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Antioxidant (Nitric oxide radical scavenging and Hypochlorous acid scavenging), Antifungal Activity and Investigation of Secondary Metabolites of Alcea Rosea L. Flowers Using GC-MS Technique

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Abstract:
Starch, gum, and extraordinarily high content of phenols show that Alcea rosea is an important medicinal plant. Thirdly, This plant has been used in ancient times to cure all kinds of ailments and is cultivated for trade. This work aimed at identifying the secondary metabolites present in Alcea rosea L. flowers using the GC-MS analysis and to assess their antioxidant activities as nitric oxide radical scavenging, hypochlorous acid scavenging activities and antifungal activity. At the state-of-the-art Botanical laboratory, the researchers used dried plantingredients that were purchased from the Babylonian markets. They were milled using an electrical grinder until reduced to powder form and then put in nylon bags and left at the laboratory temperature till required. Separation and identification was done using the GC-MS. GC-MS analysis was performed with fused silica capillary column, Trace GC Ultra/ISQ Single Quadruple MS Thermo Scientific. The antifungal activity is assessed using the diameter of the inhibition zone, in millimeters (mm), for each methanolic extract. Different antioxidant activity of Alcea rosea L. extracted in methanol, ethanol and standards using nitric oxide radical scavenging the hypochlorous acid scavenging. Different extract profiles that analysed were Crude, Ethanol fraction and Standard recorded Nitric oxide radical scavenging 33.45 ± 2.07, 49.00 ± 3.05 and Curcumin (standard) 87.60 ± 4.13 respectively. For recorded it was 176.00±5.00, 187.33±7.09 and Ascorbic acid (standard) 215.00 ± 8.93 respectively. The scavenging results demonstrated that crude and other fractions have significantly higher percentage inhibition against hypochlorous acid activity than the standard Mannitol. These results confirm other studies for the identification of Alcea rosea as potential plant source for active compounds for the treatment of some fungal diseases. Antifungal activity of secondary metabolites of Alcea Rosea L. : Effectiveness of the methanolic crude extract, ethanol fraction of Alcea rosea L. and standard antibiotics against four fungi and yeast. In vitro Alcea Rosea L. compounds displayed a high degree of effectiveness against Fusarium oxyporum ((24.08 ± 0.48 and 15.11 ± 0.49).

Keywords: Antioxidant, Radical scavenging, Antifungal Activity, Secondary Metabolites, Alcea Rosea L.

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Introduction

Most of the information on the uses of the medicinal plants is therefore, lip or word of mouth based. The ingredients found in the medicinal plants have either direct active use in treating people or are used as starting materials in the manufacture of still more expensive products that can fetch high returns in the market. This has been becoming more relevant as several times chemically developed treatments resulted to adverse drug reactions. Like all synthetic substances, Allopathic or analytic medicine CMI can be toxic or contain chemicals that can cause relatively severe or adverse side effects, and similarly with medicinal plants. Thus, the aim of present research is to identify the composition of the active plant components of the mentioned medicinal plants and their effect, as well as the unforseen side effects of these individual ingredients, in orderto establish their usage [1, 2]. A-Rose has been used for medicinal purposes since it was discovered 6000 years ago. I have only recently been able to establish that roots, seeds and flowers of plants are rich in phytochemical compounds which exhibit different actions. They are antibacterial agents; immunomodulators; hypnotic-sedative; anti-inflammatory agents; cytostatics, and analgesics, as well as antioxidants. tannins asparagine, coumarins, phytosterols and mucilage, scopolanine, flavonoids, coumarins, phytosterols, tannin, asparagine and specific amino acids. Therefore, the plant Alcea rosea is full of secondary metabolites and can be widely used in medicine and in the field of nutrition. Free radicals lead to inflammation, cancer, heart disease and osteoporosis thus, antioxidant chemicals benefit human health and reduces oxidation by lowering levels of free radicals [3-5]. The antioxidant capability on A. rosea has been described previously. The herbage of A. rosea and its flowers contained higher antioxidant activity in the methanolic extract of the aerial part and flowers than the herbage. Compared to hydroxyl radical scavenging assay, the antioxidant level in the butanol extract is found slightly higher [6, 7]. If compared to other measured fractions, the poorest results were obtained with the chloroform extract in regards to antioxidant activity. By all accounts, hydroalcoholic A. rosea extract decreases tyrosinase activity [8–10]. Variability in the effective antioxidant activity of different varieties is related to quantitative differences in SOC, flavonoids or other compounds having antioxidant activity.

