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ISOLATION, IDENTIFICATION & PRE-CLINICAL COMPUTATIONAL SCREENING OF PHYTOCHEMICALS COMPOUNDS FROM THE *ACHILLEA KAMELINII* OIL EXTRACT.

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Abstract
The proposed research was designed for the isolation and identification of anti-cancer, anti-inflammation, anti-leishmania and insomnia active compounds, accentuate cardiac and oral toxicity from natural sources. A comprehensive approach was designed to study cancer. Histone deacetylase is a relatively new class of anti-cancer agents. The recent trend prevailing nowadays is to fight cancer with natural products, which also benefit for secondary risk factor and this trend is increasing day by day. Therefore, *Achillea kamelinii* oil extract which contain heptadecanoic acid and methyl-10-methylundecanoate were selected for current study. The gas chromatography- mass spectrophotometric and NMR method was used for the identification of these compounds. Computational methods were applied to identify Histone deacetylase's inhibitory active sites, and heptadecanoic acid and methyl 10-methylundecanoate were selected for checking their anti-cancer activities. Molecular docking results revealed that heptadecanoic acid interacts with histone decetylase more effectively than methyl 10-methylundecanoate. Both ligands have the ability to merge with the active sites of histone deacetylase. Oral toxicity results, cardiac toxicity, blood brain barrier, skin sensitivity-and chemical properties of ligands were tabulated. The oil merge ingredients of plant may be treated as prospective new anti-cancer, anti-inflammation, Result leishmanial bioassay of 0.56 mg/mL IC_{50} against.

Keywords: Acoraceae; Margaric acid; antineoplastic

Introduction:

Achillea is a genus of flowering plants that includes many species, and every species has unique phytochemical properties and is known for feathery leaves and flat-topped and daisy-like flowers. These plants are primarily used in gardens for ornamental purposes. Many species haves been studied and show oil content [1] the distinctive feature of their inflorescence, which consists of multiple small flowers tightly packed together in a head. Achillea belongs to the asteraceae family [2, 3]. Members of this widely used for dermatological purpose; however the chemical contribution is still a hidden question [4]. Among those aliphatic organic compounds; we targeted two compounds, heptadecanoic acid (Molecule A) and methyl 10-methylundecanoate (Molecule B), to highlight their anti-cancer role. Cancer is a complex area of study, with every perceivable angle of attack plumbed by researchers. With changes in human lifestyle and environmental changes, cancer-causing agents can

easily disturb an individual's body mechanism to initiates disease. This change can put humans at a greater risk of cancer. Therefore, natural chemicals that cans help protect body from cancer are of keen interest. The global trend towards natural products to fight cancer is increasing now a days. Histone deacetylase (Hd) inhibitors are a relatively new class of clinically valid anti-cancer agent [5]. Histone deacetylase is a promising target for cancer chemotherapy. Histone deacetylase directly and indirectly participates in many biological processes, including development, proliferation, differentiation, and apoptosis [6]. DNA is involved in cancer development and tumour progression [7]. The function of Hd is to remove acetyl groups from histone proteins, resulting in chromatin [8]. Changes in the environment and lifestyle are considered key causes of cancer worldwide. According to the W.H.O, there we are an estimated 9.6 million deaths in 2018 across the globe. W.H.O further added that 70% of deaths from cancer occur in low- and middle-income countries and most of them are in Asia, which increases the health care budget as well[9]. Sleep disturbances in cancer survivors may be attributed to the cancer itself [10] among the most extensively researched in the treatment of cancer is inflammatory condition [11]. Herbal medicines have the same effect as lab prepared drugs; the human body has no way of distinguishing between naturally occurring compounds and synthetic or semisynthetic compounds [12].

