ejpmr, 2021,8(12), 332-340

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ISOLATION AND CHARACTERIZATION OF CONSTITUENT FROM *PARTHENIUM HYSTEROPHOROUS*

Mandeep Singh Chhina*, Dr. Manbir Kaur, Charanjit Kaur, Dr. Prabh Simran Singh and Sukhmanpreet Singh

India.

***Corresponding Author: Mandeep Singh Chhina**

India.

Article Received on 29/09/2021 **Article Revised on 20/10/2021** **Article Accepted on 10/11/2021**

ABSTRACT

Parthenium hysterophorus is an aggressive annual herbaceous weed. It is commonly known as 'altamisa', carrot grass, bitter weed, star weed, white top, wild feverfew, the "Scourge of India" and congress grass. *It* is a prolific weed belonging to *Asteraceae* family, producing thousands of small white capitul and each capitula yields five seeds on reaching maturity. It is world's most devastating and hazardous weeds. The goal of the research work is to evaluate different characteristics of *Parthenium hysterophorus* and isolate new chemical compound from methanolic extract of whole plant. The structutre of the isolated comopound was confirmed by using various spectral analytical techniques. The study revealed that based on the physical properties (crystal with white color and m.p.), steroid test and spectroscopic data (IR, NMR and MS) and comparing the data in the scientific literature; the structure of the isolated compound was confirmed to be β-sitosterol derivative. Also, the phytochemical studies show the presence of alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, carbohydrates and saponins. Being abundantly available *Parthenium hysterophorus* thus provide a vast area of research for the development of highly effective and less toxic therapeutically efficient new chemical entities. The purpose of the research is to isolate new chemical compound from *Parthenium hysterophorus* and find new pathways for its research & management so as to make it as a boon for mankind rather than a curse.

KEYWORDS: Parthenium, weed, isolation, boon.

1. INTRODUCTION

Parthenium hysterophorus is an aggressive annual herbaceous weed. It is commonly known as 'altamisa', carrot grass, bitter weed, star weed, white top, wild feverfew, the "Scourge of India" and congress grass. *It* is a prolific weed belonging to *Asteraceae* family, producing thousands of small white capitul and each capitula yields five seeds on reaching maturity. It is world's most devastating and hazardous weeds. Parthenium weed was first described in 1810 but emerged as a serious problem after 1955, when it was introduced in contaminated cereal grains.[1] Since then, it has spread like wildfire throughout India and presently occupies over 5 million hectare land.[2] It is known to cause asthma, bronchitis, dermitis and hay fever in man and livestock.

1.1 Taxonomy of *Parthenium hysterophorus.*

Table 1: Taxonomy of *Parthenium hysterophorus.*

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Asteraceae
Species	*Parthenium hysterophorus*

1.2 Chemical constituents present in plant

Isolation and structural elucidation of the active principles of *P. hysterophorus* is required to determine their chemical properties. Chemical analysis of *P. hysterophorus* has indicated that all its parts including "trichomes and pollen" contain toxins called sesquiterpene lactones (SQL).

P. hysterophorus contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic

compounds or allelochemicals are ;hysterin, ambrosin, flavonoids (such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid.). (P-hydroxy benzoin and vanillic acid), caffeic acid, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols.Parthenin, hymenin and ambrosin are found to be the culprits behind the menacing role of this weed in provoking health hazards.[3] *Parthenium hysterophorus* from different geographical regions exhibited parthenin, hymenin, coronopilin, dihydroisoparthenin, hysterin, hysterophorin and tetraneurin as the principal constituents of their sesquiterpene lactones.[4]

1.3 Health benefits and other usefull effects

It is used to treat health issues like diarrhoea, neurologic disorders, urinary tract infections dysentery, malaria and as emmenagogue.[5] Ethno-botanically, some tribes use it as remedy for inflammation, eczema, skin rashes, herpes, rheumatic pain, cold, heart trouble and gynaecological ailments. As analgesic in muscular rheumatism, therapeutic for neuralgia and as vermifuge. This weed is also reported as promising remedy against hepatic amoebiasis.

