The Journal of Phytopharmacology 2022; 11(6):378-385
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2022; 11(6): 378-385
November- December
Received: 06-10-2022
Accepted: 28-11-2022
Published: 20-12-2022
©2022, All rights reserved
doi: 10.31254/phyto.2022.11601

Bimitha Benny
MSc scholar, department of veterinary biochemistry, college of veterinary and animal sciences, Mannuthy, Kerala, India, Pin code: 680651

A Sandeshkrishna
MSc scholar, department of veterinary biochemistry, college of veterinary and animal sciences, Mannuthy, Kerala, India, Pin code: 680651

Sujith Samraj
Assistant professor, department of veterinary pharmacology and toxicology, college of veterinary and animal sciences, Mannuthy, Kerala, India, Pin code: 680651

Preethy John
Assistant professor, department of veterinary pharmacology and toxicology, college of veterinary and animal sciences, Pookode, Kerala, India, Pin code: 67357

Uma Radhakrishnan
Head, Department of Veterinary Biochemistry, College of Veterinary and animal Sciences, Mannuthy, Kerala 680651

Correspondence:
Dr. Sujith Samraj
Assistant professor, department of veterinary pharmacology and toxicology, college of veterinary and animal sciences, Mannuthy, Kerala, India, Pin code: 680651
Email: sujith@kvasu.ac.in

Differential expression of steroidogenic genes in MCF-7 cell lines by methanolic extract of *Mallotus phillippensis*

Bimitha Benny, A Sandeshkrishna, Sujith Samraj, Preethy John, Uma Radhakrishnan

ABSTRACT

The role of oestrogen in the development of breast cancer is well documented. The aberrant expression of genes involved in oestrogen hormone synthesis can be one of the causes of breast cancer. Suppression of these genes is considered a therapeutic option for the treatment of breast cancer. In the present study, we evaluated the effect of a methanolic extract of *Mallotus phillippensis* leaves on the expression of *CYP19* and *StAR* genes in MCF-7 breast cancer cell lines. The half maximal inhibitory concentration (IC_{50}) of the extract was found to be 190 µg/mL. MCF-7 cells treated with half IC_{50}, IC_{50} and double IC_{50} of the extract that is 95, 190 and 380 µg/mL, was used for evaluating the effect on the gene. Methanolic extract of *M. phillippensis* at double IC_{50} doses, upregulated the expression of *StAR* and *CYP 19* genes, whereas at half IC_{50} and IC_{50} doses downregulated *StAR* and *CYP 19* gene expression. The gas chromatography mass spectrometry analysis revealed the presence of phytosterols, fatty acid analogues and terpenoids in the extract which contributed to differential expression of *StAR* and *CYP 19* genes. The confirmation of detected compounds was carried out by Fourier transform infrared spectroscopy analysis. From this study, it is inferred that higher doses of methanolic extract of *Mallotus phillippensis* can be used for treatment of the oestrogen deficient conditions leading to infertility while at lower doses significantly reduces *StAR* and *CYP 19* gene expression can be recommended for the treatment of breast cancer.

Keywords: *Mallotus phillippensis*, Breast cancer, CYP19, StAR, Real time PCR.

INTRODUCTION

Oestrogen, a C18 steroid hormone, has a variety of functions in the body, including stimulating female sexual development, maintaining bone density and protecting against neurodegenerative diseases. The classical oestrogen receptors, ER alpha and ER beta, interact with oestrogen in target tissues, exert biological effects by activating target gene and protein expression [1-3].

Synthesis of oestrogen occurs in the theca cells of the ovary from the common steroid hormone precursor cholesterol via a series of enzymatic steps. Steroid hormone synthesis starts with the transfer of cholesterol from the outer to the inner mitochondrial membrane, which is regulated by the Steroidogenic acute regulatory protein (StAR), which is the rate-limiting step of steroidogenesis. Transformation of cholesterol into pregnenolone is mediated by the enzyme CYP11A1, the *17βhydroxylase* enzyme converts pregnenolone into androgen. Finally, aromatization of androgen generates oestrogen by the aromatase enzyme (CYP19) [4].

Long-term oestrogen exposure has been related to an increased risk of breast cancer and high levels of oestrogen in females are associated with early menarche, hormone replacement therapy and late menopause [5, 6]. The vast majority of breast cancers are oestrogen-dependent; about 388 pm/g of oestradiol was found in breast cancer tissues, while normal breast cells contain approximately 172 pm/g of oestradiol [7]. The growth of oestrogen-dependent carcinoma by oestrogen is accomplished by cell proliferation via ER receptor interaction; oestrogen increases the production of peptide growth factors, which are involved in the proliferative action of cancer cells [8].

Overexpression of enzymes associated with oestrogen synthesis, appears to be linked to the development of a more aggressive illness, as well as a prognosis and an increase in local and distant recurrences [9]. StAR, transport protein of cholesterol, aberrant expression of which, promote tumorigenesis by increased uptake of cholesterol for oestrogen production [10]. Aromatase enzyme, encoded by *CYP19* gene, responsible for the synthesis of oestrogen from androgen and is overexpressed in breast cancer cells implies significant role in tumour progression. To assess the overexpression of aromatase gene, enzyme activity assays, immunocytochemistry and reverse transcription-polymerase chain reaction (RT-PCR) studies are used [11, 12].

Plant compounds that bind to the oestrogen receptor and modify estrogenic function are known as phytoestrogens [13]. These chemicals are regarded to be useful in the prevention of breast cancer. They may act as antioestrogens or weak oestrogens by competing with oestrogens for oestrogen receptor binding. Prior studies have shown that soy isoflavone consumption may have cancer-preventive effects by lowering oestrogen synthesis, by altering aromatase enzyme activity [14]. Previous study also discovered that the flavones apigenin and quercetin, as well as the isoflavones genistein, daidzein and biochanin A, showed dose-dependent reductions in aromatase mRNA levels, with apigenin being the most potent aromatase inhibitor [15].

