EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

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ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.14 (1) pp.247- 257 (2022)*
DOI: 10.21608/EAJBSG.2022.283126

Egypt. Acad. J. Biolog. Sci., 14(1):247-257(2022)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Screening of Bio-Active Components of Egyptian *Aloe vera* Herb as Antimicrobial and Antioxidant Agents

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ARTICLE INFO
Article History
Received: 24/4/2022
Accepted:18/6//2022
Available:22/6/2022

***Keywords*:**
Antimicrobial; Aloe vera; herb; carcinogenicity; antioxidant.

ABSTRACT

Background: Antibiotic overdose is a major concern in the emergence and diversity of multidrug-resistant strains of several groups of microorganisms.**Purpose of the survey:**Screening of the bioactive constituents of the Egyptian *Aloe vera* herb as antibacterial and antioxidant agents. **Methodology:**A Botanical Screen of Bio-active Components Showed a promising Therapeutic Value of *Aloe Vera* as an Antibacterial and Antioxidant, Using In Vitro Broth and Disk Agar Diffusion Methods to Determine Antibacterial and Antifungal Efficacy. A collection of 50 herbal *Aloe vera* specimens from different grassland soil environments in Egypt was performed. Extraction of the gel leaf was finished after drying with methanol or ethanol solvent. Fractional separation of the active components of the alcoholic extract was achieved using silica gel thin-layer chromatography; then analysis of the active components was performed by mass spectrometry. Furthermore, the detection of the bioactive components responsible for the antibacterial and antifungal effects was performed by in vitro broth and disk agar diffusion methods to determine the minimum inhibitory concentration. The resulting antiviral activity was determined by detecting viral nucleic acids in a specimen obtained from human patients using PCR to exploit the inhibition of viral replication and cytopathic effect. Antioxidant activity was assessed by DPPH assay.**Result:** Twenty-one components were characterized in *Aloe vera* gel leaf extract, emodin as the major anthraquinone (26.17%) and the major component was an anthraquinone derivative (34.86%). These compounds exhibited excellent DPPH scavenging ability at an extract concentration that provided 50% inhibition (IC50) of 24.109 mcg/mL, and potent beta-carotene bleaching inhibition with an IC50 of 35.48 mcg/mL after 94 minutes of incubation. The zones of inhibition ranged from 22.7 to 25.14 mm in diameter. *Aloe vera's* antibacterial and antifungal activity was due to anthraquinone derivatives in our study, and emodin was the anthraquinone primarily responsible for antimicrobial activity against various pathogens.**Conclusion:** The current study was promising due to its ability to overcome the challenges of multi-resistant strains of multiple groups of microbes and provide alternative herbal antibacterial and anti-carcinogenic antioxidant bioactive components of A*loe vera*.

INTRODUCTION

Owing to the extended microbial resistance undertaking globally, It has become important to investigate novel origins of natural germicides. As nicely because of the presence of numerous cancers owning extra oxidative DNA harm levels, currently, the want for exploration of recent natural antioxidants that obstruct carcinogenicity and extend the shelf life ascertained important matter. The primary cornerstone for coping with microbial (bacterial and fungal) infections is stipulated with antimicrobial medications (Parveen Kumar, 2017). It became believed that the healing fraternity might be proof of the final obliteration of infectious diseases, for the reason that uncovering of antimicrobial drug treatments and their employment as chemotherapeutic factors (Caroline S, Zeind Michael G, 2018). However; overuse of antibiotics has to end up the number one thing for the emergence and dissemination of multi-drug resistant lines of numerous corporations of microorganisms (Trevor Anthony, Katzung Bertram, Kruidering-Hall Marieke, 2021). Plant screening found promising healing values in *Aloe vera as* anti-microbial agent (Bardal Stan, Waechter Jason, Martin Douglas, 2020).

