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Research Article

Isolation, Characterization And Ameliorating Effect Of *Scoparia Dulcis* Linn On Human Ovarian Cancer Cell Line And Protective Effect On PCOD

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ABSTRACT

Bioactive substances are both primary and secondary elements of medicinal plants. Numerous studies have documented the pharmacological characteristics of the Scrophulariaceae family member Scoparia dulcis Linn, a herbal medicinal plant, including antitumor, antiviral, antiproliferative, antioxidant, and antifungal effects. It is crucial to ascertain the antiproliferative effects on human ovarian cancer cell lines, such as SKOV3, which is preferable to PCOD, since the restricted research has only been done on species. EA extract was subjected to an MTT assay and IC50 value of standard drug metformin and test extract was observed and compared for further studies. Scoparia dulcis plant was collected and extracted by using hexane, ethyl acetate, and ethanol, as a solvent. After undergoing phytochemical analysis, the extract was separated using a solvent mixture of hexane, chloroform, ethyl acetate, and methanol. Three distinct fractions were analyzed using IR, NMR, and MASS spectroscopy in order to determine the compounds' structures. This study results, extract high yield in ethyl acetate. MTT assay of EA extract has a moderate effect in cell lines compared to standard metformin. After isolated and characterized 3 compounds were found to be compound 1 Neodiosmin, compound 2 6,8-dihydroxy-3-(10-hydroxyundecyl)-3,4-dihydro-1H-2-benzopyran-1-one and compound 3 [(2R,3S,4S,5R,6R)-6-[2-(3,4-dihydroxyphenyl) ethoxy]-3,4,5-trihydroxyoxan-2-yl] methyl (2E)-3-(3,4-dihydroxyphenyl) prop-2-enoate. This compound has moderate binding activity.

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INTRODUCTION

Worldwide WHO reports that PCOD (Polycystic Ovarian Disease or PCOS) affects 8-13% of reproductive-aged women, here 70% of women were undiagnosed. This causes women infertile and the level of cysts leads to cause ovarian cancer, and diabetes condition, if unaware and untreated state. It is a chronic condition and cannot be cured but lifestyle management may bring improvement in such condition of treatment1. The source of herbal medicine plays a vital role in drug development and human well-being mostly due to the latter significance of negative side effects. In the PCOD state, several herbal drugs and their formulation improve patient health by presenting active constituents of such secondary metabolites. This study looks into the plant Scoparia dulcis, which grows in tropical and subtropical areas and has a variety of medicinal uses2. In India, the stem and leaves have the properties to treat menstrual disorders and dysmenorrhea. The phytoconstituent which is present in the plant component binds with estrogen receptors and acts to inhibit cell proliferation3. Based on the literature survey and patient health records insulin resistance is one of the major causes of anovulation. Polycystic ovarian disease is an endocrine metabolic disorder of reproductive age group women as well as pre-menopausal women. The hormonal imbalance like luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen and testosterone interrupt the normal menstrual period which leads to anovulation, oligomenorrhoea and commonly known as menstrual irregularities4. The plant Scoparia dulcis commonly known as sweet broom weed, belongs to the Scrophulariaceae family of plants which can obtain from tropical and sub-tropical region of India, Myanmar, America and West Indies3,4. This plant's ethnomedical uses include the treatment of diabetes, skin conditions, kidney stones, menstrual disorders, anti-sickling, anti-cancer, and a host of other illnesses5. It also reviewed that the plant shows hypoglycaemic drug potential activity in plant source for both insulin deficiency and resistance. Here the one of major cause of polycystic ovaries in women which may lead to tumour is insulin resistance. So, the plant S. dulcis has property of improving efficiency in insulin sensitivity associated with diabetic mellitus patient6,7. Metformin is an antidiabetic drug that also has attention increased in possibility of anticancer in recent times which is prescribed for hypoglycemic activity. It is also prescribed for first line treatment with patient having PCOD. Owing to exposure to the disease and plant profile this present study aimed to extraction of plant material, investigating the effect in human ovarian cancer cell line SKOV3, identification of compounds database present in plant material and further characterization and docking studies carried out for level of effect produce in anti-proliferation in ovarian tissue which is also beneficial over polycystic ovarian disease8.

MATERIALS AND METHODS

Plant collection & Authentication

The fresh leaves of S. dulcis were collected from Taucalay, Kanyakumari District, Tamil Nadu, and India. Taxonomic identification was made from Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India), Arumbakkam, authenticated by Dr. P. Elankani, research officer (Siddha). The allotted authentication number was 640.08112302. Prior to examination, fresh plant leaves were airtightly sealed, shade-dried at room temperature, and ground into a coarse powder. They were then stored in a cold, dry location.

Chemical and materials:

Soxhlet apparatus, heating mantle, n-hexane, ethyl acetate, ethanol, chloroform of laboratory-grade solvent.

Extraction:

Initially, 250 ml of hexane was taken in the RB flask and about 100g of a well-powered plant

sample was packed in a thimble and taken in the Soxhlet apparatus. A heating mantle operating at 28°C was used to evaporate the solvent. After a day, the concentrated hexane extract from the distillation process was gathered in a container. Using the appropriate solvents, this procedure is carried out for almost 1 kg of coarse powder. Next, ethanol and ethyl acetate are used to continue the distillation process. In the ascending order polarity, this three-solvent extraction procedure was carried out progressively. Using a spinning vacuum evaporator, the liquid extracts were condensed into a semisolid extract and stored in desiccators. These three extracts were employed in multiple experiments9.

In-vitro study - MTT Assay

The result of cytotoxic of unknown compounds was identified by performing MTT assay. This method of assay measures cell viability. It is dependent upon the staining of tetrazolium dye to its insoluble form by enzymatic reaction. The other techniques that have been used for quantitative assay of cell toxicity includes measurement of radioisotope, automated counters etc. The activity of live cells is monitored by oxidoreductase enzyme of mitochondrial dehydrogenase. The MTT assay method is among the most widely used and dependable methods for determining the potential for survival and multiplication of cells. The reactant 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide a water-soluble salt which is prepared under the salt or media with lack of phenol red. The insoluble formazan is formed by mitochondrial reducatse enzyme in which tetrazolium ring was cleaved by presence of viable cells and converted as purple in color. The solubilizing agent to solubilize formazan is DMSO, acidified ethanol solution or detergents like sodium dodecyl sulfate in diluted HCl. It is possible to measure the absorbance of this coloured solution at a certain wavelength (often between 500 and 600 nm) using a spectrophotometer. The amount of formazan changes was in concordance with changes in cell number, and this variation is dependent on the metabolic rate and number of mitochondria. This interference reflects the degree of toxicity induced by the test material10,11.

