EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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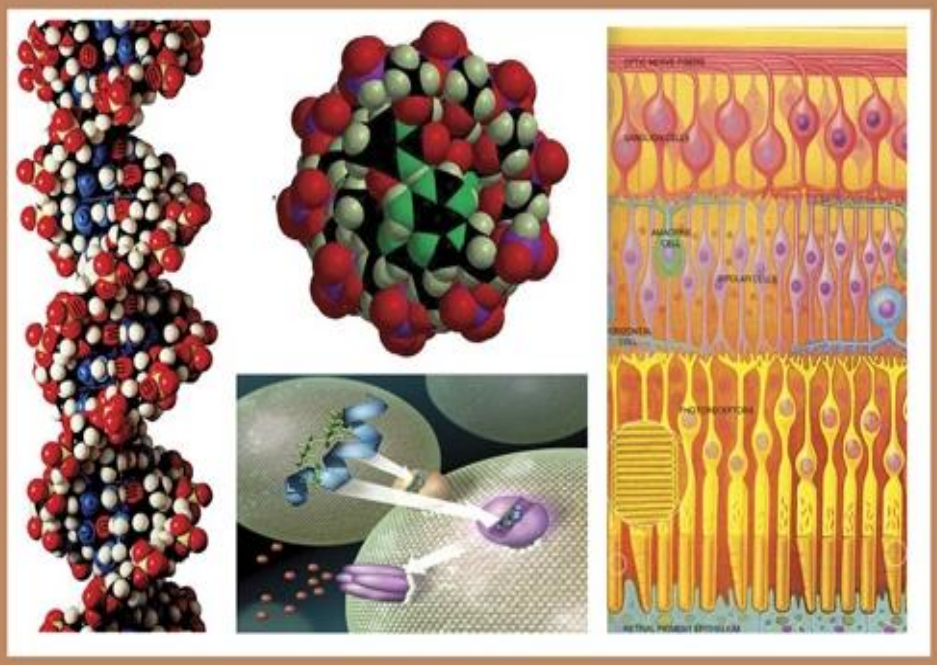

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

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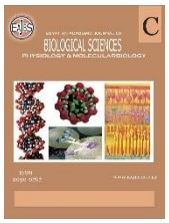
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp409-422 (2022)*
DOI: 10.21608/EAJBSC.2022.236999

Egypt. Acad. J. Biolog. Sci., 14(1):409-422 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Biological Activity and Phytochemical Analysis of Herbal Preparations from Al-Baha Region, Saudi Arabia

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ARTICLE INFO
Article History
Received:25/4/2022
Accepted:14/5/2022
Available:16/5/2022

Keywords:
Medicinal plants; GC/MS; Al-Baha; TPC; TFC; DPPH.

ABSTRACT

Background and Objective: Herbal medicinal plants are widely used in Al-Baha region, Saudi Arabia either in rural or urban areas for the treatment of several diseases. Here we investigated the phytochemical and biological effects of three herbal plant extracts, *Aloe vera*, *Jatropha* and *Ricinus communis*. **Methodology**: We have investigated the total phenolics as and total flavonoid contents of the three plant extracts. The chemical composition was determined by GC/MS. The antioxidative activity was investigated using DPPH while the antiproliferative effects were screened using the MTT assay against A549 (lung cancer, HepG2 (liver cancer) and MCF-7 (breast cancer) cell lines. **Results**: Total phenolic contents were shown to be 133.16±0.01, 131.60±0.06 and 44.73±0.02 mg GAE/g for *Ricinus communis*, *Aloe vera* and *Jatropha*, respectively, while total flavonoid contents were 19.38±0.01, 32.28±0.19 and 39.34±0.32 QE/g of dry extract. The highest DPPH scavenging potential observed was of *Ricinus communis* extract with IC_{50} of 30.98 µg/ml. Six main bioactive compounds were elucidated in the GC-MS analysis of *Aloe vera* extract including Palmatic and stearic acids with peak areas of 45.91% and 34.27%, respectively. While 10 main bioactive phytochemical compounds were elucidated in the GC/MS analysis of *Jatropha* and *Ricinus communis*. The only observed antiproliferative was *Ricinus communis* methanolic leaves extracts against the three tested cancer cell lines. **Conclusion**: This investigation presents insight into the phytochemical, antioxidant and antiproliferative activities of the three plants' methanolic extracts, *Aloe vera*, *Jatropha* and *Ricinus communis* from Al-Baha region, Saudi Arabia and provides some evidence for the medicinal properties of these plants.

INTRODUCTION

Nature has an invaluable number of natural products that humans use for the treatment of different ailments throughout life. Herbal medicinal remedy has been continuously increasing in recent decades throughout the world as complementary and alternative medicines especially in Western countries (Cheng & Leung, 2012).

WHO reports estimate the use of approximately 80% of the population still depends on the traditional use of herbal medicine for primary healthcare (Bodeker & Ong, 2005; Mukerjee, 2002). The use of traditional medicinal herbs is due to the disappointment of the patients with standard treatment and the beliefs that herbal medicines, being natural and the use of herbs are associated with a healthier lifestyle and are therefore harmless (Nemudzivhadi & Masoko, 2014).

