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Effect of Geinstein on Regulation of Estrous Cycle in Albino Rats, Isolated from *Flemingia vestita*, an Ethnomedicinally Important Plant of Meghalaya, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Flemingia vestita is a flowering plant belonging to the family of Fabaceae which is usually found in the Indian region including Khasi and Jaintia Hills of Meghalaya, India. It is cultivated for therapeutic use for its anti-microbial, anti-fungal, anti-helmintic, anticancer, anti-rheumatic, anti-inflammatory, antioxidant, and anti-histamine activities. The present study is to ascertain the phytochemical analysis of the root-tuber extract and modulating the effect of the Estrous Cycle in albino mice using

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Geinstein. The phytoestrogens present in the extract are iso-flavones and genistein which have a wide spectrum of biological activities including estrogenic effect. The number of leucocytes during the Meta-estrous at 0.1 ml/10 gm dose/body weight was maximum at ethanol extract while at 0.3 ml/10 gm dose/body weight was similar and maximum in both ethanol and methanol extract. Comparative cell number of interstitial connective tissues was maximum in ethanol followed by methanol and acetone extract. The present study concluded that the extract has a potential to modulate the estrogenic cycle in albino rats.

Keywords: Flemingia vestita; genistein; ethnomedicinal; estrous cycle; Meghalaya.

1. INTRODUCTION

Around 40-50% of women worldwide suffer from female reproductive diseases, such as endometriosis, uterine fibroids, gynecologic cancer, HIV/AIDS, interstitial cystitis, polycystic ovary syndrome (PCOS), sexually transmitted diseases (STDs). In some cases, these conditions can be fatal and hinder pregnancy. Estrogen plays a crucial role in, regulating the physiological and pathological processes in the reproductive system, cardiovascular, skeletal, endocrine, nervous, and immune systems both in male and female, it is also involved with infertility, endometriosis, polycystic ovary syndrome, and various other cancers. The role of estrogen in the female reproductive system and the development of secondary sexual characteristics are primarily recognized as the most significant function. The cellular receptors of estrogen are crucial mediators of estrogen functions, which include the nuclear receptor family (estrogen receptors (ER) alpha and ER beta) and membrane estrogen receptors (mERs; G protein-coupled receptor 30 (GPR30) (Tang et al., 2019; Revankar et al., 2005). Estrogen also important in other neurotransmitter systems: bym increasing the activity of nor-adrenaline which acts as a cholinergic agonist, and may decrease dopamine D2 receptor sensitivity (Revankar et al., 2005; Meihard et al., 2014).

Flemingia vestita commonly known as “Soh-plang” which means “Earth-nuts” is a species of flowering plant belonging to the family of Fabaceae which is usually found in the Indian region including Khasi and Jaintia Hills of Meghalaya, India. *F. vestita* is widely found in the Sichuan and Yunnan areas of China, with a very trace amount in Laos, the Philippines, Vietnam, and Nepal (Muliar et al., 2005). It grows on the slopes of mountains at high altitudes ranging between 1800–2100 m (Gavade et al., 2019). It is a seasonal tuber crop grown between mid-October to February. It is a perennial herb that has a prostrate but weak stem, several branches, and a hairy rhizome. It fixes nitrogen and has a distinctive tuberous root (Marboh and Mahanta, 2006). It was first discovered in 1991 that an isoflavone (Genistein) that was extracted from the tuber extract was the main anti-helmintic ingredient and was extremely strong against trematodes and cestodes (Madan et al., 2013). The locals of Garo, Jantia, and Khasi Hills directly consume the raw tuber or root peel to treat intestinal worms.

In-vitro efficacy of tuber peel extract against several helminth parasites was studied experimentally in 1996 (Roy and Tandon, 1996; Tandon et al., 1997). Genus *Flemingia* has been investigated for their major pharmacological effect, inclusive of antimicrobial, antifungal, anthelmintic, anticancer, anti-rheumatic, anti-inflammatory, antioxidant, and anti-histamine activities (Madan et al., 2013). (Maginnes & Owusu-Apenten, 2017). The present study is to ascertain the phytochemical analysis of the *Flemingi avestita* and modulating the effect of the Estrous Cycle in Albino Mice using Geinstein, isolated from *Flemingia vestita*, an ethnomedicinally important plant of Meghalaya, India.

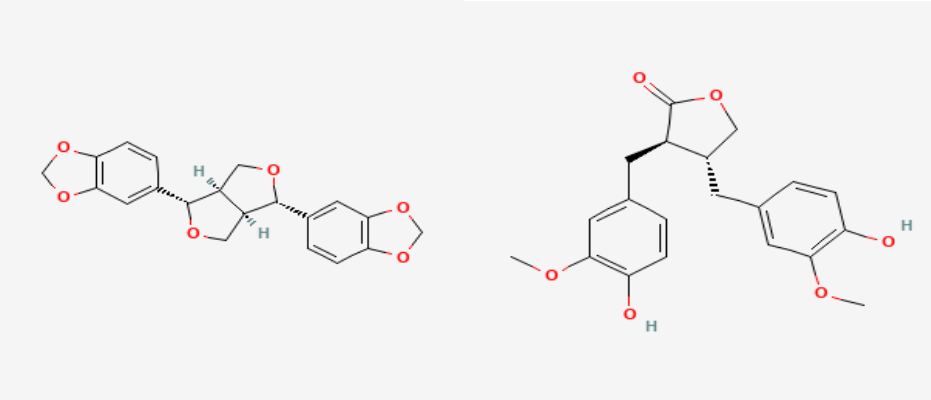
In terms of plant diversity, India stands at number four in Asia and tenth worldwide. The use of plants for their therapeutic properties had distributed among people from numerous generations (Roy and Tandon, 1996; Tandon et al., 1997). There are 16 different species of the genus *Flemingia* that have been identified, *F.strobilifera, F.chappar* and *F.paniculata* with unifoliolate leaves. The petiole was winged except for *F.chappar* and *F.vestita, whereas F. nana, F. paraecox*, and *F. sootepensis have much larger* wings while in *F.paniculata, F.strbilifera,* and *F.wightiana* they were just a small outgrowth (Thacker et al., 2021). *F. vestita* habitually known as soh-phlang is cultivated for therapeutic use, especially for its antihelmintic properties (Roy and Tandon, 1996; Tandon et al., 1997; Tandon et al., 2003).

F. vestita contain iso-flavones, and geinstein which have a wide spectrum of biological activities including estrogenic effect. These phyto-estrogens have affinity towards estrogenic steroids in human body, resembles the effects of naturally occurring estrogenic compounds especially estradiol (E2) (Roy and Tandon, 1996; Tandon, et al., 2003; Molina, et al., 2014; Shailajan, et al., 2013; Shailajan, et al., 2016).

1.1 Phytoestrogen

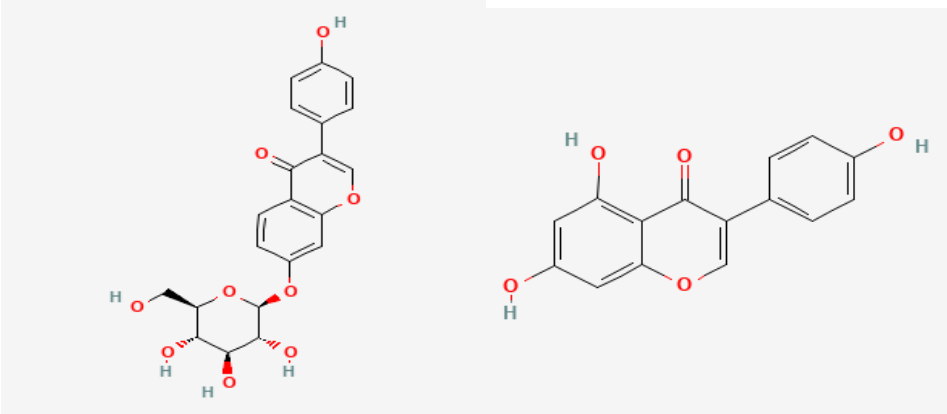
The family of exogenous estrogens, known as phytoestrogens, and represent polyphenolic and non-steroid compounds that have a similar structure and biological activity as human estrogens. There are more than 4000 phytoestrogens, they are divided into two chemical groups:a) flavonoids that are subdivided into isoflavones, coumestans and prenyl flavonoids, and b) non-flavonoids that mainly comprise lignans (Molina, et al., 2014; Nikolic, et al., 2014). The isoflavones of *F. vestita* is geinstein having a wide spectrum of biological activities includingestrogenic effect. These phytoesterogens have affnity towards estrogenic steroids in human body, resembles the effects of naturally occurring estrogenic compounds (Roy and Tandon, 1996; Tandon, et al., 2003; Molina, et al., 2014; Shailajan, et al., 2013; Shailajan, et al., 2016). The biological activity of phytoestrogens varies due to structural features and deviations in the structure. Isoflavones and flavones are the most well-known of the phytoestrogens, with isoflavones possessing a structure similar to that of E2 capable of binding to both ERs (Tandon, et al., 2003; Molina, et al., 2014; Shailajan, et al., 2013; Shailajan, et al., 2016; Turner, et al., 2007).

Non-Flavonoids

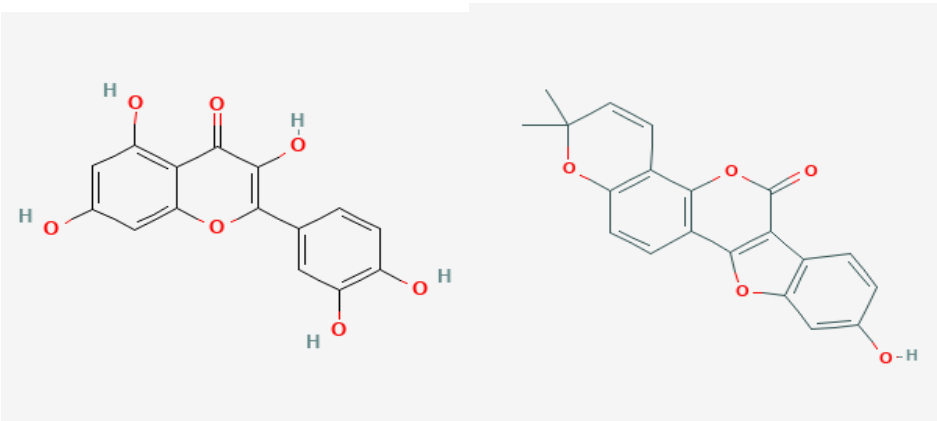


(A) Sesamin (lignans) **(B) Matairesinol (lignans)**

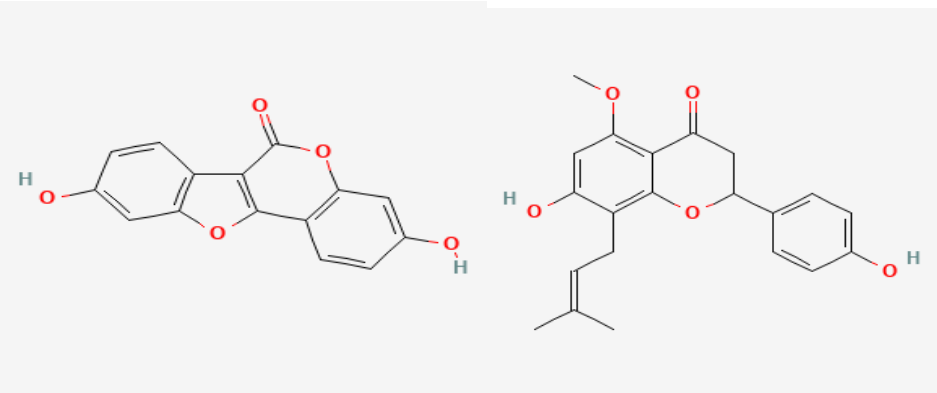
Flavonoids



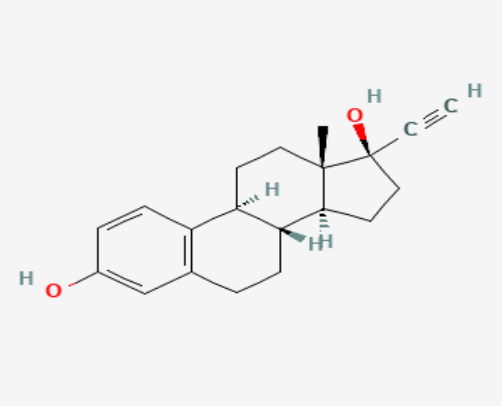
(C) Diadzein (Isoflavones) **(D) Geinstein (Isoflavones)**



(E) Quercetin (Isoflavones) **(F) Plicadin (Coumestans)**



(G) Coumestrol (Coumestans) **(H) Isoxanthohumol (Prenylflavonoids)**



(I) 17β-estradiol

Fig. 1 (A-I). ER Ligand Structure

Geinstein, a plant derivative extracted from root tuber of *Flemingia vestita*has been associated with a reduction in the risk of breast and prostate cancers, also conveys it's effect towards different biological pathway receptor including estrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR), peroxisome proliferator activated receptors (PPAR), insulin-regulated glucose transporter (GLUT), A1 adenosine receptor (A1AR) (Shailajan, et al., 2014; Taylor, et al., 2009).

