RESULTS

In the present investigation, *Rauwolfia densiflora* whole plant and *Stephania wightii* tuber have been subjected to pharma-cochemical characterization, HPTLC and GC-MS analysis to study the phytochemical profile. The anticancer, antidiabetic, hepatoprotective, antiinflammatory, CNS activities and fertility studies were carried out with a view to assess their pharmacological potential.

**Powder analysis of the drug**

**Ash and extractive values**

The results of the ash and extractive values of whole plant of *Rauwolfia densiflora* and tuber of *Stephania wightii* drug powders were depicted in Table 1. The total ash content of the powdered drugs of *R. densiflora* and *S. wightii* were 9.54% and 8.14% respectively. The extractive value in water was more than in other solvents investigated in the present study.

**Fluorescence analysis**

The results of fluorescence analysis of whole plant powder of *R. densiflora* and tuber of *S. wightii* were shown in Tables 2 and 3. 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* fluoresced pale brown in day light, brown under short UV and dark green in long UV light. The powdered whole plant of *R. densiflora* showed the characteristic fluorescent green colour when treated with 50% sulphuric acid, concentrated hydrochloric acid, aqueous 1N NaOH, 40% NaOH, HNO₃ + NH₃ and NH₃ under short UV. The tuber powder of *S. wightii* showed the characteristic fluorescent green colour when treated with 1N alcoholic sodium
hydroxide, concentrated hydrochloric acid, ferric chloride, NH₃ and benzene under short UV.

**Preliminary Phytochemical Screening**

The distribution of different phytochemical constituents in petroleum ether, chloroform, methanol and ethanol extracts of whole plant powder of *R. densiflora* and tuber of *S. wightii* were evaluated qualitatively and presented in Table 4. The phytocompounds such as alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, saponins, tannins, steroids, terpenoids, sugars and glycosides had been reported from the methanol and ethanol extracts of the above said plants.

**HPTLC analysis**

The HPTLC analysis showed the presence of alkaloids, coumarins, glycosides, phenols and steroids in the ethanol extract of whole plant powder of *R. densiflora* and tuber of *S. wightii*. The HPTLC profiles at day light, UV 254 nm, 366 nm and their densitograms, Rf values, peak areas and assigned substances were presented in Plates II - VI, Tables 5 - 9 and Figures 1 - 10. All the presently studied plant extracts showed the presence of alkaloids, coumarins, glycosides, phenols and steroids. There were two types of alkaloids found both in the whole plant powder of *R. densiflora* and tuber of *S. wightii*. In whole plant of *R. densiflora*, four types of coumarins were found and tuber of *S. wightii* showed three types. Glycosides were of six types in *R. densiflora* and nine types in *S. wightii*. Phenolics profile revealed the presence of two types of flavonoids in whole plant of *R. densiflora* and three types in *S. wightii*. Six types of steroids were found in the whole plant of *R. densiflora* while four types in *S. wightii*. 
GC - MS analysis

The chemical composition of ethanol extracts of whole plant powder of *R. densiflora* and tuber of *S. wightii* were analysed by using GC-MS. The chromatogram of whole plant powder of *R. densiflora* and tuber of *S. wightii* were shown in Figures 11 and 12. Mass spectra were used to identify the structure of the compounds found, comparing with those in NIST ver 2.1 (National Institute of Standards and Technology) library. Seven compounds were detected in the ethanol extract of *R. densiflora* whole plant (Table 10). The results revealed that 1,10-Decanediol (21.62%) was found as major compound followed by propanoic acid anhydride (10.81%), Phytol (5.41%) and 3-Pentanol,2,4-dimethyl- (5.81%). Thirteen compounds were reported in the ethanol extract of tuber of *S. wightii* (Table 11). 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%). Mass spectra of some of the detected compounds of *R. densiflora* and *S. wightii* were presented in Figures 13 and 14.

