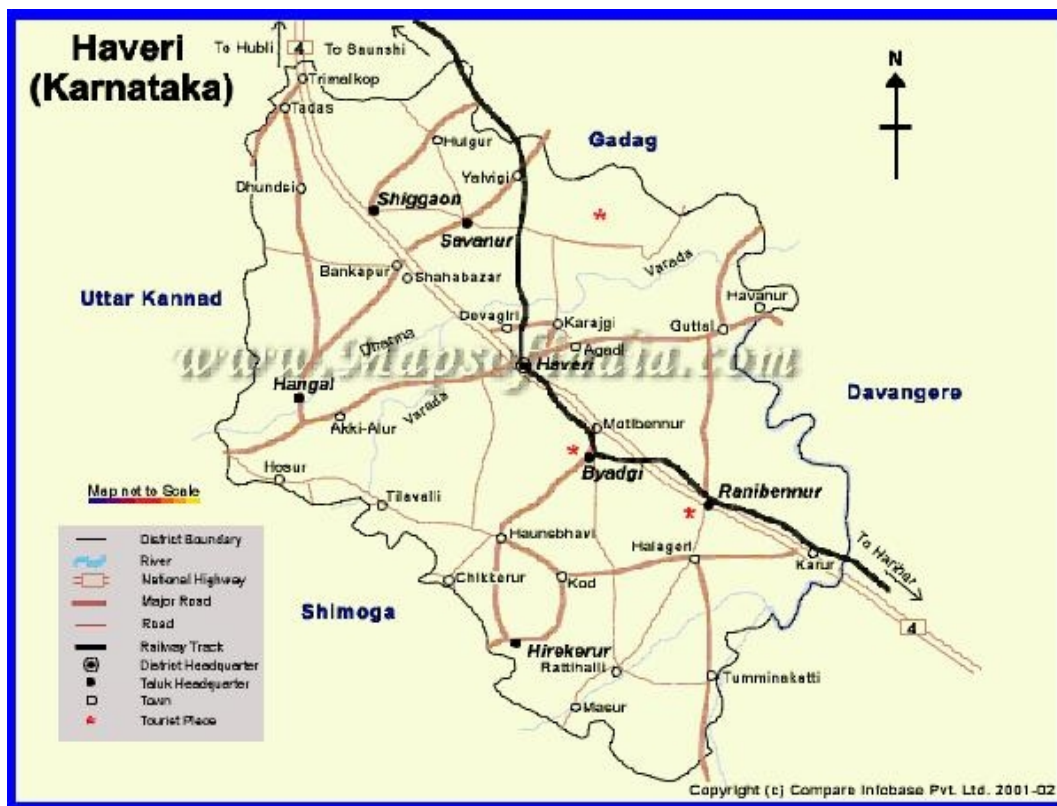


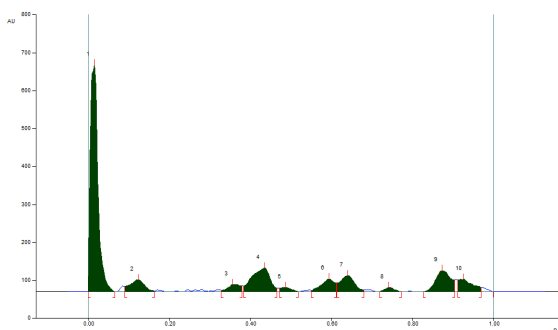
Figure 1: Map showing the study area⁸



Figure 2: *Acacia farnesiana* Linn

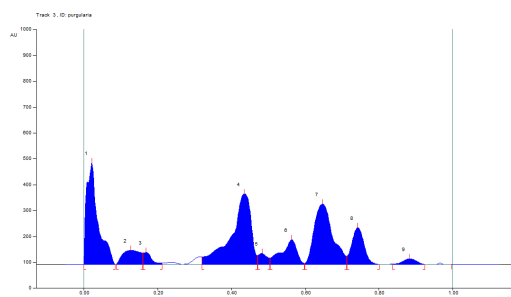


Figure 3: *Pergularia daemia* Linn



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	598.0 AU	66.68 %	0.07 Rf	0.2 AU	8173.5 AU	51.23 %
2	0.09 Rf	13.6 AU	0.12 Rf	31.7 AU	3.54 %	0.16 Rf	1.6 AU	817.4 AU	5.12 %
3	0.33 Rf	3.7 AU	0.36 Rf	18.9 AU	2.11 %	0.38 Rf	15.1 AU	430.2 AU	2.70 %
4	0.38 Rf	14.7 AU	0.44 Rf	62.6 AU	6.98 %	0.47 Rf	9.5 AU	1985.3 AU	12.44 %
5	0.47 Rf	9.4 AU	0.49 Rf	10.9 AU	1.22 %	0.52 Rf	0.0 AU	212.9 AU	1.33 %
6	0.55 Rf	3.2 AU	0.60 Rf	33.6 AU	3.75 %	0.61 Rf	22.6 AU	776.4 AU	4.87 %
7	0.61 Rf	22.7 AU	0.64 Rf	42.6 AU	4.74 %	0.68 Rf	4.9 AU	1065.4 AU	6.68 %
8	0.72 Rf	0.0 AU	0.74 Rf	10.7 AU	1.19 %	0.77 Rf	0.0 AU	177.7 AU	1.11 %
