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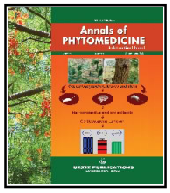

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FTIR fingerprint analysis of *Sida cordifolia* L. and *Withania somnifera* (L.) Dunal root used in Balarista formulation

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Abstract

Sida cordifolia L. and *Withania somnifera* (L). Dunal are the two most important ingredients in the preparation of Balarista formulation. The present study was aimed to assess the quality of these raw materials by FTIR fingerprint analysis. The roots of the both plants were subjected to extraction using n-hexane, chloroform, ethyl acetate and water using Soxhlet apparatus. The obtained extracts were subjected to FTIR analysis. All the spectra were recorded in the range of 400-4000 cm^{-1} using an ALPHA II FTIR spectrometer and a platinum ATR unit, using OPUS TOUCH software. The sharp characteristic peaks were observed at 2921.74, 2855.83, 1711.37, 1456.41, 750.84, 500.92 cm^{-1} of n-hexane, 1214.40, 744.24, 669.16 cm^{-1} of chloroform, 3329.88, 2933.17, 2836.30, 1019.38, 750.82 cm^{-1} of ethyl acetate, 3324.72, 2941.83, 2831.03, 1020.72, 752.84 cm^{-1} of aqueous extract of *S. cordifolia* and 3363.43, 2928.34, 1712.60, 1217.71, 1023.18, 749.81, 664.60 cm^{-1} of n-hexane, 3369.30, 2923.48, 1697.31, 1382.45, 1030.33, 753.49, 478.55, 447.39 cm^{-1} of chloroform, 3371.15, 2922.63, 1712.36, 1241.59, 1039.41, 753.68 cm^{-1} of ethyl acetate, 3272.98, 1583.77, 1379.07, 1030.21, 515.55, 447.91 cm^{-1} of aqueous extract of *W. somnifera*. The characteristic peaks in FTIR fingerprint analysis of the different extracts of the *S. cordifolia* and *W. somnifera* can be used as standard tool for the authentication of these raw materials used in Balarista formulation.

1. Introduction

Traditionally, 1500 plants have been used in systems of indigenous medicine like Ayurvedic, Siddha and Unani. Herbs have been utilized as a source of therapeutic substances for thousands of years. Currently, herbal medicines are gaining a lot of attention because they possess active chemical compounds (Kumari *et al.*, 2018). Alternative and complementary medicine commonly uses herbal medicine for the treatment of various ailments (Joos *et al.*, 2012). In developing or developed countries, over 80% of the population uses plant based medicines as preventative and therapeutic agents (Barnes, 2003). Due to increased demand, some herbal products have also been adulterated and abused, leading to consumer and producer disappointment. Health problems can also be caused by adulteration and abuse of herbal products (Bodeker and Ong, 2005). There is an emerging concern about the authenticity and discriminate use of adulterants in herbal products (Rohman *et al.*, 2019). As herbal products become more popular in primary healthcare system, discrimination and authenticity have emerged issues, particularly in countries that developed alternative medicines (Liang *et al.*, 2004). Due to economic profit motives, high quality herbal medicines are often diluted with inferior quality herbal medicine that is reasonable to defraud the consumer (Jordan *et al.*, 2010). Herbal ingredients have been identified, discriminated, and authenticated using a variety of methods, including macroscopic and microscopic analysis, chemical analyses, and some sophisticated instruments such as spectrophotometers (Kamboj, 2012). In recent years, a new strategy has been developed for distinguishing and authenticating herbal medicines by analyzing targeted components by fingerprinting (Riedl *et al.*, 2015). Quality control of herbal medicinal products has been carried out using fingerprinting approach. Using analytical methods to identify the ingredients, herbal fingerprinting represents the characteristics of the components (Razmovski-Naumovski *et al.*, 2010). A fingerprint profile typically contains large number of data that are difficult to interpret. As a result, near-IR (NIR) and mid-IR (MIR) spectroscopy have been extensively used to differentiate and authenticate herbal components for years (Rohman *et al.*, 2014). Radiation from IR sources can be divided into three primary categories, namely; NIR with wave numbers ranging from14,000-4,000 cm^{-1}, MIR at 4,000-400 cm^{-1}, and far IR at wave numbers of 400-50 cm^{-1}. A wide range of herbal medicines and pharmaceutical products have been identified, analyzed qualitatively and quantitatively using NIR and MIR spectroscopy (Lohumi *et al.*, 2015). The NIR technique is a fast, non-destructive and economical method to obtain chemical and physical data about a sample (Roggo *et al.*, 2007). *S. cordifolia* (Malvaceae) is found throughout tropical and subtropical plains of India and Ceylon up to an altitude of 1050 meters. Medicinal properties include treating facial paralysis and sciatica with the bark and treating bloody flux with its leaves (Nadkarni, 1954). Furthermore, it is useful in ophthalmia, rheumatism, and shivering fits, as well as enhancing sexual power (Prajapati *et al.*, 2003).

W. somnifera (Solanaceae) is an erect, evergreen, tomentosa shrub found throughout the drier parts of India in waste places and also

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cultivated to a limited extent for the medicinal root. Different pyrazole alkaloids (withasomnine, steroidal lactones, withaferin A, withanolides) were reported from root. It also contains starch, reducing sugars, glycosides, dulcitol and withaniol (Anonymous, 2005). The roots are astringent, bitter, stimulant and acrid in taste. It is used as aphrodisiac, diuretic and in treatment of leucoderma (Anonymous, 2004). Traditionally, the root paste and pounded leaves were applied over the carbuncles, ulcers and painful swelling (Prajapati *et al*., 2003). *S. cordifolia* and *W. somnifera* are the two important ingredients in the preparation of Balarista formulation. This traditional fermented biomedicine has been used in the treatment of rheumatoid arthritis, urinary infection, blood disorders, skin diseases and digestive impairment (Sekar and Mariappan, 2008). In the current study, the quality of these two raw materials was assessed by FTIR fingerprint analysis.

2. Materials and Methods

2.1 Plant materials

The roots of the both plants were collected from local area of Bargarh and authenticated by Professor Dr. Arun Kumar Das, Dept. of Rasa Sastra Bhaisajya Kalpana, Gopabanhu College of Ayurveda, Puri, Odisha. The voucher specimens (SPS/SOAU-22-23) were kept in the Pharmacognosy Department for further use. The roots were processed to coarse powder and stored in airtight container.

2.2 Preparation of extract

The roots of *S. cordifolia* and *W. somnifera* were subjected to successive extraction in Soxhlet apparatus using n-hexane, chloroform, ethyl acetate and water as solvent. The extracts were dried at 40ºC under reduced pressure using rotary evaporator (Harborne, 1998).

