Research article

Phytohormone synthesis for PDH activation and phytochemical screening for the treatment of endometriosis

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ABSTRACT

Introduction and Aim: Endometriosis is a challenging gynecological disorder that causes pelvic pain and infertility attributed to the prevalence of ectopic endometrial tissue outside the uterine tract. Endometrial tissue can travel to other areas of the body for inexplicable reasons. There are, nevertheless, certain patterns. Endometriosis could be treated in several ways, based on characteristics such as age, fertility, symptom severity, and disease degree. All endometriosis medications are available as contraceptives, restricting their use to pregnant mothers or intending to get children. By comparing human peritoneal mesothelial cells (HPMCs) derived from the pelvic peritoneum of women with endometriosis to HPMCs from women without endometriosis, the researchers found that HPMCs from women with endometriosis had reduced glycolysis, mitochondrial respiration, and pyruvate dehydrogenase (PDH) and improved lactate production. There is no treatment for endometriosis, even though various medications and management alternatives are available. The present study was carried out to find novel medications to treat endometriosis from medicinal plants which appear to be a promising endeavor.

Methods and Materials: Phytohormone and phytochemical screening were performed to study the medication options that could possibly treat endometriosis. Phytochemicals were determined using GC-MS.

Results: The phytohormone activity and phytochemical components of *Mentha piperita*, *Curcuma longa*, and *Andrographis paniculata* were identified using GC MS and FT-IR analysis. This initial data from the analytical methods will make it easier to perform follow-up research on finding bioactive ingredients, determining their efficacy through in vivo tests, and proving their safety and efficacy through clinical trials to treat endometriosis.

Conclusion: Gibberellin, a phytohormone obtained from natural sources, is supposed to improve PDH activity and hence inhibit PDK. The demand for more modern medicines to increase survival in such cases remains unmet. As a result, there is an increasing interest in using herbal therapy to treat endometriosis.

Keywords: Endometriosis; HPMC; pyruvate dehydrogenase; gibberellin; herbal therapy.

INTRODUCTION

Endometriosis is the term used to describe the predominance of endometrial glands and lesions that resemble stroma outside of the uterus. There are several theories as to how endometriotic lesions form, even though there is no known cause for endometriosis. A multidisciplinary strategy is necessary for the treatment of endometriosis, including (i) surgical diagnosis and debulking of the disease load, (ii) medical intervention to prevent and slow the course of the illness, and (iii) pain management techniques that result in tailored care regimens (1). In terms of prevention, diagnosis, and treatment, it's critical to understand pathophysiology.

The human endometrium, placentation, and prenatal disorders such as preeclampsia cannot be accurately modelled by any nonhuman animal. Our understanding of the pathogenesis of endometriosis is currently limited to clinical, histologic, and metabolic results as well as investigations on endometriotic tissue. Endometriosis' etiology and pathophysiology are obscure (2). One of the negative effects of endometriosis is a one-sided lower abdomen pain that is related to ovulation. It could feel like a dull cramp or a severe spasm (3). When Primolut N treatment for endometriosis is required, it should start between the first and fifth day of the cycle and consist of taking 1 tablet twice daily. Various menstruation issues, such as agonizing, heavy, or inconsistent periods, are treated with this (4). When there is a hormonal imbalance, it can lead to major hormonal diseases or disorders in women, which completely alter the quality of their lives. The best determinant of direct medical expenses and overall costs is a decline in quality of life (5).

Endometriosis HPMCs treated with (PDK) inhibitor/PDH activator undergo normalization of HPMC digestion, which reduces lactate output and stops the proliferation of endometrial stromal cells. Among commercial phytohormones, gibberellins (GAs) are a vast group of significant diterpenoid acids. They are plant growth regulators that influence a variety of developmental processes in higher plants, notably stem elongation, germination, hibernation, gender expression, and fruit senescence(6). Evaluation of extreme discomfort and infertility has been used most frequently to gauge therapy effectiveness. There are undoubtedly some successful endometriosis therapies available, but they all have drawbacks. Both medical and surgical approaches to managing pelvic

discomfort are effective. Drug treatment for endometriosis-related infertility is unhelpful, while surgical surgery appears to help women with most if not all, forms of the illness (7).

