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Research Article

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Isolation and Characterization of Chemical Compounds from *Achyranthes Bidentata* for Evaluation of Anti-Parkinson's Activity Through *In-Silico* Approach

Rahul Thapa[1*], Shikha[2], Girish Chandra Arya[3] and Saahil Arora[4]

[1,2]*Research Scholar, University Institute of Pharmaceutical Sciences, Chandigarh University, Punjab.*

[3]*Assistant Professor, University Institute of Pharmaceutical Sciences, Chandigarh University, Punjab.*

[4]*Director, University Institute of Pharmaceutical Sciences, Chandigarh University, Punjab.*

Abstract: *Achyranthes bidentata* has been used to treat asthenia of lower limbs, painful backs & knees, atherosclerosis & many other ailments for a very long time. It is found that *A. bidentata* has many pharmacological activities such as anticancer & immunomodulatory activities, antiosteoporosis, neurotrophic & neuroprotective effects. In Chinese medicine system it is used for abortion. The main chemical components of *A.bidentata* are sterones & saponins which are recognized as main active compounds. The aim of this investigation is to isolate and characterize the chemical compounds from the aerial part of *A.bidentata* for assessing the Anti-Parkinson's activity through *in-silico* studies of isolated phytoconstituents. In this present work, aerial parts of *A.bidentata* were used for the potential of anti-parkinson's activity. The phytochemical investigation revealed that it is rich in alkaloids, terpenoids and saponins. The crude ethanol extract of *A.bidentata* was partitioned with *n*-hexane, ethyl acetate, *n*-butanol, and water. Ethyl acetate fraction much revealed the presence of phytoconstituents carried out with isolation. Compounds identified by NMR, MS and UPLC analysis were oleanolic acid and 5-hydroxymethyl furfural. Molecular docking studies of designed compounds have been performed to investigate interaction with Dj-1/RS associated with PD. This research will be helpful in completing information on the identification and authentication of *A. bidentata*, and the use of aerial parts of *A.bidentata* for testing their anti-Parkinson's activity, which will aid in the development of an alternative treatment to allopathic drugs, which have serious side effects. The extraction methods for *A.bidentata* can be improved with the help of this study.

Keywords: *Achyranthes bidentata, A.bidentata, Parkinson's disease, in-silico studies.*

***Corresponding Author**

Rahul Thapa , Research Scholar, University Institute of Pharmaceutical Sciences, Chandigarh University, Punjab.

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1. INTRODUCTION

Parkinson's disease is a common neurodegenerative disorder that can cause significant disability and decreased quality of life. It is characterized by loss of neurons in substantia nigra. The neurons in this region produce a neurotransmitter, dopamine. As number of cells in substantia nigra decreases, there is less dopamine available in brain. Dopamine is important to maintain normal movement patterns. The cardinal physical signs of disease are distal resting tremor, rigidity, bradykinesia, & asymmetric onset [1]. Dopamine replacement remains standard treatment. The combination of levodopa with carbidopa, decarboxylase inhibitor, provides most significant symptomatic relief with least adverse effects, as carbidopa prevents conversion of levodopa to dopamine in peripheral tissues, allowing for a successful transport of levodopa to CNS. The major side-effects of carbidopa/levodopa are development over time of dyskinesia & fluctuating 'off-on' periods of effectiveness. The potential neurotoxicity of carbidopa/levodopa has been suggested. Recently, strong neuro-protective effect of medicinal plants extracts & phytochemicals in reduction of PD signs has been highlighted in various studies [2-4]. For many centuries, nature has been providing bioactive molecules to human kind & a remarkable number of modern medicines are derived from these molecules. WHO has identified more than 22,000 species as medicinal plants [5]. *Achyranthes bidentata* is one of medicinal plants, which belongs to amaranth family, Amaranthaceae [6], has been traditionally used for fever, asthma, rheumatism, headache, & hypertension. It is commonly known as Ox knee. Geographically, it is commonly found in India, Nepal, China & Japan [6]. *A. bidentata* is a perennial plant that can reach heights of 0.8 m (2 ft 7 in) & 0.4 m. (1ft 4in). The flowers of *A. bidentata* blooms from August to September, & seeds of *A. bidentata ripen* from September to October. The species is hermaphrodite (has both male & female organs) [7]. According to various surveys *A. bidentata* is found to be very potential herbal plant. In Chinese herbal medicine, roots, leaves, & stems are frequently used. Anodyne, anti-inflammatory, anti-rheumatic, bitter, digestive, diuretic, emmenagogue, & vasodilator are some of their medicinal properties. They are used to treat asthenia of lower limbs, painful backs & knees, & other conditions that mostly affect lower half of body. Previous studies documented that this herb should not be used in pregnancy. The herb is ingested to cure a variety of conditions, including hypertension, back discomfort, blood in urine, menstrual cramps, bleeding, etc. It is used to treat atherosclerosis because it decreases blood cholesterol levels. In Nepal, root juice is used to treat toothaches [8]. In addition to being used to treat asthma, this juice is also utilized to cure indigestion. A toothbrush made from plant's stem is reported to be helpful for teeth & can also be used to cure pyorrhea. The plant can be utilized either as fresh or in dried form. The leaves & stems are picked in summer & often either made into tinctures or crushed for their juice. The roots are taken from plants that are 1 or 2 years old in fall or winter & are typically dried, powdered, or used in decoctions [7]. Due to its anticoagulant properties, decoction from *A. bidentata* was used for female blood clots by ancient Chinese folk healers. The juice from smashed root of *A. bidentata* was injected into vagina to induce abortion. In this research, aerial part of *A. bidentata* will be studied by isolation & characterization of chemical compounds for evaluation of Anti-Parkinson's activity along with *in-silico* studies of isolated phytoconstituents.

1.1. Traditional uses

In Chinese herbal medicine, roots, leaves, & stems are frequently used. Anodyne, anti-inflammatory, anti-rheumatic, bitter, digestive, diuretic, emmenagogue, & vasodilator are some of their medicinal properties. They are used to treat asthenia of lower limbs, painful backs & knees, & other conditions that mostly affect lower half of body. Previous studies documented that this herb should not be used in pregnancy. The herb is ingested to cure a variety of conditions, including hypertension, back discomfort, blood in urine, menstrual cramps, bleeding, etc. It is used to treat atherosclerosis because it decreases blood cholesterol levels. In Nepal, root juice is used to treat toothaches [8]. In addition to being used to treat asthma, this juice is also utilized to cure indigestion. A toothbrush made from plant's stem is reported to be helpful for teeth & can also be used to cure pyorrhea. The plant can be utilized either as fresh or in dried form. The leaves & stems are picked in summer & often either made into tinctures or crushed for their juice. The roots are taken from plants that are 1 or 2 years old in fall or winter & are typically dried, powdered, or used in decoctions [9]. Due to its anticoagulant properties, decoction from *A. bidentata* was used for female blood clots by ancient Chinese folk healers. The juice from smashed root of *A. bidentata* was injected into vagina to induce abortion.

1.2. Taxonomic classification

- Kingdom: Plantae
- Subkingdom: Tracheobinota
- Super division: Spermatophyta
- Division: Mangoliophyta
- Class: Mangoliophsida
- Sub class: Caryophyllidae
- Order: Caryophyllales
- Family: Amaranthaceae
- Genus: *Achyranthes*
- Species: *Bidentata*

1.3. Morphology

- *A. bidentata* is a perennial herb that can reach heights of 0.8 m (2 ft 7 in) & 0.4 m. (1ft 4in).
- The leaves *A. bidentata* are opposite.
- The Flowers of *A. bidentata* have in elongate terminal or axillary spikes, which become deflexed & are bisexual. The bracts & bracteoles spine-tipped.
- The *A. bidentata* have 5 perianth segments which are rigid, 5 stamens, 2-celled anthers, filaments connate at base into short cup, alternating with oblong, toothed pseudostaminodes. Ovary oblong, styles filiform, stigma capitate. Fruit is 1-seeded, indehiscent, shed with perianth & bracteoles. [10]

1.4. Chemical constituents

Currently sterones, saponins, flavonoids, alkaloids, sugars & organic acids make up majority of chemical components identified from *A. bidentata*. Triterpenoid saponins, sitosterol, & sigmasterol are present in roots. The main chemical components of *A. bidentata* are sterones & saponins which are recognized as main active compounds. Several active compounds from *A. bidentata*, including phytoecdysteroids &

phytosterone [11], saponins & saccharides [12], & others, have been identified in previous phytochemical research.

1.5. Biological activity

A.bidentata is used treat cold, chronic malaria & menstrual pain, fever, diarrhea, ulcers in mouth & throat, carbuncle, fractures& toothache. The Chinese Pharmacopeia (2010 edition) currently recommends & updates *A. bidentata's root* & its medical applications as a significant herbal medication. From some reports immunomodulatory properties [13–15], Inhibitory action on cardiac ischemic/reperfusion-induced injury [16,17], anticancer & antiosteoporosis [18, 19], neurotrophic & neuroprotective effects [8,20], & antiosteoporosis are just a few of its numerous pharmacological effects of *A. bidentata* were reported.

➢ ***Neuroprtoective effects of A. bidentata***

According to research, the neuroprotective effects of a peptide named bidentatide which was isolated from *A. bidentata* was reported. In this research, by the use of MS/MS and Edman degradation, the amino acid composition and disulfide connection pattern of bidentatide were determined. In order to assess the neuroactive potential of bidentatide, its effects on N-Methyl-D-aspartic acid (NMDA)-induced excitotoxicity in vitro were investigated. It was discovered that pretreatment with bidentatide protected primary cultured hippocampal neurons from NMDA-induced cell death and apoptosis via multiple mechanisms involving intracellular Ca2+inhibition, NMDA current modulation, and apoptosis-related protein expression regulation [57]. The potential anti-Parkinson's activity of *Achyranthes bidentata* polypeptides fraction K (ABPPk) was examined in a study using in vitro and in vivo PD models. In this study, researchers pretreated SHSH5Y cells and dopaminergic neurons with ABPPk, an active fraction derived from traditional Chinese medicine A. bidentata, and found that ABPPk dramatically protected cells from apoptosis triggered by MPP+. Additionally, it is discovered that ABPPk therapy greatly enhances behavioral performances in an in vivo PD mice model and inhibits TH loss in the SNpc and striatum. ABPPk significantly reduced microglia and astrocyte activation, which in turn reduced the expression of pro-inflammatory cytokines. According to these findings, ABPPk has a strong neuroprotective effect on dopaminergic neurons and may be an effective treatment for Parkinson's disease[58]. After administering ABPPk to SHSY5Y cells, researchers exposed the cells to MPP+. As anticipated, ABPPk therapy greatly prevents apoptosis in the cells. Similar to this, ABPPk significantly reduced the MPP+-induced cytotoxity in primary dopaminergic neurons. It should be emphasized that a lower dose of MPP+ was employed in primary neurons than in SHSH5Y cells.These results were in line with earlier research that shown that ABPP had strong neuroprotective effects on a variety of neurons that had either been harmed by various toxins or had cardiac ischaemia.It proved that ABPPk might boost neuronal growth in culture and encourage peripheral nerve regeneration in response to crush injury[58,59]. In this research, aerial part of *A.bidentata* will be studied by isolation & characterization of chemical compounds for evaluation of Anti-Parkinson's activity along with *in-silico* studies of isolated phytoconstituents.

