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Extraction, Isolation And Pharmacognostical Characterization Of Components From Artemisia Vestita Wall Ex Besser

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Abstract

The aim of present study "Extraction, Isolation and Pharmacognostical characterization of components from Artemisia vestita Wall ex Besser". The whole plant collected from wild forest of 'Kotgarh' HP (India). Collected plant authenticated at YS Parmar University of Horticulture and forestry, Solan (HP) and submitted sample linked to UHF-Herbarium no. 13916. Ash values determination for total ash, water soluble and acid insoluble ash were calculated, Triplet study of plant material done for Loss on drying (LOD) and extractive value calculated for Distilled water, Methanol, Chloroform and Petroleum ether respectively. 10.95g and 2.36g crude extract obtained for Distilled water and methanol solvents by soxhlet extraction. Hydrodistillation method performed for extraction of volatile from dried powdered drug. Results concluded after pharmacognostical characterization, phytochemical, spectroscopical analysis specify purity of the plant material and Distilled water and methanol as suitable solvent from which artemisinin and thujon identified as main components and presence of flavonoids, terpenoids, alkaloids, volatile oil.

Key Words Artemisia vestita, Physicochemical, TLC, Spectroscopical screening, Artemisinin, Thujon

1. Introduction

Artemisia is a large diverse genus of plant with near 500 species belonging to family Asteraceae. Various species from the genus commonly known as mugwort, wormwood and sagebrush. The genera mainly distributed in the northern temperate regions of the world. Different species of Artemisia have been used in various treatments since ancient time as folklore medicines [1]. Different species of plant are commonly used in the treatment of gastrointestinal problems, in anorexia, indigestion, antispasmolytic, antirheumatic, in bronchitis, asthma, antibacterial, antifungal, antimalarial, in cold and cough and many more [2].

Artemisia generally known to have broad spectrum of bioactivity, because of the presence of several active ingredients, which act by different modes of action. Artemisia spp. are known to possess varieties of chemical components like essential oils, alkaloids, steroids, carbohydrates and many other. Their essential oil components mostly distilled from their flower, roots and other aerial parts mainly contain terpenoids, phenylpropanoids and other aliphatic compounds [2]. The essential oils of Artemisia annua aerial parts has been reported for the presence of germacrene, camphor, β-selinene, β- caryophyllene and artemisia ketones [3]. The volatile oil from A. absinthium has been reported for predominance of monoterpene hydrocarbon, in which chamazulene was mainly identified [4]. Two types of flavonoids namely apigenin and luteolin have been identified and reported from A. annua by using TLC method [4]. Also, the two major component from the essential oils of Artemisia vestita namely grandisol and 1,8- cineol have been reported for their antibacterial activity [2]. Other reported volatile oils components from Artemisia vestita includes caryophyllene, germacrene D, himachalol, allohimachalol, α-,γ- atlantone, yomogi alcohol, artemisia and santolina alcohol and their acetate, thujones and thujonols, borneol, ρ- cymene, α-phellandrene [5,6]. The presence and percentage of essential oils and other chemical components in any crude drug and indigenous drugs depends upon collection and processing of that drug which specify the purity of a drug. Moreover, the purity of a drug can be confirmed by certain standardization processess like pharmacognostical, phytochemical screening and also by chromatography and spectroscopical methods. Hence, the present study is the physicochemical, phytochemical and spectroscopical evaluation of Artemisia vestita Wall. ex Besser.

2. Experimental

The plant material of Artemisia vestita Wall ex Besser, was collected from the wild source from forest of Kotgarh of district Shimla of Himachal Pradesh (India). The collected plant was authenticated in Dr. YS Parmar University, Solan HP and linked to UHF-Herbarium no 13916. The collected plant material was shade dried and stored in moisture free area.

2.1 Physicochemical evaluation

2.1.1 Ash values [7-9]

Total ash- About 5g of plant powder was weighed and kept in silica crucible, the plant material was incinerated in muffle furnace at 800^{o}C till the white ash obtain. After completion, the crucible was removed from the furnace cooled and kept in desiccator. The obtained ash was then weighed and the percentage yield was calculated as total ash %w/w.

Water soluble ash- Total ash obtained from the above procedure was boiled in 25ml water for 5 minutes and filtered. The insoluble matters left was kept on the ashless filter paper was washed with hot water, ignite in tarred crucible cooled and kept in a desiccator the residue was weighed and calculated and tabulated as water ash value (Table-1).

Acid insoluble ash- Total ash obtained from the above procedure was boiled in 25ml of dilute hydrochloric acid for 5 minutes and filtered. The insoluble matters left was kept on the ashless filter paper was washed with hot water, ignite in tarred crucible cooled and kept in a desiccator the residue was weighed and calculated and tabulated as acid insoluble ash value (Table-1).

2.1.2 Loss on drying

About 5g of drug accurately weighed in different petri dishes and kept in a hot air oven at 110^{o}c for four hours after cooling in a desiccator, the losses in weight are recorded for all dishes this procedure is repeated till constant weight is obtained (Table-2).

Loss on drying (%) = loss in weight×100/W

W= weight of drug in gram (g)

2.1.3 Determination of extractive values

Preparation of the extracts [7]

The extract was prepared by cold expression method (Table-3). Total 5g of powdered crude drug of Artemisia vestita dried plant was macerate with each of 100ml of methanol, water, chloroform, petroleum ether separately in a closed flask for 24 hours. The flasks were frequently shaken for first 6 hours and then allowed to stand for next 18 hrs. Filter each extract and take 25ml of filtrate in china dish and dried at 105 ^{o}C till dryness and weighed. The percentage of extractive values were calculated for each extract with reference to air dried drug (Table-4)

3. Extraction

3.1 Extraction of phytoconstituents [10]

Total 25g coarsely powdered plant material was filled in thimble of Whatman filter paper and kept in soxhlet apparatus of 250 ml capacity. All parts of apparatus viz. 250 ml round bottom flask, soxhlet apparatus and condenser was fixed on heating mantle. Distilled water and methanol as solvent were run down through thimble to flask for two to three times. Temperature was adjusted at 55 ^{o}C and continued till complete extraction of plant materials. The starting of percolating down of clear solvent to round bottom flask is the indication of completion of extraction. The extract from round bottom flask was concentrated to semisolid and kept intact for further use.

