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Original Article

Antioxidant (Singlet Oxygen Scavenging and Hypochlorous Acid Scavenging) and Screening of Bioactive Chemical Compounds of Hollyhock (*Alcea Rosea* L.) by GCMS Method

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Abstract

As a rich source of phytochemicals i.e. flavonoids and phenolics, Alcea rosea (also referred to as Hollyhock) is a potent antioxidant. The high concentration of antioxidants present in Alcea rosea (Hollyhock) can be used to treat coughs, skin complications and possibly to cure disorders such as diabetes, since this plant contains high levels of antioxidants such as phenolic compounds and flavonoids that can be utilized to treat oxidative stress, inflammation and boosting the immune system. Extracts of the plant tested in laboratories were very active and compared to vitamin C in terms of radical scavenging and preventing cell damage. This study identified the following secondary metabolites, 3-Oxabicyclo [3.1.0] hexane-2, 4-dione, 1-Methylcyclohexa-1, 3-diene, Nonadecanedioic acid, hexadecanoic acid, thiosulfuric acid, 6-tetramethylbicyclo- [2.2.2] oct-2-ene, N-acetyl-4- nitrophenylhydroxylamine hydrobromide, benzen Antioxidant activity, that is, scavenging hypochlorous acid and singlet oxygen, of methanolic crude extract and ethanol fraction of the plant hollyhock (Alcea Rosea L.) has been determined. Some of the extracts that were recorded to possess Singlet oxygen scavenging activity included methanolic crude extract, ethanol fraction, and standard (695.00±34.09, 537.83±22.71, and 748.00±26.04 respectively). The ascorbic acid (standard) 501.06±29.14, and the hypochlorous acid scavenging (286.05±25.37) were reported respectively.

Keywords: Antioxidant, Bioactive, Compounds, Alcea Rosea L., GCMS.

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Introduction

The traditional applications have effectively demonstrated that plants contain substances that are medicinal and can be utilized in making some drugs with commercial importance as raw materials. By studying the effects of the biologically active constituents of the medicinal plants, we are able to direct our use of said plants, despite the fact that they might be causing quite adverse side effects as well. The popular name of the plant Alcea rosea is Hollyhock, which has been used as a source of pharmacological purpose by human beings over six thousand years[1]. The plant has been used extensively over a long period with multiple forms of medical use, such as fever, gastrointestinal problems, skin burns, and inflammation. Recent studies indicate that its seeds, roots, flowers, and leaves contain immunomodulatory, antitussive, antioxidant, and antiinflammatory effects. Proteins, polysaccharides, alkaloids, flavonoids, steroids, and saponins have become secondary plant metabolites that have become important due to their various pharmacological properties: hypolipidemia, analgesia, anti-inflammatory, etc. Pharmaceutical antibiotics are manufactured by industries using those drugs as being directed at human beings and animals that belong to the same health group. Nevertheless, following the health concerns of the populace on the resilience and resistance to antimicrobial agents, new ideas on alternative antimicrobial agents will inevitably be created[2,3]. The familiar Staphylococcus aureus bacteria is frequently linked to wound contaminations, other types of infection germs. These bacteria in most cases enter the deepest layer of the wound. Staphylococcus aureus is one such opportunistic human pathogen that is a Gram-positive bacterium and has been attributed to various diseases that can be acquired within medical institutions or even by the common people. Biofilm consist of water, proteins, nucleic acid and polysaccharides; it is an extracellular polymeric substance that physically disallows the medicine to reach the bacterial community enabling the bacterium to survive or at least reduce the effects of antibiotics. Since it is an environmentally friendly and safer option, nowadays the scientific community is paying a lot of attention to creating herbal medicines that can be used as antibacterial ones. The use of medicinal plants to cure different infectious illnesses is one of the significant aspects of traditional medicine [4,5,6]. They are alkoxyl radicals, and superoxide anions which are capable of damaging biological macromolecules. The ultimate result of this is several degenerative diseases like cancer and heart diseases. The importance of antioxidants and oxidation balance in maintaining a healthy biological system is considered to be outstanding [7,8].

