DISCUSSION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. They may become the base for the development of a medicine, a natural blue print for the development of new drugs and also a phytomedicine to be used for the treatment of diseases (Iwu, 1993). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world (WHO, 2002).

Medicinal plants maintain the health and vitality of individuals and cure various diseases, including cancer without causing toxicity. In this view, the pharamacochemical characterization, phytochemistry and the pharmacological potentials of the whole plant of *Rauwolfia densiflora* (Wall.) Benth. ex Hk.f and tubers of *Stephania wightii* (Arn.) Dunn. have been discussed.

**PHARMACOCHEMICAL CHARACTERIZATION**

**Physicochemical constituents**

**Ash values**

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (African Pharmacopoeia, 1986). Equally important in the evaluation of crude drugs, is the ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Musa *et al.*, 2006).
The ash values of whole plant of *R. densiflora* and tuber of *S. wightii* were 9.54% and 8.14% respectively (Table 1). These ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. In the present study, *R. densiflora* whole plant had more ash value when compared with other investigated drug sample, *S. wightii* tuber. Samples had more water soluble ash than acid insoluble ash. The ash values are generally the index of the purity as well as identity of the drug. The extractive value in water is more than that in ethanol in both the samples analysed. The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug (Anonymous, 2002).

**Fluorescence analysis**

Many phytocompounds fluoresce when suitably illuminated. The fluorescent colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyte over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al.*, 2006).

The powder from the whole plant of *R. densiflora* fluoresced green under day light, dark green under short and long UV light. The powdered tuber of *S. wightii* emitted pale brown fluorescence under day light, brown under short UV light and dark green in long UV light (Table 2).
Phytochemical studies

Natural phytochemicals are known to contain substance that can be used for therapeutic purposes or as precursor for the synthesis of novel useful drugs. Total of 50% modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry. Use of plant as a source of medicine has been inherited and is an important component of the health care system (Ahmedulla and Nayar, 1999).

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigations. Various tests had been conducted qualitatively to find out the presence or absence of bioactive compounds. Phytochemical evaluation is one of the tools for the quality assessment which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades, HPTLC has emerged as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. A HPTLC method is fast, precise, sensitive and reproducible with good recoveries for standardization of herbal drugs.

In the present study, the preliminary phytochemical evaluation on whole plant of *R. densiflora* and tuber of *S. wightii* had revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, saponins, tannins, terpenoids, steroids, sugars and glycosides in the methanol and ethanol extracts of the above said plants. HPTLC investigations also confirmed the presence of alkaloids, coumarins, glycosides, phenols and steroids which could make the plant useful for
treating different ailments as having a potential of providing useful drugs of human use. This is because, the pharmacological activity of any plant is usually traced to a particular compound. Terpenes have immunostimulant, hypocholesterolaemic and anticarcinogenic properties (Francis et al., 2002). Therapeutically, terpenoids exert wide spectrum of activities such as antiseptic, stimulant, diuretic, anthelmintic, analgesic and counter-irritant (Gokhale et al., 2003).

Tannins have been found to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Motar et al., 1985; Ruch et al., 1989; Li et al., 2003; Akinpelu et al., 2009). Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering (Handa and Kapoor, 1992). They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and antidote (Ali, 1994).

Saponins, a group of natural products occur in the whole plant of R. densiflora and tuber of S. wightii. In plants, the presence of steroidal saponins like cardiac glycosides appear to be confined to many families and these saponins have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids, vitamin D, etc. (Evans and Saunders, 2001).
From plants, sapogenin, a synthetic steroid is prepared and used to treat a wide variety of diseases such as rheumatoid arthritis, collagen disorders, allergic and asthmatic conditions (Claus, 1956). Saponins, known to produce inhibitory effect on inflammation (Just et al., 1998). Saponin reduces the uptake of certain nutrients including glucose and cholesterol at the gut through intra-luminal physicochemical interactions. Hence, it has been reported to have hypocholesterolemic effect and thus may aid lessening metabolic burden that would have been placed in the liver (Price et al., 1987). Steroidal compounds are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001).

Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2 (Li et al., 2003). Flavonoids serve as health promoting compounds as a result of their anion radicals (Haustein, 1983). Several authors reported that flavonoids, sterols/terpenoids and phenolic acids are known to be bioactive antidiabetic principles (Oliver-Bever, 1986; Rhemann and Zaman, 1989). Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats (Chakravarthy et al., 1980). Flavonoids act as insulin secretagogues (Geetha et al., 1994). Most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. which are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002).

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for development
of drugs (Harbone, 1973; Okwu, 2005). They produce analgesic, antispasmodic and bactericidal effects (Stray, 1998).

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations were compared. The comparative analysis clearly showed a correlation between a compound present in them and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are coumarins, flavonones, tannins, alkaloids and saponins. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavonones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% $\text{H}_2\text{SO}_4$ show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light (Harbone, 1976). Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Evans, 1996). Hydroxy methyl coumarin fluoresced in the 420 - 440 nm when observed in different solvents with increasing polarity (Chaltopudhyay et al., 2006). The fluorescence analysis of the crude drugs of whole plant of *R. densiflora* and tuber of *S. wightii* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

Determination of extractive values, ash residues and active components (saponins, alkaloids and essential oil content) plays a significant role for standardization of the indigenous crude drugs. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion
and to eliminate the inert material by treatment with a selective solvent known as *menstruum*. The extract formed can directly be used as a tincture or fluid extract, or be further processed for incorporation into tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine which are modern drugs (Thomson, 2007). Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug (Handa *et al.*, 2008).

