IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Isolation, Phytochemical Investigation And Anti-Inflammtory, Anti-Viral Activity Of Indian Medicinal Plant *Artemisia Pallens Walls. Ex*. DC Belonging To Family Asteraceae

Amitya Y. Ravidhone*[1], Rajanikant B. Ghotane[1], Sachinkumar V Patil[2]

Department of Pharmaceutical Quality Assurance, Ashokrao Mane College of Pharmacy, Peth

Vadgaon, Kolhapur. 416 112, India

ABSTRACT

Medicinal plants can be used to treat a wide range of medical conditions. Herbal medicines are frequently used in healthcare. *Artemisia pallens Well DC* (*Asteraceae*) are among the most significant medicinal herbs. The plant has been used as a disease treatment by numerous different tribes. The plant's antidiabetic, antifungal, anti-inflammatory, wound-healing, insecticidal, and antibacterial characteristics are only a few of the acts that have been discovered. Chemical elements present in *Artemisia pallens Well DC* species include alkaloids, glycosides, triterpenoids, phenols, flavonoids, saponins, and tannins.

The goal of the current study was to isolate, purify, and characterise the active chemical principle from the *Artemisia pallens* (Aerial parts). For the extraction process, aerial part of *Artemisia pallens* species was extracted by soxhelet extraction. The various phytochemical tests were performed. TLC and column chromatography was performed to isolate the compound. The isolated compounds were further characterized by using UV, IR, NMR and GC-MS. The anti-inflammatory activity and anti-viral activity was evaluated by using in-vitro model. In the in-vitro Anti HIV activity was carried out by using pepsin-inhibition assay, choloroform fraction II, showed good inhibition assay activity than the methanol fraction I. In the in-vitro anti-inflammatory activity was carried out by using Egg albumin protein denaturation assay, both methanol Fraction I, showed good inhibition assay activity than the choloroform fraction II.

KEYWORDS: *Artemisia pallens Well DC,* soxhelet extraction, anti HIV, anti-inflammatory, phytochemicals, Isolation

INTRODUCTION:

In order to maintain health as well as to prevent, diagnose, improve, or treat physical and mental illness, traditional medicine is the combination of knowledge, skills, and practises based on theories, beliefs, and experiences that are indigenous to various cultures. The verbal transmission of other conventional medical systems occurs from generation to generation.[1]

Ancient sages and physicians who understood the health-promoting, preventative, and therapeutic properties of herbs developed the theoretical and conceptual foundations of the Indian Systems of Medicine. These medical systems, which are fundamental to the customs, cultures, civilizations, and religions of the populace, can be broadly categorised as classical and traditional systems. The "traditional" Indian systems include naturopathy, yoga, ayurveda, siddha, and unani.[2]

The increasing use of traditional medicines and other traditional healthcare goods in both developing and developed countries of the world has increased the need for confirmation of their safety, efficacy, and quality as well as their financial benefits. However, a number of phytochemical and pharmacological studies on traditional medicines and medicinal plants have previously been conducted and are widely disseminated. The active chemical components in these experiments are being attempted to be isolated and identified in order to demonstrate the efficacy and safety of the findings.[3]

The Artemisia pallens Well, a member of the asteraceae family, is a significant plant used in conventional medicine. In the temperate Himalayas, the plant thrives in the wild. It is widespread in the Nanital Hills, Simla, and Kashmir Valley. On an area of roughly 1000 acres, it is grown commercially in Karnataka, Maharashtra, Kerala, Tamil Nadu, and Andhra Pradesh. One of the best sources of aromatic & medicinal herbs is India. About 60 cm tall and scented, davana is an upright plant with deeply split leaves and tiny yellow blooms. Tomentum that is grayish-white covers both the stem and the leaves. The leaves are lobed, alternating, and petiolate.[4]

Cis-Davanone, ascorbic acid, biocyclogermacrene, linalool, methyl cinnamate, ethyl cinnamate, 2-hydroxyisodavanone, farnesol, geranyl acetate, sequiterpene lactone, and germacranilide were all found in this species' chemical composition, according to investigations. Both the ascorbic acid and the phenolics found in the plant are potent antioxidants. Plants are shielded from microbial diseases by the presence of saponins.[4]

Davana, Davanone, and Davana-Ether The two main chemical components of davana oil comprise furan and linalol. Ethyl and methyl cinnamates, there are also other compounds like bicyclogermacrene, davana ether, 2-hydroxyisodavanone, farnesol, geranyl acetate, sequiterpene lactones, germacranolides, etc. Between the emergence of flower heads and the start of seed germination, the levels of davanone, the main component of davana oil, and linalool declined while those of (Z) and (E) methyl cinnamate, (E) ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, and farnesol increased. Five substances, including

geranyl acetate, (Z) and (E) methyl cinnamates, and (Z) and (E) ethyl cinnamates, were discovered for the first time in davana oil.[5]

Oil of Davana, a fragrant essential oil, is produced from the leaves and flowers. Several species produce essential oils, some of which are utilised as fodder santonin, a useful antihelmintic medication, is derived from several of them. Diabetes, depression, inflammatory diseases, and hypertension are all treated with Artemisia pallens Well.[6] When creating floral decorations and essential oils, the leaves and blossoms are highly prized. The flowers are little and inconspicuous, while the leaves are very small and blue green. It is a component in classic ayurvedic drug compositions. The antibacterial and disinfecting properties of Artemisia pallens essential oil are used.[7,8]

MATERIALS AND METHOD:

COLLECTION, AUTHENTICATION AND PROCESSING OF PANT MATERIAL:

