Comparative analysis of supercritical fluid and Soxhlet Prosopis juliflora leaves extract with antifungal traits

Nagaraj M Naik (nagarajnaik@uasraichur.edu.in)
University of Agricultural Sciences Raichur https://orcid.org/0000-0002-9119-9769
M Krishnaveni
University of Agricultural Sciences Raichur
M Mahadevswamy
University of Agricultural Sciences Raichur
M Bheemanna
University of Agricultural Sciences Raichur
Udaykumar Nidoni
University of Agricultural Sciences Raichur
Vasant Kumar
University of Agricultural Sciences Raichur
Tejashri K
University of Agricultural Sciences Raichur

Original Article

Keywords: Prosopis juliflora, Soxhlet extraction, Super Critical Fluid, Antifungal activity, Percent inhibition

Posted Date: June 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1676795/v1

Abstract

This study aimed to screen the bioactive compounds from *Prosopis juliflora* leaf supercritical fluid extractand to assess its antimicrobial properties. Supercritical fluid CO_2 and Soxhlet methods were used for extraction. The extract was subjected to GCMS and FTIR for the qualitative screening of the bioactive compounds.GCMS screening revealed that supercritical fluid extraction (SFE) eluted a greater number of compounds (35) compared to soxhlet extraction (20). *Prosopis juliflora* leaf SFE extract showed strong antifungal properties against *Rhizoctonia bataticola, Alternaria alternata*, and *Colletotrichum gloeosporioides* with 94.07 percent, 93.15 per cent, and 92.43 per cent, respectivelywhere as extract obtained from Soxhlet registered 55.31,75.63and45.13 percent respectively.Resultsobtained from GCMS/MS screening revealed that supercritical fluid extraction is more effective than soxhlet extraction. *Prosopis juliflora* may provide antifungal agents, probably a novel inhibitory metabolite and a natural fungicide.

Introduction

Novel drug discovery is an important challenging area where a lot of opportunities are there to exploit chemical diversity. Biomolecules or photo components present in natural products, such as plant extract could be invented as a novel drug. Many chronic and infectious diseases are cured by the wide range of active components present in plants used for traditional medicine[1]. Ethno-pharmacognosy is gaining importance, as chemically synthesized drugs exhibit adverse effects. Microorganisms are developing resistance against synthesized drugs. Thousands of phytochemicals are having antimicrobial, antioxidant, wound headlining, anticarcinogenic and antidiarrheal traits. Many studies reported plants as safe and broadly effective alternatives with fewer adverse effects[1–2].

Prosopis juliflora (Sw.) DC, (Velvet Mesquite)contains 44 species, of which 40 are native to America, three to Asia, and one to Africa and belong to the family Fabaceae, subfamily Mimosoideae[3]. The shrub *Prosopis juliflora* is known to contain different chemical compounds such as alkaloids, flavonoids, terpenoids, saponins, and phenolic compounds distributed in different parts of the plant body which makes the shrub of medicinal importance.Medically active substances have been extracted from different parts of *Prosopis juliflora* such as leaves, roots, stems, branches, bark as well as pollen. Extract from *Prosopis*is known to contain a variedclass of secondary metabolites which has unique and combined therapeutic and antifungal traits[4–5]. Lakshmibai (2018), reported that ethanolic and aqueous thorn extracts of *Prosopis juliflora* were known to contain antioxidant activity and nitric oxide scavenging activity [6].The usage of this plentiful resource imparts a good option for yielding bioactive natural products that may serve as an important rawmaterial for the pharmaceutical and chemical industries[7].

Selection of the use of solvent and the extraction procedure in obtaining the antimicrobial metabolites from various plant parts play a major role [9]. There are various conventional extraction methods to obtain an extract from the plant. Among the techniques implementedsoxhlet has been the leaching technique mostly used for a long time [8]. Solvent extraction is being practiced for extraction from

Prosopis juliflora leaves. However, this method has the considerable drawback of solvent residue leftover in the extracts. The new technique of Supercritical Fluid Extraction (SFE) has securedprime attention over the traditional techniques in the recovery of edible and essential oils in the field of natural products.At optimal conditions, SFE does not have any of the adverse effects of traditional organic solvents. Optimization of temperature and pressurehas a major impact on fluid density, improved transport properties, higher extraction yield, and shorter extraction time. The ability to control the extraction parameters is the major benefit of the supercritical fluid extraction process [10].