2.3 Fourier transform infrared spectroscopy analysis of formulation

The different extracts of *S. cordifolia* and *W. somnifera* were taken for FTIR analysis. All the spectra were recorded in 400-4000 cm^{-1} using an ALPHA II FTIR spectrometer and a platinum ATR unit, using OPUS TOUCH software. The spectra were collected at a resolution of 4 cm^{-1}. The different functional groups were observed at different intervals on the basis of peak absorption (Zou *et al*., 2005).

3. Results

FTIR analysis of n-hexane, chloroform, ethyl acetate and aqueous extract of *S. cordifolia* revealed 24, 25, 14, 18 number of peaks at different wavelengths, respectively (Tables 1, 2, 3, 4 and Figure 1).

3.1 FTIR analysis of n-hexane extract of *S. cordifolia*

The 2921.74 cm^{-1} peak was noticed with strong C-H asymmetric stretching vibration indicates presence of acyclic compound. The C-H symmetric vibration at 2855.83 cm^{-1} showed acyclic compound. The strong C=O stretching vibration at 1711.37 cm^{-1} showed carboxylic acid. The strong NO_2 stretching vibration at 1510.69 cm^{-1} represent α, β-unsaturated nitro compounds. N=N stretching vibration at 1456.41 cm^{-1} representing azothio compounds. C-H deformation revealed aldehyde at 1372.29 cm^{-1}. CH_3 rocking vibration showed 1-methoxy-phosphane at 1172.43 cm^{-1}. C-H out-of-plane ring deformation vibration at 750.84 cm^{-1} showed phenoxy group.

Table 1: FTIR spectral analysis of n-hexane extract of *S. cordifolia*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	2921.74	C-H	Stretching (asymmetric)	s	Acyclic
2	2855.83	C-H	Stretching (symmetric)	m	Acyclic
3	2740.07	C-H	Stretching	w	Aldehyde
4	2673.24	OH	Often broad and chelated	v	β-diketones
5	1711.37	C=O	Stretching	s	Carboxylic acid
6	1510.69	NO_2	Stretching (asymmetric)	s	α, β-unsaturated nitro
7	1456.41	N=N	Stretching	w	Azothio
8	1372.29	C-H	Deformation	m	Aldehyde
9	1214.92	C-H	Deformation	w	Secondary alcohol
10	1172.43	CH_3	Rocking	s-m	1-Methoxy-phosphane
11	1108.18	C-C	Stretching	w	S-alkyl methyl alkyl thioate
12	1051.11	C-C	Skeleton	w	Cyclohexanes
13	826.13	C-C	Skeletal	w	1,1 –Dimethyl
14	750.84	C-H	Deformation	s	Phenoxy
15	669.78	C-H	Wagging	w	Vinyl hydrocarbon
16	628.16	C-H	Deformation	w	Pyridines
17	589.12	C-S	Stretching	w	Aliphatic disulphide
18	564.40	C-C	Skeleton	w	Alkanes
19	549.97		Skeleton	w	Dibranched alkanes not possessing CH_3, C_2H_5
20	533.41	C-C	Skeleton	w	Alkane
21	514.20	C-C	Skeleton	w	Alkane
22	500.92	C-C	Skeleton	w	Alkane
23	468.70	C-C	Skeleton	w-m	Alkanes
24	454.49	C-C	Skeleton	w-m	Alkanes

m: Medium: m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

Table 2: FTIR spectral analysis of chloroform extract of *S. cordifolia*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3652.76	O-H	Stretching	v	Oximes
2	3604.24	O-H	Stretching	v	Oximes
3	3360.53	N-H	Stretching	m	Aliphatic primaryamine
4	3019.98	C-H	Stretching	w	1-Methoxy-phosphane
5	2926.16	CH_3	Stretching	w	Ketones
6	2857.87	C-H	Stretching	w	Aldehyde
7	1710.21	C=O	Stretching	s	Carboxylic acid
8	1629.98	$-NH_3^+$	Deformation (asymmetric)	w	Free amino acids and their hydrohalides
9	1602.20	C-H	Overtone	w	Vinylene
10	1513.80	N-H	Deformation	w	Secondary amines
11	1458.61	N=N	Stretching	w	Azothio
12	1421.29	C-H	Deformation	w	Secondary alcohols
13	1374.72	C-H	Deformation	w	Secondary alcohol
14	1315.95	C-H	Deformation	w	Amines
15	1248.79	C-C	Steletal	m	Neopentane
16	1214.40	C-O	Stretching (asymmetric)	s	Vinyl ether
17	1166.67	C-H	deformation vibration	w	Methyl zinc
18	1122.50	C-H	Deformation (symmetric)	m	1-Methylindigane
19	1035.84	C-N	Stretching	w	Primary aliphatic amines
20	944.21		Out-of-plane deformation	w	1,2-Dialkylbenzenes
21	744.24	N-H	Wagging	s	Secondary amines
22	669.16	C-C-CO	Deformation	s-m	Aliphatic aldehyde
23	629.42		Deformation	w-m	Cyclobutene
24	540.69	C-C	Skeleton	w	Dibranched alkanes not possessing CH_3, C_2H_5
25	526.54	C-C	Skeleton	w	Alkane