9	0.83 Rf	0.2 AU	0.87 Rf	55.3 AU	6.16 %	0.90 Rf	30.4 AU	1470.7 AU	9.22 %
10	0.91 Rf	30.6 AU	0.93 Rf	32.6 AU	3.63 %	0.97 Rf	11.0 AU	845.0 AU	5.30 %

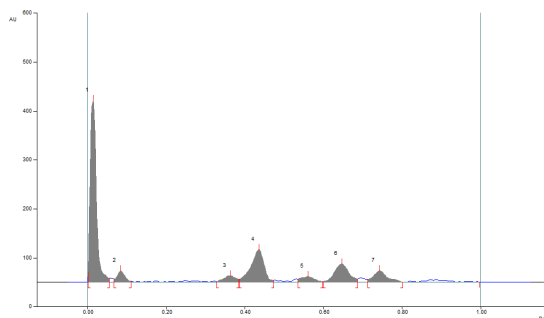
Figure 4: HPTLC photo documentation at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	393.0 AU	30.16 %	0.09 Rf	0.3 AU	8753.3 AU	23.09 %
2	0.09 Rf	1.0 AU	0.13 Rf	54.5 AU	4.18 %	0.16 Rf	44.8 AU	1917.7 AU	5.06 %
3	0.16 Rf	44.9 AU	0.17 Rf	45.9 AU	3.52 %	0.21 Rf	5.0 AU	758.9 AU	2.00 %
4	0.32 Rf	29.5 AU	0.44 Rf	272.9 AU	20.94 %	0.47 Rf	34.4 AU	10548.7 AU	27.82 %
5	0.47 Rf	34.9 AU	0.48 Rf	43.0 AU	3.30 %	0.50 Rf	25.0 AU	741.8 AU	1.96 %
6	0.51 Rf	25.3 AU	0.56 Rf	95.7 AU	7.34 %	0.60 Rf	4.6 AU	2846.9 AU	7.51 %
7	0.60 Rf	4.8 AU	0.65 Rf	233.9 AU	17.95 %	0.71 Rf	31.2 AU	8379.1 AU	22.10 %
8	0.71 Rf	31.5 AU	0.74 Rf	142.6 AU	10.95 %	0.80 Rf	0.2 AU	3393.9 AU	8.95 %
9	0.84 Rf	1.3 AU	0.88 Rf	21.7 AU	1.66 %	0.93 Rf	0.1 AU	576.2 AU	1.52 %

