Student Research project
on

QUALITATIVE ANALYSIS OF ABSORPTION OF PHENOLIC DRUGS BY ORYZA SATIVA

By

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is the bonafidework of

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QUALITATIVE ANALYSIS OF ABSORPTION OF PHENOLIC DRUGS BY ORYZA SATIVA

Introduction:

Absorption of PAOM's like drugs in plants lead to multilevel biochemical combination with phyto-chemicals which further generates series of physiological changes in plants. The interaction between plants biochemical system and drugs may cause several complex compounds, further they can involve in biosynthesis of new molecules like free radicals or bio organic conjugates. In most of the cases plants do not tolerate high concentrations of the drugs and detoxification process occurs by either bio combination with plant proteins and organic acids or bio degradation of the foreign compounds (drugs) by enzymatic action.

Quantitative analysis of absorption of drugs by plants is very important aspect to study the biological fate of absorbed drugs in plants. Qualitative analysis is helpful to characterize the bio organic conjugate of drugs, Detoxification process and precursor activity of ingested drugs. Quantitative analysis of absorbed drugs by plants was manifested by spectro analytic techniques like FTIR, H^1-NMR and Mass spectroscopy.

The present work aimed at study the biological fate of the drugs which were ingested in higher concentration to the plants and also focused on distribution of drugs in various parts of plants.

Current Experiments were performed with *Oryza sativa L.* plants in hydroponic culturing for short duration exposure of high concentration of drugs.

Material and Methods:

i. Plant material and exposure:

Oryza sativa L. plants was selected for short time exposure of APAP and ASA. 11^{th} leaf *Oryza sativa L.* plant were collected from agricultural fields and washed with 0.1% mercuric chloride and sterilized distilled water and incubated in 1000 ppm APAP and 1000 ppm ASA solution for 72 hours.

ii. Growth conditions:

The plants incubated at 25±1^{o}C under 16 / 8 hour (light/ dark cycle) photo period and irradiance provided by cool white fluorescent lamps.

iii. Extraction and sample preparation:

For the extraction of APAP and its metabolites, 5 gm of total plant material was thoroughly cleaned with deionised water and dried to remove any adhered drug on the surface of the plant. Cleaned plant material was then macerated in 50 ml methanol, and refluxed for 2hrs at 60^{0}C. The material filtered and filtrate was centrifuged to remove any micro level plants particles. The supernatant solution was evaporated to 0.5 ml sample, the content then analyzed by FTIR, H^{1}-NMR and Mass spectroscopy. Same procedure was adopted for collection of control plant's extract.

Results and Discussion:

i. Qualitative Analysis of APAP in *Oryza sativa L.* Plants:

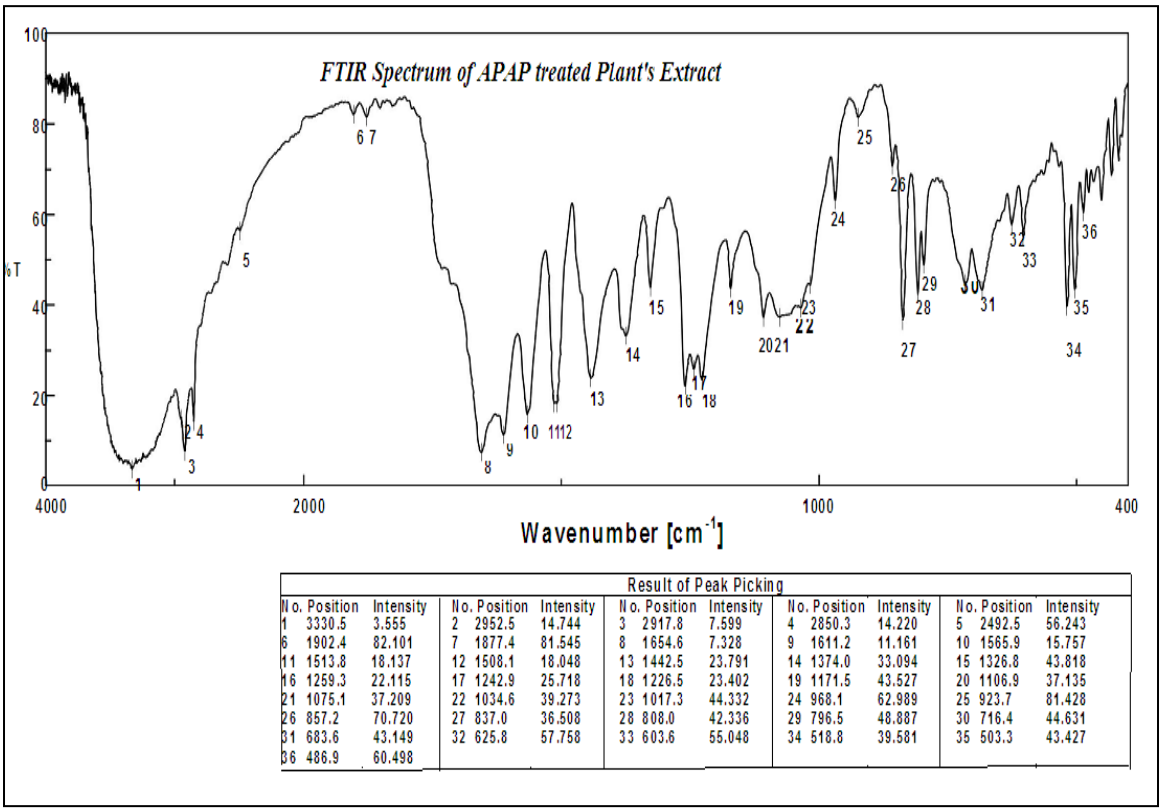
The quantitative analysis of APAP aimed at comparing the spectroscopic data of control plant extraction sample with that of extraction sample of plant grown in APAP solution.

A. FITR Analysis:

FITR spectral analysis shows that APAP was stable in aqueous conditions, and FTIR spectrum of APAP in pure state and APAP aqueous solution (suspended for 72 hours) were almost identical. This clearly suggested that plants were exposed to APAP in pure state. All the FTIR spectra were recorded using JASCO FTIR-4600 &FTIR-4100 equipments.

FITR spectrum of plants usually shows broad signals as the complex phytochemicals do not shows significant vibrations. FTIR spectrum of plant's extract usually gives broad signals due to large number of complex phytochemicals are present in the combined form, but not in Free State. Any pure form of chemical compound ingestion into the plant body will give sharp peaks in FTIR spectrum of its extract, hence FTIR spectral analysis will helpful to understand phyto-absorption and accumulation of foreign substances in the pure form.

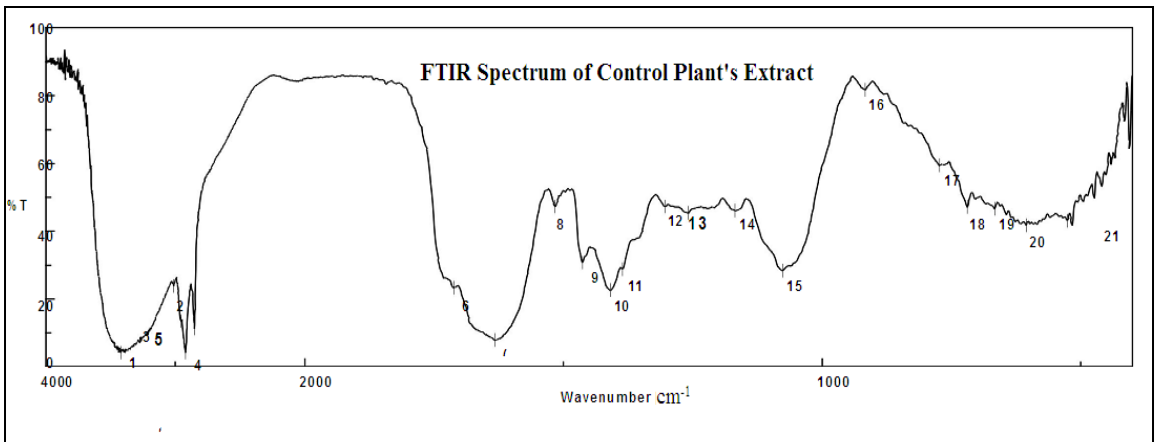
The comparison between FTIR spectrums of APAPA treated Paddy plant's extract and FTIR spectrum of APAP illustrated that all the characteristic peaks of APAP observed award wave number 1600 cm^{-1} to 460 cm^{-1} were retained in plant's extract but their intensities were different. All these sharp peaks were absolutely absent in FITR spectrum control plant extract. (No sharp peaks).



