Chapter-3

Materials and Methods

The Hazongbori village falls under Sonapur Block of Chandrapur Circle office in the district of Kamrup Metropolitan District. It is situated Eastern part of Brahmaputra. The village is one of the oldest villages. The village is surrounded by natural boundaries. In the eastern part is a village Thakurkuchi, in the West, Khankar village, South part is surrounded by the Amsing Santury and the North part is situated the mighty Brahmaputra. The other villages, Suwali Lukuwa, Kilingog and Sampothar villages are about 8 K. M. away and above from Hazongbori village, towards Southern part of Hazongbori village. The villages are inside the hilly and forest areas (Figure 3.1).

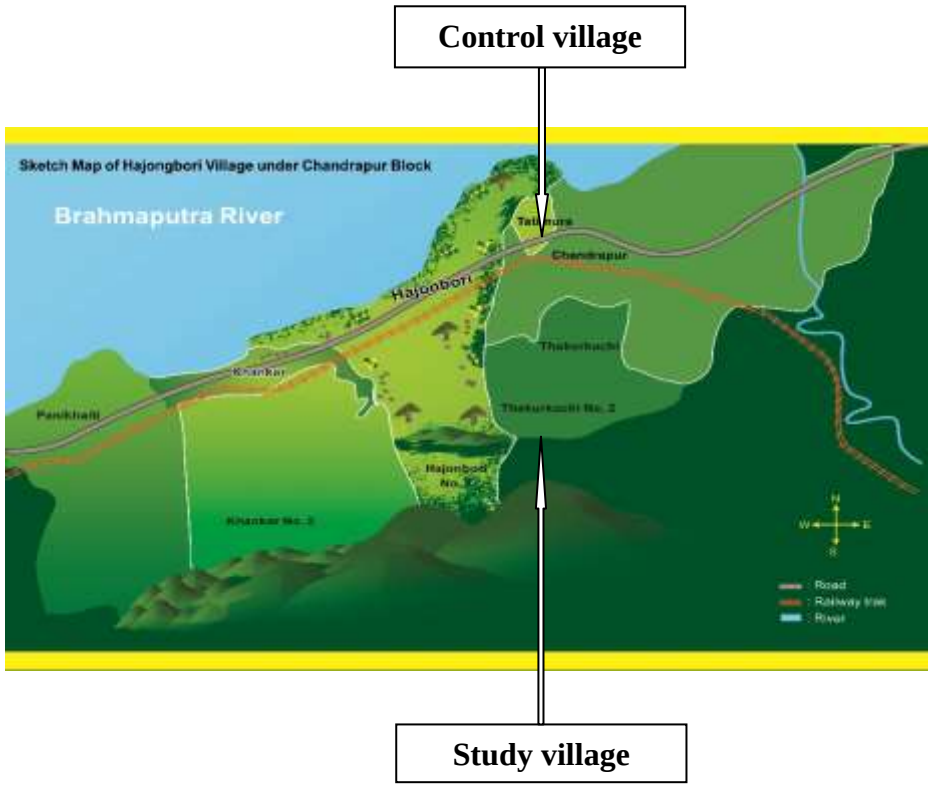

Figure 3.1: Reseach Areas (Study and Control Villages)

Prevalence is a measure of the disease burden in a population at a given location and in a particular time. Counts of the number of people affected with a disease are required to plan appropriately for their health care needs (Plate 3.2.1-3.2.6)

3.1 Study of Prevalence of Malaria among the Community

3.1.1 **Mass Blood Survey (MBS)**

The aim of house to house visits was to cover each group of the population. The rapid diagnostic test and blood slides was used for fever cases. The thick and thin smears were collected, stained with JSB by standard procedures. To study the prevalence of malaria, mass blood survey was carried out in households in both study and control villages.

3.1.2 **Asymptomatic Survey**

The present study has been carried out in three hilly, outreach, covered with dense forest areas of about 359 populations (65 families) of rural communities, around 8 KM away from Panikhaity railway station, Kamrup Metro, Assam. These areas are the natural reservoir of malaria transmission throughout the year. Hilly forest areas with natural pools, poor environmental sanitation with standing water bodies, foothills, paddy fields. Occupation and living habits of villagers which boosts a natural ecosystem for malaria transmission. The communities are developing resistant to malaria and mostly all are asymptomatic in nature. They are predominantly farmers mainly producing rice, grains, and vegetables consume in their diets, thus receive basic nutrient that required for humans to live.

