5. DISCUSSION

Among the population of *A.marmelos* found in and around Thanjavur, it was observed that many phenotypic variation exist, from that two variants of *A.marmelos* are identified, both the variants of *A.marmelos* are called by one vernacular name “Villvam”. Most of the characters are similar but the variation occurs in the leaf, spine and fruits. The two variants are used medicinally one for the other. In west Bengal, Ghosh *et al.*, (2001) noted 13 types of *A.marmelos* trees based on the size and shape of the fruits. Three variants I, II and III were identified and Pharmacognostical and Pharmacological profile of fruits these variants were studied by Amarnath Pandian (2009). For the present study var. I and var. III were selected and leaves of the two variants were subjected to Phytochemical and Pharmacological studies.

**Physiochemical study**

Physiochemical characters of leaf powders of var. I and var. III were more or less similar. However, colour of the powder and extract values in chloroform and water showed distinct dissimilarities which could be used as diagnostic tools.

Fluorescent behavior of both variants of *A.marmelos* revealed marked difference when treated with HNO$_3$ it is used as diagnostic test for the two variants. All other fluorescent behaviors showed more or less similar characters.
Qualitative and Quantitative Phytochemical studies

Qualitative phytochemical analysis of various extracts of both the variants of *A.marmelos* showed distinguished values and can be used as diagnostic values. Quantitative estimation of ethanolic extract of *A.marmelos* for total alkaloids, total tannin has shown more or less similar concentration. However, total terpenoid, total glycoside and ascorbic acid concentrations were more in variant -I. So, total terpenoid, total glycoside and ascorbic acid could be used as diagnostic feature to distinguish two variants. In TLC profiles of *A. marmelos* leaf extract showed chemical changes due to long rime storage (Chaturvedi *et al.*, 2004).

HPTLC finger print with help the identification of marker component and isolation of phytochemical in the future investigation. GC/MS analysis of ethanolic extract of both the variants of *A.marmelos* shows distinct characteristic and also reveal that the common constituents. The variant -I extracts have 13 chemical compounds with major constituents are Deconic acid methylester (41.09%), 1, 2-benzenedicarboxylic acid, diisooctylester (25.76%) and Methyl tetradecanoate (6.21). Similarly variant- III contains 11 chemical compounds with major constituents are Deconic acid methylester (40.02%), 1, 2-benzenedicarboxylic acid, diisooctylester (22.65%), Methyl tetradecanoate (6.21%) and Hexodeconoic acid methyl ester (4.74%). Hence both the variants differ both qualitatively and quantitatively.
TOXITY STUDIES

From the dose of 100mg to 3g there is no significant or abnormal changes observed in the activities rats. As there is no death even after 3gm dose to the rats (which is equal to the single dose for human dose) estimation of LD50 is terminated; the drugs being proved innocuous. Drug A.marmelos is a traditionally used Siddha Medicine. The various literature evidences proved the safety and common use by human subjects form time unknown. The present results from toxicity studies reconfirmed the safety of the traditional herbal medicine.

Veerappan et al., (2007) reported that chronic administration of A.marmelos leaf extract at a dose levels of 50,70,90 and 100 mg/kg b.w for 14 consecutive days to male and female Wistar rats did not induce any short term toxicity collectively and reported that the extracts of the leaves have a high margin of drug safety

BIOCHEMICAL AND PHARMACOLOGICAL STUDY

Ethanol induced ulcer

It is known that ethanol is among many factors increasing risk of gastric ulcer formation due to stress, use of steroids and non–steroidal anti-inflammatory drugs (NSAIDS) (Ray et al., 1990). Ethanol is widely used to induce experimental gastric ulcer in animals (Loguercio et al., 1993). Oxygen derived free radicals, primary super oxide anions, hydroxyl radicals and lipid peroxides play an important role in the
pathogenesis of acute experimental gastric lesions (Body et al., 1981; Das and Banerjee 1993)

NSAIDS are commonly used to treat pain and inflammation (Ivey, 1988). Ulcerative lesions of the gastrointestinal tract are one of the major side effects associated with the use of NSAIDS. It is of interest to know whether or not this herbal antipyretic and anti-inflammatory agent has ulcerative or anti-ulcerative activity. Therefore, the present study was undertaken to determine the effect of both the variants of *Aegle marmelos* against ethanol induced ulcerogenesis in rats.

