CHAPTER - 5

DISCUSSION

The standardization of plants fruit *Carissa carandas, Spondias mangifera* and *Solanum torvum* were carried out as per Indian Pharmacopoeia. Extraneous matter may be found in plant materials due to either faulty collection or incorrect storage. The extraneous matters are mainly found in herbaceous plant materials and plant materials which are dug out from the earth which are stored from longer time under insanitary conditions. The plant material selected for study was the fresh fruit of the plant which was free from such type of extraneous matter.

The values of loss on drying of the fruit powder *C. carandas, S. mangifera* and *S. torvum* signify a considerable amount of moister present in the fruit powder. The fruit powder of plant may be susceptible to growth of microbes, bacteria and moulds. So, after drying the plant material was kept in moisture free air tight containers. The objective of drying of fresh material is to reduce their weight and to fix their constituents i.e. to check enzymatic or hydrolytic reactions that might alter the chemical composition of the drug **[1]**.

The ash values (Total ash, acid-insoluble ash, water soluble ash and sulphated ash) of the fruit powder were determined by following the procedure of Indian Pharmacopoeia. Total ash determines quantity of inorganic materials, such as carbonate, silicates, oxalates and phosphates present in the crude drug. Ignition causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. We can detect the extent of adulteration as well as establish the quality and purity of the drug by this method. Water soluble ash is used for the estimation of amount of soluble inorganic elements. It is obtained after ignition of plant tissue itself. It is also known as physiological ash of plant tissues. Acid insoluble ash is also known as non-physiological ash. It is

determined for indicate the contamination with extraneous matter adhering to the plant surface (e.g. earthly material like sand, soil, silica, grit etc). These contaminations are mainly done during the faulty collection of plant materials. Sulphated ash determines the quantity of basic radicals in ash reactive to sulphuric acid. Crude fibre is the un-digestible tissue fibres of sclerenchymatous cells of plant in the crude drug. This is also caused by the addition of same kind of resistant woody tissues as adulterants. It also determines the measure of the content of cellulose, lignin and cork cells content in the plant tissues. Crude fibre content of *S. mangifera* was greater than the other two plants.

Water was found to be the best solvent for both hot and cold types of extraction while in case of successive extraction, methanol possessed highest extractive values. It indicates that polar constituents like, phenolic compound, flavonoids, acids and sugars are present in more extent and non polar constituents like steroid, terpenoids and caroteinoids in less extent. In a particular solvent extract of drug is often an approximate measure of the amount of certain constituents that the drug contains. Generally petroleum ether, alcohol and water extractives are taken into consideration for fixing the standard of a drug. Here the drug was extracted with different solvents in order of their increasing polarity to obtain the correct and dependable values. In successive soxhlet extractives, the extracts of petroleum ether contained fixed oil, resin and volatile substances. The resinous matter of the *C. carandas* fruits extracted out in petroleum ether may be useful as a natural polymer for the development of formulations of controlled drug release system. The obtained extractive values signify the presence of a high proportion of polar constituent than non-polar constituent. The microwave extraction under specific condition of the plant fruit was used successfully for the extraction of phytoconstituents. This type of extraction is a good alternative to

conventional extraction methods. The microwave extraction of the plant fruit was used successfully for the extraction of phytoconstituents and gave higher extractive yield as compared to the conventional methods. The results indicated that the developed microwave procedure could be used for the extraction of phytoconstituents from hard and tough plant materials.

The powdered drug exhibit different fluorescence character due to the presence of different functional groups present in the constituents of drug. The chemicals such as H_2SO_4, HCl and HNO_3 in different proportions may changes the configuration of chemical functional groups present in the powdered drug and change in colour occurs. The changes in the colour of powdered plant species specify the presence of some particular chemical constituents. So, fluorescence analyses of crude powder drug play a vital role for the determination of quality and purity of drug.

On the basis of TLC fingerprint it can be concluded that phenolic compounds are present in polar solvent fraction of *S. mangifera*. Lupeol is a triterpenoid compound and resembles with TLC fingerprints of *C. carandas* extractives in chloroform, ethyl-acetate and methanolic fractions. The chloroform and ethyl-acetate fractions of *S. torvum* showed the presence of triterpenoids that resembles with lupeol and methanolic fraction indicate the presence of flavonoids resembling with standard drug rutin.

