

**Studies on Pharmacognostic and Pharmacological properties of  
*Annona muricata* L. and *Spermacoce articularis* L.f. from Velliangiri  
hills, Coimbatore, Tamilnadu and Evaluation of their Potential as  
Anticancer Drug**

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fulfilment for the award of the degree of  
Doctor of Philosophy in Botany (Interdisciplinary - Biotechnology)*

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# *Summary*

## SUMMARY

Naturally occurring phytochemicals possess medicinal properties in the treatment of deadly diseases. Natural products attracted many researchers due to their diverse range of therapeutic properties and for its safe, ecofriendly and less toxicity. Evaluation of pharmacological and pharmacognostic properties of plants is vital to understand the complex synergistic interaction of various constituents of herbal drugs. Animal based studies ensure the accuracy and potentiality of the drug and also to ascertain the positive health effects in medical services.

These realities are taken in to consideration to evaluate pharmacological and pharmacognostic studies on selected plants such as *Annona muricata* L., *Spermacoce articularis* L.f., *Rauvolfia tetraphylla* L. and to bring out the anticancer properties.

Ethanobotanical survey of medicinal plants was done in Velliangiri hills, Coimbatore, Tamilnadu, India. Based on the traditional knowledge and frequent usage by tribes three plants were selected. The leaves of the selected plants namely *Annona muricata* L., *Spermacoce articularis* L.f., *Rauvolfia tetraphylla* L. that shows high ethanobotanical properties were tested for their macroscopic, microscopic and organoleptic characters to authenticate the plants for their use as crude drug and differentiate them from adulterants.

The macroscopic character of *Annona muricata* L. reveals that it is a small evergreen tree which grows in humid conditions. The root system is superficial, trunk is straight with dark brown characteristics where as the leaves are glossy with alternate arrangement. Flowers are inconspicuous, fruit is average about 4kg, ovoid heart shape with large spines with 127-170 black seeds embedded in scattered pulp.

On macroscopic evaluation *Spermacoce articularis* L.f. showed its procumbent, nature, scabrid herb prevalent among Coimbatore and Nilgiris district. The root commonly used for wound healing. The stem is quadrangular, leaves are subsessile, oblong 1-3.5cm long, flowers are small with stipular cup blue or white in colour. The fruit exhibits hairy characteristic capsule and seeds are granulated.

In *Rauvolfia tetraphylla* L. is a evergreen shrubs found in tropical region leaves are elliptic ovate with reticulate venation. With round stem texture fibrous and

rough, hairy surface, green outside and creamish yellow internally. The root is about 8 to 15cm long, Flowers are white bisexual, with violet colour tinge . The fruit is a drupe, 0.5 cm in diameter.

Leaves of *Annona muricata* L. are oblong, pellucid dotted, The T.S of lamina shows the presence of epidermal layers, lower surface of the leaves has stomata. The T.S of midrib shows collenchymatous cell.

T.S of *Spermacoce articularis* L.f. has only one type of trichome. The vascular bundle is crescent shaped and collateral with xylem facing adaxial side and phloem towards the abaxial side. Xylem elements are thick walled and compactly arranged. Phloem has patches of blast fibres.

In T.S of leaf of *Rauvolfia tetraphylla* L. the upper epidermis of leaf has uniseriate, multicellular trichomes. The T.S of midrib of leaves contains a single layer of upper and lower epidermis with a thin cuticle. The vascular bundles composed of xylem at the centre and phloem on both the sides.

The organoleptic character such as appearance, colour, touch, taste, odour were studied in these plants *Annona muricata* L. leaves exhibits green colour, characteristic odour with bitter taste Where as *Spermacoce articularis* L.f. leaf study exhibits the characters like dark green, characteristic odour and slightly bitter taste with coarse touch. In *Rauvolfia tetraphylla* L. the colour of the leaves shows green colour, stability of the powder exhibits pasty with the characteristic odour.

The leaves of these plants were tested for their phytochemical constituents for this purpose. Initially suitable solvents were tested to obtain higher extractive values. The phytochemicals were then analysed and identified using TLC, FTIR and GC-MS techniques.

