

Discussion

5. DISCUSSION

Survey of Medicinal Plants

The Survey of medicinal plants was done from Velliangiri hills in western ghats of Coimbatore, Tamilnadu, India. The present investigation was to study the medicinal plants used against various diseases. Based on the Traditional Aboriginal Knowledge (TAK) and from survey three medicinal plants such as *Annona muricata* L., *Spermacoce articularis* L.f. and *Rauvolfia tetraphylla* L. were selected for further investigations (Table 1).

Pharmacognostic Studies

The potency of medicinal plants and its therapeutic value depends on chemical components of the plants. Most of the pharmacognostic research basically deals with the study, authentication and identifying controversial plant species. The misconception of plant products is due to wrong identification of the plants and the common error is one common vernacular name address to two or more different species. Majority of the pharmacognostic studies ensure safety and efficacy of natural products derived from plants (Kumar, 2007). According to World Health organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

Macroscopic and Microscopic Studies

Annona muricata L.

Morphological character of *Annona muricata* L. studies shows it is a small evergreen tree with 4-10 meters tall grows in humid conditions. The root system is superficial with brown cork on surface. The stem is straight, rough, dark with fissures. The leaves are dark green, glossy of about 7.4-16cm length, 5-7cm wide alternate arrangement. Flowers with green stout stalks has narrow petals and fruit is about 14-40x10-18cm with average weight of about 7kg.

On microscopic studies the T.S of Lamina shows the presence of epidermal layers, Xylem with Xylem fibres and Xylem parenchyma has radial composed vessels. They are arranged vertically with lignified scalariform thickenings (Plate 1

&4). A number of medicinal uses ranging from the use of leaves , bark, roots, fruits to seeds have been reported ,especially the use of leaves to treat malaria in tropical countries such as Cameroon and Vietnam (Yahaya, 2014).

***Spermacoce articularis* L.f.**

It is a procumbent, scabrid herb with inflorescence which grows up to 900m in hills, dry lands etc. The leaves are sessile, oblong, elliptic with 1-3.5cm long. Flowers are very small, calyx is linear lanceolate, fruits exhibit hairy characteristics capsule and seeds are granulate oblong usually variable opaque. On microscopic examination *Spermacoce articularis* L.f. reveals that the leaf is dorsiventral with thick midrib, the epidermal cells are rectangular on adaxial and abaxial side of leaf. Xylem elements are compactly arranged and phloem seen in patches of bast fibres (Plate 2 & 6).

According to the recent report by Conserva and Jesu Costa Ferreira (2012) *Spermacoce* and *Borreira* belong to the family Rubiaceae and wide spread in America, Africa, Asia and Europe. The plant considered to be unique due to its fruits morphology, so the authors considered the two taxa under the generic name *Spermacoce*.

***Rauvolfia tetraphylla* L.**

It is a evergreen shrub found in tropical region. Leaves are elliptic ovate with reticulate venation. The base of the leaf is round, texture is smooth with characteristic odour, flowers are bisexual fruits is shiny black (Plate 3&5). Similar Macroscopic and Microscopic studies were observed in the case of *Annona reticulata*. L macroscopically the leaves were found to be lanceolate oblong, light green and pellucid with dots. On microscopic examination the T.S of lamina in leaf reveals the presence of mesophyll with palisade and spongy parenchyma. Xylem is composed of radially arranged vessels with xylem fibres and xylem parenchyma (Zaman, 2013).

Organoleptic character

It is the evaluation of the crude drugs by examining colour, odour, taste, size and shape etc.

It is considered as the important investigations to determine the identity and purity of the drug (Khandelwal, 2003).

In case of *Annona muricata* L. the leaves exhibit green colour, characteristic odour with bitter taste. In leaves of *Spermacoce articularis* L.f. also exhibits green colour, characteristic odour with coarse touch, where as *Rauvolfia tetraphylla* L. shows characteristic colour, stability and smell with green colour (Table 2) Based on the observations and evidences available in the literature the plants were identified and further authenticated by Botanical Survey of India, Coimbatore, TamilNadu (BSI/SRC/5/23/2015/tech/2215/2320/2214) (Annexure -B).

According to Amrishi and Tarasingh (2011) *Albizia odoratissima* bark shows light green, characteristic odour, astringent and sweet taste with fibrous texture and these characters could be used for authentication of the crude drug obtained from the bark which is reported as antidiabetic drug (Kumar *et al.*, 2011).

Banerjee & Ganguly 2014 by their recent observations reported that *Hemidesmus indicus* (L.) has specific odour and taste and also possess convenient characters to be differentiated from adulterants. Starch grains are the distinguishing character which is used to identify in researches on phytochemicals.

Physicochemical analysis

It is the important parameter to determine the adulterants and impurity of drugs. Ash values analyse the quantitative standards and to reveal the purity of drugs especially in powdered form (Swati *et al.*, 2012). The total ash of a crude drug exhibits the care reflected on drug preservation and the purity of crude and the prepared drug.

The physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying of *Annona muricata* L. was $11.30 \pm 0.10\%$, $0.12 \pm 0.01\%$, $11.46 \pm 0.12\%$, $0.19 \pm 0.02\%$, $8.66 \pm 0.79\%$ respectively (Table 3).