Preparation of test solutions

The MTT assay is carried out for the test solution and serial two-fold dilution in the composition of 6.25 - 100µg was processed from this MTT assay.

Cell lines and culture medium

SKOV3 cell line which comprises of human ovarian cancer cell line procured from NCCS, Pune. The culture was made from the stock cell and it was cultured in DMEM medium incorporated with the nutrient supplement with nutrients such as 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 IU/ml), and streptomycin (100 µg/ml) in a moist environment with 5% CO2 at 37°C until the media began to converge.

Procedure

Using the 10% FBS media, the trypsinization of the monolayer cell culture was continued, and the cell count was adjusted to 1.0 x105 cells/mL. A volume of 100µL of the diluted cell suspension (1 x 104 cells/well) was introduced into each well of the 96-well microliter plate. Following 24 hr there will be a partial monolayer formation, the remaining supernatant was removed, then the remnant with medium and 100µL of various concenteration of test samples were added into the partial monolayer in microliter plates. Plates were incubated in an environment containing 5% CO2 for 24 hours at 37°C. Following the incubation period, each well received 20µl of MTT (2 mg/ml of MTT in PBS) after the test solution had been removed. Then the plate was incubated at 37°C with 5% CO2 in the air for four hours. The substance formazan that had developed was gently dissolved by firmly shaking the plate after the

supernatant was removed and 100µL of DMSO was mixed12.

% viability = Total No. of. Cells absorbed in sample / Total No. of. Cells absorbed in Control x 100

Orbitrap HR-LCMS characterization (O-HRLCMS):

A strong analytical method with exceptional sensitivity and specificity is Liquid chromatography (LC) and mass spectrometry (MS) are combined to form LC-MS. Liquid chromatography (LC) can be used to separate components. Sample eluents from LC are then sent to mass spectrometry (MS) for component identification, detection, and mass determination in the presence of other components. With the understanding that the chosen plant extracts elucidated a good number of flavonoids along with some alkaloids and terpenes, the interpretation can be done using the results based on mass elucidation with structural identification of different classes of compounds and the studies provided by researchers. HR-LCMS/MS data was acquired in both positive and negative ionization modes. The whole plant's extract of ethyl acetate was collected and dried and the extracts was carried to perform analysis. LC-MS Instrument details – VANQUISH, the make of instrument is Thermo Fiseher Scientific and the run time is 35.00 min. The method carried out by Q Exactive plus – Orbitrap MS and the compound database from the software Thermo compound discoverer 3.2. The primary method of compound identification involved comparing MS data with compounds found in the mzCloud database13.

Isolation of active extract

Based on a literature survey, flavonoid content may exhibit anti-proliferative activity in human ovarian cancer cells compared to other phytoconstituents specifically for ameliorating effect in polycystic ovarian disease. Compared to other extracts, the ethyl acetate extract was selected because to its higher flavonoid content, which is active and shows better in vitro activity. As a result, gradient elution technique was used to accomplish bioactive guided isolation and subject the ethyl acetate extract to column chromatographic isolation14.

Column Packing

Wet packing was employed for the column chromatography isolation. Prior to adding pre-heated silica gel (size 100–200 mesh) to one-fourth of the column length, a cotton plug was first inserted into the bottom of the column. After dissolving the extract entirely in 20 ml of ethyl acetate using 10 gm of ethyl acetate extract, any leftover material was located and filtered. After that, silica gel with a mesh size of 60–120 was added to the clear liquid while being constantly stirred. Ultimately, it was incubated for three hours at 40˚C in order to produce a powder that flowed freely and was stacked on top of the silica gel bed. Finally, cotton was placed over the sample after a few grams of silica gel were poured to the top. The mobile phase (100% hexane) is continuously passed over the column for roughly 5–6 times in order to achieve tight packing of the column14.

Elution Process – Gradient Elution Technique

The gradient elution technique was used to elute the ethyl acetate extract utilizing several solvent systems. The elutes were collected in a beaker and labeled as fractions. In addition, TLC was run on each fraction to find the one with the closest Rf value. After mixing, the fractions with comparable Rf values vanished. Table 2's fractions, which display a single spot in the iodine chamber and at UV wavelengths of 254 and 366 nm, are regarded as pure and can be referred to as isolated compounds15.

Thin layer Chromatographic analysis:

Principle:

A technique used to separate, identify, and estimate a single or mixture of components present in the different extracts is called thin-layer

chromatography. This is a dependable method wherein the solute is distributed across the stationary and mobile phases. A solvent flow along the thin layer of the stationary phase, causing differential migration, which is the primary mechanism for separation. Depending on the stationary phase that is employed, this can be accomplished by partition and adsorption.

Selection of mobile phase:

The phytoconstituents found in each extract were taken into consideration while choosing the solvent combination. The pace at which constituents separate is influenced by variables including polarity, stationary phase, mobile phase, and component type. The optimal solvent was chosen from the extensive investigation since it demonstrated excellent separation with the greatest number of components. Methanol: chloroform is the solvent system that is employed (2:8)

Rf = Distance travelled by solute from the origin/

Distance travelled by solvent from the origin

Characterization of isolated compounds

The following analytical methods will be used to characterize the isolated compound: Mass spectroscopy, 1H-NMR, IR, and UV-visible spectroscopy.

RESULT AND DISCUSSION

In-vitro study

As illustrated in the given Table 1, the MTT assay of Ethyl Acetate extract shows moderate inhibition activity in contrast with standard drugs such as Metformin. The graphical representation of Figure 1 shows that inhibition of cell viability along with dose concenteration for the extract and dug Metformin. The drug Metformin is also used for PCOD patients and also acts as cytotoxic activity in the SKOV3 cell line. Figure 2 illustrates cell inhibition for various concentrations of the EA extract, and Figure 3 illustrates cell line inhibition at various concentration levels of the drug metformin. The IC50 value of EA extract is 41.92µg/ml and for Metformin is 12.22 µg/ml. respectively.

