Global Scientific JOURNALS

GSJ: Volume 9, Issue 9, September 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

GC MS AND PHYTOCHEMICAL ANALYSIS OF ETHANOLIC LEAF EXTRACT OF *JATROPHA CURCAS*

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ABSTRACT

INTRODUCTION: The use of medicinal plants has been in existence for a long period of time as mankind and medicine. The ethanolic leaf extract of *J. curcas* has been reported for various medicinal purposes.

AIM: This study investigated the phytochemical constituents and bioactive components present in the ethanolic leaf extract of *Jatropha curcas* collected from Akungba Akoko in Ondo State .

METHODOLOGY: The analysis of *Jatropha curcas* ethanolic leaf extract was performed using a gas chromatography – mass spectrometry (GC-MS QP2010 PLUS Shimadzu, Japan) techniques.

RESULTS: Numerically, seventy-five phytochemicals compounds were detected in *J. curcas* ethanolic leaf extracts. Some of which include; Hexadecanoic acid, phytol, 9, 12-Octadecadienoic acid, dimethyl sulfone, thunbergol, tiglic acid, 2-Imidazolidinone, 1, 3-dimethyl, alpha-guaiene, pyrogallol, and globulol.

CONCLUSION: The richness of *Jatropha curcas* ethanolic leaf extract in phytochemical compounds probably accounts for the wide application of the plant leaf for medicinal purposes. Although, further research is encouraged for their specific medicinal function.

KEYWORDS: *Jatropha curcas*, GC-MS, Medicinal plants and Phytochemicals

INTRODUCTION

Historically, the use of medicinal plants is as old as mankind and medicine. Several thousands of plants species have been claimed in possess medicinal properties and employed in the treatment of many ailments [1]. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total [2]. Medicinal plants are rich source of bioactive phytochemicals or bionutrients. Studies carried out during the past decades have shown that these phytochemicals have an important role in preventing chronic diseases (3)**.** Medicinal plants remain a great source of new compound for drug development process. *Jatropha curcas* belong to the family *Euphorbiaceae* and are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America. *Jatropha curcas* L. is becoming a useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (4, 5)**.** *Jatropha curcas* is commonly called physic nut, purging nut or pig nut. All parts of the plant: roots, barks, stems, leaves seeds and fruits have been widely used in traditional folk medicine in many parts of West Africa [6]. Previous researchers have reported that leaf extract of *Jatropha curcas* has significant wound healing activity, immunomodulatory effect, antidiabetic, anti-bacterial among others [7, 8]. The leaf extract of *Jatropha curcas* also has significant analgesic properties [9]. Considering the wide application of the leaf extract of *Jatropha curcas*, this study was designed to investigate the phytochemical component of the ethanolic leaf extract of *Jatropha curcas* using GC-MS analysis.

MATERIALS AND METHODS

2.1 Collection of plant and preparation of Extract

The plants materials were collected from different locations in Akungba Akoko between 7:00am and 11:00 am and authenticated at the Herbarium of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo state.

Selected fresh plants materials collected were cleaned with 70% ethanol. They were air dried at 28^{o}C for 10 to 15 days and grinded to powder form by grinder. 400grams of the leaves part of the plant were prepared into a 1. 2 liters of ethanol solvents and covered in air-tight container with a cork, mixed together and left on the shaker at 37^{o}C for 216hrs after which the filtrate was then evaporated with rotary evaporator at 77/78 revolution per minute (rpm) for 5 minute after which it was decanted. The extracts yielded a dark greenish residual mass. The extracts were then kept in sterile bottles at 2-4^{o}C until further experiment according to (10,11).

Phytochemical analysis of the crude plant extract

Phytochemical analysis was carried out on freshly prepared leaf extracts of the plants in order to determine the presence of secondary metabolites. Plant filtrates were prepared by boiling 20 grams of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrates were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides according to (12,13)

GAS CHROMATOGRAPHY- MASS SPECTROMETRY ANALYSIS

The GC-MS analysis of *Jatropha curcas* leaf extract was performed using a GC-MS QP2010 PLUS Shimadzu, Japan according to (14). Chromatographic conditions are given as follows; The column oven temperature and the injection temperature were 150^{0}C and 300^{0}C respectively. The injection mode was split and the flow control mode was linear velocity (47.2cm/secs). The pressure was kept at 133.3KPa, the total flow was 50.0ml/mins while the

column flow was 1.54ml/sec, and purge flow was 3.0ml/mins. Finally, the split ratio was -0.1. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The spectrum of the unknown compound was compared with that of the known component stored in NIST library (14). Confirmation of the identification of some compounds was done by comparing their molecular weight, retention times and mass spectral data with those of synthetic compounds.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The name, molecular weight, structural composition of the sample materials of *Jatropha curcas* were presented in this study.