Mallotus phillippensis, widely known as kamala, is a medicinal plant in the Euphorbiaceae family. Whole parts of plants are abundant in secondary metabolites and comprise flavonoids that have isoprenyl groups [16]. Previous studies have shown that juice prepared by *Mallotus phillippensis* leaves can control irregular menstruation [17]. Furthermore, *Mallotus phillippensis* has been claimed to have antioxidant, antibacterial, antifungal, anthelmintic and hepatoprotective properties. We have already reported the cytotoxicity of *M. phillippensis* [18] and modulation of oestrogen and progesterone secretion by the extract in MCF-7 cell lines [unpublished data]. There was an increased oestrogen secretion at double IC_{50} (380) while reduced secretion at IC_{50} (190) and half IC_{50} (95) doses. Hence the present study was undertaken to examine the effect of the extract on expression on key genes involved in steroidogenesis.

MATERIALS AND METHODS

Drugs and chemicals

Agarose-low melting, 0.25 per cent Trypsin-EDTA, bovine serum albumin, gentamicin sulphate, ceftriaxone sodium, Rosewells Park Memorial Institute (RPMI), antibiotic-antimycotic solution (100x), Foetal bovine serum (FBS), were purchased from Invitrogen Life Technologies USA. Tris buffer, dimethyl sulphoxide (DMSO), methanol, Dulbecco's phosphate buffered saline (DPBS) were purchased from Sisco Research Laboratories (SRL) Maharashtra. Polymerase Chain reaction (PCR) kit was purchased from M/s Promega. Realtime quantitative polymerase chain reaction (qRT-PCR) kit was procured from M/s Thermofischer.

Collection of plant material and authentication

The leaves of *M. phillippensis* was obtained from the campus of College of Veterinary and Animal Sciences, Pookode, Wayanad, authenticated at MSSRF

Cell lines and culture conditions

For *in vitro* studies, adherent human breast cancer cell lines, MCF7, were obtained from the National Centre for Cell Science (NCCS) in Pune, Maharashtra, India. MCF-7 was oestrogen receptor (ER) positive, progesterone receptor (PR) positive and human epidermal growth factor receptor (HER) negative. These adherent cells were cultured in RPMI1640 supplemented with 10% charcoal stripped FBS and 1% Gentamicin and kept in a humidified incubator at 37°C with 5% CO2. Trypsinization was performed on the cells using a 0.25 percent trypsin/1 mM EDTA solution. Cell suspensions with a viability of 95% (as assessed by trypan blue vital stain in an automated cell counter (CountessTM, Invitrogen, Van Allen Way, Carlsbad, California) were seeded in culture plates for different in vitro research.

Methanol extraction

M. phillippensis leaves were cleaned, shaded dried, and coarsely ground in a temperature-controlled plant sample pulveriser. At 60 ºC, the powdered plant material (500 g) was extracted with methanol in a Soxhlet extractor. After thorough extraction, the methanol extract was evaporated and extract was concentrated in a rotary vacuum evaporator (Rotavapor, Buchi, Switzerland) at 40°C and 10000 pascal pressure. The residue was first kept open at room temperature to allow the solvent to completely evaporate before being stored in an airtight container in the refrigerator for later use.

Sample preparation

To get the appropriate concentrations of the extract, a stock solution of the methanol extract was prepared in RPMI-1640 medium and then diluted with the prepared culture media.

Effect of exposure of MCF-7 cells to extract of *M. phillippensis* on expression of *CYP19* and *StAR* gene

The cells were cultured for 96 hours with IC_{50}(190), double (380) and half dosages of IC_{50} (95) extract and appropriate medium, with media replacement every 24 hours. The cells were collected by trypsinisation after 96 hours and utilised to isolate total RNA for expression experiments. Total RNA was extracted separately using the 'TRIzol' reagent (Thermo Scientific) according to the manufacturer's instructions [19].

The quality and quantity of total RNA were determined spectrophotometrically using the Nano drop (Thermo Scientific NanoDropTM1000 Spectrophotometer) technique. Using a Nano Drop spectrophotometer, the purity of total RNA was evaluated using the A260/A280 and A260/A230 ratios.

cDNA was synthesized by reverse transcription of 500 ng of RNA, the reaction was set up according to the maxima first strand cDNA synthesis kit's standard procedure (Table 1)

The product of first strand cDNA synthesis was stored at -20^{0}C until use. Then 1 µL of cDNA was used for further qPCR experiments.

Exon spanning primers (Table 2) were developed using NCBI primer design software (Primer3, http://bioinfo.ut.ee/primer3/), and the primer's specificity was confirmed using BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Gradient PCR was used to amplify the *CYP19*, *StAR*, and *GAPDH* genes and optimize the PCR condition. Table 3 shows the PCR reaction mix and cycle conditions used.

SYBR green chemistry (Maxima SYBR green qPCR master mix (Thermo scientific, USA)) was used to investigate the expression of *CYP19*, *StAR* and *GAPDH*. The reaction was performed in triplicate (Table 4). The expression of target gene *CYP19, StAR* was compared with a reference gene *GAPDH* to calculate ΔCq and the expression of same gene in treatment sample versus control samples to calculate ΔΔCq. The method used is known as comparative Δ Cq method [20].

