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Chemical Composition Using FTIR Technique, Antioxidant [Hypochlorous, Hydrogen peroxide and Superoxide Radical Scavenging] and Antimicrobial Activities of Haloxylon salicornicum Methanolic Aerial-Part Extract

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Abstract:

The objectives of the present study are identification of chemical groups present in Haloxylon salicornicum using a Fourier transform infrared spectrophotometer method and assessment of antioxidant and antimicrobial potentials. The samples H. salicornicum were washed and thereafter air dried to ensure that they were through washed. The mash was filtered employing Whatman filter sheets (no. 1, 125 mm, Cat No. 1061 135 Germany). Approximately 30 mL of each plant extract was taken for this purpose and the concentrations of the final plant extracts were also determined, and all the samples were stored at 4 degrees Celsius. In this case the sample was scanned at infrared region of 400 nm-4000 nm. The human pathogenic bacteria were isolated from Microbiology department of hilla hospital. E.coli, Salmonella, Pseudomonas, Bacillus and Staphylococcus bacteria in particular are known to be part of the major bacterial pathology. Inhibitory zone diameter (mm) were measured after 18–24 h at 37°C. FT-IR peak values of solid analysis according to Type of Intensity, Bond, Type of Vibration and Functional group assignment [Strong, =C–H, Bending, Alkenes] [Strong, C-F, Stretch, alkyl halides][Medium, C=C, Stretch, Aromatic][Bending, N-H, Stretch, Amide][Strong, C=O, Stretch, Ester][Strong, C-H, Stretch, Alkane]. Antioxidant [Hypochlorous acid, Hydroxyl and Superoxide radical scavenging] Activities of Three fractions of Haloxylon salicornicum methanolic crude extract, namely ethanol fraction and water fraction Aerial-Part Extract have been assayed. Antibacterial activity of secondary metabolites of Haloxylon salicornicum: The inhibition of five pathogenic bacteria and antibiotics by the secondary metabolites of Haloxylon salicornicum (AM-Amikacin; AP-Ampicillin; CF-Cephalothin). Pseudomonas aeruginosa (11.10 ± 0.21), Salmonella enterica (08.39 ± 0.14), Bacillus subtilis (19.00 ± 0.45), Escherichia coli (14.36 ± 0.30) and Staphylococcus aureus (12.00 ± 0.29). Haloxylon salicornicum metabolites was very highly active against Bacillus subtilis (19.00 ± 0.45).

Keywords: FTIR Technique, Hypochlorous, Hydrogen peroxide, Superoxide Antimicrobial, *Haloxylon salicornicum.*

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Introduction

Herbal and vegetative medical ingredients have often times invoked hope. It has enhanced health and convenience. Most of the infectious diseases necessitate substance research because they have the substance as part of their treatment. Plants are antibacterial. Flavonoids, tannins, terpenoids and alkaloids are laboratorialy detected plant secondary metabolites with antibacterial activity. The variation of chemical in plants is therefore underscored by microbial disease prevention. Bacterial pathogen AMR increases morbidity and mortality in human and animals due to the increased antibiotic resistance of the bacteria. Bacteria that are characterized by multi-drug resistance are sometimes unable to respond to antibiotics: Gram-positive and Gram-negative bacteria. New therapeutic and antimicrobial drugs are required because there are no therapies and no prophylactic measures, and only a few new drugs are available. Biofilms are listed as a challenging issue in infection control and multidrug resistance. Another member of the Chenopodiaceae family from which Haloxylon salicornicum is derived is distributed all over Northern Africa and Asia and includes 25 different species preferred to grow in sandy and stony desert zones [1-3]. It is a perennial herb that can be found in abundance in Egypt and consists of four distinct species: an evergreen, tall, perennial plant of the rhizophytic genus without leaves. Stems and branches of this plant are smoothly pale yellow and twisted; leaves which in fact are two small triangular cones at the joints , are tomentose on the internal side, while flowers and fruits are absent. The plant may be consumed by domestic animals and wild animals for their required nutrients, it helps in checking the movement of soil particles and provide a favorable microclimate besides, it provides protection, camouflage and shelter to many different kinds of animals. Of all the species tested, this one shows tremendous potential for fixing sand dunes and propagating vegetation. Regrettably, general knowledge on this species remains limited and vague. This species has not attracted a lot of research, this has slowed it down and it has not been utilized in a sustainable manner [4]. It has the flexibility that may allow it to grow on ground that is low in nutrient and water such as that within the desert regions. H. salicornicum is one of the most significant structural species of vegetation in Eastern Arabia [5-7]. In traditional medicine, the antmicrobial and antiinflammatory effects of H. salicornicum are established. Traditional healers are using it as medication in treatment of intestinal ulcers. Just to mention but a few, the following alkaloids have been identified in this shrub; Piperidine alkaloid, haloxynine, and haloxine. Besides that, it has: – Pyranones, – Tannins, – Saponins, – Different types of glycosides. In addition, the biological characteristics as well as the bioactive compounds of H. salicornicum are not scientifically well explored. Surprisingly little work has been done on the plant despite one's obvious relevance [8-11]. This work used Fourier transform infrared spectrophotometer for the designation of biochemical constituents of a methanol extract of H. salicornicum. The activity of the extract was evaluated using the Hypochlorous, Hydrogen peroxide and Superoxide Radical Scavenging free radical scavenging test for the antioxidant activity and Disk diffusion method for molecular antibacterial properties against some pathogenic bacteria.