Spermacoce articularis L.f. analysis showed the value of total ash content as $12.30 \pm 0.10\%$, acid insoluble ash as $17.00 \pm 0.95\%$, water soluble ash as $14.10 \pm 0.33\%$, sulphated ash as $0.20 \pm 0.03\%$, loss on drying as $9.00 \pm 0.51\%$ (Table 3).

In *Rauvolfia tetraphylla* L. the analysis showed $8.30 \pm 0.33\%$, $11.30 \pm 0.93\%$, $12.00 \pm 0.48\%$, $0.17 \pm 0.01\%$, $8.81 \pm 0.45\%$. (Table 3). In *Spermacoce articularis* L.f. water soluble ash content is higher than *Annona muricata* L. and *Rauvolfia tetraphyllum* L. and they are significant at $P < 0.01$ level.

The above studies were also reported in the plant such as *Eclipta Alba* (L.) Hassk and *Lippia nodiflora* (Linn.) The total ash content of *E. alba* was 15.91% and in *L. nodiflora* was 13.97% where as water soluble ash are 16.42% and 8.25% respectively. The extractive values obtained in *E. alba* and *L. nodiflora* gives detailed information on nature of chemical constituents present with the use of specific solvent for extraction (Joseph & George 2011).

Physicochemical evaluation in the root bark of *Caesalpinia bonducella* showed ash value of 7.78%, acid soluble ash 3.34%, water soluble ash 6.16% moisture content of 10.96% (Wadkar & Sayyad 2017). These Characteristics could therefore be useful as hallmark for ascertaining the purity of the sample.

Phytochemical Analysis

Phytochemicals are chemical compounds which are non-nutritive occurs naturally in plants with diverse medicinal properties (Minakshi, *et al.*, 2016). Secondary metabolites like Alkaloids, flavonoids, phenol, tannins, coumarins etc have numerous curative properties against several human ailments. Analysing phytochemicals will provide scientist insight for the effectiveness of the plants which can lead to development of new pharmaceuticals with less side effects.

Extractive Value

On analysis the extractive value of *Annona muricata* L. in water was $7.76 \pm 0.46 \%$, ethanol - $6.23 \pm 0.47 \%$, chloroform - $4.89 \pm 0.08\%$, acetone - $3.05 \pm 0.06\%$, hexane - 2.22% and petroleum ether was $1.34 \pm 0.08\%$. In *Spermacoce articularis* L.f. the extractive value in water was $8.83 \pm 0.45\%$, ethanol - $7.13 \pm 0.30\%$, chloroform - $6.09 \pm 0.22\%$, acetone - $2.05 \pm 0.10\%$, hexane - $1.02 \pm 0.10\%$, petroleum ether - $0.22 \pm 0.05\%$. Where as in *Rauvolfia tetraphyllum* L. the extractive values were $5.83 \pm 0.16\%$, ethanol - $5.23 \pm 0.21\%$, chloroform - $3.89 \pm 0.21\%$, acetone - $2.05 \pm 0.03\%$, hexane - $1.77 \pm 0.09\%$, petroleum ether - $0.24 \pm 0.02\%$ (Table 4).

Aqueous leaf extract of *Spermacoce articularis* L.f. showed higher results when compared to other plant and the aqueous leaf extract of three plants are not significantly different at $P > 0.05$.

Qualitative phytochemical analysis

For phytochemical investigations in these selected plants such as *Annona muricata* L., *Spermacoce articularis* L.f. and *Rauvolfia tetraphylla* L. various solvents such as water, ethanol, acetone, chloroform and hexane were used.

Aqueous Leaf extract of *Annona muricata* L. showed the presence of alkaloids, flavonoids when compare to ethanol, acetone, chloroform and hexane extract. Terpenoids are totally absent, but naphthoquinones, anthraquinone glycosides showed its presence in chloroform and hexane extract (Table 5) About 212 bioactive compounds have been reported in *Annona muricata* L. the most predominant bioactive components are acetogenins followed by alkaloids, phenols etc. The parts of this plant such as leaves and seeds have major pharmacological usage. (Gavamukulya *et al.*, 2017). The compounds such as reticuline and coreximine are found abundant in this plant (Leboeuf M *et al.*, 1981) and leaves has higher alkaloid content than root, stem and fruits (Fofana *et al.*, 2012 ; Matsushige *et al.*, 2012; Yahaya, 2014 ; Chithra *et al.*, 2016).

In *Spermacoce articularis* L. f. alkaloids, flavonoid, phenol showed its presence in water, ethanol and chloroform. Protein and aminoacid in water, ethanol, and acetone extract (Table 6). More than 60 compounds such as alkaloids, iridoids, flavonoids, terpenoids etc have been reported in *Spermacoce* and *Borreria*. These compounds possess various activities such as antioxidant, anti-ulcer, anti-tumour, antimicrobial with alkaloids and iridoids as the major active principles.

In *Rauvolfia tetraphylla* L. the alkaloids, flavonoid, phenol were present in water, ethanol, acetone, chloroform where as hexane and petroleum ether extract lacks the presence of secondary metabolites (Table 7). On Phytochemical investigation this plant exhibits more alkaloids (90%) in the bark of the roots and very less quantity are found in the wood (Iqbal *et al.*, 2013).

