Phytochemical Screening and Anti-microbial Activity of Mangrove Seedlings (Avicennia marina) Against Some Pathogens

Dr. Awatif Abdelgadir Sugga

A.Manal Bashir

Abstract:

Avicennia marina as a pioneer tree species of mangrove which belonging to the family Verbenaceae, is a cosmopolitan species widely distributed along tropical and subtropical coastlines. The barks, leaves, fruits and gum of this species have been used as traditional medicine in many parts of the world to treat skin and tooth diseases.Methanolic crude extracts of *Avicenna marina* seedlings was evaluated for its antimicrobial activity and phytochemial screening. The results have shown that the extract attempt to inhibit the growth of six species of Bacteria and fungus which were Bacteria, *Bacillus subtilis, , Escherichia coli , Pseudomonas aeruginosa* and *Staphylococcus aureus in addition to* Fungus, *Aspergillus niger* and *Candida albicans* . The methanol extract of seedlings has significant effect on bacteria and fungus with inhibition zones ranging from 10 to 12mm and 11 to 17 mm respectively whereas it showed no antimicrobial activity against all microorganisms with aqueous extract .The results of the quantitative photochemical screening of the extract showed that the extract contains more amounts of Tannins, Coumarins , Triterpenes and Saponins as compared to the concentrations of

Sterols whereas Flavonoids and Anthraquinones are not found in the extract . In addition , *Avicenna marina* seedlings primary productivity expressed Fats, Proteins, Fibers ,Ash and Carbohydrates content had been also estimated in the present study.

Key words : Mangrove ; *Avicenna marina ;* Seedlings ; Antimicrobial ; Phytochemial screening

الفحص الكيميائي النباتي والنشاط المضاد للميكروبات لبادرات المنجروف نوع أفيسينيا مارينا ضد بعض الممرضات

د. عواطف عبد القادر سقا –استاذ مشارك –قسم علوم البحار البيولوجية-معهد الابحاث البحرية –جامعة الحر الاحمر.

أ. منال سليمان بشير – قسم التخطيط والبحوث-هيئة الموانئ البحرية -السودان

المستخلص:

اشجار وشجيرات الأفيسينيا مارينا (Avicennia marina)، من الأنواع الرائدة للمنجروف (ويعرف باللغة العربية باسم القرم) وهي تابعة لفصيلة Verbenaceae، وتُعد من الأنواع واسعة الانتشار على السواحل الاستوائية وشبه الاستوائية حول العالم. ونجد ان قشورها وأوراقها وثمارها وصمغها قد استخدمت في الطب التقليدي في منذ زمن بعيد ،لعلاج أمراض الجلد والأسنان.هدفت هذه الدراسة إلى تقييم النشاط المضاد للميكروبات للمستخلص الخام الميثانولي لبادرات أفيسينيا مارينا، بالإضافة إلى إجراء فحص كيميائي نباتي لتحديد مكوناته الفعّالة. وقد أظهرت النتائج قدرة المستخلص على تثبيط نمو ستة أنواع من الكائنات الدقيقة، شملت أربع سلالات بكتيرية هي: Bacillus subtilis، وEscherichia coli ، وPseudomonas aeruginosa ، وStaphylococcus aureus، ونوعين من الفطريات هما: Aspergillus niger وCandida albicans.سجّل المستخلص الميثانولي للبادرات نشاطًا واضحًا ضد البكتيريا والفطريات، حيث تراوحت أقطار مناطق التثبيط بين 10 و12 مم للبكتيريا، و11 و17 مم للفطريات، في حين لم يُظهر المستخلص المائي أي نشاط مضاد للميكروبات تجاه جميع الكائنات المختبرة.وأوضحت نتائج الفحص الكيميائي النباتي الكمي احتواء المستخلص على نسب مرتفعة من التانينات، والكومارينات، والترايتربينات، والصابونينات مقارنة بتركيزات الستيرولات، بينما لم تُرصد أي آثار للفلافونويدات أو الأنثراكينونات.كما تم في هذه الدراسة تقدير الإنتاجية الأولية لبادرات أفيسينيا مارينا من حيث محتواها من الدهون، والبروتينات، والألياف، والرماد، والكربوهيدرات.

