5. SUMMARY AND CONCLUSIONS
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Cancer is one of the most dreaded diseases of the 20th century and spreading further with increasing incidence in 21st century. It is considered as an adversary of modernization and advanced pattern of socio-cultural life. Multidisciplinary scientific investigations are making best efforts to combat this disease, but perfect cure is yet to be brought into the world of cancer medicine. Cancer is an ailment that affects more or less 200 types of cells. There are many difficulties in treatment and more important are of them drug resistance, toxicity, and low specificity. Despite the extensive application of established cancer therapies and the new wave of biotherapies, deaths from cancer are projected to continue rising; an estimated 9 million people are expected die from cancer in 2015 (http://www.who.int/mediacentre/factsheets/fs297/en/index.html).

In recent years, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management. Several studies have been conducted on herbs under a multitude of ethno botanical grounds. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties and several of them have been used as potent anticancer drugs (Hartwell, 1969A; 1969B; 1969C; 1970A; 1970B; 1971A; 1971B; 1971C; 1971D; Pandey, 2002). Several of the anticancer drug that are in use are derived from plants. Ayurveda, the traditional Indian medicine has been successful from very early times in using plant drugs for preventing or suppressing various tumors using various lines of treatment (Pandey, 2002).

Oxygen species are key participants in tumor invasion and metastasis, injuring local tissues leading to progression of cancer. The reduction of avoidable endogenous and exogenous sources of oxidative stress is, therefore, potentially the most important means of preventing oxygen free radical related cancer. Plant derived extracts containing
antioxidant phytoconstituents have been reported to exhibit cytotoxicity towards tumor cells and antitumour activity in experimental animals. There is also supportive evidence that antioxidants enhance antitumor effects of chemotherapy, both in vitro and in vivo (John, 1999). Several members of *Ipomoea* are widely used in folk medicine. Pharmacological studies carried out on these members have reported several activities including anticancer activity.

**The objective of the present investigation was, therefore, to evaluate the plant Ipomoea leari, for its antioxidant and anticancer activity.** It was proposed to screen its successive extracts, fractions and isolated compound(s) for their in vitro and in vivo antioxidant and anticancer activity using standard procedures.

The following are some of the important conclusions made from the present study;

- Leaves of the plant, *Ipomoea leari*, were evaluated for its quality by performing ash values and extractive values. It was found that plant is of good quality as seen from their ash values and extractive values.

- Anatomical studies of the leaves were performed to identify the special micoscopical characters of the leaves. These may be helpful for further confirmation of this plant and in future these characters may be compared with new batches of the same plant material.

- Successive extracts were prepared from the plant material which was further fractionated with different solvents to get fractions.

- Preliminary phytochemical studies reveal that Alkaloids and Terpenoides are not present in any of the extract. Carbohydrates, Flavonoids, Glycosides, Steroids and Phenols are present in ILC. Carbohydrates, Flavonoids, Glycosides, Steroids and Phenols are present in ethyl acetate extract (ILE). Carbohydrates, Flavonoids, Saponins, and Phenols are present in methanolic extract (ILHM). Steroids and
phenols are present in successive chloroform extract fraction ILCF-28 and glycosides and steroids are present in successive ethyl acetate fraction ILEF-1A.

- The column chromatography of the successive chloroform extract of plant (ILC) gave two compounds, (ILC-1 and ILC-2) and column chromatography successive ethyl acetate extract (ILE) gave one compound (ILE-1). The structure of these three compounds, established by spectral and other studies and given below. **ILC-1**, (3S, 8S, 9S, 10R, 13R, 14S, 17R)-17-(E, 2R, 5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta [a] phenanthen-3-ol is **Stigmasterol**. The crystal structure data, namely ORTEP diagram also support the structure. **ILC-2**, 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17-dodecahydro-1H-cyclopenta [a] phenanthen-3-ol is **β-sitosterol**. These compounds are known compound. However, these two compounds are being reported for the first time from this plant. **Compound ILE-1** is, (Z)-17-(4-ethyl-3, 4, 6-trimethylhepta-3, 5-dien-2yl)-6, 10, 13-trimethyl-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren. It is a new compound being reported for the first time.

