CHAPTER 5

RESULTS AND DISCUSSION

Calamus tenuis Roxb. shoots are consumed as vegetables and its occasional consumption is traditionally known to have some therapeutic potential. However, there was no documentation found on consumption pattern, traditional therapeutic beliefs and practices, and health issues due to consumption of the shoot; among the inhabitants of forest villages of Dibrugarh district of Assam. Besides, tumor cell growth and cell cycle inhibition, anti-inflammatory, anthelmintic and anti-diabetic activity of some *Calamus sp.* are well documented. However, there were no reports on phytochemical composition and cytotoxic potential of *Calamus tenuis* Roxb. shoots. Hence, the study was conducted to assess the same through a series of relevant protocols.

This chapter deals with the findings, statistical analysis, interpretation and discussion of the data, obtained during the course of research. Based on the objectives, results of the present research are divided into following five phases:

Phase I: Survey for assessing the consumption pattern and traditional therapeutic practices and beliefs of *Calamus tenuis* Roxb. shoots:

5.1.1 Background information of enrolled subjects of forest villages of Dibrugarh.

- 5.1.1.1 Education
- 5.1.1.2 Occupation
- 5.1.1.3 Income
- 5.1.1.4 Socio Economic Status (SES)
- 5.1.1.5 Subjects' medical history
- 5.1.1.6 Family medical history

5.1.2 Cooking pattern of *Calamus tenuis* Roxb. shoots.

5.1.3 Consumption pattern of *Calamus tenuis* Roxb. shoots.

5.1.3.1 Frequency of consumption

5.1.3.2 Period of maximum consumption during the year

5.1.4 Sources of *Calamus tenuis* Roxb. shoots.

5.1.5 Storage of *Calamus tenuis* Roxb. shoots.

5.1.6 Therapeutic beliefs for consumption of shoots.

5.1.7 Health issue beliefs on consumption of shoots.

5.1.8 Association between consumption of *Calamus tenuis* Roxb. shoots with education, occupation, income, socio economic status (SES), therapeutic beliefs, health issues beliefs, subjects' medical condition and family medical history.

5.1.9 Collection of *Calamus tenuis* Roxb. shoots and its primary processing.

Phase II: Crude extraction of *Calamus tenuis* Roxb. shoots, cytotoxicity assay on human carcinoma cells and qualitative phytochemical screening:

5.2.1 Crude extraction of *Calamus tenuis* Roxb. shoots involving successive use of different solvents.

5.2.2 MTT assay on human lung carcinoma (A549) cells for cell viability and cytotoxicity potential evaluation of various extracts of *Calamus tenuis* Roxb. shoots.

5.2.3 MTT assay on human breast carcinoma (MCF7) cells for cell viability and cytotoxicity potential evaluation of various extracts of *Calamus tenuis* Roxb. shoots.

5.2.4 Qualitative phytoconstituents screening of the extracts of *Calamus tenuis* Roxb. shoots.

Phase III: Fractionation of Methanol Precipitate extract of *Calamus tenuis* Roxb. shoots (MPCT), cytotoxicity assay on human carcinoma cells, and qualitative phytochemical screening:

5.3.1 Fractionation of methanolic precipitate extract (MPCT) extract of *Calamus tenuis* Roxb. shoots by Column Chromatography.

5.3.2 MTT assay on human lung carcinoma (A549) cells for cell viability and cytotoxicity potential evaluation of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots.

5.3.3 MTT assay on human breast carcinoma (MCF7) cells for cell viability and cytotoxicity potential evaluation of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots.

5.3.4: Qualitative phytoconstituents screening of the selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots.

Phase IV: Cell viability assay of MPCT extract, MSCT extract and MPCT fractions (F-2, F-3 and F-8) on human normal cells for evaluation of cytotoxicity activity:

5.4.1 MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanol precipitate (MPCT) and Methanol supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoots.

5.4.2 MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of MPCT extract fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots.

Phase V: Comparison of lethal concentration (LC_{50}) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts; and methanolic precipitate (MPCT) extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot against human carcinoma and normal cells:

5.5.1 Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoot against human carcinoma and normal cells.

5.5.2 Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot extracts against human carcinoma and normal cells.

Phase I: Survey for assessing the consumption pattern and traditional therapeutic practices and beliefs of *Calamus tenuis* Roxb. shoots

Survey with a total number of 350 households was conducted in 5 forest villages of Dibrugarh district of Assam. The enrolled subjects were interviewed for various aspects such as 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. Further, association between consumption of *Calamus tenuis* Roxb. shoots with education, occupation, income, socio economic status (SES), therapeutic beliefs, health issues beliefs, subjects' medical condition, family medical history and sources of shoots was analyzed.

5.1.1 Background information of enrolled subjects

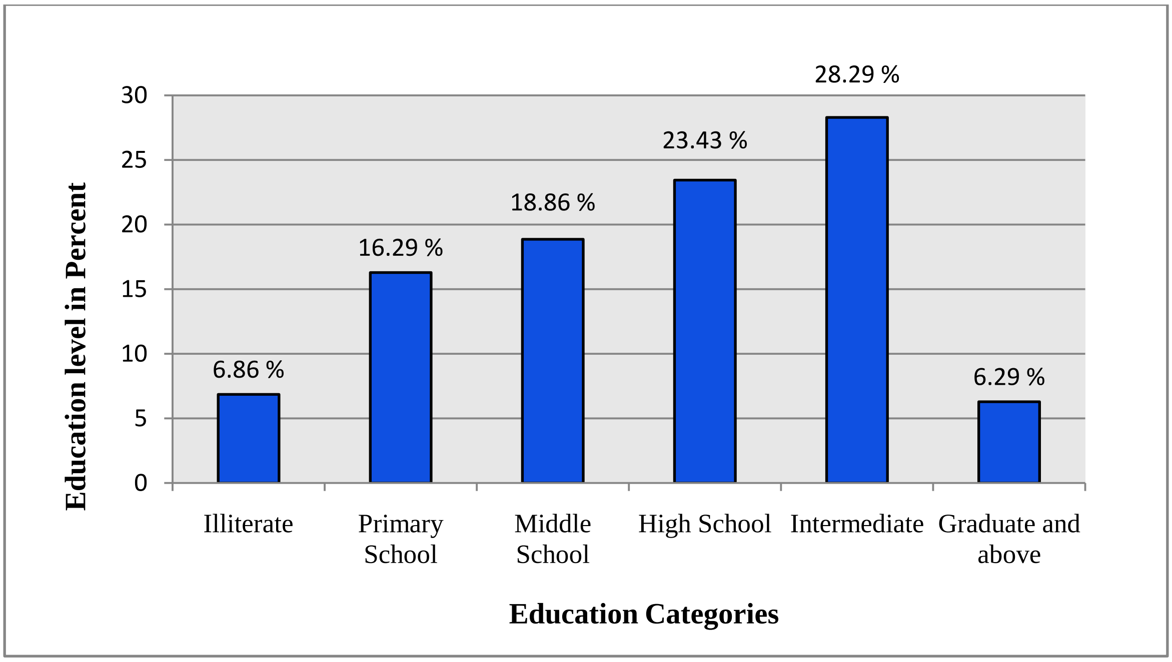
5.1.1.1 Education of subjects of surveyed forest villages of Dibrugarh, Assam

The Mean $\pm$ SD age of the subjects interviewed was 39.42 $\pm$ 11.27 years. As seen in Table 5.1.1.1, 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.

Table 5.1.1.1: Education of subjects of forest villages of Dibrugarh, Assam

SL No.	Education	N	%
1	Illiterate	24	6.86
2	Primary School	57	16.29
3	Middle School	66	18.86
4	High School	82	23.43
5	Intermediate	99	28.29
6	Graduate and above	22	6.29
Total		**350**	**100**

Figure 5.1.1.1: Education of subjects of forest villages of Dibrugarh, Assam



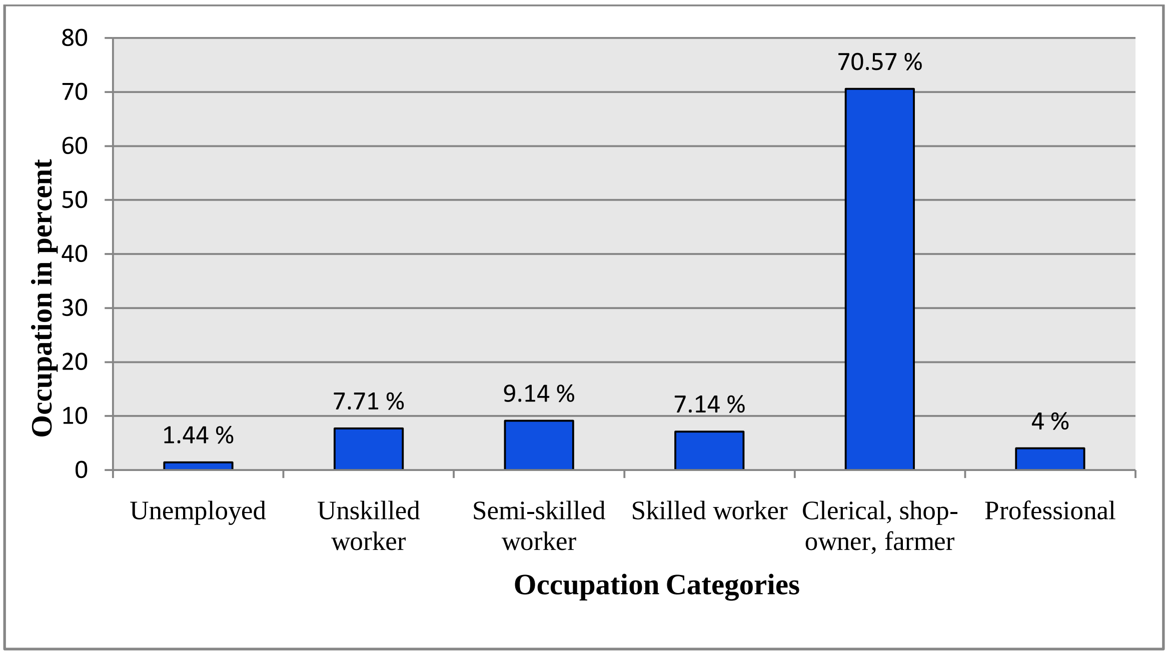
5.1.1.2: Occupation of subjects of forest villages of Dibrugarh

Table 5.1.1.2 reveals that 94.56% subjects 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.

Table 5.1.1.2: Occupation of subjects of forest villages of Dibrugarh, Assam

SL No.	Occupation	N	%
1	Unemployed	5	1.44
2	Unskilled worker	27	7.71
3	Semi-skilled worker	32	9.14
4	Skilled worker	25	7.14
5	Clerical, shop-owner, farmer	247	70.57
6	Professional	14	4.00
Total		**350**	**100**

Figure 5.1.1.2: Occupation of subjects of forest villages of Dibrugarh, Assam



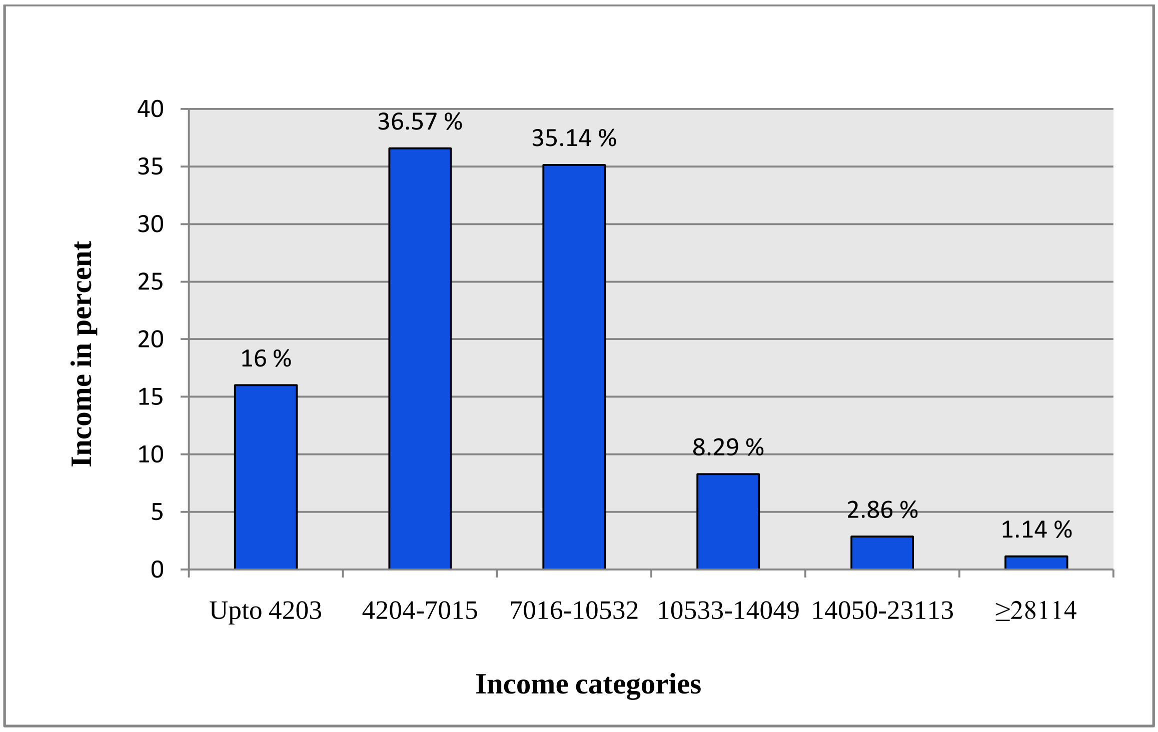
5.1.1.3: Income of subjects of forest villages of Dibrugarh

Table 5.1.1.3 shows that 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.

