5. Discussion

Botanicals or phytomedicines have always been a major component of traditional medical systems of healing in developing countries, which have also been an integral part of their history and culture. With deep concern and relevance to Indian medicinal plants and sense of realization about its medicinal value, the present research work is undertaken. In the present work, comparative pharmacognostical, phytochemical profiles and pharmacological activities like diuretic, anti-inflammatory and antimicrobial activities of whole plants of *Pedalium murex* and *Martynia annua* belonging to *Pedaliaceae* were studied.

Indian medicinal plants, *Pedalium murex* and *Martynia annua* are used in Indian Traditional medicine and in Folklore for many diseases. Though, *P. murex* is widely used, *M. annua* is not much used in Traditional or folk medicine. Since, the two species belong to the same Family (*Pedaliaceae*) and the medicinal potential of *M. annua* are not yet fully explored scientifically, the present work was undertaken with a view to compare their Pharmacognostical, Phytochemical and Pharmacological profiles, and to evolve Pharmacocophaeal standard. Whole plants of both the species are subjected to the present analytical and experimental study.
Pharmacognostical studies of *Pedalium murex* and *Martynia annua*

**Macroscopical Characters:**

Morphological studies on leaf, petiole, stem, root and flowers of *Pedalium murex* and *Martynia annua* show distinct diagnostic characters. The present study reveals that *Pedalium murex* shows morphological characters namely the presence of tap root system, soft and fleshy stem, alternate succulent leaves, pedicel with pair of yellow glands, free solitary flowers, four stamens, staminodes reddish, bilocular ovary with pendulous ovules, indehiscent drupe, conical seeds and oblong superimposed fleshy epicarp.

Morphological studies of *Martynia annua* show that the plant is a stout herb with tap root system with purplish green glandular hairy and sticky stem, leaves are opposite and palmate. Leaves with petiole of 7-14 cm long. Flowers are purple, yellowish, with one dark spot on each lobe. *Martynia annua* also possesses staminodes bud three in number, ovary globose, unilocular. The fruit is a drupe with two apical curved beaks.

**Microscopical Characters:**

Microscopical feautures of *P. murex* and *M. annua* are distinct and could be differentiated under each part of the two plant species.
Leaf:

T. S. of leaf of *P. murex* and *M. annua* reveal distinct characteristics. While the outline of leaf of *P. murex* is more or less planoconvex in shape with a shallow concavity on the adaxial side. T. S. of leaf *M. annua* show a prominent midrib with thin laminal wings. The midrib has a thick conical adaxial hump and a large circular abaxial part. It is 1.5mm thick in vertical plane and 1.15mm horizontal plane. Vascular bundle in *P. murex* is single and a two-winged planoconvex structure while that of *M. annua* is a horse-shoe shaped single large structure.

In lamina, *P. murex* has two layers of palisade cells with wider upper layer and a narrow lower layer while *M. annua* has a single layer of palisade layer with wider intercellular spaces.

Trichomes:

Morphology of trichomes in the two species show striking dissimilarity. Trichomes in *P. murex* are of two types viz. Pillar like and spherical while in *M. annua* three types of trichomes viz. club-shaped, brush-shaped and umbrella-shaped are present. Though all the trichomes are secretory in nature their size and shape have distinct features and are useful for identification. Gupta *et al.* (2006) also reported two types of glandular trichomes in *P. murex*. 
Anatomy of petioles of the two species also show distinct features. T. S. of petiole of *P. murex* show different shape at the distal and basal part. The terminal part of petiole is arc-shaped while at the basal part it is flat and wide. The vascular bundles are five in number with a large medium bundle and two lateral and two marginal bundles arranged in a horizontal row. T. S. of petiole of *M. annua* is wide, circular with a hollow central canal. Vascular bundles are numerous and arranged in a continuous cylinder.

**Stem:**

Microscopical characters of stems of *P. murex* and *M. annua* also show distinct variation and these characters could be used as diagnostic tools. T. S. of *P. murex* stem has the following zones: Epidermis, homogenous cortex, vascular ring and a homogenous pith. *M. annua* stem has the same zonal divisions, but the epidermis is beset with numerous glandular trichomes, the cortex is heterogeneous with collenchymatous peripheral zone and inner parenchymatous zone: the pith is very large.

**Root:**

Anatomical features of roots of *P. murex* and *M. annua* reveal distinct characters which could be used as diagnostic characters. Root of *P. murex* measures 1.0 – 2.5mm in diameter. T. S. reveal, outer epidermis, a narrow parenchymatous cortex (3-5 layers), secondary phloem zone (5-8 layers) and
the large central vascular cylinder. Vessels are (40-80µm dia.) solitary and diffuse. In thick root system, rays are fairly wide and straight. Root of *M. annua* varies in thickness from 3.5 – 7.0 mm. Cortex is broader and consists of 10-14 layers of tangentially elongated parenchymatous cells. Periderm is evident only in thick roots. Secondary phloem layer is wider than that in *P. murex*. Xylem vessels are larger (40 -100 µm wide) than that in *P. murex*.

