ISOLATION, ANTI-INFLAMMTORY & ANTI HIV ACTIVITY OF ARTEMISIA PALLENS WELL

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Abstract:

Medicinal plants can be used to treat a wide range of medical conditions. Herbal medicines are frequently used in healthcare. *Artemisia pallens Well* (Asteraceae) are among the most significant medicinal herbs. The plant has been used as a disease treatment by numerous different tribes.

For the extraction process, aerial part of *Artemisia pallens* species was extracted by soxhelet extraction. The various phytochemical tests were performed. The isolated compounds were further characterized by using UV, IR, NMR and GC-MS. The anti-inflammatory activity and anti-HIV activity were evaluated by using in-vitro model. 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

Keywords:

Artemisia pallens Well, soxhelet extraction, anti HIV, anti-inflammatory, phytochemicals, Isolation

Introduction:

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.* The antibacterial and disinfecting properties of *Artemisia pallens* essential oil are used. [1,2,3]

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

Materials and Method:

Collection, Authentication of Plant 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.

Preparation of Artemisia pallens Extract:

The Plant was Authenticated Plant material was crushed to get the powder and it was extracted. The extraction was done by soxhlation Method by using different Solvent.

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

Phytochemical Screening:[13]

It was done by using Different Chemical Tests.

Isolation and Purification:

The isolation and purification was done by using 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):

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

Table No 1.1: Solvent System for TLC

Isolation of Individual Compounds:

A. Column chromatography:

The details of Column Chromatography of all three extracts of Couroupita guianensis aubl as shown in (Table No 1.2)

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

Table 1.2: Details of column chromatography

B. UV–Vis Spectrophotometer:

The analysis was done between between 200 and 800 nm.

C. FT-IR:[14]

IR analysis of isolated compound was performed and Interpretation was done.

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

H^1 NMR of the purified compounds was recorded.

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

All samples of Artemisia pallens fractions were dissolved in respective solvents and GC-MS analysis was done.

In Vitro Anti-Inflammatory Activity:

1. Protein Denaturation Method was used.
2. 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:**

1. Pepsin Inhibition Assay was used.
2. 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

Sr. No.	Extract	% yield	Physical Appearance
1	Chloroform	2.65	Light green
2	Methanol	4.29	Dark greenish
3	Petroleum Ether	1.04	Dark greenish

Table No.1.3: Yield of extracts in Percentage

Phytochemical analysis:

Sr. No.	Test	Chloroform extract	Methanol extract	Petroleum ether extract
1.	Carbohydrate	+	+	+
2.	Steroids	+	-	+
3.	Glycosides	+	-	+
4.	Flavonoids	+	+	+
5.	Alkaloids	-	-	+
6.	Tannins and phenolic compounds	+	+	+

Table No. 1.4: Phytochemical analysis of different extracts of Artemisia pallens (+ indicate Present,- indicate Absent)

Isolation and Purification:

Thin-Layer Chromatography:

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

Sr. No	Solvents	Rf Value	Mobile Phase
1	Petroleum ether	0.38	Methanol : ethyl acetate : Acetic acid (5 : 2.5 : 2.5)
2	Methanol	0.60	
3	chloroform	0.52	

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

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):

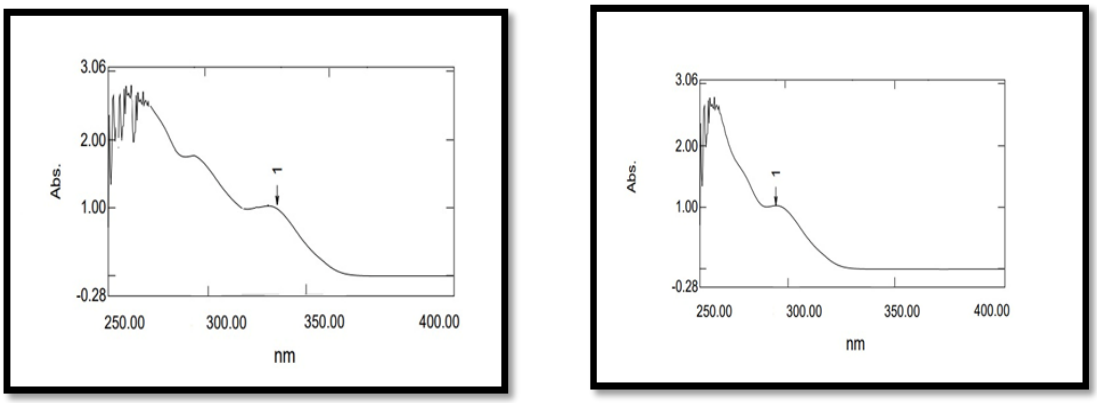


Table No.1.6: Interpretation of UV spectra of the isolated fraction of chloroform fraction II methanol fraction I

