fixed directions in all three replicates and the average values were calculated.

**Statistical Analysis** (Ghosh, 1984)

The raw data of the present study were subjected to simple statistical analysis to draw meaningful interpretation and conclusion.

1. The standardization values of study drugs were expressed in percentage (w/w).

2. For pharmacological studies:
   - The mean ± SEM and student’s ‘t’ test are computed for all the biochemical estimations, to find out statistical significance at 1% and 5% probability levels.

4. RESULTS
MORPHOLOGY

The A.marmelos found in and around Thanjavur, it was observed many phenotypic variations exist. Differences in the size, shape of the fruit, presence, absence and number of spins in the leaf, length and size of the leaflet are noted. Based on the above difference the bael tree available in Thanjavur are grouped into three types, Var. I, Var. II and Var. III (Amarnath Pandian, 2009), of these variant-I and variant-III were selected for the present study since their occurrence is common in this geographical area (Thanjavur District). (Plate -1 and Plate -2).

The following artificial key has been put forth to distinguish the two variants are:

Var. I

Armed tree to 15(m) spines axillary, straight to 1.5 cm, paired, leaves 3 foliate obovate, terminal ones 2.5 to 4 cm, lateral ones 2 to 3 cm, base cuneate, margin subcrenulate, apex obtuse, terminal petiole 2.5 to 4 cm; fruit apple shape. Berry ovoid, measuring in long axis 5.4 cm and in narrow axis 5.1 cm. Average number locules/fruit is 13; seed/fruit is 56, number of fruits/tree 300. Average weight of the fruit is 98.5(g), average weight of the pulp/fruit is 53.2(g) and percentage of pulp is 54 (Plate -1).

Var. III
Branched tree to 15(m); spines axillary, straight, single alternate 0.2 to 2 cm, leaves 3 foliate, lanceolate, terminal ones 2.5 to 6.2 cm; lateral ones 2.5 to 4 cm; terminal petiole up to 2 to 7 cm; fruit cylinder shape small in size measuring in long axis 5.3 cm and in narrow axis 3.4 cm. Average number locules/fruit is 9; seed/fruit is 24, number of fruits/tree 2000; Average weight of the fruit is 58.5(g), average weight of the pulp/fruit is 53.2(g) and percentage of pulp is 54 (Plate -2)

These two variants have distinctive features both in morphology and anatomy.

Analytical Values

Analytical values like total ash, water-soluble ash, acid-insoluble ash, sulphated ash, loss on drying, solubility in alcohol, water and extractive values of leaf of Aegle marmelos var. I and var. III are given in Table – 1 and Fig .1.

The values for leaf powders of the two variants are almost similar except that colour and extractive values in chloroform and water. Extractive value for var. I in chloroform was higher (3.238%) and that for var. III in water was higher (10.391%) than the other.

Study of Powder
Behavior of powders of leaf of the *Aegle marmelos* var. I and var. III samples on treatment with different chemical reagents and their fluorescents behavior are given in Table- 2.

The leaf powders of var. I and var. III treated with H$_2$SO$_4$ gives black colour in both UV and visible light. The leaf of both variants treated with HCl, Picric acid, Iodine, FeCl$_3$, HNO$_3$ and NH$_4$OH gives almost green colour in both UV and visible light. The leaf powder of var.I treated with HNO$_3$ gives brown colour in visible light and brownish yellow in UV light whereas var. III produce dark yellow in day light and brown colour in UV light.

**Qualitative Phytochemical Analysis**

Successive solvent extracts of leaf of *Aegle marmelos* Variant-I and Variant-III were subjected to qualitative phytochemical screening and the values are given in Table- 3.

It was observed that presences of alkaloids, carbohydrates, tannins & phenols, gums & mucilage, fixed oils & fats and saponins was noted in the leaf extracts of both variants. Appreciable and moderate amount of carbohydrate were noticed in the alcohol and water extracts, of variant -III. More amount of alkaloid was noticed in chloroform, alcohol and water extracts of variant -I, while it was present in small amount in variant -III. Tannins and phenols were present in appreciable and moderate amount in alcohol and water extract of both the variants. Moderate and small amount of fixed oils and fats were present in pet.ether and benzene extracts of variant-I compared to that of variant -III. Small amount of Saponins was noticed in alcohol and water of extracts of both variants. In water extract of
leaf of *Aegle marmelos*, moderate amount of gums and mucilage were present in both variants.

**Quantitative Phytochemical Analysis**

Quantitative estimation of Total Alkaloids, Total Terpenoids, Total Glycosides, Tannin and Ascorbic Acid for *Aegle marmelos* Variant-I and Variant-III powders were evaluated and shown in Table - 4.

