INTRODUCTION

Ever since the time of human civilization, medicinal plants have remained the primary source of healing and are widely used for the treatment of various ailments like cold, cough, fever, headache, poison bites, dog bites, skin diseases, tooth infections and many. Demand for medicinal plants is increasing in both developed and developing countries due to growing recognition of natural products, being non toxic, having no side effects and easy availability at affordable prices.

WHO has recommended the evaluation of effectiveness of plants in condition where we lack safe modern drugs. Searching for new natural drugs is still attractive although many synthetic drugs are available because they contain substances which take alternative and side effects on the diseases. There are many medicinal plants known through folklore, herbalists, traditional healers, but their introduction into the modern therapy awaits the discovery of animal test system that is closely parallel to the pathological course of diseases in human. Moreover, some medicinal plants are extinct and are at high risk of extinction due to deforestation or ignorance about its importance or indiscriminate cattle grazing or over exploitation due to huge public demand for effective medicinal use. Therefore search for more effective and safer drug has become an area of active research as medicinal plants continue to lure the world for herbal cure.

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly use insulin which causes disturbances in carbohydrate, protein and lipid metabolism and also in antioxidant system. While proteins undergo glycosylation, free radicals are being released from inflammed cells and subsequent lipid
peroxidation forms the basis of pathological processes that might initiate the development of atherosclerosis and neuromuscular problems.

As has been reported by Bailey and Day, the earliest recorded attempt to treat diabetes dates back to more than 3,500 years and the treatment used was of plant origin. Nowadays, insulin and other oral blood-glucose lowering agents are used in the clinical management of diabetes. Unfortunately the prevalence of this disease continues to rise worldwide and little, so far, can be offered to prevent or delay its secondary complications. Many traditional plant treatments for diabetes are used throughout the world even when their biologically active compounds are unknown because of their effectiveness and minimal side effects.

Several medicinal plants with potent antidiabetic activity are used to treat diabetes and *Cassia auriculata* L. is one of the well known and widely used plants in south Asian countries like India and Sri Lanka for curing diabetes. Therefore, we selected *Cassia auriculata* L. in an effort to study the active compounds responsible for hypoglycemic as well antioxidant activity.

*Cassia auriculata* L. is a sun loving perennial shrub whose habitat revolves around tropical region. In India it is found in the western, southern and central region, seen in Karnataka, Tamilnadu, Kerala, Andra Pradesh, Maharashtra, Gujarat, Rajasthan and Madhya Pradesh. As considered to the other parts of the globe it is a common plant in Western Peninsula, Burma, Ceylon, southern parts of Pakistan, certain parts of Africa. Different parts of the plant has been reported to possess ethnobotanical use for treating skin disorder, burns, body odour, fever, urinary system infections, conjunctivitis, gonorrhea, gout, rheumatism and constipation. Different solvent extracts of flowers have shown anti-inflammatory and antimicrobial properties. The survey of the literature reveals that *C. auriculata* L. flowers has anti
diabetic activity and the various extracts of the whole plant powder (Avaarai panchaga choornam) have significant pharmacological activity towards lowering of blood glucose, managing hyperlipidemic and other cardiovascular risk associated with type II diabetes.

**Common name:** Tanner's Cassia, Buttonhole, God's-Hair, Hind's Tongue, Horse Tongue, **Vernacular Name:** Sanskrit (Avartaki), Tarwar (Hindi), Tavare, Avare, Tangedi (Kannada), Tarwad (Marathi), Tangedu (Telugu), Avaram (Tamil and Kerala), Awala (Gujarati). **Botanical name:** *Senna/Cassia auriculata*  
**Family:** *Caesalpiniaceae*

**OBJECTIVES OF THE PRESENT STUDY**

The main objectives of the study were:

- To extract the plant samples using soxhlet apparatus and subjecting the same for preliminary phytochemical analysis.
- To screen the extracts for potential antidiabetic and antioxidant activities *in vitro*.
- Performing *in vivo* studies for the plant part extract showing maximum antidiabetic and antioxidant activity to confirm the *in vitro* efficacy.
- Bioactivity guided isolation of active components from fractions using column and thin layer chromatography (TLC).
- Identification of isolated active compounds by analytical techniques HPLC, HPTLC, FT-IR, LC-MS and NMR studies.

**METHODOLOGY**

- Collection of the plant and preparing herbarium for identification and authentication.
- Twigs, leaves, flower buds and flowers were separated, washed and shade dried.