3.2 Extraction of Volatile oil

The extraction of volatile oil was performed by the process of hydrodistillation [11]. Total 30g of the powdered plant material of Artemisia vestita was taken in 250ml of round bottom flask and filled the flask with water to sink the drug completely. The clevenger apparatus assembly was fixed on heating mantle and the temperature was adjusted at 55 ^{o}C. The extraction was continued for 4 hours and the amount of volatile oil obtained was noted and collected in 10ml beaker. The covered beaker was kept intactly for further use.

4. TLC (Thin Layer Chromatography (TLC) [12]

Thin layer chromatography was performed by using silica gel G plate. The volatile oil from the hydrodistillation was used as sample. One drop of volatile oil was mixed with nine drops of chloroform (1:9) to prevent the evaporation of oil sample. Two spots of sample were applied 2cm above from the bottom of TLC plate and near 1cm apart from each other, Toluene: Ethyl acetate (93:7) was used as solvent system for elution. After elution the TLC plates were removed from the chromatogram and the solvent front was marked. The plates were air dried and kept in iodine chamber to visualize the eluted components on the plate surface. The visualized spots were marked at their centre and retention factor (Rf) were calculated (Table-6, Figure 7).

Similarly, the Rf values for chloroform and aqueous extracts were calculated by spotting aqueous and chloroform extract and eluted by using Toluene: ethyl acetate in the ratio of 85:15 ml. The spots were visualized in iodine vapour and Rf values were calculated for all visualized spots (Table-7, Figure 8). Also, the Rf values for petroleum ether and alcoholic extract were performed (Table-8, Figure 9) Calculated Rf values were compared with Ayurvedic Pharmacopoea of India and the Rf values reported in literature.

5. Preliminary Phytochemical Screening

5.1 Tests for Alkaloids.

Dragendorff's test: Add few drops of Dragendorff's reagent to 2-3ml of filtrate. Orange brown ppt. is formed.

Hager's test: Add few drops of Hager's reagent to 2-3ml of filtrate. Yellow ppt. is formed.

Wagner's test: Add few drops of Wagner's reagent to 2-3ml of filtrate. Reddish brown ppt. is formed.

5.2 Tests for Tannins and Phenolic compounds.

5% $FeCl_3$ solution: Add few drops of reagent to 2-3ml of filtrate to give deep blue black color.

Gelatin solution: Add few drops of reagent to 2-3ml of filtrate to give white ppt.

Lead acetate solution: Add few drops of reagent to 2-3ml of filtrate to give white ppt.

Dilute HNO_3: Add few drops of reagent to 2-3ml of filtrate to give reddish to yellow color.

5.3 Tests for Proteins.

Biuret test: To 3 ml of extract add 4% NaOH and few drops of 1% $CuSO_4$ solution. Appearance of pink or yellow color.

Millon's test: Mix 5ml millon reagent with 3ml of extract which give white ppt. which turns brick red or get dissolves to give solution of red color.

5.4 Tests for Amino Acids

Ninhydrin test: 3 drops of 5% Ninhydrin solution with 3ml of extract for 10 minutes heat in boiling water bath gives purple or bluish color.

Tyrosine test: Appearance of dark red color while heating 3ml of extract with 3 drops of Millon's reagent.

5.5 Tests for Steroid

Salkowski reaction: Add 2ml of chloroform and conc. H_2SO_4 to 2ml extract. Shake and then appearance of red for chloroform and greenish yellow shows acid layer.

5.6 Tests for Glycosides

Foam test for Saponin Glycosides: Shake vigorously extract or powder of drug with water. Foam observed.

Borntrager's test for Anthraquinone Glycosides: Add dil. H_2SO_4 to 3ml extract, then boil and filter. Add equal volume of chloroform to cold filtrate. Shake the separate organic solvent add ammonia appearance of pink or red color layer.

5. 7 Test for Flavonoids

Shinoda Test: To small quantity of residue, add lead acetate solution. Yellow coloured precipitate is formed.

5. 8 Test for Carbohydrates

Fehling's Test: 1ml Fehling A and 1 ml Fehling B solutions mixed and boiled for 1 minute. Add equal volume of test solution. Heating in boiling water bath for 5-10 minutes gives yellow then brick red precipitate.

Benedict's test: Benedict's reagent and test solution were mixed in equal volume in test tube and then boil for 5-10minutes in water bath. Solution may appear green yellow or red.

Barfoed's Test: Barfoed's reagent and test solution were mixed in equal volume in test tube and then boil for 1-2 minutes in water bath formation of red precipitate.

Seliwanoff's test: In boiling water bath 3ml seliwanoff's reagent and 1ml test solution heated in test tube. Red colour is formed.

Iodine test: 3ml test solution and few drops of iodine solution gives blue colour and get disappears on boiling and on cooling reappears

Tannic acid Test: Test solution with 20% tannic acid gives precipitates

6. UV-Visible spectroscopy of different extracts of Artemisia vestita

UV-Visible spectroscopical analysis of different extracts of A. vestita were performed for the presence of active constituents in pure extract and their 10^{-1} dilution using UV-Vis double beam spectrophotometer (Systronoics India Limited). The spectral peaks were checked at wavelength between 190 to 800 nm.

7 Results and Discussion

7.1 Ash Values

Analysis of ash contents in crude drug is simply the burning away of organic contents, leaving inorganic minerals which in turn help to determine the physicochemical properties of the drug.

Moreover, for the determination of purity and quality of drug ash values is used, as ash contains carbonates, potassium, calcium, magnesium, phosphates etc. i.e inorganic radicals. Acid insoluble ash value is determined when sometimes silica, carbonate, calcium oxalate that are inorganic variables of the crude drug affects the 'Total ash value'. For removal of these variables, they are treated with acid as they get soluble in Hydrochloric acid. Ash values determination [8,9]

The total ash of the crude drug reveals the care taken during its preparation and the higher values of acid insoluble ash associates with the presence of silica and when the calcium oxalate contents of the drug are very high. In present study the percentage of acid insoluble ash is 0.02% very less as compared to standard value mentioned in 'Ayurvedic Pharmacopoeia of India'. Also, the obtained total ash value was calculated as 6.2% w/w which is also in the limit as given in Ayurvedic Pharmacopoeia of India (Table-1). The results of ash values here represents that the plant was collected properly and having traces of inorganic, silica and calcium oxalate as impurities. The results shows that the inorganic contents of plant is below the limits which specify the purity of powder of the plant Artemisia vestita.

