GC-MS Analysis of Bioactive Compounds of *Artemisia annua* and Assessment of its anti-proliferative activity against Human Cancer Cell Lines

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**ABSTRACT**

Plant essential oil is found to possess many beneficial effects in the field of medicine. Especially essential oil derived from different species of plants is reported to have specific activity against cancer. *Artemesia annua* is an annual herb belonging to Asteraceae family has been traditionally used for treating several ailments. It is a conventional medicinal plant used in the treatment of chloroquine-resistant and cerebral malaria. The major aim of this study was to evaluate the anticancer potential of this plant against cancer cell line. Hydrodistilled volatile oil obtained from aerial parts of *A. annua* was analysed by GCMS. The majority of the compounds derived from essential oil of *Artemesia annua* are monoterpenes and sesquiterpenes. Artemisinin, the active component of Artemesia annua is a sesquiterpenoid lactone containing unusual endoperoxide bridge that shows remarkable activity against numerous tumour growth and metastases. The pleiotropic nature of artemisinin induces oxidative stress, anti-angiogenetic effect and apoptosis triggering on cancer cells. The anti-proliferative effect of essential oil derived from *Artemesia annua* on human cancer cell lines like MCF-7, HT29 and AGS was done by MTT assay. Results shows the essential oil of artemisia annua have cytotoxic potential by inhibiting cell growth in a dose dependent manner. However, further investigations in isolating active cytotoxic components and understanding its molecular mechanisms will help in therapeutic management of cancer.

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**INTRODUCTION**

*Artemesia annua* belongs to the plant family of Astraceae is an annual short day plant with a strong fragrance origin to Asia and Eastern European countries. This aromatic species have many potent phytochemicals has been proven to possess anti-inflammatory, anti-microbial and antioxidant activities (Johnson et al., 2014). This odorous herb is the natural source of Artemisinin, a sesquiterpene lactone with an ethnoperoxide bridge responsible for its antimalarial activity (Posner and O’Neill, 2004), especially in cerebral malaria for centuries. Essential oil of *A. annua* shows characteristic sweet aromas has been describes as fresh, grassy and bitter with acamphoraceous nuance that brings more commercial value (Tellez et al., 1999).
α-terpinene and camphene. Sesquiterpenes such as β-caryophyllene, germacrene, α-copaene and α-cedrene and oxygenated terpenes such as Eucalypt, Camphor (Li et al., 2007). Most of these compounds are regarded as aroma active and show characteristic odour. The volatile oils extracted from this composite plant have high utilisation value. Here we report the extraction and identification of sesquiterpene from the leaves of A. annua extract and studies for their antiproliferative effect against to human cancer cell lines namely, MCF-7, HT29 and AGS.

Materials and Methods

Leaves of Artemesia annua were collected from Banagaluru, Karnataka. It was authenticated and voucher Specimen (No: PARC2017/3596) was retained for further reference. Leaves were clean shade dried and the dry mass of the plant was hydrodistilled-hydro distilled in a Clevenger type apparatus for 3 hours and the essential oil was isolated. The oil was separated from the aqueous phase with Dichloromethane and stored at 4°C until further use. The yield was 0.39% (V/M) on fresh weight bases.

GCMS Analysis

GC was performed on Perkin Element auto XL system using 50m×0.32mm film thickness as stationary phase and helium as carrier gas at a flow rate 2m/min. 0.1µl specimen was injected with an injector temperature of 200°C. Initially temperature was programmed from 100°C to 280°C at 3°C/min. Detector used was FID and MS was done at 70ev. Computer matched spectra with standard library were used to predict the compounds (Adams, 2017).

Cell Culture

Cell lines MCF-7, HCT116, AGS were obtained from NCCS, cell repository, Pune, India. Cells were routinely cultured in DMEH with 1% Penicillin- Streptomycin and maintained in a CO2 incubator at 37°C.

Cell Viability Assay

Cell Viability was assessed by seeding cells in 96 well plates at 1x10 densities and allowed to attach overnight. The essential oil of A. annua was dissolved in cell culture grade DMSO and dilutions made with cell culture media. Control cells had a maximum 0.1% of DMSO at any concentration. Cells were treated with an increasing range of essential oil in various doses, (initially at logarithmic concentrations and then at various increasing doses) and were treated for 24 hrs and 48 hrs. 20µl of MTT in 5mg/ml in culture media was added to the wells and incubated in dark for 3 hrs. Then so formed formazan crystal are dissolved in 15µl of DMSO and read at 540nm. The OD obtained at 540 nm was used to calculate the percentage of cell viability.

RESULTS AND DISCUSSION

Constituents of the essential oil isolated from Artemesia annua leaves

Retention indices of most constituents identified by GC were compared with authentic standards available in the laboratory or with retention indices in close agreement with reference (Adams, 2017; Ali, 2002). Further identification was achieved by GCMS. Mass spectra fragmentation patterns were compared with Wiley L. built in libraries and with those published in literature (Adams, 2017; Vernin and Petitjean, 1983; Andersen and Falcone, 1969; Jennings and Shibamoto, 1980; Stevens et al., 1981; Libbey, 1991). Greenish yellow oil with a pleasant aroma in 0.39% (V/M) obtained from hydrodistillationhydro distillation of Aannua shows different constituents which are completely volatile. Monoterpenes are found to be major (65.7%) constituents of A. annua followed by α terpineole (14%) Carvone (12.0%) and α terpenine (2.6%). Sesquiterpenes (27.3%) as monocyclic or bicyclic and in oxygenated forms occur as hydrocarbons (23.1%)and alcohols (2.5%), γ- Eumene and its derivative accounted for about 10 % of total volatile followed by Z α – bisabocene(5.4%)(Table 1).

