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Evaluation of the effects of *Ipomoea staphylina* on ovarian cancer cell line

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Abstract: Ovarine cancer is a common woman malignancy in the world. Majority of the ovarian cancers are originated in the epithelial region which are lack of symptomps and this type of cancer is often get localized, when the tumour has spread outside the pelvis. Based on this context, the present study evaluated the effects of both aqueous and ethanolic extracts of *Ipomoea staphylina* leaves on ovarian cancer cell line. The SKOV-3 ovarian cancer cell line was used for evaluation of the effects of both extracts.iBoth extracts showed IC_{50} effects on ovarian cancer cell lines as sensitivity index (SI)ifor both extracts were recorded to above 1.iFurther, ethanol extract was more effective in the moderation of gene expressions of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9)iin comparison to aqueous extract. Further, morphological changes of SKOV-3 cells after treatment with both extracts also confirmed the results. The study, therefore, concludes that the ethanolic extract of *Ipomoea staphylina* leaves is more effective against ovarian cancer cell line.

Keywords: *Ipomoea staphylina,* ethanol extract,iovarian cancer,iSKOV-3,imatrix metalloproteinase.

INTRODUCTION

Ovarian cancer has been recorded as seventh most common women malignancy in the world (De and Dey, 2019). In terms of lethality, this cancer is above all other gynaecological malignancies. However, younger women below 30 years of age, are not affected much but numbers quite higher in women with age above 60 years. Moreover, most of the ovarian itumours iare iof epithelial origin and are known as epithelial ovarian carcinomas (EOC). The prime reason for such vast prevalence of this form of cancer, is the lack of symptoms (Marí-Alexandre *et al.,* 2019). So, the ovarian cancer is often get localized, when the tumour has spread outside the pelvis.

The most commonly used treatment approach is surgery. It involves direct removal of uterus, tubes, ovaries, biopsies of peritoneum and all visible tumor lesions. Moreover, in advanced stages of ovarian cancer, extensive surgeries with multiple bowel incisions and splenectomy are preferred choices of treatment (Cortez *et al.,* 2018).

Ipomoea staphylina (Family: Convolvulaceae) is a perennial woody plant considered as an important medical plant in an Asian traditional medicine system. It is commonly used for the treatments of liver diseases, purgation, stomach disorders, pain, inflammation, and rheumatism. Experimental studies on this plant revealed that it contains sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol (Reddy *et al.,* 2013). The leaves extract of this plant possesses nalgesic (Ghosh and Firdous, 2014), anti-inflammatory (Firdous and Koneri, 2012), antiulcer (Banerjee and Firdous, 2015), hepatoprotective (Bag and Mumtaz, 2013; Ramachandran *et al.,* 2019), antidiabetic (Firdous and Koneri, 2014), nephroprotective (Bag and Mumtaz, 2013), and *in vivo* antioxidant activities (Ramachandran *et al.,* 2019). However, there is paucity of information with regard to its use in cancer. Hence, in the present study, a comparative effect of both aqueous as well as ethanolic extracts of *Ipomoea staphylina* leaves on ovarian cancer cell line have been explored.

MATERIALS AND METHODS

The SKOV-3iovarian cancer cell line was procured from Gene Copoeia, USA. Gene expressions of MMP-2 and MMP-9 were performed by using PCR kits from Sigma-Aldrich, USA. All other chemicals were commercial products of analytical reagent grade.

Collection and Authentication of the Plant

I.istaphylina plant was collected in January 2018 from the forest near Bangalore in Karnataka state. The plant was then identified and authenticated by Dr. K. Karthigeyan, Central National Herbarium (CNH), Botanic Garden,

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West Bengal. A voucher specimen was preserved with a reference No.SMF-01.

Preparation of Aqueous and Ethanolic Extracts of Leaves i

The leaves of *Ipomoea staphylina* were cleaned and dried under shade at room temperature for several days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with distilled water and ethyl alcohol to get a yield of 12.82 and 7.26% w/v of dried aqueous and ethanolic extracts. The dried extracts were stored at 4°C in a refrigerator (Firdous and Koneri, 2014). i

Experimental design i

This *in vitro* study was performed by using both aqueous as well as ethanol extracts of *Ipomoea staphylina.* The effects were quantified by exploring effects of both extracts on SKOV-3 ovarian cancer line using both extracts at their respective IC_{50} doses. Sensitivity indexes were also checked for confirmation of results. The study has been duly approved by ethical committee of our institute.

