### RESULTS DISCUSSION

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Chapter – 6

RESULTS DISCUSSION

Advancement of modernization medicine, even the herbal medicine rules.

6.1. PHARMACOGNOSTICAL STUDIES

The reliability for identification and evaluation of plant source drugs results were resulted by studying the plant pharmacognostically.

Macroscopy

The macroscopical features of the leaves of *Myxopyrum smilacifolium* and *Pamburus missionis* were studied and shown the color of the plant *Myxopyrum smilacifolium* is greeny, elliptical, petiolate, acuminate apex with obscurely serrate margin measures about 14x4 cm sized, with odor of characteristic and bitter taste. The macroscopy of *Pamburus missionis* leaves shown of dark green, oblong-ovate in shape with entire margin and measures about 10x4 cm sized. It shown aromatic odor and taste.

Microscopical study

Transverse section of leaves of both plants named *Myxopyrum smilacifolium* and *Pamburus missionis* were studied.

**Microscopy of *Myxopyrum smilacifolium***

The microscopical study of *Myxopyrum smilacifolium* were shown that the leaf is bifacial and consists of smooth thick even lamina and planoconvex. The planoconvex midrib has flat adaxial side and semicircular abaxial side. The midrib 300mm thick and the abaxial part is 350mm wide. The midrib has small, slightly concave collateral vascular bundle. The bundle consists of short, thin, vertical files of about xylem strands; the xylem elements are an angular wide and thick walled. Phloem occurs on the lower part of the xylem strand. The ground tissue of the midrib is parenchymatous. The lateral vein is also planoconve measuring 250micrometer in thickness. It had small bowl shape collateral vascular bundle which collateral with adaxial a few rows of short xylem strands abaxial phloem elements. Discontinuous segments sclerenchyma elements are seen on the lower and of the vascular bundle;
Sclerenchyma layer is also seen on the upper part of the bundle. Glandular trichomes frequently seen both on the adaxial and abaxial epidermal layers of the leaf. The gland is capitates type and located with deep epidermal pits. It consists of a thick short stalk cell and spherical or umbrella shaped head. The glands are 60 micrometer in height and the head part is 55 micrometer wide.

The lamina is fairly with even, smooth surfaces. The adaxial and abaxial epidermal layers are fairly thick, the cells being squarish or rectangular with thick cuticle. Stomata are seen only on the abaxial epidermis. The palisade cells are short, wide and single layered. The palisade zone is 30 µm in height. The spongy parenchyma cells 6 layered. They are spherical, lobed and loosely arranged with intercellular spaces and the lamina is 190 µm.

The marginal part of the lamina is slightly curved down and it is conical in shape measures about 120 µm thick. The epidermal cells along with the margin are small with thick cuticle. The palisade spongy mesophyll differentiation is suppressed and cells are circular or angular and compact.

The epidermal cells were studied from surface of paradermal sections of the lamina. The adaxial epidermal layer of cells are small, with thick wavy anticlinal walls appear amoeboid in outline and the epidermis is apostomatic. The cells surrounding the stalk of the glandular trichome form two to more circles around the cavity where the stalk is inserted. These encircling cells are called rosette cells. The abaxial epidermal cells are having tetracytic type and guard cells are elliptical in shape with wide stomatal pore and the guard cells are 30x50 µm in size.

The venation is densely reticulate and the veins are thick and straight. These are well defined vein-islets which are polygonal in outline within the islets are thick and prominent. Vein terminations which are either unbranched or branched. The petiole was studied from proximal and distal parts. The proximal parts of the petiole is semicircular with deep narrow adaxial groove and two short blunt twigs. The petiole is 1.25 mm thick and 1.2 mm wide. The epidermis consists of squarish thick walled cells where the ground tissue is homogenous and parenchymatous. The cells are circular or angular and compact. The vascular system includes are wide and large bowl shaped main strand and 2 to 3 small circular less prominent vascular strands with petiole. The main bowl shaped vascular strand consists of several long vertical
xylem elements and abaxial are of phloem elements. The wing bundles have a few xylem elements and much reduced number of phloem elements. The distal part of the petiole is semicircular with deep adaxial groove and long thick curved wings. The structure of the distal petiole is similar to that of the proximal petiole. The distal petiole has a wide bowl shaped, collateral vascular strand and 2 to 3 small circular and less prominent.