Following the reaction, a melt curve analysis was done to ensure that the amplification was specific. The melt curve analysis method included denaturation at 95°C for 15 seconds, annealing at 55°C for 15 seconds, and denaturation at 95°C for 15 seconds. During the final denaturation stage, data was collected.

Gas chromatography – Mass spectrometry (GC-MS) analysis of potent extract/fraction

The active phytochemicals present in *M. phillippensis* was analysed using Gas chromatography Mass Spectrometer (Shimadzu GC-MS, Japan, QP2010S) with a mass range of 1.5-1000 m/z was used. Helium at flow rate of one mL/ min was the carrier gas with oven temperature of 80ºC for four min and then increased to 280ºC in six min. The injector temperature was 260ºC and total analysis time was 50 min. Aliquots of extract (0.4 µL) were injected into the chromatographic column after a clear baseline was obtained. Major compounds were detected using mass spectrum library (NIST 11 and WILEY 8).

Fourier transform infrared spectroscopy (FTIR) analysis

ATR-FTIR (Attenuated Total Reflectance- Fourier transform infrared) analysis was performed, using a Perkin- Elmer spectrum two FTIR spectrometer with attenuated total reflectance. The sampling station was equipped with an overhead ATR accessory. A small quantity of the sample was carefully placed on the diamond crystal surface to cover the ATR diamond window in order to focus the laser beam. Each spectrum was recorded as absorbance under 60 N.

Statistical Analysis

All results were presented as Mean ± SE with 'n' equal to the number of replicates. SPSS software version 24 is used for the intergroup comparison was analysed using one-way analysis of variance (ANOVA) accompanied by Duncan Multiple Range Test (DMRT) for paired analysis.

RESULTS

Determination of concentration and purity of total RNA

Quality and concentration of RNA samples is chosen for gene expression studies depicted in the table 5. The A260/280 and A260/230 ratios are in the range of 1.9 to 2.2, indicating that the RNA samples are of good purity and quality.

Relative gene expression studies

A gradient PCR reaction was setup for standardization of the parameters required for the amplification of human *CYP 19* and *StAR* genes. The primer concentrations and annealing temperature with the best results were opted for the real time qPCR. The gradient PCR products were run on 1.5 per cent gel and figure 1 depict the amplification plot and melt curve of *CYP 19* and *StAR* genes in real time quantitative PCR

Effect of methanolic extract of M. phillippensis on CYP 19 gene expression

Methanolic extract of *M. phillippensis* at 380 µg/mL (double IC_{50}) increased the expression of the *CYP 19* gene in MCF-7 cells by 1.385 fold. In MCF-7 cells, extract 190 (IC_{50})) and 95 µg/mL (half IC_{50}) doses reduced *CYP 19* gene expression by 0.0729 and 0.00028-fold, respectively. In control cells, the expression of all genes was normalized to unity. Figure 2 represent the relative *CYP19* gene expression study.

Effect of methanolic extract of M. phillippensis on stAR gene expression

Methanolic extract of *M. phillippensis* showed a 61.10±3.90 fold increase in expression of the *StAR* gene at dose of double IC_{50} (380 µg/mL). Plant extract at doses of IC_{50} (190) and half IC_{50} (95) showed a 0.090±4.36 and 0.016±0.59-fold decrease in MCF-7 cell lines, respectively. In control cells, the expression of all genes was normalized to unity. Figure 3 represent the relative *StAR* gene expression study in MCF-7 cell lines.

Gas chromatography – Mass spectrometry (GC-MS) analysis of potent extract/fraction

The GC-MS analysis of *M. phillippensis* showed chromatogram with major peaks obtained at 17.169, 26.60, 27.47, 28.37, 29.21, 31.59, 31.73, 31.93, 32.15, 43.18 and 47.62 min retention time (figure.4). The major phytochemicals found using mass spectrum library were caryophyllene, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, methylpalmitate, dibutyl phthalate, linoleic acid, methyl ester, methyl 8,11,14-heptadecatrienoate, phytol, acetate, methyl isostearate, squalene, sigmast-5-en-3-ol, oleate which were belong to mainly terpenoids, precursor of vitamin synthesis, fatty acid esters and sterols. (Table. 6)

Fourier transform infrared spectroscopy (FTIR) analysis

The most probable compounds identified by comparing the spectra of the extract using FTIR with the FLUKA library are listed in table 7 and the FTIR spectra of plant extract are shown in Figure 5.

DISCUSSION

Breast cancer is one of the most frequent types of cancer in humans, with oestrogen being the most prevalent trigger. Over expression of genes involved in oestrogen production accounts for the aetiology of breast cancer and the current work aims to develop novel agents that target expression of steroidogenic genes in hormone responsive MCF-7 breast cancer cell lines.

The biosynthesis of oestrogen occurs through action of a variety of enzymes: *CYP 19* and *StAR* play critical roles in regulating oestrogen production. Endocrine therapy for breast cancer utilizes enzyme inhibitors that are involved in the synthesis of oestrogen. Phytoestrogens have been shown to have both stimulatory and inhibitory effects on steroidogenic gene expression. as a result, they can be used to modulate steroidogenic gene expression in breast cancer.

Aromatase enzyme (CYP 19) is responsible for the biosynthesis of oestrogen by converting androgen into oestrogen and hence play a key role in the development of oestrogen dependent carcinomas. *StAR* is a key regulator in steroidogenesis; impaired expression of this gene, results in the reduction of availability of cholesterol for oestrogen synthesis. In the present study, methanolic extract of *M. phillippensis* at double IC_{50} doses upregulated the expression of *CYP19* and *StAR* gene expression in MCF-7 cells. As a result of the high availability of cholesterol, oestrogen secretion was also stimulated at these doses. Increased oestrogen production at double IC_{50} doses can be utilised to treat oestrogen deficient conditions such as infertility and postmenopausal syndromes. The plant extract decreased the expression of *StAR* and *CYP 19* at IC_{50} and half IC_{50} doses, indicating that when cholesterol uptake was reduced, the synthesis of oestrogen in MCF-7 cells was significantly reduced. Negative regulation of steroidogenic gene expression at these doses can be used to treat oestrogen dependent carcinomas.

