4. Results

In this study, 102 ethnomedicinal plants species belonging to 53 families distributed in 95 genera which were commonly used by the most of the local traditional healers for the treatment of 52 types of ailments. Among the families, Euphorbiaceae has the high number of species (7) followed by Fabaceae with six species, Rutaceae and Solanaceae with each five species and Lamiaceae with four species. End of the investigation, the collected plants botanical names, specimen voucher number, local name (Tamil), life form, use value, parts used, ailment treated, methods of preparation and mode of administrations were provided in Table 2. The present study found that there 34 claims from the plants were reported for the first time from the study area (New claims were indicated with asterisk mark in Table 2). However, no plants were reported as a new medicinal plant as all plants were reported with different uses.

4.1. Life forms and parts used

Herbs (37.0%) were found to be most used plants followed by tree (28%) and climbers in descending order (12%) (Fig.6). The most frequently utilized medicinal plant parts were leaves (36%) used for the preparation of medicine solely or mixed with other plant parts followed by Fruit (12%), Root (11%), Stem (8%), Stem bark, Whole plant and Seed (each 7%), Flowers (4%), Latex (3%), tubers and Rhizome (each 2%) and Bulb (1%) as show in Fig.7.

4.2. Mode of preparation and administration of plants

Plant parts were grouped into eight categories for preparation and utilization. Among them, majority of the plant remedies were prepared by paste (39%) followed by Juice (29%), Raw (11%), Decoction (10%), Powder (5%), Soup (3%), Infusion (2%) and Inhalation (1%) (Fig. 8).
The most frequently used mode of remedy administration is oral ingestion (62%), followed by topical uses (35%), bath (2%), tooth brush (1%), and nasal applications (1%) (Fig. 9).

**4.3. Plants used for combination**

Herbal medicines prescribed by local traditional healers were either preparation based on single plant or a combination of several plant parts. In the present study reveals most of the preparation involved in multiple modes (74 plants). Only few plants (36 plants) were taken as medicine without ingredients. In some cases, the added ingredients may be other plant parts or some other products like oils, goat milk, salt and honey (Table 3).

**4.4. Statistical analysis**

**4.4.1. Use values (UV)**

The most commonly used species was *Moringa oleifera* with 21 use reports by 8 informants, giving the highest use value of 2.62. *Moringa oleifera* is attributed to its use in the treatment of various diseases and it is well recognized all the informants as an cancer followed by *Curculigo orchioides* (20 use reports by 8 informants with UV of 2.5) *Amorphophallus paeoniifolius* and *Vitex negundo* (each 19 use reports by 8 informants with a UV of 2.37), *Carica papaya* (17 use reports by 8 informants with a UV of 2.12), *Citrus hystrix* and *Tribulus terrestris* (each 16 use reports by 8 informants with a UV of 2.0), *Achyranthes aspera* and *Annona squamosa* (15 use reports by 8 informants with a UV of 1.87), *Aloe vera* and *Andrographis paniculata* (14 use reports by 8 informants with a UV of 1.75), *Acorus calamus, Azadirachta indica, Centella asiatica, Coccinia indica* and *Ziziphus zizyphus* (13 use reports by 8 informants with a UV of 1.62).

**4.4.2. Informant consensus factor (ICF)**

The reported ailments were divided into 19 categories based on the information gathered from the interview. The $F_{ic}$ values are ranged from 0.63 to 1.00 (Table 4). The highest ICF value of dermatological infections/diseases (114 use reports, 34 species)
followed by gastrointestinal ailments (89 use reports, 30 species) skeleton muscular disorder (82 use reports, 24 species) and respiratory systems diseases (73 use reports, 28 species) (Fig. 10).

**4.4.3. Fidelity level (FL)**

From the available information, fidelity level of each of the species was calculated with each category. As part of this study, 7 species have highest fidelity level of 100%, most of which were used in single ailment category with multiple informants. In this study less than three use reports were not considered for future study (Table 5).

**4.5. Edible plants**

Edible plants such as *Anacardium occidentale*, *Ananas comosus*, *Annona squamosa* and *Carica papaya*, are highly desirable in these regions. According to their growing season they are harvested by tribal community. The fresh fruits of *Mangifera indica* were used for the preparation of pickles and that are used some times for making soup. *Amorphophallus paeoniifolius* and *Solanum torvum* are used as greens and vegetables. *Alternanthera sessilis*, *Manihot esculenta*, *Moringa oleifera* and *Murraya koenigii* are taken as food and side dish. *Hemidesmus indicus* is used for juice preparation.