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3330.5	3.555	2	2952.5	14.744	3	2917.8	7.599	4	2850.3	14.220	5	2492.5	56.243
6	1902.4	82.101	7	1877.4	81.545	8	1654.6	7.328	9	1611.2	11.161	10	1565.9	15.757
11	1513.8	18.137	12	1508.1	18.048	13	1442.5	23.791	14	1374.0	33.094	15	1326.8	43.818
16	1259.3	22.115	17	1242.9	25.718	18	1226.5	23.402	19	1171.5	43.527	20	1106.9	37.135
21	1075.1	37.209	22	1034.6	39.273	23	1017.3	44.332	24	968.1	62.989	25	923.7	81.428
26	857.2	70.720	27	837.0	36.508	28	808.0	42.336	29	796.5	48.887	30	716.4	44.631
31	683.6	43.149	32	625.8	57.758	33	603.6	55.048	34	518.8	39.581	35	503.3	43.427
36	486.9	60.498												

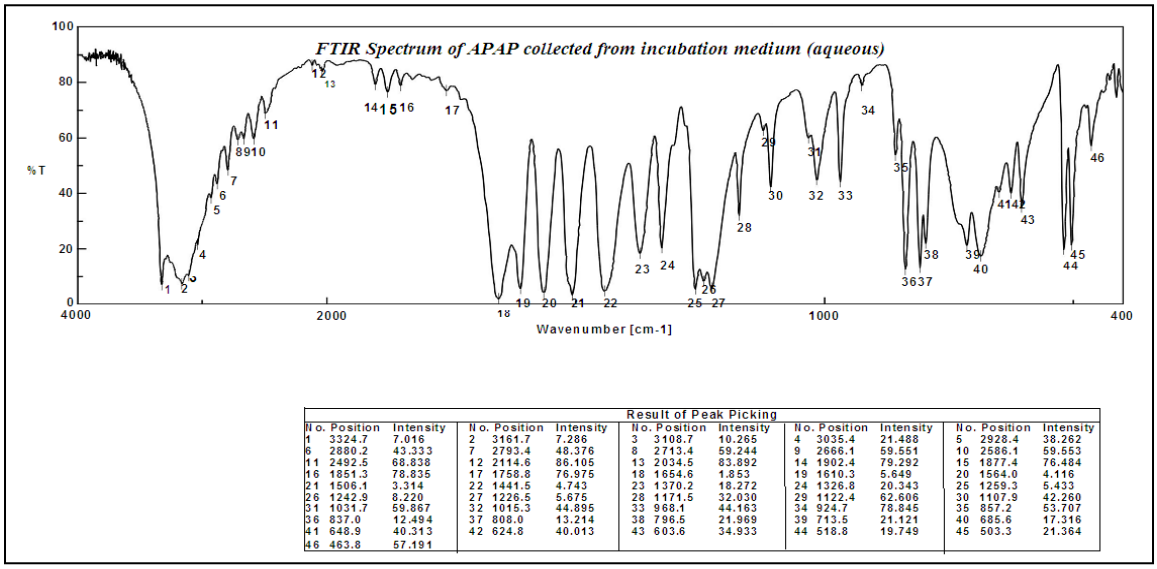
Fig3.1. FTIR spectrum of APAP collected from incubated paddy plant.



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3421.1	3.989	2	3011.3	23.824	3	2954.4	13.338	4	2918.7	4.177	5	2850.3	11.187
6	1711.5	23.241	7	1632.4	7.779	8	1515.8	47.207	9	1462.7	30.716	10	1408.7	22.524
11	1385.6	28.672	12	1303.6	47.168	13	1259.3	45.292	14	1167.7	46.015	15	1076.1	28.290
16	917.0	81.690	17	772.4	59.346	18	719.3	47.135	19	666.3	46.604	20	605.5	41.703
21	525.5	43.037												

Fig3.2. FTIR Spectrum of Control Paddy Plant's Extract.



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3324.7	7.016	2	3161.7	7.286	3	3108.7	10.265	4	3035.4	21.488	5	2928.4	38.262
6	2880.2	43.333	7	2793.4	48.376	8	2713.4	59.244	9	2666.1	59.551	10	2586.1	59.553
11	2492.5	68.838	12	2114.6	86.105	13	2034.5	83.892	14	1902.4	79.292	15	1877.4	76.484
16	1851.3	78.835	17	1758.8	76.975	18	1654.6	1.853	19	1610.3	5.649	20	1564.0	4.116
21	1506.1	3.314	22	1441.5	4.743	23	1370.2	18.272	24	1326.8	20.343	25	1259.3	5.433
26	1242.9	8.220	27	1226.5	5.675	28	1171.5	32.030	29	1122.4	62.606	30	1107.9	42.260
31	1031.7	59.867	32	1015.3	44.895	33	968.1	44.163	34	924.7	78.845	35	857.2	53.707
36	837.0	12.494	37	808.0	13.214	38	796.5	21.969	39	713.5	21.121	40	685.6	17.316
41	648.9	40.313	42	624.8	40.013	43	603.6	34.933	44	518.8	19.749	45	503.3	21.364
46	463.8	57.191												

Fig3.3. FTIR spectrum of APAP collected from aqueous drugs solution used for plant incubation.

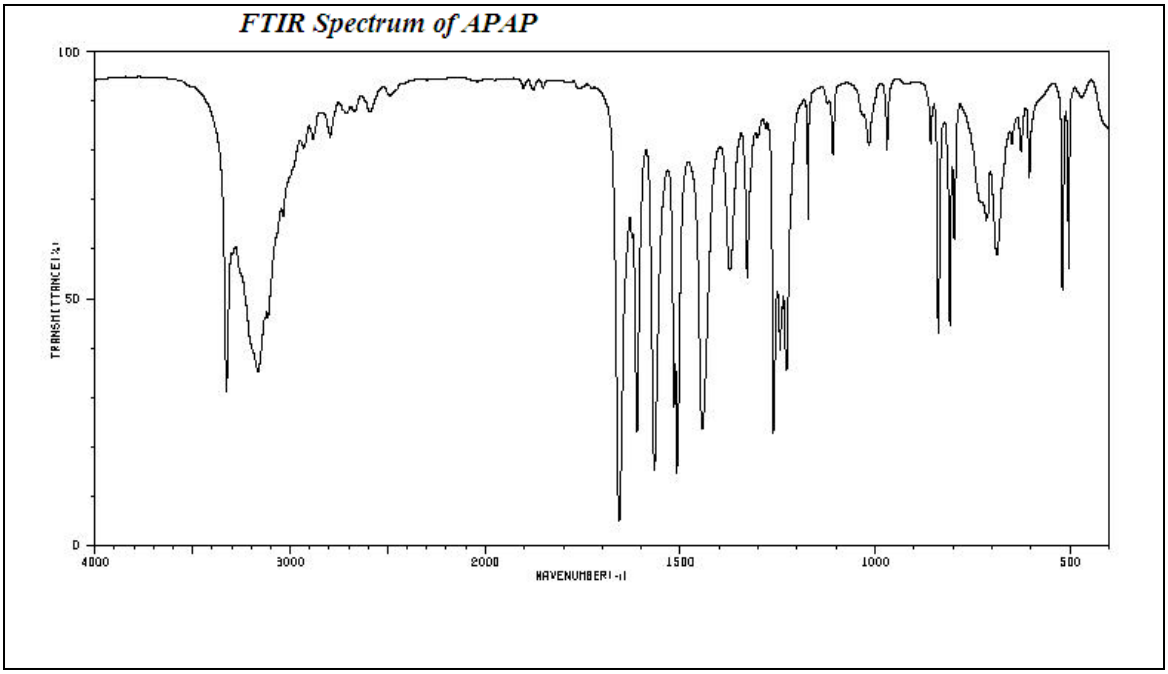


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Fig3.4. FTIR spectrum of pure APAP.

Source: Laboratory of organic chemistry,Natural products and pharmaceuticals,Anna K.Przybył, Joanna Kurek, Edited by Jan Milecki,UAM ,Poznań 2013,Laboratory ofOrganic chemistry (SERP).

B. H^1-NMR spectral Analysis:

H^1-NMR spectrum of aqueous APAP medium used for plant's hydroponic incubation and H^1-NMR spectrum of pure form of APAP were exactly same, hence APAP was stable in test conditions for great extent. But the spectral data of H^1 -NMR spectrum of APAP treated *Oryza sativa* plant's extract was not useful for qualitative analysis because NMR spectra was blurred due to interactions of phytochemicals of plants and APAP. The signal pattern of NMR spectrum of APAP treated plant's extract slightly different from NMR spectrum of control plant's extract, this might be due to bio-

conjugate of APAP and phytochemicals. All the H^1-NMR spectra were recorded by using JEOL 400MHz Spectrophotometer with multiple probe facility equipment.

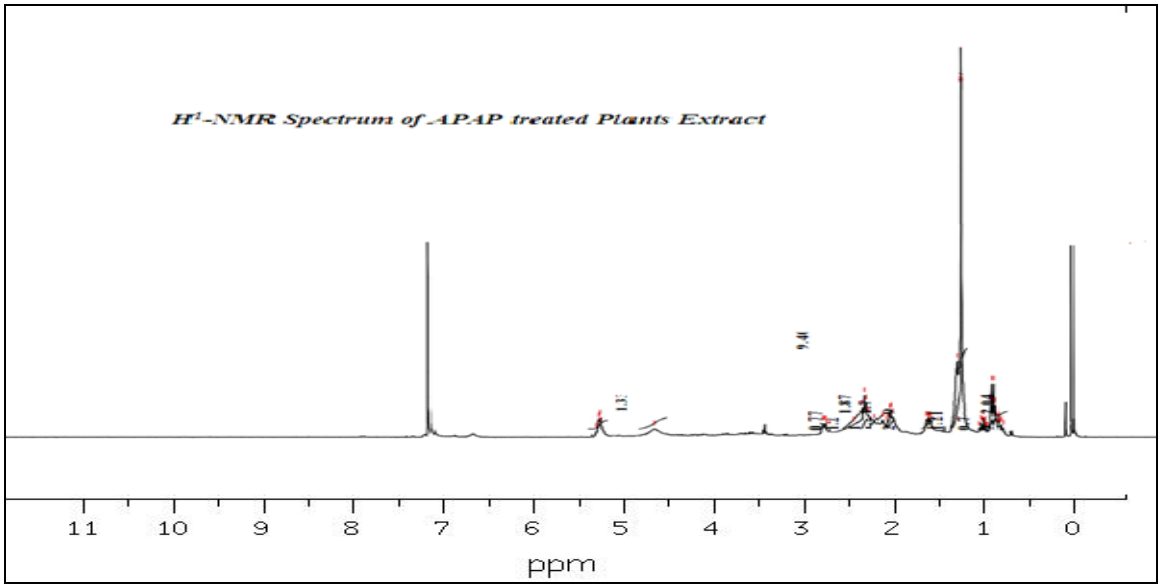


Fig.3.5. H^1-NMR spectrum APAP treated paddy plant's Extract.