Estrogens are the key regulators of cellular processes and are involved in the development and maintenance of the reproductive system. The physiological functions of estrogenic compounds are modulated largely by two estrogen receptors: alpha (ERα) and beta (ERβ)

(Molina, et al., 2014; Nikolic, et al., 2014). These types of proteins have actions in the cell nucleus, regulating transcription of specific target genes by binding to associated DNA regulatory sequences, estrogens have a neuro-protective effect and also reduce premenopausal mood fluctuations in women (El-Halawany et al., 2011; Paterni, et al., 2014). ERα is present mainly in mammary gland, uterus, ovary (thecal cells), bone, male reproductive organs (testes and epididymis), prostate (stroma), liver, and adipose tissue. By contrast, ERβ is found mainly in the prostate (epithelium), bladder, ovary (granulosa cells), colon, adipose tissue, and immune system. Both subtypes are markedly expressed in the cardiovascular and central nervous systems (Shailajan, et al., 2014; El-Halawany et al., 2011; Paterni, et al., 2014).

1.2 Swiss Albino Mice

Rats are often used to study the neural mechanisms underlying a wide variety of normal functions and patho-physiological conditions that occur in humans. The potential impact of hormonal variations between the sexes on the outcome of research as well as normal cyclic hormonal fluctuations in females requires the availability of more detailed information about the rat's reproductive cycle. The ''estrous cycle'' in female rats has four stages, proEstrous, Estrous, metEstrous and diEstrous. For the majority of animals, the estrous cycle lasts 4/5 days. The stage of the cycle can be determined by viewing at low microscopic magnification a sample of cells obtained from the surface of the vaginal epithelium. The cyclic differences in vaginal cytology occur in response to the morphological changes of the vaginal epithelium as cells desquamate (Shailajan, et al., 2014; Hubscher, et al., 2005, Ekambaram, et al., 2017). All the stages show specific cellular characteristics that can be observed in vaginal lavage. The 4 phases of the estrous cycle are characterized below:

1.2.1 Proetsrous phase

The 12-14-hour proEstrous stage is characterized by round nucleated cells of uniform size. Under the influence of estrogen the lining in the uterus (endometrium) starts to develop. During this stage, the vaginal epithelium is composed of 9-12 layers of cells with the mature cells at the surface. By the end of proEstrous stage, the surface layer of mature epithelial cells has shed and the stratum corneum is exposed (Long and Evans, 1922).

1.2.2 Estrous phase

The next stage, Estrous, lasts 25-27 hour, under the regulation of gonadotropic hormones, ovarian follicles mature and estrogen secretions exert their biggest influence, and is distinguished by the appearance of irregularly shaped, un-nucleated cornified cells (Long and Evans, 1922; Freeman, 1994).

1.2.3 MetEstrous phase

During metEstrous, lasting 6-8 hour, leukocytes infiltrate the thinned vaginal epithelium owing to a decline in estrogen secretion and pass into the vaginal canal. Vaginal secretions make the smear appear white and opaque (Long and Evans, 1922, Montes and Luque, 1988).

12.4 DiEstrous phase

The diestrous phase last for 55-57 hours, more than half of the cycle (Freeman, 1994). It is during this time the epithelium reaches its thinnest point (4-7 layers).It is during this phase the degeneration of the epithelium stops and the height of the epithelium increases again because of mitosis.

2. MATERIALS AND METHODOLOGY

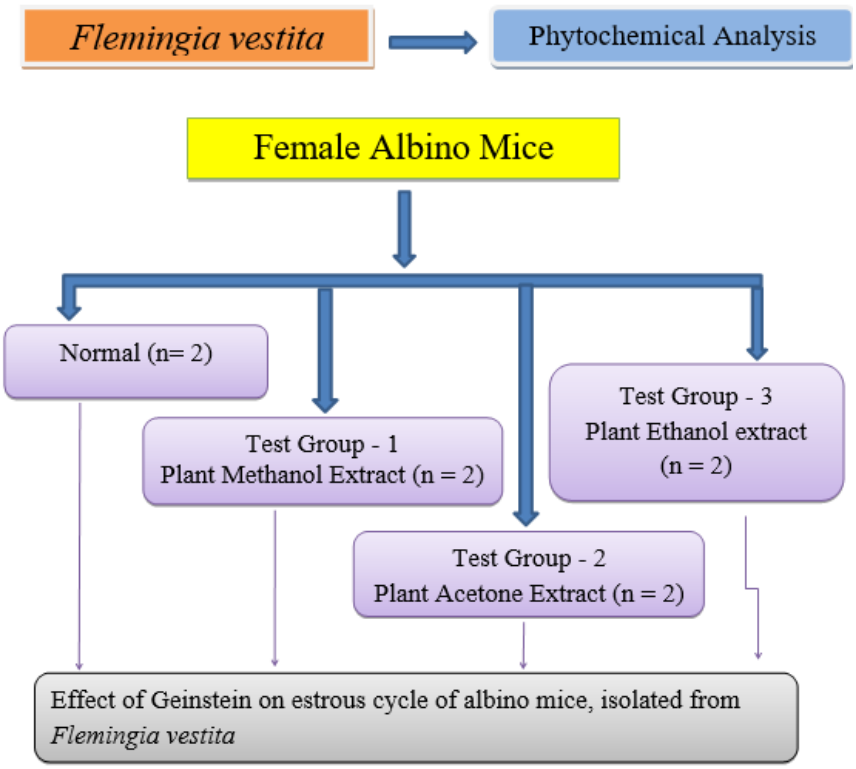
2.1 Materials

2.1.1 Parameters

1. Number of mice used: 8(Female Albino mice)
2. Weight of the mice.
3. Stool colour and quantity.
4. Amount of water intake.
5. Amount of food intake.
6. Amount of experimental solution intake.
7. Color of the vaginal secretion.
8. Smear of the vaginal secretion.
9. The physical examination of mice will be done by the veterinary doctor every week.

2.2 Collection of Plant

The north-eastern region of India has a variety of medicinal plants that are used to cure several diseases ranging from bacterial infection to cancer (Ojha et al., 2017).



Flow chart 1. Experimental Design

The fresh root-tuber of *Flemingia vestita* was collected in the month of January from Shillong. The roots were washed thoroughly to clear the dirt, and were thinly sliced. The slices are dried at 45-50℃ in a hot air oven and made into coursed into fine powder (Roy and Tandon, 1996; Tandon et al., 1997).

The general name and the taxonomical distribution of the studied plant given below

Plant profile Taxonomic classification (Muliar et al., 2022)

Kingdom: Planta
Phylum: Magnoliophyta
Class: Angiospermae
Category: Fabids
Order: Fabales
Family: Fabaceae or Leguminosae
Sub-family: *Faboideae papilionoideae*
Genus: *Flemingia*
Species: *vestita*

2.3 Experimental Animal

a. 12 Healthy female albino mice with body weight of 29 - 40gm at 8-10 weeks from birth were used for the investigation. All the animals were kept in the laboratory under the natural light and temperature. They were feed with routine diet like gram, corn and water. The experiment on mice was approved by Institutional Animal Ethics Committee **(IAHF/USTM/2023/P-5)**.
b. Animal strain used - Swiss albino mice.
c. The mice that are used for i nvestigation was obtained from University of Science and Technology, Meghalaya, India. The Mice was treated with solvent extract of *Flemingia vestita* for 25-30 days. The mice will not be killed or dissected or subjected to any kind of harm.
d. Classification of Swiss albino Mice

Kingdom - Animalia
Phylum - Chordata
Subphylum - Vertebrata
Superclass - Gnathostomata
Class - Mammalia
Order - Rodentia
Genus - *Mus*
Species - *musculus*

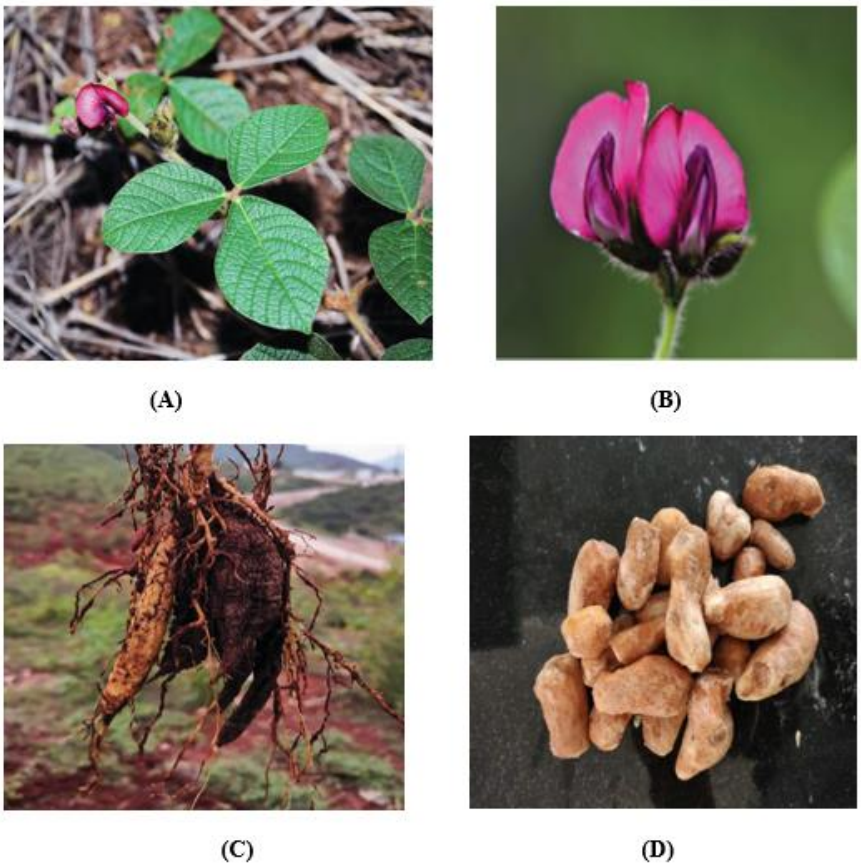


Fig. 2. *Flemingia vestita*, (A) Leaves, (B) Flower, (C) Root-Tuber (unpeeled outer skin), (D) Consumable raw tubers without the outer skin

3. METHODOLOGY

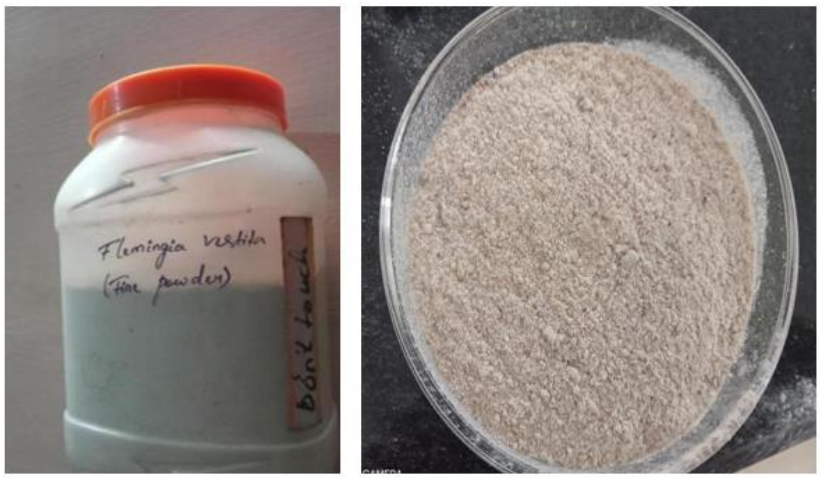
3.1 Preparation of Extract:

The fresh root-tuber of *Flemingia* was collected from Shillong in the month of January. The roots were washed thoroughly to clear the dirt, and were thinly sliced. The slices are dried at 45-50℃ in a hot air oven and made into coursed into fine powder (Roy and Tandon, 1996; Tandon et al., 1997).