BOTANICAL TAXONOMY [+]

Taxonomy of plant
Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Achillea
Species: *Achillea kamelinii*

Material and Methods:

Oil extracts:

The *Achillea kamelinii* was purchased from a local area and authenticated. The whole plant (200 g) was grounded to a fine powder. The powder was subjected to hydro-distillation using a clevenger apparatus for four hours. This procedure was used to extract oil from *Achillea kamelinii* powder, and desiccation of essential oils was conducted over anhydrous sodium sulfate. Different concentrations of oil were dissolved in the culture media (30, 60, 120 and 240 µg/ml).

Identification:

A gas chromatography-mass spectrometry (GC-MS) spectrometer was used to identify plant compounds. The 2µL sample was injected into the GC-MS spectrometer, which was coated 0.25 mm in diameter and 30m length coated with a 0.25 µm film of HP-Innowax Agilent. Split inoculation (split ratio 30:1) was achieved with nitrogen, which was used as the carrier gas at a flow rate of 1.52 bar. The conditions were maintained at 60°C for couple of minutes; after inserting the sample in the column. Then temperature was elevated to 250°C after 10 min, whereas detector temperature was 230°C mass spectra were 70ev, scanning 35 - 300 m/z. /e identification of the components of the plant was performed by matching their mass spectra with the available library.

Computational Method:

After isolation and identification of compounds, the structure of the molecule was drawn with Avogadro 1.2 [13]. Optimization of the geometry was carried out automatically. For oral toxicity, cytotoxicity and stress response calculations, ProTox-II was used [14]. A Web-based application was used to predict lipophilicity [15]. The protein structures of enzyme Hd were obtained from the online protein data bank database system. A quantitative structure–activity relationship model was used to predict the potential hERG blockage or Ether-à-go-go gene [16]. The online system is the preferred choice for the author to determine the blood brain barrier [17], Figure.3 is drawn with the help of Chimera 1.13.1 [18].

Results & Discussion:

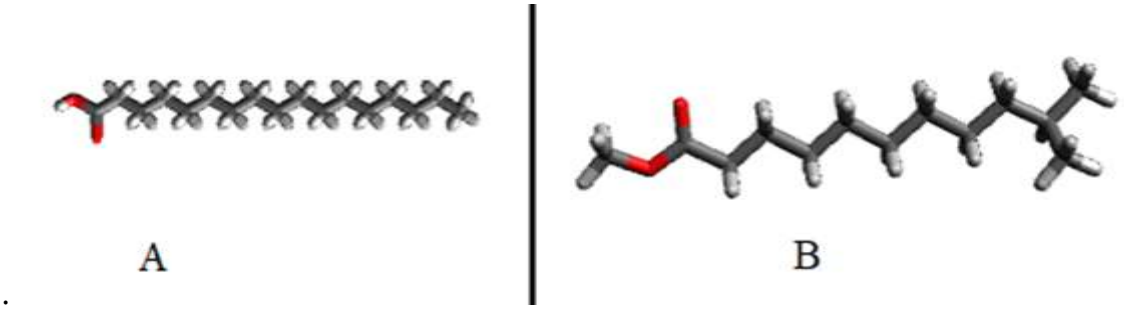


Figure 1 .Show the arrangement of atoms, A=Heptadecanoic acid & B=methyl 10-methylundecanoate. Molecular shape, atom arrangement, 3D structure.

Chemical taxonomy of molecules:

Molecule A:

The molecular formula of $C_{17}H_{34}O_2$ of Heptadecanoic acid. Thus, the molecule contained 53 atoms, as shown in (Figure .1 A). There was one double bond and no triple bond in the molecule. On the terminal side, an ester functional group was present. Its molecular weight was 270.4 g/mol. With freely rotating 15 bonds, there are 19 heavy atoms in the molecule. There are two hydrogen acceptors in the molecule and one hydrogen donor. The polar surface area of the molecule is 37.3 2. The smile notation of the molecule is CCCCCCCCCCCCCCCC(O)=O. This molecule is an aliphatic acyclic organic compounds, known as along-chain fatty acids.