- Selection of the suitable solvent for extraction by subjecting the plant parts for soxhlet extraction using different solvents and testing the extracts for the efficiency and efficacy by preliminary testing.

- Extraction of the samples using suitable solvent and concentration by rotary vacuum evaporator.

- Preliminary phytochemical analysis of the extracted samples using methodology of Harborne for the presence of alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones, steroids and terpenoids.

- The antioxidant activity of methanolic extracts of twigs, leaves, flowers and flower buds were carried out using in vitro scavenging assays of 1,1-diphenyl-2-picryl hydrazyl (DPPH), superoxide, nitric oxide, hydroxyl, H$_2$O$_2$ and their action on lipid peroxidation, reducing power, metal chelation with reference to standards.

- Screening of the extracts from different parts of *C. auriculata* for in vitro antidiabetic activity by studying the inhibitory effect on carbohydrate metabolizing enzymes, such as α-amylase and α-glucosidase.

- Further evaluation of the antidiabetic activity in vivo based on the effect of the extract on blood glucose, glycosylated hemoglobin and plasma insulin levels in streptozotocin induced diabetic animal system.

- The interaction of the plant extract with the antioxidant system (both enzymatic and non-enzymatic) studied by observing the effect on Glutathione peroxidase, Catalase, Superoxide dismutase, Glutathione-s-transferase and on the levels of reduced Glutathione and Lipid peroxidation.
SYNOPSIS

- Identification of the plant part with maximum antioxidant and antidiabetic activity and subjecting the same for isolation procedures.
- Isolation of active ingredients using preparative thin layer chromatography and column chromatography with parallel testing of fractions for in vitro antioxidant and antidiabetic activity and TLC studies.
- Identification of isolated compounds using FT-IR, LC-DAD/ESI-MS and NMR studies.

SIGNIFICANCE OF THE PRESENT STUDY

- The result of the present study initiates the search for an effective natural remedy having mechanism of action on both diabetes and oxidative stress.
- The outcome of the research contributes to the increase in competitiveness of the industry in the field of health food and functional foods by providing the opportunity to develop new health supplements of high quality.
- Disseminates the results at local, national and international level, as to initiate the people to conserve and enhance the cultivation of *C. auriculata* L. to receive adequate benefits.

RESULTS

Preliminary phytochemical analysis performed using methanolic extracts of twigs, leaves, flowers and flower buds showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, glycosides, anthraquinones and deoxy sugars in cardiac glycosides. Total phenolics and flavonoids content measured spectrophotometrically was expressed in terms of gallic acid (GAE g\(^{-1}\) of the extract) and quercetin equivalents (QE g\(^{-1}\) of the extract) respectively. Total phenolic content was found to be higher in flower buds. Leaf and twig extracts showed similar phenolic content...
while least was found in flower extract. Total flavonoids estimated in the extracts was found to be in the following order twigs > flower buds > leaves > flowers. Twigs were found to possess high tannin content compared to other plant parts, expressed in terms of tannic acid equivalents (TAE g\(^{-1}\) of the extract). Apart from this, the quantitative extraction of alkaloids and saponins from the different parts also revealed differential abundance with respect to parts of *C. auriculata* L. plant.

The inhibitory effects of the plant extracts on *in vitro* and *in vivo* assays were significant and showed direct correlation to the concentrations used. The effective *in vitro* scavenging of 1,1-diphenyl-2-picryl hydrazyl (DPPH), superoxide, nitric oxide, hydroxyl, \(\text{H}_2\text{O}_2\), lipid peroxides with reference to standards, exhibited their efficiency to combat different kinds of free radicals and was substantiated by the metal chelating and reducing properties as evident from the results. Flower and leaf extracts consistently showed effective inhibitory activity on most of the radicals.

The most significant findings of the present study are that the flower buds showed greatest inhibitory activity on hydroxyl radicals, a work reported for the first time, twigs expressed maximum inhibition on nitric oxide radicals and both extracts presented higher effect on hydrogen peroxide as compared to leaves. IC\(_{50}\) values were used to express the efficacy of the plant extracts, which represents the concentrations of the samples used to scavenge half the initial concentrations of free radicals. Plant extracts exhibited maximum antilipid peroxidative, superoxide and nitric oxide scavenging potential in many folds than compared to reference standards.

