5 RESULTS & DISCUSSION

Morphological characteristics are referred to the evaluation of herbs by colour, odour, taste, size, shape and special features like touch, texture, etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of herbs. The leaf of *Scoparia dulcis* Linn. is simple & pinnatified, green coloured, characteristics odour and sweet taste. The stem is woody type outer surface rough, fracture irregular & fibrous, odour characteristic, taste is sweet and colour is green.

In the microscopy of stem we found endodermis, cortex, chlorenchyma and needle shaped calcium oxalate crystals which may be considered as an identifying character of *S.dulcis*. The presence of xylem element with vessel, xylem elements, annular thickening vessels, bordered pitted xylem, uniseriate multicellular glandular trichomes and pith were reported.

Both upper and lower surface of *S.dulcis* leaf contains anomocytic stomata. It also contains unique sessile glandular trichome. Stem contains starch grains and calcium oxalate crystals in cortex region. Its vascular bundle contains spiral vessel, tracheid, fiber etc. The epidermis of stem has uniseriate multicellular glandular trichomes.

The root showed the presence of periderm containing brownish matter. The phellogen showed two rows of cells encircle the single layer of phelloderm. The cortex consists of 4-6 layers of tangentially oblong and radially compressed parenchyma cells. Some of the parenchyma cells contain starch grains. The phloem consists of sieve elements and companion cells. The xylem is lignified and consists of xylem elements such as parenchyma and trachieds. Cambium separates the xylem and phloem region. Medullary rays in the phloem region are non-lignified whereas lignified in the xylem region.

Leaf constant like, vein-islet number, veinlet termination number, stomatal number, number of epidermal cell, stomatal index and palisade ratio were monitored. The range of vein-islet number was found to be between 11.5-14.6, range of veinlet termination number was found to be between 16.1-20.4. The range of stomatal number was between 76- 79 (upper surface) and 82- 86 (lower surface), range of epidermal cells were between 477-532 (upper surface) and 435-491 (lower surface),
range of stomatal index was between 12.5±2 (upper surface) and 14.3±2 (lower surface) and range of palisade cells was between 6–9

Powder microscopy of the whole plant showed the presence of cork cells in surface view, epidermal cell, sclereids, xylem parenchyma, pericyclic fibre, cork cell & fibre. Starch granules, trichome, palisade layer of testa, endodermal cell and spiral vessels are also found.

The plant showed 5% total ash, 3% water soluble ash and 1.5% acid insoluble ash. Moisture content on dry weight basis was found to be 9.4%. The extractive value showed highest in methanolic extract (16.13%) and lowest in pet ether extract (1.61%). The extractive value of water soluble extract was bit closer to that of methanolic extract (15.3%). The foreign organic matter was found to be 1.2%.

The extractive value had minor difference in both cold and hot extraction process. Methanolic extract showed higher value in maceration (16%) and hot extraction (16.9%) as compared to water extracts (15% & 15.5%).

Preliminary phytochemical investigation of the whole plant was performed for its water and methanolic extracts. The study was performed to assess the presence of alkaloid, carbohydrate, glycoside, saponin, protein, amino acid, phenolic compounds, tannins and flavonoids by using suitable chemicals and reagents.

Alkaloid was present in Mayer’s & Hager’s test and absent in Wagner’s & Dragendorff’s tests for water extract. Methanolic extract showed positive result for all the above tests.

Qualitative phytochemical studies of carbohydrate & glycoside showed positive result in all the tests (Molish’s, Fehling’s, Borfoed’s, Borntrager’s and legal’s test) for water and methanolic extract.

The investigation of protein and amino acid were performed by using Millon’s, Biuret’s and Ninhydrin test and the finding showed positive result for methanolic extract in all the above tests but Biuret’s test showed negative result for water extract.

Investigation for phenolic compounds, tannins and flavonoids showed positive result for both the extracts in ferric chloride, lead acetate, gelatin and alkaline tests.
All the above preliminary phytochemical studies showed that the plant is a rich source of carbohydrates, glycosides, phenolic compound, tannins, flavonoids, saponin, proteins and amino acids. However, it also contains a modest percentage of alkaloids.

Thin layer chromatography finger print of *S. dulcis* methanolic extract was analyzed randomly by using different solvent system and visualizing media. Toluene, methanol, pet-ether, chloroform, methanol, n-hexane, ethylacetate etc. were used as mobile phase. Anisaldehyde and sulphuric acid were selected as visualizing media.

The plate one and two were run with toluene: methanol (80:20 and 75:25) solvent systems. 15 spots were shown in plate 1 and 9 spots in plate 2.

The plate 3 was run with pet-ether: chloroform: methanol (50:50:10) solvent system and 6 spots were shown.

The plate 4 was run with n-hexane: ethylacetate (90:10) solvent system and 5 spots were shown.