From the above qualitative analysis it was confirmed that the aqueous extract of all the above plants gives maximum secondary metabolites when compare to other

solvents. On quantitative analysis the total Alkaloid, flavonoid, phenol content were relatively high in aqueous extract of *Spermacoce articularis* L.f. and then in *Annona muricata* L. where as aqueous extract of *Rauvolfia tetraphylla* L. yields very less secondary metabolites.

Based on the results of Qualitative and Quantitative phytochemical analysis present findings provide experimental evidences that aqueous extract of *Annona muricata* L. and *Spermacoce articularis* L.f. are equally suitable as other extracts obtained using organic Solvents.

Quantitative Phytochemical Analysis

The total alkaloid content in aqueous leaf extract of *Spermacoce articularis* L.f. (803.00 ± 11.92) is higher than *Annona muricata* L. (433.00 ± 6.43) and *Rauvolfia tetraphylla* L. (373.00 ± 5.54) (mg/gm) and the values are compared with standard atropine (Table 8).

The flavonoid content showed higher value in aqueous leaf extract of *Spermacoce articularis* L.f. (293.60 ± 4.46), than in *Annona muricata* L. (277.60 ± 4.21) and the least value was observed in *Rauvolfia tetraphylla* L. leaf extract (51.60 ± 0.78) (mg/gm) and the above values are compared with standard quercetin solution (Table 9).

On further analysis of aqueous leaf extract of *Spermacoce articularis* L.f. the phenolic content (681.75 ± 11.97) was higher followed by the extract of *Annona muricata* L. ($254.25 \pm 4.46a$) and low in *Rauvolfia tetraphylla* L. leaf extract (231.75 ± 4.07) (mg/gm) The above analysis were compared with Gallic acid (Table 10).

In all the three plant such as *Annona muricata* L., *Spermacoce articularis* L.f., *Rauvolfia tetraphylla* L. of aqueous leaf extract showed alkaloid, flavonoid, phenolic, content. The total of 11 alkaloids which contains indole (borreCAPINE), borreCOXINE, borreLINE, borreRINE, dehydroborreCAPINE, verticillatine A, and verticillatine B, bisindole (borreVERINE), isoborreVERINE and spermacoceine and tetrahydroisoquinoline [(-)-emetine] structures have been studied from *Borreria capitata*. (Jossang *et al.*, 1977 ; Jossang *et al.*, 1981).

The plant *B. latifolia* and *B. verticillata* have been reported with thirteen alkaloids. (Vieira *et al.*, 1999 ; Moreira *et al.*, 2010 ; Sainty *et al.*, 1981) and only eight flavonoids have been reported from this plant, and it also revealed the presence of pentacyclic triterpenoids of oleanane- and ursane-types (Conserva and Jesu Costa Ferreira, 2012).

In *Annona muricata L.* thirty seven phenolic compounds have been identified and the most evident compound includes quercetin (Nawwar *et al.*, 2012) and gallic acid (Correa-Gordillo *et al.*, 2012).

According to previous reports the leaves of *Rauvolfia tetraphylla L.* showed the presence of Alkaloids, tannins, flavonoids, saponins, terpenes (Patel *et al.*, 2013 ; Archana & Jeyamanikandan, 2015 ; Nandhini& Bai, 2014).

Antioxidant activity

Therapeutic properties of the plants depends on its phytochemicals and their antioxidant effects (Akinmoladun *et al.*, 2007). Oxidative properties plays a vital role in various biological phenomenon. It can also damage the cell by aggravating the oxidative stress. Oxidative stress of free radicals are studied in many ailments such as ageing, stroke, asthma, cancer etc. Antioxidants supplements can regulate such ailments by its properties. Due to adverse effects of synthetic antioxidants there is a demand for natural anti-oxidants. It includes primary and secondary natural anti-oxidants which can reduce the oxidation process by different mechanisms (Decker *et al.*, 2005).

Free radical scavenging activity is the known mechanisms by which H₂O₂ free radical scavenging activity exhibits neutralization by either transfer of hydrogen or an electron. It is widely used to investigate the potential of the compound as free radical scavengers of hydrogen donors and to bring out the potential of antioxidant properties of the plant extract (Shimada *et al.*, 1992).

The present study demonstrates that percentage of scavenging effect on the H₂O₂ radical was increased with the increase in concentration of the plant extract.

The aqueous leaf extract of *Annona muricata* L. showed inhibition 29.13 ± 0.58 at $20\mu\text{g/ml}$ where as it increased up to 70.07 ± 0.22 at $100(\mu\text{g/ml})$ (Table - 11 & Fig - 2).

In aqueous leaf extract of *Spermacoce articularis* L.f. the percentage of inhibition increased from 23.64 ± 0.72 at $20\mu\text{g/ml}$ to 74.42 ± 2.56 at $100\mu\text{g/ml}$ (Table - 12 & Fig - 3).