The leaves of Artemisia pallens species were collected from Jotiba Dongar, Kolhapur District-Kolhapur in the month of December 2022. After collection, the plant material was identified, confirmed, and authenticated by Prof. D. G Jagtap, head department of botany, Principal of Shri. Vijayasinha Yadav Art and Science College, Peth-Vadgaon. After collection of plant material was thoroughly washed in distilled water. Drying of plant material was done using shade drying and crushed in an electrical grinder and then powdered. The plant's material was subjected to shade drying and then the shade dried plant material was crushed to get the powder and it was extracted in a soxhlet apparatus using various solvents according to their polarity viz. Petroleum ether, chloroform, methanol. About 75 gm of a powdered sample of the selected plant was weighed and added to 200 ml of solvent and extracted at 40^0 C dryness at dryness at 60^0C.[9,10]

The filtrate was evaporated to dryness at 60^0 C in a boiling water bath and the above process was repeated several times until a sufficient amount of extract was produced, the concentrated extract of selected plants was stored at 4^0C Weight until required for use.[11,12]

% Yield = Weight of Extract (Gm) / Weight Powder (Gm) × 100

Fig. No. 1.1: Aerial part of dried & fresh Davana

PHYTOCHEMICAL SCREENING:[13]

I. TEST FOR PROTEINS

1. **Biuret Test:**

To 2 ml of extract 1 ml of 4% w/v sodium hydroxide and 1 ml of 1% w/v copper sulphate were added. The change in colour of the solution to violet or pink indicates the presence of proteins.

2. **Xanthoproteic Test:**

To 2 ml of extract 1 ml of concentrated Sulphuric acid was added. The appearance of a white precipitate which turns yellow on boiling and orange with the addition of 1 ml ammonium hydroxide indicates the presence of proteins.

3. **Millon's Test:**

To 2 ml extract, 4 ml of Millon's reagent was added and heated to the appearance of a white precipitate which changed to brick red on heating indicating the presence of proteins.

II. TEST FOR FREE AMINO ACIDS

1. **Ninhydrin Test:**

To 2 ml extract 3 drops of 5% w/v lead acetate solution were added and boiled in a water bath for 10 min. the change in colour of the solution to purple or blue indicates the presence of amino acids.

III. TEST FOR CARBOHYDRATES

1. **Benedict's Test:**

Equal volumes of Benedict's solution and extracts were mixed in a test tube and heated in a boiling water bath for 10 min and observe the formation of a reddish-brown precipitate which indicates the presence of carbohydrates.

2. **Molisch's Test:**

To 2 ml of extract 2-3 drops of α-naphthalene solution in alcohol were added and shaken for 2 min and 1 ml of concentrated Sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates.

3. **Fehling's Test:**

Fehling's A and Fehling's B solutions, every 1 ml were mixed and boiled, for 1 ml and 2 ml of extracts were added and heated in a boiling water bath for 10 min, the appearance of a yellow and then brick red precipitate indicates the presence of reducing sugar.

IV. TEST FOR ALKALOIDS

To the 5 g of dry extracts, 10 ml of dilute hydrochloric acid was added, shaken well and filtered. The following tests were performed using filtrate.

1. **Wagner's Test:**

To two ml of filtrate, 1 ml of Wagner's reagent was added. The appearance of the reddish-brown precipitate indicates the presence of alkaloids.

2. **Mayer's Test:**

To 2 ml of filtrates, 1ml of Mayer's reagent was added. It shows the formation of a yellow precipitate indicating the presence of alkaloids.

V. TEST FOR PHENOLS:

1. **Ferric chloride Test:**

Extract treated with 3 ml of 5% w/v ferric chloride solution. It is resulting the formation of a blue-black colour which indicates the presence of phenols.

VI. TEST FOR TANNINS

1. **Gelatin Test:**

To 2 ml extract add 1 % gelatine solution containing sodium chloride. It is resulting the formation of a white precipitate which indicates the presence of tannins.

2. **Lead acetate test:**

To 2 ml extract, 2 ml of lead acetate solution was added. Which results in the formation of white precipitate indicating the presence of tannins.

VII. TEST FOR FLAVONOIDS

1. **Shinoda Test:**

The crude extract, 5 ml of ethanol (95% v/v), 5 drops of hydrochloride acid and 0.5gm of Magnesium turnings were added. The appearance of pink colour indicates the presence of flavonoids.

VIII. TEST FOR TRITERPENOIDS

1. **Liebermann burchard Test:**

   To 2 ml of the test solution, 10 drops of acetic anhydride were added and mixed well. 5 ml of concentrated Sulphuric acid was added from the sides of the test tube, and the appearance of greenish blue colour indicates the presence of triterpenoids.

2. **Salkowaski Test:**

   To 2 ml of extract 5 drops of concentrated Sulphuric acid was added, shaken and allowed to stand. The appearance of greenish blue colour indicates the presence of triterpenoids.

IX. TEST FOR GLYCOSIDES

1. **Keller-killiani Test:**

   To the test tubes containing 2 ml of extract and 1 ml of glacial acetic acid, 3 drops of 5% w/v ferric chloride and concentrated Sulphuric acid was added and observed, the disappearance of reddish-brown colour at the junction of two layers and bluish green in upper layer indicates the presence of cardiac glycosides.

2. **Bontrager's Test:**

   To the test tubes containing 2 ml of extract 2 ml of dilute Sulphuric acid was added, boil for 5 min and filtered. To the filtrates, equal volumes of chloroform were added and mixed well. Organic layers were separated and ammonia was added to them. The pinkish-red colour of the ammonia layer indicated the presence of anthraquinone glycosides.

ISOLATION AND PURIFICATION

The isolation and purification of plant constituents are mainly carried out by one or a combination of several fractionation procedures based on various chromatographic techniques. The most useful chromatographic techniques in phytochemical isolation include thin-layer chromatography (TLC), column chromatography (CC), and gas chromatography (GC). The choice of technique depends largely on the nature of the substances present. It is very important to note that there is considerable overlap in the use of the above techniques and often a combination of PC, TLC and GC.