**GC - MS analysis**

The major phytocomponents and their beneficial activities obtained through GC-MS study of whole plant of *R. densiflora* and *S. wightii* tubers were listed in Tables I and Table II. Seven compounds were detected in ethanol extract of *R. densiflora* whole plant. The results revealed that, 1,10-Decanediol (21.62%) was found to be the major compound followed by propanoic acid anhydride (10.81%), 3-Pentanol,2,4-dimethyl- (5.81%) and Phytol (5.41%).

**Table I: Activity of phytocomponents identified in the ethanol extract of whole plant of *Rauwolfia densiflora* by GC-MS**

<table>
<thead>
<tr>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Compound nature</th>
<th><strong>Activity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>7.37</td>
<td>Propanoic acid, anhydride</td>
<td>C₆H₁₀O₃</td>
<td>Acidic compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>11.60</td>
<td>1,10-Decanediol</td>
<td>C₁₀H₂₂O₂</td>
<td>Alcoholic compound</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>14.92</td>
<td>Phytol</td>
<td>C₂₀H₄₀O</td>
<td>Diterpene</td>
<td>Antimicrobial,Anticancer,Antioxidant,Diuretic</td>
</tr>
<tr>
<td>28.50</td>
<td>3-Pentanol, 2,4-dimethyl-</td>
<td>C₇H₁₆O</td>
<td>Alcoholic compound</td>
<td>Antimicrobial</td>
</tr>
</tbody>
</table>

**Activity Source: Dr.Duke’s Phytochemical and Ethnobotanical databases**
Table II: Activity of phytocomponents identified in the ethanol extract of tuber of *Stephania wightii* by GC-MS

<table>
<thead>
<tr>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular Formula</th>
<th>Compound nature</th>
<th><strong>Activity Source: Dr.Duke’s Phytochemical and Ethnobotanical databases</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>7.44</td>
<td>1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-</td>
<td>C4H9NO5</td>
<td>Nitrogen compound</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>13.42</td>
<td>Cyclohexaneacetic acid, α-ethyl-</td>
<td>C10H18O2</td>
<td>Acidic compound</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>15.66</td>
<td>9-Octadecenal</td>
<td>C18H34O</td>
<td>Aldehyde compound</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>25.38</td>
<td>(1H)Indolo[2,1-a]isoquinoline, 5,6,11,12-tetrahydro-2,3,8,9-tetramethoxy</td>
<td>C20H23NO4</td>
<td>Alkaloid</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>27.82</td>
<td>6H-Dibenzo[a,g]quinolizine, 5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy, (ní)</td>
<td>C21H25NO4</td>
<td>Alkaloid</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>28.97</td>
<td>Vitamin E</td>
<td>C29H50O2</td>
<td>Vitamin compound</td>
<td>Vasodilator Cancer preventive Hypoglycaemic Antitumour Antioxidant Antiinflammatory Antiaging</td>
</tr>
<tr>
<td>30.01</td>
<td>Isoquinoline, 6,7-dimethoxy-1-methyl-4-(3,4-dimethylphenyl)-</td>
<td>C20H21NO4</td>
<td>Alkaloid</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>31.25</td>
<td>4-Dodecanol</td>
<td>C12H26O</td>
<td>Alcoholic compound</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>32.52</td>
<td>3-Octanol, 3,6-dimethyl-</td>
<td>C10H22O</td>
<td>Alcoholic compound</td>
<td>Antiinflammatory</td>
</tr>
</tbody>
</table>

Phytol is detected in whole plant of *R. densiflora* which was also found to be effective at different stages of the arthritis. It was found to give preventive and therapeutic results against arthritis. The results showed that reactive oxygen species-
promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi et al., 2009).

Thirteen compounds were reported in the ethanol extract of *S. wightii* tuber. The major compounds include (1H)Indolo(2,1-a)isoquinoline,5,6,11,12-tetrahydro-2,3,8,9-tetramethoxy (59.98%), 6H-Dibenzo(a,g)quinolizine,5,8,13,13a-tetrahydro-2,3,9,10-tetramethoxy-,(ñ)- (34.86%) and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (2.89%).

Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in medicinal plants and this type of analysis will be helpful for further detailed study and further investigations in the pharmacological importance of *R. densiflora* and *S. wightii* and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

**PHARMACOLOGICAL STUDIES**

**Antioxidant activity**

**DPPH radical scavenging activity**

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002; Amarowicz et al., 2004; Wei et al., 2010). DPPH accepts an electron or hydrogen radical to become stable diamagnetic molecule (Sidduraju and Dharmesh, 2007). The use of DPPH scavenging assay among the assays involves assessing the cell membrane integrity cell membrane stabilizing capacities of plant constituents. Further, it gives explanations about the possible ways by which phytomedicine could help to reduce diseases caused by infections, inflammation and oxygen radical
generation that affects the cell membrane (Shahidi and Wanasundara, 1992). Plants with antioxidant activities have been reported to possess free radical scavenging activity (Das and Pereira, 1990). The hexane and ethyl acetate extracts of *Stephania dinklagei*, *S. rotunda* and *S. hernandifolia* showed the most pronounced DPPH scavenging activity (Gulchin et al., 2010; Sharma et al., 2010a; Udegbunam et al., 2012).

