6. SUMMARY AND CONCLUSION

6.1 Standardization of an Ayurvedic Bhunimbadi churna.

Raw materials for Bhunimbadi churna like; Swertia chirata (whole plant), Holarrhena antidysenterica (seed), Zingiber officinale (rhizome), Piper nigrum (fruit), Piper longum (fruit), Cyperus rotundus (rhizome), Picrorhiza kurroa (rhizome), Plumbago zeylanica (root), and Holarrhena antidysenterica (stem bark) were collected from local market of Vadodara, Gujarat and were authenticated by Dr. M. S. Jangid, Professor, Department of Botany, Sir P. T. Science College, Modasa.

Yellowish green chiryata, brownish yellow indraju, yellowish white sunth, greyish black mari, deep green pippali, brown nagermoth, dark brown katuki, grayish yellow chitrak and light yellow kada chhal. Pleasant odour of chiryata, nagermoth and katuki; aromatic smell of sunth, mari and pippali and characteristic odour of indraju, chitrak and kada chhal. Bitter taste of indraju, nagermoth, katuki, chitrak and kada chhal; pungent taste of sunth, mari and pippali powder and extremely bitter chiryata are the characteristic features for identification of powder drugs.

Microscopic study of Bhunimbadi churna’s ingredients revealed the whole plant powder of S. chirata shown parenchymatous cells, fibers, vessels and minute acicular crystals of calcium oxalate. Seed powder of H. antidysenterica was light yellowish-brown with fragments of endosperm, oil globules, prismatic and rosette crystals of calcium oxalate. Powder of Z. officinale rhizome shown thin-walled parenchymatous cortex cells; vessels, septate fibres; flattened, rectangular and ovate starch grains. Fruit powder of P. nigrum shown slightly sinuous epicarp, epidermis, stone cells and parenchymatous perisperm cells having a few oil globules and packed with abundant, oval to round, simple and compound starch grains with few aleurone grains and oil globules. Fruit powder of P. longum shown deep moss green, shows fragments of parenchyma, oval to elongated stone cells, vessels, oil globules and round, starch grains. Powder of C. rotundus rhizome shown circular to oval, thin-walled, parenchymatous epidermal cells, vessels. Powder of P. kurroa rhizome shown dusty grey, cork cells, thick-walled, parenchyma, pitted vessels and aseptate fibres. Root powder of P. zeylanica shown cork cells, trachieds ends and narrow lumen, parenchymatous cells, starch grains and fibres. Powder of H. antidysenterica stem bark shown cork cells, fibres, stone cells and prismatic crystals of calcium oxalate.
Maximum moisture content was found in katuki (9.34±0.04%w/w), while minimum in chiryata (6.11±0.02%w/w). pH of all ingredients were between 5.82 to 7.30. Moisture content of all the ingredients were less than 10%w/w, which help in free flowing property of churna and prevent microbial or fungal growth.

Water soluble extractives value was maximum in indraju (24.97±0.92%w/w), while minimum in pippali (8.43±0.75%w/w). Alcohol soluble extractive were maximum in kada chhal (19.32±0.10%), while minimum in sunth (3.48±0.19%). Water soluble extractives were higher than alcohol soluble extractive in all ingredients of churna.

Total ash value, acid insoluble ash and water soluble ash were found maximum in nagarmoth (6.81±0.08%w/w), (2.36±0.06%) and (3.08±0.08%) respectively. Total ash value was found minimum in chitrak powder (2.43±0.04%w/w). Acid insoluble ash and water soluble ash were minimum in mari powder (0.22±0.07%) and (0.52±0.03%) respectively.

Bhunimbadi churna was greyish yellow with characteristic odour and bitter taste. Bulk density, Tapped density, Hausner ratio and Carr's index indicates very poor flow ability and angle of repose indicates Poor-must agitate, vibrate flow property. pH of Bhunimbadi churna was 5.35±0.11 and moisture content was 8.11±0.13%w/w. water soluble extractive (21.62±0.18%w/w) was higher than others. Methanol soluble extractive (19.38±0.15%w/w) was more than alcohol soluble extractive (12.42±0.13%w/w). Chloroform extractive (3.45±0.24%w/w) was the lowest than others. Total ash value was 5.26±0.02%w/w, water soluble ash was 1.68±0.06%w/w and acid soluble ash was 0.49±0.01%w/w.