Phytoestrogens, also known as exogenous oestrogens, are secondary metabolites found in plants that have an oestrogenic or antiestrogenic effect on the human body based on their dosages. As vegetables, fruits and herbs contain phytoestrogen, consumption of these compounds in certain amounts holds the risk of induction of breast cancer. According to previous research, high dosages of phytoestrogen promoted the expression of the *CYP 19* and *StAR* genes at random. Administration of genistein, an isoflavone, induced aromatase activity and enhanced expression of *CYP 19* mRNA in hepatic cells (HepG2) [21]. About 1000 ng/mL of fusarium mycotoxin dexynivalenol suppressed expression of *stAR* [22]and quercetin also increased S*tAR* gene expression in MA-10 cells. Resveratrol and Genistein decreased *StAR* gene expression in MA-10 cells [23]. Various studies on phytoestrogen are indicated the down regulation of expression of *CYP19* mRNA at specific doses ranges. For instance, low dose (0.1 M to 10 M) phytoestrogen combinations were useful in alleviating aromatase mRNA levels in human ovarian tissues [24]. Biochanin A, an isoflavone found in red clover, was found to inhibit aromatase activity and mRNA expression in breast cancer cell lines (MCF-7 and SK-BR-3) [25]. Phytoestrogens mimic the structure of androgen substrate to interact with the active site of aromatase [26]. The differential expression of the *CYP19* and *StAR* genes in MCF-7 cell lines by methanolic extract of *M. phillippensis* is demonstrated in the present study. Previous research found that extract at low dosages considerably reduced oestrogen production while high doses increased

oestrogen synthesis; these findings are compatible with current gene expression investigations.

About 14 bioactive molecules detected in the methanolic extract of *M. phillippensis* leaves by GC-MS analysis. Majority of compounds were categorized into fatty acids, fatty acid analogues and terpenoids. Presence of phytosteroids in *M. phillippensis* accounts for the modulatory effect in the expression of steroidogenic genes. Among obtained compounds, caryophyllene possessed anticancer activity by inhibiting proliferation of MCF-7 cells and reduced cholesterol levels [27]. Methyl 8,11,14 Heptadecatrienoate, an aliphatic fatty acid found in methanol extracts of *Glycosmis mauritiana* have antioxidant, hypochloesterolemic activities [28]. The hypercholesterolemic activity of these compounds caused decrease in the expression *StAR* and *CYP 19* genes in MCF-7 cells. Stigmast-5-en-3-ol, derived from the soft *coral D. gigantea*, was found to inhibit the growth of both HL-60 and MCF-7 cancer cells in a dose-dependent manner [29]. The anticancer potential of phytol in MCF-7 and PC-3 cells were reported [30]. The presence of compounds possessing anticancer and steroidogenic activity present in the methanolic extract of *M. phillippensis* provided significant anticancer and steroid modulatory potential.

Table 1: Master Mix for cDNA synthesis

Components	Volume (µL)
5X Reaction buffer	4
dnTP mix	2
Oligo dT primer	1
Enzyme Mix	1
Ribolock	1
Total RNA	To get 500 ng
Nuclease free water	Added to make 20 µL of total mix

Table 2: Description of primers used

Gene Name	Primer sequence (5”- 3”)	Primer Length (bp)	Product Size (bp)
CYP19	TGCTTCCTCACTGGCCTTTTT CCATGATGGACCAAAATCCCAA	20 22	121
StAR	GCTGCTAGCGACATTCAAGC AGCCGAGAACCGAGTAGAGA	20 20	186
GAPDH	TCGGAGTCAACGGATTTGGT TTCCCGTTCTCAGCCTTGAC	20 20	181

Table 3: PCR conditions for amplification

Steps		Temperature	Time
Initial denaturation		95°C	5 min
30 cycles of	Denaturation	95°C	45 sec
	Annealing *CYP19* *StAR* *GAPDH*	62.1° C 58.6° C 62.9° C	30 sec
	Extension	72°C	1 min

Table 4: qRT-PCR conditions for *CYP19 and StAR* genes

Steps		Temperature	Time
Initial denaturation		95°C	3min
30 cycles of	Denaturation	94°C	30sec
	Annealing *CYP19* *StAR* *GAPDH*	62.1° C 58.6° C 62.9° C	30 sec
	Extension	72°C	30 sec

Table 5: showed the concentration and purity of RNA samples is chosen for gene expression studies

RNA samples	Concentration(µg/µL)	$A_{260\backslash 280}$	$A_{260/230}$
Untreated	2209	2.02	2.1
Cells treated with half IC_{50}	238	1.93	2.04
Cells treated with IC_{50}	353.6	1.90	2.02
Cells treated with double IC_{50}	356.7	2.03	2.2