Picture 1. Fresh root-tuber of *Flemingia*

Picture 2. Thinly sliced fresh root-tuber of *Flemingia*

Picture 3. Dried slices of root-tuber of *Flemingia*



Picture 4. Fine powder of root-tuber of *Flemingia*

3.2 Phytochemical Analysis

A different qualitative phytochemical test was performed for establishing a profile of various compound present in the extract. The following tests performed on extract to detects various phyto-constituents present in them (Tiwari et al., 2011; Raaman, 2006).

3.2.1 Detection of alkaloids

Harger's Test: 1gm of solvent free extract is stirred with dilute HCL and then filtered. After filteration, the filterate is mixed with 2ml of saturated aqueous solution of picric acid. A prominent yellow precipitate indicates the test as positive.

3.2.2 Detection of carbohydrates

Benedict's Test: 1gm of solvent free extract is dissolved in 45ml of distilled water. To 3 ml of filterate, 3ml of benedict reagent is added. A characteristic coloured precipitates indicates the presence of carbohydrates.

3.2.3 Detection of saponins

The 0.1g of extract is diluted with distilled water and made upto 40ml. The suspension is shaken in a conical flask for 15mins. A 2cm layer of foam indicates the presence of saponins.

3.2.4 Detection of proteins

Xanthoproteic Test: The 0.5g of extracts was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

3.2.5 Detection of fixed oil and fats

A Spot Test: A small quantity of extract is pressed between the two filter papers. Oil stain on the paper indicates the presence of fixed oil.

3.2.6 Detection of phenolic compounds and tanins

Ferric Chloride Test: 0.5gm of the extract is dissolved in 50 ml of distilled water. To this few drops of neutral 5% ferric chloride solution is added. A dark green colour indicates the presence of phenolic compounds.

3.2.7 Detection of gums and mucilages

The extract 0.3gm is dissolved in 30 ml of distilled water and to this 125 ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilages.

3.3 Preparation of Crude Extract

To obtain the bioactive compound from the sample, the crude extract is to be prepared following the method of Rao and Reddy (Roy and Tandon, 1996; Tandon et al., 1997). The crude extract is prepared using the sample and a solvent system of Ethanol, Methanol and Acetone at 3:10. The solution is maintained at a room temperature for 48 hrs. The extract is then filtered using Watmann filter paper 2 and the extract was prepared (Shailajan, et al., 2016).

3.4 Experimental Protocol

1. The experimental mice were divided into four groups (Test Group I-IV), n=3. 25mmol/L Na2CO3 at concentrations of 2.649 g/L (Ethanol extract, Acetone extract and Methanol extract) will be feed to the albino mice (Guo et al., 2001).
2. Doses (for first 2 weeks)
3. Group-1 (test group for methanol sample): mice feed with 0.1ml/10gm body weight using 2ml syringe and feeding tube.
4. Group-2 (test group for acetone sample): mice feed with 0.1ml/10gm body weight using 2ml syringe and feeding tube.
5. Group-3 (test group for ethanol sample): mice feed with 0.1ml/10gm body weight using 2ml syringe and feeding tube.
6. Group-4 (Control)
7. Doses (for another 2 weeks)
8. Group-1 (test group for methanol sample): mice feed with 0.3ml/10gm body weight using 2ml syringe and feeding tube.
9. Group-2 (test group for acetone sample): mice feed with 0.3ml/10gm body weight using 2ml syringe and feeding tube.
10. Group-3 (test group for ethanol sample): mice feed wth 0.3ml/10gm body weight using 2ml syringe and feeding tube.
11. Group-4 (Control)

3.5 Estrous Cycles Study

1) The mice were lifted by base of the tail with the dominant hand, then using opposite hand, the loose skin was grasped at the nape of the neck (between thumb and forefinger) the animal was lifted and restrained with one hand.
2) The mice were inverted so the remaining body of the mice rests in the palm of the

hand and exposing the vagina. A small earbud was taken and sterilized in 70% ethanol.

3) A clean slide is taken in which a 1-2 drops of water is placed centrally. Using the sterilized earbuds, vaginal lavage is gently collected in the slide.
4) The fluid is then allowed to dehydrate at room temperature.
5) After evaporation, 2-3 drops of methanol are added to the smear for fixing the cells in the fluid. After the cells are fixed and dried, then few drops of Leishmann stain are added. To remove the excess stain, after 5-7 minutes the slide is dipped into the distilled water. The slide was then observed under a binocular microscope at 10X magnification.

Picture 5. Ethanol extract of root-tuber of *Flemingia*

Picture 6. Acetone Extract of root-tuber of *Flemingia*

Picture 7. Methanol Extract of root-tuber of *Flemingia*

Picture 8. Filteration of root-tuber of *Flemingia*

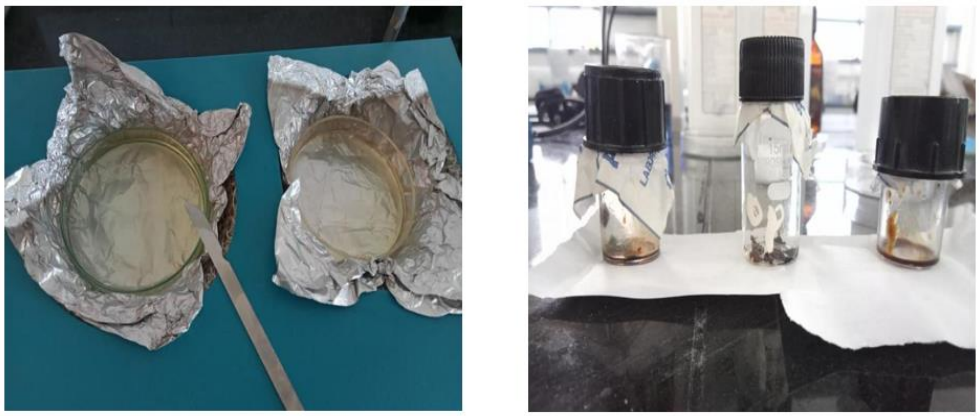
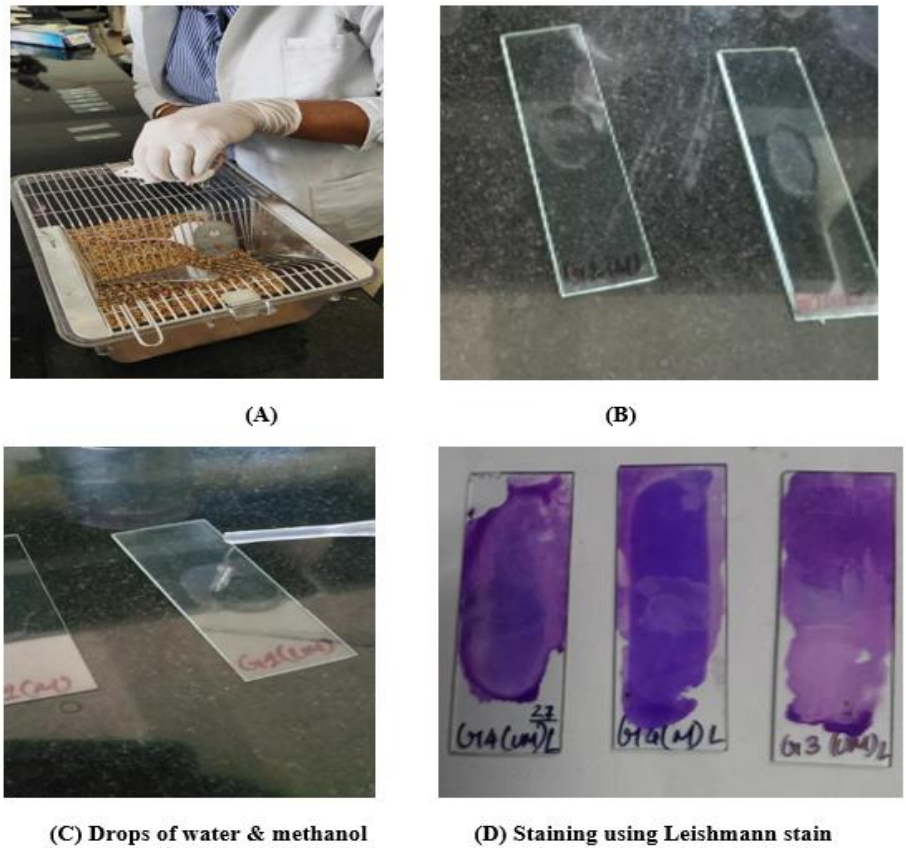


Fig. 3. Extract preparation of root-tuber of *Flemingia*

Fig. 4 (A-D). Sample feeding procedure



(A) (B)

(C) Drops of water & methanol (D) Staining using Leishmann stain

Fig. 5 (A-D). Process of estrous cycle study in albino mice

4. RESULTS AND DISCUSSION

4.1 Preliminary Phytochemical Analysis

1. Alkaloids is present in root-tuber extract of *Flemingia*
2. Carbohydrates is present in root-tuber extract of *Flemingia*
3. Saponins are found present in root-tuber extract of *Flemingia*
4. Proteins is present in root-tuber extract of *Flemingia*
5. Fixed oils and fats are absent in root-tuber extract of *Flemingia*
6. Phenols are found present in root-tuber extract of *Flemingia*
7. Gums and Mucilages are found present in root-tuber extract of *Flemingia*

Table 1. Preliminary phytochemical analysis of root-tuber of *Flemingia vestita*

Sl. No.	Phytochemical Screening	Root-tuber of *Flemingia vestita*
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Saponins	+
4.	Proteins	+
5.	Fixed oils and Fats	-
6.	Phenols	+
7.	Gums and Mucilages	+