Among the herbal plants used frequently as a cosmetic preparation as well as an herbal medicinal plant is *Aloe vera*. *Aloe vera* (L) family Aloaceae is a widely used plant in traditional medicine for several ailments (Arunkumar & Muthuselvam, 2009; Dey, Dutta, Chowdhury, Das, & Chaudhuri, 2017), as hyperglycemia in diabetic patients and hyperlipidemia in hyperlipidemic patients (Vogler & Ernst, 1999). It has been used for the healing of wounds and found to be helpful in relieving many gastrointestinal disorders as well as for its purgative effects and in the treatment of hemorrhoids (Foster, 1999). *Aloe vera* contains hundreds of active compounds and nutrients that are responsible for its medicinal properties. It has been shown to possess anti-inflammatory, antioxidant, antibacterial, anticancer, antidiabetic and antiaging properties, cellular protection, restoration, and immune and mucus stimulating activities (H. Davis, 1997; R. H. Davis, Donato, Hartman, & Haas, 1994; Hamman, 2008; Kumar, Yadav, Yadav, & Yadav, 2017). *Aloe vera* is rich in a variety of phytochemicals and compounds responsible for its remediation activity such as phenolics, flavonoids, coumarins, vitamins and amino acids (Wintola & Afolayan, 2011).

Jatropha species is a medicinal plant that belongs to the Euphorbiaceae family, among the traditional medicinal plants used in Saudi Arabia due to its anti-inflammatory, antiulcer, antioxidant, antiseptic, analgesic and wound healing properties (Omeh & Ezeja, 2010; Oskoueian *et al*., 2011). Phytochemical analysis of *Jatropha* extracts revealed to contain various phytochemical components such as glycosides, alkaloids, tannins, saponins, flavonoids and phenolics (Oskoueian *et al*., 2011; Thomas, Sah, & Sharma, 2008). The anticancer, anti-inflammatory and antimicrobial compounds and antioxidant activities are attributed to these phytochemical compounds (Rathee *et al*., 2009). It has been reported to have antiprotozoal activities against leishmania, malaria and trypanoma (Sabandar, Ahmat, Jaafar, & Sahidin, 2013). In addition, several studies have shown that *Jatropha* seeds have activity against sexually communicated diseases, mouth odor and jaundice as well as antiseptic properties (Igbinosa, Igbinosa, & Aiyegoro, 2009; Namuli, Abdullah, Sieo, Zuhainis, & Oskoueian, 2011) and molluscicidal and larvicidal activities (Rug & Ruppel, 2000). Meanwhile, *Jatropha* seeds have been known as a rich biodiesel source in many parts of the world (Belewu, 2008).

Ricinus communis Linn. plant (family Euphorbiaceae) distributed widely across the world (de Assis Junior *et al*., 2011) and found growing wild in many parts of Saudi Arabia, commonly known as castor plant. Phytochemical analysis of different parts of this plant reported containing phytosterols, proteins, fatty acids, coumarins, phenolic compounds (Williamson, 2002), flavonoids (Byamukama, Jordheim, Kiremire, & Andersen, 2008; Kang, Cordell, Soejarto, & Fong, 1985), alkaloids (Kang *et al*., 1985), terpenoid and tocopherol related compounds (Tan, Cai, Du, & Luo, 2009). In traditional medicine, *Ricinus communis* has been reported to have antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, anticancer, purgative and lubricant activities as well as a remedy for various ailments (Ilavarasan, Mallika, & Venkataraman, 2006).

Medicinal plants from Al-Baha region are widely used in traditional folklore medicine. The biological activity and

phytochemical studies of these traditionally used herbal preparations are still scarce. The flora of Saudi Arabia and in particular of Al-Baha region is extraordinarily rich and diverse. There are many plants that the Saudi people use either in rural or urban areas for the treatment of different ailments (El-Shanawani, 1996; Gushash, 2006). We have investigated previously the genotoxic effects of the three plants, *Aloe vera*, *Jatropha* and *Ricinus communis*, using Ame's bacterial inhibition assay (Al-Zubairi, 2019). This study reports the biological and phytochemical analysis results of *Aloe vera*, *Jatropha* and *Ricinus communis* plants methanolic extracts growing in Al-Baha region and used in traditional medicine.

MATERIALS AND METHODS

Plant Materials:

The plant material was collected from different places in Al-Baha region (Table 1). The plants were taxonomically identified by Dr Abdulwali Ahmed Al-Khulaidi, the botanists at the Department of Biology, Faculty of Sciences and Arts, Baljrushi, Albaha University.

Processing of the Plant Material:

Plant leaves were washed thoroughly with water. The air-dried leaves were finely grinded using an electrical grinder and stored for further use. The powdered plant material was extracted with MeOH (3x100 ml) under shaking at room temperature. The separated extracts were then filtered through Whatman's No. 1 filter paper and evaporated to dryness *in vacuo* at 40 °C. Dried extracts were collected in an air-tight container and stored at 4 °C till further analysis. Stock solutions have been prepared in DMSO at a concentration of 20 mg/mL (Al-Musayeib, Mothana, Matheeussen, Cos, & Maes, 2012).