DPPH radical scavenging system was used to evaluate the antioxidant property of ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber. The experimental data of the plant extracts revealed that the extracts are likely to have the effects on scavenging free radicals (Table 12). From the results, DPPH was observed to have a dose dependent relationship in the DPPH radical scavenging activity. The involvement of free radicals, especially their increased production, appears to be a feature of most of the human diseases including cardiovascular disease and cancer. Flavonoid and phenol compounds of *R. densiflora* whole plant and *S. wightii* tuber are possibly involved in their antiradical activity.

**Anticancer activity**

Cancer remains a life-threatening disease and a leading cause of death as its control has been difficult. Although, a range of conventional therapies based on chemotherapy, surgery, and radiotherapy are available and these approaches are in many cases of limited efficacy (Hsiao and Liu, 2010). Immunological hallmarks in cancer cells include the ability to induce chronic inflammatory response, evasion of tumour recognition, and ability to induce tolerance (Cavallo et al., 2011).

The antitumour activity of ethanol extracts of whole plant of *R. densiflora* and *S. wightii* tuber were evaluated in DAL tumour bearing mice. The ethanol extracts of
above said plants treated animals at the dose of 100 mg/kg significantly reduced the tumour volume and tumour (viable) cell count and brought back the haematological parameter to more or less normal levels.

In DAL tumour bearing mice, a regular rapid increase in ascetic tumour volume was observed. Ascetic fluid is the direct nutritional source for tumour cells and a rapid increase in ascetic fluid with tumour growth would be a means to meet the nutritional requirement of tumour cells (Prasad and Giri, 1994). Treatment with ethanol extracts of \textit{R. densiflora} whole plant and \textit{S. wightii} tuber reduced the tumour volume, viable tumour cell count and increased the life span of the tumour bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals (Clarkson and Burchenal, 1965). It may be concluded that ethanol extracts of \textit{R. densiflora} and \textit{S. wightii} decreased the nutritional fluid volume and arresting the tumour growth and thereby increasing the life span of DAL bearing mice. Thus, ethanol extracts of \textit{R. densiflora} whole plant and \textit{S. wightii} tuber have antitumour activity against DAL bearing mice.

In cancer chemotherapy, the major problems that are being encountered are of myelosuppression and anaemia (Price and Greenfield, 1958; Hogland, 1982). One major obstacle for a successful anticancer therapy is the development of resistance over time. Many aggressive tumours become refractory to anticancer therapy with hardly any chemotherapeutic alternatives (Crespo-Ortiz and Wei, 2011). The anaemia encountered in tumour bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions (Fenninger and Mider, 1954). Treatment with ethanol extracts of \textit{R. densiflora} and \textit{S. wightii} brought back the haemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicated
that ethanol extracts of whole plant of *R. densiflora* and *S. wightii* tuber possess protective action on the haemopoietic system.

The results of the present study demonstrated that the ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber increased the life span of DAL tumour bearing mice, reduced tumour volume and improved the haematological parameters. The association between flavonoids and reduced cancer risk have been reported in the previous studies that showed a decrease in cancer risk with consumption of vegetables and fruits rich in flavonoids (Ferguson *et al.*, 2004; Park *et al.*, 2008). The results of present study are in accordance with those findings since the phytochemical screening showed the presence of flavonoids in ethanol extracts of *R. densiflora* and *S. wightii*. The presence of alkaloids with flavonoids in *R. densiflora* and *S. wightii* extracts may explain their superior activity compared with other plants studied (Wamidh and Mahasneh, 2010; Gali *et al.*, 2011). Studies on flavonoids have produced the most compelling data for the antitumour activities of plant secondary metabolites in various types of cancers (Yang *et al.*, 2001) and several flavonoids had been shown to inhibit cancer development while exhibiting antioxidant activities in various animal models (Ingram *et al.*, 1997; Lahiri-Chatterjee *et al.*, 1999). The anticancer activity of flavonoids and alkaloids isolated from different plants were reported earlier (Vijayan *et al.*, 2004; Park *et al.*, 2008). Plant-derived compounds have played an important role in the development of several clinically useful anticancer agents (Cragg and Newmann, 2006). Hence, the phytochemical screening of *R. densiflora* whole plant and *S. wightii* tuber showed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, glycosides and phenols which could make these plants useful as anticancer drug. Further, steps can be taken to isolate the compounds responsible for anticancer activity which may result in a modern drug.
Antidiabetic activity

Diabetes mellitus is one of the most common chronic diseases associated with carbohydrate metabolism. It is also an indication of co-morbidities such as obesity, hypertension and hyperlipidaemia which are metabolic complications of both clinical and experimental diabetes. Alloxan, a beta cytotoxin induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by the tissues (Bierman et al., 1975; Omamoto et al., 1981; Baynes, 1991; Saravanan and Pari, 2005; Gurusamy et al., 2008). The prevention of diabetes is an urgent worldwide health concern. The period preceeding the onset of type 2 diabetes is typically characterized by obesity and insulin resistance induced by over reacting and physical inactivity.