Hence, based on the anatomical characters, *P. murex* and *M. annua* could be easily distinguished. Many medicinal plants whose identity were not known were identified based on morphological and anatomical characters (Mehrotra, 1984; Park et al., 1996; Yamaji et al., 1993; Kartnig et al., 1996; Tanker and Altun, 1997.).

**Fruit:**

The fruits of *Martynia annua* are drupes with thin epicarp, fleshy mesocarp and stony endocarp. Epidermal glandular trichomes are present on the fruit wall also. The mesocarp is well differentiated into palisade and spongy parenchyma like tissues. The palisade like zone has narrow elongated cells and is 650µm wide. The spongy zone consists of wider air chamber.
The endocarp comprises complex network of fibers and protrudes into the centre of the fruit which divides the fruit into four false chambers. The seeds are with spiny surface.

Compare to *M. annua*, epidermal fruit wall of *Pedalium murex* having single layer with rectangular cells along with unicellular trichomes of non-glandular types present. The stony endocarp of fruits of *Pedalium murex* contain schlerenchymatous cells.

**Powder analysis:**

Macerated powder of stem of *P. murex* revealed distinguished characters like, xylem fibers with wide lumen, simple pits and septate vessel elements measuring 150-300 μ in length. Root powder exhibited long vessel elements with short or no tail; perforation plate is simple and oblique or horizontal. Xylem fibers of root are wider with 500-600 μm in length.

Organoleptic and analytical values of powders of *P. murex* and *M. annua* showed both similarities and dissimilarities. Dissimilar characteristics of the powders could be used for diagnostic values. Organoleptic characters of the powders of the two species like colour and taste are of diagnostic values; *P. murex* powder is pale green and bitter while that of *M. annua* are dark green and tasteless. Loss on drying of powder of *M. annua* was more (12.86%) than that of *P. murex* (8.27%). No significant difference could be
observed in ash values of powders. Level of potassium is more in *P. murex* powder (2.78%). Extractive values in different solvents also showed distinct variation. Extractive values of *P. murex* powder in pet.ether and acetone were more (3.18% and 5.02% respectively) than that of *M. annua* whereas extractive values in Chloroform and water are more in *M. annua* than that in *P. murex*.

Fluorescent study of powders of both species also revealed distinguished characters. Though in most of the reagents the colour characteristics are similar, treatment with ammonium hydroxide, ferric chloride and Sodium nitroprusside showed diagnostic colour for powders of *P. murex* and *M. annua* which could be used as diagnostic characters.

Real problem in identification of herbal drugs comes when the drugs are in powder form. Powder microscopy is an important parameter to identify and distinguish the drug from its substitute and adulterants (Patel and Satakopan, 1979; Srivastava and Srivastava, 1988; Asif and Shafiulla, 1993; Park et al., 1996; Hashmi and Ahmad, 1997).

**Qualitative Phytochemical analysis:**

Qualitative phytochemical analysis of whole plant extracts of *P. murex* and *M. annua* showed more or less similar characteristics. Presence of alkaloids, glycosides, saponins, proteins, fixed oils, tannins and phenols,
flavonoids and gums and mucilage are present in both species. Phytosterol is present only in *P. murex* and more amount of alkaloids and gums and mucilage are present in *P. murex*.

**Fluorescent analysis:**

Florescent analysis of fruit powders of *M. annua* and *Pedalium murex* in both day and U.V light show similar characters when treated with ammonium oxalate, Iodine solution, Picric acid and Nitric acid.

Treatment of powders with acetic acid, ammonium hydroxide solution, ferric chloride and sodium nitroprusside revealed diagnostic characters.

**Mineral composition:**

Mineral composition of alcoholic extracts of *P. murex* and *M. annua* revealed presence of all the mineral elements estimated. In *P. murex* the following elements are present in higher amount than that of *M. annua*: total nitrogen, total phosphorous, potassium, manganese, alkaloids, glycosides and serpentes. Whereas, while Molybdenum is equal in amount (0.01), organic carbon, sulphur, zinc, copper, iron and boron are present in higher amount. Heavy metals were absent in both species. Nutritionists have long recognized the interaction of trace minerals with other nutrients including protein, macro elements and vitamins (Mills, 1985).
Phosphorous is estrogenic, immuno stimulant and anti-osteoporotic (Duke, 2000). Potassium is essential for smooth flow of communication signals from cell to cell and its deficiency can contribute to diseases like stroke, heart problem, diabetes and hypertension (Singhal, 2002). Copper is an important factor in the pathogenesis of human atherosclerotic cardiovascular diseases (Sandsread, 1983). Zinc plays a major role as catalytic roles in over 200 enzymes and capable of influencing immune system (Mills et al., 1982).