1.4 Harmful effects

Parthenium hysterophorus caused health problems like bronchitis, dermatitis, asthma and hay fever.[6] Parthenin and additional phenolic acids viz. caffeic acid, anisic acid, vanillic acid, chlorogenic acid, panisic acid and parahydroxy benzoic acids are the major components responsible for lethality to human beings and grazing animals.[7] Allergic eczematous, contact dermatitis and depression in humans coming in contact with this weed has also been witnessed.[8]

Clinical progression of *P. hysterophorus* induced dermatitis worsens with time and finally leads to chronic actinic dermatitis. Furthermore, weed's pollens in the air cause induction of allergic rhinitis also called hay fever.[9] Pollens comprised 44% of the total pollen load during June to September in weed infested areas.[10] The inhalation of pollens through breathing can cause allergic trinities and speed up the development of bronchitis or asthma.[11]

Parthenium hysterophorus also causes the formation of many reactive toxic compounds such as sesquiterpene lactones, which becomes the basis of weed dermatitis in India and USA.[12] Regular exposure to *P. hysterophorus* resulted in dermatitis in about 15% of individuals and another 7-15% developed respiratory problems.[13] Respiratory problems generally start with high fever and after 3-5 years of gradual exposure**,** respiratory problems become more severe as resulted into asthma and allergic bronchitis. [14]

1.5 Pharmacological activities

The plant extracts prepared from different parts shows Anticancer activity, Antibacterial activity, Antifungal activity, Analgesic, Folk remedy against skin diseases, Antioxidant activity, Antimalarial and Anti-inflammatory activities.[15]

2. MATERIALS AND METHODOLOGY

2.1 Materials

The chemicals used for the project were purchased from Sigma Aldrich, Qualikem, Himedia Laboratories, Rankem and Central Drug House Pvt.Ltd. Various solvents used: Chloroform, Ethanol, Methanol, Ethyl Acetate, Butanol, Hexane. All chemicals used were of analytical grade.

Plant used: The plant used for this research was collected from an open ground in Amritsar and was authenticated by HOD (Head of Department) Post Graduate Department of Botany, Khalsa college, Amritsar.

2.2 Morphology

Sample plant was evaluated for appearance, color, odour and taste. As these characteristics were judged subjectively and some of the substitutes or adulterants may closely resemble the original sample, so it is often necessary to verify the results by microscopy and physicochemical analysis.

2.3 Total ash value

2g of powdered sample was placed in a suitable tarred crucible of silica previously ignited and weighed. The powdered sample was spread evenly and weighed accurately. The materials were incinerated in muffle furnance by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator and weighed.[16]

It was calculated by using following formula:
Total ash value = Weight of total ash / Weight of crude drug taken × 100

2.4 Acid insoluble ash value

Total ash obtained was boiled for 5min with 25ml of dilute hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight.

The percentage of acid-insoluble ash was calculated by using following formula

Acid insoluble ash value = Weight of ash / Weight of crude drug taken × 100

2.5 Acid soluble ash value

It was determined by subtracting acid insoluble ash value from total ash value.

Fig. 1: Acid soluble ash value determination.

2.6 Water insoluble ash value

The total ash obtained was boiled with 25 ml water for 5 minutes and then filtered through an ash less filter paper. The filter paper was ignited in the silica crucible to a constant weight.

The water-soluble ash vale was calculated by formula given below:[17]

Water soluble ash value = Weight of ash / Weight of crude drug taken × 100

2.7 Water soluble ash value

It is determined by subtracting water insoluble ash value from total ash value.

Fig. 2: Water soluble ash value determination.

2.8 Loss on drying

Loss on dying is the loss of mass expressed as percent w/w. About 5g of drug sample was weighed accurately and dried in hot air oven at 105 ºC for 5 hrs. Percent w/w was calculated with reference to initial weight.[18]

Loss of drying (%) =Loss in weight x 100/W

W=Weight of drug in gm

Fig. 3: Loss on drying determination.

2.9 Determination of ph:

1% w/v or 10% w/v solution of both the samples were prepared in distilled water and pH was determined using digital pH meter.

2.10 Phytochemical studies[19-21]

A. Saponins: Froth Test - 0.1 g of powders were vigorously shaken with 2 ml of distilled water in a test tube for 30 sec and were left undisturbed for 20 min. Persistent froth indicates the presence of saponins.

B. Tannins: Test with Lead acetate – To 2-3 ml of aqueous extracts of the sample, 2 ml of 10% w/w solution of lead acetate was added. Formation of heavy dull yellowish precipitates indicates the presence of tannin.

C. Carbohydrates: Fehling's test - To 1 ml of ethanolic extract of sample, 1 ml of the Fehling solution (Fehling A + Fehling B) was added. The mixture was heated on boiling water bath for 5-10 min. Development of yellow precipitates, which changes to brick red precipitates indicates the presence of reducing sugars**.**

D. Alkaloids: Mayer's test - 2-3 ml filtrate added with few drops Mayer's reagent giving precipitation.

E. Flavonids: Shinoda test – 5ml of 95% ethanol/t-butyl alcohol, few drops. of HCL and 0.5g maganesium turning were added to extract. Orange, pink, red to purple colour appeared.