7.2 Loss on drying

Loss on drying is the method to check the percentage of moisture contents in crude drugs. The determination of loss on drying in a dried drug is necessary to check the moisture contents which can spoil the drug on storage. In conjugation with suitable temperature moisture will lead to the activation of enzymes and gives suitable conditions to the proliferation of living organism. The deterioration can cause spoiling of drug as vegetative drugs contain several essential foods for bacterial proliferation.

From the above study the result indicates for the presence of very less amount of moisture contents in the powder sample which also represent the purity of collected plant material to use in experiment.

7.3 Extractive values

Extractive value is the process of quality evaluation of crude drug when they cannot be estimated by any other method. Extractive values by different solvents are used to assess purity, quality and to detect adulteration due to exhausted and incorrectly processed drug. The methanol and water soluble extracted values are in the limit as prescribed by Ayurvedic Pharmacopeia of India. Also, the water extraction of 5g powdered drug give 0.12 g of extract which is more than methanol, chloroform and petroleum ether. This result also helps for the selection of solvent for extraction of Artemisia powder (Figure 2). The results from extractive value were found to exists in the limit as specified in Ayurvedic Pharmacopoeia of India which indicate the purity of the Artemisia plant material. The extractive value obtained from water extract (Figure 1). By considering this result distilled water can be used for further extraction of dried Artemisia plant powder.

7.4 Extraction

7.4.1 Extraction of phytoconstituents.

Both the solvents were selected on the basis of percentage of extractive values. Water as solvent gave maximum 2.4% w/w of extract as compare to all other solvents. After water soluble methanol as solvent provide 0.09 % w/w of extract.

Total 10.95 g of crude extract is obtained from the distilled water extract whereas only 2.36 g of crude drug was obtained from methanolic extract. The percentage yield gave a difference of 25.8 % between both (Table-5). Again, the result underlines the decision of the selection of distilled water as a main solvent for extraction.

7.4.2 Extraction of Volatile oil

Total 0.1ml of volatile oil were recorded on the scale of clevenger apparatus (Figure 3). The percentage yield of 0.33% v/w was calculated. The volatile oil was then collected in 10ml beaker, covering with foil and stored in refrigerator for further use.

7.5 TLC Thin Layer Chromatography (TLC)

The solvent system used for the TLC of volatile oil used was toluene: ethyl acetate in the ratio of 93:7 (figure-4) for spot volatile oil sample and standard were prepared by dissolving in chloroform in the ratio of 9:1 drop (chloroform: oil). Spots of sample and standard were spotted in the diameter of about 2mm at 2cm above from the bottom of plate. Iodine vapours were used as visualising agent. The brown spots were marked and Rf values were calculated and reported (Table 6). The Rf values from sample resemble to Rf values of standard. Similarly, the Rf values for chloroform and aqueous extracts were compared by spotting aqueous and chloroform extract and standard artemisia oil and then eluted by using Toluene: ethyl acetate in the ratio of 85: 15 ml. The spots were visualized in iodine vapour and Rf values were calculated for all visualized spots (Table 7, Figure 5). Calculated Rf values were found to be similar as that for standard spot.The Rf values petroleum ether extract and methanolic extract and compared with that of standard. The results were calculated and tabulated (Table 8, Figure 6). The Rf were calculated as 0.25, 0.95 and 0.77 near to apigenin (0.24) [13] and artemisinin (0.28) [14], A. absinthium, and near to Rf value of 0.75 (API). Only one spot was visualized from standard spot of slide I for petroleum ether with Rf value 0.87 similar to Rf value of 0.86 as mentioned in API. From the methanolic extract of A. vestita sample two spots were visualized with Rf values 0.31 and 0.5 for slide I whereas only one spot is visualized for sample spot of slide II. The calculated Rf value of 0.52 resemble to Artemisinin and similar to that of standard Rf values from API and literature. (Table **9**).

7.6 Preliminary Phytochemical Screening

The results so obtained tabulated in Table-10

7.7 UV-Visible spectroscopy of different extracts of Artemisia vestita

Five peaks were observed for chloroform extract (Figure 7) out of which three were in visible region near 680nm, 460nm and 420nm and two peaks appeared in UV spectrum near 260nm and 220nm.The wavelength 260 exist near to the wavelength of thujon at 258 nm and artemisinin. The10^{-1} dilution of chloroform extract (Figure 8) gave no clear peaks.

Methanol extract in pure form gave no clear peak (Figure 9). The 10^{-1} dilution of methanol extract gave two peaks near wavelength 660nm at maximum absorbance of 0.450 (Figure 10) and near wavelength 440nm at maximum absorbance of 0.800 (Figure 10). From the petroleum ether extract single peak was observed between the wavelength of 650-700nm and at maximum absorbance of 1.220 (Figure 11). The 10^{-1}dilution of pet ether (Figure 12) gave single peak at 230-250nm at maximum absorbance of 1.220. Distilled water extract gave the peaks between the wavelength of 250-300nm at maximum absorbance of 1.200 and 1.820. In the visible region a partial single peak was observed near the wavelength of 450nm at maximum absorbance of 0.720 (Figure 13).

8. Conclusion

Our work has describing the Extraction, Isolation and Pharmacognostical characterization of components from Artemisia vestita Wall ex Besser powder and extracts. The results indicate the confirmation of purity of the plant collected from wild source. The results from the physicochemical parameters like ash values, loss on drying, extractive values exists in the specified limit. From the results of extractive values for different solvents it is clear that distilled water (DW) is the suitable solvent for the extraction of phytoconstituents from the whole plant of A. vestita. Also, Methanol can also be opt as second option as it provided extractive values after distilled water. Preliminary phytochemical screening for DW, methanolic, petroleum ether and chloroform confirms the presence of steroids and terpenoids, saponin glycosides, carbohydrates, alkaloids and volatile oil. Terpenoids (sesquiterpenoids) has been reported to possess the maximum of medicinal activities of Artemisia species like antimalarial, antibacterial, antifungal and many more. Thus, the presence of volatile oils and terpenoids in A. vestita also confirms its uses for above activities. Double beam UV-Vis spectroscopical analysis also confirm the presence of terpenoids by giving their respective peaks at a wavelength. Hence, the whole study implies that the collected plant of Artemisia vestita is evaluated as pure and valuable medicinal plant and can be used to extract medicinally important phytoconstituents and proves to be hidden herbal fortune.

Declaration of interest.