Table 5.1.1.3: Income of subjects of forest villages of Dibrugarh, Assam

SL No.	Income (Rs.)	N	%
1	Upto 4203	56	16.00
2	4204-7015	128	36.57
3	7016-10532	123	35.14
4	10533-14049	29	8.29
5	14050-23113	10	2.86
6	≥28114	4	1.14
Total		**350**	**100**

Figure 5.1.1.3: Income of subjects of forest villages of Dibrugarh, Assam



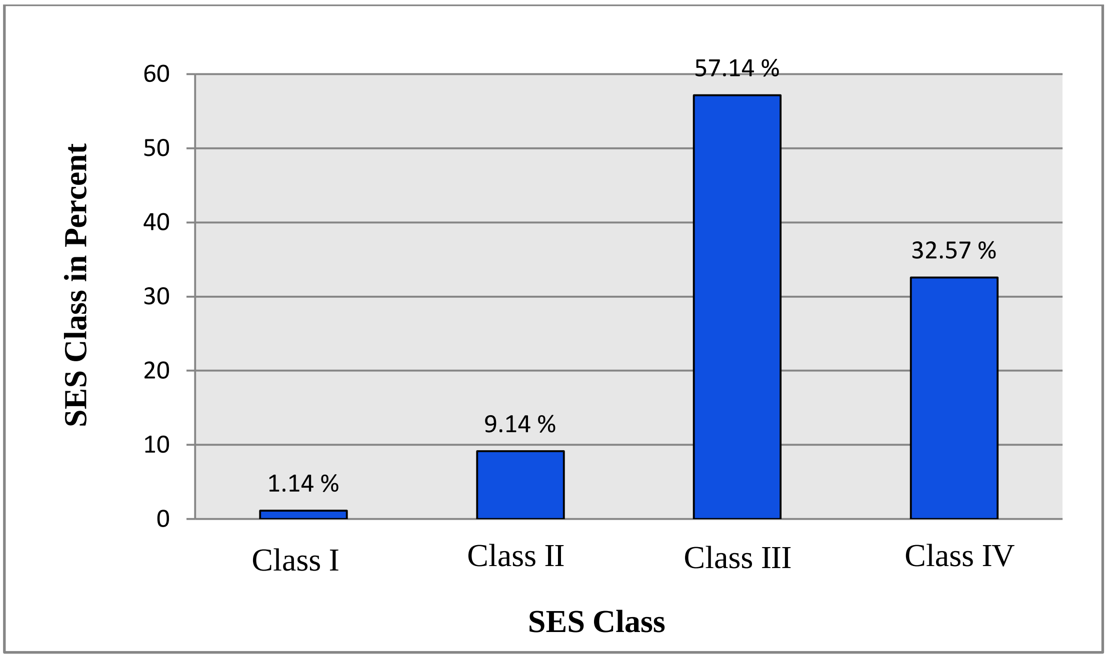
5.1.1.4: Socio Economic Status (SES) of subjects of forest villages of Dibrugarh

Table 5.1.1.4 shows that majority (89.71%) of the subjects surveyed belonged to SES class III and IV; and only a few belonged to class I (1.14%) and class II (9.14%).

Table 5.1.1.4: Socio Economic Status (SES) of subjects of forest villages of Dibrugarh, Assam

SL No.	SES: Class	N	%
1	Class I	4	1.14
2	Class II	32	9.14
3	Class III	200	57.14
4	Class IV	114	32.57
Total		**350**	**100**

Figure 5.1.1.4: Socio Economic Status (SES) of subjects of forest villages of Dibrugarh, Assam



5.1.1.5: Subjects' medical condition of forest villages of Dibrugarh

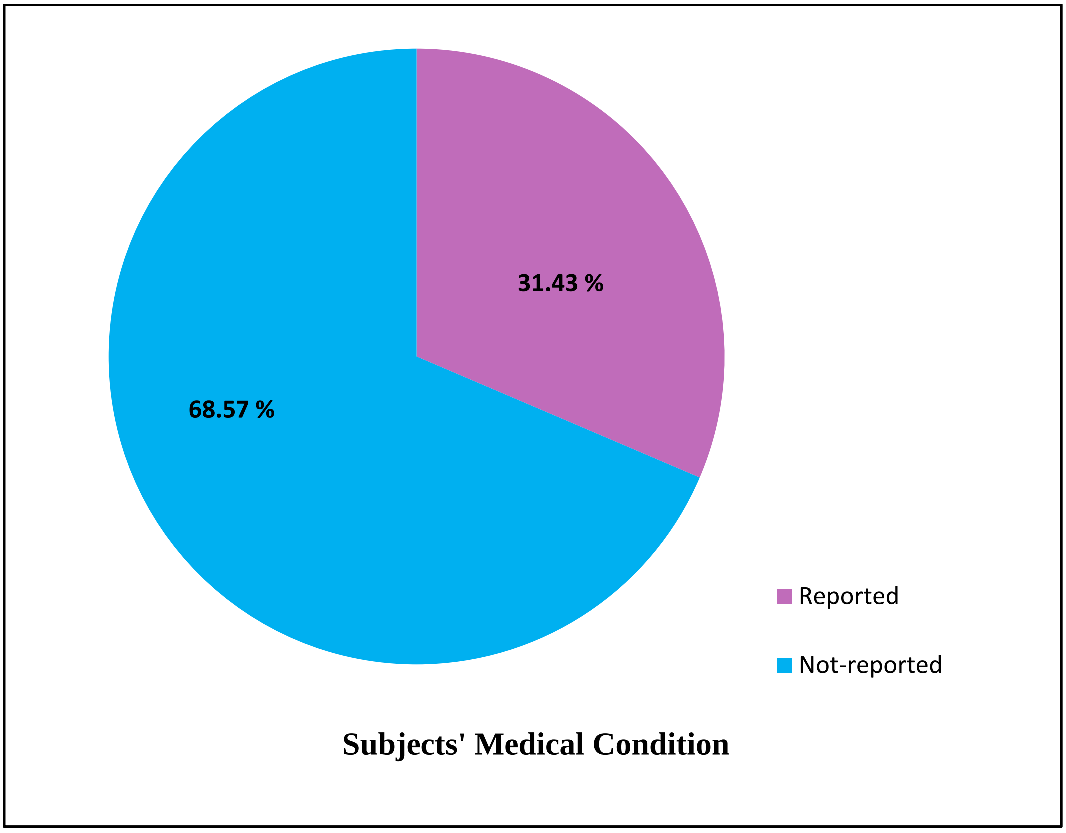
As seen in Table 5.1.1.5, majority of them (68.57%) did not report about having any illness; however 31.43% of the subjects mentioned that they suffered from some health disorder.

Table 5.1.1.5: Subjects' medical condition of forest villages of Dibrugarh, Assam

SL No.	Subjects' medical condition*	N	%
1	Reported	110	31.43
2	Not-reported	240	68.57

*Prevalence of cardio vascular disease, diabetes mellitus, gastro intestinal disorder, skin disease, kidney stone, dental disorder, eye disorder, body pain, etc.

Figure 5.1.1.5: Subjects' medical condition of forest villages of Dibrugarh, Assam



5.1.1.6: Family medical history of forest villages of Dibrugarh

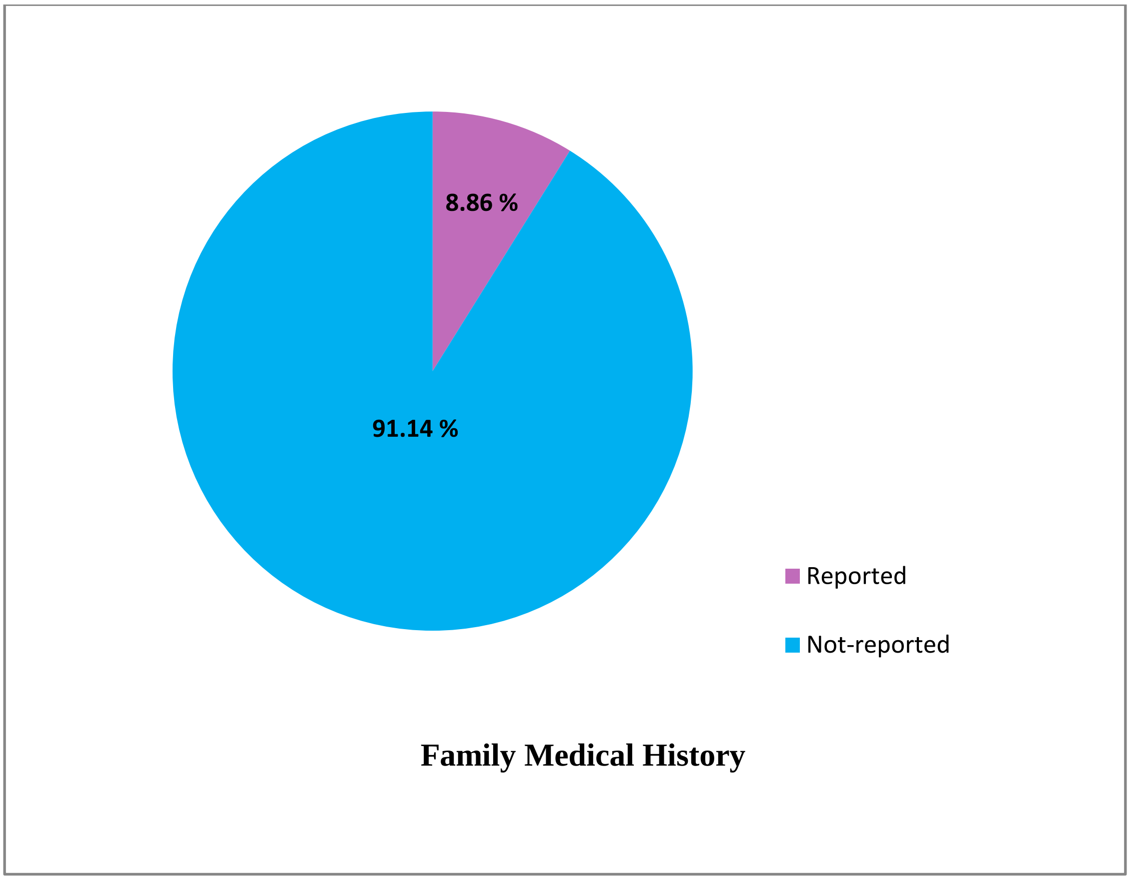
As seen in Table 5.1.1.6, subjects revealed that 91.14% did not report for any family medical history while 8.86% of them mentioned that they had some illness history in their family.

Table 5.1.1.6: Family medical history of forest villages of Dibrugarh, Assam

SL No.	Family medical history*	N	%
1	Reported	31	8.86
2	Not-reported	319	91.14

*Prevalence of cardiovascular disease, diabetes mellitus, gastrointestinal disorder, skin disease, kidney stone, dental disorder, eye disorder, body pain, etc.