F. Terpenoids: About 0.5 g of samples in separate test tubes were taken with 2 ml of chloroform; 5 ml of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish brown color interface to show positive results for the presence of terpenoid.

H. Steroids: Salkowaski test - 2ml of chloroform and 2ml conc. H_2SO_4 was added to extract and shaken and observed for chloroform layer appearing red and acid layer showing greenish yellow fluorescence.

2.11 Extraction procedure

The fresh whole plant of *Parthenium hysterophorus* was washed with distilled water to remove dust particles. The shade dried whole plants were powdered. The ground fine powder (100 gm) of the whole plant was extracted with methanol (300ml) at room temperature (30ºC) for three days after defatting. The extract was filtered through Whatman No: 1 filter paper and then concentrate using rotary vacuum evaporator to get final extract. Similarly, hexane extract, chloroform extract and ethyl acetate extract were prepared.

2.12 Fraction preparation

From the prepared methanolic extract the following fractions were prepared using solvent-solvent separation method - Hexane fraction, Ethyl Acetate fraction, Butanol fraction, Chloroform fractions and Methanol fraction.

2.13 Isolation

Isolation of compounds were done using column chromatography with isocratic elution using solvents hexane: ethyle acetate. First in the ratio of 9:1 (hexane: ethyle acetate) then polarity was increases to 8:2 (hexane: ethyle acetate).

2.14 Melting point determination

Melting point of the isolated compound was determined by melting point apparatus.

2.15 Thin layer chromatography

1. Slurry of silica gel G was prepared in distilled water and poured over a glass plate to form a thin film. The prepared plates were allowed for air-drying.
2. After air-drying, the plates were kept in an oven at 100 – 120℃ (30 min) for activation. The samples were applied to the activated plates (1 cm above from the bottom). Plates were then kept in previously saturated developing chamber containing mobile phase, and allowed to run 3/4th of the height of the plate.
3. The developed plate was removed, air dried and observed under ultraviolet light and sprayed with vanillin sulphuric acid and then R_f value was calculated using following formula:

R_f = Distance traveled by solute/Distance traveled by the solvent

4. Mobile phase – Hexane : Ethyl Acetate(8:2 v/v)

2.16 Spectral analysis

The chemical structures of plant compounds must be identified, which may provide the necessary basis for further study on the bioactivities, structure-activity relationships, metabolisms in vivo, structural modification, and synthesis of the active phytochemicals.

The structural studies are often difficult to carry out with classical chemical methods, such as chemical degradation and derivative synthesis, because of the minute amount of compound isolated from plants. Therefore, spectral analysis is mainly used. Structure of the isolated compound was determined using following spectral methods:

2.17 UV spectroscopy

Absorbance maxima of the isolated compound (IC1) was determined on UV spectrophotometer using methanol as solvent. The isolated compound was dissolved in methanol and filled in a glass cuvette. These cuvettes were then placed in UV spectrophotometer to check the absorbance maxima.

2.18 Mass spectroscopy

High-resolution electrospray ionization mass spectrometry (HR–ESI– MS) measurement was carried out for the isolated compound. Mass spectra is done to determine the molecular mass of the isolated compound.

2.19 FTIR

FTIR spectrum was recorded on a Perkin spectrometer with liquid film method. The sample was dissolved in chloroform to form a film. This solution was subjected to FTIR spectrophotometer to determine the various functional groups present in the isolated compound.

2.20 NMR spectroscopy

^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX 400 MHz and 100.06 MHz spectrometer respectively. ^{1}H NMR is used to determine the hydrogen skeleton of the sample while^{13} C NMR is used to determine the carbon skeleton of the isolated compound.

2.21 DEPT

Distortionless enhancement by polarization transfer (DEPT) is an NMR method used for determining the presence of primary, secondary and tertiary carbon atoms. The sample was dissolved in solvent and subjected to DEPT studies by setting proton pulse at 135° and 90°.

3. RESULTS AND DISCUSSION

3.1 Morphology

Morphology study reveals that plant had a green color, bitter taste, intense odour and it bears flowers of white color. The average height of the plant ranges from 3-4 ft. The following results shown in table 2 were found during morphological studies of *Parthenium hysterophorus*:

Table 2: Morphology study of plant.

S.no	Parameter	Result
1	Color	Green
2	Taste	Bitter
3	Odour	Intense
4	Av. Height	3-4ft
5	Flower color	White

3.2 PHYTOCHEMICAL SCREENING

Phytochemical screening of the powder of whole plant was carried out that shows the presence of alkaloids,

steroids, flavonoids, terpenoids, phenolic compounds, carbohydrates and saponins.