**Estimation of Total Alkaloids**

Quantitative phytochemical analysis of the leaf powders of two variants were showed that total alkaloid content were almost similar (0.27 and 0.24%).

**Estimation of Total Terpenoids**

Total terpenoids content of the both samples do not vary much and the estimated value was in the range of 0.85 and 0.68% respectively for var. I and var. III.

**Estimation of Total Glycosides**

Total glycosides content of both plant samples were found to be 2.23 and 1.89% respectively for var.I and var.III.
Estimation of Tannin

The tannin content of leaf powders of var. I and var. III were 2.47 and 2.11% respectively.

Estimation of Ascorbic Acid

Ascorbic acid (Vitamin – C) content of leaf powders of var. I and var. III were 0.92 and 0.47%.

TLC - Thin Layer Chromatography

Leaf extracts of Aegle marmelos were spotted on TLC plates. The R_f values of alcohol and water extracts were shown in Table-5. Alcohol and water extracts of leaf powders had 3 spots each.

HPTLC - High Performance Thin Layer Chromatography

Ethyl alcohol extracts of both variants of Aegle marmelos were characterised by HPTLC. The Number of major compounds was identified from its chromatogram. The percentage yields were calculated and the results were given in the Table-6.

In high performance thin layer chromatography analysis, ethyl alcohol extract of variant-I sample gave five bands of R_f value 0.21, 0.26, 0.41, 0.50 and 0.62 (Fig - 2). Similarly variant -III sample gave four bands of R_f value 0.02, 0.21, 0.26 and 0.61 (Fig - 3).
It is to be noted that variant -I and variant -III samples yielded five and four bands respectively. Both the samples gave two bands with similar R<sub>f</sub> value of 0.21 and 0.26.

All other R<sub>f</sub> values are dissimilar, among them the variant -I and variant-III sample. Thus, both the samples showed similarity and dissimilarity in both qualitatively and quantitatively.

**GC–MS STUDIES**

Gas-Chromatogram and Mass Spectral (GC-MS) studies were carried out in the EtOH extract of leaf powder of *Aegle marmelos* variant -I and variant -III.

The presence of chemical constituents with their molecular formula (MF), molecular weight (MW) and percentage of area were presented in the Table – 7 and biological activities of major chemical compounds are given in the Table – 9 and Fig. 6 to Fig. 16.

Thirteen chemical compounds belonging to different categories were identified in the leaf powder of *Aegle marmelos* variant-I are shown in Table - 7.

Dodecanoic acid, methyl ester (41.09%) C<sub>13</sub>H<sub>26</sub>O<sub>2</sub>; MW-214 and 1,2-Benzenedicarboxylic acid, diisooctyl ester (25.76%) C<sub>24</sub>H<sub>38</sub>O<sub>4</sub>; MW-390; are the major constituents in this fraction. Methyl tetradecanoate (6.21%) C<sub>15</sub>H<sub>30</sub>O<sub>2</sub>; MW-242, Hexadecanoic acid, methyl ester (4.74%) C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>;
MW-270 and 9-Octadecenoic acid (Z)-, methyl ester (4.09%) \( \text{C}_{19}\text{H}_{36}\text{O}_2 \); MW-296 are other biological compounds present in considerable quantity.

Whereas Variant -III contain eleven chemical compounds and the major compounds were; Dodecanoic acid, methyl ester (40.02%) \( \text{C}_{13}\text{H}_{26}\text{O}_2 \); MW-214 and 1, 2-Benzenedicarboxylic acid, diisooctyl ester (22.65%) \( \text{C}_{24}\text{H}_{38}\text{O}_4 \); MW-390; are the major constituents in this extracts are shown in Table - 8.

**TOXICOLOGICAL STUDIES**

**Preliminary screening and estimation of LD50**

The animals were observed for alertness, gait, posture, tremor and response to touch, pain, sound etc continuously for first 6 hours and later at intervals of 24hours for 3 days. From the dose of 100 mg of 3gm there is no significant changes observed in their activities. As there is no death even after 3gm dose to the rat, (which is equal to the single dose for human) estimation of LD50 is terminated. The drugs are being proved innocuous.

**Acute Toxicity Studies**

All the animals had normal physical activities. They gain usual body weight in the course of 14 days. Their temperature remains normal. No abnormal physical activities noted. After 14 days the animals were sacrificed. Except some petechial haemorrhagic changes due to asphyxia at the time of sacrifice (which is the common findings in this test) no changes
were observed in the histo-pathological studies. Complete details were tabulated in Table – 10.

**BIOCHEMICAL AND PHARMACOLOGICAL STUDIES**

*Ethanol induced ulcer*

The ethanolic extract of leaf of *A.marmelos* var. I and var. III exhibited concentration dependent, marked gastroprotection and neutralization in ethanol induced ulcer model at the dose of 100, 200, 300 mg/kg.