Molecule B:

Methyl 10-methylundecanoate is a chemical compound methyl, this indicates the presence of a methyl group (CH3) in the molecule, and its name provides information about the structure of the compound. 10-methyl" indicates the presence of a methyl group (CH_3) attached to the 10th carbon atom in the molecular chain. "Undecanoate" is the presence of an ester group derived from undecanoic acid. An ester group typically has the structure RCOOR', where R and R' are alkyl or aryl groups "Methyl 10-methylundecanoate" refers to a compound in which a methyl group is attached to the 10th carbon atom of an undecanoate ester molecule. The specific arrangement of atoms and bonds in a molecule determines its properties and potential uses in various applications.

The molecule contained 13 carbon atoms, two simple oxygen atoms, and 26 hydrogen atoms. A single double bond exists between carbon and oxygen atoms. Thus the molecule contains 41atoms as shown in (Figure.1B). There is one double bond and no triple bond in a molecule. On the terminal side, an ester functional group is present. Its molecular weight was 214.3 g/mol. With freely rotating bonds ten, there are two hydrogen acceptors in the molecule. The polar surface area of the molecule was 10A.The smile notation of the concerned molecule is O=C(CCCCCCCCC(C)C)OC, with a molecular formula of $C_{13}H_{26}O_2$.

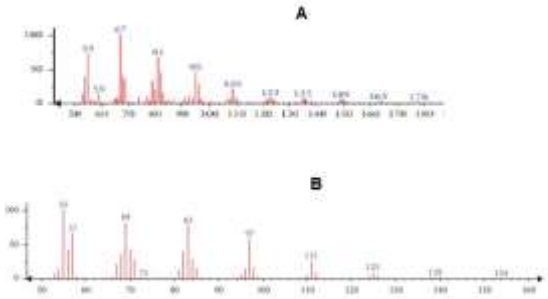
Mass Spectrogram result:

Following remarkable peaks are observed in molecule A

294(M+), 95(450), 82(450), 81(670), 79(330), 69(350), 68(400), 67(999), 55(720), 54(380), 41(910)

Following remarkable peaks are observed in molecule B
242(M+), 97(573), 83(760), 82(394), 70(425), 69(822), 57(653), 56(410), 55(999), 43(710), 41(758)

Figure 2A



Show the mass spectrogram of molecule A, B

NMR:

