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Phytochemistry, GC-MS, and FTIR analysis of ethanolic extract of alfalfa (*Medicago sativa*) leaves from Cold Desert Ladakh, India

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Abstract
This study was done to examine the phytochemistry of *Medicago sativa* (Alfalfa) leaves extracted with ethanol. Alfalfa leaves were analysed for their proximate nutritional properties, qualitative, quantitative phytochemistry as well as its antioxidant properties. Identification of various compounds and their functional groups present in the extract was done using GC-MS and FTIR techniques. The results revealed alfalfa leaves have a very good nutritional property with 25% crude protein, 12.91% moisture, 4.35% crude oil and 2.13% crude fiber. Alkaloids, flavonoids, saponins, terpenoids, proteins and phenolic compounds were found in the qualitative phytochemical analysis. The quantitative analysis results showed a good amount of phenolics and flavonoids present in the plant leaves. The free radical scavenging activity by the DPPH method showed a good antioxidant property of the extract. A total of 30 compounds were identified by GC-MS analysis and the FTIR spectrum revealed the presence of amines, alkanes, alkenes, alcohols and sulfoxides.

Keywords: alfalfa, pharmaceutical property, functional groups, phytochemistry

Introduction
Alfalfa, (*Medicago sativa*) commonly known as Lucerne is a perennial blooming herb belonging to the Fabaceae family [1]. It is grown as major forage crop in number of countries throughout the globe [2]. Alfalfa is rich in protein, calcium, and high-fiber feedstuff and is broadly utilized because of its adaptability, high yield capacity and good forage quality attributes high intake and edibility, rich amino aicd profile and comparatively less fiber [3]. The Arabians claimed that feeding alfalfa to their horses made them quick and strong and they named the plant "Al-fal-fa" which means "father of all foods". It is also used by humans in many countries as a sprout and dietary supplement in the form of tablets and powders. Besides its use as forage for animals and homeopathic medicine for humans, alfalfa has been also used as a feed supplement for various farming animals such as lambs, poultry, pig farm and fisheries sector. A comprehensive literature survey shows that alfalfa constitutes flavonoids, phenolic compounds, tannins, saponins, alkaloids, phytoestrogens, amino acids, vitamins, terpenes and digestive enzymes as major classes of compounds [1, 2, 4]. It is these compounds which shows a broad range of medicinal and nutraceutical application including anti-microbial, anti-inflammatory, anti-cancer and anti-oxidant activities [5, 6]. Phenolic compounds and flavonoids are the main anti-inflammatory and antioxidant compounds as they act as free radical scavengers or metal chelators [7]. Traditionally alfalfa has been used in Ayurved and Homeopathy medication particulary for disorders related to nervous system and digestive system, as well as for number of other diseases. With recent advancement in analytical techniques, powerful tools like GC-MS and FTIR are now available to identify the phytochemical compounds and their relative concentration. The present study has been done to investigate the preliminary phytochemical analysis complemented with GC-MS and FTIR analysis and antioxidant properties of this plant to highlight the use of this plant in the nutraceutical, pharmaceutical and aquaculture industry as a nutritive supplement.

Material and methods
Plant collection: The plant alfalfa (*Medicago sativa*) has been collected from Kargil, Ladakh in August 2018. The alfalfa leaves were collected, washed with distilled water and shade dried before grounded to powder. Samples of the plant part were submitted to the Botany Department of Panjab University, Chandigarh for authentication and voucher number was acquired (21690).

Proximate composition: The nutritional value of alfalfa leaves has been evaluated by proximate analysis of dry powdered leaves [8].
Crude protein was determined by Kjeldahl method, moisture percentage by oven drying for 24 hours at 104ºC, crude oil was determined by Soxhlet apparatus, crude fiber by acid/alkali digestion, ash content by incineration at 650ºC for 4 hours in a muffle furnace and Nitrogen-free extract (NFE) was calculated by subtracting protein, lipid, fiber, moisture and ash values from 100.

Plant extraction: Alfalfa leaves were grounded to powder using an electric grinder. The powdered alfalfa leaves were extracted with ethanol using a Soxhlet extractor for 48 hours. The extracts were concentrated under a vacuum using a rotary evaporator. The semi-solid leftovers have been collected and lyophilized to remove the remaining water

contents. The sticky extract was then stored at 4ºC till further usage.

