CHAPTER 6

SUMMARY AND CONCLUSION

Wild plants play an important role in community nutrition and treatment of diseases worldwide with minimal cost and side effects. Edible plants serve the purpose of food during scarcity due to its availability throughout the year. Some of the Rattan species including *Calamus tenuis* Roxb. are edible and used as ingredient in many delicious and nutritious dishes, and also as traditional medicine. The ethnic communities of North East India have immense knowledge on utilization of wild plants and shoots of *Calamus tenuis* Roxb. is traditionally believed to be used by the natives in this region including Dibrugarh district of Assam, in the forms of vegetable and for treating certain diseases, and no report was available on its consumption pattern, traditional therapeutic practices, health issues beliefs, storage and sources. It has been found that the uses of medicinal plants are linked to factors like education, knowledge, health, cultural issue, environment, etc. In recent years ethnic knowledge has been increasingly studied due to depletion of such important information, to identify and conserve the use of indigenous plants with high nutritional, medical and/or commercial potentials to contribute for improvement of livelihood. Herbal medicine remains one of the common forms of therapy available for most of the world's population. Among various diseases, cancer is one of the leading causes of death worldwide. Undergoing treatment for cancer automatically leads to disastrous side-effects caused by chemotherapy and radiotherapy. The information obtained on identification, preparation, clinical use, gathering, and preservation of medicinal plants dramatically facilitates the search for new drugs. Medicinal plants and its ethnic knowledge about their therapeutic uses serve as a raw material for such

research. As per laboratory and epidemiological researches, whole edible plants or their active components like flavonoid, tannin, volatile oil, glycoside, alkaloids, etc. have substantial and protective effect on human carcinogenesis. Besides other indigenous plants, there are some *Calamus sp*. which have been reported to have some therapeutic activities. However, data on scientific evidence about phytoconstituents and cytotoxic potential against human carcinoma and human normal cells of *Calamus tenuis* Roxb. edible shoots extracts was not available. In view of the stated rationale present study entitled **"Consumption pattern of *Calamus tenuis* Roxb. shoots of the forest village natives of Dibrugarh, Assam and investigation of its cytotoxicity activity on cancer and normal cells (A549, MCF7 and L132)"** was planned and conducted and can be summarized with certain conclusion in following five phases of the study.

6.1: Under **Phase I** of the study, plant identification, sample collection and its primary processing, survey of *Calamus tenuis* Roxb. shoots consumers regarding their background information, cooking and consumption pattern, sources and storage of *Calamus tenius* Roxb. shoots, therapeutic and health issues beliefs due to consumption of shoot and, association between consumption of the shoot with education, occupation, income, socio economic status (SES), therapeutic beliefs, health issues beliefs, subjects' medical condition, family medical history and sources of shoots was evaluated. The survey was conducted in five selected forest villages of Dibrugarh district of Assam with a total number of 350 households. A structured and pretested proforma was used for the survey. The obtained data was entered in a Microsoft excel (2007) spreadsheet. To interpret the results of Phase I, data was cleaned and verified for appropriate statistical analysis. Number and percent calculation was done by using Microsoft excel (2007) and Chi Square was calculated

for obtaining association between consumption pattern and other parameters by using Epi Info (7.0). Following are the result highlights of Phase-I.

Result highlights of Phase I:

- The plant was identified as *Calamus tenuis* Roxb. (Aracaceae family).
- The Mean ± SD age of the subjects interviewed was 39.42 ± 11.27 years.
- Most subjects (86.87%) were educated upto secondary level and only 6.86% were illiterate and a very few (6.29%) were graduates and postgraduates.
- Most subjects (94.56%) were engaged as unskilled worker, clericals, shop owners and in farming. Only 4% had high professions and very few (1.44%) were found to be unemployed.
- Total monthly income of most subjects (71.17%) was in the range of Rs. 4204-10532, 16% of the subjects earned upto Rs. 4203 and a few subjects (12.29%) earned above Rs. 10532.
- Majority of the subjects (89.71%) surveyed belonged to SES class III and IV; and only a few belonged to class I (1.14%) and class II (9.14%).
- About medical condition, 31.43% of the subjects mentioned that they suffered from some common health disorder while 68.57% subjects did not report about having any illness.
- For family medical history, 8.86% of subjects revealed that they had some illness history (cardio vascular disease, diabetes mellitus, gastro intestinal disorder, skin disease, kidney stone, dental disorder, eye disorder, etc.) in their family whereas, 91.14% did not report for the same.
- The subjects followed frying, roasting and boiling as most preferred methods of cooking. Some subjects (32.87%) also practiced cooking the shoots in combination with other food items like fish, meat, red ant eggs, elephant

apple, mustard flakes, wrapped in edible leaves and with black gram pulses. Few consumed in raw form.

- Most of the subjects resorted to occasional (53.71%) or monthly (34.86%) consumption of *Calamus tenuis* Roxb. shoots.
- Consumption of shoot was maximum (68.57%) during the month of March-May.
- The preferred sources of *Calamus tenuis* Roxb. shoots for the subjects were forest followed by local market and some grew shoots in their kitchen garden.
- Most of the subjects (95.72%) mentioned that the shoot can be stored on cold and dry floor at room temperature for about 7 to 10 days, while some other (3.99%) revealed that it could be stored for about 5-7 days only.
- Most of the subjects (71.14%) did not believed that the consumption of *Calamus tenuis* Roxb. shoots treat illness while 28.86% of the subjects believed that it may be helpful against some illness like cough, intestinal worms, small pox, tooth ache, wound healing and low blood pressure.
- Most of the subjects (81.14%) did not believe that the consumption of the shoots cause any side effect whereas, 18.86% of them believed that consumption of shoot at night and in empty stomach cause stomach problem.
- The subjects, who had education upto primary level, consumed more shoots as compared to others.
- A significant association of consumption pattern of the shoots with occupation of the subjects was found. The unemployed and unskilled workers consumed shoot more as compared to others.

- The income of subjects had high impact on amount of consumption. The subjects who had low income, consumed shoot more than those of other income groups.
- The subjects belonging to upper class of SES consumed shoots in less amounts while lower SES group consumed in more quantity.
- No association was found between consumption of *Calamus tenuis* Roxb. shoot and therapeutic beliefs, health issues beliefs, subjects' medical condition and family medical history of the subjects.
- The preference of sources of the shoot had significant impact on consumption of shoots. The subjects, who choose forest as a source of shoots, consumed more.

***Calamus tenuis* Roxb. by and large still remains as a forest crop and is not freely available in the market and confined to traditional delicacy of the region. The shoot of the plant is consumed in various forms of preparations either in combination with other food items or individually. The beliefs and practices of the folks do not confirm the therapeutic potential of the plant, in extensive way. Although toxicity studies on *Calamus tenuis* Roxb. shoots have not been carried out for its safety and dosage, some of the subjects reported to experience gastritis and stomach disorder specifically when consumed at night and on empty stomach. Researchers have also reported that some vegetable species are potentially toxic to humans and animals when consumed in high amount or in inappropriate manner. Therefore, indigenous edible plants like *Calamus tenuis* Roxb. need to be proved for its therapeutic value, safety and dosage. The findings imply towards therapeutic potential and safety of the shoots and encourage for scientific scrutiny for its consumption as a functional food.**

6.2: Under **Phase II** of the study, raw 14.09 kg *Calamus tenuis* Roxb. shoots were collected and fresh edible portion of the shoot was obtained. The moisture content of the edible portion of the shoots was evaluated. The fresh edible shoots were processed to obtain dry powder sample. Crude extraction of the powdered shoot was done using successive solvents (Hexane, Ethyl acetate and Methanol). The methanolic extract was sticky in nature; therefore it was partitioned with distilled water and lyophilized, which yielded amorphous powder (MPCT) and sticky paste (MSCT). MTT assay was done on human lung (A549) and breast (MCF7) carcinoma cells for cell viability and cytotoxicity potential evaluation of Methanol precipitate extract (MPCT), Methanol supernatant extract (MSCT), Hexane extract (HECT) and Ethyl acetate extract (EACT) of *Calamus tenuis* Roxb. shoots. Qualitative biochemical analysis was done for assessment of active phytoconstituents present in the crude extracts of *Calamus tenuis* Roxb. shoots. Following are the result highlights of Phase-II.