In the preliminary phytochemical screening methanolic and water extracts of the fruit, show positive results for reducing sugar, protein, flavonoids, phenolic compounds and acids. The ethyl acetate extract shows positive results for phenolic contents, flavonoids and terpenoids. Steroids were found only in the chloroform extracts. It indicates that terpenoids and steroids are non-polar in nature and it can be isolated in non-polar solvent only. Petroleum ether extracts of

Carissa carandas have latex type resinous matter which was not dissolved in other solvents.

The compound Cc-01 was obtained as amorphous brownish white powder. It gave positive Salkowiski test for triterpenoid and showed IR absorption band for hydroxyl groups (3450, 3260 cm^{-1}), un-conjugated C=C group (1637 cm^{-1}) and aliphatic chain (929 cm^{-1}). A review of the literature reveals that the non hydrogen bonded or "free" hydroxyl group of alcohols absorbs strongly in the 3600 - 3384 cm^{-1} region. In the spectra presented here, a strong band occurs around 3450 cm^{-1} indicating the presence of –OH group. Methyl groups have been found to give rise to absorption bands at two distinct region 2924 and 2845 cm^{-1}. These bands are interpreted as arising from vibration of the angular methyl groups at C_{l0} and C_{13}. An un-conjugated C=C group has been found to give rise to an absorption band 1637 cm^{-1} **[2].** The C-C bending vibration occurs at 754 cm^{-1} and C-C stretching vibrations was found at 929 cm^{-1}. Based on the ^{1}H and ^{13}C-NMR data the structure of the compound, Cc-01 is shown in (**Fig.** 4.20). The chemical shift values of the various signals and their functional group of compound was given in (**Table** 4.16). On the basis of mass and ^{13}C-NMR spectra the molecular peak of Cc-01 was found m/z 458.39 $[M]^{+}$ consistent to the molecular formula $C_{30}H_{50}O_3$. It may be due to loss of CO_2 molecule during the ionization of the compounds in position C_{21} the molecular ions found in the mass spectra were corresponding the mass peak at m/z 412. The compound is fragmented in two parts between C_{11} and C_{12} and between C_8 and C_{14}. These fragments have m/z 191 and 248 with molecular formula $C_{14}H_{22}$ and $C_{18}H_{32}$ respectively. The ^{1}H-NMR spectrum of the Cc-01 showed a series of proton signal (1.25 to 1.90 ppm) attributed to resonance of overlapping of methylenes and methines a characteristic frame work of triterpenoid **[3]**. The double doublet

signal at 5.28 ppm indicates the presence of ethylenic proton at C_6 and coupled with H-7a and H-7b **[4 & 5]**. The doublet at 0.95 ppm indicates the presence of methyl protons (-CH_3) in highly shielded environment. The methynic proton which is in a deshielded (-CH) environment gives a double doublet signal at 3.21 and another methynic proton gives singlet peek at 1.50 ppm. The gem dimethyl protons (-CH_3) give a broad multiplete signal at 0.88 ppm. The hydroxy proton (-OH) is identified with the singlet signal at 2.27 ppm. The spectrum further displaced signal at 0.78 ppm (C_{18}), 1.08 ppm (C_{19}) and 0.90 ppm (C_{26} and C_{27}) characteristic of 5-ene-3-*β*-hydroxy terpenols. The ^{13}C-NMR showed peaks at δ_C 18.02, δ_C 21.03, δ_C 182.15 corresponding to C_{18}, C_{19} and C_{21}, respectively. The C_3 and C_{23} resonated at δ_C 66.17 & 22.68, respectively. C_5 resonated at δ_C 138.01 while C_6 resonated at δ_C 123.16. On the basis of these evidences the structure of Cc-01 has been established as Lanost–5–en–3*β*–ol–21–oic acid **[6]**.

The compound Sm-01 is propane tricarboxylic diglucoside is chocolate colour sticky mass. It gave positive Fehling solution test for glycosides and showed IR absorption band for hydroxyl groups (3426, 3255 cm^{-1}), ester group (1752 cm^{-1}), acidic group (1485 cm^{-1}) and aliphatic chain (772 cm^{-1}) **[2 & 3]**. The IR-Spectra of Sm-01 showed a strong band around 3255 and 3426 cm^{-1} arises from polymeric structures of hydrogen in alcoholic hydroxyl groups and an another strong band appears around 2925 cm^{-1} assigned to the linear vibration of hydrogen in C-H groups of the compound. Carbonyl groups, whether present in ketones, aldehydes, acids, esters, or anhydrides, absorb in the region between 1835 and 1653 cm^{-1}. The carbonyl absorption band in the compound for ester occurs at 1752 and for acid at 1628 cm^{-1}. Vibration of a C—O linkage has been shown to produce an absorption band at 1485 cm^{-1} supporting the presence of pyranosyl group in the compound. The C—C bending vibrations occurs772 cm^{-1}