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

Table 3: FTIR spectral analysis of ethyl acetate extract of *S. cordifolia*

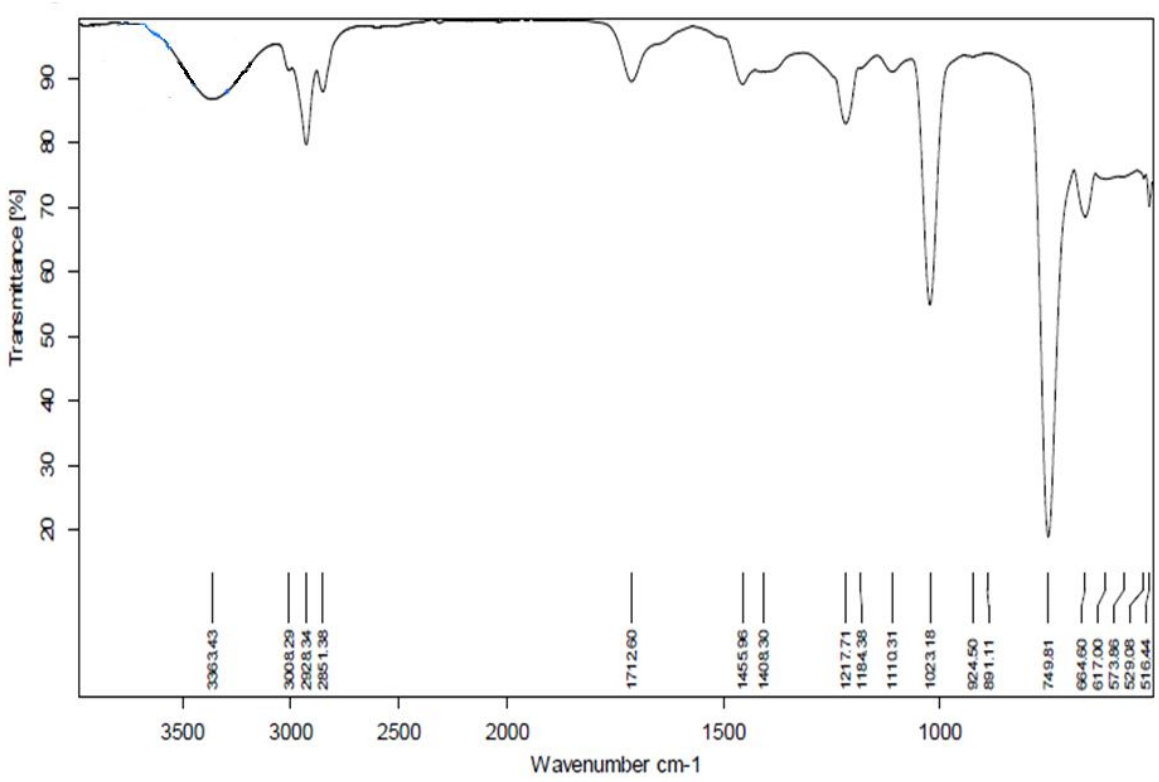
Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3329.88	O-H	Stretching	s-b	Alcohol
2	2933.17	C-H	Stretching (asymmetric)	s-m	Ethylene
3	2836.30	C-H	Stretching (symmetric)	m	Ethers
4	1654.04	C=C	Stretching	w-m	Ethylene
5	1452.82	N=N	Stretching	w	Azothio compounds
6	1411.42	C-H	In-plane deformation	w	Vinylene
7	1227.59	C-O	Stretching	s	Alkyl aryl ether
8	1110.04	C-O	Stretching	s	Saturated secondary alcohol
9	1019.38	C-S	Stretching	s	Monothio ester
10	750.82	CH	Rocking	s-m	Methyl-plumbane
11	650.12		Skeletal	v	1-alkyl ethylene
12	588.40		Broad, two bands	m-w	Propionates
13	552.55	C-C	Skeleton	w	Dibranched alkanes
14	519.21	C-C	Skeleton	w	Straight chain alkanes

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

Table 4: FTIR spectral analysis of aqueous extract of *S. cordifolia*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3324.72	N-H	Stretching	m	Secondary amine
2	2941.83	C-H	Stretching (asymmetric)	s-m	1-Methylargon
3	2831.03	C-H	Stretching (symmetric)	m	Ethers
4	1653.39	C=N	Stretching	m	Imine / oxime
5	1603.32	C=C	Stretching	m	Conjugated alkene
6	1545.75	N-H	Deformation	w	Secondary amines
7	1449.87	CH_3	Deformation	w-m	Ethoxy-phosphane
8	1411.61	O-H	Bending	m	Carboxylic acid
9	1229.78	C-H	Deformation	w	Secondary alcohol
10	1112.64	C-N	Stretching	m-w	Primary aliphatic amine
11	1020.72	C-O	Stretching	s	Alicyclic secondary alcohol
12	752.84		Butyl symmetrical skeletal	w-m	2-methoxypropane
13	653.50	C-H	Wagging	w	Vinyl hydrocarbon
14	616.54	C-H	Deformation	w	3-subst.pyridine
15	527.11	C-C	skeleton	w	Straight chain alkane
16	471.86	C-C	Skeleton	w	Branched alkane
17	445.31	C-C	Skeleton	w-m	Monobranched alkane
18	428.72	SO_3	Rocking	w	Primary alkyl sulphate salt

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.