Percentage Yield: The percentage yield of the extract were estimated using the following formula: % Yield = Weight of the extract / Initial weight of sample x 100

Phytochemical analysis: Qualitative phytochemistry of ethanolic extract of alfalfa leaves were evaluated using standard procedures [9] to find out the presence of flavonoids, alkaloids, saponins, terpenoids, phenolic compounds, tannins, glycosides, amino acids, carbohydrates and fixed oils.

Quantitative phytochemistry

The total phenolic content of ethanolic extract of *Medicago sativa* leaves were determined by the standard folin-ciocalteau method [10] with slight modification by using standard gallic acid curve to calculate the total phenolics and an UV-visible spectrophotometer was used to measure the absorbance at 765 nm. Total flavonoid content was determined by an aluminum chloride complex-forming assay by measuring the absorbance at 510 nm using quercetin standard curve to calculate the flavonoid content in extract [11, 12]. The DPPH radical scavenging activity was carried out by measuring the absorbance at 517 nm using ascorbic acid as standard [13].

GC-MS analysis

The GC-MS analysis was performed on a GC (Thermo Trace 1300) system coupled with Thermo TSQ 800 Triple Quadrupole MS equipped with a TG 5MS (30m X 0.25mm, 0.25µm) composed of 5% diphenyl; 95% dimethyl polysiloxane. Helium was used as the carrier gas constantly flowing at a rate of 1.5 ml/min. Split mode was used to inject a 1.0 µl sample at 250°C. The temperature programming was set with an initial temperature of 60ºC for 2 minutes then increasing at a rate of 15°C per minute upto 200°C with a holding time of 6 minutes, ending with 6 minutes isothermal at 220°C. The total GC running time was 21.78 minutes. Mass spectra were collected spanning in the range of 50 to 700 m/z. Using XCalibur 2.2SP1 with Foundation 2.0SP1, the relative percent amount of each component was computed by comparing its mean peak area to the total areas [14]. The National Institute of Standards and Technology (NIST) database, containing over 62,000 patterns, was used to interpret the mass spectrum of the GC-MS. The known component's spectrum was compared to the spectrums listed in the NIST 2.0 database. The components of the test material were determined by their name, molecular weight, and structure.

FTIR analysis

The characteristic functional group of compounds present in the extract was determined by using Fourier Transform Infrared (FTIR). The extract was dissolved in dichloromethane (DCM) solvent before using for FTIR analysis. The IR spectrum was obtained using Perkin-Elmer FTIR spectrometer and was scanned from 4000 to 500 cm^{-1}.

Results and Discussion

The proximate nutritional analysis of dried leaves revealed a high amount of crude protein (25.92%) with moisture content (12.91%), crude oil (4.35%), crude fiber (2.13%), ash content (11.84) and NFE (42.85%) (Figure 1). Earlier studies conducted on alfalfa seeds also reported a high content of crude protein (33.97%) and crude oil (8.11%) [15]. The presence of high protein content and moisture along with comparatively low fat signifies the plant leaves as a healthy food that can be used in nutraceutical and cattle farming industries [16].