Four chief mechanisms intercede bacterial resistance to antimicrobial medicines (Olson James, 2020). (I) Bacteria create enzymes that deactivate the drug such as betalactamases can deactivate penicillins and cephalosporins via way of means of cleaving the betalactam ring of the drug (Levinson Warren, 2021). (II) Bacteria synthesize changed objectives in opposition to which the drug has no influence (Swanson Larry N, Souney Paul F, Muntnick Alan H, Shargel Leon, 2019) such as a mutant protein with-inside the 30S ribosomal subunit can bring about resistance to streptomycin, (Fisher Bruce, Champe pamela, Harvey Richard, 2021) and a methylated 23S rRNA can bring about resistance to erythromycin (Dipro Cecily, Schwinghammer Terry, Dipro Joseph, Well Barbara, 2021). (III) Bacteria lower their permeability such that a powerful intracellular awareness of the drug isn't achieved (Golderg Stephen, 2020) such as adjustments in porins can lessen the quantity of penicillin coming into the bacterium (Wilson Golder N., 2019). (IV) Bacteria actively export medicine via the employment of a multi-drug resistance pump (Metting Patricia J, 2019). The multidrug resistance pump imports protons and, in an exchange-kind reaction, exports a lot of overseas molecules such as quinolones antibiotics(Safia Arbab *et al.*, 2021). In the present study, we aimed toward looking for a new natural *Aloe vera* herb augmenting antimicrobial and antioxidant activities to defend in opposition to distinctive deadly infections and to avert carcinogenicity thru minimizing DNA harm via way of means of dangerous oxidizing agents consisting of gamma radiation and others.

MATERIALS AND METHODS

Chemicals:

All chemical materials used were of analytical reagent grade. All reagents were purchased from Algomhoria pharmaceutical company in Cairo, Egypt and Alnasr pharmaceutical company in Qalyobia, Egypt.

Place and the Date of The Study:

The present study was carried out in the faculty of pharmacy, at Cairo university between January 2021 and November 2021.

Type of study:

Screening pharmaceutical experimental study.

Equipment:

Table 1. List of instruments.

Instrument	Model and manufacturer
Autoclaves	Tomy, Japan
Aerobic incubator	Sanyo, Japan
Digital balance	Mettler Toledo, Switzerland
Oven	Binder, Germany
Deep freezer -80	Artiko
Refrigerator 5	whirlpool
PH meter electrode	Mettler-toledo, UK
Deep freezer -20	whirlpool
Gyratory shaker	Corning gyratory shaker, Japan
190-1100nm Ultraviolet-visible spectrophotometer	UV1600PC, China
Light(optical) microscope	Amscope 120X-1200X,China

Methodology:

A collection of 50 herbal plant *Aloe vera* samples from different grassland soil

environments in Egypt was carried out. Extraction of gel leaf after drying by methanol or ethanol solvent. Fractional separation of active components of alcoholic extract exploiting silica gel thin layer chromatography; then analysis of active components by mass spectrometer was performed (Marzanna Hes *et al.,* 2019). Furthermore, the detection of bioactive components responsible for anti-microbial effect victimizing in vitro broth and disc agar diffusion methods for determination of antibacterial and anti-fungal effects and utilizing inhibition of viral replication and cytopathic effect for determination of antiviral activities was achieved:

I- Determination of Minimum Inhibitory Concentration MIC:

Broth Dilution Method:A known concentration of the tested agent was distributed by 2-fold serial dilution in wasserman tubes containing double-strength nutrient broth. The tubes were inoculated with a fixed volume of the test organism about 10^6cfu/ml and then incubated at a suitable temp for a proper time. After incubation MIC was determined visually, where MIC is the lowest concentration Of antimicrobial agent (AA) that inhibits microbial growth.

Agar Dilution Method: Test microbes were: *Eschreshia coli, Klebsella pneumonae,Pseudomonas aeruginosa, Haemophilus influenza, Staphyllococcus aurus, Streptococcus pyogenes, Candida albicans, Aspergillus niger.*

Serial dilution of the test agent was prepared in molten agar and then poured into sterile plates. The plate surface was inoculated with broth culture containing 10^5 cfu/ml of the test organism. Growth inhibition was recorded after incubation MIC was the lowest concentration of AA that inhibited microbial growth of each m.o.

Advantages: Many m.o. can be tested at once in the same plate i.e this method is useful for determining the spectrum of AA. Used for evaluation of turbid agents. Resistant cells can be easily detected.

Disadvantages: No one culture medium can support the growth of all types of bacteria. Tested m.o. may show differences in their growth rate or incubation temp.

II-Agar Diffusion Methods:

These methods depend on the diffusion of AA into the agar. They include:

D**itch Plate Technique:** This method allowed a single agent or preparation to be tested against a range of microorganisms. So it was utilized for screening of Antimicrobial activity of new agents especially those presented in semisolid dosage forms e.g. ointments and creams. The test agent was filled in a ditch cut in a nutrient agar plate. Various test organisms are streaked on the surface of nutrient agar. After incubation at a suitable temp and for a suitable time, the length of growth inhibition was measured.

***sensitive microorganisms were grown far from the ditch, while resistant ones were grown near the ditch. The extent of inhibition was a reflection of the activity of the test agent.