**Powder microscopy**

The powder preparation of the leaf was examined under microscope and the following inclusions were observed. Adaxial epidermal peeling of the lamina is common in the powder. The peeling is surface view shows cells with wavy anticlinal walls and buried one to three circles of rosette cells. The glandular trichome has a circle of 6, secretary body cells which stain dark due to dent protoplast.

Abaxial epidermal peelings are seen in surface view. The epidermis has dense stomat which are tetracytic type, having four subsidiary cells surrounding the guard cells. Sclereids are foliar, which are long, narrow filiform, are seen mostly associated with veins of the leaf. Isolated from the veins are also seen in the powder. The sclereids have very thick lignified walls and reduced narrow lumen. The sclereids are about 40 µm long and 15 µm thick.

Fragments of palisade layer with epidermis are occasionally seen in the powder. The epidermal cells are cylindrical and rectangular. The palisade cells are vertical, pillars and compactly arranged.

**Microscopy of Pamburus missionis**

The leaf of *Pamburus missionis* shown dorsiventral symmetry, rigid midrib and the epidermal cells are of thick walled cells. Vascular bundle is of collateral. Xylem cells are of scalariform type which is of lignified. At the cortex portion it consists of Secretary cavities. The elongate polygonal cells are of lignified. Anamocytic type stomata was observed. Phloem fibres were not observed. It consists of unicellular trichome.
Quantitative microscopy of both plants leaves were performed for leaf constants namely Stomatal number, stomatal index, Vein islet number, Vein termination number and palisade ratio where it can establish the platform for the identification of future use.

**Physicochemical constant values**

In order to assess the purity and quality of plant drug it is needed to check the physicochemical parameters. An ash value is an earthy matter of the drug or it may of other impurities. Calcium oxalates are naturally occurring inorganic salts which were get stored in parts of the plant in different shapes of crystals. An incineration at controlled operations the crude drug resulted in ash residue of inorganic substances such as silica and salts of metals.

The evaluation of drug at its purity level is necessary as the parts of the plant may be adhered with extraneous matter or soil. The determination of both physiological and non-physiological combinely called as total ash. The total ash value of the sample was found to be around 1.74 and 2.36 % w/w respectively. The total ash differs in wider range due to variability in regard of natural or physiological ash as the ash is of digested acid in which the greater part of the ash solubilized where the silica is remained as the acid insoluble ash.

The acid insoluble ash of the sample was around 0.0034 and 0.086 % w/w respectively and water soluble ash was found around 0.16 and % w/w respectively. The deviations in these values represents in adulteration of the drug.

Extractive values give the report about the chemical constituent’s presence and their nature of solubility in the drug and it also useful for the determination of adulteration of the drug. The results were constrained in the Table No. 5.5 and it suggest that both the plant drugs were shown high water soluble extractive values i.e., of about 15.14 and 31.86% w/w indicates the presence of various constituents such as sugars, acids and inorganic substances and the ethanol extractive values were found to be around 11.36 and 37.60 % w/w indicates that the drugs contains polar constituents more.
The loss on drying for both the plants were shown of about 1.01 and 0.9 % w/w as these values represents that both the drug contains the moisture content very limited. If the drug contains more amount of moisture it leads to deterioration due to microbial growth or decomposition.

Evaluation of physic chemical constants for plant drug was useful to establish the standards to determine the quality of the plant drug.

The crude fibre content of both plants were tabulated in Table No. 5.7 and it was found to be around 32 and 36. The crude fibre content was more in the leaves of *Myxopyrum smilacifolium* than *Pamburus missionis*. The crude fibre content were essential as it prevents the constipation and enhances the stimulation of peristalsis.