The application of chromatographic techniques for the determination of various bioactive components (qualitatively and quantitatively) present in the extract is of prime importance. Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in any matrix sample.GC-MSwith triple quadrupole is suitable equipment for an important role in the analysis of photo components and chemotaxonomic studies of medicinal plants containing biologically active components [11].

To date, any research article on comparative analysis of extraction methods such as SFE and Soxhlet through GC-MS determination of bioactive components of *Prosopis juliflora* leaf extract has not been reported. Keeping because of these facts, the investigation on “GCMS screening of bioactive compounds in *Prosopis juliflora* leaf supercritical fluid extract with antimicrobial traits” was undertaken at the Pesticide Residue and Food Quality Analysis Laboratory, University of Agricultural Sciences, Raichur, Karnataka (India).

Materials And Methods

Raw materials

Fresh leaves of *Prosopis juliflora* were collected around the campus of the University of Agricultural Sciences (UAS), Raichur, Karnataka State.Leaves were cut dried in dehumidified air dryer (make: Bry Air Asia; model: FSD-600) at 45 °C and 15% relative humidity. The dried leaves were ground in a laboratory hammer mill to obtain a fine powder.

Chemicals

The solvents, chemicals, and reagents (analytical grade) used throughout the experiment were procured from M/s. Sigma Aldrich Chemicals.

Fungal culture

Authentic pure cultures of*Rhizoctonia bataticola*, *Alternaria alternata,* and *Colletotrichum gloeosporioides*were collected from the Department of Plant Pathology, UAS, Raichur. Procured cultures were maintained in the appropriate media for further use.

Extraction of *Prosopis juliflora* leaf extract

Supercritical fluid and Soxhlet extraction methods were employed for obtaining an extract from *Prosopis juliflora* leaves.

Supercritical fluid extraction

The supercritical carbon dioxide (SC-CO_2) extraction system (Thar; SFE 500 system) was used for the extraction of *Prosopis juliflora* leaf powder. Deionized water (5 °C) was used for cooling different zones in the SC-CO_2 extraction system. Fifty grams of *Prosopis juliflora* leaf powder were placed into the extractor vessel. The flow rates of supercritical CO_2 and co-solvent (ethanol) were maintained at 20 and 2 g/min, respectively [12]. The static extraction process was performed for 30 min. After attaining desired pressure (200 bar) and temperature (45^0C) dynamic extraction time (90 min) was started by opening the exit valve of the SC-CO_2 extraction system. The static extraction time allowed the sampleto soak in the CO_2 and co-solvent to equilibrate the mixture at desired pressure and temperature. During the dynamic extraction time, CO_2 carrying the crude extract flowed out of the extraction vessel and then into a collection vessel, where the CO_2 was separated through the vent connected to the fume hood [13].

Soxhlet extraction

Prosopis juliflora leaves extraction was carried out by the soxhlet extraction method using SOCS- PLUS apparatus (Make: Gerhardtz, model: SOX-416) with hexane as solvent. Accurately, 50 g of the *Prosopis juliflora* leaf powder was taken into the thimble and placed in the sample compartment of the extractor. The sample compartment was attached to a 500 ml round bottom flask containing 300 ml hexane. The SOCS- PLUS apparatus was run at 85 ^{o}C for 90 min. Hexane is the oil extract that was distilled out by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) [14].

Gas Chromatography-Mass Spectrometry Triple Quadrupole (GCMS-MS) analysis

GCMS-MS analysis was carried out in a combined 7890B gas chromatograph system Agilent make and mass spectrometer triple quadrupole fitted with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m X 250 µm, film thickness 0.25 µm, interfaced with 7000D Agilent mass detector with (TQD) triple detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0ml/min.

Other GC oven conditions are 60^0 C @ initial temperature @7^0 C /min reaching to 270^0 C with splitless, injection volume 1µl and Mass condition ion source temperature, 280 ^{0}C with injection temperature of 250 ^{0}C. Mass range 50 to 600amu, scan time 0.39sec interscan delay 0.01sec by Electron ionization (EI) mode. Mass Hunter software provided by the supplier was used to control the system and acquire data. The data can be integrated by using software and compiling the compounds with the provided NIST Library software to identify unknown compounds and structures.