The results are shown in table 3. This helps in determining the type of constituents presents in the plant.

Table 3: Phytochemical screening of plant powder.

S.no	Test	Inference
1.	Saponins	Absent
2.	Tannins	Present
3.	Alkaloids	Present
4.	Steroids	Present
5.	Flavonoids	Present
6.	Carbohydrates	Present
7.	Phenolic Compounds	Present
8.	Terpenoids	Present

Fig. 4: Phytochemical screening.

3.3 PHYSICOCHEMICAL STUDIES

Total ash value, acid soluble ash value, acid insoluble ash value, water soluble ash value, water insoluble ash value, loss on drying and pH of the powdered plant was evaluated. The results are shown in table 4.

Table 4: Results of physicochemical parameters studied for *Parthenium hysterophorus.*

S.no	Parameter	Result
1	Total Ash Value	9%
2	Acid soluble ash value	3.05%
3	Acid insoluble Ash Value	5.95%
4	Water Soluble Ash Value	3.7%
5	Water Insoluble Ash Value	6.3%
6	Loss On Drying	7.5%
7	pH	1.93

3.4 ISOLATED COMPOUND STUDY RESULTS

Compound (IC1) was isolated and various phytochemical tests were performed on it:

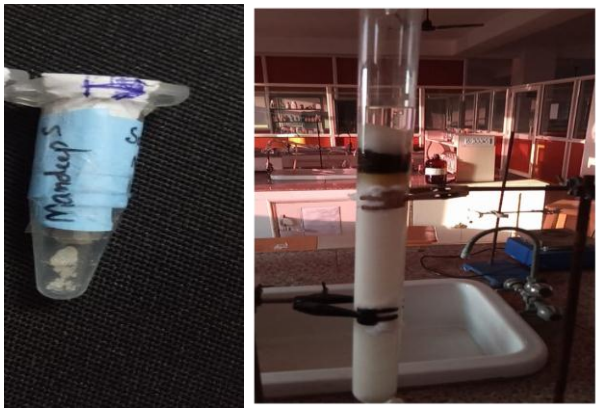

Fig. 5: Compound isolation.

3.4.1 Phytochemical study of IC1

Various phytochemical tests were performed on isolated compound which shows the presence of alkaloids, steroids, phenols and terpenoids. This helps us in confirming that the nucleus of our isolated compound is of steroid. Table 5 shows the results of phytochemical screening of isolated compound.

Table 5: Phytochemical study results of isolated compound.

S.no	Phytoconstituent	Test	Inference
1	Alkaloids	Picric acid Test	Present
2	Terpenoids	Sulphuric acid test	Present
3	Flavonoids	Ferric chloride test	Absent
4	Phenols	Ferric chloride test	Absent
5	Phytosterols	Salkowski Test	Present

3.4.2 Absorption maxima of isolated compound

U.V spectroscopy was done using methanol as solvent. It shows that absorption maxima was found at 298nm shown in fig 6.

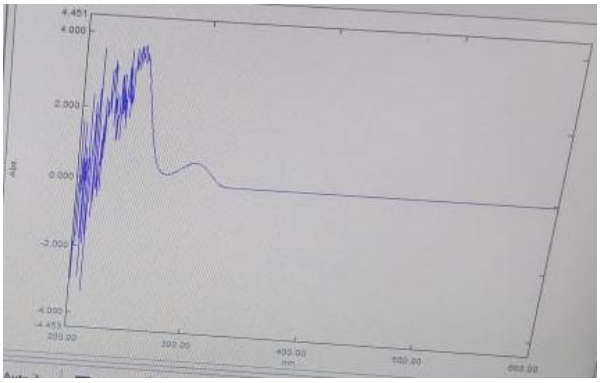

Fig. 6: UV results.

3.4.3 Melting point study

Melting point of isolated was found to be 134-136°C. using melting point apparatus.

3.4.4 TLC study

TLC study was performed for isolated compound using Hexane : Ethyl Acetate(8:2 v/v) as mobile phase. Rf value of IC1 was found to be 0.53 shown in fig 7.

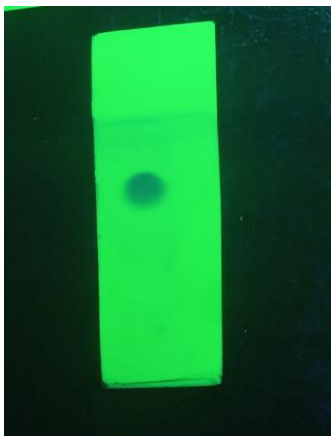

Fig. 7: TLC study of IC1.