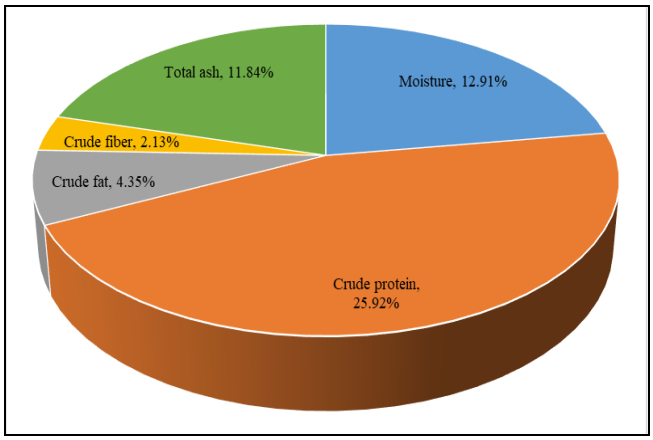


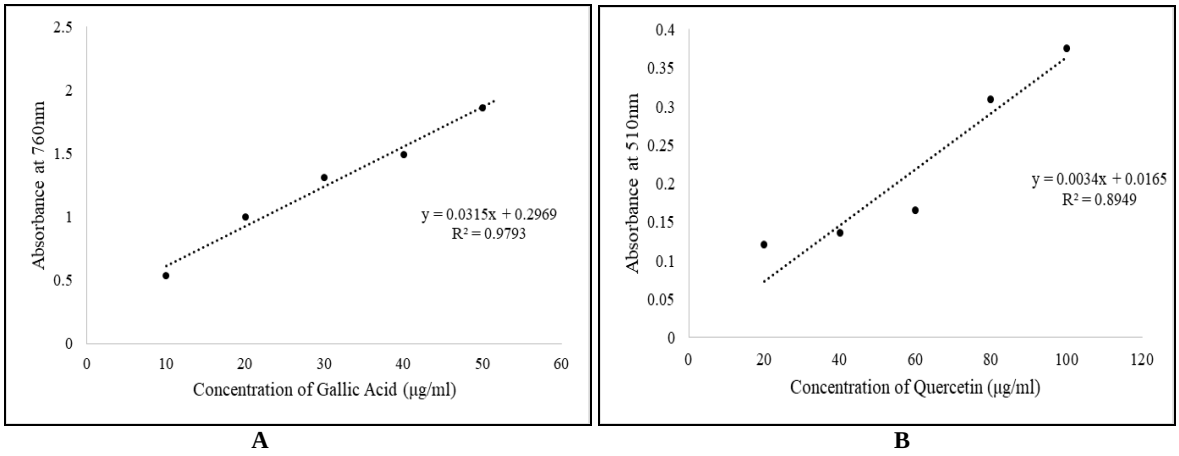
Fig 1: Pie-chart showing the chemical composition of alfalfa leaves.

After extraction, the resultant extractive was weighed for percentage yield which was found to be 10%. The preliminary phytochemical screening of ethanolic extract of alfalfa leaves revealed that it contains several organic compounds like flavonoids, alkaloids, saponins, terpenoids, proteins, phenolic compounds and fixed oils (Table 1). An earlier investigation [17] using hydroethanolic extract of alfalfa leaves collected from Indore, India also reported the presence of carbohydrate, glycosides, tannins, and proteins. Saponins are known to have anti-microbial activity when taken in appropriate dosage in extremely in cold-blooded animals [18]. Saponins also show hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory and anti-cancer activities [19]. Alkaloids and phenolic compounds have been long known to have antib-acterial and anti-diabetic properties [20]. Phenolic compounds along with flavonoids and tannins are the major compounds contributing to the antioxidant property of a plant which attributes to the anti-inflammatory, anti-bacterial and anti-cancer activities [16]. The nutraceutical and pharmaceutical potential of medicinal plants is perhaps due to the phytocompounds present in them such as alkaloids, flavonoids, phenolics, saponins, tannins and phytosterols [21].

Table 1: Qualitative phytochemical analysis of ethanolic extract of alfalfa leaves

Phytocompounds	Methods	Results
Flavonoids	Shinoda test	+
Phenolic compounds	Lead acetate test	+
Protein	Biuret test	+
Fixed oils	Filter paper	+
Alkaloids	Wagner's test	+
Terpenoids	Salkowski test	+
Saponins	Foam test	+
Tannins	Ferric chloride test	-
Reducing sugars	Fehling's test	-
Glycosides	Borntrager test	-

Further, the quantitative phytochemical analysis revealed a good amount of flavonoids and phenolic compounds in the alfalfa leaves extract. Gallic acid and quercetin were used as standards for flavonoids and phenolic compounds (Figure 2). Total phenolic compounds were 47.590 ± 10.004 mg gallic acid equivalent/g dry weight of the sample, and the total flavonoid content was 117.402 ± 2.716 mg quercetin equivalent/g dry weight of the sample. The DPPH % inhibition activity of alfalfa extract along with ascorbic acids as a standard was evaluated and the highest percentage of inhibition was at a concentration level of 200 ug/ml (50.48%) and lowest at the concentration level of 50 ug/ml (22.82%) (Figure 3). The free radical scavenging ability of extract can be attributed to the presence of high quantity flavonoids because flavonoids are the main source of antioxidants in plants [22, 23].