**Flourescence analysis**

Illumination of phytoconstituents produces fluorescence and these illuminated fluorescence were specific for each and every compound. The fluorescence analysis of leaf of *Myxopyrum smilacifolium* shown Green color with distil water, Dark green with 1N HCl and Ethanol, moderate green with 50% HNO₃, Bluish green with FeCl₃, Blackish green with CHCl₃, Light greenish with picric acid. The fluorescence analysis of leaf of *Pamburus missionis* shown green with distilled water, Flourescent green with 1 N NaOH IN Methanol, picric acid and Ethanol, pale green with IN HCl and CHCl₃, Moderate green with 50% HNO₃, Olive green with FeCl₃ at long UV 366nm which has proven the presence of chromophore in the plant drug.

**6.2. PHYTOCHEMICAL STUDIES**

**Extraction**

The percentage yield and the nature of the extract of leaves of *Myxopyrum smilacifolium* and *Pamburus missionis* were tabulated in the Table No. 5.10. and the ethanolic extracts of both the plants have the yield high.

**Preliminary phytochemical analysis**

Preliminary phytochemical analysis was studied for the various solvent extracts of *Myxopyrum smilacifolium* and *Pamburus missionis* and the study revealed the
presence of phytoconstituents. The leaves of *Myxopyrum smilacifolium* consists of alkaloids, tannins, phenols, saponins, terpenoids, carbohydrates and glycosides. The leaves of *Pamburus missionis* consists of flavonoids, alkaloids, tannins, phenols, terpenoids, carbohydrates and glycosides.

**Quantitative estimation of phytoconstituents**

Quantitative estimation of phytoconstituents for alkaloids, tannins, glycosides, flavonoids, terpenoids for both drugs were performed and the results declared that the presence of high glycosidal content in *Myxopyrum smilacifolium* and high flavonoidal content in *Pamburus missionis*. These have shown to pick the suitable extract to study pharmacologically.

**6.3. PHARMACOLOGICAL STUDIES**

**6.3.1. Acute toxicity study:**

According to OECD-423 guidelines the acute oral toxicity study was progressed with the extracts. Healthy female adult rats were used and made fastened. The dose of 5, 50, 300, 500, 1000 and 2000mg/kg body weight of ethanol extract of leaves of *Myxopyrum smilacifolium* and *Pamburus missionis* were administered. After the drug treatment to the rats, they were observed for days of 14. The observations include intake of food, locomotion, body weight, grip strength of muscles, urination, faecel, water and food intake, tail erections etc was examined and it was observed that there is no changes in all behavior and no mortality rate was seen with dose of 2000mg/kg by oral route. By considering dose of 1/10th of the maximum tolerated dose, the effective dose of 200 and 400 mg/kg were selected for progressing the pharmacological studies.

**6.3.2. In-vitro antioxidant study:**

**1,1-Diphenyl-2-Picryl Hydrazyl radical scavenging activity**

The amount of DPPH reduction was quantified by measuring the antioxidant compound absorbance at 517nm. The extract quantity which is needed for 50% inhibition (IC50) of DPPH free radical shows better scavenging property. By observing the results, the ethanolic extract of *Myxopyrum smilacifolium* and
*Pamburus missionis* conquer the DPPH radical. At the concentration of 51.25µg/ml, 59.9 µg/ml shown antioxidant activity when compared with standard which had shown at 55.9 µg/ml depicted in Table No. 5.17 and Fig no.5.21. The results indicated that the extracts had potent antioxidant activity but the EEPM extract have more capacity to scavenge the DPPH radical than EEMS extract.

**Nitric oxide radical scavenging activity**

Nitric oxide is the free radical involves in relaxation of smooth muscle, platelet aggregation inhibition and toxicity regulation in cells. Over production of free radicals of nitric oxide results in pathogenesis of inflammatory diseases. The nitric oxide radical generation from sodium nitroprusside and measures by reduction of Greiss. Greiss reagent estimates the production of nitrate ions.