Pergularia daemia at 6 µl

Figure 5: HPTLC photo documentation at 366nm

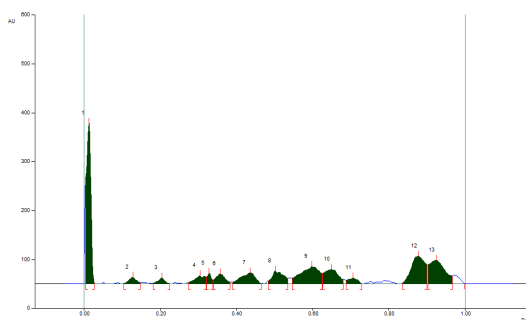


Track 3, ID: pergularia

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	20.6 AU	0.01 Rf	370.7 AU	68.00 %	0.06 Rf	7.7 AU	4274.6 AU	51.33 %
2	0.07 Rf	6.4 AU	0.08 Rf	23.2 AU	4.25 %	0.11 Rf	1.8 AU	328.1 AU	3.94 %
3	0.33 Rf	1.2 AU	0.36 Rf	13.2 AU	2.42 %	0.39 Rf	6.2 AU	292.4 AU	3.51 %
4	0.39 Rf	6.3 AU	0.44 Rf	66.5 AU	12.19 %	0.47 Rf	3.8 AU	1584.0 AU	19.02 %
5	0.54 Rf	6.4 AU	0.56 Rf	11.3 AU	2.08 %	0.60 Rf	1.0 AU	285.4 AU	3.43 %
6	0.60 Rf	1.1 AU	0.65 Rf	37.1 AU	6.80 %	0.69 Rf	6.4 AU	973.7 AU	11.69 %
7	0.71 Rf	5.9 AU	0.74 Rf	23.1 AU	4.24 %	0.80 Rf	0.0 AU	589.1 AU	7.07 %

Pergularia daemia at 6 µl

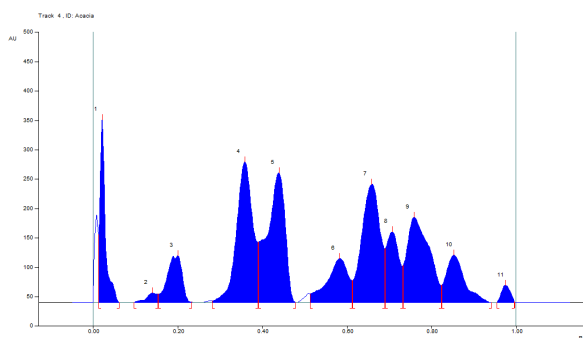
Figure 6: HPTLC photo documentation at 540nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	214.6 AU	0.01 Rf	328.5 AU	51.37 %	0.03 Rf	0.9 AU	2809.1 AU	27.47 %
2	0.10 Rf	0.5 AU	0.13 Rf	13.4 AU	2.09 %	0.15 Rf	2.2 AU	185.6 AU	1.82 %
3	0.18 Rf	1.1 AU	0.20 Rf	12.1 AU	1.89 %	0.23 Rf	0.3 AU	144.6 AU	1.41 %
4	0.27 Rf	2.4 AU	0.30 Rf	16.5 AU	2.58 %	0.32 Rf	14.4 AU	317.3 AU	3.10 %
5	0.32 Rf	14.4 AU	0.33 Rf	20.9 AU	3.28 %	0.34 Rf	7.7 AU	166.0 AU	1.62 %
6	0.34 Rf	7.8 AU	0.36 Rf	20.4 AU	3.19 %	0.38 Rf	3.5 AU	346.9 AU	3.39 %
7	0.39 Rf	2.9 AU	0.44 Rf	22.7 AU	3.54 %	0.47 Rf	0.3 AU	593.7 AU	5.81 %
8	0.48 Rf	5.5 AU	0.50 Rf	26.0 AU	4.06 %	0.54 Rf	10.7 AU	562.9 AU	5.50 %
9	0.55 Rf	11.1 AU	0.60 Rf	34.9 AU	5.45 %	0.63 Rf	20.5 AU	1240.3 AU	12.13 %
10	0.63 Rf	21.0 AU	0.65 Rf	28.9 AU	4.51 %	0.68 Rf	9.1 AU	754.5 AU	7.38 %
11	0.69 Rf	6.6 AU	0.71 Rf	11.5 AU	1.80 %	0.73 Rf	0.1 AU	208.9 AU	2.04 %
12	0.84 Rf	1.4 AU	0.88 Rf	56.0 AU	8.75 %	0.90 Rf	38.8 AU	1464.7 AU	14.32 %
13	0.90 Rf	38.9 AU	0.93 Rf	47.8 AU	7.48 %	0.97 Rf	16.4 AU	1432.0 AU	14.00 %

