Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

PHYTOCHEMISTRY SCREENING, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *EUPHORBIA INARTICULATA* SCHWEINF PLANT EXTRACT

Ghaliah Almalki[1]*, Samar Rabah[2], Zarraq Al-Faifi[3], Asmaa Alharbi[1], Mukul Sharma[3]

1. *Department of Biology, Faculty of Science, Jazan University, 42145, Saudi Arabia.*
2. *Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.*
3. *Center of Environment Research and Studies, Jazan University, 42145, Saudi Arabia.*

ARTICLE INFO

Received:
10 Nov 2021
Received in revised form:
04 Feb 2022
Accepted:
10 Feb 2022
Available online:
28 Feb 2022

Keywords: *Euphorbia inarticulate*, Antioxidant, Antimicrobial, Phytochemistry, Radical scavenging

ABSTRACT

Nowadays, infectious diseases are subsequently increasing with the ongoing pandemic and thus there is a basic need for treatment. As medicines are quite expensive and not easily available. Thus, scientists are exploring new ways to develop inexpensive and resistant drugs. As we know plants extract are a rich source of secondary metabolites and consist of very valuable chemical properties. Considering all these facts in mind the present work for at first phytochemical screening by GC–MS of the active components of fractionated extractions, ethyl acetate, hexane, and methanol plant extracts of *Euphorbia inarticulate* Schweinf collected from the Jazan region were investigated, followed by a screening of its antioxidant and antimicrobial activity. The antioxidant activity of the methanolic extract revealed high inhibitory activity with the IC_{50} value of ascorbic acid, and the methanolic extract was found to be 36.67µg/mL, and 52.94µg/mL respectively. The antimicrobial activities of these extracts were examined on microorganisms such follows: *Streptococcus pneumoniae* and *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *and Aspergillus fumigants* by the agar diffusion method. Methanol extract showed against *B. subtilis* and *S. pneumoniae* (Gram-positive bacteria) MIC values of 1.95 and 0.98µg/mL, respectively as that recorded for the standard Ampicillin. While against *Pseudomonas aeruginosa* and *E. coli* (Gram-negative bacteria) MIC values were 1.95 and 0.98µg/mL, respectively. Moreover, MIC values recorded for the standard were 1.95 and 0.49µg/mL, respectively. The MIC value recorded for methanol extract against *A. fumigatus* only (Fungi) was 1.95µg/mL, and that for the standard Amphotericin B were 0.98 and 0.49µg/mL respectively.

To Cite This Article: Almalki G, Rabah S, Al-Faifi Z, Alharbi A, Sharma M. Phytochemistry Screening, Antioxidant and Antimicrobial Activities of *Euphorbia inarticulata* Schweinf Plant Extract. Pharmacophore. 2022;13(1):91-9. https://doi.org/10.51847/S7eqwFEBAX

Introduction

The advent of a new viral infection due to the outburst of a coronavirus-associated with an acute respiratory disease named coronavirus disease (COVID-19) which has spread to >210 nations [1] has become a global threat to public health. Thus, prevention and precautions are the most important as to date no medicine has been reported. Due to these plants are withdrawing global attention as they are widely known to have an important effect in the detection of drug and growth of therapeutic agents for their exceptional medicinal uses, antimicrobial activity, and multiple biological activities such as antioxidant, anticancer, antilithiatic, hepatoprotective, antihyperlipidemic, antibacterial, antidiabetic and nephroprotective potential. These activities are found due to the presence of compounds synthesized in the secondary metabolism of plants. Therefore, more studies for using plants as therapeutic agents are in need to be designed. The use of traditional medicine for the management of various conditions is a common practice in most developing countries [2] including the Kingdom of Saudi Arabia, especially in rural areas.

In Saudi Arabia, Euphorbiaceae is represented by 15 genera (Andrachne L., Flueggea Willd, Phyllanthus L., Clutia L., Chrozophora Neck. ex Juss., Ricinus L., Mercurialis L., Erythrococca L., Micrococca Benth., Acalypha L., Tragia L., Dalechampia L., Jatropha L., Croton L., and Euphorbia L.) [3]. Euphorbiaceae are recorded throughout Saudi Arabia, from

Corresponding Author: Ghaliah Almalki; Department of Biology, Faculty of Science, Jazan University, 42145, Saudi Arabia. E-mail: g.almaleki@gmail.com.

the desert wadi of the northern border region of Saudi Arabia [4], the central region [5], and the eastern province, central region, southwestern region, and throughout of Saudi Arabia [6]. A study recorded ninety-five species that boleng to seventy five genera and 31 families from Wadi Wasaa, Jazan, Saudi Arabia. The floristic analysis [7] showed four families of Poaceae, Malvaceae, Euphorbiaceae, and Apocynaceae abundant in the wadi.