Synthetic antioxidants are very harmful and toxic to health hence a general agreement was that it should be abandoned and adopt natural antioxidants. The discovery of new natural sources of active antioxidant molecules has given much emphasis on antioxidant search based on natural sources. Alcea rosea can be utilized in many dietary as well as pharmaceutical research applications due to its abundance of secondary metabolites. Various pharmacological researches have shown the analgesic and bacterial anti-inflammatory characteristics of this herb[9,10]. Ethnopharmacological studies and ethnobotanical studies into the properties of a large number of species of Alcea rosea have shown that it has the potential to treat numerous diseases. There is an increased interest in the correlation between antioxidants and diseases and therefore it becomes important to determine the total antioxidant activity of Alcea rosea. The objective of this study was to establish the number of various Alcea rosea extracts that performed well as regards total protein content, antioxidant, and free radical scavenging properties. It is considered that the presence of antioxidants and the preservation of the balance of oxidants are the key to the maintenance of a healthy biological system. Dietary antioxidants have the potential to protect cell components against oxidative stress and reinforce cell defences[11]. The toxicity and health issues of the synthetic antioxidants had the potential to be toxic and this made a broad consensus to replace them with natural

antioxidants. Nature as a source of antioxidants has received a significant amount of attention and this has questioned the efforts to find new sources of natural active antioxidant molecules. Several pharmacological studies have recorded the anti-inflammatory, antimicrobial, and analgesic effects of this plant. Ethnopharmacological and ethnobotanical studies on most species of Alcea rosea have demonstrated a good outcome in the treatment of many diseases[12,13]. The importance of measuring the total antioxidant activity of Alcea rosea is based on the increasing interest of the relationship between antioxidants and diseases. Total content, antioxidant and free radical scavenging activity of the Alcea rosea extracts were thus evaluated in this study.

Materials and Methods

Collection, identification, and processing of amalcea rosea material

In December 2023, a complete collection of Kia pink papers were collected in the Babylon governorate in Iraq. The sample was then documented and kept at the College of Science, Babylon University to be used later. The leaves were ruffled up after being well sprayed under the sun to cleanse them of all dust. The dried leaves were milled into a coarse powder and reserved to use in future.

Approaches to Extracting

The soaking and digestion procedures were applied at the same time and across each other in producing the A.rosea aqueous extract. The preparation of 10 grams of A.rosea leaf powder was done and after weighing it, it was immersed in 250 ml of water in a beaker and left to soak for one day with a shaker. The extract was filtered with Whatman after heating it to 30 o C in an hour with a magnetic stirrer.

Oxygen Sparing In Singlets

To measure the generation of singlet oxygen (1O2), a spectrophotometric method had been previously reported to measure the bleaching of N, N-dimethyl-4-nitrosoaniline (RNO). An observation of bleaching of RNO was made at 440 nm and NaOCl and H2O2 were reacted to form singlet oxygen. The absorbance of RNO, at 440 nm, was taken after 40 minutes incubation period at 30C in order to establish the decrease. Lipoic acid was used as a reference chemical and used to compare the scavenging activity of the sample. We ran each test six times.

Hypochlorous acid degradation

The hypochlorous acid (HOCl) concentration was determined by the molar extinction coefficient which was 100 M-1 cm-1 through the absorbance at 235 nm. A 10 percent (v/v) solution of sodium hydroxide was titrated to a pH of 6.2 using 0.6 M H 2 SO 4 just before the experiment. The scavenging activity was determined by watching the decrease in catalase absorbance at 404 nm. The reaction mixture was placed in a final volume of 1 ml, containing 50 mM phosphate buffer (pH 6.8), 7.2 0m catalase, 8.4 mM HOCl, and different plant extract (0-100 0g/ml). Measurement of the absorbance was done using an appropriate blank following 20 minutes incubation of the test mixture at 25C. We ran each test six times. The material that was used as a reference was the ascorbic acid that is a good HOCl scavenger.