A **B**

Fig 2: Standard curve for Gallic acid (A) and Quercetin (B) to quantify the total phenolic and total flavonoids.

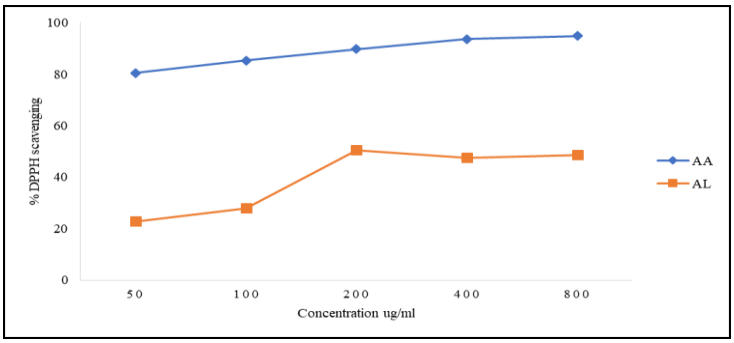


Fig 3: DPPH free radical scavenging activity of alfalfa ethanolic extract at different concentrations. AA = Ascorbic acid, AL= Alfalfa extract.

The GC-MS analysis revealed a total of 30 compounds in the *M. sativa* extract as shown in the chromatogram (Figure 4). The identified compounds along with their molecular weight, chemical formula and biological activity as reported from the literature are presented in Table 2. The major compounds identified along with their peak percentage area were Dasycarpidan-1-methanol, acetate (ester) (7.94%), Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester (4.32%), 2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether (3.95%), 1-Monolinoleoylglycerol trimethylsilyl ether (5.51%), 5-Bromoadamantan-2-one (4.89%), Phorbol (12.34%), Alpha-tocopherol (3.56%) and 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone (7.05%). The compounds possess anti-bacterial, anti-viral, anti-fungal, anti-oxidant, anti-inflammatory, anti-cancer, diuretic, renal disease treatment, heart failure treatment and anti-epileptic properties and can be used in the treatment of these many diseases. Among the identified compounds à-Bisabolol, Rhodopin, Oleic acid, 3-(octadecyloxy)propyl ester, Alpha-tocopherol and 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester have anti-oxidant, anti-bacterial, anti-inflammatory, hypocholesterolemic, nematicide, pesticide, lubricative and hemolytic activity [24]. Hexadecanoic acid derivatives can be a potent anti-oxidant, nematicide, pesticide, lubricant and hypocholesterolemic agent [25] which is one of the 30 compounds found in alfalfa leaves extract. Presences of octadecenoic acid in the alfalfa extract were also reported in earlier studies [26, 27, 28] which has various biological activities like anti-oxidant, anti-inflammatory, anti-arthritic and hypocholesterolemic. The presence of 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, Docosanoic acid, 1,2,3-propanetriyl ester, 9,12,15-Octadecatrienoic acid methyl esters and hexadecanoic acid methyl ester were also reported predominantly in methanolic extract of alfalfa seed [15]. It has been reported that the major compounds in *M. sativa* from the deserts of Oman are protchaechenic acid (3.22%), hydroxyl benzoic acid (1.05%), β-Phenyl caffate (0.97%), kaempherol (0.89%) and Pterostilbene which are mainly responsible for antioxidant and cholesterol-lowering properties [29].

However, none of these compounds were found in the current study which might be attributed to the fact that different species, geographical conditions, extraction techniques and extraction solvent can result in the detection of different compounds by GC-MS.