الكلمات المفتاحية: منجروف (القرم)؛ Avicennia marina؛ بادرات؛ مضادات الميكروبات؛ الفحص الكيميائي النباتي.السودان.

Introduction:

Mangroves are delicate complex and dynamic ecosystems located between land and marine waters along the tropical and the sub-tropical coastal lines of the globe, few people could determine what mangroves are, and still also undervalued (Zahran 2010) Mangrove shows various types of vegetative growth ranging from shrubs to well-developed high trees , mangrove ecosystems are threatened worldwide due to aspects such as reclamation of mangrove lands for agriculture, aquaculture, horticulture, human settlement, industries and salt production. Due to natural stress-es projected from climate change and desertification coupled with coastal pollution with heavy metals and petroleum recently, the stress increased upon these fragile ecosystems(Sugga&Nasr 2024). Mangroves all over the world have countless uses for both nature and human beings as source of lively hood, they could be used as material in building houses, ships and boats. (Zahran,2009). In Sudan mangrove stands occur in the intertidal protected estuar-ies along the Rea Sea coasts ,occupying more than 26 locations showing luxuriant growth in some locations such as Ashat Hkor Strab Shinaab, Halout, Kilo Tammanina, Maroub, Inkaifal ,Salak and Hosheri ,Mangroves are known as fresh fodder for camels, nurseries for many species of commercial fishes, and source of some medicinal products (Mouafi et al,2014,).

This study is conducted mainly to fill-in the gap of knowledge in the field of mangrove seedlings information, and lightened the opportunities of using such endangered marine plants as source

of safe natural marine products . It also offer information about the distribution mode and seasonality abundance of seeds and naturally growing seedlings in mangrove forests in Sudan .

Objectives of the study:

1. Screening the chemical and phytochemical constituents of Mangrove seedlings *(Avicenna marina sp.)*which grows under high stress due to high temperature and salinity of Red Sea region .
2. Assessment of qualitative effects of the abstract prepared from whole A. marina seedlings (roots cotyledons leaves and stems) potency as antibiotic and growth regulating factor ,on selected some Bacteria an fungi pathogens (both aqueous and ethanol Abstracts).
3. Identify the physical characteristics of soil used in the mangrove nurseries to identify suitable requirements needed for seedling production. .
4. Assessement of socioeconomic value of mangroves to the coastal community.

Literature Review:

Mangroves are essentially land plants that can tolerate salinity where the nature of mangrove establishment depends on tidal movements, inundation frequency, soil type, salt contents, water salinity and light intensity (Mohamed1984) mentioned that mangroves exist since the early Oligocene about 55 million years ago which means that mangroves are prehistoric plants. Phonological characteristic of mangrove flowering and seeding is relat-

ed directly to the degree of air temperature where the maximum flowering peaks were observed at 25 ˚C and the minimum at 18 ˚C for most of mangrove species *Avicenna. marina* under the family *Avicenniaceae* is the most globally spread mangrove species .It shows high tolerance to temperature and salinity and optimum growth between 20˚C and 12˚C, although it exists along the Red Sea coast where the average temperature exceeds 42 ˚C in summer (Abdul Fatah,2015)., *A.tomentosa, A.tonduzii*, *A.cicolor,*and *A.nitida* xxx . *A.marina* (family *Avicenniaceae)* is a mangrove species that wildely spread, This species produces numerous erect pencil-like (pneumatophores) with lenticels covering the root surface. Flowering may occur throughout the year. Usually the seeds starts germination before detaching from the mother tree, it's then called propagule. *A. marina* propogules are greenish-grey and heart-shaped with and apex that is rounded or having a short peak (Shigeyuki Baba, et al 2016). The global mangrove coverage has been variously estimated at 10^6 hectares where Luxuriant mangrove forest flourish along muddy or sandy shores protected from strong waves and storms (Prabhu .et al,2012 .Mangrove massive root system or the pneumatophores impede the tidal movement thus accelerating deposition of silt and fine clay material (Mohamed,1984). They are good breeding and nursery grounds for fishes and many other marine fauna and flora. The root systems of such unique trees provide ideal breeding, nursery and feeding shelter for a diverse range of marine aquatic lives (Zahran,2007) . Mangrove plants are rich in a wide diversity of phytochemical