The authors are declaring no conflict of interest

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References

1. Abad J, Bedoya LM, Bermejo P.2013. Essential oil from the Asteraceae family active against multidrug resistant bacteria. Fighting multidrug resistance with herbal. Essential oils and their components. 205-221.
2. Nigam MM, Atanassova M, Mishra AP, Pezzani RHP, Devkota, Plygun S, Salehi B, Setzer WN, Rad JS.2019. Bioactive compounds and health benefits of Artemisia species. Natural product communication.1:17
3. Juteau, Massotti V, Bessiere JM, Dherbomez M, Viano J. 2002. Antibacterial and antioxidant activity of Artemisia annua essential oil.Fitoterpia. 73:532-535
4. Yang C, Hui Hu D, Feng Y.2015. Essential oil of Artemisia vestita exhibits potent in vitro and in vivo antibacterial activity: investigation of the effect of oil on biofilm formation, leakage of potassium ions and survival curve measurement. Molecular Medicine Reports. 5762-5770
5. Weyerstahl P, Kaul VK, Weirauch M, Weirauch H, Weyerstahl M.1987. Volatile constituents of Artemisia vestita oil. Planta med. 57(1): 66-72.
6. Msaada K, Salem N, Bachrouch O, Bousselmi S,Tammar AA, Sane KAS, Ammar WB, Azeiz S, Brahim AH, Hammami M, Selmi S, Limam BM.2015.Chemical composition and antioxidant and antimicrobial activity of wormwood (Artemisia absinthium L) “Essential oils and phenolics”. Journal of chemistry Vol.
7. Chaudhari RK et al, 2015, Girase NO. 2015.Determination of soluble extractive values and physicochemical studies of bark of Sesbania sesban (L) Merr. Journal of chemical and pharmaceutical research.**7**(8):657-660.
8. The Ayurvedic Pharmacopoeia of India.Government of India, Ministry of Health and family welfare, Department of Ayush New Delhi. 2008, Part I, Volume- VI, First Edition, page-77-80
9. Abubakar AR, Haque M.2020. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and bioallied sciences., 12 (1):1-10.
10. Khandelwal KR. Practical Pharmacognosy, Techniques and experiments, Nirali Prakashan, Fifth Edition.
11. Sharma D., Vashist H.2015. Hydrodistillation and comparative report of percentage yield on leaves and fruit of peels from different citrus plants of Rutaceae family. Journal of plant sciences.10(2): 75-78. DOI:10.3923/jps.2015.75.78
12. Li Cai.Thin layer chromatography. Current protocols essential Laboratory techniques. (2014). DOI: 10.1002/9780470089941.et0603s08

13. Ansari S, Siddiqui MA, Maaz M, Khan QA, Ahmad I. 2017. Physicochemical standardization and HPTLC of Artemisia absinthum, Linn collected from Khari Baoli Delhi. Indo American journal of Pharmacetical research.,**7**(2):1-8.
14. Mishra H, Mehta D, Mehta BK, Jain DC. 2014. Extraction of Artemisinin an active antimalarial phytopharmaceutical from dried leaves of Artemisia annua L., using microwaves and validated SHPTLC- visible method for its quantitative determination.
15. Gyorgyi H, Kamilla ACS. 2013. TLC-Direct bioautography for determination of antibacterial activity of Artemisia adamsii essential oil. Journal of AOAC international.96:1209.
16. Wagner H, Bladt S. Plant drug Analysis. A thin layer chromatography atlas,2nd Ed., Springer, berlin, Germany. 90-91.
17. Phadungrakwittaya R, Chotewuttakorn S, Piwtong M, Thamsermsang O, Laohapand T, Akaraserenont P.2019. Identification of Apigenin and Luteoline in Artemisia annua L. for the Quality control. Siriraj medical journal.71 (3):241-245.
18. Roberts MF, B.N.Timmermann, T.J. Mabry. 1989. .6-Methoxyflavonol from Brickellia veronifolia (compositae). Phytochemistry. 19: 127-129.

Table1. Showing percentage yield of total, acid insoluble and water soluble ash values

Sr.No.	Ash values	Drug in 'g'	Total ash obtained in 'g'	Test % w/wage value	Standard value from API
1.	Total Ash	5	0.310	6.2	Note more than 14 %
2.	Acid insoluble ash	5	0.80	0.02	Not more than 7%
3.	Water soluble ash	5	0.001	1.6	------

API = Ayurvedic Pharmacopoeia of India

Table 2. Loss on drying (LOD) at 110°c in four hours

Plate. no.	Total amount of dried drug (in g)	Weight of drug after drying (in g)	Loss on drying (in g)	% of LOD (in %w/w)
1.	3g	2.410	0.59	0.59/3×100 = 19.66
2.	3g	2.409	0.591	0.591/3×100 = 19.7
3.	3g	2.410	0.59	0.59/3×100 = 19.66

Table 3. Colour of Artemisia vestita extracts during maceration with different solvents.

Sr. No.	Solvents	Colour
1.	Methanol	Emerald green
2.	Water	Brownish
3.	Chloroform	Dark green
4.	Petroleum ether	Light green

Table 4. Extractive Values of methanolic, water, chloroform and petroleum ether extract.

Sr. No.	Solvent	China dish wt. (in g)	Amount of drug in g	China dish + drug wt. (in g) After drying	Remained drug (in g)	% w/w	Standard value API
1	Methanol	80.410	5	80.500	0.09	0.09/5×100=1.8	Not more than 5%
2	Water	55.570	5	55.690	0.12	0.12/5×100=2.4	Not more than 11
3	Chloroform	59.310	5	59.350	0.04	0.04/5×100= 0.8	--------
4	Pet. Ether	73.470	5	73.490	0.02	0.02/5×100=0.4	---------

Table 5. Showing total amount and percentage of crude extract from different solvents at same time interval.

Sr.No.	Name of extract	Amount of plant powder taken in gram (g)	Time of extraction	Amount of crude extract in gram (g)	Percentage yield(% w/w)
1.	Distilled Water	25	16 hrs	10.95	43.8
2.	Methanolic	25	16 hrs	4.50	18

Table 6. **Showing Rf values for Artemisia vestita volatile oil and standard Artemisia oil.**

	Slide 1	Rf value	Slide 2	Rf value
Solvent front	9.5		11.5	
Sample	8	0.84	10.8	0.93

Standard	8.3	0.87	11	0.95

Table 7. Rf values for chloroform and aqueous extract after visualising in iodine vapour

	Chloroform extract				Aqueous extract			
Solvent front	8.5 (slide 1)				10 (Slide II)			
	Sample A	Rf value	Standard	Rf value	Sample A	Rf value	Standard	Rf value
Spot 1	2.5	0.29	2.6	0.30	2.3	0.23	2.4	0.24
Spot 2	6.6	0.77	6.7	0.78	3.2	0.32	3.4	0.34
Spot 3	8.3	0.97	8.2	0.96	4.7	0.47	4.6	0.46

Toluene: ethyl acetate (85:15) was used as solvent system for elution

Table 8. Rf values for Petroleum ether and alcohol extract after visualizing in iodine vapour.