THIN-LAYER CHROMATOGRAPHY (TLC):

Preparation of chromatographic plates:

1. 1. The silica gel-G (30gm) was weighed out and a suspension was prepared by agitating with 60 ml of distilled water.
2. The slurry was poured into a TLC applicator, which was adjusted to 0.25mm thickness on a flat glass plate.
3. The applicator was rolled over the glass plates set adjacent to each other to form uniform coated plates which were allowed to dry in air for 15-20 mins, followed by heating in an oven at 120^0C for one hour, cooled and protected from moisture.
4. The plates were stored in a dry atmosphere.
5. Whenever required the plates were activated by heating in a hot air oven at 100^0C for 30 min and used for TLC.

Application of extract :

1. For separation of spots Petroleum ether extract, methanol extract and chloroform extract have been dissolved in appropriate solvents where maximum solubility is shown in the sample.
2. The solution of extract was spotted on a TLC plate with the help of a narrow capillary tube 1 cm above the bottom of the plate.
3. The spots were equally sized as far as possible and separated equidistant to each other and the edges of the plate. The diameter of spots should not exceed 0.25 cm.

Development of chromatogram:

1. The developing chamber was prepared by lining the inner wall of the chamber with filter paper and the developing solvent (the volume of the developing solvent should be sufficient to cover with a lid in order to provide chamber saturation.
2. The chamber saturation avoids the edge effect and tailing effect.
3. Immediately after chamber saturation (1Hr approx), the spotted silica gel plate was placed in the developing chamber with caution, the lid was closed and the solvents were allowed to run up to ¾ the of the chromatographic plate.
4. The plate is marked and allowed to dry in hot air.
5. The spot on the plate is detected by placing the plate in a UV chamber, an iodine chamber or by spraying chromatographic reagents.
6. Hence on a trial and error basis, several combinations of solvent systems were tested, and the following system was selected.

Table No 1.1: Solvent System for Extract

Sr.No.	Extracts	Solvent system
1.	Cholroform	Methanol : Ethyl Acetate : Acetic acid (5 : 2.5 : 2.5)
2.	Methanol	
3.	Petroleum Ether	

ISOLATION OF INDIVIDUAL COMPOUNDS:

A. Column chromatography:

WET PACKING TECHNIQUE:

1. The bottom end of the column is packed with cotton after which the stationary phase is packed.
2. After packing the column, a paper disc is placed on the top to avoid the disturbance of the stationary phase during the introduction of the sample or mobile phase.
3. The slurry of crystal silica gel was prepared in the mobile phase and poured into the column.
4. The sample was prepared in the mobile phase and introduced into the column from the top.
5. Through the elution process, the samples are collected in the test tube.
6. About 140-150 gm of silica for column chromatography was activated in a hot air oven at 110^0 C for one hour and filled in a column.
7. The various mobile phase solvent used to build the column for three different extracts.
8. The small quantity of solvent system was allowed to remain on the top of a column of about (2cm).
9. The air bubbles present in the column were removed by gentle tapping to get a uniform bed of adsorbent phase.
10. About 2 gm of all 3 extracts were dissolved in chloroform, methanol and Petroleum ether respectively.
11. The sample solutions were then applied on the bed of silica with the help of a pipette.
12. For chloroform extract the column was then eluted with a mixture of **Methanol : Ethyl Acetate : Acetic acid(5 : 2.5 : 2.5)**
13. For methanol extract the column was then eluted with a mixture of **Methanol : Ethyl Acetate : Acetic acid(5 : 2.5 : 2.5)**
14. For Petroleum Ether extract the column was then eluted with a mixture of **Methanol : Ethyl Acetate : Acetic acid(5 : 2.5 : 2.5)**
15. The details of Column Chromatography of all three extracts of Couroupita guianensis aubl as shown in (Table No 1.2)

Table 1.2: Details of column chromatography

Length of column	40 cm
Diameter of column	Outer 3 cm, inner 2.8 cm
Adsorbent	Silica gel for column chromatography activated at 1100C for 1 hour
Length of adsorbent	25cm
Rate of elution	12-15 drops/ min
The volume of each fraction collected	40-45 mL

B. UV–Vis Spectrophotometer:

1. The purified compounds were dissolved separately in methanol and chloroform at 2–10 µg/mL concentrations and their UV–Vis spectra were recorded using a UV–Vis spectrophotometer (Cary 60 Agilent technologies) & (Shimadzu, Japan) between 200 and 800 nm.
2. Methanol & chloroform was used as blank respectively.

C. FT-IR:[14]

1. IR analysis of isolated compound was performed and Interpretation of IR was done.
2. An IR spectrum was recorded using ATR on an FTIR Spectrophotometer.
3. All the interpretation was made illustrative by the prominent peaks, vibrational class, possible group and environment, strength and nature of absorption intensities along with an identified portion of the expected compound.
4. The interpretation was satisfactory and all the compounds conform to their expected structure. IR spectra for the purified compounds were recorded on a Brucker FTIR spectrometer using KBr pellets.

D. Nuclear Magnetic Resonance Spectroscopy (NMR):[14]

1. H^1 NMR of the purified compounds was recorded in deuterated DMSO with tetramethylsilane (TMS) as an internal standard solution using a 400 MHz Bruker machine (Bruker, MA, USA).

E. Structural elucidation of the isolated compound by GC-MS:

1. All samples of *Artemisia pallens* fractions were dissolved in respective solvents and GC-MS analysis was done at Shivaji University, Kolhapur (CFC) using a GC-MS model; Shimadzu GC-2010 Plus, Tokyo, Japan equipped with a VF-5ms fused silica capillary column of 30m length, 025mm internal diameter and 0.25µm film thickness.
2. For GC-MS detection quadrupole system was used. 1µl of respective samples manually injected in split less mode with scan ranges up to 650 amu total running time of GC-MS is 20 min.