C**up-Plate Technique:**The agar is inoculated with the m.o.The test agents are filled in cups.

After incubation at suitable temp and time, zones of inhibition were measured which represented the extent of activity of the tested agents.

Gradient Plate Technique: The technique was characterized by exploiting a double agar layer:

The first layer of plain agar containing AA was first poured and allowed to harden in a sloping manner. The second layer of nutrient agar was poured over the first layer to give a homogeneous layer.

The test microorganisms were then streaked over the agar surface in a direction running from the highest to the lowest concentration of the test agent. At incubation of the plate, AA diffused through the agar giving a gradient concentration leading to growth inhibition of microorganisms.

MIC could be determined according to the following equation:-

MIC=CY/X

C=concentration of antimicrobial agent mg/ml
Y=total length of actual growth in cm
X=total length of streak in cm.

Determination of Synergism and Antagonism:

Put-upon to determine the result of a combination of 2 antimicrobial agents filter paper strips impregnated with AA were placed at right angles to each other on the surface of inoculated agar. After incubation, the extent of growth inhibition was recorded. Synergism was observed as an inhibition band at the intersection.

Antagonism was observed as a growth band at the intersection.

Viral Nucleic Acids Determination:

With complementary RNA or DNA like a probe, viral mRNA or the viral genome could be sensed in the patient's human tissues or blood. If solely tiny viral nucleic acid quantities were existing in the long-suffering, the chain reaction of polymerase could be victimized to magnify the viral nucleic acids. *Herpes viruses* (HSV1, HSV2 and cytomegalovirus), Influenza virus, Human immunodeficiency virus and corona viruses such as SARS-COV2 were utilized as the screening test viruses for the in vitro assessment of antiviral activity.

Assessment of antimicrobial consequences of bio-active components of *Aloe vera* herb collected from different locations in Egypt was performed via a comparison with standard antimicrobial drugs such as ciprofloxacin, cephradine antibiotics, fluconazole, amphotericine anti-fungal drugs, acyclovir and zanamivir antiviral drugs via the previously mentioned techniques. Moreover, formulation of the main antimicrobial bio-active components of *Aloe vera* as an emulsion for the treatment of different topical and superficial infections was carried out.

Estimation of the Antioxidant Activity of *Aloe vera*:

This was determined according to the beta-carotene bleaching assay and by 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

Antioxidant Testing Assays: They were done through either:

DPPH Radical Scavenging Activity: Using DPPH radical as a reagent, the Radical scavenging activity of the different fractions was determined. Briefly, 600 µl of sample solutions (different concentrations) was blended with 2 ml of a 5% (w/v) DPPH radical solution in ethyl alcohol. For 30 minutes in the dark at room temperature, The inter-mixture was incubated. Scavenging capacity was interpreted using UV spectrophotometer by observing the drop-off of the optical density at 517 NM. Higher free radical scavenging activity was indicated by Lower absorbance of the mixture of reaction. Ascorbic acid was utilized as a standardized antioxidant. According to the formula: DPPH radical scavenging activity (%) = $[(OD_{blank} - OD_{sample})/ OD_{blank}] \times 100$, DPPH radical scavenging action was measured. OD_{blank} was the optical density of the control reaction bearing all reagents. OD_{sample} was the optical density of the tested compound. Extract concentration rendering fifty percent inhibition (IC50) was measured from the graph plotting extract concentration against inhibition percentage. Tests were performed in triplicates.

β-Carotene Bleaching Assay: The antioxidant action was dictated accordant to the β-carotene bleaching assay. We prepared a stock solution of linoleic/ β-carotene acid inter-mixture as postdate: 0.6 mg of β-carotene was liquefied in 1.1 ml of chloroform and 190 mg of Tween-20 with 26 µl of linoleic acid. Chloroform was wholly evaporated, utilizing an evaporator of vacuum. And so, we saturated 100 ml of distilled water with oxygen and the acquired solution was smartly agitated. 5 ml of that reaction inter-mixture was distributed into test tubes and 199 µl of each sample, processed at various concentrations, was added. The emulsion system was incubated for 1 h at 45 °C. We repeated the identical procedure with a blank as a negative control and Butylated hydoxytoluene (BHT) as a positive control. Later this incubation period, the optical density of each mixture was calculated at 480

nm. The activity of antioxidants in β-carotene bleaching form in percentage (A %) was measured with the succeeding equation: A % = 1 − (A0 – At/A' 0−A' t) × 100, where A0 and A'0 were optical densities of the blank and the sample, respectively, calculated at zero time, and A t and A't were optical densities of the sample and the blank, respectively, measured after 1 h. Every test was performed in triplicates.

