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HPLC analysis of Ethynylestradiol from seed extract of *Moringa oleifera* Lam

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Abstract

Moringa oleifera Lam is common Indian plant belongs to monogeneric Moringaceae. The research investigated the presence ethynyl estradiol in 'Drumstick' (*Moringa oleifera* Lam.) seed samples. The major compounds found in *Moringa oleifera* Lam. seeds were further analyzed by HPLC technique. High pressure liquid chromatography (HPLC) has been found to be an efficient and sensitive method for identification and quantification of different classes of Ethynylestradiol. HPLC studies showed that analysis of Ethynylestradiol derivatives extracted in petroleum ether and aqueous seed extracts of *Moringa oleifera* Lam. revealed significantly high levels. The calculated value of ethynyl estradiol was obtained 108.199 µg/ml of petroleum extract while it was obtained 655.26 µg/ml of *Moringa oleifera* aqueous extract. As the concentration of the samples increased, the peak area was also increased.

Keywords: *Moringa*, Ethynylestradiol, HPLC, Petroleum ether extract

1. Introduction

Moringa oleifera Lam is common Indian plant belongs to monogeneric Moringaceae. *Moringa oleifera* is referred to as "Drumstick", which is considered one of the world's most useful trees. Almost every part of moringa tree is beneficial. The plant is also well recognized in India, Pakistan, Bangladesh and Afghanistan as a folkloric medicine (Mughal *et al* 1999) [5]. The study revealed that *Moringa oleifera* stem bark is being used traditionally as an abortifacient (Kadam *et al* 2013) [3] and antifertility activity (Zade and Dabhadkar 2015) [8]. During the recent past the interest has developed again in study and use of traditional medicinal plants. More people are realizing that the natural is better. Most of the traditional plant based remedies are back in use and find increasing as therapeutic agent, raw material for new synthetic compound and a major molecule for the discovery of molecule. The use of herbal crude drugs, extracts and their remedies have significantly increased throughout the world and the green revolution in terms of herbal medicine has now achieved astonishing popularity. Natural drugs are being used antiviral and contraceptive agents (Suhagia *et al.* 2011) [6]. The study showed that more than 80% percent of women have accepted natural traditional herbal contraceptives (Kadam *et al* 2013) [3]. Since many years workers are trying to find out innovative plant based drugs containing medicinally valuable phytochemicals. These are alkaloids, glycosides, phenolic compounds, steroids, and terpenoid derivatives. However, the phytochemical composition of the aqueous and petroleum ether extracts of *Moringa oleifera* Lam. used for medicinal purposes has not been sufficiently studied with respect to contraceptive activity. Hence, it becomes essential to evaluate the phytochemicals of pharmaceutical importance in relation to contraceptive activity. The qualitative and quantitative phytochemical study was carried out from *Moringa oleifera* Lam. The seeds of *Moringa oleifera* Lam. were analyzed by HPLC technique. High pressure liquid chromatography (HPLC) has been found to be an efficient and sensitive method for identification and quantification of different classes of Ethynylestradiol.

2. Material and Methods

2.1 Collection of plant material

Seed samples of *Moringa oleifera* Lam. (Moringaceae) were collected from various areas of Ahmednagar District, India in their natural habitat. It was identified and authenticated from Botanical Survey of India, Western Circle, Pune. The voucher specimens were deposited in the Herbarium, BSI, Pune as well as Herbarium of Department of Botany, New Arts, Commerce and Science College, Ahmednagar. (ABK 003).

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2.2 Extraction of the Plant Materials

The seed were air dried at room temperature followed by pulverization to powder from using a mortar and pestle. The powdered seeds were subjected to aqueous extraction as well as extraction of active components from seed powder was performed with petroleum ether by using Soxhlet. Polar and Non polar solvent were taken into consideration for extraction. Solvent was removed by vacuum rotary evaporator at room temperature. The remaining residue was collected and preserved at 4°C for further experiment. The non-polar Petroleum ether was used which being more effective than methanol extracts (Mawahib 2015) [4], so the extracts were made in this non polar solvent.

3. Results and discussion

The medicinal importance of plants is due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, Saponins, Steroids, and Tannins etc. So the seeds were analyzed to find out the novel phytochemical which may responsible and correlate with contraception.