Figure: 5.1.1.6: Family medical history of forest villages of Dibrugarh, Assam



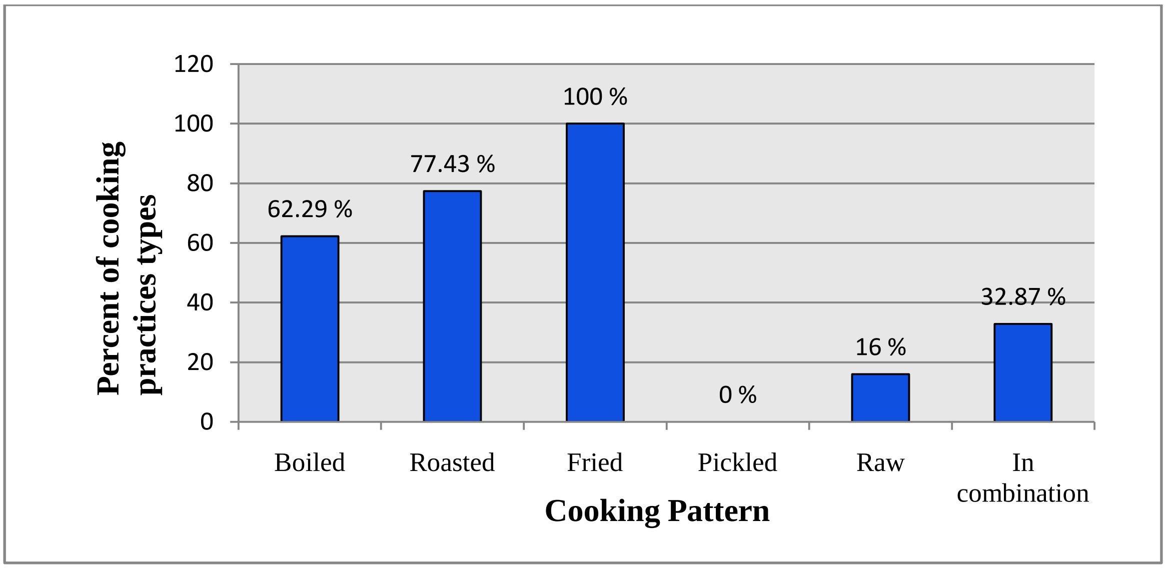
5.1.2 Cooking pattern of *Calamus tenuis* Roxb. shoots

According to Table 5.1.2 cooking pattern of *Calamus tenuis* Roxb. shoots revealed that most subjects followed frying, roasting and boiling as most preferred methods. 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.

Table 5.1.2: Cooking pattern of *Calamus tenuis* Roxb. shoots followed by subjects of forest villages of Dibrugarh, Assam

SL No.	Cooking Pattern types	N	%
1	Boiled	218	62.29
2	Roasted	271	77.43
3	Fried	350	100.00
4	Pickled	0	0.00
5	Raw	56	16.00
6	In combination with other food items	115	32.87

Figure 5.1.2: Cooking pattern of *Calamus tenuis* Roxb. shoots followed by subjects of forest villages of Dibrugarh, Assam



5.1.3 Consumption pattern of *Calamus tenuis* Roxb. shoots

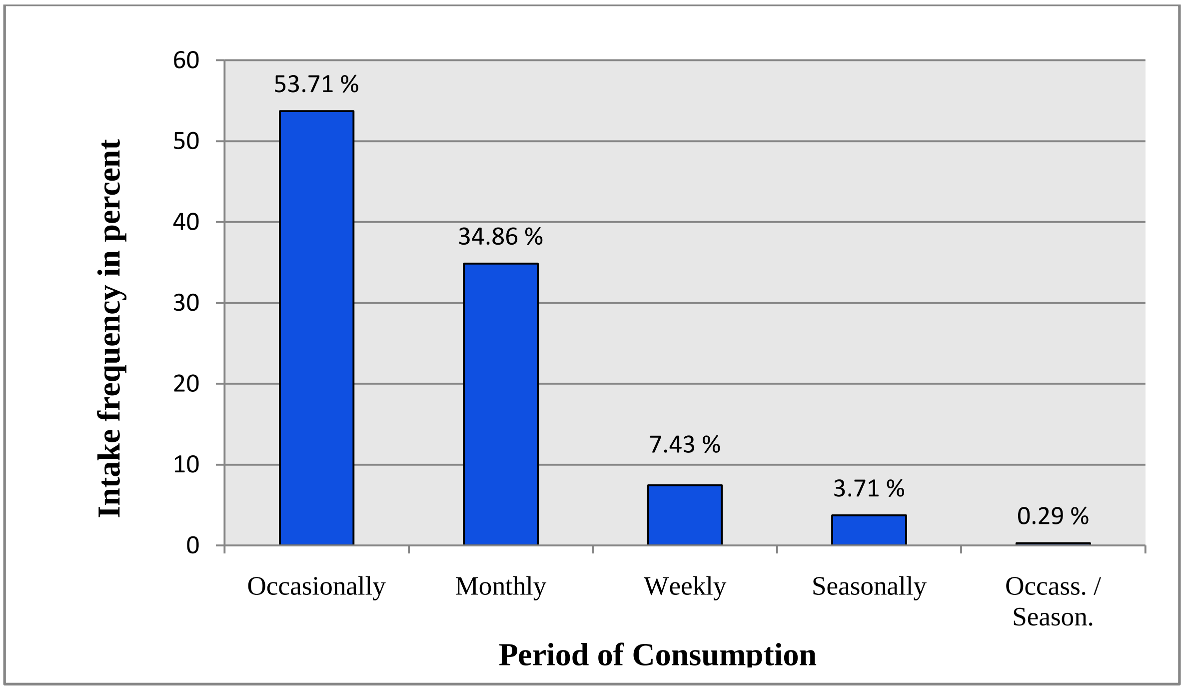
5.1.3.1: Frequency of consumption

As seen in Table 5.1.3.1, most of the subjects resorted to occasional or monthly consumption of *Calamus tenuis* Roxb. shoots.

Table 5.1.3.1: Frequency of *Calamus tenuis* Roxb. shoots consumption by the subjects of forest villages of Dibrugarh, Assam

SL No.	Intake Frequency	N	%
1	Occasionally	188	53.71
2	Monthly	122	34.86
3	Weekly	26	7.43
4	Seasonally	13	3.71
5	Occass. / Season.	1	0.29
Total		**350**	**100**

Figure 5.1.3.1: Frequency of *Calamus tenuis* Roxb. shoots consumption by the subjects of forest villages of Dibrugarh, Assam



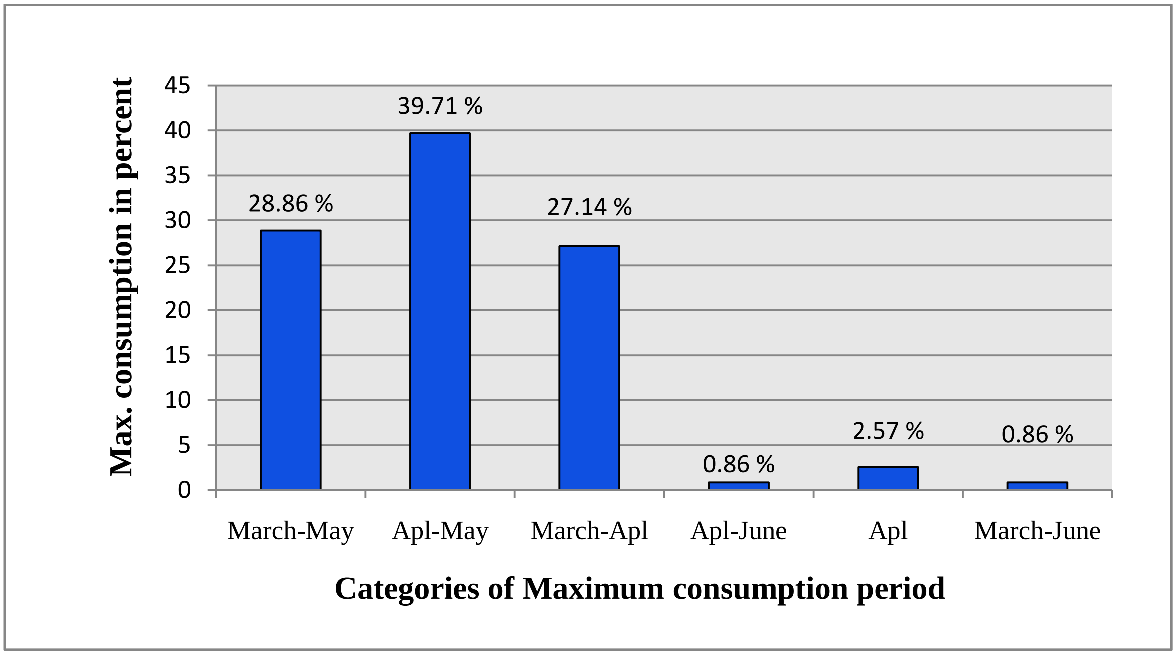
5.1.3.2: Period of maximum consumption during the year

Most of the subjects consumed *Calamus tenuis* Roxb. shoots traditionally since childhood. As seen in Table 5.1.3.2, Consumption of the shoot was maximum (68.57%) during the month of March-May.

Table 5.1.3.2: Period of maximum consumption of *Calamus tenuis* Roxb. shoots during the year by subjects of forest villages of Dibrugarh, Assam

SL No.	Max. Consumption Period	N	%
1	March-May	101	28.86
2	Apl-May	139	39.71
3	March-Apl	95	27.14
4	Apl-June	3	0.86
5	Apl	9	2.57
6	March-June	3	0.86
Total		**350**	**100**

Figure 5.1.3.2: Period of maximum consumption of *Calamus tenuis* Roxb. shoots during the year by subjects of forest villages of Dibrugarh, Assam



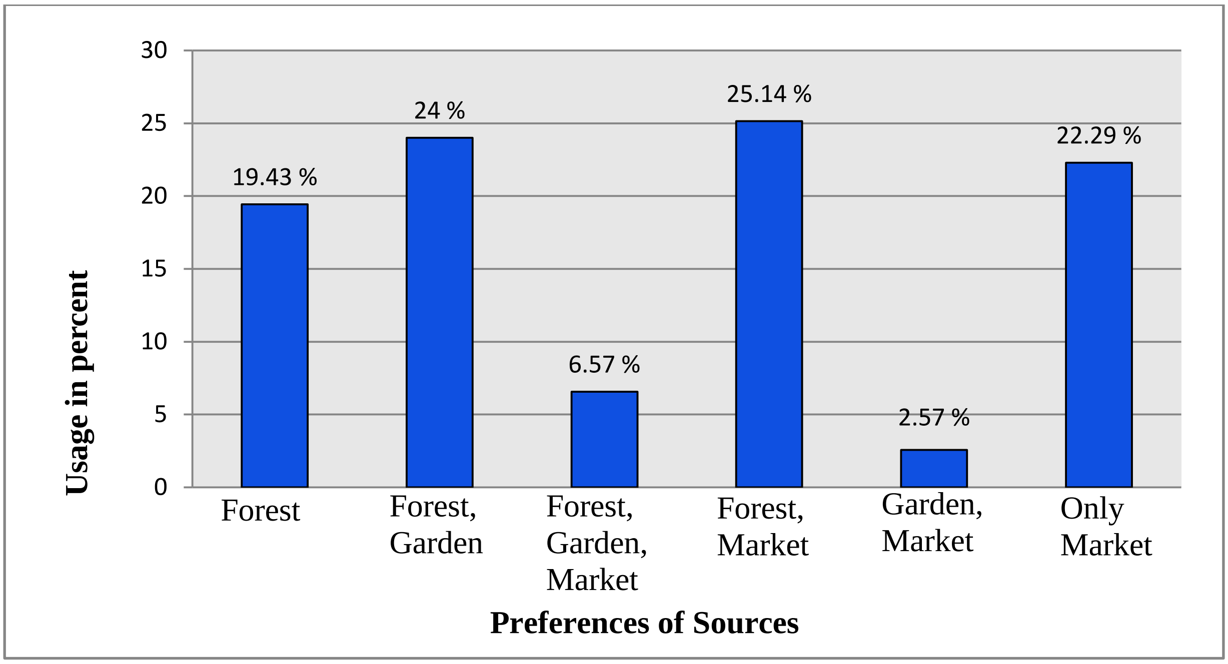
5.1.4 Sources of *Calamus tenuis* Roxb. shoots

As seen in Table 5.1.4, the sources of *Calamus tenuis* Roxb. shoots for most subjects were forest followed by the local market and some grew shoots in their kitchen garden.