Fig.1. *A.bidentata*

Fig.2. Air dried *A. bidentate*

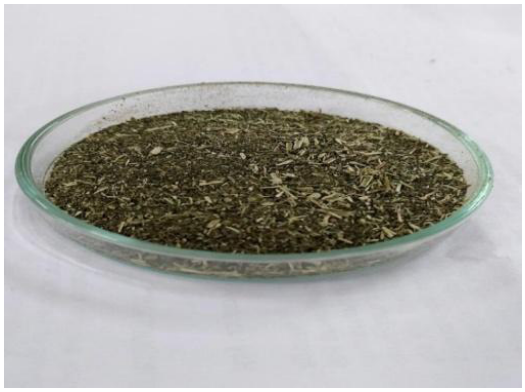


Fig.3. Grounded *A. bidentata*

The perennial or annual herb Achyranthes L. (Amaranthaceae), often known as Chaff Flower (Common Name) or by its Chinese name Niuxi , is primarily found growing on edges of forests, slopes, & riverbanks There are roughly 21 species in genus, all of which are found in tropical & subtropical areas. The two most popularly used medicinal herbs among these species, *A. bidentata*, have been utilized for thousands of years, mostly in India & China. The dried root of *A. bidentata* has been formally listed in Chinese, Japanese, & Korean Pharmacopoeias; among other components of these plants that can be used medicinally include leaves, juice, & seeds. *A. bidentata* is non-toxic for oral & external uses, as demonstrated by numerous ancient medical texts & a number of modern toxicity tests; commonly emphasized contraception in pregnancy is due to their abortion-causing abilities. As an upper herb, The liver & kidney meridians are affected by neutral plant *A. bidentata*, which is employed in TCM. It's noteworthy to note that *A. bidentata* is frequently made with salt water or yellow rice wine to boost benefits of nourishing liver, enhancing renal health, & strengthening sinews & bones. The contribution of *A.bidentata* has been crucial in folk medicine & culture of all Indian traditional medicinal systems; Ayurveda, Unani, Siddha, & homoeopathy. It is pungent, bitter, laxative, carminative & stomachic, according to Ayurveda *A.bidentata* has been utilized in southeast coastal China to increase silkworm age in addition to its usage in human health. The CFDA has also green-lit a slew of Chinese patent medicines (such as Mailuoning granule/injection/oral liquid & Danxi granule) that use *A. bidentata* as a sovereign, minister, assistant, or courier medicinal. Many older, *A. bidentata*-based recipes have been updated for modern use. Additionally, *A.bidentata* has been used all over world as active ingredients in a variety of products, including health wine, sanitary products, cream, sex pills & toothpaste. The four primary groups of bioactive substances are polypeptides, ketosteroids, triterpenoid saponin & polysaccharides. β- Ecdysterone is listed as index component for *A. bidentata's* quality control in Chinese Pharmacopoeia. [23]

2. MATERIALS & METHODS

A. Collection of plant

The aerial part of *A. bidentata* was gathered from campus of CSIR IHBT, Palampur, Himachal Pradesh, India & was authenticated by Department taxonomist of CSIR IHBT, Palampur (H.P)[40].

B. Extraction

Shade dried & powdered aerial part of *A.bidentata* (1.6 kg) was extracted with ethanol[41]: water (80:20 v/v) by percolation (24 h x 3).The percolate was dried with help of rotary evaporator to yield 960 g crude extract.

C. Partitioning

The partitioning of crude extract (960 g) was carried out by using *n*-hexane[42], ethyl acetate, *n*-butanol & water to yield fractions of 321.3 g, 315.1 g, 315.7 g & 5.8 g respectively.

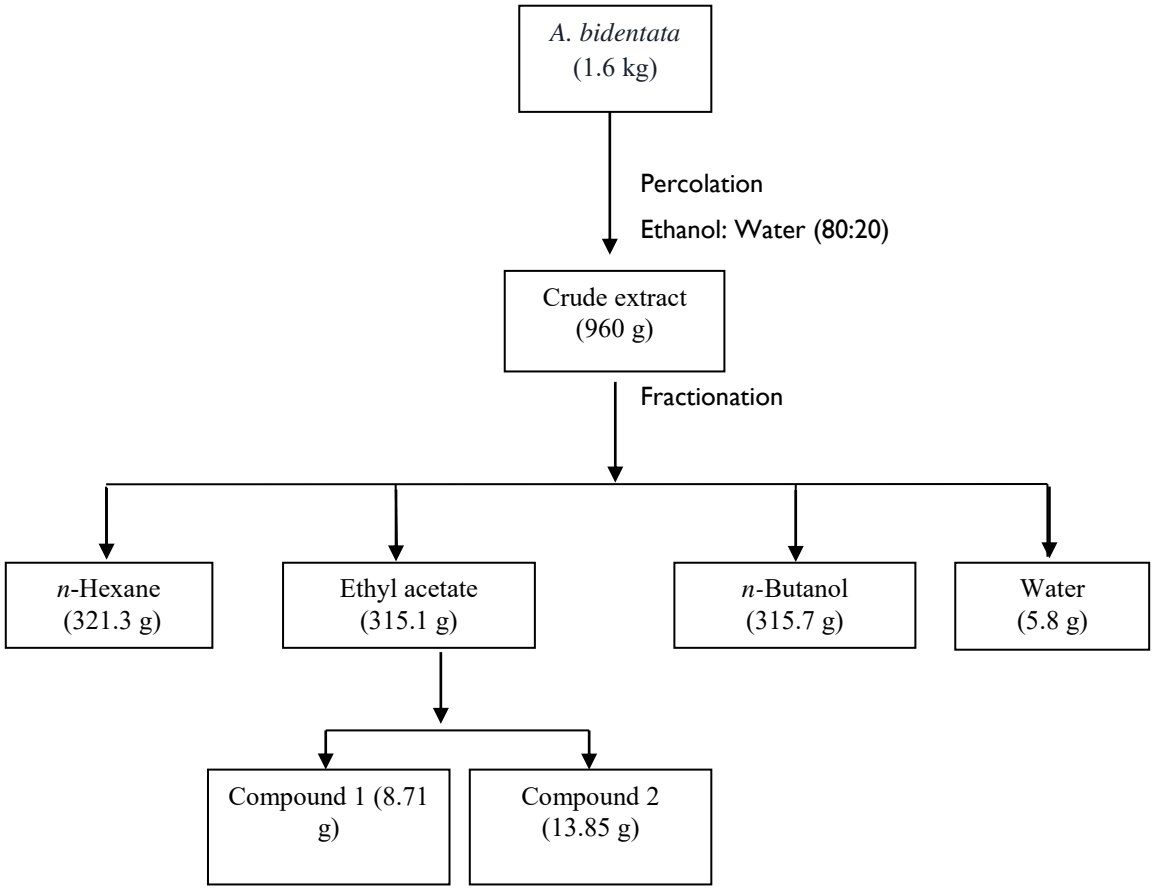


Chart-1: Flow chart of Extraction & Isolation process

D. Preliminary phytochemical studies

(Table no.1)- An important step in identifying chemical components of plant materials is preliminary phytochemical screening of plants. The ethyl acetate extract was used for the preliminary phytochemical studies.The following types of preliminary tests were conducted-

- ***Test's for saponins***[43]

After rapidly shaking 0.25 g of crude plant extract with 20 ml of distilled water, presence of saponins was revealed by formation of foam (Fig.4.a.a).

- ***Test's for phenols***[44]

The ferric chloride test involved adding 0.25 g of crude extract to 4 drops of $FeCl_3$ to create a blue-black colour that indicated presence of phenols (Fig.4.b.).

- ***Test's for flavonoids***[45]

Alkaline reagent testing revealed existence of flavonoids by seeing production of a bright yellow colour in crude extract, which then became clear with addition of only a few drops of NaOH solution ((Fig.4.c.).

- ***Test's for terpenoids***[46]

The crude extract mixed with chloroform & conc. sulphuric acid, which leads to form a layer. The presence of terpenoids was shown by formation of a reddish brown colour(Fig.4.d.).

- ***Test's for alkaloids***[47]

By following procedure of Dragendroff's test, addition of 1 ml of dragendroff's reagent to 2 ml of crude extract, an orange red precipitate was formed, indicating presence of alkaloids (Fig.4.e.).

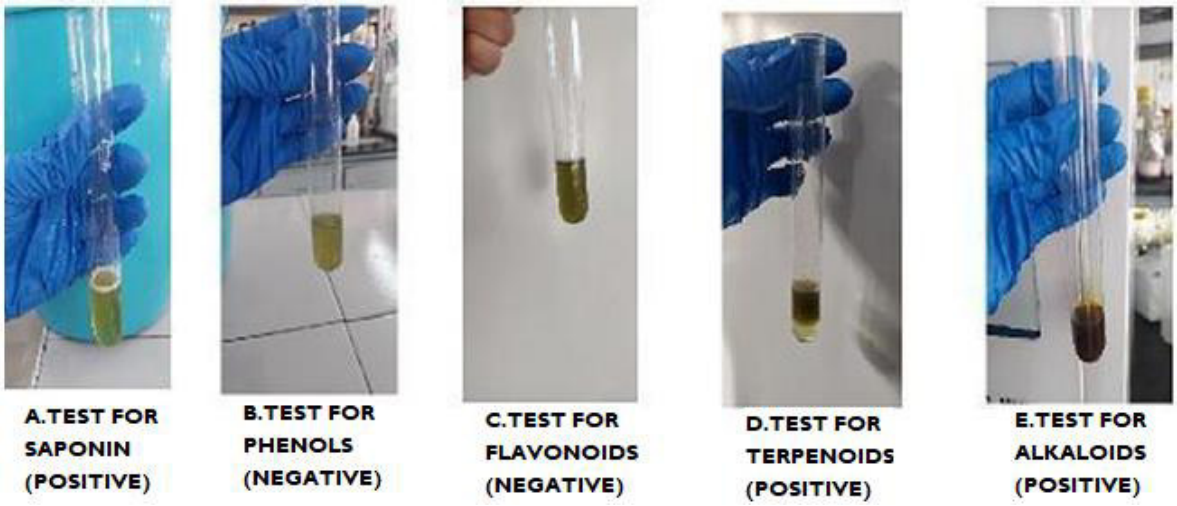


Fig.4. Preliminary Phytochemical tests

These tests are used to identify the presence of certain compounds in plants. They can help determine the medicinal value of plants and provide information on the active components. They are also used to detect the presence of toxic compounds. By running these tests, researchers can gain a better understanding of the potential benefits and dangers of plants, enabling them to make informed decisions about their use. Through these tests, researchers can gain an insight into the properties of plants, and accurately assess their medicinal potential and potential toxicity. This knowledge can then be used to make informed decisions about the use of plants for therapeutic and other purposes.

Table 1: Preliminary Phytochemical tests

Types of test's	Results
Saponin	Positive
Phenols (Ferric chloride test)	Negative
Flavonoids	Negative
Terpenoids	Positive
Alkaloids (Dragendroff's tests)	Positive

E. Isolation of compounds from A.bidentata[48]

Column chromatography is a scientific method for separating & purifying chemical compounds from a mixture of constituents. The phenomenon of differential adsorption of chemicals to adsorbent serves as foundation for separation of constituent. The key benefits of column chromatography are stationary phase's disposability & comparatively low cost. The stationary phase in chromatography is a column adsorbent (such as silica or alumina), & mobile phase is a liquid eluent. Formerly, ethyl acetate fraction was isolated using ethyl acetate: *n*-hexane [0:100 to 100:0] as mobile phase to obtain different fractions according to their polarity. Further fractions were mixed on basis of TLC profile & dried.

❖ ***Selection of Polar Phase and Fraction for Isolation***

The selection of polar phase and fraction for isolation was done by optimizing the resolution on TLC. It was found that the ethyl acetate fraction was showing the best resolution which shows that when ethyl acetate is taken as a polar phase the more separation was taking place.