Statistical Analysis

We analyzed the data with the help of SPSS 19.0 (IBM, New York, NY, USA) and the honestly significant differences (HSD) test to compare the mean values of the average values with a 95% or 99% confidence interval. ANOVA test of variance was done. The p-value of less than 0.05 was used to establish the statistical significance.

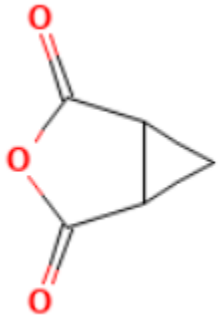
Results and Discussion

The subsequent secondary metabolites were identified in the study: 3-Oxabicyclo[3.1.0]hexane-2,4-dione, 1-Methylcyclohexa-1,3-diene, Nonadecanedioic acid, hexadecanoic acid, thiosulfuric acid, 6-Tetramethylbicyclo -2,2,2-hex-carboxylate, N-Acetyl-4-nitrophenylhydroxylamine
hydrobromide, benzen Alcea roseais was used ethnopharmacologically in a number of diseases. Medicinal herbs are attributed to antioxidant properties. They can either maintain antioxidant defense mechanisms or they scavenge reactive

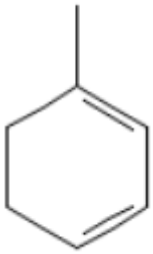
oxygen species, thus acting in this way. In vitro measurements of the antioxidant activity of various extracts of Alcea rosea were determined by using superoxide radical scavenging, total phenolic content, DPPH, and reducing power assays[14,15,16,17]. The transfer of hydrogen is believed to be the reason why antioxidants act on DPPH. Radical scavenging activities are important in a bid to prevent cancer and other illnesses brought by free radicals. The DPPH free radical scavenging assay is the assay most frequently used to determine the antioxidant properties of many plant extracts. In DPPH assay, addition of an extract in violet-colored DPPH solution leads to a reduction of the solution in a yellow-colored product, diphenylpicryl hydrazine, in a concentration-dependent manner. The reason behind this is that the method has gained popularity due to its fast analysis time to predict the antioxidant activity. Our results showed that the methanolic extract of Alcea rosea had a similar free radical scavenging activity as normal BHT [18,19]. Polyphenols and tocopherols prevent radicals of DPPH because of the ability to donate hydrogen. According to the study, the findings of the study suggest that all the Alcea rosea extracts possessed radical scavenging activity, either electron transfer or hydrogen donation. Reducing power is also commonly used to determine the antioxidant activity of plant polyphenols (Abdel-Hameed, 2009).There is a high correlation between the number of total polyphenols and scavenging activity of free radicals by antioxidants. It is a common belief that power reduction occurs when the substances are available since they are antioxidants releasing a hydrogen atom to destroy the radical chains of free radicals. In this test, the reductants of the antioxidant sample convert Fe3+/ferricyanide complex to Fe2+/ferrous one. Consequently, the sample reducing power may be confirmed by the fact that Perl Prussian blue at 700 nm was produced. The reducing capacity of the extracts at 700 nm in methanol and ethyl acetate was found to be 0.09-0.41 and 0.07-0.33 respectively. Their declining power ability of all the extracts was good and concentration-dependent[20,21,22,23] We conclude that the presence of the polyphenols in the extracts was what allowed them to exhibit the antioxidant effects and reduce the power capacity because they are capable of scavenging free radicals by donating an electron or hydrogen.