Physicochemical analysis such as determination of total ash, sulphated ash, acid insoluble, water soluble ash and moisture content were done. The yield of water soluble extractive values of *Spermacoce articularis* L.f. was more than *Annona muricata* L. and *Rauvolfia tetraphylla* L.

The leaf powder of *Annona muricata* L., *Spermacoce articularis* L.f., *Rauvolfia tetraphylla* L. were extracted with water, ethanol, chloroform, Acetone,

Hexane and petroleum ether. The results suggested that water could be used as the solvents for secondary metabolites extraction in the above three plants.

The presence of various phytochemicals in the above three plants were confirmed by qualitative tests. The result showed the presence of Alkaloids, Flavanoids in Water, Ethanol, Acetone, Chloroform, Hexane extract of *Annona muricata* L. Coumarin shows its presence only in water and hexane extract.

In the plant extract of *Spermacoce articularis* L.f. it showed the presence of Alkaloids, flavonoid, phenol shows its presence in water, ethanol and chloroform protein and aminoacid is present in water, ethanol, acetone extract.

In *Rauvolfia tetraphylla* L. water, ethanol, acetone, chloroform shows the presence of alkaloids, flavonoid, phenol where as hexane and petroleum ether extract lacks the presence of secondary metabolites.

The amount of phytochemicals which are found in the selected plants was quantitatively determined by standard procedures. The total alkaloid, flavonoid and phenolic contents in aqueous extract of *Spermacoce articularis* L.f. was found to be high when compare to *Annona muricata* L. and least content showed in *Rauvolfia tetraphylla* L.

Aqueous extraction yield maximum result in all the three plants when compared to other extracts. Based on the qualitative and quantitative results the plant *Annona muricata* L., *Spermacoce articularis* L.f. were selected as suitable for further studies.

The extracts were tested for their biological activity by *Invitro* and *Invivo* tests and possible compounds responsible for such activity were assessed using TLC, UV spectroscopy, FTIR and GC-MS techniques.

Antioxidant property of aqueous extract of *Annona muricata* L., *Spermacoce articularis* L.f. was investigated by H<sub>2</sub>O<sub>2</sub> radical scavenging activity. The results suggested that the free radical scavenging activity were found to be increasing with increase in extract concentration from 20µg/ml to 100µg/ml. The result clearly revealed that the aqueous leaf extract of *Spermacoce articularis* L.f. showed higher free radical scavenging activity compare to *Annona muricata* L.

The results of antimicrobial activity assay demonstrated that aqueous leaf extract of *Spermacoce articularis* L.f. showed good antimicrobial activity against most of the bacterial strains. *Staphylococcus aureus* showed maximum zone of inhibition and *K. pneumoniae* showed inhibition in hexane extract of *Spermacoce articularis* L.f. Where as aqueous extract of *Annona muricata* L. exhibited good inhibition against *Staphylococcus aureus*, hexane extract exhibited good inhibition against *Klebsiella pneumoniae*. Fungal strain such as *Aspergillus niger*, *Aspergillus flavus* showed very less inhibition. The antimicrobial potential was comparatively high in *Spermacoce articularis* L.f. compared to *Annona muricata* L.

*In vitro* anti-inflammatory activity of aqueous extract of *Annona muricata* L., *Spermacoce articularis* L.f. were determined by Human Red Blood Cell membrane stabilization method (HRBC) Effectiveness was tested in comparison with the standard drug Diclofenac. The results indicated that membrane stabilizing profiles at the concentration of 50 µg/ml are higher in the aqueous leaf extract of *Spermacoce articularis* L.f. compared to *Annona muricata* L.

Possible phytochemicals present in the extracts when tested by standard methodologies. TLC results confirmed the presence of alkaloid, flavonoid and phenol with two distinct spots in aqueous leaf extract of *Spermacoce articularis* L.f. and with one spot in *Annona muricata* L.

The FTIR analysis of aqueous leaf extract of *Spermacoce articularis* L.f. showed broad absorption spectrum at various position indicating the spectral evidences of the compounds such as amine, phenol, carboxylic acid, aldehydes, saturated aliphatic, sulphur compound, alkanes and  $\alpha$ ,  $\beta$  unsaturated aldehydes and ketones. Aqueous leaf extract of *Annona muricata* L. showed the presence of alcohol, phenols, amine, alkanes aldehydes, saturated aliphatic acid, aryl nitro compounds,  $\alpha$ ,  $\beta$ -unsaturated aldehydes, ketones and sulphur compounds.