Table 6: GC-MS analysis of phytochemicals in *M. Phillippensis* leaves

Retention Time (Min)	Name of the Compound	Molecular formula	MW(g/mole)	Peak area (%)	Class	Probability (%)
17.169	Caryophyllene	$C_{15}H_{24}$	204.35	1.08	Bicyclic sesquiterpene	81
26.607	Neophytadiene	C20H38	278.5	15.11	Diterpene	94
27.477	3,7,11,15-Tetramethyl-2 hexdecen-1-ol	$C_{20}H_{40}O$	296.5	4.28	Acyclic diterpene alcohol	90
28.376	Methyl Palmitate	$C_{17}H_{34}O_2$	270.45	8.42	Fatty acid ester	95
29.214	Dibutyl Phthalate	$C_{16}H_{22}O_4$	278.34	22.96	Phthalate ester	96
31.599	Linoleic acid	$C_{18}H_{32}O_2$	280.4	4.72	Fatty acid	88
31.599	Methyl ester	$C_{19}H_{36}O_2$	296.5	4.72	Fatty acid ester	88
31.731	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.4	5.76	Aliphatic fatty acid	89
31.937	Phytol	$C_{20}H_{40}O$	296.53	19.60	Acyclic diterpene alcohol	92
31.937	Acetate	$C_2H_3O_2^-$	59.04	19.60	Fatty acid ester	92
32.158	Methyl isostearate	$C_{19}H_{38}O_2$	298.5	3.01	Fatty acid methyl ester	84
34.181	Squalene	$C_{30}H_{50}$	410.7	11.38	Triterpene	95
47.622	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414.7	3.67	Phytosterol	78
47.622	Oleate	$C_{18}H_{34}O_2$	282.5	3.67	Fatty acid	78

Table 7: enlisted Functional groups presented in *M. phillippensis* after FTIR analysis

Wave number cm^{-1}	Bond	Functional group
3259	O-H	Carboxylic acids
2917	N-H	Amine salts
1710	C=O	Carboxylic acids, esters
1442	C-H	Alkanes
1104	C-O	Secondary alcohol
515	C-I	Halo compound

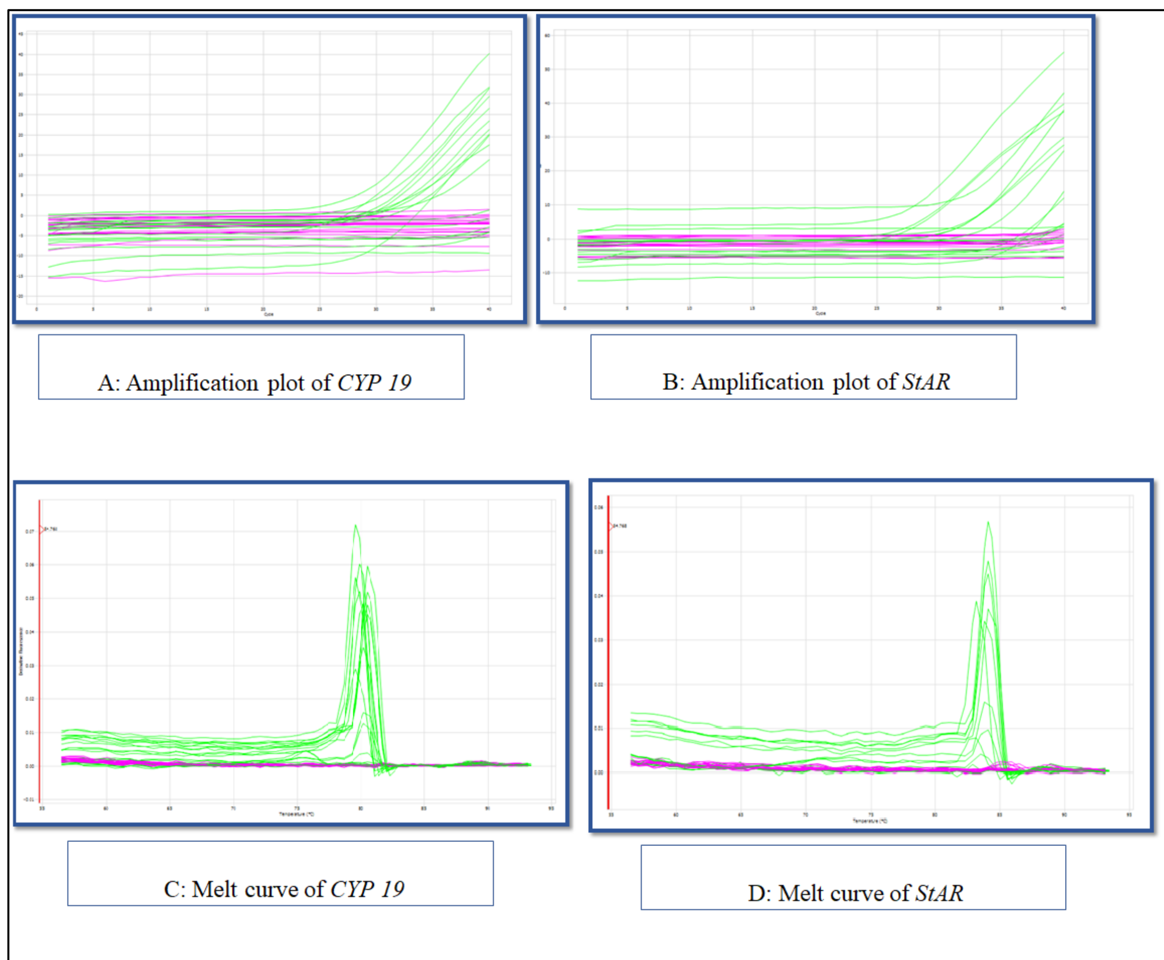

Figure 1: representing Amplification plot and melt curve of *CYP19* and *StAR* genes respectively A: Amplification plot of *CYP19* gene. B: amplification plot of *StAR* gene. C: melt curve of *CYP 19* gene. D: melt curve of *StAR* gene