IN VITRO ANTI-INFLAMMATORY ACTIVITY (PROTEIN DENATURATION METHOD):

1. The anti-inflammatory activity was determined by using the inhibition of protein denaturation method.
2. The reaction mixture (1 mL) consisted of 0.1 mL of egg albumin (from fresh hen's egg), 0.5 mL of phosphate buffered saline (PBS, pH 6.4) and 0.4 mL of given Sample fraction I and II similar volume of double-distilled water served as control.
3. Then the mixtures were incubated at (37^0c ±2) in an incubator for 15 min and then heated at 70 degree celecious for 5 min.
4. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at concentration (1 mg/ml) was used as reference drug and treated similarly for determination of absorbance.[15]
5. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

IN VITRO PUTATIVE ANTI HIV ACTIVITY (PEPSIN INHIBITION ASSAY):

AIDS is a wide spread immunosuppressive illness that can cause cancer and opportunistic infections with a high mortality rate. Human immunodeficiency virus (HIV), which is classified as a retrovirus, has been unmistakably recognised as the disease's main cause.[16,17] There are twelve phases in the HIV replication cycle that could be used as potential targets for chemotherapy.[18]

The creation of antiviral medicines that stop HIV at various phases of viral replication is a major focus of several laboratories.[19,20] HIV has a high mutation rate, which typically causes the quick emergence of treatment resistance, and attempts have been made to get around this issue by employing drug combinations.[21]

1. For this assay, 50µg pepsin, 800µg hemoglobin and Fraction I and Fraction II were taken in 500µl of reaction mixture.[22,23,24]
2. The mixture was allowed to incubate at 37^0 C, after 20 min 700µl of 5% TCA was added to stop the reaction. It was then centrifuged at 14000 rpm for 5 min and the supernatant was collected.
3. Optical Density (OD) was recorded spectrophotometrically at 280 nm.
4. Distilled water is used as a blank

5. For negative control, enzyme and substrate were taken and followed the above procedure and for negative control protease was taken as a well-known inhibitor of HIV-protease, pepstatin was taken.
6. Each sample was taken in triplicate, so this assay gives reproducible results. Percentage of inhibition was calculated by using a formula.

Inhibition (%) = [(OD of negative control - OD of sample) /OD of negative control] ×100

RESULT AND DISCUSSION:

Yield of extraction:

Extractions of all three plants were carried out by using the solvent mixture of methanol (70%)-water (30%). The physical nature, color characteristic and percentage yield of each individual extracts are found as given in the Table-1.3

Table No.1.3: Percentage yield of extracts

Sr. No.	Extract	% yield	Physical Appearance
1	Chloroform	2.65	Light green
2	Methanol	4.29	Dark greenish
3	Petro. Ether	6.04	Dark greenish

Phytochemical analysis:

Table No. 1.4: Phytochemical tests for various extracts of *Artemisia pallens*

Sr. No.	Test	Chloroform extract	Methanol extract	Peetroleum ether extract
1.	Test for carbohydrate	+	+	+
2.	Test for steroids	+	-	+
3.	Test for Glycosides	+	-	+
4.	Test for Flavonoids	+	+	+
5.	Test for Alkaloids	-	-	+
6.	Test for Tannins and phenolic compounds	+	+	+

ISOLATION AND PURIFICATION:

THIN-LAYER CHROMATOGRAPHY:

Fig. No. 1.2 : TLC of a) Methanol extract b) Petroleum Ether extract c) chloroform extract

Table No. 1.5: Mobile phase used for separation of various extracts of *Artemisia pallens*

SR.NO	EXTRACTS	RF VALUE	MOBILE PHASE
1	Petroleum ether	0.78	Methanol : ethyl acetate : Acetic acid (5 : 2.5 : 2.5)
2	Methanol	0.60	
3	chloroform	0.52	

(a) (b) (c)

Fig. No. 1.3: Column Chromatography of a) chloroform extract b) Methanol extract c) Petroleum Ether extract

For the column chromatography silica gel was used as an adsorbent and different mobile phases are used for different extracts (Table10.9). The chloroform extract gives three isolated fractions as fractions of CF1, CF2, and CF3. The methanol extract gives three isolated fractions such as fractions MF1, MF2, MF3. The petroleum ether extract gives three isolated fractions such as fractions PF1, PF2, PF3.

SPECTRAL DATA:

A. UV Spectra (Determination Of Wavelength):

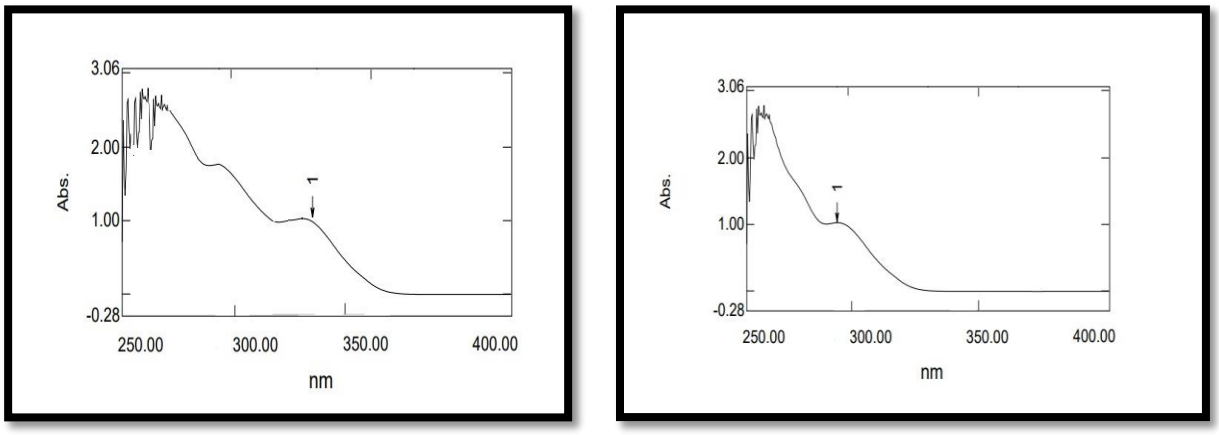


Table No.1.6: Interpretation of UV spectra of the isolated fraction of chloroform friction II methanol friction I