RESULTS

The qualitative phytochemical properties of *Jatropha curcus* were revealed from this study (Table 1). Phytochemical study of *Jatropha curcas* leaf extracts obtained using various extraction solvents revealed the presence of anthraquinones, terpenoid, phenol, steroids, saponin, and cardiac contents and absence of phlobatannins, proteins, fixed oil and phytosterons using various solvents (Table 1). Saponin, anthraquinones, terpenoid, phenol and steroids were observed. The structural elucidation of the bioactive components was carried out using the GC – MS analysis. The GCMS chromatogram (Figures 1) revealed that fifty two distinct peaks were recorded in *J. curcas* ethanolic leaf extract. The bioactive compounds identified are shown in Table 2 while different structures representing the bioactive compounds are represented in Figures 2. The analysis of the bioactive compound from GCMS chromatogram of *J. curcas* ethanolic leaf extract also showed phytol was the highest peak at 33 peak with retention time 15.815. The phytochemicals detected in *J. curcas* were seventy-five; some of which include

Hexadecanoic acid, 9, 12-Octadecadienoic acid, phytol, dimethyl sulfone, thunbergol, tiglic acid, 2-Imidazolidinone, 1, 3-dimethyl, alpha-guaiene, pyrogallol, globulol and others.

DISCUSSION

The result of this study is in consonance with (15) who reported the identification of 2-hexadecane-1-ol (phytol) from the methanolic crude extract of the *J. curcas* leaves. Similarly, [16,17] both reported phytol as a compound found in the leaves extract of *J. curcas* in agreement with this present study. A study by (18) also reported the presence of phytol in the crude extract of the plant although not in the leaves but the seeds oil.

Furthermore, in agreement with this study, the presence of hexadecanoic acid from the methanolic crude leaf extract was earlier reported by (1,19). Hexadecanoic acid was also found in the seed oil of *J. curcas* according to (20).

Various studies have reported the presence of 9,12- Octadecadienoic acid from the seed oil extract of *J. curcas* (1,15). However, (19) found 9,12- Octadecadienoic acid in the methanolic leaf extract in agreement with this study.

The presence of tiglic acid has been reported in the leaves extract (2) and in the stem bark and root extracts (21) of *J. curcas* plant. The presence of thunbergol in the residues of nodes, leaves, stem and root of Egyptian *J. curcas* has been reported by (21).

In this study, guaiene, was observed to be present in the ethanol extracts of the leaves and have been reported to be present in the stem according to (22). It is not amazing that abundant Pyrogallol was found in this study because pyrogallol can be formed by heating garlic acid and in this experiment, samples were heated during the extraction process and hence the amount of garlic acid was expectedly lower than the pyrogallol content. In this study, Bicyclo (3.3.0) octane was found in the leave and previously reported in the seed (23, 24). Also beta.-D-allose earlier

reported by (21) was identified is this study. Cyclohexadecanone was not detected in the leave although the presence of the compound in kernel meal has been reported by (23, 25).

Cyclohexadecanone are lipophilic in nature, hence they are soluble in organic solvents only. Other compounds in different parts of *J. curcas* plant reported by other authors but were not observed in this study include 9-Octadecenamide, corilagin and ellagic acid in the leaves (2, 21). The types of compounds detected may vary according to the MS in the three solvent extracts of different plant parts and the extraction method, the solvents used, well as the type of standards in the GCMS analysis.

CONCLUSION

The richness of *Jatropha curcas* ethanolic leaf extract in phytochemical compounds probably accounts for the wide application of the plant leaf for medicinal purposes. Although, further research is encouraged for their specific medicinal function.

Table 1: Results of qualitative phytochemical analysis of *Jatropha curcas*.