The antioxidant activity of hollyhock (Alcea Rosea L.) plant in terms of hypochlorous acid and singlet oxygen scavenging of the methanolic crude extract and ethanol fraction sample has been studied. Some of the extracts recorded under the Singlet oxygen scavenging included methanolic crude extract, ethanol fraction, and standard (748.00±26.04, 537.83±22.71 and 695.00±34.09, respectively). It was reported to have ascorbic acid (standard) 501.06±29.14 and hypochlorous acid scavenging (286.05±25.37), respectively. The significance of nitric oxide in several inflammatory events is also familiar. The unremitting production of this radical is directly detrimental to tissues and is associated with the vascular failure evident in septic shock. Conversely, the expression of nitric oxide radical is associated with several malignancies and inflammatory diseases, including ulcerative colitis, juvenile diabetes, multiple sclerosis, arthritis, and juvenile diabetes. The toxicity of NO is increased when it interacts with superoxide radical to give the highly reactive peroxynitrite anion (ONOO-). The oxygen is reacted with the singlet oxygen produced by sodium nitroprusside and forms nitrite. The extract inhibits formation of nitrite by competing better than oxygen in the singlet oxygen reaction. This study demonstrated that the extract of interest had a significantly greater ability regarding singlet oxygen scavenging capacity than conventional curcumin [24,25].

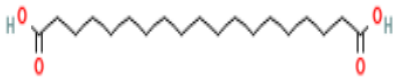
3-Oxabicyclo[3.1.0]hexane-2,4-dione
MF: C5H4O3
MW: 112.08 g/mol



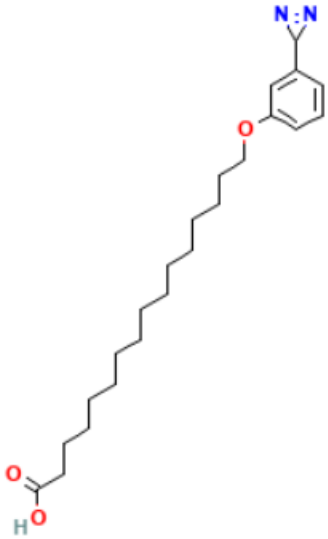
1-Methylcyclohexa-1,3-diene
MF: C7H10
MW: 94.15 g/mol



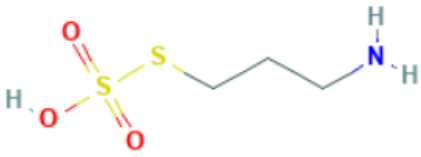
Nonadecanedioic acid
MF: C19H36O4
MW: 328.5 g/mol



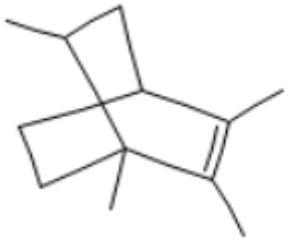
Hexadecanoic acid
MF: C23H36N2O3
MW: 388.5 g/mol



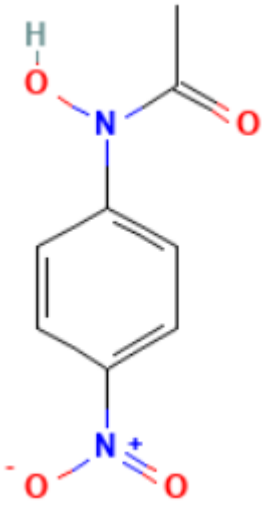
Thiosulfuric acid
MF: C3H9NO3S2
MW: 171.2 g/mol



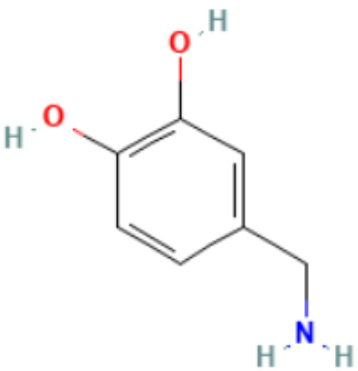
6-Tetramethylbicyclo-[2.2.2]oct-2-ene
MF: C12H20
MW: 164.29 g/mol



