CHAPTER-4
MATERIALS AND METHODS

4.1. SELECTION OF MEDICINAL PLANTS

The selection of medicinal plants was done based on estimated consumption by herbal industries, estimated annual trade, bio-physical requirement suitability in context to the climatic conditions of the Punjab, the feasibility of cultivation, prior traces of their cultivation in the state, expert consultations from industry experts, subject experts, and officials from NMPB, FITM, Ministry of AYUSH. Total 13 medicinal plants were selected for the agro-climatic zoning studies, out of these 13 medicinal plants 05 medicinal plants *viz. A. vera, P. emblica, O. sanctum, C. longa,* and *R. serpentina* were cultivated by the farmers of Punjab so these plants were selected for survey-based study and among the 05 medicinal plants *A vera*, *O. sanctum* and *C. longa* were chosen for detailed GAP monograph study based on the first harvest of the crop cycle as *P. emblica* and *R. serpentina* gave harvest after 4 years and 18 months respectively.

4.2. AGRO-CLIMATIC FEASIBILITY ANALYSIS INCLUDING AGRO-CLIMATIC MAPPING AND ZONING TO FIND BEST SUITABLE ZONE OF THE SELECTED MEDICINAL PLANTS

4.2.1. Collection of Meteorological Data

To define temperature and rainfall zones, meteorological data was collected from the available meteorological research stations in Punjab. Annual temperature and rainfall records were analysed after obtaining the climatic data from IMD and PAU meteorological research stations present. The temperature records were collected from 08 research stations similarly; rainfall data was collected from 11 research stations present in Punjab**.** Depending upon the availability of meteorological data in the research stations, the period for the meteorological record was selected for the study.

4.2.2. Preparation of Base Maps

A methodology was developed and implemented to create suitability maps for medicinal plants. Because of the generally limited information about medicinal plant adaptation, a methodology, based on assessing temperature, rainfall, soil parameters was utilized to create maps for the introduction of species in the state. The base maps were digitalized online and digital information layers were created using GIS Arc.

GIS 10.3 software. The climatic data obtained from the different meteorological stations were used for the preparation of temperature and rainfall digital base maps. The thermal regime was defined by the average annual temperature and similarly, the moisture regime was plotted based on annual rainfall data. The different agro-climatic zones of Punjab were assigned temperature ranges based on the meteorological data collected from the different stations present in the specific zones. The research stations from where meteorological data was collected were geo-tagged using their latitude and longitude specifications on digital maps using GIS. Likewise, base maps for rainfall were prepared utilizing rainfall data and assigning the rainfall ranges to different agro-climatic zones. In Punjab, 17 benchmark soils have been established as benchmark soils as per studies conducted by Department of Soils, PAU (Singh *et al.*, 2021a,c). The benchmark soil represented other soils present in small proportion of that area. Therefore, an agro-eco-subregion based benchmark soils network was utilized for the preparation of digital soil maps based on texture and pH (Kumar *et al.* 2008; Chahal, 2005; Saini *et al.* 1995).

4.2.3. Agro-climatic Zoning Model

The base maps for temperature, rainfall, and soil were integrated with agro-ecological zones map of Punjab delineated by ENVIS centre, Punjab (Singh *et al*., 2021a,b). All the base maps were superimposed to highlight optimally suitable zone (having all the climatic and edaphic parameters common with the bio-meteorological requirements of selected medicinal plants), suitable zone (having only two parameters common with the bio-meteorological needs of selected medicinal plants), and lesser suitable zone (having only one parameter common with the bio-meteorological need of the plant).

4.3. IDENTIFICATION OF MEDICINAL PLANTS CULTIVATORS EXPLORING DIFFERENT CHANNELS IN PUNJAB

4.3.1. Collection of Medicinal Plants Cultivators Data from Various Public and Private Channels

Different government channels (RCFC-North-NMPB, State Forest Department, etc.), private (farmer groups, NGO's, herbal *mandi* traders, farmer-producer organization, industries) were explored to identify farmers involved in medicinal plants cultivation for geo-tagging and conduct of survey throughout Punjab. The channels explored are represented in Fig. 4.1.

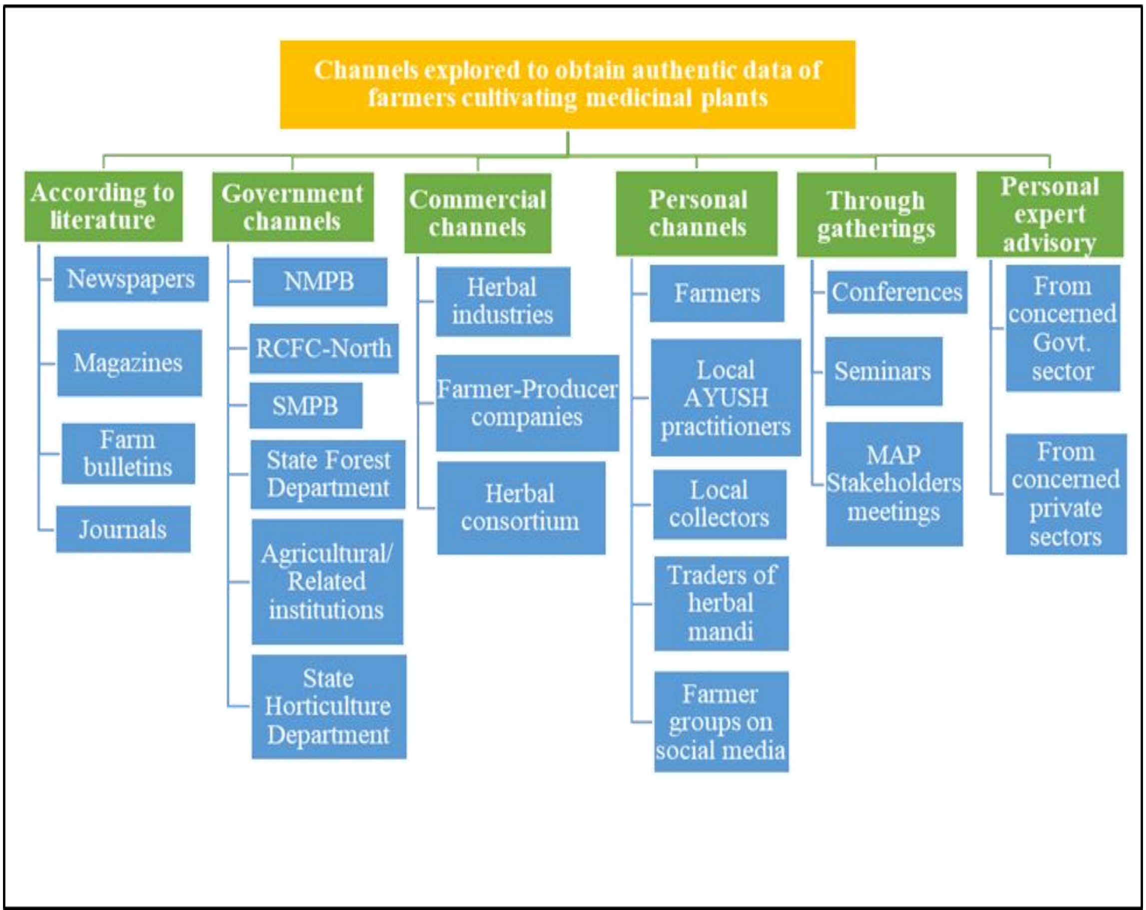

Figure 4.1: Channels explored to obtain data of farmers cultivating medicinal plants in Punjab.

4.4. GEO-TAGGING OF MEDICINAL PLANTS CULTIVATORS USING GIS

Personal field visits were made to the farmer's fields, and the exact location of farmers was marked on the digitalized maps using their latitude and longitude coordinates using GIS. A manual digitization method was used, through which X and Y coordinate values were assigned to describe the locations of points. The first layer comprised of basic geophysical structures viz. major roads, built-up and district boundaries (Deshpande *et al*., 2004). Maps were marked considering the pre-determined five agro-climatic zones of Punjab using GIS software Arc. GIS 10.3.

4.5. SURVEY FOR IDENTIFICATION OF CONSTRAINTS IN MEDICINAL PLANT CULTIVATION BY FARMERS OF PUNJAB

4.5.1. Study Area

The present study was conducted in the areas having medicinal plants cultivation in Punjab (latitudinal extent from 29º 33’ to 32º 34’ N and longitudinal extent from 73º 53’ to 76º 56’ E) as represented in Fig. 4.2.

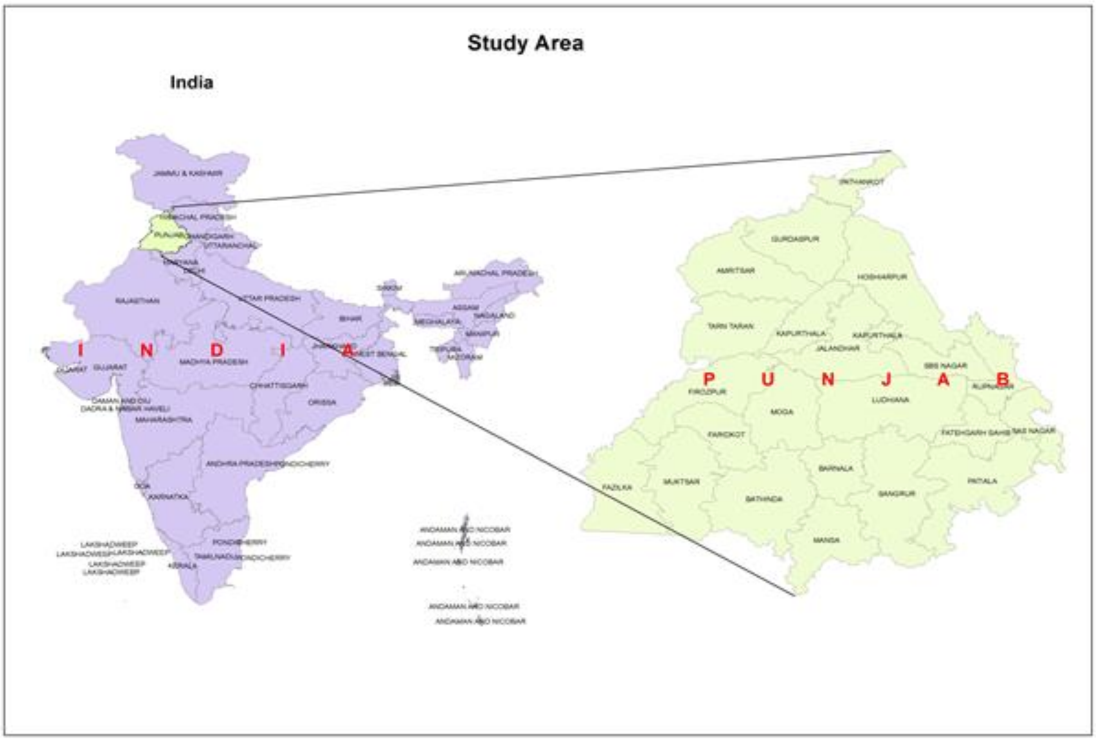

Figure 4.2: Study area selected for the survey.

4.5.2. Sample Size

Based on the information gathered from the concerned channels such as state forest department, RCFC North-NMPB, farmer-producer companies, herbal industries, farmers groups, traders, local herbal practitioners, and local herbal *mandi*, total 68 farmers were selected for the study and data was collected through questionnaire (attached as Appendix-I). The list of farmers, participated in the study is given in Appendix-II.

4.5.3. Inclusion and Exclusion Criteria

The farmers cultivating medicinal plants in Punjab and the farmers willing to participate in the study were selected for the study. The farmers outside the jurisdiction of Punjab, farmers not involved in the cultivation of medicinal plants, and cultivators who were not ready to participate in the study were excluded from the study.

4.5.4. Mode of Survey

Personal interviews were conducted to collect the primary data using a questionnaire.

4.5.5. Data and Sampling Techniques

The data was collected from April 2019 to August 2019 throughout Punjab and purposive sampling was followed as a sampling technique (Tongco, 2007).

4.5.6. Design and Validation of Questionnaire

The questionnaire was drafted after expert consultations and pre-testing of the questionnaire was carried out by interviewing at least 40 medicinal plant cultivators in Medicinal Plants Stakeholders Meet conducted by RCFC-North (Regional-cum-Facilitation Center), NMPB. Subsequently, modifications were made and it was tested again on ten other farmers. The reliability of the Likert scale was measured using Chronbach's Alpha value. The value measured the reliability of the questions and internal consistency (Singh *et al.,* 2021c). The questions having Chronbach's alpha value of more than 0.6 were selected for further analysis.

4.5.7. Statistical Tools

Different constraints related to technical, trade, social participation, awareness and farmer attitude and policy were studied based on their respective MPS (Mean Percentage Score). Constraints were ranked according to their MPS values. As the data was collected in the form of categorical variables and skewness was observed in the numerical value, the Chi-sqaure test was selected for the study. The Chi-square is distribution-free or a non-parametric tool to analyse the categorical data and differences among the groups measuring the dependent variable at a nominal level. This method is commonly applied for checking relationships among the categorical variables (Franke *et al*., 2012; Cain and Diehr, 1992; Hoffman, 1976). In the present study, it was hypothesized that no significant relationship existed between the farmer's landholdings on the constraints faced by the farmers. So, to validate the null hypothesis, the Chi-square test was applied to find significant relationships between the farmer's land holdings with every statement of technical, trade, social participation, awareness, and attitude related constraints (Sharpe, 2015; Mchugh, 2013). SPSS version. 22 software was used for statistical calculations. The following statistical measures were applied to come to the conclusion:

i. **Percentage:** Comparisons were made on the basis of percentage

ii. **Mean score:** Total score of each item divided by total number of respondents. It was calculated by the formula:

$$Mean\ Score = \frac{W_1 + W_2 + ------- W_n}{n}$$

n = Number of respondents

W = Weight of the respondent

iii. **MPS (Mean Percentage Score):** It is defined and calculated as total score got by the respondent for one item, which is divided by maximum obtained score for an item multiplied by 100. The formula of MPS is:

$$\frac{Total\ score\ obtained}{Maximum\ score\ that\ can\ be\ obtained} X100$$

iv. **Chi-square test:** It is estimated for goodness of fit values. This test is used to access the association or independency of two variables.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Σ = Sum up

O = Each observed actual value

E = Each expected value

v. **S.D. (Standard deviation):**

$$S.D. = \frac{1}{n} \sqrt{\left(\sum x^2 - \frac{(\Sigma x)^2}{n}\right)}$$

Σx^2 = Sum of squares of observations

(Σx^2) = Squares of sum of observations

n = Number of observations

4.6. DRAFTING OF COMPREHENSIVE GACP GUIDELINES AFTER CRITICALLY ASSESSING AND COMPARING GAP OF WHO AND OTHER COUNTRIES LIKE AMERICA, JAPAN, CHINA, EUROPEAN UNION, NMPB INDIA FOR SELECTED PLANTS

A comparative study was carried out after exploring the GACP developed by the WHO, America, Europe, China, Japan, and India to draft robust and comprehensive GACP guidelines for the medicinal plants. Point-wise comparisons were made for 12 parameters such as seeds, site selection, soil, water, cultivation, crop management, crop nutrition, harvest, post-harvest, personnel, equipment, and documentation described in various GAP guidelines were analysed to develop robust GACP guidelines possessing 12 heads and 108 sub-heads.

4.7. CRITICAL VARIABLE ALIGNMENT STUDY TO UNDERSTAND SIGNIFICANT VARIABLES AFFECTING THE CRITICAL QUALITY ATTRIBUTE IN QUESTION BASED ON SCIENTIFIC RATIONALE AND SUGGESTIVE IMPROVEMENTS IN AGRO-PRACTICES FOR QUALITY COMPLIANCE WITH SPECIFIC REFERENCE TO CONTENT OF ACTIVE CONSTITUENT(S), HEAVY METAL RESIDUE AND PESTICIDE CONTAMINATION

4.7.1. Design of the Study Based on Critical Variables

Critical alignment study is a scientific approach that begins with pre-defined quality materials and processes based on sound science and literature to pre-determine the risks to the quality of the herbs (Sangshetti *et al.*, 2017). This study has pre-defined three set of variables such as CQA, CPP and CMA that are linked to each other defining the quality of the herbs in a scientific manner (Rathore and Winkle, 2009). The critical quality attributes were selected based on empirical data in context to industrial importance and sound literature exploration (Singh *et al.*, 2021,a,b,c,d 2020b; Singh and Baldi, 2018;). Critical alignment to quality was evaluated based on scientific knowledge, farmer's inputs allowing selection of critical material attribute (any physical, biological or chemical property of an input material i.e. water, soil, seed, fertilizer, pesticides) and critical process parameter (processes such as plant identity, soil and water treatment processes, sowing, harvesting, fertilizer application, harvest, drying, storage, etc. whose variability impacts the quality of the herbs) from the potential list of agricultural materials and processes. The critical quality attributes for the study were selected based on the parameters that were highly important to the industry and such as active chemical constituents, toxicity indicators (heavy metals and pesticide limits), microbial load (aflatoxin levels, microbial contamination related disease), physicochemical ranges (ash values, extractives) and crop productivity, etc. (Krull *et al.,* 2017; Patel *et al.,* 2013; Rathore, 2009). The critical quality material or process having the highest relation to the critical quality attributes was termed as very high followed by high, moderate, less, very less, and negligible. Suggestive improvements were highlighted as descriptive study particularly in the results section of this objective in context to quality of material and agro-processes parameters affecting active constituents, toxicity indicators (heavy metals and pesticide contamination), and other important quality related attributes.

4.8. STANDARDIZATION OF FARMER'S PRODUCE FOR SAME QUALITY PARAMETERS AND GAP/VARIABILITY ANALYSIS IN COMPARISON TO INDUSTRIAL STANDARDS

In the present study, Failure Mode Effect Analysis (FMEA) approach was used to devise in quality and reliability in the early phase of medicinal plants production. The FMEA was adapted for standardization of the farmers produce to get rich-quality herbs and avoid widespread testing of medicinal plants which is mostly costly (Reddy *et al.,* 2014; Dasgupta, 2003). The method was implemented by identifying the failure mode, the ways in which a process could fail with respect to the requirements of customer/industry. The method was devised in a manner that it measured risks associated with each agricultural process to prevent in-appropriate physicochemical ranges, active constituents, toxicity indicators (heavy metals, pesticide residues), and microbial content at the delayed phases. The process acted by not only by identifying the potential failure mode, but also considering the effect on the process to categorize actions to diminish the failure in form of checklists to lower the Risk Priority Number (RPN) of each agricultural process (Namdhari *et al.* 2011).

4.8.1. Descriptions of FMEA Model

For calculating the risk in the FMEA model, there were three components which were multiplied to get an RPN for each process (Lipol and Haq, 2011):

- Severity (SEV): It numerically rated the intensity of the severity of the failure effect on the customer. In the present study, presence of toxicity indicators (heavy metals, pesticides residues) in medicinal plants was described as most severe followed by microbial load (microbial content, aflatoxins content), quantity of active constituents, crop yield and physicochemical ranges. The severity was described on a 6 point scale.
- Occurrence (OCC): It numerically rated the chances of the occurrence of the failure cause. More the chances, more was the occurrence rate. Occurrence was described on a 6 point scale where 6 was the highest.
- Detection (DET)*:* It numerically rated the potential of process controls in detecting the cause of the failure. Higher the numerical value for detection, most difficult it is detected. Detection rate was described on 6 point scale in the present study.

$RPN = SEV \times OCC \times DET$

$RPN_{min}=1$ and $RPN_{max}=216$

The minimum and maximum RPN ranking is mentioned in Table 4.1.

Table 4.1: Minimum and maximum RPN rankings.

Potential failure mode	SEV	OCC	DET	RPN
Min	1	1	1	**1**
Max	6	6	6	**216**

4.8.2. Criteria for Severity, Occurrence and Detection Ranking for Agricultural Processes

In the present study, the rating scales for severity, occurrence and detection ranged from 01 to 06 (higher number represented the highest seriousness). The severity score was rated against the impact of the effect caused by the failure mode on the agricultural process (Xiao *et al.*, 2011; Van Leeuwen *et al.* 2009). The severity rankings were classified considering toxicity to environment and health, qualitative, quantitative and economic factors for the farmers. A non-linear scoring scale was applied to augment the effect of the severity criteria as shown in Table 4.2.

Table 4.2: Severity ratings for processes related to medicinal plants.

06	Dangerously high	Failure could lead to the toxicity of environment and health of consumers and farmer. Subject to financial penalty from regulatory agencies.
05	Extremely high	Failure could lead to the rejection of produce from all the buyers (Industry, mandi, local market, trade, etc.). Financial: (Economic loss more than the input costs of crop).
04	High	Failure could lead to the rejection of produce from only potential buyers (industry and herbal mandis, trade, etc.). Financial: (Economic loss equivalent to the input costs of crops)
03	Moderate	Failure apparent to all the buyers (industry, herbal mandi, local market, trade, etc.) with lower chances of rejection. Financial: (Price of crop decided by the consumer; little benefit to farmer)
02	Low	Failure apparent only to the potential buyer (industry, herbal mandi) with no chances of rejection. Financial: (Price of the crop decided by the farmer with lower price than expected; moderate benefit to the farmer)
01	None	Failure not noticeable to the buyers. Financial: (Price of crop decided by the farmer with good economic benefit)

The probability of occurrence was evaluated on the basis of factor's occurrence in per cent of medicinal plants cultivators per crop cycle. The score was rated against the probability of the effect occurred as a result of a failure mode. More the numerical value for occurrence, more were its occurrence rate in the farmer population as mentioned in Table 4.3.

Table 4.3: Occurrence ratings of failure in population.

06	Extremely high	Failure inevitable. Expected failure in more than 90% of the total farmers per crop cycle
05	Very high	Failure almost inevitable. Expected failure in 71-90% of the total farmers per crop cycle
04	High	Expected failure in 51-70% of the total farmers per crop cycle
03	Moderate	Expected failure in 31-50% of the total farmers per crop cycle
02	Low	Expected failure in 10-30% of the total farmers per crop cycle
01	Remote	Expected failure in less than 10% of the total farmers per crop cycle

Detectability was the probability of the failure being detected before the impact of the failure to the system or process being evaluated was detected (Mariajayaprakash and Senthilvelan, 2013). In the present work, the detectability ratings were assigned based on the ability of the farmers to detect the cause of failures through process control methods. Higher the score for detectability, higher were the chances of process parameters to fail the detection. A non-linear scoring scale was applied to augment the detectability effects of the process parameters as shown in Table 4.4.

Table 4.4: Detection ratings of the failure.

06	Impossible to detect	The product is not inspected or impossible to detect or detected at 6th level.
05	Remote	The product is thoroughly sampled in laboratory and released on acceptable quality level or detected at 5th level.
04	Low	The product is morphologically inspected by regular field visits by an expert or detected at 4th level.
03	Moderate	The defect is detected by closely evaluating the features of the crop by the farmer or detected at 3rd level.
02	High	The defect is easily detected by the farmer during regular field visits or detected at 2nd level.
01	Certain	The defect is certain and pre-determined by the farmer or detected at 1st level.

4.8.3. Assigning RPN

RPN was calculated based on the multiplication factor of the severity, occurrence and detection values for each agricultural process involved in the medicinal plants cultivation. The basic idea behind calculating the RPN was to determine the intensity of the risk involved in particular agriculture process and take suitable process control measures and actions. Higher the RPN number, more was the intensity of the risk factor involved in the agricultural process and quick and concrete actions needed to be taken (Cassanelli, *et al.* 2006). In the present FMEA model, RPN more than 65 was described as critical, 38-65 was termed as major and less than 38 was termed as minor as described in Table 4.5.

Table 4.5: Actions to be taken based on RPN.

RPN	**Level**	**Action**
More than 65	Critical	Take quick action
38-65	Major	Take action
Less than 38	Minor	To be acted upon later

In case, if the RPN of two potential failure modes was same, then the first priority would be failure mode having highest severity ranking. Similarly, if the two failure modes had same severity the priority would be given to the mode having high occurrence value (Lipol and Haq, 2011).

4.9. DRAFTING OF MONOGRAPH OF SELECTED MEDICINAL PLANTS FEATURING GAP RELATED DOCUMENTATION FOR ON-FIELD CULTIVATION

On-field cultivation analysis was conducted to develop GAP monographs of *A. vera, O. sanctum,* and *C. longa* that comprised of land selection, seeds, land preparation, crop management, fertilization, harvest, post-harvest conditions of medicinal plants after conducting field visits, farmer interaction and corroborating the inputs with reported literature to avoid un-predicted yield and quality of the selected medicinal plants (World Health Organization, 2006). Scientific rationale was developed considering farmer's collection/harvest practices and its corroboration with the literature (Jayashree *et al.*, 2015; Jat *et al.*, 2014). The monographs were divided into three parts *viz.* botanical and pharmacological characteristics, good agricultural practices, and standard quality parameters/certifications for herbal materials. The SQC's of *A. vera, O. sanctum,* and *C. longa* comprising of morphological, microscopical, physico-chemical, qualitative, quantitative, microbial load, toxicity indicators, etc. along with their acceptable limits prescribed by API, ICMR, FSSAI, WHO, have been drafted as an intuitive document for the farmers involved in the commercial utilization of these medicinal plants (FSSAI, 2015; Tandon, 2011; Indian Herbal Pharmacopoeia, 1998; Ayurvedic Pharmacopoeia of India, 1989).

4.10. ECONOMIC FEASIBILITY STUDIES OF SELECTED MEDICINAL PLANTS

The agro-economics study was conducted for the selected medicinal plants *viz: A. vera, O. sanctum,* and *C. longa.* The primary data collected through semi-structured questionnaire was thoroughly checked, compiled and tabulated. The cost-return analysis following descriptive statistics such as average prices of the crops to calculate total variable costs, yield, gross returns, returns over variable costs was conducted (Kaur *et al*., 2018a). The per acre cost and returns involved in the cultivation were calculated at current prices. The cost A1 concept considering actual expenses in cash and kind incurred in production by owner which included human labour, machine labour, value of seeds, insecticides, pesticides, manures, fertilizers, irrigation, miscellaneous expenses was followed in the present study. Subsequently, the comparative agro-economics analysis was conducted between traditional crops (wheat and rice) and selected medicinal plants.

4.11. TO DEVELOP FARMING MANUAL FOR SELECTED PLANTS IN LOCAL LANGUAGE FOR WIDER BENEFITS

The GAP based farming manuals have been translated in vernacular language (Punjabi) for wider translation of the study to the farmers of Punjab.

4.12. TO SUBMIT THE PROPOSED COMPREHENSIVE GAP FOR SELECTED MEDICINAL PLANTS TO REGULATORY AGENCIES

Developed training manuals, monographs, and other relevant material related to the present work have been submitted to the FITM/Ministry of AYUSH/RIS in form of book chapters.