and C—C stretching vibrations appear 1086 cm^{-1}. Based on the ^{1}H and ^{13}C NMR data the structure of the compound is shown (**Fig.** 4.26). The chemical shift values of the various signals and their functional group of compound Sm-01 was given in (**Table** 4.18). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of Sm-01 was determined at m/z 500 $[M^{+} H]^{+}$ consistent to the molecular formula of propane tricarboxylic diglucoside, $C_{18}H_{28}O_{16}$. The ion peak appearing at m/z 191$[C_7H_{10}O_6]^{+}$ indicated that propane tricarboxylic acid attached to diglucoside unite. It may be due to loss of H_2O molecule the glycosidic bond of original compound is break down during ionization. So the peak of original compound is not appeared in mass spectra. The compound is fragmented in two parts with molecular weight 394 and 193 and molecular formula $C_{16}H_{26}O_{11}$ and $C_7H_{14}O_6$. The compound shows a characteristic fragmentation pattern with only a small molecular ion and a few abundant fragment ions. The ^{1}HNMR spectrum of Sm-01 displayed two one proton doublet at δ_H 5.01 (J =10.8 Hz) and 5.38 (J = 10.5 Hz) assigned to anomeric H-1′ and H-1″ respectively and others sugar protons from δ_H 3.79 to 3.20. Other methylene protons as a two proton multiplate at δ_H 4.35 and as a broad singlet at δ_H 3.28 and δ_H 3.20. The ^{13}C NMR spectrum exhibited two acids carbon at δ_C 176.01 (C-4) and 175.21 (C-5), two anomeric sugar carbon at δ_C 101.52 (C-1′) and 105.98 (C-1″), aglycone ester carbons at δ_C 173.81(C-6) and other sugar carbons between δ_C 86.35 to 64.07 **[7]**. On the basis of these evidences the structure of Sm-01 has been established as Propan–1, 2–dioic acid–3–carboxyl–*β*–D–glucopyranosyl–(6′→1″)–*β*–D–glucofuranoside.

Modern life style has enhanced the exposure of human beings to stressful conditions resulting in the physical, psychological abnormalities. Therefore, there is a need to enhance the adaptability of human beings to stressful conditions. In the present study an attempt was made to evaluate the adaptogenic property of

traditionally used fruits *Carissa carandas, Spondias mangifera* and *Solanum torvum*. Anoxia stress, swimming endurance test and Cyclophosphamide induced immunosuppression models were used for evaluation of adaptogenic activity. Anoxia is a very severe form of stress. All the body functions including cellular respiration depend on oxygen supply to them. The lack of this vital element will play havoc on all body mechanisms and increase in adaptation during this stress by a drug could be considered as its major adaptogenic effects. Pretreatment with EEFCC, EEFSM, Cc-01 and Sm-01 observed that increase in anoxia stress tolerance time indicating the significant adaptogenic activity. Non-specific adaptogens facilitate the conversion of energy in cellular system of the organism and helps in adaptations **[8]**. Hence we suggest that EEFCC, EEFSM, Cc-01 and Sm-01 facilitated conversion of energy in cellular system of organisms which could help adaptive process during stress.

The swim endurance test and post-swimming motor function results indicate clearly that the pretreatment with EEFCC, EEFSM, Cc-01 and Sm-01 have the properties whereby they increase the physical swimming endurance time as well as stay on rota rod in mice. In stressful event blood glucose level will drop below the normal level. In this situation adrenalin and noradrenalin increases the blood glucose level by preventing the entry of glucose molecule into cells. Adaptogens work at the cellular level to help the body cope up with stress-related situations **[9].** Since pretreatment with EEFCC, EEFSM, Cc-01 and Sm-01 have enhanced swimming time and shown significant adaptogenic activity. The enhanced swimming endurance in mice as compared to the normal animals may be attributed to the glucopyranosides, flavonoids, acids glycosides and the triterpenoids **[10]** which are identified in chemical test of the extracts.

