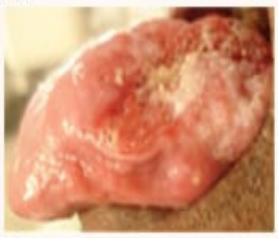
IN- VITRO ANTICANCER EFFECTS OF CINNAMOMUM VERUM J. PRESL , CINNAMALDEYDE, 4 HYDROXYCINNAMIC ACID AND EUGENOL IN ORAL SQUAMOUS CELL CARCINOMA CELL LINE

INTRODUCTION

Despite several recent advances in therapeutic strategies, the five-year survival rate has not seen much improvement with 62% worldwide and 35% in Indian and South East Asian countries. A therapeutic approach that would be cost effective and would possess anticancer properties would aid in management of the disease and reducing Cancer burden in our nation



Cinnamon is indigenous to Sri Lanka and has been cultivated in India , Seychelles, Madagascar, Brazil, South East Asia and other tropical countries in the early times

Cinnamon Quills

→ CINNAMALDEHYDE, CINNAMIC ACID, EUGENOL, POLYPHENOLS, TANNINS

AIM

To assess the in-vitro anticancer effect of C. verum J. Presl extract, cinnamaldehyde, 4 hydroxyxinnamic acid and eugenol in oral squamous cell carcinoma cell line

OBJECTIVES

To quantify 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)

To quantify saponins, polyphenols and tannins in Cinnamomum verum J. Presl (bark).

To assess the in-vitro anticancer effects of Cinnamomum verum J. Presl (bark), active compounds cinnamaldehyde, 4 hydroxycinnamic acid, eugenol, and compare it with standard cisplatin in oral squamous cell carcinoma cell line by

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) cytotoxicity assay

Assessment of apoptosis by Acridine orange/ Ethidium bromide staining by fluorescence microscopy

- Assessment of DNA fragmentation by gel electrophoresis
- Assessment of cell cycle analysis by flowcytometry
- Determination of mitochondrial membrane potential

METHODOLOGY

Aqueous, ethanol and hydroalcoholic extracts (70 ethanol: 30 water; v/v) of C. verum J. Presl (bark) was prepared by maceration. Cinnamaldehyde, 4 hydroxy cinnamic acid and eugenol was quantified by HPTLC; saponins was quantified by HPLC; tannins and polyphenols were quantified by UV method. SCC25 (ATCC® CRL1628 TM) cell line was cultured according to standard protocol. MTT assay was used to determine the IC 50 concentration of the extract, active compounds and cisplatin. Apoptosis was assesed by Acridine orange/ Ethidium bromide staining and DNA fragmentation assay. Cell cycle analysis was done by flowcytometry. Effect of C. verum J. Presl and cisplatin on mitochondrial membrane potential was assesed by JC-1 staining.

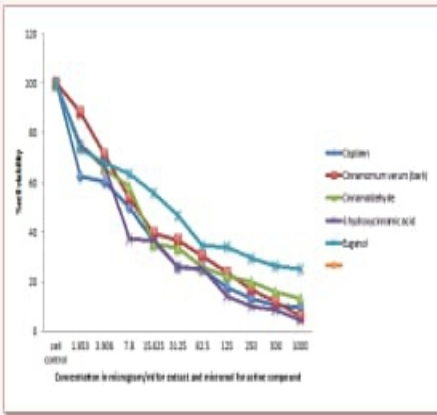
RESULTS

QUANTIFICATION OF PHYTOCHEMICALS

ACTIVE COMPOUND	PLANT MATERIAL	PERCENTAGE w/W OF PHYTOCHEMICAL CONSTITUENT PRSENT IN		
		ETHANOL EXTRACT	ETHANOL: WATER EXTRACT	AQUEOUS EXTRACT
Cinnamldehyde	Cinnamomum verum J. Presl (bark)	0.05727	0.17238	Nil
4 hydroxy Cinnamic acid	Cinnamomum verum J. Presl (bark)	1.6959	1.5667	Nil
Eugenol	Cinnamomum verum J. Presl (bark)	0.238	0.3971	Nil

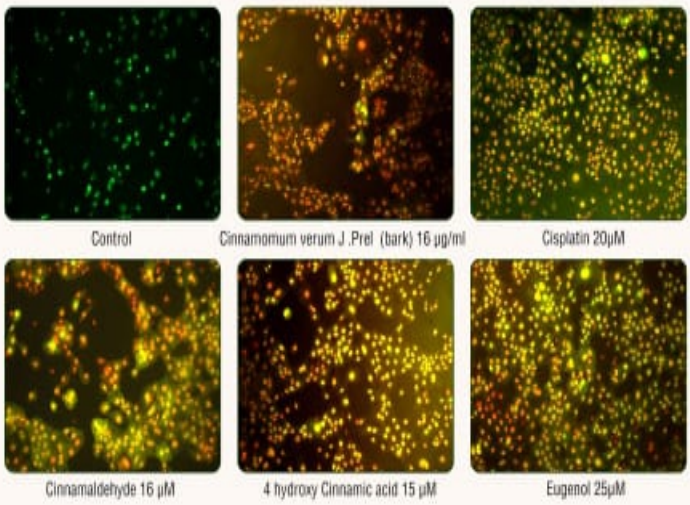
Phytochemical constituent	% w/W of the phytochemical constituent present in Cinnamomum verum J. Presl (bark) extract
Saponins	0.31
Polyphenols	22.5
Tannins	16.88