Materials and Methods

Following washing and rinsing the H. salicornicum samples were allowed to dry under ambient conditions. It was done using 30 milligrams of the dried plant material which was stirred together with 300 milliliters of methanol in 500 ml conical flask. Thereafter, the mixture was shaken for 2 hours at room temperature on a Memnert WB18 water bath shaker, Schwabach Germany. The mash was funnelned using Whatman filter sheets (no.1, 125 mm catalogue no.1061 135 made in Germany) and there was spontaneous nystagmus. The final concentration of plant extracts was also found to be similar and was maintained at 4 degree Celsius.

Fourier transform infrared spectrophotometer [technique known as the FTIR]

Identification of FTIR spectroscopy was done on Haloxylon salicornicum powered sample in Shimadzu, IR Affinity, Japan. The sample was scanned in the region of infrared wavelength ranging from 400 nm to 4000 nm..

Antimicrobial Activity Procedure

The antibacterial activity of H. salicornicum extract was the agar well diffusion method. These included anticoccus; the standard antibiotics were defined as A-AMP (Ampicillin), AM-Amikacin, AP-Ampicillin, and CF-Cephalothin. Human pathogenic bacteria were kindly provided by the Microbiology Department at hilla hospital. Escherichia coli, Salmonella enterica [12, 13], Pseudomonas aeruginosa, Bacillus cereus, and Staphylococcus aureus. Inhibitory zone diameter (mm) was determined after 18–24 h incubation at 37 °C as of the end of the experiment.

Superoxide radical scavenging

The reduction of NBT was used to evaluate this activity following a previously published method [14, 15]. It has been noted that when exposed to superoxide radicals derived from non-enzymatic PMS/NADH system NBT gets reduced to purple formazan. Different quantities (0-20 µg/mL) of the sample solution. The quantity of formazan formed was quantified by measuring the absorbance at 562 nm against an appropriate blank at the end of 5 minutes of incubation at 37°C. Every test was run six times. Positive control was used in this experiment, with quercetin.

Hydrogen peroxide scavenging

This activity was determined by a somewhat different approach of a method described earlier [13]. For 30 minutes at room temperature, a mixture of 50 mM H2O2 and samples at concentrations ranging from 0 to 2 mg/ml was incubated in a 1:1 v/v ratio. After incubation, the samples/blank were made by adding 90 µl of the H2O2-sample solution and 10 µl of methanol, HPLC grade. In addition, 9 volumes of 4.4 mM BHT in HPLC grade methanol, one volume of 1mM xylenol orange and 2.56 mM ammonium ferrous sulfate in 0.25M H2SO4 was added to the mixture. Subsequently the reaction mixture was allowed to mix well and was kept at room temperature for half an hour. An absorbance reading was taken at 560 nm of the ferric-xylenol orange complex [16, 17].