3.1.3 **Fortnight Fever Survey**

Regular fortnight fever survey is carried out by the health workers of the National Vector Borne Disease Control Program (NVBDCP) and are recorded in their malaria formats. All the fever cases reported by the villagers were checked by blood slides and rapid diagnostic kits. Cases found positive were immediately treated as per treatment guidelines of the program. Severe malaria cases and pregnant women were referred to hospitals.

3.2 Study of Socio-economic Status of the Community

A total of 200 hundred people (men and women), 100 from each village were selected in this study. A household questionnaire was directed to the head of the household. The questionnaire included demographic information and data on socioeconomic conditions, Knowledge of malaria, awareness and preventive measures.

3.3 Evaluation of Nutritional Properties

3.3.1 BMI Status of the Children below =<15 years

Weight was measured using Nova BGS-1231 for all the children. For children, less than 2 years recumbent length was measured and for the older children standing heights were taken. The age was calculated by the birth certificate shown or by the knowledge of birthday by family members (Plate 3.3.1-.3.3.2).

3.3.2 Food Varieties and Methods of Preparation

A household questionnaire was directed both to the head of the household and to the woman who had prepared the food in the previous day. In addition, it included a simplified food frequency questionnaire, the women had to answer the food items that had been used in the meal preparation or eaten by any of the household members the previous day. The list of food items were elaborate based on in-depth interviews and local knowledge of the area (Plate 3.4.1-3.4.6 and 3.5.1-3.512).

Random samples were collected for the villages to a different period of time. Cooked samples (food prepared by them as per their choice and normally consumed), were collected in a sterilized container. Refrigerated at 4 degrees for overnight or delivered by hand within 4-5 hrs. in good condition (As per Laboratory Guidelines). The sample analysis part was outsourced to the State Public Health Laboratory, Bamunimaidam, Assam, India. Each type of dish/item was analyzed separately. The moisture content and chemical analysis were carried out by the Food Safety and Standards Act (FSSAI).

Table 3.1: List of dishes consumed in Study and Control villages

Sl. No	Common Name	English Name	Local name (Study village)	Local name (Control village)	Scientific name
1	Chawal	Rice	Mirong	Mairong	*Oryza sativa*
2	Bhat	Cooked Rice	Mii	Aakham	*Oryza sativa*
3	Masur Dal	Lentil	Masur dali	Masur dali	*Lens culinaris*
4	Arhar Dal	Tuvar Dal	Rahar dali	Rahar dali	*Cajanus cajan*
5	Mati dali	Black gram	Bangsi	Sabai dali	*Vigna mungo*
6	Kasuthuri	Colocasia	Sigi or Thagong	Thaso	*Colocasia affinis*
7	Tita phul	Justicia	Alat	Gatha bibar	*Phlogacanthus thyrsiflorus*
8	Ada	Ginger	Ising	Haijeng	*Zingiber officinale*
9	Rasun	Garlic	Asinggupak	Sambrang Gufur	*Allium sativum*
10	Piyaj	Onion	Nasing Kisak	Sambrang Guja	*Allium cepa*
11	Dhekia	Blechnum (hard fern)	Mikhongchek	Dhingka	*Diplazium esculentum*
12	Jatilao	Gurd	Sebong	Panilao	*Lagenaria siceraria*
13	Rangalao	Pumpkin	Kamanda	Jugunath	*Cucurbita pepo*
14	Kol	Banana	Sirik	Thali	*Musa acuminate*
15	Sojina	Drumstick	Sojna	Sojna	*Moringa oleifera*
16	Dangbodi	Bean	Khasrek	Lesera	*Phaseolus vulgaris*
17	Morisa Xak/Datha	Amaranth	Denga	Datha	*Amaranthus caudatus*
18	Begena	Brinjal	Barung	Fanthao	*Solanum melongeana*
19	Teteli	Tamarind	Thimfli or ching ching	Thingkhali Mukhoi	*Tamarindus indica*
20	Kekura	Crab	Sangkhi	Khangkhrang	*Brachyura*
21	Mas	Fish	Nathak	Naa	
22	Murgi	Chicken	Duu	Daw	*Gallus gallus domesticus*
23	Gahori	Pig	Wak	Ama	*Sus scrofa domesticus*