The nitric oxide scavenging activity of EEMS and EEPM was found to be around 89.8 µg/ml and 77.52 µg/ml where standard shown at 63.10 µg/ml. Both the extracts were shown the potent antioxidant activity but comparatively they were less activity with standard. The extract EEPM had shown good antioxidant activity when compared with that of EEMS which is depicted in Table no. 5.18 and illustrated in Fig. No. 5.21

**Hydrogen peroxide scavenging activity:**

The hydrogen peroxide scavenging activity of EEMS and EEPM were shown of about 130.20 µg/ml and 52.2 µg/ml the standard had shown at 53.12 µg/ml. Both the extracts EEMS and EEPM had shown potent antioxidant activity when compared with standard but EEMS had shown less potent when compare with EEPM and as well as standard which is depicted in Table no. 5.19 and illustrated in Fig. No. 5.21
6.3.3. Anti-arthritic study:

Acute Study

Carrageenan induced paw oedema

The acute study was made for the extracts of EEMS and EEPM by using Carrageenan Induced Paw Odema. The inflammatory parameters were observed which is as follows

Paw volume:

Paw volume is an index of paw inflammation, measured by plethysmograph on alternate days till 28th day. Both extracts of 200mg/kg and 400mg/kg of EEMS and 200mg/kg and 400mg/kg of EEPM decreased the paw volume from day 14th. Both the extracts of lower dose i.e., 200mg/kg of EEMS (p<0.01) and 200mg/kg of EEPM (p<0.01) had shown the anti-inflammatory activity but they were less effective when compared with that of the standard and 200mg/kg of EEPM had shown good anti-inflammatory activity when compared with that of 200mg/kg of EEMS. On 28th day the 400mg/kg of EEMS and EEPM (p<0.001) were shown significant activity when compared with that of the standard drug Diclofenac sodium at a dose of 10mg/kg.

Knee diameter:

Inoculation of CFA, increases knee diameter, signs as an inflammation for the ankle joint. On 28th day, decrease in the ankle joint were observed to the group treated with 200mg/kg of EEMS (p<0.01) and EEPM (p<0.01) but 400mg/kg of EEMS and EEPM (p<0.001) had shown decrease in knee diameter from 14th day.

Paw thickness:

On the 21st day, there was decrease in the paw thickness to the group treated with 200mg/kg of EEMS (p<0.01) and EEPM (p<0.01) but 400mg/kg of EEMS and EEPM (p<0.001) had shown decrease in paw thickness on 14th day.

Cotton pellet method

The results revealed that the extract at the dose of 400mg/kg of EEMS and EEPM had shown 35.96 and 33.06% inhibition in weight of dry cotton pellets, while
the extract at the dose of 200mg/kg had shown 31.81 and 23.44% inhibition in weight of dry cotton pellets when compared to that of control group animals as shown in the following Table no.5.25. Therefore the decrease in granuloma weight indicates suppression of the proliferative phases, which was effectively inhibited by extracts of EEPM and EEMS.

**Chronic study**

**Complete friends adjuvant method**

Adjuvant arthritis in rats is considered to be an immunologically mediated and most frequently investigated model of chronic inflammation. This model depicts the very close similarity with the clinical RA. The arthritis is induced by s.c. injection of CFA. The sub plantar injection of 50-100μl of this suspension results in primary, non immune, localized inflammatory response in the paw within 24 h, reaches a peak on day 4 or 5 and becomes stabilized on day 6-11. The systemic disease as usually starts on day 7. Foot thickness and body weight changes can be monitored in drug treated group and compared with that of untreated control group. The loss in body weight in arthritic condition has been reported to be associated with reduced absorption of glucose and leucine in rat intestine. This model allows evaluation of chronically administered drug against inflammation. There is a delayed systemic response to the CFA, which makes this model superior to other models in assessing the efficacy of all types of potential anti-rheumatic drugs.

