CHAPTER I

INTRODUCTION
CARCINOMA is the term which encompasses a series of about 300 types of disorders, which may generally affect different parts of the human system irrespective of their age, region and culture, and are characterised by the undesirable growth of cells which mass together resulting, what is known as CARCINOMA. This unarrested growth of mass ultimately compells the organism to collapse.

References about the carcinoma has been made in the ancient Egyptian, Greek and Sanskrit records of medical writings. In fact the word cancer (which implies "CRAB" meaning cancerous growth) comes from Romans.

Until the first half of the twentieth century the communicable diseases were the main cause of concern and medical researches devoted exclusively towards those researches which may furnish information leading to the better understanding of the secrecy of the cause of communicable diseases.

However during the second half of the present century, due to changing pattern of the living and
dining habits, carcinoma

has started engulfing a major

segment of the humanity and naturally compelled the

custodian of medical researches all over the globe
to divert their attention towards such researches
which have often yielded clues for the discovery of
drugs, which could ultimately lead to the solution
to this dreaded problem on which no country of the
globe, however advance it may be, can yet claim to
have obtained complete expertise in controlling this
unconquered disease.

PLANTS AS CYTOTOXIC AGENTS: Mention about the use

of plants for curing CARCINOMA dates back to ancient
time. Of course due to enormous advances made in
the field of modern purification and special techniques,
the phytochemical investigations of the flora having
reputed history of their use in folklore has resulted
in the discovery of cytotoxic agents which are compounds
of plants origin having cytotoxic activity.

Few such plants constituents have already

established their reputation as anticancer drug e.g.
Vincristine and Vinbastine isolated from Catharanthus
roseus (Apocynaceae).

A deep sweep and comprehensive survey of
the available literature on carcinoma has enlisted
about 1400 anticancerous plants\(^1\), for example, podophyllum
was the first to be used as an anticancer drugs about
2000 years ago by the ancient chinese.
The resin from this plant Podophyllum\textsuperscript{2,3}hexandrum and related American species have yielded a number of lignans and their glycosides having cytotoxic activity along with the active tumor inhibitory alkaloid glycoside \(\beta\)-Solammarine.

The most widely cytotoxic agents of higher plant origin used in cancer chemotherapy are the alkaloids of Catharanthus roseus. A number of dimeric indole alkaloids showing antileu'emic active have subsequently been isolated e.g. Vincalekoblastine and Leunocristine which are now commercially extracted from Catharanthus roseus and used for cancer.

Systematic phytochemical investigations have resulted in the isolation of many new natural products exhibiting antitumor activity. Camtothecin and its derivatives, alkaloids from the Chinese tree Camptotheca acuminata showed broad spectrum activity and it is reported to be used for the treatment of cancer in China.

Brucea antidysenterica is used in Ethiopia for the treatment of cancer, compounds isolated from this plant is Bruceantin which showed high antitumor activity at low dosages. Maytenus serrata (N.O. Celastraceae) and other species of Maytenus contain Maytansine an ansamacrolide, which are regarded as antitumor agents. Other studies on natural products as models for anticancer drugs are discussed by Cassady and Douros\textsuperscript{4}.
In India various workers\textsuperscript{5-11} have published the data on biological activity of number of indigenous plants which also incorporate the anticancer properties of plants detected during investigations.

A good number of cytotoxic sesquiterpene lactones have been isolated and characterised during the period of continuing search for antitumor agents of plant origin\textsuperscript{12}.

Sesquiterpene lactone may be divided into three classes: monofunctional, those having conjugated cyclopentanone, and those with either a conjugated side chain ester or $\alpha$-methylene $\gamma$-lactone.

The structure of cytotoxic sesquiterpene lactone required deep knowledge of special degradation skill and an expertise in modern spectral technique due to their diverse nature in respects that they have considerable variation in carbon skeleton and contain variety of combination of functional groups. The first problem in their structural elucidation is that which functional group contributed the cytotoxicity. Preliminarily evidence suggested that $\alpha$-methylene $\gamma$-lactone was of major importance. It has been possible to demonstrate that the cytotoxicity is critically dependent upon the presence of this functional group.