24	Kosu	Kosu	Colocasia corms	Kosu	*Colocasia esculenta*
25	Hah	Duck	Gagak	Hangsu	*Anas platyrhynchos*
26	Jika	Ridge gourd	Zingfa	Jingkha	*Luffa acutangula*
27	Jaluk	Black paper	Gul moris	Jati Jaluk	*Piper nigrum*
28	Amita	Papaya	Mudu	Aamutru	*Carica papaya*
29	Kolful	Banana flower	Sobok	Thali bibar	*Musa ornate*
30	Patal	Pointed gourd	Su	Patal	*Trichosanthes dioica*
31	Bah gaj	Bamboo shoot	Miyan	Baa	*Bambusa vulgaris*
32	Anaras	Pinaple	Tegasu	Anaras	*Ananas comosus*
33	Kothal	Jackfruit	Tibrong	Khanthal	*Artocarpus heterophyllus*
34	Gonkasu	Elephant ear	Gongmentry	Gonthasu	*Xanthosoma roseum*
35	Titamora	Jute plant	Fanbada	Titamora	*Corchorus olitorius*
36	Chengmora	Lasia	Sungi	Chengmora	*Lasia spinosa*
37	Mosundari	Fish Mint	Gong	Mosundari	*Houttuynia cordata*
38	Jalakia	Chilli	Humrila	Fun Julu	*Capsicum annuum*

Table 3.2: Food Varieties frequently used by Study Village and methods of preparation

No.	Name of food	Form	Composition of food
1	Meska Tenga/ Menda(Roselle)	Curry	Roselle+ Green Chilies + Salt + Dry Fish
2	Mudu biber (Papaya Flower)	Solid	Papaya Flower + Green Chilies + Salt + Dry Fish + Soda
3	Barang+ Sigi (Brinjal + Colocasia)	Solid	Colocasia Stem + Brinjal + Ginger + Salt + Soda + Dry Fish
4	Sobok chatany (Banana flower Salad)	Solid	Banana Flower + Ginger + Onion + Burnt Dry Fish + Salt
5	(Papaya Flower) Mudu biber Sabji	Curry	Papaya Flower + Maan Dhania + Ginger + Garlic + Green Chilies + Salt + Soda
6	Sigi (Colocasia)	Curry	Colocasia Leaves + Thai coriander + Ginger _Garlic + Raw Turmeric + Salt + Chilies
7	Hridal (Fermented fish)	Solid	Dry fish + mustard oil + salt + soda
8	Dhekia Sabji (Blechmim)	Curry	Blechnum + Fermented fish+ Chilies + Salt + Soda
9	Mudu biber (Papaya Flower)	Curry	Papaya Flower + Green Chilies + Salt + Dry Fish + Soda
10	Wak (Pork)	Curry	Pork + Brassica Leaves + Ginger + Garlic + Rice flour + Salt
11	Mirong (Rice)	Solid	Rice +water
12	Jola (Green chilies)	Raw	Raw/cooked
13	(Bitter gourd) Kerala	Raw/Curry	Boil/cooked with ginger + garlic +chilies + fermented fish
14	Kasu (Taro)	Raw/Curry	Boil/cooked with vegetables
15	Ising (Ginger)	Raw/Curry	Raw/salad/cooked with other vegetables
16	Thali biber (Banana flower)	Raw/Curry	Raw a/cooked salad with fermented fish
17	Thali (Riped banana)	Raw	Fruit
18	Simalu Alu (Cassava)	Raw	Boil/cooked
19	Kosu (Colocasia)	Raw/Curry	Boil/cooked with vegetables + fermented fish
20	Lesera (Bean)	Raw/Curry	Cooked with fish + ginger + garlic + chilies + fermented fish
21	Haldhi (Turmeric tuber)	Raw/Curry	Raw as salad/cooked other vegetables with fermented fish
22	Til (Sesame)	Raw/Curry	Raw as salad/cooked with other vegetables
23	Mosundari (Fish mint)	Raw	Raw as salad/cooked other vegetables with fermented fish
24	Gongmentry chatany (Elephant earSalad)	Raw/Curry	Gon Kosu + Ginger + Garlic + Chilies + Burnt dry fish + Salt
25	Ama+ Ikara (Pork +Jute leaves)	Curry	Jute + Soda(banana) + Ginger + Garlic + Chilies + Salt + Fish