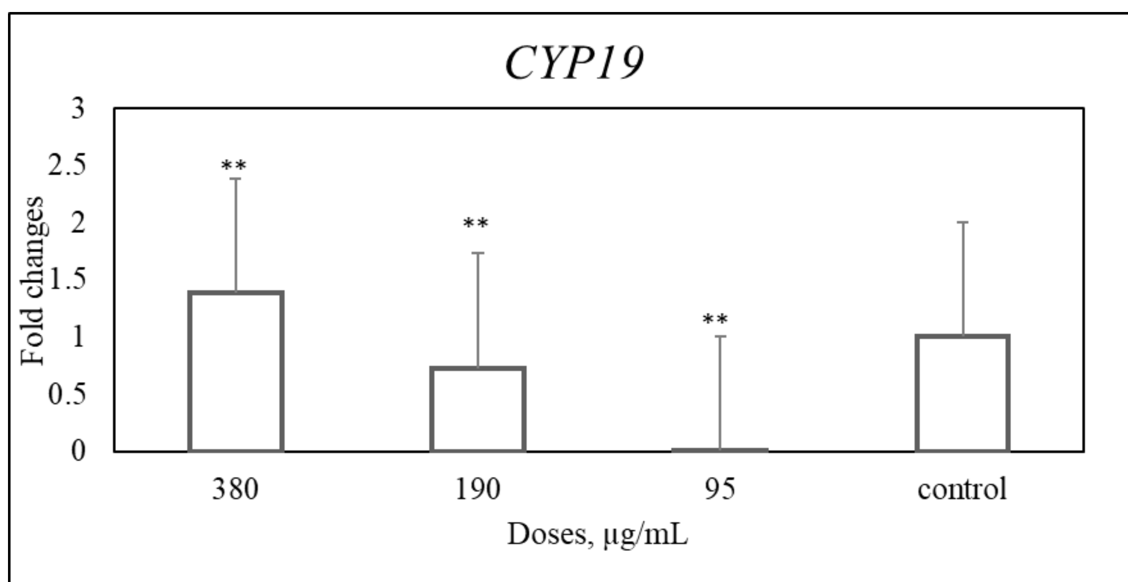

Figure 2: Relative expression of *CYP 19* gene in MCF-7 cell line in response to treatment with different doses of *M. phillippensis*. Values expressed as Mean ±standard error(n=3) **denotes statistically significant (P>0.05) difference compared to control

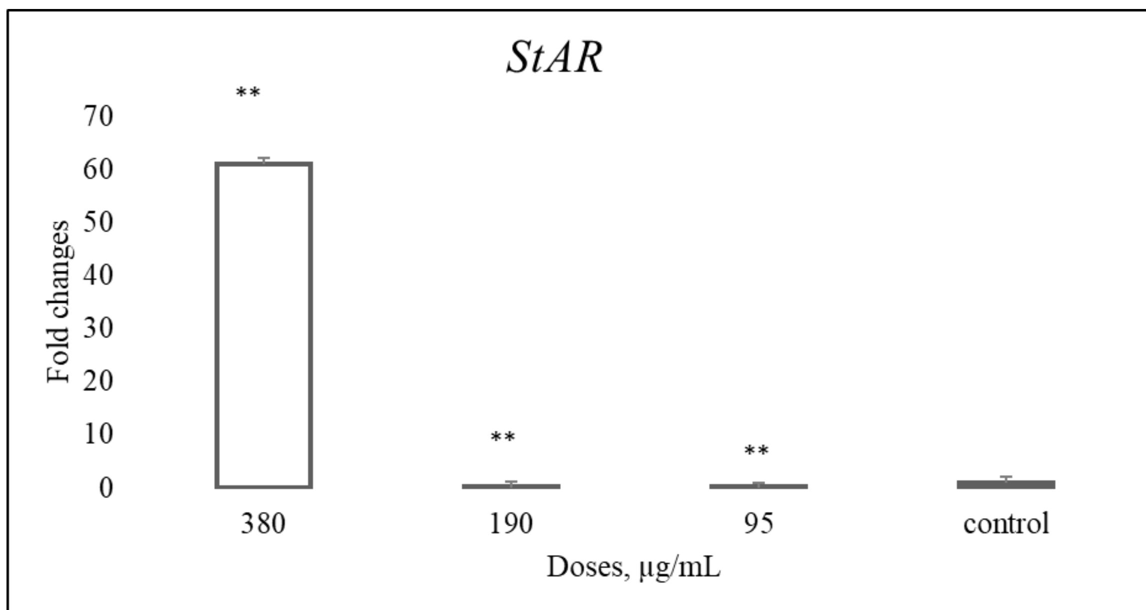

Figure 3: Relative expression of *StAR* gene in MCF-7 cell line in response to treatment with different doses of *M. phillippensis*. Values expressed as Mean ±standard error(n=3)**denotes statistically significant (P>0.05) difference compared to control

