



# مجلة التربوي

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## Phytochemical, Heavy Metals and Antimicrobial Study of the Leaves of *Amaranthus viridis*

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*Amaranthus viridis* belongs to Amaranthaceae family. *Amaranthus*, communally known as Green amaranth. *Amaranthus viridis* L decoctions are traditionally used in the treatment of various ailments including lessen labor pains and as an antipyretic, antiulcer, analgesic, laxative, as an antileprotic, as anti-inflammatory agents of urinary tract, against high blood sugar as well as against high cholesterol. In the present study phytochemicals present in the leaves of *Amaranthus viridis* were extracted with ethanol and the extract was used to investigate the phytochemical constituents. Based on results supported by different studies, Several secondary metabolites were found such as Alkaloids, Tannins, Phenols, Flavonoids, Proteins & amino acids, Glycosides, Saponins and Steroid. Our study also included the antibacterial activity of *Amaranthus viridis* against the bacterial pathogens using Ethanolic extract. The selected pathogens were *E.coli*, *Staphylococcus aureus* and *Klebsiella*, the ethanolic extract of *Amaranthus viridis* showed antibacterial activity against the *E.coli*, *Staphylococcus aureus* and *Klebsiella*. Finally, A study was conducted to analyse the presence of heavy metals in the *Amaranthus viridis*. AAS was used to examine the presence of heavy metals by acid digestion method. Overall the results of analysis showed that heavy metals were present in varied concentrations in *Amaranthus viridis*. The results obtained revealed that the leaves of *Amaranthus viridis* have some therapeutic values and could be exploited in the preparation of herbal drugs for the treatment of various ailments

**Keywords:** *Amaranthus viridis*, Leaf Extract, phytochemical, Antibacterial, Heavy Metal, Atomic Absorption Spectroscopic.

### Introduction

Plants are the major sources of drugs or molecule which demonstrate mild to significant pharmacological activity against tremendous organisms and diseases. Plant extracts represents a complex mixture containing both organic and inorganic chemical species, being trace metals one group the inorganic components present in this type of matrix. Information on trace element concentrations in Plant extracts is getting increasingly important for the clinical characterization of diseases and also to allow interferences actions during Plant extractions processing (U.S.L 2004). *Amaranthus viridis* belongs to Amaranthaceae family. communally known as Green amaranth or locally in libya as “shamosh”, is a multinational genus of herbs. *Amaranthus viridis* widely distributed all over the world, growing under a wide range of climatic conditions and among the vegetables employed in traditional medicine (Brenan , 1981)(Paulpati, 2014). *Amaranthus viridis* L was commonly used to lessen labor pains and as an antipyretic.( Kirithikar , 1987). The fruits, leaves, and flowers of *Amaranthus viridis* serve as vermifuge in venereal diseases, antiulcer, analgesic, laxative, as an antileprotic, as anti-inflammatory agents of urinary tract, against high blood sugar as well as against high cholesterol (Burkill, 1995) (Ross, 2001). Other traditional uses are as ananti-inflammatory of the urinary tract, vermifuge, diuretic,



antirheumatic, antiulcer, analgesic, antiemetic, laxative, improves appetite, antileprotic, respiratory problems, eye treatment and for asthma (Ashok Kumar, 2011).

Metal ions are essential for growth, more or less in all types of living cells but at some sort of extent or in very small amount. After and above required degree of necessity, metal ions are termed as heavy metal, responsible to cause toxicity in cells of living plant and animals. Those heavy metals include Copper (Cu), Lead (Pb), Zinc (Zn), Cadmium (Cd), Chromium (Cr) etc. These metal ions are also responsible to raise environmental pollution. These metal ions are generated in very high concentration through industrial effluent, petroleum products, pesticides, refineries and various types of chemical fertilizers. Metal ions are absorbed by the plants through soil and enter into the human body after consumption as food article. High amount of consumption and accumulation in living organism is termed as Bioaccumulation.

In light of this, the goal of this study was to identify the phytochemical components of *Amaranthus viridis* leaves, measure the concentration of heavy metals contained in the leaves, and then assess the plant leaves' antimicrobial effectiveness against a variety of human infections

## EXPERIMENTAL

### Plant Material

Fresh leaves of *Amaranthus viridis* was collected from AL KHUMS, LIBYA during the month of Marsh in the year 2022. The identification of the plants was carried out at Biology Department, Faculty of Science, El-Mergheb University. Alkhums, Libya.