Test	Ethanol	Hexane	Cold water	Hot water
Flavonoids	+++	+	-	-
Tannins	+	-	-	-
Saponins	-	-	+++	+
Quinones	++	+	+	+
Anthraquinones	++	-	-	-
Terpenoids	+	+	-	-
Coumarin	++	-	+	+
Phenol	+	-	+	+
Steroids	+	++	+	+
Phytosteroids	+	+	-	-
Glycosides	+++	++	+	+
Alkaloids	+	++	-	-
Carbohydrates	+	+	+	+

Phlobatannins	-	-	-	-
Proteins	-	+	-	-
Fixed oil	-	-	-	-
Cardiac glycoside	+	+	-	-

Legend:
- absent
+ present in small quantity
++ moderately present
+++ resent in large quantity

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Sample Information

Analyzed by : $Ronald Ibia$
Analyzed : 3/1/2020 11:47:23 AM
Sample Type : Unknown
Level # 1
Sample Name : JAF
Sample ID : JAF
IS Amount : [1]=1
Sample Amount 1
Dilution Factor 1
Vial # 3
Injection Volume : 1.00
Data File : C:\07032020\JAF.QGD
Org Data File : C:\GCMSsolution\Extract\JAF.QGD
Method File : C:\GCMSsolution\Extract\Assay of Extract1.qgm Org Method File : C:\GCMSsolution\Extract\Assay of Extract1.qgm Report File :
Tuning File : C:\GCMSsolution\System\Tune1\A022920.qgt [Comment] JAF
Modified by : Admin
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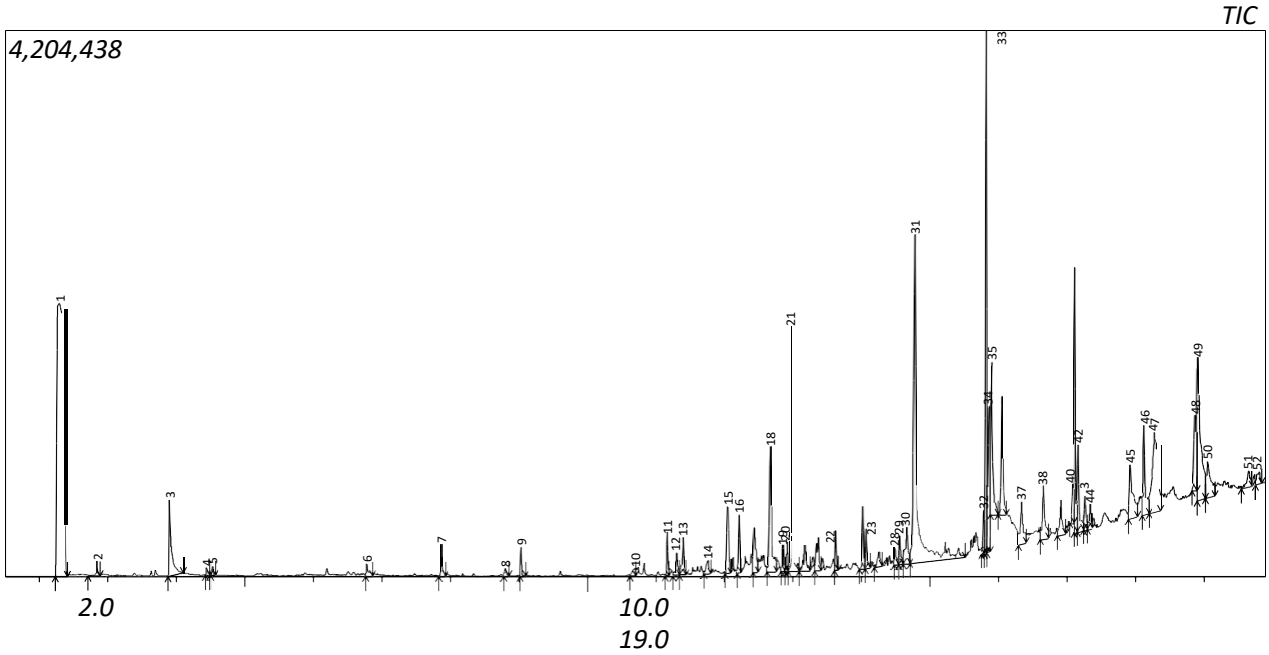