Table 3.3: Food Varieties frequently used by Control Village and methods of preparation

Sl No	Name of food	Form	Composition of food
1	Xaak (Vegetables mustard+ Chinese Mellow)	Curry	Vegetables mustard+ Chinese Mellow+ Soda + Turmeric powder + Chilies + Salt
2	Sojna (Drumstick)	Curry	Drumstick + Potato + Raw Banana + Salt + Chilies + Turmeric powder
3	Xaak (Green Leaves)	Curry	Vegetables mustard+ Chinese Mellow+ Soda + Turmeric powder + Chilies + Salt
4	Dal (lentil)	Liqid	Masur Dal + Ginger + Garlic + Turmeric powder + Onion + Mustard Oil
5	Mix sobji (Mix Veg)	Solid	Mustard Green + White Goosefoot + Beans + Dry fish + Soda + Salt + Chilies + Mastard Oil
6	Akham (Rice)	Solid	Rice + water
7	Ama (Pork)	Gravy	Pork + Fermented fish + Banana soda + Rice Flour + Salt + Chilies + water
8	Duu (chicken)	Gravy	Chicken + Potato + Meat masala + Turmeric + Salt
9	Bahaka ful(Justicia)	Gravy	Justicia + Banana soda + Salt + Chilies
10	Ama + ful kobi (Pork+Cauliflower)	Gravy	Pork + Cauliflower + Masala Powder + turmeric powder + Chilies + Salt + Mastard oil
11	Ama (Pork)	Gravy	Pork + Potato + Curry leaves + Turmeric powder + Salt + Chilies + Turmeric Powder + Masrad oil
12	Masur dal+ ouu (Lentil+ Elephant fruit)	Gravy	Lentil + Elephant fruit + salt
13	Ama (Pork)	Curry	Pork + Potato + Tomato + Ginger + Garlic + Chilies + Turmeric Powder + Salt + Mustard oil
14	Ama (Pork + Ficus carica)	Curry	Pork + ficus + Curry leaves + Fern + Ginger + Garlic + Chilies + Salt
15	Hridal (Fermented fish)	Gravy	Dry fish + Banana soda + Ginger + Garlic + Sat
16	Xaak bhaji (Mustard Green)	Dry	Peas + Mustard leaves + Onion + Potato + Chilies + Turmeric powder + Salt + Mustard oil
17	Nafaap naa+ Thaso (Fish+Colocasia)	Curry	Fresh Fish + Colocasia Stem + Elephant Fruit + Ginger + Garlic _ Chilies + Salt + Mustard Oil

18	Sabai dali (Black Gram)	Curry	Black Gram + Papaya + Ginger + Garlic + Onion + chilies + Masala powder + Mustard oil
19	Xaak bhaji (Green leaves)		Mustard Green + Radish leaves + Soda + Chilies + Salt
20	Ama+Ikar (Pork +Jute leaves)	Curry	Jute leaves + Soda + Pork + Onion + Ginger + Garlic + Chilies + Salt + Fish
21	Hridal (Fermented fish)	Gravy	Dry Fish + Banana soda + Ginger + Garlic + Sat
22	Ginger	Raw	Raw/Salad/ mix with other vegetables
23	Thali biber+ Alu (Banana Flower+Potato)	Dry	Banana Flower + Potato + Onion + Garlic + Salt + Chilies + Mustard oil
24	Green chilies	Raw/Cooked	Raw/Salad/ mix with other vegetables
25	Gonkosu (Elephant ear)	Raw/Cooked	Salad with dry fish +chilies+ ginger+ Garlic

3.3.3 Phytochemical Analysis

3.3.3.1 Extraction and Photochemical Screening

300gm of powdered plant were extracted successively with solvents like petroleum ether, benzene, chloroform, acetone, methanol respectively in a Soxhlet apparatus (Wallis, 1967). Each solvent extract was then concentrated by distilling off the solvent under reduced pressure. The aqueous and methanolic extracts along with other solvent extracts of plant materials were studied for various phytochemicals like alkaloids, flavonoids, phenols, tannins, and terpenoids by using precipitation and coloration reactions (Trease and Evans, 1996).