Cytotoxicity and Selectivity Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye uptake assay was used to determine the cytotoxicity effects of aqueous and ethanolic extract of *Ipomoea staphylina* in SKOV-3 cell line. In 96-well plates SKOV-3 cell line was seeded containing 5×10^3 cells in 150µL of completed medium per well. Cells were allowed to adhere for 16h. After that cells were treated with the extracts diluted to 5-80µg/mL concentration in a medium. Besides, DMSO alone was used as a control. The cells were incubated for 72h and then washed with phosphate buffer saline (PBS) prior to the addition of 100µL of 0.5mg/mL MTT solution into each well. Then 150µL DMSO was used to dissolve the formazan crystals and the absorbance was measured at 570nm was measured on a microplate reader. The whole experiment was replicated in three independent times, and cell viability determination and fitting of response curves followed the previously described method (Srisawat *et al.,* 2014). The 50% inhibitory concentration (IC50) of the crude extracts was calculated from fitted response curves and determined according to the US National Cancer Institute. The selectivity index (SI) was determined by the ratio of the IC50 value of the extracts on normal cells (L929) to the IC50 value of the extracts on cancer cell (Prayong *et al.,*2008; Mahavorasirikul *et al.,*i2010).

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

The cells were lysediand total RNA was isolated with an easy-BLUE total extraction kit (Intron, Seoul, Korea) according to the manufacturer's instructions. For cDNA synthesis, 1µg of total RNA was mixed with Maxime RT premix (Intron) containing oligo dT primers and DEPC-treated water to a final volume of 20µl and incubated at 45°C for 60 min. The reaction was stopped by heat inactivation at 95°C for 5imin. RT-qPCR was performed using a Rotor-Gene 3000 system (Corbett Research, Sydney, Australia)iwith SYBR Green Master mix (Qiagen, Tokyo, Japan). All reactions were carried out according to the following protocol: 94°C for 2 min, followed by 35 cycles of 94°C for 20 sec,i60°C for 20 sec and 72°C for 30 sec. The percentage of target gene expression (MMP-2 and 9) relative to the control was normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH; internal control) using 2-ΔΔCt analysis (Koressaar *et al.,* 2007). Primers for the target genes were designed using Primer 3 software (table 1).

Table 1: Sequences of The Primers used in Reverse Transcription Quantitative Polymerase Chain Reaction analysis.

Gene	Forward	Reverse
MMP-2	CAGAATACCATCGAGACCAT	ATGTGATCTGGTTCTTGTCC
MMP-9	CCACTACTGTGCCTTTGAGT	TCCCATCCTTGAACAAATAC

STATISTICAL IANALYSES

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). One-way analysisiof variance was followed by Dunnett's post-hoc test. $P<0.05$ was considereditoiindicate aistatistically significant difference.

RESULTS

The results obtained from various experiments conducted in this study are depicted in tables 2-4 and fig. 1. The data from various treatment groups have been compared with the normal control animals.

IC_{50} of Aqueous and Ethanolic Leaves i i *i i*

The IC_{50} ¡values (table 2) of ethanol leaf extract of *I.istaphylina* was observed to be higher in comparison to IC_{50} value of aqueous extract of *I.staphylina.*

Table 2: IC_{50} of aqueous and ethanolic leaves extract of *I. istaphylina*.

Cell type	IC_{50} value for cytotoxicity (µg/ml)		
SKOV-3	Aqueous Extract	Ethanolic Extract	Cisplatin
	9.08±0.11	12.02±0.28	1.05±0.10
L929	17.80±0.22	25.92±0.16	1.78±0.14

Values are presented as the mean ± standard deviation (n=3)

Sensitivity Index (SI) of Aqueous and Ethanolic Leaves

The sensitivity indexes (SI) (table 3)ifor both extracts were noticed to be above 1.iThe results confirmed IC_{50} effects of both leaf extracts on ovarian cancer cell line.

Table 3: Sensitivity index (SI) of aqueous and ethanolic leaves extract iof *I. staphylina*.

Cell type	Sensitivity index (SI)		
SKOV-3	Aqueous Extract	Ethanolic Extract	Cisplatin
	1.96±0.17	2.15±0.21	1.69±0.12

Values are presented as the mean ± standard deviation (n=3)

Gene Expressions of MMP-2 and MMP-9

The mRNA expressions of both MMP-2 and MMP-9 showed significant decrease after treatment with both the extracts of *I.istaphylina* (table 4). However, IC_{50} effects were more prevalent in the case of ethanol extract of *I.istaphylina.*

Table i4: iAqueous iand iethanolic ileaves iextract iof i*I. staphylina i*alter imRNA ilevel iof iMMP-2 iand iMMP-9.

