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Investigation of Phytoconstituents Present in Methanolic Extract of Portulaca Lutea, Family- Portulacaceae.

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KEYWORDS

Portulaca lutea, Phytoconstituents, GC-MS, FT-IR

ABSTRACT:

Pharmacological activities exerted by plants are due to presence of specific phytoconstituents. In the present study, whole aerial parts of Portulaca lutea, family- Portulacaceae was procured and subjected to soxhlation using the solvents constituting petroleum ether (40 - 60 oc), chloroform, methanol and purified water. Out of the four extracts, methanol extract contained major phyto-constituents including alkaloids, flavonoids, triterpenoids. Methanol extract was subjected to isolation. Initial TLC and subsequent column chromatography and again confirmation by TLC showed four distinct fractions with profound Rf values. The isolated four fractions obtained from column chromatography were further subjected for characterization through GC-MS technique to know the chemical nature of the compounds. The isolated fraction-1showed presence of about 13 compounds including triterpenoids. The isolated fraction-2 showed presence 8 major compounds mostly triterpenoids. The isolated fraction-3 showed presence of 12 compounds mostly flavonoids. The isolated fraction-4 showed presence of 14 major compounds mostly alkaloids and higher fatty acids. Isolated fraction-1 & 3 which showed potent antidiabetic activities were characterized through FTIR. The FT-IR fingerprint spectra at 3360.20 to 3362.20 cm-1 bands showed presence of phytols, stigmasterol, inositol, tocopherols, cholesterol, octadecanamide, Luteolin and docosenamide; at 2979.65 to 2834.99 cm-1 showed presence of neophytadiene, squalene, nonadecene and tetrapentacontane; at 1651.10 to 1104.41 cm-1 showed presence of cyclopentadione, octadecanoic acid and guanosine; and at 1019.17 to 1019.90 cm-1 showed presence of pyrrolidine etc. Fraction-1 contained triterpenoids and fraction-3 contained a good number of flavonoids (Sitostenone and Luteolin) which were having antidiabetic activities as well as antioxidant activities.

INTRODUCTION:

Traditionally many plants and herbs are used by the tribal people and local vaidyas for treatment of many diseases. Plant parts extracted in liquid form as raw juice or decoction may be used extensively.[1,2] Compounds of natural origin play a major role as drugs and as lead structures for the development of synthetic molecules which has been reported to possess many biological activities like neuropharmacological effect, anti-inflammatory and analgesic effect, antimicrobial effect, antihypertensive activity, antioxidant activity, anti-hyperglycemic activity. In most of the research, the objective is to identify the phytochemical compounds present in plant as secondary metabolites.[3,4]

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This study was carried out to know the presence of various phytoconstituents[5] possessing anti-hyperglycemic activity and antioxidant properties. *Portulaca lutea* belongs to the family Portulacaceae. The herb is commonly known as Nunia Sag. The plants are branched, reddish green to purplish green, main root tuberous and fleshy. All the aerial parts of the plant are nontoxic. It is well grown in Wetland, moist place, irrigation channel of all parts of India.This herb species contain steroids, terpenoids, flavonoids, glycosides, alkaloids, and tannins.

MATERIALS AND METHODS:

Collection of plant:

The whole aerial part of *Portulaca lutea* was collected during the month of January'2018 from Bhubaneswar, Odisha. The plant specimen was identified by Dr. P. C. Panda, Principal Scientist, Regional Plant Resource Centre, Bhubaneswar, Odisha. The plant specimen (VN- UIH/22549) was deposited in the Pharmacognosy Department of Hi-Tech College of Pharmacy, Bhubaneswar.

Extraction:

The freshly collected whole aerial part of *Portulaca lutea* was shade dried and powdered. One kilogram of the powder was subjected to soxhlation using petroleum ether (40 - 60 ^{o}C), chloroform, methanol and purified water respectively. [6,7] Extraction was done for 24 hours by using Soxhlet apparatus. After 24 hours, the extract was filtered, evaporated and vacuum dried. %Yield of extracts were calculated. Petroleum ether (40 - 60 ^{o}C)

Study of extracts:

Extracts [chloroform, methanol and purified water] were subjected to phytochemical analysis followed by study of antioxidant and anti-hyperglycemic properties. Methanol extract of *Portulaca lutea* was found to contain most of the phytoconstituents such as alkaloids,phenols, saponins, flavonoids, triterpenoids etc. Methanol extract was found to be more potent towards antioxidant and anti-hyperglycemic activities. Considering the above facts methanol extract was subjected to thin layer chromatography, column chromatography and the collected fractions were analysed by GC-MS to isolate active phytoconstituents.