(+) = Presence, (-) = Absence

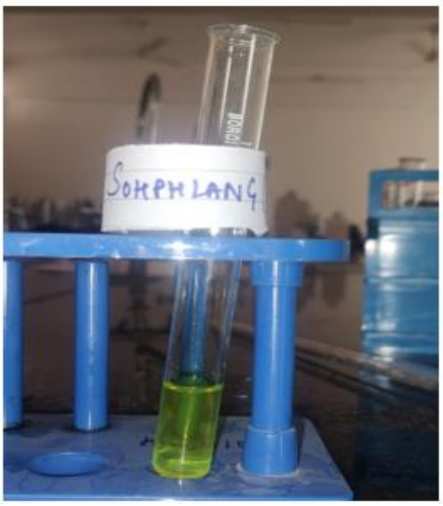


Fig. 6. Alkaloids test positive

Fig. 7. Carbohydrate test positive

Fig. 8. Fats and fixed oils test negative

Fig. 9. Protein test positive

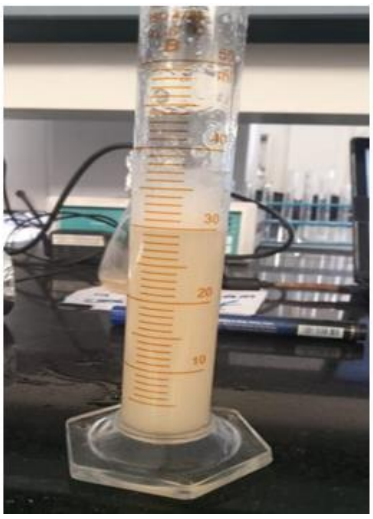


Fig. 10. Saponin test positive

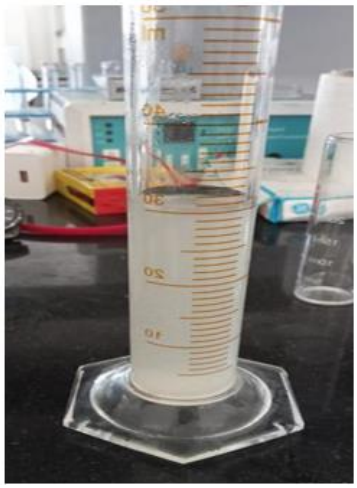


Fig. 11. Gums and mucilages test positive

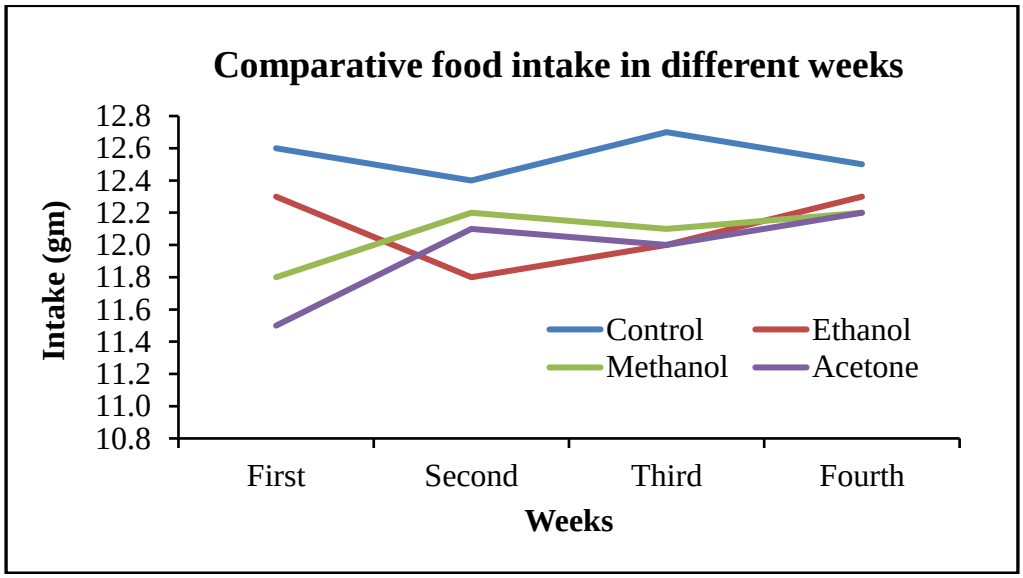


Fig. 12. Graphical representation of comparative food intake in different weeks

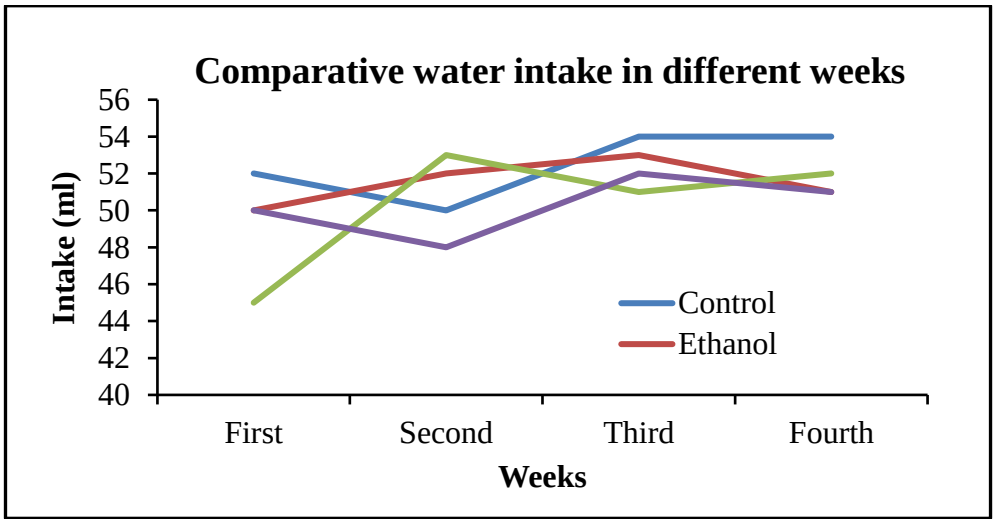


Fig. 13. Graphical representation of comparative water intake in different weeks

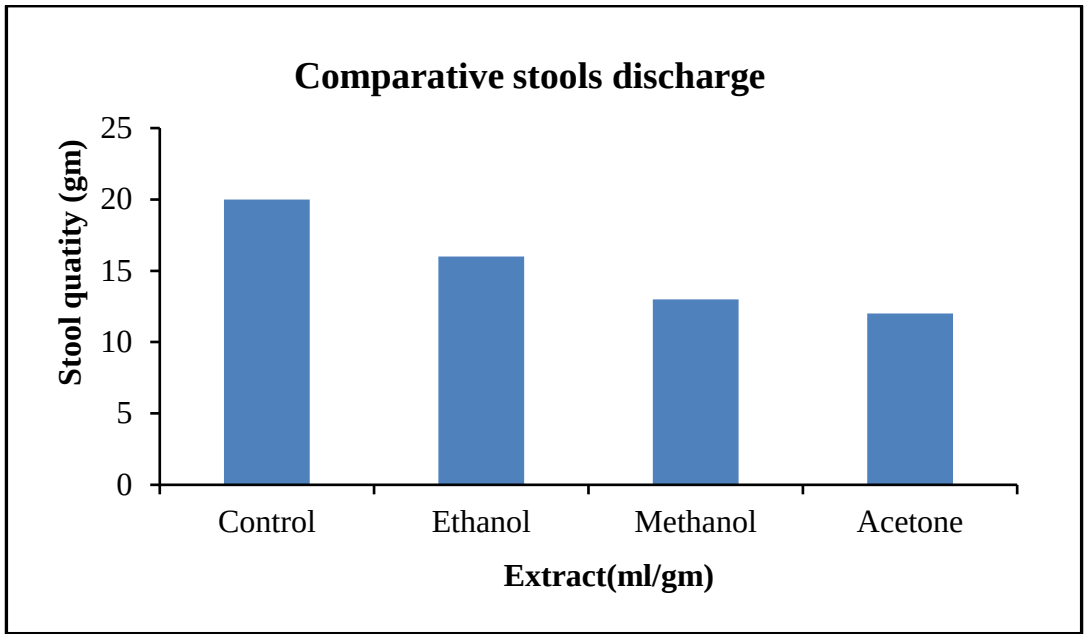


Fig. 14. Graphical representation of comparative stool discharge

Fig. 15. Stool coloration of mice after treating with different extracts

The preliminary phytochemical analysis of root tuber of *Flemingia vestita* shows presence of alkaloid, carbohydrate, saponins, proteins, phenols, gums and mucilages. During the course of experiment, the food and water intake was found to be highest in ethanol followed by methanol and acetone group. The comparative stool discharge was observed in the ethanol

group. The weight variation of both groups was found to be the maximum in methanol based food extract. The total cell number during pro-estrous at 0.1 ml/10 gm dose/body weight and 0.3 ml/10 gm dose/body weight was maximum in ethanol extract and minimum in acetone extract. The cell number during the estrous at 0.1 ml/10 gm dose/body weight and 0.3 ml/10 gm dose/body weight was maximum in methanol extract while the cell number during the Meta-estrous at 0.1 ml/10 gm dose/body weight and 0.3 ml/10 gm dose/body weight was maximum in methanol extract. The number of leucocytes during the Meta-estrous at 0.1 ml/10 gm dose/body weight was maximum at ethanol extract while at 0.3 ml/10 gm dose/body weight was similar and maximum in both ethanol and methanol extract. The cell number observed during the Di-estrous at 0.1 ml/10 gm dose/body weight and 0.3 ml/10 gm dose/body weight was found to be maximum in methanol extract. Comparative cell number of interstitial connective tissues was maximum in ethanol followed by methanol and acetone extract.

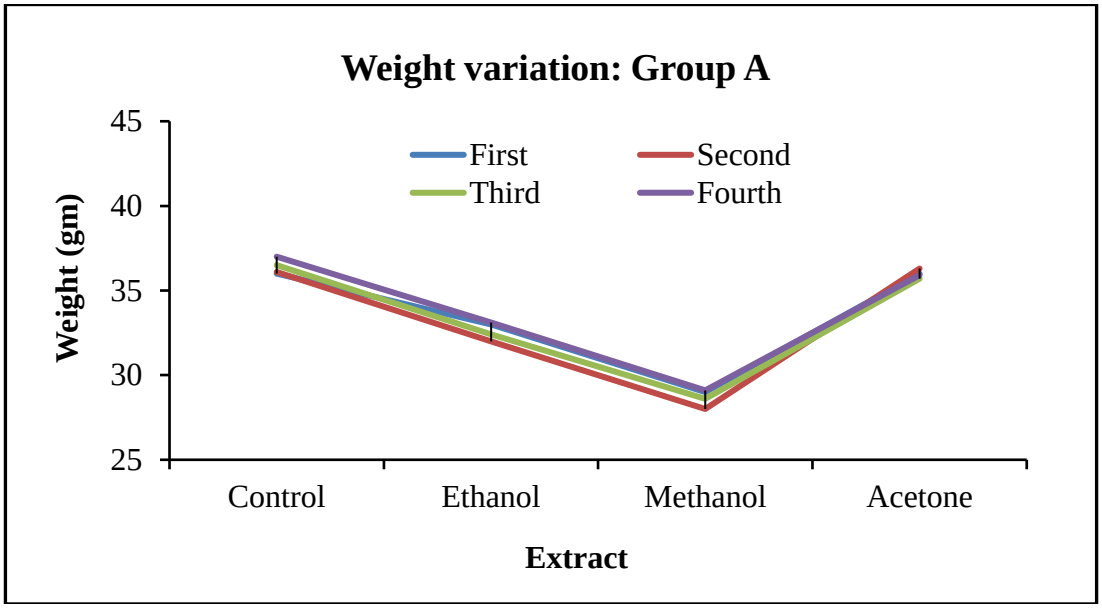


Fig. 16. Graphical representation of weight variation (after experiment)

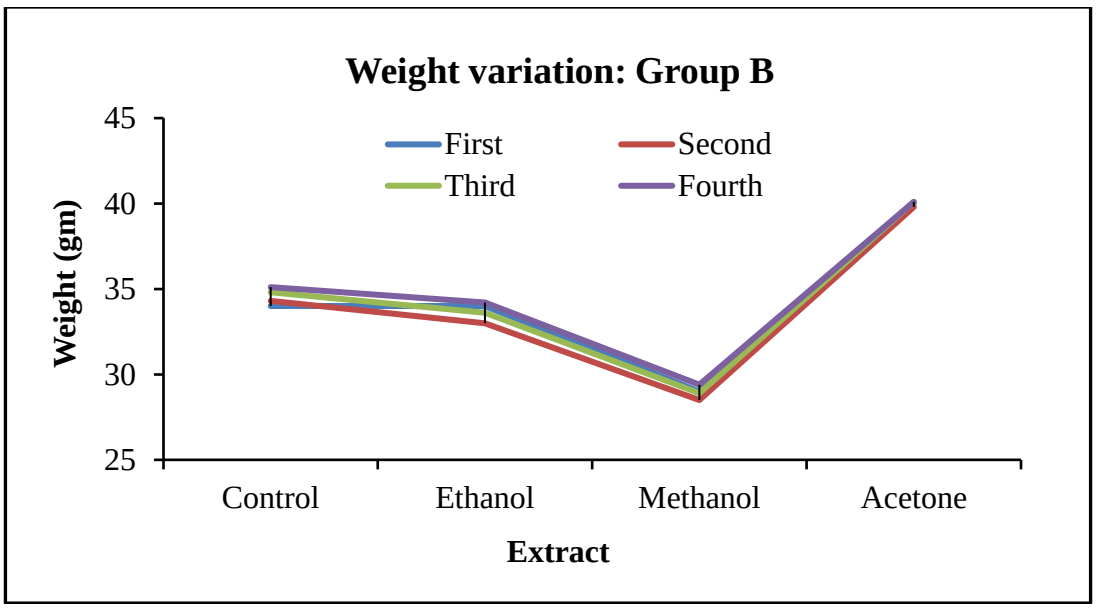


Fig. 17. Graphical representation of weight variation (after experiment)

