CHAPTER X

10. SUMMARY

*Dodonaea viscosa* Linn. (Sapindaceae) is an evergreen woody perennial shrub distributed throughout India. This plant is commonly known as Hop bush plant in English and Sinatha in Hindi. The stem, leaves, seeds, roots, bark and aerial parts are used in traditional medicine. Traditionally, leaves are used in the treatment of fever, malaria, ulcers, diarrhoea, dysmenorrhoea, rheumatism, sprains, bruises, burns and wounds. It is proved to have antibacterial, antiviral, analgesic, anti-inflammatory, antiulcer and antioxidant activity. Literatures showed the presence of flavonoids, diterpenoid acids, saponins, P-coumarin acid ester, sterols, essential oils and tannins. Based on the folklore claim and the phytoconstituents present, the plant *Dodonaea viscosa* was selected for the study to explore the wound healing potential of this plant.

**Pharmacognostical studies**

- The leaves of *Dodonaea viscosa* was collected from Algarkoil hills, Madurai, Tamil nadu during the period of August 2010 and authenticated.

- Pharmacognostical studies such as macromorphology and microscopy (transverse section, powder microscopy and quantitative microscopy) and physico chemical parameters were studied to establish the salient diagnostic features. The morphological evaluation depicts that leaves are alternate, simple with short petiole and elliptic to oblong or oblong-lanceolate in shape.

- Microscopical analysis shows that a leaf consists of abaxial epidermis with cyclocytic stomata and subsessile peltate glandular trichomes. Mesophyll cells constitute palisade cells and spongy parenchyma along with concentric vascular bundles surrounded by sclerenchyma. The diagnostic features of
powder microscopy include lignified fibres, large spherical masses of druses of calcium oxalate crystals, foliar sclereids and cyclocytic stomata which could be useful in the identification of the plant.

- Physico-chemical analysis was performed to standardize the plant material. Total ash, acid insoluble, water soluble and sulphated ash was found to be 2.52% w/w, 0.46 % w/w, 0.87 % w/w and 0.82 % w/w respectively. Ethanol soluble extractive (16.89 %) was found to be high than water soluble extractive (12.51 %) value.

- Heavy metals such as lead, cadmium, mercury and arsenic were estimated and it was found to be within the limits of WHO standard. The elemental analysis of powdered drug was carried out to determine the levels of copper, zinc, sodium, potassium, selenium and iron.

- Physico-chemical and elemental analysis of powdered drug was carried out which will act as a standardization tool for future identification of the plant.

**Phytochemical studies**

- Different fractions were prepared from *Dodonaea viscosa* leaves using solvents like n-hexane, chloroform, ethyl acetate, n-butanol and water. The percentage yield was calculated and it was found that ethyl acetate fraction showed high yield of 11.3 % w/w compared to other fractions.

- Preliminary phytochemical analysis of powdered leaf material and fractions revealed specific phytoconstituents such as phenols, flavonoids, tannins, saponins, terpenoids, steroids, glycosides and carbohydrates.

- The evaluation of fluorescence character of powdered leaf material and various fractions of *Dodonaea viscosa* revealed that specific fluorescence with alkali indicates the presence of flavonoids.
Quantification of phenols and flavonoids were carried out in chloroform, ethyl acetate and n-butanol fractions. The results indicated that ethyl acetate fraction contains more amount of phenols and flavonoids than chloroform and n-butanol fractions.

Quantification of quercetin and kaempferol were performed by HPLC method in ethyl acetate fraction and the amount was found to be 6.46 % w/w and 0.132 % w/w respectively.

**In vitro pharmacological studies**

The various fractions such as n-Hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions were subjected to in vitro antioxidant studies using DPPH radical and Nitric oxide radical scavenging assay. The results of DPPH radical scavenging assay revealed that ethyl acetate fraction at 1000 µg/0.1ml showed significant inhibition of 91.78 % which is comparable with that of standard curcumin (93.81 %).

The nitric oxide radical scavenging assay showed that among the various fractions, ethyl acetate fraction at 1000 µg/ml (83.11 %) showed maximum activity which is comparable with that of standard curcumin (89.91 %).