Euphorbiaceae family is known to be the third-largest genus of angiosperm plants composed of nearly 2000 subgenera and sections. The Euphorbiaceae plants are shrubs, herbs, trees, etc. [8]. It provides food [9, 10] and varied medicinal features employed in ethnobotany [11-14]. Its plants are used in managing several human diseases like respiratory infections, ulcers, venereal diseases, wounds, cough, rheumatism, and toothache [15]. They were also utilized as traditional medicine against wart removers, trichiasis, and venomous bites. The effectiveness of this family is thought to be due to constituents responsible for different types of therapeutic activities such as flavonoids, polyphenols, alkanes, triterpenes, phytosterols, essential oils, and tannins. Due to the presence of chemical components, this family has long-term recognition and is reported to exhibit antioxidant and antimicrobial features.

the aim of this research was: (1) to phytochemical screening by GC–MS of the active components of fractionated extractions, ethyl acetate, hexane, and methanol extracts of *E. inarticulata* from the Jazan region, and (2) Analyze the antioxidant and antibacterial effects of methanol, ethyl acetate and hexane extracts. The antibacterial activity of these extracts was tested with microorganisms as follows: *S. pneumoniae* and *B. subtilis, E. coli, C. albicans, and A. fumigants* by the agar diffusion method. The MIC values were determined and the extracts of methanol were found to exhibit the minimum value.

Materials and Methods

Collection of Plant Material

The plant, *E. inarticulata* Schweinf was collected between May 2018 to June 2019, from different locations in Al'Aridah, Jazan region, Saudi Arabia. 17°03'45.6"N 43°03'03.7"E. The plant was identified by taxonomist Professor Yahya Masrahi, Department of Biology, Faculty of Science, Jazan University, Jazan, Saudi Arabia.

Extraction of Plant Material

The dried *E. inarticulata* Schweinf whole plant (150g) was ground and extracted with hexane for 24h and then filtered. The filtrate is then concentrated by a rotary evaporator (BÜCHI, Switzerland). The concentrated extract was individually fractionated with methanol and ethyl acetate. The residue from every fractionation step became used to acquire the following fraction. The extracts from each fractionation step were evaporated to dryness under a vacuum. These extracts were dissolved in dimethylsulfoxide (DMSO) [16] and then diluted with a cell culture medium. The final DMSO concentration was below one percent of the total volume of the medium in all managements and controls.

Botanical Description

E. inarticulata Schweif. showed Qasas much-branched. spiny succulent shrub to 2cm, usually with a short trunk but often trunkless and with branches arising at ground level. Branches ascending 3-5 angled, the angles are slightly winged but not lobed. Spines paired, 5-15mm long, the spine shields long, decurrent, Cyathia in sessile cymes at the top of the branches. Capsule glabrous, trigonous, rounded, 3-4mm diameter, purple-black when ripe. Flowers in autumn and early winter usually begin a week or two after *E. cactus* [17].

Chromatographic Analysis

To each 10mL filtrate of Hexane, Ethyl acetate and Methanol add 2.5gm of Na_2SO_4 to remove H_2O. Further analysis of the filtrate was done on Gas chromatography coupled to a mass spectrometer (GC-MS) equipped with TG-5MS SIL fused-silica capillary column (‘Resets’) (30 m x 0.25mm internal diameter, 0.25m film thickness). Helium (1.0mL/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10-1:10. The injector was kept at 230 ºC and the transfer line at 250 ºC. The column was maintained at 50 ºC for 2min and then programmed to 150 ºC at 8 ºC /min and held for 20min at 280 ºC. The MS was operated in the EI mode at 70 eV, in the m/z range of 40-500. The detection of the compounds was done by Thermo Scientific Trace GC Ultra ISQ shown as percentages attained by peak area normalization; all relative response factors being taken as one.

DPPH radical Scavenging Assay

Free radical scavenging activity was tested based on [18, 19]. Many concentrations of the extract were intermixed with 80mM of 1, 1-diphenyl-2- picrylhydrazyl (DPPH) in methanol. later, the solution was incubated for 30min at room temperature. Ascorbic acid was used as the positive control. The absorbance of the solution was measured at 517nm using a double-beam spectrophotometer. The DPPH radical scavenging activity was calculated using the equation:

$$\text{Inhibition (\%)} = [(AB - AA)/AB] \times 100, \quad (1)$$

where AA, absorption of test sample, AB, absorption of blank sample.

The percentage of DPPH radical scavenging activity was calculated. The amount of extract required to react with half of the DPPH radicals is a 50% inhibitory concentration (IC_{50}).