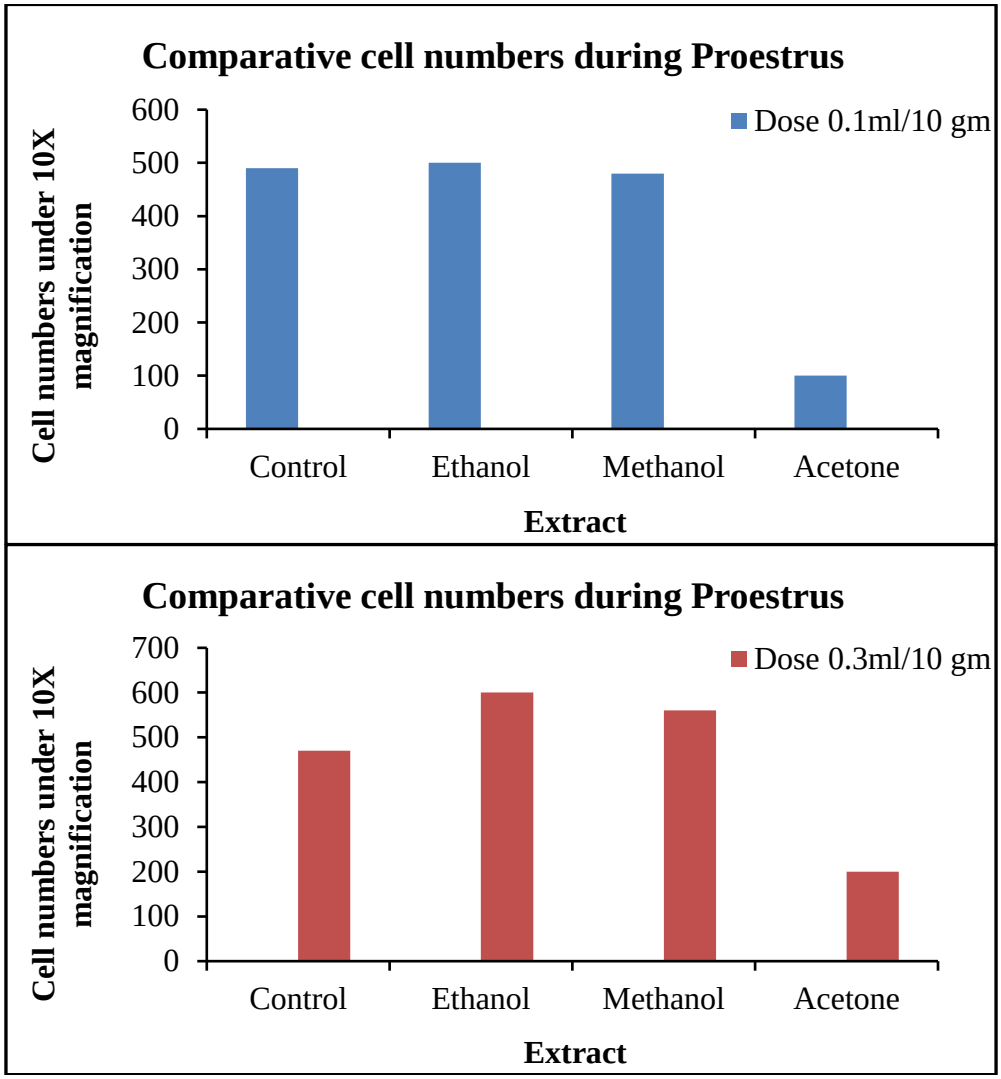
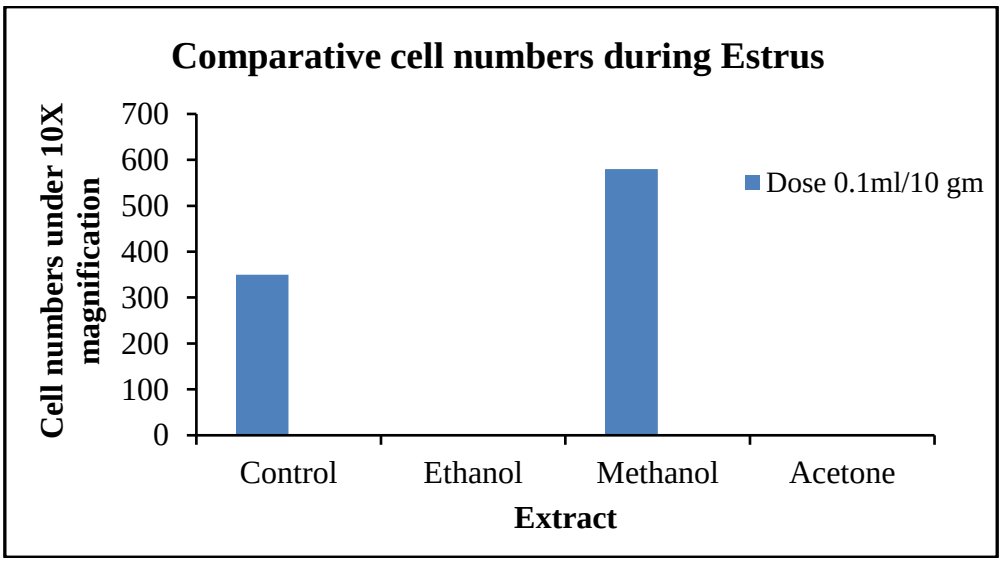


Fig. 18. Graphical representation of Comparative cell numbers during Pro-estrous phase



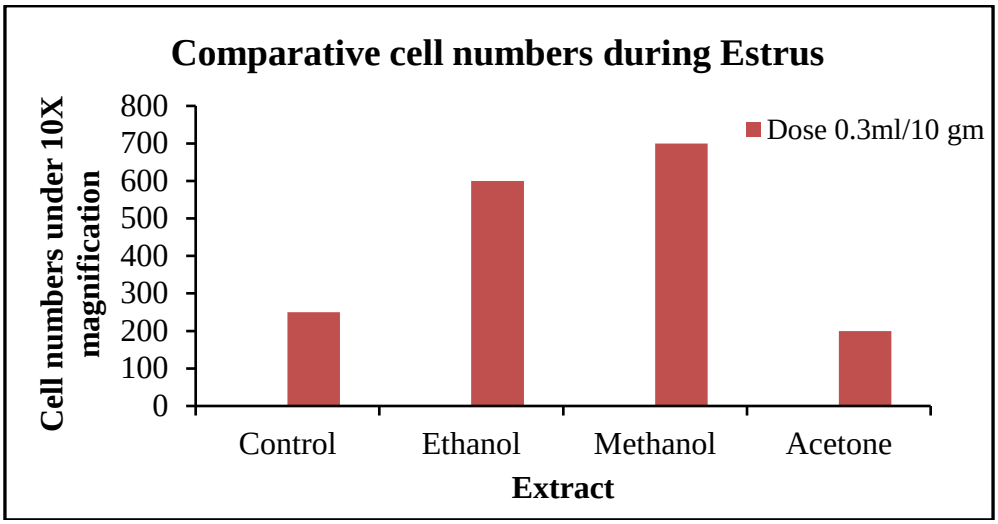


Fig. 19. Graphical representation of comparative cell numbers during Estrous phase

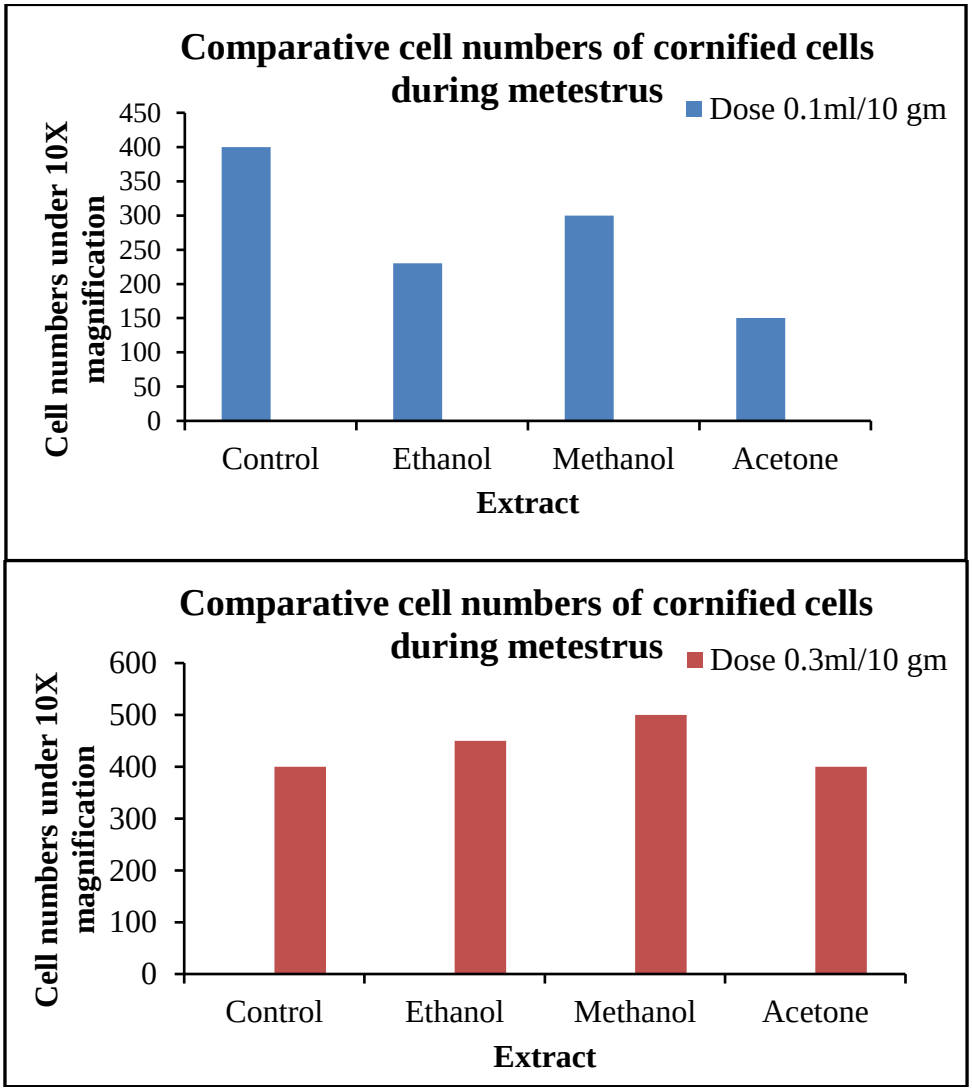


Fig. 20. Graphical representation of comparative cell numbers of cornified cells during Met--Estrous phase

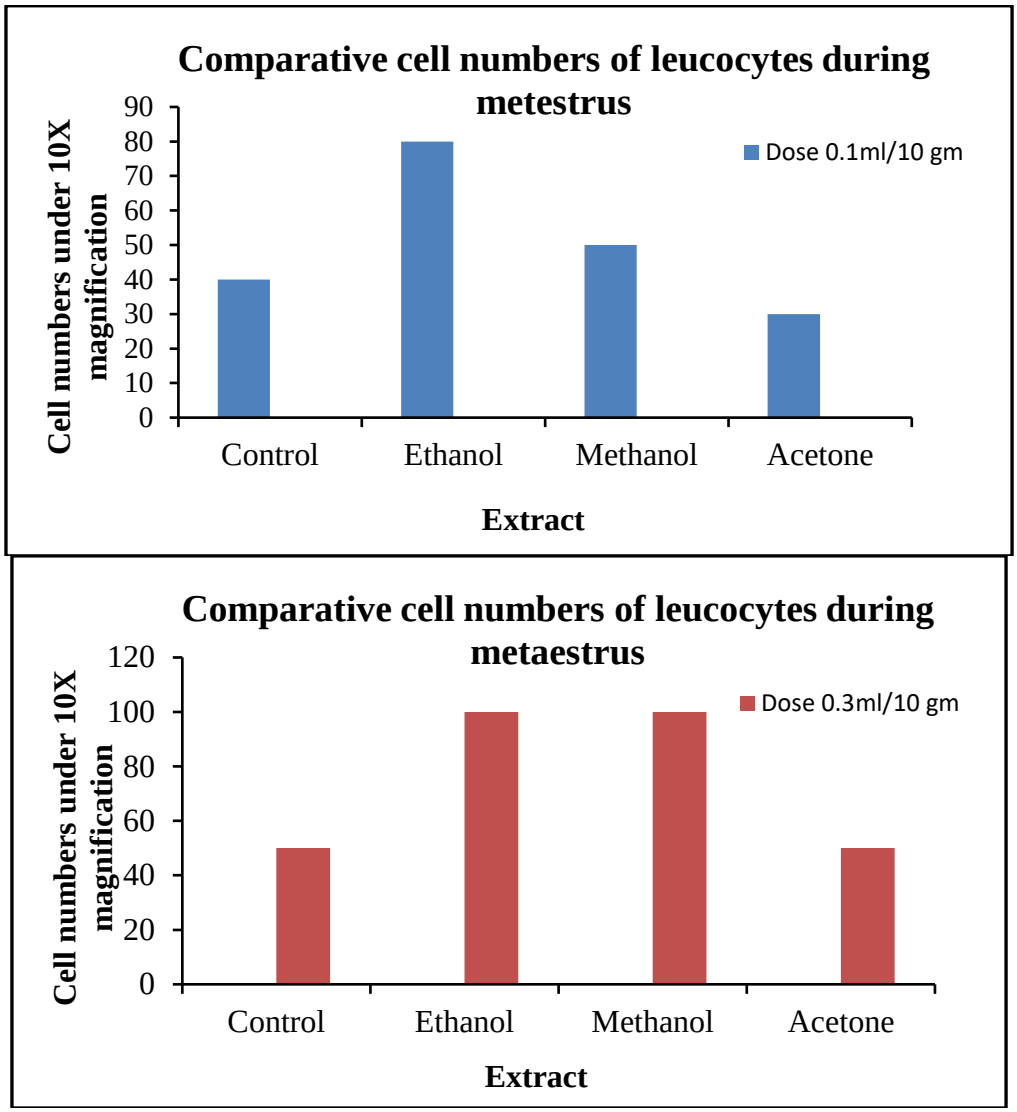
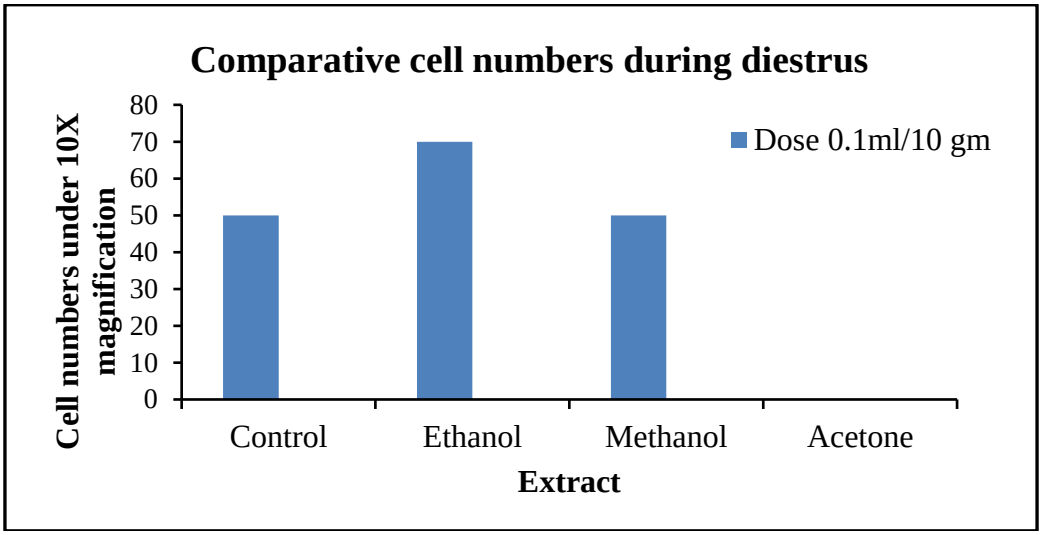