Due to the irrational structural and biological variety, nature offers a vast resource for the discovery of novel bioactive compounds. The most potential source for achieving this goal is folk treatments. In vitro activity- or in vivo bioassay-guided fractionation and isolation techniques have been used to identify hundreds of active substances in traditional medicine. There hasn't been a successful endometriosis treatment up to now. Therefore, research seems to be required to find novel pharmaceutical compounds to treat endometriosis. Herbal remedies, phytochemicals, and multi-ingredient herbal formulations are used in the treatment of endometriosis. Because the bulk of studies on the effectiveness of herbal remedies in the management of endometriosis is at cellular and laboratory levels, well-designed drug trials are encouraged to provide clear evidence about the effectiveness of medicinal plants in this issue (8).

Antiangiogenic, antioxidant, sedative and pain-relieving capabilities are present in medicinal herbs and their bioactive components, and the effects have been seen to advocate their use for endometriosis management up to this point. Because of this, we view medicinal plants and phytochemicals as great supplemental therapies for the management of endometriosis but not yet sufficient as stand-alone therapeutics (9). The chance of synergistic interactions within specific plants or plant combinations is a possible advantage of herbal medicine (10).

Natural flora is being used to extract a huge variety of therapeutic plants for the economic production of medicines. The development of new medicinal medications with effective prevention and therapeutic effects against many diseases, including cancer, has resulted in the screening of active chemicals from plants (11). Over the past ten years, the general population has used herbal remedies more frequently since they are generally affordable, provide an option where other therapies often don't and are generally believed to always be safe (12). Plants' chemical and biological variety is a potentially limitless renewable source for the development of new pharmaceutical industries (13).

The herbal drugs can increase female ovarian reserve by controlling blood flow to the uterus and ovaries. Because they have fewer negative effects and side effects than pharmaceutical medicines, medicinal herbs, and Ayurvedic medications are frequently employed in serious conditions (14).

Endometriosis and estrogen are closely related, and it is obvious that estrogen regulates the production and function of a number of ion channels, which alter during the menstrual cycle. Their connection to endometriosis is still unknown, though. The information that is now available points to a crucial involvement of the cystic fibrosis transmembrane conductance regulator (CFTR), aquaporins, and the chloride channel CLC-3 in the etiology of endometriosis (15). Endometriosis has a significant chance of altering a person's life path because symptoms typically appear from menarche through menopause, a period when many decisions that can alter one's life and trajectory must be taken. The influence of these ailments and environmental/social circumstances at different ages or life stages, as well as the degree, to which certain symptoms have an effect on the course of one's life, are all in question. The first step in assisting women in realizing their full potential in life is gaining an understanding of the repercussions of endometriosis that go beyond the manifestations of pain and infertility (16).

Endometriosis is a chronic, aggressive, estrogen-dependent illness that results in discomfort and infertility in about 10 percent of the total women of reproductive age. Medical treatment has not yet been able to cure the illness, and stopping the medication causes symptoms to return (17). New biomedical targets must be developed in an effort to fight the cellular and molecular origins of endometriosis. Non-coding RNAs are being studied in the context of endometriosis treatment because they are emerging as key players in a number of human disorders, including cancer and endometriosis (18).

The distinctive functional groups in any plant extracts were located using Fourier transform infrared (FTIR). It explains how a molecule's structure can frequently be determined from its absorption spectra. The utilization of plant extracts by global beings is supported by the knowledge that they contain bioactive chemicals. It also applies to the creation of new medications through the isolation of particular substances (19).