The antibacterial activity of various fractions was screened against *Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using agar well diffusion method. The ethyl acetate fraction of *Dodonaea viscosa* at 1000µg/ml was more effective in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the inhibition zones of 23 mm and 22 mm respectively compared with other fractions. Thus based on the potency, ethyl acetate fraction was selected for further studies.
• Fibroblast proliferation assay was carried out with ethyl acetate fraction of *Dodonaea viscosa* at concentrations upto 100 μg/ml on human dermal fibroblast cell line. The results revealed that increase in concentration of ethyl acetate fraction causes increase in proliferation and maximum at 100 μg / ml. Among the various tested groups, EFDV at the dose of 100 μg / ml treated group produced significant proliferation similar to the growth stimulation control 10% FBS.

• The effect of ethyl acetate fraction on the fibroblast cells were determined using scratch assay. It indicates that the migration of fibroblast cells into denuded area was markedly increased in the presence of 50 μg/ml and 100 μg/ml of ethyl acetate fraction of *Dodonaea viscosa* compared to the migration of cells in untreated control at 12 h and 24 h after the creation of scratch wound. The ethyl acetate fractions has better efficiency in inducing cell migration in fibroblast cell line and thereby mediate the process of wound healing.

• Fibroblasts which are involved in granulation and collagen maturation are stimulated by ethyl acetate fraction of *Dodonaea viscosa* resulting in proliferation and migration within the wound site. So it could be an effective source for treatment of wounds.

**Development and evaluation of topical formulation**

• Drug excipient compatibility study was carried out for the selected formulation by FT-IR & DSC studies for fraction, base and formulation. The results indicated that no interaction between the fraction and the excipients.

• Topical formulations (F1 - F6) were prepared with ethyl acetate fraction (2.5 % w/w and 5 % w/w) using different ointment bases and were evaluated
for pH, viscosity, spreadability and diffusion study. The results indicated that F5 and F6 were found to be satisfactory.

- Stability study was carried out for the selected formulations (F5 & F6) at 25°C / 65% RH and 40°C / 75% RH for six months and the ointment (F5 & F6) was found to be stable.

**In vivo pharmacological studies**

- Acute dermal toxicity study was performed for the formulation using *Sprague dawley* rats (OECD 402 guideline). The results showed that LD$_{50}$ of the DVFO was found to be > 2000 mg/kg b.w when applied once dermally and it was found to be safe. Hence, the formulation “DVFO” falls in the “category-5 or unclassified” in accordance to the Globally Harmonized System of classification of chemicals.

- The developed ointment (DVFO 2.5 % w/w & 5 % w/w) was subjected to *in vivo* wound healing activity using excision and incision model in *Sprague dawley* rats. The results revealed that the formulation at 2.5 % w/w & 5 % w/w enhanced the rate of wound contraction and tensile strength of skin similar to that of standard treated group.

- The levels of hydroxyproline and hexosamine in connective tissue were studied. The formulation (DVFO 2.5 % w/w & 5 % w/w) significantly increased the levels of hydroxyproline and hexosamine indicated faster collagen turnover and stabilization of collagen fibres leading to rapid healing of treated wounds compared with untreated group.

- The present study on antioxidants and free radical levels revealed that DVFO (2.5 % w/w & 5 % w/w) had significant antioxidant activity, reduced free
radicals stress thereby helped to prevent inflammation, oxidative damage and promoted the healing process.

- Histopathological findings also showed enhanced fibroblast proliferation, angiogenesis, fibrosis and complete epithelialization in standard and DVFO (2.5 % w/w & 5 % w/w) treated group which supported faster wound contraction and elevation of hydroxyproline content in DVFO treated groups.

- Formulation (DVFO 2.5 % w/w & 5 % w/w) also exhibited up-regulation of COL3A, bFGF and VEGF in western blot analysis indicating that the formulation stimulates angiogenesis and collagen production thereby accelerate the process of wound healing.

- The \textit{in vivo} study revealed that the developed formulation (DVFO) showed significant wound healing potential in a dose dependent manner compared with untreated group and the activity was comparable with standard treated group (Povidone iodine ointment).

- The healing effect of DVFO seemed to be due to decreased free radical generated tissue damage, promoting effects on antioxidant status, faster collagen deposition and other connective tissue constituent formation, enhanced growth factors and antibacterial activity.

- The wound healing potential of the formulation (DVFO) may be attributed to the presence of phenols, flavonoids and tannins present in the ethyl acetate fraction of \textit{Dodonaea viscosa}. 