### Sampling of plant material

*Amaranthus viridis* leaves were separated, washed under distilled water and dried at room temperature. The sample was dried in an oven at 40°-50° C till a constant weight was obtained. The dried sample was then ground and powdered by an electrical blender. Finally, the Sample was labelled and stored in cleaned bottles for further analysis.

### Preparation of Plant Extract

10 g of ground sample was used to make ethanolic extract (80:20, ethanol: water, v/v). was carried out using Soxhlet apparatus. The extraction was carried out for 4 hrs at 60°C . A finely the extract was concentrated at 45°C using Rotary vacuum evaporator and used to determine qualitatively the presence of the phytochemical components.

### Phytochemical screening

#### Qualitative Analysis

Phytochemical evaluates for various phytoconstituents of the extract were undertaken using standard qualitative methods. Tannins, phlobatannins, alkaloids, saponins, flavonoids, steroids, terpenoids, glycosides, phenolic compounds and proteins were screened on the leaf extract.

#### Test for Alkaloids (Mayer's reagent)

1ml of Mayer's reagent was added to 1ml of each of the extract in a test tube and a creamy precipitate indicated the presence of alkaloid.

#### Test for Tannin

2 ml of extract was mixed with an equal volume of bromine water. The formation of greenish to red precipitates was taken as positive result for the presence of tannins.



**Test for Phenols:** Phloroglucinol test was used. One percent of  $\text{FeCl}_3$  was added to 2 mL of the extract and the presence of blue, violet, purple, green or red-brown colour was taken as positive result for phenolic compounds.

**Test for Flavonoids (Sodium hydroxide Test)** Two drops of 10% NaOH solution were added separately to 1 ml of extract in test tube, and the presence of a yellow colour revealed the presence of flavonoids.

**Test for Saponins (Frothing Test)**

8 ml of distilled water was used to dilute 2 ml of the extract, and the content was vigorously shaken for 2 min. Persistent frothing indicated the presence of saponins.

**Test for proteins and amino acids**

5 ml were added 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

**Test for Steroid (Salkowski test)**

Salkowski test was used for the screening of steroids. 1 ml of the extract was dissolved in 2.0 ml of chloroform in a test tube. Through one side of the test tube, 1 ml of concentrated  $\text{H}_2\text{SO}_4$  was carefully added. A reddish brown colour at the interphase was taken as positive test for steroid nucleus.

**Test for Glycosides**

2 ml of each extract were added: 5 ml of distilled water, 5 ml of concentrated  $\text{H}_2\text{SO}_4$  and boiled in a water bath for 15 min. The test tube was then allowed to cool and each was neutralized with 20% NaOH, after which 1 ml of Fehling's solution was added and boiled for another 15 min. A brick-red precipitate indicated the presence of glycosides.

**Test for phlobatannins**

5 ml of the extract was added to 2.0 ml of 1% Hydrochloric acid (HCl), and red precipitate was then as evidence for the presence of phlobotannins.

**Test for Terpenoids**

To 2ml of of the extract, 2ml of acetic anhydride and 2ml of concentrated  $\text{H}_2\text{SO}_4$  were added, and the formation of blue-green rings showed a positive test for terpenoid.

**Antimicrobial Screening test**

The antimicrobial activity of ethanol extract of the leaves of *Amaranthus viridis* was tested against human pathogens using the disk diffusion. Three organisms were used, pathogenic bacteria, namely *Staphylococcus aureus*, *E.coli* and *Klebsiella*.

The sensitivity test of the ethanol extract was determined using disc diffusion method. Disc impregnated with the plant extract were placed on the swabbed plate. The plates were incubated over night at 37 °C for 24 hours. After incubation, the clear zone around the disc were measured and expressed in mm as a measure of their antibacterial activity (Balakrishnan, 2003).

**Quantitative Analysis**

**Determination of Heavy Metals**

**Digestion of plant samples**

The extraction of heavy metal was done through acid digestion method. The dried plant sample of 5 g was added to conical flask (100 ml). A mixture of acid ( $\text{HNO}_3$  and  $\text{HClO}_4$ ) with a ratio of 4:1 was added, respectively. The flask was then heated gradually up to 160°C followed by gradual cooling. The samples was then filtered and



added with double distilled water to make the final solution of 50 ml and kept at room temperature for further analysis(Khan, 2011). The sample was estimated for four metal ions Cd, Pb, Cu and Cr by using Atomic Absorption Spectrophotometer (AAS).