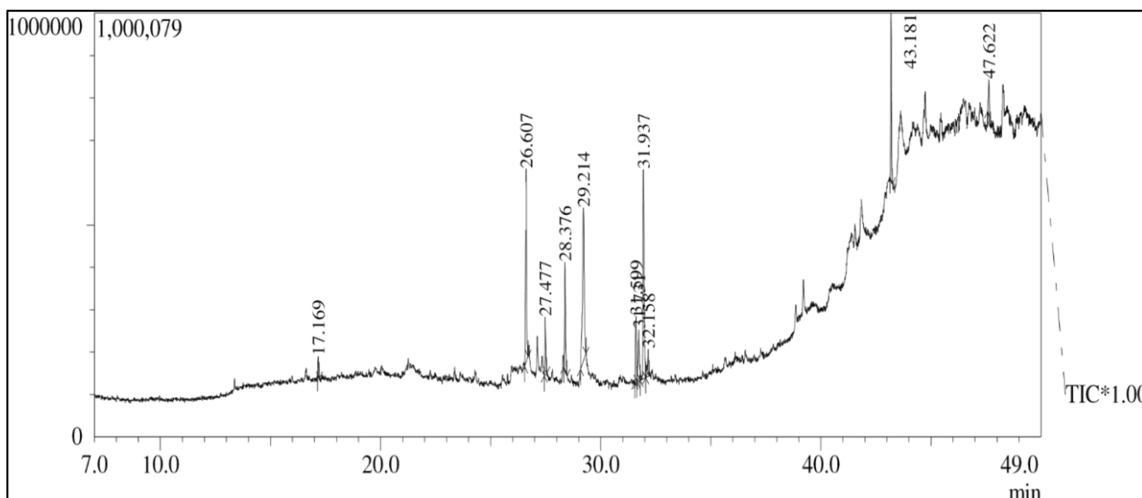

Figure 4: Gas chromatography mass spectroscopy chromatogram of methanolic extract of *M. phillippensis* leaves

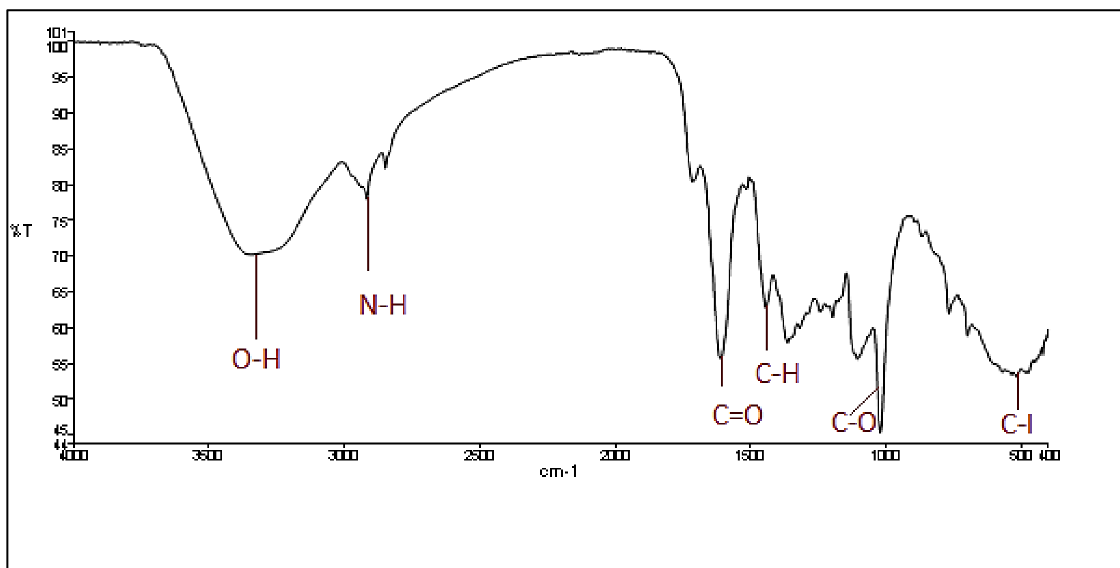

Figure 5: FTIR spectrum of methanolic extract of *M. phillippensis* leaves

CONCLUSION

The current study evaluated the modulatory potential of methanolic extract of *M. phillippensis* leaves on steroidogenic gene expression in hormone responsive MCF-7 breast cancer cell lines. The extract upregulated the expression of the *CYP 19* gene (aromatase) and the *StAR* gene (key regulator in steroidogenesis) at high doses, while it downregulated the expression of these steroidogenic genes at low doses. Oestrogen deficient conditions like infertility can be treated by using higher doses of the plant extract. Cytostatic nature of lower doses of *M. phillippensis* could be used as a novel agent that targets aberrant expression of steroidogenic genes in the treatment of breast cancer. Presence of potent phytochemicals like fatty acids, phytosterols and terpenoids, accounted for the differential expression of steroidogenic genes in MCF-7 cell lines. Future research will be required to isolate bioactive compounds that are responsible for the modulatory steroidogenic activity of *M. phillippensis* and assess the toxicological safety of bioactive compounds in vivo animal models.

Acknowledgements

The authors are thankful to the College of Veterinary and Animal Sciences, Mannuthy, under Kerala Veterinary and Animal Sciences University for providing the facilities financial assistance provided.

Conflict of Interest

None declared.