Essential oil of Artemesiaannua induced cytotoxicity in cancer cell lines

Essential oil obtained from Artemesia annua showed cytotoxicity against the cancer cell lines MCF-7 HT29 and AGS. Cytotoxicity of Artemesia annua was evaluated by MTT assay, a simple and reliable experiment which measures cell viability and cytotoxicity for screening cytotoxic agents (Mosmann, 1983). Finding of this experiment demonstrated that essential oil of A annua was strongly cytotoxic on MCF-7, HT-29, and AGS in a dose dependent manner. Here, we found that essential oil of A.annua showed cytotoxic potential at doses ranging from (0-160 µg/ml) in the Figure 1, it implies that the IC50 value of plant oil in HT 29 cell line is 40 µg/ml, in MCF-7 cells showed cytotoxic potential at doses ranging from (0-40µg/ml) with an IC50 value of 35 µg/ml, in AGS cell line with cytotoxic potential at doses ranging from (0-100 µg/ml) with an IC50 value of 38 µg/ml respectively. Previous reports show that the anti-tumour mechanism of Artemesia annua is due to cleavage of ethanoperoide ring.
Table 1: Shows the qualitative analysis of phytochemicals present in essential oil of A. annua characterised using GC-MS analysis

| S.No | Constituent       | RI  | Molecular formula | Biological activity          |
|------|-------------------|-----|-------------------|------------------------------|
| 1.   | α-pinene          | 928 | C_{10}H_{16}       | Bactericidal activity        |
| 2.   | Camphene          | 939 | C_{10}H_{16}       | Hypolipidemic activity       |
| 3.   | β-pinene          | 964 | C_{10}H_{16}       | Bactericidal activity        |
| 4.   | Myrcene           | 992 | C_{10}H_{16}       | Antibacterial activity       |
| 5.   | γ-Terpinene       | 1001| C_{10}H_{16}       | Antiproliferative activity   |
| 6.   | Linalool          | 1006| C_{10}H_{18}O     | Anti-inflammatory activity    |
| 7.   | 1,8-Cineole       | 1016| C_{10}H_{18}O     | Antimicrobial activity       |
| 8.   | Pinicamphone      | 1142| C_{10}H_{16}O     | Antimicrobial activity       |
| 9.   | α-Terpinene       | 1181| C_{10}H_{18}O     | Anticonvulsant activity      |
| 10.  | Artemisia ketone  | 1192| C_{10}H_{18}O     | Antimalarial activity        |
| 11.  | Carvone           | 1223| C_{10}H_{14}O     | Antimicrobial activity       |
| 12.  | Ascaridol         | 1276| C_{10}H_{18}O     | Antitumor activity           |
| 13.  | Undeane           | 1390| C_{11}H_{24}      | Antiviral activity           |
| 14.  | 3,5-Cycloheptadieny1-one | 1403 | C_{7}H_{6}O_{2}    | Antihypertensive activity    |
| 15.  | α-Guainine        | 1419| C_{15}H_{24}      | Antimicrobial activity       |
| 16.  | Z-α-Bisabolene    | 1490| C_{15}H_{24}      | Antioxidant activity         |
| 18.  | β-Selinene        | 1531| C_{15}H_{24}      | Antioxidant activity         |
| 19.  | Junipene          | 1598| C_{15}H_{26}O     | Antibacterial activity       |
| 20.  | γ-Elemene         | 1626| C_{15}H_{24}      | Antimicrobial activity       |
| 21.  | Germacrene B      | 1636| C_{15}H_{24}      | Antibacterial activity       |
| 22.  | Humulene isomer   | 1640| C_{15}H_{24}      | Antineoplastic activity      |
| 23.  | Caryophyllene oxide | 1675 | C_{15}H_{26}O    | antimicrobial activity       |
| 24.  | α-Cadinol         | 1681| C15H_{26}O       | Anti-fungal activity         |

Figure 1: MTT Assay of essential oil of A. annua treated cancer cell lines.

which is characteristic of sesquiterpenes by iron in cancer cells and formation of free radicals. Free radicals induce oxidative stress that cause cellular alterations like modulator of nuclear receptor responsiveness, DNA damage, apoptosis, tumor-tumour invasion and metastasis (Ivanescu et al., 2015). However, our study implies about cytotoxic potential of Artemesia annua, further investigations are needed to isolate bioactive constituents from essential oil of A.annua for investigation of the molecular mechanisms as well as understanding the effects of A.annua on other various tumor cell line models to explore its anticancer potential.

CONCLUSIONS

The molecular docking studies are one of the most influential techniques to find out new ligand for known protein and facilitates in treating dreadful diseases. In this present work, we have carried out molecular docking to analyze the binding properties of the mediator called 3HNG with Hexade-
cane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl) reported from Dictyotabartayresiana and 5-fluorouracil used as standard. The wet analysis carried out by us showed the best result with 3HNG and proven anti-colon cancer property. Among the various compound, of Hexadecanoic acid methyl ester and Quinoline, 1,2-dihydro-2,2,4-trimethyl) has higher binding energy than standard. So the present study may strongly conclude that anti-colon cancer property of the seaweed extract.

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