Acacia frnasiana at 6 µl

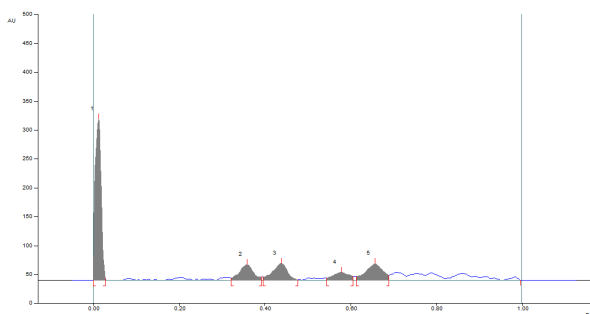
Figure 7: HPTLC photo documentation at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	115.7 AU	0.02 Rf	310.8 AU	20.46 %	0.06 Rf	0.2 AU	2941.1 AU	7.64 %
2	0.10 Rf	0.1 AU	0.14 Rf	16.2 AU	1.07 %	0.15 Rf	14.3 AU	297.0 AU	0.77 %
3	0.15 Rf	14.4 AU	0.20 Rf	79.4 AU	5.23 %	0.23 Rf	0.5 AU	2018.3 AU	5.25 %
4	0.28 Rf	3.0 AU	0.36 Rf	239.7 AU	15.78 %	0.39 Rf	02.6 AU	6641.5 AU	17.26 %
5	0.39 Rf	102.9 AU	0.44 Rf	220.7 AU	14.52 %	0.48 Rf	0.1 AU	6704.0 AU	17.42 %
6	0.51 Rf	15.0 AU	0.58 Rf	75.2 AU	4.95 %	0.61 Rf	37.2 AU	2662.7 AU	6.92 %
7	0.61 Rf	37.6 AU	0.66 Rf	201.5 AU	13.26 %	0.69 Rf	90.4 AU	6232.5 AU	16.20 %
8	0.69 Rf	92.1 AU	0.71 Rf	120.5 AU	7.93 %	0.73 Rf	62.1 AU	2535.3 AU	6.59 %
9	0.73 Rf	63.2 AU	0.76 Rf	145.4 AU	9.57 %	0.82 Rf	29.3 AU	5469.2 AU	14.21 %
10	0.83 Rf	30.0 AU	0.85 Rf	80.6 AU	5.30 %	0.94 Rf	0.0 AU	2530.8 AU	6.58 %
11	0.95 Rf	0.4 AU	0.98 Rf	29.3 AU	1.93 %	1.00 Rf	2.4 AU	443.2 AU	1.15 %

Acacia farnaesana at 6 µl

Figure 8: HPTLC photo documentation at 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	279.2 AU	73.93 %	0.03 Rf	2.6 AU	2800.3 AU	52.65 %
2	0.32 Rf	4.2 AU	0.36 Rf	27.0 AU	7.15 %	0.39 Rf	5.6 AU	636.9 AU	11.98 %
3	0.40 Rf	5.4 AU	0.44 Rf	29.1 AU	7.71 %	0.48 Rf	0.9 AU	707.4 AU	13.30 %
4	0.55 Rf	4.0 AU	0.58 Rf	13.6 AU	3.61 %	0.61 Rf	7.2 AU	368.6 AU	6.93 %
5	0.61 Rf	6.8 AU	0.66 Rf	28.7 AU	7.61 %	0.69 Rf	8.5 AU	805.5 AU	15.14 %

Acacia farnesiana at 6 µl

Figure 9: HPTLC photo documentation at 540nm

Table 1: Comparison between *Staphylococcus aureus* (Test group I) and *Pseudomonas aeruginosa* (Test group II) (Zone of inhibition)

Groups	Mean ± SEM <i>S. aureus</i>	Mean ± SE <i>P. aeruginosa</i>	T Value	P Value
G2 Standard	25.33 ± 0.33	25.31 ± 0.33	0.405	P > 0.05
G3 Aqueous extract of <i>Acacia farnesiana</i>	24.07 ± 0.67	23.57 ± 0.67	0.705	P > 0.05
G4 Alcoholic extract of <i>Acacia farnesiana</i>	23.00 ± 0.00	22.00 ± 0.05	0.703	P > 0.05
G5 Aqueous extract of <i>Pergularia daemia</i>	22.67 ± 0.33	21.90 ± 0.00	1.05	P > 0.05
G6 Alcoholic extract of <i>Pergularia daemia</i>	21.07 ± 0.24	20.07 ± 0.34	0.702	P > 0.05