Materials and Methods

Samples Collection

After acquiring dried plant parts from Babylonian marketplaces, they underwent cleaning and foreign substance isolation before being studied in the state-of-the-art Botanical laboratory at the University of Babylon's Faculty of Science. Following their crushing by an electrical grinder, the powder was gathered in nylon bags and stored at room temperature in the laboratory until needed.

Gas chromatography – mass spectrometry analysis (GC-MS)

For separation and identification, one used a GC-MS. The of GC-MS was performed on a GC-MS using fused silica capillary column from Thermo Scientific; model, Trace GC Ultra/ISQ Single Quadruple MS, TG-5MS (30 m × 0.251 mm × 0.1 mm film thickness. Identification of GC-MS was carried out using a 70 eV electron ionization equipment and He gas flow rate at 1 mL/min. With regard to the injection volume, the analysis requires the consideration of the sample volume of 1 µL. For the injector and MS transfer a temperature of 280 ◦C was selected. For the first run of the experiment, the temperature programme opted was 40 ◦C hold for 3 min and heat at 5 ◦C/min to 280 ◦C The percentage relative peak area was used to determine the quantification of all the observed species as advised by reference [13, 14]. By comparing the retention time and the mass spectra of the compounds with the data from the GC-MS system as well as with the spectra in the NIST and Wiley libraries, we made the first identification.

The Antifungal Activity of Phenolic Compound Extracts: A Research Study

The antifungal activity of the methanolic extract has been investigated by using the mixing method with sabouraud dextrose agar. The procedure that followed was placing 0/1mL of stock solution in each of the concentrations into the Petri dish. Once the SDA medium was poured on top of the dishes, the dishes were allowed to polymerize.

Subsequently, a 5 mL disc using a sterile cork borer was utilized to transfer each fungus on the surface of the culture medium. Afterwards the petri dishes are incubated at a temperature of 25°C ± 2 while the other parameters are maintained at the mentioned earlier for seven days [15, 16]. Data obtained from the biochemical test for antifungal activity include the diameter of the inhibition zone in millimeters (mm).

Nitric oxide radical scavenging

At physiological pH, nitrite ions are produced when oxygen, nitrate oxide interacts with an aqueous sodium nitroprusside (SNP) solution using the Griess-Illosvoy assay. These were composed of 3 mL phosphate buffered saline (PBS) at pH 7.4, the test solution in different concentrations (0-70 µg/mL), and 10 mM of SNP. After 150 min of incubation at 25°C 1 ml of the incubated solution was mixed with 1 ml of sulfanilamide 0.33% in 20% glacial acetic acid. The mixture was allowed to stand at room temperature for 5 minutes. Then, 1 mL of napthylethylenediamine dihydrochloride (NED) (0.1 % w/v) was added, the final was incubated at 25°C for 30 minutes [17, 18]. For this measurement, the spectrophotometric reading was taken at 540 nm using blank sample to estimate the quantity of pink chromophore that is formed when nitrite ions is diazotized with sulphanilamide then coupled with NED. Every test was run six times. A standard was curcumin.

Hypochlorous acid scavenging

Hypochlorous acid (HOCl) was prepared just before the experiment, by diluting a 10% NaOCl solution to pH 6.2 with 0.6 M H2SO4. The concentration of HOCl was derived from the absorbance obtained at 235 nm, employing an extinction coefficient of 100 M -1 cm -1. For this test, with slight modification, we followed the same procedure described in the work of Aruoma and Halliwell. The extent of the suppressive effect on the catalase activity was determined from the changes in absorbance at 404 nm. The reaction mixture consisted of 50 mM phosphate buffer at a pH of 6.8, 7.2 µM of catalase, 8.4 mM HOCl and different concentrations of plant extract ranging from 0µg/mL to 100µg/mL in 1 mL of the solution [19]. The absorbance of the resulting coloured complex was obtained by diluting aliquots of the combination and incubating for 20 minutes at 25°C, against a suitable blank. All the tests were carried out six times. Because it is known that ascorbic acid readily reacts with HOCl and has the ability to interrupt its synthesis we decided to use it as a reference.