CYTOTOXICITY ASSAY



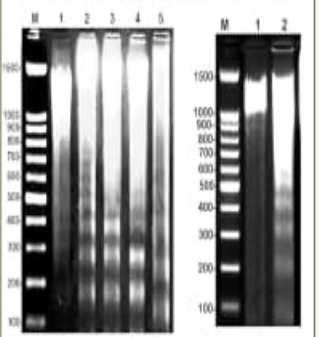
TEST SUBSTANCE	IC50
Cisplatin	20.21µM
Cinnamomum verum J. Presl (bark)	16.36µg/ml
Cinnamaldehyde	15.64µM
4 hydroxycinnamic acid	24.51µM
Eugenol	24.71µM
Benzylisothiocyanate	29.80µM

ACRIDINE ORANGE AND ETHIDIUM BROMIDE STAIN



DNA FRAGMENTATION ASSAY

Agarose gel image internucleosomal DNA fragmentation in cancer cells on treatment with test substance

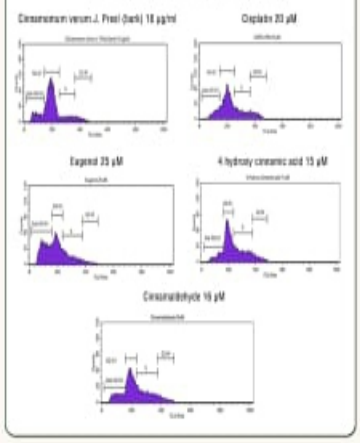


Lane-1 control (Cells without drug)
Lane 2 Cisplatin -20 uM,
Lane 3 4 hydroxy Cinnamaldehyde- 16 uM
Lane 4 Cinnamic acid –15 uM
Lane 5 Eugenol -25 uM

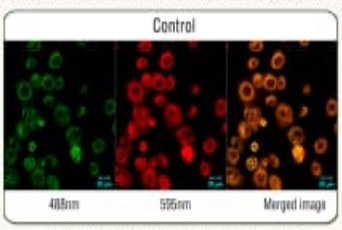
Lane 1 control
Lane 2 Cinnamomum verum J. Presl (bark) 16 µg/ml

CELL CYCLE ANALYSIS BY FLOWCYTOMETRY

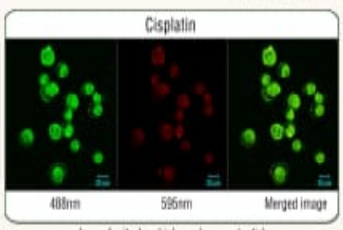
Comparison of the effects of the extract, active compounds and cisplatin at IC 50 concentration



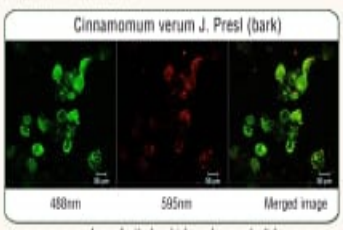
MITOCHONDRIAL MEMBRANE POTENTIAL BY CONFOCAL MICROSCOPY



Intact mitochondrial membrane potential in untreated cells



Loss of mitochondrial membrane potential (indicated by predominant green fluorescence)



Loss of mitochondrial membrane potential (indicated by predominant green fluorescence)

5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1)

DISCUSSION

The present study is based on the fact that indigenous herbal extracts exert anticancer effects.

Cinnamon bark was chosen as it is easily cultivable and can be procured cost effectively and grows well in our country.

The hydroalcoholic extracts demonstrated higher quantity of cinnamaldehyde, 4 hydroxy cinnamic acid and eugenol. C. verum J. Presl bark extract demonstrated 0.31% saponins, 22.5 % polyphenols and 16.88% tannins.

Cisplatin, C. verum J. Presl, cinnamaldehyde, 4 hydroxycinnamic acid, eugenol exhibited an IC 50 concentration of 20 µg/ml, 16µg/ml, 15.64µM, 14.51 µM, 24.71 µM respectively and were found to induce apoptosis in oral squamous cell carcinoma cell line as demonstrated by acridine orange and ethidium bromide staining and confirmed by DNA fragmentation. Flowcytometry results revealed S phase arrest.

Cisplatin and C. verum were found to induce apoptosis via the mitochondrial pathway by alteration of mitochondrial membrane potential as demonstrated by the JC-1.

The anticancer effects of C. verum J. Presl could thus be attributed to the above mentioned compounds as well as other compounds saponins, polyphenols and tannins.

CONCLUSION

This evidence emphasises on the importance of medicinal properties of plant extracts for oral cancer management, as herbal extracts are less likely to produce side effects since they have been a part of diet for several years.