3.3.3.2 Determination of Ash Values

3.3.3.2.1 Determination of Total Ash Content

5 gms of a sample was weighed accurately in a tared silica dish. It was then charred carefully on a burner and transferred the dish to a muffle furnace and ash at a temperature of 550 ±100° C until the ash was free of Carbon. The dish was heated at 550 ± 100° C for 30 minutes and was cooled in a desiccator. It was weighed and was

repeated the process and heating for 30 minutes. It was cooled in a desiccator and weighed until the difference between two successive weightings is less than 1 mg and noted the lowest weight (Kashi *et al.*, 2012).

.

$$\text{Total ash (\% on dry weight)} = \frac{(W2 - W) \times 100 \times 100}{(W1 - W) \times (100 - M)}$$

Where, W1 = Weight in Silica dish(gms) + sample,
W2 = Weight in Silica dish (gms)+ ash,
W = Weight empty Silica dish(gms),
M =% of moisture of the sample

3.3.3.2.2 Acid-Insoluble Ash

The total ash was boiled for five minutes with 25 ml of diluted hydrochloric acid. The insoluble matter was collected in a Gooch crucible, washed with hot water, ignited, and weighed. The percentage of acid- insoluble ash was calculated with reference to the air-dried drug (Kashi *et al.*, 2012).

3.3.3.2.3 Water-Soluble Ash

Boiled the total ash for 5 minutes with 25 ml of water. The insoluble matter was collected in a Gooch crucible then washed with hot water and ignited to constant weight at a low temperature. Subtracted the weight of insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage was calculated with reference to the air-dried drug (Kashi *et al.*, 2012).

3.3.3.3 Determination of Alcohol Soluble Extractive

5 grams of air dried macerated coarse powder of drug was mixed with 100 ml of 95% alcohol and kept for 24 hours in a closed flask. Shacked frequently during the first 6 hours and then allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to

dryness in a tared dish, dried at 105^{o} C and weighed. The percentage was calculated with reference to the air-dried drug (Trease and Evans, 1996, Kokate *et al*., 2016).

3.3.3.4 Determination of Water-Soluble Extractive

About 5 gm of powder was accurately weighed, placed in a petri-dish and dried in a hot-air oven at 110° C for four hours. It was placed in a desiccator. The loss in weight was recorded. It was repeated untill constant weight was obtained. The % Loss on Drying was calculated with reference to the air-dried sample (Trease and Evans, 1996, (Kokate *et al*. 2016).

3.3.3.5 Qualitative Analysis of Alkaloids

About 50 mg of solvent-free extract was stirred with little quantity of diluted hydrochloric acid and filtered. The various alkaloid tests viz., Mayer's Test, Wagner's Test, Hager's Test, Dragendroff's Test (Kokate *et al*. 2016) were done for the filtrate.

3.3.3.6 Detection of Glycosides

About 50 mg of extract was hydrolyzed with concentrated hydrochloric acid. It was placed on a water bath for 2 hours. The filtered and the hydrolysate was subjected to the Glycoside tests viz., Borntrager's Test, Legal's Test (Kokate *et al*. 2016).

3.3.3.7 Qualitative Analysis of Terpenoids

About 100mg of dried plant extract was taken. It was soaked in 9 ml of ethanol for 24 hours. The filtration was extracted with 10 ml of petroleum ether using separating funnel and was separated in pre-weighed glass vials and waited for its complete drying.

Ether was evaporated and yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100) (Indumathi *et al*., 2014 and Ahmed *et al*. 2017).

3.3.3.8 Estimation of Phenolic Compounds and Tannins

Alcoholic and aqueous extracts in water are tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5 percent),1 percent solution of gelatin containing 10 percent sodium chloride,10 percent lead acetate and aqueous bromine solutions (Kokate *et al*. 2016).

3.3.3.9 Estimation of Flavonoid

1 ml of stock solution of alcoholic extract was added with few drops of dilute NaOH. The intense yellow colour became colourless on addition of a few drop of dilute acid that indicated the presence of flavonoids (Kokate *et al*. 2016).

To extract flavonoid the method as per Subramanian and Nagarjan, (1969) was followed. 100 grams of finely powdered plant material was taken. Soxhlet extracted with hot 80% methanol (500 ml) on a water bath for 24 hours and filtered. The filtrate was re-extracted with petroleum ether, ethyl ether, and ethyl acetate. Petroleum ether fraction was discarded as it is rich in fatty substances and ethyl ether fractions (free flavonoids) were collected. Ethyl acetate fractions were analyzed for bound flavonoids and were hydrolyzed in 7% H_2SO_4 for 2 hours. Resultant was filtered. The filtrate was extracted with ethyl acetate, washed with distilled water and collected. The ethyl ether (free flavonoids) and ethyl acetate (bound flavonoids) fractions were dried in a vacuum. Weighed and stored in glass vials at 4°C.