Figure 1: Chromatogram of *Jatropha Curcas* showing the different spectra

Table 2: Bioactive Compounds Identified From GCMS Analysis of Ethanolic Leaf Extract of *Jatropha curcas*

S/N	Compound	MW	Formula
1	Hydroxylamine, O-methyl-	47	CH_3ONH_2
2	Acetic acid	60	CH_3COOH
3	Dimethyl Sulfoxide	78	C_2H_6OS
4	Dimethyl sulfone	94	C_2H6O_2S
5	Tiglic acid	100	$C_5H_8O_2$
6	2-Imidazolidinone, 1,3-dimethyl-	114	$C_5H_{10}N_2O$
7	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	114	$C_6H_8O_4$
8	Benzene, 1,3-bis (1,1-dimethylethyl)-	190	$C_{14}H_{22}$
9	Phenol, 2-methyl-5-(1-methylethyl)-	150	$C_{10}H_{14}O$
10	beta.-D-Glucopyranose, 1,6-anhydro	162	$C_6H_{10}O_5$
11	alpha.-Guaiene	204	$C_{15}H_{24}$
12	Phenol, 2,4-bis(1,1-dimethylethyl)	206	$C_{14}H_{22}O$
13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro	180	$C_{11}H_{16}O_2$
14	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl	180	$C_{15}H_{26}O$
15	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	220	$C_{15}H_{24}O$
16	5-Azulenemethanol, 1,2,3,4,5,6,7,8-octahydro	222	$C_{15}H_{26}O$
17	Methyl 10,12-pentacosadiynoate	388	$C_{26}H_{44}O_2$
18	3,9-Dibromo-(+)-camphor	308	$C_{10}H_{14}Br_2O$
19	Globulol	222	$C_{15}H_{26}O$
20	Aromadendrene oxide	220	$C_{15}H_{24}O$
21	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl	248	$C_{14}H_{16}O_4$
22	cis-Z-.alpha.-Bisabolene epoxide	220	$C_{15}H_{24}O$
23	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl	222	$C_{13}H_{18}O_3$
24	6-(1'-Hydroxyethyl)-7-methoxy-2,2-dimethyl	234	$C_{14}H_{18}O_3$
25	2-Pentadecanone, 6,10,14-trimethyl	268	$C_{18}H_{36}O$
26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$
27	7-Hexadecenal	238	$C_{16}H_{30}O$

28	Nerolidyl acetate	264	$C_{17}H_{28}O_2$
29	Cyclopentanetridecanoic acid, methyl ester	296	$C_{19}H_{36}O_2$
30	Tricyclo [5.4.3.0(1,8)]tetradecan-6-one, 4-ethenyl	304	$C_{20}H_{32}O_2$
31	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
32	1-Heptatriacotanol	536	$C_{37}H_{76}O$
33	Phytol	256	$C_{20}H_{40}O$
34	9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$
35	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	212	$C_{11}H_{16}O_4$
36	7-Hexadecenal	238	$C_{16}H_{30}O$
37	Cinnamic acid	652	$C_{31}H_{40}O_{15}$
38	Bicyclo[4.1.0]heptan-2-ol, 3,7,7-trimethyl-	154	$C_{10}H_{18}O$
39	9-Octadecenamide	281	$C_{18}H_{35}NO$
40	2H-Pyran-2-one, tetrahydro-6-tridecyl-	282	$C_{18}H_{34}O_2$
41	Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-	222	$C_{15}H_{26}O$
42	Cholestan-7-one, cyclic 1,2-ethanediyl acetal, (5.alpha.)-	430	$C_{29}H_{50}O_2$
43	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$
44	Thunbergol	290	$C_{20}H_{34}O$
45	14-Oxabicyclo[10.3.0] pentadecane, 2-chloro-	224	$C_{14}H_{25}ClO$
46	i-Propyl 9-tetradecenoate	268	$C_{17}H_{32}O_2$
47	Hydrazine	60	$C_2H_5NHNH_2$
48	Bicyclo[3.3.0]octane	143	$C_6H_{14}BNO_2$
49	N(2)-Ethyl-4-methyl-1,2-pentanediamine	144	$C_8H_{20}N_2$
50	2H-Imidazole-2-thione, 1,3-dihydro-1-methyl-	114	$C_5H_{10}N_2O$
51	Methacrylic acid, ethyl ester	114	$C_6H_{10}O_2$
52	p-Pentylacetophenone	190	$C_{13}H_{18}O$
53	beta.-D-Allose	180	$C_6H_{12}O_6$
54	Tricycloundec-5-ene, 1,5,9,9-tetramethyl- (isocaryophyllene-I1)	204	$C_{15}H_{24}$
55	Cyclohexane, (2-ethyl-1-methylbutylidene)	180	$C_{13}H_{24}$
56	3-Thia-4-azatricyclodecane, 4,5-epoxy-10,10-dimethyl-, 3,3-dioxide	229	$C_{10}H_{15}NO_3S$
57	3,5-di-tert-Butyl-4-hydroxyacetophenone	28	$C_{16}H_{24}O_2$
58	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethylbenzopyran-8-yl)	28	$C_{14}H_{16}O_4$