Pretreatment with Cyclophosphamide have shown significant decrease in weight of mice, indicating that doses of 25 mg/kg were toxic for these mice. The results demonstrate that EEFCC, EEFSM, Cc-01 and Sm-01 treatment were able to reverse the Hb, RBC and WBC count significantly and they are able to reduce leucopenia and anemia induced by Cyp. The present findings in the experiment suggest that EEFCC, EEFSM, Cc-01 and Sm-01 have ability to stimulate haemopoietic system and to reverse the Cyp induce haematological changes. The body weight alterations are a usually observed in mice indicative of Cyp induced toxicity. Major side effects of chemotherapy observed in clinics are impairment and suppression of immune system **[11 & 12]**. In such clinical situations the adaptogenic effect of EEFCC, EEFSM, Cc-01 and Sm-01 may be of therapeutic importance in the treatment of patients with severely impaired or suppress immune system. Although the study did not include the tests for elucidating the mechanism of action EEFCC, EEFSM, Cc-01 and Sm-01, the finding that it increases resistance to stress against diverse aversive stimuli in a non-specific manner indicates that it could have adaptogenic activity. The study affirms that the EEFCC, EEFSM, Cc-01 and Sm-01 are the effective adaptogenic agent.

Free radicals cause oxidation of nucleic acids, proteins and also damage bio-membranes, reflected by an increased lipid peroxidation **[13]**. The lipid peroxidation of the membrane lipids, cause damage of the cell membrane of phospholipids, lipoprotein by propagating a chain reaction cycle initiated by reactive oxygen species (ROS), such as superoxide anion (O^{-2}), perhydroxy radical (HOO^{-}) and hydroxyl radical (OH^{-}) **[14]**. During this process, the ability of the body's defence system to combat with oxidative stress may diminish due to reduced anti-oxidants. Thus, antioxidants defence systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Phenolic

compounds are very important plant constituent because of their scavenging ability due to their hydroxyl group. Many plant materials such as vegetables, fruits, spices and herbs containing phenolic and flavonoids contents, are established sources of natural antioxidants **[15]**. As ethanolic and water extractive of *C. carandas, S. mangifera* and *S. torvum* fruit contained considerable amount of phenolics and flavonoids compounds, it may be responsible for their antioxidant activity. DPPH is one of the free radicals generally used for testing preliminary radical scavenging activity of a compound of a plant extract. In present study ethanolic extract of fruit showed a good antiradical activity by scavenging DPPH radicals. Nitric oxide (NO) is a free radical produced in mammalian cells, exhibits numerous physiological properties and also implicated in several pathological states **[16]**. The interaction of (NO) with other radical leads to formation of more hazardous radical such as peroxy nitrite anion and hydroxyl radical. Chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis and other pathological conditions **[17]**. In this study the nitric oxide was reduced by extracts which is produced by the incubation of sodium nitropruside solution in standard phosphate buffer. The extracts of spices may well act as electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reactions and it has been shown that the antioxidant effect exponentially increases as a function of the development of the reducing power **[18]**. Iron is an essential element for normal physiology, but excess can result in cellular injury. If they undergo the Fenton reaction, these reduced metals may form highly reactive hydroxyl radicals and thereby contribute oxidative stress. The resulting oxy radicals cause damage to cellular lipids, nucleic acids, proteins, and carbohydrates and lead to cellular

impairment. For the measurements of the reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of the extracts **[15]**. When free radicals attack membranes, which are high in lipids, they form lipid peroxides **[19]**. Hence, we initially used an iron-dependent system (ferrous chloride: thiocyanate) for induced lipid peroxidation to investigate the antioxidant activities of the extract using linoleic acid micelles as lipid phase model systems Increasing concentrations of the ethanolic fruits extract were tested for antioxidant activity in the linoleic acid medium.

Conclusion:

The authenticity of *Carissa carandas*, *Spondias mangifera* and *Solanum torvum* fruits were assessed by their morphological, microscopical, physicochemcal and phytochemical characteristics.

The ethanolic extracts of *C. carandas* and *S. mangifera* showed significant adaptogenic activities against selected mice models, whereas *S. torvum* not showed significant activity against the same.

A lanostane triterpenoid compound Lanost-5-en-3*β*-ol-21-oic acid (Cc-01) and an acidic glycoside Propan-1,2-dioic-3-carboxyl-*β*-D-glucopyranosyl-(6′→1″)-*β*-D-glucofuranoside (Sm-01) were isolated from the ethanolic extracts of *C. carandas* and *S. mangifera* respectively. Both Cc-01 and Sm-01 showed significant adaptogenic activities against the selected mice models.

The ethanolic fruits extracts of all the selected plants possessed antioxidant activities and or free radical scavenging effects in *in-vitro* models.

Although adaptogens can normalize the plasma level of catecholamines and monoamine oxidase in stress conditions still further study may confirm this hypothesis by determining these biochemical markers.

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