Extract i(µg/ml)	MMP-2 i(Gene/GAPDH imRNA)	MMP-9 i(Gene/GAPDH imRNA)
Extract i(0 µg/ml)	85.27±1.82	94.36±2.43
Aqueous extract (Half iIC_{50})	54.05±1.37**	64.93±1.71**
Aqueous extract (IC_{50})	36.63±0.96**	52.41±1.03**
Ethanol extract (Half iIC_{50})	49.61±1.31**	61.82±1.05**
Ethanol extract (IC_{50})	34.25±0.74**	44.09±1.12**

Values areipresented as the mean ± standard deviation (n=5). ***P<0.01* when treatment groups were compared with extract (0µg/ml)

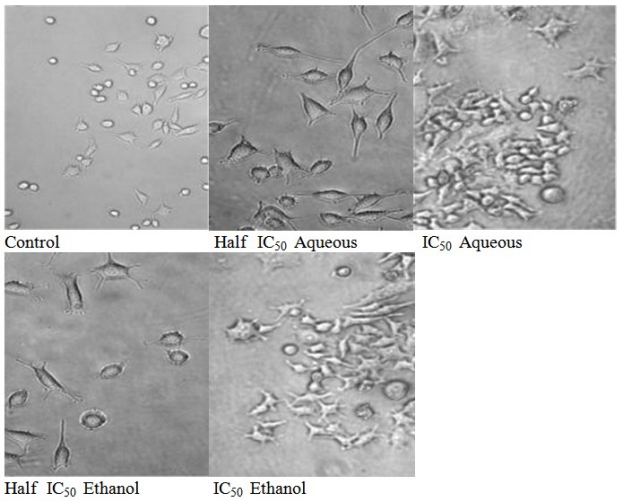

Fig. 1: Morphological changes of SKOV-3 cells treated with aqueous and ethanolic leaves extract of *I. staphylina* at Half IC_{50} and IC_{50} for 48h.

Morphological Changes of SKOV-3 Cells

Both aqueous as well as ethanol leaf extracts of *I.staphylina* showed prominent morphological changes in morphology of SKOV-3 cells. The cells were undergone shrinkage with IC_{50} concentration of the extracts. However, morphological changes were significantly higher in the cells treated with ethanolic extract in comparison to aqueous extracts confirming efficacy of ethanolic extract over aqueous extract.

DISCUSSION i

The present study evaluated the effects of aqueous as well as ethanolic extracts of *I. staphylina* on SKOV-3 ovarian cancer cell line. Based on the obtained data, the cytotoxicity of both extracts was comparable with the standard drug cisplatin. The study clearly indicated both extracts have significant effects on the SKOV-3 ovarian cancer cell iline. The SI of the extracts was more than 1, indicating the less toxicity of the extracts. However, overall efficacy of ethanol leaf extract of *I. staphylin* outshines aqueous extract in terms of IC_{50} effects on cancer cell line. On the other hand we found that the SI of cisplatin was 1.69 in SKOV-3 cells.

The present study clearly demonstrated that ethanol extract has significantly higher IC_{50} effects as significant decreases in the gene expressions of both MMP-2 and 9 were noticed. MMPs having an essential role in tissue remodeling in pathological condition. MMP-2 and MMP-9 are gelatinases, which obliterate the fence surrounds tumor, including collagen IV in the basement membrane and the extracellular matrix (ECM) which aid in invasion of tumor cells in the surrounding tissue. Reported studies explained about the up-regulation of MMPs in epithelial ovarian cancer and also the role of MMP-2 and MMP-9 as prospective markers in the malignancy of ovarian cancer and survival of the patients (Jia *et al.,* 2017). Besides, MMP-9 is known for its ability to cleave many extracellular matrix (ECM) proteins to regulate ECM remodelling (Jia *et al.,* 2017). It could also cleave many plasma surface proteins to release them from the cell surface. In this way, it allows cancer cells to invade and promote processes. Moreover, they have been reported to be elevated in cancer cell lines as observed in the present study. Further, treatment with ethanol extract significantly decreased MMP-9 expression and the effects more prominent in comparison to aqueous extract. The possible justification for above observation could be presence of certain active phytochemicals in ethanolic extract.

Similar, observations were also noticed in the case of MMP2 expression. Altered expression and activity levels of MMPs have been strongly implicated in the progression and metastasis of many forms of cancer (Xu *et al.,* 2005). Increased MMP-2 activity has also been linked with a poor prognosis in multiple forms of cancer

including colorectal, melanoma, breast, lung, ovarian and prostate (Gómora *et al.,* 2018). Moreover, morphological changes noticed in the present study also supported the expression studies as results were in sync with mRNA expression results. The ethanol extract resulted in more prevalent effects morphologically too in comparison to aqueous extract.