The ethanol extracts of *R. densiflora* and *S. wightii* (Group III and IV) were treated on alloxan induced diabetic rats (Group II). The results were compared with control (Group I) and the positive control glibenclamide (Group V) after fourteen days of treatment based on biochemical parameters. After the alloxan induction, glucose, insulin, lipid profiles, protein and antioxidant were restored to control levels with the administration of the known drug glibenclamide and extracts of *R. densiflora* whole plant and *S. wightii* tuber. The present study showed significant changes in biochemical parameter during the experimentally induced diabetes. Blood glucose, serum insulin, urea, creatinine levels were determined in control, ethanol extracts and glibenclamide treated rats. The standard drug Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans (Rajkumar et al., 2011).
The administration of ethanol extracts of *R. densiflora* and *S. wightii* decreased the blood glucose level whereas serum insulin level was increased in glibenclamide treated rats compared to control rats. The hypoglycemic ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber were found to be inducing insulin release from pancreatic cells of diabetic rats (Sharma and Garg, 2009). It is evident from this study that there was an increase in insulin level in diabetic rats treated with plant extracts. Many plants have been studied for their hypoglycemic and insulin release stimulatory effects (Shanmugasundaram *et al.*, 2011; Maruthupandian *et al.*, 2011; Maruthupandian and Mohan, 2011; Kala *et al.*, 2012a, b; Shajeela *et al.*, 2012). The possible mechanism by which the plant extract decreases the blood glucose level may be by potentiation of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of langerhans or by increase in peripheral glucose uptake (Rajkumar *et al.*, 2011).

Extensive research had been conducted in the last few decades on plants mentioned in ancient literature and used traditionally for antidiabetic activity. Grover *et al.* (2002) have reported 45 medicinal plants and their products have been used in the Indian traditional system of medicine and shown experimental or clinical antidiabetic activity. The most effective and commonly studied antidiabetic plants are *Allium cepa, A. sativum, Aloe vera, Gymnema sylvestre, Syzygium cumini, Ficus benghalensis, Rubia cordifolia* and *Tinospora cordifolia* (Ziyyat *et al.*, 1997; Grover *et al.*, 2002; Mohana Rao *et al.*, 2005).

A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II) when compared to control rats (Table 18). The ethanol extracts of *R. densiflora* and *S. wightii* were administered orally to rats for fourteen days and this reversed the urea and creatinine level to near
normal. The administration of glibenclamide also decreased the levels of urea and creatinine to some extent.

Alloxan is taken as an indication of an abnormal glomerular function where a single injection of cisplatin at a dose of 5 mg/kg body weight in rabbits caused a marked reduction in the glomerular filtration rates which was accompanied by an increase in the serum creatinine level, indicating the induction of acute renal failure. It was confirmed that there was a significant increase in serum creatinine in albino rats 14 days after alloxan administration. The present result showed that the treatment with ethanol extracts of *R. densiflora* and *S. wightii* were effective in preventing alloxan induced increase in serum creatinine level when compared with the control.

A significant reduction in serum protein, albumin and globulin was observed in alloxan induced diabetic rats (Group II) when compared to control (Group I) and glibenclamide treated rats (Group V) (Table 19). On administration of ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber to the diabetic rats, protein, albumin and globulin levels were found to be restored to normal. The increased level of serum protein, albumin and globulin in alloxan induced diabetic rats were presumed to be due to increased protein catabolism and gluconeogenesis during diabetes (Palanivel *et al.*, 2001).

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C, PL and LDL /HDL in control and experimental animals were investigated (Table 20). The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Pre-treatment with ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber and glibenclamide resulted in a decrease of transaminase activities in alloxan treated rats.
The serum AST and ALT levels increased as a result of metabolic changes in the liver such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes (Chalasani et al., 2004). Similarly in the present study, it was observed that the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely et al., 1999). AST and ALT were used as markers to assess the extent of liver damage in streptozocin induced diabetic rats (Hwang et al., 2005).

In this study, the ethanol extracts of *R. densiflora* and *S. wightii* regulated the activity of SGPT, SGOT and ALP in liver of rats intoxicated with alloxan. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi and Kuttan, 2009). The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, VLDL-C, PL and LDL/HDL in control and experimental animals were investigated. Alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL-C, when compared with normal rats. The glibenclamide and ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber treated rats showed a significant decrease in the content of lipid profiles, when compared with diabetic induced rats. Similarly HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. On administration of ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. The level of serum lipid profiles was high in diabetic rats in the present study and such elevation represents a risk factor for coronary heart diseases (Mironova et al., 2000). Lowering the serum lipid level through dietary or drug
therapy seems to be associated with a decrease in the risk of vascular disease (Scott and Grundy, 1999).