Table 1: MTT assay of Ethyl acetate extract of Scoparia dulcis

Sample	Concentrations	% viability			Mean	IC50µg/ml
		Singlet	Duplicate	Triplicate		
Control	0	100	100	100	100	
EA extract	6.25	93.67541766	90.85510689	89.98835856	91.50629437	41.92
	12.5	74.5823389	77.0783848	74.27240978	75.31104449	
	25	62.76849642	63.06413302	63.67869616	63.17044186	
	50	46.8973747	44.41805226	44.35389988	45.22310895	
	100	30.7875895	31.11638955	31.43189756	31.11195887	
Metformin	6.25	58.35322196	54.98812352	55.29685681	56.21273409	12.22
	12.5	52.38663484	49.52494062	49.12689173	50.34615573	
	25	45.70405728	43.58669834	43.18975553	44.16017038	
	50	34.00954654	32.5415677	33.76018626	33.43710017	
	100	26.73031026	24.58432304	25.14551804	25.48671712	

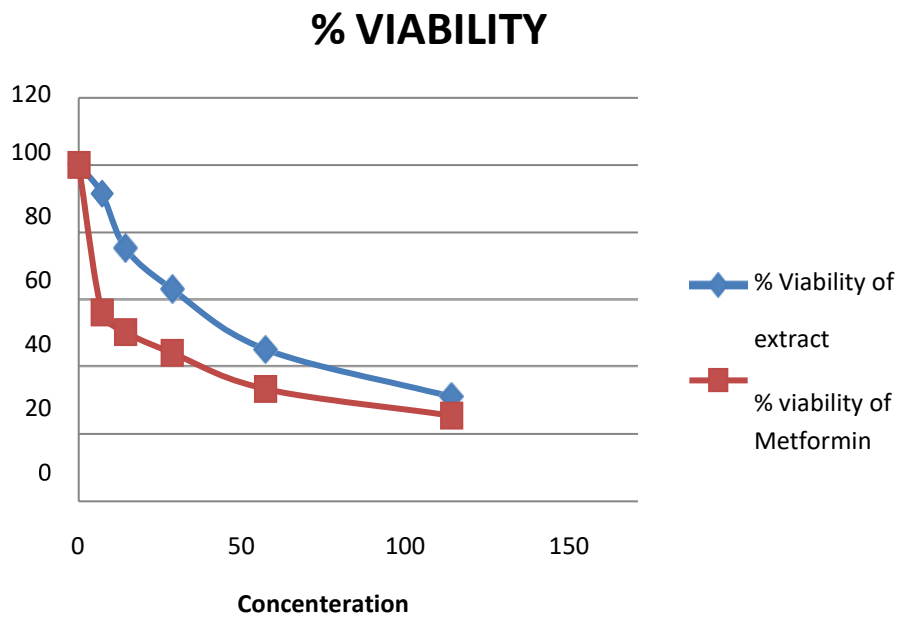


Figure 1: Graphical representation of viability of cell in extract and drug against concenteration

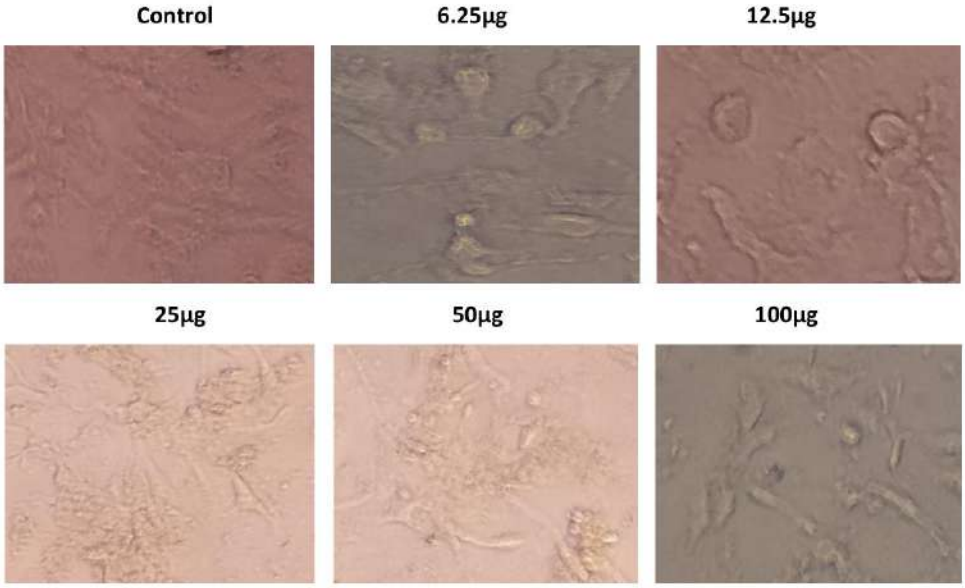


Figure 2: Ethyl acetate extract in cell line SKOV3

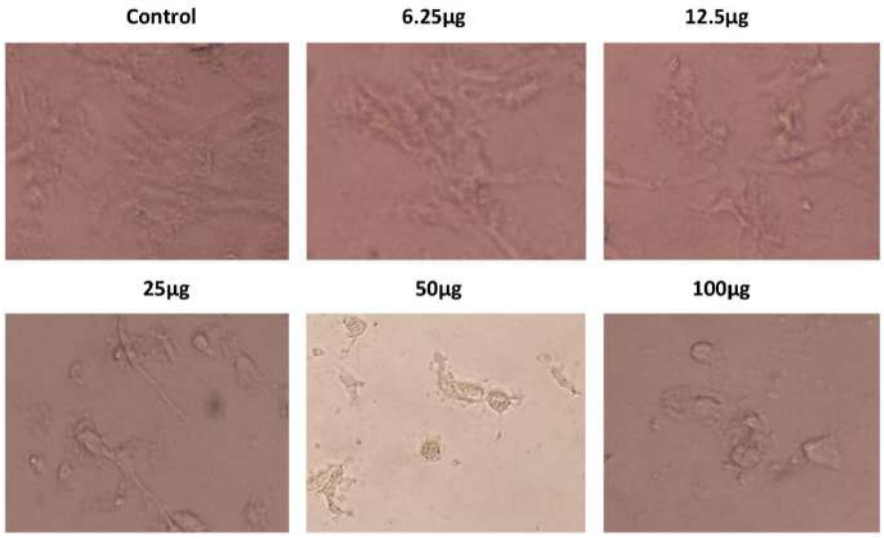


Figure 3: Metformin in cell line SKOV3

Orbitrap HR-LCMS

HRLC-MS analyses demonstrated the close proximity of various phytoconstituent components from the extract, In Figure 4 displayed the LC-MS chromatogram in both positive and negative modes. The isolated compounds from the column chromatography technique was coincide with the library database as includes Neodiosmin, (2R,2'R,4'aS,6'S,7'R,8'aS)-4,6',7'-trihydroxy-2',5',5',8'atetramethyl-3,3',4',4'a,5',6,6',7,7',8,8',8'adodecahydro-2'Hspiro[furo[2,3-e] isoindole-2,1'-naphthalene]-6-one and [(2R,3S,4S,5R,6R)-6-[2-(3,4-dihydroxyphenyl) ethoxy]-3,4,5-trihydroxyoxan-2-yl] methyl (2E)-3-(3,4-dihydroxy phenyl) prop-2-enoate shown in Table 2