Chromatographic study:

Thin layer chromatographic study:[8]

TLC Plates were prepared by silica gel G. The solvent composition (toluene: n-hexane: ethyl acetate: methanol:: 3:2:1:1) with 2-3 drops of diethylamine was found to be the suitable solvent system with good resolution. Solution of methanol extract was spotted at base line of plates and plates were put inclined in the chamber. The spots were visualized under UV Cabinet with both short and long. (Fig.1)

Column chromatographic study:

A glass column (500mm X 40mm) was packed with silica gel (60-120 mesh), after packing of the column, the dried methanol extract (2g) was mixed with the developed solvent and then silica gel (80 g) was added to make the extract to get adsorbed in the silica gel. The solvent was then evaporated slowly in water bath to get the dried sample. The prepared sample was charged over the packed column and eluted with the developed solvent system to collect fractions at each 20ml interval and used for further GC-MS studies with the same solvent system. The collected fractions were tested for presence of the phytoconstituents. (Table.1)

GC-MS Analysis:

GC-MS analysis on the collect fractions of *Portulaca lutea* was carried out in the State Pollution Control Board, Odisha. GC-MS (Ultima 1310 & Thermo TQS 8000) with auto sampler (Triplus RSH) instrument was used.

Identification of Components:

Interpretation on mass spectrum obtained from GC-MS study[9] was conducted by using database of National Standard and Technology (NIST) library, containing 62,000 more patterns. The spectrum of unknown component was compared with the spectrum of the known components stored in the NIST library. Identification of compound by compound name and structure of the test materials were ascertained.

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Selection of fraction for further FTIR study:

Out of the four fractions, fraction 1 & 3 showed potent antidiabetic activities and both were subjected for characterization through FTIR. About 1 mg of plant fraction was mixed with 100 mg potassium bromide (1% w/w) of spectroscopic grade and pressed at 8 MPa via a transparent disk (1 mm) and functional groups of plant samples were analyzed by FT/IR-4600type A spectrometer (Serial Number E139561786, JASCO and Accessory ATR PRO ONE) School of Pharmaceutical Sciences, Siksha O Anusandhan (Deemed to be University), within 7800.65 to 399.193 cm^{-1} frequency range of scanning. The experimental conditions were set at 21 ºC temperature and 50 % RH. The FT-IR fingerprint spectra of both fractions are shown in Fig-6 and Fig-7.