Mechanism of PDH enzyme in endometriosis

The endometriosis condition is caused due to various unknown reasons but some of the pathogenesis is alleged to be retrograde menstruation, embryonic remnants, etc. Human peritoneal mesothelial cells (HPMC) from the pelvic peritoneum of a patient with endometriosis produce more lactate due to increased glycolysis, decreased mitochondrial respiration, and lower pyruvate dehydrogenase (PDH) enzyme activity. An enzyme called pyruvate dehydrogenase (PDH) transforms pyruvate during glycolysis into acetyl CoA, which ultimately moves into the TCA cycle. Pyruvate from glycolysis cannot be converted to acetyl CoA if this crucial PDH enzyme is blocked or reduced. Pyruvate uses another pathway to acquire energy (ATP) by converting NADH to NAD+ and producing lactic acid. Lactic acidosis occurs when there is too much lactic acid in the bloodstream. When the phytohormone gibberellin (GAs) is administered to HPMC, it is

thought to restore HPMC metabolism while also suppressing endometrial growth. For women with endometriosis, this approach of administering gibberellin can be utilized as a non-contraceptive treatment.

Theories relating to endometriosis's pathogenesis

Even though endometriosis is typically thought of as a steroid-sensitive illness, one of the potential causes of its development may be the immune system. Numerous immune system components, including the number of activated macrophages and the various subtypes of lymphocytes and their activity, have been observed to vary, pointing to the role of immunity. On the other hand, the development of endometriosis may be the only factor contributing to the immunological alterations around the endometriotic lesion (20). Endometriosis can be brought on by bacterial infections, immunological issues, epigenetics, and abnormal DNA methylation, among other things. The CD16 and CD56 molecules are expressed by the natural killer cells in the peritoneal fluid. They also express inhibitory and activating natural killing cell receptors and often function to get rid of endometrial cells during retrograde menstruation (21).

However, in endometriosis-affected women, alterations to these receptors and NK cell production of cytokines are what trigger the development and spread of endometriosis (22). Endometriosis' pathophysiology has been explained by four theories: lymphatic and vascular dispersion, lymphoid metaplasia, and transplantation of endometrial tissue. However, no one explanation can explain the location of ectopic endometrium in every instance of endometriosis. A high rate of obstructions in the genital canal has been linked to the onset of endometriosis in the first few years following menarche (23).

The idea that endometriosis is a condition linked to an epigenetic issue is being supported by mounting research. Numerous illnesses are caused by the disruption of such alterations (epigenetic aberration). Numerous gene transcriptions connected to chromatin alterations that characterize the states of illnesses are regulated by epigenetic mechanisms, which include DNA methylation and histone modifications (24). In endometriosis-affected women, the endometrium has increased proliferation as well as the capacity to implant and persist in ectopic sites. Since angiogenesis is necessary for the endometrium to survive outside of the uterus, the idea of increased endothelial proliferation is appealing. In addition to endometriosis, numerous disorders exhibit excessive angiogenesis (25).

METHODS AND MATERIALS

Phytochrome synthesis

Potential *Pseudomonas aeruginosa* was utilized from the stock purchased (ATCC - 424) and stored and the culture was inoculated in the Luria-Bertani medium. The culture was developed for 72 hours after being inoculated in the LB medium. *Pseudomonas aeruginosa* produces gibberellic acid in nutritional broth (LB medium). The supernatant was retrieved using a liquid-liquid extraction technique (ethyl acetate/$NaHCO_3$). In 100 ml of culture, 458 mg of cell residue was obtained. The supernatant obtained 61.5% alpha-amylase activity, confirming the presence of gibberellin in the *pseudomonas sp*.

Extraction of plant compounds with polar and non-polar solvents

Mentha piperita, Curcuma longa, and Andrographis paniculata plant samples were extracted using an ultrasonic technique utilizing polar (ethanol, acetone, and propanol) and non-polar (chloroform, diethyl ether, and hexane) solvents. Sonication for the extraction of plant material is increasingly being applied with low-frequency ultrasound emitters (20–40 kHz). By breaking cellular barriers and causing cavitation, ultrasonic waves allow target components to flee. To limit the heat gained in the samples, the ultrasonication process involves 15 seconds of off time followed by 10 seconds of on time. During the treatment, hundreds of tiny vacuum bubbles occur in the solution due to pressure being applied. The created bubbles crumble into the solution because of concussion.