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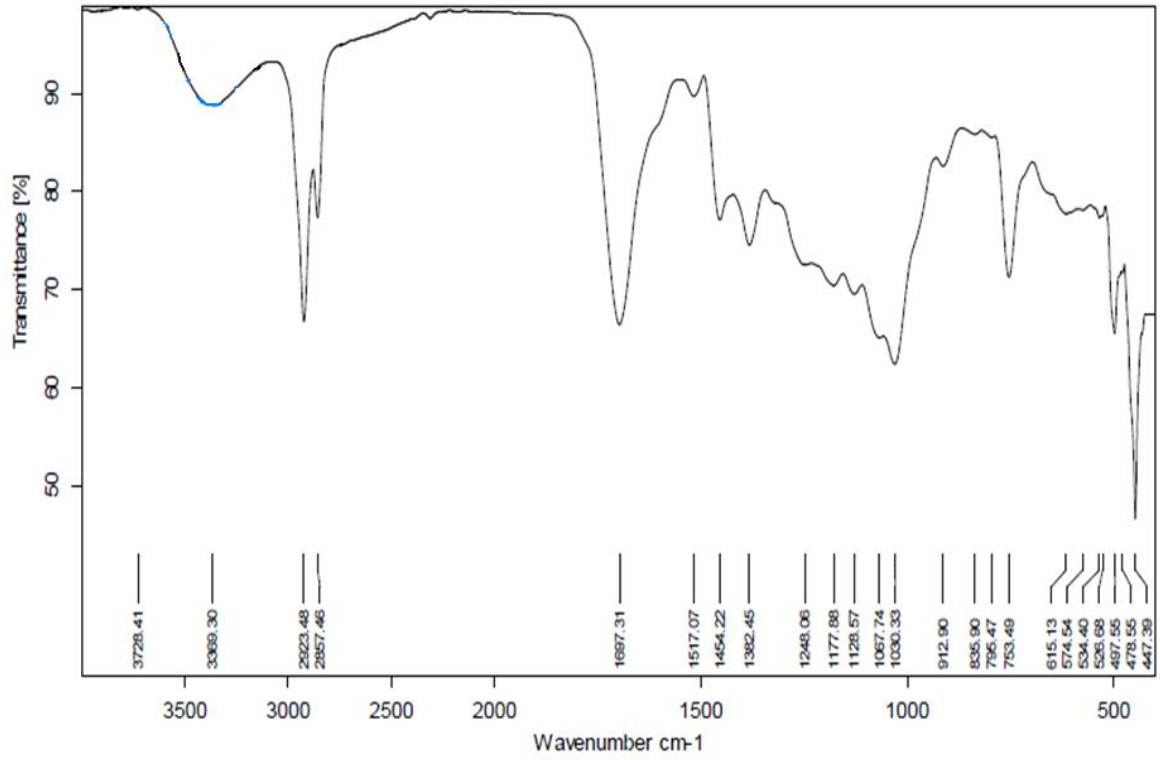
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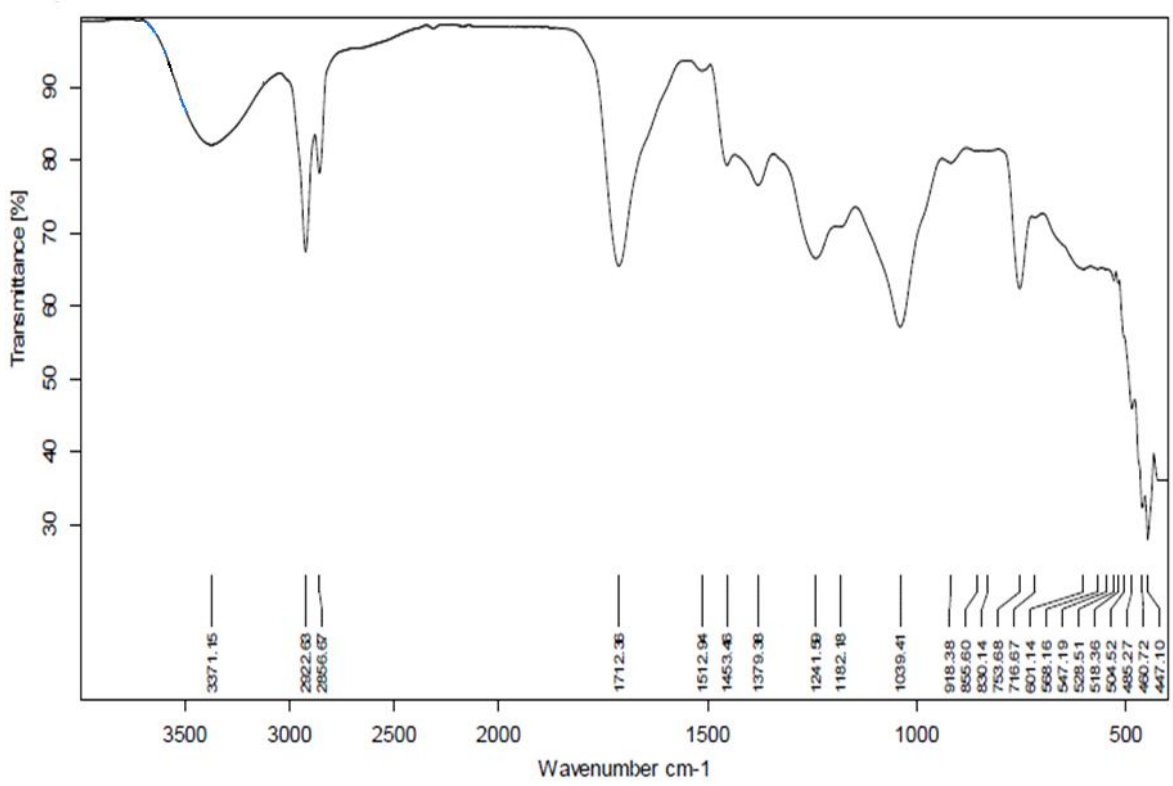
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1039.41
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753.68
716.67
601.14
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Wavenumber cm-1

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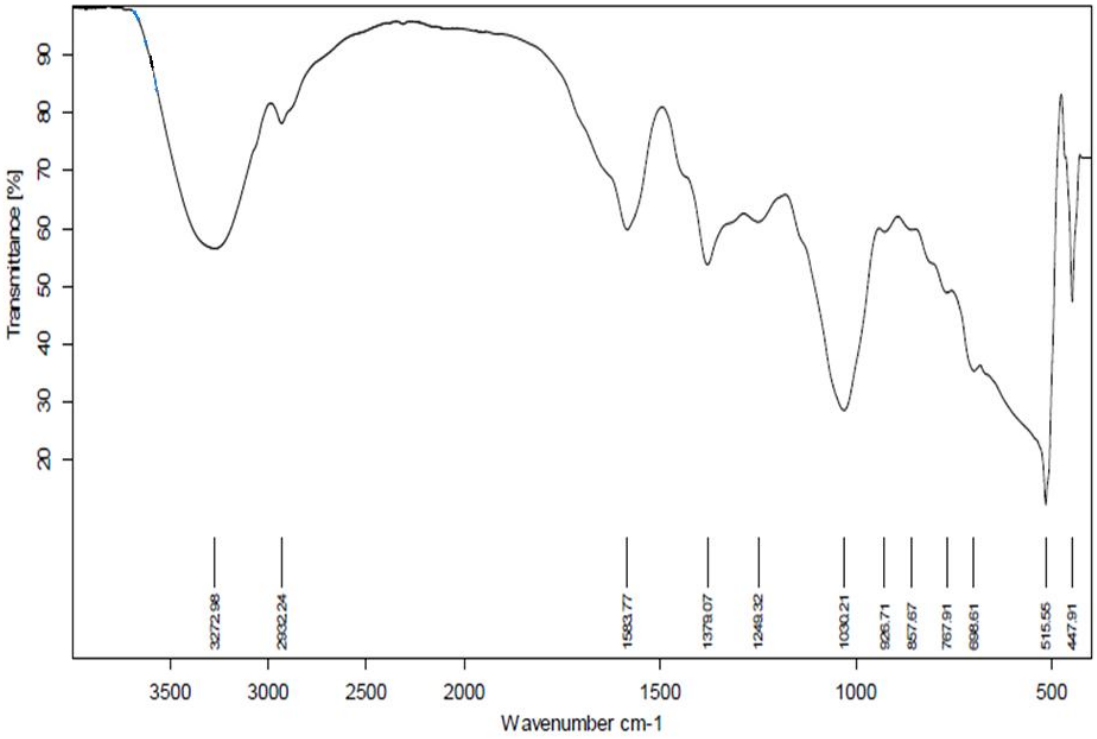


(d)

Figure 1: FTIR spectral analysis of (a) n-hexane, (b) chloroform, (c) ethyl acetate, (d) aqueous extract of *S. cordifolia*.

3.2 FTIR analysis of chloroform extract of *S. cordifolia*

The N-H stretching vibration at 3360.53 cm^{-1} showed aliphatic primary amine. Strong C=O stretching vibration revealed carboxylic acid at 1710.21 cm^{-1}. The N=N stretching vibration displayed azothio compounds at 1458.61 cm^{-1}. Strong C-O asymmetric stretching vibration showed vinyl ether at 1214.40 cm^{-1}. The medium C-H deformation vibration showed 1-methylindigane at 1122.50 cm^{-1}. C-N stretching vibration showed primary aliphatic amines at 1035.84 cm^{-1}. Strong N-H wagging vibration showed secondary amines at 744.24 cm^{-1}. C-C-CO in-plane deformation vibration at peak 669.16 cm^{-1} showed aliphatic aldehyde.