1. Phytochemical Analysis:

Total Phenolic Content:

Phenolic contents of crude extracts were estimated by the method of Singleton *et al*., (1999) with some modifications. Briefly, 100 µl of an aliquot sample(2mg/ml) or gallic acid, a standard phenolic (31.25-1000 µg/ml), were mixed with 1.5 ml of distilled water and 100 µl Folin–Ciocalteu reagent and allowed to stand for 8 min at room temperature, then 300 µl of sodium carbonate (20 %) were added (Singleton, Orthofer, & Lamuela-Raventós, 1999). After incubation, the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. The absorbance of all the sample solutions was measured at 765 nm using a spectrophotometer (Phenolic content is expressed as Gallic acid equivalent per gram).

Total Flavonoid Content:

The total flavonoid content was determined based on the formation of flavonoid-aluminium as described by Djeridane *et al*. (2006) with some modifications (Djeridane *et al*., 2006). Briefly, 1 mL of sample extract (2mg/mL) was mixed with 1 ml of 2% aluminium chloride solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured using a spectrophotometer at 430 nm. Quercetin (3.125-100 µg/ml) was used as a standard to plot the calibration curve. The number of flavonoids was expressed as Quercetin equivalents (QE) per gram.

2. GC-MS Analysis:

The chemical constituents of methanolic extract was determined using gas chromatography and a mass spectrometer (Turbomass, PerkinElmer, Inc., Waltham, MA, USA). The temperature program 4 was set to 40 °C, followed by a 2 min hold, then raised to 200 °C at a rate of 5 °C min−1, which was also then put on hold for 2 min. From 200 °C, the temperature was raised by 5°C min−1 to 300 °C and held for another 2 min. The chemical composition was determined by comparing the mass spectra obtained with the mass spectra from the National Institute of Standard and Technology Spectral library. The mass spectra compounds were also compared with those of similar compounds in the Adams Library (Adams, 2007) and the Wiley GC/MS Library (McLafferty and Stauffer, 1989).

3. Biological Studies:

1. Antioxidant Evaluation:

***DPPH Radical Scavenging Activity*:**

Crude extracts were screened for antioxidative activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Brand *et al*. (1995). In brief, 100 µL (1mg/ml) of each sample at various concentrations was mixed with 100 µL DPPH solution (1 mM) and incubated for 30 min. Finally, UV-spectrophotometer absorbance measurement at $\lambda = 517$ nm was applied to demonstrate the anti-DPPH activity using the following formula:

% Antiradical activity = Abs of control − Abs of sample /Abs of control × 100

2. MTT Assay:

In order to test the anticancer activity of extracts, MTT assay was performed. The A549 (lung cancer, HepG2 (liver cancer) and MCF-7 (breast cancer) cell lines were plated in 24 cell culture plates (10^5 per well) and allowed overnight for adherence. Cells were then treated with various concentrations of extracts (500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml). Doxorubicin was used as a positive control. Following 48h of treatment, 0.01mL of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrasodium bromide (MTT), was added to each well and incubated at 37°C in 5% CO_2 for 2-4 hours. Isopropanol (0.1mL) with 0.1 N HCL was then added to solubilize formazan products and placed on a shaker for 10 min. Absorbance measurements with an ELISA plate reader (Bio-Tek, USA) were read at 570 nm. The IC_{50} (concentration of tested extract needed to inhibit cell growth by 50%) was calculated from a dose-dependent curve and cell viability was calculated using the following equation: Cell Viability (%) = (O.D of the treated sample)/ (O.D of the untreated sample) ×100%

RESULTS AND DISCUSSION

The return to the use of folk medicine is largely due to the disappointment of patients due to the side effects caused by modern medicines, which are difficult to avoid, and the fact that the use of a natural product is less likely to cause side effects or no side effects since the natural product is a raw extract. Saudi people either in rural or urban areas, commonly use traditional medicine for the treatment of different ailments (El-Shanawani, 1996; Gushash, 2006). In this study, we tried to emphasize the phytochemical composition, and the antioxidant and anti-proliferative effects of three herbal plants grown in Al-Baha region, namely, *Aloe vera*, *Jatropha* and *Ricinus communis*.

Total Phenolic and Flavonoid Contents:

Phytochemical analysis performed on the whole leaf methanolic extracts of the three plants, *A. Vera*, *Jatropha sp*. and *Ricinus communis*, revealed the presence of phenol and flavonoids (Table 1). The highest phenolic content was shown by *Ricinus communis* extract at 133.16 ± 0.01 mg GAE/g, followed by *Aloe vera* extract at 131.60 ± 0.06 mg GAE/g and finally, the lowest total phenolic content was shown by *Jatropha sp* extract at 44.73 ± 0.02 mg GAE/g. Different extracts of *Ricinus communis* have shown previously to have phenolic contents of 121mg GAE/g to 147mg GAE/g (Saeed, Khan, & Shabbir, 2012) and the aqueous, n-butanol and ethyl acetate extracts showed activity of 131 mg/mL, 127 mg/mL and 117 mg/mL respectively by (Ahmed & Iqbal, 2018), while, total phenolic content obtained with ethanol varied from 72.03 ± 1.92 to 135.06 ± 1.69 mg GAE/g of extract as reported by (Santos *et al*., 2018) and 52 ± 7 to 89 ± 6 mg/g extracts (Iqbal *et al*., 2012). Results of this study of total phenolic contents of *Ricinus communis* methanolic extract from Al-Baha region, Saudi Arabia, were found to be comparable to previous reports from other sources. On the other hand, the *Aloe vera* phenolic contents of samples from India, have been reported by Fareeha I. and Ambreen A., 2021, to vary between 53.6 mg QE/g to 398.0 mg QE/g with a marked presence of the phenols in methanolic extract while the lowest content of phenols was found in Acetone (Fareeha I. and Ambreen A., 2021). The phenolic content of methanolic extract of whole *Aloe vera* leaves was found to be 30.53±0.30 mg GAE/g as