The ethanolic extracts of both the variants of *Aegle marmelos* showed maximum gastroprotective activity against ethanol induced ulcer at a dose of 300mg/kg. The activity was comparable with the reference drug Ranitidin.

The anti-ulcer activity was also supported by the decrease in the aggressive factors like pepsin, protein and increase in the resistance factors like pH, hexose, hexosamine, any drug which increases gastric juice pH is considered as antacid (Jain and Santani, 1994). Protection of experimental ulcers may be due to effect of prostaglandin in the parietal cells (Takevchi and Nobubara, 1985; Laurisen and Madsen, 1986). As prostaglandin enchance the mucosal resistance, perhaps by increasing the secretion of mucous and bicarbonates (Hogan et al., 1994). Strengthen the mucosal barrier, decrease the gastric motility (Szabo et al., 1984). The mucosal defense mechanism may be due to the epithelial cell of the gastric mucosal which is impermeable to $H^+$ there by forming
a physical barrier (Davenport et al., 1964). The antiulcer activity of the
drugs may be enhanced due to the presence of saponins, terpenoids and
amino acids (Pasquale et al., 1995). Presence of β-sitosterol in the
samples may also enhance the antiulcer activity and it is not toxic as
reported earlier (Malini and Vanithakumari, 1989).

Previous workers also reported anti-ulcer potential of A. marmelos. The aqueous ethanolic extract from the fresh unripe fruits of
Aegle marmelos showed potent antiulcer and anti diarrhoeal activity
(Ammresh et al., 2003). Amarnath Pandian (2009) reported antiulcer
activities of fruit pulps of three variants of Aegle marmelos.

Garg et al (2003) studied the “Bowel care” formulation consisting
of Aegle marmelos, Plantago ovata and Lipidium sativum, which are
known gastro friendly.

The anti ulcerogenic activity of the extracts of Rhizophora mangle
L. was due to the presence of tannins (Sanchez Perera et al., 2001). The
higher amount of tannins was present in both variants of
Aegle marmelos, it might be the reason for antiulcer activity. However,
more antiulcer activity of leaf extract of var. III compared to var. I could
not be accounted in terms of biological active compounds. Identification
and characterization of biological compounds in TLC and HPTLC
would through more light on this aspect. However TLC and HPTLC
profiles of leaf extracts of variant-I and variant-III have been
established.
Anti-inflammatory and anti pyretic activity

In the present study, the anti inflammatory activity of the leaf extracts of *A.marmelos* has been established. The extracts of *A.marmelos* were found to significantly inhibit the carrageenan-induced rat paw oedema, a test, which has significant predictive value for anti inflammatory agents acting by inhibiting the mediators of acute inflammation (Mossa *et al*., 1995). Oedema formation due to carrageenan in the rat paw is the biphasic event (Vinegar *et al*., 1969). The initial phase is attributed to the release of histamine and serotonin (Crunkhon and Meacock, 1971). The second phase of is sensitive to most clinically effective anti inflammatory drugs (Vivegar *et al*., 1969 and DiRosa *et al*., 1971).

In chronic studies, the inflammatory granuloma is a typical feature of established chronic inflammatory reaction (Olajide *et al*., 2000). The dry weight of the cotton-pellets correlated well with the amount of granulomatous tissue (Swingle and Shideman.1972).

The extract derived from the *A.marmelos* exhibited significant anti inflammatory activity in the cotton-pellet test. This effect showed the ability of the extract in reducing the number of fibroblasts, and the synthesis of collagen and mucopolysaccharide, which are natural proliferative events of granulation tissue formation. However, the effect was less when compared to ibuprofen.

In addition the ethanolic extract *A.marmelose* leaves of variant -I and variant -III shows significant antipyretic activity in mice.
Variant -III shows more significant than Variant -I. The response was almost half of the response compare to that of paracetamol.

**Antioxidant activity**

**Free radical scavenging activities**

The powerful oxidant, including superoxide anions, hydroxyl radicals, hydrogen peroxide are known as free radicals. Free radicals are unguided missiles that bounce around and attack healthy cells, tearing the cell membranes and spilling cytoplasm and subjecting the cells to infection, genetic damage and mutations. They react with serum lipoprotein (LDL) and cause formation of atheromatous plagues or react with the cell membrane lipid and cause peroxidation of polyunsaturated fatty acid and cause generation of further free radicals. Some disorders in which free radicals are attributed are: Alzheimer’s arthritis, hemorrhoids, Parkinson’s, rheumatism, heart attack, AIDS, stroke, cancer, phlebitides, immune system disorder, and a long list of degenerative diseases including aging. Free radicals are not entirely bad. The macrophages and neutrophils use them to destroy bacteria and other foreign invaders.