3.3 FTIR analysis of ethylacetate extract of *S. cordifolia*

The strong and broad O-H stretching vibration revealed alcohol at 3329.88 cm^{-1}. C-H stretching vibration showed ethers at 2836.30 cm^{-1}. C=C stretching vibration showed ethylene at 1654.04 cm^{-1}. N=N stretching vibration revealed azothio compounds at 1452.82 cm^{-1}. Weak C-H deformation vibration showed vinylene at 1411.42 cm^{-1}. Strong C-O stretching vibration represent alkyl aryl ether at 1227.59 cm^{-1}. Strong C-O stretching vibration revealed saturated secondary alcohol at 1110.04 cm^{-1}. Strong C-S stretching vibration showed monothio ester at 1019.38 cm^{-1}. C-C skeleton vibration showed dibranched alkanes at 552.55 cm^{-1}.

3.4 FTIR analysis of aqueous extract of *S. cordifolia*

The N-H stretching vibration at 3324.72 cm^{-1} showed secondary amine group. C-H stretching vibration at 2941.83 cm^{-1} showed 1-methyl argon group. Sharp C-H symmetric stretching vibration at peak 2831.03 cm^{-1} showed ether group. The O-H bending vibration showed carboxylic acid at 1411.61 cm^{-1}. C-N stretching vibrations at 1112.64 cm^{-1} displayed primary aliphatic amine. Strong C-O stretching vibration revealed alicyclic secondary alcohol at 1020.72 cm^{-1}.

FTIR analysis of n-hexane, chloroform, ethyl acetate and aqueous extract of *W. somnifera* revealed 19, 23, 24, 11 number of peaks, respectively (Tables 5, 6, 7, 8 and Figure 2).

3.5 FTIR analysis of n-hexane extract of *W. somnifera*

The N-H stretching vibration revealed aliphatic primary amine at 3363.43 cm^{-1}. Strong to medium C-H stretching vibration denoted acyclic compound at 2928.34 cm^{-1}. N=N stretching vibration showed azothio compounds at 1455.96 cm^{-1}. Strong C-O stretching vibration at peak 1217.71 cm^{-1} showed vinyl ether compound. C-O stretching vibration at peak 1023.18 cm^{-1} showed alicyclic secondary alcohol. C-C skeleton vibration showed cyclopentane at 924.50 cm^{-1}. N-H wagging vibrations at peak 749.81 cm^{-1} showed secondary amines.

3.6 FTIR analysis of chloroform extract of *W. somnifera*

The N-H stretching vibration at 3369.30 cm^{-1} displayed aliphatic primary amine. Strong to medium C-H stretching vibration represents 1-methylargon at 2923.48 cm^{-1}. C-H stretching vibration showed acyclic compound at 2857.46 cm^{-1}. Strong C=O stretching vibration at 1697.31 cm^{-1} showed conjugated aldehyde. The N=N stretching vibration revealed azothio compounds at 1454.22 cm^{-1}. C-H deformation vibration presented secondary alcohol at 1382.45 cm^{-1}. C-N stretching vibrations at peak 1128.57 cm^{-1} showed primary aliphatic amines. C-N stretching vibration showed primary aliphatic amines at 1030.33 cm^{-1}. The strong C-H out-of-plane ring deformation vibration at peak 753.49 cm^{-1} showed phenoxy group.

Table 5: FTIR spectral analysis of n-hexane extract of *W. somnifera*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3363.43	N-H	Stretching	m	Aliphatic primary amine
2	3008.29	C-H	Stretching (asymmetric)	w	1-Methoxy-phosphane
3	2928.34	C-H	Stretching (asymmetric)	s-m	Acyclic
4	2851.38	C-H	Stretching	w	Aldehyde
5	1712.60	P-D	Stretching		Phosphane-d
6	1455.96	N=N	Stretching	w	Azothio
7	1408.30	C-H	Deformation	w	Secondary alcohol
8	1217.71	C-O	Stretching	s	Vinyl ether
9	1184.38	C-H	Deformation	w	Methyl zinc
10	1110.31	C-C	Stretching	w	S-alkyl methyl alkyl thioate
11	1023.18	C-O	Stretching	s	Alicyclic secondary alcohol
12	924.50	C-C	Skeleton	w	Cyclopentane
13	891.11	C-H	Deformation	w	3-subst.pyridines
14	749.81	N-H	Wagging	s-b	Secondary amines
15	664.60	C-H	Wagging	w	Vinyl hydrocarbon
16	617.00	C-H	Deformation	w	3-subst.pyridines
17	573.86	C-C	Skeleton	m	Propyl and butylbenzene
18	529.08	C-C	Skeleton	w	Alkanes
19	516.44	C-C	Skeleton	w	Alkanes

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

Table 6: FTIR spectral analysis of chloroform extract of *W. somnifera*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3369.30	N-H	Stretching	m	Aliphatic primary amine
2	2923.48	C-H	Stretching (symmetric)	s-m	1-Methylargon
3	2857.46	C-H	Stretching (symmetric)	m	Acyclic compound
4	1697.31	C=O	Stretching	s	Conjugated aldehyde
5	1517.07	N-H	Deformation	w	Secondary amines
6	1454.22	N=N	Stretching	w	Azothio
7	1382.45	C-H	Deformation	w	Secondary alcohol
8	1248.06	C-H	Deformation	w	Secondary alcohols
9	1177.88	C-H	Deformation	w	Methyl zinc
10	1128.57	C-N	Stretching	m-w	Primary aliphatic amines
11	1067.74	C-N	Stretching	m-w	Primary aliphatic amines
12	1030.33	C-N	Stretching	w	Primary aliphatic amines
13	912.90	C-C	Skeleton	w	Cyclopentane
14	835.90	C-C	Skeletal	w	1,1 –Dimethyl
15	795.47	C-C	Skeletal	w	1,1 –Dimethyl
16	753.49	C-H	Deformation	s	Phenoxy
17	615.13	C-H	Wagging	w	Vinyl hydrocarbon compound
18	574.54		Deformation	w	1- vinyl-silane
19	534.40	C-C	Skeleton	w	Alkanes
20	526.68	C-C	Skeleton	w	Alkanes
21	497.55	C-C	Skeleton	w	Alkanes
22	478.55	C-C	Skeleton	w	Branched alkane
23	447.39	Si-C-C	Bending	s	Trialkyl (phenyl) silane