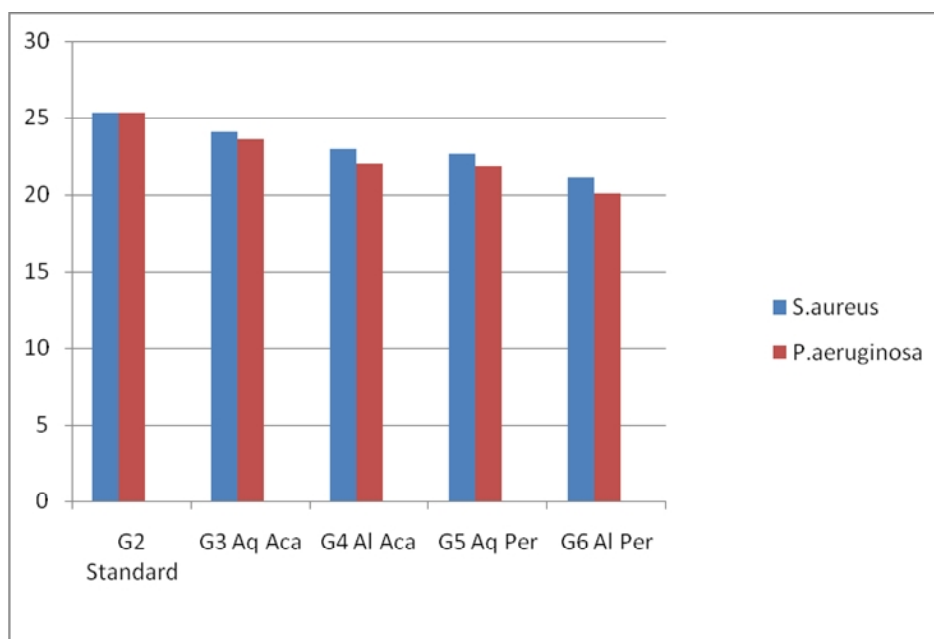


Figure 10: Comparison between zone of inhibition of *Staphylococcus aureus* (Test group I) and *Pseudomonas aeruginosa* (Test group II)
X- axis – Test groups, Y- axis- Zone of inhibition in mm

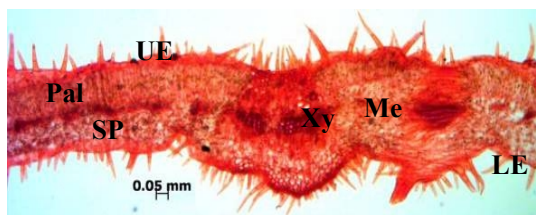
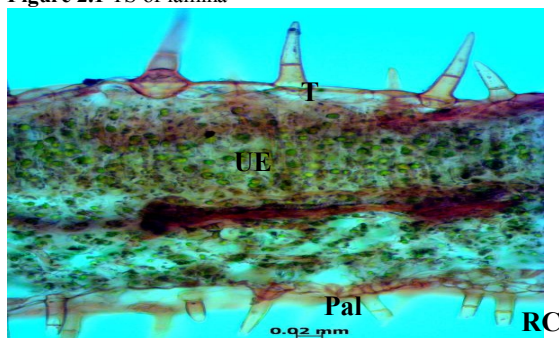


Figure 2.1 TS of lamina



LC, latex cell; LE, Lower epidermis; Me, mesophyll; Pa, parenchyma; Pal, palisade; Per, pericycle; Ph, phloem; RC, rosette crystal; SP, spongy parenchyma; St, stomata; T, trichome; UE, upper epidermis; VB, vascular bundle; Ve, vessel; Xy, xylem.

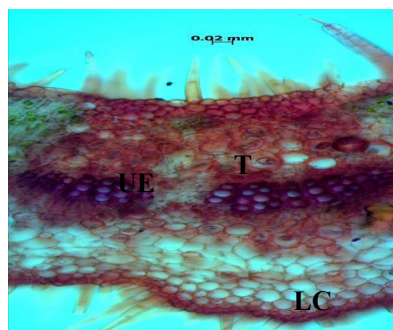


Figure 2.3 Portion of TS of midrib enlarged

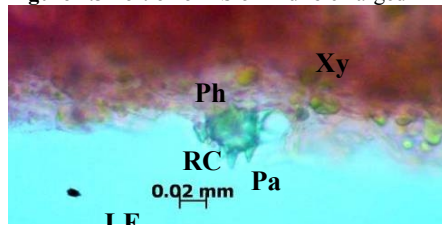


Figure 11: Detailed microscopic features of midrib and lamina of *Pergularia daemia*