**4.6. Economic plants**

*Acacia nilotica* is used as a good fuel source (wood for fire) by the tribal people. *Areca catechu*, *Curcuma domestica*, *Piper betle*, *Piper nigrum* and *Zingiber officinale* are harvested by the tribal community and that are sold in local markets and villages. Eleven species (14.5%) of 102 medicinal plant species were reported in present study which also used in field of technology and craft. In kerala, the local Women’s are very famous for wearing the cotton sarees with bordered silk materials. These handicrafts exported all over the world and are very good sources of income for weavers. This has yarns with natural dyes prepared from different plants like *Curcuma domestica*, *Phyllanthus emblica* and
Lawsonia inermis. Pongamia glabra and Santalum album are used as good air freshener (Table 2).

4.7. Evaluation of potential medicinal plants to treat venereal diseases

In this ethnobotanical survey, 102 plants were recorded from different plant families. Among them, 12 plants (Anacardium occidentale. L., Ananas comosus (L) Merr., Coccinia indica. L. (Voigt), Curculigo orchioides S. Gaerth, Ficus benghalensis Linn. Ficus racemosa. L. Hibiscus rosa-sinensis. L. Moringa oleifera. Lampk. Odina wodier. Roxb. FL, Pedalium murex L. Ricinus communis. L. and Tribulus terrestris. Linn) were treated for venereal diseases (Table 6).

4.8. Antimicrobial activity

In the present study, antimicrobial activity of various extracts of A. occidentate, A. comosus, C. indica, C. orchioides, F. Benghalensis, F. racemosa, H. rosa-sinensis, M. oleifere, O. Wodifer, P. murex, R. communis, and T. Terrestris were investigated against venereal disease causing pathogens. These plants antimicrobial efficiencies were shown in Table 3. B. subtilis has shown remarkable susceptibility in acetone extract of A. occidentale, A. comosus, C. indica, C. Orchioide, F. Benghalensis, H. rosa-sinensis, M. oleifera and T. terrestris. S. aureus was showed notable inhibition activity in all the solvent extracts, except aqueous extracts. Methanol extracts of A. occidentate, A. comosus, C. indica, F. Benghalensis, F. racemosa, H. rosa-sinensis, M. oleifera and O. Wodifer were exhibited excellent antibacterial activity against N. gonorrhoea when compare with standard drug of cefotaxime. Likwise, the methanol extracts of A. occidentale, A. comosus, F. racemosa, H. rosa-sinensis and M. oleifera were represented significant antifungal activity against C. albicans. Where as, the H. rosa-sinensis extracts have also showed notable activity than standard drug. The acetone and chloroform extracts of A.
comosus, C.indica, F. Benghalensis were shown better antibacterial activity against C.trachomatis. Among the selected methanolic extract, the extracts of A.ocidentale, C.indica, H. rosa-sinensis and M.oleifera have exhibited good antimicrobial activity against tested pathogens (Table 7).

4.9. Gas chromatography and Mass spectroscopy (GC-MS)

GC-MS analysis has performed flower of Hibiscus rosa-sinensis methanolic extract (Fig. 3). The peaks of compounds and retention time present in methanolic extracts of hibiscus rosa -sinensis identified by GC-MS analysis were reported in chromatogram. The active principle, retention time (RT), Molecular Weight (MW), Molecular Formula (MF) and chemical structure is presented in Table 8. More than nine compounds were identified in the plant flower extract. The qualitative analysis of the extract, showed the presence of nine compounds in methanolic extract. Phthalic acid with retention time (19.20), Octadecanoic acid with retention time(21.79), 3-N-Hexylthiolane with retention time (29.98), 1,2-Benzenedicarboxylic acid with retention time (35.90), 1 – Iodoundecane with retention time (49.12), 2, 2, 4 - Trimethyl 3-pentanone with retention time (49.50), 1 2-benzenedicarboxylic acid with retention time (56.23) 2-Propenamide with retention time (57.85) and Amylnitrite with retention time (58.33).