The aqueous leaf extract of *Spermacoce articularis* L.f. showed high antioxidant activity than the extract of *Annona muricata* L. and they are significant at $P < 0.001$ level Both the plant showed potential antioxidant activity. So it is considered as good source of natural antioxidants in treating various diseases.

Annona muricata L. had gain more significance for antioxidant activities. Many antioxidant activites have been screened against oxygen derived from free radicals which results in development of many diseases such as arthritis, cardiovascular diseases, cancer and degenerative health hazards such as Parkinson and Alzheimer (Almeida *et al.*, 2011). Different plant parts of *Annona muricata* L. and solvents have been evaluated for various antioxidant assays. The major components were mainly lipophilic and the mechanism of action is by hydrogen. (Correa-Gordillo *et al.*, 2012). The various extracts such as ethanolic, n-butanolic, methanolic and aqueous extract possessed antioxidant activity assayed by DPPH activity. The aqueous extract showed 1000 times less activity than butylated hydroxytoluene (Alitonou *et al.*, 2013). A positive correlation was observed between antioxidant and the total polyphenol content (Gavamukulya *et al.*, 2014 ; George *et al.*, 2012).

Biological activities of crude extracts and fractions of *Borreria* and *Spermacoce species* have also been reported (Conserva & Jesu Costa Ferreira, 2012).

In *Rauvolfia tetraphylla* L. the plant parts such as root, leaf and fruit indicates antioxidant activity. The methanolic leaf extract of *Rauvolfia tetraphylla* L. were found effective in DPPH scavenging activity (Nair *et al.*, 2012 ; Archana and Jeyamanikandan, 2015 ; Shyma *et al.*, 2013).

Antimicrobial activity

Medicinal plants extract has wide usage as potential antimicrobial activity due to its bioactive molecules. Many bioactive molecules are screened as raw material for medicinal applications (Renisheya *et al.*, 2011). Due to hazardous effects on synthetic drugs many scientists focused on natural products. It is estimated that about 35,000 to 70,000 plants have been in use as medicinal usage out of 4, 22, 127 plants reported world wide (Bibi *et al.*, 2011).

Antimicrobial activity of the plant extracts such as *Annona muricata* L. and *Spermacoce articularis* L.f. was tested against the microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Aspergillus niger*, *Aspergillus flavus*. Various solvents such as water, chloroform, ethanol, acetone, petroleum ether, hexane extracts were used for this purpose.

The aqueous extract of *Annona muricata* L. showed inhibition against *Staphylococcus aureus* (11.00 ± 0.21) where as its hexane extract exhibited good inhibition against *Klebsiella pneumonia* (12.00 ± 0.74). However fungal strains showed very less inhibition by this plant extract (Table 13) (Plate -7 A-E).

In aqueous leaf extract of *Spermacoce articularis* L.f. most of the strains experienced antimicrobial effect. *Staphylococcus aureus* showed maximum zone of inhibition (12.00 ± 0.74) and *K. pneumoniae* showed inhibition of 12.00 ± 0.74 in hexane extract. Fungal strain such as *Aspergillus niger*, *Aspergillus flavus* showed very less inhibition. From the above studies it is concluded that aqueous extract of *Spermacoce articularis* L.f. has higher potential antimicrobial activity compared to the aqueous extract of *Annona muricata* L. (Table 14) (Plate- 8 A-E).

The plant *A. muricata* L. exhibited antimicrobial activity against gram positive and gram negative bacteria. Ethanolic and methanolic extracts of this plant showed potential antimicrobial activity against *Staphylococcus aureus* but the aqueous extract of *A. muricata* L. exhibits no activity.

The antibiotic activity exhibited by this plants is attributed to the presence of bioactive compounds such as steroids, flavonoids and alkaloids (Radji *et al.*, 2015)

The compound 6-methyl-5-cyclodecen-1-ol (54) was extracted from *B. articularis*. The compound has potential antimicrobial activity with the inhibition zone of MIC 500 - 2000 µg/mL against *S. typhi*, *S. paratyphi*, *P. aeruginosa*, *S. dysenteriae*, *E. coli*, *S. aureus*, *B. megaterium*, *Bacillus cereus*, *S. sonnei* and *V. cholera* and it also exhibited antifungal activity with the inhibition zone of MFC 1500 - 3000 mg/mL) against *Aspergillus niger*, *A. ochraceus* and *A. ustus* (Sultana *et al.*, 2008).

The antimicrobial activities are therefore attributed to the presence of bioactive molecules such as Saponins, tannins, Alkaloids and phenolic compounds which lead to therapeutic usage (Javid *et al.*, 2015).

***In vitro*-anti inflammatory activity**

Anti-inflammatory drugs are commonly called as NSAID (non-steroidal anti-inflammatory drugs), Inflammation occur due to the autocoid potential of prostaglandin which release extracellularly and initiate pain. Anti-inflammatory drugs inhibits the autocoid by synthesing CO_x cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandin. Inflammatory drugs protect the lysosomal membrane to prevent inflammation (Chowdhury *et al.*, 2013).

The anti-inflammatory action of the aqueous extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were tested against HRBC membrane as the HRBC membrane is similar to the membrane of lysosomal membrane.