like tannins, Terpenoids, Flavonoids, alkaloids, found to have antimicrobial activities,(in addition medical plants have been used for centuries for remedies of human disease because they contain components of therapeutic importance (Mouafi et.al, 2014).In many countries mangroves were used either as raw plant material, or after conversion to other product. Prabhu et al,2012). Mangroves also serve as a good locations for bees to produce honey Mangrove provide nutritional material directly or indirectly for fish, crabs, shellfish, prawns and edible snakes and worms. (Abdulrazzaq,1994) Mangroves are richest source of phytochemical due to presence of important chemical compounds such as carbohydrates, alcohols, amino acids, many fatty acids, lipids and phenolic compounds (Prabhu et al ,2012). Mangrove leaves contain amino acids, vitamins and minerals which help in the growth and development of marine organisms. (Khafagi et al,2003). In traditional medicine, the leaves fruits and bark of *A.marina* are used to treat skin diseases while the stems are used to treat rheumatism, smallpox and ulcers (Abeysinghe,2010). Seedlings of mangrove contain large amounts of aerenchyma. In the first year of growth, they do not produce pneumatophores, and the only access route for oxygen transportation from the root to the gas spaces through the stem. Seedlings of *A.marina* have been shown highly resistant (Skelton et al, 2006)In many countries mangrove seeds and seedlings are cooked and eaten as vegetable, coastal populations in Gujarat, an India, collect the foliage of *A.marina* as fodder for their cattle. It serve as lives tock forage for camels (Bashir,1998).These

conspicuous Mangrove eco-systems have been under stress due to many factors including, overgrazing, cutting, pollution, physical modification of the coastal habitat, and land use changes , so There is amassing need for their conservation (Arun Kumar et al,2010). Based on available directory, of all the climate change outcomes, relative sea level rise might be the greatest threat to mangrove., standards and indicators of change in response to effects from climate change, while regional observation networks are needed to observe these responses to enable adaptation.Adaptation measures should consider expected mangrove losses and improve resistance to climate change. (Arun Kumar et al,2010).Mangrove extracts could be a probable source for stuffs against mosquito larvae, bacterial, fungal, ulcers, plasmodia and tumors. *A. marina* has proved to have numerous medicinal properties and show anti-cancer effects (Prabhu et al,2012 and Mouafi et al,2014).

Pathogenic bacteria includes many harmful species which infect plants and animals causing damage and serious complications on life performance ,among those pathogenic bacteria were *Pseudomonas aeruginosa*:-***Bacillus subtilisStaphylococcus*** aureus و***, Escherichia coli ,-Candida albicans ,***and ***spergillus niger*** .Sudanese have mass of curative methods, techniques and recipes. The Atlas of medicinal plant published in 2000 reported many of these Sudanese medicinal plants. In different regions of Sudan as common practice, people collect medicinal plants from their natural habitats depending on their traditional experience for home consumption and/or commercial issue(Baba,2016). Mangrove leaf extracts are non toxic to humans and are source for regulating Antioxidant ,choosing the best solvents for extraction of valuable amino acids from mangroves concluded that it con-

tains seven kinds of essential amino acids within its constituents, (mouafi et al ,2014). In the future, it would be preferable to promote the use of traditional herbal remedies by conversion of raw plant material into more sophisticated products instead of completely replacing the traditional remedies with synthetic products from industrialized countries. (Prabhakaran, 2012).Herbalists in Sudan for different purposes have used many plants as the unique geographical position of Sudan offer great opportunity for research in plant fields, the most important of which is medicinal and aromatic plants. About 80% of the world population still count on medicinal plants for health care. Around 20% of the drugs in modern pharmacopoeias are plant-driven either as pure phyto-pharmaceuticals extracted from plants or as semi-synthetic derivatives. (Hassan et al, 2012).Natural products have played a necessary role in antibiotic drug discovery with most antibacterial drugs being derived from natural sources.. (Parekh et al,2007).Mangrove ecosystem are used as source of flavoring agents, textiles mats, paper, housing, baskets, boats and tap cloth and also used as staple food in many countries (Abdulrazzaq,1994;Prabhu et al,2012)..The new concept of blue economy is not just about market chance it also provides for the protection and development of more insensible 'blue' resources such as mangroves which is a carbon sequestration ecosystem that increase , and coastal resilience to support vulnerable states and mitigate the often(IGAD ,2023)