	Petroleum Extract				Alcoholic extract			
Solvent font	8 (Slide 1)				8 (Slide II)			
	Sample A	Rf value	Standard	Rf values	Sample A	Rf values	Standard	Rf values
Spot 1	2	0.25	7	0.87	2.5	0.31	4.2	0.52
Spot 2	7.6	0.95	--	--	4	0.5	--	--
Spot 3	6.2	0.77	--	--	--	--	--	--

Table 9. Rf Values of Artemisia species Ayurvedic Pharmacopoea of India and from literature

Visualizing Method	Ayurvedic Pharmacopoeia of India [API]	Visualizing Methods	Literature Rf values [15]	References [15-18]
UV at 366nm	0.47,0.64,0.70 and 0.82	By GC and	0.56	Thujon,

On exposure to Iodine Vapour	0.28,0.31,0.41,0.55, 0.75 and 0.86	GC-MS	0.45, 0.15 to 0.45, 0.37 0.33	1,8 – cineol, Terpene zone, Terpene-4-ol components, Linalool
In white light after derivatization From A. nilagirica leaf volatile oil (Shakila et al, 2013)	0.31 (Blue spot) 0.38 (Blue spot) 0.57 (Purple) 0.69 (Blue spot) 0.85 (Purple)		0.52 0.67 (Singh et al) 0.326 0.486 0.24,0.21,0.09 0.10,0.13,0.15 0.82	Artemisinin Artemisinin (brown spot) Artemisinic acid Alpha-thujone Apigenin Luteolin. Artemisinin (using BuOH:H_2O:OHAc)

Table 10. Showing Preliminary phytochemical screening for different solvents extract

S.No.	Constituents	Tests	Aqueous Extract	Alcoholic Extract	Chloroform Extract	Petroleum ether Extract
1.	Carbohydratee	Fehling's test	+	+	+	-
		Benedict's test	+	+	+	+
		Barfoed's test	-	-	-	-
		Seliwanoff's test	+	+	-	-
2.	Non- Reducing Polysaccharides (Starch)	Iodine test	-	-	-	-
		Tannic acid test for starch	-	+	+	+
3.	Proteins	Biuret test	+	-	-	-
		Millon's test	+		-	-
4.	Amino acids	Ninhydrin test	+	+	-	-
		Tyrosine test	+		-	-
5.	Steroid and terpenoids	Salkowski test	+	-	+	+
6.	Glycosides	Borntrager's test	-		-	-
		Foam test	+	+	+	+
7.	Flavonoids		+	+	-	-
8.	Alkaloids	Dragendorff's test	+	+	+	-

		Hager's test	+	+	+	-
		Wagner's test	+	+	+	+
9.	Tannins and Phenolic compounds	5% $FeCl_3$ solution	+	+	-	-
		Lead acetate solution	+	+	+	+
		Gelatin solution	+	+	-	-
		Dilute HNO_3	+	+	-	-
10.	Volatile oil		+			

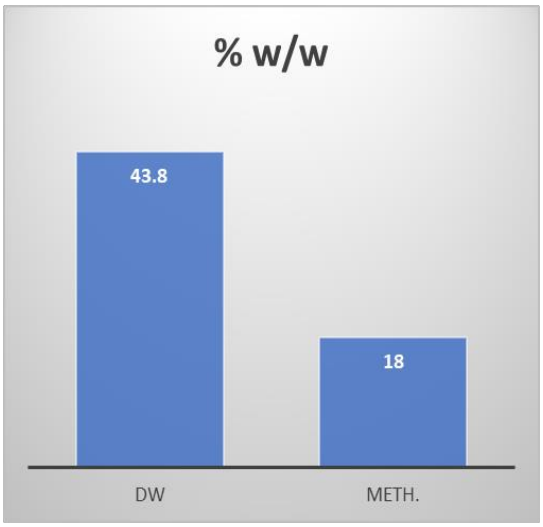


Figure 1. Percentage of distilled water and methanolic extract of Artemisia vestita.

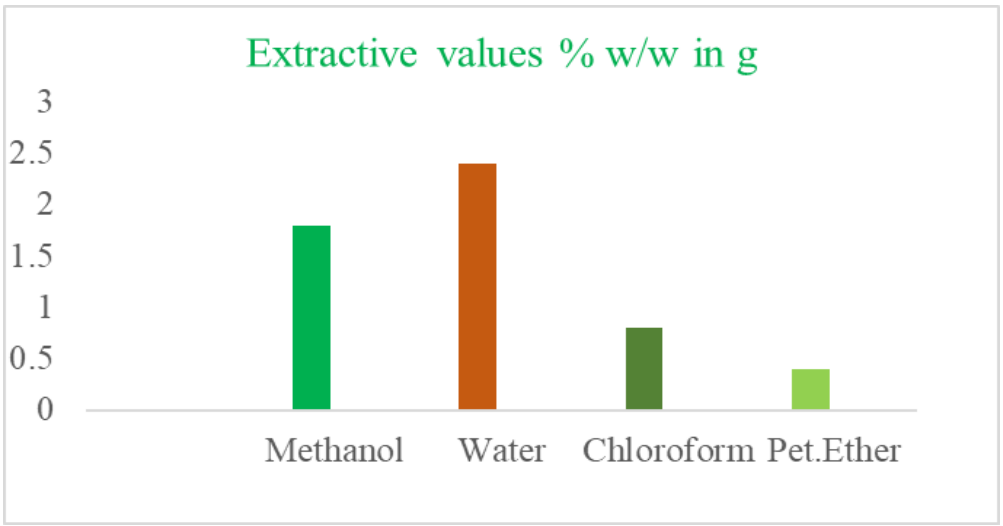


Figure 2. showing percentage of extractive values w/w in g.