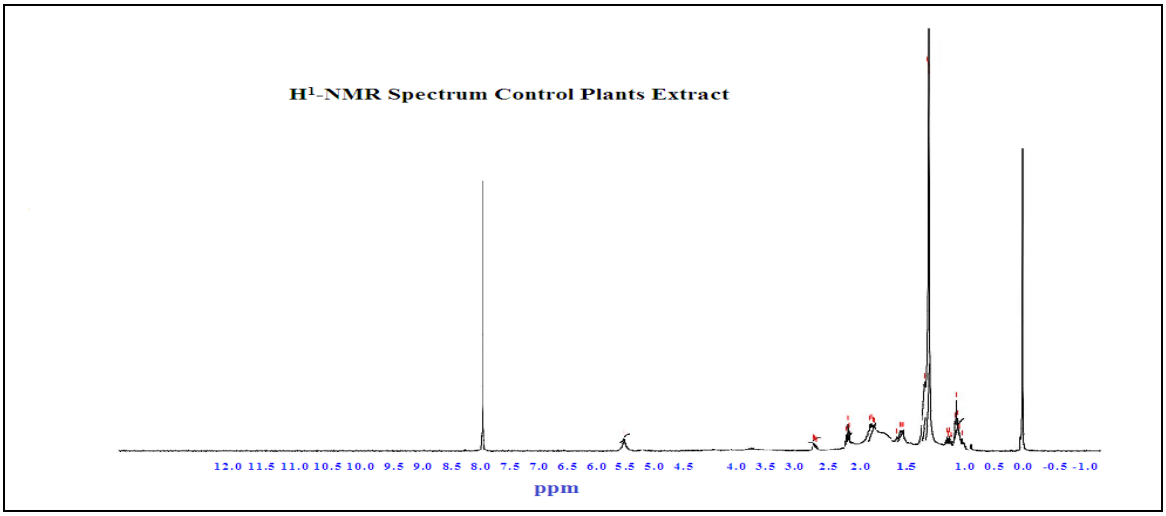


Fig3.6. H^1-NMR Spectrum of control Paddy Plant's Extract.

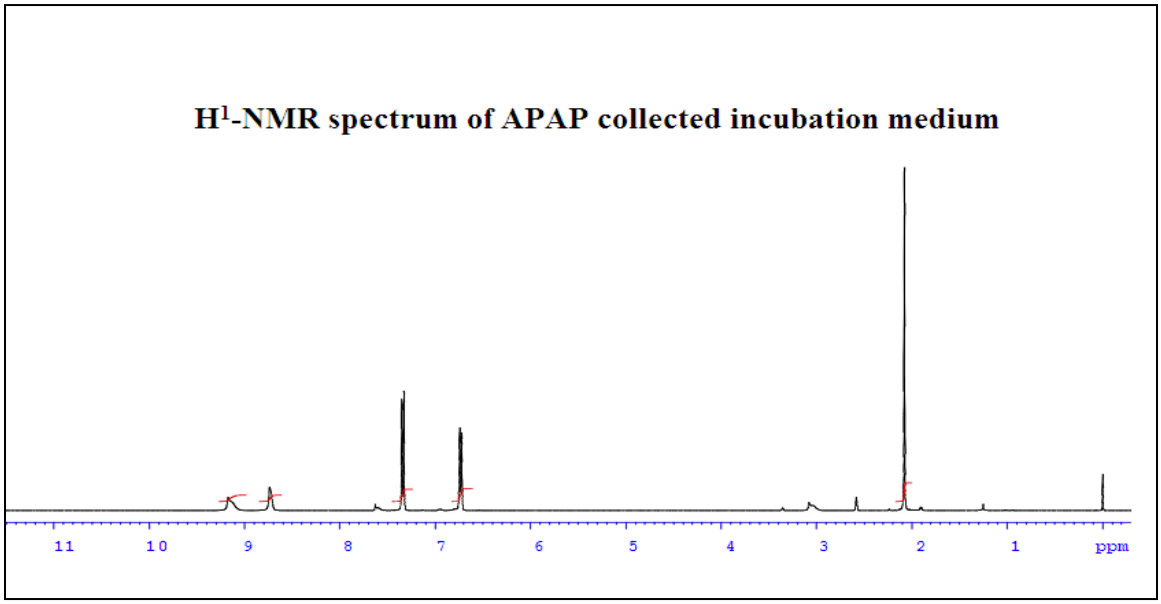


Fig3.7. H^1-NMR spectrum of APAP collected from aqueous drugs solution used for plant incubation.

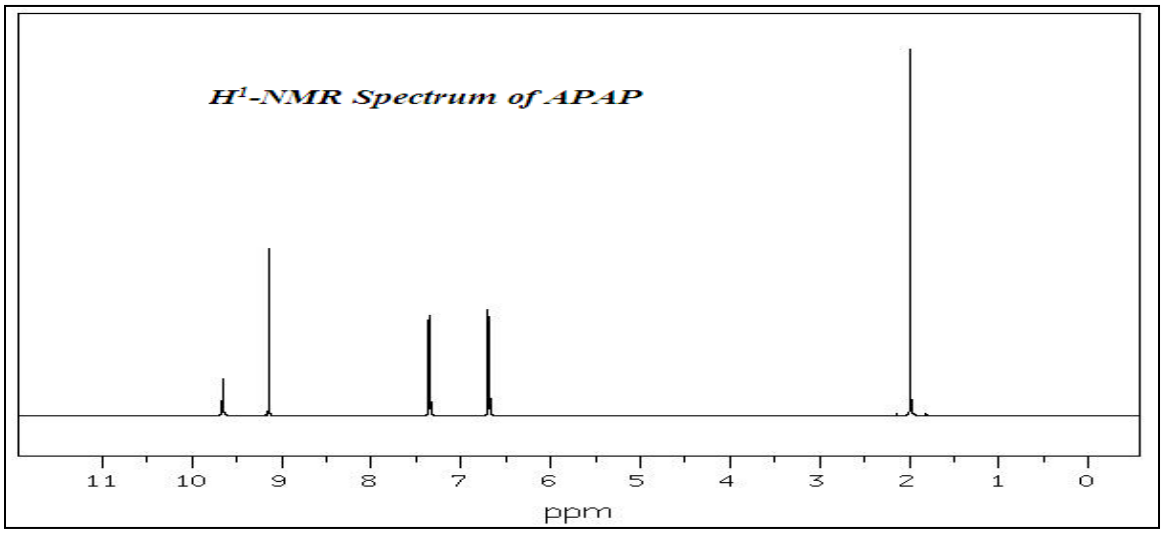


Fig3.8. H^1-NMR spectrum of pure APAP.

Source: *Laboratory of organic chemistry,Natural products and pharmaceuticals,Anna K.Przybył, Joanna Kurek, Edited by Jan Milecki,UAM ,Poznań 2013,Laboratory of Organic chemistry (SERP).*

C. Mass spectral Analysis:

Mass spectral analysis is an excellent analytical tool to identify and characterize biodegradation and biological combination of absorbed molecules by plants from their extracts. In the current study, Mass spectrum of Plant's extracts provided all the essential aspects to suggest biochemical combination of drug with plant's biomolecules like sugars, proteins, amino acids and other organic acids etc.

Mass spectrum of APAP treated Oryzasativa plant's extracts consists fragments corresponding to APAP (m/z : 150.27,151.27, 152.23, 110,109) along with some peculiar fragments around m/z values 613 to 1053 which were absent in mass spectrum of control plant's extract. Few fragments found around m/z : 278 to 329 in APAP treated plant's were absent in control plant's extract. All these fragments indicated the Bioconjugates of APAP with phytochemicals. The mass fragments of m/z 152.23, 110 supports the presence of APAP in the sample of plant extract and low intensity fragments at m/z around 314, 152,110, 328, 311 indicated the formation of Glucothionyl-conjugate of APAP.(P.Schröder et al.,2009). [3,4]

Whereas the fragments m/z 253.48, 383.51, 491.55 and 579.39 which were found in control plant's extract were absent in APAPA treated plant's extract. All these peaks were absent in pure APAP, which might be due to biochemical combination. All the Mass Spectra were recorded by using WATER-SQD-2 equipment.