Qualitative phytochemical examination in ingredients of Bhunimbadi churna revealed the presence of alkaloid except katuki and chitrak. All ingredients had shown the presence glycoside, flavanoid, steroids & triterpenoids, tannins and carbohydrate, while absence of protein and amino acid. Whereas Bhunimbadi churna had shown the presence alkaloid, glycoside, flavanoid, steroids & triterpenoids, tannins, carbohydrate and devoid of protein and amino acid.

Thin layer Chromatographic of all ingredient of churna and churna carried out using silica Gel 60F254 plate; S. chirata alcohol extract using mobile phase toluene: ethyl acetate: formic acid (5:4:1) and after derivatization by anisaldehyde sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.25 (light
violet), 0.40 (orange), 0.48 (violet), 0.68 (blue). *H. antidysenterica* seeds methanol extract treated with 2ml 30% ammonium solution using mobile phase toluene: ethyl acetate: diethyl ether (70: 20: 10) and after derivatization by dragendorff’s reagent showed major spots at Rf: 0.30, 0.45, 0.50 and 0.62 (all orange). *Z. officinale* alcohol extract using mobile phase toluene: ethyl acetate (93:7) and after derivatization by vanillin sulphuric acid heating the plate for about 10min at 105°C showed all major spots at Rf: 0.16, 0.22, 0.28 (all violet) 0.50 (red), 0.54 (brown), 0.65, 0.68 and 0.78 (light violet). *P. nigrum* methanol extract using mobile phase toluene: ethyl acetate (7:3) and after derivatization by vanillin sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.20 (light blue), 0.48 (green) 0.50, 0.52 and 0.68 (light blue). *P. longum* alcohol extract using mobile phase toluene: ethyl acetate (90:10) and after derivatization by vanillin sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.28 (green), 0.30, 0.42, 0.52 and 0.80 (light brown). *C. rotundus* methanol extract using mobile phase toluene: ethyl acetate (9:1) and after derivatization by vanillin sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.34 (Brown), 0.74 (light brown). *P. kurroa* methanol extract using mobile phase chloroform: methanol (95:5) and after derivatization by anisaldehyde sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.18 (light blue), 0.48 (blue) and 0.70 (violet). *P. zeylanica* chloroform extract using mobile phase toluene: ethyl acetate (3:1) and after derivatization by anisaldehyde sulphuric acid heating the plate for about 10min at 105°C showed major spots at Rf: 0.20 (light blue), 0.35 (light green), 0.45 (violet) and 0.84 (yellow). *H. antidysenterica* stem barks methanol extract treated with 2ml 30% ammonium solution using mobile phase toluene: ethyl acetate: diethyl ether (70: 20: 10) and after derivatization by Dragendorff’s reagent showed major spots at Rf: 0.32, 0.45, 0.52 and 0.62 (all orange).

The developed HPTLC method was simple accurate, precise, economic and can be utilised for estimation of mangiferin, 6-gingerol, piperin and conessine in an ayurvedic polyherbal formulation *Bhunimbadi churna* as well as raw material used in *churna*. It could be used as a valuable analytical tool in the routine analysis. The total concentration of mangiferin in the raw material of *Swertia chirata* and *Bhunimbadi churna* determined using calibration curve of mangiferin were found to be 0.21%w/w and 0.076%w/w respectively. The total concentration of 6-gingerol in the raw
material of Zingiber officinale and Bhunimbadi churna determined using calibration curve of 6-gingerol were found to be 0.54%w/w and 0.06%w/w respectively. Piperin content in the raw material of Piper nigrum, Piper longum and Bhunimbadi churna determined using calibration curve of piperin were found to be 2.87%w/w, 1.53%w/w and 0.24%w/w respectively. The total concentration of conessine in the raw material of H. antidysenterica seed and stem bark and Bhunimbadi churna determined using calibration curve of conessine were found to be 0.19%w/w, 0.90%w/w and 0.083%w/w respectively.