Fig.5. Column Chromatography

Column Chromatography is a preparative technique used to purify compounds depending on their polarity or hydrophobicity. In column chromatography, a mixture of molecules is separated based on their differentials partitioning between a mobile phase and a stationary phase. The stationary phase is usually a solid support, such as silica, onto which different molecules in the mixture are adsorbed. The mobile phase is usually a liquid, such as water, which passes through the column and interacts with the stationary phase. The different molecules in the mixture will interact differently with the stationary phase and will move through the column at different rates, leading to separation.

F. Characterization

➢ ***Thin layer chromatography[49]***

It is an analytical method to identify phytoconstituents found in plant material or plant extracts.

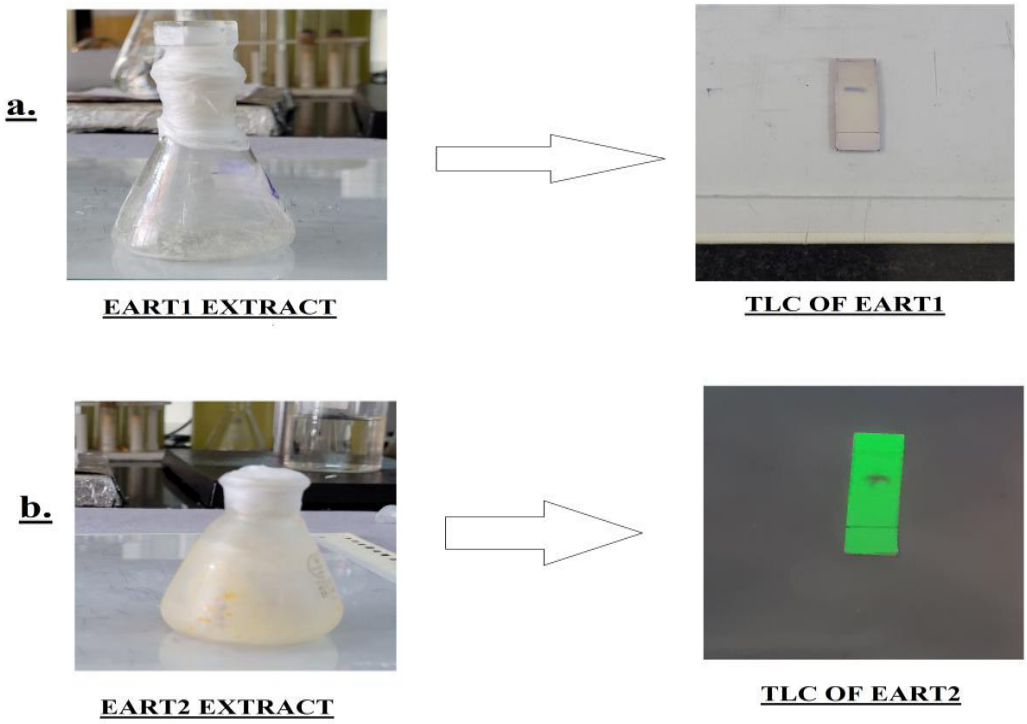


Fig.6. TLC

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel. The sample is then applied to the adsorbent material, and the mixture is passed over the surface of the adsorbent material. As the mixture moves across the surface, the components separate because of their different affinities for the adsorbent material. This allows for the individual components of the mixture to be isolated. The process of separation is known as chromatography, and the isolated components can be collected and further analyzed.

➢ ***UPLC [Ultra high pressure liquid chromatography][50]***

UPLC, a type of column chromatography used to identify, quantify & separate compounds. It enables quick & efficient separation & analysis of analytes. (Fig.9.))

➢ ***UPLC sample preparation***

The obtained crude extract & fractions of ethyl acetate were dissolved (1 mg) separately in methanol (1 ml) & filtered through a 0.22 µm syringe filter & subjected to UPLC analysis.

➢ ***UPLC Conditions***

The Waters Acquity UPLC system (Milford, MA, USA) was used for chromatographic analysis, & a BEH C18 column (2.1 100 mm, 1.7 m) was used with a flow rate of 0.30 mL/min. 1 µL was injection volume. At 344 nm, absorbance was measured. Solvent A (0.1% formic acid in water) & Solvent B (acetonitrile) made up mobile phase.

➢ The isolated compounds were analyzed & retention time (RT) of EART1 & EART2 was 13.733 & 2.087 minutes respectively.

➢ ***Mass spectrometry[51]***

With help of analytical method of mass spectrometry, chemical compounds are according to their *mass-to-charge* ratios.(Fig.10.). The molecular mass of "EART1" & "EART2" was found to be 455.4 $[M-H]^-$ & 127.1 $[M+H]^+$ respectively.

➢ ***Nuclear magnetic resonance (NMR) spectroscopy[52]***

NMR spectroscopy is utilised in research to determine a sample's contents & purity as well as its molecular structure. (Fig. 7 &8.)

➢ ***In-silico Studies***

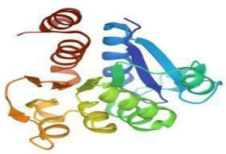
i.Computational tools & database source (ligands & proteins)[53]

Auto Dock Vina (version 1.1.2) was used to conduct this investigation. The PDB data base held appropriate ligands. In Auto Dock Vina, these molecules were changed into three-dimensional structures. The protein data bank was used to retrieve DJ-1/Rs protein molecules.

ii.Protein preparation[54]

Using PDB database, protein structure for docking was created. Numerous physiological processes; Parkinson's disease, cancer, & male fertility, are influenced by a protein known as DJ-1. DJ-1/Rs is a helix-strand-helix sandwich that forms a dimer, according to crystal structure of DJ-1/Rs at 1.6 resolution. The R-Value Free is 0.202 & R-Value Work is 0.191 at 1.6 Å resolutions. (Table no.2)

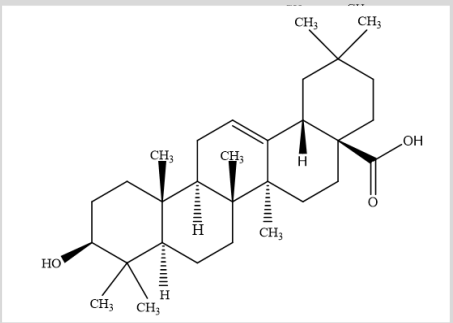
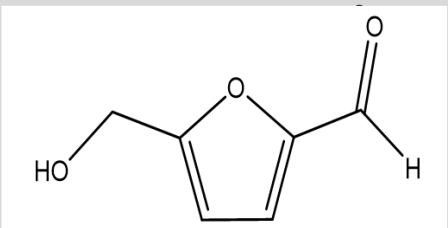
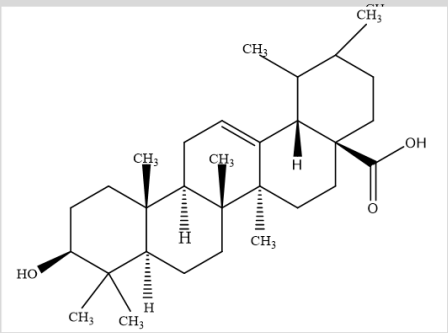
Table 2 : PDB database

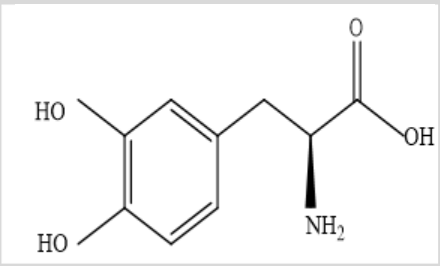
Name of the Target	PDBID	Structure	Reference
Dj-1/RS	1Q2U		19

iii.Ligand preparation[55]

The PubChem database was used to prepare ligand. With goal of generating structures from both 1D (Smiles) & 2D (SDF) representations, a number of tools have been combined & drawn ligands were converted to SDF format in Chemdraw professionals. (Table no.3)

Table 3: PubChem Database

Name of the Ligands	Pubchem Cid	Structure	Smile Format
Oleanolic Acid	10494		CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C(=O)O)C
5-Hydroxymethylfurfural	237332		C1=C(OC(=C1)C=O)CO
Ursolic Acid	64945		CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O

Levodopa	6047		C1=CC(=C(C=C1CC(C(=O)O)N)O)O

iv.Molecular docking & binding evaluation[56]

A docking study was carried out through Auto Dock VINA 1.1.2. The ligand was able to attach to target proteins' active sites thanks to system's automation. A flexible ligand was docked into an enzyme with a stiff active site. The energy-minimized target ligand is what kicks off docking process. The MOL file containing molecular structure was created using Chem draw programme. After importing acquired molecule into software, its energy was optimised. The molecule with stable conformation was allowed to dock in active site. Biovia discovery studio visualize 2021 was used to visualize molecular docking results & thus through docking score observation, antiparkinson's activity of compound can be studied. The final docking results were represented in Kcal/mol. H-bonding or hydrophobic interaction of docked ligand having amino acid residues of active site was utilised to interpret complex produced by Oleanolic acid & 5-hydroxymethylfurfural and chosen targets. (Table no. 4)

Table 4: Molecular property

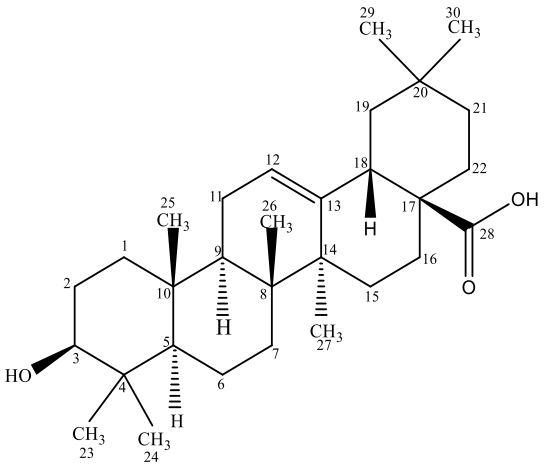
Descriptor	Value(oleanolic acid)	Value(5-hydroxymethylfurfural)
Molecular weight	456.11	126.11
LogP	7.2336	0.5844
Rotational Bonds	1	2
Acceptors	2	3
Donors	2	1
Surface Area	201.354	51.911

Clinical growth of novel biologically active compounds relies heavily on pharmacokinetic & cell membrane permeability investigations to characterise compounds' in vivo behaviour & determine most effective dosing strategy. Oleanolic acid is a pentacyclic triterpenoid formed by adding a -hydroxy group to third position of olean-12-en-28-oic acid. It has a role as a plant metabolite. It is a pentacyclic triterpenoid & a hydroxy monocarboxylic acid. It has molecular wt. - 456.11g/mol, & has Log P value 7.2336. Oleanolic acid is soluble in organic solvents such as ethanol, DMSO & DMF. The solubility of oleanolic acid in these solvents is approximately 5, 3 & 30mg/ml, respectively. Oleanolic acid is sparingly soluble in aqueous buffers. In a research it is found that OA significantly reduced permeability of BBB & relieved brain edema by increasing protein expression of TJs & AJs, & decreased SAH grades by increasing protein expression of heme oxygenase-1 (HO-1) in SAH rats [20]. 5-hydroxymethylfurfural is a member of class of furans that is furan which is substituted at positions 2 & 5 by formyl & hydroxymethyl substituents, respectively. Virtually absent from fresh foods, it is naturally generated in sugar-containing foods during storage, & especially by drying or cooking. It has molecular weight of 126.11 g/mol, & has LogP value 0.5844 which predicts drug likeliness of compound for good absorption. It is soluble in water, alcohol, ethyl acetate, acetone, dimethylformamide, benzene, ether, carbon tetrachloride, chloroform & other conventional solvent.