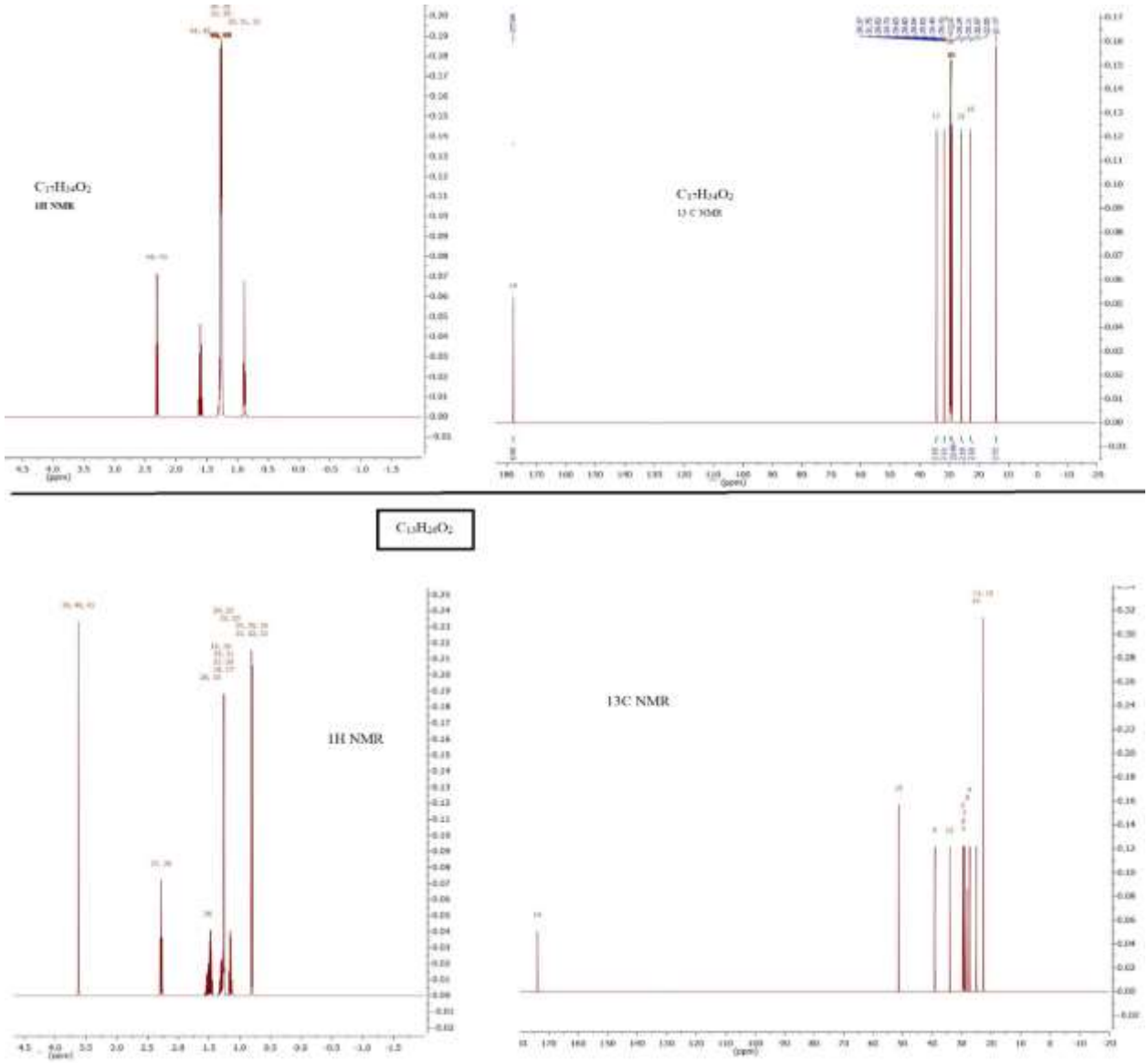


Figure 2B. Show the NMR spectra of Molecule A and B

For NMR analysis, approximately 2 mL of each sample was introduced into a standard 5 mm disposable NMR tube, along with approximately 2 mL of non-deuterated chloroform. The sample temperature inside the spectrometer was 37°C. 1H-NMR measurements were performed with a 500 MHz NMR spectrometer at 298K. 1H and 13C nuclear resonance frequencies were 500 and 125 MHz, respectively. Resolution enhancement methods were not applied. Empty region were discarded; the region around the chloroform reference peak was discarded, and the data were re-aligned by simple linear sideways shifting using the glyceride-peak maxima as the reference point. Each resulting spectrum was normalized to unit integrated area.

Anti-cancer evaluation at molecular level:

Molecule A (Heptadecanoic acid):

The virtual molecular docking of ligand is studied with Hd in Swiss docking online web system which shows the interrelating amino acids (Table 1). The mentioned extract is binding with amino acids, interrupt the mechanism of further growth of cell, which are considered as the main cause of cancer in animals. As Shown in Figure. 3.

Table.1. Interaction of amino acid with Hd.

Name of Amino Acid which interact with A.					
Arg	Ser	Val	Phe	Hsd	Gly
Position of Amino acid in Hd enzyme					
27	29	28	198	131	295

Molecule B:

The predicting results score, which is calculating from [19] shows that it's a good candidate for Hd inhibitor. After obtaining best score result, the above mentioned ligand is studied in Swiss docking online web system[20], which shows the Interrelating amino acids i.e valine 28 which is used in protein synthesis.