Material and Method:

Samples of seeds and sediment were collected from (Kilo-Tamania stand at Amwage park) .Then transferred in plastic bags to be cultivated in the nursery of the forestry administration. Survey and visual observation and literature review at KiloTammania ,Klanaeib and Halout during field visits were carried in summer and winter to offer information about availability ,distribution

mode and seasonality abundance of seeds and naturally growing seedlings at mangrove forests. The **Study area** is affected by the Red Sea large saline water body which gives the region its peculiarity in the local characteristics of climate, especially temperature, rainfall, atmospheric, humidity and nature of wind. The temperature is generally high as 46 C˚ may be experienced in seaside especially during July and August. The mean temperature is between mean 23.0 C˚ and 33 C˚. The total Annual rain fall of the Red Sea coast is generally very low and in-consistent (Abdulfatah ,2015). **Material preparation ,**the seeds were weighed using assertive scale some of small seeds medium size and large seeds were weighed . The material used for experimental plant material was harvested after 45-60 days from the forestry nursery where they were grown in plastic bags ,watered with brackish water. The air temperature, salinity of Red Sea water during the season of cultivation was reported from literature.The harvested seedlings were dried under shade and then grinned. for further microbiological tests.Plastic bags were used for sediment and seeds collection .Photographic prints of different vegetation was taken using mobile camera .Experimental work was supplemented by two questionnaires addressed to candidates selected randomly from Port Sudan community.

Extraction Was carried using distilled water and ethanol (aqueous and ethanol) by the suxolite device. Method.(Parekh and Chanda, 2007). **The Phytochemical screening of the seedlings contents was conducted as for Phenolic Flavonoids:** One ml of

filtrate with 2 ml of 10% lead acetate gives brown precipitate **Test for Terpenoids:** One ml of the filtrate with 2ml CHCl is carefully added to few drops of concentrated H_2SO_4. An interface with a reddish brown coloration will be formed. **Test for Steroids:** To one ml of the filtrate, 10 ml. chloroform and 10 ml of H SO is slowly added by the sides of the test tube. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. **Test for Saponins:** One ml of filtrate with 2 ml distilled water, is shaken vigorously and allowed to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins. (.mouafi et al ,2014). **DPPH** (2,2-diphenyl-1-picryl-hydrazyl-hydrate 2,2-diphenyl- 1-picryl-hydrazl-hydrate) **Methodwas used for detecttion antioxidants**. The DPPH radical scavenging was determined according to the method of (Farzana et.al ,2005) with some modification. In 96-wells plate , the test samples were allowed to react with 2.2Di (4-tert-octylphenyl) -1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37˚c.The concentration of DPPH was kept as (300µM).The test samples were dissolved in DMSO (Dimethyl sulfoxide) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group (Farzana et al,2005) . **As for Cytotoxicity screening:-**Micro-culture-tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of plants. This colorimetric assay is based on

the capacity of mitochondria-succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into an insoluble, blue colored-formazan, a product measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel et al,2009).Cell Line and Culture Medium:Vero (Normal, African green monkey kidney) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37 º C. The cells were sub cultured twice a week.Cell counting: Cells were counted using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. (Parekh and Chanda, 2007). MTT assay:Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer walls of the plate were filled with 250 µl of in-complete culture medium except the last row 6 middle wells (B - G), which were used for the negative control receiving 50 µl of culture medium and 2µl of sterile 0.5% Triton X. 50 µl/wells complete culture medium (CCM) were add(

ed and 30 µl more were added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of c suspension extract were added to the 80 µl. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 µl to the next well after proper mixing. From the last dilution wells (B-11), 50 µl were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5 X 105 /ml was properly mixed, and 150 µl of it were transferred into each well of the plate. The plate was covered and placed in 5% CO2 incubator at 37 ˚C for three-five days (72 hours-120 hours). On the third/fifth day, the supernatant was removed from each well without detaching cells. MTT ((3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) stock (5 mg/ml) was prepared earlier in 100 ml PBS (phosphate buffer saline). MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. The clear suspension was filter sterilized with 0.2 µ Millipore filter and stored at 4 ˚C or – 20 until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µl of diluted MTT were added. The plate was incubated further at 37˚ C for 2 to 3 hours in CO2 incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO were added to each well. The plate was agitated at room temperature for 15 minutes then read at 540 nm using micro plate reader. The percentage growth inhibition was calculated using the formula below:

% cell inhibition = 100-{(Ac-At)/Ac} × 100

Where, At = Absorbance value of test compound; Ac =Absorbance value of control.