Hypochlorous acid scavenging

A 10 % (v/v) sodium hypochlorite solution NaOCl prepared earlier was titrated with 0.6 M H2SO4 to pH 6.2 immediately before the experiment in order to produce hypochlorous acid HOCl. Based on the absorbencies at 235 nm using the molar absorptivity of 100 M cm-1. Modifying the experiment slightly, the experimental design of the study corresponded to the method proposed by Aruoma and Halliwell [19]. Scavenging activity was vibrated, through measuring absorbance at 404 nm and recording the decrease in catalase. The preparation of the reaction mixture included one milliliters of 50 millimolar phosphate buffer (pH 6.8), 7.2 microgram of catalase, 8.4 millimolar hydrochloric acid and depending with the concentration of the plant extract ranged from 0 microgram per millile to 100 microgram per milliliter. The absorbance was thereafter read against a suitable blank after allowing the test mixture to stand at 25°C for 20 minutes. Every test was run six times. The one in references was ascorbic acid which is a HOCl quencher [18].

Statistical Analysis

IBM statistical software and Tukey's HSD were used for the analysis of variance (ANOVA) averaging the mean values, included a 95% CI or a 99% CI. The $p < 0.05$ was used to define statistical significance.

Results and Discussion

StatisticalAnalysis

The infrared spectroscopy has to be accepted as a tool for the analysis of herbal and the structure of the compounds present can also be elucidated as well as in the determination of drug concentration.Fourier transform infra-red spectrometry is designated as a physico-chemical analytical technique and in general is one of the most

widely used method of identification of unknown component or chemical group, and the degree of absorption spectra, related to composition and content of chemical The present work encompasses comparative analysis by employing the GC-MS and FT-IR spectroscopy approaches to examine the traceability of the drug by profiling [20-25]. using FT-IR spectroscopy assays to assess the quality of the commercial sample of the herbal drug by fingerprinting. The characterization of the Dry Methanolic extract of the aerial part of Haloxylon salicornicum by FTIR Spectral analysis showed the existence of all the corresponding FTIR peak values to the solid analysis of the compound by the intensity, bond, type of vibration and functional group assignments [Strong, =C–H, Bending, Alkenes][Strong, C-F, Stretch, alkyl halides][Medium, C=C, Stretch, Aromatic][Bending, N-H, Stretch, Amide][Strong, C=O, Stretch, Ester][Strong, C-H, Stretch, Alkane] (Table 1; Figure 1). Among there we have antibacterial and antioxidant phytocompounds. The use of antimicrobials derived from plants is an effective one because such agents are clinically inactive. There is however a need for more researches today on plant based antimicrobials. According to the authors of the study they the main compounds present in the oil included 1,8-cineole, camphor, β-pinene and α-pinene. A characterization of one sample of an essential oil extracted from the Spanish rosemary leaves in according to the chemical compounds [26-29]. The identified major compounds were camphene, and 1, 8 – cineole. Identification of the chemical compounds of the extracted rosemary essential oils from three regions of Iran was done using GC/MS. The oils primarily contained the following compounds: Amongst the sesquiterpenes that were present the following were identified α-pinene, 1, 8-cineole, camphene, camphor, myrcene and broneol the 1, 8-cineole together with other useful compounds. That, hypochlorous acid, hydroxyl, and superoxide the radical scavenging effect are attributes of antioxidants. Hydrophobic crude extract, ethanol fraction, and methanolic extract of *Haloxylon salicornicum*: The activities they make Parts captured on recording dev from above 106.03 ± 5.00, 123.87 ± 5.82, 145.92 ± 6.71 and Ascorbic acid (standard) 219.17 ± 8.00 respectively of Hypochlorous acid radical scavenging, recorded 141.54 ± 4.00, 168.74 ± 3.21, 203.00 ± 5.71 and Mannitol (standard) 551.09 ± 18.36 respectively of Hydroxyl radical scavenging. While recorded 26.60 ± 1.11, 34.00 ± 1.97, 41.97 ± 1.72, 49.00 ± 2.83 and Quercetin (standard) 49.00 ± 2.83 respectively of Superoxide radical scavenging Haloxylon salicornicum secondary metabolites against five harmful bacteria and drugs (amikacin, ampicillin, and cephalosporin): of an antibacterial activity study. Pseudomonas aeruginosa (11.10 ± 0.21), Salmonella enterica (08.39 ± 0.14), Bacillus subtilis (19.00 ± 0.45), Escherichia coli (14.36 ± 0.30) and Staphylococcus aureus (12.00 ± 0.29). Haloxylon salicornicum metabolites was very highly active against Bacillus subtilis (19.00 ± 0.45).