Fraction no. 90 was eluted with 80% chloroform and 20% ethylacetate which showed single spot on TLC plate (ethylacetate: n-hexane 90:10). Fraction no. 116 was eluted with 50% chloroform and 50% ethylacetate, which showed 2 spots on TLC plate (toluene: methanol 75:25). Fraction no. 117 was eluted with 50% chloroform and 50% ethylacetate showed 1 spot on TLC plate (toluene: methanol 75:25).

In the TLC plate p-anisaldehyde/sulphuric acid was used for detection of phenolic compound, sugars, steroids and terpenes. The constituents such as phenols, terpenes, sugars and steroids turn violet, blue, red, grey or green colours respectively when exposed to p-anisaldehyde /sulphuric acid as TLC detecting reagent.

Total carbohydrate percentage was analyzed by phenol sulphuric acid method, which showed that the plant contains 11.89 % of sugar.

The carbohydrate percentage was also analyzed by Anthrone method, which showed 9.87 % of sugar which was the same as the previous method.

The protein content was estimated by Lowry’s method and was found to be 6.23%.

Total phenol was estimated to be 4.9 % by using Folin-Ciocalteau reagent.
The ascorbic acid percentage was analyzed by colourimetric method. The percentage of ascorbic acid was found to be 0.81%.

Tannin was estimated by Folin-Denis method. The content of tannin was found to be 1.0%.

Chlorophyll was estimated in a spectrophotometer using the absorption coefficients and the calculated amount of chlorophyll was 1.35 mg total Chlorophyll /g tissue.

The percent α-amylase inhibition of MESD showed positive results. It inhibits the activity of α-amylase in the conversion of starch into maltose by slowing its activity. It showed 50% inhibition at a concentration of 80 (µg/ml) (Tab. 4-25). The percentage inhibition of α-glucosidase by MESD also showed 50.4% inhibition at a concentration of 80 (µg/ml) (Tab. 4-26). The IC$_{50}$ was found to be 80.35 which is 50% inhibition showed in Fig-4.19.

*In-vivo* study of MESD was done on streptozotocin induced diabetic rats. The effect of the treatment with extract and Glibenclamide on blood glucose concentration in diabetic rats after post-treatment days was shown in Table 4-27. The study was conducted on the 7$^{th}$, 14$^{th}$ and 21$^{th}$ days after induction of diabetes and analyzed for the determination of blood glucose level. All the values were found significant in both treated doses (200 and 400mg/kg) as compared to control and standard.

The TEP of MESD & WESD was determined as gallic acid equivalent and the results were found to be 32.24 & 38.32 mg/g of gallic acid respectively.

The TEP of MESD & WESD showed a concentration dependent antiradical activity by inhibiting DPPH radical as equivalent to gallic acid. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidants due to the formation of the non radical form DPPH-H by the reaction DPPH to the yellow coloured diphenylpicrylhydrazine. It has been found that cystine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (e.g., hydroquinone, pyrogallol, gallic acid) and aromatic amines (e.g., p-phenylene diamine, p-aminophenol) reduced and decolorized 1, 1-Diphenyl-2, picrylhydrazyl by their hydrogen donating ability. It appears that the TEP of MESD & WESD possess
hydrogen donating capabilities and act as an antioxidant. The scavenging effect increased with increasing activity of gallic acid, a known antioxidant used as positive control gave similar result as compared to TEP of MESD & WESD.

The defence mechanism of our body prevents us from the hazardous effects of free radicals by reducing these molecules with the antioxidant substances. The Fig.4.21 & 4.22 showed the reductive properties of sample extracts as compared to gallic acid, an established standard. The reductive ability was measured by Fe$^{3+}$-Fe$^{2+}$ transformation in the presence of TEP of MESD & WESD. Earlier we have observed a direct correlation between antioxidant activities and reducing power of certain plant extract \[^{[19]}\]. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant activity. The reductones break the free radical chain by donating a hydrogen atom \[^{[48]}\]. The present data on the reducing power of TEP of MESD & WESD suggested that it was an active antioxidant like gallic acid.

The effect of MESD on urine volume, electrolyte balance and creatinine clearance Glomerular filtration rate (GFR) were determined.

The diuretic effect of MESD was confirmed, when it caused significant increase in the urine volume (diuresis) in rats; this effect was similar to that of furosemide, a standard diuretic \[^{[7]}\]. In parallel with increase in the volume of urine, MESD also enhanced the urinary excretion of Na$^+$ and K$^+$. The mechanism of action by which diuresis was induced by MESD was also assessed by comparing the effect with that of furosemide, a high-ceiling loop diuretic. \[^{[49]}\] The reference drug, furosemide, increases urine output and urinary excretion of sodium by inhibiting Na$^+$/K$^+/2$Cl$^-$ symporter (co-transporter system) in the thick ascending limb of the Loop of Henle, while the thiazide diuretics inhibit the Na$^+$/Cl$^-$ symporter (co-transporter system) in the distal convoluted tubule, by competing for the Cl$^-$ binding site, and increasing the excretion of Na$^+$ and Cl$^-$ \[^{[49]}\]. Hydrochlorothiazide has been reported to increase the urinary excretion of both Na$^+$ and K$^+$ by 50–60% over controls after a single oral dose in normal rats \[^{[51]}\].