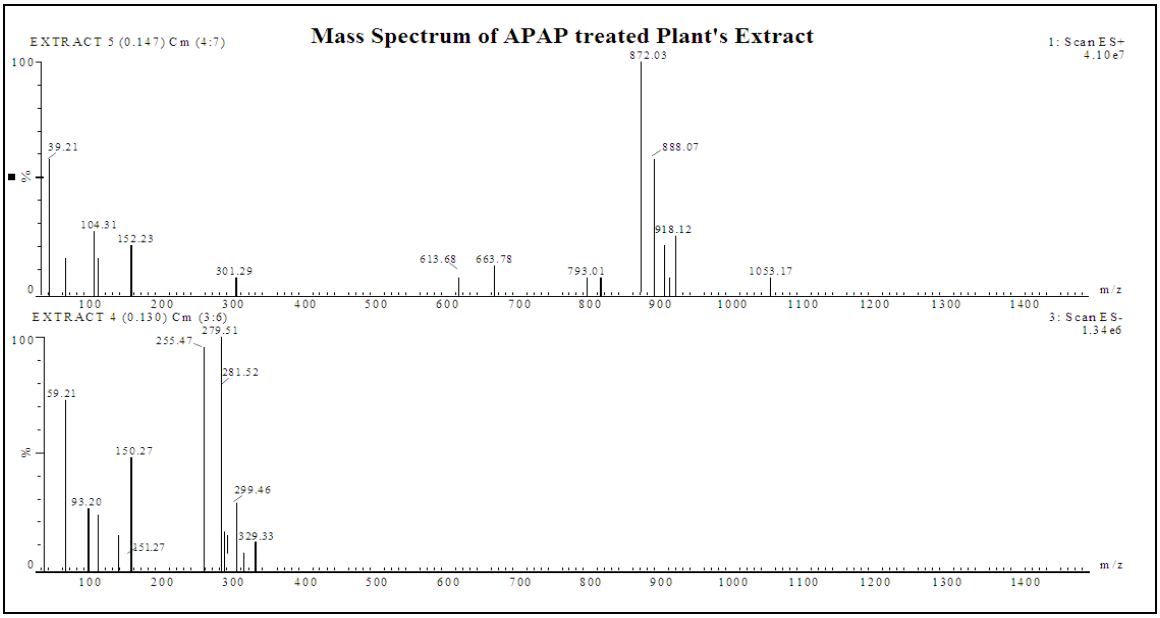


Fig.3.9. Mass Spectrum of APAP treated Paddy Plant's Extract.

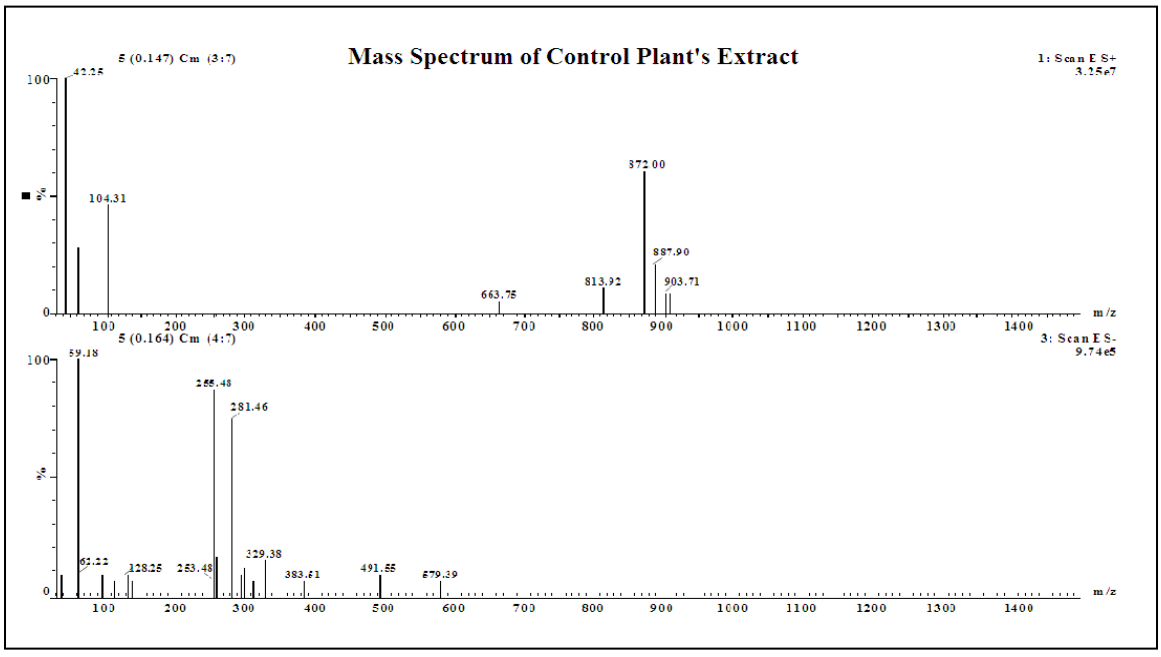


Fig.3.10. Mass Spectrum of Control Paddy Plant's Extract.

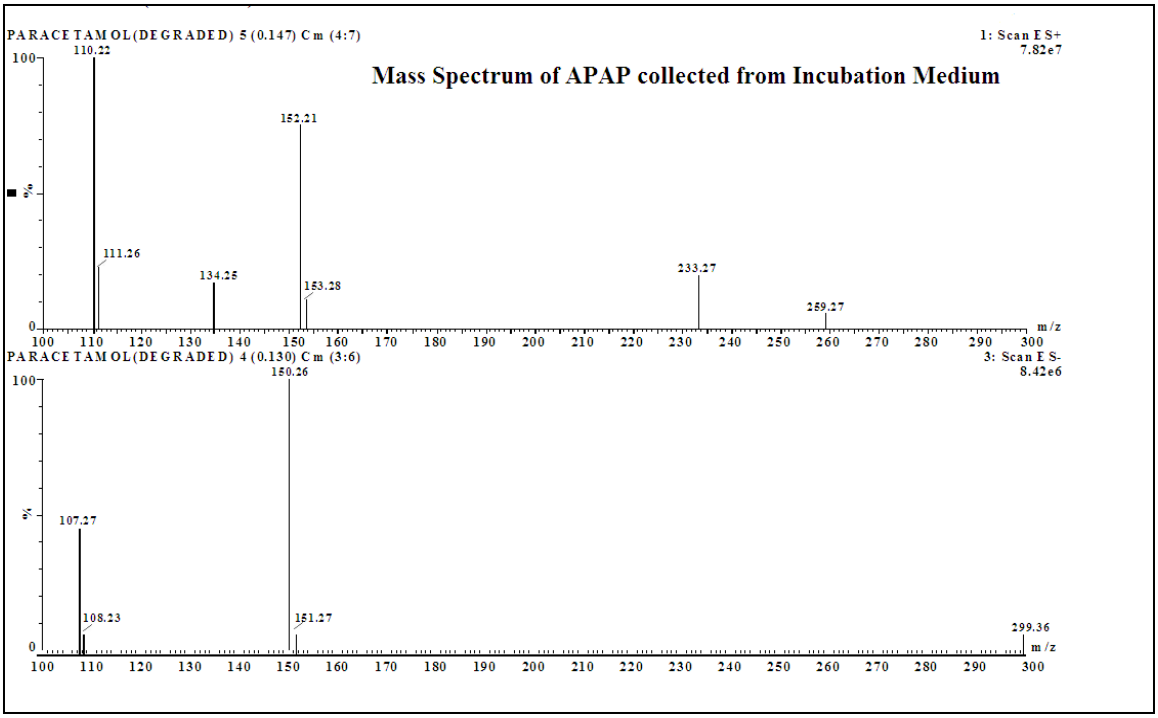


Fig3.11. Mass Spectrum of APAP collected from aqueous drugs solution used for plant incubation.

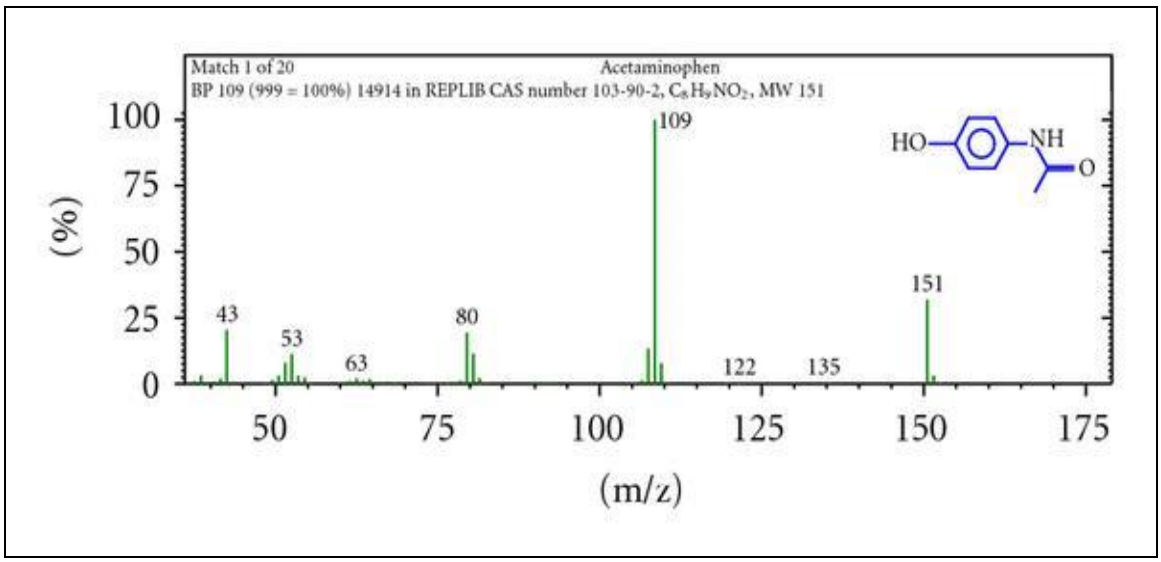


Fig.3.12. Mass Spectrum of pure APAP.