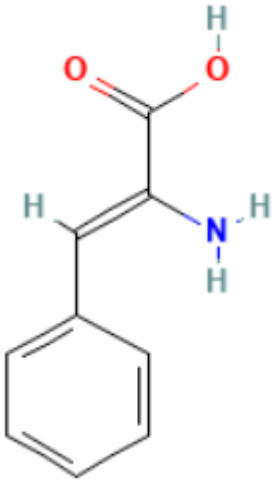
N-Acetyl-4-nitrophenylhydroxylamine
MF: C8H8N2O4
MW: 196.16 g/mol



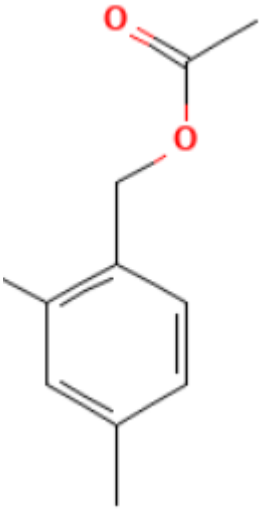
3,4-Dihydroxybenzylamine hydrobromide
MF: C7H10BrNO2
MW: 220.06 g/mol



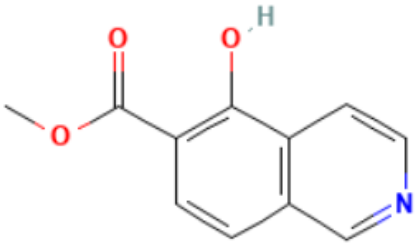
alpha-Aminocinnamate
MF: C9H9NO2
MW: 163.17 g/mol



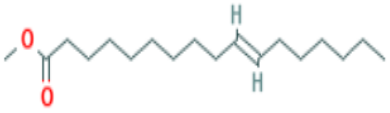
Benzenemethanol, 2,4-dimethyl
MF: C11H14O2
MW: 178.23 g/mol