Table 5.1.4: Sources of *Calamus tenuis* Roxb. shoots of the subjects of forest villages of Dibrugarh, Assam

SL No.	Preferred *Calamus tenuis* Roxb. shoots sources	N	%
1	Only Forest	68	19.43
2	Forest, Garden	84	24.00
3	Forest, Garden, Market	23	6.57
4	Forest, Market	88	25.14
5	Garden, Market	9	2.57
6	Only Market	78	22.29
Total		**350**	**100**

Figure 5.1.4: Sources of *Calamus tenuis* Roxb. shoots of the subjects of forest villages of Dibrugarh, Assam



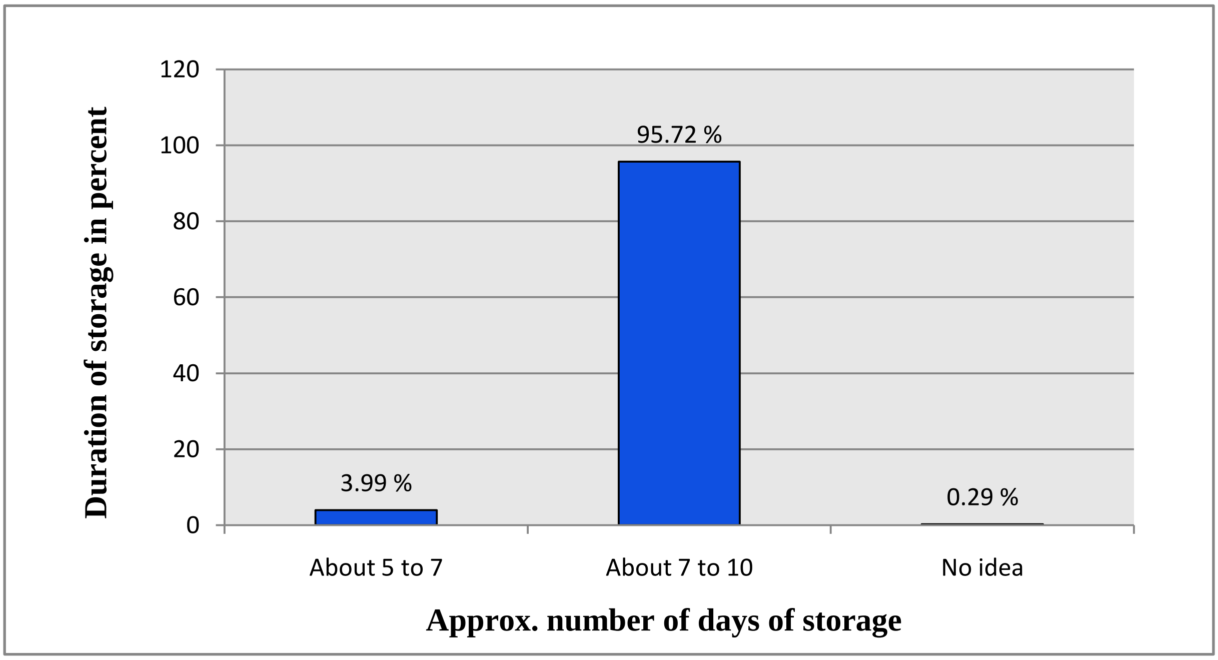
5.1.5 Storage of *Calamus tenuis* Roxb. shoots

According to Table 5.1.5, 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%) said that it could be stored for about 5-7 days only.

Table 5.1.5: Storage of *Calamus tenuis* Roxb. shoots by the subjects of forest villages of Dibrugarh, Assam

SL No.	*Calamus tenuis* Roxb.. storage (days)	N	%
1	About 5 to 7	14	3.99
2	About 7 to 10	335	95.72
3	No idea	1	0.29
Total		**350**	**100**

Figure 5.1.5: Storage of *Calamus tenuis* Roxb. shoots by the subjects of forest villages of Dibrugarh, Assam



5.1.6 Therapeutic beliefs for consumption of shoots

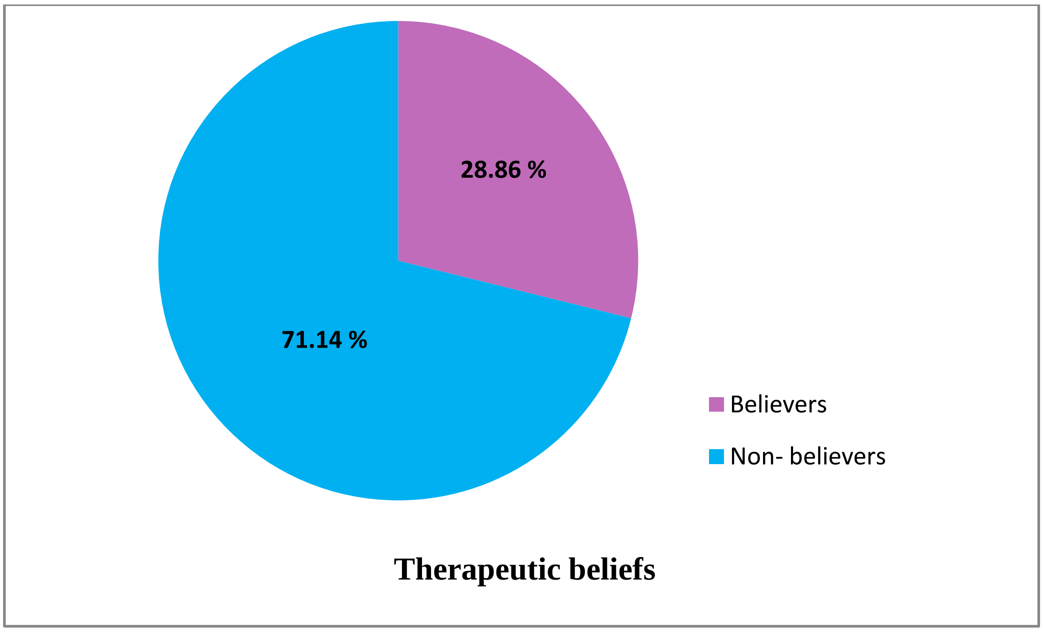
As seen in Table 5.1.6, most of the subjects (71.14%) did not believed that the consumption of *Calamus tenuis* Roxb. shoots treat illness while 28.86% of the subjects believed that it may be helpful against some illness like cough, intestinal worms, smallpox, toothache, wound healing and low blood pressure.

Table 5.1.6: Therapeutic beliefs of the subjects of forest villages of Dibrugarh, Assam

SL No.	Therapeutic beliefs*	N	%
1	Believers	101	28.86
2	Non- believers	249	71.14

*Therapeutic beliefs against intestinal worms, cough, prevention of smallpox, blood purification, wound healing, improvement of low blood pressure and toothache.

Figure 5.1.6: Therapeutic beliefs of the subjects of forest villages of Dibrugarh, Assam



5.1.7 Health issues beliefs on consumption of shoots

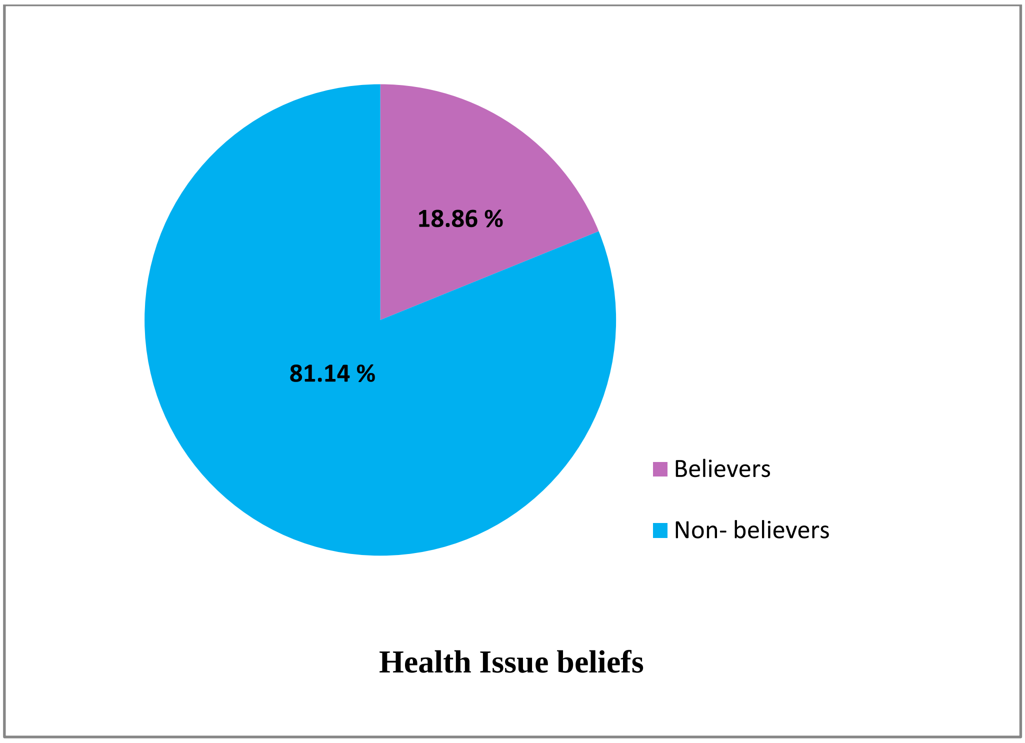
As seen in Table 5.1.7, most of the subjects (81.14%) did not believe that the consumption of the shoots cause any side effects whereas, 18.86% of them believed that consumption of shoot at night and in the empty stomach cause stomach problem.

Table 5.1.7: Health issues beliefs on consumption of shoots of the subjects of forest villages of Dibrugarh, Assam

SL No.	Health issue beliefs*	N	%
1	Believers	66	18.86
2	Non- believers	284	81.14

*Stomach disorder on consumption of the shoots at night and in empty stomach

Figure 5.1.7: Health issues beliefs on consumption of shoots of the subjects of forest villages of Dibrugarh, Assam



5.1.8 Association between consumption of *Calamus tenuis* Roxb. shoots with education, occupation, income, socioeconomic status (SES), therapeutic beliefs, health issues beliefs, subjects' medical condition and family medical history

According to Table 5.1.8, 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 the occupation of the subjects was found. The unemployed and unskilled workers consumed shoot more as compared to others. The income of subjects had a high impact on the amount of consumption. The people who had low income consumed shoot more than those of other income groups. A significant association was seen between consumption of the shoots and socio-economic status of the subjects. The people belonging to the upper class of SES consumed shoots in fewer 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 people. The preference of sources of the shoot had a significant impact on consumption of shoots. The people, who chose forest as a source of shoots, consumed more.

Table 5.1.8: Association of consumption of *Calamus tenuis* Roxb. shoots with education, occupation, income, socioeconomic status (SES), therapeutic beliefs, health issue beliefs, subjects' medical condition, family medical history and sources of the shoots

Parameters		**Total Consumption (≤ 80g) N (%)**	**Total Consumption (≥ 80g) N (%)**	**Chi sq. (χ^2)**
Education	Upto Primary	30 (37.04)	51 (62.96)	54.72***
	Middle – High school	86 (58.10)	62 (41.90)	
	Intermediate and above	89 (73.55)	32 (26.45)	
Occupation	Unemployed – Unskilled worker	13 (40.62)	19 (59.38)	19.04***
	Semi skilled – Skilled worker	36 (63.16)	21 (36.84)	
	Clerical – Professional	156 (59.77)	105 (40.23)	
Income (Rs)	≤1406 - 4203	12 (21.43)	44 (78.57)	39.50***
	4204 - 10532	161 (64.14)	90 (35.86)	
	10533 - ≥28114	32 (74.42)	11 (25.58)	
Socio Economic Status (SES)	C I	2 (50.00)	2 (50.00)	46.72***
	C II	24 (75.00)	8 (25.00)	
	C III	141 (70.50)	59 (29.50)	
	C IV	38 (33.34)	76 (66.66)	
Therapeutic beliefs	Believers	54 (53.47)	47 (46.53)	1.52^{NS}
	Non-believers	151 (60.64)	98 (39.36)	
Health issues beliefs	Believers	39 (59.09)	27 (40.91)	0.01^{NS}
	Non-believers	166 (58.45)	118 (41.55)	
Subjects' Medical Condition	Reported	66 (60.00)	44 (40.00)	0.13^{NS}
	Not reported	139 (57.92)	101 (42.08)	
Family Medical History	Reported	18 (58.06)	13 (41.94)	0.00^{NS}
	Not reported	187 (58.62)	132 (41.38)	
Calamus tenuis Roxb. Shoot sources	Only Forest	22 (32.35)	46 (67.65)	46.12^{***}
	Forest, Garden	44 (52.38)	40 (47.62)	
	Forest, Garden, Market	9 (39.13)	14 (60.87)	
	Forest, Market	61 (69.32)	27 (30.68)	
	Garden, Market	5 (55.56)	4 (44.44)	
	Only Market	64 (82.05)	14 (17.95)	

***- Significant at p<0.001 NS- Non Significant

Total consumption is the amount of shoot consumed in total number of times during the entire season, where 80g is the mean of the total consumption

Figures in parenthesis indicates percentage

Result highlights of Phase I

- The plant was identified as *Calamus tenuis* Roxb. (Aracaceae family).
- The Mean $\pm$ SD age of the subjects interviewed was 39.42 $\pm$ 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.

Result highlights of Phase I (Contd.)

- 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 cool 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 believe 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 affects whereas, 18.86% of them believed that consumption of shoot at night and in empty stomach cause stomach problem.

Result highlights of Phase I (Contd.)

- 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 chose forest as a source of shoots, consumed more.

Phase II: Crude extraction of *Calamus tenuis* Roxb. shoots, cytotoxicity assay on human carcinoma cells and qualitative phytochemical screening:

Crude extraction of the powdered dry shoots of *Calamus tenuis* Roxb. was done using different solvents (Hexane, Ethyl acetate and Methanol). The extracts were tested on human lung (A549) and breast (MCF7) carcinoma cells by Cell viability assay [MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to evaluate the cytotoxicity potential of the extracts. Qualitative biochemical analysis was done to assess phytoconstituents of the extracts.