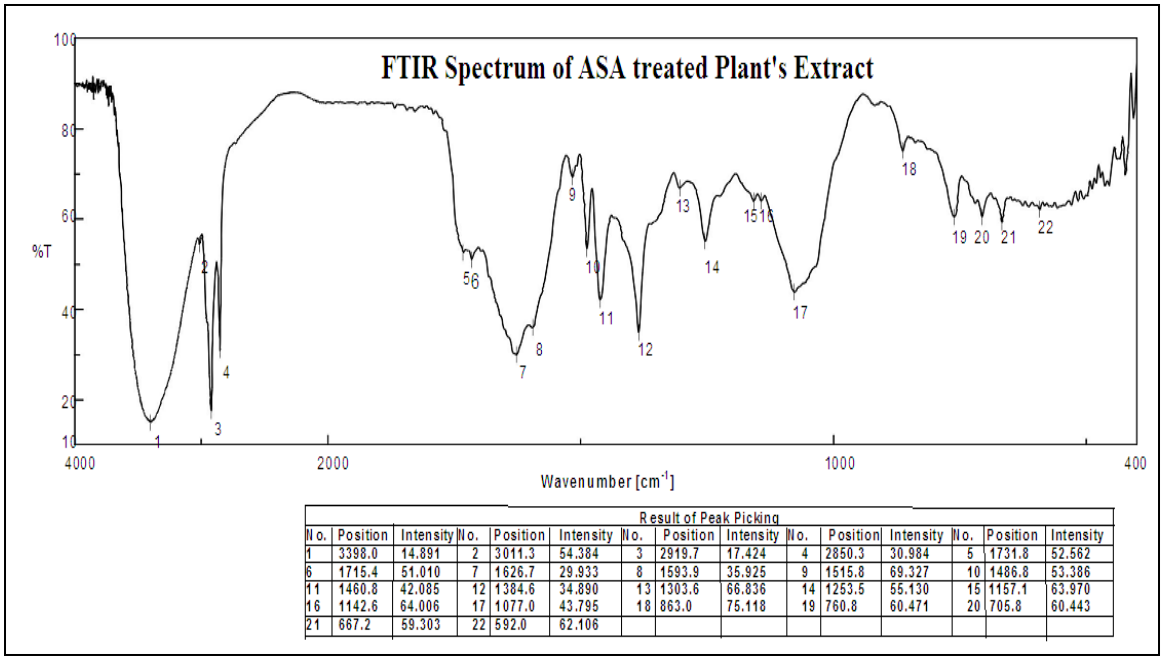
Source: www.hindawi.com/journals/ijelc/2011/171389/fig10/.

ii. Qualitative Analysis of ASA in *Oryza sativa L.* Plants:

As ASA is closely related to SA, which is a phyto-hormone, the qualitative Analysis of absorbed ASA by *Oryza sativa L.* will help to understand the biological significance of ASA in plants in terms of biochemical combinations, biodegradation, bio-conjugation, and bio-stimulation of natural product synthesis. Salicylates content was negligible in *Oryza sativa L.* (Swain et.al., 1985)[5] therefore ingestion of ASA in paddy plant was used to focus on the interaction of ASA with plant's biochemical system and biological fate of ASA within the plant. These aspects were clarified and analyzed by spectro-analytical techniques likes FITR, NMR and Mass spectroscopy.

A. FTIR-spectral Analysis:

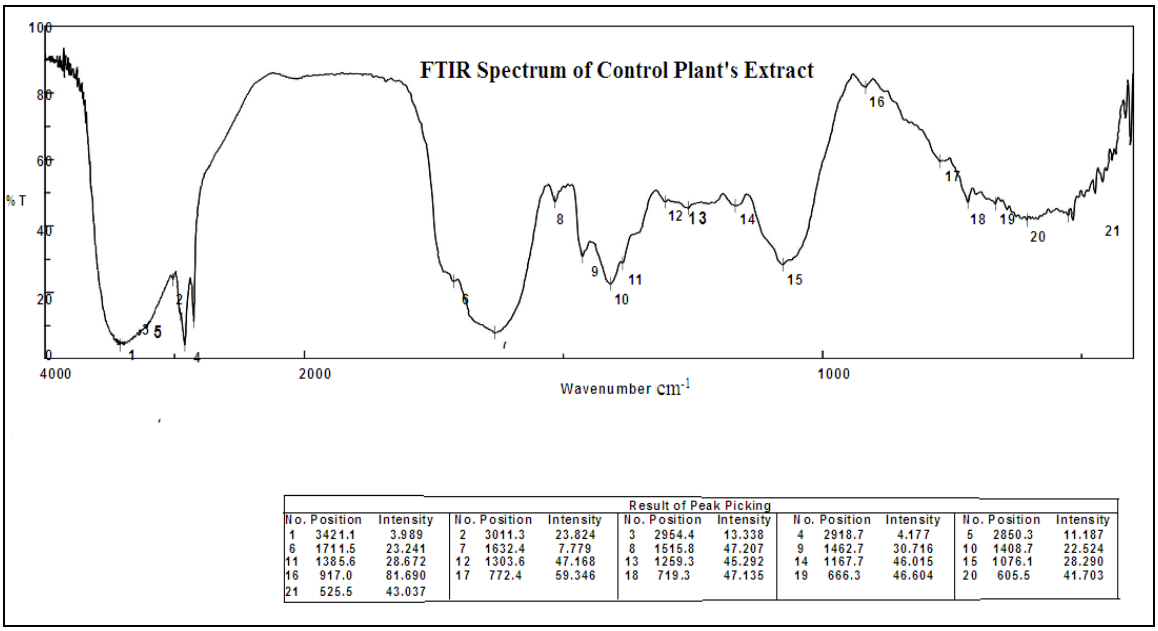
FITR spectrum of aqueous ASA medium used for *Oryza sativa L.* culturing slightly different from FTIR spectrum of pure ASA due to hydrolysis of ASA for slight extent. When the FITR spectrum of ASA, only few sharp peaks were observed in ASA treated in plant's extract and those were corresponding to neither pure ASA nor control plant's extract. Although FTIR spectrum of ASA treated plant's extract shows considerably sharp peaks which is usually not found normal phytoextracts as phytoextracts donot consists free compounds in pure state. Most of the phyto extracts show broad peaks due to complex interactions of phyto chemicals. All the FTIR spectra were recorded using JASCO FTIR-4600 &FTIR-4100 equipments.



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3398.0	14.891	2	3011.3	54.384	3	2919.7	17.424	4	2850.3	30.984	5	1731.8	52.562
6	1715.4	51.010	7	1626.7	29.933	8	1593.9	35.925	9	1515.8	69.327	10	1486.8	53.386
11	1460.8	42.085	12	1384.6	34.890	13	1303.6	66.836	14	1253.5	55.130	15	1157.1	63.970
16	1142.6	64.006	17	1077.0	43.795	18	863.0	75.118	19	760.8	60.471	20	705.8	60.443
21	667.2	59.303	22	592.0	62.106									

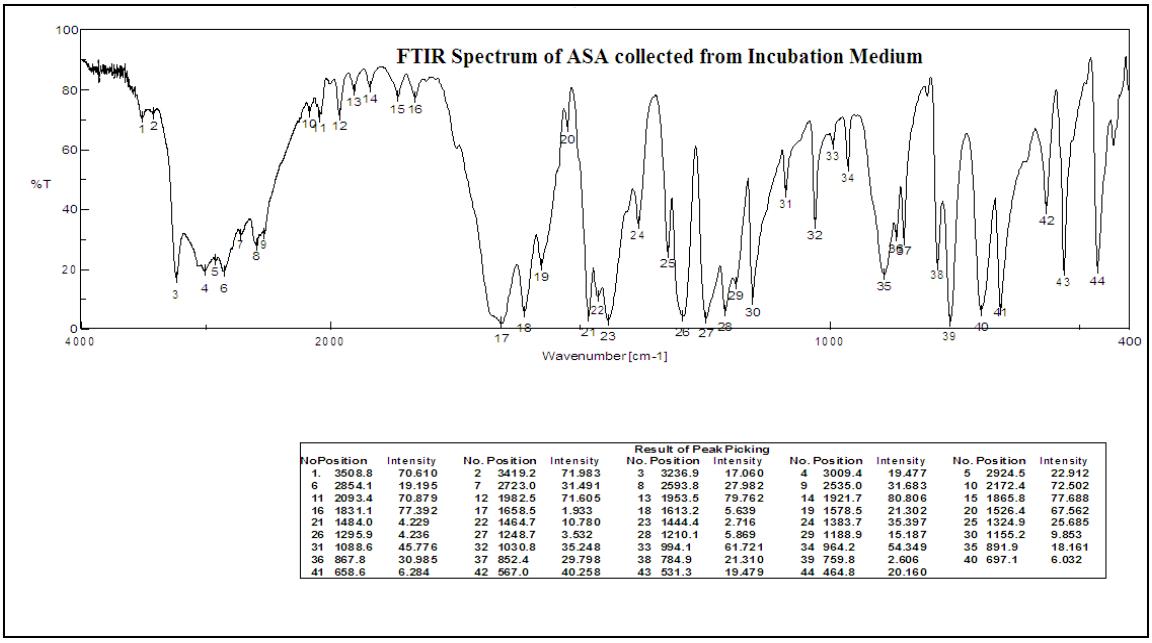
Fig.3.13. FTIR Spectrum of ASA treated Paddy plant's Extract.