Fig. 21. Graphical representation of comparative cell numbers of leucocytes cells during Met--Estrous phase



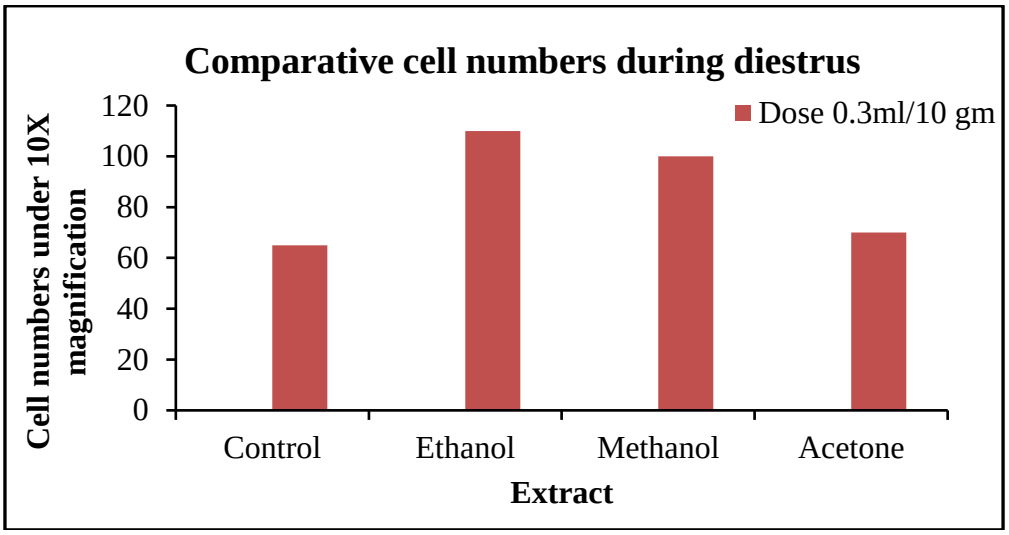


Fig. 22. Graphical representation of comparative cell numbers Di-Estrous phase

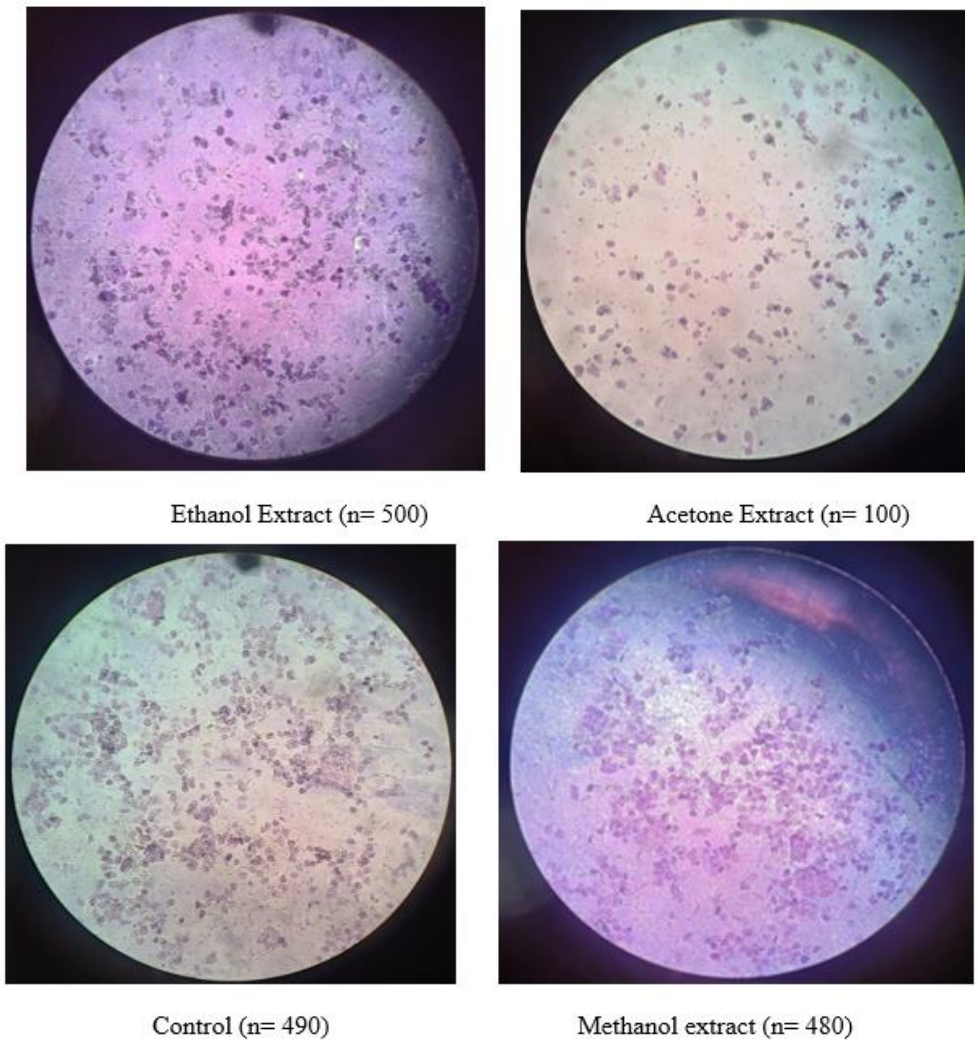


Fig. 23. Number of cells during Pro-Estrous phase under 10X magnification after treatment with dose 0.1ml/10gm of body weight

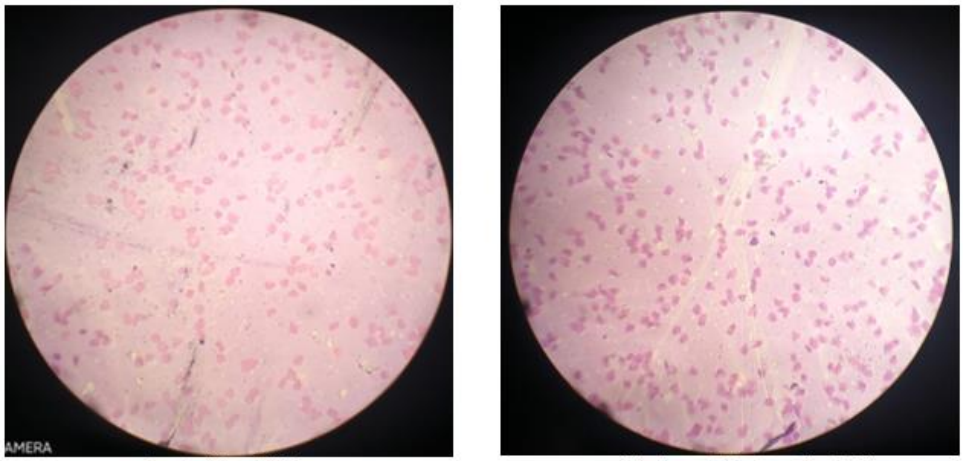


Fig. 24. Number of cells during Estrous phase under 10X magnification after treatment with dose 0.1ml/10gm of body weight

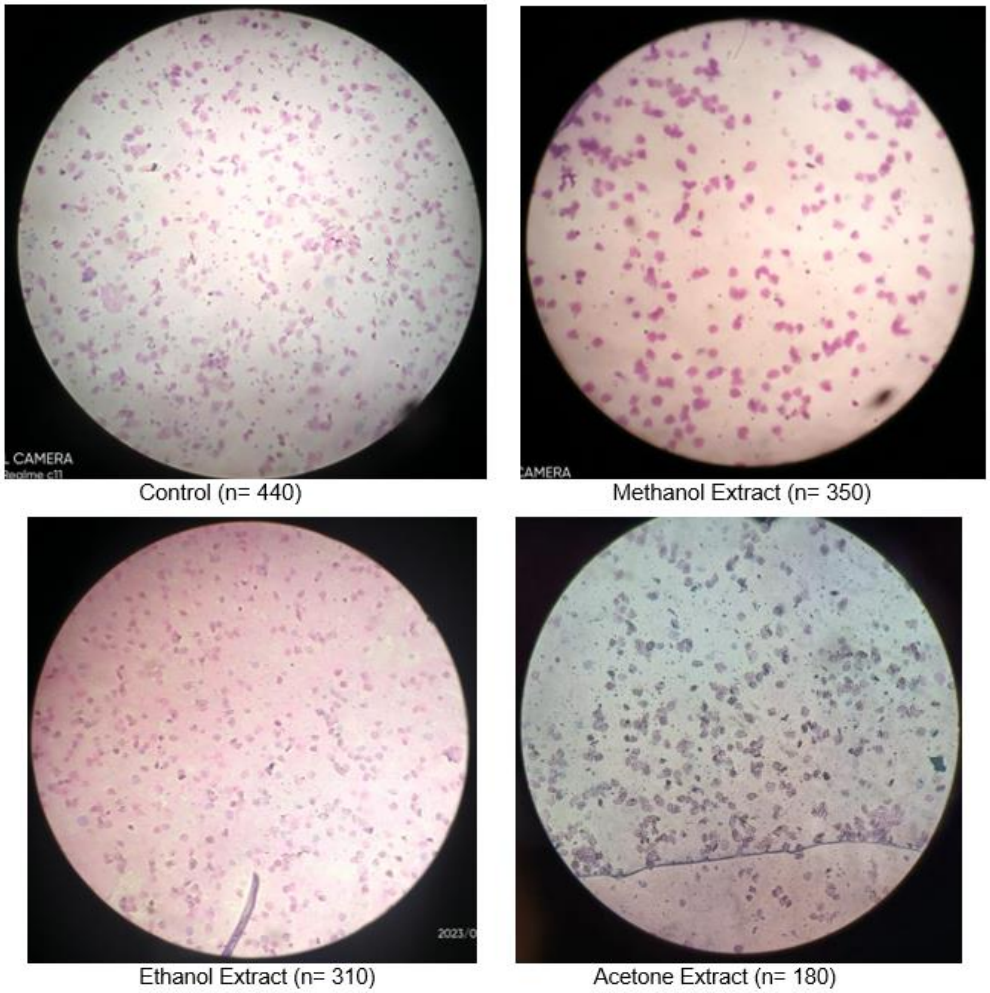


Fig. 25. Number of cells of Met--Estrous phase under 10X magnification after treatment with dose 0.1ml/10gm of body weight