The in-situ effect of *Aloe vera:*

Bioactive components of *Aloe vera* were assessed for preservative and prevention of carcinogenicity activities through physicochemical parameters (PH and thiobarbituric acid reactive substance (TBARS); as well against *Listeria monocytogenes* in minced beef meat model (Prakash P Athiban *et al.*, 2012).

Formulation of *Aloe vera* as Topical Oil in Water(O/W) Emulsions:

Medication Order: Leaf extract of *Aloe vera* 20 ml; Acacia q.s.; Distilled water, q.s. a.d. 90 ml.and Sig: 1 table spoon q.d.

Manufacturing Procedure: An initial emulsion (primary emulsion) was settled with the dry gum method, utilizing one part (4.5 g) of powdered acacia, two parts (7 ml) of water, and four parts (20 ml) of oil extract from the leaf. In a Wedgwood mortar, the acacia was emulsified with mineral oil.

The 7 ml of water was added all at one time and, with fast broiement, settled the primary emulsion, which for about 5 mins was triturated. The leftover water was merged in small amounts with broiement. To a 90-mL prescription bottle, the emulsion was transferred and to the container, a "shake well" label was affiliated.

DSC and FTIR spectroscopy were exploited in the drug and the polymer compatibility examination.

Compatibility study:

We characterized *Aloe vera* extract gel leaf and different excipients utilized in the preparation of emulsion formulations by FT-IR (Perkin-Elmer 1600 FTIR spectrophotometer) spectroscopy and DSC (Shimadzu-DSC 50) to see the compatibility. The optimized formulation was blended with 200 mg KBr; then comressed into discs which were scanned at 5mm/sec with a resolution of 1 cm^{-1} at a range of 4000-200 cm^{-1}. Experiments of thermal analysis were carried out utilizing various scanning calorimeter (DSC). We heated the samples of the optimized formulation in hermetically sealed Aluminium pans at a temperature range 0-4000 ^{0}C at a constant rate of 110 ^{0}C/minute under a purge of nitrogen (35 ml/min).

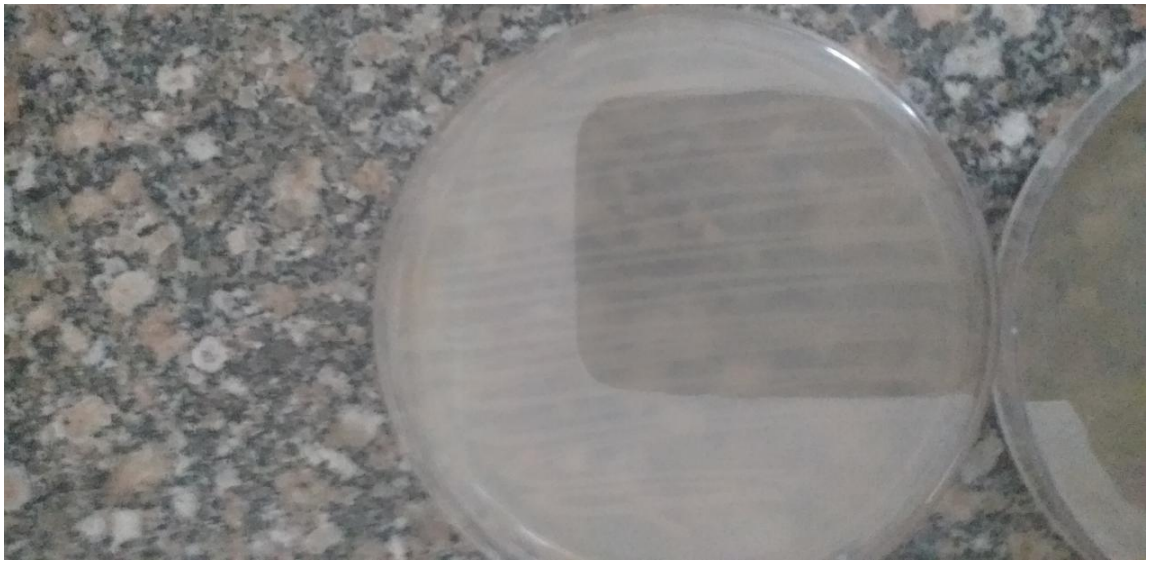


Fig. 1. It shows the antibacterial activity of Egyptian *Aloe vera* against S*almonella typhi and paratyphi*.