Statistical Analysis

Statistical software from IBM (New York, NY, USA) and Tukey's test for statistically significant differences (HSD) were utilized to conduct an analysis of variance (ANOVA) on the average mean values, with a confidence interval of 95% or 99%. Statistical significance was determined by a p-value lower than 0.05.

RESULTS and DISCUSSION

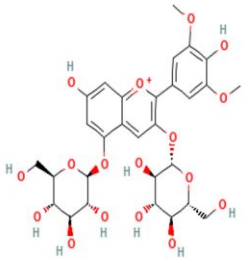
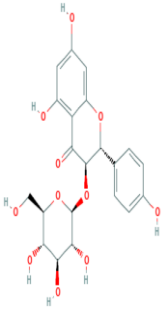
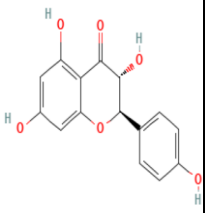
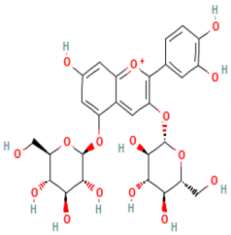
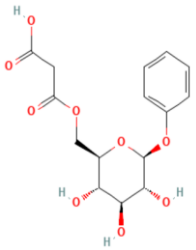
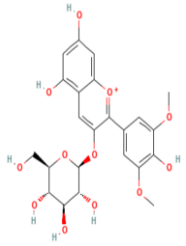
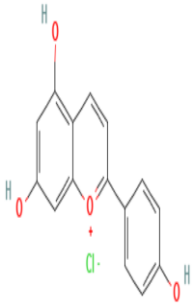
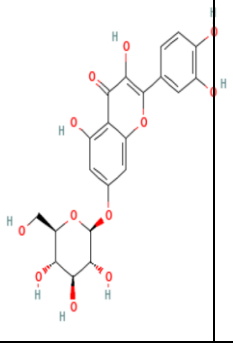
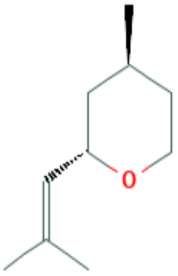
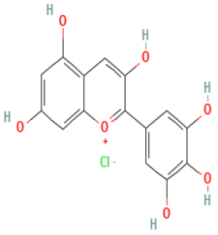
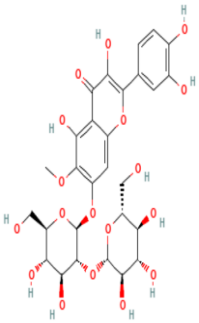
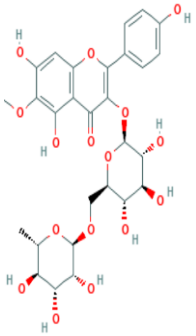
Especially in the regions where people can rarely afford modern pills, traditional medicine has always been the main component of the primary healthcare system's least expensive remedy. Individuals in the poor countries especially those in the developing world have lived cultures that involved the use of herbs as medicine. Increased significance of new technologies and scientific investigation procedures is evident as continues research in actions and medical values in phyto-active compounds of plants [20–23]. Single herbal extracts in the form of plant galenicals are marketed in different pharmaceutical dosage forms and packaging as per the pharmacopoeia.

The nitric oxide radical scavenging and hypochlorous acid scavenging of Alcea rosea L. in methanol, ethanol and standards, antioxidant activity of different standards. The various types of extracts found included crude, ethanol fraction and standard Curcumin extracts at Nitric oxide radical scavenging 33.45 ± 2.07, 49.00 ± 3.05 and Curcumin (standard) 87.60 ± 4.13 respectively. The percentage of Sequestration of hypochlorous acid were recorded 176.00±5.00, 187.33±7.09 and Ascorbic acid (standard) 215.00 ± 8.93 respectively The results are depicted in figure 2 indicating a higher % inhibition of crude and other fractions as compared to standard mannitol ($P<0.05$) against Hypochlorous acid activities. Contribution of nitric oxide in several inflammatory events is known well. This radical is directly toxic to tissues and the production of such a radical leads to the vascular collapse noted in septic shock. In

contrast, inflammatory diseases such as ulcerative colitis, juvenile diabetes, multiple sclerosis, arthritis and carcinomas are associated with chronic nitric oxide radical [24, 25].

