UGC MAJOR RESEARCH PROJECT
(2013 – 2017)
FINAL REPORT

"Purification, Crystallisation and Pharmacological Evaluation of isolated compounds from *Leptadenia reticulata wight* and *arn* and *Lawsonia inermis* Linn for abortifacient activity"

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SUMMARY OF THE FINDINGS

INTRODUCTION

Fertility control is an issue of global and national public health concern. Control of fertility with hormonal preparations containing estrogens and progesterone has been proved to be effective. The safety of long term use of these contraceptives, however, is controversial. To this effect, World Health Organization (WHO) has given much attention in the search for safe, affordable and socially acceptable alternatives. Part of this vital work has focused upon use of anti-fertility herbs. The need from new pregnancy prevention options that improve on currently available methods also has important public health dimensions of the estimated 210 million pregnancies that occur worldwide each year, 40% are unplanned. Half of all pregnancies are unplanned and a quarter certainly unwanted. Many unwanted pregnancies result in abortion. It is estimated that around 50 million abortions are performed each in developing countries and many a pregnancy is terminated in unsafe conditions. These factors expose women to a high risk of mortality and morbidity. In this new environment, it may be necessary to introduce a new safe herbal abortifacient drug with minimal side effects successfully. Keeping this factor as the background, the present study was under taken to screen some selected medicinal plants for abortifacient activity in albino rats.

OBJECTIVES OF THE PROJECT

This study is mainly focused to screen and analyze abortifacient activitiy of two selected medicinal plants; ***Leptadenia reticulata*** **Wight and Arn** [Fam: Asclepiadaceae] and ***Lawsonia inermis*** **Linn.**[Fam: Lythraceae]. The abortifacient activity *of Leptadenia reticulata* and *Lawsonia inermis* plants were measured in order to verify the traditional uses of these ethnomedicinal plants.

The specific objectives of this study are the following:

- Selection, identification and collection of whole plant of *Leptadenia reticulata* (**LR**) and root of *Lawsonia inermis* (**LI**).
- Extraction of the dried powdered plant materials by hot continuous extraction method using ethanol solvent.
- Preliminary photochemical screening of the crude extracts of *Leptadenia reticulata* and *Lawsonia inermis* (LREE & LIEE).

- To analyze the chemical constituents of the whole plant of *Leptadenia reticulata* (**LR**) and root of *Lawsonia inermis* (**LI**) using GC-MS
- Separation of the phytoconstituents by column chromatography and identification by Thin Layer Chromatography (TLC).
- Characterization of the isolated compounds by using modern analytical techniques (IR, NMR & MASS).
- Acute toxicity studies of isolated compound LR S1 and LI S1 as per **OECD Guidelines 423** (Organization for Economic Cooperation and Development).
- Evaluation of anti-implantation, abortifacient activities of LRS1 and LIS1 in animal model.
- Studying the effect of LRS1 and LIS1 on biochemical parameters.
- Studying the effect of LRS1 and LIS1 on haemotological parameters
- Studying the effect of LRS1 and LIS1 on Histopathology of isolated animal organs of Uterus and Ovary.

SUMMARY OF THE WORK

A detailed study of phytochemical and pharmacological investigations on dried whole plant of *L. recticulata* (LR) *and* dried roots of *L. inermis* (LI) were carried out. The powdered plant materials were extracted with ethanol by soxhlation for 24 hrs.

Systematic photochemical screening was performed for both (LR) and (LI) to detect the presence of biologically active phytocompounds. The LR showed the presence of tannins, phenolic and flavonoid compounds while LI showed the presence of carbohydrates, glycosides, tannins, phenolic and flavonoid compounds.

GC-MS analysis was carried out to investigate the phytoconstituents present in ethanolic extract of *Leptadenia reticulata* and *Lawsonia inermis.* The GC spectrum of *Leptadenia reticulata* confirmed the presence of thirty one compounds in ethanolic extract. The GC chromatogram of *Lawsonia inermis* confirmed the presence of forty one compounds in ethanolic extract.

In Dockling studies in *L.inermis* the results showed that D-allose is having the highest binding affinity with the progesterone receptor having a GOLD score of 32.08. D-allose present in *L.inermis* inhibits the activity of progesterone and induces abortion, and it also satisfies ADME parameters.

In *L.reticulata* the results showed that á-d-Allopyranoside, methyl 6-deoxy-2-O-methyl- is having the highest binding affinity with the progesterone receptor having a GOLD score of 32.76. á -d-Allopyranoside, methyl 6-deoxy-2-O-methyl- inhibits the activity of progesterone and induces abortion, and it also satisfies ADME parameters.

LREE and LIEE were subjected to column chromatography and TLC analysis by using various solvent systems for isolation and identification of compounds from the extracts.

One compound from each plant extract, were isolated, purified and characterized. These compounds were characterized by physical methods like nature of compound, solubility, melting point and retention factor value (R_f value). The structural elucidation was done by Infra Red Spectroscopy (KBr press pellet technique), Proton and Carbon NMR spectroscopy and Mass spectrometry. The compound were named as LRS1 and LIS1

The toxicological studies of isolated compound, which were performed as per OECD guidelines). Dose was fixed from acute toxicity dose, $1/10^{th}$ dose was taken for the purpose of Pharmacological screening. (antiimplantation and abortifacient activity.)

Throughout the experiment period, the stages of estrous cycle were monitored by preparing the vaginal smears.

In the present study, both the LRS1and LIS1 exhibited Anti-Implantation and abortifacient activity.

The study revealed that both LRS1 and LIS1 inhibited the process of implantation. The loss of implantation caused by LRS1 and LIS1 may be because of antizygotic, blastocytotoxicity, estrogenic and antiestrogenic properties and as well as anti-implantation activity. It is well known that for implantation and sustenance of pregnancy, exact equilibrium of estrogen and progesterone hormone level is essential and any disturbance in the level of these hormones may cause antiimplantation or can induce abortion.

The study indicated that LRS1 and LIS1 may have anti-progesterone like effects and this activity may be responsible to terminate the pregnancy in earlier stages.

Both LRS1 and LIS1 have no toxic effect on haematology. The biochemical investigations revealed that LRS1 treatment exerted estrogenic activity while LIS1 exerted and antiestrogenic activity in female rats.

Histopathological studies of the present investigations, it was observed that animals treated with both LRS1 and LIS1 did not produce any remarkable changes in uterus and ovary.

CONTRIBUTION TO THE SOCIETY.

Hence, in this present investigation, the isolated herbal compounds from *Leptadenia reticulata* and *Lawsonia inermis* have been proved to be more potent in abortifacient activity. Efforts are being undertaken globally to develop abortifacient products from plants. Synthetic drugs for abortion will produce major side effects but these isolated compounds will have safe effects and the health of the mother will not be affected. Knowledge addition on the effective use of herbal drugs for Abortion, which will help the society in managing the health aspect of the women.

ACHIEVEMENTS FROM THE PROJECT

In this project work compounds which are isolated from plants may be a alternative medicine for synthetic drugs and it gives a balanced biological effect than a synthetic drugs. *L. recticulata* and *L. inermis* can be safely used as abortifacient drugs after undertaking necessary clinical screening. The various experimental findings of this research work have been proved use of *L. recticulata* and *L. inermis* as the safe, orally active, affordable and socially acceptable alternative abortifacient herbal drugs free from side effects.