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program (2010)..

Antimicrobial Tests:-

The analysis was done at the National Research Center in Khartoum test microorganisms

Bacterial microorganisms:

The tested organisms include *Bacillus subtilis* (Gram + ve bacteria) ,*Staphylococcus aureus* (Gram +ve Bacteria),*Escherichia coli* (Gram -ve bacteria),*Pseudomonas aeruginosa* (Gram -ve bacteria)

Fungal microorganisms: *Aspergillus Niger* and *Candida albicans* .

Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37 ° C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4 °C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.

Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

Testing of antibacterial susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.

1. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active.
2. The results were expressed in terms of the diameter of the inhibition zone:
3. < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active;
4. >18 mm, very active. (Mukhtar et al 2012) and (Maria et al ,2000).

3 .11Crude fats:-

The required test was carried out by using official method, the analysis result are showed bellows as average value and expressed

per dry weight of the sample (w\w %). (Zimmermann et al, 1994)

3.12 Minerals :-

Was conducted accrdey to(Ong.et al.,1985).

3.13 Sediment Characteristics :-

For sediment texture and grain size sieve was used to determine sediment particle size that ranges between <2mm to 0.023 mm(Ong.et al.,1985).the weight of each fraction is then recorded.

3.14Statistical Analysis :

Collected data was analyzed using the most widely used statistical methods including the analysis of variance (test ANOVA). All values were represented in tables and plotted in histogram

Results and Discussion:

seedlings and young vegetation occupies the coastal zone land side and spread under the canopy of the mother tree. Discretion of *A. marina* seeds and seedlings

A. marina seeds as shown in plate (1) are yellowish greenish at maturity, they are elliptical average weight is between 10.0 to 15.0 gm and average dimension is (6.2 x4.3 cm) the seedlings resemble bean pods (Zahran ,2007).

4.1Phytochemical screening of A. marina:-

The results of the study proved that *A. marina* seedlings constitutes of many important phytochemicals. Its relevant to mentioned that the study findings were in agreement with (Foukia etal2014 ; Prabhu et al ;2012 and Khafagi etal,2003) where Tritepenoids, Glycoside, Steroids were reported, and disagreed with(Foukia etal,2014and Khafagi etal,2003). in the presence of

Sapoones ,Coumarens ,and absence of Anthroquines and Alkaa loide in the present study (Table 1).

Table (1) phytochemical screening of *A. marina* seedlings

Component	Coexist
Tannins	+++
Spoonines	++
Conumarins	++
Steroids	+
Glycoside	+
Triterpenes	++
Flavonoids	-
Anthroaquinones	-
Alkaloide	-

Key:-

+++ = Strongly present

++ = Moderatel present

+ = Poorly present

- = Absent

4.2 Antioxidants evaluation:-

The result as shown in table No. (2) Reveled that *A. marina* seedlings constituent of Antioxidant compared to Propyl gallate (standard) was 92 ±0.02 this is in agreement with (Parekh et al,2007)

Table (2) Anti-Oxidant contents of *A.marina* seedlings

DPPH Assay

No	Sample cod	DPPH%
1	Mangrove	92 ±0.02
2	Propyl gallate (standard)	950.02±

4.3 Cytotoxicity Screening of *A.marina* seedlings:-

Micro-culture-tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of *A.* marina seedlings. The result proved that the Ethanol extract is not toxic as shown in table . (3)

Table (3) Cytotoxicity Screening of *A.marina* seedlings ethanol abstract

No	Name of Extracts	Concentration Inhibition(%) SD			IC50(µg/ml)	IC50
		500	250	125		
1	Mangrove	68.3	60.3	49.7	124.2	>100
2	Control	96.3				<30

Key:-

SD :- standard deviation.