4.10. Computational analysis

4.10.1. Molecular docking

In this study, we intended to explore the overlapped N. gonorrhea inhibitory molecule of 1, 2, Benzenedicarboxylic acid. Our research get a target protein having the length 208 amino acid residues with the resolution of 1.67 Å. Thereby the computational tools the effective ligand molecule has find out driven by molecular docking. It also showing potential binding pocket on protein and their hydrogen bond interactions. Totally, nine bioactive compounds that are all generated complex for dock with the N. gonorrhea protein. Here suitable ligand molecules only produced docking score, glide energy and so
on. And also shows that molecule hydrogen bond distances and their binding affinities with a target.

Outcome this computational experiment 1, 2, Benzenedicarboxylic acid has showed effective binding affinities and had superior docking score than other ligands. Our computational finding shows lot of bioactive ligands having neutral docking score above -4.0. Here, the lead docking score has received the molecule of 1, 2, Benzenedicarboxylic acid and it score was displayed in Table 9.

This finding displayed phytocompound was taking part to the further experimental designing like as cell culture, animal model and so on. It was checked by binding affinities of protein to ligand molecules. This molecule also shows contribution to the ligand flexibility of target. There that the ligand molecule also generated their consequent glide energy after completion of docking analysis. These are all enzymes have been docked with the bioactive molecules as well as conventional drugs for future drug development process in Pharmacy industry.

4. 10.2. Molecular interactions of 1, 2, Benzenedicarboxylic acid with functionally important residues of Neisseria gonorrhea.

The Neisseria gonorrhea protein residues were interacts with ligand groups. Such interactions are depending on both specific interactions and non-specific forces to the outside of the target binding pocket. The interaction pattern between N.gonorrhoea and 1, 2, Benzenedicarboxylic acid were examined the site to which the target residues. The 1, 2, Benzenedicarboxylic acid was robustly interacting with the target residues of His 210, Gly266, and Gly209. These are all formed hydrogen bonds like both side chain and back chain formation to the ligand groups (Fig. 15).

4.10.3. Analysis of docking

This computational finding demonstrated the ligand efficiency. Entire result of this research outcomes were listed in Table 9.
4.10.3.1. 1, 2, Benzenedicarboxylic acid

Through this molecular docking we found that 1, 2, Benzenedicarboxylic acid glide Gscore (-7.955 kcal/mol) and their binding energy (-38.692 kcal/mol). Analysis of this docked complex showed that the residues His 210 (1.89), Gly266 (2.10), Gly209 (2.25) were involved in hydrogen (H) bindings with 1, 2, Benzenedicarboxylic acid. The residue Lys 173 was involved in n-cation with the ligand (Fig. 15a).

4.10.3.2. 2, 2, 4 - Trimethyl 3-pentanone

2, 2, 4 - Trimethyl 3-pentanone had the second best Glide G score (-7.082 kcal/mol) and binding energy score (-39.610 kcal/mol). Analysis of the docked complex showed that the residues Asp232 (1.84) was involved in hydrogen bonding with 2, 2, 4 - Trimethyl 3-pentanone (Fig. 15b).

4.10.3.3. 3-N-Hexylthiolane

3-N-Hexylthiolane had the third best Glide G score of -6.040 kcal/mol with well binding energy score (-20.612 kcal/mol). Analysis of this docked complex showed that the residues Asp232 (1.65) and NH2 (2.28). These are involved in hydrogen bond formation with 3-N-Hexylthiolane (Fig. 15c).

4.10.3.4. Octadecanoic Acid

Octadecanoic Acid had the fourth best Glide G score of -5.849 kcal/mol with good binding affinity energy (- 19.251 kcal/mol) (Fig. 3d). This not received any hydrogen bond formation (Fig. 15d).

4.10.3.5. Iodoundecane

Iodoundecane had the fifth best Glide G score (-5.156 kcal/mol) and get well binding energy score -23.551 kcal/mol. There is did not show any binding affinities on ligand to target (Fig.15e).
4.10.3.6. 2-Propenamide

2-Propenamide is received lowest docking score when consider with only Iodoundecane ligand. There not shown any hydrogen bond formation with a target (Fig. 15f).

4.11. Isolation of phytocompound

The potential phytocompounds was isolated from Hibiscus rosa-sinensis flower by extraction, preparative thin layer chromatography and column chromatography (Fig. 16 & Fig. 17). Elucidated sample was checked by $^1$H NMR, $^{13}$C NMR, Mass Spectra and UV analyses. The functional groups of 1, 2-Benzenedicarboxylic acid were then analysed with FTIR.