3.3.3.10 Determination of Carotenoid Content

The total amount of carotenoids was determined using a spectrophotometer at 450 nm. Carotenes were analysed with high-performance liquid chromatography and scanned between 350 to 600 nm using Empower software. A C_{30} column was purchased from Waters. The mobile consisted of 8:2 (methanol: *t*-butyl methyl ether, v : v). The mobile phase flow rate was 0.8 mL/min, and 25 µL of an ether extract sample was injected. Analysis temperature was 30 °C with a total analysis time of 60 min.

To determine the total amount of carotenoids, approximately 15 g of the samples, plus 3 gm of celite 454 were weighed in mortar on a digital balance. For the carotenoid extraction, successive addition of 25 ml of acetone were made to obtain a paste, which was then transferred into a sintered funnel (5 μm) coupled to a 250 mL Buchner flask and filtered under vacuum. This procedure was repeated three times or until the sample became colourless. It was transferred to a 500 mL separatory funnel containing 40 mL of petroleum ether. With the slow addition of ultrapure water (Milli-Q - Millipore), the acetone was removed. The aqueous phase was discarded and was repeated four times until no residual solvent remained. It was transferred to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate and was made up to 50 ml by petroleum ether. The samples were read at 450 nm (Mukharjee, 2002).

3.3.3.11 Determination of Moisture Content

Determination of moisture content was carried out both for phytochemicals and nutritional analysis of food varieties by similar methods.

The sample was dried at 98 –100^0 C and weighed. Then it was cooled in a desiccator and weighted with cover soon after attaining room temperature. It was placed in the oven and was heated to constant weight (about 5 1/2 hrs) at 98 – 100^0 C at pressure to 25 mm Hg. During heating, slow current of air was admitted through H2SO4 into the oven to bring to atmospheric pressure. The dish was covered and transferred to desiccator and was weighed soon after room temperature was attained. The moisture weight was calculated by the percent of loss in weight. (A.O.A.C 17^{th} edition, 2000 Official Method 968.11 Moisture (Loss on Drying) in Roasted Coffee, Vacuum Oven method 1)

3.3.3.12 Determination of Tannins

10 gms of previously powdered herbs to a known fineness sample was weighed accurately into a conical flask. 150 ml of water was added and heated over a boiling water bath for 30 minutes. The mixture was cooled and transferred to a 250 ml volumetric flask and was diluted to volume with water. The solid material was allowed to settle and filtered the liquid through a filter-paper, diameter 12 cm,

discarding the first 50 ml of the filtrate. To determine the total amount of material that was extractable into the water, evaporated 50.0 ml of the plant material extracted was evaporated to dryness. The residue was dried in an oven at 105 °C for 4 hours and weighed (T1). To determine the amount of herbal material not bound to hide powder that was extractable into the water We had taken 80 ml of the herbal material extract and was added 6 g of hiding powder R and shacked well for 60 minutes. It was filtered and 50 ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105 °C and weighed (T2). To determine the solubility of hide powder, 6 gm of hide powder R was taken and 80 ml of water was added and shacked well for 60 minutes. It was filtered and 50 ml of the clear filtrate was evaporated to dryness. The residue was dried in an oven at 105 °C and weighed (T0). The amount of tannin was calculated as a percentage using the following formula (Kokate *et al*. 2016):

$$[T1 – (T2 – T0)] \times 500/ w$$

where w = the weight of the herbal material in grams.

3.3.3.13 Direct Detection of Saponins

The standard procedures of plant constituent's identification as described by Edeoga *et al*., 2005 was followed for detection of saponins. 3 grams of each dry plant powder were weighed. It was extracted with 300 ml of hot distilled water in a beaker. After filtration, the aqueous extracts were cooled and stored in a refrigerator at 4° C until needed for the test. 5 ml of each plant extract were placed in a test tube and diluted with 5 ml of distilled water. The mixture was shaken vigorously for two minutes. The presence of saponins was done by adding olive oil.