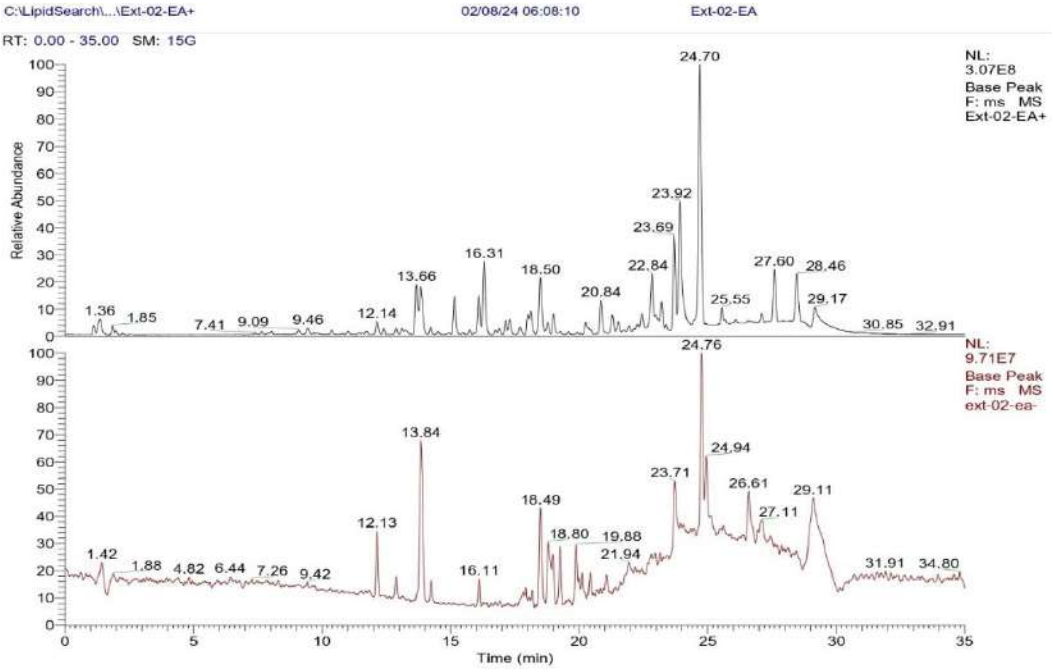


Figure 4: LC-MS Chromatograph

Table 2: Compound profile present in Ethyl acetate extract

Sr. NO	Name of structure	Molecular formula	Molecular weight	+ve/-ve mode
1.	Neodiosmin	$C_{28}H_{32}O_{15}$	608.1714	+VE
2.	6,8-dihydroxy-3-(10- hydroxy undecyl)-3,4-dihydro-1H-2-benzopyran-1-one	$C_{20}H_{30}O_5$	401.2202	+VE
3.	[(2R,3S,4S,5R,6R)-6-[2-(3,4-dihydroxyphenyl) ethoxy]-3,4,5-trihydroxyoxan-2-yl] methyl (2E)-3-(3,4-dihydroxyphenyl) prop-2-enoate	$C_{23}H_{26}O_{11}$	478.1475	+VE

Isolation & TLC

The gradient elution method was used to elute the EA extract using different solvents. A 100 ml collection of each fraction was made. Thin Layer Chromatography (TLC) was used to evaluate the fraction and determine its Rf value. The fractions F5 - F70 in the solvent composition Hexane: Ethyl acetate, Ethyl acetate: Chloroform, and Chloroform: Methanol are where the yellow and yellow tinted color band was eluted and produced (see table 3). When exposed to UV light (254 nm and 366 nm), the collected yellow fractions displayed a single spot in TLC. In Table 4 displays iodine vapour with the same Rf value in several solvent compositions, including methanol and chloroform.

Table 3: Collection of Fraction from isolation of ethyl acetate extract

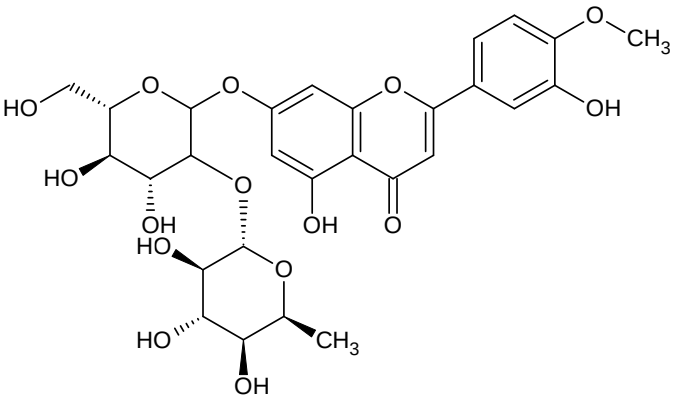
Sr. No	FRACTONS	SOLVENT SYSTEM & RATIO
1.	F1 – F3	Hexane (100%)
2.	F4 – F33	Hexane: Ethyl acetate (95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90)
3.	F34 & F35	Ethyl acetate (100%)
4.	F36 – F46	Ethyl acetate: Chloroform (90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90)
5.	F47	Chloroform (100%)
6.	F48 – F79	Chloroform: Methanol (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 40:60, 30:70, 20:80, 10:90)
7.	F80	Methanol (100%)