Screening of phytochemicals

It involves extracting, examining, and locating medicinally useful plant components. Phytochemicals are non-nutritive compounds from plants that have medicinal, disease-preventing properties (26, 27). Over 900 phytochemicals have already been identified as food additives, and many more are still undiscovered. Some of the bioactive chemicals derived from plants include flavonoids, alkaloids, carotenoids, tannins, and phenolic compounds (28). The term "phytochemical" refers to compounds that are regarded as required nutrients, meaning that they are naturally present in plants and are essential for healthy physiological functions; hence they must be consumed by humans through food. A crucial step in the discovery of a new medication is qualitative phytochemical analysis, which reveals whether any specific primary or secondary metabolites of clinical relevance are present in the plant's extracts. In any instance, it is required to use appropriate chromatographic technology to extract the chemical from the mixture of compounds if any substantial natural bioactive product is present.

FTIR (Fourier transform infrared) analysis

FTIR analysis is a method for distinguishing organic, inorganic, and polymeric compounds. For the characterization and validation of chemicals or chemical linkages contained in an unknown blend of plant extract, FTIR has emerged as an effective approach (29, 30). The three fundamental IR-spectroscopic sampling modes are transmission,

transflection, and attenuated total reflection (ATR). Each model works well for some examples, while others provide challenges. The x-axis, or horizontal axis, represents the infrared spectrum, which shows the intensity of infrared spectra. The peaks sometimes referred to as absorbance bands correspond to the sample's various atomic vibrations when subjected to the infrared region of the electromagnetic spectrum.

GC-MS (gas chromatography-mass spectroscopy) analysis

The molecular constituents of a studied mixture are separated using the separation science method of gas chromatography (GC), which is then used to identify the components to ascertain whether or not they are there and how much each component is. Conversely, peak regions are related to the amount of the relevant molecule. A complicated sample separated by GC-MS will result in numerous distinct peaks, each of which generates a distinct mass spectrum utilized for compound identification. Unknown substances and target analytes could be located and measured using large commercial libraries of mass spectra (31, 32).

RESULTS

Phytochemical screening

Tables 1 and 2 mentioned below show the process for examining phytochemical ingredients and their corresponding observations.

Table 2 shows the phytochemical constituents' presence in the plant samples extracted with different polar and non-polar solvents. The phytochemical phenol and tannin were absent in any plant samples – solvent combinations.

Table 1: Screening of phytochemicals

Constituents	**Test**	**Observations**
Phenols and tannins	To the crude extract, 2 ml of 2% ferric chloride is added	Black coloration indicates the presence of phenols and tannins
Glycosides	To the crude extract, 2 ml of glacial acetic acid, a few drops of ferric chloride, and 1 ml of concentrated sulfuric acid are added	The formation of a brown ring at the interface indicates the presence of glycosides
Flavonoids	The crude extract added 2 ml of 2% sodium hydroxide. Then 2 drops of diluted acid will be added	The sample turns to concentrated yellow color upon NaOH addition and becomes colorless upon the addition of diluted acid. This indicates the presence of flavonoids
Alkaloids	To the crude extract, a few drops of Wagner's reagent are added	Reddish-brown precipitates indicate the presence of alkaloids
Saponins	To the crude extract, 5 ml of distilled water is added and mixed vigorously	Foam appearance indicates the presence of saponins
Terpenoids	To the crude extract, 2 ml of chloroform is added which was warmed and cooled. To the cooled sample, 3 ml of concentrated sulfuric acid is added along the sides	Reddish-brown precipitates indicate the presence of alkaloids
Steroids	To the crude extract, concentrated sulfuric acid is added	The red color at the lower layer indicates the presence of steroids