Antimicrobial Bioassay by Using the Agar Diffusion Cylinder Method

The approach of Akujobi *et al*., (2004); Balouiri *et al*., (2016) were implemented [20, 21]. The crude extracts were dissolved in thirty percent dimethylsulphoxide (DMSO) and then diluted to get 250, 200, 150, 100, and 50mg/mL concentrations. These were kept at 150 °C till its need arise. The microbial strains altogether were obtained from the Regional Center for Mycology and Biotechnology culture collection center (RCMB), Al-Azhar University, Cairo, Egypt. The plant extracts were tested *in vitro* against different types of bacteria, such as *S. pneumoniae* and *B. subtilis* as examples of Gram-positive bacteria, and *P. aeruginosa* and *E. coli* as examples of Gram-negative bacteria. Fungi, *A. fumigates* and *C. albicans* was used for testing the antifungal activity of plant extracts. The plates were incubated at 37 °C for 24h for bacteria and yeast, and 48–72h for fungi. Tetracycline changed into used as the usual antibacterial drug, whilst amphotericin B changed into used as the usual antifungal drug. The diameters of the inhibition zones (mm) had been measured and used because the criterion for antimicrobial activity. The plates were gathered so also the zones of inhibition which were established were measured as previously described at the end of incubation [21, 22]. The average of the zones of inhibition was calculated. The by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition, the calculation of the minimum inhibitory concentration (MIC) was done. A regression line has been drawn through the points. The antilogarithm of the intercept on the logarithm of the concentration axis gave the MIC values.

Experimental Condition for Antimicrobial Activity

The equipment used was autoclaved and sterilized for 15min at 120 °C. The assay was started by pouring 20mL molten agar media into the sterilized petri dish (9cm) and was let to solidify at room temperature. The media used was Mueller Hinton Agar for antibacterial and Muller Hinton agar media with methylene blue -glucose (used to enhance zone diameter visualization). Further concentrations of 250, 200, 150, 100, and 50µg/mL were prepared from *E. inarticulate* extract.
S. pneumoniae and *B. subtilis* were used for testing gram-positive bacteria and *P. aeruginosa* and *E. coli* were used as gram-negative bacteria. Fungi *A. fungates* and *C. albicans* were used for antifungal activity.

Results and Discussion

Chemical Composition of Hexane Extract

The Hexane extract of whole plant of *E. inarticulate* was analysed using GC-MS and the compounds were identified using Helium at 230 ºC to 250 ºC. The GC-MS chromatogram obtained from GC-MS analysis shows the retention time (R.T.) peaks of 28 compounds. The compounds identified from the hexane extract of *E. inarticulata* Schweinf using GC–MS analysis are listed in **Table 1**.

Table 1. Chemical composition of Hexane Extract.

No.	R.T.	Name of compound	Molecular formula	Molecular weight	%Area
1	22.34 29.57 45.58	9-Octadecen-12-ynoic acid, methyl ester	$C_{19}H_{32}O_2$	292.5 g/mol	0.15 0.29 0.11
2	25.15	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34 g/mol	1.44
3	27.27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	0.47
4	29.06	Phytol	$C_{20}H_{40}O$	296.5 g/mol	0.41
5	29.33	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5 g/mol	0.41
6	30.67	6-Methyloctadecane	$C_{19}H_{40}$	268.5 g/mol	0.28
7	31.14	2-Methylhexadecan-1-ol	$C_{17}H_{36}O$	256.5 g/mol	0.12
8	33.25 34.46	Tetratriacontane	$C_{34}H_{70}$	478.9 g/mol	1.05 2.46
9	33.73	Erucylamide (13-Docosenamide, (Z)	$C_{22}H_{43}NO$	337.6 g/mol	3.12
10	34.02 41.23 50.08	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6 g/mol	0.86 5.07 0.13
11	34.84	Oleic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{76}O_3$	593 g/mol	0.33
12	35.19	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7 g/mol	0.42
13	36.16	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697 g/mol	5.40

