

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 hydroxycinnamic 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

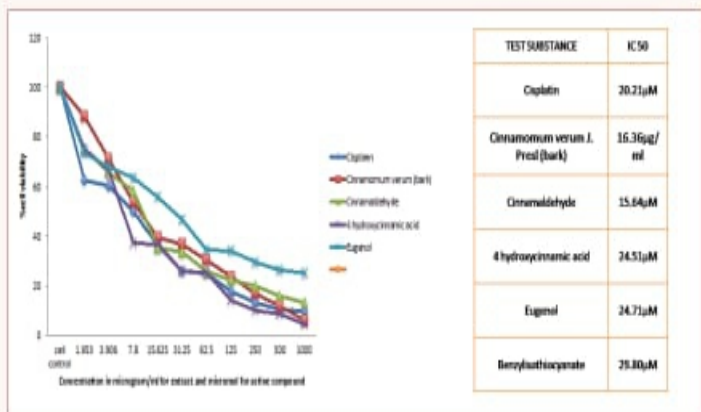
Aqueous, ethanol and hydroalcoholic extracts (70 ethanol: 30 water; v/v) of C. verum J. Presl (bark) was prepared by maceration. Cinnamaldehyde, 4 hydroxy cinnamic acid and eugenol was quantified by HPTLC; saponins was quantified by HPLC; tannins and polyphenols were quantified by UV method. SCC25 (ATCC® 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 assessed 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 assessed by JC-1 staining.

RESULTS

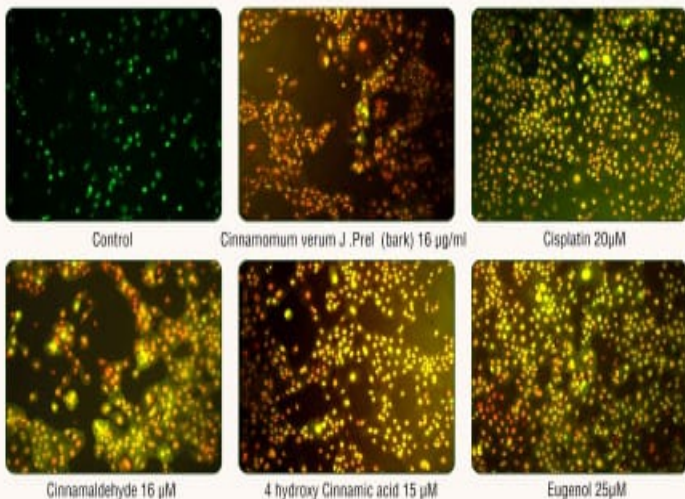
QUANTIFICATION OF PHYTOCHEMICALS

ACTIVE COMPOUND	PLANT MATERIAL	PERCENTAGE w/W OF PHYTOCHEMICAL CONSTITUENT PRESENT IN			Phytochemical constituent	% w/W of the phytochemical constituent present in Cinnamomum verum J. Presl (bark) extract
		ETHANOL EXTRACT	ETHANOL: WATER EXTRACT	AQUEOUS EXTRACT		
Cinnamaldehyde	Cinnamomum verum J. Presl (bark)	0.05727	0.17238	Nil	Saponins	0.31
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	Polyphenols	22.5
					Tannins	16.88

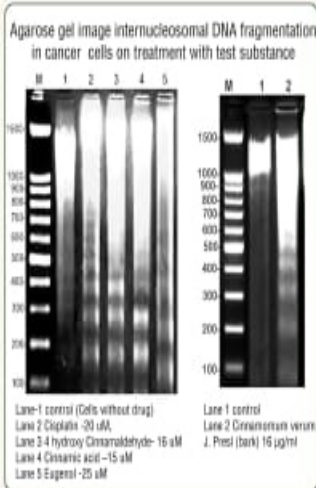
CYTOTOXICITY ASSAY



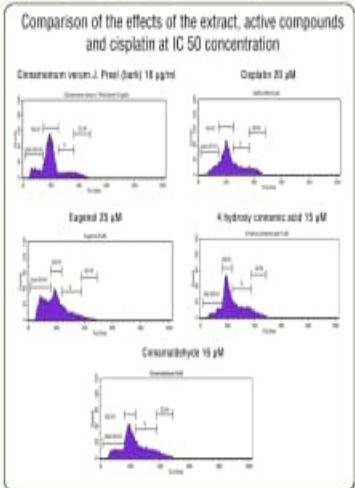
ACRIDINE ORANGE AND ETHIDIUM BROMIDE STAIN



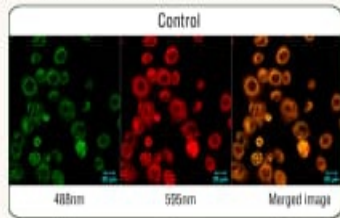
DNA FRAGMENTATION ASSAY



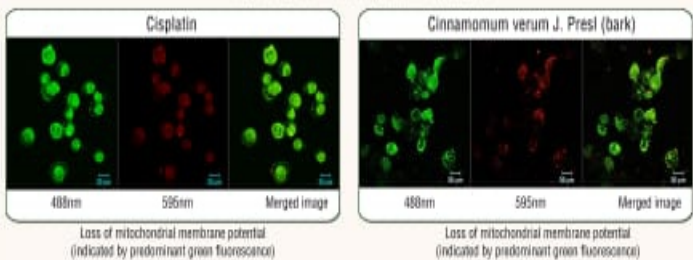
CELL CYCLE ANALYSIS BY FLOWCYTOMETRY



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'-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.