The $\beta$-unsubstituted cyclopentanone ring and $\gamma$-lactone functions play an important role in cytotoxic activity.
Using the data available at the time, Hartwell and Abbott, \(^{13}\) surveyed the structural activity of the cytotoxic sesquiterpene lactones and attempted a statistical treatment of the structural activity problem. It was noted that all the cytotoxic sesquiterpenes were lactone of which all but one were \(\alpha - \beta\) unsaturated and that the \(\beta\)-ethlenic linkage was exocyclic in every case.\(^{13}\)

Phytocemical and pharmacological investigations concerning cytotoxic activity of sesquiterpene lactones have revealed that the presence of \(\alpha\) - methylene - \(\gamma\) - lactone is essential for significant cytotoxic activity among the sesquiterpene lactone. The cytotoxicity has been found to be enhanced by the presence of certain additional \(\alpha - \beta\) unsaturated carbonyl functions. Those sesquiterpene which demonstrated in-vivo antitumor activity have common feature which set them apart from the majority of the sesquiterpene lactones.

Evaluation of the cytotoxic activities of the transformation products was often informative of since it gave a clear indication that which functional group contributed to cytotoxic activity. In this way so called active functional groups were identified from amongst the wide variety present in thesesesquiterpenes.

The systematic studies have also resulted in the isolation of many new terpenoidal lactones exhibiting antitumor activity. The plant desert marigold BAILGYA-MULTIRADIATA(N.O. Compositae) has been found
to yield a series of sesquiterpenes that markedly inhibit
the growth of the "Murine Lymphotic Leukemia p-388"
and the corresponding ,In-Vitro cell line separation
guided by blossary,which has let to the isolation of several
cytotoxic sesquiterpene lactones;

(1) Baileyin,(2) Fastigilin-B, (3) Fastigilin-C,
(4) Radiatin, (5) Multiradiatin,(6) Plearadin

A survey of literature revealed that some
plants when worked up phytochemically by earlier workers
have resulted in the discovery of new potential antitumor
agents. Their phytochemical work on antitumor agents
is tabulated below;

TABLE

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Class</th>
<th>Compound</th>
<th>Plant</th>
<th>Family</th>
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<tbody>
<tr>
<td>1.</td>
<td>Sesquiterpene lactone</td>
<td>Ovatifolin</td>
<td>Podonthus-mitiqui</td>
<td>Compositae</td>
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<td>2.</td>
<td>-----&quot;------&quot;</td>
<td>Arturin</td>
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<td>3.</td>
<td>-----&quot;------&quot;</td>
<td>Costunolide</td>
<td>Liriodendron tutilpi fera</td>
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<td>4.</td>
<td>-----&quot;------&quot;</td>
<td>Baileyin</td>
<td>Bailey mul- Compositae tiradiata</td>
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<td>5.</td>
<td>-----&quot;------&quot;</td>
<td>Fastigilin-B</td>
<td>-----&quot;----&quot;</td>
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<td>6.</td>
<td>-----&quot;------&quot;</td>
<td>Fastigilin-C</td>
<td>-----&quot;----&quot;</td>
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<td>7.</td>
<td>-----&quot;------&quot;</td>
<td>Pléniradin</td>
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<td>8.</td>
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<td>Radiatin</td>
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<td>Multiradiatin</td>
<td>Bailey mul tiradiata</td>
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<td>10.</td>
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<td>Tenuin</td>
<td>Helinium amarum</td>
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<td>11.</td>
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<td>Amperboin</td>
<td>Amberboa-lippi</td>
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<td>12.</td>
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<td>Lipidiol</td>
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<td>13.</td>
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<td>Gorosshe-limin</td>
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<td>14.</td>
<td></td>
<td>Vulgarin</td>
<td>Artemisia canariensis</td>
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<td>15.</td>
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<td>Tabarin</td>
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<td>16.</td>
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<td>Cnicin</td>
<td>Centaurea-cacitrapa</td>
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<td>17.</td>
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<td>Cynaropicrin</td>
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<td>Deacylecynaropicrin</td>
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<td>19.</td>
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<td>Melitensin</td>
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<td>20.</td>
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<td>Dihydro-estafionate</td>
<td>Centaurea-webbiana</td>
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<td>Picridin</td>
<td>Picribium-cristallinum</td>
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<td>22.</td>
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<td>Piptocar-phins A-F</td>
<td>Piptocarpha-chontaenesis</td>
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<td>Helenalin</td>
<td>Helinium-amarum</td>
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<td>24.</td>
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<td>Elephantopin</td>
<td>Elephantopus-elatus</td>
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<td>25.</td>
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<td>Vernolipin</td>
<td>Vernonia-hymenolepis</td>
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<td>Compound</td>
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<td>26.</td>
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<td>Elephant opus-elatus</td>
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<td>28.</td>
<td>Lupane-lactone</td>
<td>3-Ox1up-20 (29)en 30-21 α-olide</td>
<td>Kokoona-ochracea</td>
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<td>29.</td>
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<td>20,29, epoxy3 oxolupan30, 21 α-olide</td>
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<td>Ileostylus</td>
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<td>2,3dihydro-2 hydrolxypodolide</td>
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<td>-F podolactone -E</td>
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<td>Milanji-lactone-A</td>
<td>Podocarpus</td>
<td>Taxaceae milanjianus</td>
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<td>Podolactone -C</td>
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<tr>
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<td>Nagilactone</td>
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<td>Sellowin-A</td>
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<td>42.</td>
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<td>nagilactone -F</td>
<td>Podocarpus Podocarpaceae sellowii</td>
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<td>43.</td>
<td></td>
<td>Nagilactone -G</td>
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</table>
The plants belonging to compositae family are rich in terpenoidal lactones and are abundantly available in India.