14	36.65	17-(1,5-Dimethylhexyl)-4,10,13-trimethyl-4,5,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-4-ol	$C_{28}H_{48}O$	400.7 g/mol	2.27
15	37.23	Pollinasterol	$C_{28}H_{48}O$	400.7 g/mol	4.29
16	37.82	14alpha-Methyl-5alpha-ergosta-8,24(28)-dien-3beta-ol	$C_{29}H_{48}O$	412.7 g/mol	3.01
17	38.15	Stigmasterol	$C_{29}H_{48}O$	412.7 g/mol	4.31
18	38.65	Stigmasta-5,22-dien-3-ol, (3beta,22E)-(Stigmasterol methyl ether)	$C_{30}H_{50}O$	426.7 g/mol	10.32
19	39.05	β-Sitosterol	$C_{29}H_{50}O$	414.7 g/mol	5.76
20	39.61	Amyrin	$C_{30}H_{50}O$	426.7 g/mol	15.18
21	40.34	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	18.17
22	42.27	D:A-Friedooleanan-3.alpha.-ol	$C_{30}H_{52}O$	428.7 g/mol	6.17
23	42.75	Friedlein	$C_{30}H_{50}O$	426.7 g/mol	2.70
24	43.05	9,19-Cyclolanostan-3-ol, 24,24-epoxymethano-, acetate	$C_{33}H_{54}O_3$	498.8 g/mol	0.85
25	43.59	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502.8 g/mol	0.94
26	44.61	A-Friedooleanan-7-ol	$C_{30}H5_2O$	428.7 g/mol	2.04
27	45.13	9,19-Cyclo-27-norlanostan-25-one, 3-(acetyloxy)-24-methyl-, (3beta,24R)	$C_{32}H_{52}O_3$	484.8 g/mol	0.14
28	46.28 48.18	1,1-Bis(dodecyloxy)hexadecane	$C_{40}H_{82}O_2$	595.1g/mol	0.22 0.11

Chemical Composition of Ethyl Acetate Extract

The ethyl acetate extract of whole plant of *E. inarticulate* was analysed using GC-MS and the compounds were identified using Helium at 230 ºC to 250 ºC. The GC-MS chromatogram obtained from GC-MS analysis shows the retention time (RT) peaks of 39 compounds. The compounds identified from the ethyl acetate extract of *E. inarticulata* Schweinf using GC–MS analysis are listed in **Table 2.**

Table 2. Chemical composition of ethyl acetate extract.

No.	R.T.	Name of compound	Molecular formula	Molecular weight	%Area
1	17.21	9-Octadecen-12-ynoic acid, methyl ester (Isopropyl laurate)	$C_{15}H_{30}O_2$	242.4 g/mol	0.40
2	22.43	2,8-Decadienedioic acid, diethyl ester	$C_{14}H_{22}O_4$	254.32 g/mol	0.30
3	25.19	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34 g/mol	7.47
4	27.30	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	2.00
5	27.61	5,8,11-Heptadecatriynoic acid, methyl ester	$C_{18}H_{24}O_2$	272.4 g/mol	1.70
6	28.47	2(1H)-Naphthalenone, octahydro-4a-phenyl-, trans	$C_{16}H_{20}O_2$	228.33 g/mol	0.59
7	29.34	9, 12, 15 Octadecatrienoic acid, (Z,Z,Z)-(Linolenic acid)	$C_{18}H_{30}O_2$	278.4 g/mol	2.02
8	29.57	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5 g/mol	0.47
9	30.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	478.9 g/mol	0.39
10	30.44	(4-Isopropylidenebicyclo [3.2.0] hept-2-en-6-ylidene) acetic acid, methyl ester	$C_{13}H_{16}O_2$	204.6 g/mol	0.26
11	31.13	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5 g/mol	0.41
12	31.55	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methy	$C_{23}H_{32}O_2$	340.5 g/mol	0.93
13	32.17	Phenol, 2,2'-methylenebis [6-tert-butyl-4-ethyl-	$C_{25}H_{36}O_2$	368.6 g/mol	0.83
14	32.47	Docosanoic acid	$C_{22}H_{44}O_2$	340.6 g/mol	0.38
15	33.22	Tetracosane, 11-decyl-	$C_{34}H_{70}$	478.9g/mol	0.22
16	33.67	17-Pentatriacontene	$C_{35}H_{70}$	490.9 g/mol	0.36
17	34.01	2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene	$C_{30}H_{50}$	410.7g/mol	0.44
18	34.44	Tetratriacontane	$C_{34}H_{70}$	478.9 g/mol	2.16
19	34.81	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-	$C_{30}H_{50}O$	426.7 g/mol	0.22