Pharmacological studies

DPPH Radical Scavenging activity

The DPPH radical scavenging activity of ethanol extracts of *R. densiflora* whole plant and tuber of *S. wightii* was comparable to ascorbic acid. The IC<sub>50</sub> values were found to be 33.71, 39.46 and 34.47 µ/ml for ethanol extracts of *R. densiflora* whole plant and tuber of *S. wightii* and ascorbic acid respectively (Table 12).
Anticancer activity

Antitumour activity of ethanol extracts of *R. densiflora* whole plant and tuber of *S. wightii* against DAL tumour bearing mice was assessed by the parameters such as relative organ weights, solid tumour volume, viable and non-viable cell count, mean survival time and % increase of life span. The results were shown in Tables 13 - 15. The tumour volume and viable cell count were found to be significantly increased and non-viable cell count was significantly low in DAL control animals. Administration of ethanol extracts of *R. densiflora* (200 mg/kg) and *S. wightii* (200 mg/kg) significantly (p<0.01) decreased the tumour volume, viable cell count (p<0.05). Non-viable cell count was significantly (p<0.05) higher in *R. densiflora* and *S. wightii* treated animals when compared with DAL control animals. The mean survival time was increased to 29.14±0.14 (% ILS=58.11), 34.81±0.26 (% ILS=88.87) and 31.74±0.31 (% ILS=72.21) on administration of ethanol extracts of *R. densiflora*, *S. wightii* and vincristine respectively.

Haematological parameters (Table 16) of tumour bearing mice (Group II) on day 30 were found to be significantly altered from normal group (Group I). The total WBC count was found to be increased with a reduction of Hb content of RBC. The total number of RBC showed a moderate change. In differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time, administration of *R. densiflora* and *S. wightii* treatment also recovered these altered depleted parameters towards near normal.

Antidiabetic activity

Table 17 depicted the effect of whole plant of *R. densiflora* and tuber of *S. wightii* extracts and glibenclamide on body weight and fasting blood glucose level.
Table 18 showed the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin of normal and experimental rats. There was a significant elevation in blood glucose, urea, creatinine and glycosylated haemoglobin levels, while the plasma insulin level decreased significantly in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Administration of whole plant of *R. densiflora* and tuber of *S. wightii* (Group III and IV) and glibenclamide (Group V) tends to bring the parameters significantly towards the normal. The effect of whole plant extract, at the dose of 200 mg/kg body weight was highly significant in restoring normalcy.

The levels of total protein, albumin, globulin and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats were presented in the Table 19. The diabetic rats (Group II) had decreased levels of serum total protein, albumin, globulin and elevated level of liver marker enzymes such as SGPT, SGOT and ALP when compared with normal control rats (Group I). After treatment with whole plant extracts of *R. densiflora* and tuber of *S. wightii* (200 mg/kg body weight) and glibenclamide (Group III, IV and V), total protein, albumin, globulin and liver marker enzymes were brought back to near normal levels.

Table 20 showed the levels of TC, TG, HDL-C, LDL-C, VLDL-C, PL and LDL/HDL in the serum of diabetic rats. The diabetic rats had elevated levels of serum TC, TG, LDL-C, VLDL-C and PL and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with whole plant extracts of *R. densiflora* and tuber of *S. wightii* and glibenclamide reversed serum lipid profiles to near normal levels.
The activities of LPO, GPx, GSH, SOD and CAT in the serum, liver and kidney of alloxan induced diabetic rats were illustrated in Tables 21, 22 and 23. In the present study, the alloxan induced diabetic rats had shown increased activities of LPO and decreased activities of SOD, CAT and GPx in the serum, liver and kidney. Treatment with *R. densiflora*, *S. wightii* and glibenclamide showed reversal of all these parameters to near normal levels.

**Hepatoprotective activity**

The effect of ethanol extracts of whole plant of *R. densiflora* and tuber of *S. wightii* at 100 and 200 mg/kg body weight doses were compared with that produced by silymarin, a known hepatoprotectant. Table 24 depicted the effect of whole plant of *R. densiflora* and tuber of *S. wightii* extracts and silymarin on body weight. The hepatotoxicant group (CCl₄) (Group II) when compared to control (Group I) showed an elevation in the levels of SGOT, SGPT and ALP. The levels of SGOT, SGPT and ALP in whole plant extracts of *R. densiflora* and tuber of *S. wightii* treated rats were found to be lower when compared with CCl₄ treated group (Table 25). The decrease in the serum activity of SGOT was found to be greater in Group IV whereas, decrease in SGPT was found to be greater in Group VI when compared to standard drug treated group (Group VII). The levels of total protein, albumin and globulin (Table 25) concentration were found to be significantly (p<0.05) reduced in CCl₄ treated rats when compared to normal control (Group I). After treatment with *R. densiflora* (Group III and IV) and *S. wightii* (Group V and VI) extracts and silymarin (Group VII), total protein, albumin and globulin were brought back to near normal levels.