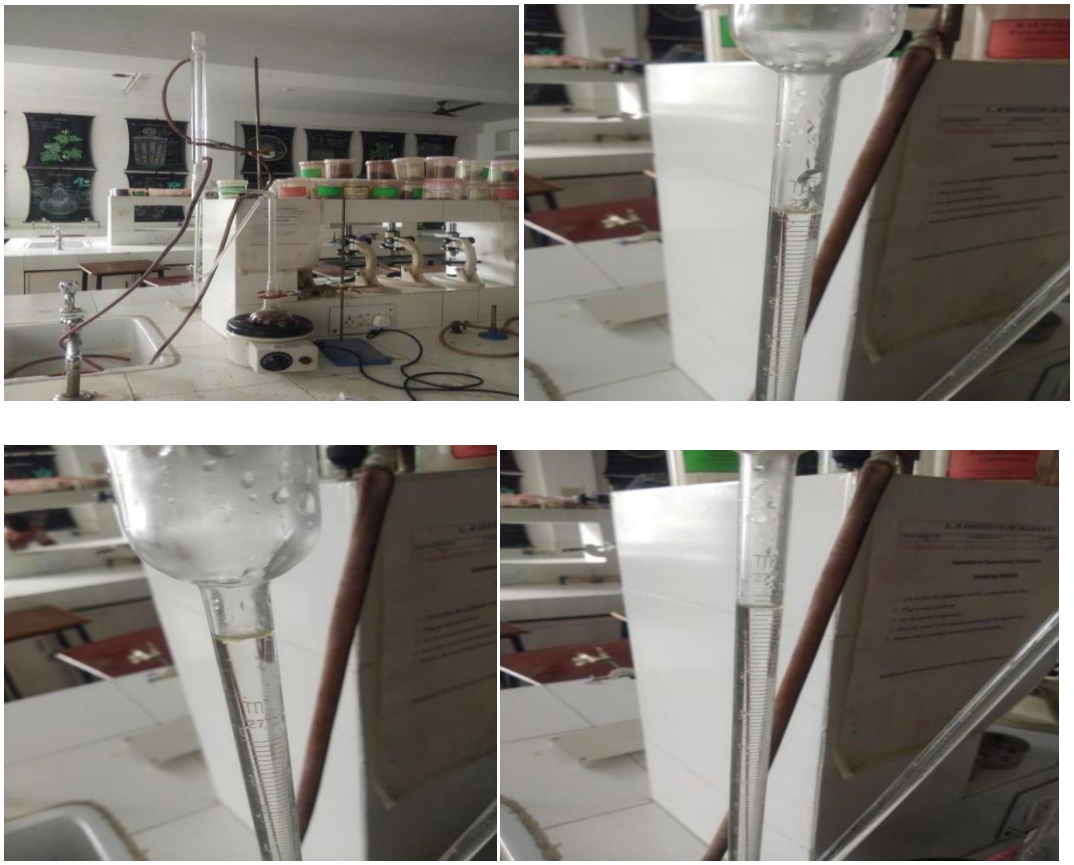
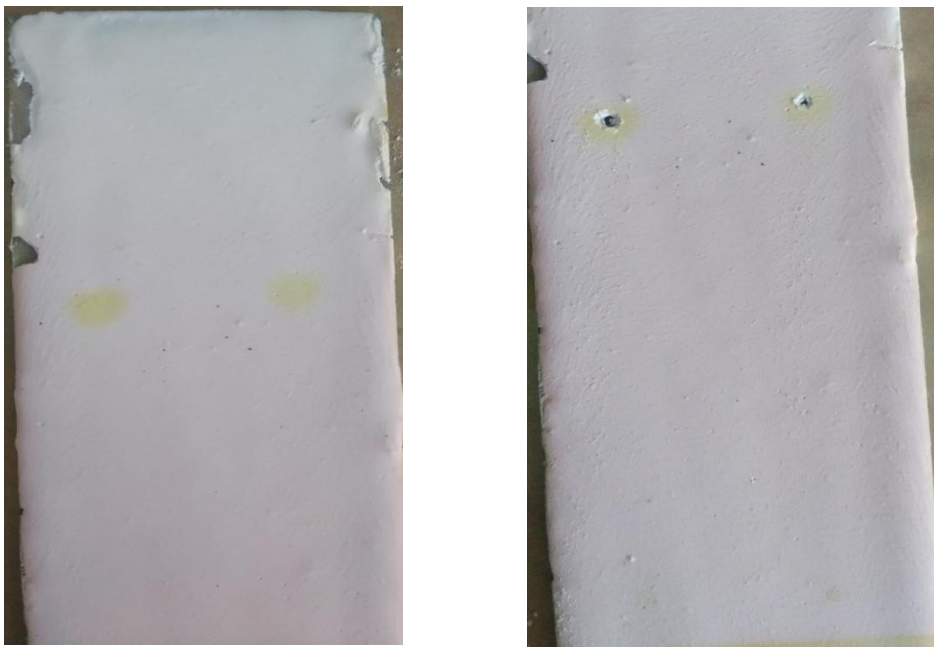


Figure 3. Showing hydrodistillation of powdered whole plant of Artemisia vestita powder



1. **2.**

Figure 4. TLC plates of volatile oils of Artemisia vestita after elution in toluene: ethyl acetate (93:7)

Figure 5. Showing TLC plates for Chloroform and Aqueous extract from Artemisia vestita

Figure 6. Showing TLC for (I) Petroleum ether (left) and (II) Methanolic(right) extract of A. vestita