3.3.3.14 Macroscopic and Microscopic Examination

The material was measured by a graduated ruler aligning 10 of them on a sheet of calibrated paper, with 1 mm spacing between lines, and the result was divided by 10. The untreated sample was examined under diffuse daylight and was compared with that of a reference sample. The untreated sample was examined and wetted with water, as per requirement. The material was touched to determine if it was soft or hard; bent and ruptured it to obtained information on brittleness and the appearance of

the fracture plane to observe whether it was fibrous, smooth, rough, granular, etc. (Mukharjee, 2002).

If the material was expected to be innocuous, a small portion of the sample was placed in the palm of the hand or in a beaker of suitable size and slowly and repeatedly inhaled the air over the material. If no distinct odour was perceptible, the sample was crushed. If the material was known to be dangerous, mechanical means was used and then a small quantity of boiling water was poured on the crushed sample in a beaker. First, the strength of the odor (none, weak, distinct, strong) was determined and then the odor sensation (aromatic, fruity, musty, moldy, rancid). A direct comparison of the odor with a defined substance was advised (e.g. peppermint should have an odor similar to menthol, cloves should have an odor similar to eugenol) (WHO, 1998).

3.3.3. 15 Inspection by Microscopy

Selected representative pieces were cut into suitable lengths. Prepared cross or transverse sections by cutting with a razor blade. Prepared cross or transverse sections by cutting with a razor blade. Prepared longitudinal sections and was transferred with a brush moistened with ethanol (~150 g/l) TS to a dish containing ethanol (~150 g/l) TS. Satisfactory sections were selected for the preparation of the slides (Plate 4.24 and 4.25) (Kokate *et al*., 2005).

3.3.4 Nutritional Analysis of Food Contents

3.3.4.1 Estimation of Total Fat content

A few drops of NH_4OH was added to 4 gm of sample. The mixture was warmed on steam bath. 10 ml of conc. HCl was added and boiled for 30 mins. It was allowed to cool and then filtered through a wetted filter paper. Filter paper was washed with hot water and dried, rolled and inserted in an extraction thimble and fat content was determined by a Soxhlet apparatus using ethyl ether and transferred to another flask. The solvent was removed and the flask in an air oven maintained at 100°C for 30 minutes to remove residual solvent if any. The flask was transferred to a

desiccator and allowed to cool. The residue was weighed and total fat was calculated. (ISO 1443- 1973 Codex approved method - Extraction / gravimetry Type I method).

3.3.4.2 Determination of Total Protein content

1.5 gm of prepared sample was weighed and transfer to a Kjeldahl digestion flask and added 5gm of Pot sulfate, 0.5gm of copper sulfate and 25-40ml of Sulphuric acid. The flask was gently heated in an inclined position until frothing ceased and then boiled briskly for 2 hours and allowed to cool. Approx. 200ml of water and 25ml of Sodium thiosulphate solution (80gm/l) was added and mixed. A piece of granulated zinc was added carefully poured down the side of the flask with 110 ml sodium hydroxide sol (1+1) to make the contents strongly alkaline. The flask was connected to a distillation apparatus incorporating an efficient splash head and condenser which was fitted to the condenser and was boiled until about 150ml of the distillate had been collected. Then 5 drops of methyl red indicator was added and was titrated with 0.1N NaOH and also a blank was carried out. l ml of 0.1 HCl is equivalent to 0.0014 of N. Total protein is equal to N X 6.25.
(A.O.A.C, 17th edition, 2000, Official Method 928.08 Nitrogen in Meat (Alternative II).

3.3.4.3 Determination of Total Carbohydrates

Estimation of total carbohydrate content was done by the method of Hedge and Hofreiter, 1962. About 100 mg of the sample was weighed in a boiling tube and hydrolyzed for three hours with 5.0 ml of 2.5 N HCl and was cooled to room temperature. It was neutralized with solid sodium carbonate and made up the volume to 100 ml, centrifuged and then collected the supernatant. 0.2 to 1.0 ml was taken for analysis and working standard .1.0 ml of distilled water (blank), then 4.0 ml of anthrone reagent was added. It was heated in a boiling water bath and cooled rapidly. Green to dark green colour was developed and reading was taken at 630 nm. The standard was taken as glucose.