4.11.1. Ultraviolet visible spectroscopy
To identify the presence of the conjugated $\pi\text{-}285$
Peak I – Conjugated $\pi\text{-}285$
Peak II – Enone with external conjugated group–higher in texting.
Product colour–Pale yellow
Chromophoric $=\text{O}$ attach in the compound (Fig. 18).

4.11.2. FTIR Interpretation:
780 Cm$^{-1}$ - Meta di-substituted (very strong) = C-H
1067 Cm$^{-1}$ - Aromatic Methane (Weak)
1248 Cm$^{-1}$ - Aromatic C-C
1654 Cm$^{-1}$ - Aromatic acid (Medium to Strong) $=\text{C-O}$
1872 Cm$^{-1}$ - Vinyl terminal (Medium)
(Fig. 19)

4.11.3. Mass Spectra:
EI- Ms m/z: The Molecular ion peak is (M+) 166.

The other fragments are 57, 69, 104, 121, 149, 205, 223 & 321
HREI – Ms ÷ gave Molecular formula as C_6H_4 (COOH) 2
(Fig. 20).

**4.11.4. **$^{13}$C NMR Interpretation:

- 128.60- C in aromatic Rings
- 131.40- C=C (in alkenes)
- 132.90- C=C (in alkenes)
- 168.80- c=o (in acid & esters)
(Fig. 21)

**4.11.5. **$^1$H NMR Interpretation:

- 6.72 - Aromatic (Ar H)
- 7.59 - Phenolic (Ar OH)
- 7.80 - Amide (RC = o NH R)
(Fig. 22)

**4.11.6. Product characterization**

The Preliminary identification of the various product of solvents has been accomplished by Thin layer Chromatographic technique. The Compound is Separates by using Colum chromatography. The FTIR, $^1$H NMR, $^{13}$CNMR, Mass Spectra and UV analyses have enabled to confirm the set of product for 1-2, benzenedicarboxylic acid above investigations. FT-IR, NMR and mass spectral analysis be concludes that the compound is 1-2, benzenedicarboxylic acid molecular formula is C_6 H_4 (COOH) 2 (or) C_8 H_6 O_4. The molecular weight is 166.14. The colour of the Compound is pale yellow crystalline and melting point is 230º C and it is insoluble in water but soluble in methanol.
The structure of the compound is 1-2, benzenedicarboxylic acid is very much useful in further study (Fig. 23 & 24).

**Compound analysis:**

**1-2, Benzenedicarboxylic acid**

- **Molecular Formula:** $C_6H_4(COOH)_2$ (or) $C_8H_6O_4$
- **Molecular Weight:** 166.14
- **Colour:** Pale yellow
- **Melting Point:** 230°C
- **Boiling Point:** 378.27 ± 25.00
- **No of Hydrogen Bond Donors:** 2
- **No of Hydrogen Bond Acceptors:** 4
- **TPSA:** 74.6
- **No of Rotatable Bond:** 2
- **Monoistic Mass:** 166.026609 Da
- **Nominal Mass:** 166 Da
- **Molar refractivity:** $40.11 \pm 0.3 \text{ cm}^3$
- **Vapour Pressure:** $\log_{10}(P) = -5.669 \pm 0.91$ (Pin mm Hg)
- **Enthalpy of Vaporization:** $\Delta_{vap}H(T \text{ boiling point}) = 66.05 \text{ KJ/Mole}$
- **Other names:** Phthalic acid, 1-2, benzenedicarboxylic Acid; Benzene 1,2-dicarboxylic acid; O- Phthalic acid; O- Discarboxy benzene
- **Solubility:** Soluble in alcohol; sparingly soluble in ether

4.12. **Antimicrobial activity of Hibiscus rosa-sinesis extracts and 1,2 Benzenedicarboxylic acid**
Identification and characterization of antimicrobial compounds from selected ethnomedical plants of Silent Valley (Western Ghats, Kerala) with emphasis on Venereal diseases

