2.1 NEED FOR THE STUDY

- The research project is based on the fact that indigenous herbal extracts have antioxidant, antimicrobial and anticancer effects.
- The herbs used in this study are fenugreek seeds, cinnamon bark and papaya leaf and seed that are common, easily cultivable and can be procured cost effectively. They grow well in India and the sources are abundant.
- The present study has an *in-vitro* design to minimize ethical issues.
- Phytochemical parameters and microscopic characteristics were assessed as a part of routine assay performed for herbal research.
- Oxidative stress plays a vital role in carcinogenesis and moreover the antioxidant capacity is lowered in patients diagnosed with oral squamous cell carcinoma. Hence the antioxidant properties of the herbs were determined.
- Recent studies have also shown the role of microorganisms and fungi in carcinogenesis. Hence the present study assessed the antimicrobial and antimycotic effects of the herbal extracts against *Streptococcus mutans* and fluconazole resistant *Candida albicans.*
- Research on major non-communicable diseases like cancer, drug research including traditional remedies, developing alternative strategies for health care delivery are some among the research priorities of the Indian Council of Medical Research. Hence the

present study was conducted to assess the *in-vitro* anticancer effects of the herbal extracts on oral squamous cell carcinoma cell line with a view to reduce the total burden of the disease and to promote health and well-being of the population.

2.2 AIM

To assess the *in-vitro* anticancer and antimicrobial effects of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (leaves of male and female plant), and *Carica papaya* L. CO.2 strain (seeds) extracts in SCC 25 (ATCC CRL 1628 (oral squamous cell carcinoma cell line) and MTCC 890 (*Streptococus mutans*), MTCC 227 (*Candida albicans*) respectively along with their antioxidant potential.

2.3 HYPOTHESIS

NULL HYPOTHESIS

Extracts of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (leaves of male and female plant) and *Carica papaya* L. CO.2 strain (seeds) do not exert anticancer, antimicrobial and antioxidant effects.

ALTERNATE HYPOTHESIS

Extracts of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (leaves of male and female plant) and *Carica papaya* L. CO.2 strain (seeds) exert anticancer, antimicrobial and antioxidant effects.

2.4 OBJECTIVES

- To determine the phytochemical constituents and to assess the *in-vitro* antioxidant activity of hydroalcoholic extracts of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (leaves of male and female plant) and *Carica papaya* L. CO.2 strain (seeds) by
  1. Total antioxidant activity assay
  2. Reducing power assay
  3. Diphenyl picryl hydrazyl assay
  4. Nitric oxide radical scavenging activity assay
  5. Superoxide radical scavenging activity assay

- To assess the *in-vitro* antimicrobial and antimycotic activity of hydroalcoholic extracts of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (leaves of male and female plant) and *Carica papaya* L. CO.2 strain (seeds) against *Streptococcus mutans* and fluconazole resistant *Candida albicans* by agar well diffusion method and to assess minimum inhibitory concentration.

- To quantify trigonelline in aqueous, ethanol and hydroalcoholic (60 ethanol: 40 water; v/v) extracts of *Trigonella foenum-graecum* L. (seeds), cinnamaldehyde, 4 hydroxy cinnamic acid and eugenol in aqueous, ethanol and hydroalcoholic extracts (70 ethanol: 30 water; v/v) of *Cinnamomum verum* J. Presl (bark) and benzyl isothiocyanate

in aqueous, ethanol and hydroalcoholic (60 ethanol: 40 water; v/v) extracts of CO.2 strain *Carica papaya* L. (seeds) by HPTLC (High Performance Thin Layer Chromatography)

- To isolate alkaloid fraction and quantify alkaloid content in CO.2 strain *Carica papaya* L. (leaves of male and female plant) by UV precipitation method.

- To assess the *in-vitro* anticancer effects of hydroalcoholic extracts of *Trigonella foenum-graecum* L. (seeds), *Cinnamomum verum* J. Presl (bark), *Carica papaya* L. CO.2 strain (seeds) and alkaloid fraction of CO.2 strain *Carica papaya* L. (leaves of male and female plant); their respective active compounds trigonelline, cinnamaldehyde, 4 hydroxy cinnamic acid, eugenol, benzyl isothiocyanate and standard cisplatin in oral squamous cell carcinoma cell line by
  1. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) cytotoxicity assay
  2. Assessment of apoptosis by Acridine orange/ Ethidium bromide staining by fluorescence microscopy
  3. Assessment of DNA fragmentation by gel electrophoresis
  4. Assessment of cell cycle analysis by flowcytometry
  5. Determination of mitochondrial membrane potential

- Quantification of saponins, polyphenols and tannins in the extract having pronounced anticancer activity by HPLC (High Performance Liquid Chromatography) and UV method.

2.5 PLAN OF WORK