Table 4: Rf value of isolated compounds

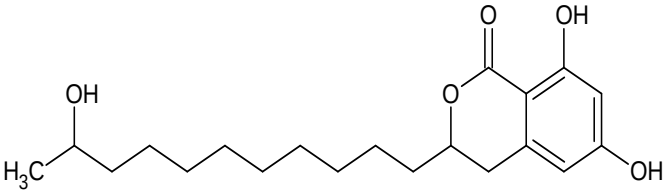
Mixture Of Compounds	Isolated Compounds In Fraction	TLC Solvent System	No. Of. Spots	Rf Value
M14	F40	Chloroform: Methanol(2:8)	1	0.871
	F41	Chloroform: Methanol(2:8)	1	0.871
	F42	Chloroform: Methanol(2:8)	1	0.846
M15	F51	Chloroform: Methanol(2:8)	1	0.611
	F58	Chloroform: Methanol(2:8)	1	0.657
	F59	Chloroform: Methanol(2:8)	1	0.687

Characterization of Isolated compounds

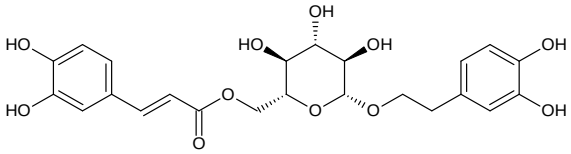
Compound 1



Compound 2



Compound 3



Compound 1 was isolated as green dried powder with molecular formula C28H32O15 by TOF-ESI -US+ at m/z – 609.2737 (M+1) adduct ions which is shown in **Figure 6.** The chemical structure of this substance has a set of aromatic and aliphatic protons, as shown by 1H NMR spectroscopic properties.

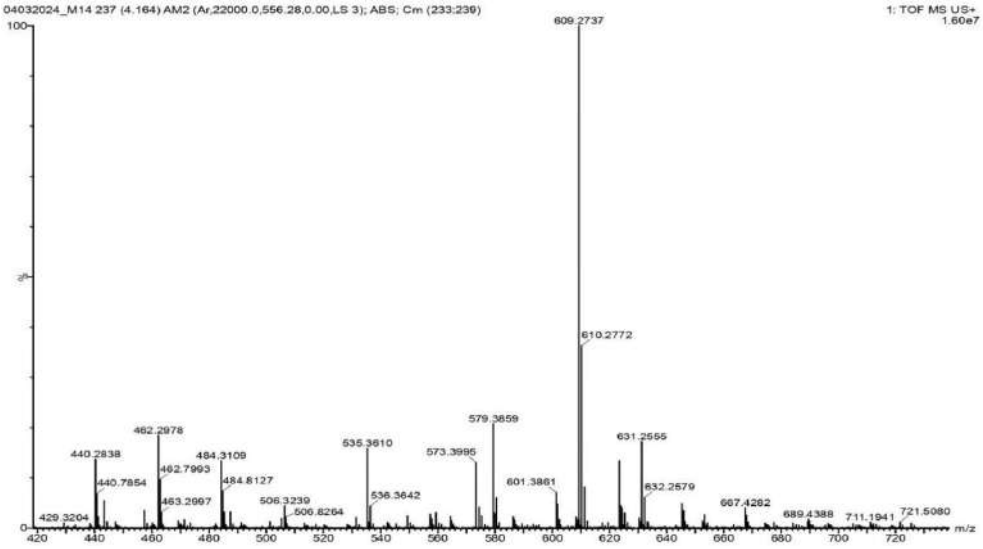


Figure 5: Mass spectra of Compound 1

From the observed Figure 7 1H-NMR (CDCl3): 6.7 (1H, =CH), 9.0-10 (2H, O-H aromatic), 3.5-4.0 (6H, O-H aliphatic), 3.57 (2H, -CH2), 1.11 (3H, -CH3), 3.8-5.0 (10H, -CH), 3.6 (3H, -CH3), 7.0-7.4 (5H, -CH aromatic).

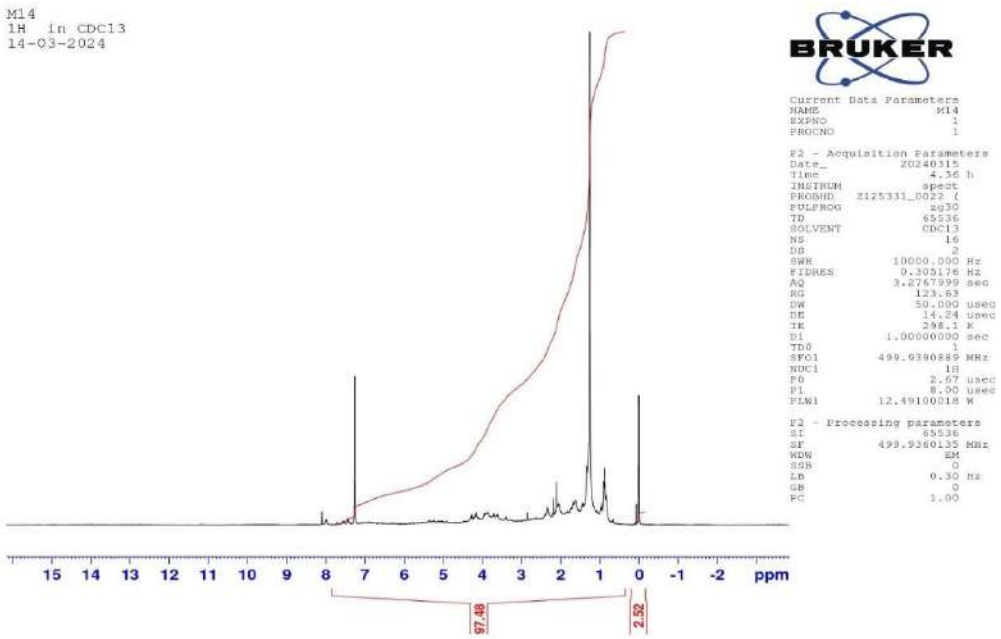


Figure 6: NMR of Compound 1

Neodiosmin was closely resembled by the spectroscopic features mentioned above and described in Table 5

Table 5: Interpretation of NMR for compound 1

Types of Proton	Chemicalshift	No. of. Protons
HC=CH	6.7	1
O-H Aromatic	9.0-10	2
O-H Aliphatic	3.5-4.0	6
O-CH2	3.57	2
CH3	1.11	3
O-CH	3.8, 5	10
C-H Aromatic	7.0-7.4	5
O-CH3	3.6	3

Compound 2 was separated by TOF-ESI -US+ at m/z –401.2895, yielding a green dried powder with the chemical formula C28H32O15, as illustrated in Figure 8. The chemical structure of this substance has a set of aromatic and aliphatic protons, as shown by 1H NMR spectroscopic properties.