While the terpenes obtained from Cytisus multiflorus, Reichardia tingitana, Filipendula ulmaria and Rumex vesicarius contain antioxidants which would help to recover the free radicals in the solution. They further realised that the ability of the plants to inhibit free radicals and over all antioxidant effects against annihilation is proportional to the phytochemical substances present. This comprises carotenoids, Vitamin C, flavonoids, and phenolic acids are a component of phenolic ingredient. The chemical make-up further showed that the plant does contain non-volatile products known as Tannins, Flavonides and Phenolics. For antibacterial potency we observed that Haloxylon Salicornicum essential oil has negligible effect on bacteria [30-33]. Several Petri dishes were examined to illustrate the inhibition halos around the Haloxylon Salicornicum disks soaked in HE and these varied in width from 9 – 12 mm; The Haloxylon Salicornicum essential oil is effective against these strains as indicated in [34, 35]. Enterococci Faecalis, Salmonella Typhimurium, staphylococcus endiole are resistant but there are no zone of inhibition around the discs and all the bacterial strains namely Listeria monocytogenes, Klebsiella pneumoniae, Bacillus subtilis G+ and Salmonella Typhimurium. Haloxylon salicornicum, is also used as antibacterial agents even in its essential oil but they pose a big problem of drug resistance among bacteria in spite of this. The present study results are in concordance with the study of R14 which established that methanol extract of Haloxylon Salicornicum has exhibited effectiveness against antibacterial sensitive towards Staphylococcus aureus and E.coli. Similarly, work done earlier that stated that these strains were sensitive to the phenolic extract of Haloxylon Salicornicum is in agreement with this conclusion. Nevertheless, these same authors have also discovered that Bacillus subtilis is resistant because it is sensitive to our experimental conditions. We employed pure essential oil which produced other effects to those who employed phenolic extract or plain methanol extract [36-37]. These other extracts include apolar flavonoids which are chemical compounds which synergistically pull off the intended impact.