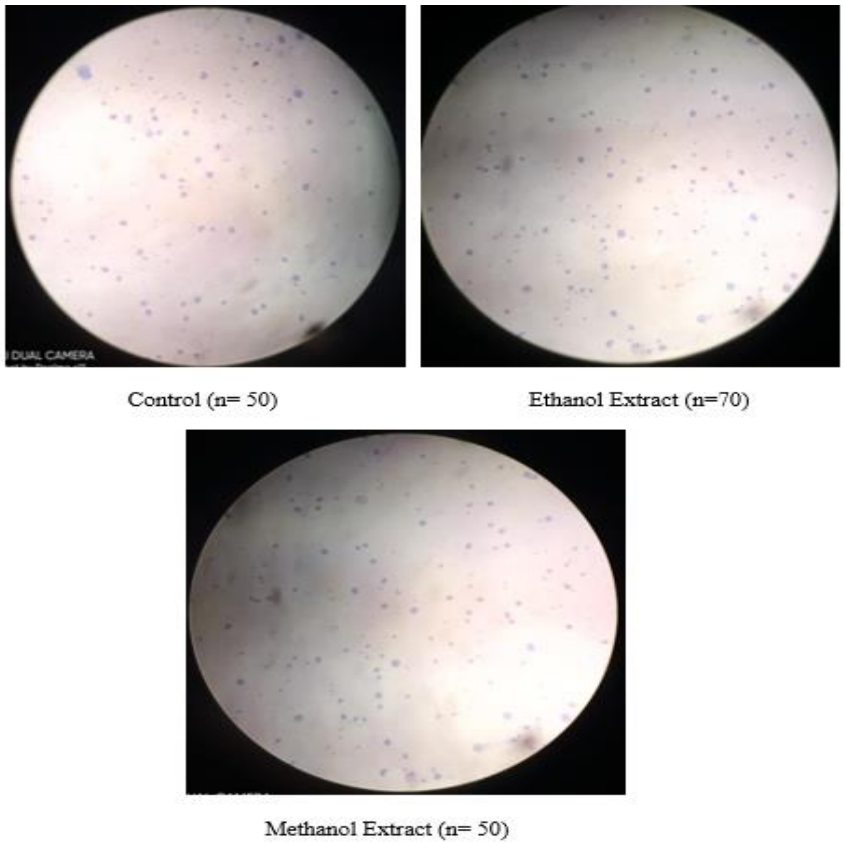


Fig. 26. Number of cells of Di-Estrous phase under 10X magnification after treatment with dose 0.1ml/10gm of body weight

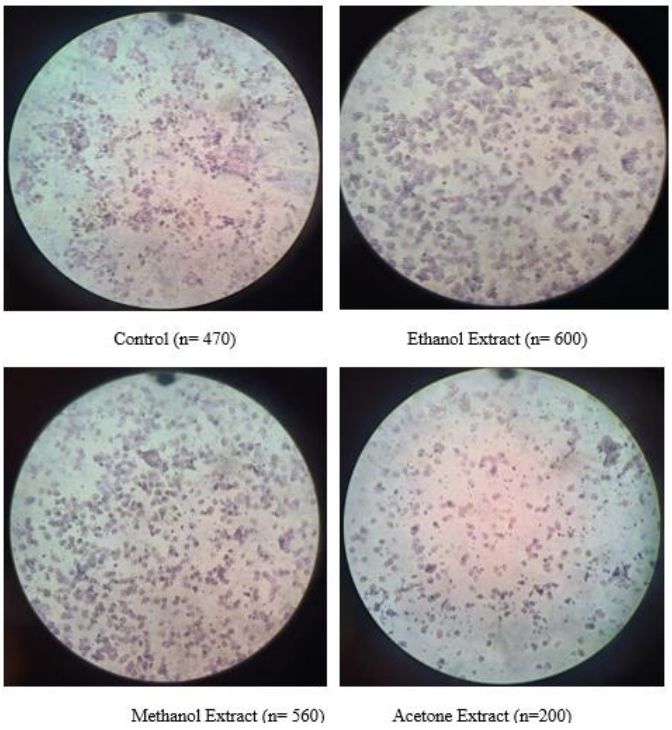


Fig. 27. Number of cells of Pro-Estrous phase under 10X magnification after treatment with dose 0.3ml/10gm of body weight

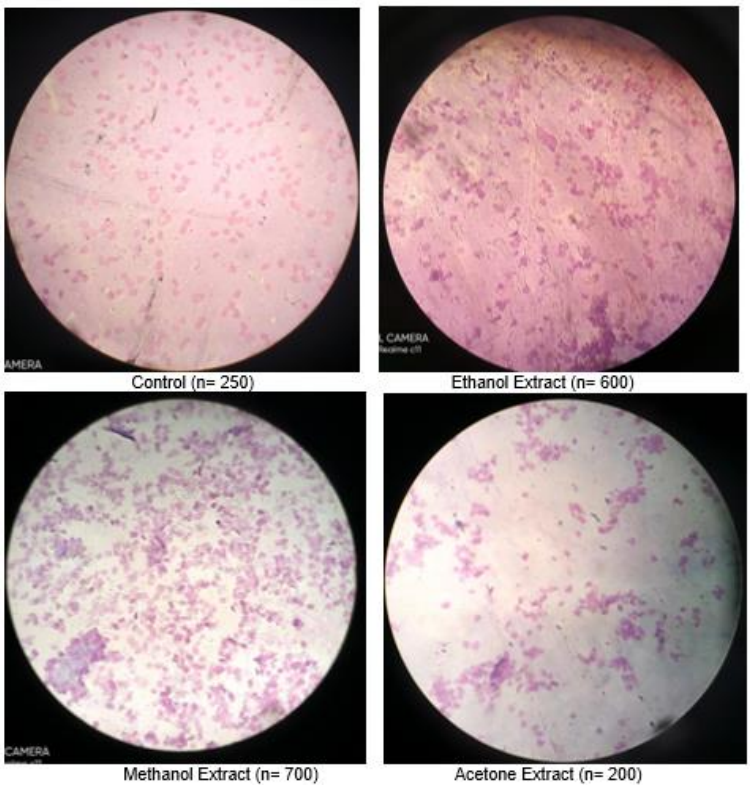


Fig. 28. Number of cells of estrous phase under 10X magnification after treatment with dose 0.3ml/10gm of body weight

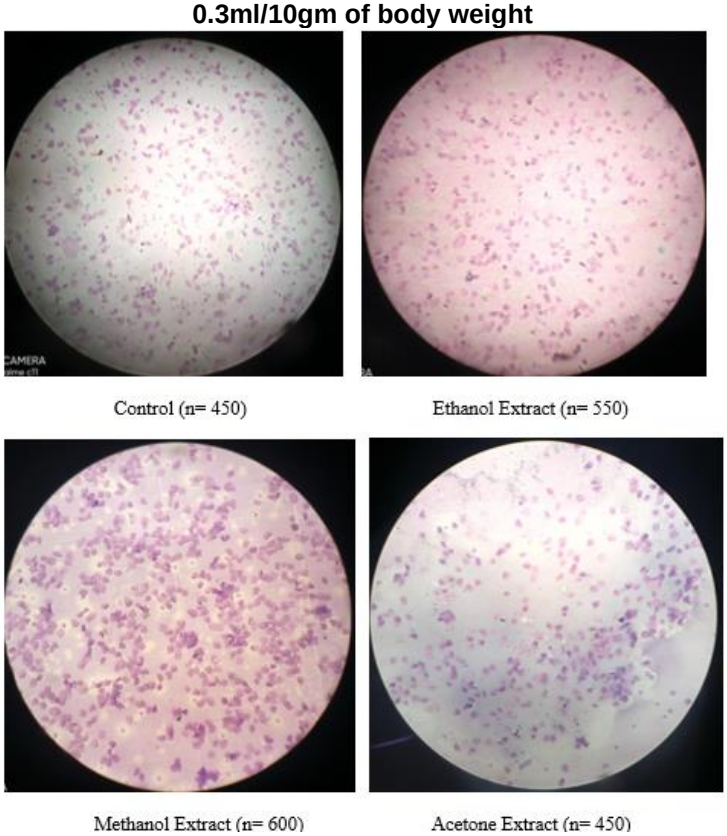


Fig. 29. Number of cells of Met-Estrous phase under 10X magnification after treatment with dose 0.3ml/10gm of body weight

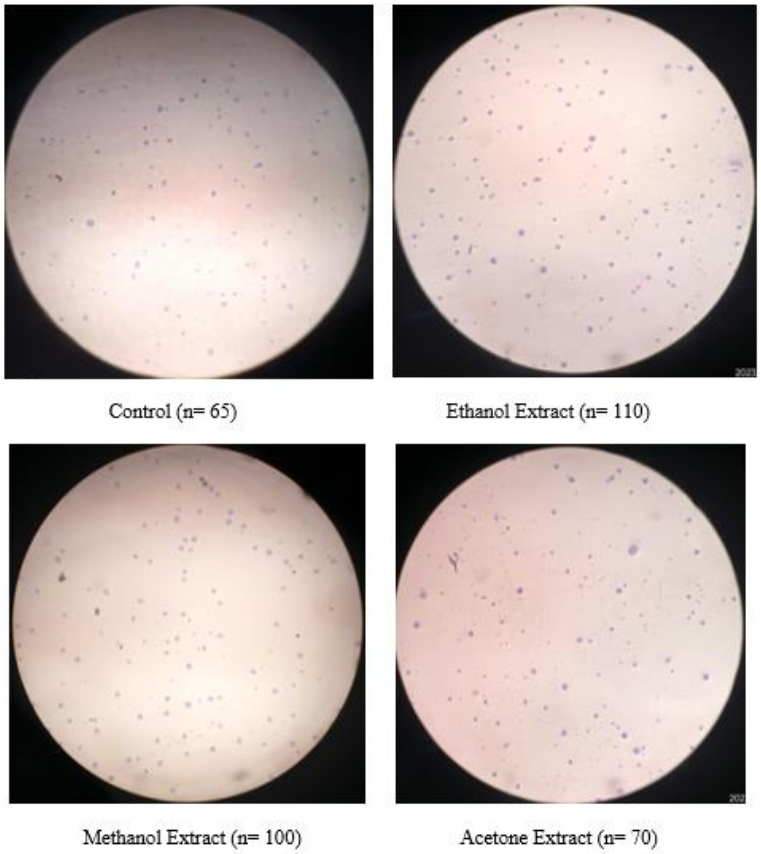


Fig. 30. Number of cells of Di-Estrous phase under 10X magnification after treatment with dose 0.3ml/10gm of body weight