3.4.5 Mass spectroscopy results

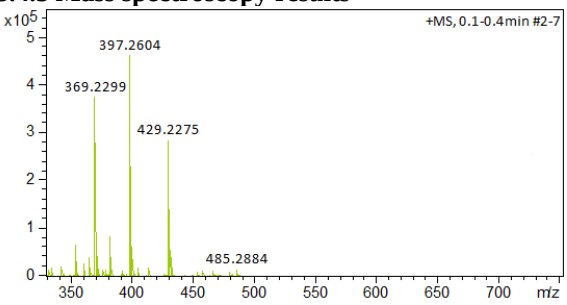

Fig. 8: Mass spectra of isolated compound.

The mass molecular ion of the compound appeared in HR–ESI–MS spectrum at m/z 485.212. This indicated that the isolated compound with molecular weight of 485.22, in good agreement with the theoretical value (calculated for $C_{32}H_{54}O_3$, 486.41). The characteristic peak was given at m/z 429.2449 that corresponds to (M-1) or loss of H. The spectrum showed the most intense peak at m/z 397.2. Other ion peak at m/z 369.22.

3.4.6 ^{13}C-NMR study results

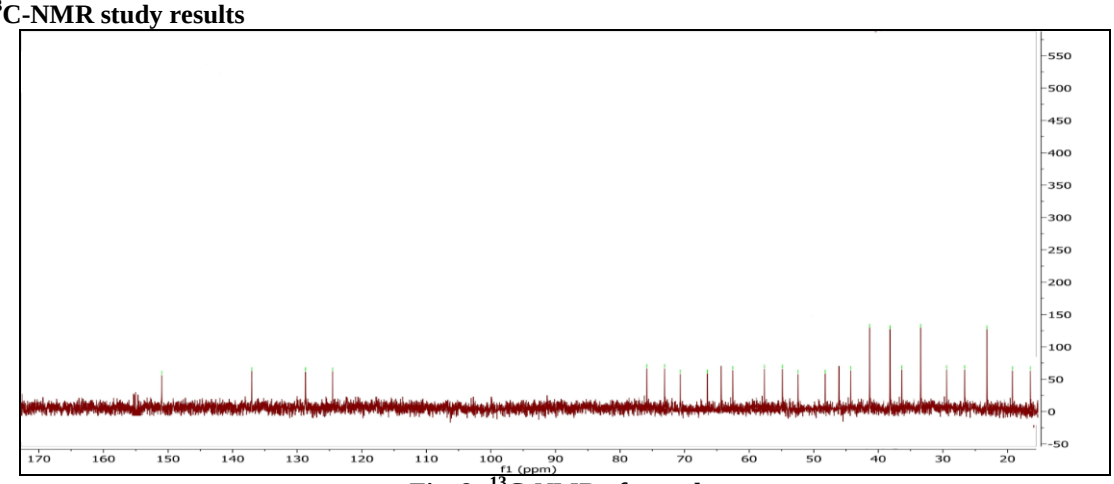

Fig. 9: ^{13}C-NMR of sample.

3.4.7 ^{1}H-NMR study results

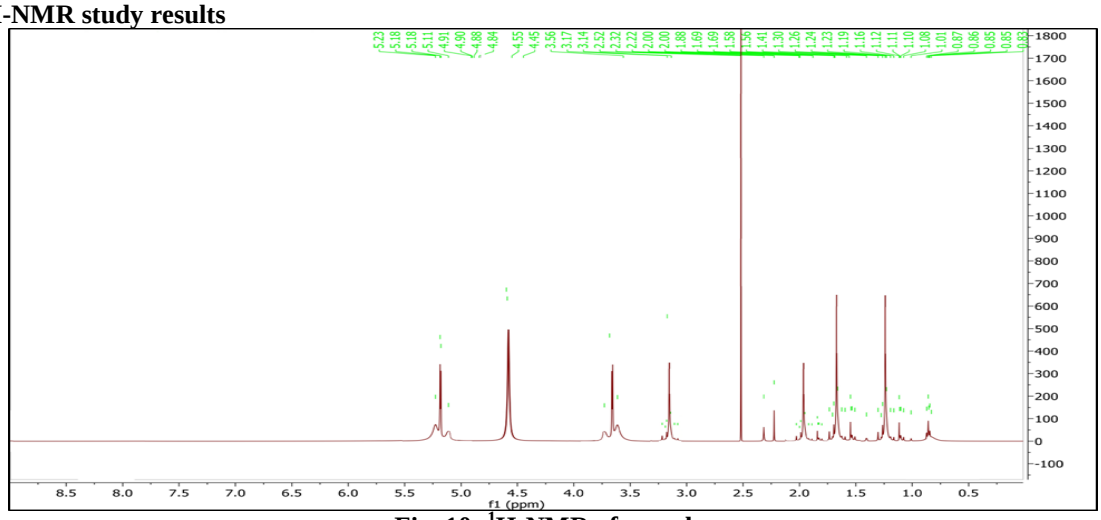

Fig. 10: ^{1}H-NMR of sample.