Table - 11 and Plate – 3, shows the effect of *A.marmelos* var. I leaf extract on ulcer index, gastric volume and all other biochemical parameters. The extract significantly reduced the gastric volume, ulcer score, total acidity and protein and increased protective factors like hexose, hexosamine etc, when as compared to control. The leaf extract found to exert antiulcer activity in all the parameters, with close value to that of the standard drug Ranitidine (Fig. 17& 18).

The ethanolic extract of *A.marmelos* var. III also exhibited more antiulcer activity as observed by all the parameters when compared to var. I and are given in Table- 12 & Plate – 4; Fig. 19 and Fig. 20.

**Anti-Inflammatory Activity**

*Carrageenin Induced Rat-Paw Oedema Model*
Anti-inflammatory activity of ethanolic extract of *A. marmelos* variant-I and variant-III in Carrageenin induced rat-paw oedema model is given in Table -13 and induced inflammation is shown in plate 5a & 5b.

Anti-inflammatory activity of ethanolic extract of variant-I was dose dependently increased. The percentage inhibition at 300mg/kg b.w. was 67.74% and that of standard drug was 79.03%.

In case of variant-III, the percentage of inhibition was 27.41%, 34.67 and 40.32 for 100mg/kg, 200mg/kg and 300mg/kg b.w respectively.

Maximum percentage inhibition was exhibited by variant-I at 300mg/kg b.w. that is 67.74% in Fig.21.

**Cotton-Pellet Granuloma Model**

In sub-acute studies100 mg, 200 mg and 300 mg/kg doses of *A.marmelos* variant-I produced significant sub-acute anti-inflammatory effect. The inhibition percentage of *A.marmelos* variant-I at the dose of 300 mg/kg was 62.65% and the effect was comparable to standard drug, 65.89%. It was closer to the value of the Ibuprofen. Drug *A.marmelos* variant-III showed 21.05%, 27.12% and 32.79% inhibition at the dose of 100, 200 and 300 mg/kg respectively. And it was tabulated in Table-14. Leaf extracts of both variants dose dependently decreased inflammation of the two variant var. I showed more anti-inflammatory activity. 300mg/kg b.w was found to be effective dose in Fig.22.

**Antipyretic Activity**
The alcoholic extracts of leaf of *A.marmelos* variant-I and variant-III at the dose of 300 mg/kg were administered to hyperpyrexia induced rat by dried yeast injection. Within 45 min after administration of the extracts the effects are shown in the Table 15.

**Antioxidant activity**

Antioxidant activity of ethanolic extract of leaf of *Aegle marmelos* variant -I and variant -III was studied by LPO method and All doses 100mg, 200mg and 300mg/kg produced significant antioxidant activity in Table- 16 and Fig.23. The maximum antioxidant activity was exhibited at dose 300mg/kg in *Aegle marmelos* variant-III. Among the two variants var. III exhibited more antioxidant activity. The activity was dose dependent.

The alcoholic extract of *Aegle marmelos* variant-I and variant-III was studied for antioxidant activity by nitric oxide method & GSH method (Table. 17 and 18). In this two methods are all the doses like 100 mg, 200mg and 300mg/kg possessed significant antioxidant activity. Both leaf extracts of variants showed dose-dependent anti-oxidant activity. More and significant activity was exhibited by var. III. The maximum antioxidant activity was identified at the dose of 300mg/kg in *Aegle marmelos* variant-III (Fig.24 and Fig. 25).

**ANTI-MICROBIAL ACTIVITY**

Anti- microbial activity of EtOH extracts of leaf of *Aegle marmelos* at different concentration (10, 20, 30 mg %) against *Escherichia coli*,

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Streptococcus pyogenes, Helicobacter pylori, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans, Trichoderma viride and Fusarium spp. were given in Table 19.

**Variant-I**

Ethanol extract of leaf of Aegle marmelos var. I showed antimicrobial activity in a concentration dependent manner. The drug gave positive result against Helicobacter pylori, Pseudomonas aeruginosa, and Trichoderma viride. No zone of inhibition was found in Streptococcus pyogenes, Aspergillus niger, Candida albicans, and Fusarium spp.

**Variant-III**

Ethanol extract leaf of Aegle marmelos var. III also showed antimicrobial activity in a concentration dependent manner. The drug gave positive result against Streptococcus pyogenes, Helicobacter pylori, Pseudomonas aeruginosa, Aspergillus niger, Trichoderma viride and Fusarium spp. No zone of inhibition was found in Candida albicans and Escherichia coli.

Among the leaf extracts of Aegle marmelos Variant-I and Variant-III showed the broad spectrum of antimicrobial activity in tested organism in well diffusion assay method.