## RESULTS AND DISCUSSION

### Preliminary Phytochemical screening:

Phytochemical screening of ethanolic extract of *Amaranthus viridis* L was carried out. In general, test for the presence or absence of phytochemical compounds using standard methods involves the addition of an appropriate chemical agent to the extract. The extract yield from the leaves ranged 85. 371% The ethanolic extract of *Amaranthus viridis* revealed the presence of all the phytochemicals tested for except for Terpenoids and phlobatannins, the concentrations of tannins and saponins were high, Saponins are known to possess bitter taste, and are foamy in nature (Adeoti, 2012). Saponins have reported to possess antibacterial properties(Wadood, 2013). while the concentrations of flavonoids, alkaloids, steroids, phenolic compounds, Proteins and glycosides were low. Phlobatannins and terpenoids, were not found in *A. viridis* leaf extract. Flavonoids have anti-microbial potential and can be used in the treatment of dropsy, hay fever, and ulcers (Finar, 2006). In addition, Alkaloids possess reductive effect towards fever and headache (Adeoti, 2012). They also possess analgesic and antibacterial properties. Thus the presence of these phyto-compounds in the leaves of *A. viridis* confers on it its medicinal value. The results of phytochemical screening of leaves of *Amaranthus viridis* were depicted in Table 1.

**Table 1** Results of phytochemical screening results of crude extract of leaves of *Amaranthus viridis*

Name s' plant	Name of part	Percentage Yield (%)	
<i>Amaranthus viridis</i>	leaves	Ethanolic extract	85. 371%

  

Chemical Component	Crude Extracts of Leaves of <i>Amaranthus viridis</i>	The Tests Names and Resulted in Colours
	Ethanolic extract	
Alkaloids	+	Mayer's reagent: creamy Precipitate
Tannins	++	bromine water: greenish to red precipitates
Phenols	+	Phloroglucinol test: blue, violet, purple, green or red-brown colour
Flavonoids	+	Alkaline Reagent: (Sodium Hydroxide test): yellow colour
Saponins	++	Frothing Test: Persistent foam
Proteins & amino acids	+	Ninhydrin: Violet , blue color
Steroid	+	Salkowski test: A reddish brown colour
Glycosides	+	conc. H <sub>2</sub> SO <sub>4</sub> , 20% NaOH Fehling's solution,: A brick-red precipitate



phlobatannins	-	1% Hydrochloric acid (HCl): red precipitate
Terpenoids	-	acetic anhydride, concentrated H <sub>2</sub> SO <sub>4</sub> : blue-green rings

+ = Present in low concentration; ++ = present in high concentration; and - = absent.

### Antibacterial Activities

The plant *Amaranthus viridis* L. is known for various medicinal uses. The medicinal properties of this plant due to the phytochemicals present in them, which have antimicrobial activity. The result on Antibacterial activity of *Amaranthus viridis* using ethanol extract showed that the maximum inhibitory zone was observed against the pathogen *Klebsilla* (15mm), and minimum activity was found against the *Staphylococcus aureus* (7mm). (Balakrishanan, 2003) reported that Amaranthaceae family comprises many species with biological activities, which are used in nutrition and alternative medicine. The results are presented in Table 2 of antibacterial activity of *Amaranthus viridis* L.

Table 2 Inhibition Zone in *Amaranthus viridis* using Ethanol Extract using Disc Diffusion Method

Part of plant	Ethanol Extract	Bacterial pathogens		
		Zone of Inhibition (mm)		
		<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Klebsiella</i>
leaves	100%	7 mm	12 mm	15 mm

### Analysis of metals by atomic absorption spectrophotometer

Some of the metal ions are essential for living cells for proper functioning and rest of them are responsible to cause toxicity. But the quantity in terms of concentration is a major factor, which derives their specific role inside living cell of each metal ion. Determination of quantity of metal ion in food material can reveal their effect on human health. The Atomic Absorption Spectrometer was used to test for the presence of four heavy metals (cadmium, copper, lead and chromium) in *Amaranthus viridis*. The Results obtained in the present study showed that the *A. viridis* analysed contain concentration of heavy metals ( Cu, Cr, Pb, and Cd). Cd and Pb are toxic elements which occur naturally in plants as a result of uptake, generally in places with high concentration caused by atmospheric and industrial fallout. However, the low concentration of Pb in the leaf extract of *Amaranthus viridis* implies that the plant is relatively safe, either to be used as fodder for livestock or for the preparation of herbal drugs for the treatment of said ailments.