3.4.8 Chemical shift values of sample from ^{1}H NMR and ^{13}C NMR graphs

Table 6: Chemical shift values.

Position	Type	Chemical shift δ (ppm) value	
		^{1}H-NMR	^{13}C-NMR
1	CH_2	1.46(m)	37
2	CH_2	1.56(m)	32
3	CH(OH)	3.54(m)	72
4	CH_2	2.32(m)	42
5	QC(=)	-	140
6	CH(=)	5.37(t)	121`
7	CH_2	2.04(m)	32
8	CH	1.69(m)	32
9	CH	1.55(m)	48
10	QC	-	36.6 QC
11	CH_2	1.52(m)	21
12	CH_2	1.57(m)	40
13	QC	-	43
14	CH	1.5(m)	56
15	CH_2	1.5(m)	24
16	CH_2	1.8(m)	28
17	CH	1.4(m)	56
18	CH_3	0.7(s)	17
19	CH_3	10.3(s)	19
20	CH	1.6(m)	30
21	CH_3	0,94(d)	19.1
22	CH_2	0,93(m)	34
23	CH_2	1.15(m)	26
24	CH	1.38(m)	46
25	CH	1.57(m)	29
26	CH_3	0.84 (d)	20
27	CH_3	0.80(d)	20
28	CH_2	1.10(m)	23
29	CH_2	0.82(t)	12
-	OH	1.98(s)	-
-	C=O	-	152
-	CH_3	0.93(s)	38
-	OCH_3	3.5(s)	50

Where m is multiplet, d is doublet, s is singlet, t is triplet and QC is quaternary carbon.

3.4.9 DEPT results

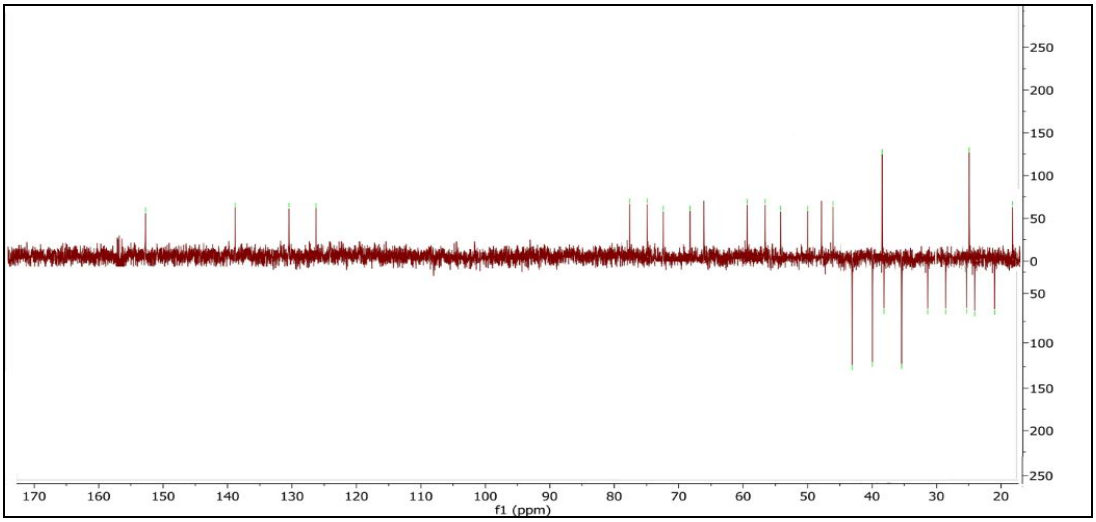

Fig. 11: DEPT spectra of sample.

The integration of ^{1}H NMR spectrum showed the presence of fifty four hydrogens: six CH_3, eleven CH_2, nine CH and one ether group. The appearance the singlets at δ 0.70 and 1.03 confirming the presence of two CH_3 attached to quaternary carbons. The appearance of the complex multiplets at δ 2.29 and 2.32 is revealed that the two CH_2 adjacent to carbon attached to ether group. The ^{13}C NMR spectrum exhibited the existence of 32 carbons.