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

Table 7: FTIR spectral analysis of ethyl acetate extract of *W. somnifera*

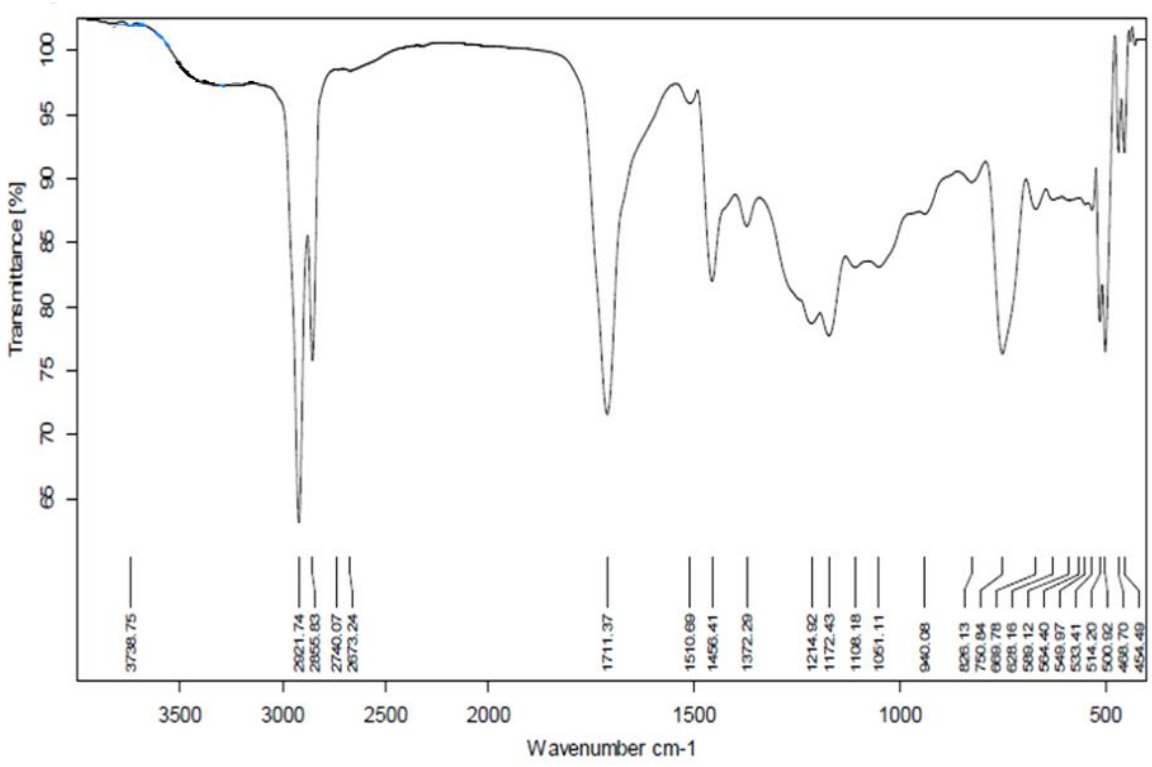
Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3371.15	N-H	Stretching	m	Aliphatic primary amine
2	2922.63	C-H	Stretching (symmetric)	s-m	Methylargon
3	2856.67	C-H	Stretching	w	Aldehyde
4	1712.36	C=O	Stretching	s	Carboxylic acid
5	1512.94	N-H	Deformation	w	Secondary amines
6	1453.46	N=N	Stretching	w	Azothio
7	1379.38	C-H	Deformation	w	Secondary alcohol
8	1241.59	C-O	Stretching	s	Alkyl aryl ether
9	1182.18	C-H	Deformation	w	Methyl zinc
10	1039.41	C-O	Stretching	s	Alicyclic secondary alcohol
11	918.38	C-C	Skeleton	w	Cyclopentane
12	855.60	O-O	Stretching	w	Peroxides
13	830.14	C-C	Skeletal	w	1,1 –Dimethyl
14	753.68	C-H	Deformation	s	Phenoxy
15	716.67		Twisting	w	Diaryl dihydridosilanes
16	601.14	C-S	Stretching	w	Aliphatic disulphide
17	568.16	C-C	Skeleton	w	Branched alkanes
18	547.19	C-C	Skeleton	w	Branched alkanes
19	528.51	C-C	Skeleton	w	Alkanes
20	518.36	C-C	Skeleton	w	Alkanes
21	504.52	C-C	Skeleton	w	Alkanes
22	485.27	C-C	Skeleton	w	Alkanes
23	460.72	C-C	Skeleton	w-m	Alkanes
24	447.10	C-C	Skeleton	w-m	Alkanes

m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.

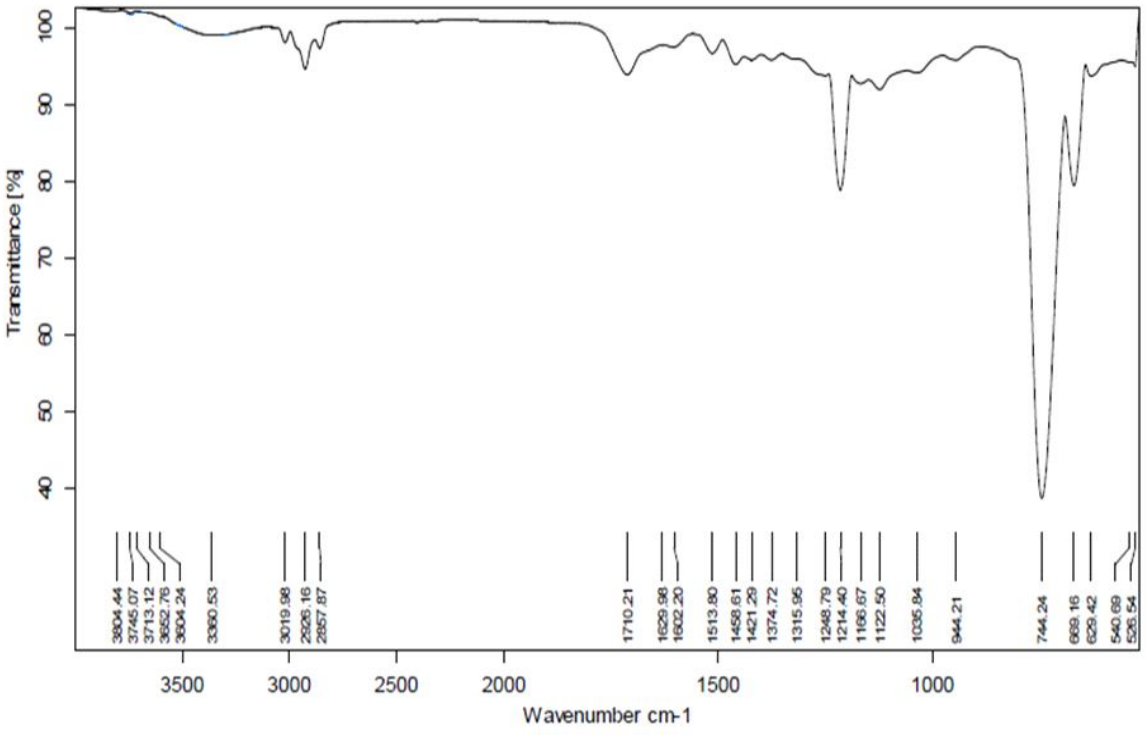
Table 8: FTIR spectral analysis of aqueous extract of *W. somnifera*