methyl 5-hydroxyisoquinoline-6-carboxylate
MF: C11H9NO3
MW: 203.19 g/mol



10-Heptadecenoic acid, methyl ester
MF: C18H34O2
MW: 282.5 g/mol



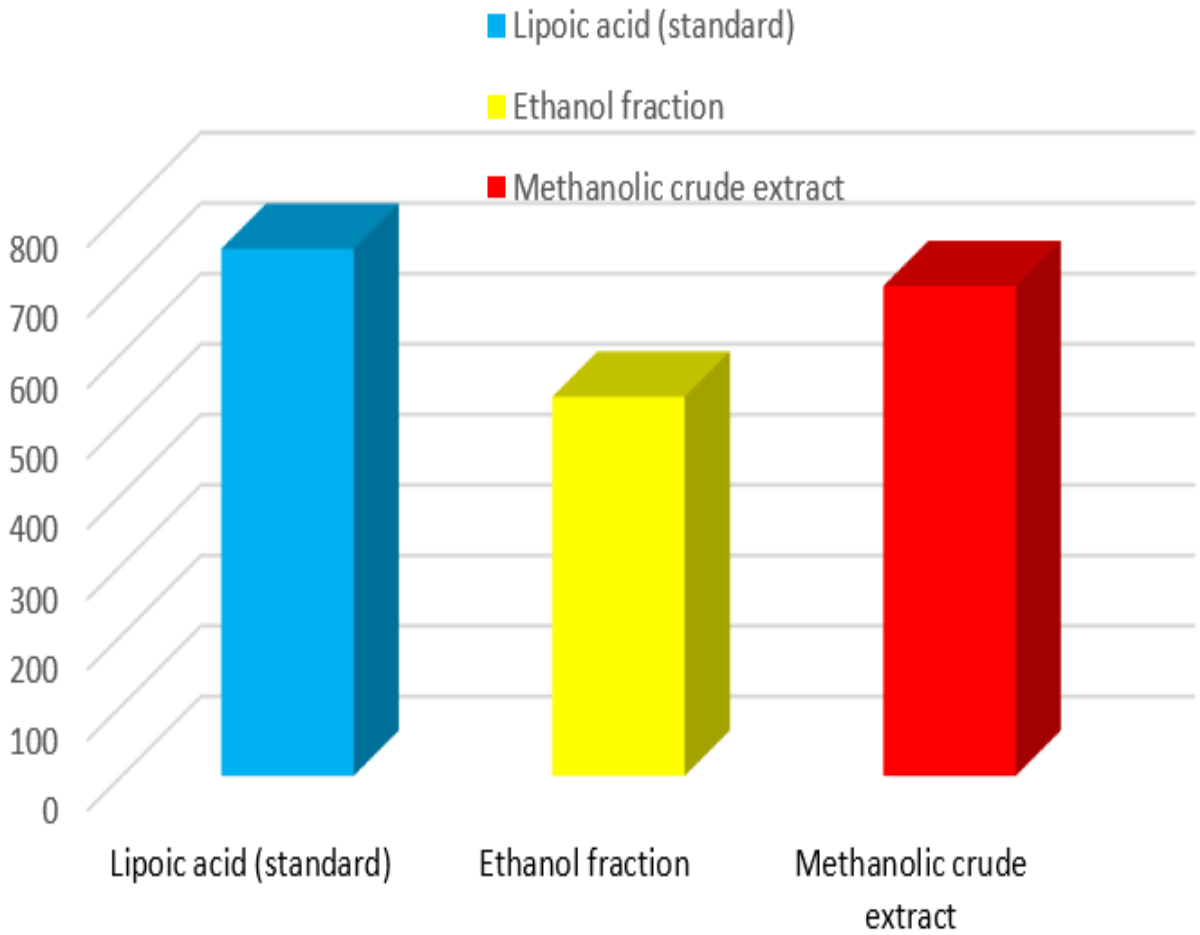


Figure 1. Antioxidant activity (Hypochlorous acid scavenging) of extract (methanolic crude extract and ethanol fraction) of Hollyhock (*Alcea Rosea* L.).