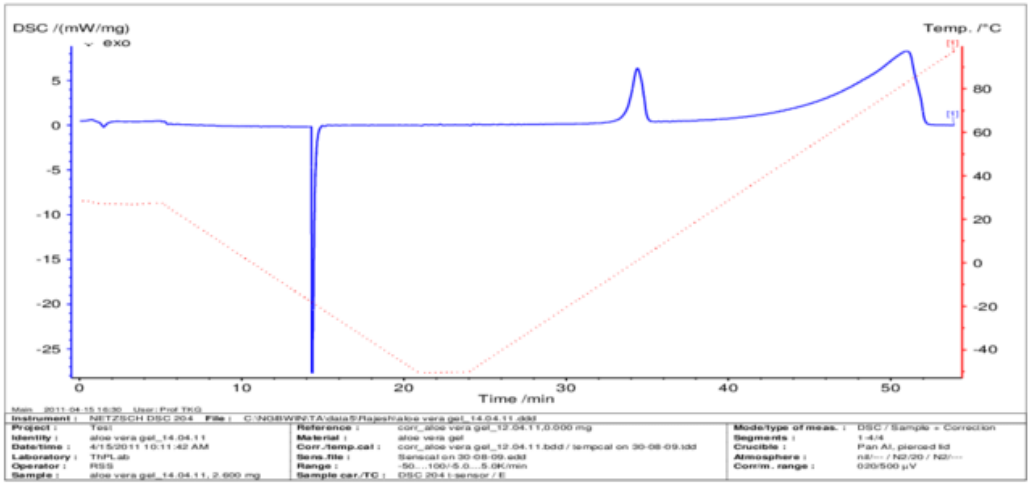


Fig. 2. It represents DSC of *Aloe vera* bioactive components. DSC study demonstrated no drug-drug interaction or drug incompatibility of *Aloe vera* extract with other formulation ingredients.

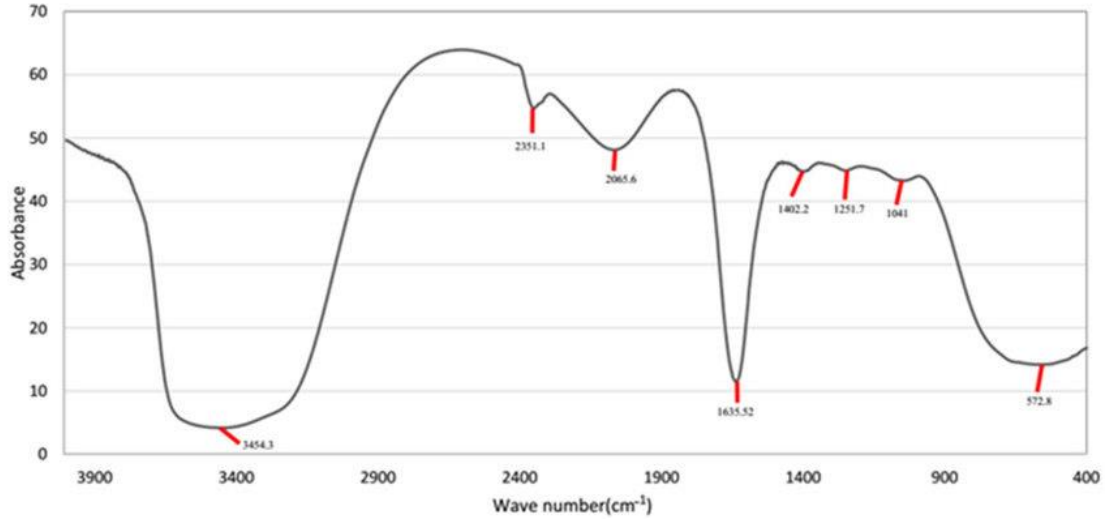
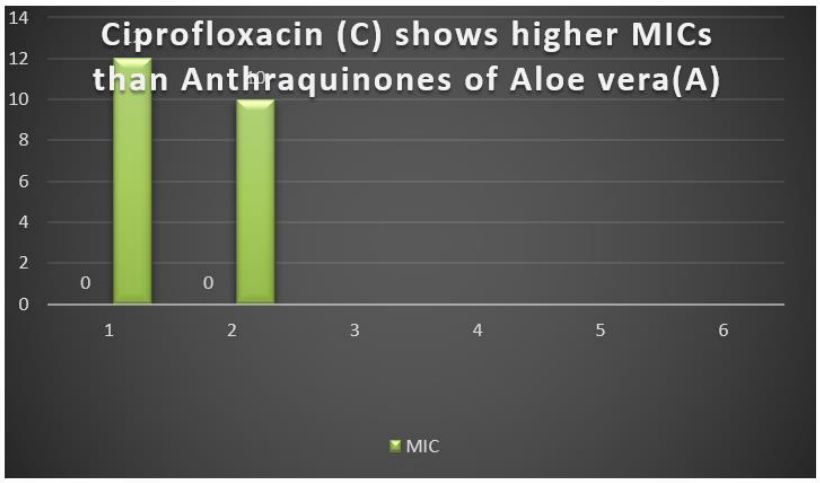


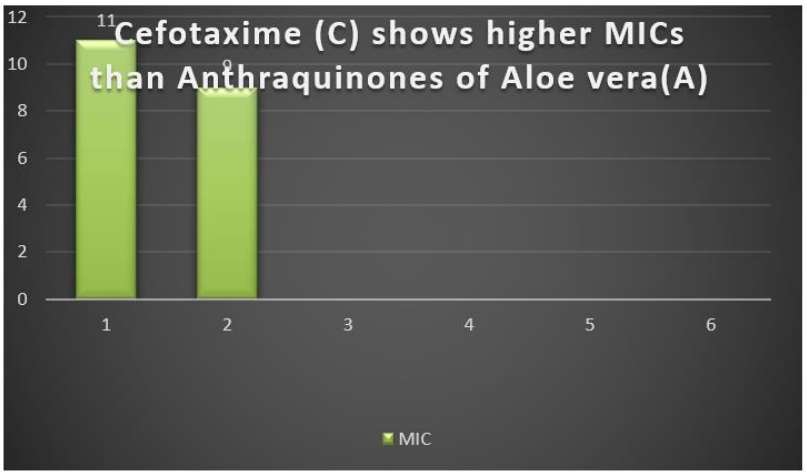
Fig. 3. It represents FTIR of *Aloe vera* bioactive components. FTIR study demonstrated no drug-drug interaction or drug incompatibility of *Aloe vera* extract with other formulation ingredients.

Statistical Analysis:

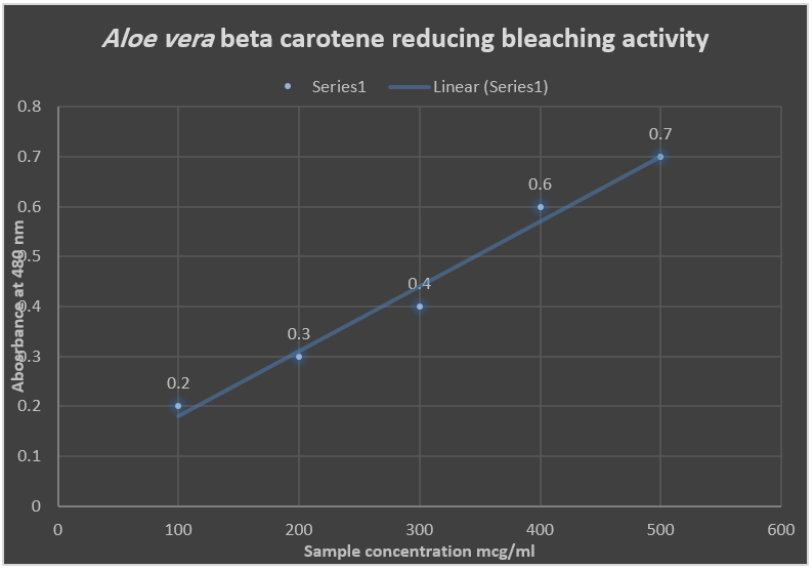
All cultures were conducted in triplets. Their presentation was by means and standard deviation. One-way analysis of variance (p value≤.05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software.



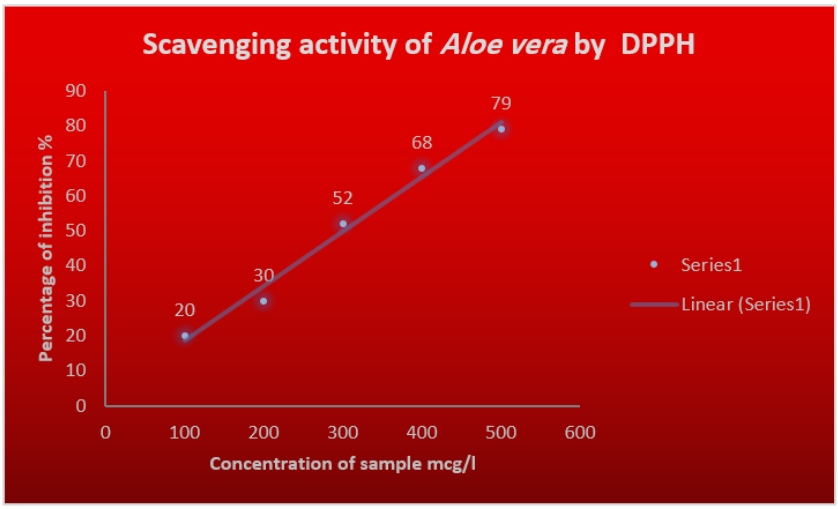
Graph 1. It represents MIC of ciprofloxacin(C) versus anthraquinones of *Aloe vera*(A) against *Salmonella typhi* and *paraptyphi*.



Graph 2. It represents MIC of cefotaxime(C) versus anthraquinones of *Aloe vera*(A) against *Salmonella typhi* and *paraptyphi.*



Graph 3. It displays Beta carotene bleaching reducing action(antioxidant power) of *Aloe vera*.



Graph 4. It represents the scavenging activity of *Aloe vera.*

RESULTS

Twenty-one constituents were characterized in the gel leaf extract of *Aloe vera* and the dominant components were anthraquinone derivatives (34.86%) with emodin as the principal anthraquinone (26.17%). These compounds showed an excellent scavenging DPPH ability with an extract concentration providing 50% inhibition (IC50) of 24.109 mcg/ml and a strong beta-carotene bleaching inhibition after 94 minutes of incubation with an IC50 of 35.48 mcg/ml. Zones of inhibition ranged from 22.7 to 25.14 mm in diameter. The minimum inhibitory concentrations varied from 0.062 to 1.73 mg/ml for Gram-positive and Gram-negative bacteria and fungi. The meat-preserving potential of *Aloe vera* was investigated against *Listeria monocytogenes*. They successfully inhibited the arising of *Listeria monocytogenes* in minced beef meat at concentrations 0.051 and 0.264 mg/g when stored at 4 C. Additionally during the storage period, physicochemical values (PH and TBARS) were higher in control meat than treated meat with *Aloe vera* proposing efficient antioxidant activeness of *Aloe vera* gel extract. They displayed no carcinogenicity or mutagenicity while chemical preservatives showed mutagenicity and carcinogenicity. They presented antiviral action aside from the abstinence of the cytopathic consequence of *Herpes simplex* viruses. Gel extract of *Aloe vera* prohibited the carcinogenicity of Gama rays in minced beef suggesting that they were excellent anticancer agents against gamma rays mediated cancers in humans. No realizable fundamental interactions were sensed between the medicine and the polymer via FTIR spectroscopy and DSC survey. Figure 1 represents antimicrobial activity against *Salmonella typhi and paratyphi.* Graphs 1 and 2 represents MIC of gel leaf extract of *Aloe vera* compared to quinolone and cephalosporin antibiotics respectively.

Tables (2 and 3) represent MICs of cefotaxime and ciprofloxacin versus essential oils of *Citrus*(E) against *Listeria monocytogenes*. Graph 3 and 4 represent antioxidant and scavenging activities of *Aloe vera* gel leaf extract. Figures 2 and 3 shows absence of drug drug interaction between gel leaf extract and excipients of the formulated emulsion.

Table 2. It represents MIC of ciprofloxacin(C) versus anthraquinones of *Aloe vera*(A) against *Salmonella typhi* and *paraptyphi:*

Description	C	A
MIC	13	10

Table 3. It represents MIC of cefotaxime(C) versus anthraquinones of *Aloe vera*(A) against *Salmonella typhi* and *paraptyphi.*

Description	C	A
MIC	11	9

Table 4. Beta carotene bleaching reducing activeness(antioxidant power) of Aloe vera.

Concentration mcg/ml	Absorbance at 480 nm wavelength
100	0.2
200	0.3
300	0.4
400	0.6
500	0.7

Table 5. Scavenging activity of Aloe vera via DPPH assay: sample concentration(mcg/l) versus percentage of inhibition (%).

Concentration of sample(mcg/l)	Percentage of inhibition(%)
100	20
200	30
300	52
400	68
500	79

DISCUSSION

Anti-Microbial Activity of Egyptian *Aloe vera:*

Aloe vera's antimicrobial properties were attributed to anthraquinone derivatives, with emodin serving as the primary anthraquinone with antimicrobial properties against numerous infections. Anthraquonones were aromatic compounds with glycosidic connections that gave them their distinctive properties.