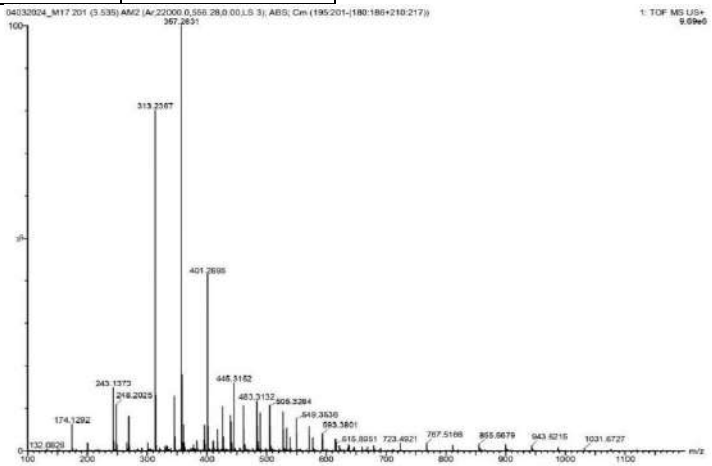


Figure 7: Mass spectra of Compound 2

From the observed Figure 9 1H-NMR (CDCl3): 9.0-10 (2H, O-H aromatic), 4.2 (1H, O-H aliphatic), 1.11 (3H, -CH3), 4.0-4.1(2H, -CH2), 7.0-7.4(2H, -CH aromatic), 2.3(20H, -CH2). The above spectroscopic characteristics closely resembled to 6,8-dihydroxy-3-(10-hydroxyundecyl)-3,4-dihydro-1H-2- benzopyran-1-one and shown in Table 6.

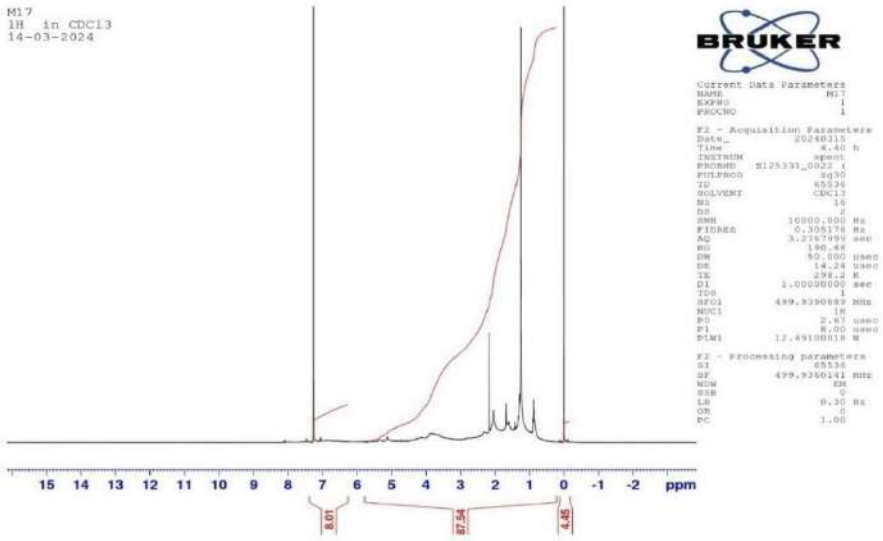


Figure 8: NMR of Compound 2

Table 6: Interpretation of Compound 2

Types of Proton	Chemicalshift	No. of. Protons
O-H Aromatic	9.0-10	2
O-H Aliphatic	4.2	1
CH3	1.11	3
O-CH	4.0-4.1	2
C-H Aromatic	7.0-7.4	2
CH2	2.3	20

Compound 3 was isolated as green dried powder with molecular formula C28 H32 O15 by TOF-ESI -US+ at m/z – 477.0350 (M+1) adduct ions which is shown in Figure 10. The chemical structure of this substance has a set of aromatic and aliphatic protons, as shown by 1H NMR spectroscopic properties.

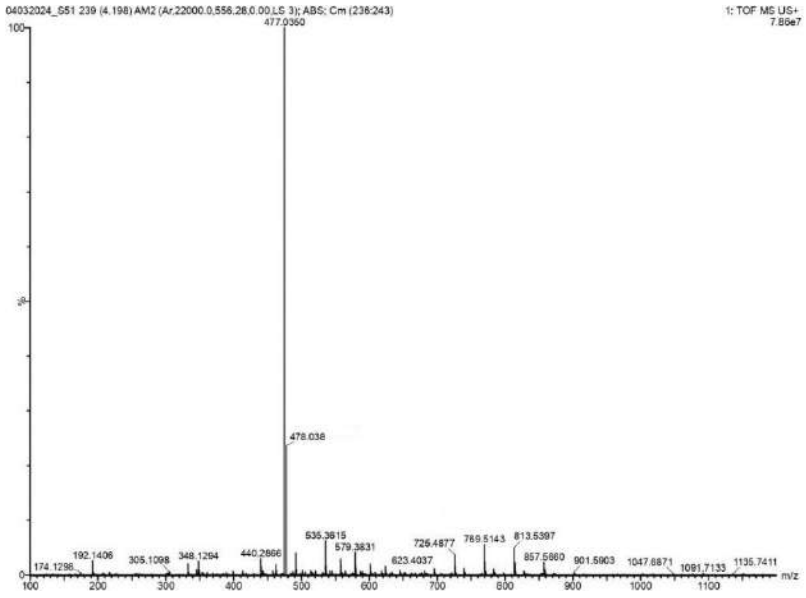


Figure 9: Mass spectra of Compound 3

From the observed Figure 11 1H-NMR (CDCl3): 5.3 – 7(2H, =CH), 9.0-10(4H, O-H aromatic), 4.2(3H, O-H aliphatic), 4.1(2H, O-CH2), 2.72(2H, -CH2), 3.8-5(5H, O-CH), 7.0-7.4(6H, -CH aromatic) The above spectroscopic characteristics closely resembled to [(2R,3S,4S,5R,6R)-6-[2-(3,4- dihydroxyphenyl)ethoxy]-3,4,5-trihydroxyoxan-2-yl] methyl (2E)-3-(3,4-dihydroxyphenyl) prop-2-enoate were shown in Table 7.