5.2.1 Crude extraction of *Calamus tenuis* Roxb. shoots involving successive use of different solvents

Raw 14.09 kg *Calamus tenuis* Roxb. shoots were collected which yielded 3.58 kg of fresh edible shoots. The moisture content of the edible portion of the shoots was found to be 92%. The fresh edible shoots were processed and 275 g dry powder sample was obtained. As seen in Table 5.2.1, the successive solvent extraction of 275g of powdered dry *Calamus tenuis* Roxb. shoots, on removal of excess solvent by rotary vacuum evaporator yielded 3.83g, 1.38g, and 31.63g of crude extracts of hexane (HECT), ethyl acetate (EACT) and methanol (sticky paste) respectively. The methanolic extract (sticky paste) was partitioned with distilled water and lyophilized which yielded 4.64g of amorphous powder (MPCT) and 15.16 g of sticky paste (MSCT).

Table 5.2.1: Crude extracts yield of *Calamus tenuis* Roxb. shoots

<table>
<tr><th>SL No.</th><th colspan="2">Crude Extract</th><th>Amount (g)</th></tr>
<tr><td>1</td><td colspan="2">Hexane (HECT)</td><td>3.83</td></tr>
<tr><td>2</td><td colspan="2">Ethyl acetate (EACT)</td><td>1.38</td></tr>
<tr><td>3</td><td colspan="2">Methanol</td><td>31.63</td></tr>
<tr><td>4</td><td rowspan="2">Methanolic water portioned and lyophilized extracts</td><td>from methanolic precipitate (MPCT)</td><td>4.64</td></tr>
<tr><td>5</td><td>from methanolic supernatant (MSCT)</td><td>15.16</td></tr>
</table>

5.2.2 MTT assay on human lung carcinoma (A549) cells for cell viability and cytotoxicity potential evaluation of various extracts of *Calamus tenuis* Roxb. shoots

MTT assay was done on human lung carcinoma cells (A549) 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. Results of cell viability assay of *Calamus tenuis* Roxb. shoots extracts on human lung carcinoma cells (A549) given in Table 5.2.2 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.2.2, with the increase in concentration of the extracts from 10 to 200ug/ml, the percent viability of lung carcinoma cells (A549) decreased for all the four extracts in the order of MPCT, MSCT, HECT and EACT and this percent viability decrease was found to be statistically significant (minimum $p<0.01$). The results revealed that even at higher concentration (≥200ug/ml) HECT and EACT were not able to induce 50% cells mortality whereas, MPCT and MSCT showed ~50% cytotoxicity at 20ug/ml and 100ug/ml respectively. Hence, among various extracts MPCT was found to be the most cytotoxic to lung carcinoma cells (A549). The same data is graphically presented in figures 5.2.2 (a-d).

Table 5.2.2: Mean percent viability of Lung carcinoma cells (A549) treated with different concentrations of various *Calamus tenuis* Roxb. shoots extracts

Extract	Concentration (ug/ml)											'F' Value (Significance)
	10	20	30	40	50	60	70	80	90	100	200	
MPCT (n=3) (Mean ±SD)	59.02 ±0.08	46.18 ±0.31	33.08 ±1.42	28.14 ±0.47	23.00 ±0.63	10.74 ±0.88	8.78 ±0.49	9.04 ±0.94	9.10 ±0.95	9.39 ±0.49	10.17 ±0.52	**1979.5 *** **
MSCT (n=3) (Mean ±SD)	82.74 ±1.62	80.88 ±1.14	72.03 ±0.53	68.04 ±1.38	63.57 ±0.96	62.16 ±0.52	62.35 ±2.91	60.67 ±1.36	58.85 ±0.85	50.61 ±0.56	43.32 ±0.03	**242.3 *** **
HECT (n=3) (Mean ±SD)	99.16 ±0.73	92.44 ±2.09	95.91 ±1.57	95.12 ±1.72	95.63 ±1.57	96.30 ±2.38	95.48 ±1.11	89.27 ±1.65	89.21 ±5.33	85.64 ±0.60	76.45 ±2.05	**24.89 *** **
EACT (n=3) (Mean ±SD)	94.35 ±3.89	95.24 ±1.19	95.60 ±2.04	94.37 ±4.10	95.00 ±1.05	97.78 ±1.19	95.32 ±1.08	97.71 ±1.54	94.24 ±0.92	93.52 ±3.59	85.11 ±6.32	**3.81 ** **
'F' Value (Significance)	**209.92*** **	**856.05*** **	**1311.47*** **	**542.07*** **	**2878.14*** **	**2450.15*** **	**1800.13*** **	**2450.68*** **	**594.38*** **	**1283.81*** **	**315.57*** **	

Mean values represent the average of triplicates. Level of significance in increasing order (** p<0.01, ***, p<0.001).

Figure 5.2.2 (a-d): Percent viability of Lung carcinoma cells (A549) treated with different concentrations of various *Calamus tenuis* Roxb. shoots extracts

Fig. 5.2.2 (a): MPCT-A549

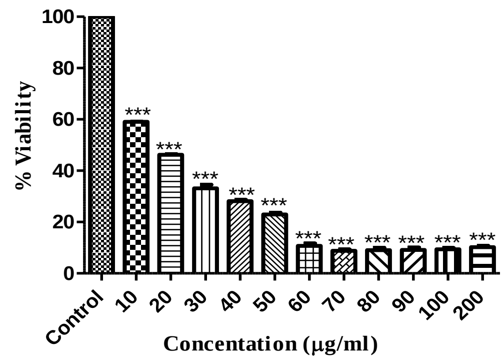
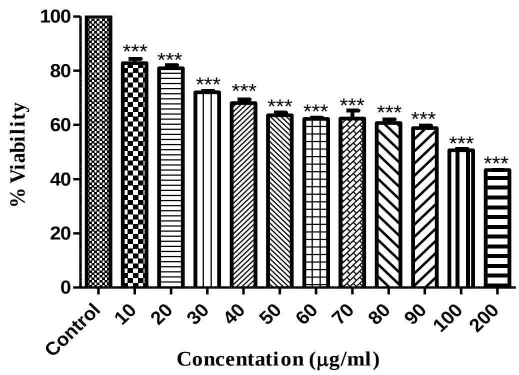
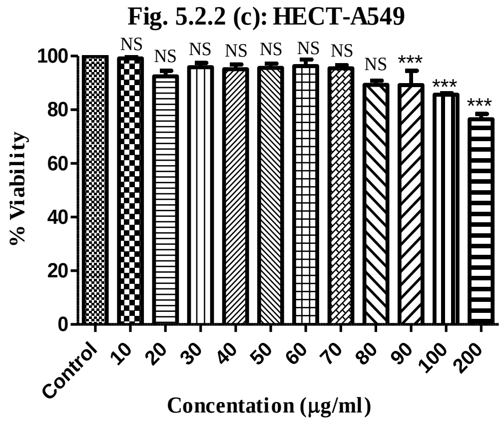
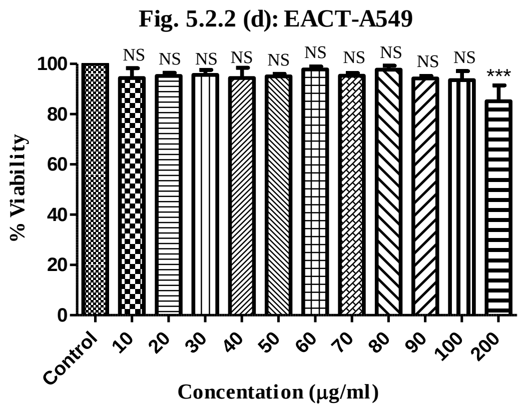


Fig. 5.2.2 (b): MSCT-A549







Where ***p<0.001 denotes power of significance as compared to control and NS denotes non-significance.

5.2.3 MTT assay on human breast carcinoma (MCF7) cells for cell viability and cytotoxicity potential evaluation of various extracts of *Calamus tenuis* Roxb. shoots

MTT assay was done on human breast carcinoma cells (MCF7) 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. Results of cell viability assay of *Calamus tenuis* Roxb. shoots extracts on human breast carcinoma cells (MCF7) given in Table 5.2.3 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.2.3, with the increase in concentration of the extracts from 10 to 200ug/ml, the percent viability of breast carcinoma cells (MCF7) decreased for all the four extracts in the order of MPCT, MSCT, HECT and EACT and this percent viability decrease was found to be statistically significant (minimum p<0.05) except at 10ug/ml. The results revealed that HECT, EACT and MSCT were not able to induce 50% cells mortality even at higher concentration (≥200ug/ml), whereas MPCT showed ~50% cytotoxicity at 40ug/ml. Hence, among various extracts MPCT was found to be the most cytotoxic to breast carcinoma cells (MCF7). The same data is graphically presented in figures 5.2.3 (a-d).

Table 5.2.3: Mean percent viability of Breast carcinoma cells (MCF7) treated with different concentrations of various *Calamus tenuis* Roxb. shoots extracts

Extract	Concentration (ug/ml)											*'F' Value (Significance)*
	10	20	30	40	50	60	70	80	90	100	200	
MPCT (n=3) (Mean± SD)	86.48 ±1.92	59.04 ±1.03	54.40 ±0.55	48.72 ±0.69	48.18 ±3.20	26.19 ±2.39	10.66 ±0.93	10.01 ±0.76	11.30 ±0.62	10.66 ±0.88	10.23 ±0.65	**1235.5 *****
MSCT (n=3) (Mean± SD)	93.46 ±0.44	83.26 ±3.10	86.78 ±1.79	82.25 ±2.02	81.29 ±8.04	79.16 ±2.36	75.01 ±2.95	82.32 ±4.82	77.80 ±4.19	74.67 ±3.48	61.65 ±2.34	**13.76 *****
HECT (n=3) (Mean± SD)	96.19 ±3.17	96.67 ±2.98	95.53 ±3.17	97.80 ±0.43	98.22 ±1.11	96.80 ±2.72	93.23 ±2.74	92.56 ±3.07	89.47 ±4.75	84.62 ±0.88	73.34 ±1.75	**22.61 *****
EACT (n=3) (Mean± SD)	91.99 ±7.66	91.34 ±7.14	95.14 ±3.86	89.30 ±7.82	90.83 ±7.10	96.55 ±2.13	88.57 ±5.89	95.06 ±3.29	89.92 ±6.70	90.99 ±5.82	73.87 ±6.15	**2.97 ***
'F' Value (Significance)	**2.82^{NS}**	**47.33 *****	**160.10 *****	**84.33 *****	**46.16 *****	**654.66 *****	**338.80 *****	**442.98*****	**199.26 *****	**344.27 *****	**234.14 *****	

Mean values represent the average of triplicates. Level of significance in increasing order- (* p<0.05, *** p<0.001) and NS denotes non-significance.

Figure 5.2.3 (a-d): Percent viability of Breast carcinoma cells (MCF7) treated with different concentrations of various *Calamus tenuis* Roxb. shoots extracts

Fig. 5.2.3 (a): MPCT-MCF7

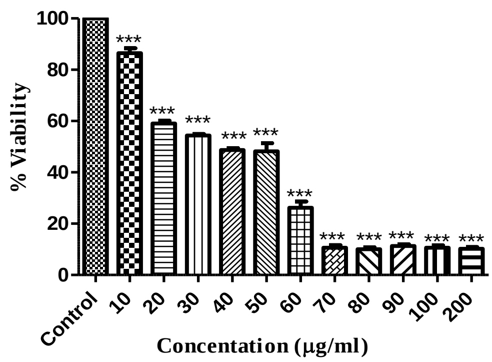


Fig.5.2.3 (b): MSCT-MCF7

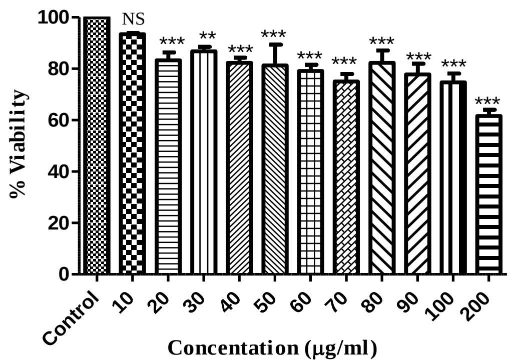


Fig. 5.2.3 (c): HECT-MCF7

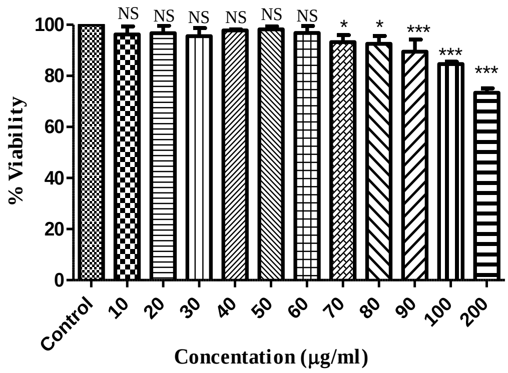
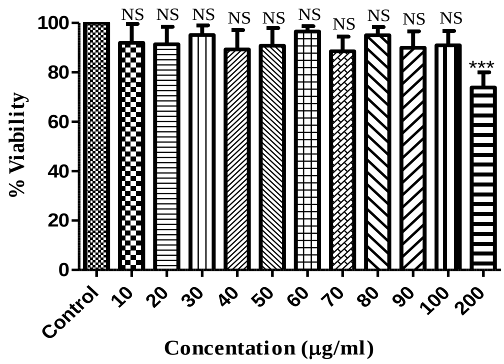


Fig. 5.2.3 (d): EACT-MCF7



Where *p<0.05, **p<0.01 and ***p<0.001 denotes power of significance as compared to control and NS denotes non-significance.