During diabetes, there is an enhanced activity of the enzymes, increased lipolysis and release of more fatty acids into the circulation (Agarth et al., 1999). The increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl Co-A and cholesterol during diabetes. In normal condition, insulin increases receptor-mediator removal of LDL-cholesterol and decreased activity of insulin during diabetes, causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceremia have been reported to occur in diabetic rats (Mironova et al., 2000). The increased concentration of free fatty acid may be due to lipid break-down and this may cause increased generation of NADPH-dependent microsomal lipid peroxidation. Phospholipids were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth, 1996). Increased phospholipid levels in tissues were reported by Venkateswaran et al. (2002) and Pari and Satheesh (2004) in streptozocin diabetic rats. Administration of ethanol extracts of R. densiflora whole plant and S. wightii tuber and glibenclamide decreased the levels of phospholipids.

The results (Table 21, 22 and 23) showed increased lipid peroxidation (LPO) in serum, liver and kidney of alloxan induced diabetic rats. Earlier studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari, 2003a; Ananthan et al., 2004). This may be because the tissues contain relatively high concentration of early peroxidizable fatty acids. In the present study, an increase in the levels of LPO was found and these levels were
significantly reduced after the supplementation of the ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber and glibenclamide. These indicated that, plant extracts inhibit oxidative damage due to the antiperoxidative effect of ingredients present in ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber. This could be correlated with previous study which reported that the extracts of *Cassia auriculata* flower (Pari and Latha, 2002), *Syzygium cuminii* (Prince and Menon, 1998; Prince *et al.*, 2004), *Tinospora cordifolia* (Prince *et al.*, 1999) and *Scoparia dulcis* (Latha and Pari, 2003b) have antiperoxidative and antihyperlipidaemic effects on diabetic animals. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the lipid metabolism. Insulin is a potent inhibitor of lipolysis, since it inhibits the activity of hormone sensitive lipase in adipose tissue and suppresses the release of free fatty acids (Loci *et al.*, 1994).

The level of serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and reduced glutathione (GSH) in control and experimental rats were studied on serum, liver and kidney. A highly significant reduction in the activity of scavenging mitochondrial enzymes is observed in alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber treated groups (Group III and IV) as well as glibenclamide treated rats (Group V).

Mitochondria are the energy reservoir of the cell and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death (Sohal and Dubey, 1994). Subcellular membrane, associated with thiol bearing enzymes, represents sensitive sites for detoxification causing perpetuation of cellular function (Kyu and Byung, 1997). Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. The
ethanol extracts of whole plant of *R. densiflora* and *S. wightii* tuber have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

Free radical reacts with lipids causing peroxidation, resulting in the release of products such as malondialdehyde, hydroperoxide and hydroxyl radicals. These extracts have the capacity to scavenge free radicals directly or interfering with generation of free radicals (Reddy and Lokesh, 1992; Dhuley *et al.*, 1993). Thus, the inhibitory effects of these extracts on oxidative damage may be attributed to the suppression induced peroxidation (Selvendiran *et al.*, 2004). It is well known that CAT, SOD and GPx play an important role as protective enzymes against free radical formation in tissues (Oberly and Buettner, 1974). Several investigators reported that, the reduced activities of CAT and SOD were due to the induction of CAT and SOD genes by free radicals and also by certain humoral factors (Anderson *et al.*, 1994; Slaga, 1995). The present study indicated the reduction in the activity of SOD, CAT, GPx and GSH in alloxan induced rats (Group II). These results revealed that protective role of plant extracts in decreasing lipid peroxidation and by normalizing antioxidant system.

It is concluded that, medicinal plants have been reported to possess antihyperglycemic activity (Rajkumar *et al.*, 2011). These preliminary investigation on the antidiabetic efficacy of ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber will be significant to proceed further in this path for the isolation of active principles responsible for antidiabetic activity. Of the studied plants, whole plant of *R. densiflora* showed more hypoglycemic activity.
Hepatoprotective activity

Liver diseases remain as one of the most serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders. A number of plants showed hepatoprotective activity (Malhotra et al., 2001). Based on the promising results shown by the plant extracts in the in vitro studies, in vivo hepatoprotective studies were carried out in experimental rats using CCl₄ induced hepatotoxicity.

Any increase in the level of serum AST, ALT, ALP, ACP and LDH activity is an indication of hepatic disease. Defect in protein metabolism, evidenced by changes in total protein and/or albumin level, are used to indicate the severity of the hepatic disease (Henry, 1984; Ibrahim et al., 2008). In the present investigation, when the rats treated with hepatotoxicant CCl₄, transaminases (AST and ALP), were increased remarkably in plasma by the release of these enzymes from hepatic parenchyma cells, which indicated a considerable hepatocellular injury (Bishayee et al., 1995). Oral treatment with drug silymarin, ethanol extracts of R. densiflora and S. wightii attenuated these increased enzyme activities produced by CCl₄. The results of the present investigation coincided with the reports of Shah et al. (2002) which showed that an elevation in the levels of AST, ALT and ALP in hepatotoxic rat models and their restoration by Phyllanthus debilis plant extract. An elevation of LDH levels in hepatotoxic rats and its restoration by curcumin in in vitro liver slice cultures was reported by Naik and Ghaskadbi (2004).