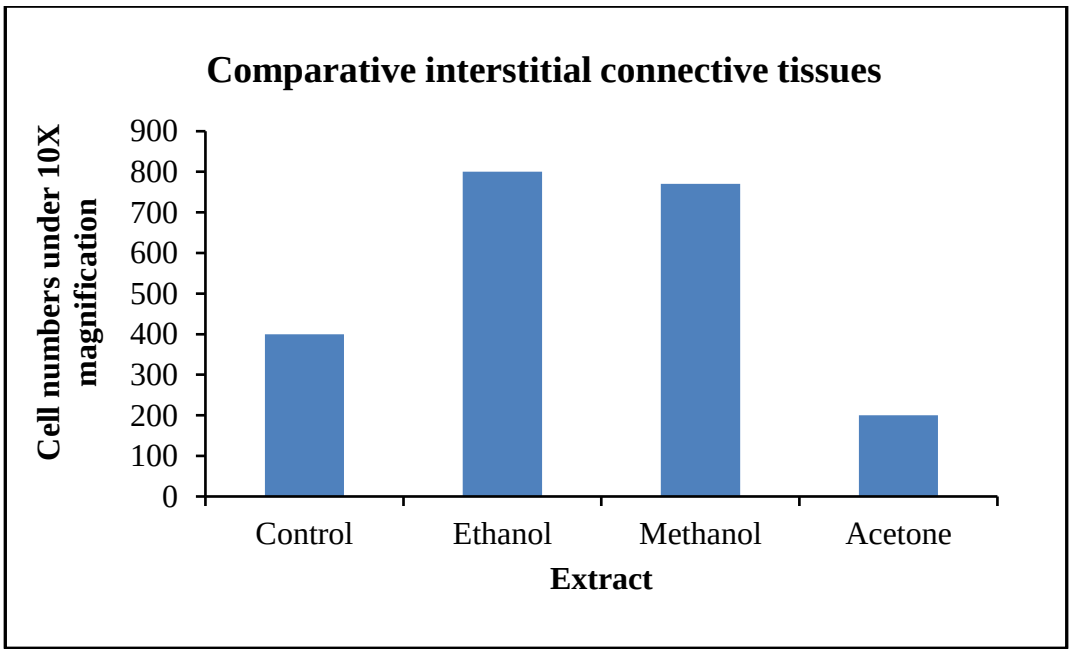


Fig. 31. Graphical representation of comparative interstitial connective tissues

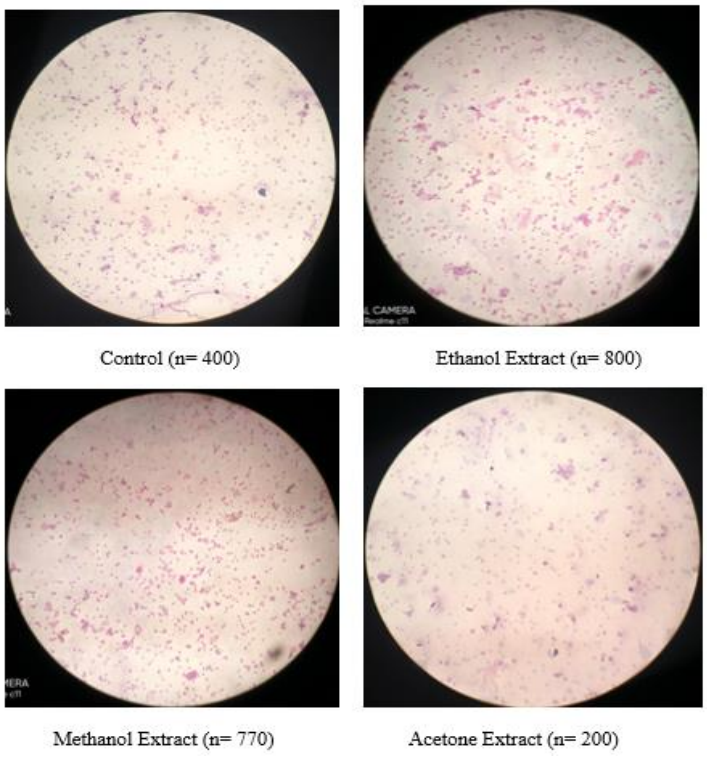


Fig. 32. Number of cells of interstitial connective tissue under 10X magnification

5. CONCLUSION

F. vestita contains isoflavones, and genistein which have a wide spectrum of biological activities including estrogenic effect. Geinstein, a plant derivative extracted from root tuber *Flemingia vestita*has been associated with a reduction in the risk of breast prostate cancers, also conveys its effect towards different biological pathway receptors including estrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR), peroxisome proliferator-activated receptors (PPAR), insulin-regulated glucose transporter (GLUT), A1 adenosine receptor.

Alkaloids are present in the root-tuber extract of *Flemingia.* Carbohydrates are present in the root-tuber extract of *Flemingia.* Saponins are found present in the root-tuber extract of *Flemingia.* Proteins are present in the root-tuber extract of *Flemingia.* Fixed oils and fats are absent in the root-tuber extract of *Flemingia.* Phenols are found present in the root-tuber extract of *Flemingia.* Gums and Mucilages are found present in the root-tuber extract of *Flemingia.*

In comparison to the control, the food intake for consecutive four weeks was efficient in the case of Methanolic Extract. In comparison to the control, the water intake for consecutive four weeks was efficient in the case of Ethanolic Extract. In comparison to the control, the Stool for consecutive four weeks was efficient in the case of Ethanolic Extract. No distinctive changes are seen in the total body weight variation for both Groups A & B albino rats.

In comparison to cell number during ProEstrous Cycle, Acetonic extract showed the least cell count as compared to Ethanol and Methanol for both the doses 0.1ml/10gm and 0.3ml/10gm.In comparison to cell number during Estrous Cycle, Methanolic extract showed the highest cell count as compared to others for both the doses 0.1ml/10gm and 0.3ml/10gm.In comparison to cell number during MetEstrous Cycle, Methanolic extract showed the highest cell count followed by Ethanolic and Acetone extract for both the doses 0.1ml/10gm and 0.3ml/10gm.In comparison to cell number during Leucocytes Cycle, Ethanolic extract showed the highest cell count as compared to others for both the doses 0.1ml/10gm and 0.3ml/10gm.In comparison to cell number during DiEstrous Cycle, Ethanolic extract showed the highest cell count as compared to others for both the doses 0.1ml/10gm and 0.3ml/10gm.Interstitial

connective tissues were found highest in the Ethanol extract.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICAL APPROVAL

The work has been carried out after review and under the institutional animal ethics committee (IAHF/USTM/2023/P-5).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Ekambaram, G., Kumar, S. K. S., & Joseph, L. D. (2017). Comparative study on the estimation of estrous cycle in mice by visual and vaginal lavage method. *Journal of Clinical and Diagnostic Research, 11*(1), AC05–AC07. https://doi.org/10.7860/JCDR/2017/23977.9148

El-Halawany, A. M., Dine, R. S. E., Chung, M. H., Nishihara, T., & Hattori, M. (2011). Estrogenic and anti-estrogenic activities of *Cassia tora* phenolic constituents. *Pharmacognosy Research, 55*(10), 1476–1482. https://doi.org/10.4103/0974-8490.81958

Freeman, M. E. (1994). The neuroendocrine control of the ovarian cycle of the rat. In E. Knobil & J. D. Neill (Eds.), *The Physiology of Reproduction* (pp. 613–658). Raven Press.

Gavade, S. K., Surveswaran, S., van der Maesen, L. J. G., & Lekhak, M. M. (2019). Taxonomic revision and molecular phylogeny of *Flemingia* subgenus *Rhynchosioides* (Leguminosae). *Blumea, 64*(3), 253–271. https://doi.org/10.3767/blumea.2019.64.03.06

Guo, T. L., McCay, J. A., Zhang, L. X., Brown, R. D., You, L., Karrow, N. A., Germolec, D. R., & White, K. L., Jr. (2001). Genistein modulates immune responses and increases host resistance to B16F10 tumor in adult female B6C3F1 mice. *The Journal of Nutrition, 131*, 3251–3258.

Hubscher, C. H., Brooks, D. L., & Johnson, J. R. (2005). A quantitative method for assessing stages of the rat estrous cycle. *Biotechnic & Histochemistry, 80*(2), 79–87. https://doi.org/10.1080/10520290500138422

Long, J. A., & Evans, H. M. (1922). The estrous cycle in the rat and its associated phenomena. *Mem. Univ. California, 6*, 1–148.

Madan, S., Gullaiya, S., Singh, G. N., & Kumar, Y. (2013). *Flemingia strobilifera*: Review on phytochemistry and pharmacological aspects. *International Journal of Phytopharmacology, 4*(4), 255–262.

Marboh, V., & Mahanta, C. L. (2020). Characterisation and antioxidant activity of sohphlang (*Flemingia vestita*), a tuberous crop. *Journal of Food Science and Technology, 57*(10), 3533–3544. https://doi.org/10.1007/s13197-020-04344-2

Meinhard, N., Kessing, L. V., & Vinberg, M. (2014). The role of estrogen in bipolar disorder: A review. *Nordic Journal of Psychiatry, 68*(2), 81–87. https://doi.org/10.3109/08039488.2013.775341

Molina, L., Bustamante, F. A., Bhoola, K. D., Figueroa, C. D., & Ehrenfeld, P. (2018). Possible role of phytoestrogens in breast cancer via GPER-1/GPR30 signaling. *Clinical Science, 132*, 2583–2598. https://doi.org/10.1042/CS20180885

Montes, G. S., & Luque, E. H. (1988). Effects of ovarian steroids on vaginal smears in the rat. *Acta Anatomica, 133*, 192–199.

Muliar, G., Paul, A., & Zaman, M. K. (2022). *Flemingia vestita* Benth—a highly valued medicinal and edible tuber of Meghalaya. *Current Trends in Pharmaceutical Research, 9*(1), 35–46.

Nikolic, I. L., Savic-Gajic, I. M., Tacic, A. D., & Savic, I. M. (2017). Classification and biological activity of phytoestrogens: A review. *Advanced Technologies, 6*(2), 96–106.

Ojha, A., Tak, N., Rathi, S., Chouhan, B., Rao, S. R., Barik, S. K., Joshi, S. R., Sprent, J. S., James, E. K., & Gehlot, H. S. (2017). Molecular characterization of novel *Bradyrhizobium* strains nodulating *Eriosema chinense* and *Flemingia vestita*, important unexplored native legumes of

the sub-Himalayan region (Meghalaya) of India. *Systematic and Applied Microbiology, 40*, 334–344. https://doi.org/10.1016/j.syapm.2017.06.003

Paterni, I., Granchi, C., Katzenellenbogen, J. A., & Minutolo, F. (2014). Estrogen receptors alpha (ERα) and beta (ERβ): Subtype-selective ligands and clinical potential. *Steroids, 90*, 13–29. https://doi.org/10.1016/j.steroids.2014.06.012

Raaman, N. (2006). Phytochemical techniques. New India Publishing Agency.

Revankar, C. M., Cimino, D. F., Sklar, L. A., Arterburn, J. B., & Prossnitz, E. R. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science, 307*(5715), 1625–1630. https://doi.org/10.1126/science.1106943

Roy, B., & Tandon, V. (1996). Effect of root-tuber extract of *Flemingia vestita*, a leguminous plant, on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski*: A scanning electron microscopy study. *Parasitology Research, 82*, 248–252.

Shailajan, S., Kumaria, S., Pednekar, S., Menon, S., Choudhury, H., & Matani, A. (2016). Estrogenic potential of *Flemingia vestita* Benth tubers in ovariectomized rat model. *Pharmacognosy Journal, 8*(1). https://doi.org/10.5530/pj.2016.1.10

Shailajan, S., Kumaria, S., Pednekar, S., Menon, S., Joshi, H., & Matani, A. (2014). Chromatographic evaluation of a phytoestrogen genistein from *Flemingia vestita* Benth: An endemic plant of Northeast India. *Pharmacognosy Communications, 4*(4). https://doi.org/10.5530/pc.2014.4.2

Tandon, V., Das, B., & Saha, N. (2003). Anthelmintic efficacy of *Flemingia vestita* (Fabaceae): Effect of genistein on glycogen metabolism in the cestode, *Raillietina echinobothrida*. *Parasitology International, 52*, 179–183.

Tandon, V., Pal, P., Roy, B., Rao, H. S. P., & Reddy, K. S. (1997). In vitro anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. *Parasitology Research, 83*, 492–498.

Tang, Z.-R., Zhang, R., Lian, Z.-X., Deng, S.-L., & Yun, K. (2019). Estrogen- receptor expression and function in female reproductive disease. *Cells, 8*(10), 1123. https://doi.org/10.3390/cells8101123

Taylor, C. K., Levy, R. M., Elliott, J. C., & Burnett, B. P. (2009). The effect of genistein aglycone on cancer and cancer risk: A review of in vitro, preclinical, and clinical studies. *Nutrition Reviews, 67*(7), 398–415. https://doi.org/10.1111/j.17534887.2009.00213.x

Thacker, K. D., Gavade, S. K., Lekhak, M. M., Gondaliya, A. D., & Rajput, K. S. (2021). Comparison of petiole anatomy in *Flemingia* and its potential for delimitation of species. *Flora, 278*. https://doi.org/10.1016/j.flora.2021.151790

Tiwari, P., Kaur, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia, 1*(1), 98–106.

Turner, J. V., Agatonovic-Kustrin, S., & Glass, B. D. (2007). Molecular aspects of phytoestrogen selective binding at estrogen receptors. *Journal of Pharmaceutical Sciences, 96*(8), 1879–1885. https://doi.org/10.1002/jps.20987

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