4. RESULTS

In the present investigation, *Pleiospermium alatum* (leaf & bark) and aerial part extract of *Balanites aegyptiaca* have been subjected to pharmacochemical characterization, GC-MS analysis, antidiabetic, antihyperlipidaemic, 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 leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* drug powders are depicted in Table -1 & 2. The total ash content of the powdered leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* are 8.34%, 9.56% and 10.66% 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 leaf and bark powder of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* are shown in Table – 3, 4 & 5. The powder from the leaf of *Pleiospermium alatum* fluoresced green under day light, dark green under short UV light and dark blue in long UV light. The powdered bark of *Pleiospermium alatum* fluoresced yellow in day light, greenish yellow under short UV and dark blue in long UV light. The powdered aerial part of *Balanites aegyptiaca* emitted greenish yellow under day light and short UV light and violet in long UV light. The powdered leaf of *Pleiospermium alatum* shows the
characteristic fluorescent green colour treated with aqueous sodium hydroxide, 50% sulphuric acid, concentrated hydrochloric acid, 40% sodium hydroxide+10% lead acetate and ammonia. The bark powder of *Pleiospermium alatum* shows the characteristic fluorescent green colour treated with 50% sulphuric acid and acetone. The aerial part of *Balanites aegyptiaca* shows the characteristic fluorescent green colour treated with concentrated hydrochloric acid, 40% sodium hydroxide + 10% lead acetate, acetic acid, ferric chloride, ammonia, benzene, petroleum ether, methanol and ethanol.

**Preliminary Phytochemical Screening**

The distribution of different phytochemical constituents in petroleum ether, chloroform, methanol and ethanol extracts of leaf and bark powder of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* were evaluated qualitatively and presented in Table-6. 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 leaf and bark powder of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca*. 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 2 - 6, Table - 7 - 11 and Figures 1 - 25. 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 leaf of *Pleiospermium alatum*, four in bark of *Pleiospermium alatum* and five in
Balanites aegyptiaca aerial part. Flavonoids were found in more amounts in leaf of Pleiospermium alatum than in the investigated other plant samples of bark of Pleiospermium alatum and aerial part of Balanites aegyptiaca. Flavonoids profiles revealed the presence of ten types of flavonoids in leaf of Pleiospermium alatum, eight in bark of Pleiospermium alatum and six in aerial part of Balanites aegyptiaca. Glycosides profiles revealed the presence of nine types of glycosides in leaf of Pleiospermium alatum, twelve in bark of Pleiospermium alatum and six in aerial part of Balanites aegyptiaca. Fourteen types of saponins were found in the leaf of Pleiospermium alatum. Four types of steroids were found in the bark of Pleiospermium alatum whereas three were found in leaf of Pleiospermium alatum and aerial part of Balanites aegyptiaca.

**GC – MS analysis**

The chemical composition of ethanol extracts of leaf and bark powder of Pleiospermium alatum and aerial part of Balanites aegyptiaca were analysed by using GC – MS. The chromatogram of leaf and bark powder of Pleiospermium alatum and aerial part of Balanites aegyptiaca were shown in Fig.26, 27 & 28. 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. The results revealed that, E-2-Hexenylbenzoate (13.84%) was found as major compound in Pleiospermium leaf extract followed by 2,6,10,14,18,22- Tetracosahexane, 2,6,10,15,19,23-hexamethyl- (all-E) – [All-trans-squalene] (13.57%), 2H,8H-Benzo [1,2-b:5,4-b’] dipyran-2-one, 8,8-dimethyl- (11.12%), Cyclohexanemethanol, 4-ethenyl-qá,á,4-trimethyl-3-(1- methylethenyl)- [1R-91á,3á,4á–[Elemol] (10.96%), 2-Hydroxymethyl-5- (1-hydroxy-1-isopropyl)-2-Cyclohexen-1-one (10.35%), Lupeol (8.81%), 9,12-Octadecadienoic acid (Z,Z)- (8.36%), Acetyl turicine (8.15%), n-Hexadecanoic acid (6.11%), Phytol (5.13%) (Table - 12). Eleven compounds were
reported in the ethanolic extract of *Pleiospermium alatum* bark (Table - 13), the major compound includes 9, 12-Octadecadienoic acid (Z, Z)- (18.81%), All-trans-Squalene (17.55%), 1, 2-Benzenedicarboxylic acid, diisoctyl ester (13.38%), n-Hexadecanoic acid (12.61%), Diisoctyl adipate (11.80%).