20	35.16	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7 g/mol	0.34
21	36.12	Octacosyl heptafluorobutyrate	$C_{32}H_{57}F_7O_2$	606.8 g/mol	4.05
22	36.62	Cholest-4-ene, 3beta-(methoxymethoxy)-	$C_{29}H_{50}O_2$	430.7 g/mol	2.45
23	37.30	5,beta,6,beta-Epoxy-7.alpha-bromocholestan-3.beta-ol	$C_{27}H_{43}BrO_2$	481.5 g/mol	6.47
24	37.77	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-	$C_{28}H_{44}O_2$	412.6 g/mol	3.45
25	38.11	Stigmasterol	$C_{29}H_{48}O$	412.7 g/mol	4.47
26	38.59	Stigmasta-5,22-dien-3-ol, (3beta,22E)-(Stigmasterol methyl ether)	$C_{30}H_{50}O$	426.7 g/mol	7.97
27	38.96	β-Sitosterol	$C_{29}H_{50}O$	414.7 g/mol	6.77
28	39.48	Amyrin	$C_{30}H_{50}O$	426.7 g/mol	10.0
29	40.20	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	13.77
30	40.60	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	$C_{31}H_{52}O_3$	472.7 g/mol	1.41
31	41.14	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.7g/mol	4.49
32	42.14 44.52	D:A-Friedooleanan-3.alpha.-ol	$C_{30}H_{52}O$	428.7 g/mol	4.63 0.90
33	42.64	Friedelan-3-one	$C_{30}H_{50}O$	426.7 g/mol	2.25
34	42.94	9,19-Cyclolanostane-3,7-diol	$C_{30}H_{52}O_2$	444.7 g/mol	0.97
35	43.50	Cholesta-5,17(20),24-trien-3-ol, acetate, (3beta)-	$C_{29}H_{44}O_2$	424.7 g/mol	0.72
36	43.76	17-(1,5-Dimethyl-hexyl)-4,4,9,13,14-pentamethylhexadecahydrocyclopenta[a]phenanthren-3-one	$C_{30}H_{52}O$	428.7 g/mol	0.71
37	44.98	Propanoic acid, 3,3'-thiobis-, didodecyl ester	$C_{30}H_{58}O_4S$	514.799 g/mol	1.21
38	45.40	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502.8 g/mol	0.28
39	48.21	17-Pentatriacontene	$C_{32}H_{54}O_4$	502.8 g/mol	0.56

Chemical Composition of Methanol Extract

The ethyl acetate extract of whole plant of *E. inarticulate* was analyzed using GC-MS and the compounds were identified using Helium at 230 ºC to 250 ºC. The GC-MS chromatogram obtained from GC-MS analysis shows the retention time (RT) peaks of 33 compounds. The compounds identified from the ethyl acetate extract of *E. inarticulata* Schweinf using GC–MS analysis are listed in **Table 3.**

Table 3. Chemical composition of Methanol Extract.

No.	R.T.	Name of compound	Molecular formula	Molecular weight	%Area
1	8.72	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	115.13 g/mol	0.33
2	17.15	Ribitol	$C_5H_{12}O_5$	152.15 g/mol	1.44
3	17.74	DL-Arabinitol	$C_5H_{12}O_5$	152.15 g/mol	0.43
4	19.12	Oxazolidine, 2-(1,1,4,8-tetramethylnona-3,7-dienyl)-	$C_{16}H_{29}NO$	251.41 g/mol	0.33
5	21.73	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl	$C_{13}H_{18}O_3$	222.28 g/mol	0.34
6	25.15	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34 g/mol	5.14
7	26.60	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	374.6 g/mol	0.31
8	27.31	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	4.80
9	27.61	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans-	$C_{28}H_{44}O_4$	444.6 g/mol	0.92
10	28.01	1-Aminocyclopropanecarboxylic acid, 2,6-di-t-butyl-4-methoxy-phenyl ester	$C_{19}H_{29}NO_3$	319.4 g/mol	0.41
11	28.93	d-Mannose	$C_6H_{12}O_6$	180.16 g/mol	3.61
12	29.33	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.4 g/mol	2.50
13	29.57	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5 g/mol	1.25
14	31.12	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5 g/mol	0.99

15	31.54	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methy	$C_{23}H_{32}O_2$	340.5 g/mol	0.50
16	32.19	Cholesterol margarate	$C_{44}H_{78}O_2$	639.1 g/mol	0.85
17	32.46	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	340.6 g/mol	0.31
18	33.23	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.5 g/mol	1.30
19	33.88	9H-Naphtho[2,1-b] pyran-9-one, 3-ethenyl dodeca hydro-3,4a,7,7,10a-pentamethyl-, (3R, 4aR, 6aS, 10aS, 10bR)	$C_{20}H_{34}O_2$	304.5 g/mol	4.84
20	34.44	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	498.8 g/mol	1.56
21	34.90	Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]-5-one,3'a,4',5',5'a,6',7',8',9',9'a,9'b-decahydro-3,3'a,6',6',9'a-pentamethyl-,(2S,3'aR,5'aS,9'aS,9'bR)-	$C_{20}H_{30}O_3$	318.45 g/mol	0.46
22	35.69	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6 g/mol	0.80
23	36.11	1-Octacosanol	$C_{28}H_{58}O$	410.8 g/mol	2.49
24	36.59	Chol-8-en-24-al, 3-hydroxy-4,4,14-trimethyl-	$C_{27}H_{44}O_2$	400.6 g/mol	1.69
25	37.26	5.alpha.-Ergost-8-en-3.beta.-ol, 14-methyl-	$C_{29}H_{50}O_2$	414.7 g/mol	5.09
26	37.75	8-Androsten-3-ol, 17-(2-methylallyl)-4,4,14-trimethyl-	$C_{28}H_{44}O_2$	412.6 g/mol	3.22
27	38.08	Stigmasterol	$C_{29}H_{48}O$	412.7 g/mol	5.49
28	38.57	9,19-Cyclolanost-24-en-3-ol, acetate, (3beta)-	$C_{32}H_{52}O_2$	468.8 g/mol	6.55
29	38.90	β-Sitosterol	$C_{29}H_{50}O$	414.7 g/mol	8.39
30	39.44	Amyrin	$C_{30}H_{50}O$	426.7 g/mol	8.56
31	40.14	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	10.99
32	40.56 42.89 44.08 44.93	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502.8 g/mol	1.15 0.53 0.33 0.58
33	41.11	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.7g/mol	4.96
34	42.10 42.59	Friedelan-3-one	$C_{30}H_{50}O$	426.7 g/mol	3.47 1.43
35	43.46	Cholestano[7,8-a]cyclobutane, 3-methoxy-6-oxo-2'-methylene-	$C_{31}H_{50}O_2$	454.7 g/mol	1.10
36	42.27	D:A-Friedooleanan-3.alpha.-ol	$C_{30}H_{52}O$	428.7 g/mol	6.17