Result highlights of Phase II:

- The collected raw 14.09 kg *Calamus tenuis* Roxb. shoots yielded 3.58 kg of fresh edible tender shoots.
- The fresh shoots on further processing and drying yielded 275g shoot powder.
- The moisture content of the edible portion of the shoots was found to be 92%.
- The successive solvent extraction of 275g of powdered dry *Calamus tenuis* Roxb. shoots, on removal of excess solvent by using a rotary vacuum evaporator yielded 3.83g, 1.38g, and 31.63g of crude extracts of hexane (HECT), ethyl acetate (EACT) and methanol respectively.
- The water partitioned and lyophilized methanolic extract yielded 4.64 g of amorphous powder (MPCT) and 15.16 g of sticky paste (MSCT).

- Among the extracts tested for cell viability on human carcinoma cells (A549 and MCF7), the partitioned methanolic precipitate (MPCT) extract showed highest potential against both carcinoma cell types; followed by MSCT, HECT and EACT.
- Qualitative biochemical analysis showed that methanolic extracts (MSCT and MPCT) exhibited presence of saponin, flavonoid, steroid, tannin and glycoside.
- Qualitative biochemical analysis of hexane extract (HECT) showed presence of saponin and steroid only whereas ethyl acetate (EACT) emerged as a steroid rich fraction that lacked all the other said ingredients.

***Calamus tenuis* Roxb. is rich in saponin, flavonoid, steroid, tannin and glycoside. Such important bioactive constituents of plants are known to contribute in drug development. The methanolic extract of the plant was found to have higher cytotoxicity against human carcinoma cells as compared to other extracts; with higher cytotoxicity potential against human lung cancer (A549) than breast cancer (MCF7) cells.**

6.3: Under **Phase III** of the study, the most potent extract MPCT (partitioned methanolic precipitate) among all tested extracts assayed against human carcinoma cell lines which also constituted all the biochemically analyzed phytoconstituents (assessed in Phase-II of the study) was fractionated using Column Chromatography and Thin Layer Chromatography (TLC) for further investigation on carcinoma cells (A549 and MCF7). Qualitative biochemical analysis of fractions selected for cell viability (showing majority of phytoconstituents on TLC) was done for identification of important phytoconstituents. Following are the result highlights of Phase-III.

Result highlights of Phase III:

- Among all the 14 fractions (F-1 to F-14) obtained from methanolic precipitate (MPCT) extract using Column Chromatography and Thin Layer Chromatography (TLC); Fraction No-2, 3 and 8 (F-2, F-3, F-8) were found to have majority of phytoconstituents (observed on TLC).
- Both fractions F-2 and F-8 were able to induce ~50% cells mortality at 10ug/ml of concentration in lung carcinoma cells (A549).
- Fraction F-3 showed ~50% cytotoxicity between 10 and 25ug/ml in lung carcinoma cells (A549).
- Among the tested fractions, fraction F-8 was most potent against lung carcinoma cells (A549).
- Fractions F-2 and F-3 were able to induce ~50% cells mortality at 25ug/ml concentration against breast carcinoma cells (MCF7).
- Fraction F-8 showed ~50% cytotoxicity at 10ug/ml in breast carcinoma cells (MCF7).
- Qualitative biochemical analysis showed that methanolic extract fractions No-2, 3 and 8 (F-2, F-3, F-8) exhibited presence of saponin, flavonoid, steroid, tannin and glycoside.