reported by Bista *et al*. (2020) which was similar to the results obtained by Kumar *et al*. (2017) (Bista, Ghimire, & Subedi, 2020; Kumar *et al*., 2017). *Jatropha* methanolic extract was found to have less total phenolic content, however, it was found to be in agreement with the results of (Osman, Abdullah, & Ahmad, 2017), who reported the total phenolics of *Jatropha* Ethyl acetate and n-butanol fractions to have the highest phenolic content of 34.0 ± 0.02 µg GAE/g DW and 33.1 ± 0.01 µg GAE/g dry weight. There are reports of other *Jatropha* species showing a low content of phenolic compounds (Vega-Ruiz *et al*., 2021).

Total flavonoid contents of the A. vera, *Jatropha* and *Ricinus communis* are shown in Table 1. Total flavonoid content in *Ricinus communis* methanolic extract was found to be 39.34 ± 0.32 mg QE/g of dry weight. These results are in agreement with the findings of Chakarborthi (2008), who reported total flavonoid as catechin equivalent activity to be 23 to 37 µg/ml (GS, 2008), as well as the finding of Faheem A. and Moshin I., who reported the total flavonoids to be 32 µg/ml dry weight (Ahmed & Iqbal, 2018). *Jatropha sp* methanolic extract yielded 32.28 ± 0.19 mg QE/ g dry weight in this study, while Zengin *et al*., 2021 reported *J. gossypiifolia* extract to contain total flavonoid contents of (17.63 ±0.34 mg RE/g and 6.97 ± 0.32 mg RE/g) in stem bark and leaf extracts respectively (Zengin *et al*., 2021). The total flavonoid content of ethyl acetate and n-butanol of *Jatropha curcas* was reported by (Osman *et al*., 2017), to be 9.2 ± 0.04 µg CE/g DW and 10.1 ± 0.01 µg CE/g DW, respectively. *Aloe vera* methanolic extract was found to contain 19.38 ± 0.01 mg QE/ g dry weight in this study and was in agreement with that published by (Iqbal & Ahmed, 2021). Meanwhile, the total flavonoid content of methanolic extract of *A. vera* was found to be 1.60 ± 14.10mg catechin equivalents/g (Masaldan & Iyer, 2011). In contrast, the finding of the total flavonoid content of *Aloe vera* was found to be 73.26 ± 2.46 mg QE/g of dry weight in the report by (Bista *et al*., 2020), which is more than that observed here in *Aloe vera* collected from Al-Baha region, Saudi Arabia (Bista *et al*., 2020). Additionally, the highest flavonoid content was observed by Alseini, Abdulbasit II, 2014) who reported total flavonoid to be 1958.27 mg QE/100g when compared with 10 different Arabian herbs and spices (Alseini, 2014).

Table 1: Total phenolic and Total flavonoid contents of *Aloe Vera*, *Jatropha* sp. and *Ricinus communis*, leave extracts

Samples± STD)	Total phenol (mg GAE/ g of dry extract) ± STD)	Total flavonoid (mg QE/ g of dry extract
Aloe vera	131.60 ± 0.06	19.38 ± 0.01
Jatropha	44.73 ± 0.02	32.28 ± 0.19
Ricinus communis	133.16 ± 0.01	39.34 ± 0.32

GC-MS Analysis:

The capacity of medicinal plants to synthesize bioactive compounds is unlimited and has shown to have various biological activities. These compounds have many beneficial diseases relieving effects with few or no side effects compared to synthetic drugs. The present study carried out on *Aloe vera* collected from Al-Baha region, Saudi Arabia, revealed the presence of biologically active constituents. Figure 1 highlight the identification of the bioactive compounds present in the methanolic extracts of the *Aloe vera*. The extracts were subjected to Gas Chromatography-Mass Spectroscopy. The bioactive compounds shown in the chromogram of the GC/MS are listed in Table 2. According to the results shown in Figure 1, 6 main bioactive phytochemical compounds were elucidated in the GC-MS analysis of *Aloe vera*. The identification of phytochemical compounds is based on the

peak area, molecular weight and molecular formula as shown in the table. Palmatic acid ($C_{16}H_{32}O_2$) with RT 20.19 min has a peak area of 45.91% followed by stearic acid ($C_{18}H_{36}O_2$) with RT 21.94 has a peak area of 34.27% and 9-Octadecenoic ($C_{18}H_{34}O_2$) acid with RT 22.14 min has peak area of 8.53%. Pentacosanoic acid methyl ester ($C_{26}H_{52}O_2$) with RT 19.66 min and a peak area of 6.46%, 3,7,11,15-Tetramethyl-2-hexadecene ($C_{20}H_{40}$) with RT 18.78 min and a peak area of 4.58% and Octyl acrylate ($C_{11}H_{20}O_2$) with RT 27.07 min and a peak area of 0.26 %. A. vera has been reported to contain organic acids, mono- and polysaccharides, sterols, tannins, enzymes, saponins, vitamins and minerals (Newall *et al*., 1996).

