DISCUSSION

The observations presented in the preceding pages of the present investigation are discussed in the light of "review of literature". Cancer is considered as one of the dreaded diseases of human race. Research still continues to find out suitable drugs. The Siddhas of the Siddha System of Indian Medicine have categorically stated that the medicine for a disease is available in the environment of the diseased itself. So, it is fitting to identify the medicine from the environment in which the diseased lives and administer the medicine to the diseased so as to get a cure from the disease.

The medicinal plants *Ludwigia prostrata* and *Hibiscus vitifolius* are available in plenty in the area of study. These two plants were also known to be less investigated so far for their anticancer activities.

Ecology of the habitat plays an important role in the availability of medicinal plants for human and animal use (Sethi *et al.*, 1991; Gasic *et al.*, 1992). Utilisation of the easily available medicinal plants has also been considered to be a step towards their conservation. Otherwise the medicinal plants will become extinct. Cultivation may be taken up if they are needed in large quantities. If environment based therapy is undertaken, cultivation of these plants will become unnecessary and the plants may be collected from their natural habitats as and when they are required.
Taxonomical investigation is important to ascertain the correct identity of medicinal plants. The study plants were identified with the help of institutional and regional herbaria. Herbarium specimens were deposited in the departmental herbarium for verification by future investigators. Morphological characters were also given in detail.

Pharmacognostical studies were undertaken to avoid spurious and adulterated drugs available in the place of genuine plants (Figs.1, 7). Microscopic analysis alone will assist the correct identification of medicinal plants (Figs.5, 6, 8, 9, 10). With the help of given morpho-taxonomical and anatomical characters *L. prostrata* and *H. vitifolius* can be easily and correctly identified among the other species of the genus.

Preliminary morphological, pharmacognostical and phytochemical studies were also done for the identification and proper use of plants. Pharmacognostical standards such as macro and microscopic characters, physicochemical standards of the drug yielding plants are necessary for the correct identification of genuine samples (Siddiqui et al., 1991).

In the present study on anticancer activities of the extracts of various parts of *L. prostrata* and the flavonoid extracted from the petals of *H. vitifolius* show significant antitumor activities at different grades. For the present investigation on anticancer activity phytochemical studies were conducted to identify the presence of carbohydrates, glycosides, fixed oil, protein, saponin, tannin, alkaloid, flavonoid, fatty acids and terpenoid in the study materials (Table 5). Phytochemical studies
reveal the presence of flavonoid in the root, stem, leaf and seed of *L. prostrata*. Previous works reveal the effectiveness of flavonoids in cancer chemotherapy. So, to give more emphasize, the flavonoid alone was extracted from the petals of *H. vitifolius*. The reliable criterion for judging the value of any anticancer drug is the prolonging of life span of the animal and disappearance of leukemic cells from the blood (Feninger, 1954). The extracts of *L. prostrata* and flavonoid of *H. vitifolius* show significant antitumour activities by reducing the number of WBC, increasing the haemoglobin and decreasing the protein content of cells (Tables 10-15; Figs.41-43). Moreover, the life span of DAL induced mice was increased by the treatment with extracts of the study materials (Table 6; Figs.30-39). The results are more or less nearer to the results of the treatment using 5FU (5-flurouracil), a standard drug used in cancer chemotherapy (Kavimani *et al.*, 1996). Park *et al.* (1995) isolated a cytotoxic principle from *Dalbergia odorifera* with similar activity to 5-fluorouracil.

Earlier studies have shown the antitumour activity of flavonoid against different cancer types both in vitro and in vivo. Pomilio *et al.* (1994), Woerdenvag *et al.* (1994), Klimeck *et al.* (1994), Pettit *et al.* (1996) and Ryu *et al.* (1997) have reported that the flavonoids were responsible for the cytotoxicity against different tumour cell lines.

Hidehiko *et al.* (1994) reported an increase in the survival period of tumour bearing mice administrated with apigenin, a flavonoid. Chen *et al.* (1990), Coward (1993), Ryu *et al.* (1994) and Shi *et al.* (1995) have also reported the anticancer activity of flavonoids. Through the present investigation *H. vitifolius* is considered to be a valuable addition to the list of plants existing already for cancer therapy.
Among the various plant parts of *L. prostrata*, screened for phytochemicals, seed material alone was found to possess alkaloids. The root, stem and leaves were devoid of alkaloids. Treatment with the seed extract increased the percentage of mean survival time (152%) which is the highest when compared with other plant parts. This significant antitumour activity may be due to the presence of alkaloids in the seed extract. The anticancer activity of alkaloids has already been reported by Petricic *et al.* (1991), Vhen *et al.* (1991), Perrone *et al.* (1993), Li *et al.* (1993), Antoun *et al.* (1993), Rates (1993), Lopez-Meyer *et al.* (1994), Takano *et al.* (1996) and Kavimani *et al.* (1996). The result of the present work is supported by the findings of the above authors.