GCMS results also confirmed the presence of phytocompounds in *Annona muricata* L. The first compound identified with less retention time (6.81 min) and peak area % was 2.02. It was Dodecane (CAS) with molecular formula  $C_{12}H_{26}$  and Molecular Weight 170 and the last compound identified with less retention time 33.18 min and peak area % 7.63 was Di-(2-ethylhexyl) phthalate with molecular formula  $C_{24}H_{38}O_4$  and molecular weight 390.

In the aqueous extract of *Spermacoce articularis* L.f. the first compound identified with the retention time 9.31 min and peak area % 9.23 was General with molecular formula  $C_{10}H_{18}O$  and with molecular weight 154 and the last compound identified with less retention time 33.18 and peak area % 7.63 was Di-(2-ethylhexyl) phthalate with molecular formula  $C_{24}H_{38}O_4$  and molecular weight 390. The compounds identified by GC-MS have various pharmacological properties.

On heavy metal analysis it was understood that the metals such as Cadmium, Lead, Zinc, Chromium, Iron in the aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were at the permissible limits and they were significant at  $P < 0.001$  level.

The *Invivo* therapeutic potential of the leaf extracts were studied using swiss albino rats for various biological activities showed the following results. The study of assessment of the toxic potential after oral administration of aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. did not cause any mortality up to 2000mg/kg and was considered as safe as per OECD-423 guide lines.

The analgesic activity of aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. was carried out on swiss albino mice by Eddy's Hot Plate method. The pain threshold was measured at different time intervals. Aqueous leaf extract of *Spermacoce articularis* L.f. at a dose of 400mg/kg showed more significant ( $P < 0.001$ ) results at 60 minutes time in swiss albino mice and this effect was comparable to standard drug Pentazocine. *Spermacoce articularis* L.f. showed maximum activity when compared to *Annona muricata* L.

The aqueous leaf extract of *Spermacoce articularis* L.f. at 400mg/kg showed moderate significant results at ( $P < 0.01$ ) in yeast induced pyrexia in rats, in lowering the hyperthermia (antipyretic) than the aqueous leaf extract of *Annona muricata* L. but found to have similar effects as the standard drug aspirin administration.

The anti-inflammatory activity of aqueous extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were studied in albino rats by observing its anti-inflammatory activity induced by carrageenan. In case of *Annona muricata* L. at 400 mg/kg exhibited more significant result within 5 hr of administration of the extract.

Where as the aqueous leaf extract of *Spermacoce articularis* L.f. at 400 mg/kg showed more significant result .

From the above analysis it was confirmed that the aqueous leaf extract of *Spermacoce articularis* L.f. has potential antioxidant, anti-inflammatory activity which is essential for anticancer effect. Further studies were then enhanced to check anticancer potential of these plants.

MTT calorimetric assay against HeLa cells using the aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. were tested. The assay determines the cell cytotoxicity and cell proliferation. The results indicated that the given test compound *Spermacoce articularis* L.f. showed IC 50 concentration (The Concentration of the Compound have the capacity to kill 50% of Viable Cells) against the HeLa Cells at the 410.63 /mL after the treatment of 24hours of incubation at 37°C temperature, where as the extract of *Annona muricata* L. relatively showed less activity. The observations strongly suggested *Spermacoce articularis* L.f. can be used as potential anticancer agent against Human Cervix Cancer Cells.

DNA fragmentation assay was also carried out to check for apoptosis against HeLa cell lines using the aqueous leaf extract of *Annona muricata* L. and *Spermacoce articularis* L.f. DNA migrated as discrete bands when compared to DNA markers giving a ladder appearance. Such ladders indicate hall mark of apoptosis. The result also confirmed that extract of *Spermacoce articularis* L.f. showed high apoptosis activity than *Annona muricata* L.

To conclude the aqueous leaf extracts of *Annona muricata* L. and *Spermacoce articularis* L.f. have potential anticancer property and suggested the possibility of their aqueous leaf extract as crude drugs for oral administration.