Sodium nitroprusside releases nitric oxide, this can be reduced to formulate nitrite, upon exposure to oxygen. Since it outcompets for oxygen in the process of nitric oxide, the extract reduces the production of nitrite. In the present study it was shown that the investigate extract possessed the higher nitric oxide scavenging activity in comparison with the standard curcumin [26-29]. Bioactivity of the methanolic crude extract, ethanol fraction, and conventional antibiotics against four fungi and yeast was assessed in the study of the antifungal activity of secondary metabolites of *Alcea Rosea* L. *Trichophyton rubrum* (16.00 ± 0.49 and 20.74 ± 0.41), *Fusarium oxyporum* (24.08 ± 0.48 and 15.11 ± 0.49), *Cladosporium herbarum* (14.79 ± 0.28 and 19.00 ± 0.36), *Candida albicans* (21.00 ± 0.43 and 18.41 ± 0.35), and standard antibiotics Voriconazole (VCZ) and Amphotericin B (AmB) (23.31 ± 0.48 and 27.09 ± 0.51) respectively. Alcea rosea L. metabolites were exceptionally potent against Fusarium oxyporum (24.08 ± 0.48 and 15.11 ± 0.49). In particular, regarding chemotaxonomic markers with high content of polyprenols, Malvaceae family occupies a prominent position among the many other plant families, particular these with the foliage plants. Besides, the plants contain high cyclopropane acids which have not been identified in plants belonging to any other family. Alcea L. Malvaceae is one among the most acknowledged species in this family; the subspecies belonging to this category blossom into unique flowering plants and the flowers they bear are large and highly tined [30-33]. There are over 70 different species of Alcea all of which are found in the Mediterranean, as well in the Iran-Turanian region. Currently, there are eighteen species Alcea reported in Turkey. It was identified that members of the genus Alcea has numerous uses from medicinal point of view such as antioxidant, hepatoprotecting, antimicrobial and antiviral activity. It is also important to say that no medicinal properties or applications of marijuana discussed in this article are connected to psychoactive effects. This could be so because Alcea has comparatively lower concentrations of the alkaloids and other such chemicals that cause toxicity. Alcea species are also reported in traditional medicine in some way or another.

Alcea species have been analyzed by numerous scientists for botanical purposes, and they also are well documented for their traditional medical applications. However, studies on the chemical composition, as well as pharmacology and usage, of some Alcea species have not been done for others. The present studies explain that A. setosa shows potential as a chemotherapeutic agent for breast and colon cancers because of its specificity for cancer cells and its anticancer activity concerted with antioxidant effects. More, upon phytochemical screening of the alcoholic extract of A. rosea flowers, chemical entity identified as kaempferol-3-O-[6”-(Ecoumaroyl)]-β-d-glucopyranoside. This flavonoid showed potent cytotoxic effect on the HepG-2 cell, with high specificity for hepatocellular carcinoma, in a culture system. Dihydrokaempferol-40 -Oβ-d-glucopyranoside and dihydrokaempferol displayed good antioxidant properties, while kaempferol-3-O-β-d-glucopyranoside sample had fairly good immunostimulating effect. Nonetheless the economic value of the medicinal Alcea rosea cannot be refuted. The usage of synthetic pharmaceuticals which affects consumers’ immunity can be limited by active elements of the medicinal A. rosea and its possible and potential uses are numerous due to its medicinal compounds. Thus, the usage of these plant parts as a medicine for human diseases elevates the value of researches on this subject and should prompt more researches on the other beneficial and unknown aspects of this plant. Due to the possibility of this plant, it has been recommended for use in development of cosmetic-medicinal products. Plant origin natural and synthetic compounds are the most important focus of existing scientific research in the development of new antifungal drugs. This type of plant characterizations has been informed by evidence proposing that extracts derived from different plants display biological activity in vitro and on living organisms within antifungal studies of traditional medicine. This indicates that, leaf extract of Alcea rosea possesses antioxidant properties because it is capable of donating electron to free radicals. Flavonoids are the plant’s major secondary metabolites in majority of the plants besides providing the medicinal plants high pharmacological activity [34]. As a major component, its redox characteristic aspect and scavenging functions give it high antioxidant function. The total phenolic content of Alcea rosea showed relatively high phenolic contents in the following order. Essentially more radical scavenging is likely to be associated with the high phenolic component content in the plant. Since galic acid is fairly mass-equivalent to other phenolic compounds and is in fact a major phenolic compound, it was used as the reference for this test. There is phytochemical content, Flavonoids are also a secondary metabolite of plants and possess the antioxidant activity of plants as an antiradical. The findings that were realized suggest that the plant contains flavonoids and it has anti-radical activity and used in treatment of various diseases.