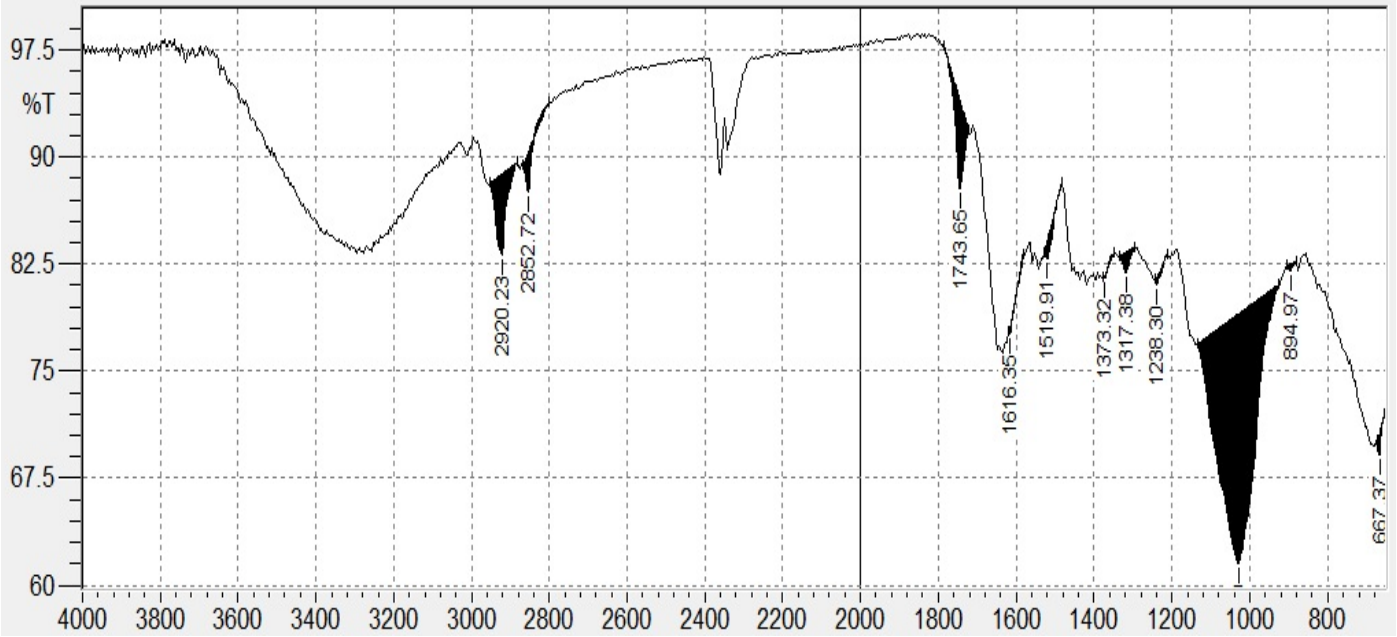

Figure 1. Interpretation of fourier transform infrared spectroscopic profile solid analysis to Haloxylon salicornicum.

Table 1. FT-IR peak values of solid analysis of *Haloxylon salicornicum*.

No.	Peak (Wave number cm-ˡ)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	69.147	1.522	673.16	653.87	2.915	0.063	Strong	=C–H	Bending	Alkenes	650-1000
2.	894.97	82.045	0.457	904.61	881.47	1.958	0.030	Strong	=C–H	Bending	Alkenes	650-1000
3.	1029.99	61.548	17.442	1134.14	925.83	32.156	10.810	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1238.30	81.092	0.518	1242.16	1211.30	2.645	0.042	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1317.38	81.874	1.459	1334.74	1296.16	3.182	0.136	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1373.32	81. 514	0.203	1375.25	1348.24	2.255	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1519.91	82.843	1.227	1527.62	1483.26	3.086	0.127	Medium	C=C	Stretch	Aromatic	1400-1600
8.	1616.35	77.669	0.321	1618.28	1579.70	3.636	0.027	Bending	N-H	Stretch	Amide	1550-1640
9.	1743.65	87.838	6.121	1786.08	1720.50	2.211	0.667	Strong	C=O	Stretch	Ester	1735-1750
10.	2852.72	87.591	2.845	2868.15	2802.57	2.629	0.191	Strong	C-H	Stretch	Alkane	2850-3000
11.	2920.23	83.176	5.651	2951.09	2883.58	4.259	0.812	Strong	C-H	Stretch	Alkane	2850-3000

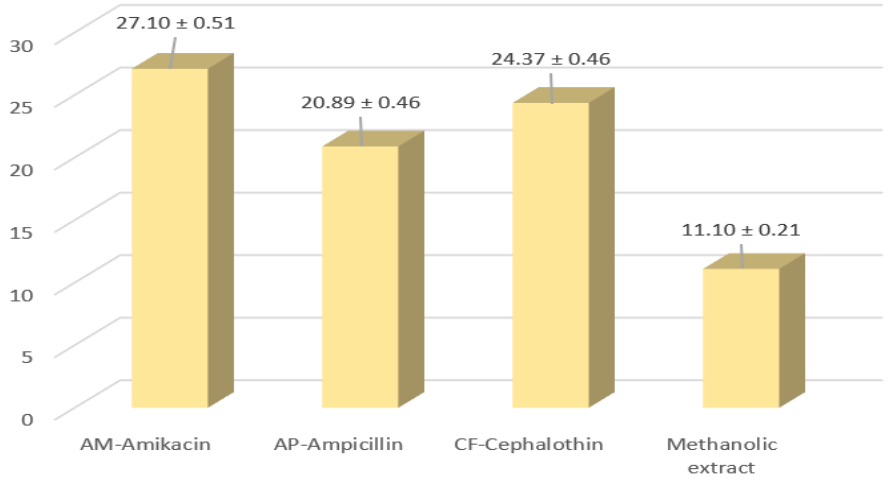

Figure 2. Antibacterial activity of secondary metabolites of *Haloxylon salicornicum* against pathogenic bacteria *Pseudomonas aeruginosa* and antibiotics (AM-Amikacin; AP-Ampicillin; CF-Cephalothin).