Antifungal activity of *Prosopis juliflora* leaf extract

The antifungal activity of Soxhlet and SFE*Prosopis juliflora*leaf extract against the plant pathogens viz., *Rhizoctonia bataticola, Alternaria alternata,* and*Colletotrichum gloeosporioides*was carried out.The potato dextrose media was prepared and sterilized. A volume of 0.5 ml of plant extract was aseptically poured into a Petri plate followed by the addition of 9.5 ml of melted PDA and was gently mixed. The inoculum disc of test fungus was aseptically inoculated upside down at the center of the Petri plate and incubated at 25 °C for 7days

The media plate without extract was set as a negative control and the media plate supplemented with 0.1% hexaconazole was considered a positive control. The average radial growth of the fungal mycelia was measured on the 7^{th} day of incubation.

$$\text{Mycelial Inhibition (\%)} = \frac{\text{DC- DT}}{\text{DC}} \times 100$$

Where,

DC - Average diameter of the colony in the control plate

DT - Average diameter of the colony in treatment plate

FTIR analysis of *Prosopis juliflora*leaf powder extract

Supercritical fluid*Prosopis juliflora*leaves extract was used for FTIR (Fourier Transform Infrared Spectroscopy) analysis. FTIR analysis was performed using Shimadzu FTIR spectrometer 8000 series, between 4000 – 750 cm-1 which was used to detect the characteristic peaks and their functional groups. The peaks values and the functional groups of *Prosopis juliflora*leaf SFE extract were recorded.

Results And Discussion

Gas Chromatography-Mass Spectrometry Triple Quadrupole (GCMS-MS) analysis:

As expected, a wide variety of bioactive compounds could be found in Soxhlet and SFE extract.Twenty compounds were detected from the GCMS-MS analysis of soxhlet extract of *Prosopis juliflora*leaves whereas thirty-five compounds were identified from the GCMS-MS analysis of a supercritical fluid extract of *Prosopis juliflora*leaves. The chromatogram is depicted in Fig. 1, while the name of bioactive components with their retention time (RT), molecular formula,height, and area are presented in Tables 1 and 2. The major compounds identified in the *Prosopis juliflora* soxhlet extract are Phenol, 3,5-bis(dimethyl ethyl), Benzene dicarboxylic acid, and Squalene. The major components detected in SFE extract of *Prosopis juliflora* leaves are Pentanoic acid 5hydroxy 2,4, dibutyl phenyl ester, Phytol, Tetramethyl heptadecane, Neophytadiene, hexadecanal. Many other compounds were traced as low levels.All these major plant metabolites have a role as anti-inflammatory agents, anti-oxidants, and antimicrobial agents. More number of compounds are eluted in SFE extract compared to Soxhlet extract

may be due to the special features of SFE. Special features of SFE such as efficient extraction efficiencyand selectivity aredue to its gas-like mass transfer traits and liquid-like solubility traits.Extraction of analytes present in low concentrations, cleaner extracts and preservation of bioactive constituents could be achieved through SFE.Even higher extraction yield can be achieved by providing close contact between sample and extractant [15].

Antifungal activity of *Prosopis juliflora* leaf extract

The antifungal activity of Soxhlet and SC-CO_2 *Prosopis juliflora*leaf extract against the plant pathogens viz., *Rhizoctonia bataticola, Alternaria alternata*and*Colletotrichum gloeosporioides*is presented in Table 3. The zone of inhibition for *Rhizoctonia bataticola*was recorded 94.07, 79.58, and 94.60 percent in plates treatedwith SFE extract, Soxhlet extract and positive control (0.1% hexaconazole) respectively. For *Alternaria alternata,* 93.15, 85.07, and 94.57 percent zone of inhibitionwere registeredwhereas 92.42, 66.57, and 93.77per cent zone of inhibition were found in *Colletotrichum gloeosporioides*platestreated with SFE extract, Soxhlet extract and positive control respectively.

Results of the present investigation were in agreement with Raghavendra *et al.*[16] that the activity of aqueous extract of *Prosopis juliflora* against *Alternaria alternata showed* 71.59 per cent inhibition of mycelial growth.Deressaaand associates [17] used methanol, acetone and aqueous extract of *Prosopis juliflora* leaves against *Colletotrichum gloeosporioides* the results showed radial growth inhibition of 100 percent, 100 percent, 79.60 percent, respectively. Bazie*et al.*(2014) [18] reported the activity of methanolic extract of *Prosopis juliflora* against *Colletotrichum musae*, which showed a 30.70 mm zone of inhibition.

