Results
4. RESULTS

4.1. Study on the survey of ethnomedical plant knowledge and practices with reference to microbial infectious diseases in Javadhu hills, Tamil Nadu

4.1.1. Medicinal plants and uses reported by informants

The traditional information regarding the medicinal uses of plants are represented in Table:2. A total of 40 plants belonging to 24 families and 36 genera were reportedly used by the Malayali community in traditional health care system to treat and cure different ailments/diseases; 42.5% of which are infectious, 45% bacterial and 12.5 influenza/viral diseases (Fig. 5). Family Euphorbiaceae was represented by the highest number of four plant species, followed by three plant species each from families Asclepiadaceae and Solanaceae and constituted the most cited botanical families reported in this study. The habit of the species revealed that 40% were Herbs, 30% were Trees, 20% were Shrubs and 10% were Climbers (Fig. 6).

Among the various plant parts used in the preparation of the medication, the leaves represent the major part used with 60%, while the use of other parts as stem (rhizome/bark)- 20%, roots-5%, fruits-5%, seeds-5%, flowers- 3% and latex-2% (Fig. 7).

4.1.2. Modes of preparation, administration and dosage of remedies

The phyto-remedies in the study area were mostly prepared from freshly harvested plant parts except for Curcuma longa L. and Zingiber officinale Rosco, where the dry rhizomes were used, either singly (67%) or in combination (33%) with other plant species or mixed with milk, honey, coconut milk or coconut oil (Fig. 8).

Some plants as Achyranthes aspera Linn (Acanthaceae), Carthamus roseus G.Don (Apocynaceae), Gymnema sylvestre R.Br. (Asclepiadaceae), Eclipta alba Linn. (Asteraceae), Acalpha indica L. and Euphorbia hirta L. (Euphorbiaceae), Pongamia pinnata (L).Pierre and Sesbania grandiflora (L) Pers. (Fabaceae), Azadirachta indica A. Juss. (Meliaceae), Eugenia jambolana L. (Myrtaceae), Aegle marmelos Correa Roxb. And Murraya koenigii (L).Sprengel (Rutaceae) Solanum nigrum L. (Solanaceae) Vitex negundo L. (Verbenaceae) and Zingiber officinale Rosco (Zingiberaceae), were used for the treatment of more than one illness with a variation
in the mode of preparation and administration. The remedies were mostly processed using a locally made mortar and pestle and the weighing balance though used rarely, consisted of two small aluminium plates suspended with strings from a short bamboo stick. Plant material thus used was difficult to quantify and was indicated approximately. The mode of administration was mostly through oral consumption - 68%, tropical application -26.75%, steam inhalation -1.75%; gargle- 1.75%; drops - 1.75% (Fig.9). The dosage depended on the age and the physical status of the individual. The dosage differed from children to adults and depended on the type of illness and its intensity. Children under the age of 12 were administered only half the regular dosage and so also adults above the age of 60. There were no side effects reported in any of the treatments.

4.1.3. Traditional medical practices and beliefs

The traditional plant practitioners were usually men in the age group of 58-65 years whose traditional healing practices were trusted by the people in the community. A large number of informants reported that most of the ailments were treated effectively through the methodology that they followed. They maintained a diet during the treatment of certain illnesses as Malaria and Jaundice. They mostly consumed vegetarian food, milk, curds, buttermilk, groundnuts and leafy vegetables during the period of treatment and drank only boiled water.

4.1.4. Informant’s Consensus Factor (ICF) for Therapeutic purposes

The medicinal plants were reported to be used for treating 16 different ailments and broadly grouped into seven categories (Table:3). The highest Informant’s Consensus Factor (0.80) was for stomach disorders as gastroenteritis, nausea, worms, indigestion, dysentery and diarrhoea, followed by dermatological diseases such as wounds, snake/dog/insect bites, eruptions as rash and cracks in the heel region (0.70); Respiratory disorders as Asthma, cold, cough (0.63); Viral fever, hepatitis, jaundice, malaria (0.60); Diabetes (0. 58); Rheumatic pains and body pains (0.50); General health and well being (0.42) and hair loss (0.36). A high ICF reflects a high citation of plants used in the treatment of a particular disease category. On the other hand a low ICF reflects on the lesser use or disparity among the informants on the plant species used in the treatment of a particular disease category.
4.1.5. Fidelity Level (FL)

The Fidelity level (FL) refers to the healing efficiency of the most commonly used medicinal plants by the community. The fidelity level of a plant species for a specific disease in the study area varied between 50% and 100% (Table: 4). The maximum Fidelity Level of 100% was expressed by *Adhatoda vasica*, *Gymnema sylvestre* and *Andrographis paniculata* and indicated 100% success in the choice of plant species used by Traditional Healing Practitioners in the treatment of a particular disease. On the other hand, moderate FL of 50-80% indicated a lesser preferred choice, though normally used by many in the community for the treatment of a particular disease.