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3421.1	3.989	2	3011.3	23.824	3	2954.4	13.338	4	2918.7	4.177	5	2850.3	11.187
6	1711.5	23.241	7	1632.4	7.779	8	1515.8	47.207	9	1462.7	30.716	10	1408.7	22.524
11	1385.6	28.672	12	1303.6	47.168	13	1259.3	45.292	14	1167.7	46.015	15	1076.1	28.290
16	917.0	81.690	17	772.4	59.346	18	719.3	47.135	19	666.3	46.604	20	605.5	41.703
21	525.5	43.037												

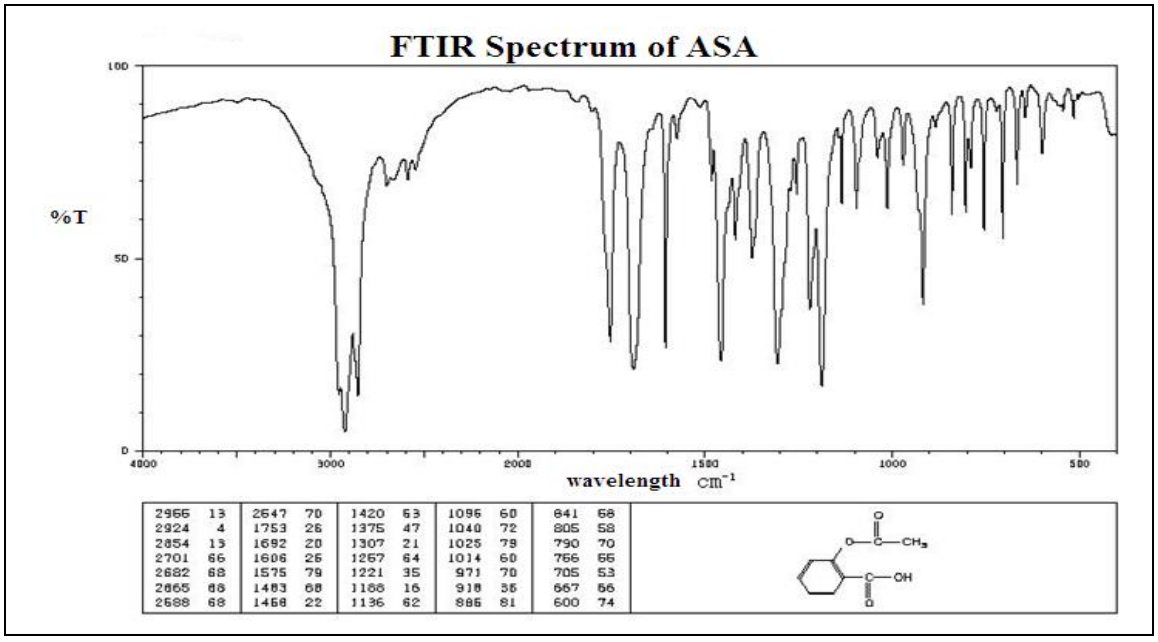
Fig3.14. FTIR Spectrum of Control Paddy Plant's Extract.



Result of Peak Picking

No	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3508.8	70.610	2	3419.2	71.983	3	3236.9	17.060	4	3009.4	19.477	5	2924.5	22.912
6	2854.1	19.195	7	2723.0	31.491	8	2593.8	27.982	9	2535.0	31.683	10	2172.4	72.502
11	2093.4	70.879	12	1982.5	71.605	13	1953.5	79.762	14	1921.7	80.806	15	1865.8	77.688
16	1831.1	77.392	17	1658.5	1.933	18	1613.2	5.639	19	1578.5	21.302	20	1526.4	67.562
21	1484.0	4.229	22	1464.7	10.780	23	1444.4	2.716	24	1383.7	35.397	25	1324.9	25.685
26	1295.9	4.236	27	1248.7	3.532	28	1210.1	5.869	29	1188.9	15.187	30	1155.2	9.853
31	1088.6	45.776	32	1030.8	35.248	33	994.1	61.721	34	964.2	54.349	35	891.9	18.161
36	867.8	30.985	37	852.4	29.798	38	784.9	21.310	39	759.8	2.606	40	697.1	6.032
41	658.6	6.284	42	567.0	40.258	43	531.3	19.479	44	464.8	20.160			

Fig.3.15. FTIR spectrum of ASA collected from aqueous drugs solution used for plant incubation.



2965	13	2647	70	1420	53	1096	60	841	68
2924	4	1753	26	1375	47	1040	72	805	58
2854	13	1692	20	1307	21	1025	79	790	70
2701	66	1606	25	1267	64	1014	60	766	66
2682	68	1575	79	1221	35	971	70	705	53
2665	68	1483	68	1168	16	918	36	667	66
2588	68	1458	22	1136	62	886	81	600	74

Fig.3.16. FTIR Spectrum of pure ASA.

Source: organic spectroscopyint.blogspot.com.

B. H^1- NMR Spectral Analysis:

H^1-NMR spectrum of ASA – treated *Oryza sativa L.* plant's extract was complex and no useful information obtained from it, but the signal pattern of H^1-NMR spectrum of ASA treated plant's extract was similar to spectrum of control plant's extract, and different from spectrum of pure ASA. The complex and blurred vision of spectrum was due to several interactions of phytochemicals of plant with absorbed ASA. All the H^1-NMR spectra were recorded by using JEOL 400MHz Spectrophotometer with multiple probe facility equipment.

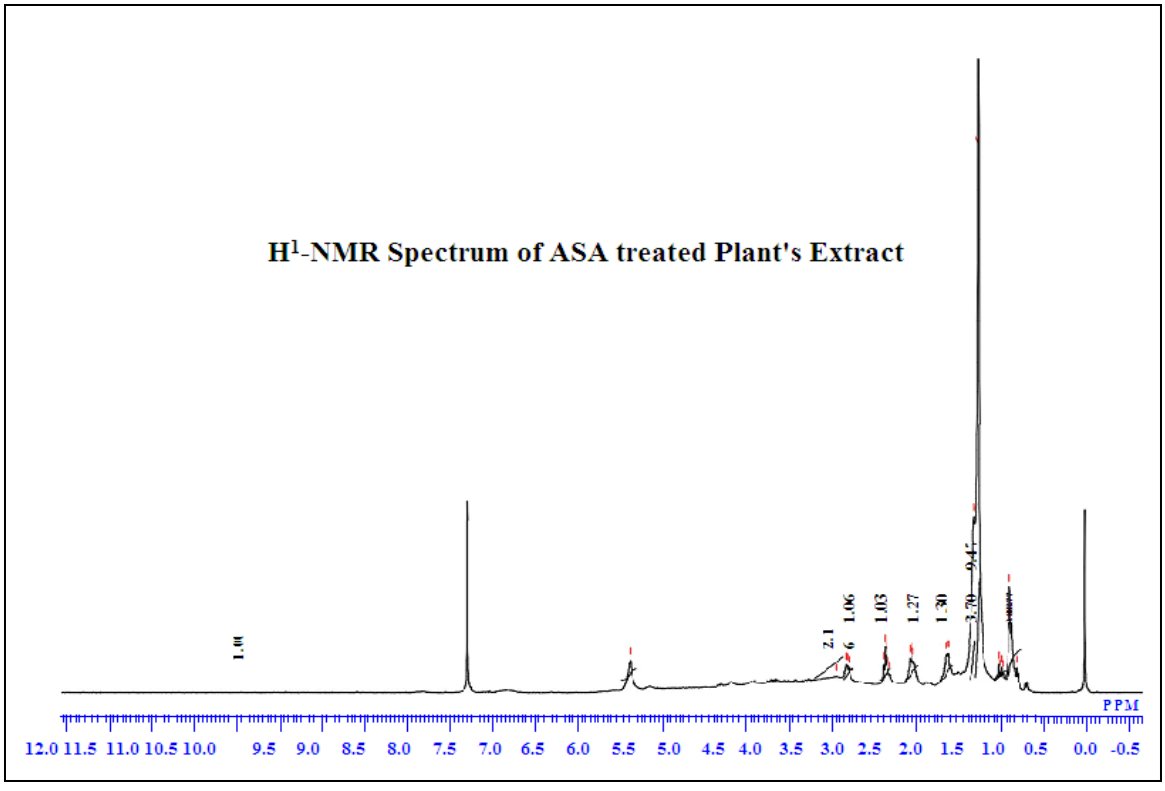


Fig.3.17. H^1-NMR Spectrum of ASA treated Paddy plant's Extract.

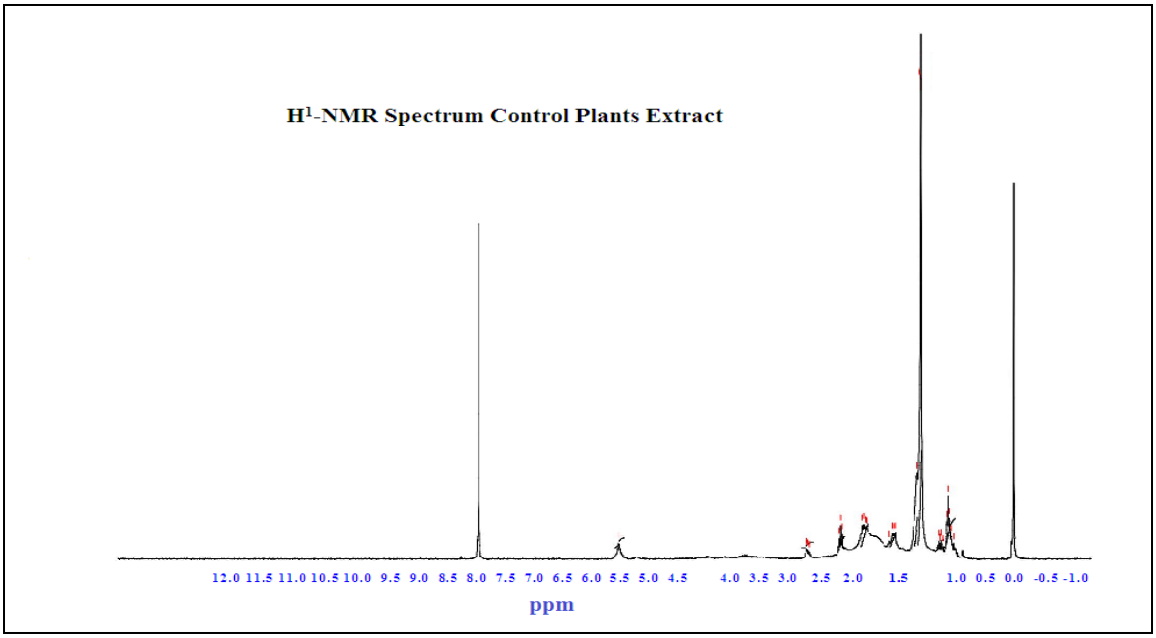


Fig.3.18. H^1-NMR spectrum Control Paddy plant's Extract.