Five compounds were reported in the ethanol extract of aerial part of *Balanites aegyptiaca* (Table - 14). The result revealed that 3-O-Methyl-d-glucose (83.75%) was found as major compound followed by All - trans - Squalene (8.11%), 9,12-Octadecadienoic acid (Z,Z)- (3.92%), n-Hexadecanoic acid (3.17%), and Phytol (1.05%). Mass spectra of some of the detected compounds of *Pleiospermium alatum* and *Balanites aegyptiaca* are presented in Fig.29, 30 & 31.

**Pharmacological studies**  
**Antidiabetic activity**

Table - 15 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 *Balanites aegyptiaca* (Group VI) and glibenclamide (Group VII) tends to bring the parameters significantly towards the normal when compared with leaf and bark extract of *Pleiospermium alatum*. The effect of *Balanites aegyptiaca* aerial part 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 - 16.
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 leaf and bark extracts of *Pleiospermium alatum* (300mg/kg body weight) and *Balanites aegyptiaca* (100 and 200 mg/kg body weight) extracts and glibenclamide (Group III, IV, V, VI & VII), total protein, albumin, globulin, and liver marker enzymes were brought back to near normal levels.

Table - 17 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 leaf and bark extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* extracts and glibenclamide reversed serum lipid profiles to near normal levels.

The activities of LPO, GPx, GSH, SOD and CAT in the Alloxan induced diabetic rats were illustrated in Table - 18. In the present study, the Alloxan induced diabetic rats had shown increased activities of LPO, SOD, CAT and GPx in the serum. On the other hand, an opposite trend was noted in the activity of serum GSH. Treatment with Pleiospermium *alatum* leaf and bark, *Balanites aegyptiaca* aerial part extract and glibenclamide showed reversal of all these parameters to near normal levels.

**Antihyperlipidaemic activity**

Table - 19 shows that in group II, Triton X-100 induced a significant increase (p<0.05) in serum total cholesterol (TC), glycerides (TG), low density lipoprotein-
cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and phospholipids (PL) and a significant decrease in the serum high density lipoprotein-cholesterol (HDL-C) levels in comparison with the control (Group I). Thus injection of Triton X-100 (100mg/kg) (Group V) has successfully induced hyperlipidaemic in rats. Treatment with ethanol extracts of leaf (Group III) and bark (Group IV) of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* at the doses of 100 mg/kg (Group V) significantly reduced the serum TC, TG, LDL-C, VLDL-C and PL levels and significantly increased the serum HDL-C levels when compared to the hyperlipidaemic control group. The changes in lipid profiles levels in group II, III, IV and V were comparable with group VI of Atorvastatin treated rats.

Total lipid, total cholesterol and triglycerides levels in liver tissue and fecal matter showed a significant increase in Triton X-100 treated rats (Table 20 & 21). There were significant reduction in liver and fecal total lipids, total cholesterol and triglycerides levels at the dose of 100 mg/kg of leaf and bark extracts of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* respectively.