Peak No.	Wave No. (cm^{-1})	Bond type	Vibration mode	Intensity	Functional group
1	3272.98	O-H	Stretching	Strong broad	Alcohol
2	2932.24	CH_3	Stretching (symmetric)	w	Ketones
3	1583.77	N-H	Deformation	s-m	Primary amines
4	1379.07	O-H	Bending	m	Phenol
5	1249.32	C-H	Rocking	m-w	Vinyl group
6	1030.21	C-O	Stretching	s	Acyclic secondary alcohol
7	926.71	C-C	Skeleton	w	Cyclopentane
8	857.67	O-O	Stretching	w	Peroxides
9	698.61	CH_2	Deformation	w	1,1-Dialkylethylene
10	515.55	C-C	Skeleton	w	Alkane
11	447.91	C-C	Skeleton	w-m	Alkanes

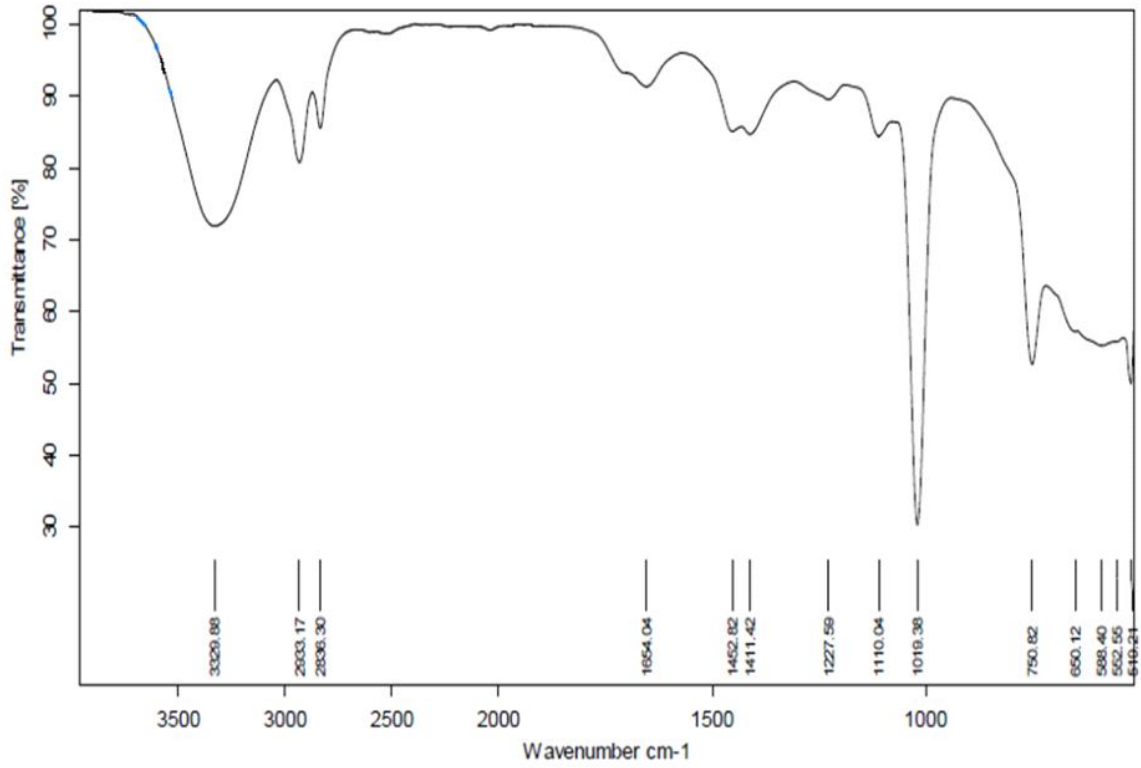
m: Medium, m-w: Medium to weak, w: Weak, s-m: Strong to medium, s-b: Strong and broad, s: Strong, w-m: Weak to medium.


Transmittance [%]
100
95
90
85
80
75
70
65
3738.75
2921.74
2855.83
2740.07
2573.24
1711.37
1510.69
1456.41
1372.29
1214.92
1172.43
1108.18
1051.11
940.08
826.13
750.84
669.78
628.16
588.12
564.40
548.97
533.41
514.20
500.92
468.70
454.49
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

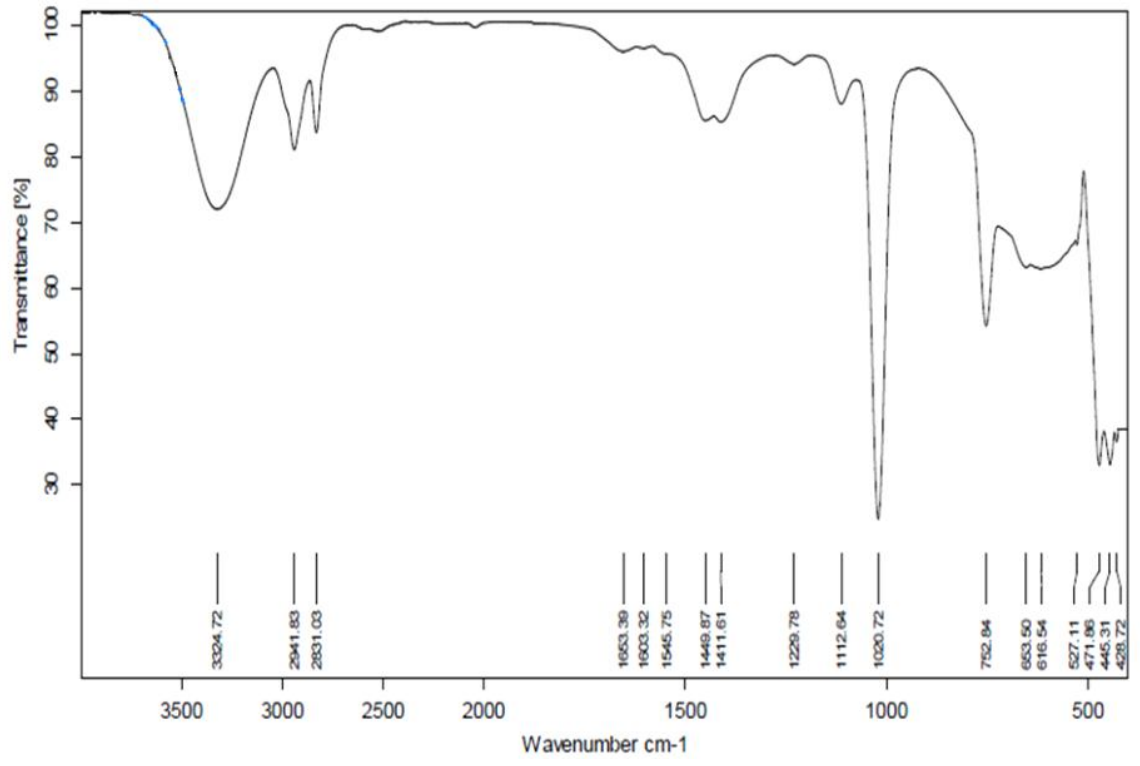
(a)