RESULTS

From TLC, four spots were observed with different R_f values (Table.2). The fractions were further separated by column chromatography and tested for presence of the phytoconstituents (Table.1). It was found that the fraction-1(PL-M-01) contained sterols, Fraction-2 (PL-M-02) contained sterol and terpinoids, Fraction-3 (PL-M-03) contained flavonoids, glycosides and sterol, whereas Fraction-4 (PL-M-04) contained alkaloids and tannins. The fractions were subjected to analysis by the GC-MS. The GC-MS analysis is one of the determination methods which has been widely applied for the analysis and identification of phytochemical present in medicinal plants like non-polar components, volatile essential oil, fatty acids and alkaloids etc.[10] 13 major phyto-components were isolated from fraction-1 such as Nonanedioic acid, dimethyl ester, 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester, Tetradecanoic acid, 9-Octadecenoic acid (Z)-, phenylmethyl ester, (3β,5α)-Cholestan-3-ol,2-methylene, Heptadecanoic acid, 16-methyl-, methyl ester, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-, Hexadecanoic acid, 14-methyl-, methyl ester, Oleic acid, 3-(octadecyloxy)propyl ester, Lupeol,24-nor-cholest-22-ene, Sitostenone,9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-.(Fig-2 and Table.3). Eight major phyto-components were isolated from fraction-2, such as Ethanol, 2-(octadecyloxy)-, Stigmastanol, Phytylpalmitate, Phytyldodecanoate, Tetracosanoic acid, methyl ester, Daucosterol, Portuloside A, Friedelane (Fig-3 and Table.4). Twelve major phyto-components were obtained From fraction-3, such as Methyl palmitate, Linoleic acid, 6,9,12,15-Docosatetraenoic acid, methyl ester, Catechol, Desulphosinigrin, Genistein, Apigenin, Kaempferol, Luteolin, Quercetin, Calcitriol, 1-Monolinoleoylglycerol trimethylsilyl ether (Fig-4 and Table.5). Fourteen major phyto-components were isolated From fraction-4 such as, Dopamine, Noradrenalin, Indole-3-carboxylic acid, 3-Quinolinecarboxylic acid, Cyclo (L-tyrosinyl-L-tyrosine), n-cis-Feruloyltyramine, Lonchocarpic acid, Linoleic acid, Eicosapentaenoic acid, Aurantiamide Oleraceins A,B,C&D. (Fig-5 and Table.6). FTIR analysis of fraction 1 and 3 of methanol extract of *Portulaca lutea* divulged the presence of several functional groups of minor and major phytoconstituents by generating spectral profile (Fig-6 and Fig-7) with absorption bands between 7800.65 to 399.193 cm^{-1} for both the fraction. The broad peaks between 3360.20 to 3362.20 cm^{-1} represent free hydroxyl (−OH: Ar−OH stretching) group of alcohols, water, organic acids and phenols, and amine and amide (N−H: stretched) group of proteins and water, respectively; smaller peaks between 1879.65 to 1534.99 cm^{-1} represent alkanes, alkenes and alkynes (C−C, C=C and C≡C: symmetric and asymmetric of fatty acids and lipids; broad peak array between 1651.10 to 1104.41 cm^{-1} represent carbonyl (C−O) group of esters and ketones (−C=O), carboxylic acids (−COOH), aldehydes (−CHO), aliphatic amines (C−N), phosphoryl groups (P=O), nitro compounds (−NO) and nucleic acids; and lower intense peaks between 1019.17 to 1019.90 cm^{-1} represent aryl and ether hydrocarbons n(C−O−C), di and tri-substituted aromatics due to C=C stretching. The FT-IR fingerprint spectra of both fractions further invigorate different phytochemicals revealed through GC-MS analysis. The functional groups falling under 3360.20 to 3362.20 cm^{-1} bands may represent phytols, stigmasterol, inositol, tyrosinol, lupeol, lanosterol, tocopherols, cholesterol, glycerin, eicosanol, octadecanamide, oleic acid amide, Luteolin and docosenamide; 2979.65 to 2834.99 cm^{-1} may include neophytadiene, squalene, amyrin, norursadiene, hexamethyldodecane,

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nonadecene and tetrapentacontane; 1651.10 to 1104.41 cm^{-1} may specify cyclopentadione, octadecanoic acid, methyl commate-D, sitostenone, and guanosine; and 1019.17 to 1019.90 cm^{-1} may comprise pyrrolidine, etc. Fraction-1 which contained triterpenoids and esters were more active. The antioxidant activity and blood glucose level lowering activity were due to these phytochemicals. Similarly the fraction-3 contained a good number of flavonoids which were having antidiabetic activities as well as antioxidant activities.

DISCUSSION

Phyto-chemicals are an important source of medicinal plants and for naturopathy. Plant extracts are active against various diseases. [11] The present study revealed that the whole aerial parts of *Portulaca lutea* contained various phyto-chemicals including flavonoids, alkaloids, glycosides, sterols, terpenoids and tannins. The results supported similar findings observed by Samer. R.A. AL-Mosawi.[12] The GC-MS analysis of all four fractions and further FTIR analysis of fraction 1 & 3 revealed the presence of various different phyto-chemicals; including Flavonoids and other observed structured compounds.[13] This analysis indicated that collected plant materials from local place are rich in various phytocomponents due to presence of genetic variability and variation of edaphoclimatic condition from their ancestor wild ecotypes.

CONCLUSION

The present investigation on the phyto-components of whole aerial parts of *Portulaca lutea* showed that the plant is rich in flavonoids and it represents an interesting species with potent anti-oxidant activity.[14] However, further studies are still necessary to screen for more phyto-components and isolate those.

ACKNOWLEDGEMENTS

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DECLARATION OF CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

Table.1: Phytochemical Present in different isolated fraction:

Phyto-constituents	GC-MS isolated fractions			
	PL-M-01	PL-M-02	PL-M-03	PL-M-04
Flavonoids	-	-	++	-
Alkaloids	-	-	-	+
Glycosides	-	-	+	-
Sterols	+	+	+	-
Terpenoids	-	+	-	-
Tannins	-	-	-	-

(++) Presence, (+) Week Presence, (-) absent

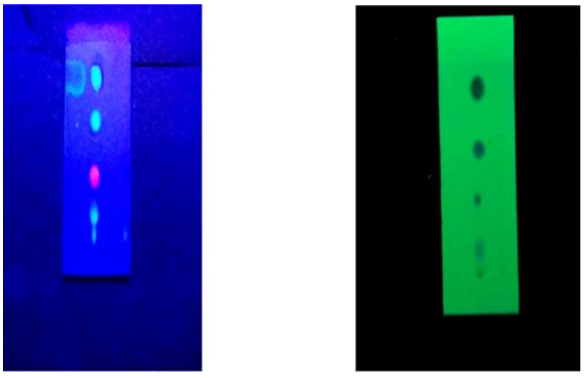

Fig-1: TLC plates showing the spots.