The carbons could be classified as representing CH_3, CH_2, CH or quaternary carbon (QC) by DEPT-135. The DEPT-135 spectrum indicated the presence of 26 carbons: six peaks appeared up due CH_3 groups, nine peaks up for CH groups and peaks appeared down indicated the presence of eleven CH_2 groups. The absence of three signals in the DEPT-135 spectrum confirmed the presence of three QC atoms. In ^{13}C NMR spectrum, the recognizable signals at 140.77 and 121.73 are assigned for double bond between carbon atoms in position 5 and 6 (C5 = C6), respectively. The signal at δ 71.8 is assigned for C3 β-OH group, and the signals at δ 11.89 and 19.42 are assigned for angular methyl carbons for C19 and C18, respectively.

3.4.10 IR results

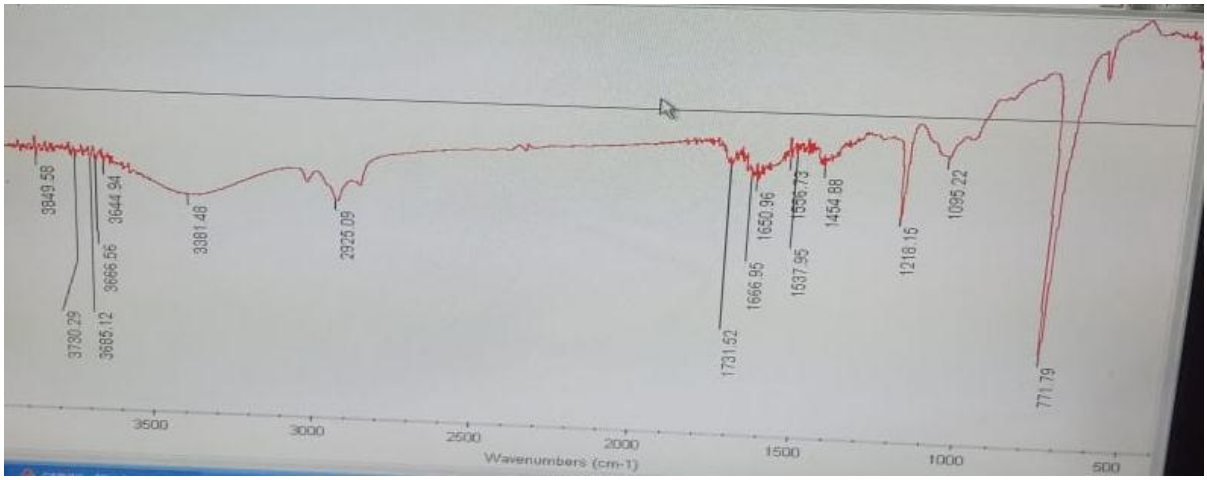

Fig. 12: IR spectra of sample.

Table 7 - Functional group with peak values.

S.no	Frequency range cm^{-1}	Functional group
1	771.79	C-H bending
2	1059.22	C-O stretching primary alcohol
3	1218.15	C-O stretching alkyl aryl ether
4	1454.88	C-H bending methyl
5	1650.96	C=C stretching conjugated alkene
6	1666.95	C=C stretching alkene
7	1731.52	C=O stretching aldehyde
8	2925.09	C-H stretching alkane
9	3381.48	O-H stretching alcohol

The IR spectral analysis reveals a broad peak at 3381 cm^{-1} for the OH group, 2925 cm^{-1} for the CH stretching, 1731.52 $^{cm-1}$ for C=O, 1666,95 cm^{-1} for the C=C group, and 1059.25 cm^{-1} for the C–O group.

The observed chemical shift values in NMR spectra are very close to values reported in the literature for β-sitosterol derivative. Based on ^{1}H NMR, ^{13}C NMR, DEPT-135 and HR–ESI–MS data, molecular formula of the isolated compound was determined to be $C_{32}H_{54}O_3$. Since the isolated compound gave positive test for steroids, all of the other structures other than steroids were rejected.

Hence, the following structure was confirmed for $C_{32}H_{54}O_3$, 486.14.