	Solvent	Observed Value (λ Max)	Literature Value (λ Max)
1-eicosanol	Methanol	295	297
18, 19-secoyohimban-19-oic acid	Choloroform	338	340

B. IR SPECTRA:

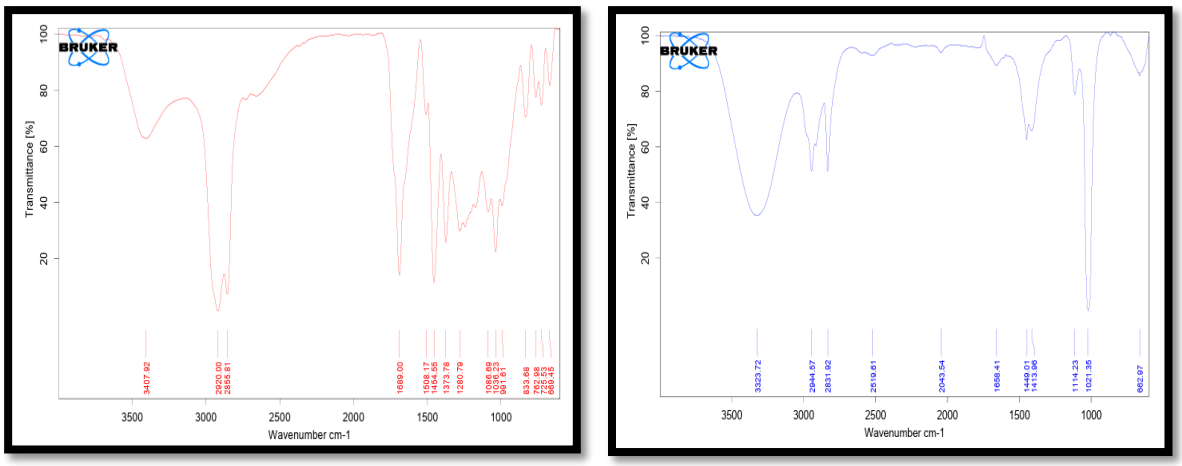


Fig No. 1.4: IR spectra of chloroform friction II & Methanol Fraction I

Table No.1.7: IR interpretation of chloroform friction II & Methanol Fraction II

Compound Code	Functional group	Standard value (cm-1)	Observed value (cm-1)
Chloroform Fraction II	O-H	3000-2500	3323
	C-CH3	2500-3000	2831
	C-O	1250-1050	1021
	C-C	1600-1400	1449
	C-H	3300-2700	2944
Chloroform Fraction II	N-H	3500-3300	3407
	C-N	1350-1280	1280
	C=O	1750-1700	1689
	C-O	1250-1050	1036
	C=C	1600-1300	1373
	C-H	3300-2700	2920
	C-C	1600-1400	1454

C. NMR SPECTRA:

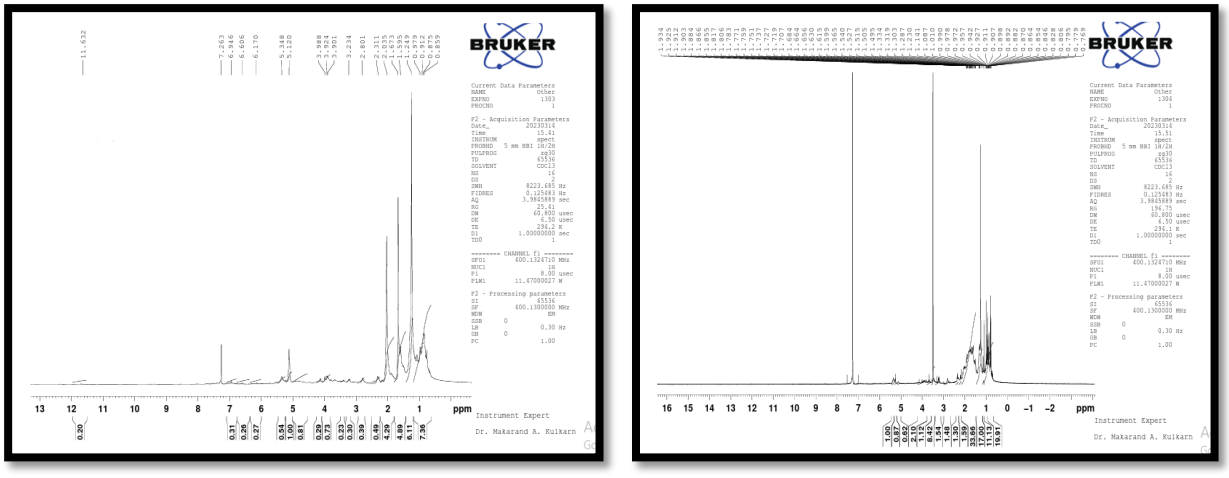


Fig No. 1.5: NMR spectra of chloroform friction II & Methanol Fraction I

Table No.1.8: NMR interpretation of chloroform friction II & Methanol Fraction II