The phytoplankton *Prosopis juliflora*demonstrated significant antifungal activity and may be used to identifybioactive natural products that can serve as leads for developing new pharmaceuticals that address previously unmet needs. The results indicate that leaves extract from *Prosopis juliflora* are a promising source of antifungal agents and may have therapeutic potential. *Prosopis juliflora* leaf extract was found to contain antifungal compounds based on the results of the study conducted.Cellular energy in ATP form may eventually dissipate due to degrading the cell wall, disrupting cytoplasmic membranes, damaging membrane proteins, or causing interference with the active transport of metabolic enzymes due to these bioactive compounds. [19]. A deeper study of this plant with its pure compounds may lead to the development of natural antioxidants and alternative antifungals against plant pathogens.A **s**imilar study was carried out byRizwana et al in 2018,[20]who isolated two pentanoic acid compounds from *Bluejack Oak* and tested for their antimicrobial potential and showed promising antifungal activity against *Aspergillus niger* and *Aspergillus flavus*.

FTIR spectroscopy analysis of *Prosopis juliflora*leaves extract

Phytochemical screening is an important step that leads to the isolation of new and novel compounds.The results of FTIR analysis of the *Prosopis juliflora*leafSFE extractrevealed the presence of different functional groups in the extract (Fig 2). Major peaks in the FTIR analysis showed the presence of alcohol, phenols, alkanes, aromatic, ether, carboxylic acid, aliphatic amines, primary and secondary

amine.Similar preliminary phytochemical screening of the*Prosopis juliflora*extract through FTIR alsorevealed that the plant contains Alkaloids, Flavonoids, Saponins, Tannins, Anthraquinone Glycoside and Coumarins [21-22].

Conclusion

The supercritical fluid extraction process is superior compared to the soxhlet extraction process in recovering the bioactive components present in *Prosopis juliflora* leaves. *P. juliflora* SFE extract is effective in inhibiting the growth of *Rhizoctoniabataticola, Alternaria alternata* and *Colletotrichum gloeosporioides* as it is containing antimicrobial components such as Pentanoic acid 5hydroxy 2,4, dibutyl phenyl ester, Phytol, Tetramethyl heptadecane, Neophytadiene, hexadecanal. *Prosopis juliflora* could be a potential source for antifungal agents, probably a novel inhibitory metabolite and can be used asa potential natural fungicide.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Competing interests : The author(s) declare(s) that they have no competing interests".

Funding: Experiment has been carried out under the Post Graduate Research garnt as it is a part of Masters degree research program.

Authors' contributions:

Nagaraj M Naik: Conducting experiment and Manuscript preparation

Krishnaveni M.: Conducting experiment

Mahadevswamy M: Head and Member of advisory team (Microbiology Part)

M. Bheemanna: Professor and Head, Member of advisory team (Monitoring)

Udaykumar Nidoni: Member of advisory team (Extraction Part)

Vasantkumar: Analysis part

Tejashri K : Analysis part

Acknowledgment :

The authors are thankful to the Pesticide Residue and Food Quality Analysis Laboratory(PRFQAL) Lab, University of Agricultural Sciences, Raichur for providing the necessary facilities in accomplishing the

research work.

References

1. Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S., 2006, Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary Altern. Med.*, 6: 35-41. doi: 1186/1472-6882-6-35
2. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. and Latha, L. Y., 2011, Extraction, isolation and characterization of bioactive compounds from plants extracts. *African J. Tradit Complement Altern. Med.,* 8(1).
3. Prasad, M. N. V. and Tewari, J. C., 2016, *Prosopis juliflora* (Sw) DC: potential for bioremediation and bioeconomy. *In Bioeconomy*, pp. 49-76. https://www.researchgate.net/publication/313651776
4. Solanki, D. S. Kumar S, Parihar K and Tak A, Gehlot P Patk R and Singh S K, 2018. Characterization of a novel seed protein of *Prosopis cineraria* showing antifungal activity. Int. J. Biol. Macromol. 116, 16–22. https://doi.org/10.1016/j.ijbiomac.2018.05.018
5. Saleh Iman and Mohammed H.Abu-Dieyeh 2021, Novel P*rosopis julifora* leaf ethanolic extract as natural antimicrobial agent against food spoiling microorganisms , Scientific Reports. 11:7871 | https://doi.org/10.1038/s41598-021-86509-3
6. Lakshmibai, R. 2018, Phytochemical analysis and antioxidant activity of Prosopis Julifora thorn extract. Malaya J. Biosci. https://doi. org/10.13140/RG.2.2.35189.88806
7. Prabha, D. S., Dahms, D. and Malliga, P., 2014, Pharmacological potentials of phenolic compounds from *Prosopis* -a review. *J. Coastal Life Medicine.*, 2(11): 918-924. doi: 10.12980/JCLM.2.2014J27
8. De Castro, M. L. and Garcıa-Ayuso, L. E., 1998, Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *chim. acta*., 369(1-2): 1-10.https://pharmacy.hebmu.edu.cn/trywhx/resources/43/2019520154130.pdf
9. Adhikari, P., Pandey, A., Agnihotri, V. and Pande, V., 2018, Selection of solvent and extraction method for determination of antimicrobial potential of *Taxus wallichianaRes. Pharm.*, pp.01-09. doi: 10.25081/rip.2018.v8.3487
10. ShazanaAzfarRadzali, MasturahMarkom and Noorashikin Md Saleh, 2020, Co-Solvent Selection for Supercritical Fluid Extraction (SFE) of Phenolic Compounds from Labisia pumila, Molecules 25, 5859; doi:10.3390/molecules25245859
11. Olivia U N, Umesh C, G and Ogugofor M O, 2021, Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of Hibiscus asper leaves, Future Journal of Pharmaceutical Sciences, 7; 59 https://doi.org/10.1186/s43094-021-00208-4
12. Pradhan, R. C. A., Meda, P. K. A., Rout, B. V., Naik, S. A., Ajay. K. & Dalai, C., 2010, Supercritical CO_2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. *Fd. Engg,* 98(1): 393-397. doi :10.1016/j.jfoodeng.2009.11.021