The CCl₄ treated group showed an elevation in the levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin when compared to control (Table 26). The administration of ethanol extracts of R. densiflora and S. wightii (Group III and
IV) showed significant restoration of levels of total bilirubin, conjugated bilirubin and unconjugated bilirubin. The present results were in accordance with the results of Rajkapoor et al. (2002), who showed that there was an elevation in AST, ALT, ALP and total bilirubin, in hepatotoxic rats and their restoration to near normal levels by Nigella sativa extract administration. Sethuraman et al. (2003) have also shown a similar curative effect of Sarcostemma brevistigma against CCl₄ induced hepatic damage in rats.

The efficacy of any hepatoprotective drug is essentially dependent on its capability of either reducing the harmful effect or in maintaining the normal hepatic physiological mechanism, which have been imbalanced by a hepatotoxin (Hukkeri et al., 2003).

Aspartate and alanine transaminases were present in high concentrations in liver, due to hepatic necrosis or normal membrane permeability. These enzymes are released from the cells and their levels increase in the blood. It is a sensitive indicator of acute liver damage.

Alkaline phosphatase is a membrane bound enzyme and its elevation in the plasma indicates membrane disruption in the organ. The level of this enzyme increases in cholestasis (Shah et al., 2002).

Hypoproteinemia is most frequent in the presence of advanced chronic liver diseases (Venukumar and Latha, 2002). Hence, the decline in total protein content can be deemed as an useful index of the severity of cellular dysfunction in chronic liver diseases. The lowered level of total proteins recorded in the serum of CCl₄ treated rats revealed the severity of hepatopathy. The attainment of near normalcy in total protein content of serum of the treated rats confirmed their hepatoprotective nature.
γ- glutamyl transferase (GGT) is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum γ - glutamyl transferase is generally elevated as a result of liver disease, since γ - glutamyl transferase is a hepatic microsomal enzyme. Serum γ - glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in γ - glutamyl transferase is parallel to those of aminotransferases (Anitha et al., 2012). The acute damage caused by CCl₄ increased the γ - glutamyl transferase level but the same attained the normal level after *R. densiflora* and *S. wightii* treatment due to their antioxidant activity.

The increase in malondialdehyde (MDA) levels in plasma suggested the enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extracts of *R. densiflora* whole plant and *S. wightii* tuber significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extracts of *R. densiflora* and *S. wightii* was due to the antioxidant effect.

The recovery observed in various serum biochemical parameters after the treatment with ethanol extracts of *R. densiflora* and *S. wightii* indicated that these plant extracts were effective in the treatment of CCl₄ induced liver dysfunction in animal models.

**Fertility studies**

The rats treated with *R. densiflora* whole plant and *S. wightii* tuber extracts (200 mg/kg body weight) for fourteen days showed little changes in the body weight. The weight of the testis and other accessory sex organs of *R. densiflora* treated rats
were decreased significantly and that of *S. wightii* treated rats were increased during the experiment (Table 28). Among the accessory sex organs, a significant weight reduction was seen in the testis, caput and caudal epididymal segments of *R. densiflora* treated rats and significant weight gain was seen in *S. wightii* and the weight reduction was dose dependent. Reduction in the weight of testis and other accessory sex organs might be due to low level of androgen, which was not enough to maintain the weight of gonads and accessories (Sharma and Jacob, 2001). It was known that the accessory sex organs viz., epididymis and vas deferens are androgen dependent target organs and manifest differential sensibility to androgens for maintenance of their structure and function. It was also known that any change in circulating androgens would affect the internal micro environment of epididymis and thereby lead to alternation in sperm motility and metabolism (Khan and Awasthy, 2003).

In the present study, there was a decrease in the sperm motility and sperm density in cauda and caput epididymal segments in the rats treated with *R. densiflora* extract whereas, extract of *S. wightii* tuber increased the sperm motility and sperm density in cauda and caput epididymal segments in treated rats. Drastic effect on the nature of the normal sperms in the caput and caudal region was observed in ethanol extracts of *R. densiflora* and *S. wightii* treated rats (Table 29). Further, head region of the sperm in all the treated groups (Groups II and III) of *R. densiflora* were much affected than the tail region. The development of normal and mature sperm is the key to optimum for male fertility. The production of the sperm cells (spermatozoa) and testosterone in the testis are mainly regulated by the follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are released from the anterior pituitary (Steinberger, 1971). FSH stimulates spermatogenesis in the sertoli cells, while LH
stimulates the production of testosterone in the Leydig cells of the testis (Kerr and Klester, 1975). Many studies on the testis of rat treated with plant extracts have also revealed the inhibitory activity on the proliferation of spermatogonia in mammals (Steinberger et al., 1964; Mancini et al., 1967; Krueger et al., 1974). Spermatogenesis is therefore, a complicated process, covering proliferation of the spermatogonia, long-lasting process of the tissue meiosis and numerous changes in the spermatids during their pre-formation (Steinberger, 1971; Kerr and Klester, 1975). The result of the present study suggested that, ethanol extracts of S. wightii enhanced the fertility activity and that of R. densiflora may affect the normal function of the Sertoli and Leydig cells on continuous oral administration for fourteen days.