Sr. No.	Chemical Shift (ö)	Interpretation
1.	0.979	OH group is present
2.	0.912	
3.	0.875	
4.	2.311	C-H (aliphatic hydrogen)
5.	3.924	
6.	7.263	Aromatic H is present (i.e. H is on Phenyl ring)
7.	11.632	Aldehyde is present
1.	0.927	OH group is present
2.	0.911	
3.	0.907	
4.	0.898	
5.	1.527	Aromatic H is present (i.e. H is on Phenyl ring)
6.	1.010	C-H (aliphatic hydrogen)

D. GCMS:

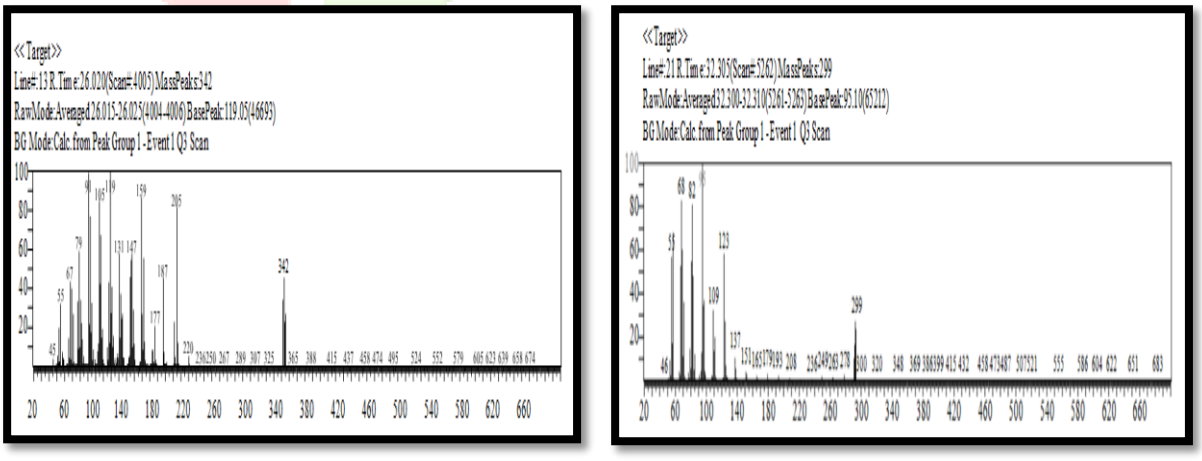
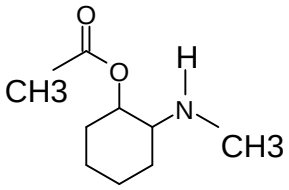
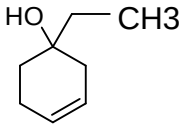
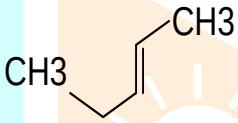


Fig No. 1.6: GCMS spectra of chloroform friction II & Methanol Fraction I

Table No.1.9: GCMS interpretation of chloroform friction II & Methanol Fraction II

SR.NO	STRUCTURE	MASS PEAK (M/Z)
1		**154**
2		**121**
3		**68**
1.		134
2.		161

PHARAMACOLOGICAL SCREENING:

IN VITRO PUTATIVE ANTI HIV ACTIVITY BY PEPSIN-INHIBITION ASSAY:

Table 1.10: anti HIV activity by pepsin-inhibition assay

SR. NO.	COMPOUND	CONCENTRATION	READING 1	READING 2	READING 3	MEAN	% INHIBITION
1	CONTROL		0.058	0.048	0.066	0.057	
2	PEPSTATIN (STD)	100 µG/ML	0.007	0.006	0.005	0.006	89.47
3	METHANOL FRACTION I	1MG/ML	0.009	0.008	0.087	0.034	40.35
4	CHOLOROFORM FRACTION II	1MG/ML	0.003	0.004	0.009	0.005	91.22

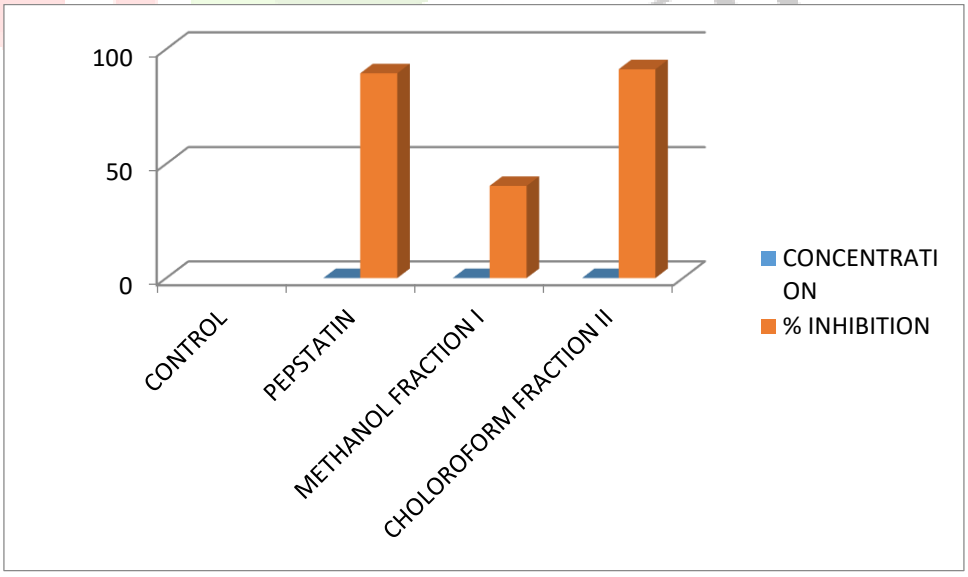


Fig No.1.7: Graphical representation of anti HIV activity by pepsin-inhibition assay

In the in-vitro Anti HIV activity was carried out by using pepsin-inhibition assay, choloroform fraction II, showed good inhibition assay activity than the methanol fraction I. Choloroform fraction II showed activity due to presence of active constituents in the given sample.