Phytochemical analysis of *Jatropha* has been performed in this study on methanolic extract. Figure 2 highlight the identification of the bioactive compounds present in the methanolic extracts of *Jatropha*. The bioactive compounds shown in the chromogram of the GC/MS are listed in Table 3. According to the results shown in Figure 3, 10 main bioactive phytochemical compounds were elucidated in the GC/MS analysis of *Jatropha*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula as shown in the table. The phytochemical compounds identified in the methanolic extract of *Jatropha* leaves were found to be in the RT range from 18.85 to 25.69. The compounds identified were Cholest-5-en-3-ol ($C_{27}H_{46}O$) with RT 25.69 and peak area of 22.950% followed by Phytol ($C_{20}H_{40}O$) with RT 21.57 and peak area of 16.790%, then 2-Decen-1-ol ($C_{10}H_{20}O$) with RT 18.78 and peak area 14.100% and Decanoic acid ($C_{10}H_{20}O_2$) with RT 20.17 and peak area, 12.710%, 6,6-Dimethyl-2-(4,8-dimethyl-3,7-nonadienyl)-bicyclo(3.1.1)hept-3-ene ($C_{20}H_{32}$) with RT 24.33 and peak area 8.430%, 3,5,24-Trimethyltetracontane ($C_{43}H_{88}$) with RT 23.72 and peak area 6.340%, finally 2-Tridecen-1-ol ($C_{13}H_{26}O$) with RT 19.05 and peak area 5.250%. In addition to other phytochemical compounds with less proportion such as cis-2,4-Diisopropyl-5,5-dimethyl-1,3-dioxane ($C_{12}H_{24}O_2$), Palmitic acid ($C_{16}H_{32}O_2$) and 9-Octadecenoic acid ($C_{18}H_{34}O_2$) with a peak area of 2.850%, 1.340% and 1.340%, respectively. The compounds with maximum peak area were Cholest-5-en-3-ol ($C_{27}H_{46}O$) at 22.950%, Phytol ($C_{20}H_{40}O$) at 16.790% and 2-Decen-1-ol ($C_{10}H_{20}O$) at 14.100%.

Ricinus communis methanolic extract phytochemical analysis has been performed on leaf extract in this study. Figure 3 shows the identification of the bioactive compounds present in the methanolic extracts of *Ricinus communis*. The bioactive compounds shown in the chromogram of the GC/MS are listed in Table 4. Ten main bioactive phytochemical compounds were elucidated in the GC/MS analysis of *Ricinus communis* presented as shown in Figure 5. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula as shown in the table. The phytochemical compounds identified in the methanolic extract of *Ricinus communis* leaves were found to be in the RT range from 18.00 to 21.91. The phytochemical compounds of *Ricinus communis* as shown in the chromatogram were ordered in decreasing order according to the area of the peak, Ricinine ($C_8H_8N_2O_2$) with RT 20.97 and peak area 46.530%, Alpha-Linolenic acid ($C_{18}H_{30}O_2$) with RT 21.91 and peak area 18.410%, Palmitic acid ($C_{16}H_{32}O_2$) with RT 20.17 and peak area 10.650%, Heptadecanoic acid methyl ester ($C_{18}H_{36}O_2$) with RT 19.73 and peak area 7.290%, 3,6-Dodecadien-1-ol, (3Z,6Z)- ($C_{12}H_{22}O$) with RT 21.47 and peak area 7.190%, 1-Hydroxylinalool ($C_{10}H_{18}O_2$) with RT 21.58 and peak area 3.160%, 2-Decen-1-ol ($C_{10}H_{20}O$) with RT 18.79 peak area 2.700%, DL-3,4-Dimethyl-3,4-hexanediol ($C_8H_{18}O_2$) with RT 18.24 and peak area 2.550%, Valeric acid ($C5H_{10}O_2$) with RT 18.00 and peak area 0.810% and 2-Dodecen-1-ol ($C_{12}H_{24}O$) with RT 19.26 and peak area 0.710%. The major phytochemical compound identified was Ricinine (figure 6) with a peak area of 46.530%, to which the

bioactivity of the plant is attributed as has been reported previously (Bigi *et al*., 2004), followed by Alpha-Linolenic acid, Palmitic acid, Heptadecanoic acid methyl ester and 3,6-Dodecadien-1-ol, (3Z,6Z) with peak area 18.410%, 10.650%, 7.290% and 7.190%, respectively.