Table 26 showed the levels of total bilirubin, conjugated unconjugated bilirubins and GGTP. A significant elevation of total bilirubin, conjugated
unconjugated bilirubins and GGTP in the serum of CCl₄ treated group when compared to normal control (Group I) were noted. In all the other treated groups (III to VII), the above biochemical parameters were found to decrease when compared to Group II. The decrease in the concentration of total bilirubin, conjugated bilirubin unconjugated bilirubin and GGTP were found to be greater in Group V, followed by Group IV (Table 26).

Table 27 showed the levels of plasma LPO, GPx, GRD, SOD and CAT. CCl₄ treated rats had elevated level of LPO and decreased level of GPx, GRD, SOD and CAT compared to normal control rats. CCl₄ treated rats treated with ethanol extracts of whole plant of *R. densiflora* and tuber of *S. wightii* and silymarin reversed the LPO, GPx, GRD, SOD and CAT to near normal levels.

**Fertility studies**

**Body and reproductive organ weight**

The administration of ethanol extracts of whole plant of *R. densiflora* and tuber of *S. wightii* to rats did not cause any significant change in the body weight (Table 28) and on the libido of treated rats, weights of testis and other accessory sex organs of *S. wightii* treated rats were increased significantly whereas weights of testis and other accessory sex organs of *R. densiflora* treated rats were decreased significantly (p < 0.05) (Table 28). Among the accessory sex organs, a significant weight reduction was seen in the caput and caudal epididymal segment in *R. densiflora* treated rats and a significant weight gain was seen in the caput and caudal epididymal segment in *S. wightii* treated rats. Slight decrease was observed in vas deferens (VD), seminal vesicle (SV) and prostrate of *R. densiflora* treated rats and
slight increase was observed in vas deferens (VD), seminal vesicle (SV) and prostrates of *S. wightii* treated rats.

**Sperm count and sperm motility**

Sperm motility and sperm density in both caput and caudal epididymal segment significantly decreased in *R. densiflora* treated rats (Group II and III) (Table 29) and increased sperm motility and sperm density were observed in the epididymis of *S. wightii* treated rats (Group IV and V) and the same trend was seen in the caput epididymal sperm density when compared to control (Group I).

**Sperm abnormality**

Sperm abnormality in caput and caudal region was drastically affected by ethanol extract of *R. densiflora* treated rats (*p* < 0.05) (Table 29). Among the studied plants, whole plant extract of *R. densiflora* had shown significant and drastic abnormality in the sperm morphology, further tail region of the sperm in all the treated groups were much affected than the head region.

**Serum biochemical profile**

Serum protein, albumin, globulin, urea and creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and treated rats were depicted in Table 30. No significant changes were noted in the serum biochemical and liver marker enzymes in the entire drug treated groups except Group III when compared to control group. Significant weight reduction was seen in *S. wightii* treated rats at the dose of 200 mg/kg body weight.
Reproductive hormone profile

Serum testosterone level

The treatment with ethanol extract of whole plant of *R. densiflora* (100 and 200 mg/kg body weight) daily for 14 days caused a significant decrease in the serum level of testosterone in male rats whereas increased serum level of testosterone were seen in the ethanol extract of *S. wightii* at the doses of 100 and 200 mg/kg body weight (Table 31).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the *R. densiflora* extracts at the dosage of 100 and 200 mg/kg body weight for 14 days caused a dose related decrease in the serum level of LH whereas increase in the serum level of LH was observed in *S. wightii* extracts at the dosage of 100 and 200 mg/kg body weight (Table 31). The level of decrease was statistically significant (p < 0.05).