IC50<30µg/ml: high toxic,>100µg/ml: no toxic.

IC: inhibition concentration.

50: means the concentration that killed more than 50 % of the normal cell line.

Control= Triton-×100 was used as the control positive at 0.2µg/ml.

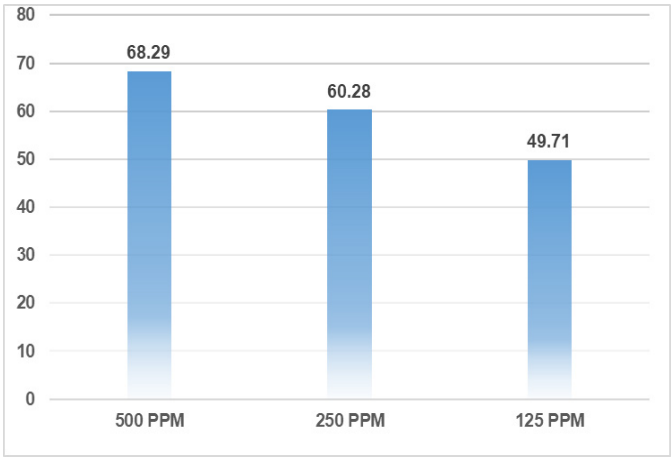


Fig (1) Cytotoxicity Screening of *A.marina*seedlings eth-anol abstract

4.4 chemical composition of *A. marina* seedlings:-

Minerals content of the *A. marina* seedlings was shown in Table (4) high concentration of Potassium (K) were recorded while low concentration of Magnesium (Mg) were reported this is disagrees with (Zhran, 2007) who report high concentration of Calcium (Ca) and low concentration of phosphorus (P) .

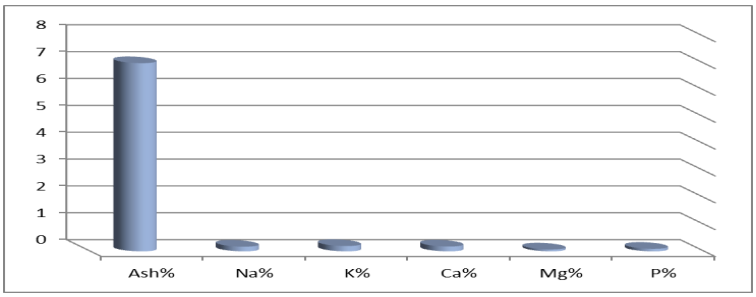


fig (2) chemical composition of *A.marina* seedlings .

4.5Crude fats in *A. marina* seedlings tissue:-

As for the Crude fats the result of *A. marina* analysis

showed that the seedlings contains about 4.706 % and this is in disagreement with (Zhran,2007) where they found average percentage of crude fats up to 13.9 in the mature mangrove leaves .

4.6 Anti-microbial activity of *A. marina* Seedlings:-

Assessment of qualitative effects of the extract prepared from whole *A. marina* seedling (roots cotyledons leaves and stems) shows its potency as antibiotic and growth regulating factor on selected pathogens(both aqueous and)ethanol extracts this is in agreement with (Foukia etal2014)where the least effect was on *Bacillus subtilis* bacteria . The highest effect was on *Pseuomonas aeruginos* bacteria,.

As for fungi this study findings showed that the highest effect is *on A spregillus niger* .

While the extract using ethanol had positive effects on the selected pathogens the water extract showed negative results .