Based on these results, all the metals are below the WHO permissible limits. Hence, the plant can be used for the development of drug and herbal products. Thus, on the basis of experimental outcome, it can be accomplished that the plant The result of the heavy metals determination of the leaf of *A. viridis* is shown in Table 3.



Table: 3 Heavy metal analysis of leaf of *Amaranthus viridis*

Plant s´name	Common Name in libya	Elements analyzed				
		Family	Pb	Cd	Cr	Cu
<i>Amaranthus viridis</i>	shammosh	Amarantahcae	0.002	0.0047	0.034	1.6240

### Conclusion

The plant kingdom has proved to be the most useful in the treatment of diseases, and plants provide an important source for pharmaceuticals. The phytochemical and antibacterial activities of ethanolic extract achieved from *Amaranthus viridis* To justify the use of this plant in treatment of diseases. Furthermore The presence of high amount of phytochemical compounds put forward that the *Amaranthus viridis* plant has higher medicinal value and can be extensively studied to extract the natural compounds which are beneficial to human beings and that could be commercialized for higher production. As well as, A. viridis which has vast ethnomedicinal values estimated are safe and may not produce any harmful effect of metal toxicity during their remedial application and could also develop new drug entities from the plant.

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## الفهرس

الصفحة	اسم الباحث	عنوان البحث	رت
1-10	Manal Mohammed bilkour	An optimal fuzzy zero point method for solving fuzzy transportation problem	1
11-24	Mohamed Bashir M. Ismail	Assessing the Adaptability of Students and Teachers in the Faculty of Arts at Alasmarya Islamic University to the Sudden Transition to Online Teaching and Learning Processes during the COVID- 19 Pandemic	2
25-34	Dawi Muftah Ageel	Environmental study for Cyanobacteria Blooms using Envisat data at the western coastal of Libya	3
35-53	Nuria Mohamed Hider	Possible solutions to ensure data protection in cloud computing to avoid security problems	4
54-60	Gharsa Ali Elmarash Najla Mokhtar	A printed book or an e-book? Student Preferences & Reasons	5
61-75	هدية سليمان هويدي نادية عطية القدار دعاء عبد الباسط باكير	التشهير الإلكتروني عبر مواقع التواصل الاجتماعي من وجهة نظر طلبة كلية طب الأسنان بمدينة زليتن	6
76-89	Hamza A. Juma Saif Allah M. Abgenah Mustafa Almahdi Algaet Munayr Mohammed Amir	Designing an Autonomous Embedded System for Temperature Monitoring and Warning in Medical Warehouses	7
90-101	Salem Msaoud Adrugi Tareg Abdusalam Elawaj Milad Mohamed Alhwat	The effect of using electronic mind maps in learning visual programming through e-learning platforms An experimental study of computer departments students at Elmergib University	8
102-110	Suad Mohamed Ramadan Zainab Ahmed Dali Ahlam Mohammad Aljarray Zenoba Saleh Shubar	Performance analysis of different anode materials of double chamber Microbial Fuel Cell technology using different types of wastewater	9
111-116	Faiza Farag Aljaray Saad Belaid Ghidhan	Evaluation of Hardness for Electroless Ni-P Coatings	10
117-128	Saleh Meftah Albouri Hadya S Hawedi Mansur Ali Jaba	Using Smartphone in Education: How Smartphone has impacted in Education, A Review Paper	11
129-139	Ibrahim O, Sabri	The Concept of Illegal Immigration and Its Causes in North Africa Region	12
140-151	A.S. Deeb I.A.S. Gjam	Solution of a problem of linear plane elasticity in region between a circular boundary with slot by boundary integrals	13