3.1 HPLC analysis of *Moringa oleifera* Lam.

The major compounds found in *Moringa oleifera* Lam. seeds were further analyzed by HPLC technique. High pressure liquid chromatography (HPLC) has been found to be an efficient and sensitive method for identification and quantification of different classes of Ethynylestradiol. It is an estrogen that is active when taken by mouth and is used in almost all formulation of combined birth control pills, and is nearly the exclusive estrogen used for this purpose. Estrogen contributes to ovulation suppression (Evans and Sutton 2015) [2]. Analysis of Ethynylestradiol derivatives extracted in petroleum ether and aqueous seed extracts of *Moringa oleifera* Lam. revealed significantly high levels. Three peaks

were elucidated in the chromatogram developed in Petroleum ether extract. The highest peak area was obtained by the peak having retention time 3.092 minute with 82.43% peak area. Second higher peak was obtained at retention time 6.206 minutes which has covered 11.20% peak area. It was observed that the third peak of the Moringa sample has showed about same retention time 6.206 minutes for Ethynylestradiol with less peak area 11.20%. Simultaneously, the standard Ethynyl estradiol peak area and retention time was compared with the Sample peak. The peak obtained in standard was at retention time 6.161 minute with 62.77% peak area. The spectra 6.206 obtained in petroleum ether extract of Moringa indicate that Ethynyl estradiol may be present in more amounts and may be associated with its derivatives. So retention time of standard and observed spectra of sample may be different on chromatogram. The peaks obtained in aqueous extract were totally different with respect to petroleum ether extract due to the concentration.

The calculated value of ethynyl estradiol was obtained 108.199 µg/ml of petroleum extract while it was obtained 655.26 µg/ml of *Moringa oleifera* aqueous extract. As the concentration of the samples increased, the peak area was also increased. Hence the above values for the amount of Ethynyl estradiol in petroleum ether and aqueous extract are variable. From these results it was confirmed that *Moringa oleifera* Lam. contains Ethynyl estradiol. It is a steroidal estrogen and a derivative of estradiol (Elks, 2014) [1]. It is very essential to evaluate observed (Retention Time 6.206 minute) compound by more sensitive technique for characterization and quantification of Ethynylestradiol. It has high estrogenic potency when administered orally, and is often used as the estrogenic component in oral contraceptive (www.ncbi.nlm.nih.gov/mesh/68004997).

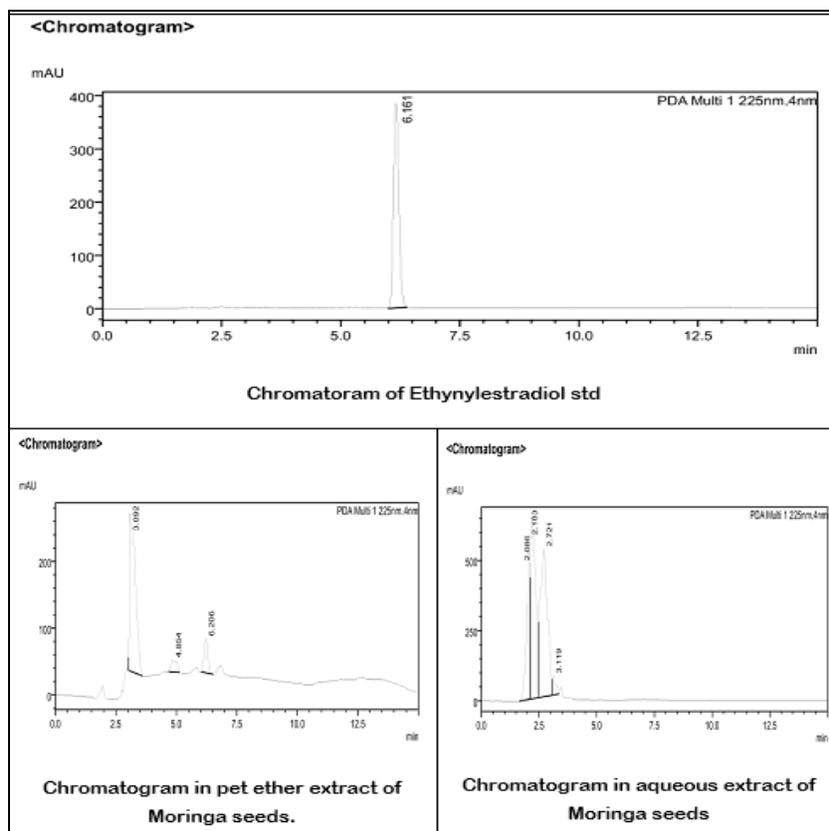


Fig 1: HPLC Chromatogram of Ethynylestradiol std and Moringa seed extract

4. Conclusion

The present study provides evidence that solvent extract of plants contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. The present work investigated the properties of Ethynylestradiol with HPLC technique. *Moringa oleifera* Lam. contains Ethynyl estradiol. It is one of the important ingredients of contraceptive preparations.

5. References

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