PLAN OF WORK AND METHODOLOGY

1. Exhaustive literature survey

   Literature will be search through Journals, e-Journals, Reference books, news etc.

2. Procurement and authentication of plant materials:

   The plant materials will be collect from the vicinity of Nallamalai forests. The authentication will be carry out by the botanist, Department of Botany, SKU, Anantapur.

3. Extraction Technique:

   The extraction technique includes extraction of (Secondary metabolites) crude extract from plant material and extraction of volatile oil from plant material.

4. Extraction of volatile oil from plant material:

   The extraction of volatile will be done by hydro distillation process with the help of clevenger apparatus.

5. GC-MS Studies:

   The GC- MS analysis will be carry out on Shimadzu GC-MS-2010 (quadruple) instrument.

6. Herbal activity:

   Herbal products will evaluate for their antimicrobial, antilarval activity and their efficiency could be analyzed by testing them against microorganisms and on biological systems.

7. Antimicrobial Studies:

   *Invitro* testing of the sensitivity of bacterial and fungal isolates against antimicrobial agents will be carried out using the disc diffusion assay.
8. **Antimicrobial Susceptibility Test:**

*In vitro* testing of the sensitivity bacterial and fungal isolates to antimicrobial agents will carry out using the disc diffusion assay, according to the guidelines set by the national committee for clinical laboratories standards [NCCLS, 1997].

9. **Minimal Inhibition Test (MIC):**

The volatile oils will be tested for MIC using the macro broth dilution method in broth media Muller- Hinton (Difco). [Lucia KH Souza, 2003].

10. **Larvicidal Bioassay Test:**

The larvicidal bioassay test will be carried out by following the standard procedure i.e. W.H.O. larval susceptibility test method.

11. **Adulticidal Bioassay Test:**

The adulticidal bioassay test will be performed by following the standard W.H.O. adult susceptibility test method [WHO 1981b].

12. **Repellency Bioassay Test:**

The experiment will carried out 4 times and then compare with control bite. The percentage of repellency will calculate with the standard method [Dua and Gupta, 1996; Oyedele and Orafidiya, 2000].

13. **Data management and statistical analysis:**

The obtained data will be subjected to statistical analysis of percentage larvicidal, probit analysis and Dragestedt and Behrens Equation to calculate lethal dose.