The phytochemical evaluation showed that the root, stem, leaf and seed of *L. prostrata* contain polysaccharide (Table 5). Polysaccharides are also anticancerous as observed by Chung *et al.* (1990), Wang *et al.* (1993), Tomshich *et al.* (1997) and Kim KiHwan (1998).

Qualitative phytochemical studies reveal the presence of terpenoid in the root, stem, leaf and seed of *L. prostrata* selected for the present study on anticancer activity (Table 5). Terpenoid has already been reported to be possessing tumouricidal property by Vhen *et al.* (1991), Umchara *et al.* (1992), Zheng *et al.* (1992), Wang *et al.* (1996) and Rodrigues *et al.* (1997). Triterpenoid extracted from *Aster lingulatus* showed inhibitory activity of DNA synthesis in human leukemia HL-60 cells.

Seeds of *L. prostrata* were found to contain unknown fatty acids (Table 5). Fatty acids have been reported to be prolonging the life span of tumour bearing
animals by Siegel et al. (1987), Zhu et al. (1989) and Numata et al. (1994). Hence, it is evident that fatty acids play a very important role in the tumouricidal activity of L. prostrata.

Seeds of L. prostrata have been found to contain fixed oil (Table 5). Fixed oil of Nigella sativa has already been reported to prevent non-enzymatic lipid peroxidation and thereby help to inhibit tumour growth (Houghton et al., 1994). Hence, the anticancer activity of seed extracts of L. prostrata may also be attributed to its fixed oil. The root, stem, leaf and seed of L. prostrata were found to contain glycosides. These natural glycosides may possess antitumour property. Cytotoxicity of steroidal glycoside from Solanum nigrum was evaluated. Lyubov et al. (1999) reported the cytotoxic property of natural glycosides from Ginseng. This was proved in six cultured human solid tumour cell lines.

**Cytological studies**

The ascitic fluid of tumour induced mice treated with the extracts of L. prostrata and the flavonoid of H. vitifolius showed considerable cytological changes. Cells with multiple vacuoles and eccentric nuclei (Fig. 14), cells forming syncytium, a feature of degeneration (Fig. 19), nuclear degeneration and cytoplasmic blebs (Fig. 21), clear lymphophagocytosis (Fig. 22), irregular nuclear border due to degeneration of nucleus (Fig. 24a, 24b), increased number of nuclear and cytoplasmic vacuoles (Fig. 25a, 25b) and fragmentation of nuclei (Fig. 28) are some of the observed features which are the indications of death of cancerous cells.
The results of the statistical analysis of the haematological parameters also show significant regression of cancer by the extracts of *L. prostrata* and the flavonoid of *H. vitifolius* (Tables 8-23).

**Mechanism of action**

In the present work, the pharmacological experiments with albino mice were done using different extracts of *L. prostrata* which contain more than one compound and the flavonoid of *H. vitifolius*. Their mechanism of action may differ. The antitumour principles are known to exercise their effects through a variety of mechanism of action. Alkaloids from *Thalictrum glandulosissium* were reported to lock the cell cycle transfer from G1 to S phase (Xu et al., 1990). The mechanism of action of plumbagin was to disturb mitosis (Mohana and Purushothaman, 1980). Terpenes were reported to hasten the differentiation of cells in carcinoma cells (Lee et al., 1994). The fatty acid expressed its cytotoxicity by altering the rigidity of tumour cell membrane and causing cell death (Numata et al., 1994). The tumour regression may be due to reduction in DNA and protein synthesis in the presence of polysaccharide (Wang et al., 1993).

Hence, it is concluded that the phytochemical constituents in different extracts of *L. prostrata* may be responsible for their anticancer properties. The anticancer activity of flavonoids has been reviewed exhaustively in the present work. *H. vitifolius*, so far uninvestigated for anticancer activity has been investigated for the first time in the present work and found to be possessing significant anticancer activity.
From the above discussion it is concluded that different compounds act differently and finally the tumour regression is resulted. The mode of action of each compound may be different at different stages during cell division. The combined effect of the compounds is supposed to give more favourable results during the treatment. In the present investigation the extracts of *L. prostrata* and the flavonoid of *H. vitifolius* used for the treatment of DAL has shown significant regression of tumour cells. This may be due to the combined chemotherapeutic activity of the phytochemical constituents of the medicinal plants *L. prostrata* and *H. vitifolius*.

The ultimate aim of the present work is to find out whether *L. prostrata* and *H. vitifolius* are possessing anticancer activities. It is evident from the present investigation that the extracts of *L. prostrata* and the flavonoid of *H. vitifolius* at 50 mg/kg and 100 mg/kg doses have shown significant anticancer activities against DAL in albino mice. Hence, the drug standardization has been left for future pharmaceutical investigations.