Malvoside, C29H35O17, 655.6 g/mol 	Dihydrokaempferol 3-glucoside, C21H22O11, 450.4 g/mol 	Aromadendrin, C15H12O6, 288.25 g/mol 	Cyanidin 3-O-glucoside, C27H31O16, 611.5 g/mol 	4-Phenyl-6-O-malonylglucoside, C15H18O9, 342.30 g/mol 	malvidin 3-O-beta-D-glucoside, C23H25O12, 493.4 g/mol 
Apigeninidin, C15H11ClO4, 290.70 g/mol 	Quercetin3-O-β-D-glucuronopyranoside-8-C-β-Dglucopyranoside, C21H20O12, 464.4 g/mol 	trans-(+)-rose oxide, C10H18O, 154.25 g/mol 	Delphinidin, C15H11ClO7, 338.69 g/mol 	Patuletin-7-diglucoside, C28H32O18, 656.5 g/mol 	6-Methoxykaempferol 3-O-rutinoside, C28H32O16, 624.5 g/mol 

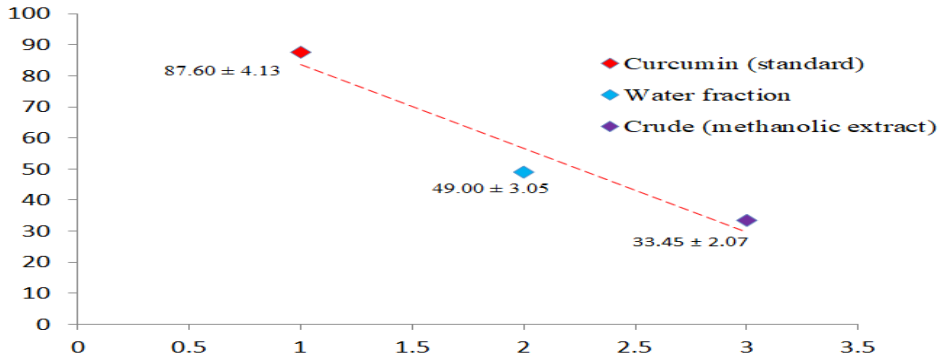


Figure 1. Nitric oxide radical scavenging antioxidant activity of Crude, Water extract and Curcumin (standard) of *Alcea Rosea* L.

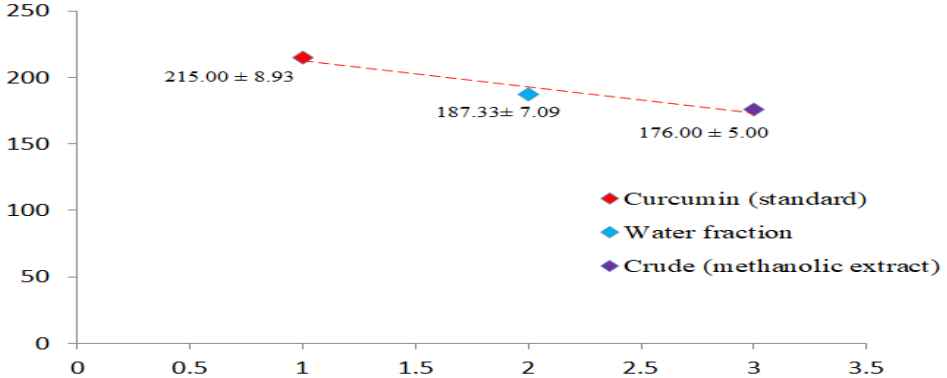


Figure 2. Hypochlorous acid scavenging antioxidant activity of Crude, Water extract and Ascorbic acid (standard) of *Alcea Rosea* L.