IN VITRO ANTI-INFLAMMATORY ACTIVITY BY PROTEIN DENATURATION METHOD :

Table 1.11: Anti-inflammatory activity of different formulation by Protein denaturation method

COMPOUNDS	CONC.	O.D.	MEAN	% INHIBITION
CONTROL	-	1.96 1.98 1.78	1.90	
STANDARD (DICLOFENAC SODIUM)	1MG/ML	0.50 0.46 0.48	0.48	74.73
METHANOL FRACTION I	1MG/ML	0.90 0.94 0.95	0.93	51.50
CHOLOROFORM FRACTION II	1MG/ML	1.29 1.35 1.30	1.31	31.05

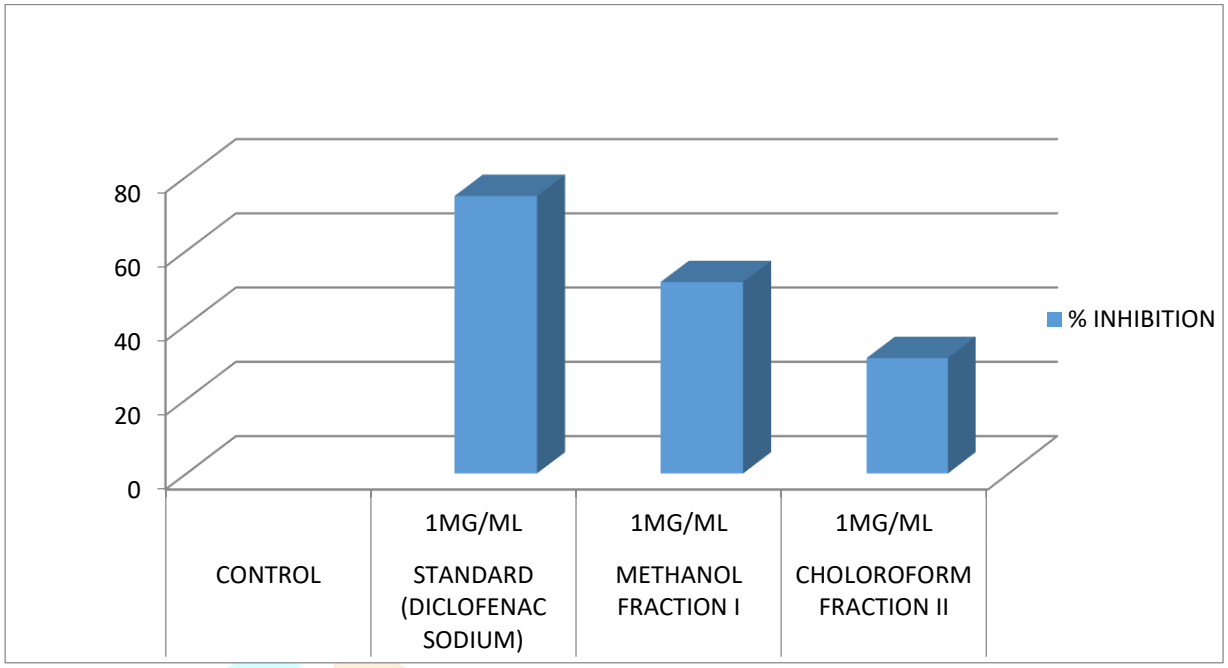
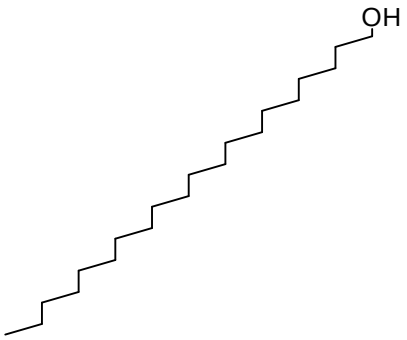


Fig 1.8: Graphical representation of Anti-inflammatory activity of different formulation by Protein denaturation method

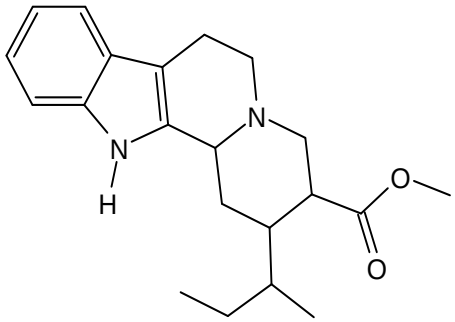
In the in-vitro anti-inflammatory activity was carried out by using Egg albumin protein denaturation assay, both methanol Fraction I, showed good inhibition assay activity than the choloroform fraction II. methanol Fraction I showed activity due to presence of active constituents in the given sample.

CONCLUSION:

The objective of the current study was to investigate the active chemical principles of fruits of ***Artemisia pallens*** extracts, as well as their anti-inflammatory and anti-Viral activity. The plant belong to ***Asteraceae*** family is a rich source of bioactive compounds with therapeutic potential for a variety of medical conditions, including diabetes, infectious disorders, anaemia, stomatitis, etc. TLC, column chromatography, UV, FTIR, NMR and GCMS study reveal that this drug mainly contains **1-eicosanol**, **18, 19-secoyohimban-19-oic acid** in methanol, chloroform extract respectively.



Structure of Icosan-1-ol



Structure of 18, 19-secoyohimban-19-oic acid

The anti-inflammatory activity of various extracts of ***Artemisia pallens*** was carried out **by Protein denaturation method**. All the extracts of ***Artemisia pallens*** showed significant anti-inflammatory activity. According to the results the std. diclofenac sodium shows 74.73% inhibition while the methanol and chloroform extract shows 51.50 and 31.05 % inhibition respectively. so the methanol extract shows the significant anti-inflammatory activity when compared to standard diclofenac sodium.

