6. Summary and Conclusions

Diabetes mellitus is an important risk factor for atherosclerosis, and coronary heart disease is the most frequent cause of mortality in these patients. One of the pathogenic mechanisms that can explain this increased risk in diabetes is the imbalance between pro-oxidants and antioxidants, which results in oxidative stress (OS). Hyperglycaemia results in glucose auto-oxidation, nonenzymatic glycation and monocyte dysfunction, which lead to increased production of free radicals. This is further aggravated by the decreased levels of antioxidants and leads to oxidative damage, illustrated by the high levels of lipid and DNA peroxidation products found in these patients. All these diabetes-related abnormalities can intensify the endothelial dysfunction, oxidation of LDL and foam cell formation, which ultimately lead to the formation of the atheroma plaque.

One way of combating this increased oxidative stress (OS) would be to increase antioxidant defences. Epidemiological evidence demonstrating the benefits of the 'diet rich in fruits, vegetables, whole-grain cereals and nuts' supports the dietary approach, but the elements responsible and their interactions have not been convincingly identified yet.

Rational of the study was to evaluate the herbal plants for their antioxidant and antidiabetic properties.

The objectives of the present study were as follows:

- Phytochemical evaluation of the *Cassia absus* and *Sida spinosa*
- To evaluate the *Cassia absus* and *Sida spinosa* solvent extract for antioxidant activity via invitro and invivo methods.
- To evaluate safety & antidiabetic property of the *Cassia absus* and *Sida spinosa*
- To isolate & characterize bioactive constituents (antioxidant) from *Cassia absus* and *Sida spinosa* extracts.
- To develop & validate HPTLC method for isolated compounds from selected plants.
The study involved

1. Collection and authentication of plant materials
2. Pharmacognostical evaluation of plants
3. Extraction of plant material
4. Phytochemical evaluation,
5. *in vitro* & *in vivo* antioxidant activity
6. Isolation of phytoconstituents from extracts showing good *in vitro* & *in vivo* antioxidant activity
7. Characterization of and structural elucidation
8. Antidiabetic activity
9. Safety studies
10. HPTLC method development,
11. Comparison of antioxidant activity with conventionally used plants.
12. Docking studies

Detailed pharmacognostical study, physicochemical parameters and preliminary phytochemical screening of *Cassia absus* and *Sida spinosa* were carried out. *Cassia absus* and *Sida spinosa* showed presence of anthraquinones, phenolics, flavonoids and tannins. Foreign organic matter, ash values, extractive values, moisture content, heavy metals, arsenic, residual solvent (ethyl acetate) of *Cassia absus* and *Sida spinosa* were found to be within Pharmacopoeial limits. Various *in vitro* and *in vivo* antioxidant assay were carried out for various solvent extracts of *Cassia absus* & *Sida spinosa*. Ethyl acetate extract of *Cassia absus* & *Sida spinosa* were found to have prominent antioxidant potential.

Three compounds were isolated from Ethyl acetate extract of *Cassia absus* which were Chrysophanol, Rhein, Emodin. Two compounds were isolated from Ethyl acetate extract of *Sida spinosa* which were Apigenin, Kaempferol. Characterization and structural elucidation of isolated marker compounds were carried out by different spectroscopic methods: UV, IR, NMR, Mass.
A densitometric HPTLC method for simultaneous qualitative and quantitative analysis of **Chrysophanol, Rhein and Emodin** in the ethyl acetate extract of *Cassia absus* is established. Hexane–ethyl acetate–formic acid 8.0:1.0:0.5 (v/v) mobile phase was used for separation. The method is simple, precise, specific, sensitive, and accurate. The method established in this work can be used as quality-control method for quantification of **Chrysophanol, Rhein and Emodin** in the ethyl acetate extract of *Cassia absus*. This method is validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug.

A densitometric HPTLC method for simultaneous qualitative and quantitative analysis of **Apigenin and kaempferol** in the ethyl acetate extract of *Sida spinosa* is established. Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2) (v/v) mobile phase was used for separation. The method is simple, precise, specific, sensitive, and accurate. The method established in this work can be used as quality-control method for quantification of **Apigenin and kaempferol** in the ethyl acetate extract of *Sida spinosa*. This method is validated for good accuracy, repeatability and precision, and can be used to evaluate the quality of the drug.

Ethyl acetate extracts of *Cassia absus* and *Sida spinosa* were found safe in acute and repeated dose toxicity studies. Ethyl acetate extracts were tested for their antidiabetic activity using streptozotocin induced diabetes model and showed antidiabetic activity in the selected model.

Docking studies were carried for isolated compounds from *Cassia absus* & *Sida spinosa* (**Chrysophanol, Rhein, Emodin, Apigenin and kaempferol**). Apigenin was identified as best and docked well into the active site of 1IR3. From the binding energies obtained its propose that Apigenin can be considered as insulin mimetic since they effectively interact with the active site region of insulin receptor by hydrogen bonding interactions. The present study also provides an insight into the mechanism of action of oral insulin mimetics.

**Comparision of invitro antioxidant activity of Cassia absus & Sida spinosa** with conventionally used plants like *Coccinia indica, Momordica charantia, Moringa oleifera* was carried out. *Cassia absus & Sida spinosa* were found to be more potent antioxidant over conventional plants.
In conclusion of this work

**Conclusion:**

- Invitro and Invivo antioxidant assay of Cassia absus & Sida spinosa were carried out
- Ethyl acetate extracts of Cassia absus and Sida spinosa showed good antioxidant activity.
- Phytochemical evaluation of Cassia absus & Sida spinosa ethyl acetate extract showed the presence of anthraquinones, flavanoids and phenolic compounds
- Acute and Subacute antidiabetic assay of ethyl acetate extracts of CA & SS were carried out, it showed promising result.
- Acute and Sub acute toxicity studies established safety of the extracts of Cassia absus and Sida spinosa.
- 3 compounds viz, Chrysophanol, Emodin, Rhein were isolated from the Ethyl acetate extracts of Cassia absus.
- 2 Compounds Kaempferol, Apigenin were isolated from Sida spinosa.
- HPTLC method was developed and validated for isolated compounds as per ICH guidelines.
- Docking studies were carried for isolated compounds. Apigenin was identified as best and docked well into the active site
- **Comparision of invitro antioxidant activity of Cassia absus & Sida spinosa** with conventionally used plants like Coccinia indica, Momordica charantia, and Moringa oleifera was carried out. **Cassia absus & Sida spinosa were found to be more potent antioxidant over conventional plants**