Table 2: Qualitative analysis of phytochemicals

Plant Sample and Solvent	**Phytochemical test**						
	Phenol and tannins	**Glycosides**	**Flavonoids**	**Saponins**	**Terpenoids**	**Steroids**	**Alkaloids**
Ethanol - Peppermint	-	-	-	-	-	-	-
Ethanol - Turmeric	-	+	-	-	+	+	-
Ethanol - Nilavembu	-	+	+	-	-	-	+
Acetone - Peppermint	-	+	-	-	+	-	-
Acetone - Turmeric	-	-	-	-	+	+	-
Acetone - Nilavembu	-	+	+	-	-	+	+

Propanol - Peppermint	-	+	-	+	-	+	-
Propanol - Turmeric	-	+	-	+	+	+	-
Propanol - Nilavembu	-	+	+	+	+	+	+
Chloroform - Peppermint	-	-	-	-	-	-	-
Chloroform - Turmeric	-	+	-	+	-	+	-
Chloroform - Nilavembu	-	-	-	+	-	+	+
Diethyl ether - Peppermint	-	-	-	-	+	-	-
Diethyl ether - Turmeric	-	+	+	+	+	+	-
Diethyl ether - Nilavembu	-	-	-	+	-	-	+
Hexane - Peppermint	-	+	-	-	+	-	-
Hexane - Turmeric	-	+	-	-	+	+	-
Hexane - Nilavembu	-	+	-	+	+	-	+

FTIR (Fourier Transform Infrared) analysis

The wavenumber for mid-range IR is shown to be between 4,000 and 400 cm-1 in the infrared spectrum. The analysis of the FT-IR spectroscopy was done to investigate the acetone and diethyl-ether extracts of three medicinal plants: *Mentha piperita, Curcuma longa, and Andrographis paniculata*. The FT-IR experiments normally show the different functional groups in the extracts with varied characteristics of peak values. The functional groups of the active components discovered in the extract were characterized using the FT-IR spectrum using the peak values in the IR radiation band. After being processed by FT-IR, the elements were categorized into functional groups based on the extract's peak ratio. The functional groups O-H, C-C, C=C, C-H, O-H, C-N, R-COO, C-O, and CH3 were confirmed by the results of the FT-IR analysis. Evidence suggests that FTIR spectroscopy is a reliable and sensitive method for figuring out the makeup of biomolecular systems.

GC-MS (Gas chromatography-mass spectroscopy) analysis

From the GC spectrum analysis, the GC fractions of the methanolic extracts of *Mentha piperita, Curcuma longa, and Andrographis paniculata* show peaks of several compounds. specifically, 22 different bioactive compounds, namely, N,N',2,2-Tetramethyl-1,3-propanediamine, urea propane nitrile, N-methyl-N-nitroso, 3-(Octyl amino), 2,3-Dihydro-1-Ethyl-1H-Cyclopenta [b], N-[3-[N-Aziridyl] propylidene] quinoxaline DL-citrulline, Asarone, Curlone, Ar-tumerone, 3-Dimethylaminopropylamine, 1-(1-propynyl), Propanoic acid, 2,7-octanedione, z-1,9-Hexadecadiene, 2-Oxo-, Methyl Ester 2,6,6-Trimethyl-Bicyclo [3.1.1] 3-Decyn-2-ol, Hept-3- Ylamine 2,6,9, and 11-dodecatetraenal, also known as cis-5-methyl-2-isopropyl-2-hexen-1-al, 2,6,10-Trimethyl-, 1-Di (Tert-butyl) silyloxytridecane, (e, e, e), hydroxy-3-keto-12-ketobisnorcholanic acid, 2,6-Lutidine 3,5-Dichloro-4-dodecylthio. Table 3 shows some biomolecules that have been identified. The separated substances could be used to treat endometriosis.