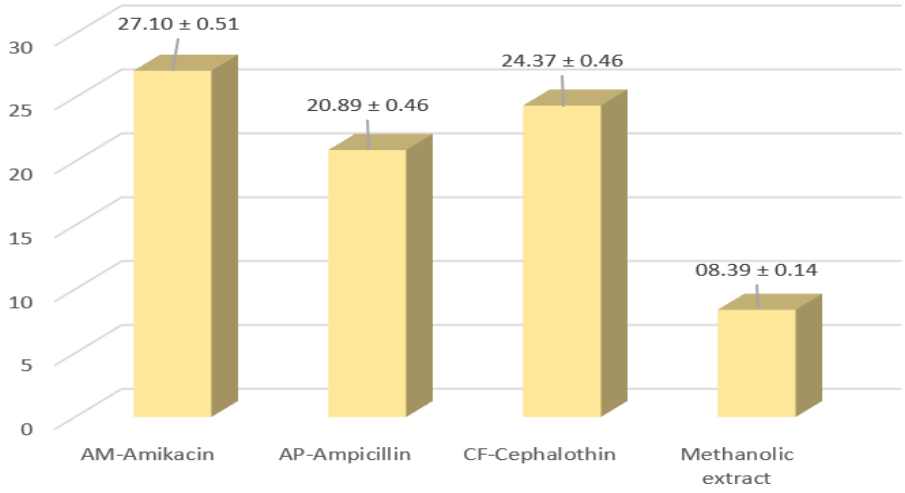

Figure 3. Antibacterial activity of secondary metabolites of *Haloxylon salicornicum* against pathogenic bacteria *Salmonella enterica* and antibiotics (AM-Amikacin; AP-Ampicillin; CF-Cephalothin).

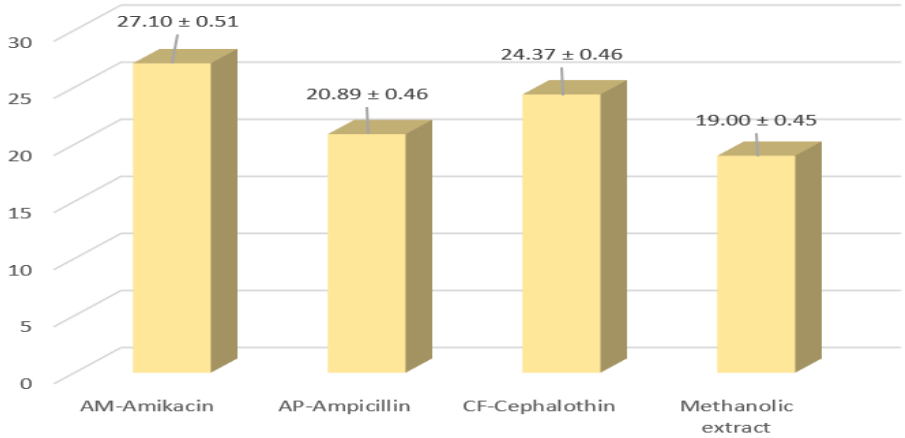

Figure 4. Antibacterial activity of secondary metabolites of *Haloxylon salicornicum* against pathogenic bacteria *Bacillus subtilis* and antibiotics (AM-Amikacin; AP-Ampicillin; CF-Cephalothin).

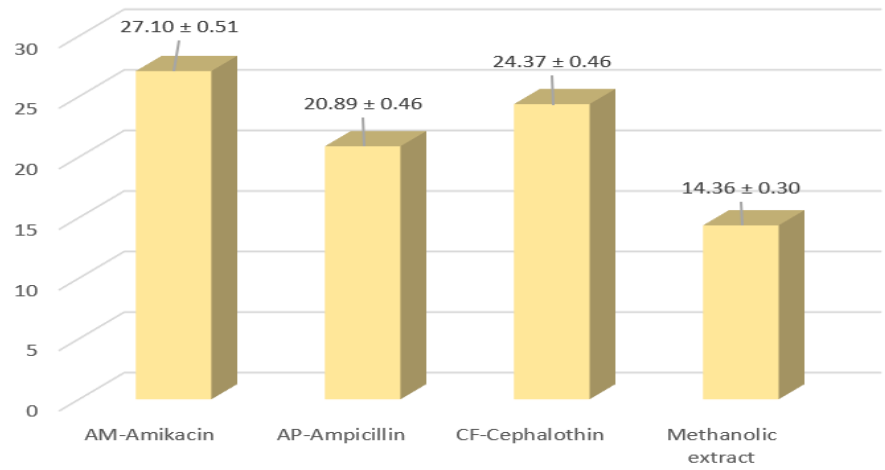

Figure 5. Antibacterial activity of secondary metabolites of *Haloxylon salicornicum* against pathogenic bacteria *Escherichia coli* and antibiotics (AM-Amikacin; AP-Ampicillin; CF-Cephalothin).