13. Palafox, J. O., Navarrete, A., Julio, C., Rivero, S., Atoche, C. R., Escoffie, J., Antonio, P. A. & Uribe, R., 2012, Extraction and characterization of oil from *Moringa oleifera*using supercritical CO_2 and traditional solvents. *American J. Analytical .Chem.,* 3(1): 946-949. doi: 4236/ajac.2012.312A125
14. Malapit, C. A., 2010, Report on the extraction of moringa oil from moringa oil seeds*. Lipid Sci*., 1(1): 1-5.
15. Elsa Autor, Alfonso Cornejo , Fernando Bimbela, MaitaneMaisterra, Luis M. Gandía and Víctor Martínez-Merino, 2022 Extraction of Phenolic Compounds from *Populus salicaceae* Bark Biomolecules, 12, 539. https://doi.org/10.3390/biom12040539
16. Raghavendra, M. P., Satish, S. and Raveesha, K. A., 2009, Alkaloids isolated from leaves of *Prosopis juliflora* against *Arch. Phytopathol. Pl. Protec*., 42(11): 1033-1041. https://doi.org/10.1080/03235400701621644
17. Deressaa, T., Lemessab, F. and Wakjirab, M., 2015, Antifungal activity of some invasive alien plant leaf extracts against mango (Mangifera indica) anthracnose caused by Colletotrichum gloeosporioides. Int. J. Pest Manage., 61(2): 99-105
18. Bazie, S., Amare, A. and Woldetsadik, K., 2014, Antifungal activity of some plant extracts against (*Colletotrichum musae*) the cause of postharvest banana anthracnose. *Pl. Pathol. Microbiol.,* 5(2): 2157-7471.DOI: 10.4172/2157-7471.1000226
19. Davidson, P. M., 2012, Chemical preservatives and natural antimicrobial compounds. Food Microbiology: Fundamentals and Frontiers, ASM Press, Washington, 593-627.https://doi.org/10.1128/9781555818463.ch30
20. Rizwana Sarwar, Um,ar Farooq Sadia Naz, Nadia Riaz. Syed Majid Bukhari, Abdur Rauf, Yahia N. Mabkhot, and Salim S. A, 2018 Isolation and Characterization of Two New Antimicrobial Acids from*Quercus incana (Bluejack Oak)* BioMed Research International :5 https://doi.org/10.1155/2018/3798105
21. Badri, A. M., Garbi I M.,, Sara M. G, Amira A. Magzoub, Ibrahim T. Ibrahim, Mahmmoud S. Saleh, Ahmed S. Kabbashi and Sameer G. Mohamed. 2017 Antioxidant activity and phytochemical screening of *Prosopis julifora* leaves extract. Adv. Med. Plant Res. https:// doi.org/10.30918/AMPR.53.17.020
22. Singh, S., 2012, Phytochemical analysis of different parts of *Prosopis juliflora*. *J. Curr Pharm Res*., 4(3): 59-61.