Biological Activities

Antioxidant Activity Using DPPH Radical Scavenging Assay

The results of antioxidant activity using DPPH radical scavenging assay by *E. inarticulata* extracts (methanol, ethyl acetate, and hexane) in associstion with ascorbic acid are recorded in **Table 4.**

Table 4. Radical scavenging activity of various concentrations of *E. inarticulata* plant methanolic extract (n= 24, % Inhibition ± SD.).

Item µg/mL	**Percentage inhibition**			
	Ascorbic acid	**Methanolic extract**	**Ethyl acetate extract**	**Hexane extract**
50	31.89±0.39	28.7±0.21	5.1±0.02	6.8±0.32
100	45.5±0.23	47.0±0.25	34.2±0.34	14.0±.033
150	65.1±1.20	57.1±0.34	37.0±0.23	28.3±.059
200	87.0±0.45	80±0.35	89.0±0.52	86.2±0.76
250	93.6±0.5	91.2±0.45	92.0±0.52	90.2±0.26
500	96.0±0.53	95.5±0.56	93.1±0.60	91.0±.032
IC_{50} µg/mL	110.50	115.50	165.00	171.00

Antimicrobial Screening

The results study **Table 5,** showed that the methanol extract at a concentration of 250 ug/mL,

Table 5. *In vitro* antimicrobial activity of the different extracts of *E. inarticulata* at 250 (µg /mL) by well diffusion agar assay

Extract	Zones of inhibition (mm)					
	B. subtilis	***S. pneumoniae***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. fumigatus***
Methanol	25.3±0.62	22.6±0.72	28.3±0.73	24.2±0.57	NI	23.4±1.5
Ethyl acetate	17.1±0.41	19.1±1.2	21.0±.37	17.3±0.49	NI	17.3±0.49
Hexane	15.2±0.63	18.2±1.1	19.3.4±0.32	19.2±1.4	NI	15.4±0.98

*Values are means± SD of triplicate readings. NI means no inhibition.

Antimicrobial Activity

Results of lowest inhibitory concentrations of 3 extracts were determined **(Table 6)**. The results showed high variation in MIC among the extracts and the methanol extract exhibited the minimum.

Table 6. Minimal Inhibitory Concentration (MIC) of methanol, ethyl acetate, and hexane extracts of *E. inarticulate*

Microorganism	Minimum Inhibitory Concentration (µg/mL)			
	Methanol	**Ethyl acetate**	**Hexane**	**Standard**
Gram-Positive Bacteria:				Ampicillin
Streptococcus pneumonia	1.95	3.9	7.81	1.95
Bacillus subtilis	0.98	3.9	3.9	0.98
Gram-negative Bacteria:				Gentamycin
Pseudomonas aeruginosa	1.95	14.63	60.50	1.95
Escherichia coli	0.98	3.9	3.9	0.49
				Amphotericin B
Aspergillus fumigatus	1.95	3.9	7.81	0.98
Candida albicans	NA	NA	NA	0.49

* NA: No activity.