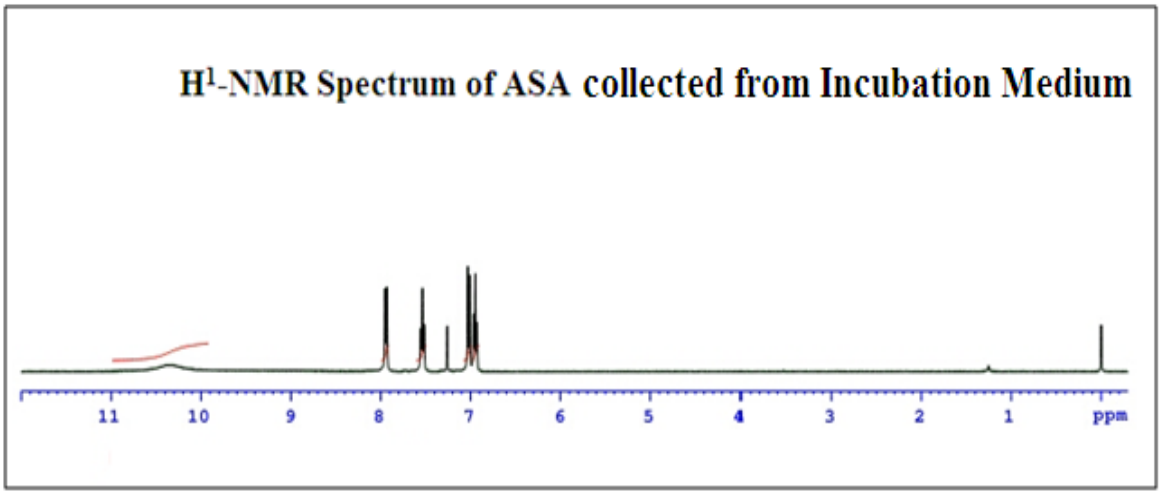


Fig.3.19. H^1-NMR spectrum of APAP collected from aqueous drugs solution used for plant incubation Medium.

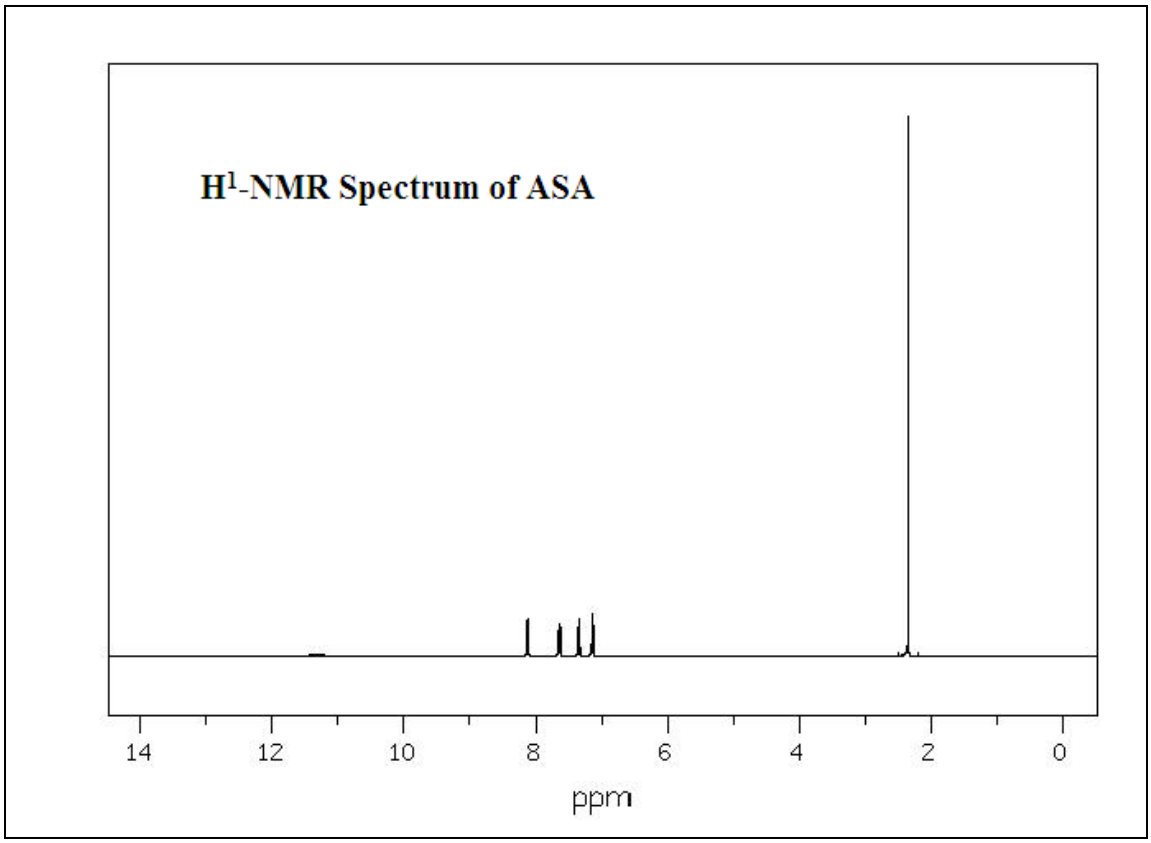


Fig.3.20. H^1-NMR Spectrum of pure ASA.

Source: Laboratory of organic chemistry,Natural products and pharmaceuticals,Anna K.Przybył, Joanna Kurek, Edited by Jan Milecki,UAM ,Poznań 2013,Laboratory ofOorganic chemistry (SERP).

C. Mass Spectral Analysis:

Mass spectrum of ASA treated *Oryza sativa L.* plant's extract was more or less similar to mass spectrum of control plant's extract expect few fragments of control plant's extract (m/z : 62.22, 128.25, 253.48, 329.38, 383.5, 491.5, 579.39, 663.75, 813.92). Most of the characteristic fragments of ASA was absent in ASA treated plant's extract. All the Mass Spectra were recorded by using WATER-SQD-2 equipment.

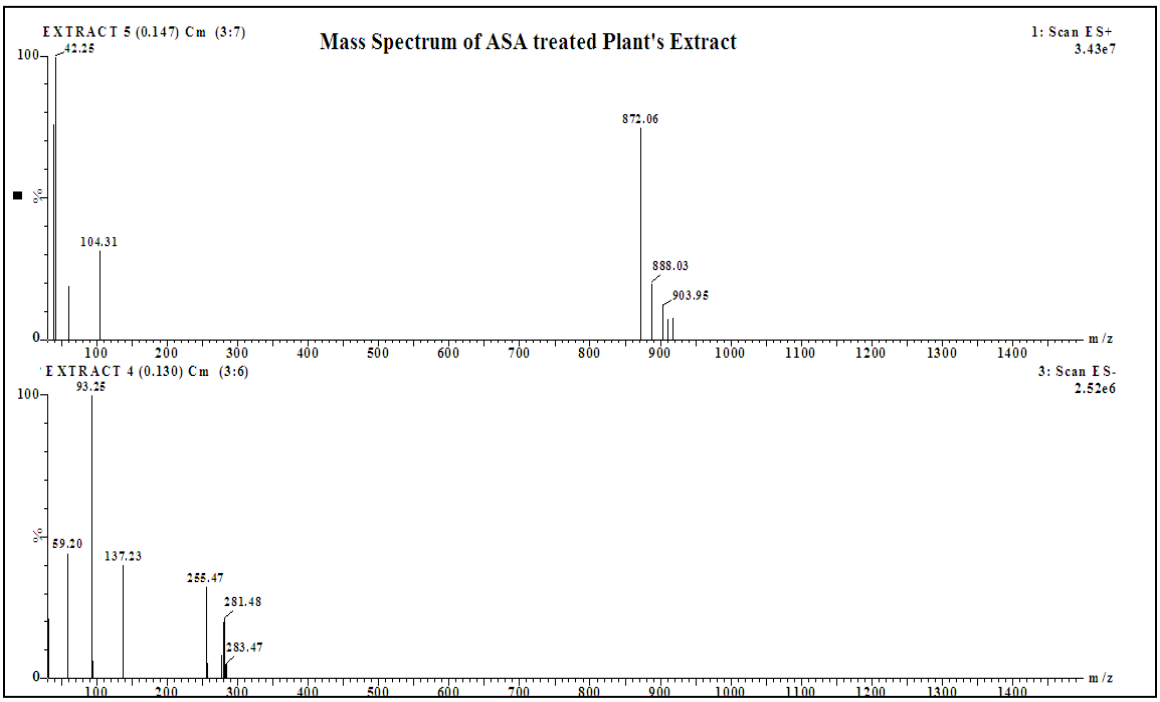


Fig.3.21. Mass Spectrum of ASA treated Paddy plants Extract.