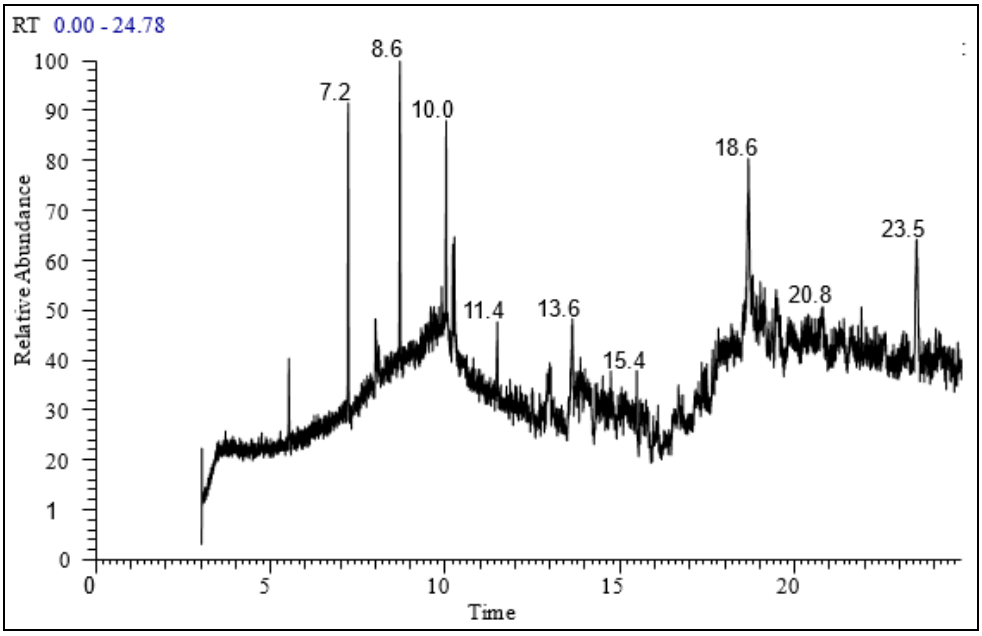


Fig 4: GC-MS chromatogram of ethanolic extract of alfalfa leaves.

Table 2: GC-MS analysis of ethanolic extract of alfalfa leaves

Sr. No.	Compounds	RT	MW	Formula	Biological activity
1	Dasycarpidan-1-methanol, acetate (ester)	3.45	326	C20H26N2O2	Antimicrobial, antioxidant,
2	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	3.5	355	C20H13N5O2	Renal disease treatment, Heart failure treatment, Antiepileptic
3	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	3.96	496	C27H52O4Si2	
4	Acetic acid, [bis[(trimethylsilyl)oxy]phosphinyl]-, trimethylsilyl ester	7.21	356	C11H29O5PSi3	
5	Methyleugenol	7.99	178	C11H14O2	
6	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	8.69	296	C14H24O3Si2	
7	1-Monolinoleoylglycerol trimethylsilyl ether	10.01	498	C27H54O4Si2	Antimicrobial, antioxidant, anti-inflammatory
8	Ethyl iso-allocholate	10.2	436	C26H44O5	Antimicrobial, Diuretic, Anti-inflammatory
9	à-Bisabolol	10.25	222	C15H26O	Antimicrobial, Anticancer, Anti-inflammatory
10	1-Monolinoleoylglycerol trimethylsilyl ether	11.48	498	C27H54O4Si2	
11	Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a methanocyclopenta [a] cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aà,2à,5á,5aá,6á,8aà,9à,10aà)]-	13	586	C36H58O6	Antioxidant, hypocholesterolemic, nematicide, lubricant
12	5-Bromoadamantan-2-one	13.62	228	C10H13BrO	
13	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta[a]phenanthren-2-one	13.87	488	C35H52O	Antibacterial
14	Glycine, N-[(3à,5á,7à,12à)-24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	13.87	695	C36H69NO6Si3	
15	Hexa-t-butylselenatrisiletane	14.08	506	C24H54SeSi3	
16	Rhodopin	14.73	554	C40H58O	Antioxidant
17	Hematoporphyrin	15.47	598	C34H38N4O6	
18	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,7,9-trimethyl-1-[[(2-methyl-1-oxo-2-butenyl)oxy]methyl]-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester	15.58	594	C35H46O8	
19	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	16.67	488	C30H52O3Si	
20	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	17.82	322	C21H38O2	
21	7,8-Epoxylanostan-11-ol, 3-acetoxy-	17.98	502	C32H54O4	Antimicrobial
22	Phorbol	18.67	350	C19H26O6	Antiviral, Antimicrobial
23	.psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	19.13	600	C42H64O2	
24	Docosanoic acid, 1,2,3-propanetriyl ester	19.46	1058	C69H134O6	Antimicrobial
25	Oleic acid, 3-(octadecyloxy)propyl ester	19.82	592	C39H76O3	Antitumor, anti-inflammatory
26	1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-, dimethyl ester, (1à,2á,4á)-	19.92	242	C13H22O4	
27	2-Myristynoyl pantetheine	20.3	484	C25H44N2O5S	Used to treat diabetes mellitus
28	Alpha-tocopherol	20.76	430	C29H50O2	Antioxidant, Vitamin E type
29	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	22.56	444	C28H44O4	Anti-inflammatory, antibacterial, antioxidant
30	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	23.49	487	C28H25NO7	Anticancer, Anti-inflammatory