Table.2: R_f value of components present in methanol extract:

Fraction No.	No. of spots	Colour of the fraction	Rf. value
1	1	Colorless	0.72
2	2	White	0.67 & 0.58
3	1	Light Yellowish	0.57
4	1	Brownish Yellow	0.51

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5	1	Deep Brown	0.4

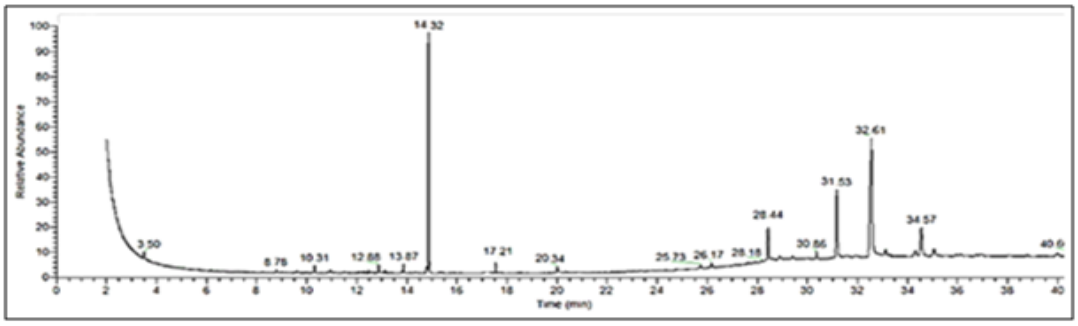

Fig-2: GC-MS spectrum of Fraction-1(PL-M-01).

Table.3: Compound present in PL-M-01isolated fraction:

Peak No	Compound Name	Formula	RT
1	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	10.31
2	1,2,4-Trioxolane-2-octanoic acid, 5-octyl-, methyl ester	$C_{19}H_{36}O_5$	12.45
3	Tetradecanoic acid	$C_{14}H_{28}O_2$	12.88
4	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	13.12
5	(3β,5α)-Cholestan-3-ol,2-methylene	$C_{28}H_{48}O$	14.32
6	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	17.55
7	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	20.34
8	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	28.44
9	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	28.44
10	Lupeol	$C_{30}H_{50}O$	30.56
11	24-nor-cholest-22-ene	$C_{26}H_{44}O$	31.53
12	Sitostenone	$C_{29}H_{48}O$	32.61
13	9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-	$C_{34}H_{64}O_2$	34.57

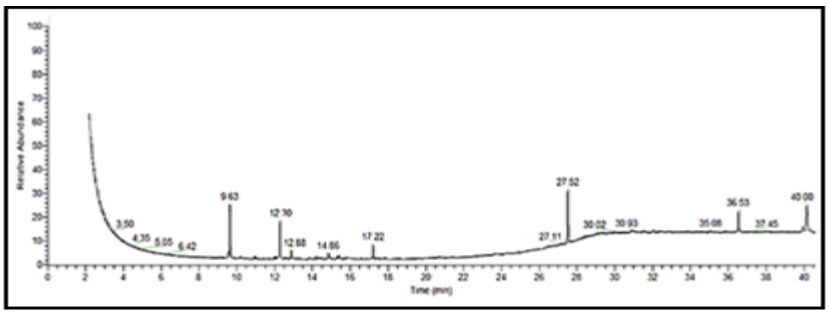

Fig-3: GC-MS spectrum of Fraction-2(PL-M-02).

