RESULTS

In the present investigation, *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* whole plant have been subjected to pharmacochemical characterization, GC-MS analysis, anticancer, antidiabetic, hepatoprotective, antifertility and antiinflammatory activity 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 *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* drug powders are depicted in Table 1. The total ash content of the powdered whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* are 8.36%, 9.54% and 10.28% respectively. The extractive value in water is more than in other solvents investigated in the present study.

**Fluorescence analysis**

The results of fluorescence analysis of whole plant powder of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* are shown in Table 2, 3 & 4. The powder from the whole plant of *P. javana* fluoresced light green under day light, yellowish green under short UV light and dark brown in long UV light. The powdered whole plant of *P. chinensis* fluoresced yellow in day light, light yellow under short UV and dark blue in long UV light. The powdered whole plant of *P. rosmarinifolia* emitted yellow under day light, dark green under short UV light and violet in long UV light. The powdered whole plant of *Polygala javana* shows the characteristic fluorescent green colour treated with 50% sulphuric acid, concentrated hydrochloric acid, ammonic acid and acetone. The whole plant powder of *polygala chinensis* shows the characteristic fluorescent green colour treated with 1N alcoholic sodium hydroxide,
50% sulphuric acid, 50% nitric acid, concentrated nitric acid + ammonia and ethol. The whole plant powder of *Polygala rosmarinifolia* shows the fluorescent green colour treated with 1N alcoholic sodium hydroxide, 50% sulphuric acid, 50% nitric acid, concentrated nitric acid + ammonia and acetone.

**Preliminary Phytochemical Screening**

The distribution of different phytochemical constituents in petroleum ether, chloroform, methanol and ethanol extracts of whole plant powder of *Polygala javana, P. chinensis* and *P. rosmarinifolia* were evaluated qualitatively and presented in Table 5. The phytocompounds such as alkaloid, anthraquinone, catechin, coumarin, flavonoid, phenol, saponin, tannin, terpenoid, sugar, and glycosides have been reported from the methanol and ethanol extracts of the above said plants.

**HPTLC analysis**

The HPTLC analysis showed the presence of alkaloids, flavonoids, glycosides, saponins and steroids in the ethanol extracts of whole plant powder of *Polygala javana, P. chinensis* and *P. rosmarinifolia*. The HPTLC profiles at day light, UV 254 nm, 366 nm and their densitograms, Rf values, peak areas and assigned substances are presented in Plate II - VI, Tables - 6 - 10 and Figures 1 - 15. All the presently studied plant extracts showed the presence of alkaloids, flavonoids, glycosides, saponins and steroids. There were three types of alkaloids found in the whole plant of *Polygala javana* four type in *P. chinensis* and five type in *P. rosmarinifolia*. Flavonoids were found in more amounts in whole plant of *Polygala javana* eight type in *P. chinensis* and six types in *P. rosmarinifolia* nine types of glycosides in *polygala javana*, twelve type in *P. chinensis* and six types in *Polygala rosmarinifolia*. Flavonoids profiles revealed the presence of ten types of flavonoids in whole plant of *Polygala javana, P. chinensis* and *P. rosmarinifolia*. Fourteen types of saponins were found in the whole plant of *Polygala javan*; nine types in *P. chinensis* and ten types in
Three types of glycosides in *Polygala javana*, four type in *P. chinensis* and three types in *Polygala rosmarinifolia*.