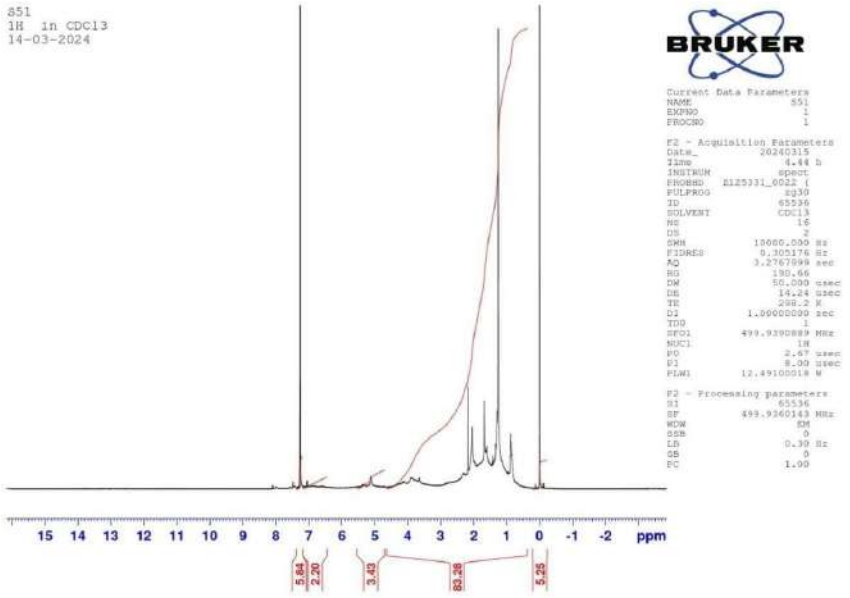


Figure 10: NMR of Compound 3

Table 7: Interpretation of NMR for Compound 3

Types of Proton	Chemicalshift	No.of.Protons
HC=CH	5.3, 7.4	2
O-H Aromatic	9.0 - 10	4
O-H Aliphatic	4.2	3
O-CH2	4.1	4
CH2	2.72	2
O-CH	3.8, 5	5

The IR spectroscopic studies were shown in Table 8,9 & 10 for the compound 1 in figure 5, compound 2 in figure 8 and compound 3 in figure 11.

Table 8: Interpretation of IR for compound 1

Sr. No	Stretchingfrequency	Functional group
1	3456.18 cm^{-1}	O – H
2	2923.87 cm-1	C – H (Aliphatic)
3	1720.38cm-1	C = O
4	1650.95cm-1	C = C (Aromatic)
5	794.61cm-1	C = H alkenebending
6	1172.63cm-1	C - O

Table 9: Interpretation of IR for Compound 2

Sr. No	Stretching frequency	Functional group
1	3448.47 cm^{-1}	O – H
2	2923.87 cm-1	C – H (Aliphatic)
3	1712.66 cm-1	C = O
4	1643.23 cm-1	C = C (Aromatic)
5	1164.92 cm-1	C = H alkenebending
6	756.04 cm-1	C - O

Table 10: Interpretation of IR for compound 3

Sr. No	Stretching frequency	Functional group
1	3448.47 cm^{-1}	O – H
2	2923.87 cm-1	C – H (Aliphatic)
3	1712.66 cm-1	C = O
4	1658.66 cm-1	C = C (Alkene)
5	748.33 cm-1	C = C alkenebending
6	1164.92 cm-1	C - O