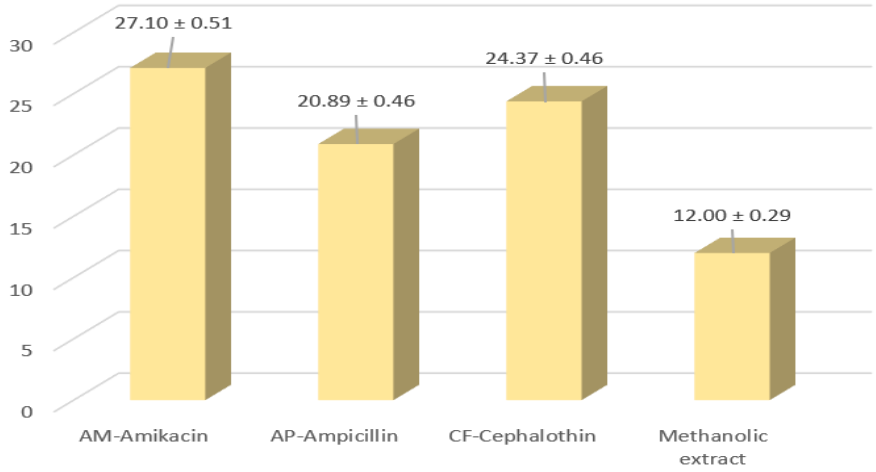

Figure 6. Antibacterial activity of secondary metabolites of *Haloxylon salicornicum* against pathogenic bacteria *Staphylococcus aureus* and antibiotics (AM-Amikacin; AP-Ampicillin; CF-Cephalothin).

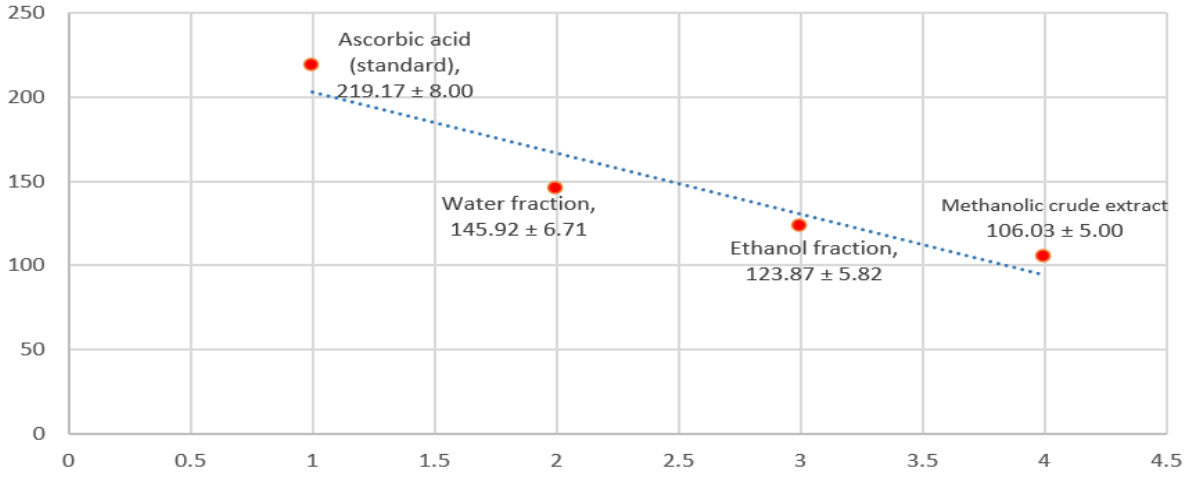

Figure 7. Antioxidant [Hypochlorous acid radical scavenging] activities of *Haloxylon salicornicum* methanol, ethanol, and water fractions aerial-part extract