**Phytochemical studies**

Estimation of minerals in *Pedalium murex* confirmed more amount of total nitrogen, potassium, magnesium, manganese, alkaloids than that of *Martynia annua*. But in *Martynia annua*, amount of ash, organic carbon, calcium, zinc, iron, flavonoids are more than that in *Pedalium murex*.

**TLC Profile:**

TLC profiles showed difference in number of bands and $R_f$ values in mobile phases like BAW, TBA, Water, Ferosol confirm that the species differ from one another. And in 60% alcohol presence of a band in *P. murex* (0.88) and in *M. annua* (0.86) with more or less similar $R_f$ values confirm that this two species relate one another.
The \( R_f \) value of aqueous extracts of *Pedalium murex* and *Martynia annua* lend support for establishing phytochemical affinities and differences between them. The varying spots of *Martynia annua* and *Pedalium murex* in TLC confirmed that the biological active principles in those two species differ from one another.

TLC profiles of aqueous extracts of *P. murex* and *M. annua* showed distinct characteristic with only a common spot with similar \( R_f \). Hence, TLC profile could be used as a diagnostic tool to identify the two species. Major active principle or marker compound present in Pedaliaceae member, Pedalisin and Pedalin (Subramanian Sankara and Nair, 1972) and diosgenin (Mangle and Jolly, 1998) have to be estimated and compared in further study.

**GCMS Studies:**

GC-MS studies on both aqueous and alcohol extract revealed the presence of 28 compounds, where Oleic acid, constituted the major part in both *Pedalium murex* and *Martynia annua* but it is far appreciable amount in *Pedalium murex* than *Martynia annua*. In phytochemical profiles, qualitative and quantitative difference is found between the two species.

GC MS studies of alcohol and aqueous extracts of *P. murex* and *M. annua* have shown both similar and dissimilar biological active compounds,
within and between the two species. Many biological active compounds are present in both alcoholic and water extracts of *P. murex* and *M. annua*. Common major biological compounds present in both the species are oleic acid and n-hexa-deconic acid. Presence of linoleic acid, malvolic acid, oleic acid, palmitic acid and stearic acid in seeds of *M. annua* are reported earlier (Tayal and Dutt, 1939). Oleic acid has anti-inflammatory activity, n-Hexadeconic acid (Palmitic acid) has anti-oxidant, hypocholesterolemic, nematicide and pesticide activities. Pedalitin (3,4′,5,6 tetrahydroxy-7-methoxyflavone), diosmetin and dinatin isolated from the leaves of *Pedalium murex*. 5,7- dimethoxy -2′. 4′. 5’ – trimethoxyflavone and tricontanyl dotriacontanoate along with lutcolin, rubusic acid and nonacosane-β-D-glucoside isolated from fruits of *Pedalium murex* (Rastogi and Mehrotra, 1992).

**Pharmacological studies**

**Anti-inflammatory activity**

Pharmacological evaluation of whole plant alcoholic and water extracts of *P. murex* and *M. annua* was carried out for anti-inflammatory, diuretic and anti-microbial activities. In both acute and sub-acute anti-inflammatory experiments. *P. murex* extracts showed higher anti-inflammatory activity than that of *M. annua*. Out of both species, *P. murex*
showed higher anti-inflammatory activity. Alcoholic extract of *P. murex* at 500 mg/Kg B.W. showed the highest reduction (38.46 per cent) in inflammation. The activity was dose dependent.

Higher anti-inflammatory activity of *P. murex* extracts could be attributed to the presence of higher amount of linoleic acid and oleic acid which are anti-inflammatory and anti-oxidant in activities. Alcoholic extracts of PM and MA contain both linoleic acid and oleic acid whereas water extracts have only oleic acid.

**Diuretic activity**

Diuretic activity of alcohol and water extracts of *P. murex* and *M. annua* was evaluated. Significant diuretic activity of alcohol extracts of *P. murex* and *M. annua* was observed. Alcoholic extracts of both species showed dose dependent diuretic activity. Maximum diuretic activity was exhibited by alcoholic extract of *P. murex* at 500 mg/Kg B.W. dose. At this dose *P. murex* exhibited aquaretic, kaliuretic and chloroureteric activity. The dose is also safer as Na+/K+ ratio is 0.94 which is almost equal to control and standard drug. High potassium content of *P. murex* and other diuretic biological compounds are responsible for the safer diuretic effect. Further activity guided fractionation, separation, isolation and chemical characterization are needed to confirm this effect.
Antimicrobial activity

Antimicrobial activity of the both the extract of *P. murex* and *M. annua* have marked effects only on bacteria but not effective against fungi. *P.murex* shows positive results in benzene and chloroform extracts but *M. annua* shows positive effects in pet.ether, chloroform and alcoholic extracts.