Occurrence of Quercetin, rutin, cinnamic acid and ferulic acid in different extracts as evident from HPTLC analysis, supplements the bioactivity of the plant extracts. Quercetin and rutin are not only known antioxidants which brings about the
formation of considerably less reactive species from free radicals by its reactivity but also hypoglycemic in function by regulating carbohydrate hydrolyzing enzymes. Two phenolics identified, ferulic acid and cinnamic acid are potentially antioxidant by nature and their antihyperglycemic nature is attributed to their effect on peroxisome-proliferator activated (PPAR)γ receptors.

Based on the observation of the in vitro antioxidant efficiency of the extracts, further investigation for the antidiabetic activity by evaluating inhibitory effect on carbohydrate metabolizing enzyme α-amylase and α-glucosidase was studied with reference to the standard drug acarbose. The maximum inhibition of enzyme activity by the flower extract compared to extracts from different parts of *C. auriculata* plant and acarbose clearly indicated its efficacy as hypoglycemic and antioxidant agent. Therefore, further in vivo studies and isolation procedure were restricted only to the flower extract.

The diabetic condition induced in the experimental animals by streptozotocin, was treated with the flower extract for a month and reference standard glibenclamide. The elevated blood sugar and decreased insulin level in diabetic rats were normalized by the flower extract and marked increase in insulin level supports the hypoglycemic effect. Further studies on the antioxidant enzyme system also expressed the increased activity of superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GPx) in flower extract administered diabetic rats, indicating the antiradical properties of flower components on diabetic induced lipid peroxidation. The restored activities of antioxidant enzymes shown in extract treated animals were correlated to increased glutathione content and decreased lipid peroxidation (LPO) levels.
Presence of abundant polyphenolics not only explains the relaxing effect on oxidative stress but also antihyperglycemic potential as they are known antioxidants and also reported to inhibit carbohydrate hydrolyzing enzymes. HPLC analysis of the flower extract showed the presence of mixed polyphenols and catechins. Polyphenols and flavonoids are well recognised superoxide, hydroxyl and hydrogen peroxide scavengers. Occurrence of polyphenolics in the methanolic extract, typical of tea extract like (+)catechin, (-)epigallo catechin,(-)epicatechin gallate, (-)epicatechin have been reported to have antihyperglycemic activity and potent S-GLUT-1 mediated glucose inhibitory action. Presence of these along with other phytoconstituents suggests synergistic/antagonistic antihyperglycemic and antiradical action of the flowers.

Further evaluation of fractionated flower extract i.e., petroleum ether, chloroform, ethyl acetate, acetone and methanolic extracts for hypoglycemic and antioxidant effect showed the methanolic extract to have maximum activity compared to ethyl acetate, acetone and chloroform. DPPH radical scavenging and reducing power of the extract shown at micro gram concentration was well supported by the results of $\alpha$-amylase and $\alpha$-glucosidase inhibition. The results clearly indicated the expression of activity becoming more effective and enhanced with purification. Twenty fractions collected at various percentages, from the methanolic extract using column showed remarkable inhibitory effect both on radical scavenging and enzyme activity. Repeated column and TLC finally yielded three compounds two flavonoids, quercetin and rutin and a hydroxyanthraquinone emodin. FT-IR, LC-DAD/ESI-MS and NMR results were used to identify the three compounds isolated from the most active fraction from flower part of *C.auriculata* L.
Flavonoids and anthraquinones are the most widely used dietary supplements that possess perceived benefits on human health. Epidemiological studies with flavonoid supplement such as quercetin and its derivatives have produced remarkable effect on type 2 Diabetes mellitus by lowering blood glucose level as a consequence of inhibitory activity on enzymes of pharmacological importance. Prevention of diabetes related oxidative stress by flavonoids and anthraquinones is due to presence of multiple hydroxyl groups that presents antioxidant property towards range of oxidizable compounds.

CONCLUSION

The result of the present study provides scientific evidence for antioxidative and antidiabetic activities of flowers in various *in vitro* and *in vivo* models and hence supports the therapeutic usage of flowers in traditional medicines for treating diabetes, inflammation, burns, urinary disorder and skin diseases. Further, the presence of diversified biomolecules such as phenolics, flavonoids, tannins, anthraquinones suggest the use of these phytomedicines for developing drug formulation for treating and combating both radical mediated oxidative damage and complications associated with diabetes.