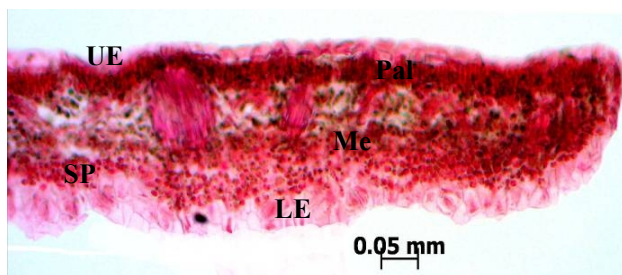


Figure 3.1 TS of lamina

E, epidermis; LE, lower epidermis; Me, mesophyll; Pal, palisade; SP, spongy parenchyma; St, stomata; UE, upper epidermis;

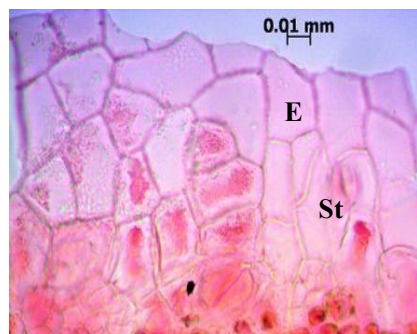


Figure 3.3 TS through midrib

Figure 12: Detailed microscopic features of lamina of leaflet of *Acacia farnesiana*

RESULTS

During the research work it was found that among 44 folk people the most popular and repeated disease was the septic condition and also treated commonly by using leaves of *Acacia farnesiana* Linn and *Pergularia daemia* Linn. Hence based on RPL and fidelity level the above two drugs were selected for further study. Phyto chemical screening of aqueous extract and alcoholic extract of leaves of *Acacia farnesiana* reveals the presence of phenol, flavonoids, alkaloids, steroid, triterpenoids and tannins. Phyto chemical screening of aqueous extract and alcoholic extract of leaves of *Pergularia daemia* showed the presence of phenol, flavonoids, alkaloids, steroid and tannins. Except triterpenoids all other phyto constituents are similar in both the plant extracts.

HPTLC photo documentation of alcohol extract of *Acacia farnesiana* Linn showed:

At 254nm aqueous extract of *Acacia farnesiana* exhibited 13 peaks having highest peak covering an area of 27.47% and lowest having

an area of 1.41%. where as alcoholic extract showed 9 peaks having highest area under peak as 7.30%. At 366 nm aqueous extract of *Acacia farnesiana* showed 11 peaks covering highest area of 17.42% and alcoholic extract showed 7 peaks with maximum area under peak as 6.93%.

At 540 nm aqueous extract of *Acacia farnesiana* showed 5 peaks with maximum area under peak as 52.65% and alcoholic extract showed three small peaks with highest area under peak as 6.93%. At 254nm aqueous extract of *Pergularia daemia* exhibited 10 peaks having highest peak covering an area of 51.23% where as alcoholic extract showed 7 peaks having highest area under peak as 10.52%. At 366 nm aqueous extract of *Pergularia daemia* showed 9 peaks covering highest area of 27.82% and alcoholic extract showed 6 peaks with maximum area under peak as 9.87%. At 540 nm aqueous extract of *Pergularia daemia* showed 7 peaks with maximum area under peak as 51.33% and alcoholic extract showed 4 peaks with highest area under peak as 7.07%.

Determination of total phenols

Results shows the highest phenol content in the aqueous extract of *Acacia farnesiana* (224.24GAE/g) followed by its alcoholic extract (217.09 GAE/g). The total content of the phenol is higher in the aqueous extract of *Pergularia daemia* (209.13 GAE/g) when compared to its alcoholic extract (203.56 GAE/g).

In vitro study

In vitro studies was conducted for the antibacterial activity of *Acacia farnesiana* Linn and *Pergularia daemia* Linn on selected two bacterial stains.