In the study we found that MESD 250 mg/kg BW increased the sodium concentration in urine from day 1 to day 8 but the potassium concentration in urine was increased less starting from day 4 continues till day 8. When MESD 500 mg/kg BW was
administered it showed significant increase in sodium concentration in urine but there was no increase in potassium in urine. Furosemide which was used as reference standard showed increase in sodium concentration in urine but not that of potassium unlike Hydrochlorothiazide which has been reported to increase the urinary excretion of both Na\(^+\) and K\(^+\).

In the sub-chronic study MESD and furosemide induced significant diuresis from day 1, urine output continued to increase until Day 3 (maximum effect), in case of MESD-250 mg/kg BW, until Day 5 in case of MESD-500 mg/kg BW and until Day 6 in case of furosemide after that it stayed stable until the last day of the study. Tolerance did not seem to develop to the diuresis stimulating activity of the plant extracts.

Both MESD and furosemide increased the sodium excretion. On the other hand, repeated administration of MESD 250 mg/kg caused a significant increase in urinary excretion of K\(^+\) beginning on day 1 and continued throughout the study period contrarily, where as furosemide and MESD 500mg/kg virtually had no effect on urinary K\(^+\) excretion for the entire 8 days.

Further, there was decrease in plasma electrolyte levels both of sodium and potassium in the sub-chronic studies, suggesting that probably the active principle(s) in MESD do not act like potassium-sparing diuretics.

The results of the present studies suggest that the effect of one or more of the active components of MESD have a furosemide like action.

The GFR is one important parameter to understand mechanistic regulation in the kidney. The MESD at 500 mg/kg dose and furosemide increased GFR significantly. The increase in GFR by MESD is possibly due to (a) a detergent like interaction with structural components of glomerular membranes affecting fluid filtration \(^{[56]}\), (b) a decrease in renal perfusion pressure, attributable to decrease in the resistance of the afferent arteriole, and/or increase in the resistance of the efferent arteriole \(^{[57]}\) and/or (c) direct effect on arterial pressure affecting glomerular blood flow \(^{[58]}\).

The present studies also support the ethnomedicinal use of *Scoparia dulcis* for its diuretic effect. Although, the active components remain unidentified, based on the pattern of excretion of water, sodium and potassium, it appears that the active
principles present in its methanolic extract may have a furosemide like activity. The plant extracts do not seem to have renal toxicity in rats at the dose studied. These findings suggest for the first time the mechanism(s) of diuretic action of *Scoparia dulcis* used in traditional medicine.

On the basis of above results it can be concluded that the methanolic extract of *Scoparia dulcis* produce dose dependent diuretic effect. The present data support the ethanomedical application of *Scoparia dulcis* as diuretic.

The potential antihypertensive activity of *Scoparia dulcis* was evaluated by the inhibition of the angiotensin converting enzyme (ACE), using a UV Visible spectroscopy. The IC$_{50}$ value of MESD was found to be 0.102mg/ml and WESD 0.124mg/ml (Table 4-37 and Figure 4.31 & 4.32). These values indicate that *Scoparia dulcis* has a good ACE inhibitor activity.

The MESD showed higher IC$_{50}$ in comparison to WESD and for this reason we have selected methanolic extract for its in-vivo study. It was observed that the *Scoparia dulcis* drug have the activity on the systolic blood pressure in case of DOCA induced hypertension in rats. All the value of tested MESD (200 and 400mg/kg) was found significant (p<0.01) systolic BP value in comparison to DOCA induced hypertension (Table 4-38 and figure-4.33).

The ACE inhibitor activity of *Scoparia dulcis* may be due to the presence of its chemical constituents like coumarins, phenols, saponins, tannins, amino acids, flavonoids, terpenoids and catecholamines [88]. Compounds belonging to these classes of natural products have been described to possess ACE in vitro inhibiting activity and, for this reason, *Scoparia dulcis* was selected for antihypertensive activity [89]. It contains terpenoids, which are responsible for numerous medicinal effects. Scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol and scopadulin are all biologically active. Additional identified terpenoids of broomweed include alpha-amyrin, betulinic acid, dulcioic acid, friedelin, glutinol and ifflaionic acid [90].

The *Scoparia dulcis* showed presence of tannins, flavanoids and triterpene that have good antioxidant activity, might be due to the antioxidant ability of this compound shown to reduce hypertension in experimental animal models. Several cohort studies have suggested that high intake of flavonoids may decrease the risk of coronary heart
diseases \cite{91, 92}. These findings suggest that systolic blood pressure has been remarkably controlled by treatment of MESD in DOCA salt hypertensive rats.

6 CONCLUSIONS

The claim made by the traditional healers has been confirmed by using modern technique. So, our phyto-pharmacological approach in search of a potent antidiabetic and antihypertensive drug has been contented. But further study may need to have investigation in detail about the characterization of the individual compounds responsible for particular disease. A formulation may also be developed for convenient route of administration.