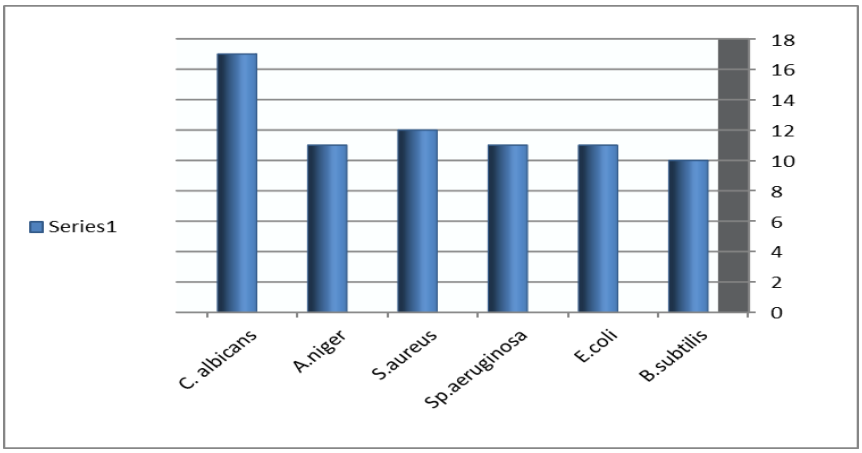


Fig (3) anti-microbial activity of *A. marina* seedlings

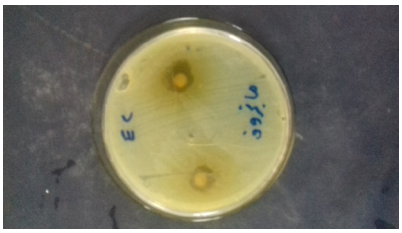
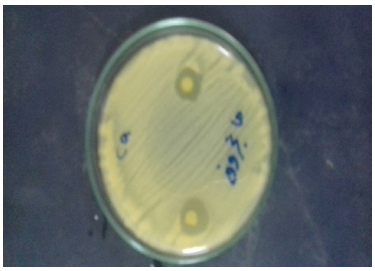


Plate (2) showing anti-microbial *Escherichia coli* of mangrove seedlings



Plate(3) showing Anti-microbial *Candida albicans.* of Mangrove Seedlings.

4.7 Characteristics of Mangrove nurseries Sediment:-

physical characteristics of the nurseries sediment used in cultivation for seedling production is shown in Table (5).

The result proved that this is the suitable type and it is in agreement with(Usife,2019) .

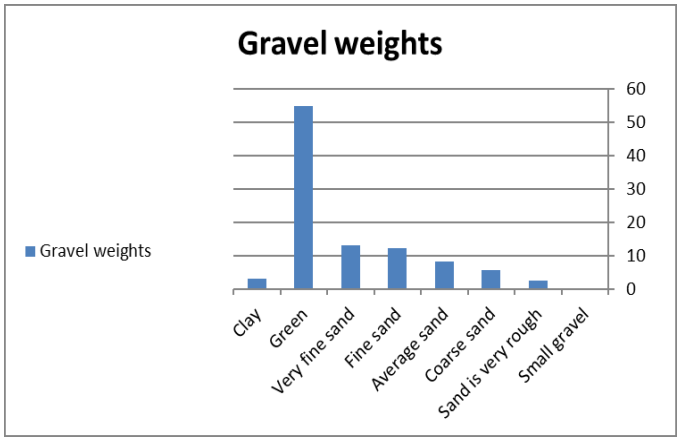


Fig (4) Sediment texture characteristics at Kilo – Tammania

4.8 Age of *A.marina* seedlings used for extraction:-

The results showed that *A. marina* seedlings suitable for harvest to be used for extraction, were with average height (15) cm, age was (54) days while the average fresh weight was (2.89) g, and the dry weight was (1.54) g as shown.

The planting material was observed to be available in sufficient amounts during December to February although the seedlings were observed to be available almost during the year round.

The difference in the investigated parameters such as Phytochemical screening, Anti-Oxidant , Cytotoxicity Screening, chemical composition, Anti-Microbial, analysis, between the study results for *A. marina* seedlings growing along the Red Sea coasts and other studies might be due to environmental characteristics of the Red Sea coast where *A. marnia* grows under stress of high temperature , high salinity and low income of fresh water (Sugga &Nasr 2024).Finally, the results the questionnaire adoted to evaluate the level of awareness with the socioeconomic values of

mangrove among the local communities revealed that (63.5%) of the interviewed interviewee were aware by mangroves medicinal value,(35.4%)used it in periodontal diseases,(24.2%) in liver diseases, while (51.5%) of interviewees acknowledge it's leaves uses in treatment, (22.2%) use all the plant in treatment, (30.3%) use seedlings in treatment, (40.4%) use mangrove contents as anti – oxedant and(24.2%) anti- tumor.