1. *Staphylococcus aureus* (Gram +ve) MTCC 737
2. *Pseudomonas aeruginosa* (Gram -ve) MTCC 1688

- Ciprofloxacin showed maximum inhibition at 10µg against both the bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
- Against *Staphylococcus aureus* MTCC 737 aqueous extract of *Acacia farnesiana* showed maximum inhibition at 175 µg and its alcoholic extract at 200 µg. whereas aqueous extract of *Pergularia daemia* showed maximum inhibition at 225 µg and its alcoholic extract showed maximum inhibition at 250 µg.
- Against *Pseudomonas aeruginosa* MTCC 1688 aqueous extract of *Acacia farnesiana* showed maximum inhibition at 200 µg and its alcoholic extract at 225 µg. whereas aqueous extract of *Pergularia daemia* showed maximum inhibition at 225 µg and its alcoholic extract showed maximum inhibition at 250 µg.
- Based on MIC against *Staphylococcus aureus* MTCC 737 aqueous extract of *Acacia farnesiana* showed maximum inhibition at least concentration compared to other extracts and lowest response is showed by alcoholic extract of *Pergularia daemia*.
- Based on MIC against *Pseudomonas aeruginosa* MTCC 1688 again it was found that aqueous extract of *Acacia farnesiana* showed maximum inhibition at least concentration compared to other extracts and equally moderate response is showed by alcoholic extract of *Acacia farnesiana* and aqueous extract of *Pergularia daemia*. The lowest response is showed by alcoholic extract of *Pergularia daemia*.
- Based on zone of inhibition, *Staphylococcus aureus* MTCC 737 has shown highest sensitivity to aqueous extract of *Acacia farnesiana* with zone of inhibition 24.07 ± 0.67 (mean \pm SEM), followed by its alcoholic extract 23.00 ± 0.00 and aqueous extract of *Pergularia daemia* 22.67 ± 0.33 . Least sensitivity is shown by the alcoholic extract of *Pergularia daemia* as 21.07 ± 0.24 .
- *Pseudomonas aeruginosa* MTCC 1688 has also shown the highest sensitivity to aqueous extract of *Acacia farnesiana* with zone of inhibition 23.57 ± 0.67 followed by its alcoholic extract 22.00 ± 0.05 and aqueous extract of *Pergularia daemia* 21.90 ± 0.00 . Least sensitivity is shown by the alcoholic extract of *Pergularia daemia* as 20.07 ± 0.34 .
- Based on 't' value it can be derived that the difference between the sensitivity of both the bacterial stains to all the four trial extracts is not significant.
- Both the bacterial stains *Staphylococcus aureus* MTCC 737 and *Pseudomonas aeruginosa* MTCC 1688 have commonly shown highest sensitivity to the aqueous extract of *Acacia farnesiana*.

DISCUSSION AND CONCLUSION

The present research paper deals with the ethno botanical survey of plants used by folklore practitioners of Haveri district, Karnataka in treating septic conditions with special reference to *Acacia farnesiana* Linn and *Pergularia daemia* Linn. The presence of phenol, steroid, tannins, flavonoids and triterpenoids in extracts of both the plants exhibits their antimicrobial activity.⁵ Tannins have been found to form irreversible complexes with praline rich protein resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerative tissues. Plants are complex chemical store houses of undiscovered biodynamic compounds with unrealized potential use in medicine. Several antimicrobial agents are isolated from secondary metabolites such as terpenoids, phenols, alkaloids, and flavonoids. The probable mode of action of phytoconstituents against microorganisms are because of these phyto constituents.⁶ Terpenoids, phenols, alkaloids, and flavonoids are compounds whose hydrophobicity induces partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable. Flavonoids may act through inhibiting cytoplasmic membrane function as well as by inhibition of DNAgyrase and β -hydroxyacyl-acyl carrier protein dehydratase activities. It has been suggested that terpenes promote membrane disruption. Tannins act on microorganism membranes as well as bind to poly saccharides or enzymes promoting inactivation. The HPTLC findings showed more peaks for aqueous extract of *Acacia farnesiana* than its alcoholic extract indicating more number of phytoconstituents in aqueous extract. The HPTLC findings showed more peaks for aqueous extract of *Pergularia daemia* than its alcoholic extract indicating more number of phytoconstituents in aqueous extract. *In vitro* study when compared between the test drugs extracts of *Acacia farnesiana* have shown more potent antibacterial activity against both the selected bacterial stains than the extracts of *Pergularia daemia*. Highest sensitivity of both the bacterial stains for the aqueous extract of *Acacia farnesiana* may be due to the presence of phenol, flavonoids, alkaloids, steroid, triterpenoids and tannins which are said to have potential antimicrobial action. Further research is needed to conserve and to develop some selected species of medicinal plants along with their sustained utilization.

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