**Hepatoprotective activity**

The effect of ethanol extracts of *Pleiospermium alatum* leaf and bark (Group IV and VI) and *Balanites aegyptiaca* aerial part (Group VIII) at 200 mg/kg body weight dose were compared with that produced by silymarin, a known hepatoprotectant (Group IX). 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 leaf and bark extracts of *Pleiospermium alatum* and *Balanites aegyptiaca* aerial part extracts treated rats were found to be
lower when compared with CCl₄ treated group (Table - 22). The decrease in the serum activities of SGOT, SGPT and ALP were found to be greater in Group VII and VIII (Balanites aegyptiaca) and followed by group IV and VI (Pleiospermium alatum) when compared to standard drug treated (Group IX). The levels of total protein, albumin and globulin (Table - 22) concentration were found to be significantly (p<0.05) reduced in CCl₄ treated rats when compared to normal control (Group I). After treatment with Pleiospermium alatum leaf (Group III and IV) and Pleiospermium alatum bark (Group VI) and Balanites aegyptiaca aerial part (Group VII) extracts and silymarin (Group IX), total protein, albumin and globulin were brought back to near normal levels.

Table - 23 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 VIII, followed by group VI (Table - 23). (The total protein concentration was found to be significantly reduced in CCl₄ treated rats when compared to normal control. The same was restored back in group IV, group VI, VIII and IX).

Table - 24 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 *Balanites aegyptiaca* (Group VIII) aerial part and silymarin (Group IX) 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 leaf and bark of *Pleiospermium alatum* and *Balanites aegyptiaca* aerial part to rats did not cause any significant change in the body weight (Table - 25) and on the libido of treated rats whereas weights of testis and other accessory sex organs were decreased significantly (p < 0.05) (Table - 25). Among the accessory sex organs, a significant weight reduction was seen in the caput and caudal epididymal segment. No change was observed in vas deferens (VD), seminal vesicle (SV) and prostrate.

**Sperm count and sperm motility**

Sperm motility and sperm density in caudal epididymal, significantly decreased (Table - 26) and the reduction was severe in bark extract of *Pleiospermium alatum* (Group III) followed by aerial part extract of *Balanites aegyptiaca* (Group IV) 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 *Pleiospermium alatum (leaf and bark)* and aerial part of *Balanites aegyptiaca* (p < 0.05) (Table – 26). Among the studied plants, bark extract of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* 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 – 27. 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 leaf and bark of *Pleiospermium alatum* and *Balanites aegyptiaca* aerial part (150 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 bark extract of *Pleiospermium alatum* (Group III) (Table - 28).

**Serum luteinizing hormone (LH) level**

Repeated treatment of male rats with the *Pleiospermium alatum* and *Balanites aegyptiaca* extracts for 14 days caused a dose related decrease in the serum level of LH (Table - 28). The level of decrease was statistically significant (p < 0.05).

**Serum estrogen level**

The ethanol extracts of Pleiospermium alatum and *Balanites aegyptiaca* (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 - 28). Highest level of estrogen increase was shown in bark extract of *Pleiospermium alatum* (Group III).

**Serum follicle stimulating hormone (FSH) level**

Pretreatment with ethanol extracts of leaf and bark of *Pleiospermium alatum* and *Balanites aegyptiaca* aerial part for 14 days caused an increase in the serum level of FSH in male rats compared to control (Table - 28). The increase in the serum level of FSH in male rats statistically significant when treated with *Pleiospermium alatum* and *Balanites aegyptiaca* (p < 0.01 and p < 0.05 respectively).

**Fertility test**

The results presented in Table - 29 shows that intragastric administration of extracts of leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* (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 to significant values (p< 0.05) in female impregnated by treated male rats when compared to controls.

**Antiinflammatory activity**

Table - 30 shows that the antiinflammatory activity of ethanol extracts of the leaf and bark of *Pleiospermium alatum* and aerial part of *Balanites aegyptiaca* significantly inhibited the rat paw oedema at 3rd hr post carrageenan were 72.16% and 56.33% for 100 and 200 mg/kg ethanol extracts of leaf of *Pleiospermium alatum*
respectively and 79.53% and 77.36% for 100 and 200 mg/kg ethanol extracts of bark of *Pleiospermium alatum* respectively and 50.31% and 42.51% for 100 and 200 mg/Kg ethanol extracts of *Balanites aegyptiaca* respectively.