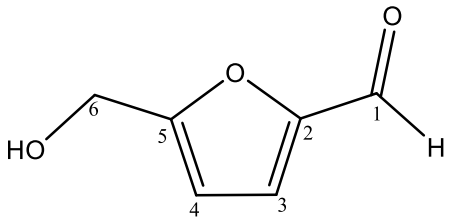
3. RESULTS & DISCUSSIONS

From aerial part of *A.bidentata,* two single compounds were isolated. Column chromatography of ethyl acetate fraction yielded these two single compounds from different fractions of concentration 25 % & 35 % EtOAC/hexane & they were named "EART1" & "EART2" respectively.[24,25] "EART1" was found to be white & UV-inactive & was visualized under Vanillin–sulphuric acid spraying reagent. [26] It is believed to be a triterpene saponin glycoside that can be isolated from the fruits of the plant species *Erythronium americanum.* [27] The saponin glycoside was also found to have antifungal and anti-inflammatory properties. [28] Tests of the compound revealed it to be a triterpene saponin glycoside, meaning it is composed of three-carbon molecules linked together in a special pattern. [29]This type of molecule is commonly found in plants and is known to possess antifungal and anti-inflammatory properties. The compound was isolated from the fruits of the plant species Erythronium americanum, giving it its name "EART1". [30] It was also found to be white and UV-inactive, meaning it does not absorb light at ultraviolet frequencies, which is why it is visible under Vanillin–sulphuric acid spraying reagent. [31-33]. "EART2" was found to be orange & showed single spot under UV. These single compounds were then further investigated by UPLC, NMR spectroscopic & mass spectrometry methods. [34] The chemical structure of "EART2" was determined to be a new compound. The synthesis of "EART2" was then successfully completed. Finally, the anti-inflammatory and anti-bacterial activity of "EART2" was evaluated. [35]The UPLC, NMR spectroscopy, and mass spectrometry methods helped to further investigate the single compounds and determine their chemical structure. Isolated compounds' structures were elucidated by ^{1}H NMR, ^{13}C NMR, mass spectrometry, UPLC & characterised as oleanolic acid & 5-hydroxymethylfurfural (Fig.10.). *In-silico* molecular studies performed with isolated compound for potential of anti-Parkinson's activity. The docking parameters were determined by interacting phytocompounds & commercial medicines with target. [36] The results showed that the isolated compound was able to interact with the target, suggesting potential anti-Parkinson's activity. Further studies are needed to evaluate the efficacy of

this compound. [37]The findings also offer promise in the search for new treatments for Parkinson's disease. To further validate the efficacy of the compound, the researchers conducted in vitro experiments and animal studies. [38]The results showed that the compound was able to inhibit the activity of the target, implying that it may be effective in treating Parkinson's disease. Moreover, the findings suggest that the compound could be used as a lead compound for the development of new, more effective treatments for Parkinson's disease. [39]



Compound 1 (EART1)



Compound 2 (EART2)

Table 5: ^{1}H & ^{13}C NMR (400 MHz) data of Compound 1

C/H	δH	δC
1	0.97, 1.62	38.54
2	1.35, 1.61	27.3
3	3.22	79.19
4	---	38.9
5	0.74	55.37
6	1.54, 1.37	18.44
7	1.77, 1.28	32.75
8	---	38.9
9	1.55	47.78
10	---	37.24
11	1.97, 1.62	23.05
12	5.29	122.7
13	---	143.7
14	---	41.73
15	1.72, 1.08	27.83
16	1.88, 1.61	23.54
17	---	46.68
18	2.82	41.11
19	1.62, 1.15	46.02
20	---	30.82
21	1.35, 1.22	33.95
22	1.59, 1.30	32.59
23	0.77	15.68
24	0.99	28.24
25	0.91	15.46
26	0.75	17.29
27	1.13	26.09

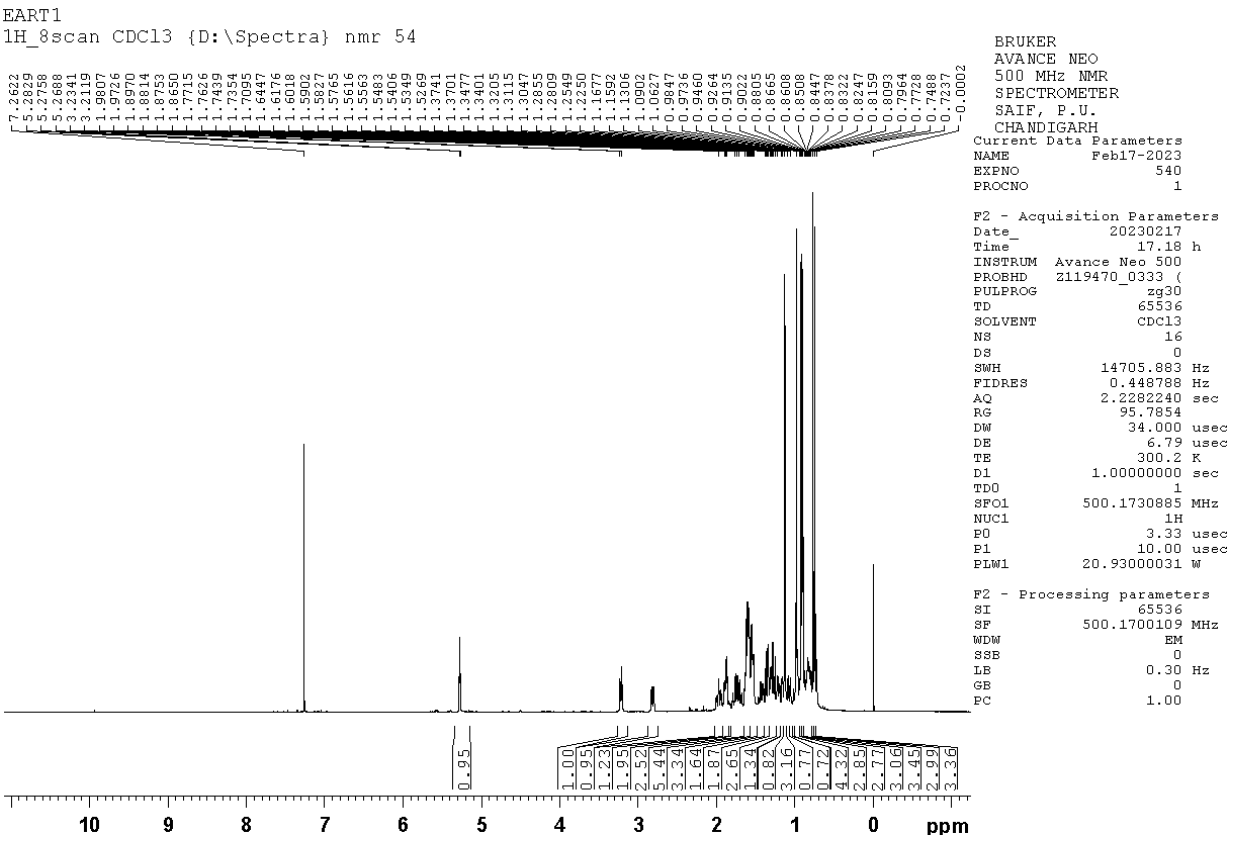
28	180	183.6
29	0.92	23.72
30	0.90	33.21

The spectrum of OA's ^{1}H-NMR was shown in Table 5. demonstrates a number of tertiary methyl groups at positions δ0.77, 0.99, 0.91, 0.75, 1.13, 0.92, and 0.90, as well as a distinctive olefinic proton of a C12-C13 double-bonded pentacyclic triterpenoid at position δ5.29 (1H, brs, H-12). This suggests that the skeleton is olea-12-ene. At least one hydroxyl group on the OA olean-12-ene skeleton is compatible with one methine proton at δ2.82 (1H, t, J = 8.2 Hz, 3H). On the other hand, the ^{13}C-NMR spectrum displays signals related to an oxygenated carbon signal at δ79.19(C-3), one tri-substituted double bond at δ122.7(C-12) and 143.7 (C-13) and one carboxyl group at δ183.6 (C-28). Further, 13C-NMR signals from C-18 to C-22 at δ(41.11 (C-18), 46.02 (C-19), 30.82 (C-20), 33.95 (C-21) and 32.59 (C-22)) indicates that OA derives from the oleanyl carbocation.

Table 6- ^{1}H & ^{13}C NMR (400 MHz) data of Compound 2 (EART2)

C/H	δH	δC
1	9.5	177 (1)
2	---	152
3	7.4	123 (3)
4	6.5	110
5	---	161
6	4.6	57

^{1}H NMR (400 MHz) δ: 9.5 (s, 1H, H-6), 7.4 (d, J=3.5 Hz, 1H, H-3), 6.5 (d, J=3.5 Hz, 1H, H-2), 4.5 (s, 2H, H-5). ^{13}C NMR (δ: 178.44 (s, C-6), 162.64 (s, C-1), 152.19 (s, C-4), 124.91 (s, C-3), 110.15 (s, C-2), 56.40 (s, C-5).



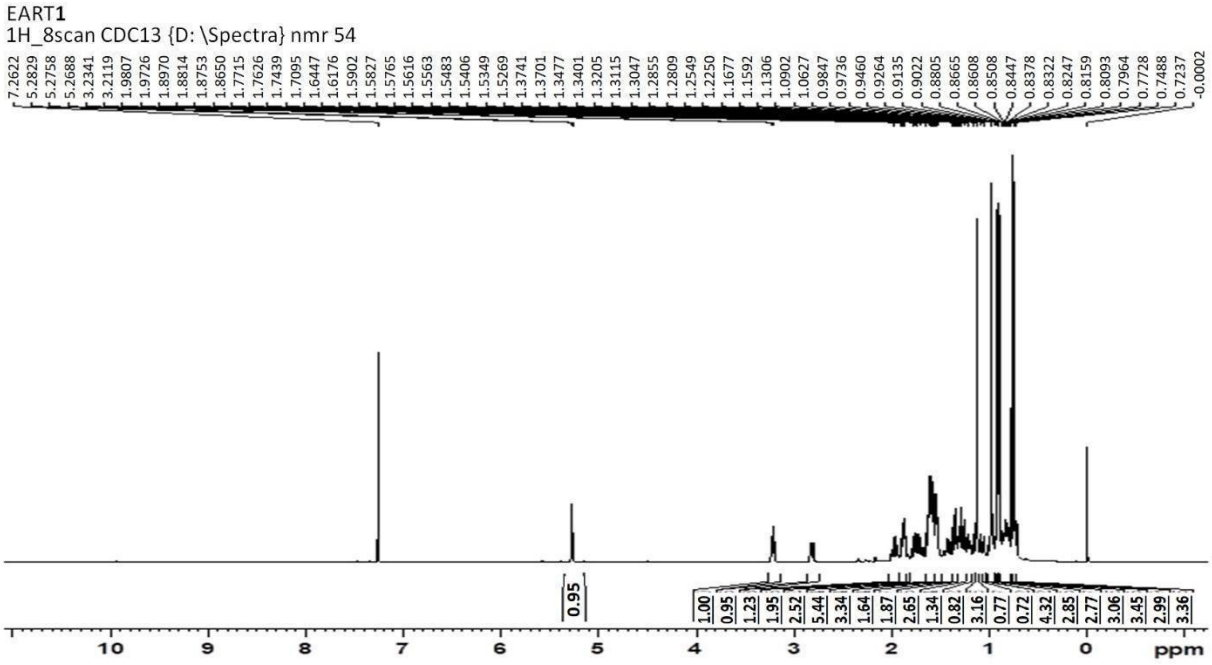


Fig 7a: Compound 1's ^{1}H NMR spectra (EART1)

Compound 1's H NMR spectra (EART1) It is an analytical technique used to identify and quantify the structure of molecules. It provides information on the types of atoms present in a molecule, as well as their relative positions in the molecule. The technique is used to study the properties of compounds, such as their reactivity and stability. H NMR spectra (EART1) is a powerful tool, allowing researchers to unlock the secrets of the molecular world and gain insights into the behavior of chemicals and their interactions with each other.