4.2. Percentage yield

The percentage yield of various extracts of *Adhatoda vasica*, *Gymnema sylvestre* and *Andrographis paniculata* were calculated (Table: 5).

The values on percentage yield showed the maximum obtained from the methanol extract followed by aqueous, chloroform and lastly hexane.

4.3. Preliminary phyto-chemical investigation

Phyto-chemical analysis of the leaf extracts of *Adhatoda vasica* (Table: 6) revealed the presence of alkaloids, phenol compounds and steroids in the aqueous extract; anthroquinones, flavonoids, tannins, saponins and steroids in the methanol extract and tannins and flavonoids in the Chloroform and hexane extracts.

The leaf extracts of *Gymnema sylvestre* (Table: 7) revealed the presence of alkaloids, phenol compounds, tannins and saponins in the aqueous extract; alkaloids, flavonoids, phenol compounds and anthroquinones in the methanol extract; alkaloids, terpenoids, phenol compounds and steroids in the chloroform extract and phenol compounds, terpenoids and tannins in the hexane extract.

The leaf extracts of *Andrographis paniculata* (Table: 8) revealed the presence of flavonoids, phenol compounds, saponins and tannins in the aqueous extract; flavonoids, steroids and tannins in the methanol extract and steroids, alkaloids and saponins in the Chloroform and Hexane extracts.
4.4. Antimicrobial Activity

In the present study the aqueous, methanol, chloroform and hexane extracts of *Adhatoda vasica* Nees (Table: 9-a, Fig.: 10-a; Table: 9-b; Fig. 10-b; plates: 1-a to 1-h), *Gymnema sylvestre* R.Br (Table: 10-a, Fig.: 11-a; Table: 10-b, Fig. 11-b; plates: 2-a to 2-h) *Andrographis paniculata* (Burm.f.) Wall.ex Nees (Table: 11-a, Fig.: 12-a; Table: 11-b, Fig. 12-b; plates: 3-a to 3-h) were utilized to evaluate their antimicrobial effect against Bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Klebsiella pneumonia* with Chloromphenicol as the reference drug and Fungal strains: *Candida albicans*, *Candida tropicalis*, *Candida kefyr* and *Candida krusei*, with Ketoconazole as the reference drug.

The results showed varying inhibitory effects indicated by clear zones of inhibition around the wells of plant extracts and were recorded in mm and were in comparison to the reference drugs Chloromphenicol for bacteria and Ketoconazole for fungi used as controls.

4.4.1. Anti-bacterial activity

The aqueous extract of *Adhatoda vasica* showed the highest inhibitory effect on the growth of *Staphylococcus aureus* (10.45±0.31 mm) followed by *Bacillus cereus* (8.51±0.20 mm) and *Escherichia coli* (8.25±0.51 mm); *Gymnema sylvestre*, only on *Staphylococcus aureus* (7.86±0.31 mm), while *Andrographis paniculata* showed no inhibitory effects on any of the bacterial strains tested.

The methanol extract of *Adhatoda vasica* showed the highest antibacterial activity against *Staphylococcus aureus* (9.7±0.62 mm), followed by *Bacillus cereus* (8.10±0.42 mm); *Gymnema sylvestre* showed the highest antibacterial activity on *Escherichia coli* (15.1±0.55 mm), followed by *Bacillus cereus* (7.95±0.31 mm); *Andrographis paniculata* showed antibacterial on all the bacterial strains tested, ranging from *Staphylococcus aureus* (16.01±0.01 mm), *Bacillus cereus* (10.85±0.56 mm), *Escherichia coli* (9.02±0.42 mm) and *Klebsiella pneumonia* (5.5±0.31 mm).

There was no anti-bacterial activity recorded with the chloroform - plant extracts on any of the bacteria tested, except for *Adhatoda vasica* (8.18±0.17 mm) against *Escherichia coli*. 
There were wide zones of inhibition in the hexane extracts of *Adhatoda vasica* against *Klebsiella pneumonia* (15.67±1.45 mm), followed by *Staphylococcus aureus* (9.02±0.63 mm); *Gymnema sylvestre* against *Bacillus cereus* (9.58±0.57 mm), followed by *Staphylococcus aureus* (8.05±0.56 mm) and *Andrographis paniculata* against *Escherichia coli* (13.93±0.82 mm), followed by *Staphylococcus aureus* (12.07±1.43 mm) and *Klebsiella pneumonia* (7.02±0.21 mm).