The extract treatments either had influence on mitotic division of the spermatogonia or during the maturation of the spermatozoa, thereby affecting the number and quality of the sperm cells produced in the testis. Among the ethanol extract treated groups, Group II and III (200 mg/kg body weight) produced a significant reduction in total sperm count and viable sperms. This may be due to the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or with the activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis (Bowman and Rand, 1985; William, 2000). The presence of immature sperms was also observed in the experimental rats treated with 200 mg/kg body weight of ethanol extracts of R. densiflora. It was suggested that the 200 mg/kg body weight dose level could affect the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the decrease in the mean total sperm count. The data generated in the present study confirmed to those already reported in studies with various plant extracts (Njar
et al., 1995; Raji and Bolarinwa, 1997; Parveen et al., 2002). The decrease in the caudal epididymal sperm counts was a clear indication that ethanol extract of *R. densiflora* can affect one or more aspects of spermatogenesis as well as spermiogenesis. Though a direct effect of *R. densiflora* and *S. wightii* on the cellular mechanisms of spermatogenesis cannot be concluded, it is likely that the impairment of the hormonal mechanisms concerned with the regulation of spermatogenesis may be the underlying cause.

The various other sperm abnormalities, like sluggish motility, coiled tail and sperm maturation were also due to ethanol extracts of *R. densiflora* toxicity. The hitherto unreported abnormal sperm morphology, coiled tail and malformed head could be attributed to both testicular and epididymal effects of *R. densiflora* extract. Coiling of the sperm tail is usually the product of abnormal axoneme and/or the outer dense fibril. The outcome of the present study affirmed the male reproductive toxic effects of *R. densiflora* extract when applied as a therapeutic agent. Since male reproductive toxicology and male contraception are two sides of the same coin, the negative consequence of *R. densiflora* and *S. wightii* extracts on the sperm may be taken as an advantage for further study. By the treatment employed, in the study, no toxic effect was produced in the liver and kidney, neither was it directly involved on the development and functioning of the male reproductive system nor in the reproductive organs.

In the present study, a significant decrease in the sperm density and motility was observed in the cauda epididymis in *R. densiflora* treatment group, which leads to prove the impairment of fertility in the treated groups. The results of the present investigation also indicated that the treatment with the ethanol extract of *R. densiflora* in adult male rats reduced the number of female’s impregnation. In addition, the
number of implantations and the number of viable fetuses were also deeneared, this
decrease could be a reflect and may be due to the decrease in sperm motility and
sperm density observed in this study. Hence, this may be due to the effects of the
given plant extracts on the enzymes involved in the oxidative phosphorylation
process.

The present study revealed a decrease in the serum level of testosterone in
*R. densiflora* treated rats. This observation was similar to the earlier findings of Udoh
and Kehinde (1999), Udoh and Ekpenyong (2001) and Udoh *et al.* (2005a). The
reduction in the serum level of testosterone could be probably due to the decrease of
serum levels of LH/ICSH observed in this investigation. Leydig cells secrete
testosterone by the stimulatory effect of LH (Udoh and Udoh, 2005; Udoh *et al.*, 2005b). In males, reduction in testosterone level may impair spermatogenesis and
cause male infertility. This study further revealed a dose dependent increase in the
serum estrogen level. This increase might probably be due to the conversion of
testosterone to estrogen (Carr and Blackwell, 1993; Chinoy and Padman, 1996).

Treatment with the ethanol extracts of *R. densiflora* and *S. wightii* (200 mg/kg
body weight) was highly effective in producing reversible functional sterility. The
drug treated male rats clearly indicated structural and functional alteration in testis,
epididymis and seminal vesicle. Depletion of sperm count and sperm motility in
*R. densiflora* treated rats suggested alteration in sperm production in the testis and
maturation in the epididymis. Changes in both sperm count and motility resulted in
partial infertility within twenty one days. This resulted in abnormal sperm functions
which ultimately gave rise to complete male sterility. Among the plant based
contraceptives, inhibition of male fertility after administration of natural substances
has been related to decreased spermatozoa density (Watcho *et al.*, 2001). For male
contraception, it is not necessary to stop spermatogenesis, but it is enough to eliminate the fertilizing ability of the spermatozoa by causing changes in the morphology or in the function of the sperm (Dwivedi et al., 1990).

The results of this study also indicated that the treatment with the ethanol extract of *R. densiflora* to male adult rats reduced the number of female’s impregnation. In addition, the number of implantations and the number of viable fetuses were also decreased; and it may be due to the decreased sperm motility and sperm density observed in this study. In contrast, administration of *S. wightii* tuber extract to adult male rats increased the number of impregnated females. The number of implantations and the number of viable fetuses were increased. This effect may be due to increase in sperm motility and sperm density. From the present study, it can be concluded that *R. densiflora* is capable to suppress male fertility without altering general metabolism. Long term administration of *S. wightii* showed enhanced effect on fertility and reproductive system in adult male rats. However, the exact mode of action requires further study.