The present study provides the first information on the phytochemical constituents of *E. inarticulata* Schweinf extracts utilizing GC–MS. Overall, our findings are in agreement with many literatures previously in which they supported using methanol as a better solvent to recuperate greater extractable active compounds from several medicinal plants [23, 24]. The yield in hexane and ethyl acetate extracts was comparable while it was higher in methanol. This variation in the yield of extract from different solvents could be due to the availability of extractable constituents of different polarities [24]. The polarity of the solute of interest determines the solvent used to extract the biomolecule from the plant. A solvent with the same polarity as the solute will properly dissolve the solute. Several solvents can be used sequentially to extract different phenolic compounds for antioxidant extraction from plants that exhibit greater ratio of accuracy to set boundary to the number of analogous compounds in the preferred yield. The polarity, from least polar to most polar, of a few common solvents, is as, Hexane < Ethylacetate < Methanol as in the present study extraction preparation [25, 26].

Methanol is a strong polar solvent and might be considered highly efficient in extracting the different constituents from *E. inarticulate*. In the current study, the number of active compounds recovered by ethyl acetate was similar to that by methanol (39 constituents each). Nonetheless, our results showed that higher concentrations of lupeol and β-amyrin were recovered using hexane and then ethyl acetate extracts compared to the methanolic extract. Thus, the selection of solvent for extracting *E. inarticulata* will depend on the targeted constituents.

Interestingly, the present study demonstrated that lupeol was found to be the most abundant compound detected in all three extracts, with the highest amount in hexane (18.4%) followed by ethyl acetate (13.72%) and then the methanol (10.7%). Lupeol is a pharmacologically active pentacyclic triterpenoid observed in many medicinal plants worldwide. Triterpenes are strong antioxidants, and the majority of triterpenes found in nature are produced by higher plants. Many previous studies have demonstrated significant protective effects of lupeol. For instance, it possesses hepatoprotective effects against aflatoxin B1-induced damage in rats [27] and CCl4 intoxication [28]. Likewise, it is effective in ameliorating the kidney injury associated with hypercholesterolemia [29] and minimizing the lipid biochemical abnormalities induced by cholesterol and cholic acid-fed rats [30]. Moreover, lupeol has cardioprotective effects represented by preserving membrane permeability, a protective effect against cyclophosphamide-induced cardiotoxicity [31].

Conclusion

From our understanding, this study represents first of its kind regarding the antioxidant, cytotoxic, and antimicrobial effects of *E. inarticulata* Schweinf whole plant extracts. more literatures are needed to evaluate the active ingredients of *E. inarticulata*

Schweinf, involved in the cytotoxic or antiproliferative effects of this plant. These kind of literatures should include the establishment of an in vivo cancer model and treatment with a natural crude extract or purified active ingredient from this plant. More efficient extraction techniques and instruments have to be used to obtain the crude extracts or their derivatives in convenient quantities and concentrations.

Significance Statement

This research established for the first time, the antioxidant and antimicrobial activities of methanolic extract of *E. inarticulata* Schweinf sampled from the Jazan region, Saudi Arabia. Therefore, this research will allow more future studies on characterizing the different effects of *E. inarticulata* Schweinf plant extract It can serve as a successful drug guideline.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, et al. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS, Cent Sci. 2020;6:315-31. doi:10.1021/acscentsci.0c00272
2. Abdel-Kader MS, Hazazi AM, Elmakki OA, Alqasoumi SI. A survey on traditional plants used in Al Khobah village. Saudi Pharm J. 2018;26(6):817-21. doi:10.1016/j.jsps.2018.04.001
3. Aldhebiani A, Jury S. Anatomical studies on the genus Euphorbia L. Saudi Arabia (Subgenera: Triucalli, Ermophyton, Esula and Chamaesyce). Int Res J Plant Sci. 2013;4(6):168-91.
4. Osman AK, Al-Ghamdi F, Bawadekji A. Floristic diversity and vegetation analysis of Wadi Arar: A typical desert Wadi of the Northern Border region of Saudi Arabia. Saudi J Biol Sci. 2014;21(6):554-65. doi:10.1016/j.sjbs.2014.02.001
5. Thomas J, Sivadasan M, Al-Ansari AM, Alfarhan A, El-Sheikh M, Basahi M, et al. New generic and species records for the flora of Saudi Arabia. Saudi J Biol Sci. 2014;21(5):457-64. doi:10.1016/j.sjbs.2014.07.001
6. Thomas J, El-Sheikh MA, Alfarhan AH, Alatar AA, Sivadasan M, Basahi M, et al. Impact of alien invasive species on habitats and species richness in Saudi Arabia. J Arid Environ. 2016;127:53-65. doi:10.1016/j.jaridenv.2015.10.009
7. Al-Gifri AN, Kasem WT, Shalabi LF. Vegetation Structure and Diversity of Wadi Wasaa, Jazan, Saudi Arabia. J Adv Biol Biotechnol. 2018;18(4):1-6. doi:10.9734/JABB/2018/43463
8. Satya K, Srinivas K, Srilakshmi BJ, Lakshmi CM, Krishna SA. Phytochemical and pharmacoligical evaluation of Euphorbiaceae family plant leaves Acalypha indica, Croton bonplandianum Baill. Mintage J Pharm Med Sci. 2015;4(3):17-22.
9. Pandey BP. A textbook of botany: Angiosperms, taxonomy, anatomy, embryology and economic botany. Chand and Co., Ltd., Ram Nagar, New Delhi; 2006.
10. Etukudo I. Ethnobotany: Conventional and Traditional Uses of Plants. The Verdict Press: Uyo, Nigeria; 2003.
11. Betti JL. An ethnobotanical study of medicinal plants among the Baka pygmies in the Dja biosphere reserve, Cameroon. Afr Study Monogr. 2004;25(1):1-27. doi:10.14989/68229
12. Gill LS. Taxonomy of flowering plants. Africana-Fep Publishers Limited. 1988;1067.
13. Agbovie T, Amponsah K, Crentsil OR, Dennis F, Odamtten GT, Ofusohene-Djan W. Conservation and sustainable use of medicinal plants in Ghana: Conservation Report. UNEP-WCMC, Cambridge UK; 2002. pp. 42.
14. Kubmarawa D, Ajoku GA, Enwerem NM, Okorie DA. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotechnol. 2007;6(14):1690-6.
15. Oliver BE. Medicinal plants in Nigeria. Nigerian College of arts. Sci Technol. 1960;21(37):52-3.
16. Wang ZY, Liu HP, Zhang YC, Guo LQ, Li ZX, Shi XF. Anticancer potential of Euphorbia helioscopia L extracts against human cancer cells. Anat Rec. 2012;295(2):223-33. doi:10.1002/ar.21517
17. Wood JRI. A Handbook of the Yemen Flora. Royal Botanic Gardens, Kew; 1997.
18. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995;28(1):25-30.
19. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001;15(2):127-30. doi:10.1002/ptr.687
20. Akujobi C, Anyanwu BN, Onyeze C, Ibekwe VI. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. J Appl Sci. 2004;7(3):4328-38.
21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-9. doi:10.1016/j.jpha.2015.11.005