The plant extract was subjected to FTIR analysis to define the functional groups and the resultant spectrum showed 8 bond stretches (Figure 5) corresponding to various functional groups. Results were analysed by comparing the peak values with standard IR spectrum chart by Sigma-Aldrich and identified amines, alkanes, alkenes, primary alcohols and sulfoxide as major functional groups (Table 3) in ethanolic extract of *M. sativa* leaves which probably represents different classes of phytocompounds including alkaloids, flavonoids and phenolic compounds. Total 8 peaks were recorded and the peaks at 2921.7 cm^{-1}, 2850 cm^{-1} & 1462.7 cm^{-1} showed C-H stretch corresponds to alkane groups [30] while the peaks at 3376.1 cm^{-1} and 3013.4 cm^{-1} attributed to N-H and C-H/O-H which assigned to the stretching vibration of amines moieties and primary alcohols [31]. The functional groups corresponding to alcohol moities are assigned to be phenols which are known to have an ani-inflammatory effect by reducing the production of reactive oxygen and nitrogen species [32]. Some of the other studies too had reported the presence of different classes of compounds in some selected plants representing amines and carboxylic groups, which attributes to alkaloids, polyphenols and steroids which are also reported in the present study [33].

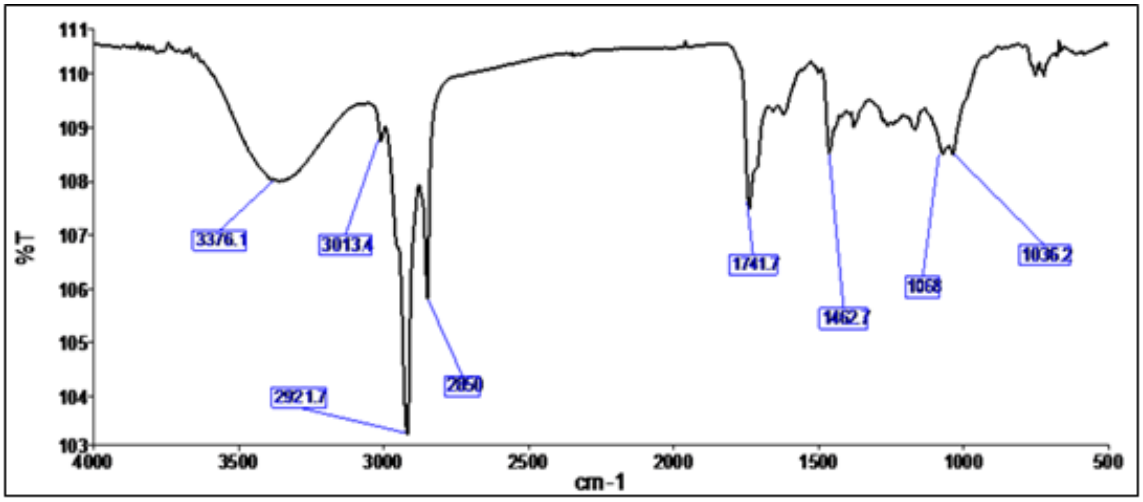


Fig 5: FTIR chromatogram showing peaks corresponding to various functional groups.

Table 3: FTIR analysis of ethanolic extract of alfalfa leaves.