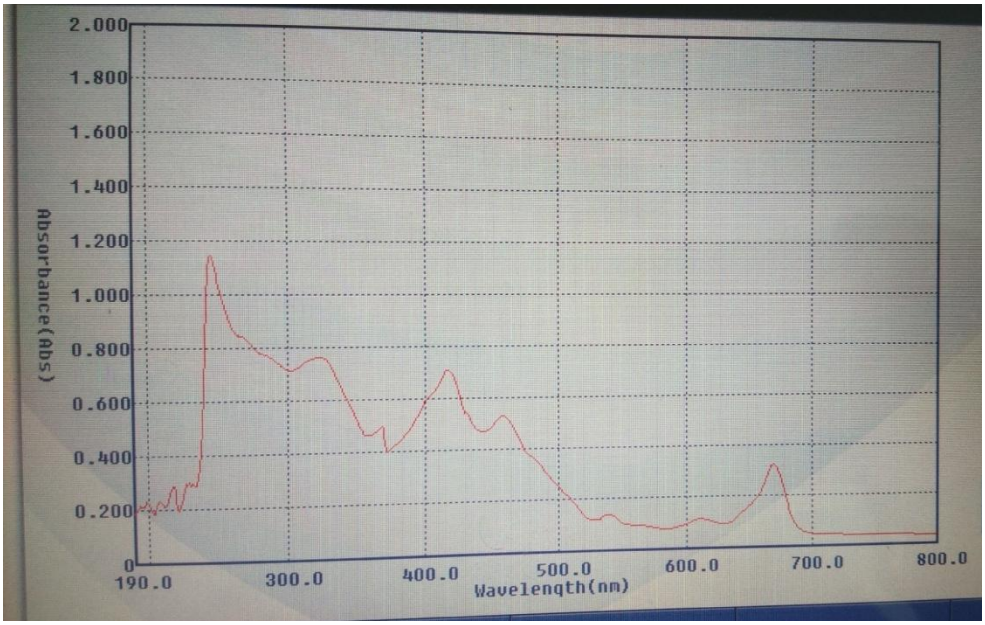


Figure 7. UV-visible spectroscopy of chloroform extract of Artemisia vestita

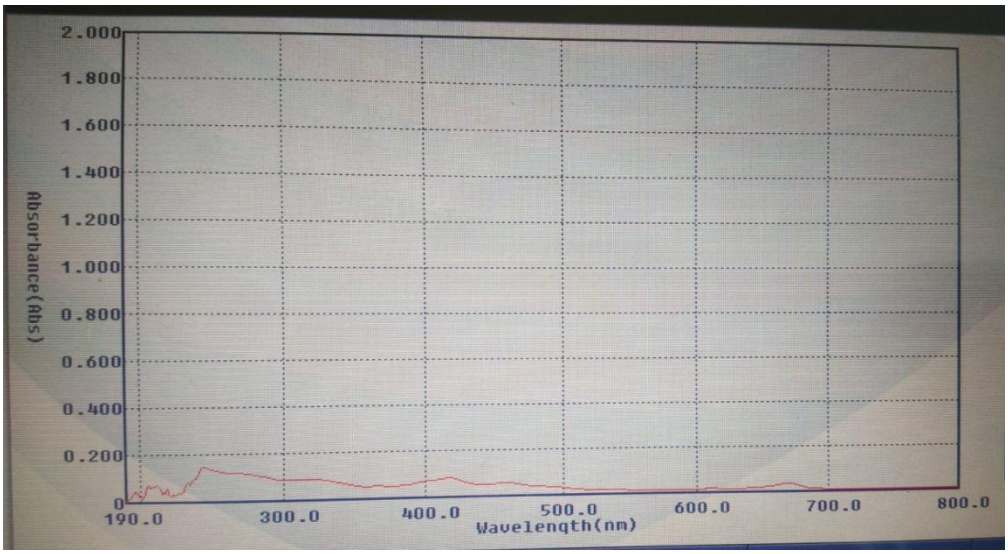


Figure 8. UV-visible spectroscopy of Chloroform 1 extract 10^{-1} dilution of Artemisia vestita

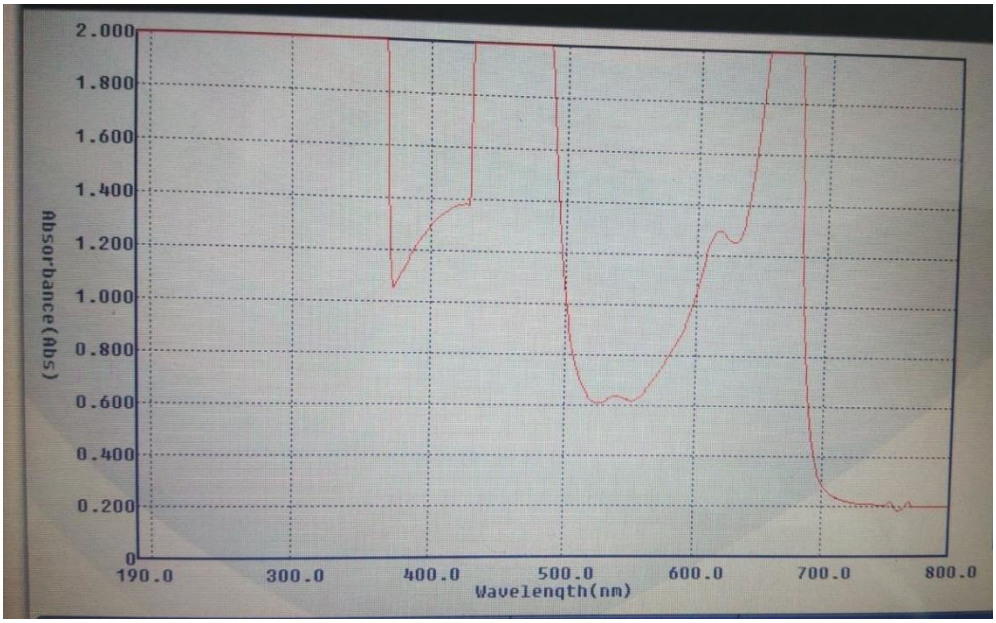


Figure 9. UV-visible spectroscopy of Methanol extract of Artemisia vestita

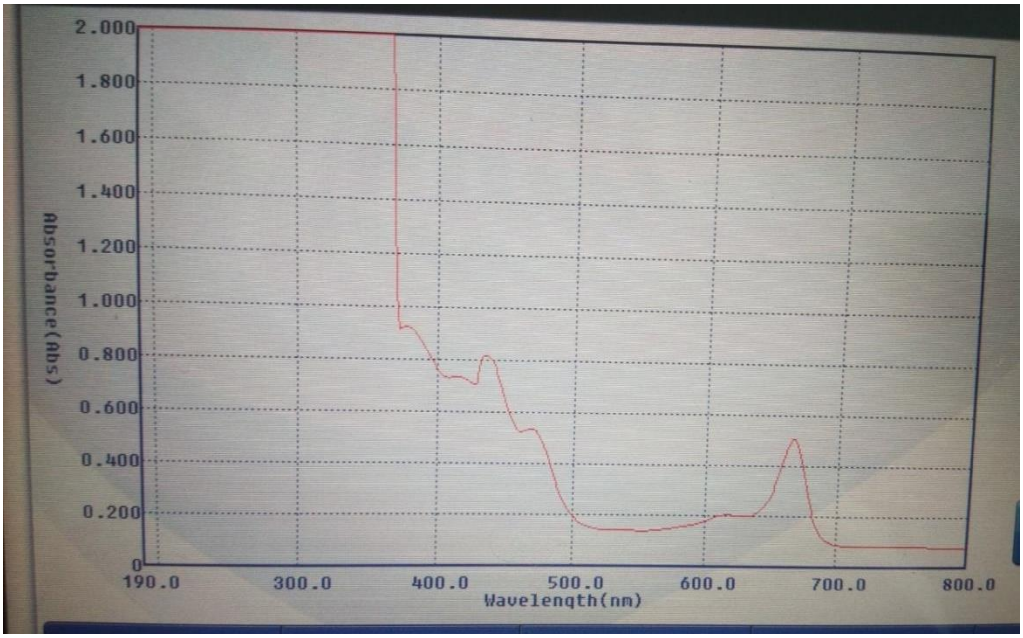


Figure 10. UV-visible spectroscopy of Methanol extract 10^{-1} of Artemisia vestita