The methanolic precipitate extract fractions (F-2, F-3 and F-8) showed significant higher cytotoxicity at low concentration to human lung carcinoma cells (A549) as compared to breast cancer cells (MCF7). Among all fractions, F-8 was most potent against lung carcinoma cells (A549) and breast carcinoma cells (MCF7). Qualitative biochemical analysis revealed the presence of all the tested active phytoconstituents (saponin, flavonoid, steroid, tannin and glycoside) in all the fractions (F-2, F-3 and F-8).

6.4: Under **Phase IV** of the study, MTT assay was done on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanolic precipitate (MPCT), Methanolic supernatant (MSCT) and MPCT extract fractions F-2, F-3 and F-8 of *Calamus tenuis* Roxb. shoots. Following are the result highlights of Phase-IV.

Result highlights of Phase IV:

- MPCT induced ~50% cells mortality at 9ug/ml to normal cell (L132).
- MSCT showed ~50% cytotoxicity between 10 to 50ug/ml in normal cell (L132).
- MPCT was found to be more cytotoxic to normal cells (L132) than MSCT.
- Fraction F-2 showed ~50% cytotoxicity at 8ug/ml in normal cell (L132).
- Fraction F-3 induced 50% cytotoxicity between 10 and 25ug/ml in normal cell (L132).
- Fractions F-8 was found highly cytotoxic to normal cell (L132) and induced ~50% cells mortality at 4ug/ml.

Among the methanolic partitioned crude extracts tested for cytotoxicity, the methanolic precipitate extract (MPCT) showed higher cytotoxicity to human lung normal cells (L132) as compared to methanolic supernatant extract (MSCT). The methanolic precipitate extract fractions (F-2, F-3 and F-8) showed significant higher cytotoxicity at low concentration to human lung normal cells (L132) as compared to crude extracts. Among the methanolic precipitate extract fractions, fraction F-8 was found most cytotoxic to human lung normal cells (L132) as compared to other fractions.

6.5: Under **Phase V** of the study, Lethal Concentration value (LC_{50}) was calculated as per MTT assay done (in previous phases) for cell viability and cytotoxicity assessment on exposure of Methanolic precipitate extract (MPCT), Methanolic supernatant extract (MSCT) and MPCT fractions against both human carcinoma (A549 and MCF7) and normal (L132) cells. Following are the result highlights of Phase-V.

Result highlights of Phase V:

- MPCT extract showed LC_{50} at 20, 40 and 9ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.
- MSCT extract showed LC_{50} at 100, >200 and 10ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.
- The MSCT extract fraction F-2 showed LC_{50} at 10, 25 and 10ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.
- The MSCT extract fraction F-3 showed LC_{50} at 10-25, 10 and 10-25ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.
- The MSCT extract fraction F-8 showed LC_{50} at 10, 10 and 4ug/ml of concentration against human lung carcinoma (A549), breast carcinoma (MCF7) and normal (L132) cells respectively.

As compared to MSCT, MPCT extract was found more potent against all assayed cell lines and achieved LC_{50} at lower concentration. Fraction F-2, F-3

and F-8 attained LC_{50} value at lower concentration against lung carcinoma cells (A549) as compared to breast carcinoma cells (MCF7). All the fractions (F-2, F-3 and F-8) showed higher cytotoxicity to all the assayed cell lines than crude extracts. However, fraction F-8 was found most potent against all the assayed cell lines but showed highest cytotoxicity to normal cell line (L132).

Overall results revealed that the crude extracts and fractions were more toxic to normal cells than to carcinoma cells, which means that the studied extracts and fractions of *Calamus tenuis* Roxb. shoots do not have anticancer potential against human lung (A549) and breast (MCF7) carcinoma cells. However, consumption of *Calamus tenuis* Roxb. shoots has not been reported to have serious health complications and the results of the present study revealed that the extracts of these plant are more toxic to normal cells, it may be presumed that this plant may be useful against non-cancerous benign tumors for controlling cell proliferation or eradication of such tumors by the process of cytotoxicity.