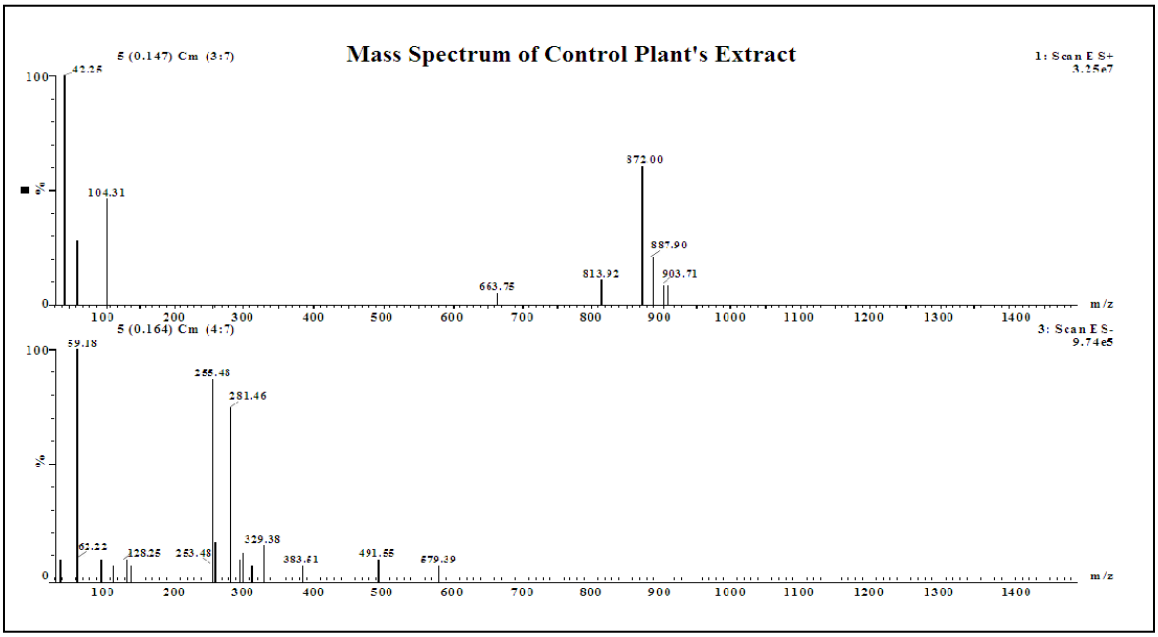


Fig.3.22. Mass Spectrum of Control paddy Plant's Extract.

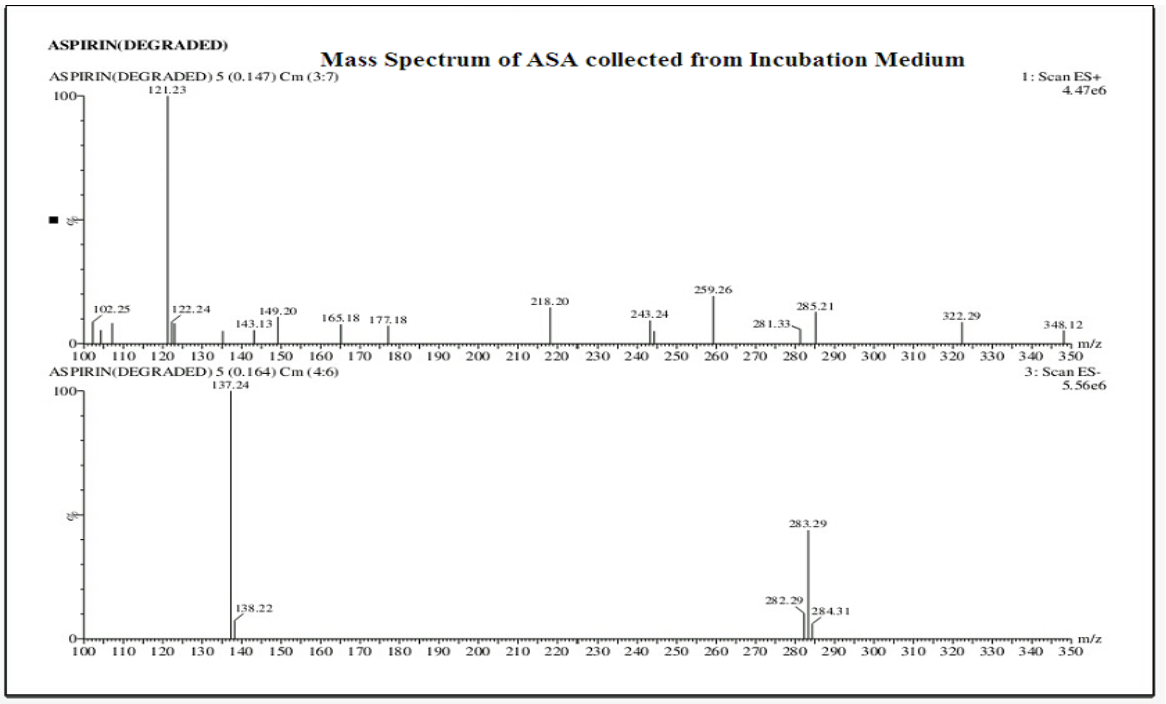


Fig.3.23. Mass Spectrum of ASA collected from aqueous drugs solution used for plant incubation Medium.

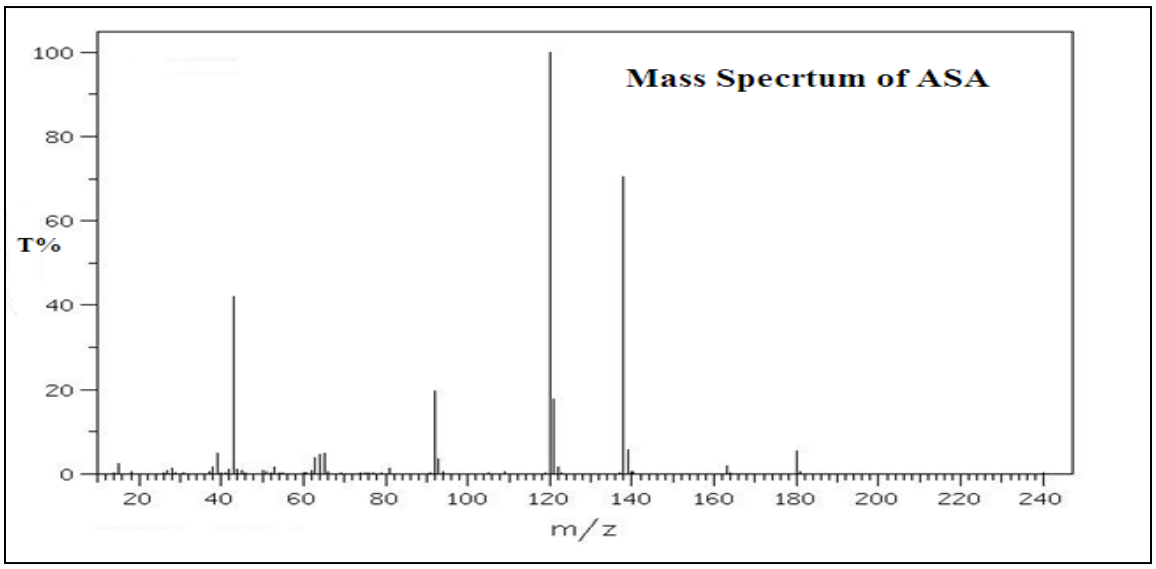


Fig.3.24. Mass Spectrum of Pure ASA.

Source: Organic Spectroscopy International/orgspectroscopyint.blogspot.com.

3.3. Conclusions:

The selected plant, *Oryza sativa L.* absorbs and metabolizes the drugs APAP and ASA. Extracts of *Oryza sativa L.* plants (which were treated with APAP and ASA hydroponically) were analyzed spectroscopically. The traces of APAP and ASA were detected in these extracts along with unknown metabolites of these drugs. The reports showed that drugs were absorbed by plants degraded into fragments and these fragments combined with phytochemicals to form bio-conjugates. The analytical data was not prominent to suggest any precursor activity of these drugs in the plant.

References:

1. Jensen, M.H. 2010. Controlled environment agriculture in deserts, tropics and temperateregions – a world review. Supported by CEAC, the Controlled Environment Agricultural Center, College of Agriculture and Life Sciences, and the University of Arizona; 2010.
2. *Joseph, A. and I. Muthuchamy,2014.* Productivity, Quality and Economics of Tomato (Lycopersicon esculentum Mill.) Cultivation in Aggregate Hydroponics – A Case Study from Coimbatore Region of Tamil Nadu, Indian journal of Science and Tech.,Vol 7(8), 1078–1086, August 2014 ISSN (Print) : 0974–6846 ISSN (Online) : 0974–5645.
3. Schröder, P., C. Huber, B. Bartha and R. Harpaintner 2009. Metabolism of acetaminophen (paracetamol) in plants—two independent pathways result in the formation of a glutathione and a glucose conjugate, Fate of pharmaceuticals in plants, Environmental Science and Pollution Research, March 2009, 16:206;

4. Schröder, P., C. Huber, B. Bartha and R. Harpaintner 2009. Metabolism of acetaminophen (paracetamol) in plants—two independent pathways for detoxification, Proc. Of 11th International on Environmental science and technology, Chania, Crete, Greece, 3-5 september, 2009.

5. Swain, A.R., S.P. Dutton and A.S. Truswell.1985. J. of the American Dietetic Association, vol.85, Number 8,pp.7.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