The antifertility activity of *R. densiflora* has been attributed to the action of various steroidal saponin. Saponins are important mainly because of their steroid structure. They are precursors for the production of birth control pills (with progesterone and estrogens) as well as similar hormones and corticosteroids (Crabbe, 1979). Recently many laboratories are engaged in developing male contraceptives from plants (US National Academy of Sciences, 1992). Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high. Recently, extensive efforts had been made to study the antifertility
drugs from plants (Upadhyay et al., 1993; Handelsman, 1994; Khan and Awasthy, 2003).

**Antiinflammatory activity**

In the present study, the antiinflammatory activity of extract of *R. densiflora* and *S. wightii* tuber has been established. The extracts were found to be significantly inhibiting the carrageenan-induced rat paw oedema, a test which has significant predictive value for antiinflammatory agents acting by inhibiting the mediators of acute inflammation. Carrageenan-induced inflammation is useful in detecting orally active antiinflammatory agents (Di Rosa et al., 1971; Ismail et al., 1997; Muhammad et al., 2012). The development of carrageenan-induced oedema is believed to be biphasic (Vinegar et al., 1969; Udegbunam et al., 2012). The initial phase is attributed to the release of histamine and serotonin. The oedema produced at the peak 3 hours is thought to be due to the release of Kinin-like substances, especially bradykinin (Crunkhon and Meacock, 1971; Ruangsang et al., 2010). The second phase of oedema is due to the release of prostaglandins, protease and lysosomes and it is sensitive to most antiinflammatory drugs (Vinegar et al., 1969; Di Rosa et al., 1971; Nair et al., 1988; Paschapur et al., 2009; Sharma et al., 2010; Jothimanivannan et al., 2010). Fangchinoline and tetrandrine, major alkaloids from *Stephania tetrandrae* have been used traditionally to treat inflammatory diseases in Korea. Both fangchinoline and tetrandrine showed antiinflammatory effects on the mouse (Choi et al., 2000). Bhattacharya et al. (2005) have reported antiinflammatory potential of methanol extract of *Stephania glabra* of Menispermaceae family. Similarly, antiinflammatory activity of *S. wightii* may be due to the presence of certain chemicals present in it.
Results of the present study revealed that, the drugs under investigation predominantly inhibit the release of prostaglandin like substances. The whole plant of *R. densiflora* and *S. wightii* tuber extracts possessed varying degree of antiinflammatory activity when tested at two different doses (200 and 400 mg/kg) (Table 33). The whole plant extracts of *R. densiflora* and *S. wightii* tuber at the dose of 200 mg/kg showed high significant antiinflammatory activity at 3rd hour, where it caused 66.50% and 52.69% inhibition respectively, as compared to that of 10 mg/kg of Indomethacin (65.19%).

**CNS activity**

Public concern on mental health has noticeably increased, given the high prevalence of neuropsychiatric disorders especially anxiety and depression. Most of the drugs for these conditions used nowadays have adverse side effects so the need for newer, better-tolerated and more efficacious treatments is remaining high. Growing attention is being paid to traditional herbal medicines.

In the present study, the ethanol extract of *R. densiflora* and *S. wightii* on CNS activity has been evaluated. The result indicated that among the plants studied, the ethanol extract of *R. densiflora* showed much better activity when compared to *S. wightii*. The ethanol extract of *R. densiflora* influence the general behavioral profiles, as evidenced in the spontaneous activity, righting reflex, pinna reflex, grip strength and pain responses. Reduction of awareness and depressant action may be due to the action of the extract on CNS. Reduction of pinna reflex may be due to blocking synapses of the afferent pathway (Kumar *et al.*, 2008).

The different doses of ethanol extract of *R. densiflora* produced a significant increase in the hypnotic effect induced by the phenobarbitone, in a dose dependent
manner, thus suggesting a profile sedative activity. It should be emphasized that the method employed for this assay is considered in a very sensitive way and denote agent with depressor activity on the CNS. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and had been identified in certain plant extracts (Jha et al., 2011).

A myorelaxant effect was observed only with the higher dose of ethanol extract of *R. densiflora* which resulted in an increase in the number of falls and a decrease in the time on the bar as detected by the rotarod test. The intensity of reduction in exploratory behaviours in the treated animal groups which reflected the same line of action like the standard reference drug diazepam, which acted as an anxiolytics (at low doses), anticonvulsants, and also produce sedation and a myorelaxant effect at higher doses (Onaivi et al., 1992). The reduction in exploratory behaviour in animals treated with methanol extract of *R. densiflora* was similar with the action of other CNS depressant agents.

It was studied that terpenoids, flavonoids, coumarins, saponins and tannins were present in the extract of *R. densiflora*. A number of scientific reports indicated that terpenoids produced CNS depressant action (Chattopadhyay et al., 2003). Therefore, the presence of terpenoids in ethanol extract *R. densiflora* may be responsible for the CNS activity. Since the pharmacological profiles of the present investigation of the ethanol extract of *R. densiflora* was similar to that of benzodiazepine, it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of ethanol extract of *R. densiflora* in folkloric medicine may be due to its CNS action and relief of pain validated by the present findings.
Since the plants, *R. densiflora* and *S. wightii* are useful in traditional medicine for the treatment of various ailments, it is important to standardize its use as a drug. Further studies can be made on this investigation to correlate the pharmacological and the phytochemical principles to elucidate the exact mechanism in their activity.