Sr. No.	Peak Vaue	Bond	Functional group
1.	3376.1	N-H	Primary amine
2.	3013.4	C-H/O-H	Alkene/Alchohol
3.	2921.7	C-H	Alkane
4.	2850	C-H	Alkane
5.	1741.7	C=O/C-H	Esters/Aromatic compound
6.	1462.7	C-H	Alkane
7.	1068	C-N/C-O	Amine/Primary alchohol
8.	1036.2	S=O	Sulfoxide

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References

1. Sadowska B, Budzyńska A, Więckowska-Szakiel M, Paszkiewicz M, Stochmal A, Moniuszko-Szajwaj B *et al*. New pharmacological properties of *Medicago sativa* and *Saponaria officinalis* saponin-rich fractions addressed to Candida albicans. Journal of Medical Microbiology,2014:63(8):1076-1086. https://doi.org/10.1099/jmm.0.075291-0.
2. Rafińska K, Pomastowski P, Wrona O, Górecki R, Buszewski B. *Medicago sativa* as a source of secondary metabolites for agriculture and pharmaceutical industry. Phytochemistry Letters,2017:20:520-539.https://doi.org /10.1016/j.phytol.2016.12.006.
3. Dale N. National Research Council: Nutrient Requirements of Poultry–9th revised edition (1994). Journal of Applied Poultry Research,1994:3(1):101.
4. Bora KS, Sharma A. Phytochemical and pharmacological potential of *Medicago sativa*: A Review. Pharmaceutical Biology,2011:49(2):211-220. https://doi.org/10.3109/13880209.2010.504732.
5. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: A Review. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)(Discontinued),2009:8(3):229-235. https://doi.org/10.2174/187152809788681029.
6. Bora KS, Sharma A. Evaluation of antioxidant and cerebroprotective effect of *Medicago sativa* Linn. against ischemia and reperfusion insult. Evidence-Based Complementary and Alternative Medicine, 2011, 9. https://doi.org/10.1093/ecam/neq019.
7. Oskoueian E, Abdullah N, Hendra R, Karimi E. Bioactive compounds, antioxidant, xanthine oxidase inhibitory, tyrosinase inhibitory and anti-inflammatory