59	6-Methyl-cyclodec-5-enol	18	$C_{11}H_{20}O$
60	4,7,7-Trimethylbicyclo[4.1.0]heptan-2-ol	14	$C_{10}H_{18}O$
61	1,3-Ditert-butyl-2-methoxy-5-methylbenzene	24	$C_{16}H_{26}O$
62	2,6-Ditert-butyl-4-(hydroxymethylene)-2,5-cyclohexadien-1-one	24	$C_{15}H_{22}O_2$
63	Cyclohexadecanone	238	$C_{16}H_{30}O$
64	cis-11,12-Epoxytetradecen-1-ol	270	$C_{16}H_{30}O_3$
65	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	304	$C_{16}H_{32}O_3S$
66	beta.-D-Mannofuranoside, farnesy	384	$C_{21}H_{36}O_6$
67	Dodecanoic acid, 10-methyl-, methyl ester	228	$C_{14}H_{28}O_2$
68	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
69	Pentadecanoic acid	242	$C_{15}H_{30}O_2$
70	Retinal	284	$C_{20}H_{28}O$
71	Octadecanoic acid	284	$C_{18}H_{36}O_2$
72	1-Heptadec-1-ynyl-cyclopentanol	30	$C_{22}H_{40}O$
73	4,7,7-Trimethylbicyclo[4.1.0]heptan-2-ol	154	$C_{10}H_{18}O$
74	9-Octadecenamide	281	$C_{18}H_{35}NO$
75	15-Chloro-13-oxabicyclo[9.3.1] pentadecane	224	$C_{14}H_{25}ClO$