![Diagram of ILC-1](image-url)
Compound ILC-1 and ILC-2 have been shown to exhibit anticancer activity by earlier investigators. ILE-1 could not be screen for anti cancer activity for want of the required quantity of the sample.

Among the extracts and the fractions tested, the successive chloroform extract (ILC) exhibits antioxidant activity in DPPH, nitric oxide, ABTS, reducing power, p-NDA, and hydrogen peroxide with IC<sub>50</sub> value of 25.02±0.05, 37.97±2.11, 65.19±0.21, 195.34±2.01, 402.01±0.22 and 516.21±3.11, respectively. Its fraction, ILCF-28, however, exhibits better antioxidant activity with IC<sub>50</sub> value of 19.31±0.91, 26.72±0.72, 54.22±1.12, 125.15±1.04, 341.01±0.32 and 480.21±2.01 µg/ml, respectively. There are comparable with those of standards ascorbic acid, rutin, butylated and hydroxyl anisole indicating its potent antioxidant nature.
Summary and Conclusions

- Among the extracts tested, successive chloroform extract (ILC) shows high total phenol content while ILE shows high flavonoid content. In *in vivo* antioxidant studies, ILC significantly (p<0.001) restored all the biochemical parameters towards normal in a dose dependent manner indicating its potent antioxidant activity.

- Among the extracts and fraction screened for *in vitro* cytotoxicity, the chloroform extract fraction (ILCF-28) shows potent toxicity against normal and cancer cell cultures. All the four extracts show specific toxicity towards cancer cell cultures.

- Among the extracts and the fraction tested, successive chloroform extract (ILC) and its fraction (ILCF-28) show considerable reduction in the regenerative capacity, both in normal and cancer cell cultures.

- In the studies conducted using DLA model for *in vivo* anticancer activity, treatment with successive chloroform extract (ILC) and its fraction (ILCF-28) at 400 mg/kg significantly (p<0.001) increases the average life span of DLA bearing mice. It also shows a significant and dose dependent reduction in the percentage increase in the body weight and significant reversal of haematological parameters towards near normal values, when compared to DLA control (p< 0.001). The anticancer potential of successive chloroform extract fraction, (ILCF-28) and 5-FU are comparable at their respective dose levels.

- In studies using EAC model, treatment with successive chloroform extract (ILC) and its fraction (ILCF-28) at 400 mg/kg significantly (p<0.001) increases the average life span of EAC bearing mice. It also shows a significant and dose dependent reduction in the percentage increase in body weight and a significant reversal of haematological parameters towards near normal values, when
compared to EAC control (p<0.001). The anticancer potential of 5-FU and successive chloroform extract fraction (ILCF-28) is evident at their respective dose levels.

- In DLA induced solid tumor model, treatment with successive chloroform extract fraction (ILCF-28) shows a significant reduction in the solid tumor volume at 200 mg/kg dose level, when compared to DLA control. This fraction is thus more active in reducing the tumor volume than chloroform extract.

- The successive chloroform extract (ILC) shows the presence of two compounds, namely stigmasterol and β-sitosterol. Both these compound have been reported to possess antioxidant and antitumor and anticancer activities by earlier workers. In the present study chloroform extract fraction (ILCF-28) shows potent in vitro cytotoxicity and in vivo anticancer activity in DLA, EAC and solid tumor models. The compounds, namely stigmasterol and β-sitosterol could, therefore, be relevant contributors for the observed anticancer activities of the successive chloroform extract (ILC).

The results of the present study clearly reveal that among all the four extracts studied, the successive chloroform extract fraction (ILCF-28) has potent anticancer activity. The three two phytoconstituents present in the plant obviously contribute to this activity. Further studies such as creating a “peak library” where in the crude extract (ILC) is prefracionated in to a series of almost pure compounds, to use of cell based assays targeted toward specific mechanism of action and dereplication or the rapid identification of known compounds in the plant will be carried out in future.