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Table. 4: Compound present in PL-M-02:

Peak No.	Compound Name	Formula	RT
1	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	09.63
2	Stigmastanol	$C_{29}H_{52}O$	12.30
3	Phytylpalmitate	$C_{36}H_{70}O_2$	12.88
4	Phytyldodecanoate	$C_{32}H_{62}O_2$	14.66
5	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	17.22
6	Daucosterol	$C_{35}H_{60}O_6$	27.52
7	Portuloside A	$C_{16}H_{26}O_7$	36.53
8	Friedelane	$C_{30}H_{52}$	40.00

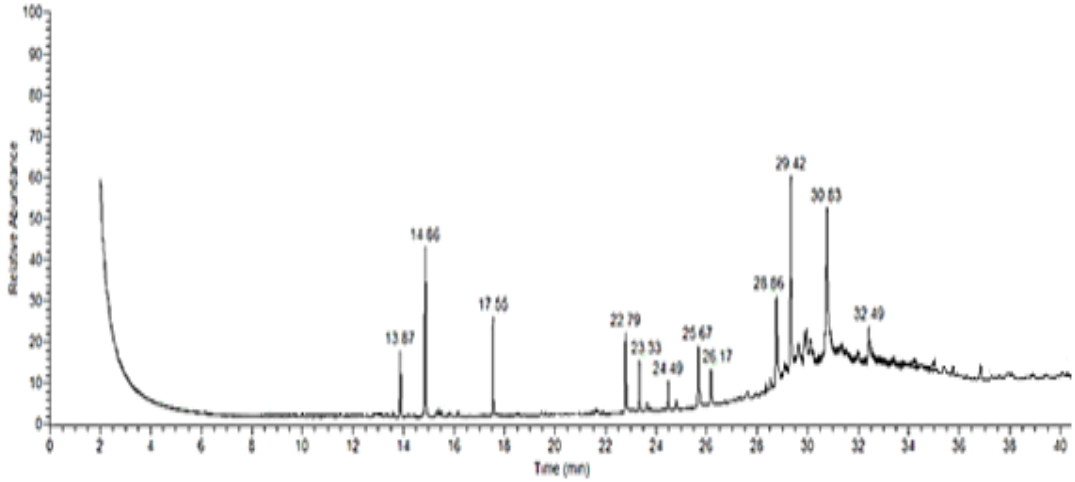

Fig-4: GC-MS spectrum of Fraction-3(PL-M-03).

Table. 5: Compound present in PL-M-03:

Peak No.	Compound Name	Formula	RT
1	Methyl palmitate	$C_{17}H_{34}O_2$	13.87
2	Linoleic acid	$C_{18}H_{32}O_2$	14.56
3	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	17.55
4	Catechol	$C_6H_6O_2$	22.29
5	Desulphosinigrin	$C_{10}H_{17}NO_6S$	23.33
6	Genistein	$C_{15}H_{10}O_5$	24.9
7	Apigenin	$C^{15}H_{10}O_5$	25.67
8	Kaempferol	$C_{15}H_{10}O_6$	26.17
9	Luteolin	$C_{15}H_{10}O_6$	28.86
10	Quercetin	$C_{15}H_{10}O_7$	29.42
11	Calcitriol	$C_{27}H_{44}O_3$	30.83

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12	1-Monolinoleoyl glycerol trimethylsilyl ether	$C_{27}H_{56}O_4Si_2$	32.49

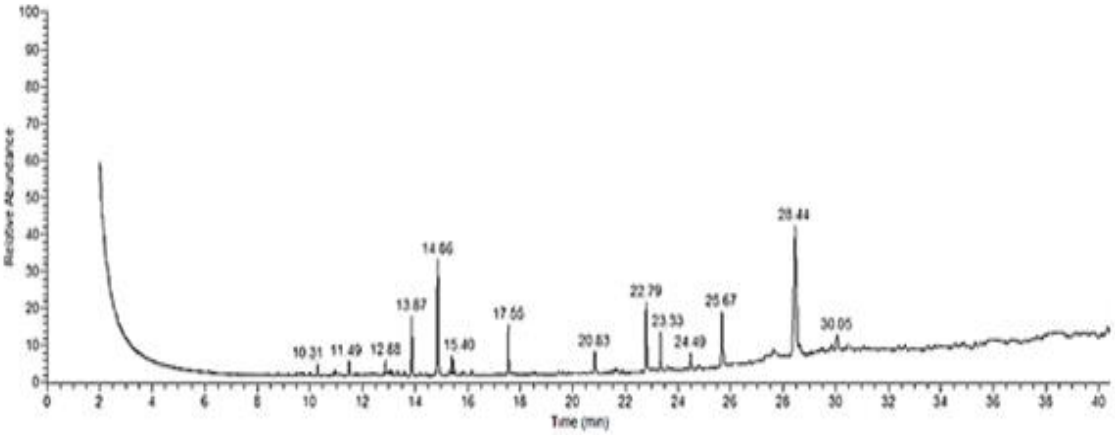

Fig-5: GC-MS spectrum of Fraction-4(PL-M-04).