A deep sweep in the available literature on some indigenous plants by the author has revealed that still there was much scope of phytochemical investigations on the plants;

(1) SAUSSUREA LAPP A C.B. CLARKE$^{28-30}$
(2) SOLIDAGO VIRGAUREA LINN$^{31-33}$

The author as such got very much fascinated and decided to phytochemically investigate plants having reputation as cytotoxic agents to ameliorate human sufferings.

The herb of Saussurea lappa C.B. Clarke$^{28-30}$ is reported to be used in cough and asthma particularly of vagotonic type, while other species of the genus$^{30}$ are employed as carminative, purgative and antisyphilitic.

Semmler and Feldstein$^{14}$ (1914) were the first to investigate the composition of essential oil of Saussurea lappa and more recently Ukita$^{35}$ (1939), Carbolana$^{36}$ (1948) and Naves$^{37}$ (1948) have investigated it which revealed the presence of 6% α-Costene, 14% Costus acid, 0.2% Terpene alcohol, 11% Costus lactone and 15% Dihydrocostus lactones in it.
Ukita\(^3\) (1939) reported the presence of lactone \(\text{C}_{15}\text{H}_{18}\text{O}_{2}\) in low yield in it. Carbolana isolated the same lactone from the oil of Saussurea lappa root. This lactone was identical with Dehydrocostus lactone isolated by Naves Rao\(^4\) (1960) has reported the occurrence of new sesquiterpene lactone Costunolide (44) from Saussurea lappa. Chopra and Prem ankur\(^5\) (1939) reported an aikaloid Saussurine. Salooja et al.\(^6\) (1950) examined the roots of Saussurea lappa and reported the presence of Kusthin. Kulkarni et al.\(^7\) (1961) reported B-sitosterol, Stigmasterol and Betulin from the roots of Saussurea lappa. Saussurea lactone was isolated by Rao et al.\(^8\) (1951) from costus roots oil. Subsequently Rao et al.\(^9\) (1958) suggested the tentative bicyclic structure (45) for this lactone.

Mathur et al.\(^10\) (1965) confirmed the structure of Dehydrocostus lactone (46) which was isolated by Naves.\(^11\)

Jain et al.\(^12\) (1970) prepared Dehydrosaussurea lactone (47) from Costunolide. Govindan et al.\(^13\) (1977) reported the presence of Alantolides (48) and Cyclocostunolides (49), novel sesquiterpene lactones from costus root. Kalsi et al.\(^14\) (1983) have reported the isolation of two new sesquiterpene lactones; Isodhydrocostus lactone (50) and Isolazaluzamin (51) from Saussurea lappa.
The another plant chosen by the author is Solidago virgaurea Linn\textsuperscript{31-33} which is reported by Ayurvedic system of medicine to be used as carminativ, antiseptic and diuretic. The flowers of the plant are used as tonic, astringent, diaphoretic, carminative and vulnerary Robert et al.\textsuperscript{48} (1944) isolated the Quercitrin and Quercetin from Solidago virgaurea.

It is thus clear that these plants are rich in sesquiterpenoidal lactones. Since costunolide\textsuperscript{15} and new germacranoalide isolated from Liriodendron tulipra have shown reproducible inhibitory activity against the cell culture of human carcinoma of the nasophrynix and therefore the above deliberations clearly transpire that there is still enough scope for adequate phytochemical and pharmacological investigations on the forage; Saussurea lappa C.B. Clarke and Solidago virgaurea Linn (N.O. Compositae) and hence the author thought it worthwhile to carry out furture systematic investigations on these plants.
PROBLEM TAKEN AND WORK DONE:

Higher plants have been used for the treatment of malignant diseases for centuries. A comprehensive survey of the literature, both old and new, describing plants finding use against cancer is no doubt adequately high. In view of growing demand for Vincristine and looking to the fact that the plant produces a higher proposition of Vinblastine, it became imperative to convert Vinblastine into Vincristine.