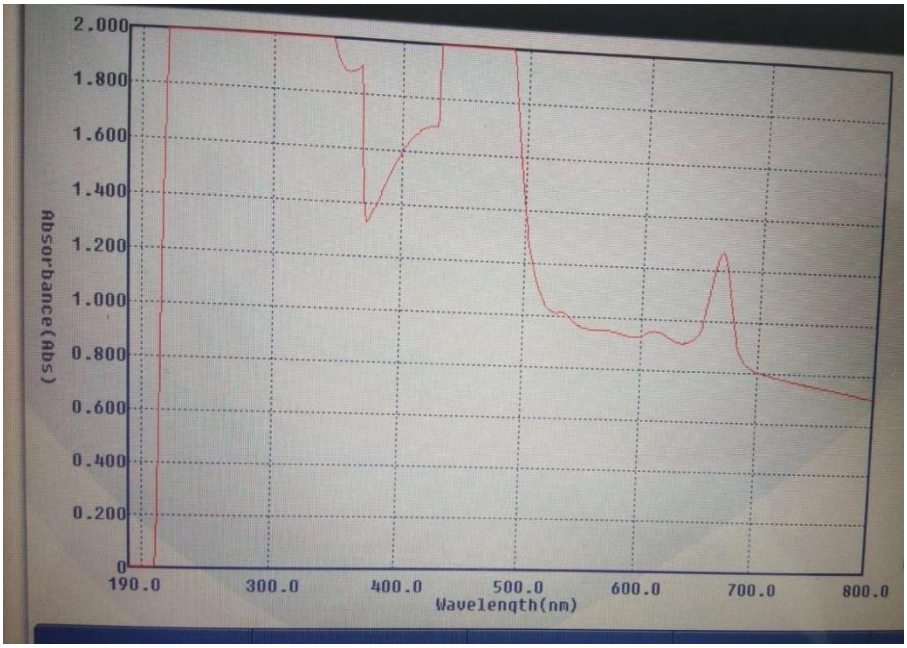


Figure 11. UV-visible spectroscopy of Petroleum ether extract of Artemisia vestita

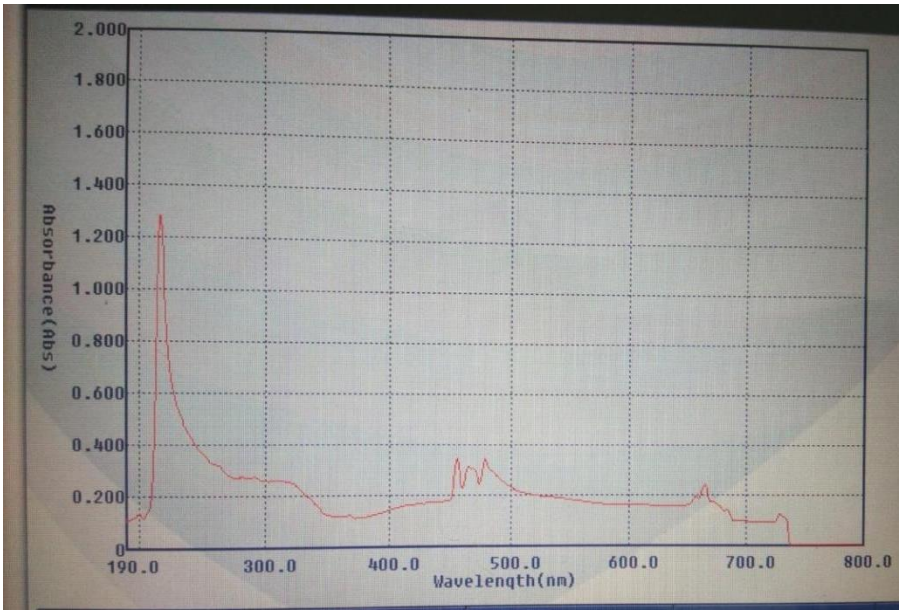


Figure 12. UV-visible spectroscopy of Petroleum ether extract 10^{-1} dilution of Artemisia vestita

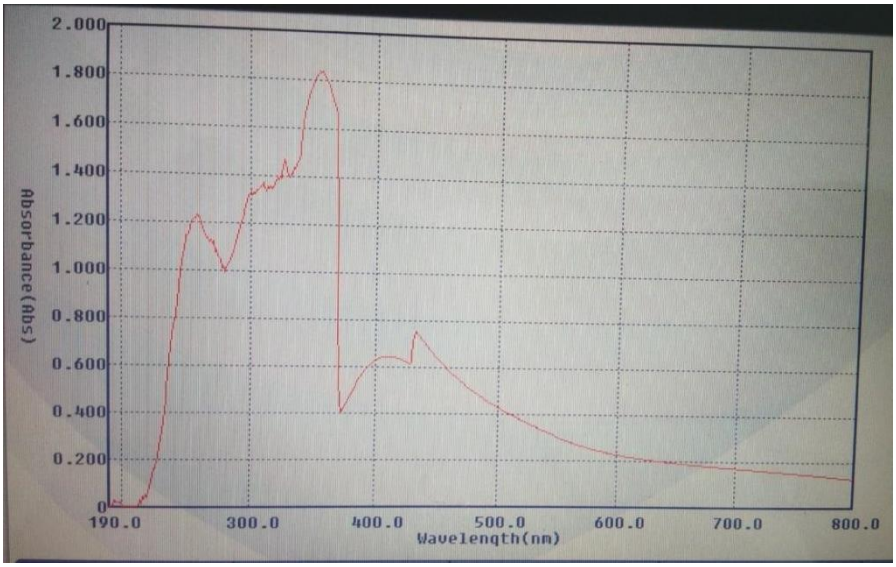


Figure 13. UV-visible spectroscopy of Distilled water extract of Artemisia vestita