Antibacterial and Anti-Fungal Activities Determination:

This was accomplished by calculating the MIC against the test bacterial and fungal pathogens by measuring the inhibition zones, which measured between 21 and 23.4 mm in diameter.

Synergism And Antagonism:

The bioactive components showed synergism in antibacterial activity with quinolone and cephalosporin antibiotics.

Antiviral Activity Determination:

The bio-active components demonstrated antiviral activity against a variety of viruses, including *SARS COV-2* mutant strains, *Herpes viruses*, *Influenza viruses*, and *HIV*. By inhibiting the cytotoxic effect and viral replication, this was made clear.

Formulation of Aloe Vera as Oil in Water Emulsion:

In comparison to conventional topical antimicrobial ointments and creams, they were able to demonstrate superior antimicrobial efficacy against burns, ulcers, various topical fungal infections like *Candida albicans*, and topical viral infections including *herpes simplex virus type 1 and 2* infections.

Determination of *Aloe vera* Anti-Oxidant Anti-Cancer Activities:

Gel leaf extracts outperformed conventional chemical preservatives in terms of antioxidant activity, extending the shelf life of minced beef while also slowing the spread of some malignancies. Gama strangely induced tumours in ground beef. *Aloe vera* gel leaf extract's anthraquinone and vitamin C components were primarily responsible for these effects.

Comparison With the Previous Study:

compared to a prior study (Safia Arbab *et al.*, 2021) that was carried out in China. Our current investigation revealed stronger antioxidant and greater germicide activeness than standard ones, contrary to the earlier study which claimed that the bioactive components of *Aloe vera* had less antioxidant and antimicrobial effects than standard antioxidants and antimicrobial agents.

CONCLUSION

Crops of *Aloe vera* gathered from several parts of Egypt have shown antimicrobial efficacy and synergy with other antimicrobial agents.

Acknowledgement:

A patent 1357/2021 was approved by the ministry of scientific research and high education.

Conflict of interest: There is no conflict of interest.

Fund: This study was carried out in a research project number 46362/2021 funded by STDF.

Data availability: Raw data were generated at faculty of pharmacy, Cairo university,Egypt. Derived data supporting the findings of this study are available from the

corresponding author Dr. Mohammed Kassab up on request.

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ARABIC SUMMARY

استخلاص المكونات النشطة الحيوية من عشب الصبارالمصري كمضاد ميكروبي ومضاد للاكسدة

محمد كساب
قسم علوم المايكروبيولوجي والمناعة كلية الصيدلة جامعة القاهرة

الاستعمال الزائد للمضادات الحيوية بدون داعي مشكلة تؤرق العالم كله لانه تسبب في ظهورسلالات بكتريا مقاومة لانواع كثيرة من المضادات الحيوية مما يستلزم استكشاف مصادر جديدة من المضادات الحيوية كالاعشاب والطحالب.
الهدف من الدراسة الحالية هو استكشاف المكونات الحيوية لنبات الصبار كمضاد ميكروبي ومضاد للاكسدة **خطوات العملي**: كشفت دراستنا للمكونات النشطة بيولوجيًا عن القيمة العلاجية الواعدة للصبار كعامل مضاد للجراثيم ومضاد للأكسدة عن طريق استعمال طرق المرق المختبري وطرق نشر أجار القرص لتحديد فعالية المكونات كمضادات للميكروبات والفطريات وتثبيط التكاثر الفيروسي والتأثير الخلوي لتحديد النشاط المضاد للفيروسات. النتيجة: كان نشاط الصبار المضاد للميكروبات ناتجًا عن مشتقات الأنثراكينون في دراستنا ، وكان إيمودين هو الأنثراكينون الرئيسي المسؤول عن النشاط المضاد للبكتيريا ضد مسببات الأمراض المختلفة. الأنثراكينونات هي مركبات عطرية تتميز بروابط جليكوسيدية.
اظهرت النتائج ايضا احتواء الصبار على مضادات للاكسدة كفيتامين ج والانثراكينون الخلاصة: كانت الدراسة الحالية بحثًا واعدًا نظرًا لقدرتها على التغلب على صعوبة السلالات المتعددة المقاومة لمجموعات متعددة من الميكروبات وتوفير مكونات عشبية بديلة مضادة للبكتيريا ومضادة للسرطان ومضادة للأكسدة نشطة بيولوجيًا من عشب الصبار بمصر .