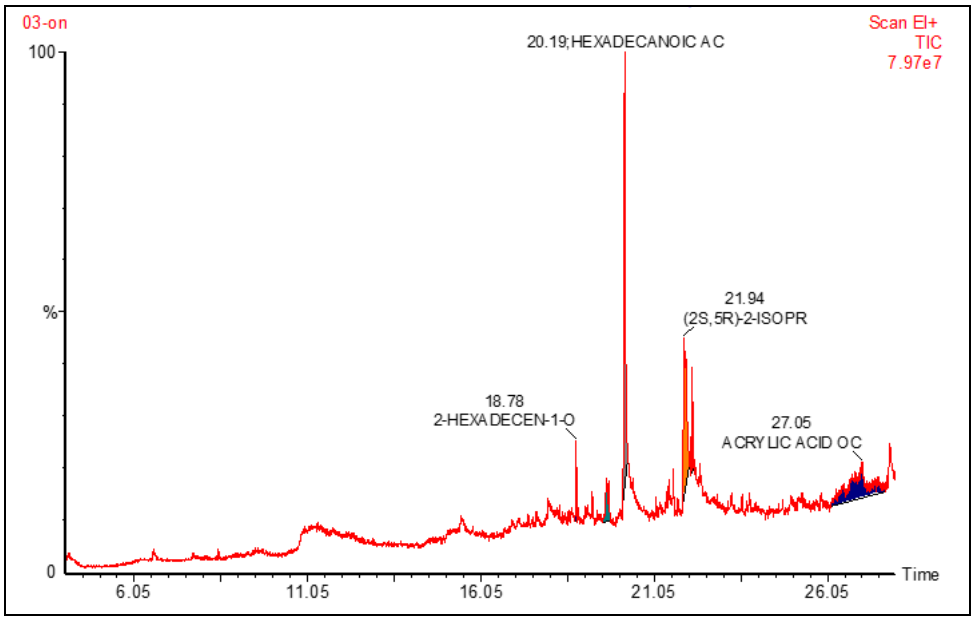
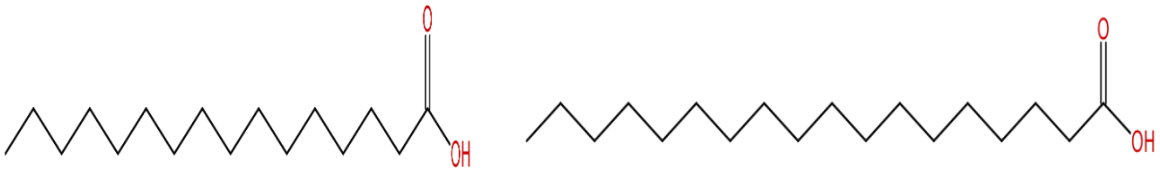


Fig. 1: GC-MS chromatogram of *Aloe vera*

Table 2: GC-MS analysis of *Aloe vera*

Compound Name	Chemical Formula	Molecular weight(g/mol)	RT (min)	Area%
3,7,11,15-Tetramethyl-2-hexadecene	C20H40	280.5	18.78	4.580
Pentacosanoic acid methyl ester	C26H52O2	396.7	19.66	6.460
Palmitic acid	C16H32O2	256.42	20.19	45.910
Stearic acid	C18H36O2	284.5	21.94	34.270
9-Octadecenoic acid	C18H34O2	282.5	22.14	8.530
Octyl acrylate	C11H20O2	184.27	27.07	0.260



Palmitic acid Stearic acid

Fig. 2: Major compounds Found in *Aloe vera*

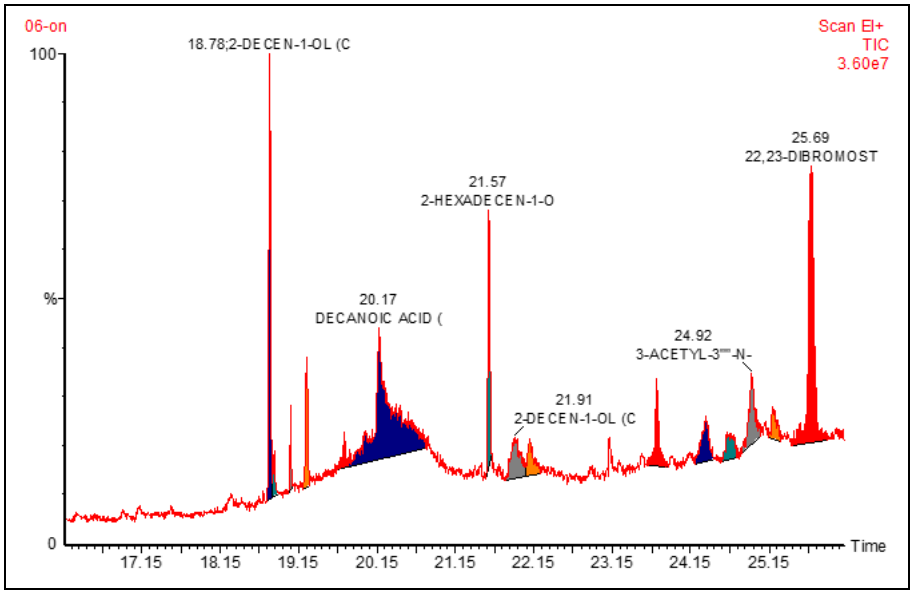
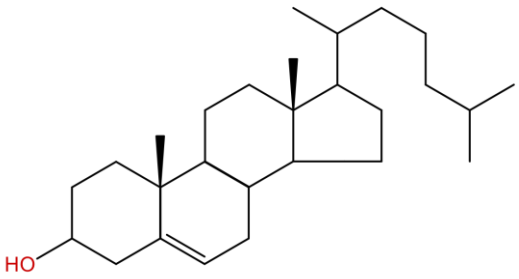