22. Osadebe PO, Okide GB, Akabogu IC. Study on anti-diabetic activities of crude methanolic extracts of Loranthus micranthus (Linn.) sourced from five different host trees. J Ethnopharmacol. 2004;95(2-3):133-8. doi:10.1016/j.jep.2004.06.029
23. Haleyur N, Shahsavari E, Mansur AA, Koshlaf E, Morrison PD, Osborn AM, et al. Comparison of rapid solvent extraction systems for the GC–MS/MS characterization of polycyclic aromatic hydrocarbons in aged, contaminated soil. MethodsX. 2016;3:364-70. doi:10.1016/j.mex.2016.04.007
24. See I, Ee GC, Mah SH, Jong VY, Teh SS. Effect of solvents on phytochemical concentrations and antioxidant activity of Garcinia benthamiana stem bark extracts. J Herbs Spices Med Plants. 2017;23(2):117-27. doi:10.1080/10496475.2016.1272523
25. Wong PY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (Petroselinum crispum) and cilantro (Coriandrum sativum) extracts. Food Chem. 2006;97(3):505-15. doi:10.1016/j.foodchem.2005.05.031
26. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4):42. doi:10.3390/plants6040042
27. Preetha SP, Kanniappan M, Selvakumar E, Nagaraj M, Varalakshmi P. Lupeol ameliorates aflatoxin B1-induced peroxidative hepatic damage in rats. Comp Biochem Physiol C Toxicol Pharmacol. 2006;143(3):333-9. doi:10.1016/j.cbpc.2006.03.008
28. Donfack HJ, Kengap RT, Ngameni B, Chuisseu P, Tchana AN, Buonocore D, et al. Ficus cordata Thunb (Moraceae) is a potential source of some hepatoprotective and antioxidant compounds. Pharmacologia. 2011;2(5):137-45. doi:10.5567/PHARMACOLOGIA.2011.137.145
29. Sudhahar V, Ashok Kumar S, Varalakshmi P, Sujatha V. Protective effect of lupeol and lupeol linoleate in hypercholesterolemia associated renal damage. Mol Cell Biochem. 2008;317(1):11-20. doi:10.1007/s11010-008-9786-5
30. Sudhahar V, Kumar SA, Sudharsan PT, Varalakshmi P. Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vascul Pharmacol. 2007;46(6):412-8. doi:10.1016/j.vph.2006.12.005
31. Sudharsan PT, Mythili Y, Selvakumar E, Varalakshmi P. Lupeol and its ester inhibit alteration of myocardial permeability in cyclophosphamide administered rats. Mol Cell Biochem. 2006;292(1):39-44. doi:10.1007/s11010-006-9171-1