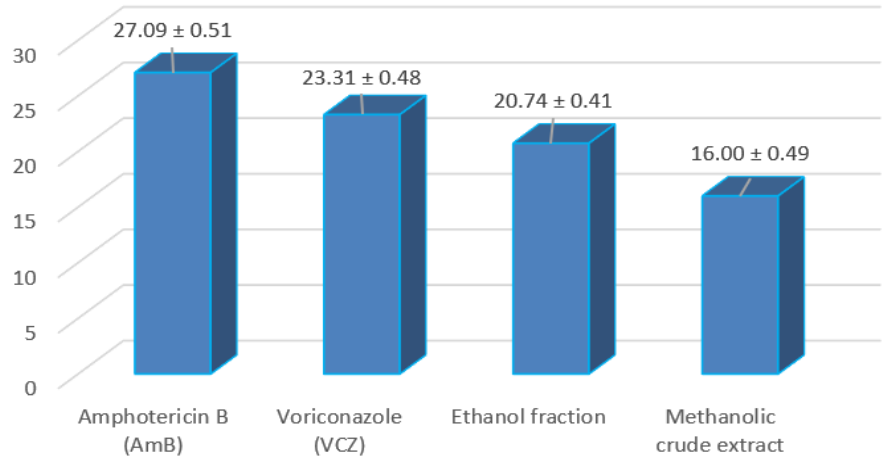


Figure 3. Bioactivity of the methanolic crude extract, ethanol fraction of *Alcea rosea* L. and standard antibiotics as antifungal activity against *Trichophyton rubrum*

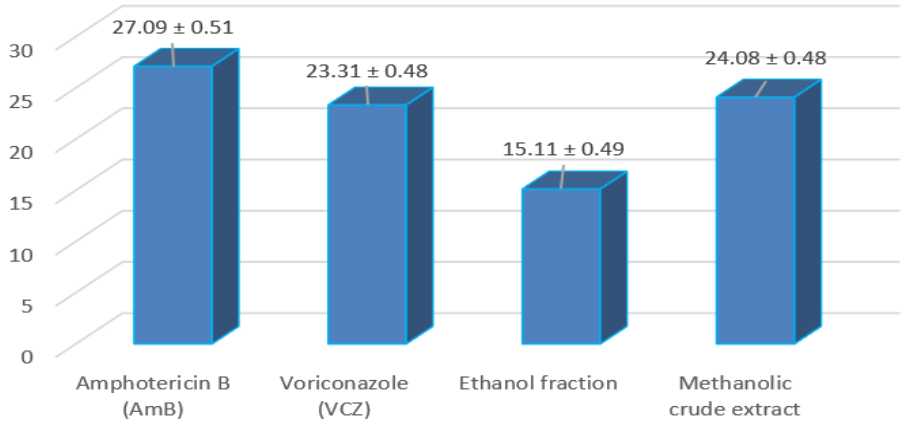


Figure 4. Bioactivity of the methanolic crude extract, ethanol fraction of *Alcea rosea* L. and standard antibiotics as antifungal activity against *Fusarium oxyporum*

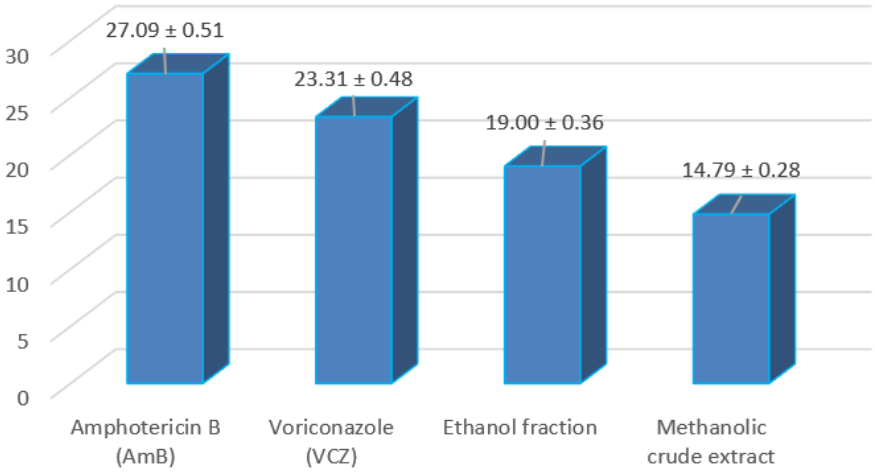


Figure 5. Bioactivity of the methanolic crude extract, ethanol fraction of *Alcea rosea* L. and standard antibiotics as antifungal activity against *Cladosporium herbarum*