From the analysis the aqueous leaf extract of *Annona muricata* L. at 50 µg/ml showed 20.26 ± 1.52% prevention of lysis, where as at the concentration of 200 µg/ml it exhibits 69.68 ± 1.49% prevention of lysis. In *Spermacoce articularis* L.f. the percentage of prevention of lysis at 50 µg/ml showed 22.36 ± 0.62% where as at the concentration of 50 µg/ml it exhibits 74.23 ± 0.70% prevention of lysis. In standard drug of Diclofenac at 500 µg/ml the prevention of lysis percentage was 87.56%. The above evidence clearly emphasis that *Spermacoce articularis* L.f. has higher anti-oxidant and anti-inflammatory activity and both the plant sample are significant at P < 0.001 level (Table 15).

It has been indicated by (Laksmiawati *et al.*, 2016) that *Annona muricata* L. extract possess anti-inflammatory potential that could be attributed to mediators including TNF - α , IL - 6 and NO.

Till date more than 60 compounds have been identified from *Spermacoce* and *Borreria* that includes iridoids, alkaloids, flavonoids, terpenoids etc. These compounds have diverse biological properties such as anti-inflammatory, antitumour, antimicrobial and hepatoprotective with iridoids, alkaloids as major biological components.

Thin Layer Chromatography TLC

TLC and column Chromatography have been used for identification and extraction of acetogenin from *Annona muricata* L. leaves by Vinothini and Growther (2016). These acetogenins are nontoxic to normal cells but toxic to cancer cells.

TLC profiling reveals the presence of different bioactive compounds such as alkaloids, flavonoids, phenols and tannins in plants. Different Rf values of the compound provides an idea about their polarity. Present study confirms the presence of many important phytochemicals which has medicinal significance that generate characteristics physiological actions against different ailments.

On TLC analysis, alkaloids with the Rf value 0.85 and 0.72 with deep violet colour were noted in aqueous extract of *Spermacoce articularis* L.f. where as *Annona muricata* L. showed one spot with mild violet (Rf value 0.64) (Table 16).

The presence of flavonoids were confirmed by one distinct spots with yellow in colour in *Spermacoce articularis* L.f. (Rf value 0.71), where as *Annona muricata* L. showed one spot with yellowish green (Rf value 0.64) (Table 17).

The presence of phenols were confirmed by two distinct spots with different intensities and bluish black in colour (Rf value 0.51 and 0.62) in *Spermacoce articularis* L.f. where as *Annona muricata* L. showed one spot with blue colour (Rf value 0.44) (Table 18).

Thus the above TLC profiling shows the presence of alkaloids, flavonoids, phenol and methanol was found the best suited solvent for extraction purpose.

These studies have been employed to identify the two species of *Spermacoce* namely *Spermacoce articularis* L.f. and *Spermacoce exilis* by Keat *et al.*, (2010).

FTIR

Fourier Transform Infrared Spectroscopy is one of the technique to provide structural features of large range of compounds (Agatonovic *et al.*, 2013). It has been widely used analytical method to identify the phytochemicals in the plants. It is a reliable and sensitive method for finding the functional groups present in the plant and confirmed with the aid of IR region in the range of 400 - 4000cm⁻¹ (Florence and Jeeva 2015).

The present study was undertaken to identify the functional groups and elucidate the chemical structure present in the aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. . On analysis *Annona muricata* L. aqueous leaf extract shows the presence of alcohol, phenols, alkanes, aldehydes, saturated aliphatic acid, aryl nitrocompounds, sulphur compounds with major peaks at 3981.25 and 3957.93, 3471.87, 2399.45, 1749.08 and 1728.23, 1689.34, 1442.75, 1304.90 and 1210, 667.40 and 655.83, 457.15 (Table 19 & Figure - 4).

In case of *Spermacoce articularis* L.f. the FTIR confirms the presence of amine, phenol, carboxylic acid, aldehyde, saturated aliphatic sulphur compound, α , β unsaturated aldehydes, ketones which shows major peaks at 2538.32, 2365.59, 2314.58, 2252.82, 1982.82, 1882.52 and 1759.08 (Table 20 & Figure- 5).

The above results also confirms the presence of many bioactive components in both the plant extracts. The aqueous extract of the *Spermacoce articularis* L.f. has more bioactive compounds than the other extract.

Preliminary phytochemical screening and FTIR studies of *Annona muricata* L. bark has been done by Manigandan *et al.*, (2015). Phytochemical screening by FTIR analysis of leaf extracts of selected Indian medicinal plants has also been done by Ashokkumar & Ramaswamy (2014).

UV-Spectral Analysis

UV-Visible spectrophotometer analysis of leaf extracts of *Annona muricata* L. showed narrow absorption band at 472 nm. In the present study, the formation of

silver nanoparticles was confirmed by the bands observed in the range of 200-800nm as shown in the Figure-6.

In the aqueous leaf extract of *Spermacoce articularis* L.f. the two narrow bands were observed in 206 and 472 nm in water extract and the formation of silver nanoparticles were confirmed by the bands observed in the range of 200-800nm were shown in the Figure- 7.

GC-MS

Gas chromatography and mass spectroscopy (GC-MS) has been widely used technique for identification of bioactive compounds in plants. The combination of separation (GC) and identification technique (MS) made GC-MS an ideal process to analyse volatile and semi-volatile bioactive compounds (Grover and Patni 2013).