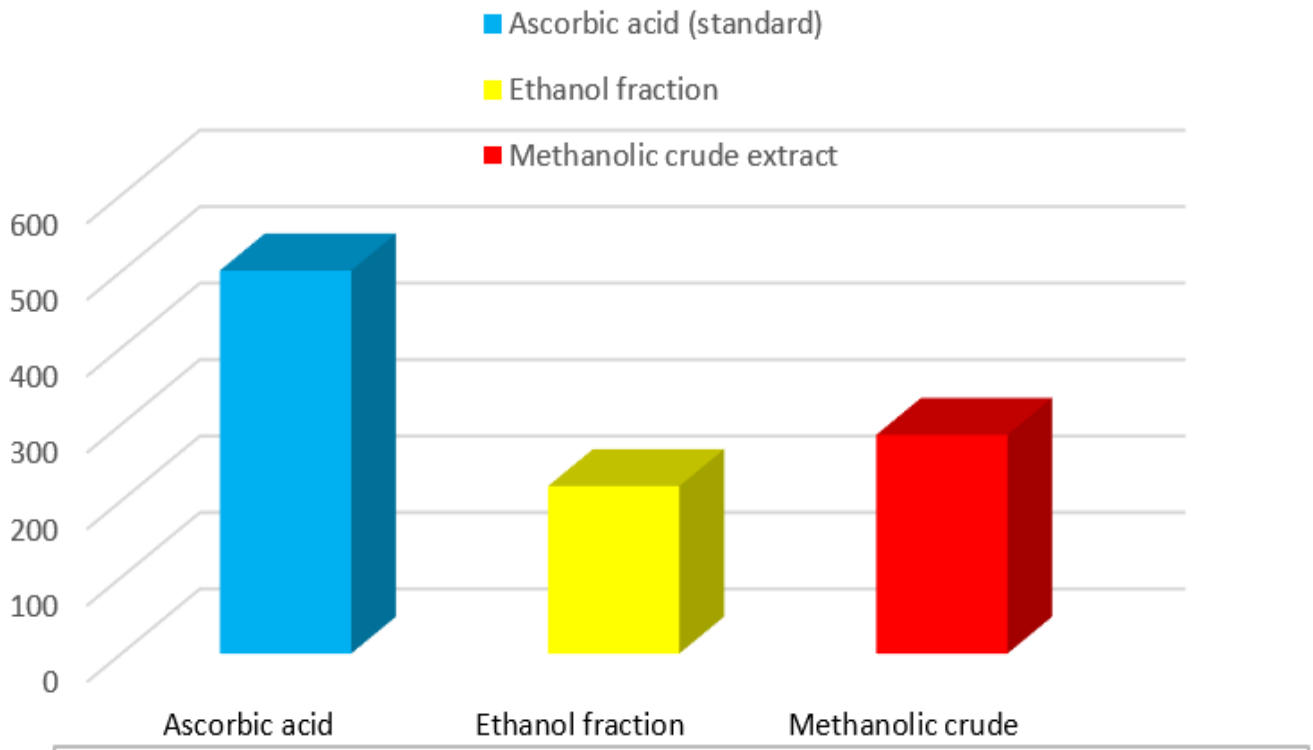


Figure 2. Antioxidant activity (Singlet oxygen scavenging) of extract (methanolic crude extract and ethanol fraction) of Hollyhock (Alcea Rosea L.).

The study of the biological properties of A. rosea indicated good potentials such as antioxidant, analgesic, and anti-inflammatory properties. These actions were due to the numerous chemically active chemicals of the plant. The chemical analysis identified the plant to be having tannins, saponins, flavonoids, terpenoids, and cardiac glycosides. The data provided by these compounds indicate that the plant would be viable in the biomedical work; moreover, the analysis of the GCMS findings supported the findings of the previous studies on the topic. The usage of herbs in traditional medicine does not have a new history and their capability to accelerate the healing process following surgery as well as enhance the skin activity is paramount. The repeated interactions between cells and matrix pass through a consecutive sequence of interconnected steps and processes accelerating the healing process. These are inflammation, contractions of the wound, re-epithelialization, tissue remodeling, and formation of angiogenic granulation tissue. Immunosuppression and contact with various microbes causes wound infection which is one of the greatest clinical barriers to wound healing. S. aureus is among the key factors that lead to delays in wound healing, but other bacteria may also result in wound infections[26]. Infection of acute wounds is one of the most common causes of poor wound healing. Topical antimicrobials as a part of wound care are, therefore, a crucial element. Besides reducing the inflammation and tissue destruction of the locality, herbal remedies used in wound healing have the prospect of direct killing of bacteria making them a perfect element in the prevention and management of wound infections. According to the previous studies, egg albumin denaturation assay is a handy instrument to identify whether a medicine or a chemical has anti-inflammatory effects or not in relation to its capacity to inhibit or reduce the egg albumin denaturation. Such findings indicate that Alcea rosea extract has the potential of being used as an antioxidant and as a possible cure to various clinical conditions that are related to oxidative stress due to the ability to scavenge free radicals. Ethnopharmacology uses alcea rosea to treat many types of diseases[27]. The antioxidant properties of medicinal plants tend to boost their healing qualities. Antioxidants shield

us against a number of diseases by counteracting the free radicals.

Conclusion

They have discovered that A. rosea harbours secondary metabolites with high antioxidant functions. We therefore encourage further studies of the plant components to establish its molecular mechanism of wound healing ability and the mechanism that mediates its effects and to establish, identify and purify such components. The meteoric popularity of the antioxidants has been aided by their positive effects on human health despite the presence of other products that embody the same benefits. This is coupled with preserving various bio-elements in the human body against free radical attack, which may cause oxidative stress-related illnesses. We discovered that Alcea rosea possesses antioxidant characteristics hence can be employed in pharmaceuticals, as a natural cure to diseases due to free radical reactions, and as a free radical scavenger. This study can therefore be used as a reference point by future studies in the field of biology.

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