A. IR Spectra:

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

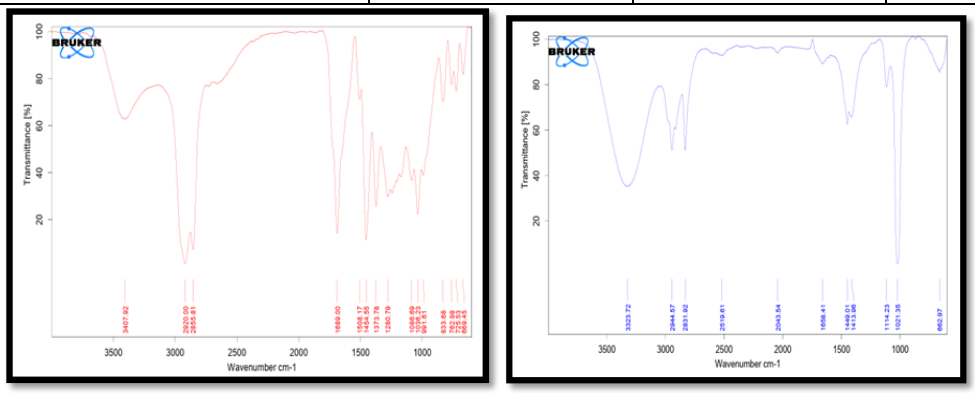


Fig No. 1.4: IR spectra of chloroform fraction II & Methanol Fraction I

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

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

B. NMR Spectra:

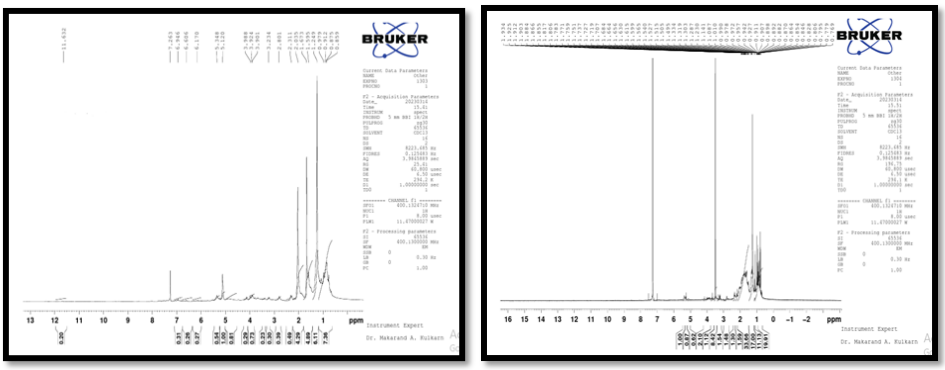


Fig No. 1.5: NMR spectra of chloroform fraction II & Methanol Fraction I

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)

Table No.1.8: NMR interpretation of i) chloroform fraction II ii) Methanol Fraction II

C. GCMS:

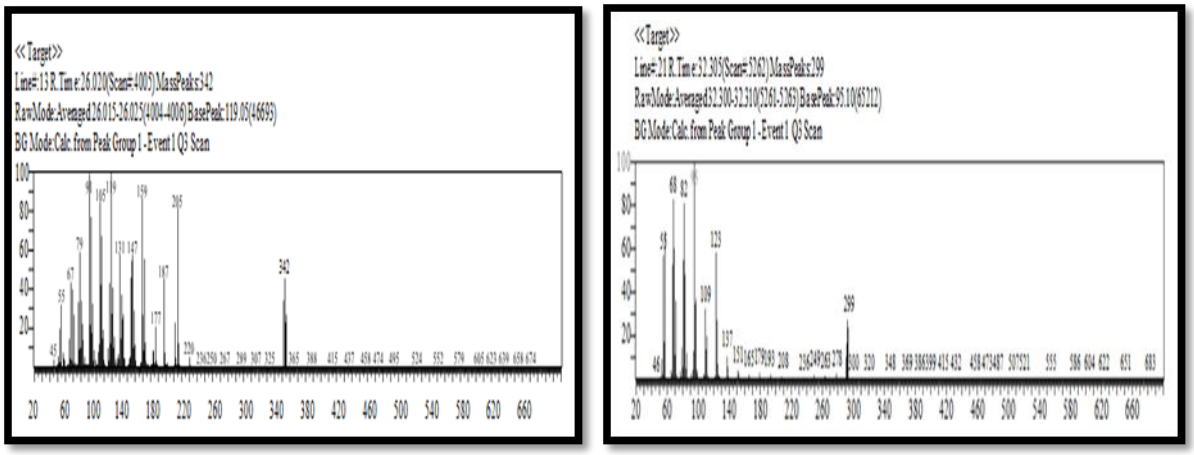
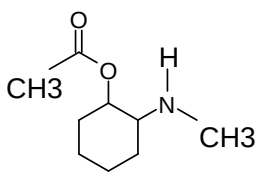