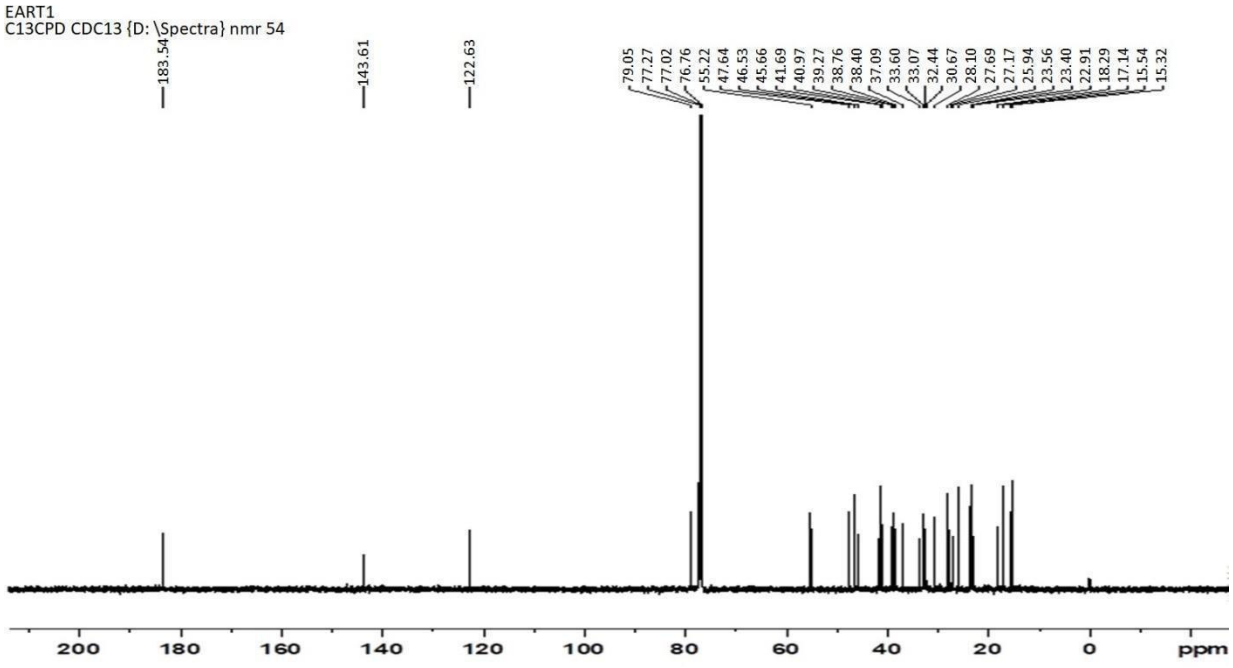


Fig.7.b: Compound 1's ^{13}C NMR spectra (EART1)

Compound 1's ^{13}C NMR spectra (EART1) The spectrum consists of multiple peaks that represent the different chemical environments of the nuclei present. The peak positions and intensities can be used to identify the different atomic environments in a molecule. The spectrum also provides an insight into the structure of the molecule. By understanding the chemical environments and structure of a molecule, C NMR spectra can be used to gain valuable insight into its properties and behavior.

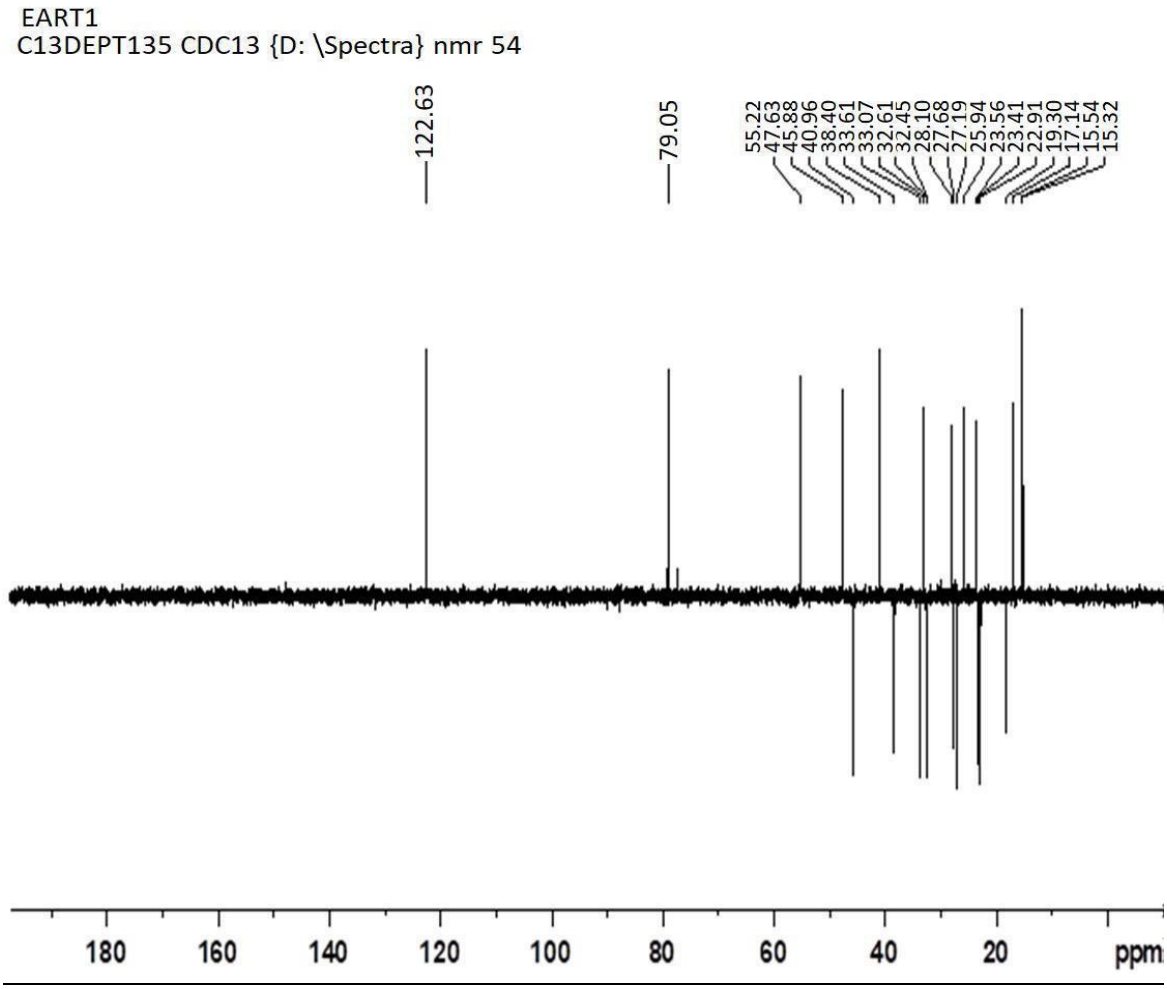


Fig.7.c: [13]C-DEPT NMR spectra of compound 1 (EART1)

[13]C-DEPT NMR spectra of compound 1 (EART1) The 1H and 13C NMR spectra of EART1 showed the presence of aromatic and aliphatic protons and carbons. The spectrum also showed the presence of an oxygenated functional group. The chemical shifts of the protons and carbons were consistent with the proposed structure of EART1. Furthermore, C-DEPT analysis confirmed that EART1 has a unique connectivity between the aromatic and aliphatic protons and carbons, as well as the oxygenated functional group.

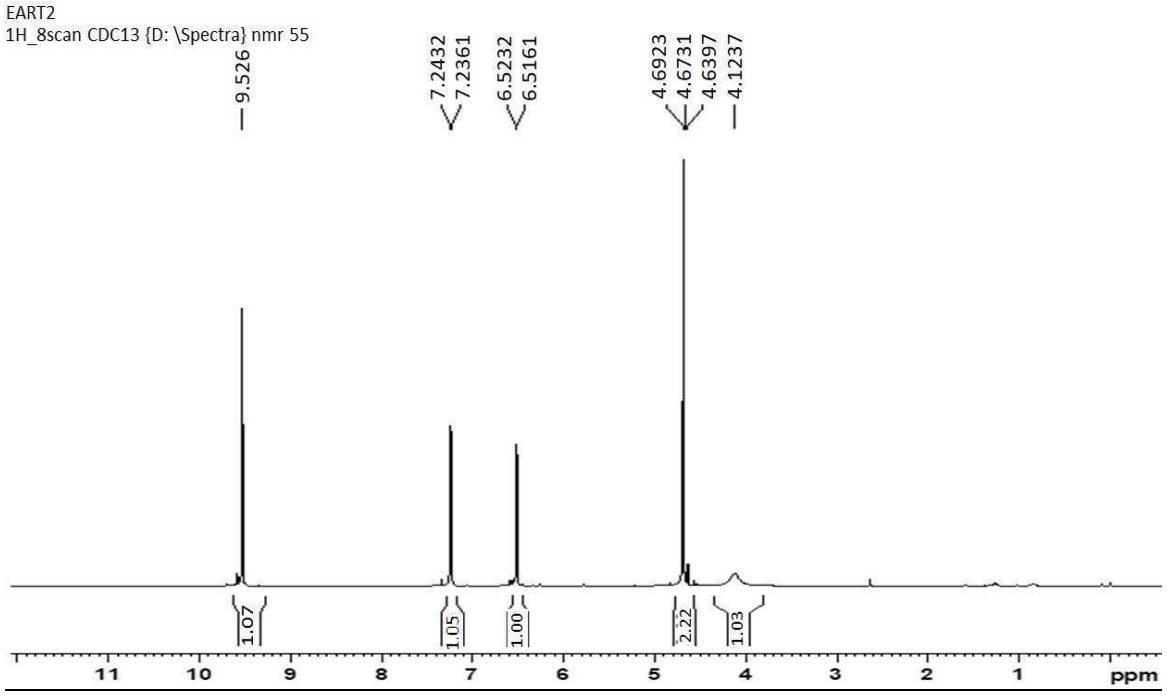


Fig.8.a: [1]H NMR spectra of compound 2 (EART2)

The compound exhibited a single peak at 7.2 ppm, indicating a single proton on the molecule. The 1H NMR spectra of compound 2 (EART2) showed vinylic protons at 6.1 and 6.3 ppm, methoxy protons at 3.8 ppm, allylic protons at 1.8 ppm, and a methyl proton at 1ppm. The two aromatic protons appeared in the region between 6.8 and 7.2 ppm. The spectrum also revealed that the molecules have five atoms and one double bond, suggesting a 1,3-diphenyl-2-propene structure. This structure was further confirmed by comparison of the 1H NMR spectrum of compound 2 (EART2) to an authentic sample of 1,3-diphenyl-2-propene, verifying the compound's identity.