152-173	Musbah Ramadan Elkut	Transforming TESOL Pedagogy: Navigation Emerging Technology and Innovative Process	14
174-192	سالم علي سالم شخطور	آراء أبي محمد القيسي في خزانة الأدب "دراسة وتحليل"	15
193-217	نورية صالح إفريج	اعتراضات النحاة على حجية الشواهد في مسألة إعادة حرف الجر مع حتى العاطفة	16
218-238	نجاة صالح اليسير	الازدواجية اللغوية وأثرها في تعليم اللغة العربية الصفوف الأولى من المرحلة الابتدائية (أنموذجاً)	17
239-256	محمود محمد رحومة الهوش	الرضا الوظيفي وأثره على الاداء المهني لدى معلمي ومعلمات التربية البدنية ببلدية العجيلات	18
257-272	إبراهيم رمضان هدية	السرد الروائي عند إبراهيم الكوني في رواية الدنيا أيام ثلاثة	19
273-279	ابراهيم علي احمدودة ابراهيم علي ارحومة	التحليل الاستراتيجي لشركة الخطوط الجوية الليبية دراسة تطبيقية على الشركة باستخدام النماذج	20
280-294	Ismail F. Shushan Emad Eldin A. Dagdag Salah Eldin M. Elgarmadi	Petrography of Abushyba Formation columnar-jointed sandstones (Triassic-Jurassic) from Jabal Nafusa- Gharian, NW-Libya	21
295-307	Samera Albghil	Multimodal discourse analysis of variations in Islamic dress code in Bo-Kaap, Cape Town	22
308-317	عبداللطيف بشير المكي الديب رجب فرج سالم اقنيير	( استخدام نظم المعلومات الجغرافية والاستشعار عن بعد في تقدير النمو العمراني وأثره على البيئة المحلية بمنطقة سوق الخميس - الخمس / ليبيا)	23
318-331	حنان عبد السلام سليم عائشة حسن حويل	تطوير الخدمات العقارية باستخدام تقنية المعلومات ( تطبيق أندرويد للخدمات العقارية أنموذجاً)	24
332-338	Mahmoud Mohamed Howas	Hepatoprotective Potential of Propolis on Carbontetrachloride-Induced Hepatic Damages in Rats	25
339-352	نورية محمد النائب الشريف	البناء العشوائي في مدينة الخمس (مفهومه - أسبابه - تأثيره على المخطط)	26
353-371	إسماعيل حامد الشعاب معمر فرج الطاهر سالم العامري	اختلاف القراء السبعة في البناء للفاعل وغير الفاعل وأثره في توجيه المعنى "نماذج مختارة"	27
372-376	عبد السلام صالح أبوسديل عطية رمضان الكيلاني	دراسة على مدى انتشار Gnathia sp. في بعض الأسماك البحرية المصطادة من شواطئ الخمس- ليبيا	28
377-392	الصغير محمد المجري	(بيان فعل الخير إذا دخل مكة من حج عن الغير) للملا علي القاري المتوفي سنة 1014هـ دراسة وتحقيق	29
393-421	نجيب منصور ساسي	فضل المواهب في شرح عيون المذاهب لعبد الرؤوف الأنطاكي (1009هـ) (الاستنجا ونواقض الوضوء من كتاب الطهارة) دراسة وتحقيقا	30
422-439	حنان ميلاد عطية	برنامج ارشادي معرفي سلوكي في خفض مستوى الوحدة النفسية لأبناء النازحين الليبيين	31
440-457	Hanan A. Algrbaa,	Speaker recognition from speech using Gaussian mixture model (GMM) and (MFCC)	32
458-467	هشام علي مرعي	علاقة المنطق بالعلوم الشرعية عند الغزالي	33