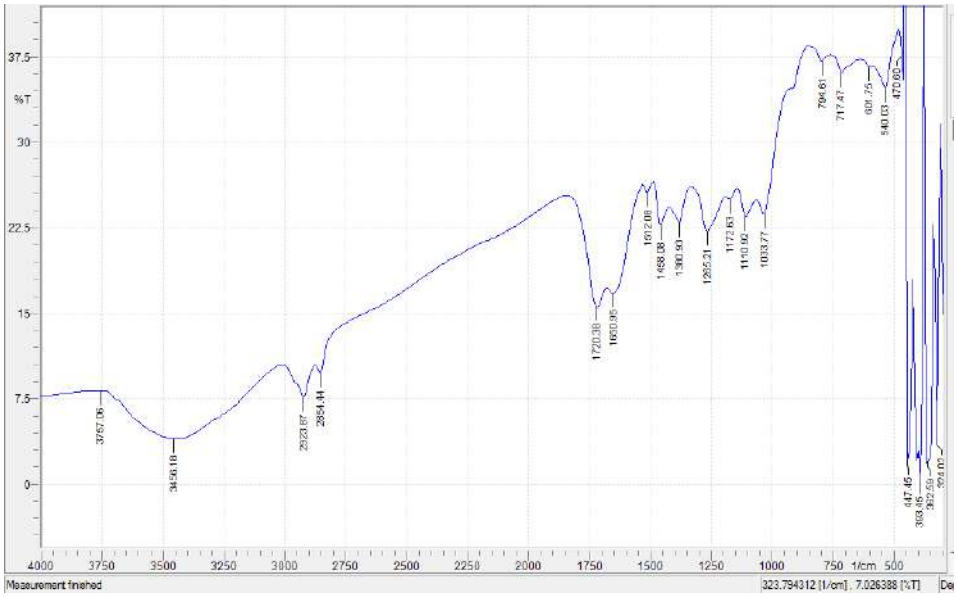


Figure 11 IR for compound 1

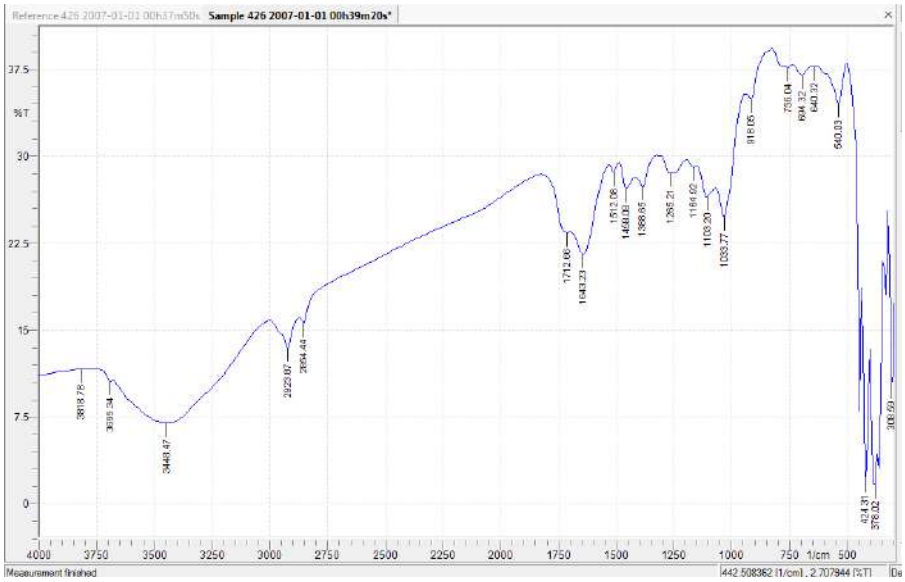


Figure 12: IR for compound 2

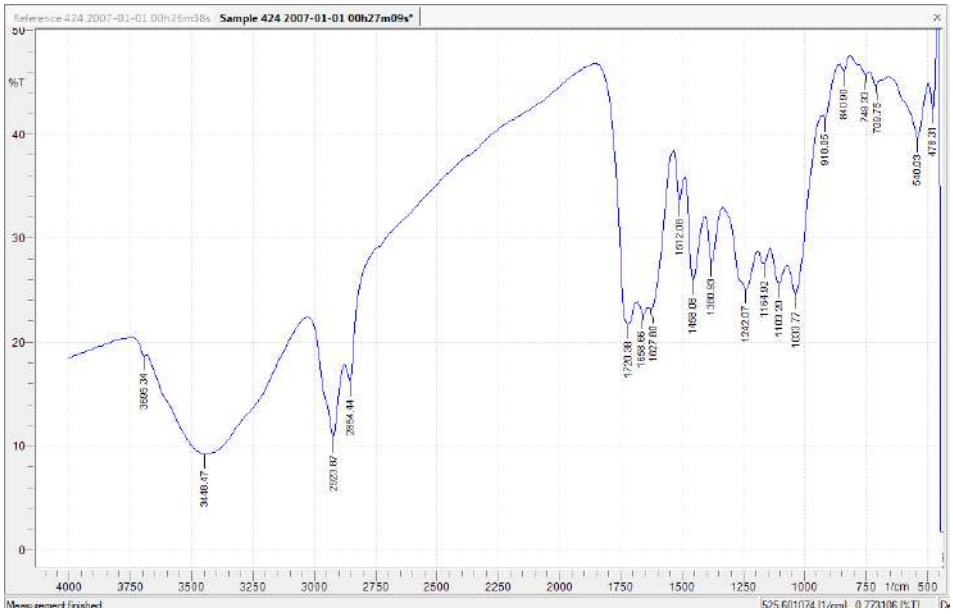


Figure 13: IR for compound 3

DISCUSSION

In Polycystic ovarian disease most of the time it was associated with overgrowth of cell leads to benign or ovarian cancer in case of unaware and also associated with diabetic mellitus, cardiac disorder. In the current study, the leaves of Scoparia dulcis were selected and the ameliorating effect of polycystic ovarian disease (PCOD) in a human ovarian cancer cell line of SKOV3. In the first attempt of the research, the leaves (aerial parts) of Scoparia dulcis were extracted one at a time using the hot continuous percolation process with solvents such ethanol, hexane, and ethyl acetate. It was observed that the higher yield of ethyl acetate extracts comparatively than other extracts possess essential phytoconstituents for this study which contain more glycosides, flavonoids, terpenoids, phytosterol, and phenolic compounds. Thus, investigating the possible benefits of the chosen plant, Scoparia dulcis Linn., for the treatment of PCOD is the goal of the current investigation. It also exhibited moderate anti-cancer activity in human ovarian cell line SKOV3 which was assessed by in-vitro methods by using

the standard drug metformin. For the treatment and management of PCOD, the drug mefenamic acid is also used for inducing ovulation. To evaluate the effectiveness of studies, the compounds should be isolated in column chromatographic technique and also a collection of library database of such compounds through high-resolution LC-MS analysis was interpreted. These results were observed in both positive and negative modes. The isolation technique was proceeded by solvents from the range of high polarity to low polarity composition in hexane, ethyl acetate, chloroform, and methanol. This results in a collection of fractions which is yellow in colour and indicates the presence of phytoconstituent. The collected fraction was subjected to TLC analysis. Fraction that possesses similar Rf values was mixed and dried to get compounds such 1(M14), 2(M17) & 3(S51) at the different composition of chloroform and methanol. The Characterization studies such as FT-IR, Mass, and NMR spectroscopic studies were carried out for isolated compounds 1, 2 & 3.

CONCLUSION:

The presence of chemical structure facilitated the isolation and structural identification of Scoparia dulcis, according to examination of the ethyl acetate extract. The moderate effect of cell inhibition in respective ovarian cancer cell of SKOV3 was due to presence of flavonoids and some phenolic compounds. The structural database of such compounds present in EA extract was obtained by HR-LCMS technique. Furthermore, the isolation was carried out to elucidated and characterized by 1D-NMR, Mass and IR analyses. In conclusion, the in-vitro study exhibits that moderate effect when compared with reference drug as Metformin. The database of HR-LCMS technique reveals numerous compounds such as glycosides, flavonoids and polyphenolic compounds. The isolated compound was identified as Neodiosmin, (2R,2'R,4'aS,6'S,7'R,8'aS)-4,6',7'-trihydroxy-2',5',5',8'atetramethyl-3,3',4',4'a,5',6,6',7,7',8,8',8'adodecahydro-2'Hspiro[furo[2,3-e]isoindole-2,1'-naphthalene]-6-one and [(2R,3S,4S,5R,6R)-6-[2-(3,4-dihydroxy phenyl) ethoxy]-3,4,5-trihydroxyoxan-2-yl] methyl (2E)-3-(3,4-dihydroxy phenyl) prop-2-enoate.

FUTURE PROSPECTUS

In future in-vitro and in-vivo research, the other n-hexane and ethanol extract will be regarded as having an anti-proliferative activity (ovarian cancer) which may also beneficial over PCOD. The library database reports shows that numerous novel compounds and some may synthetically effective on synthetic scheme. To conduct the animal study based on experimental design of PCOD or ovarian cancer through in-vivo study in Animal. To synthesis the possibility of novel compound or basic nucleus derivative which possess the in-silico study criteria.

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CONFLICT OF INTEREST

None to declare

ETHICS APPROVAL

None to declare

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