4.4.2. Anti-fungal activity

The aqueous extract of *Gymnema sylvestre* showed wide zones of inhibition with *Candida krusei* (10.0±0.57 mm) and *Candida kefr* (8.43±0.57 mm) and *Andrographis paniculata* against *Candida tropicalis* (8.01±0.21 mm). There was no anti-fungal activity reported in the aqueous plant extract of *Adhatoda vasica* against any of the fungal strains tested.

The Methanol extracts of *Adhatoda vasica* exhibited anti-fungal effects on the growth of *Candida tropicalis* (12.26±0.81 mm), followed by *Candida krusei* (11.01±0.13 mm) and *Candida kefr* (10.20±0.57 mm); *Gymnema sylvestre* against *Candida albicans* (12.48±0.82 mm) and *Candida krusei* (11.05±0.13 mm) and *Andrographis paniculata* against *Candida krusei* (14.12±1.23 mm) followed by *Candida albicans* (13.08±1.72.82 mm) and *Candida kefr* (6.6±0.13 mm).

There was no anti-fungal activity recorded with any of the chloroform - plant extracts on any of the fungal strains tested.

There were wide zones of inhibition in the hexane extracts of *Andrographis paniculata* against *Candida kefr* (15.6±0.76 mm), followed by *Candida tropicalis* (10.98±0.86 mm) and *Candida albicans* (9.98±1.1 mm). The hexane extracts of *Adhatoda vasica* and *Gymnema sylvestre* showed no inhibitory effects on any of the fungal strains tested.

The inhibition in the growth of micro-organisms recorded using different plant extracts was less than the corresponding reference drugs used in both the above antimicrobial studies.
4.5. Minimum Inhibitory Concentration (MIC)

In the present study the minimum concentration of aqueous, methanol, chloroform and hexane extracts of the leaves of *Adhatoda vasica* Nees (Tables: 12-a; 12-b; plates: 1.1-a,b; 1.2-a,b; 1.3-a,b; 1.4-a,b; 1.5-a,b; 1.6-a,b; 1.7-a,b); *Gymnema sylvestre* R. Br (Tables: 13-a; 13-b; plates: 2.1-a,b; 2.2-a,b; 2.3-a,b; 2.4-a,b) *Andrographis paniculata* (Burm.f.) Wall.ex Nees (Table: 14-a; 14-b; plates: 3.1-a,b; 3.2-a,b; 3.3-a,b; 3.4-a,b; 3.5-a,b; 3.6-a,b; 3.7-a,b; 3.8-a,b; 3.9-a,b; 3.10-a,b) at which growth of the Bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Klebsiella pneumonia*, with Chloromphenicol as the reference drug and Fungal strains: *Candida albicans*, *Candida tropicalis*, *Candida kefyr* and *Candida krusei*, with Ketokonazole as the reference drug is totally arrested / inhibited and death of the cell occurs is recorded.

The MIC values ranged from 12.5 (reference drugs used) to 100 mg/ml for the various plant extracts used to inhibit the growth of micro-organisms. The minimum concentration at which growth was arrested was at 50 mg/ml.

4.5.1. Minimum Bactericidal Concentration (MBC)

The MIC/ MBC of the aqueous extract of *Adhatoda vasica*, on *Staphylococcus aureus* and *Bacillus cereus* and *Andrographis paniculata* on *Staphylococcus aureus* was 50 mg/ml and *Adhatoda vasica* on *Escherichia coli* and *Gymnema sylvestre*, on *Staphylococcus aureus*, 75 mg/ml.

The MIC/MBC of the methanol extract of *Adhatoda vasica* on *Staphylococcus aureus*, *Gymnema sylvestre* on *Escherichia coli* and *Andrographis paniculata* on *Bacillus cereus* was 50 mg/ml; *Adhatoda vasica* on *Bacillus cereus* and *Klebsiella pneumonia*; *Gymnema sylvestre* on *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pneumonia* and *Andrographis paniculata* on *Escherichia coli* and *Klebsiella pneumonia* was 75 mg/ml.

The MIC/MBC of the Chloroform extract of *Adhatoda vasica* on the growth of *Escherichia coli* and *Andrographis paniculata* on the growth of *Bacillus cereus* was 75 mg/ml.
The MIC/MBC of the hexane extract of Adhatoda vasica on the growth of Klebsiella pneumoniae, and Andrographis paniculata on the growth of Staphylococcus aureus and Escherichia coli was 50 mg/ml; Adhatoda vasica on Staphylococcus aureus, Gymnema sylvestre on Staphylococcus aureus and Bacillus cereus and Andrographis paniculata on Klebsiella pneumonia was 75 mg/ml.