468-476	خالد الهادي الفيتوري زينب أحمد زوليه	الحلول العددية للمعادلات التفاضلية الملزمة باستخدام ب-سبلين التكعيبية	34
478-500	خميس ميلاد الدزيري	تأثير نظم معلومات التسويقية على توزيع السلعة " دراسة تطبيقية على إدارة مصنع إسمنت المرقب "	35
501-517	منصور عمر سالم فرعون	إدارة الوقت في الإدارة المدرسية في ضوء مهامهم الإدارية	36
518-533	فائزة محمد الكوت	أراء العلامة الدماميني النحوية في باب الظروف في كتاب خزانة الأدب ولب لباب لسان العرب	37
534-547	محمد محمد مولود الأنصاري حمزة مسعود محمد مكاري	"فوائد الفرائد في الاستعارة " عبد الجواد بن إبراهيم بن شعيب الأنصاري (1073هـ)	38
548-559	عبدالرحمن بشير الصابري إبراهيم عبد الرحمن الصغير أبوبكر أحمد الصغير	حروف الجر بين التناوب والتضمين دراسة تطبيقية على آيات من القرآن الكريم "دراسة وصفية تحليلية"	39
560-565	Ayda Saad Elagili Abdualah Ibrahim Sultan	An Application of "Kushare Transform" to Partial Differential Equations	40
566-598	أمل إجمد إقميع فاطمة محمد ابوراس	الأداء الوظيفي للمعلم وأثره على العملية التربوية دراسة سوسولوجية على عينة من معلمين ومعلمات مرحلة التعليم الأساسي	41
599-623	خيري عبدالسلام كليب عبدالسلام بشير اشتوي طارق أبوفارس العجيلي محمد عبدالسلام الأسطي فتحية خليل طحيشات	مدى التزام المصارف التجارية بتطبيق مبادئ إدارة الجودة الشاملة (دراسة ميدانية على مصرف الجمهورية فرع المرقب)	42
624-633	Abdulrhman Iqneebir Khaled Muftah Elsherif	Determination of Some Physical and Chemical Parameters of Groundwater in Ashafyeen-Masallata Area	43
634-650	أحمد على معتوق الزائدي	أحكام الأهلية وعوارضها عند الإنسان	44
651-671	عمر مصطفى النعاس السيد مصطفى السنباطي	الثقة بالنفس وعلاقته بالتوجه نحو الحياة لدى طالبات كلية الآداب	45
672-700	فاطمة جمعة الناكوع	معايير جودة آليات التدريب الميداني	46
701-718	إيمان عمر بن سعد بثينة علي أبو حليقة عمر محمد بشينه وليد حسين الفقيه	أثر المخاطر المالية في الأداء المالي للمصارف التجارية الليبية للفترة من (2011-2017)	47
719-730	هدي الهادي عويطي	دور مداخل ادارة المعرفة في تحسين ادارة الموارد البشرية في المؤسسات الحديثة	48
731-739	Khaled Abdusalam B. A Eman Mohammed Alshadhli Tasnim Adel Betro Amera Lutfi Kara Mawada Almashloukh	Antimicrobial Activities of Methanol Extract of Peganum harmala Leaves and Seeds against Urinary Tract Infection Bacteria	49
740-750	فتحية زايد شنيبه نجاة بشير الصابري	الصور البيانية في سورة الواقعة	50



751-757	Afifa Milad Omeman	Phytochemical, Heavy Metals and Antimicrobial Study of the Leaves of Amaranthus viridis	51
758-765	أسماء جمعة القلعي	قواعد المنهج عند ديكرت	52
766-777	فرج مجد صالح الدريع	النفط والاقتصاد الليبي 1963م – 1969م	53
778-789	عمر عبدالسلام الصغير رضا القدافي الأسمر	تقويم دية القتل الخطأ بغير الأصل	54
790-804	أبو عجيبة رمضان عويلي أحمد عبد الجليل إبراهيم	مناقشة المسألة الأربعين من كتاب المسائل المشككة للفارسي	55
805-823	فتحية أبو عجيبة جبران صالحة عمر الخرارزة	في منطقة سوق الخميس التلوث البيئي الناتج عن محطات الوقود (بحث مقدم للحصول على ترقية عضو هيئة تدريس)	56
824-856	هنية عبدالسلام البالوص	بعض المشكلات الضغط النفسي وعلاقتها بالصحة النفسية	57
857-871	احمد علي عزيز علي مفتاح بن عروس	تطبيقات البرمجة الخطية ونماذج صفوف الانتظار في مراقبة وتحسين الأداء دراسة إحصائية تطبيقية على القطاع الصحي بمدينة الخمس	58
872-879	Mona A. Sauf Fathi Shakurfow Sana Ali Soof Abdel-kareem El-Basheer	Isolation of Staphylococcus Aureus From Different Clinical Samples And Detects on Its Antibiotic Resistance	59
880-885	Wafa Mohamed Alabeid Omar Alamari Alshbaili	Combined Method of Wavelet Regression with Local Linear Quantile Regression in enhancing the performance of stock ending-prices in Financial Time Series	60
886-901	خالد مجد بالنور خالد أحمد قناو	حجم الدولة الليبية وأثره عليها طبيعياً وبشرياً	61
902-918	Amna Ali Almashrgy Hawa Faraj Al-Burrki Khadija Ali AlHebshi	EFL Instructors' and Students' Attitudes towards Using PowerPoint Presentation in EFL Classrooms	62
919-934	سالمة عبد العالی السيليني	اضطرابات الشخصية الحدية وعلاقتها بالجمود المعرفي	63
935-952	Samah Taleb	Common English Pronunciation Difficulties Encountered by Third Year Students at the Faculty of Education- English Department- Elmergib University	64
953-958	Hassan M. Krifa	A Study on Bacterial Contamination of Libyan Currency in Al-Khoms, Libya	65
959-964	Jamal Hassn Frjani	A New Application of Kushare Transform for Solving Systems of Volterra Integral Equations and Systems of Volterra Integro-differential Equations	66
965-978	Ismail Elforjani Shushan Saddik Bashir Kamyra Hitham A. Minas	Study of chemical and biological weathering effects on building stones of the Ancient City of Sabratha, NW-Libya	67
979-991	مجد عبد السلام دخيل	الآثار الاجتماعية والثقافية المصاحبة للتغير الاجتماعي في المجتمعات النامية	68