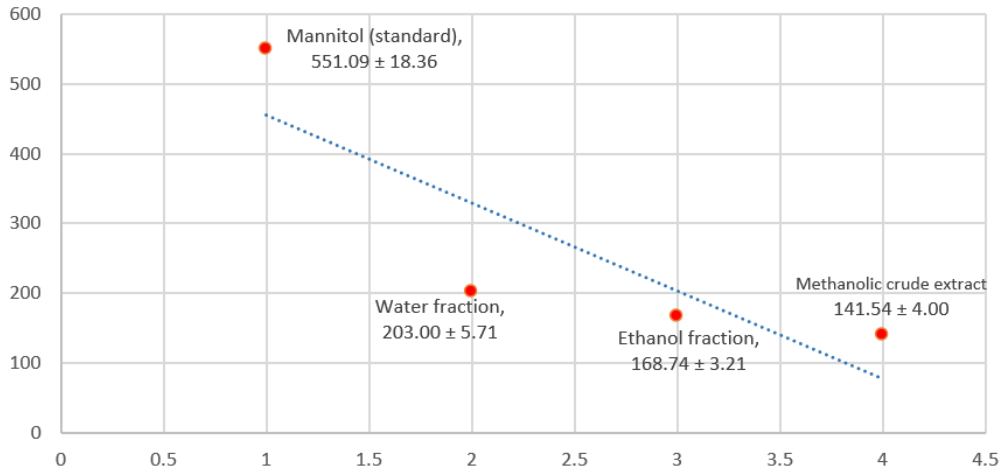

Figure 8. Antioxidant [Hydroxyl radical scavenging] activities of *Haloxylon salicornicum* methanol, ethanol, and water fractions aerial-part extract

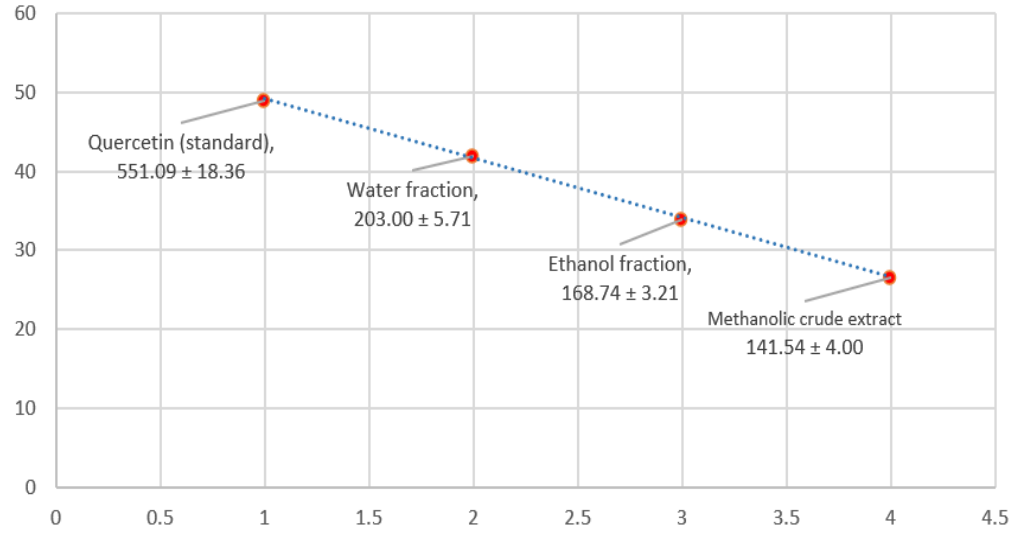

Figure 9. Antioxidant [Superoxide radical scavenging] activities of *Haloxylon salicornicum* methanol, ethanol, and water fractions aerial-part extract

Conclusions

Mean solid sample IR peak values by functional group alkanes C-H alkenes C-C "C-F alky halides C=C extending amine amid A-H bending stretching stiff C=O elastik strong C-H bonds in an alkane ring set. The antioxidant properties of Haloxylon salicornicum were tested in three different fractions: The samples tested were ethanol extract, water extract and methanolic crude extract respectively. The fractions included; Hydroxyl radical scavenging, hypochlorous acid and superoxide radical scavenging fractions. In serious inhibition, the growth of Bacillus subtilis by the metabolites of Haloxylon salicornicum was found to be at 19.00 ± 0.45. Due to the content and possible activity of the bioactives, the H. salicornicum extract should be investigated further to the area of natural products for medicine.

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