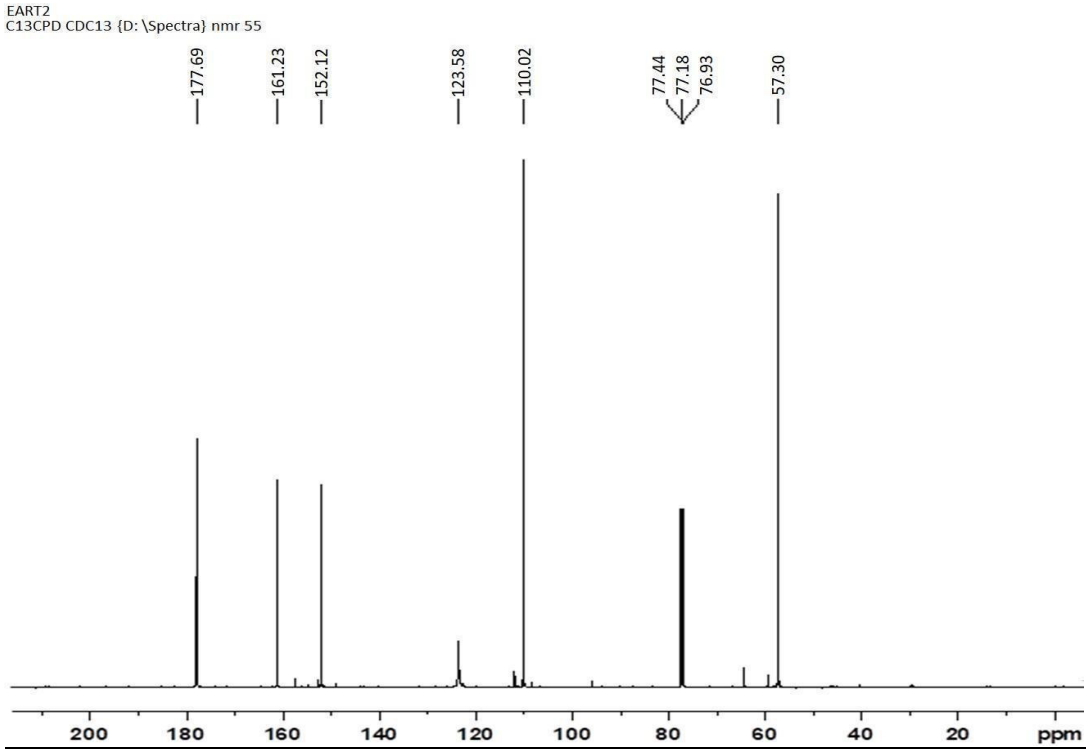


Fig.8.b: ^{13}C NMR spectra of compound 2 (EART2)

The spectrum showed the presence of two methyl groups and two methoxy groups. The spectrum also showed a quaternary carbon atom, indicating that the structure was a tertiary amine. A peak at 175 ppm confirmed the presence of an aromatic ring. This suggests that compound 2 (EART2) is a tertiary amine with two methyl groups, two methoxy groups, and an aromatic ring.

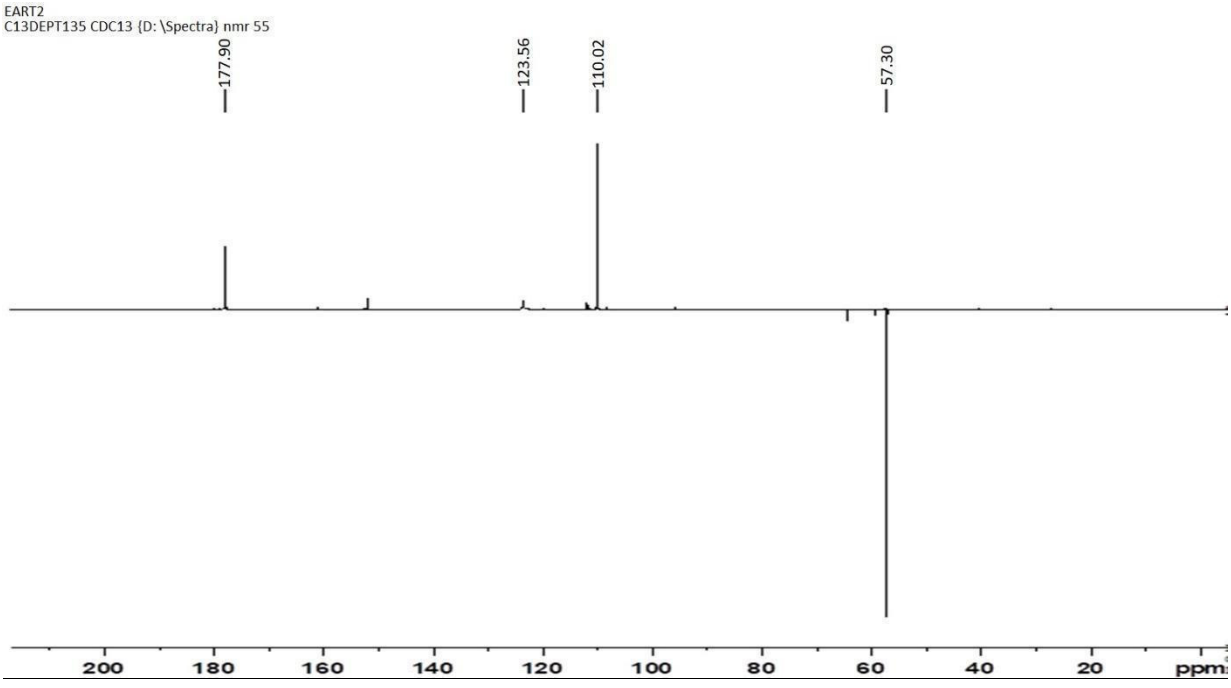
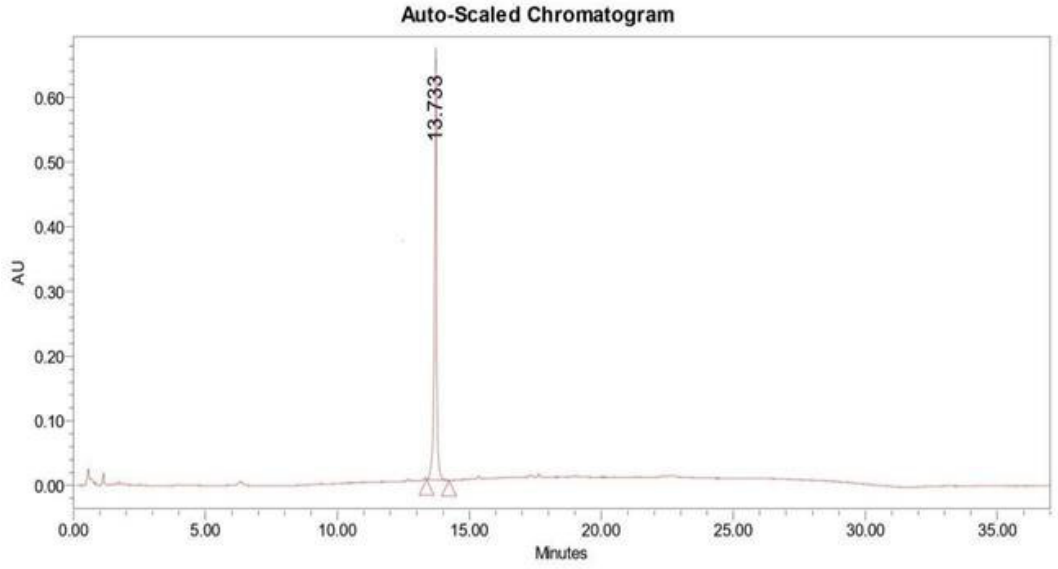


Fig.8.c: ^{13}C-DEPT NMR spectra of compound 2 (EART2)

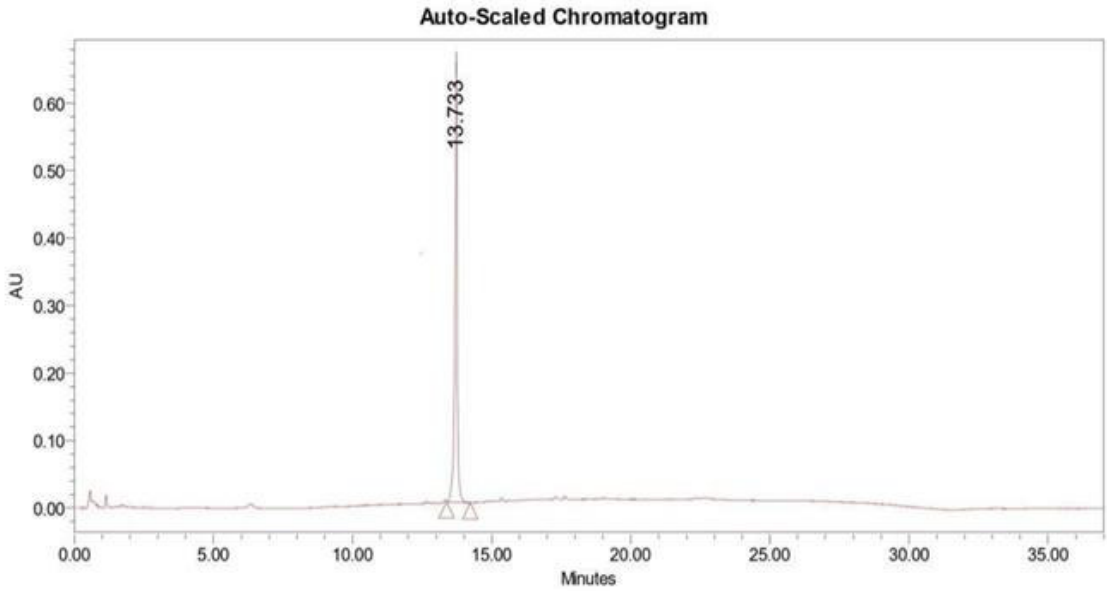
The spectrum showed a well-resolved proton-coupled DEPT signal pattern with the amide proton and methylene protons of the pyrrolidine ring at chemical shifts of 2.83 and 2.48 ppm, respectively. The carbonyl carbons resonated at 171.1 ppm, indicating the presence of an ester group. This is supported by the observation that the chemical shift of the carbonyl carbon is shifted

downfield compared to a carbonyl carbon of an amide, which usually resonates around 170 ppm. Additionally, the proton-coupled DEPT signal pattern is consistent with the presence of an ester group, since the amide proton and methylene protons of the pyrrolidine ring are well-resolved. These results suggest that the compound is most likely an ester, rather than an amide.



Peak Results

	Name	RT	Area	Height	Amount	Units
1		13.733	3775683	652581		



Peak Results

	Name	RT	Area	Height	Amount	Units
1		13.733	3775683	652581		

Fig.9.a: UPLC chromatogram of compound 1(EART1)

The compound was detected at 13.733 min with a peak area of 0.02%. It was identified as EART1 by comparison of retention time and mass spectra to the reference standard. The peak purity was found to be 99.9%. The peak had a very high purity, indicating that its identity as EART1 was reliable and confirmed.