CONCLUSION i

The present study concludes that ethanolic leaf extract of *I.istaphylina* is more efficient against ovarian cancer cells. However, further studies are required to ascertain mechanistic information behind these effects.

REFERENCES i

Bag iAK iand iMumtaz iSMF (2013). Hepatoprotective and inephroprotective iactivity iof ihydroalcoholic extract iof i*Ipomoea istaphylina* ileaves. *Bangladesh. J. Pharmacol*. **8**(3):i263-268.

Banerjee A and Firdous SM (2015). Antiulcer activity of hydroalcoholic extract of *Ipomoea staphylina* plant in rats. *Bangladesh. J. Pharmacol*., **10**(3): 652-653.

Cortez AJ, Tudrej Patrycja, Kujawa KA and Lisowska KM (2018). Advances in ovarian cancer therapy. *Cancer. Chemother. Pharmacol*., **81**(1): 17-38.

De P and Dey N (2019). Mutation-driven signals of ARID1A and PI3K pathways in ovarian carcinomas: Alteration is an opportunity. *Int. J. Mol. Sci*., **20**(22): 5732.

Firdous iSM iand iKoneri iR (2012). *In ivivo* iand i*in vitro* antiinflammatory activity iof ileaves iof *Ipomoea staphylina*. *Int.iJ. Pharm.iPharm.iSci*. **4**(5): i339-343.

FirdousiSMiandiKoneri Ri(2014). Antidiabeticiand antioxidant iactivities iof i*Ipomoea istaphylina i*leaves in iStreptozotocin (STZ) iinduced idiabetic imice. *J. Pharma. Sci. Tech*. **3**(2): i77-84.

Ghosh iS iand iFirdous iSM (2014). Evaluation iof analgesic iactivity iof ihydroalcoholic iextract iof *Ipomoea istaphylina*. *Thai. J. iPharm. Sci*., **38**(2): 57-60.

Jia ZH, Jia Y and Guo FJ (2017). Phosphorylation of STAT3 at Tyr705 regulates MMP-9 production in epithelial ovarian cancer. *PLoS. One*. **12**(8): e0183622.

Koressaar iT iand iRemm iM (2007). Enhancements and modifications iof iprimer idesign iprogram iPrimer i3. *Bioinformatics*., **23**(10): 1289-1291.

Mahavorasirikul W, Viyanant V, Chaijaroenkul W, Itharat A and Na-Bangchang K (2010). Cytotoxic activity of Thai medicinal plants against human cholangiocarcinoma, laryngeal and hepatocarcinoma cells *in vitro*. *BMC. Complement. Altern. Med*., **10**(55): 1-8.

Marí-Alexandre J, Carcelén AP, Agababyan C, Moreno-Manuel A, García-Oms J, Calabuig-Fariñas S and and Gilabert-Estellés J (2019). Interplay between MicroRNAs and oxidative stress in ovarian conditions with a focus on ovarian cancer and endometriosis. *Int. J. Mol. Sci*., **20**(21): 5322.

Gómora MJ, Morales-Vásquez F, Pedernera E, Perez-Montiel D, López-Basave H, Villa AR, Hernández-Martínez A, Mena E and Mendez C (2018). Sexual steroid hormone receptors profiles of ovarian carcinoma in Mexican women. *Endocr. Connect*, **7**(9): 1006-1012.

Prayong iP, iBarusrux iS iand iWeerapreeyakul iN (2008). Cytotoxic iactivity iscreening iof isome indigenous Thai iplants. *Fitoterapia*., **79**(7-8): 598-601.

Ramachandran J, Devanesan AA and Thilagar S (2019). Hepatoprotective and antioxidant activity of *Ipomoea staphylina* Linn. *Clin. Phytosci*. **5**(18): 1-11.

Reddy DP, iKota iR, iRenuka iS, iAnarthe iSJ iand Raghavendra iNM (2013). Isolation, characterization of iphytoconstituents iand pharmacological iscreening of i*Ipomoea istaphylina*. *Asian. J. iPharm. Clin. Res*., **6**(1): 30-33.

Srisawat iT, iSukpondma iY, iChimplee iS, iKanokwiroon K, iTedasen A iand iGraidist iP (2014). Extracts from *Vatica idiospyroides* itype iSS ifruit ishow ilow dose activity iagainst iMDA-MB-468 ibreast icancer cell-line ivia iapoptotic iaction. *BioMed. Res. Int*. **2014**: 479602.

Xu iX, iWang iY, iChen iZ, iSternlicht MD, iHidalgo iM and iSteffensen B (2005). Matrix imetalloproteinase-2 icontributes ito icancer icell imigration ion icollagen. *Cancer. Res*. **65**(1): 130-136.