**GC – MS analysis**

The chemical composition of ethanol extracts of whole plant powder of *Polygala javana, P. chinensis* and *P. rosmarinifolia* were analysed by using GC–MS. The chromatogram of whole plant powder of *Polygala javana, P. chinensis* and *P. rosmarinifolia* were shown in Fig.16,17 & 18. Mass spectra were used to identify the structure of the compounds found, comparing with those in NITS ver 2.1 (National Institute of Standards and Technology) library. Sixteen compounds were detected in ethanol extracts of *Polygala javana* whole plant (Table 11). The results revealed that, polygalitol (84.79%) was found as major compound followed by 1H- perimidine, 2,3-dihydro – 2 (2,4,5- trimethoxyphenyl – (6.33%). 4H – 1 – benzopyran – 4 – one, 5-hydroxyl – 2- (4- hydroxypheryl)were reported in the - 3,7 – dimethoxy – (1.53%), ledene oxide – (1), (1.43%) and phytol (1.28%). Fourteen compounds were reported in the ethanol extract of *Polygala chinensis* whole plant (Table 12) the major compounds include 1,5- Antydro – d- mannitol (92.30%), 9H- furo [2,3-H] chromene – 2,8 – dione, 4- methyl 1-9- (3,4,5- trimethoxybenzylidene)- (1.80%). Twelve compounds were reported in the ethanol extract of *Polygala rosmarinifolia* whole plant (Table13). The results revealed that 1,5 – Antydro - d - mannitol (73.35%), was the major compound followed by Benzene 1,2 – dimethoxy – 4[(4-methylphenyl) sulfonyl methyl]- (9.80%) d- mannitol 1- decylsulfonyl (5.12%), 9-octadecenoic acid (Z) – phenylmethyl ester (4.72%), Squalene (3.22%) and propane 1,1,3- triethoxy- (2.21%).

Mass spectra of some of the detected compounds of *Polygala javana, P. chinensis* and *P. rosmarinifolia* are presented in Fig.19-25.
Pharmacological studies

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of methanol extracts of *Polygala javana*, *P. chenensis*, *P. rosmarinifolia* whole plant was comparable to ascorbic acid. The IC 50 values were found to be 48.26, 59.19, 41.58 and 22.51 µ/ml respectively for methanol extract of whole plant of *Polygala javana*, *P. chinensis*, *P. rosmarinifolia* and ascorbic acid. (Table 14).

Anticancer activity

Antitumour activity of ethanol extracts of whole plant of *Polygala javana*, *P. chinensis* and *P. rosmarinifolia* 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 are shown in tables (Table 15, 16, & 17). 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 *P. javana* (200 µg/kg), *P. chinensis* (200mg/kg), and *P. rosmarinifolia* (200mg/kg), significantly (p<0.01) decrease the tumour volume, viable cell count Non-viable cell count was significantly (p<0.05) higher in *P. javana*, *P. chinensis* and *P. rosmarinifolia* treated animals when compared with DAL control animals. The mean survival time was increased to 27.58±0.44 (% ILS=60.02), 32.74±0.23 (% ILS=90.23), 28.85±0.78 (% ILS=73.44) and 30.68±1.34 (% ILS=78.26) on administration of ethanol extracts of *P. javana P. chinensis* and *P. rosmarinifolia* vincristine respectively.

Haematological parameters (Table. 18) of tumour bearing mice (Group II) on day 14 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 modest change. In differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time, administration of *P. javana* *P. chinensis, Prosmarinifolia* treatment also recovered these altered depleted parameters towards near normal.

**Antidiabetic activity**

Table 19 shows 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 aerial part of *P. javana* *P. chinensis, rosmarinifolia* (III, IV, & V) and glibenclamide (Group VI) 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 normally.

The levels of total protein, albumin, globulin, and liver marker enzymes such as SGPT, SGOT and ALP in the serum of diabetic rats are presented in the Table 20. 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 *P. javana P. chinensis, Prosmarinifolia* (200mg/kg body weight) and glibenclamide (Group III, IV, V & VI), total protein, albumin, globulin, and liver marker enzymes were brought back to near normal levels.
Table 21 shows 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 *P. javana, P. chinensis, P. rosmarinifolia* 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 22, 23, & 24. 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 *Pjavana P.chinensis, Prosmarinifolia* and glibenclamide showed reversal of all these parameters to near normal levels.

