

Executive summary of the UGC Minor Research Project

Entitled

“Evaluation and Characterization of *Eulophia* extract effects on Physiological and Reproductive activities of Albino Rat”

A MINOR RESEARCH PROJECT CARRIED OUT UNDER THE FINANCIAL ASSISTANCE

OF

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Submitted by

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INTRODUCTION

Orchids such as *Eulophia herbacea* and *Eulophia ochreatea* from the family *Orchidaceae* have attracted the worldwide researchers towards their ethnomedicinal potential. Parallel to traditional medicines, several ethnobotanical medicinal plants have also been validated for their therapeutic efficacy with the help of modern scientific tests. Some of these plants are receiving merits as both food and medicine (Jagtap et al., 2006) and *Eulophia* is one of the best examples of this. Despite the popular use and numerous therapeutic benefits of *Eulophia* species (Patil and Mahajan, 2013) little is known about the safety profile of crude extract for animal model and its effects on male reproduction. Besides there is paucity of information on its reproductive, pharmacological, physiological efficacy. Three medicinal plants namely *Mucuna pruriens* (Linn) (Amin et al., 1996), *Chlorophytum borivillianum* (Sant and Fernand)(Thakur and Dixit, 2006), and *Eulophia campestris* (Wall) as aphrodisiac and adaptogenic have been evaluated for enhancing sperm density in experimentally induced sterility in rat (Mahajan et al, 2012). Although, *Eulophia* is a constituent of number of herbal formulations that are known for aphrodisiac nature and improving fertility, there is no scientific report on its usage as sexual tonic or stimulant. According to the growing popularity and market interest for the drug, present studies were undertaken to provide scientific support for its purported folkloric usage.

Origin of the Research Problem

Researchers are exploring ethnobotanically important plants to find out potential natural products of Orchids which are biologically active and orchids contain bioactives or phytoconstituents like Alkaloids-steroids, Amino Acids, Carbohydrates, Flavonoids, Glycosides, Lipids, Phenol, Saponin, anthocyanin, stilbenoids, triterpenoids, Sugar and Tannin. So it becomes important to study and elaborate their effects on the human being.

Looking to above literature glimpses, we proposed to study the bioprospectives of crude extracts obtained from the tubers of *Eulophia*, in various organic solvents. Emphasis will be given to the biological activities of the extracts obtained. Highlight of literature survey focus on the folk-claim of benefits of *Eulophia* species for the activities viz. rejuvenating, antioxidant, antirheumatic, aphrodisiac and fertility. Hence it is thought to evaluate some of these activities experimentally to validate the claims made of local tribals, healars and vaidyas.

Many *Eulophia* species are used medicinally by tribals of various parts of India and such folklore claim needs proper formulation, investigation and its validation by experimentation especially with respect to aphrodisiac, spermatogenic and fertility activities, as the human reproductive potential declining because of fast changing living standards, hybrid intake and environmental changes.

Objectives of the Study

- ✓ To extract phytochemicals from the tubers of *Eulophia* species by using various organic solvents.
- ✓ To study qualitative phytochemical tests for detection of alkaloids, phytosterols, saponine, flavanoids, glycosides, tannins etc. from the various extracts.
- ✓ To isolate bioactives by column chromatography, partial character value of natural product by HPTLC, HPLC, UV, IR, NMR and MS.
- ✓ To study the various biological activities of the extracts in different solvents.

METHODOLOGY

Identification of collected material

The tubers of *E. herbacea* and *E. ochreatea* collected from district Nasik and Jalgaon respectively in the month of October were identified, authenticated and the voucher specimens (PCA/BOT H.S.1641 and 1642) were deposited at the herbarium of Department of Botany, Pratap College, Amalner.

Preparation of extracts

The tubers of both the plants were washed, chopped into small pieces and shade dried. After complete drying, the material was powdered and extracted with 5 solvents viz. petroleum ether (60-80°C), benzene (80.3°C), chloroform (61°C), methanol (65°C) and water in a Soxhlet extractor. Each extract was concentrated and analyzed phytochemically. On the basis of promising results on phytochemical investigation, methanolic extracts of *E. herbacea* (79.7% β -sitosterol) and *E. ochreatea* (94.6% β -sitosterol) were screened for their biological activities. The protocol for experimentation was approved by Institutional Animal Ethical Committee (IAEC/22/CPCSEA/MJ/2014-15) and the guidelines of CPCSEA, India, were strictly adhered to during the whole animal experimentation protocol.

Phytochemical studies

The extracts were analyzed for the preliminary and secondary metabolites, qualitatively and quantitatively. The ash content, moisture content and foreign organic matters in % w/w; solvent extract in %, Fluorescence analysis in both the *Eulophia* species tuber powder is determined. β -sitosterol is detected in *E. herbacea* and *E. ochreatea* on the basis of HPLC (High performance Liquid Chromatography) analysis.

Biological activities of *Eulophia* species- Experimental design

After one week of acclimatization, 24 adult male rats weighing about 200-250 g each, were randomized into 6 groups comprising of 4 animals each (n = 4). Control, Reproductive disfunctioned and Test plants extracts treatments were administered to respective groups from the study day 1 to day 28. Here lead acetate was used as reproductive disfunctioning agent (Ramah et al., 2015).