Tables

Table 1: Bioactive components with retention time (RT), molecular formula, height, and area of Soxhlet *Prosopis juliflora* extract

Sl. No	Compound Label	RT	Formula	Height	Area
1	Ethyl Acetate	3.112	C4H8O2	18029329	112121755
2	Butanoic acid	3.591	C4H8O2	3404926	20695565
3	Acetic acid, 2-phenylethyl ester	11.48	C10H12O2	1546234	2540370
4	1-Tetradecene	13.43	C14H28	2887070	4421657
5	Phenol, 3,5-bis(1,1-dimethylethyl)-	15.06	C14H22O	8732153	13235692
6	1-Hexadecanol	16.01	C16H34O	4684995	6681963
7	2-Piperidinone, N-[4-bromo-n-butyl]-	18.31	C9H16BrNO	5038996	10916233
8	Phthalic acid, butyl tridec-2-yn-1-yl ester	20.57	C25H36O4	2281893	4653279
9	1-Decanol, 2-hexyl-	20.90	C16H34O	3221931	7927989
10	2-Methyltetracosane	20.98	C25H52	1149186	6149745
11	7-Hexadecenal, (Z)-	23.51	C16H30O	381911	3442115
12	5-Eicosene, (E)-	23.94	C20H40	1579729	3884158
13	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	24.37	C24H38O4	10537558	103719281
14	7-Hexadecenal, (Z)-	26.11	C16H30O	435929	3787460
15	1-Decanol, 2-hexyl-	27.14	C16H34O	1059557	7287544
16	Carbonic acid, eicosyl vinyl ester	27.29	C23H44O3	1856158	12056569
17	Squalene	28.57	C30H50	34034322	241424474
18	7-Hexadecenal, (Z)-	29.48	C16H30O	348577	3521799
19	Benzeneethanol, .beta.-methoxy-.beta.-(trifluoromethyl)-, (S)-	31.10	C10H11F3O	1127897	11254787
20	Heneicosane	31.35	C21H44	10675295	77709695

Table 2: Bioactive components with retention time (RT), molecular formula, height, and area of SFE *Prosopis juliflora* extract

Sl. No	Compound Label	RT	Formula	Height	Area
1	Ethyl Acetate	3.11	C4H8O2	**47481915**	**335863804**
2	p-Xylene	4.734	C8H10	2047946	13150162
3	2,4,4-Trimethyl-1-pentanol, 2-methylpropionate	10.72	C12H24O2	1022902	4743155
4	Benzaldehyde, 2,4-dimethyl-	10.86	C9H10O	2364457	4564856
5	2-Bromotetradecane	11.54	C14H29Br	1190642	3445335
6	2-Isopropyl-5-methyl-1-heptanol	12.20	C11H24O	2967242	6067812
7	2-Isopropyl-5-methyl-1-heptanol	12.33	C11H24O	3149993	6015443
8	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	13.07	C13H18	3034830	5203641
9	1-Tetradecene	13.42	C14H28	2895992	4230978
10	Naphthalene, 1,5-dimethyl-	13.73	C12H12	2967620	5952515
11	Heptadecane, 2,6,10,15-tetramethyl-	14.83	C21H44	1711282	4427511
12	Pentanoic acid, 5-hydroxy-, 2,4-di-tbutylphenyl esters	15.05	C19H30O3	11211181	16136111
13	1,3-Butanedione, 1-phenyl-	15.22	C10H10O2	2199453	5042320
14	Nonadecane	15.39	C19H40	2420507	4941251
15	5-Nitro-4,6-pyrimidinediol	15.82	C4H3N3O4	2143562	5595180
16	1-Hexadecanol	16.00	C16H34O	4547223	6717343
17	1-Octadecanesulphonyl chloride	17.23	C18H37ClO2S	1762504	5391876
18	1,4-Naphthalenedione, 2,3,6-trimethyl-	18.16	C13H12O2	2551195	4274909
19	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7atetrahydrobenzofuran-2(4H)-one	18.21	C11H16O3	5829047	10165591
20	1-Nonadecene	18.31	C19H38	4635481	7611077
21	Neophytadiene	18.88	C20H38	39026264	71644378
22	Phytol	18.96	C20H40O	3027518	5477980
23	3,7,11,15-Tetramethyl-2-hexadecen-1-	19.18	C20H40O	7543136	13776914

	ol				
24	Neophytadiene	19.42	C20H38	13242210	25270940
25	L-Lysine, N(6)-[(1,1-dimethylethoxy)carbonyl]-N(2)-[(9Hfluoren-9-ylmethoxy)carbonyl]-	20.00	C26H32N2O6	1982886	9153357
26	n-Hexadecanoic acid	20.54	C16H32O2	33264532	146691362
27	11-Methyldodecanol	20.90	C13H28O	2574190	5428167
28	Oxirane, hexadecyl-	21.31	C18H36O	2036065	3927815
29	Phytol	22.72	C20H40O	32397714	90470552
30	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	23.24	C18H30O2	36602649	269599504
31	9,12-Octadecadienoic acid (Z,Z)-	23.50	C18H32O2	8490307	26042217
32	7-Hexadecenal, (Z)-	23.93	C16H30O	1287334	3935590
33	4,8,12,16-Tetramethylheptadecan-4-olide	26.67	C21H40O2	1417112	3594837
34	7-Hexadecenal, (Z)-	29.30	C16H30O	952950	4230944
35	Tetradecane, 2,6,10-trimethyl-	30.85	C17H36	1067075	3518551