5.2.4 Qualitative phytoconstituents screening of the extracts of *Calamus tenuis* Roxb. shoots

A qualitative biochemical analysis was done for assessment of active phytoconstituents present in the crude extracts of *Calamus tenuis* Roxb. shoots, used for cell viability assay. As shown in Table 5.2.4, the biochemical analysis revealed that methanolic extracts (MSCT and MPCT) exhibited presence of saponin, flavonoid, steroid, tannin and glycoside. Hexane extract (HECT) showed presence of saponin and steroid, whereas ethyl acetate (EACT) emerged as only steroid rich extract that lacked all the other said ingredients.

Table 5.2.4: Biochemical analysis of the extracts of *Calamus tenuis* Roxb. shoots for phytoconstituents screening

SL No	Constituents	Name of the test	Results			
			Hexane (HECT)	Ethyl acetate (EACT)	Methanol supernatant (MSCT)	Methanol Precipitate (MPCT)
1	Saponin	Frothing test	+	-	+	+
2	Flavonoid	HCl acid test	-	-	+	+
3	Steroid	Salkowski's test	+	+	+	+
4	Tannin	Ferric chloride test	-	-	+	+
5	Glycoside	General test	-	-	+	+
		Fehling's test	-	-	+	+

The symbols '+' shows present and '-' shows absent

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.

Phase III: Fractionation of Methanol Precipitate extract of *Calamus tenuis* Roxb. shoots (MPCT), cytotoxicity assay on human carcinoma cells, and qualitative phytochemical screening:

Among the tested extracts (Cell viability assay) in Phase-II of the study, the partitioned methanolic precipitate extract (MPCT) was found to be most potent against all assayed human carcinoma cell lines, besides constituted all the biochemically analyzed phytoconstituents. Therefore, MPCT was fractionated by Column Chromatography for further investigation. Fractions selected for Cell viability assay were biochemically analyzed for their qualitative phytoconstituents.

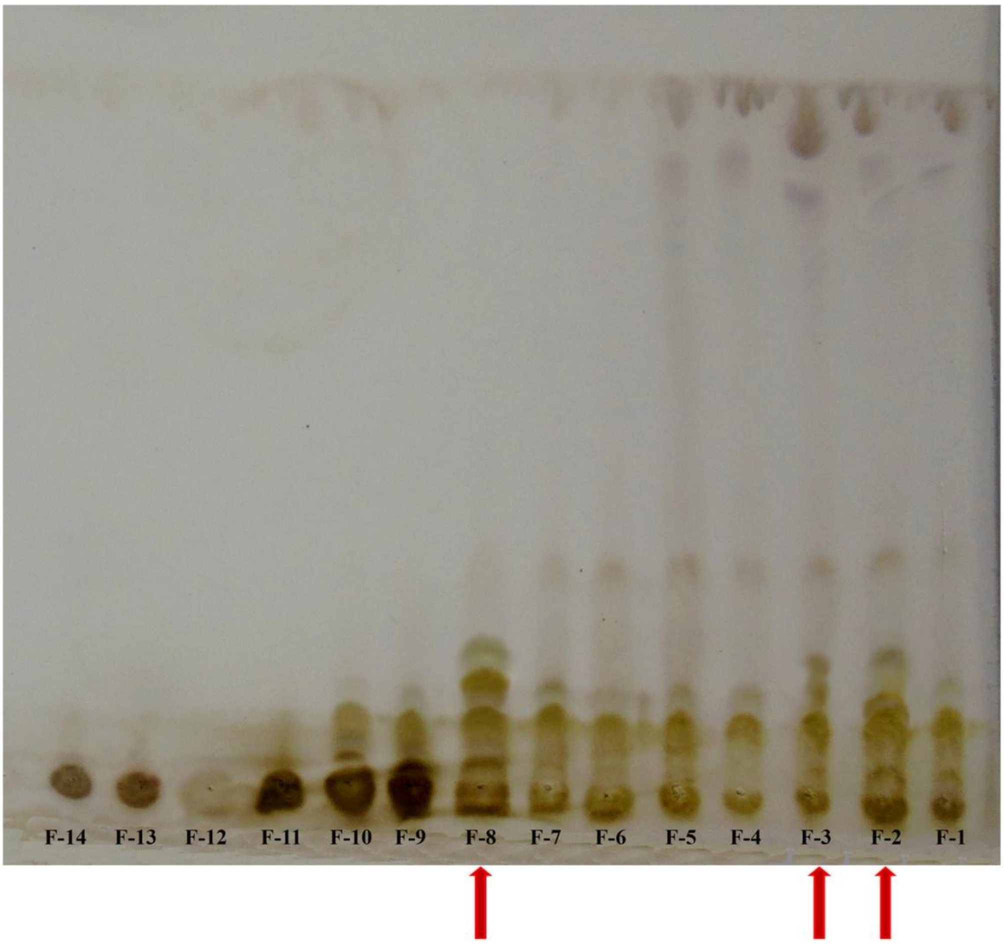
5.3.1 Fractionation of methanolic precipitate extract (MPCT) extract of *Calamus tenuis* Roxb. shoots by Column Chromatography

Methanolic precipitate extract (MPCT) was fractionated by Column Chromatography and 14 fractions (F-1 to F-14) were obtained in total (Table- 5.3.1). Fraction No- 2, 3 and 8 (F-2, F-3, F-8) were found to constitute and include the similar TLC spots as other fractions. Besides, fractions F-2, F-3 and F-8 showed a majority of phytoconstituents among all the fractions, as per TLC observation (Plate- 5.3.1). Therefore, fractions F-2, F-3 and F-8 were selected for further Cell viability assay and cytotoxicity assessment.

Table 5.3.1: Fractions of MPCT extracts obtained by Column Chromatography

MPCT Fractions obtained by Column Chromatography		
Sl No.	**Fraction No.**	**Amount (mg)**
1	F1	231
2	F2	284
3	F3	261
4	F4	213
5	F5	218
6	F6	214
7	F7	221
8	F8	313
9	F9	244
10	F10	231
11	F11	206
12	F12	114
13	F13	177
14	F14	183

Plate 5.3.1: Thin Layer Chromatography showing TLC spots of various fractions



F-1 to F-14 are the fraction numbers of MPCT extract fractions. Arrows pointing to fractions F-2, F-3 and F-8 are showing a majority of phytoconstituents spots as compared to other fractions.

5.3.2 MTT assay on human lung carcinoma (A549) cells for cell viability and cytotoxicity potential evaluation of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

MTT assay was done on human lung carcinoma cells (A549) for cell viability and cytotoxicity potential evaluation of methanol precipitate extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoots. Results of cell viability assay of *Calamus tenuis* Roxb. shoots MPCT fractions on human lung carcinoma cells (A549) given in Table

5.3.2 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.3.2, with the increase in concentration of the extract fractions from 2 to 200ug/ml, the percent viability of lung carcinoma cells (A549) decreased for all the three fractions in the order of F-2, F-3 and F-8 and this percent viability decrease was found to be statistically significant (minimum p<0.01). The results revealed that both fractions F-2 and F-8 were able to induce ~50% cells mortality at 10ug/ml concentration, whereas F-3 showed ~50% cytotoxicity between 10 and 25ug/ml. However results showed that among the fractions, fraction F-8 was most potent against lung carcinoma cells (A549). The same data is graphically presented in figure 5.3.2 (a-c).

Table 5.3.2: Mean percent viability of Lung carcinoma cells (A549) treated with different concentrations of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

Fractions	**Concentration (ug/ml)**									***'F' Value (Significance)***
	2	**4**	**6**	**8**	**10**	**25**	**50**	**100**	**200**	
F2 (n=3) Mean±SD	94.75 ±2.21	90.70 ±2.08	79.47 ±2.16	63.88 ±1.52	55.03 ±1.59	39.12 ±1.01	28.58 ±1.65	11.31 ±0.67	9.08 ±0.56	**1222.01*** **
F3 (n=3) Mean±SD	98.59 ±1.27	99.08 ±0.82	68.14 ±1.33	72.68 ±1.75	63.10 ±1.30	40.28 ±0.83	20.49 ±0.71	14.84 ±0.92	8.18 ±0.10	**2955.67*** **
F8 (n=3) Mean±SD	91.48 ±0.10	78.44 ±1.22	60.57 ±1.26	59.70 ±0.91	54.55 ±1.14	28.55 ±1.36	9.62 ±1.15	8.77 ±0.33	9.29 ±0.38	**3072.38*** **
'F' Value (Significance)	**17.47 ****	**148.93 *****	**101.03 *****	**63.17 *****	**37.34 *****	**104.47 *****	**178.55 *****	**58.23 ****	**6.59 ****	

Mean values represent the average of triplicates. Level of significance in increasing order (** p<0.01, *** p<0.001).

Figure 5.3.2 (a-c): Percent viability of Lung carcinoma cells (A549) treated with different concentrations of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

Fig. 5.3.2 (a): F2-A549

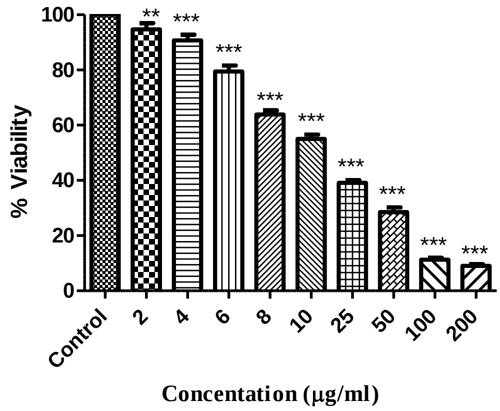


Fig. 5.3.2 (b): F3-A549

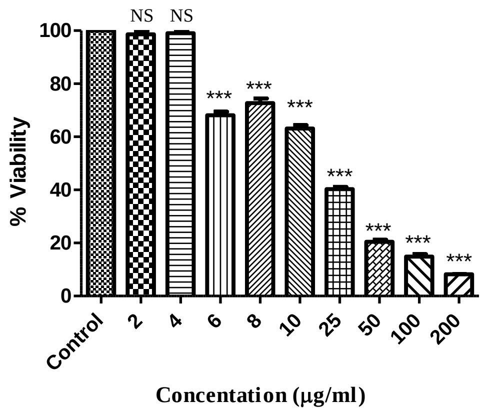
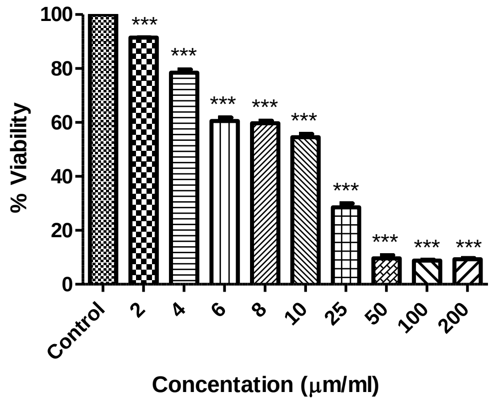


Fig. 5.3.2 (c): F8-A549



Where **p<0.01, ***p<0.001 denotes power of significance as compared to control and NS denotes non-significance.

5.3.3 MTT assay on human breast carcinoma (MCF7) cells for cell viability and cytotoxicity potential evaluation of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

MTT assay was done on human breast carcinoma cells (MCF7) for cell viability and cytotoxicity potential evaluation of methanol precipitate extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoots. Results of cell viability assay of *Calamus tenuis* Roxb. shoots MPCT fractions on human breast carcinoma cells (MCF7) given in

Table 5.3.3 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.3.3, with the increase in concentration of the extract fractions from 2 to 200ug/ml, the percent viability of breast carcinoma cells (MCF7) decreased for all the three fractions in the order of F-2, F-3 and F-8 and this percent viability decrease was found to be statistically significant (minimum p<0.01). The results revealed that both fractions F-2 and F-3 were able to induce ~50% cells mortality at 25ug/ml concentration, whereas F-8 showed ~50% cytotoxicity at 10ug/ml. Hence, among all the fractions, fraction F-8 was found to be most potent against breast carcinoma cells (MCF7). The same data is graphically presented in figure 5.3.3 (a-c).

Table 5.3.3: Mean percent viability of Breast carcinoma cells (MCF7) treated with different concentrations of selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

Fractions	**Concentration (ug/ml)**									***'F' Value (Significance)***
	2	**4**	**6**	**8**	**10**	**25**	**50**	**100**	**200**	
F2 (n=3) Mean±SD	98.75 ±1.60	92.70 ±1.53	87.73 ±3.01	79.44 ±1.72	71.46 ±2.33	55.92 ±2.59	43.61 ±2.25	39.28 ±2.08	31.76 ±2.38	**377.77 *****
F3 (n=3) Mean±SD	97.88 ±1.50	96.99 ±1.63	76.98 ±2.43	64.61 ±2.43	60.57 ±1.98	53.75 ±1.72	44.28 ±1.91	31.59 ±2.22	18.76 ±2.42	**521.64 *****
F8 (n=3) Mean±SD	94.88 ±1.24	86.77 ±1.01	75.83 ±1.61	53.76 ±1.49	53.12 ±1.83	40.97 ±1.22	31.87 ±0.52	24.32 ±1.21	16.19 ±1.61	**1255.66 *****
'F' Value (Significance)	**5.79 ****	**39.15 *****	**22.05 ****	**134.20 *****	**60.05 *****	**52.457 *****	**48.67 *****	**46.87 *****	**44.14 *****	

Mean values represent the average of triplicates. Level of significance in increasing order (** p<0.01, *** p<0.001).

Figure 5.3.3 (a-c): Percent viability of Breast carcinoma cells (MCF7) treated with different concentrations of various *Calamus tenuis* Roxb. MPCT shoot extract fractions

Fig. 5.3.3 (a): F2-MCF7

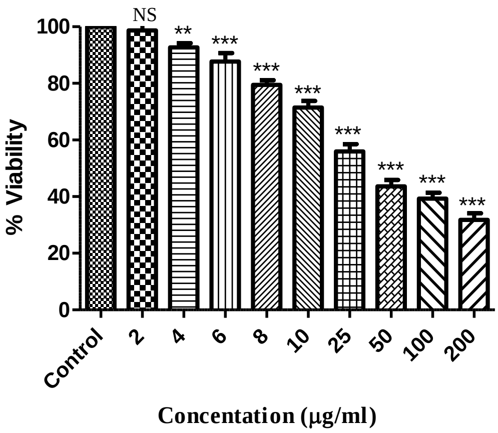


Fig. 5.2.6 (b): F3-MCF7

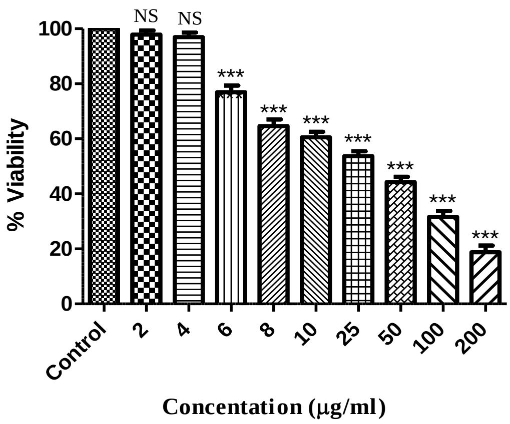
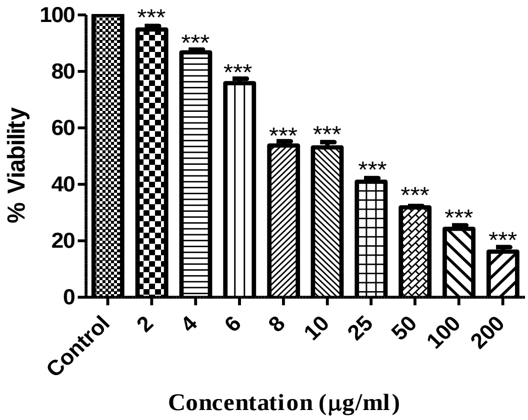


Fig. 5.3.3 (c): F8-MCF7



Where **p<0.01 and ***p<0.001 denotes power of significance as compared to control and NS denotes non-significance.

5.3.4: Qualitative phytoconstituents screening of the selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

A qualitative biochemical analysis was done for assessment of active phytoconstituents present in the fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots, used for MTT assay. As shown in Table 5.3.4, the biochemical analysis revealed that the fractions exhibited presence of saponin, flavonoid, steroid, tannin and glycoside.

Table 5.3.4: Biochemical analysis of the selected MPCT fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots for phytoconstituents screening

Sl No	Constituents	Name of the test	Results		
			F-2	F-3	F-8
1	Saponin	Frothing test	+	+	+
2	Flavonoid	HCl acid test	+	+	+
3	Steroid	Salkowski's test	+	+	+
4	Tannin	Ferric chloride test	+	+	+
5	Glycoside	General test	+	+	+
		Fehling's test	+	+	+

The symbols '+' shows presence of phytoconstituents

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.

Phase IV: Cell viability assay of MPCT extract, MSCT extract and MPCT fractions (F-2, F-3 and F-8) on human normal cells for evaluation of cytotoxicity activity

Since MPCT followed by MSCT among all the crude extracts, were found to most potent against carcinoma cells and as the MPCT fractions showed potential cytotoxicity against carcinoma cells at lower concentrations, they were tested on human lung normal cells (L132) for cytotoxicity by cell viability MTT [3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide] assay and results are as follows:

5.4.1 MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanol precipitate (MPCT) and Methanol supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoots

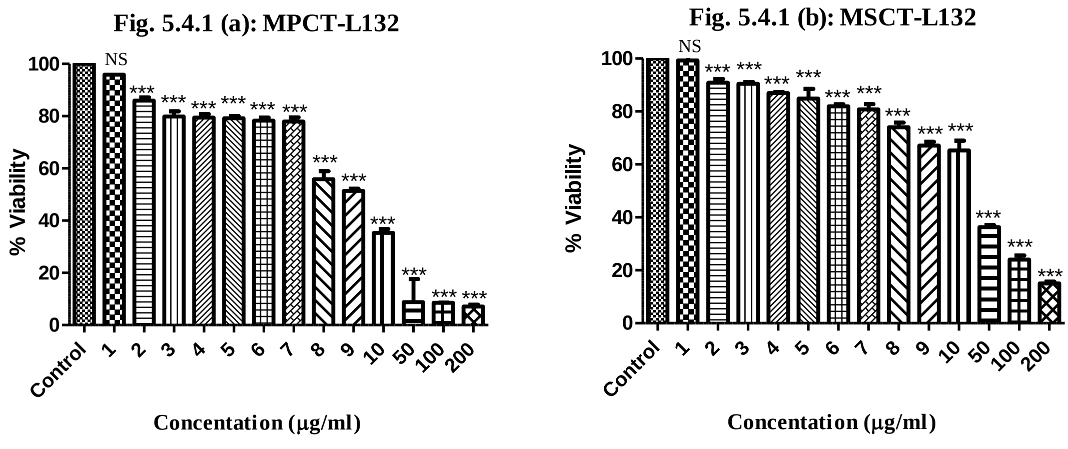
MTT assay was done on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanol precipitate (MPCT) and Methanol supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoots. Results of cell viability assay of *Calamus tenuis* Roxb. shoots extracts on L132 given in Table 5.4.1 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.4.1, with the increase in concentration of the extracts from 1 to 200ug/ml, the percent viability of human lung normal cells (L132) decreased for both the extracts and this percent viability decrease was found to be statistically significant (minimum $p<0.05$) except at 5 and 7ug/ml concentration levels. The results revealed that MPCT induced ~50% cells mortality at 9ug/ml, whereas MSCT showed ~50% cytotoxicity between 10 to 50ug/ml. Hence, MPCT was found to be more cytotoxic to normal cells (L132) than MSCT. The same data is graphically presented in figure 5.4.1 (a-b).

Table 5.4.1: Mean percent viability of human lung normal cells (L132) treated with different concentrations of MPCT and MSCT extracts of *Calamus tenuis* Roxb. shoots

Extract	Concentration (ug/ml)												
	1	2	3	4	5	6	7	8	9	10	50	100	200
MPCT (n=3) (Mean ±SD)	95.90 ±0.01	85.92 ±1.28	79.92 ±1.93	79.43 ±1.35	79.19 ±0.81	78.31 ±1.13	77.98 ±1.56	55.84 ±3.12	51.38 ±0.8	35.32 ±1.44	8.82 ±0.54	8.52 ±0.14	7.12 ±0.69
MSCT (n=3) (Mean ±SD)	99.33 ±0.91	90.87 ±1.41	90.49 ±0.62	86.93 ±0.34	84.86 ±3.64	81.95 ±0.75	80.76 ±1.99	74.01 ±1.74	67.09 ±1.47	65.32 ±3.61	36.27 ±0.74	24.03 ±1.56	15.01 ±0.68
't' value (Significance)	6.50 **	4.51 *	9.02 **	12.82 ***	2.63 NS	4.65 *	1.90 NS	8.81 **	16.32 ***	13.35 ***	51.97 ***	17.14 ***	14.08 ***

Mean values represent the average of triplicates. Level of significance in increasing order (* p<0.05, ** p<0.01, ***, p<0.001) and NS denotes non-significance.

Figure 5.4.1 (a-b): Percent viability of human lung normal cells (L132) treated with different concentrations of MPCT and MSCT extracts of *Calamus tenuis* Roxb. shoots



Where ***p<0.001 denotes power of significance and NS denotes Non-significance

5.4.2 MTT assay on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of MPCT extract fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

MTT assay was done on human lung normal cells (L132) for cell viability and cytotoxicity potential evaluation of Methanol precipitate (MPCT) extract fractions F-2, F-3 and F-8 of *Calamus tenuis* Roxb. shoots. Results of cell viability assay of MPCT extract fraction of *Calamus tenuis* Roxb. shoots on L132 given in Table 5.4.2 are expressed as Mean±SD for n=3 (replicates). As seen in Table 5.4.2, with the increase in concentration of the extracts from 2 to 200ug/ml, the percent viability of human lung normal cells (L132) decreased for all the three fractions in the order of F-2, F-3 and F-8 and this percent viability decrease was found to be statistically significant (minimum $p<0.05$). The results revealed that fractions F-8 was highly cytotoxic to normal cell and induced ~50% cells mortality at 4ug/ml. Fractions F-2 showed ~50% cytotoxicity at 8ug/ml, whereas F-3 induced between 10 and 25ug/ml. Hence, fraction F-8 was found most cytotoxic to normal cells (L132) among all the fractions. The same data is graphically presented in figure 5.4.2 (a-c).

Table 5.4.2: Mean percent viability of human lung normal cells (L132) treated with different concentrations of MPCT extracts fractions (F-2, F-3 and F-8) of *Calamus tenuis* Roxb. shoots

Fractions	**Concentration (ug/ml)**									***'F' Value (Significance)***
	2	**4**	**6**	**8**	**10**	**25**	**50**	**100**	**200**	
F2 (n=3) Mean±SD	95.15 ±2.46	86.08 ±2.87	74.85 ±2.61	52.08 ±1.65	44.17 ±1.27	12.43 ±0.71	8.96 ±1.41	5.33 ±0.89	4.85 ±0.46	**1239.86 *****
F3 (n=3) Mean±SD	92.23 ±2.65	86.72 ±1.74	73.91 ±1.94	74.94 ±1.88	68.45 ±0.42	17.44 ±1.29	8.17 ±0.99	7.73 ±0.31	4.66 ±0.17	**1875.83 *****
F8 (n=3) Mean±SD	73.32 ±1.15	46.28 ±1.67	43.35 ±2.81	25.04 ±1.22	12.23 ±1.54	8.99 ±0.89	5.69 ±1.51	5.59 ±0.91	4.76 ±0.71	**783.04 *****
'F' Value (Significance)	**87.76 *****	**343.05 *****	**156.71 *****	**724.23 *****	**1714.33 *****	**54.82 *****	**4.988^{NS}**	**8.972***	**0.112^{NS}**	

Mean values represent the average of triplicates. Level of significance in increasing order (* p<0.05, *** p<0.001) and NS denotes non-significance.