The GC-MS analysis in the aqueous extract of *Annona muricata* L. revealed the presence of various bioactive compounds such as Dodecane (CAS), Trans-caryophyllene, 2-propenoic acid, 3-(4-methoxyphenyl)-ethylester, 2-hexadecen-1-ol, 3, 7, 11, 15-tetramethyl, [R-[R*,R*-(E)]]-(CAS) (Table 21 & Figure- 8). Where as in *Spermacoce articularis* L.f. more predominant bioactive compounds were found such as Generol, Geranyl acetate, 2-propenoic acid, 3-(4-methoxyphenyl)-, ethylester, Benzenemethanol, a-ethyl-4-hydroxy-(CAS). The compounds present in the above plant extract shows various pharmacological actions such as anticancerous, anti-inflammatory, anti-microbial activity, antioxidant properties (Table 22 & Figure- 9).

Soosairaj *et al.*, (2013) have also analysed the phytochemicals of *Spermacoce articularis* L.f. by GC-MS. Similar analysis of bioactive phytochemicals present in ethanolic extracts of leaves of *Annona muricata* L. has been studied by Gavamukulya *et al.*, 2015.

To conclude, the plant has new persepective in pharmaceutical research for the development of potential drugs for anti-inflammatory, antimicrobial, anticancer as revealed by the bioactive components (Table 30 &31).

Table -30**Bioactivity of phytocomponents identified in the aqueous extract of*****Annona muricata* L. by GC-MS**

| S. No. | RT | Name of the compound | Nature of compound | Activity |
|--------|-------|-------------------------------------------------------------|--------------------------------|---------------------------------------------------------------------------------------------------------|
| 1. | 6.81 | Dodecane (CAS) | Alkane hydrocarbon | No activities reported |
| 2. | 11.38 | Trans-caryophyllene | Natural bicyclic sesquiterpene | Anti-inflammatory anxiolytic and antidepressant |
| 3. | 16.07 | 1-tetradecanamine, N, N-dimethyl-(CAS) | Tertiary amines | Anticancer |
| 4. | 21.70 | 2-propenoic acid, 3-(4-methoxyphenyl)-ethylester | Alkene compound | Anticancerous activity, antidiabetes, |
| 5. | 25.22 | 2-hexadecen-1-ol,3,7,11,15-tetramethyl,[R-[R*,R*-(E)]-(CAS) | Phytol | Antimicrobial Anticancer Antiinflammatory |
| 6. | 33.18 | Di-(2-ethylhexyl) phthalate | diester of phthalic acid | most common phthalate plasticizer in medical devices such as intravenous tubing and bags, IV catheters, |

Table-31**Bioactivity of phytocomponents identified in the aqueous extract of***Spermacoce articularis* L.f. by GC-MS

| S. No. | RT | Name of the compound | Nature of compound | Activity |
|--------|-------|---------------------------------------------------------|--------------------------|---------------------------------------------------------------------------------------------------------|
| 1. | 9.13 | Geranol | Geranyl alcohol | Antitumor, antioxidant and anti-inflammatory properties |
| 2. | 11.06 | Geranyl acetate | Monoterpene | Use in fragrances |
| 3. | 16.21 | 1 tetradecanamine N, N-dimethyl-(CAS) | tertiary amines | Anticancer |
| 4. | 21.62 | 2-propenoic acid, 3-(4-methoxyphenyl)-, ethylester | Alkene compound | Anticancerous activity, |
| 5. | 26.22 | Benzenemethanol, a-ethyl-4-hydroxy-(CAS) | Benzyl Alcohol | Anticancer |
| 6. | 27.30 | (Z)-Ethyl6-oxabicyclo [3,0] hexan-2-ylidenecyanoacetate | acetate | No activities reported |
| 7. | 33.19 | Di-(2-ethylhexyl) phthalate | diester of phthalic acid | most common phthalate plasticizer in medical devices such as intravenous tubing and bags, IV catheters, |

Heavy metal analysis

Plants play a vital role in transferring heavy metals to humans from contaminated soil. Heavy metal accumulation results in hazardous effects on human. Metals such as copper, zinc, iron, manganese, nickel, chromium are vital elements for physiological and biological functions of the body. If it exceeds permissible limits it leads to many ailments such as cardiac dysfunction, immunological diseases, foetal malformation, neurological behavior etc. (Korfali *et al.*, 2013) Heavy metal contamination occurs due to several factors such as oxidation reduction potential of the soil, chemical and physical factors, storage at the point of sale (Verma *et al.*, 2007).

From the present analysis in aqueous leaf extract of *Annona muricata* L. the cadmium limits were 0.078 ± 0.01 ppm and in *Spermacoce articularis* L.f. result showed 0.048 ± 0.01 ppm, both the plants have permissible limit of cadmium.

The amount of Lead concentration in both the plants analysed were found to be 0.671 ± 0.03 ppm in *Annona muricata* L. and 0.058 ± 0.01 ppm in *Spermacoce articularis* L.f. and the result showed both the plant exhibits lead within permissible limit.