The development of tests for compounds having specific anti arthritic activity has been hindered by inadequate knowledge of the etiology of RA. Most of the tests involve the production of inflamed or granulated tissue in animals, and have limited value because these tissue conditions have only a superficial relation to the RA in man. The tests are unapt to detect compounds that modify the arthritic process at a stage before or after development of inflammation.

The present method is based on a syndrome believed to be more closely related to RA than any other tests. Anti inflammatory activity of most classes useful in medication is detected.

In this model of immunological mediated chronic synovial inflammation and
arthritis, macrophages play a central role. After activation, they are capable of synthesizing mediators such as PGE$_2$ and cytokines such as TNF-$\alpha$ and IL-1 in turn these synthetic products induce a production of variety of enzymes which initiates the bone and cartilage destruction.

In autoimmune disease, pro-inflammatory cytokines plays an imp role in RA and scientist intended to design drugs for suppression of this disease via anti-inflammation. Currently, therapeutically controlling inflammation is essential for the clinical management of many high prevalence human diseases. Drugs that block pro-inflammatory cytokines such as TNF-$\alpha$ and IL-1$\beta$ can improve outcomes of RA.

For the assessment of anti rheumatic activity of test compounds, the inflammatory parameters, heamatological parameters and radiological parameters were assessed.

**Inflammatory parameters:**

**Paw volume:**

It is an index of paw inflammation which is measured by plethysmograph on alternate days till 28$^{th}$ day. Both the extracts of 200mg/kg and 400mg/kg of EEMS and 200mg/kg and 400mg/kg of EEPM decreased the paw volume on day 14$^{th}$. Both the extracts of lower dose i.e., 200mg/kg of EEMS ($p<0.01$) and 200mg/kg of EEPM ($p<0.01$) had shown the antiarthritic activity but they were less effective when compared with that of the standard and 200mg/kg of EEPM had shown good antiarthritic activity when compared with that of 200mg/kg of EEMS. On 28$^{th}$ day the 400mg/kg of EEMS and EEPM ($p<0.001$) were shown good significant activity when compared with that of the standard drug Diclofenac sodium at a dose of 10mg/kg. Comparatively among both the extracts EEPM at 400mg/kg had shown equivalent decrease in paw volume with that of standard drug and shown significant statistically

**Knee diameter:**

Due to inoculation of CFA, there was an increase in the knee diameter which signs as an inflammation for the ankle joint. On the 21$^{st}$ day, there was decrease in the ankle joint to the group treated with 200mg/kg of EEMS ($p<0.01$) and EEPM ($p<0.01$) but 400mg/kg of EEMS and EEPM ($p<0.001$) had shown decrease in knee
diameter on 14\textsuperscript{th} day and comparatively 400mg/kg of EEMS ($p<0.001$) had shown less effective compared with that of standard drug.

**Paw thickness:**

On the 21\textsuperscript{st} day, there was decrease in the paw thickness to the group treated with 200mg/kg of EEMS ($p<0.01$) and EEPM ($p<0.01$) but 400mg/kg of EEMS and EEPM ($p<0.001$) had shown decrease in paw thickness on 14\textsuperscript{th} day and comparatively 400mg/kg of EEMS ($p<0.001$) had shown less effective compared with that of standard drug.

**Haematological parameters:**

There will be moderate rise in WBC count and reduction in Hb count in arthritis results in reduced erythropoietin levels. The current study reveals that extracts of 200mg/kg and 400mg/kg of both EEMS and EEPM and standard drug significantly decreased the WBC count, increased Hb level and RBC count, decreased arthritic index and reduced the levels of RF factor.

**Radiological analysis:**

All the above parameters were made supported by making out of x-rays at the joints and made reported in Fig no. 5.38

**6.4. Spectral analysis**

The spectral analysis of isolated compounds from *Myxopyrum smilacifolium* Blume and *Pamburus missionis* Swingle revealed the details of molecular formula and structure elucidated and it may be of apigenin and irridoid glycoside in *Pamburus missionis* *Myxopyrum smilacifolium* respectively.