The anti-viral i.e. anti-HIV activity of various extracts of ***Artemisia pallens*** was carried out **by pepsin-inhibition assay**. All the extracts of ***Artemisia pallens*** showed significant anti-HIV activity. According to the results the std. pepstatin shows 89.47% inhibition while the methanol and chloroform extract shows 40.35 and 91.22 % inhibition respectively. so the chloroform extract shows the significant anti- inflammatory activity when compared to standard pepstatin.

Hence, it is concluded that the ***Artemisia pallens*** is potential member for the anti-inflammatory activity and anti-viral i.e. anti-HIV activity. As a result, our subsequent goals are to thoroughly examine isolated molecules for their derivatives, conduct docking studies to establish the mechanism of action, and other tasks that may open up a new area of study for the creation of drugs from plants.

REFERENCES:

1. Nwafor EO, Lu P, Liu Y, Peng H, Qin H, Zhang K, Ma Z, Xing B, Zhang Y, Li J, Liu Z. Active components from traditional herbal medicine for the potential therapeutics of idio- pathic pulmonary fibrosis: a systemic review. *The American Journal of Chinese Medicine*.2021;49(05):1093-114.
2. Jeyalalitha T, Murugan K, Umayavalli M. Preliminary phytochemical screening of leaf extracts of *Anthocephalus cadamba*. *International Journal of Recent Scientific Research*. 2015;6(10):6608-6611.
3. Ghani A. Traditional medicines: Present status, future prospects. Pharmabiz.com, 2017.
4. Suresh J., Vasavi A., Ihsanullah M., Mary S. Phytochemical and pharmacological properties of artemisia pallens. International journal of pharmaceutical sciences and research. 2011; 2(12): 3081-3090.

5. Ruikar AD, Kamble GS, Puranik VG, Deshpande NR, Ingle TR. Antimicrobial screening of medicinal plant-Artemisia pallens. Int. J. Pharmtech. Res. 2009;1:1164-6.
6. Misra LN, Chandra A, Thakur RS. Fragrant components of oil from Artemisia pallens. Phytochemistry. 1991 Jan 2;30(2):549-52.
7. Ruikar AD, Kamble GS, Puranik VG, Deshpande NR, Ingle TR. Antimicrobial screening of medicinal plant-Artemisia pallens. Int. J. Pharmtech. Res. 2009;1:1164-6.
8. Mallavarapu GR, Kulkarni RN, Baskaran K, Rao L, Ramesh S. Influence of plant growth stage on the essential oil content and composition in Davana (Artemisia pallens Wall.). Journal of agricultural and food chemistry. 1999 Jan 18;47(1):254-8.
9. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants.* 2015;4(196):2167- 0412.
10. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC.; Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry.* 2017; 6(1):32-6.
11. Harborne JB. Methods of Plant Analysis; Methods of seperation; Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Springer International Publishing; 3rd ed.; 2013; 7.
12. Rangari VD. Pharmacognosy and aphytochemistry. Career Publication; 3rd ed. Vol. 2; 2015;446-47.
13. Khandalwal KR, Sethi VK. Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan. 1999: 23.6-25.9.
14. Dyer JR. Applications of absorption spectroscopy of organic compounds. Prentice - Half of India; 2006; 33-38.
15. Datatraya Raut et al (2022) synthesis, molecular docking and biological evaluation of 2- (2-hydrazinyl) thiazoles as potential Antioxidant, Anti-inflammatory and significant anticancer agent, Recent advances in inflammation and allergy drug discovery, 2022, 16, 96-106.
16. Cherman JC, Rey F, Chamaret S, Gruest J, Isolation of T-lymphotropic retrovirus from a patient at risk for AIDS. Science. 220: 868-871, 1983.
17. Gallo RC, Salahudin SZ, Popovic M, Kaplan M, Frequent detection and isolation of cytopathic retrovirus from patients with AIDS and at risk for AIDS. Science. 224(4648): 500-503-1984.
18. De Clercq E, Antiviral therapy for HIV infections. Clinical Microbiology Review, 8 (2): 200 239-. 1995.
19. Balzarini J, Mitsuya H, De Clercq E, Broder S,. Comparative inhibitory effects of suramin and other selected compounds on the infectivity and replication of human T cell lymphotropic virus. International Journal of Cancer. 37(3): 451-457-1986.
20. Sarin PS,. Molecular pharmacological approaches to the treatment of AIDS. Annual Review of Pharmacology and Toxicology. 28: 411-428, 1998.

21. Tantillo C, Ding J, Nanni RG, Boyer PL,. Location of Anti-AIDS drug binding sites and resistance mutations in the three dimensional structure of HIV-1 reverse transcriptase implications for mechanism of drug inhibition and resistance. Journal of Molecular Biology. 243(3): 369-387, 1994.
22. Suneetha T.B, S M Gopinath, Divya T.K, Amarshankar, Narasimha Murthy.T.P, Anti HIV and Antibacterial property of coumarinsisolated from Sonchus oleraceus, International Journal of Innovative Research in Science, Engineering and Technology, Vol. 2, Issue 10, October2013, 5253-5258.
23. Aoyagi, T., Umezawa, H., Takita, T. and Shiba, T., In Bioactive Peptides Produced by Microorganisms. Eds.; Halsted Press,1978; 129-151.
24. Seelmeier S., Schmidt H., Turk V., Vonderhelm K., Human Immunodeficiency Virus Has an Aspartic-Type Protease That Can Be Inhibited By Pepstatin-A. Proc. Natl. Acad. Sci., 1988; 85: 6612-6616.
25. Singh KP., Upadhyay B., Prasad R., Kumar A., Screening of Adhatoda vasica nees as a putative HIV-protease inhibitor,Journal of Phytology, 2010; 2(4): 78–82.