The amount of Zinc concentration in an aqueous extracts of *Annona muricata* L. 0.184 ± 0.01 ppm whereas in *Spermacoce articularis* L.f. aqueous extract it was 0.120 ± 0.02 ppm.

The aqueous leaf extract of *Annona muricata* L. showed 0.372 ± 0.01 ppm of nickel whereas in *Spermacoce articularis* L.f. leaf aqueous extract 0.453 ± 0.01 ppm of Nickel was found.

Iron concentration in aqueous extract of *Annona muricata* L. was 0.032 ± 0.01 ppm and in *Spermacoce articularis* L.f. aqueous extract 0.042 ± 0.01 ppm was recorded all these concentrations were found to be below the permissible level (Table 23).

These results suggest that use of aqueous leaf extract of these as crude drugs may not pose any hazards results as they do not accumulate heavy metals. However care should be taken to grow the plants in areas with no heavy metal contamination

and if cultivated the tendency of heavy metal accumulation in these plants must be checked before their use as crude drugs.

Pharmacology Study

Acute Toxicity studies

Phytotherapy has more significant ethnomedicinal use and insist safety evaluation of herbal medicines. The current study was to assess the toxicity of plants which is determined by acute toxicity method for screening mortality or safe use of drugs. The present study has been undertaken to prove the safe use of *Annona muricata* L. and *Spermacoe articularis* L.f. medicinal plants extract in mice.

In the aqueous leaf extract of *Annona muricata* L. and *Spermacoe articularis* L.f. the results of acute toxicity at the concentration of 2000mg/kg is found to be safe as per OECD-423 guide lines. On oral administration of aqueous extract of both the plants no mortality or lethality was observed after 24hrs and 72hrs. To conclude both the above leaf extract was considered to be safe for human consumption (Table 24 &25) (Plate 9). Hepatoprotective activity of methanolic extract was found to be 2g/kg by Parameshwar *et al.*, 2010.

However the acute toxicity of the aqueous extract of *Annona muricata* L. was determined to be < 5000mg/kg body weight by Larbie *et al.*, 2011, where as ethanolic extract when given at this concentration the mortality rate was 100 % (Gbaguidi *et al.*, 2017).

Analgesic activity

Pain is an unpleasant sensation and considered as a public health problem with socioeconomic effects. It indicates several illness and has adverse effects in humans. The pain requires treatment using analgesics but current therapy leads to adverse effects such as gastrointestinal problems, irritations etc. Many scientist have suggested that phytotherapy can be a significant source of natural medicines.

The present study was conducted using the aqueous leaf extract of *Annona muricata* L. and *Spermacoe articularis* L.f.. In swiss albino mice using Eddy's Hot plate method at the dosage of 400mg/kg dose of aqueous leaf extract of *Spermacoe*

articularis L.f. 12.0 ± 0.94 at 60 minutes showed more significant activity than *Annona muricata* L. (10.6 ± 0.72). The above effect are comparable to standard drug Pentazocine. The experiments showed that extracts of *Spermacoce articularis* L.f. has significant analgesic activity which could be of use in pharmaceutical industries. (Table 26 and Figure - 10).

Analgesic activity of *Spermacoce hispida* aqueous extract has also been reported by Vinayak Meti *et al.*, (2013)

Anti-pyretic effect

Pyrexia is an unpleasant condition which elevate body temperature. It imparts secondary infection or other diseased states. It is a natural defense mechanism against pathogen survival. Many scientist depends on herbal drugs for less side effects. Most of the anti-pyretic drugs inhibit CO_{x-2} which are highly toxic to hepatic cells, glomeruli, cortex, heart muscles etc. where as plant based CO_{x-2} inhibitors exhibits very less side effects (Luo and Bohlin, 2005).

Kumar and Venkatarathanamma 2015 have showed that the methanolic extracts of leaves of *Annona* plants are nontoxic and possessed significant antipyretic activity which they have attributed to the flavonoids, saponins in the extracts.

This study was oriented to evaluate the antipyretic activity of aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. using albino rats by observing its antipyretic activity induced by brewer's yeast. In case of aqueous leaf extract at the concentration of 400 mg/kg *Annona muricata* L. showed significant result (*P < 0.05) where as concentration of *Spermacoce articularis* L.f. at 400 mg/kg the plant extract showed significant results at P < 0.01 which is higher than *Annona muricata* L. and this effect is comparable to standard Aspirin. To conclude the present report indicates that *Spermacoce articularis* L.f. can be suggested for medical practitioners as an antipyretic remedy (Table 27 and Figure - 11).

Similarly Antipyretic activity of ethanolic root extract of *Asparagus racemosus* showed significant antipyretic effect compare to aqueous extract on yeast induced pyrexia in rats. The results indicated high antipyretic activity in ethanol

extract which may be due to high flavonoid and tannin content (Vasundra & Divya, 2013).

Anti-inflammatory activity

Inflammation is the response against harmful stimuli to localize or eliminate the harmful agents. Inflammation if left untreated leads to adverse effects such as rheumatoid arthritis, atherosclerosis etc. Certain synthetic drugs such as opioids indicate the blocking mechanism of prostaglandins by irreversibly inhibiting synthesis of CO_{X-2} therefore resulting in more side effects. Biological researchers focused on natural products for anti-inflammatory activity which has yielded numerous compounds extracted from plants for the development of modern drugs.