However too much production or production in the wrong place can be harmful, both acutely and chronically. This is why body needs antioxidant compounds. Antioxidants function by offering easy electron targets for free radicals. In absorbing a free radical, antioxidant ‘trap’ the lone free-radical electron and make it stable enough to be transported to an enzyme which combines two stabilized free radicals
together to neutralize. Antioxidant compounds must be constantly replenished, since they are “used up” (converted) in the process of neutralizing free radicals. Therefore, one has to continuously produce more of the antioxidants in the body or ingest them either in diet or by supplementation. Fortunately, the body has, throughout the course of millions of years of evolution become accustomed to coping with free radicals and have evolved various schemes for doing this. The most effective natural antioxidants are tocopherol (vitamin E) and vitamin C. These vitamins are efficient in mapping up free radicals.

β-carotene is a precursor of vitamin A(retinol) which is another antioxidant present in spinach, carrots, jams, tomato and peaches. Since β-carotene is converted to Vitamin A by the body there is no set requirement. The hormone melatonin is an important antioxidant. It detoxifies the highly reactive hydroxyl radical and neutralizes other toxic species, including single oxygen, hydrogen peroxide, nitric oxide and peroxynitrite anions, and stimulates several antioxidative enzymes. The building block nutrients that the body requires for making SOD, catalase and glutathione peroxidases include the minerals Mg, Zn, Cu. Oligomeric proanthocyanidin complexes from grape seeds and pine bark, turmeric, resveratrol from grapes, soya isoflavones and garlic flavonoids are preventive against heart diseases and cancer. The lycopene of tomato is one of the latest extracts in the antioxidant group. A study by Harvard Medical School estimated that consuming tomato products twice a week was associated with a reduced risk of cancer.

The methanolic extract of A.marmelos decrease alloxan induced lipid peroxidation (LPO) significantly in serum and liver. Liver
superoxide dismutase (SOD) activity was increased significantly when compared with alloxan induced diabetic rats (Sabu and Kuttan, 2001). The present investigation was aimed to evaluate the \textit{in vitro} antioxidant activity of ethanolic extract of \textit{A.marmelos} both variants against LPO, NO and GSH method.

From the result it was observed that the extracts of \textit{A.marmelos} two variants where found to act as radical scavengers against different free radicals under the conditions of oxidative stress. Most non enzymatic anti oxidative activity like scavenging is meditates by redox reactions. The reducing power determine in the present study depends on the redox potentials of the compounds present in the variant I. The significance $p\leq 0.001$ inhibition was noted in all the extracts of both the variants. In conclusion, the present study, all the extracts at a different concentration were found to have potent free radical scavenging activity. The observed activity may be mainly due to their chemical constitutes like Oleic acid, Hexadeconic acid, Octadeconic acid and Squalene.

\textbf{Anti microbial activity}

Anti microbial activity of ethanolic extracts of \textit{A.marmelos} showed positive result against tested organism in a concentration dependent manner. Phenolic, aldehyde, ester, flavonoid and alkaloid compounds are known to be antimicrobial in action.

Presence of diterpene, sesquiterpene, plasticizer compound, olic acid ester, alkaloid, flavonoid and steroid compounds in the leaf of \textit{A.marmelos} of lend support to their antimicrobial action.
Higher concentration of Dodecanoic acid, methyl ester (40.02%), 1,2-Benzene dicarboxylic acid, diisooctyl ester (22.65%), Hexadecanoic acid, methyl ester (4.74%) in the leaf of Var.III might be responsible for more antibacterial activity than the leaf of other variant.

Anti microbial activity of ethanolic extract of A.marmelos shows positive result against tested organisms Klebsiella spp., Pseudomonas aeruginosa, Salmonella typhi and Streptococcus. Aegle marmelos shows potent antibacterial activity of Pseudomonas aeruginosa and Salmonella typhi (Balakrishnan et al., 2006).

Previous work also reported antimicrobial activity of fruits and leaves of A.marmelos (Valsraj et al., 1997). Rind extracts of A.marmelos showed significant antimicrobial activity (Kalkar et al., 2005). Anti fungal activity was also observed in the leaves of A.marmelos (Kaushik et al., 2004). Ethanol extracts also showed antifungal activity (Jain et al., 1998).