Serum estrogen level

The ethanol extracts of *R. densiflora* (100 and 200 mg/kg body weight) caused an increase in the serum level of estrogen in male rats and that of *S. wightii* (100 and 200 mg/kg body weight) caused decrease in the serum level of estrogen. Dose of 200 mg/kg body weight administered daily for 14 days caused a sharp rise in the serum level of estrogen in *R. densiflora* treated rats (Table 31).

Serum follicle stimulating hormone (FSH) level

Pre-treatment with ethanol extracts of whole plant of *R. densiflora* for 14 days at the dosage of 100 and 200 mg/kg body weight caused an increase in the serum level of FSH in male rats and decreased serum level of FSH in *S. wightii* treated rats for 14
days at the dosage of 100 and 200 mg/kg body weight compared to control (Table 31). The increase in the serum level of FSH in male rats statistically significant when treated with *R. densiflora*.

**Fertility test**

The results presented in Table 32 showed that intragastric administration of extract of whole plant of *R. densiflora* (100 and 200 mg/kg body weight) for 14 days to male rats caused a significant decrease (p< 0.05) in the number of females impregnated by male treated rats when compared to that of *S. wightii* tuber. The number of viable fetuses calculated after cesarean sections were significantly decreased (p< 0.05) in female rats impregnated by treated males when compared with female impregnated with untreated male rats. On other hand, the number of resorption sites was found to be increased in female impregnated by *S. wightii* treated male rats when compared to control.

**Antiinflammatory activity**

Table 33 showed that the antiinflammatory activity of ethanol extracts of *R. densiflora* and *S. wightii* significantly inhibited the rat paw oedema at 3\textsuperscript{rd} hr. post carrageenan. The inhibition percentage of carrageenan induced paw oedema were 66.50% and 73.50% for 200 and 400 mg/kg ethanol extracts of whole plant of *R. densiflora* respectively and 52.69% and 73.28% for 200 and 400 mg/kg ethanol extracts of tuber of *S. wightii* respectively.

**Central Nervous System (CNS) activity**

The results obtained from the effect of whole plant extract of *R. densiflora* and tuber of *S. wightii* in different experiments were presented in Tables 34 - 38. The
ethanol extract of *R. densiflora* affected spontaneous activity, alertness, awareness, sound response, touch response, pain response, righting reflex and pinna reflex at a dose of 150 mg/kg whereas the tuber extract of *S. wightii* showed slight or moderate depression related to all the activities (Table 34). However, the standard drug Diazepam caused a significant depression of all these responses compared with the ethanol extract of *R. densiflora*.

**Effect on Phenobarbitone sodium-induced sleeping time**

The ethanol extract of *R. densiflora* whole plant significantly potentiated the phenobarbitone sodium-induced sleeping time in a dose dependent manner. While the ethanol extract of *R. densiflora* at 100 and 150 mg/kg doses showed much better results when compared to tuber of *S. wightii* extract (Table 35).

**Exploratory behaviour potentials**

In Y-maze test, the mouse treated with ethanol extract of *R. densiflora* at the doses of 100 and 150 mg/kg showed marked decrease in exploratory behaviour compared with control. The other plant *S. wightii* tested for Y-maze test did not show any change in exploratory behaviour compared with control (Table 36). In case of head dip test, the mice treated with different doses of ethanol extract of *R. densiflora* showed marked decrease in head dip response when compared to other plant extract, *S. wightii* and control (Table 37).

**Effect on muscle relaxant activity**

In the Traction test, the mice treated with ethanol extract of *R. densiflora* showed a significant failure in traction in all doses tested when compared with other plant tested.
The result obtained from the rotarod test showed that ethanol extract of *R. densiflora* at 100 and 150 mg/kg significantly reduced the motor co-ordination of the test animals when compared with other plant tested (Table 37).

**Cocaine induced hyperactivity experiment**

Among the studied plants, the ethanol extract of *R. densiflora* produced partial inhibition of hyperactivity induced by cocaine (40 mg/kg) in rats. This suppression by ethanol extract of *R. densiflora* was evident from 40 through 90 minutes after the cocaine injection and the results were tabulated in Table 38.