activities of selected agro-industrial by-products. International Journal of Molecular Sciences,2011:12(12):8610-8625. https://doi.org/10. 33 90/ijms12128610.
8. AOAC I. Official Methods of Analysis. Washington, DC, USA, 1990, 233-234.
9. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri district (MP). International Journal of Life Science Scientific Research,2017:3(1):844-847.
10. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of *Moringa oleifera*. Asian Pacific Journal of Tropical Biomedicine,2013:3(8):623-627. https://doi.org/10.1016/S2221-1691(13)60126-4.
11. Dulf FV, Vodnar DC, Socaciu C. Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. Food Chemistry,2016:209:27-36. https://doi. org/10.1016/j.foodchem.2016.04.016.
12. Madhu M, Sailaja V, Satyadev TN, Satyanarayana MV. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry,2016:5(2):25.
13. Krakowska A, Rafińska K, Walczak J, Kowalkowski T, Buszewski B. Comparison of various extraction techniques of *Medicago sativa*: yield, antioxidant activity, and content of phytochemical constituents. Journal of AOAC International,2017:100(6):1681-1693. https://doi.org/10.5740/jaoacint.17-0234.
14. Hema R, Kumaravel S, Alagusundaram K. GC/MS determination of bioactive components of *Murraya koenigii*. Journal of American Science,2011:7(1):80-83.
15. Tepe HD. Qualitative Analysis of Alfalfa Seed Methanol Extract by GC-MS and Determination of Antioxidant Properties. Celal Bayar University Journal of Science,2019:15(2):175-180. https://doi.org/10.18 466/cbayarfbe.493017.
16. Sharma S, Satpathy G, Gupta RK. Nutritional, phytochemical, antioxidant and antimicrobial activity of *Prunus armenicus*. Journal of Pharmacognosy and Phytochemistry,2014:3(3):23-28.
17. Kumar H, Jain S, Shukla K. Evaluation of alfalfa (*Medicago sativa*) leaves for wound healing activity. Journal of Pharmacognosy and Phytochemistry,2020:9(5):1164-1169.
18. Verma S, Mohanta T, Revathy T, Suthindhiran K, Jayasri MA. Phytochemical and pharmacological evaluation of selected plants. American Journal of Biochemistry and Biotechnology,2013:9(3):291-299. doi:10.3844/ajbbsp.2013.291.299.
19. Murugan M, Mohan VR. Phytochemical, FTIR and antibacterial activity of whole plant extract of *Aerva lanata* (L.) Juss. Ex. Schult. Journal of Medicinal Plants Studies,2014:4(3):51-57.
20. Akinyeye AJ, Solanke EO, Adebiyi IO. Phytochemical and antimicrobial evaluation of leaf and seed of *Moringa oleifera* extracts. International Journal of Research in Medicine and Health Sciences,2014:4(6):2307-2083.
21. Britto JD, Sebastian SR. Biosynthesis Silver Nanoparticles and its Antibacterial Activity against Human Pathogens. International Journal of Pharmaceutical Science,2012:5:257-259.
22. Singh Gill N, Sood S, Muthuraman A, Garg M, Kumar R, Bali M *et al*. Antioxidant, anti-inflammatory and analgesic potential of *Cucumis sativus* seed extract. Latin American Journal of Pharmacy, 2010, 29. http://sedici.unlp.edu.ar/handle/10915/8006.
23. Egbung GE, Atangwho IJ, Iwara IA, Odey MO, Ebong PE. Chemical composition of root and stem bark extracts of *Nauclea latifolia*. Archives Applied Scientific Research,2013:5(3):193-196.
24. Parthipan B, Suky MG, Mohan VR. GC-MS analysis of phytocomponents in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle, (Rutaceae). Journal of Pharmacognosy and Phytochemistry, 2015, 4(1).
25. Kumar PP, Kumaravel S, Lalitha C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African Journal of Biochemistry Research,2010:4(7):191-195. https://doi.org/10.5897/AJBR.9000213.
26. Lalitharani S, Mohan VR, Regini GS, Kalidass C. GC-MS analysis of ethanolic extract of *Pothos scandens* leaf. Journal of Herbal Medicine Toxicology,2009:3:159-160.
27. Uma B, Prabhakar K, Rajendran S, lakshmi SY. Studies on GC/MS spectroscopic analysis of some bioactive antimicrobial compounds from *Cinnamomum zeylanicum*. Journal of Medicinal Plants,2009:8(31):125-131.
28. Ponnamma SU, Manjunath K. GC-MS Analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (nees) T. anders. International Journal of Pharma and Bio Science,2012:3(3):570-576.
29. Hanif MA, Al-Maskari AY, Al-Sabahi JN, Al-Hdhrami I, Khan MM, Al-Azkawi A *et al*. Chemical characterisation of bioactive compounds in *Medicago sativa* growing in the desert of Oman. Natural Product Research,2015:29(24):2332-2335. https://doi.org/10.1080/14786419.2015.1008474.
30. Maitera ON, Chukkol IB. Phytochemical and Fourier transform infrared spectroscopy analysis of *Faidherbia albida* (Del) as a preservative agent. World Journal of Research and Review,2016:3(3):25-29.
31. Poojary MM, Vishnumurthy KA, Adhikari AV. Extraction, characterization and biological studies of phytochemicals from *Mammea suriga*. Journal of Pharmaceutical Analysis,2015:5(3):182-189. https://doi.org/10.1016/j.jpha.2015.01.002.
32. Pontiki E, Hadjipavlou-Litina D, Litinas K, Geromichalos G. Novel cinnamic acid derivatives as antioxidant and anticancer agents: Design, synthesis and modeling studies. Molecules,2014:19(7):9655-9674. https://doi.org/10.3390/molecules19079655.
33. Meyre-Silva C, Niero R, Bolda Mariano LN, Gomes do Nascimento F, Vicente Farias I, Gazoni VF *et al*. Evaluation of antileishmanial activity of selected Brazilian plants and identification of the active principles. Evidence-Based Complementary and Alternative Medicine, 2013. https://doi.org/10.1155/ 2013/265025.