Molecular docking score is -5.4.

Cytotoxicity inactive

Carcinogencity inactive

Predicted LD_{50}: 5kg/kg and belong to Toxicity Class: 5.

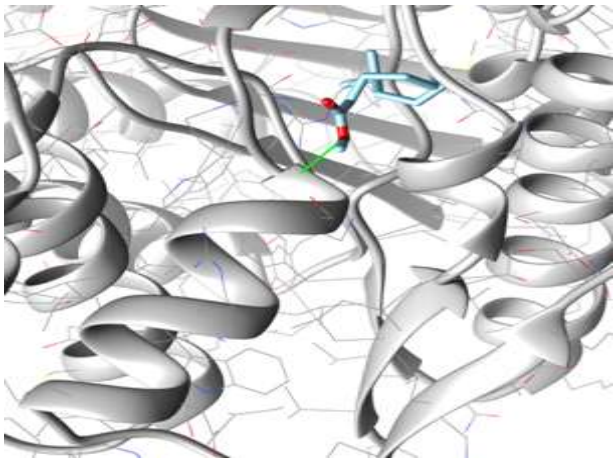


Figure 3. Show the binding of methyl 10-methylundecanoate and enzyme.

Blood-brain barrier permeability:

The blood brain barrier act as a barrier to prevent brain to uptake pharmaceuticals. Blood brain barrier analysis were carried out through online method [21].The results of the analyses of blood brain barrier showed that said Heptadecanoic acid and methyl 10-methylundecanoate as an active compound for blood-brain barrier.

Cyclooxygenase-2:
Cyclooxygenase-2 (C0X-2) is an enzyme that plays a key role in promoting inflammation. In contrast, when cyclooxygenase-2 activity is blocked, it results in reduction of inflammation. Cox-2 becomes active only at the site of inflammation i.e peripheral tissues. These are propitious curative molecules not only for the treatment of inflammation, and also for cancer treatment. On the basis of scientific result Heptadecanoic acid and Methyl 10-methylundecanoate are blocked Cox-2 activity.

Sleep aid:
Cancer and sleep disorders can often be interconnected [22], as cancer and its treatments can significantly impact a person's sleep patterns and overall sleep quality. Cancer itself can be painful and discomfort, which can make it difficult for patients to fall asleep. Pain is the key reason which lead to emotional distress, anxiety, and depression [23]. These mental health issues can contribute to sleep disorders. The stress and worry associated with a cancer diagnosis can make it difficult for patients to relax & fall asleep due to constantly thinking about their health [24]. Managing sleep disorders can contribute to better overall well-being and quality of life during cancer treatment and recovery [25]. To manage sleep disorders in cancer patients, various strategies [26], including natural [27] vegetable oil were used to improve overall health and sleep quality [28,29].Traditional diet contains a lots of constituents, making it difficult to identify the active components and define their exact mechanism of action [30]. GABA Role in sleep and mode of action are explain and define [31,32] GABA is the main inhibitory neurotransmitter of brain. Activation of GABA (A) receptors favors sleep [33]. The blood-brain barrier is not an absolute barrier, and some substances can cross it under specific conditions or with the assistance of transport mechanisms. The blood-brain barrier is a highly selective and semipermeable barrier that separates the circulating blood from the brain and tissues. It plays a crucial role in protecting the brain from harmful substances. Enzymes working within the blood-brain barrier can metabolize or break down certain chemicals, rendering them less harmful or less able to cross the barrier. As mention *Achillea kamelinii* extract cross blood brain, molecular docking is performed between the obtain chemical (Heptadecanoic acid & Methy10-methylundecanoate) against GABAA receptors which were obtain from PDB 4S0V an orexin receptor, after this select chain A and remove all unnecessary residue. The molecular docking result reveal that the above mention compounds are suitable to regulate sleep mechanism in human body.

Human ether-a-go-go related gene:
hERG predation was carried out through an online method [34] the result revealed that the molecule did not contribute to hERG blockage.