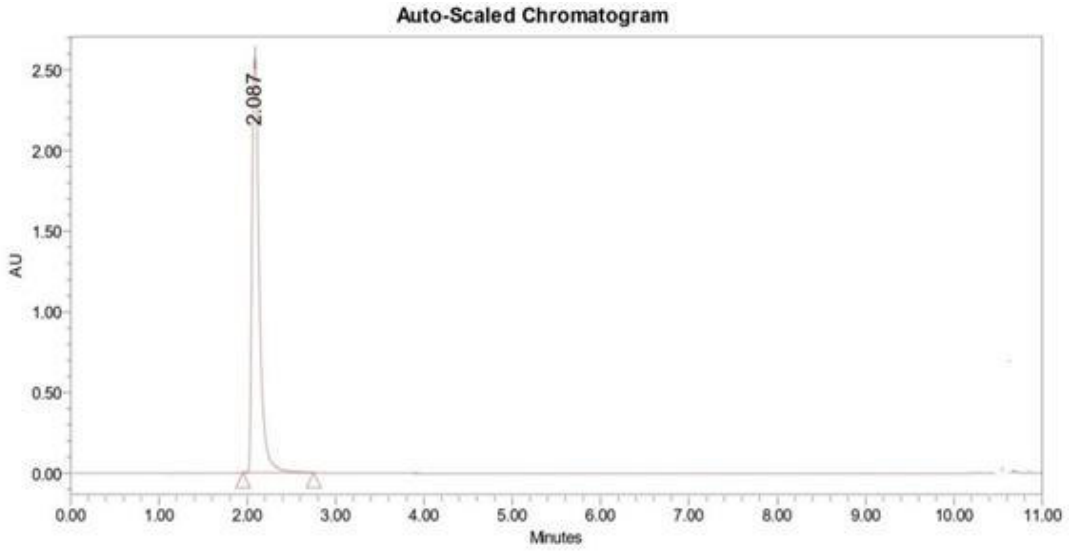


Fig.9.b: UPLC chromatogram of compound 2(EART2)

The Compound 2 showed a retention time of 3.6 minutes. Compound 2 was identified by its mass spectral fragment ions at m/z 186.1 and 149.1. The compound was also confirmed by its ^{1}H-NMR spectrum. The retention time of 2.087 minutes for Compound 2 was consistent with the retention times of its derivatives, indicating that it is the same compound. Additionally, its mass spectral fragment ions at m/z 186.1 and 149.1 were consistent with those of its derivatives, indicating that it is the same compound. The ^{1}H-NMR spectrum was also consistent with the spectrum of its derivatives, further confirming that Compound 2 is the same compound.

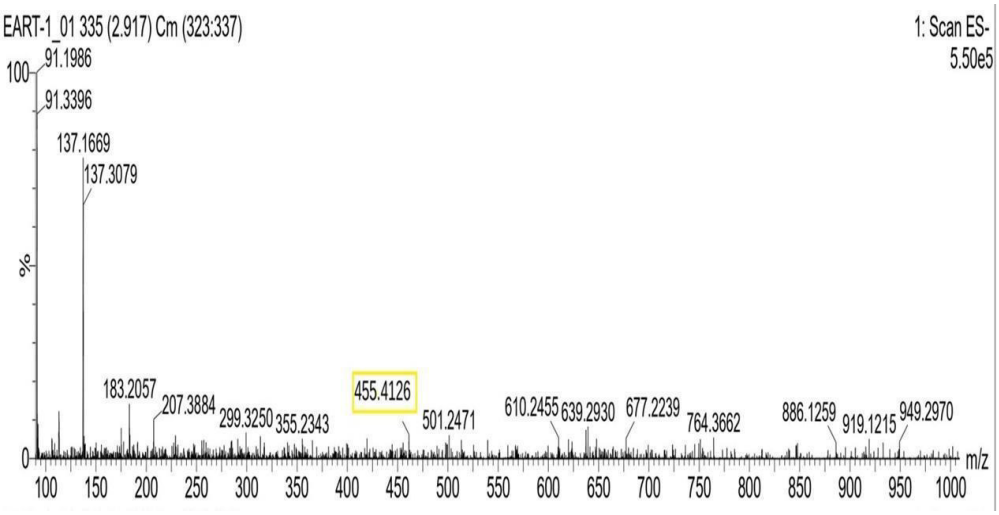


Fig.10.a: Compound 1's ESI-MS spectrum (EART1)

It showed a peak between m/z 300-, suggesting the presence of the desired product. The product's elemental composition was further confirmed by a high-resolution ESI-MS spectrum (EART2). The product's purity was assessed to be greater than 95%. The high-resolution ESI-MS spectrum (EART2) showed a clear peak corresponding to the expected elemental composition, confirming the presence of the product with a purity of greater than 95%.

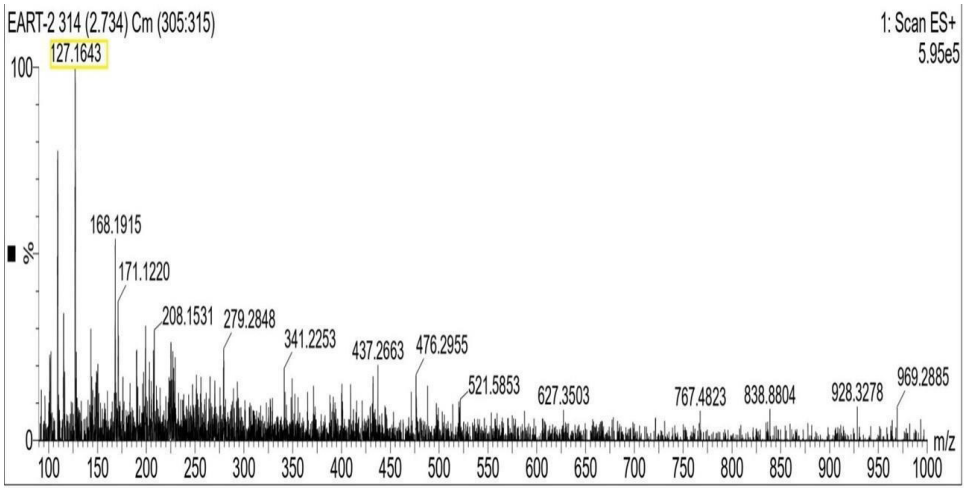
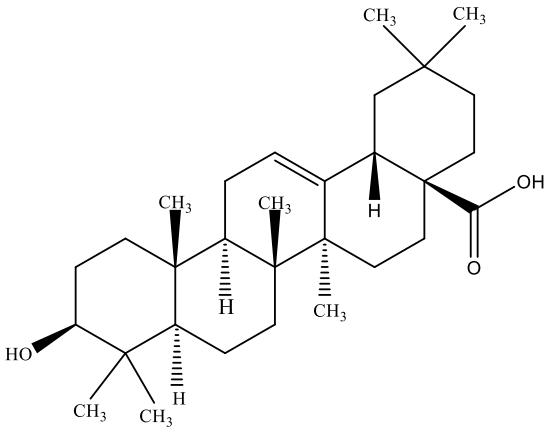


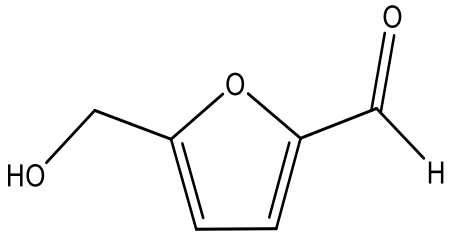
Fig.10.b: Compound 2's ESI-MS spectrum (EART2)

This indicates it is an organic compound with molecular weight of127.1 $[M+H]^+$. The fragmentation pattern is consistent with a molecular structure with two tertiary amines, two esters, and two ethers, indicating that it is an ester-containing polyether. This structure is further confirmed by the detection of an alkene, a phenyl ring, and a terminal alkane, suggesting that Compound 2 is a complex organic polymer with a molecular architecture not previously observed.

- ***Chemical structures of isolated compound***



A. Oleanolic acid (EART1)



B. 5-hydroxymethyl furfural (EART2)

Fig.10: Chemical structure of isolated compound

3. ADMET STUDIES

In order to discover molecules with drug potential, molecular docking & ADMET analysis are crucial. The lowest affinity score between molecular interactions between ligand & receptor complex structure is produced via molecular docking. Drug candidates' distribution, absorption, metabolism, elimination, and toxicity may all be evaluated with use of ADMET analysis assays (Table no. 6 & 7). An ADMET research, which stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity, evaluates the pharmacokinetics of a medicine. Predicting how a drug will be used and its effects on the body, such as how much will be taken orally and how much will be absorbed in the digestive system, is a crucial step in the development of new drugs. It could cause neurotoxicity and nephrotoxicity, much like how insufficient absorption would affect distribution and metabolism. The aim of the study is to understand the behavior of a drug molecule inside an organism. Consequently, ADMET research is among the most important elements of computational drug design.

Table no.6: Drug likeliness of Compound 1 & 2[21,22]

Property	Model name	Predicted value of Oleanolic acid	Predicted value of 5-Hydroxy methyl furfural	Unit
Absorption	Water solubility	-3.074	-0.59	Numeric (log mol/L)
Absorption	$CaCO_2$ permeability	1.17	1.172	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption(human)	99.931	95.848	Numeric(% Absorbed)
Absorption	Skin Permeability	-2.735	-3.416	Numeric (log P)
Absorption	P-glycoprotein Substrate	No	No	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	No	No	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	No	No	Categorical (Yes/No)
Distribution	VDss (human)	-1.085	-0.146	Numeric(log L/Kg)
Distribution	Fraction unbound (human)	0	0.744	Numeric (Fu)
Distribution	BBB permeability	-0.14	-0.361	Numeric (log BB)
Distribution	CNS permeability	-1.157	-2.914	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	Yes	No	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitor	No	No	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitor	No	No	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitor	No	No	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitor	No	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitor	No	No	Categorical (Yes/No)
Excretion	Total clearance	-0.081	0.614	Numeric (logml/min/Kg)
Excretion	Renal OCT2 substrate	No	No	Categorical (Yes/No)

Table no.7: Toxicity results of Compound 1 & 2 [21, 22]

	Model name	Predicted value of Oleanolic acid	Predicted value of 5-Hydroxy methyl furfural	Unit
Property	AMES toxicity	No	No	Categorical (Yes/No)
	Max. tolerated dose(human)	0.203	0.77	Numeric (log mg/kg/day)
	hERG I inhibitor	No	No	Categorical (Yes/No)
	hERG II inhibitor	No	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.349	2.283	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	2.085	2.488	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	Yes	No	Categorical (Yes/No)
	Skin Sensitisation	No	No	Categorical (Yes/No)
	T.Pyriformis toxicity	0.285	-0.767	Numeric (log ug/L)
	Minnow toxicity	-0.823	2.836	Numeric(log mM)

4. OUTCOMES FROM ADMET STUDIES

The molecular weight of Oleanolic acid is 456.70g/mol. It has Log P value 7.2336. Oleanolic acid is soluble in organic solvents such as ethanol, DMSO & DMF. The solubility of oleanolic acid in these solvents is approximately 5, 3 & 30mg/ml, respectively. Oleanolic acid is sparingly soluble in aqueous buffers. It has 2 H-bond donors and 3 H-bond acceptors. The molar refractivity of OA is 136.65. So as per the Lipinski's rule of five, OA shows 75% compliance with LogP being the only term that reaches a value above the permissible value (greater than 5), while the other four rule comply adequately. Regarding absorption properties, oleanolic acid has good absorption in the intestine and the BBB. 5-hydroxymethylfurfural has molecular weight of 126.11 g/mol, & has LogP value 0.5844. It is soluble in benzene, ether, carbon tetrachloride, water, alcohol, ethyl acetate, acetone, dimethylformamide, chloroform, and other common solvents. It has one H-bond donor and one H-bond acceptor. So as per the Lipinski's rule of five, 5-hydroxymethylfurfural shows 100% compliance, which predicts drug likeliness of compound for good absorption.

5.1. Docking Interaction of Oleanolic acid & 5-Hydroxymethyl furfural against Dj-1/RS (Table no. 8))

Levodopa is a popular medication used to treat Parkinson's disease, while numerous studies have also shown that ursolic acid has anti-Parkinson's effects [64,65]. For the purpose of determining the anti-parkinson's activity of isolated

compounds, levodopa and ursolic acid are utilized as a standard compound for comparison.