Table 3: Bio-compounds retrieved from GC-MS analysis

Compound Name	**Molecular Weight**	**Formula**	**Retention Time (min)**
1,3-Propane diamine, n, n',2,2-Tetramethyl	130	$C_7H_{18}N_2$	3.03
Urea, n-methyl-n-nitroso-	103	$C_2H_5O_2N_3$	3.56
Propanenitrile, 3-(octylamino)-	127	$C_{11}H_{22}N_2$	10.34
2,3-dihydro-1-ethyl-1h-cyclopenta[b]quinoxaline	198	$C_{13}H_{14}N_2$	15.44
N-[3-[n-aziridyl] propylidene]-3-dimethylaminopropylamine	183	$C_{10}H_{21}N_3$	28.06
Dl-citrulline	175	$C_6H_{13}O_3N_3$	31.35
Asarone	208	$C_{12}H_{16}O_3$	14.85
Curlone	218	$C_{15}H_{22}O$	15.48

Ar-tumerone	216	$C_{15}H_{20}O$	15.53
Cyclohexene, 1-(1-propynyl)-	120	C_9H_{12}	15.92
2,7-octanedione	142	$C_8H_{14}O_2$	2.56
Propanoic acid, 2-oxo-, methyl ester	102	$C_4H_6O_3$	3.56
Z-1,9-hexadecadiene	222	$C_{16}H_{30}$	20.37
2,6,6-trimethyl-bicyclo [3.1.1] hept-3-ylamine	153	$C_{10}H_{19}N$	20.49
3-decyn-2-ol	154	$C_{10}H_{18}O$	20.59
Cis-5-methyl-2-isopropyl-2-hexen-1-al	154	$C_{10}H_{18}O$	25.90
2,6,9,11-dodecatetraenal, 2,6,10-trimethyl-,(e, e, e)-	218	$C_{15}H_{22}O$	27.26
1-Di(tert-butyl) Silyloxytridecane	342	$C_{21}H_{46}OSi$	27.51
3-hydroxy-12-ketobisnorcholanic acid	362	$C_{22}H_{34}O_4$	27.62
2,6-lutidine 3,5-dichloro-4-dodecylthio	375	$C_{19}H_{31}NCl_2S$	29.13
Methyl 10,12-pentacosadiynoate	388	$C_{22}H_{44}O_2$	29.83
2-myristynoyl-glycinamide	280	$C_{16}H_{28}O_2N_2$	30.99

DISCUSSION

In accordance with several theories implicated in its pathogenesis, the etiology of endometriosis is complex and multifactorial, comprising hormonal, genetic, immunological, and ambient variables. One of the hallmarks of endometriosis is pelvic inflammation, which is brought on by endometriotic sores and contributes to the ectopic proliferation and expansion of endometrial tissue. The outcomes of the research show that GA3 was generated by *Pseudomonas* species. *Pseudomonas* sp. has a shorter incubation period and less complicated culture conditions for GA3 production than other species. These results imply that *Pseudomonas* sp. can be used as a high-yielding bacterial source and an effective replacement for the synthesis of GA3. It is expected that feeding HPMC with the phytohormone gibberellin (GAs) will increase HPMC metabolism while reducing endometrial growth. This type of gibberellin administration can be utilized as a non-contraceptive therapy for endometriosis-affected women. Recurrence of endometriosis after treatment discontinuation is common and most patients require medical assistance to maintain low estrogen control until conception. Even with recent developments in computational and chemical techniques, conventional medicine is still regarded as the most reliable source for finding new drugs. Plants are believed to have therapeutic or healing properties. Various traditional medicines and herbal compounds have been investigated for the treatment of gynecological diseases, especially endometriosis using analytical techniques.

CONCLUSION

Endometriosis is a well-known health problem that commonly leads to gynecological diseases. In the current study, the genus Pseudomonas was discovered. It can be used as a high-yield microbial source and as an effective alternative to GA3 synthesis. This kind of gibberellin infusion can be utilized as a non-contraceptive treatment for endometriosis-afflicted women. The use of medicinal herbs to heal endometriosis has grown in recent years. Traditional treatments for endometriosis and other gynaecological diseases include a number of herbs. As a result, the utilization of medicinal plants and their phytochemical components has been recognized as a novel method for treating endometriosis and promoting good health. Thus, comprehensive and systematic research, alongside clinical evaluation, derivatization, and formulation studies, can yield products that benefit human health.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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