In the present study, antimicrobial activity of various flower extracts of *H. rosa-sinensis* and isolated compound 1,2 Benzenedicarboxylic acid were investigated by using agar well diffusion method against selected pathogens (Table 11). Of them, the methanol flower extracts showed maximum inhibition activity against gram positive bacteria. The inhibition zones are 22±2.88, 20±4.23, 17±2.65, and 15±0.18mm in methanol extracts, 17±1.43, 15±0.21, 14±2.42 and 9± 0.11mm in chloroform extracts 16± 1.71, 14±1.21, 12± 1.54, 10±2.6mm in acetone extracts and 14±0.42, 11±0.62, 10±1.21 and 8±0.78mm in aqueous extracts of 100,75,50,25µg/ml to *S.aureus*. Whereas, the same concentration in methanol extracts more or less inhibited of same in *B.subtilis* such as 18±1.31, 16±0.41, 13±0.88 and 12 ± 0.57mm .On the other hand, inhibition at 10, 7.5, 5 and 2.5µg/ml was 26±1.08, 22±1.92, 19±2.36 and 15±0.09mm followed by 22±2.31, 19±1.36, 15±1.71 and 11±1.04 *S.aureus* and *B.subtils* respectively.

Similarly to be concentrations of gram positive bacteria, gram negative bacteria also exhibited maximum zone of inhibition .Such as 24±8.54, 22±6.23, 20±0.34 and 17±2.32 mm in methanol extracts 17±1.34, 15±0.71, 12±3.12 and 10±1.54mm to chloroform extract to *N.gonorrheae*. Parallel concentration produced 19±1.94, 17±1.98, 14±3.34 and 12±1.98mm to methanol extracts against *C. trachomatis* and 18±1.68, 17±2.46, 15±3.22, and 11±1.67mm to acetone extracts against *T.pallidum*.Isolated compound of 1, 2 Benzene dicarboxylic acid at various concentrations like 10, 7, 5, 5 and 2.5µg/ml exhibited maximum inhibited of 28±2.34, 25±1.36, 22±0.82, 23±0.22, 19±1.93, and 15± 1.24mm to *C.trachomatis* and 24±1.91, 20±1.27, 16±1.07 and 11±3.01mm to *T. pallidum*. Pathogenic fungi at 100,75,50 and 25µ g/ml, maximum zone was observed such as 19±1.69, 17±2.13, 14±1.89, and 11±2.6mm to methanol extract 18±1.36,16±3.23, 12±2.13 and 11±2.13mm to chloroform extract 17±1.23, 15±3.21 , 13±4.56 and 11±2.51mm to acetone and 14±1.34, 13±0.58, 11±2.77and 10±1.99mm to respectively. Maximum inhibition at 10,7.5, 50 and 2.5µg/ml for 1,2 Benzene dicarboxylic acid were 28± 1.34, 24±2.17, 22±1.08 and 19±1.82mm against *C.albicans*.The above extracts were also compared with standard drugs of Chloramphenicol, Cefotaxime, Azithromycin,
Doxycycline and Clotrimazole. This research observed the antimicrobial activity of methanolic flower crude extract of *H. rosa-sinensis* and 1,2 Benzene discarboxylic acid were showed better inhibition activities than corresponding drugs.

5. Discussion

5.1. Medicinal plants

Medicinal plants have been used for the treatment of diverse ailments for thousands of years. Even with the advent of allopathic medicine, using plants for medicinal purposes is still prevalent in many parts of the world (Siew et al., 2014). According to the World Health Organization estimates that up to 80% of the people in developing countries still depend on local medicinal plants to fulfil their primary healthcare needs (WHO, 2002). Historically, plants have been traditionally used for ages to cure ailments. Today, over 70% proportion of patients and healthcare providers in the world rely on herbal medicines directly or indirectly for meeting their health care needs (Sofowora et al., 2013).

The indigenous medicinal practices can provide valuable information for discovery of new and more effective drugs. A historical perspective on the use of medicinal plants for the treatment and cure of disease shows that traditional medicinal practices have been associated with humanity since ancient times (Halberstein, 2005).

Since the dawn of civilization, plants are in use as sustenance end medicine. Statistics reflect that nearly 80% of the global population uses plants for their primary health care (Barkatullah et al., 2015) Plants are extensively being screened for therapeutic phytochemicals and lead compounds (Katiyar et al., 2012). An estimated 25% of prescription drugs and 11% of drugs considered essential by WHO are derived from plants and a large number of synthetic drugs are obtained from precursor compounds isolated...