In recent years in the programmes of cancer drug development a number of clinically useful agents have been developed by screening high plants extract by coordinating with the pharmaceutical industry and research institutes. The efficiency of uncovering effective antitumor agents from higher plants strongly depends on the pre-screening/ screening methodology which is utilized for guiding the fractionation and separation of the crude extracts.

Systematic phytochemical and pharmacological screening of the plant kingdom having reputed therapeutic values for the treatment or cure of cancer may be regarded as an empirical approach to the biggest threat to the human race.

Important anti-malignant obtained from higher plants have been recognized in cancer chemotherapy not only in their isolated forms but also as templets
for the formation of analogues having greater therapeutic activity. As such, the author felt it as a dire need for making concentrated attempts to uncover the presence of cytotoxic agents and his findings are summarised below:

**ISOLATION AND STRUCTURAL STUDY OF NOVEL SESQUITERPENE LACTONE; 7-α- 14- HYDOXY COSTUNOLIDE; FROM THE STEMS OF SAUSSUREA LAPPA C.B. CLARKE.**

Isolation and structural elucidation of a new sesquiterpene lactone, molecular formula C_{15}H_{20}O_{3}, m.p. 105-107°C, M^+ =248 (EIMS), yield (0.068%) obtained by column chromatography from the unsaponifiable fraction of concentrated petroleum ether extract of the stems of Saussurea lappa C.B. Clarke and identified as; 7-α- 14-hydroxy costunolide (SG) by various chemical degradation and HNMR, CNMR and mass spectral studies, has been dealt in it.
(2) ISOLATION AND STRUCTURAL STUDY OF NOVEL SESQUITERPENE LACTONE; 15-HYDROXY DEHYDROCOSTUS LACTONE; FROM THE ROOTS OF SAUSSUREA LAPPA C.B. CLARKE

The concentrated acetone extract of 95%ethanolic extract of the roots of Saussurea lappa C.B. Clarke, when worked up by column chromatography, yielded a novel sesquiterpene lactone (Sg), yield (0.087%), molecular formula C_{15}H_{19}O_{3}, m.p. 60^0 and M=247(EIMS) and its identity as 15-hydroxydehydrocostus lactone was confirmed by various colour reactions, degradative processes and spectral analysis.

(II)

(3) ISOLATION AND STRUCTURAL STUDY OF NOVEL SESQUITERPENE LACTONE; 12-HYDROXY-α -CYCLOCOSTUNOLIDE; FROM THE ROOTS OF SAUSSUREA LAPPA C.B. CLARKE.

The concentrated ethyl acetate extract of the 95%ethanolic extract of the roots of Saussurea lappa C.B. Clarke when worked up, by column chromatography gave a novel sesquiterpene lactone (SLG), yield (0.072), molecular formula C_{15}H_{21}O_{3}, m.p. 82-83 and M=249(EIMS). It was identified as 12-hydroxy-α -cyclocostunolide by various colour reactions, degradations and spectral studies.

(III)
(4) This chapter comprises of two parts:

PART-I: - ISOLATION AND STUDIES OF SESQUITERPENE LACTONES;
COSTUNOLIDE AND DIHYDROCOSTUNOLIDE; FROM THE ROOTS
OF SAUSSUREA LAPPA C.B. CLARKE;

The petroleum ether soluble fraction of 95% 
ethanolic extract when worked up yielded two sesquiter-
pene lactones which were identified as: Costunolide(SC)  
\[ \text{C}_{15}\text{H}_{20}\text{O}_{2}, \text{m.p.} 105-108, M^+ 232(\text{EIMS}) \]
and yield (0.076%) and Dihydrocostunolide (SD) molecular-
\[ \text{C}_{15}\text{H}_{22}\text{O}_{2}, \text{m.p.} 80, M^+ 234(\text{EIMS}) \]
yield (0.058%) by chemical and spectral studies.

PART-II:- ISOLATION AND STUDY OF SESQUITERPENE
LACTONE; PARTHENOLIDE, FROM THE ROOTS OF SOLIDAGO VIRGAUREA
LINN;

The concentrated hexane extract of 95% ethanolic 
extract of the roots of Solidago virgaurea Linn, when 
worked up gave a sesquiterpene lactone molecular formula 
\[ \text{C}_{15}\text{H}_{20}\text{O}_{2}, \text{m.p.} 113, M^+ 248(\text{EIMS}) \]
yield (0.039%). It was identified as: Parthenolide by its chemical 
degradations and spectral.
REFERENCES