ORCID ID

Bimitha Benny: NA

A Sandeshkrishna: NA

Sujith Samraj: NA

Preethy John: NA

Uma Radhakrishnan: NA

REFERENCES

1. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P. Functional domains of the human estrogen receptor. *Cell*, 1987; 51:941-51.
2. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science*, 1988; 240:889-95.
3. Weisz A, Cicatiello L, Persico E, Scalona M, Bresciani F. Estrogen stimulates transcription of c-jun protooncogene. *Mol. Endocrinol*, 1990; 4:1041-50.
4. Kristensen VN, Borresen-Dale AL. Molecular epidemiology of breast cancer: genetic variation in steroid hormone metabolism. *Mutat. Res*, 2000; 462:323-33.
5. Russo J, Lareef MH, Balogh G, Guo S, Russo IH. Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. *J Steroid Biochem Mol Biol*, 2003; 87:1-25.
6. Chen S. Aromatase and breast cancer. *Front Biosci*, 1998; 3:d922-33.
7. Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J. Steroid Biochem. Mol. Biol, 2000; 72:23-7.
8. Green S. Modulation of oestrogen receptor activity by oestrogens and anti-oestrogens. The Journal of Steroid Biochemistry and Molecular Biology. J. Steroid Biochem. Mol. Biol, 1990; 37:747-51.
9. Subramanian A, Salhab M, Mokbel K. Oestrogen producing enzymes and mammary carcinogenesis: a review. Breast cancer research and treatment. Breast Cancer Res Treat. 2008; 111:191-202.
10. Clewell RA, Campbell JL, Ross SM, Gaido KW, Clewell III HJ, Andersen ME. Assessing the relevance of in vitro measures of phthalate inhibition of steroidogenesis for in vivo response. *Toxicol In Vitro*, 2010; 24:327-34.
11. Miller WR, O'neill J. The importance of local synthesis of estrogen within the breast. *Steroids*, 1987; 50:537-48.
12. Zhou C, Zhou D, Esteban J, Murai J, Siiteri PK, Wilczynski S, Chen S. Aromatase gene expression and its exon I usage in human breast tumors. Detection of aromatase messenger RNA by reverse transcription-polymerase chain reaction. J. Steroid Biochem. Mol. Biol,1996; 59:163-71.
13. Clarke R, Hilakivi-Clarke L, Cho E, James MR, Leonessa F. Dietary Phytochemicals in Cancer Prevention and Treatment. In: American Institute for Cancer Research, editors. Estrogens, phytoestrogens and breast cancer, 1 nd ed. Springer New York, NY. 1996, 63-85.
14. Adlercreutz H, Bannwart C, Wahala K, Makela T, Brunow G, Hase T, Arosemena PJ, Kellis Jr JT, Vickery LE. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J. Steroid Biochem. Mol. Biol, 1993; 44:147-53.
15. Rice S, Mason HD, Whitehead SA. Phytoestrogens and their low dose combinations inhibit mRNA expression and activity of aromatase in human granulosa-luteal cells. J. Steroid Biochem. Mol, 2006; 101:216-25.
16. Furusawa M, Ido Y, Tanaka T, Ito T, Nakaya KI, Ibrahim I, Ohyama M, Iinuma M, Shirataka Y, Takahashi Y. Novel, complex flavonoids from Mallotus philippensis (Kamala tree). *Helvetica Chimica Acta*, 2005; 88:1048-58.
17. Patil MV, Patil DA. Plants used in reproductive ailments by tribals of Nasik district (Maharashtra). *Anc Sci Life*, 2004 ;23:37.
18. Benny B, Krishna AS, Samraj S, John P, Radhakrishnan U. Cytotoxic and antiproliferative potential of methanolic extract of *Mallotus phillippensis* in MCF-7 cell line. J. Phytopharmacol, 2022; 11:60-63
19. Gautam A, Donohue D, Hoke A, Miller SA, Srinivasan S, Sowe B, Detwiler L, Lynch J, Levangie M, Hammamieh R, Jett M. Investigating gene expression profiles of whole blood and peripheral blood mononuclear cells using multiple collection and processing methods. PLoS One, 2019; 14(12):e0225137.
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods*, 2001; 25:402-8.
21. Ye L, Chan MY, Leung LK. The soy isoflavone genistein induces estrogen synthesis in an extragonadal pathway. *Mol. Cell. Endocrinol,* 2009; 302:73-80.
22. Chi F, Wu R, Zeng YC, Xing R, Liu Y, Xu ZG. Post-diagnosis soy food intake and breast cancer survival: a meta-analysis of cohort studies. APJCP, 2013; 14:2407-12.
23. Cormier M, Ghouili F, Roumaud P, Martin LJ, Touaibia M. Influence of flavonols and quercetin derivative compounds on MA-10 Leydig cells steroidogenic genes expressions. *Toxicol In Vitro*, 2017; 44:111-21.
24. Lephart ED. Modulation of aromatase by phytoestrogens. Enzyme research. Enzyme Res. 2015, 1-11.
25. Ranzenigo G, Caloni F, Cremonesi F, Aad PY, Spicer LJ. Effects of Fusarium mycotoxins on steroid production by porcine granulosa cells. Anim. Reprod. Sci. 2008; 107:115-30.
26. Kao YC, Zhou C, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site-directed mutagenesis study. Envir Health Perspect. 1998; 106:85-92.
27. Park KR, Nam D, Yun HM, Lee SG, Jang HJ, Sethi G, Cho SK, Ahn KS. β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer Lett*, 2011; 312:178-88.
28. Vivekraj P, Vinotha S, Vijayan A, Gideon VA. Preliminary phytochemical screening and GC-MS analysis of methanolic extract of Turnera subulata Smith (Passifloraceae). J. phytopharm. 2017; 6:174-177.

29. Fernando IS, Sanjeewa KA, Ann YS, Ko CI, Lee SH, Lee WW, Jeon YJ. Apoptotic and antiproliferative effects of Stigmast-5-en-3-ol from Dendronephthya gigantea on human leukemia HL-60 and human breast cancer MCF-7 cells. *Toxicol In Vitro*. 2018; 52:297-305.
30. Swantara MD, Rita WS, Suartha N, Agustina KK. Anticancer activities of toxic isolate of Xestospongia testudinaria sponge. vet world. 2019; 12:1434-1440.

HOW TO CITE THIS ARTICLE
Benny B, Sandeshkrishna A, Samraj S, John P, Radhakrishnan U. Differential expression of steroidogenic genes in MCF-7 cell lines by methanolic extract of *Mallotus phillippensis*. J Phytopharmacol 2022; 11(6):378-385. doi: 10.31254/phyto.2022.11601

Creative Commons (CC) License
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (http://creativecommons.org/licenses/by/4.0/).