➢ ***Ursolic acid***

Ursolic acid exhibits docking score of -8.0kcal/mol. Here in interaction if Ursolic acid against Dj-1/RS it shows van der walls interaction with various amino acid residues like LEU166(A), ALA167(A), GLU163(A), VAL146(A), ARG145(A), ASN144(A), ALA178(A), ALA179(A) & a conventional bond with GLU170(A) amino acid. It also shows an alkyl interaction with LYS182 (A) amino acid. The backbone of protein is represented by ribbon structure. (Fig.11.a)

➢ ***Oleanolic acid***

Oleanolic acid exhibits docking score of -7.9kcal/mol. Here in interaction of Oleanolic acid against Dj-1/RS it shows van der walls interaction with various amino acid residues like VAL177(A),MET1(A),ALA29(A), SER3(A), ALA2(A), LYS4(A),LEU172(A),GLY174(A) & conventional hydrogen bond interaction with ASN173(A), GLY301(A). The backbone of protein is represented by ribbon structure. (Fig 11.b)

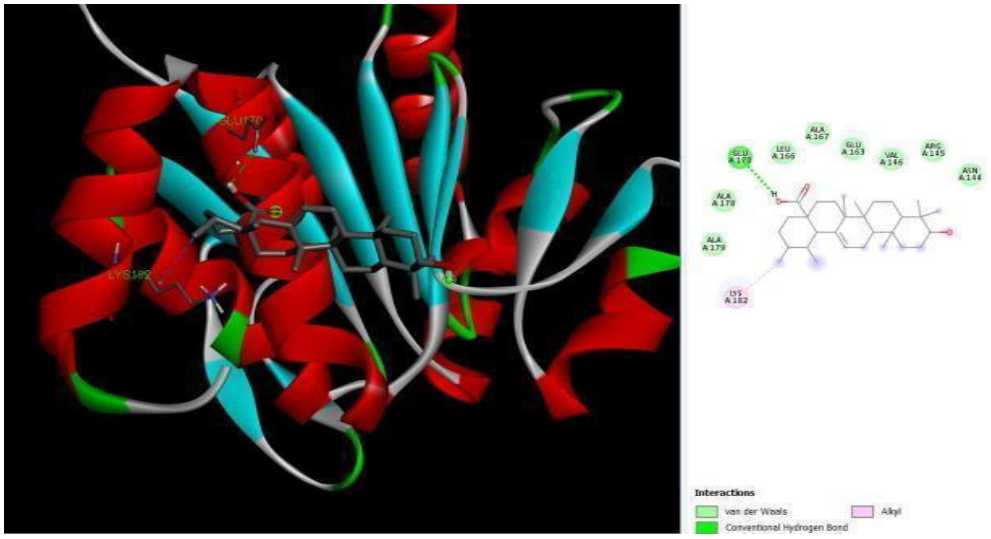
➢ ***5-hydroxymethyl furfural***

5-hydroxymethyl furfural exhibits docking score of -5.2kcal/mol. Here in interaction of 5-hydroxymethyl furfural against Dj-1/RS it shows van der walls interaction with amino acid ASP55 (A) as well as conventional hydrogen bond interaction with ARG27 (A), ASP24 (A). Besides this it shows on Pi-sigma bond VAL23 (A) & three Pi-alkyl bonds VAL35 (A), VAL20 (A) & ILE52 (A). The backbone of protein is represented by ribbon structure. (Fig.11.c)

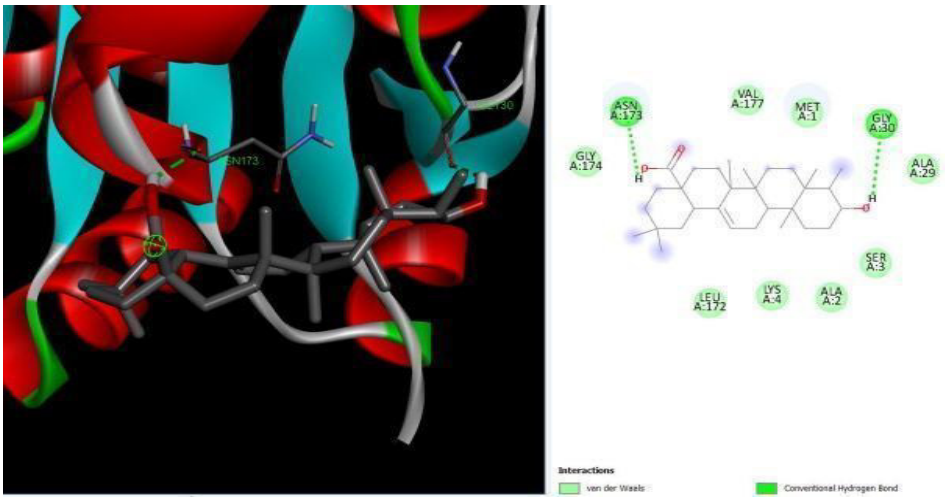
➢ ***Levodopa***

Levodopa exhibits -5.3 Kcal/mol. Here in interaction of Levodopa against Dj-1/RS it shows van der walls interaction with various amino acid residues like GLN45(A), ASP49(A), ASN76(A),GLY78(A),LYS12(A) and GLY75(A) and conventional hydrogen bond with CYS46(A), LEU77(A), GLY13(A). It also shows an unfavorable donor- donor bond with ARG48 (A). The backbone of protein is represented by ribbon structure.

A.



B.



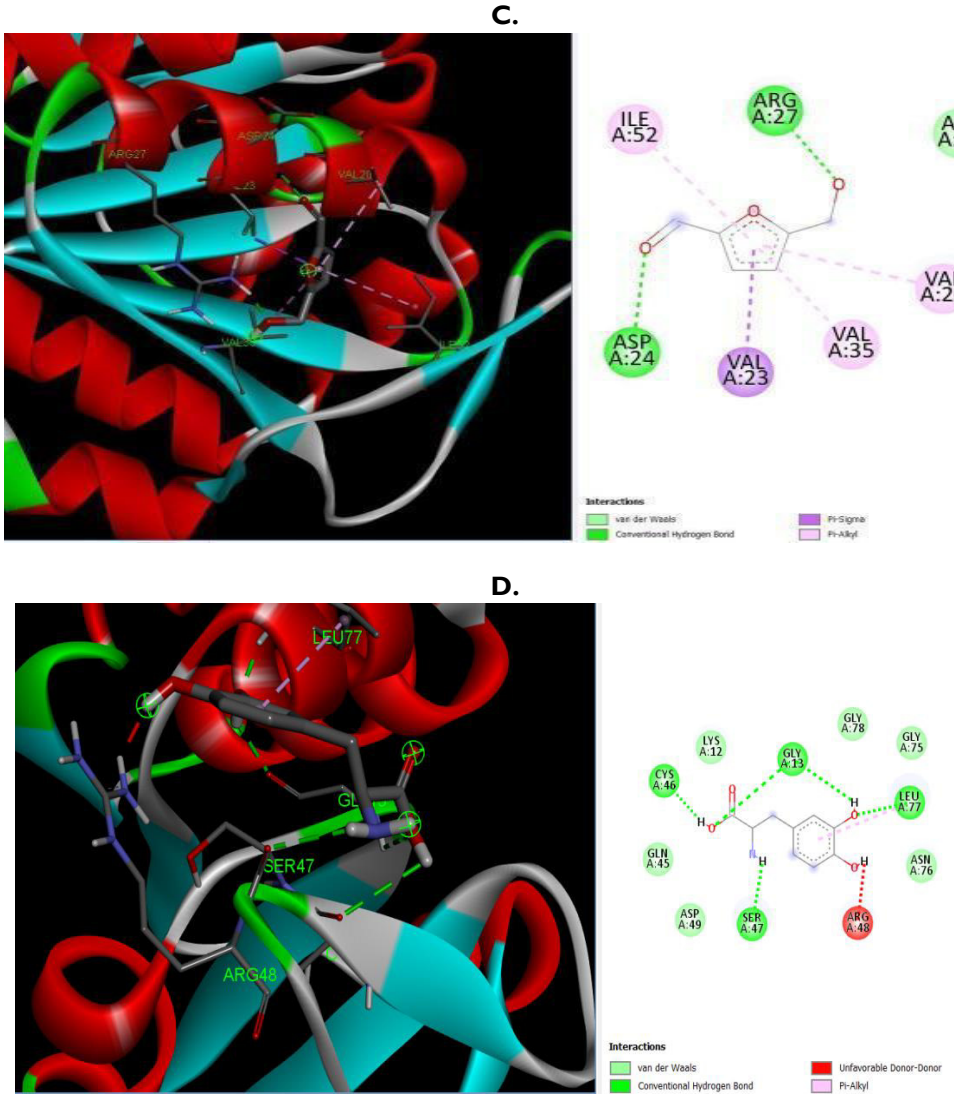


Fig.11: 3D & 2D binding pose within active site DJ-1/RS

A.Ursolic acid;B.Oleanolic acid;C.5-hydroxymethyl furfural; D. Levodopa

3D & 2D binding pose within active site DJ-1/RS A.Ursolic acid;B.Oleanolic acid;C.5-hydroxymethyl furfural Docking simulations were performed to determine the binding affinity for each ligand. Results showed that oleanolic acid had the highest binding affinity. In addition, 5-hydroxymethyl furfural had the lowest binding affinity. Furthermore, the 3D and 2D binding poses of oleanolic acid and 5-hydroxymethyl furfural within the active site of DJ-1/RS were analyzed in detail to gain a better understanding of the observed binding affinities.

Table no. 8: Docking Interaction of Oleanolic acid & 5-Hydroxymethyl furfural against Dj-1/RS

		Vander walls interactions	Hydrogen bond interactions	Alkyl bond interactions	Pi-sgma interactions	Unfavorable donor-donor
Target Protein	Ursolic acid (Standard compound)	LEU166(A)	GLU170(A)			
		ALA167(A)				
		GLU163(A)				
		VAL146(A)				
		ARG145(A)				
		ASN144(A)				
		ALA178(A)				
		ALA179(A)				
	Levodopa (Standard Compound)	GLN45(A)	CYS46(A)			ARG48(A)
		ASP49(A)	GLY13(A)			
		ASN76(A)	LEU77(A)			
		GLY78(A)				
		GLY75(A)				
		LYS12(A)	SER47(A)			

DJ-1/RS	Oleanolic acid (Compound1)	VAL177(A)	ASN173(A)		
		MET1(A)	GLY301(A)		
		ALA29(A)			
		SER3(A)			
		ALA2(A)			
		LYS4(A)			
		LEU172(A)			
		GLY174(A)			
	5-hydroxymethyl furfural (Compond2)	ASP55 (A	ARG27 (A)	VAL35 (A)	VAL23 (A)
			ASP24 (A)	VAL20 (A)	
				ILE52 (A)	

From the above docking result (Table 8), it can be concluded that in comparison with Levodopa and Ursolic acid, oleanolic acid has a higher docking score with DJ-1/RS. Oleanolic acid interacts with DJ-1/RS amino acid residue GLY301(A) and ASN173(A). The docking score of the other phytocompound 5-hydroxymethyl furfural is lower than that of Oleanolic acid, but it also has good binding affinity when compared to Levodopa. Molecular docking studies helped us determine the efficacy of, 5-hydroxymethyl furfural and Oleanolic acid, found in *A. bidentate,* for Parkinson's disease treatment.

6. CONCLUSION

Two compounds named Oleanolic acid & 5-hydroxymethyl furfural were isolated from *A.bidentata*. From Above docking results it can be concluded that in comparison with Ursolic acid and levodopa, the isolated compounds Oleanolic acid and 5-hydroxymethylfurfural shows a very good affinity with DJ-1/RS ligand which leads to show potent antiparkinson's activity. Hence, it can be concluded that *A. bidentata* can be further explored & used for Parkinson's disease's management.

7. AUTHORS CONTRIBUTION STATEMENT

Rahul Thapa and Shikha designed the whole study including collection of plant, drying of plant, extraction, isolation and characterization of phytoconstituents and prepared the manuscript. Girish Chandra Arya conducted the *in-silico* studies of isolated compounds for their Anti-Parkinson's activity. Finally, Rahul Thapa wrote the whole manuscript. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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