Table.6: Compound present in PL-M-04:

Peak No.	Compound Name	Formula	RT
1	Dopamine	$C_8H_{11}NO_2$	10.31
2	Noradrenalin	$C_8H_{11}NO_3$	11.49
3	Indole-3-carboxylic acid	$C_9H_7NO_2$	12.48
4	3-Quinolinecarbox ylic acid	$C_{10}H_7NO_2$	13.67
5	Cyclo(L-tyrosinyl-L-tyrosine)	$C_{18}H_{18}N_2O_3$	14.66
6	n-cis-Feruloyltyramine	$C_{18}H_{19}NO_4$	15.40
7	Lonchocarpic acid	$C_{26}H_{26}O_6$	17.66
8	Linoleic acid	$C_{18}H_{32}O_2$	20.83
9	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	22.79
10	Aurantiamide	$C_{25}H_{26}N_2O_3$	23.33
11	Oleraceins A	$C_{24}H_{25}NO_{11}$	24.49
12	Oleraceins B	$C_{25}H_{27}NO_{12}$	25.67
13	Oleraceins C	$C_{30}H_{35}NO_{16}$	28.44
14	Oleraceins D	$C_{31}H_{37}NO_{17}$	30.05

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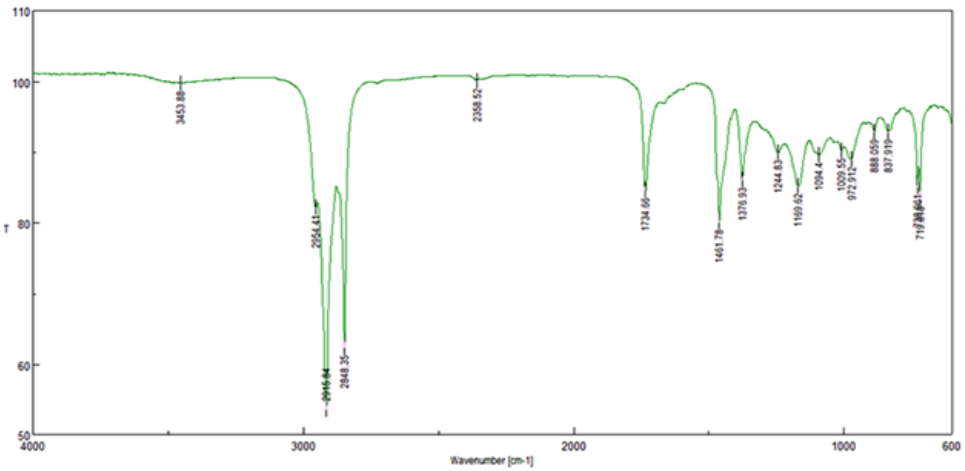

Fig-6: FT-IR spectra of fraction 1(PL-M-01).

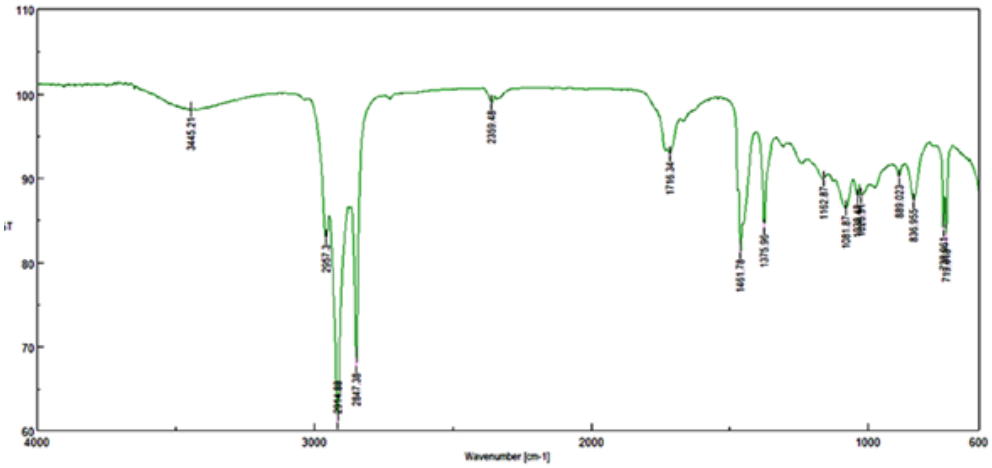

Fig-7: FT-IR spectra of fraction 3(PL-M-03).

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