Fig No. 1.6: GCMS spectra of chloroform fraction II & Methanol Fraction I

Sr .No	Structure	Mass Peak (M/Z)
1.		**154**

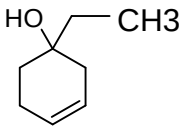
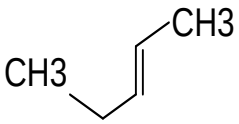
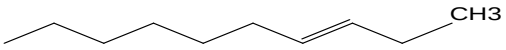
2.		**121**
3.		**68**
4.		**134**
5.		**161**

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

PHARAMACOLOGICAL SCREENING:
IN VITRO PUTATIVE ANTI HIV ACTIVITY
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	1 mg/ml	0.009	0.008	0.087	0.034	40.35
4	Choloroform Fraction II	1 mg/ml	0.003	0.004	0.009	0.005	91.22

Table 1.10: Anti HIV activity by pepsin-inhibition assay

All the extracts of **Artemisia pallens** showed significant anti-HIV activity. According to the results the standard pepstatin shows 89.47% inhibition while the methanol and chloroform extract shows 40.35 and 91.22 % inhibition respectively.

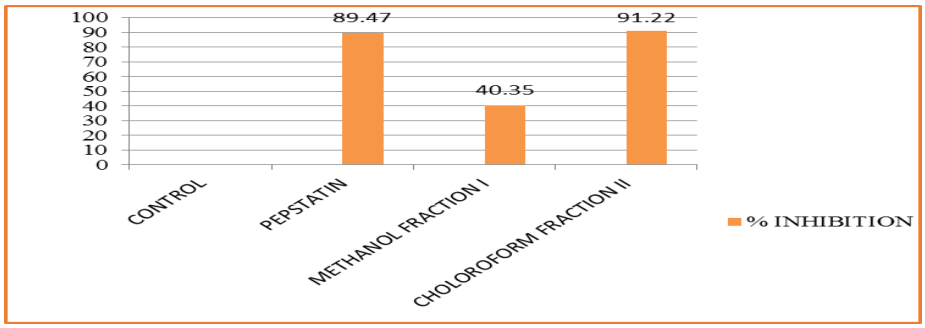


Fig No.1.7: Graphical representation of anti HIV activity by pepsin-inhibition assay

IN VITRO ANTI-INFLAMMATORY ACTIVITY:
PROTEIN DENATURATION METHOD:

Compounds	Conc.	O.D.	Mean	% Inhibition
Control	-	1.96	1.90	-
		1.98		
		1.78		
Standard (Diclofenac Sodium)	1mg/ml	0.50	0.48	74.73
		0.46		
		0.48		
Methanol Fraction I	1mg/ml	0.90	0.93	51.50
		0.94		
		0.95		
Choloroform Fraction II	1mg/ml	1.29	1.31	31.05
		1.35		
		1.30		

Table 1.11: Anti-inflammatory activity of different formulation 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.

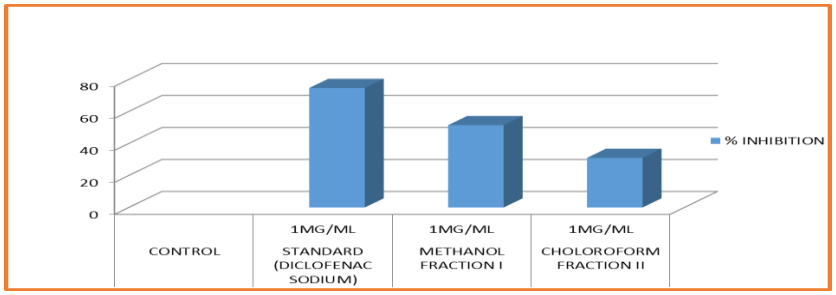
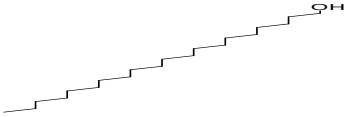


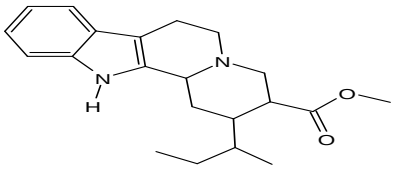
Fig 1.8: Graphical representation of Anti-inflammatory activity of different formulation by Protein denaturation method

Conclusion:

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 1-eicosanol,



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

From above study it is concluded that the **Artemisia pallens** shows significant 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 molecular 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.

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