Table 3: Antifungal activity of supercriticalfluid and soxhlet Prosopis juliflora leafextract against fungal test organisms

Treatments	*Rhizoctonia bataticola*		*Alternaria alternata*		*Colletotrichum gloeosporioides*	
	Mycelia growth (mm)	Inhibition (%)	Mycelia growth (mm)	Inhibition (%)	Mycelia growth (mm)	Inhibition (%)
SFE	5.30	94.07	6.10	93.15	6.37	92.43
Soxhlet	18.10	79.58	13.30	85.07	28.13	66.57
Negative control	90.00	0.00	90.00	0.00	88.00	0.00
Positive control	5.00	94.60	4.90	94.57	5.10	93.77

SFE: Supercritical fluid extraction

Figures

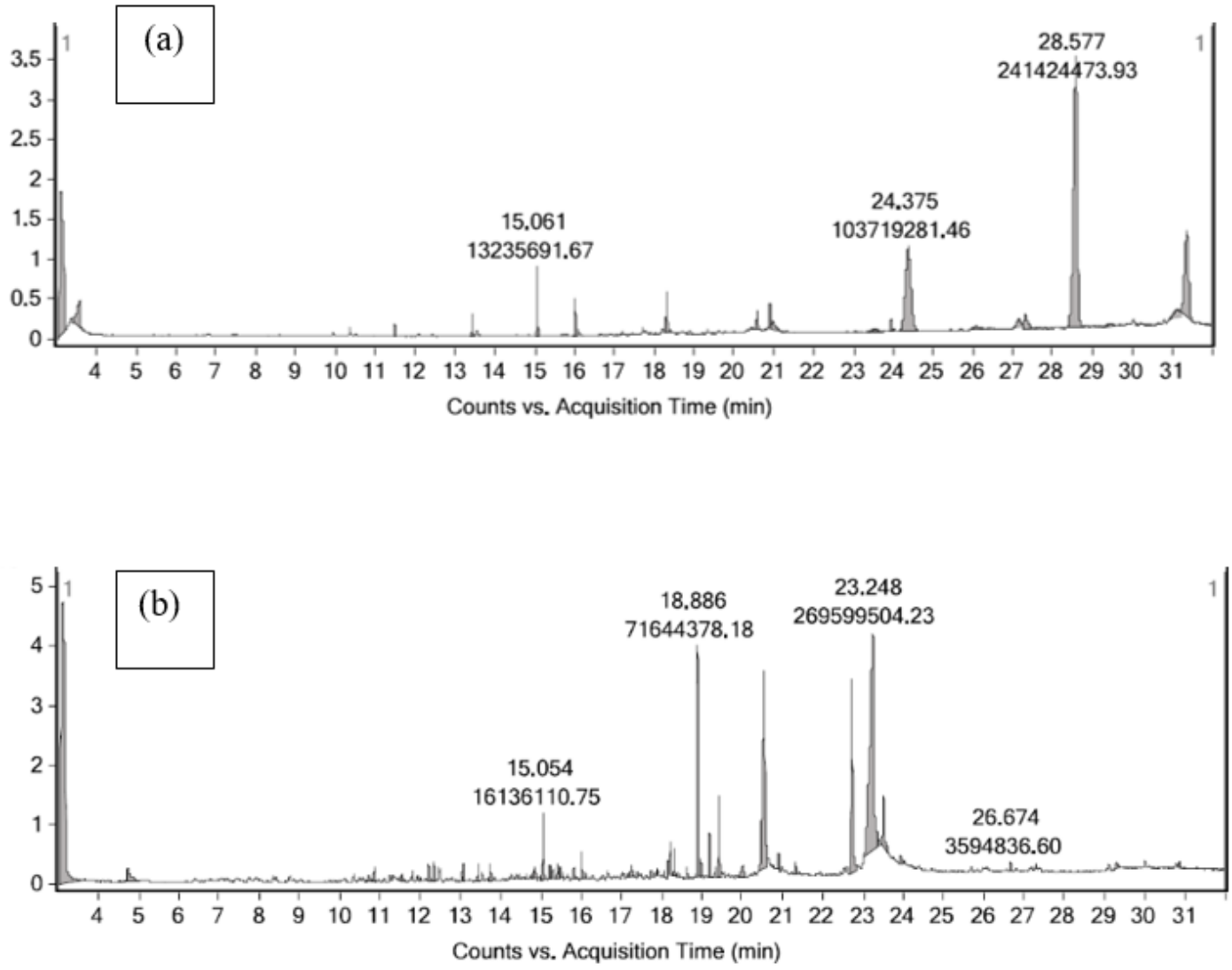

Figure 1

GCMS-MS chromatogram (a) Soxhlet extract (b) SFE extract

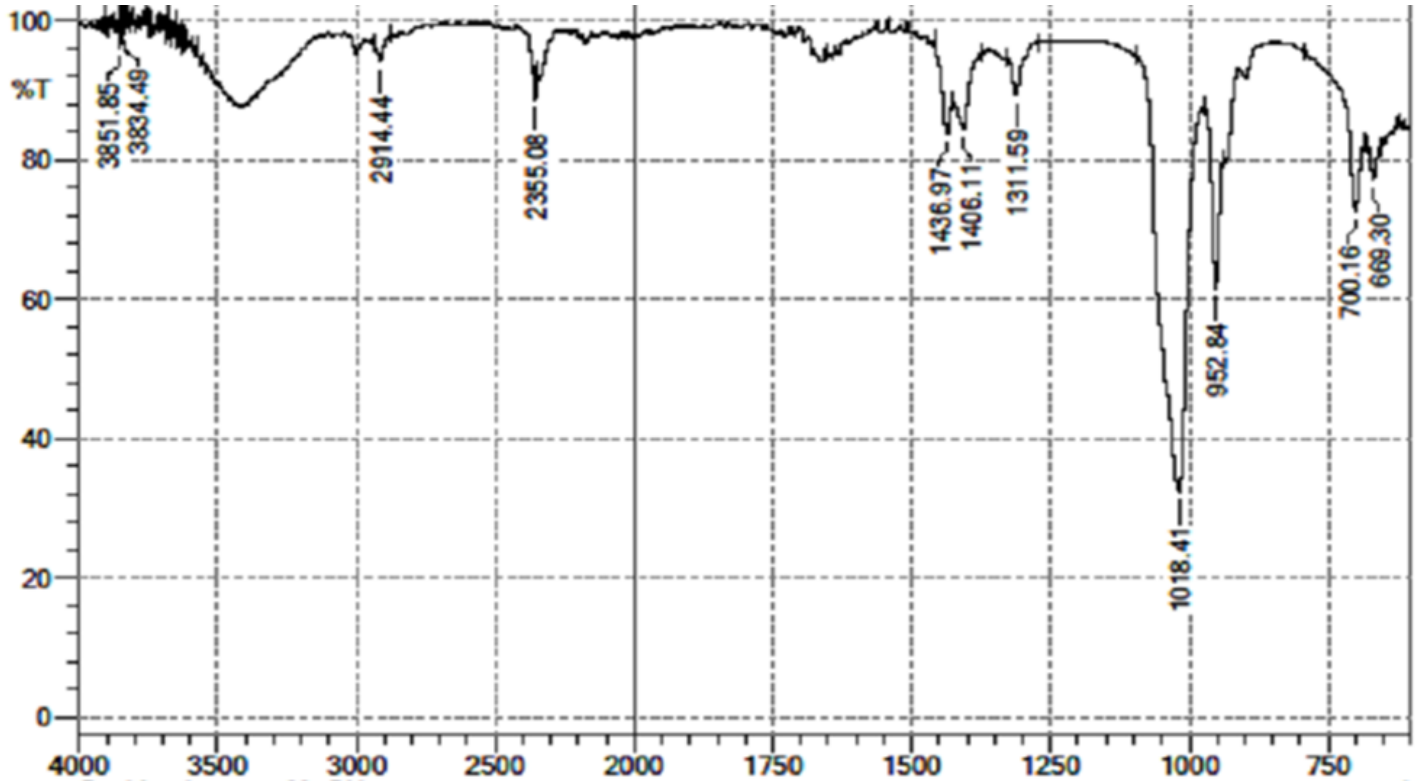

Figure 2

FTIR spectra of *Prosopis juliflora* leaves SFE extract