HPTLC finger printing profile of Bhunimbadi churna developed in toluene: ethyl acetate: formic acid (5: 1.5: 0.5 v/v) solvent system. under 254nm showed 13 spots at Rf 0.25, 0.33, 0.45, 0.56, 0.59, 0.62, 0.73, 0.75, 0.82, 0.85, 0.87, 0.95 (all blue colour); under 366nm showed 8 spots at Rf 0.25, 0.37 (Purple), 0.44, 0.56 (Dark green), 0.56, 0.62 (Purple), 0.73, 0.83, 0.88 (Black). After derivatization with the anisaldehyde sulphuric acid reagent showed 11 spots at Rf 0.05 (dark purple), 0.09 (yellow), 0.19 (light purple), 0.25 (purple), 0.45 (green), 0.56 (dark green), 0.62 (purple), 0.68 (dark purple), 0.73 (black), 0.82 (light pink), 0.95 (black).

Heavy metal analysis of Bhunimbadi churna showed lead, cadmium and arsenic content were 9.40, 0.3 and 2.15ppm respectively, while mercury was absent.

Results of microbial limit in Bhunimbadi churna indicated 410CFU/gm total aerobic viable count while total yeast and mould, E. coli and Salmonella species were absent.

6.2 Antioxidant activity of Bhunimbadi churna.

Methanol extracts of churna and ascorbic acid had showed significant dose dependents reduction in free radical to the corresponding hydrazine in the antioxidant principles of DPPH, scavenging of H$_2$O$_2$ and ferric reducing power model indicated dose dependent antioxidant activity of Bhunimbadi churna.

The IC50 values in DPPH model were found to be 62.80 and 14.26 µg/ml for methanol extracts of churna and ascorbic acid respectively. IC50 values in H$_2$O$_2$ scavenging model were found to be 35.64 and 75.45 µg/ml for ascorbic acid and methanol extract of churna respectively.
6.3 Antidiabetic Activity of Bhunimbadi churna In Alloxan Induced Diabetic Rat.

All the animals were alive, healthy and active during the observation period at any of the doses selected indicating the non-toxic nature of churna up to 2000mg/kg, hence, 200mg/kg and 400mg/kg doses were selected for further study.

Result of change in body weight of animals, after first, 7th and 14th days, showed significant increased in body weight in normal control group, reference standard group, group-IV and V, while decreased in diabetic control group (p<0.05).

Blood glucose level in diabetic control group animals was significantly increased. While decreased in diabetic animals receiving Bhunimbadi churna (p<0.05).

There was significant decreased in serum cholesterol level, serum triglyceride level, serum VLDL level and serum LDL level, while increased in HDL level in diabetic animal treated with Bhunimbadi churna indicated antidiabetic activity (p<0.05).

Bhunimbadi churna (200 and 400 mg/kg) significantly reduced elevated serum urea level, serum creatinine level, while raised serum albumin and serum total protein in Diabetic control rats (p<0.05) indicated improving renal profile.

Antidiabetic activity of Bhunimbadi churna was concluded by exhibiting regaining body weight, lowering blood glucose level, improving lipid profile by decreasing serum cholesterol, triglyceride, VLDL & LDL level and increasing serum HDL level and improving renal profile by decreasing serum urea and creatinine level and increasing serum albumin and total protein.

Antioxidant activity, Antidiabetic activity, improvement in lipid profile and renal profile exhibited by Bhunimbadi churna may be due to phytoconstituents present like; alkaloid, glycoside, flavanoid, steroids and triterpenoids, tannins and carbohydrate.

Present investigation justifies the traditional therapeutic claim of Bhunimbadi churna as antidiabetic activity. However further phytochemical investigation may provide isolation and identification of specific compound/s from Bhunimbadi churna which may be responsible for antioxidant activity, antidiabetic activity and improving lipid and renal profile.

Results of pharmacognostical, phytochemical and pharmacological study will help in routine checking the quality, purity and efficacy of Bhunimbadi churna and its ingredients.