3.4 Statistical Analysis

Data were expressed as mean ± SE. For both control and study villages were recorded for 3 independent biological replicates and were analyzed statistically by analysis of variance (ANOVA) as per Kruskal–Wallis test with Dunn's post-test (Dunn, O J., 1962) to compare three or more unmatched groups using GraphPad Prism (version 5.03 for Windows; CA, U.S.A.). For all statistical analyses, significance was set to * = $0.01 \leq p \leq 0.05$, ** = $0.001 \leq p \leq 0.01$, and ***/ΔΔΔ = $p \leq 0.001$ throughout the experiments and accordingly plotted in the tables for clear understanding. For comparative study t-test was done.

Plate 3.2.1:BS/RDK test at Control Village

Plate 3.2.2:BS/RDK test at Control Village

Plate 3.2.3: BS/RDK test at Study Village

Plate 3.2.4: BS/RDK test at Study Village

Plate 3.2.5 : BS/RDK test at Study Village

Plate 3.2.6 : BS/RDK test at Control Village

Plate 3.2.1-3.2.6: Prevalence of Malaria in Study and Control Villages

Plate 3.3.1: Measurement of Height in Study Village

Plate 3.3.2: Measurement of Weight in Control Village

Plate 3.3.1-3.3.2: Measurement of Height and Weight (BMI) in Study and Control villages

Plate 3.4.1: ***Capsicum sp***

Plate 3.4.2: ***Zingiber officinale***

Plate 3.4.3: ***Colocasia esculenta***

Plate 3.4.4: ***Manihot esculenta***

Plate 3.4.5: ***Curcuma longa***

Plate 3.4.6: ***Musa sp.***

Plate 3.4.1-3.4.6: Food materials of Study Village

Plate 3.5.1: ***Houttuynia cordata***

Plate 3.5.2:*Colocasia schott*

Plate 3.5.3: ***Colocasia esculenta***

Plate 3.5.4: ***Phaseolus vulgaris***

Plate 3.5.5: ***Momordica charantia***

Plate 3.5.6: ***Solanum melongena***

Plate 3.5.1-3.5.6: Food materials of Study Village

Plate 3.5.7
Phlogacanthus thyrsiflorus

Plate 3.5.8
Corchorus olitorius

Plate 3.5.9
Colocasia esculenta

Plate 3.5.10
Ficus carica

Plate 3.5.11
Diplazium esculentum

Plate 3.5.12
Brassica juncea

Plate 3.5.7-3.5.12: Food material of Control Village

Questionnaire

A. Personal Information

1. Sex of the respondent.
    a. Male
    b. Female
2. What is your age?
    a. 25-35
    b. 36-45
    c. 46- 55
    d. 56-above
3. ST/non-ST status of the respondent.
    a. ST
    b. Non-ST
4. What is the Marital status of the respondent?
    a. Unmarried
    b. Married
    c. Widowed
    d. Separated
5. What is the Family type of the respondent?
    a. Nuclear
    b. Joint
    c. Not known

B. Education, Income and Occupation of the respondent

6. What is your Educational qualification?
    a. Elementary
    b. Upto Class X
    c. Matriculate
    d. H.S

e. Graduate & above

7. What is the Occupational pattern of the respondents?
    a. Cultivation
    b. Unemployed/Students
    c. Service
    d. Retired
    e. Business
    f. Housewife
8. What is the category of income level of the respondent?
    a. UPTO 3000
    b. 3001-6000
    c. 6001-9000
    d. 9001-12000
    e. 12000 and above

C. Awareness about Malaria

9. Have you heard about Malaria?
    a. Yes
    b. B. No
10. What is the source of Malaria?
    a. Mosquito
    b. Other
11. How do you know about Malaria?
    a. Health provider
    b. Mass media.
    c. Other

12. Do you know about availability of treatment?
    a. Yes

b. No

13. Do you know about Preventive measures for malaria?
    a. Yes
    b. No
14. What are the preventive measures adopted by you?
    a. Medicated mosquito nets
    b. Long Lasting Bed Nets(LLIN)
    c. Smoking
    d. Cleanliness
    e. Heath & hygiene
    f. Traditional
15. What do you do if your family member suffers from Malaria?
    a. Hospitalize/ sub centers
    b. Traditional
    c. Self-medication
    d. Without treatment
    e. Heath provider
    f. Private fractioned

D. About Food Items of the respondent

16. Type of food eaten previous night
17. Composition of food items
18. Methods of Cooking

Name of the Investigator:

Place:

Date: