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Anticancer, Anticollagenase and in Silico Docking Studies of *Anethum sowa* L. Herb Oil against HCT 116 Human Colorectal Cancer Cell Line

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Authors' contributions

This work was carried out in collaboration between all the four authors. Author DJP designed the study, performed the anticancer , anticollagenase, antimicrobial studies, author BSMK performed the in silico docking studies, author PK designed and wrote the protocol of insilico studies, STmanaged the analyses of the study literature searches and results. All the authors read and approved the final manuscript.

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ABSTRACT

Aim: *Anethum sowa* L. isan aromatic plant with pharmacological potential. The chemical composition and the therapeutic of *Anethum sowa* L. herb oil grown in South Karnataka is very few; moreover, its essential oil and extract together is not being studied and compared for its effects on colon cancer cell lines HCT -116 and anti collagense study .
Methods: The current investigation was intended to sight see the incidence of components present in the herb oil examined by (GC-MS), antioxidant , antimicrobial , anticancer & anticollagenase potential was investigated and further the insilco docking studies to unleash the potential drug like molecules in the therapeutic plant was studied .
Results: 5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester, (17.41%),beta-Amyrin(8.20),ritodrine(6.49),1-Naphthalenol,decahydro-1,4a-dimethyl-7-

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(methylethylidene)-,[1R-(1.alpha.4a.beta,8a.alpha)](2.39%),meta-Cymene(1.95%),trans-z-alpha-Bisabolene epoxide (1.80), and Viridiflorol(0.77%) were the new compounds isolated from this therapeutic plant, and *Anethum sowa* L. herb ethanolic extract contained many potential phytochemicals. The total phenol and flavonoid of the herb extract were 0.136mg/ml. and 0.108mg/ml respectively . *Anethum sowa* L. herb extract ABTS antioxidant assay showed excellent activity with an IC_{50} of 540µg/ml which was in power with gallic acid which showed an IC_{50} of 393µg/ml. Essential oil of *Anethum sowa* L. herb exhibited potent antimicrobial activity against all the three microorganisms *E-coli* strain (MTCC 433), *Klebsiella pneumoniae* strain (MTCC 3384*) Streptococcus*mutants strain (MTCC 497) with a minimum inhibitory concentration of 20% herb oil . Cytotoxicity of *Anethum sowa* L.herb essential oil and ethanolic extract against Colon cancer cell lines – HCT -116 , showed that herb oil and herb ethanolic extract repressed the cell growth of the cell . Herb oil with an IC_{50} 79.75µg/ml was more effective than the herb extract . Herb essential oil showed the maximum capacity in inhibiting the collagenase when compared to ethanolic extract of herb, the percentage of inhibition of *Anethum sowa* L. herb essential oil was found to be 60.89% and that of herb extract was (15.18%).

Conclusion: Herb oil showed very good anticancer, antimicrobial and anticollagenase activity and by the In silico docking performed between the compounds present in the herb oil, MAI-150 and APC of *Homo sapiens*, it was found that lupeol showed the highest binding affinity with APC when compared with MAI-150 and rest other compounds present in the herb oil.

Keywords: Human Colorectal cancer cell line HCT-116; herb oil; Anticancer; Anethum sowa L; Lupeol.

ABBREVIATIONS

GC-MS : Gas Chromatography–Mass Spectrometry
IC_{50} : The half maximal Inhibitory Concentration
RCSB-PDB : Research Collaboratory for Structural Bioinformatics Protein Data Bank
APC : Adenomatous polyposis coli FALGPA: N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala

1. INTRODUCTION

Universally, non-communicable diseases (NCDs) reportedto cause 71% of total losses of human life and in India, NCDs account to 63% of all deaths, and cancer has been one of the chiefreasons (9%) [1].The latest statistics on cancer reported that the regular yearly figure of affected role in Bangalore district was (122) in males and (146.8) in females with a mortality rate high in females. The cumulative risk of people to develop cancer in Bangalore was found to be 96.8 in males and 125.1 in females. One of every 7 males &6 females in Bangalore is expected to progress in cancer between 0-74 age group [2]. The third most prevalent cancer reported after prostate or breast and lung cancer is the colorectal cancer [3]. The Colorectal cancer's mortality rate is advanced in males than females, with the occurrence growing with oldness, significantly people overhead 50 years; the new trend which is very alarming is that the incidence of the disease even on younger age group people below the age of 50 [4,5]. Colon cancer risk rate in India is 1 in 298.

From the beginning of life medicinal and aromatic plants have been used for various diseases, as these serve as source of molecules with potential to be used as drug for the world's population [6] due to its bioactive constituents [6-9]. Health care with nutrition is interrelatedand florae have been expende dboth for nutritive diet and for various the rapeuticpurposes in the traditional societies [10].The the rapeuticplants are efficious, safe, less toxic andeasily available with no side effects which gives immense demand for plant based remedies supported by the WHO and reliable naturalresources [11].

Essential oils are highly volatile secondary metabolites produced mainly by aromatic plants. The extraction and the various uses with these secondary metabolites were much familiar among the Greeks, Arabs and Egyptians.An essential oil contains around 20-60 aromatic compounds, which offers the oil its characteristics fragrance and flavour [12]. Various food preservative, pharmacological, alternative medicine and natural therapies were some of the important uses of the volatile oil and extracts of the aromatic plants [13,14].

Anethum sowa L.(Indian Dill) an aromatic and spice herb which occurs in India is cultivated for

its foliage which belongs to family Apiaceae (Umbelliferae) and has been grown as a cold weather crop. It is a variant of *Anethum graveolens*(European Dill).The Foliage and also the fruits of *Anethumsowa* L. are used extensively for culinary and medicinal purposes [15].Anethum has being listed as the plant with little information regarding its anticancer activity [16].There is no studies reported till now with the anticancer property of the essential oil from herb and extract of *Anethumsowa* L. grown in south Karnataka on HCT-116 colon cancer cell line .Hence this study done is unique as it gives an understanding on the comparative effect of essential oil and extract on various pharmacological properties like antioxidant , antimicrobial and anticancer .

2. METHODS

2.1 Sample Procurement

Anethum sowa L.herb procured from South Karnataka and was authenticated by Dr. M .Vasundhara , Professor , Department of Horticulture , University of Agricultural Sciences , Bangalore.

2.2 Extraction of Essential oil by Clevenger Apparatus

Anethum sowa L. entire herb was taken without washing and mud was just removed by gentle tapping and the herb was cut in to small pieces and was loaded in to the round bottom flask of the Clevenger apparatus and one litre of water was added in to the two litre Clevenger round bottom flask and the machine was set to 20 heating units and was subjected to hydro distillation for 2 hours and 30 mins and the oil was collected. Sodium sulphate was added to remove the water and kept at 4 °C till further analysis.

2.3 Soxhlet Extraction

The plant material with roots were taken, only the mud was removed from the roots by washing and the herb was cut in to small pieces and was put in to hot air oven at 60 degree Celsius for drying 5 hours. After which was taken and powered and taken for further analysis.

2.3.1 Ethanol and acetone extraction

Dried herb powder weighing 10 grams each was loaded in to two different thimbles, two different soxhlet apparatus were rinsed with the respective solvents, and the thimbles were placed inside the soxhlet, 200 ml each of the solvents were poured in to two different soxhlet and the apparatus was set to 20 heating units. Using rotary evaporator and hot air oven the extract was concentrated and further preserved by freezing at 4 degree Celsius.

2.3.2 Aqueous extracts using indirect heating method

15 grams of dried *Anethum sowa* L. powder and 80 ml of water needed just to soak the leaf powder were taken in conical flask and was subjected to heating with a water bath set to 90 °Cand the plant powder was heated ,till the water inside the conical flask had been boiled for a time duration of 10 mins .Thecooled content filtered using double muslin cloth and later was kept in the hot air oven for the evaporation of water which was set to 60 °C. The paste form of the extract was kept for further analysis.

2.4 Phytochemical Analysis

Preliminary phytochemical analysis was performed by colour differentiation method byadopting the methods of Solomon Charles Ugochukwa et al. [15].

2.4.1 Alkaloids

Wagner's reagent(few drops)was mixed to 2 ml of extract . Precipitate which was reddish brown colour indicated the incidence of Alkaloids.

2.4.2 Flavonoids

2 ml of the herb extract was treated with few drops of 20% NaOH. An intense yellow colour appearance, followed by the accumulation of 70% dilute HCl, and vanishing of the yellow colour after addition of few drops of 70% dil HCl indicated flavonoids.

2.4.3 Saponins

Extract (2 ml) distilled water (6 ml) was add-on, mixed thoroughly and vigorously. Formation of bubbles or persistent foam showed positive result for Saponins.

2.4.4 Tannins

10% alcoholic ferric chloride mixed with 1ml of extract. Formation of brownish blue colour showed Tannins.

2.4.5 Phenols

Mix extract (1 ml) and 5% aqueous ferric chloride (1ml). Formation of blue solution in the tube showed positive for Phenols.

2.4.6 Proteins

1ml of 40% NaOH and 1% copper sulphate (few drops) was mixed to the extract .The presence of peptide linkage molecule in the extract was identified with the formation of violet colour.

2.4.7 Cardiac Glycosides

Anhydrous acetic acid (0.5 ml), 1% aqueous iron (lll) chloride solution (3 drops) was added to the extract which is 1 ml .Interface ring which was brown indicated positive for cardiac glycosides test.

2.4.8 Terpenoids

Chloroform (0.5 ml) was added after few drops of sulphuric acid to 1ml of the plant extract . A reddish brown precipitate indicatedterpenoids.

2.4.9 Carbohydrates

The plant extract was mixed with Molisch's reagent (few drops)and with H_2SO_4 (1 ml) added to tubes. The mixture was boiled fortwo to three minutes. Red or dull violet colour specified carbohydrates.

2.4.10 Quinones

Extract mixed with HCl (few drops). Precipitate or yellow coloration showed Quinones.

2.5 Total Phenol and Flavonoid Content in Ethanolic Extract of Herb

FC reagent (800µl), 200 µl of the plant extract and 7.5% sodium carbonate (2 ml) were supplemented and with 7 volumes of distilled water the contents were diluted, followed by incubation in dark for 2 hr under dark conditions. At 765 nm the absorbance was noted using a UV Spectrophotometer. As standard solutions Gallic acid dilutions (0 to 1.0mg/ml) were taken. Phenol content were stated as Gallic acid in mg/ml of extract.

5 ml of 2% $AlCl_3$ mixed with equal volume of the plant extract with incubation period of 10 minutes, at 415nm absorbance against blank using UV Spectrophotometer. Blank was prepared as 5ml of extract mixed with 5ml of methanol without $AlCl_3$. Catechin was used to prepare standard graph [16].

2.6 Antioxidant Effect

2.6.1 Assay-DPPH

Different concentrations (0.1mg – 0.5 mg) of the sample were made up to 100µl with methanol.3ml of DPPH solution (whose absorbance was pre-set to 1) was supplemented to all the tubes and was in dark condition for 15minutes. After incubation, at 517nm the absorbance was measured , spectrophoto metrically with methanol as a blank [17].

2.6.2 Antioxidant activity (ABTS ASSAY)

Different concentrations (100µg - 500µg) of the sample taken , volume in each test tube was made upto 1ml with methanol, 3ml of ABTS solution added kept in dark conditions for 30minutes. At 734nm spectrophotometrically with methanol as a blank absorbance was read [18].

2.7 Antimicrobial Effect of Extracts and Oil

The *Anethum sowa* L. herb oil and ethanolic extract taken for the antimicrobial studies by well diffusion method. *E-coli* strain (MTCC 433), *Klebsiella pneumoniae* strain (MTCC 3384) *Streptococcus* mutants strain (MTCC 497) taken for the study . Microbial culture collection centre , India provided all the stock cultures for the study.

2.7.1 Preparation of Media and conservation of bacteria

Luria Bertani plates containing bacterial cultures sub cultured and kept overnightin LB Broth, to obtain turbidity comparable to McFarland (0.9) standard.

2.7.2 Antibacterial activity of herb extract and oil

Well diffusion method, against three bacteria *E-coli* strain (MTCC 433), *Klebsiella pneumoniae* strain (MTCC 3384) *Streptococcus*mutants strain (MTCC 497). 100 mg of Herbal ethanolic extract were dissolved in 1mL of Dimethyl sulfoxide (DMSO). Different aliquots of the herb extract (1-4mg) and (20 % - 80%) for herb essential oil were taken for study and the final volume were

made upto 50µL by adding DMSO. 24hrs cultured, inoculum(100µl) of *E-coli*, *Klebsiella pneumoniae* and *Streptococcus*mutants added into the plates containing media and spread throughout the plate using spreader. Six wells were made using well borer and 50µL of prepared extracted aliquots and prepared oil sample aliquots were loaded into the respective plates, 50µL of DMSO as negative control and 50µL of tetracycline (10µg) as positive control were also loaded into the wells respectively and wereset aside for 24hrs at 37 degree Celsius and the MIC was calculated.

2.8 Anticancer Studies

2.8.1 Culturing of cell lines

The HCT -116 procured from NCCS, Pune was preserved in Dulbecco's Modified Eagle Medium high glucose media containing 10 % Foetal Bovine serum along with 1% antibiotic followed which was kept in carbondioxide incubator(5%), also provided with 18 – 20% O_2 and temperature set to 37°c . The cells were maintained by changing the mediafor every two days.

2.8.2 MTT assay

MTT test done to study anticancer potential and the method adopted was with slight modifications from Alley and Mosmann [19,20]. The HCT – 116 cell line (monolayer) was trypsinised and the cell count of 20,000 cells per well was accustomed. Without the test agent, only 200µl of cell suspension were grown for 24 hours. *Anethum sowa* L . herb oil (62.5, 125 ,250 , 500,1000 µg / ml) another separate set of herb extract (50, 100 , 200, 400 , 800 µg / ml) were added and incubated after which the MTT reagent 0.5mg/ml) added . The plates werekept inside for 3 hours in an incubator. After that 100µl ofDMSO was supplemented and was subjected to gentle stirring. At 630 nm the absorbance was measured with an ELISA reader followed by determination of IC_{50}value.

2.9 Anticollagenase Activity

Anticollagenase by stein enhanced also by Moore method [21] with alterations by Mandle [22]. 1mg/ml of enzyme (25µl,) 50Mm TES buffer with 0.36Mm $CaCl_2$ (pH7.4) and assessment sample (1.4mg/ml)taken in vials . 75µl (TES buffer), 25µl enzyme+TES buffer(50µl),25µl collagenase, 25µl TES buffer +25µl EDTA(1mg), (25µl) of collagenase + TES buffer +30% DMSO were taken as blank , negative and positive and solvent control respectively. Incubation of the vials in a water bath for 20 mins at 37℃. 100 µl FALGPA further supplemented and for 60 mins at 37℃ incubation .200 µl of 200Mm sodium citrate-citric acid buffer along with ninhydrin was supplemented to the vials and were kept in water bath(100℃)for 5min and was brought to room temperature followed by addition of 200µl of 50 % isopropanol .At 540 nm using a Tecan infinite spectrophotomter absorbance was detected and the percentage inhibition determined by $[(A_{control}-A_{sample})/A_{control}]X100$,where $A_{control}$wasthe absorbance of buffer+collagenase+30%DMSO andA_{sample}wasthe absorbance of buffer +collagenase + assessment sample.

2.10 GC- MS

GC-MS electron impact ionization method on GC-17 a gas chromatograph (Shimadzu) with FID detector coupled to a GC-MS QP2010S mass spectrometer (Shimadzu).,fused silica capillary column (30m x0.25mm film thickness .Column temperature 40°c(3 min) was raised to 250 with hold time 10.00(at the rate of 5 °c/min).Injection port temperature was 250°c and the injection volume 1µl. Carrier gas helium used at constant pressure of 52.2Kpa ,flow rate 24.7ml/min. Compounds were confirmed by computer matching with their mass spectral fragmentation pattern with those of compounds in NIST 11&Wiley 8 .

2.11 In Silico Docking Studies on Herb Oil

In silico docking was performed between the compounds present in the herb oil, MAI-150 and APC of *Homosapiens*. RCSB-PDB was used in order to retrieve the protein structure of APC with PDB id 5IZ8 in .pdb format. Further Auto Dock vina [23] of PyRX 0.9 was used for the docking studies onto which the protein was loaded. The structure of ligands 5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester, anethofuran , 2-methyl-5-propan-2-ylcyclohexa-1,3-diene, Apiol and Lupeol, MAI-150) was retrieved from PubChem and saved in .sdf format. Initially the most stable ligands were generated using Open Babel [24] through the process of energy minimization available in PyRX. The grid box was customary to the XYZ coordinates of 32.620 78.271 and 334.857 respectively and box dimensions (Angstrom) were 100.512, 98.853 and 108.309 along the XYZ axis respectively to cover the entire protein. Protein- ligand interaction of the conformation

complex was visualized by PyMOL2.4 with the lowest Auto Dock vina score and LIGPOLT+ [25] software was used to study its interaction.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of *Anethum sowa* L. Herb

Phytochemical analysis showed, that the herb extractswererich source of secondary metabolites. Ethanolic extract of the *Anethum sowa* L. herb was found to be best when compared to aqueous and acetone. Except terpenoids and tannins all the other phytochemicals were present like alkaloid , flavonoid , saponins , phenols , proteins , cardiac glycosides , carbohydrates and Quinones.

3.2 Antioxidant Action

Spectrophotometrically by the DPPH and ABTSmethod the antioxidant potential was measured .In comparison to the *Anethum sowa* L. herb essential oil (IC_{50}1483.1µg)herb ethanolic extract(IC_{50}540.1µg)of *Anethum sowa* L. gave good antioxidant activity for ABTS as well as for DPPH method(herb extract IC_{50} – 2356µg&herb oil IC_{50} – 11835µg).The ABTS method has beenstrong in detecting the antioxidant present in fruits , vegetables and were absolutely linked with the absorbance of the oxygen radical potential. Hydrophilic and high- pigmented antioxidants were detected by ABTS assay . ABTS assay more suitable in noticing antioxidant bulk infood [26].*Anethum sowa* L. herb extract showed potent antioxidant activity while the essential oil showed moderate activity. *Anethum sowa* Kurz herb microwave oven boiled water exhibited the highest percentage of inhibition with DPPH , with an half inhibitory concentration of 5.59µg/ml [27].

3.3 Total Phenolic and Flavonoidcontent

The phenolic , flavonoid content of the*Anethum sowa* L. extract of herb was resolute through a linear gallic acid standard graph and catechin standard graph .The phenol content in the ethanolic extract of herb was found to be 0.136mg/ml and the flavanoid content was 0.108mg/ml.

3.4 Antimicrobial Studies

The essential oil repressed the growth of all the three organisms *Klebsiella pneumoniae* strain MTCC 3384, *Streptococcus* mutants strain MTCC 497 and *E-coli* strain MTCC 433 producing an inhibition zone of 10-22mm diameter . The zone found against, *Streptococcus*mutantswere greater after which was the *Klebsiellapneumoniae* strain MTCC 3384 and *E-coli* strain MTCC 433 and the effect in the case of *streptococcus*where in power with that of the standard tetracycline(10 µg).*Anethum sowa* L.ethanolic herb extract inhibited the growth of *Klebsiella pneumoniae* strain MTCC 3384 &*Streptococcus* mutants strain MTCC 497 , which produced zone diameter measuring 10 to 25mm ,and the minimum inhibitory concentration was found to be 1mg /ml ,but *E-coli* strain MTCC 433 was found resistant to the herb ethanolic extract.50µL of DMSO was used as the negative control and 50µL of tetracycline (10µg) as the positive control. The herb of *Anethum sowa*Kurz. microwave oven boiled water exhibited lowest Minimum inhibitory concentration of 250-500µL/ml which proved its antimicrobial activity against negative and positive bacteria [27].

3.5 MTT Assay

The results of MTT assay of *Anethum sowa* L. herb essential oil and ethanolic extract were tabulated and shown in, fig 1,2 ,3&4 . *Anethum sowa* L. herb oil and herb extract showed decrease in the percentage of cell viability and exhibited a dose dependent effect , herb oil with an IC_{50} value of 79.75µg/ml was more effective when compared to herb extract which had an IC_{50} value of 194.76µg/ml.10µM of Camptothecin (standard) showed an IC_{50} of 3.5 µg/ml. Anethum species has been listed among the herbs investigated least for its Anticancer potential , even though few cytotoxic studies have been carried out [27]. Among the Iranian and also Arabic herbal medicines*Anethum graveolens* is being listed as natural cancer agent [28]. The herb volatile oil of *Anethum graveolens* grown in Tajikistan showed toxic activity to human cervical cancer, Colon & human breast cancer cell lines. The colon cancer cell lines Caco –2 was found to possess an IC_{50} of 216µg/ml which was much higher dose when compared with *Anethum sowa* L. herb essential oil taken for the study [29].Brine Shrimp Lethality bioassay studies on *Anethum sowa* L. root ethyl acetate extracts showed potent cytotoxic activity with LC_{50}=5.03±0.0805µg/ml when compared to Oncovin(0.46±0.05µg)which was the standard [30].Physcion and bergapten in the *Anethum sowa* L. root extracts testified to possess cancer inhibition which was investigated by insilico molecular docking studies [31].

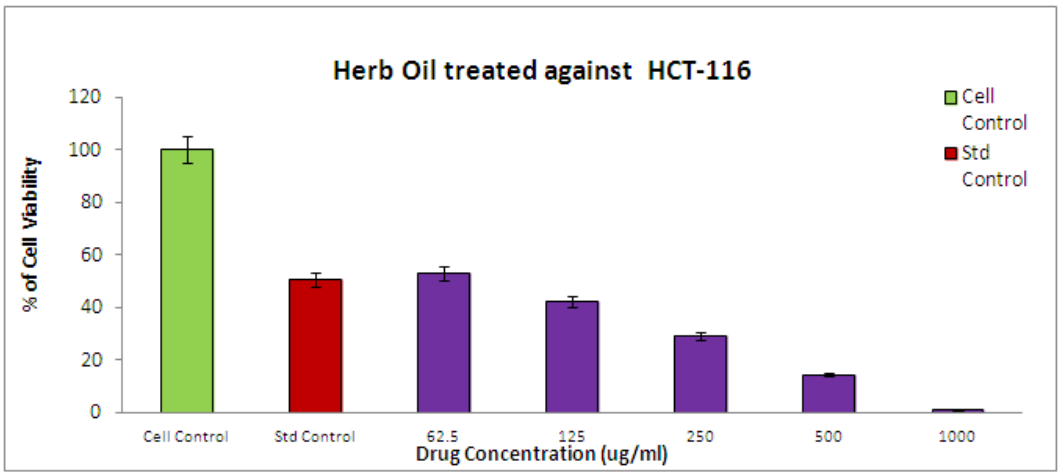


Fig. 1. Cell viability percentage of herb oil treated against HCT – 116 cells

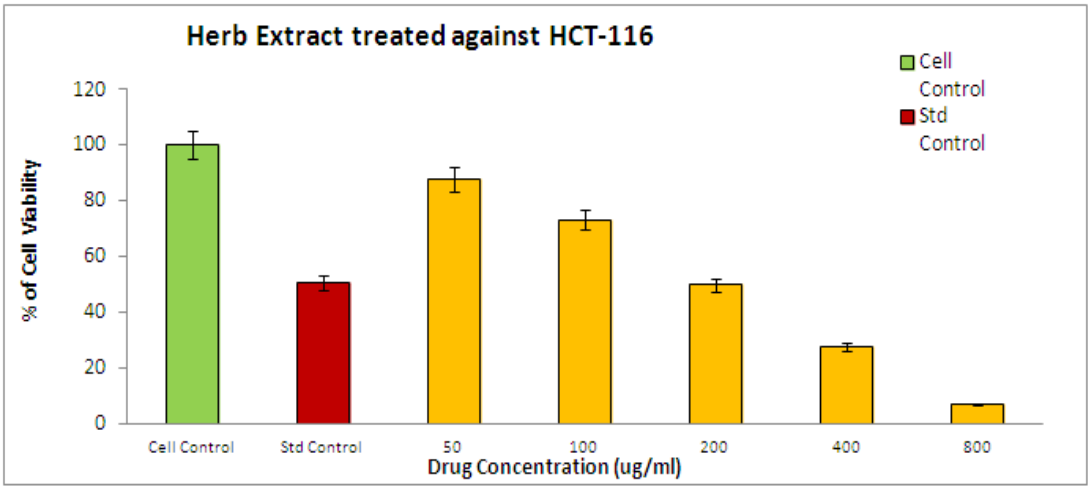
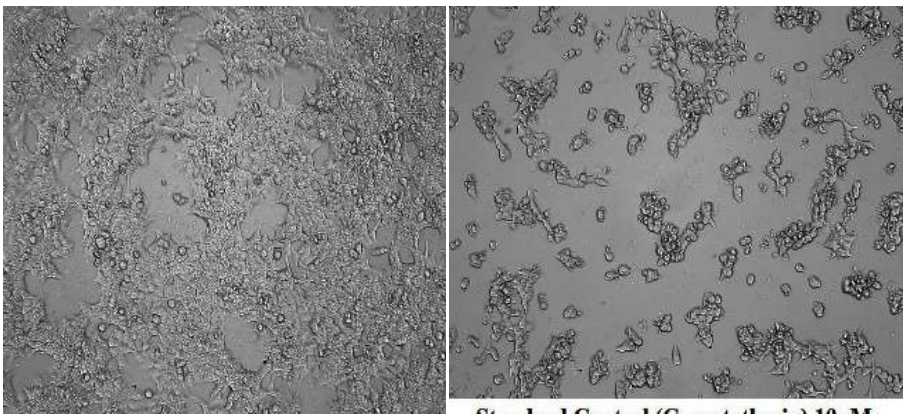


Fig. 2. Cell viability percentage of herb extract treated against HCT -116 cells



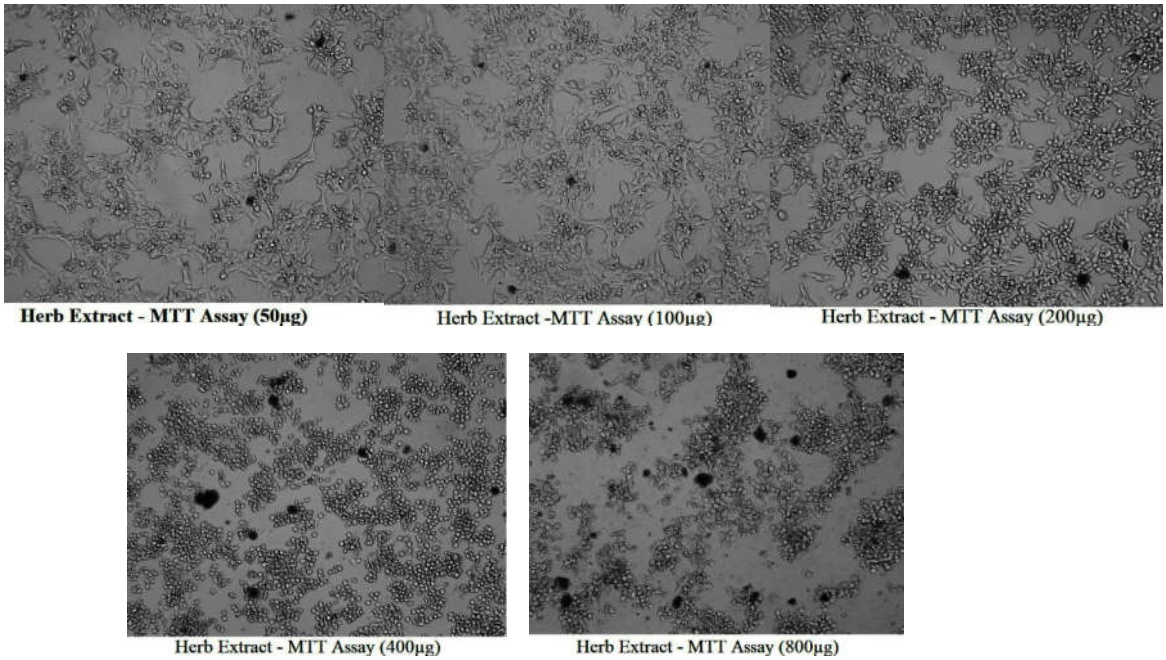


Fig. 3. MTT assay of HCT – 116 cells treated with herb extract

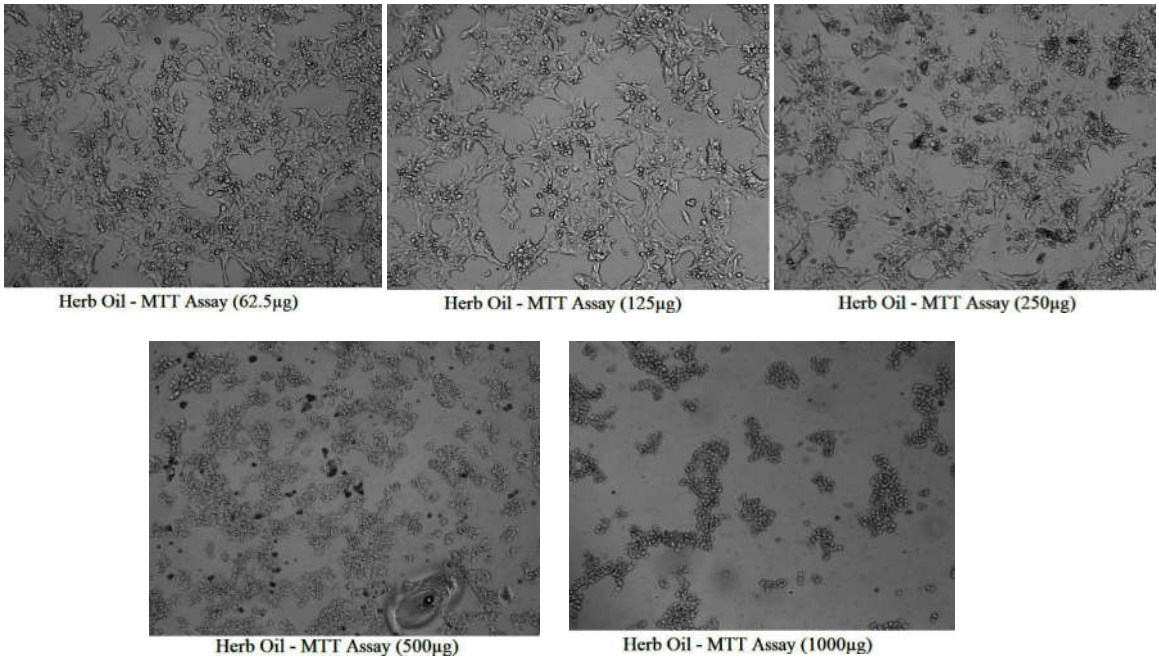


Fig. 4. MTT assay of HCT – 116 cells treated herb oil

3.6 Anticollagenase Activity

Anethum sowa L. herb essential oil andherb ethanolic extract was subjected to anticollagense test . *Anethum sowa* L. has not been investigated for its potential to inhibit collagenase enzyme. Herb Essential oil showed potential anticollagenase activity when compared to that of the extract.*Anethum sowa*L. Herb essential oil showed the maximum capacity in inhibiting thecollagenase when compared to ethanolic extract of herb , the percentage of inhibition of

Anethum sowa L. herb essential oil was found to be 60.89% and that of herb extract was (15.18%).

3.7 GC-MS Analysis

Anethum sowa L. herb oil chemical composition was determinedby GC-MS.19different compounds were found ,and 5 new compounds not reported before in this herb was also found which were5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo [2,3-c]pyridine-3-propionicacid methyl ester,(17.41%),beta.Amyrin(8.20%),Ritodrine(6.49%),1-Naphthalenol,decahydro-1,4a-dimethyl-7-(1-methylethylidene),[1R(1.alpha.,4a.beta.,8a.alpha.)]-(2.39%),Viridiflorol (0.77%). The other major and minor components found were Apiol (98.14%), Lupeol (27.70%), anethofuran (23.26%),2-methyl-5-propan-2-ylcyclohexa-1,3-diene,(17.85%),beta.-Pinene(8.08%),gamma.-Sitosterol(4.16%),Lup-20(29)-en-3-ol, acetate, (3.beta.)- (2.61%), meta-Cymene(1.95%),D-Germacrene(1.86%),beta.-copaene(1.79%),Stigmasterol(1.59%),Phytol, acetate(0.32%),beta.-Elemene(0.12%),7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(2-methyloxiranyl)-(0.01).

3.8 Insilico Docking Studies on Herb Oil

Anticancerous activity of the herb oil on HCT-116 cell line was carried out and from the in vitro results it was observed that herb oil showed great potency for anticancerous activity with an IC_{50} value of 79.75 µg/ml . The same protein whose interaction with Asef is inhibited by MAI-150 is taken into consideration for docking analysis. APC contains Phenylalanine and Arginine at positions 510 and 463 respectively, in the APC-Asef binding pocket which is found function as an interaction site for Alpha phellandrene and MAI-150. Similarly Asparagine and Tryptophan at the positions 594 and 553 was conserved in both anethofuran and MAI-150. And Methionine, Aspartic acid, Valine and Glutamine at positions 503, 539, 543 and 542 respectively was also found to be conserved in both Apiol and MAI-150.

Greater number of hydrogen bond ensures better specificity of the ligand with the target protein. 5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester,forms 6 hydrogen bond with Gln663 (3.11 Å), Leu662 (3.99Å), Cys661 (3.05Å), Asp651 (3.02Å) , Arg653 (3.87Å) and also Asn691 (3.79Å).(Fig. 5). Anethofuran forms an hydrogen bond with Arg554 (3.29Å) (Fig. 6). Apiol forms 3 hydrogen bonds with Arg499 (3.22Å) (3.16Å) (2.97Å) and two hydrogen bonds with Ser678 (3.21Å) (3.01Å) (Fig. 7). Lupeol forms 5 hydrogen bonds with Glu3 (3.12Å), Ala1 (2.98Å), Arg549 (3.26Å), Leu674 (3.17Å) and Ser678 (3.01Å) (Fig. 8).

Whereas MAI-150 forms two hydrogen bonds with Asn594 (3.33 Å) (3.13Å), Arg549 (3.19Å) (3.04 Å), Gln542 (3.28Å) (2.47Å) and single hydrogen bonds with Asn550 (2.94Å), Asn507 (2.70Å), Lys516 (4.12Å) and Gly511 (3.39Å) (Fig. 9).

Hence, from and docking studies and the anticancer studies done , lupeol showed the highest binding affinity with APC when compared with MAI-150 and rest other compounds present in the herb oil.

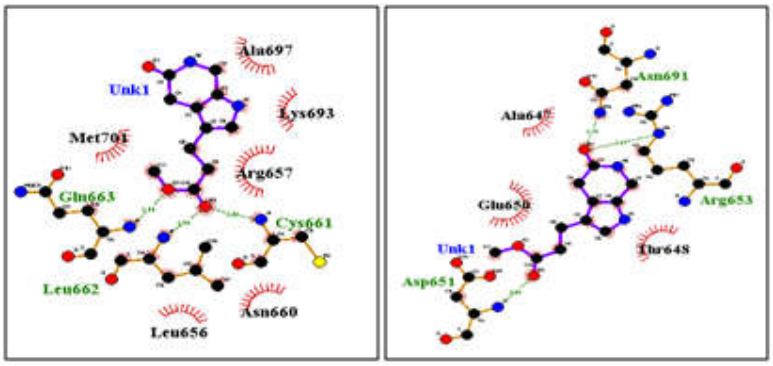


Fig. 5. Interaction of APC a 2D schematic representation with a)5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester,Red semi-circles with spokes represent hydrophobic interactions and dotted lines depicts Hydrogen bonds

Table 1. Docking interaction analysis of APC with the herb oil compound and MAI-150

Ligands	Vina score (kcal/mol)	Binding site residues
5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester	-5.0	Gln663 (3.11 Å), Leu662 (3.99Å), Cys661 (3.05Å), Asp651 (3.02Å) , Arg653 (3.87Å) , Asn69 (3.79Å).Met701, Ala679, Lys693, Arg657, Asn660, Leu656, Thr648, Glu650, Ala647
Anethofuran	-5.6	Arg554 (3.29Å), Ser552, Asn594, Try553, His598, Ala597, Glu536
2-methyl-5-propan-2-ylcyclohexa-1,3-diene	-5.3	Met701, Leu662, Asp694, Cys661, Arg657, Lys693, Ala697, Gln663, Phe510, Arg463, Cys483, Glu460, Ala528, Arg527, Glu484.
Apiol	-5.1	Arg499 (3.22Å) (3.16Å)(2.97Å) , Ser678(3.21Å)(3.01Å),Gly430, Met431, Asp7, Met503, Asp539, Val543, Gln543, Asp539, Tyr500, Pro429, Met717, His715, Leu674, Asp491, Thr675, His492
Lupeol	-8.6	Glu3 (3.12Å), Ala1 (2.98Å), Arg549 (3.26Å), Leu674 (3.17Å), Ala4, Met438, Met485, Tyr486, Ala440, Val442, Asp491, Thr489, Gly2, His492, Lys716, His715, Met717, Thr675, Asn490, Glu536
MAI-150	-7.0	Asn594 (3.33 Å)(3.13Å), Arg549 (3.19Å)(3.04 Å), Glu542 (3.28Å)(2.47Å) Asn550(2.94Å), Asn507 (2.70Å), Lys516 (4.12Å) and Gly511 (3.39Å), Asn641, Trp593,Ala597, Ser590, Val543, Met503, Phe458, Thr506, Arg463, Phe510,

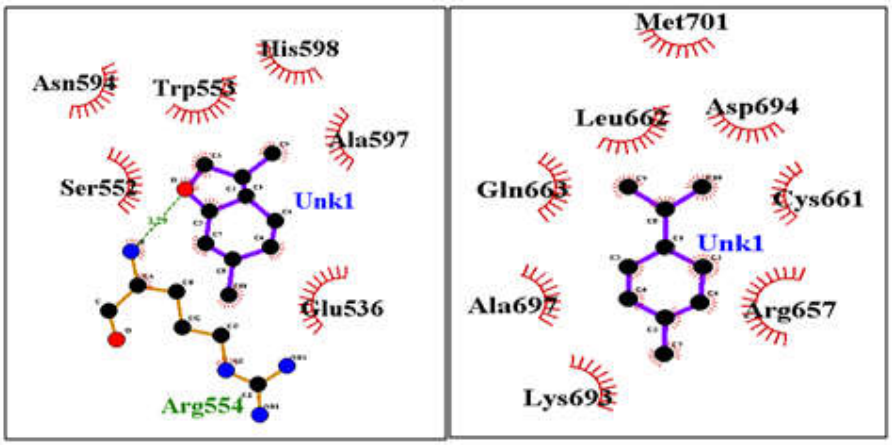


Fig. 6. Interaction of APC a 2D schematic representation with (A)Anethofuran (B) 2-methyl-5-propan-2-ylcyclohexa-1,3-diene. Red semi-circles with spokes represent hydrophobic interactions and dotted lines depicts Hydrogen bonds

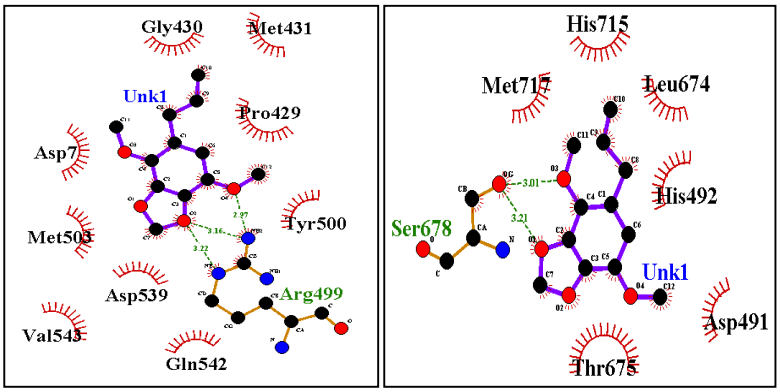


Fig. 7. Interaction of APC with Apiol - 2D schematic representations of two best interacting conformations . Red semi-circles with spokes represent hydrophobic interactions and dotted lines depicts Hydrogen bonds

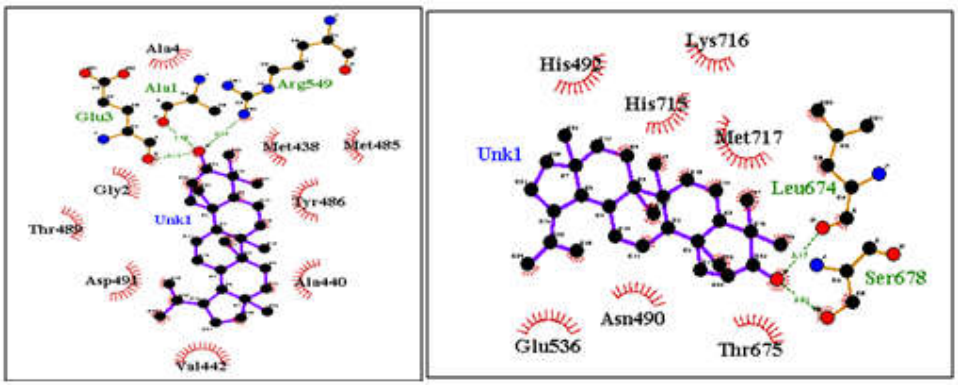


Fig. 8. Interaction of APC with Lupeol - 2D schematic representations of two best interacting conformations. Red semi-circles with spokes represent hydrophobic interactions and dotted lines depicts Hydrogen bonds

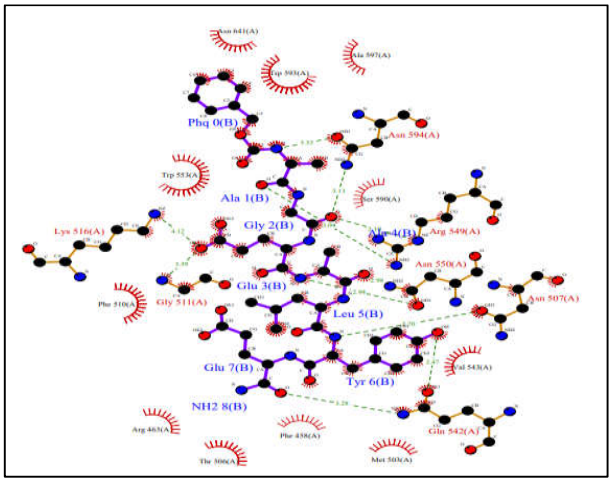


Fig. 9. Interaction of APC with MAI-150 - 2D schematic representations of two best interacting conformations. Red semi-circles with spokes represent hydrophobic interactions and dotted lines depicts Hydrogen bonds

4. CONCLUSION

Even though there are many scientific research on this species, this study is highly unique as it is the first study to be done with the *Anethum sowa* L. grown in South Karnataka, as based on the geographical region the oil content as well as the phyto chemicals present in the plant may vary. Anticancer potential of *Anethum sowa* L. herb grown in South Karnataka both the herb oil and herb extract has not been studied with respect to Colon Cancer cell line HCT116 as majority of the study done was with Brine Shrimp lethal toxicity studies.The study also highlights on the pharmacological potential of *Anethum sowa* L. herb oil , ethanolic extract of this plant ,which is an unique element of this study and the various studies done were antioxidant , antimicrobial , anticancer ,anticollagense and in silico docking studies. *Anethum sowa* L. Herb essential oil was more effective in its antimicrobial , anticancer and anticollagense properties when compared to the ethanolic herb extract , but the antioxidant activity was more in Herb extract when compared with that of Herb oil of *Anethum sowa* L. *Anethum sowa* L. has not been investigated for its potential to inhibit collagenase enzyme . Herb essential oil showed potential anticollagenase activity when compared to that of the extract.

Based on our findings, further insilico docking studies was performed with the APC (5IZ8) and major compounds present in Herb oil 5-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[2,3-c]pyridine-3-propionic acid methyl ester,Anethofuran,2-methyl-5-propan-2-ylcyclohexa-1,3-diene, Apiol and Lupeol) considering standard MAI-150 as a control. Lupeols showed the highest binding affinity with APC when compared with MAI-150 and rest other compounds present in the herb oil shown by docking investigations.

DISCLAIMER

The plant used for this research are commonly and predominantly found in our country. There is absolutely no conflict of interest between the authors. Also, the research was not funded by any organization rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

AVAILABILITY AND DATA AND MATERIAL

All data and material are available upon request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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