Transmittance [%]
100
90
80
70
60
50
40
3804.44
3745.07
3713.12
3652.76
3604.24
3360.53
3019.98
2926.16
2857.87
1710.21
1629.98
1602.20
1513.80
1458.61
1421.29
1374.72
1315.95
1248.79
1214.40
1166.67
1122.50
1035.84
944.21
744.24
668.16
629.42
540.69
526.54
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

(b)



(c)



(d)

Figure 2: FTIR spectral analysis of (a) n-hexane, (b) chloroform, (c) ethyl acetate, (d) aqueous extract of *W. somnifera*.

3.7 FTIR analysis of ethyl acetate extract of *W. somnifera*

The C-H stretching vibration at peak 2922.63 cm^{-1} showed methyl argon. Strong C=O stretching vibration at peak 1712.36 cm^{-1} displayed carboxylic acid. Weak N=N stretching vibration showed azothio compounds at 1453.46 cm^{-1}. Strong C-O stretching vibration showed alkyl aryl ether at 1241.59 cm^{-1}. Strong C-O stretching vibration at peak 1039.41 cm^{-1} presented alicyclic secondary alcohol. The strong C-H out-of-plane ring deformation vibration at 753.68 cm^{-1} showed phenoxy group.

3.8 FTIR analysis of aqueous extract of *W. somnifera*

The strong and broad O-H stretching vibration showed alcoholic group at 3272.98 cm^{-1}. CH_3 symmetric stretching vibration showed ketones at 2932.24 cm^{-1}. N-H bending vibrations at peak 1583.77 cm^{-1} displayed primary amines. Medium O-H bending vibration revealed phenol at 1379.07 cm^{-1}. C-H rocking vibration showed vinyl group at 1249.32 cm^{-1}. Strong C-O stretching vibration revealed acyclic secondary alcohol at 1030.21 cm^{-1}. C-C skeleton vibration showed cyclopentane at 926.71 cm^{-1}. O-O stretching vibration showed peroxides at 857.67 cm^{-1}. Tertiary butyl symmetric skeletal vibration showed 2-methoxypropane at 767.91 cm-^{1}.

4. Discussion

Due to the rapidness, non-destructive, low cost and easy in sample preparation, the NIR and MIR spectroscopies have been employed in the identification and authentication of plant extracts (Mazivila and Olivieri, 2018). The characteristic peaks noticed in the aqueous extract of *W. somnifera* were compared with previously reported work, *i.e.*, the presence of alcoholic group at 3272.98 cm^{-1} was also noticed previously at 3422.4 and 3294.42 cm^{-1}, respectively (Uddin *et al*., 2018; Aryal *et al*., 2020). The presence of ketone at 2932.24 cm^{-1} was also observed earlier at 2930 cm^{-1} (Trivedi *et al*., 2021), 2924.09 cm^{-1} (Aryal *et al*., 2020) and 2929 cm^{-1} (Trivedi *et al*., 2021). The phenol group noticed at wavelength 1379.07 cm^{-1} in the present study was reported previously at 1373.32 cm^{-1} (Aryal *et al*., 2020). The wavelength 1249.32 cm^{-1} revealed vinyl group; however, carboxylic acid was reported earlier at same wavelength as reported by Uddin *et al*. (2018). The appearance of acyclic secondary alcohol at 1030.21 cm^{-1} was also found at 1030 cm^{-1} (Trivedi *et al*., 2021). The wavelength 857.67 cm^{-1} revealed O-O stretching vibration; however, C-H aromatic bending was seen at same wavelength as reported by Trivedi *et al*. (2021).

The sharp characteristic peaks observed at 2921.74, 2855.83, 1711.37, 1456.41, 750.84, 500.92 cm^{-1} of n-hexane, 1214.40, 744.24, 669.16 cm^{-1} of chloroform, 3329.88, 2933.17, 2836.30, 1019.38, 750.82 cm^{-1} of ethyl acetate, 3324.72, 2941.83, 2831.03, 1020.72, 752.84 cm^{-1} of aqueous extract of *S. cordifolia* and 3363.43, 2928.34, 1712.60, 1217.71, 1023.18, 749.81, 664.60 cm^{-1} of n-hexane, 3369.30, 2923.48, 1697.31, 1382.45, 1030.33, 753.49, 478.55, 447.39 cm^{-1} of chloroform, 3371.15, 2922.63, 1712.36, 1241.59, 1039.41, 753.68 cm^{-1} of ethyl acetate, 3272.98, 1583.77, 1379.07, 1030.21, 515.55, 447.91 cm^{-1} of aqueous extract of *W. somnifera*, respectively can be used for standardization.

5. Conclusion

The sharp characteristic peaks observed at 1711.37, 750.84 cm^{-1} of n-hexane, 1214.40, 744.24, cm^{-1} of chloroform, 1019.38, 750.82 cm^{-1} of ethyl acetate, 1020.72, 752.84 cm^{-1} of aqueous extract of *S. cordifolia* and 1023.18, 749.81 cm^{-1} of n-hexane, 753.49, 478.55, 447.39 cm^{-1} of chloroform, 1039.41, 753.68 cm^{-1} of ethyl acetate, 1030.21, 515.55 cm^{-1} of aqueous extract of *W. somnifera*, respectively revealed different functional groups; carboxylic acid, phenoxy, vinyl ether, secondary amines, monothio ester, methyl-plumbane, alicyclic secondary alcohol, 2-methoxypropane, trialkyl (phenyl) silane, alkane which can be used for standardization. Thus, the developed characteristics peaks in FTIR fingerprint analysis of the different extracts of the *S. cordifolia* and *W. somnifera* can be used as standard tool for the authentication of these raw materials used in Balarista formulation.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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