4.5.2. Minimum Fungicidal Concentration (MFC)

The MIC/ MFC of the aqueous extract of Gymnema sylvestre on Candida krusei was 50 mg/ml. The MIC/ MFC of Gymnema sylvestre on Candida kefyr and Andrographis paniculata on Candida tropicalis was 75 mg/ml.

The MIC/MFC of the methanol extract of Adhatoda vasica on Candida krusei, Candida kefyr and Candida tropicalis; Gymnema sylvestre on Candida krusei and Andrographis paniculata on Candida krusei was 50 mg/ml; Andrographis paniculata on Candida kefyr was 75 mg/ml.

The MIC/MFC of the chloroform extract of Gymnema sylvestre on Candida albicans was 50 mg/ml.

The MIC/MFC of the hexane extract of Andrographis paniculata on Candida albicans, Candida tropicalis and Candida kefyr was 50 mg/ml and Adhatoda vasica on Candida tropicalis, 75 mg/ml.

The other extracts were effective on the test micro-organisms at concentrations exceeding 100 mg/ml.

4.6. Wound healing efficacy

4.6.1. Acute Toxicity studies

The rats of either sex were fed with increasing doses (1,2,4 and 8 gm/Kg body weight) of methanol extract of Andrographis paniculata for 14 days. The doses went up to 8 gm/Kg body weight but there were no signs of toxicity and mortality. The animals were physically active and consumed food and water as normal and also did not exhibit any abnormal behavior. However in the current study, a dosage of 5 gm/Kg body weight was utilized for the preparation of the Andrographis paniculata methanol-impregnated ointment.
4.6.2. Wound healing study

Studies on *Andrographis paniculata* methanol-impregnated ointment for its wound healing property on excised wound showed significant results in terms of epithelialization period and wound contraction and was close to the standard (Povidone iodine ointment) used. (Table: 15; Fig.: 11)

4.6.2.a. Epithelialization period

The epithelialization period for the wound treated with the methanol leaf extract of *Andrographis paniculata* - impregnated ointment (5% w/w) was 16 days, when compared to wound treated with povidone iodine ointment (5% w/w) which was 14 days and the simple ointment, 23 days.

4.6.2.b. Measurement of wound contraction

The percentage of wound contraction was measured and calculated on 6th, 9th, 12th and 18th day. On the 18th day, the animals treated with the methanol leaf extract of *Andrographis paniculata* - impregnated ointment (5% w/w), showed a wound area of 47.26 ± 1.9 and wound contraction of 81.01% when compared to the animals treated with povidone iodine ointment (5% w/w), which showed a wound area of 41.64 ± 2.1 and a wound contraction of 83.21%, whereas the wound treated with simple ointment showed an area of 67.77 ± 1.4 and a contraction of 72.45%.

4.7. GC-MS analysis of Phyto-chemical compounds

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of *Adhatoda vasica* Nees. (Family Acanthaceae), *Andrographis paniculata* (Burm.f.) Wall.ex Nees. (Family Acanthaceae) and *Gymnema sylvestre* R.Br (Family Asclepiadaceae). These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in (Tables: 16, 17 and 18). The compound structures prediction was based on NIST Library.

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure.
of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library.

The GC-MS analysis of the methanol leaf extracts of *Adhatoda vasica* and *Andrographis paniculata* (Table 16 & 17; Figs. 12 & 13) revealed the presence of six different phytochemical compounds, viz. *z,z*-6,8-heptatriactontadien-2-one (with retention time i.e. 16.90, 17.34 for *A. vasica* and 16.894, 17.339 for *A. paniculata*), *Cis*-1-chloro-9-octadecene (with RTs 16.96 for *A. vasica* and 16.959 for *A. paniculata*), *trans, cis*-1,8-dimethyl spiro(4,5)decane (with RT 17.15 for *A. vasica* and 17.149 for *A. paniculata*), Phytol (with RT 19.57 for *A. vasica* 19.575 for *A. paniculata*), cyclotrisiloxane hexamethyl (with RT 25.22, 25.49, 26.64, 29.58 for *A. vasica* and 25.22, 25.488, 26.64, and 26.938 for *A. paniculata*). Cyclohexanone, 2-methyl-5(1-t-butyldimethylilyloxymethyl) ethen (with RT 26.94 for *A. vasica* and 26.938 for *A. paniculata*).

Further, the GC-MS analysis of methanol leaf extract of *Gymnema sylvestre* (Table 18 & Fig. 14) showed the presence of four phytochemical compounds viz. *z,z*-6,8-heptatriactontadien-2-one (with RT 16.894), 3 methyl-2-(oxopropyl) furan (with RT 19.575), bicycle (4-1-0) heptan, 7 phenyl- (with RT 19.485) and cyclotrisiloxane hexamethyl- (with RT 25.213).