The anti-inflammatory activity of aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were observed in albino rats by carrageenan induced paw oedema test.

In control (Distilled Water) carrageenan elevated paw volume in each hour. In reference control of indomethacin less paw volume was noticed. In case of *Spermacoce articularis* L.f. at the end of 5th hr paw volume showed least volume 9.04 ± 0.61 which is more significant at $P < 0.001$. In the aqueous leaf extract of *Annona muricata* L. at 400 mg/kg at the end of 5th hr of administration of extract showed the paw volume of about 13.06 ± 0.90 which is also more significant at $P < 0.001$ and it is comparable to the standard drug Indomethacin with the paw volume of 8.74 ± 0.62 .

To conclude the aqueous extract of *Spermacoce articularis* L.f. exhibited potent anti-inflammatory activity. Compared to other plant extract this plant can be effectively used as an anti-inflammatory drug in future (Table 28 and Figure - 12).

Analgesic and anti-inflammatory property of *Annona muricata* L. fruit was attributed to epidergic pathway and inhibition of chemical mediators of antiinflammation respectively (Ishola et al., 2014).

It was also suggested that oedema inhibitory effect of various solvent extracts of *Spermacoce articularis* L.f. may be due to the presence of ursolic acid in the extracts (Vadivelan et al., 2007).

***Invitro* Evaluation of anticancer activity by MTT assay**

Medicinal plants play a significant role in novel drug research. The search for new anticancer agents is a long term goal followed by isolation, identification of bioactive compounds. Screening of natural bioactive components from plants are considered as excellent anticancer agents.

The biological investigations of cytotoxicity using plant extract is essential to determine the aptitude of the compound. The WHO promoted the use of medicinal plants for their efficacy, affordability and safety. More than 50% of drugs extracted from natural products were in clinical trials for anticancer activity.

MTT assay is a technique to determine the viability of cells. The assay was carried out using MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5 diphenyl tetrazolium bromide). MTT is cleaved with mitochondrial dehydrogenases and gives measurable purple colour product formazan. The purple colour formation is inversely proportional to the cytotoxic activity. It indicates the antiproliferative activity based on the principle of formazan colour measurement which is formed by the enzymes in the mitochondria. (Stockert *et al.*, 2012).

In the present study the aqueous leaf extract of *Spermacoce articularis* L.f. and *Annona muricata* L. were investigated for cytotoxic studies. The aqueous leaf extract of *Annona muricata* L. showed very less activity with IC₅₀ values at 365.60/mL (Plate 10).

The aqueous extract of *Spermacoce articularis* L.f. against HeLa cells (Human Cervix Cancer Cells) showed IC₅₀ concentration values at 410.63/mL after incubation of 24hrs at 37° C (Plate 11).

The observations strongly suggest that the test compound *Spermacoce articularis* L.f. possess potential anticancer activity against HeLa cell lines and further drug design is required to enhance its potential (Table 29).

DNA fragmentation assay

Cancer is the dreadful disease due to free radical damage resulting in degeneration of lipids, protein DNA etc. Cancer cell leads to abnormal growth due to defective caspases mediated cell death (apoptosis) (Zimmerman *et al.*, 2013). Many

herbs exerts anticancer effect on abnormal cancerous cells. Synthetic drugs target normal cells and results in hazardous effects such as hair loss, gastrointestinal problem, canal distress etc. Plant based cytotoxic studies leads to the discovery of new drugs in pharmaceutical industries.

The aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were treated with HeLa cell lines (Human carcinoma cervical cancer cell). The trypsinized cell were subjected to DNA fragmentation analysis. The DNA migrated as discrete bands giving ladder of 100 base pairs due to endogenous cleavage activity (Plate 12).

From the analysis it is concluded that *Spermacoce articularis* L.f. showed high apoptosis activity compare to *Annona muricata* L. Anti-leukemia properties of *Spermacoce* species have been reported by Sukari *et al.*, 2013.

According to the report by Hasrat *et al.*, 1997 ; Matsushige *et al.*, 2012 ; Lannuzel *et al.*, 2002 ; Nawwar *et al.*, 2012, the alkaloids from *Annona* stimulates antidepressant, neurotoxic by apoptosis and cytotoxic effects. The anticancer property of *Annona muricata* L. is due to cytotoxic effect. The hydroalcoholic extracts of *Annona muricata* L. at the concentration of 1.6 mg/mL and 50 mg/mL increase the viability of normal cells while 100 mg/mL didn't alter the viability. This selective studies confirms the healing with less side effects.

Anticancerous compounds analysed by GC-MS studies (Table - 21, 22) could be attributed for this property. Anticancerous effect of aqueous extracts of leaves of *Annona muricata* L. and *Spermacoce articularis* L.f. studied in the present work suggests us the possibility of using such extracts against cancer cell as crude drug with out being extracted using other solvents.

To conclude aqueous leaf extracts of these plants could be used as anti-inflammatory, antipyretic, analgesic and anti cancer drug.