Studies Performed

- 1) **Anabolic Effect of MEEH and MEEO** –Effect of MEEH and MEEO treatment on relative body weight, reproductive organ weight was carried out (Sharma et al, 2009).
- 2) **Sperm parameters – sperm density, motility and viability**- Sperm density per mL of the diluted

sperm suspension was evaluated by Dorostghoal method (2013). Motion parameters included total percentage of motile sperm, percentage of progressively motile sperm and sperms showing circular motion. The sperm viability (% live sperms) was determined using Eosin/ Nigrosin stain as described by Raji et al., 2005.

3) Serum biochemical profile- Serum testosterone, cholesterol, blood glucose were estimated using commercial kits (Erba Pvt. Ltd., Mumbai).

4) Histological examinations of testis- Formalin fixed testes of each group of rat were embedded in paraffin wax and sections of 5 μm thickness were cut, deparaffinized and stained with hematoxyllin - Eosin stain.

ACHIEVEMENTS FROM THE PROJECT

- ✓ The literature on the techniques, plant content and the studied activities is updated.
- ✓ The phytochemical analysis of tuber extract from the *Eulophia* species by using various organic solvents.
- ✓ The study pertaining to qualitative phytochemical tests for detection of alkaloids, phytosterols, saponine, flavanoids, glycosides, tannins etc. from the various extracts.
- ✓ Anabolic effect of methanolic extracts of *E. herbacea* (MEEH) and *E. ochreatea* (MEEO) on Albino rats with respect to body and reproductive organs weight.
- ✓ The effect of MEEH and MEEO on sperm parameters like sperm density, motility and viability from epididymus and testes of different groups of rats.
- ✓ The serum biochemical profile with respect to the level of testosterone, cholesterol and glucose in different groups of rats treated with MEEH and MEEO.
- ✓ Histopathological examination of testes of reproductively dysfunctional rats concomitantly treated with MEEH and MEEO.

CONTRIBUTION TO THE SOCIETY

1. These findings provide a scientific basis for acclaiming *E. herbacea* and *E. ochreatea* as 'Male Fertility Agents'. *E. ochreatea* is proved to be a good alternative for management of infertility due to reduced spermatogenesis than *E. herbacea*.
2. A drug designer / biochemist or an Ayurvedic medical practitioner may take the help of the results of reproductive study in the present work or suggested the biological principles which may be active.
3. Same study may be used by chemists to develop some newer compounds of future use which will serve the society in their development.

No. of Publications out of the Project: One publication is accepted, one communicated and other will be ready to communicate.

- 1) Manisha C. Patil, C. J. Patil and Raghunath T. Mahajan, **Evaluation of the Anabolic and Reproductive Activity of *Eulophia herbacea* Lindl. in Male Rats**, (accepted to British J. Med. Helth Res., Jan. 2017).
- 2) Manisha C. Patil, C. J. Patil and Raghunath T. Mahajan, **Extraction and Characterization of the Phyto-markers from *Eulophia herbacea* Lindl. in different Organic Solvents**, (communicated to Int. J. Green Herbal Chem., Jan. 2017).

Glimpses of Characterization of *Eulophia* extract

Qualitative screening of secondary metabolites showed presence of Flavonoids, Glycosides,

Phenols, Phytosterol or Steroids, Saponins, Tannins and Triterpenes in different solvents. Alkaloids were absent in all solvents of both the test samples.

In quantitative screening, in both the test plants Crude fibres, carbohydrates, mucilage, total proteins, total phenolic and flavonoids were obtained in different concentrations. Swelling index of *E. ochreata* was higher than *E. herbacea*.

In HPLC analysis the percentage of β -sitosterol in MEEH was 79.7% and in MEEO was 94.6%. Secondly, both these extracts contain some phytophenols like gallic acid 11.6% in MEEH and 0.8% in MEEO whereas MEEO contains additional phenol, tannic acid (0.8%).

Glimpses of Evaluation of *Eulophia* extract effects on Physiological and Reproductive activities of Albino Rat

Methanolic extract of *E. herbacea* (MEEH) and *E. ochreata* (MEEO) showed dose dependent anabolic effect on rats with respect to body and reproductive organs weight.

The effect of MEEH and MEEO showed increased sperm density (92.9 and 95.5% respectively) at higher dose as compare to reproductive dysfunctioned group. The higher doses of MEEH and MEEO significantly increased the live sperm count ($p < 0.001$). The concurrent administration of MEEH and MEEO ameliorated the sperm motility 3, 7 folds and 7, 10 folds more than reproductive dysfunctioned group than at their low and high dose respectively. The concomitant groups of MEEH and MEEO treatment showed increased viability of sperms by 156.4%, 219.23% and 170.51%, 235.9% respectively.

The treatment with MEEH and MEEO group increased the serum testosterone levels even when the rats received concomitant effect of Rd was highly significant ($p < 0.001$).

High dose of MEEH (G-IV) successfully lowered the cholesterol as compare to normal.

MEEH and MEEO have significantly updated serum glucose levels up to the control group especially in their high doses.

Histopathological examination of testes of concomitant treatment with MEEH and MEEO recovered all the deformities in spermatogenic series and showed focal regeneration of seminiferous tubules thereby to attain its normal structure as in control group.

CONCLUSION

High sperm count, increased viability and low percentage of abnormal spermatozoa each has been associated with improved fertility (Raji et al., 2005). *Eulophia ochreata* is comparatively more effective in restoration of sperm viability than *Eulophia herbacea*. The folk claimed efficacy of *E. herbacea* and *Eulophia ochreata* as male fertility agents is proved experimentally in rat and thus provide an alternative for management of infertility due to reduced spermatogenesis.

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