Fig.3: GC-MS chromatogram of *Jatropha*

Table-3: GC-MS analysis of *Jatropha*

Compound Name	Chemical Formula	Molecular weight(g/mol)	RT (min)	Area%
2-Decen-1-ol	C10H20O	156.26	18.78	14.100
cis-2,4-Diisopropyl-5,5-dimethyl-1,3-dioxane	C12H24O2	200.32	18.85	2.850
2-Tridecen-1-ol	C13H26O	198.34	19.05	5.250
Palmitic acid	C16H32O2	256.42	19.73	1.340
Decanoic acid	C10H20O2	172.26	20.17	12.710
Phytol	C20H40O	296.5	21.57	16.790
9-Octadecenoic acid,	C18H34O2	282.5	22.10	1.340
3,5,24-Trimethyltetracontane	C43H88	605.2	23.72	6.340
6,6-Dimethyl-2-(4,8-dimethyl-3,7-nonadienyl)-bicyclo(3.1.1)hept-3-ene	C20H32	272.5	24.33	8.430
Cholest-5-en-3-ol	C27H46O	386.7	25.69	22.950



Cholest-5-en-3-ol

Phytol

Figure-4: Major compounds Found in *Jatropha*

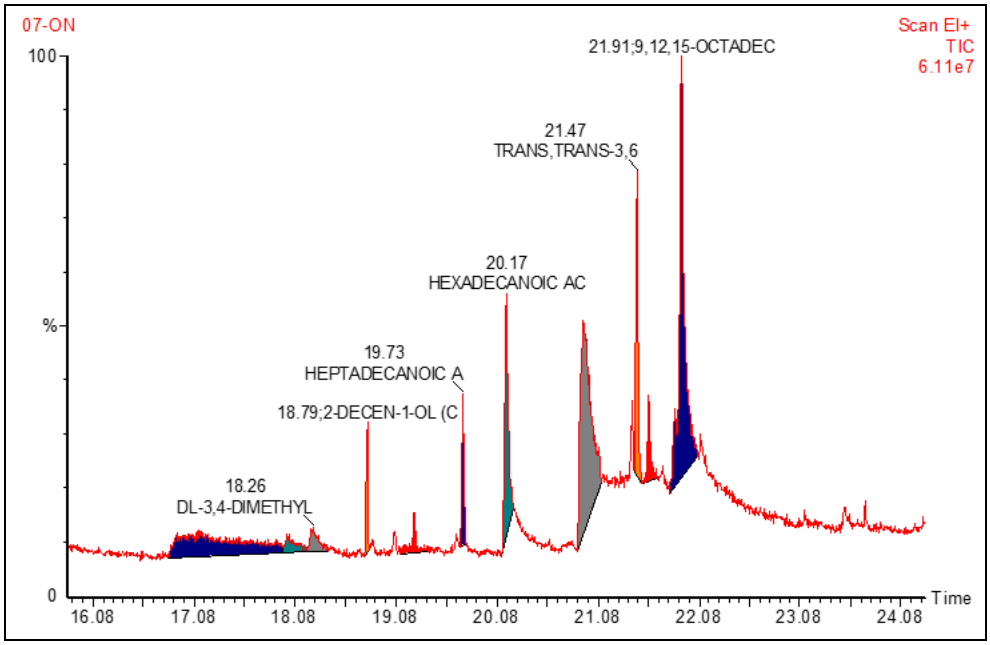
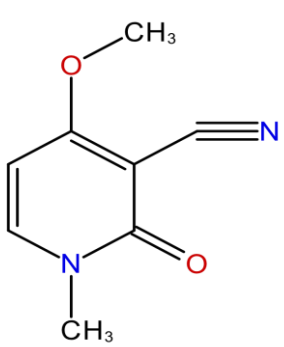


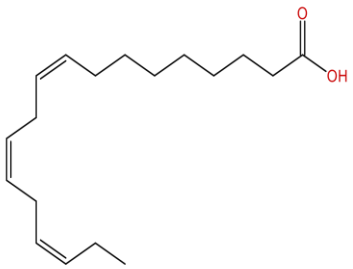
Figure-5: GC-MS chromatogram of *Ricinus communis*

Table 4: GC-MS analysis of *Ricinus communis*

Compound Name	Chemical Formula	Molecular weight(g/mol)	RT (min)	Area%
Valeric acid	C5H10O2	102.13	18.00	0.810
DL-3,4-Dimethyl-3,4-hexanediol	C8H18O2	146.23	18.24	2.550
2-Decen-1-ol	C10H20O	156.26	18.79	2.700
2-Dodecen-1-ol	C12H24O	184.32	19.26	0.710
Heptadecanoic acid methyl ester	C18H36O2	284.5	19.73	7.290
Palmitic acid	C16H32O2	256.42	20.17	10.650
Ricinine	C8H8N2O2	164.16	20.97	46.530
3,6-Dodecadien-1-ol, (3Z,6Z)-	C12H22O	182.30	21.47	7.190
1-Hydroxylinalool	C10H18O2	170.25	21.58	3.160
Alpha-Linolenic acid	C18H30O2	278.43	21.91	18.410



Ricinine



Alpha-Linolenic acid

Fig. 6: Major compounds Found in *Ricinus communis*

Anti-Proliferative Activity:

Growth inhibition studies for the three tested methanolic extracts have been done on three cancer cell lines, A549, HepG2 and MCF-7 cell lines. Different concentrations (500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml), have been tested against the cancer cell lines from which the IC50 has been calculated. Table-5 shows the antiproliferative activity of the three extracts against the three cancer cell lines. *Aloe vera* and *Jatropha* methanolic extracts showed no antiproliferative activity against the tested cancer cell lines. *Aloe vera* methanolic extract antiproliferative activity in this study seems to be in agreement with the study. Similarly, the antiproliferative activity of *Jatropha* methanolic extracts against the tested cancer cell lines has not been seen in this study. This observation of the lack of inhibition of proliferation has been also reported on BRL-3A cells in the MTT test (Calabrone *et al*., 2019). In contrast, Jatrophone, an isolated compound from *Jatropha* gossypifolia has been reported to have an inhibitory effect on the liver cancer cell lines HepG2 (Sukohar *et al*., 2017). *Ricinus communis* methanolic leaves extracts were found to have antiproliferative activity against the three tested cancer cell lines A549, HepG2 and MCF-7 with IC50 of 189.2 ± 1.8, 227.6 ± 2.4 and 123.6 ± 1.4 µg/ml. The most cytotoxic effects were observed against MCF-7. These cytotoxic effects of *Ricinus communis* methanolic extracts have also been observed by other researchers with comparable concentrations (Nemudzivhadi and Masoko, 2014; Morobe *et al*., 2012).

Table 5: Anti-proliferative effects of A. Vera, *Jatropha* and *Ricinus communis*

Plant	A549	HepG2	MCF-7
Aloe vera	-	-	-
Jatropha	-	-	-
Ricinus communis	189.2 ± 1.8	227.6 ± 2.4	123.6 ± 1.4
Doxorubicin	0.9±0.1	1±0.2	0.98±0.1

IC_{50} (µg/ml) Values for Plant Extracts Obtained by MTT Assay with Different Cancer Cell Lines Treated for 48 h:

Antioxidant Activity:

Antioxidant activity of the methanolic plant leaves extracts was performed against 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by Brand *et al*. (1995). The percentage radical scavenging activity of different extracts concentrations of *Aloe vera*, *Jatropha* and *Ricinus communis* were determined against DPPH and was concentration-dependent with the highest percentage of inhibition were 75.25% for *Ricinus communis* extract, 73.16% for *Aloe vera* extract and 60% for *Jatropha* extract, of DPPH was observed. The IC_{50} values (which represent the concentration of the extract necessary to decrease the initial absorbance of the DPPH solution by 50% (Bittencourt *et al*., 2015) are shown in Table 6. *Ricinus communis* methanolic extract was found to have the highest activity with IC_{50} of 30.98 µg/ml, while the IC50 values of *Aloe vera* and *Jatropha* were 559.91 µg/ml and 666.67 µg/ml, respectively. This high antioxidant activity of *Ricinus communis* methanolic axtract could be attributed to the high phenolic content of the methanolic extract as shown in table-1. It has been reported that the aerial part of *Ricinus communis* has remarkable antioxidant activity (Iqbal *et al*., 2012) same as in the present study, the antioxidant activity was observed to be high in contrast to the DPPH radical scavenging activity of *Aloe vera* and *Jatropha*. The DPPH radical scavenging activity of *Aloe vera* methanolic extract was found to be consistent with the activity reported by

Sultana *et al*., (2009) and Bista *et al*., (2020), where the DPPH scavenging activity of the methanolic extract was shown to be 80.1±2.3% and 81.91±0.04%, respectively. The high phenolic contents of *Aloe vera* methanolic extract could contribute an important role in its high antioxidative activity.

Table 6: Antioxidant effects of A. Vera, Jatropha and Ricinus communis methanolic extracts expressed as DPPH activity.

Plant extract	**IC50**
Aloe vera	559.91
Jatropha	666.67
Ricinus communis	30.98

Conclusion

Among the plants that are widely grown in Al-Baha region and used for medicinal purposes are *Aloe vera*, *Jatropha* and *Ricinus communis*. The TPC, TFC, antioxidant activity and phytochemical composition have been investigated for these plants, in addition to the antiproliferative activity against the cancer cell lines, A549, HepG2 and MCF-7. TPC was studied and expressed in mg GAE/g extract, with high activity for *Jatropha* spp. While the TFC were investigated and found to be comparable with the three extracts. The antioxidant activity was estimated as a percentage of radical scavenging activity of different extracts concentrations. *Aloe vera*, *Jatropha* and *Ricinus communis* methanolic extract antioxidant activity, were determined against DPPH and was concentration-dependent with the highest percentage of inhibition were 75.25% for *Ricinus communis* extract that could be attributed to the high phenolic contents, 73.16% for *Aloe vera* extract and 60% for *Jatropha* extract, of DPPH was observed. *Aloe vera* and *Jatropha* methanolic extracts showed no antiproliferative activity against the tested cancer cell lines, while *Ricinus communis* extracts were found to have antiproliferative activity against the three tested cancer cell lines A549, HepG2 and MCF-7 with IC50 of 189.2 ± 1.8, 227.6 ± 2.4 and 123.6 ± 1.4 µg/ml, respectively. GC/MS analysis of the three methanolic extracts revealed the presence of numerous phytochemical compounds that could convey the medicinal properties of herbal plants.

Acknowledgment

The author would like to express the sincerest gratitude and appreciation to the Deanship of Scientific Research– Al Baha University which supports this project number (5/1441).

Conflicts of Interest: The authors declare no conflict of interest.

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