**Hepatoprotective activity**

The effect of ethanol extracts of *P.javana, P.chinensis, P.rosmarinifolia* whole plant at 100 and 200 mg/kg body weight doses were compared with that produced by silymarin, a known hepatoprotectant. 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 *P. javana, P. chinensis* and *P. rosmarinifolia* 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 VI whereas, decrease in SGPT was ALP was found to be the greater in Group VIII when compared to standard drug treated (Group IX). 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 *P. javana, (Group III&IV) P. chinensis*
(Group V&VI) and *P. rosmarinifolia* (Group VII&VIII) extracts and silymarin (Group IX), total protein, albumin and globulin were brought back to near normal levels.

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

Table 27 showed the levels of plasma LPO, GPx, GRD, SOD and CAT level. 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 *P. javana*, *P. chinensis* and *P. rosmarinifolia* whole plant and silymarin reversed the LPO, GPx, GRD, SOD and CAT to near normal levels.

**Antifertility activity**

**Body and reproductive organ weight**

The administration of ethanol extracts of whole plant of *P. javana*, *P. chinensis*, and *P. rosmarinifolia* to rats did not cause any significant change in the body weight (Table 28) and on the libido of treated rats whereas weights of testis and other accessory sex organs 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. Slight increase was observed in vas deferens (VD) and decrease seminal vesicle (SV) and prostrate.
Sperm count and sperm motility

Sperm motility and sperm density in caudal epididymal, significantly decreased (Table 29) and the reduction was severe in whole plant extract of *P. chinensis* (Group III) followed by whole plant extract of *P. javana* (Group II) 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 extracts of *P. javana*, *P. chinensis* and *P. rosmarinifolia* whole plant and (*p* < 0.05) (Table 30). Among the studied plants, whole plant extract of *P. chinensis* have shown significant and drastic abnormality in the sperm morphology, further tail region of the sperm in all the treated groups 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 when compared to control group.

Reproductive hormone profile

Serum testosterone level

The ethanol extract of whole plant of *P. javana, P. chinensis, P. rosmarinifolia* (200 mg/kg body weight) repeated treatment daily for 14 days caused a significant decrease in the serum level of testosterone in male rats. The level of testosterone decrease was shown in whole plant extracts of *P. javana* (Group III) (Table 31).
Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the P. javana, P. chinensis and P. rosmarinifolia extracts for 14 days caused a dose related decrease in the serum level of LH (Table - 31). The level of decrease was statistically significant (p < 0.05).

Serum estrogen level

The ethanol extracts of P. javana, P. chinensis and P. rosmarinifolia (200 mg / kg body weight) caused an increase in the serum level of estrogen in male rats. Dose of 200 mg / kg body weight administered daily for 14 days caused a sharp rise in the serum level of estrogen (Table - 31). Highest level of estrogen increase was shown in whole plant of P. chinensis (Group III).

Serum follicle stimulating hormone (FSH) level

Pretreatment with ethanol extracts of whole plant P. javana, P. chinensis and P. rosmarinifolia of for 14 days caused an increase in the serum level of FSH in male rats compared to control (Table 31). The increase in the serum level of FSH in male rats statistically significant when treated with P. javana, P. chinensis and P. rosmarinifolia (p < 0.05 respectively).

Fertility test

The results presented in Table - 32 shows that intragastric administration of extracts of whole plant of P. javana, P. chinensis and P. rosmarinifolia (200 mg/Kg body weight) for 14 days to male rats causes a significant decrease (p< 0.05) in the number of females impregnated by male treated rats. The number of viable foetuses 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 treated male rats when compared to controls.
Antiinflammatory activity

Table 33 shows that the antiinflammatory activity of ethanol extracts of whole plant of *P. javana*, *P. chinensis* and *P. rosmarinifolia* significantly inhibited the rat paw oedema at 3rd hr post carrageenan were 32.45% and 58.79% for 100 and 200 mg/kg ethanol extracts of whole plant of *P. javana* respectively and 57.36% and 65.26% for 100 and 200 mg/kg ethanol extracts of whole plant of *P. chinensis* respectively and 49.29% and 60.40% for 100 and 200 mg/Kg ethanol extracts of whole plant *P. rosmarinifolia* respectively.