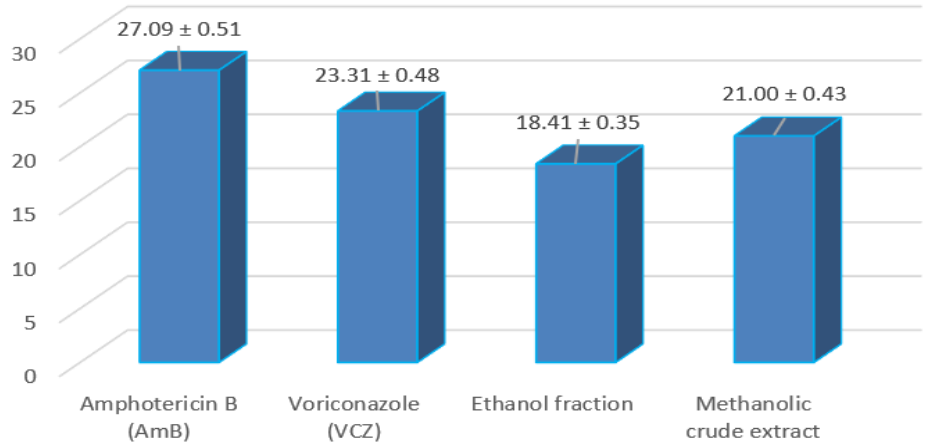


Figure 6. Bioactivity of the methanolic crude extract, ethanol fraction of *Alcea rosea* L. and standard antibiotics as antifungal activity against *Candida albicans*

The search for medicines from natural products is then emerged in medical concern and health issues for many pathogenic fungi which have developed antibiotic resistance. Different researchers have provided evidence that show that the mortality rate among these fungi due to its ability to develop drug-resistance and cross-resistance among the isolated species has increased in the recent decade despite the presence of numerous antifungal medicines. The resistance in fungi especially the common class of Aspergillus and Candida species has changed patterns of susceptibility that denies the traditional drugs their effectiveness and leads to constant fungal infections. The fungitoxic properties can be attributed to the compounds of water-soluble glucosides and resins and anti-enzyme action on the cytoplasmic membrane. This in turn leads to depolarization of the cytoplasmic membrane of microorganisms through effecting on lipids and proteins. Fungi may not be killed by plant extracts because they possess an enzyme that inactivates the active ingredient, because they have a molecule that extracts the active constituents, because the site of the molecule where it is attempting to go is refractive, or the genes governing characteristics of the target and or enzyme features maybe mutated. Further, some of these substances can dissolve in the cytoplasmic membrane and may therefore limit the availability of enzymes which these microbes require for replication. These chemicals are the ones that gives the plant its microbial, fungal, and inflammation reducing properties.

Conclusion:

In conclusion, from the result of this study, it is proper to conclude that Alcea rosea is rich in plant chemicals that have medicinal use which may be employed for managing some of the fungal illnesses. This study revealed that the metabolites of Alcea Rosea L. possesses an exceptionally high antic-Myling activity against Fusarium oxyporum f. sp. oryzae, with activities of 24.08 ± 0.48 and 15.11 ± 0.49, respectively. Perhaps, you will get more information about this plant and its perspective in medicine, agriculture, food industry and other spheres with the help of this information. Our research shows that A. rosea is among the most valuable and widely used medicinal plants. This is because the plant is known to have numerous therapeutic uses in different diseases and in addition to this, the plant is widely used in landscapes by appearance and other beneficial chemical constituents. Consequently, this article contains data that may benefit overall further study and the creation of herbal and natural prodigy remedies. Not only is this therapeutic plant and its secondary metabolites more important than before, but cultivating and producing it is more necessary than before as well.

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