992-998	Ismael Abd-Elaziz Fatma Kahel	Molecularly imprinted polymer ( poly-pyrrole ) modified glassy carbon electrode on based electrochemical sensor for the Sensitive Detection of Pharmaceutical Drug Naproxen	69
999-1008	خالد رمضان الجربوع علي إبراهيم بن محسن صلاح الدين أبوغالية	علي الجمل وقصيدته (اليوم الأربعاء في رثاء النورس الكبير)	70
1009-1014	نادية مجد الدالي ايمان احمد اخميرة	Comparing Review between Wireless Communication Technologies	71
1015-1024	Khairi Alarbi Zaglom Foad Ashur Elbakay	The importance of Using Classroom Language in Teaching English language as a Foreign Language	72
1025-1042	حمزة بن ربيع لقرون	الأدلة المختلف فيها التي نُسب الاختصاص بها إلى مذهب مُعَيَّن (دراسة تحليلية مقارنة)	73
1043-1052	أسماء السنوسي لحيو	معدل انتشار بعض الأوليات المعوية الطفيلية في مدينة الخمس، ليبيا	74
1053-1067	برنية صالح إجمد صالح	استعمالات (ما) النافية في سورة البقرة	75
1068-1085	اسماعيل عبدالكريم اعطية	عوامل نجاح وفشل نظام المعلومات دراسة تطبيقية على شركة الأشغال العامة بني وليد	76
1086-1098	نجوى الغويلي	"الرعاية الاجتماعية والدعم الاجتماعي والتربية الإيجابية للطفل"	77
1099-1105	Seham Ibrahim abosoria Fatheia Masood Alsharif Abdussalam Ali Mousa Hamzah Ali Zagloun	The Error Correction in second language writing	78
1106-1128	ميسون خيري عقيلة	أساليب المعاملة الوالدية وعلاقتها بالتحصيل الدراسي لدى عينة من طلبة كليات جامعة المرقب بمدينة (الخمس)	79
1129-1135	Majdi Ibrahim Alashhb Mohammed Alsunousi Salem Mustafa Aldeep	Quality of E-Learning Learning Based on Student Perception Al Asmarya University	80
1136-1150	Ekram Gebрил Khalil	The Importance of Corrective Feedback in leaning a Foreign Language	81
1151-1164	سكينه الهادي الحوات فوزي مجد الحوات سلمية رمضان الكوت	شكل العلاقات الاجتماعية في ظل انتشار الأوبئة والأمراض السارية (جائحة كوفيد 19 نموذجاً)	82
1165-1175	Salma Mohammad Abad	A comparative study of the effects of Rhazya stricta plant residue on Raphanus sativus plant at the age of 15 and 30 days	83
1176-1191	مجد عمر مجد الفقيه الشريف	توظيف الاعتزال عند الزمخشري وانتصاره له من خلال تفسيره	84
1192	الفهرس		