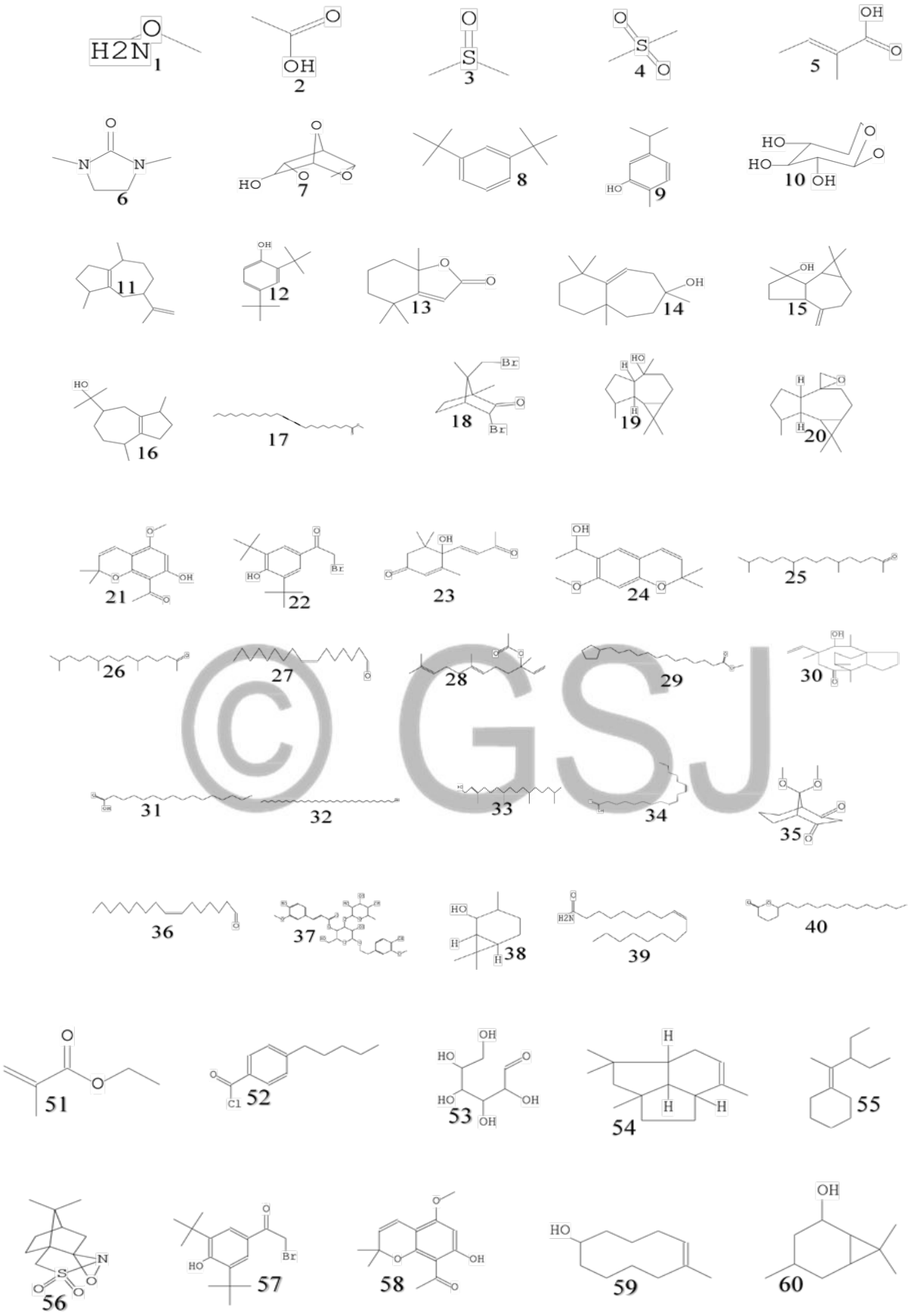

Figure 1: Molecular structures of bioactive compounds in Ethanolic Leaf Extract of Jatropha curca

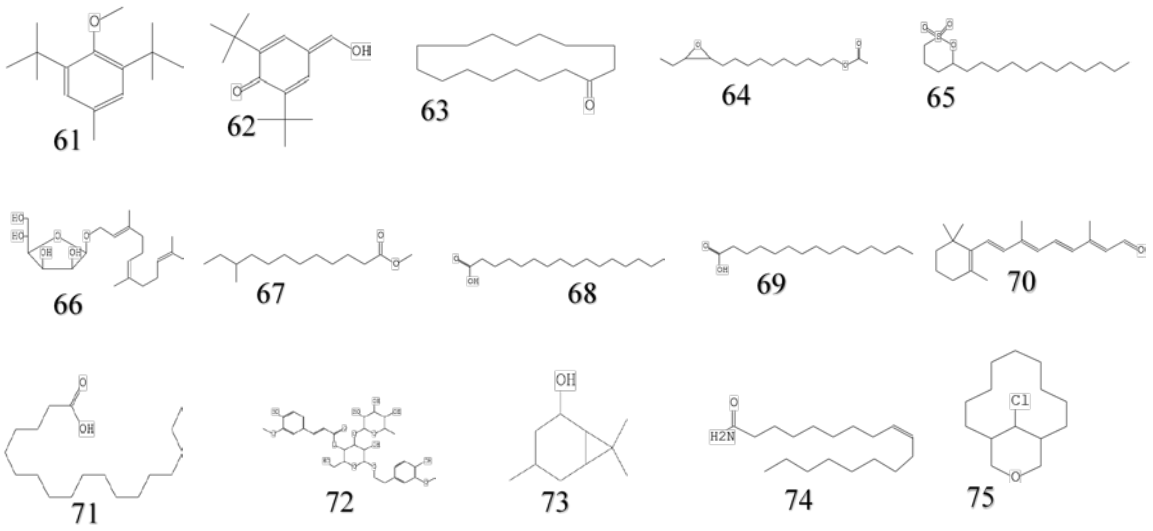

Figure 2: Molecular structures of bioactive compounds in Ethanolic Leaf Extract of *Jatropha curcas* (contd).

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