Figure 5.4.2 (a-c): Percent viability of human lung normal cells (L132) treated with different concentrations of various *Calamus tenuis* Roxb. MPCT shoot extract fractions

Fig. 5.4.2 (a): F2-L132

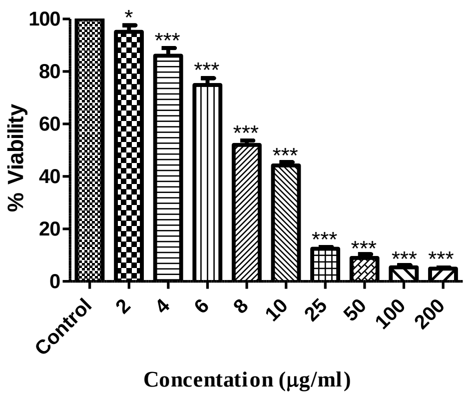


Fig. 5.4.2 (b): F3-L132

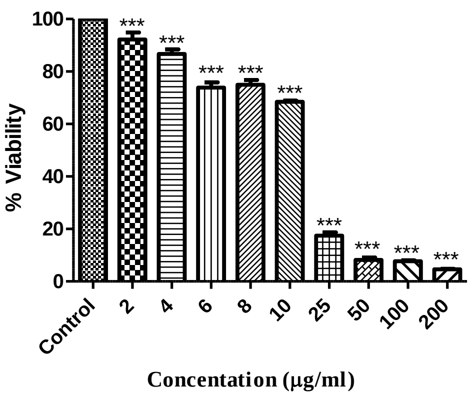
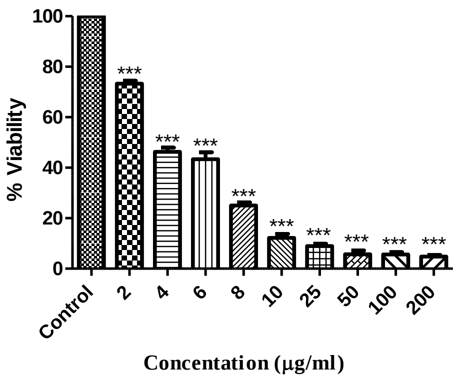


Fig. 5.4.2 (c): F8-L132



Where *p<0.05 and ***p<0.001 denotes power of significance as compared to control.

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.

Phase V: Comparison of lethal concentration (LC_{50}) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts; and methanolic precipitate (MPCT) extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot against human carcinoma and normal cells

Lethal Concentration value (LC_{50}) was calculated as per MTT assay done for cell viability and cytotoxicity assessment against both human carcinoma (A549 and MCF7) and normal (L132) cells.

5.5.1 Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoot against human carcinoma and normal cells

Table 5.5.1 given below shows the lethal concentration (LC_{50}) value of MPCT and MSCT extracts against human carcinoma (A549 and MCF7) and normal (L132) cells. Results revealed that MPCT was more potent and achieved LC_{50} at lower concentration as compared to MSCT against all assayed cell lines. LC_{50} value against lung cancer cells (A549) was found at 20ug/ml and 100ug/ml whereas against breast cancer cells (MCF7) it was found at 40ug/ml and 100ug/ml for MPCT and MSCT respectively. Hence, MPCT was found to be more cytotoxic to normal cells as compared to MSCT extract.

Table 5.5.1: Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) and supernatant (MSCT) extracts of *Calamus tenuis* Roxb. shoot extracts against human carcinoma and normal cells

Extracts	Cell Lines		LC_{50} (ug/ml)
MPCT	Lung cancer	A549	20
	Breast cancer	MCF7	40
	Normal	L132	9
MSCT	Lung cancer	A549	100
	Breast cancer	MCF7	>200
	Normal	L132	10-50

5.5.2 Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot extracts against human carcinoma and normal cells

Table 5.5.2 given below shows the lethal concentration (LC_{50}) value of MPCT extract fractions (F2, F3 and F8) against human carcinoma (A549 and MCF7) and normal (L132) cells. Results revealed that fraction F-2 (10ug/ml) and F-8 (10ug/ml) attained LC_{50} value at lower concentration as compared to fraction F-3 (10-25ug/ml) against lung carcinoma cells (A549). Against breast carcinoma cells, fraction F-2, F-3 and F-8 showed LC_{50} value at 25ug/ml, 25ug/ml, and 10ug/ml of concentration respectively. Fraction F-8 was highly active against normal cells (L132) and showed LC_{50} value at 4ug/ml, whereas fractions F-2 and F-3 showed LC_{50} value at 8ug/ml and 10-25ug/ml respectively. Hence, fraction F-8 was found to be most cytotoxic to normal cells (L132) among all the fractions.

Table 5.5.2: Lethal Concentration (LC_{50}) of methanolic precipitate (MPCT) extract fractions (F2, F3 and F8) of *Calamus tenuis* Roxb. shoot extracts against human carcinoma and normal cells

Extracts	Cell Lines		LC_{50} (ug/ml)
F-2	Lung cancer	A549	10
	Breast cancer	MCF7	25
	Normal	L132	8
F-3	Lung cancer	A549	10-25
	Breast cancer	MCF7	25
	Normal	L132	10-25
F-8	Lung cancer	A549	10
	Breast cancer	MCF7	10
	Normal	L132	4

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.

DISCUSSION

The present study has highlighted some important unexplored views on various aspects of *Calamus tenuis* Roxb. edible shoots which were not yet explored, in spite of having a major role in the field of nutrition and therapeutics. The highlights include factors like current consumption pattern, traditional therapeutic practices, health-related beliefs, storage and sources of *Calamus tenuis* Roxb. shoots followed by the forest villagers of Dibrugarh district of Assam. The study also attempts to give an insight of qualitative phytoconstituents of shoot extracts of the plant and its cytotoxicity potential against human lung and breast carcinoma, and lung normal cells.

Most societies recognize that food, medicine and health are interrelated. Food is typically associated with cultural identity and social well-being. Traditional food systems typically draw on local biodiversity and are based on local production and management of land and specific environments [Johns, 2006]. In earlier days, in absence of modern medicine people were mostly dependent on the indigenous plant for their therapeutic benefits [Payyappallimana, 2006] which may have proved to be beneficial for curing illnesses. However, by the discovery and adoption of modern medicine, consumption of these plants has reduced [Kong *et al.,* 2003; Shrestha and Dhillion, 2003]. The nutrition transition associated with industrialization and the modernization of diets poses challenges to public health worldwide [Popkin, 1998]. The replacement of wild foods by store-bought products is linked to reduced dietary diversity, rising rates of chronic lifestyle-related conditions such as obesity and type II diabetes, poor intake of micronutrients [Batal and Hunter, 2007] and malnutrition [Erikson *et al.,* 2008]. Traditional species become undervalued and underused as exotic ones become available, as has been found in India [Rathore, 2009] and Amazon [Byron, 2003]. Yet, the importance of wild foods towards nutritional security means that they are not necessarily altogether replaced by store-bought foods [Johns and Maundu, 2006].

Calamus tenuis Roxb., is one such edible wild plant and has been scrutinized for its shoot consumption and other factors in the present study. The study has shown a significant negative association between consumption of the *Calamus tenuis* Roxb. shoots and education, occupation, income, socio economic status (SES) and sources of the shoot. The finding of Patel (2014) also supports the results of present study that the use of traditional plant is highest among the people who have lower education upto primary level. The present study also agrees with previous reports that consumption of medicinal plant varies with factors such as income and access to public health facilities [Kayombo *et al.,* 2012; Adera, 2013], ethnicity [Lim *et al.,* 2005] and ethnobotanical knowledge [Wehi and Wehi, 2010]. More than one-fourth of the world's population relies on forest resources for livelihood [Shrivastava, 2014] and in the present study also forest was found to be the most preferable source of the *Calamus tenuis* Roxb. shoots and forest dependent subjects consumed shoots in more quantity than those who depended on other sources like market and garden; which may be due to its availability, easy access and cost effectiveness. Payyappallimana (2006) also mentioned that plants which are locally available, easily accessible and cost-effective are primarily used by communities. Various other studies have reported that aspects like gender, age, educational level, profession [Ayantunde *et al.,* 2008; Ladio and Lozada, 2004; Reyes-Garcia *et al.,* 2005; Arias-Toledo *et al.,* 2007; Nascimento *et al.,* 2012] and distance from urban centers as well as the cultural issues inherent in local communities and increased agricultural activities can influence the use of indigenous wild plant [Ladio and Lozada, 2003; Ali-Shtayeh *et al.,* 2008].

Hence, *Calamus tenuis* Roxb. shoots remain the food mainly of rural inhabitants belonging to low socio-economic group and are consumed mostly by the local unemployed or unskilled workers living by the forest villages. This shoot seems to be primarily consumed for food purpose by most of the village dwellers rather than for therapeutic purpose as there was no statistical significance difference observed between the subject who believed in its therapeutic values as compared to those who did not believe. Also, beliefs about the healing properties of the shoot did not show any significant association with various medical condition suffered by them. Thus, such beliefs about therapeutic potential of plants may not be always treated as true without confirmed scientific scrutiny [Sutaryadi, 1986]. Therefore, *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.

Although toxicity studies on *Calamus tenuis* Roxb. shoots have not been carried out for its safety and dosage, some of the subjects reported experiencing gastritis and stomach disorder specifically when consumed at night and on an empty stomach. Researchers have also reported that some vegetable species are potentially toxic to humans and animals when consumed in high amount [Kofi-Tsekpo, 1997; Pfander, 1984] and there are several reports that show wild edible plants (WEPs) may contain undesired qualities, such as anti-nutritional factors that interfere with absorption of nutrients [Kebede *et al*., 1995; Ogle and Grivetti, 1985] and other undesirable activities. Therefore, indigenous edible plants like *Calamus tenuis* Roxb. need to be proved for its therapeutic value, safety and dosage.

Ethnomedicines have a proven track record of being most useful in the treatment of diseases worldwide with minimal cost and side effects [Yabesh *et al.,* 2014]. Most of the important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides that have greatly contributed towards drug development [Ajayi *et al.,* 2011]. Phytoconstituents in plants such as saponins, glycosides, flavonoids, alkaloids phenols and anthraquinones have been reported to exhibit antimicrobial, antifungal, anti-inflammatory, fungistatic and molluscicidal properties [Ajayi *et al.*, 2011]. Secondary metabolites of some ethnomedicinal herbs such as resins, oleoresins, lactones and volatile oils have also been reported for their therapeutic potentials [Dubey *et al.,* 2004] but a majority of the herbs lack complete phytochemical investigation. Hence it is imperative to validate their traditional claims and characterize biologically active compounds present within. *Calamus tenuis* Roxb. has been extensively reported for the therapeutic potential of its fruit that is rich in flavonoid, steroid, tannin and alkaloid [Hossain, 2013]. Though shoots of *Calamus tenuis* Roxb. is consumed as vegetable and in other forms, there was no report on its phytochemical constituents. Preliminary studies of the shoot have revealed that methanolic extracts (MSCT, MPCT) and MPCT fractions (F-2, F-3, F-8) are rich in saponin, flavonoid, steroid, tannin and glycoside. Hexane extract (HECT) of *Calamus tenuis* Roxb. shoots showed presence of saponin and steroid whereas, ethyl extract (EACT) showed presence of steroid.

MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] is a tetrazolium dye that undergoes reduction by the mitochondrial enzymes to form a blue colored formazan. Hence, it is a useful tool to detect cytotoxicity and antiproliferative potential of various compounds or nano formulations [Thakore *et al.,* 2015]. Cells with viable mitochondria retain the ability to carry out this reaction; therefore the color intensity is directly proportional to cell viability. Results obtained herein revealed that MPCT was highly cytotoxic as compared to MSCT and other extracts against both carcinoma cells (A549 and MCF7). In therapeutic assessment of plants, studies have reported that the initial crude extract of herbs under scrutiny may be less-toxic but the resultant bio-assay guided fractions or pure compounds may show significant toxicity [Jain *et al.,* 2012]. Therefore, the methanolic precipitate extract (MPCT) was fractionated by Column Chromatography for further study and it was found that the MPCT extract fractions (F-2, F-3, F-8) were more cytotoxic to carcinoma cells as compared to crude extracts. Most researchers screen for cytotoxicity against cancer cells lines over normal cell lines to determine anticancer property of a phytoconstituent or compound [McCauley *et al.,* 2013]. Therefore, human normal cell line (L132) was assayed for cytotoxicity against methanolic partitioned crude extracts (MSCT and MPCT) and methanolic precipitate (MPCT) extract fractions (F-2, F-3, F-8) and it was found that they were highly toxic to normal cells as compared to carcinoma cells. MPCT among all the extracts and fraction F-8 among all the fractions showed highest cytotoxicity to both carcinoma and normal cell lines. However, the 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. Since, 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 assumed 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.

The anti-mutagenic and anti-carcinogenic potential of polyphenol-rich functional foods has been extensively reported by various research groups [Hossain, 2013]. The toxicological profile of any herb is dependent on its phytochemical constituents [Ekpenyong *et al.*, 2014]. Phenolic group of compounds such as flavonoids, phenolic acids and tannins has been reported to be protective against allergies, inflammation, platelet aggregation, microbes, ulcer and tumour [Hour *et al.*, 1980]. High content of these phyto-ingredients have been reported in *Calamus tenuis* Roxb. fruits [Hossain, 2013] and it was also reported that *Calamus tenuis* Roxb. fruits have antioxidant and cytotoxic potentials [Ahmed *et al.*, 2014]. Hence, the present study assumes that the cytotoxic potential recorded in MPCT extract and fractions (F-2, F-3, F-3) is attributable to the assessed phytoconstituents.