Leishmania:
Leishmaniasis is considered a neglected protozoa disease and can have a significant impact on the health and well-being of affected populations, particularly in resource-limited areas. Leishmaniasis is a vector-borne disease that can affect humans [35].It is transmitted through the bite of infected female sandflies. The treatment of leishmaniasis typically involves anti-parasitic medications. The choice of medication and duration of treatment depend on the specific species causing the infection and the clinical form of the disease [36]. Pteridine reductase 3JQ7 obtain from pdb is a target for drug development against Leishmania parasites that cause serious tropical diseases [37]. The molecular docking result reveal that the above mention both molecule A&B are suitable for lesihmina in human body and also call anti-parasitic on the bases of scientific evidence. Bioassay for Antileishmanial. The Whole extract of plant were analysis against (promastigotes) Leishmania major through culture in microplates. Leishmania major were grown in NNN biphasic medium. The Leishmania major were harvested for 10 min at 2000 rpm, the leishmanial organisum were centrifuged. Leishmania major was washed three times with saline. Under the microscope with help of Neubauer chamber, the Leishmania major were counted. The addition of fresh culture medium to develop the leishmanial organisms with a final density of 106 cells/mL. The extracts and fractions

with concentration of 20 µL were introduced in wells. Further serially diluted to develop working solution with 1–100 µg/mL range. The parasite culture with 100 µL was introduced in all wells. In well microtiter plate, 180µl of the parasite culture (1×106 parasite/ml) was added in different wells in which 20µl of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound was 1µg/ml. Negative control received medium with a parasite density1×106 cells/ml. The positive control contained varying concentration of standard antileishmanial compound e.g., Amphotericine B, Pantamidine. The plate was incubated between 21-22 C for 3 days. The culture was seen under microscopically (Neubauer chamber and IC_{50} values of compound) possessing anti-leishmanial activity were counted.The IC_{50} values of extracts and fractions with anti-leishmanial activities were calculated [38].Result is 0.56 mg/mL IC_{50} against lesishnmainal major. The molecule B show superior anti-inflammatory effect as compare to molecule A.

Skin predication: QSAR online models based skin response results [39]. Molecule A and B are non-sensitive, whereas molecule B is less sensitive than molecule A.

Achillea kamelinii contain many compounds, which have been linked to various health benefits, including antioxidant and anti-inflammatory properties. Some scientific studies suggest that these compounds could potentially contribute to a lower risk of certain types of cancer, such as colorectal and stomach cancers. However, the evidence is not conclusive, and more research is needed to fully understand for cancer prevention. The key goal of molecular docking is to understand how molecules interact at a molecular level, which is crucial for discover of new anti-cancer drugs candidate, understanding enzyme-substrate interactions, and predicting protein-ligand binding affinities. That molecular docking is a predictive tool, and its accuracy depends on various factors. It's often used in combination with experimental validation to gain a comprehensive understanding of molecular interactions. As computational methods continue to evolve, molecular docking remains a valuable tool in the field of structural biology and drug development. Heptadecanoic acid are commonly found in various natural sources, including animal fats, dairy products, and some plant oils. Heptadecanoic acid can serve as a building block for more complex lipids and can be metabolized by the body for energy. In biological systems, heptadecanoic acid can be incorporated into cell membranes and other lipid structures. It's important to note that while some saturated fatty acids are essential for various physiological functions. The naturally obtain organic compound disuses in current study also applicable to other natural obtaining heptadeconic acid and molecule B which also give same benefits.

Conclusion:

A current study revealed that *Achillea kamelinii* shows significant anticancer activity at the molecular level in ideal situations. In the near future, the demeanour of constituent fat soluble can be revealed as an alternative drug for cancer due to its natural product with the least toxicity and side effects.The hepadecanoic acid & Methyl 10-methylundecanoate was confirmed through GC-MS investigation.

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