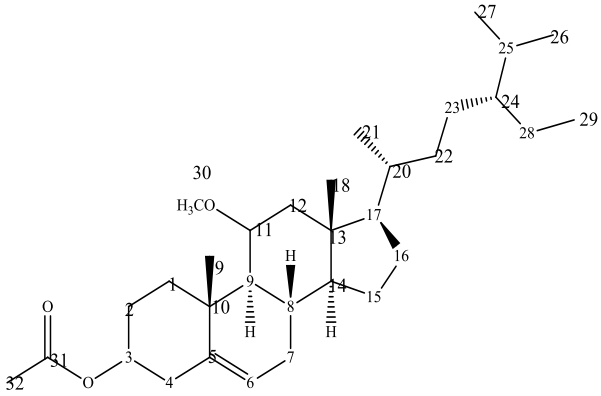

Fig. 13: Structure of isolated compound.

4. CONCLUSION

Based on the physical properties (crystal with white color and m.p.), steroid test and spectroscopic data (IR, NMR and MS) and comparing the data in the scientific literature, the structure of the isolated compound was determined to be β-sitosterol derivative.

The phytochemical studies shows the presence of alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, carbohydrates and saponins. Further *in-vivo* and *in-vitro* experiments and the isolation & identification of active compounds present in the plant should be focused to identify a potential chemical entity for clinical use in the prevention and treatment of various diseases.

Being abundantly available Parthenium hysterophorus thus provide a vast area of research for the development of highly effective and less toxic therapeutically efficient new chemical entities.

REFERENCES

1. Rao R. (Parthenium hysterophorusLinn). A new record for Indian J Bombay Nat Hist Soc., 1956; 54: 218-20.
2. Kumar M, Kumar S. (Effect of Parthenium hysterophorus ash on growth and biomass of Phaseolus mungo). Academia Arena, 2010; 2(1): 98-102.
3. Maishi AI, Ali PS, Chaghtai S, Khan G. (A proving of Parthenium hysterophorus, L). British Homoeopathic Journal, 1998; 87(1): 17-21.
4. www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=38164#
5. Gurib-Fakim A, Sewraj MD, Gueho J, Dulloo E. (Medicinal plants of Rodrigues). International Journal of Pharmacognosy, 1996; 34(1): 2-14.
6. Kololgi P, Kololgi S, Kololgi N. (Dermatologic hazards of Parthenium in human beings). Proceedings of the First International Conference on Parthenium Management, Dharwad, India, 1997; 6-9.
7. Mahadevappa M. (Ecology, distribution, menace and management of Parthenium). Proceedings of the 1st International Conference on Parthenium Management, 1997; 1-12.
8. Oudhia P, Tripathi R. (Allelopathic effects of Parthenium hysterophorus L. on kodo, mustard and problematic weeds). Proceedings of the First International Conference on Parthenium Management, University of Agricultural Sciences, Dharwad, India, 1997; 136-9.
9. Rao M, Prakash O, Rao PS. (Reaginic allergy to Parthenium pollen: evaluation by skin test and RAST). Clinical Experimental Allergy, 1985; 15(5): 449-54.
10. Seetharamiah A, Viswanath B, Rao P. (Atmospheric survey of pollen of Parthenium hysterophorus). Annals of allergy, 1981; 47(3): 192-6.
11. Evans HC. (Parthenium hysterophorus. a review of its weed status and the possibilities for biological control). Biocontrol News Information, 1997; 18: 89N-98N.
12. Towers G, Subba Rao P. (Impact of the pan-tropical weed, Parthenium hysterophorus L. on human affairs). Proceedings of the first international weed control congress, Weed Science Society of Victoria Melbourne, 1992; 2: 134-8.
13. McFadyen RC. (Biological control against parthenium weed in Australia). Crop protection, 1992; 11(5): 400-7.
14. Ayele S. (The impact of Parthenium (Parthenium hysterophorus L.) on the range ecosystem dynamics of the Jijiga rangeland, Ethiopia). Department of Animal Sciences, School of Graduate Studies, Haramaya University, 2007; 134.
15. Bezuneh TT. (Phytochemistry and antimicrobial activity of Parthenium hysterophorus L.: a review). Sci J Anal Chem., 2015; 3(3): 30-8.
16. Padmaja D, Kannappan N. (Standardization of traditionally designed neutraceutical). International Current Pharmaceutical Journal, 2012; 1(9): 288-93.
17. Subrahmanyam C. (Textbook of physical pharmaceutics). Vallabh prakashan, 2000; 223-4.
18. Johansen DA. (Plant microtechnique). McGraw-Hill Book Company, Inc., 1940; 16-63.
19. (Indian Medicinal Plants. International Book Distributors, Dehradun), 1999; 2(1): 3128.
20. Khandelwal KR. (Practical Pharmacognosy Techniques And Experimental). Nirali Prakashan, 2011; 25.3.
21. Yadav N, Dixit V. (Recent approaches in herbal drug standardization). Int J Integr Biol., 2008; 2(3): 195-203.