CONCLUSION:

According to the field surveys to estimate the distribution and seasonality abundance of naturally growing *A. marina* seedlings in different locations, the planting material was reported to be available in sufficient amounts during December to February in 2019 although the seedlings were observed to be available almost during the year round.

Screening of phytochemical constituents of mangrove seedlings *A. marina sp* which grows under natural conditions shows that it contains, very important chemicals this include(Spooning-Steroids Glycoside -Conumarins - Triterpenes –Tannins .)

While, Assessment of qualitative effects of the abstract prepared from whole *A. marina* seedling (roots ,leaves and stems) potency as antibiotic and growth regulating factor on selected pathogens(both aqueous and ethanol extracts) proved that there is adverse effects on four species of Bacteria namely (*Bacillus subtilis, Pseuomonas aeruginosa, Escherichia coli, Staphloccus aureus*,)and two species of fungi *Candida albicans and Aspregillusniger*

The Study proved that the physical characteristics of the nurseries soil required for seedling production consists of (small gravel(0.32), rough sand (2.55g), coarse sand(5.74g), average sand(8.32g), Fine sand(12.12g), very fine sand(13.11g), mud (54.72g) , clay(3.12g) .

The awareness level among society about the different uses of mangroves shows that (53.5%) of the interviewed were aware by its medicinal value.

Recommendations

1. Awareness among society about the economic and medical importance of the mangrove plant should be raised.
2. Extracting the active components from the seedlings of mangroves with technical methods and testing extracts for pharmaceuticals and other uses.
3. Establishing a data bank with the participation of all relevant research centers

References :

(1)Abdul fatah, A. A. (2015). The Ecological and Biological Characteristics of Mangrove Ecosystem at Mersa Daroor. Thesis submitted to Red Sea University for M.Sc. (Unpublished).

(2)Abdulrazzaq, A. A. (1994). Seedling Emergence and Growth of Mangrove *A. marina* (Forssk.) Vierh. Under Different Environmental Conditions in the United Arab Emirates.

(3)Arun Kumar, M., Asif Khan, & Abdul Muqtadir. (2010). Distribution of Mangroves along the Red Sea Coast of the Arabian Peninsula: Part-I: the Northern Coast of Western Saudi Arabia. *e-Journal Earth Science India*.

(4)Baba.S., Chan ,H.K , Oshiro .N; Maxwell ,G; Inoue,T and Chan,E W. (2016): Botany, uses, chemistry and bioactivities of mangrove plants IV: A.marinaISME/GLOMIS Electronic Journal

(5)Dias, et al. (2018). In vitro antifungal and antibacterial potentials of organic extracts of *Avicennia marina* collected from Rabigh Lagoon, Red Sea Coasts in Saudi Arabia. *Brazilian Journal of Biology*. https://www.scielo.br

(6)El-Keblawy, A., et al. (2019). Bioactive compounds and antioxidant activity of mangrove plants from the Arabian Gulf. *Industrial Crops and Products*, 138, 111460. https://www.sciencedirect.com

(7)Gurudeeban, S., Ramanathan, T., & Satyavani, K. (2013). Antimicrobial and radical scavenging effects of alkaloid extracts from *Rhizophora mucronata*. *Pharmaceutical Chemistry Journal*, 47, 50–53. https://link.springer.com

(8)Hegazy, M. E., et al. (2018). Antimicrobial diterpenes from the Red Sea mangrove *Avicennia marina*. *Phytochemistry Letters*, 26, 98–103. https://www.sciencedirect.com

(9)Ibrahim, et al. (2022). Ethyl acetate extract of *Avicennia marina* from Egypt: Antibacterial efficacy, flavonoid and phenolic content analysis. *Brazilian Journal of Biology*. https://www.scielo.br

(10)Khattab, A. R., et al. (2022). Leaf extracts from the Red Sea of *Avicennia marina*: Phytochemical composition and antibacterial efficacy. *Brazilian Journal of Biology*. https://www.scielo.br

(11)Mahady, G., Huang, Y., Doyle, B., & Locklear, T. (2008). Natural products as antibacterial agents. *Studies in Natural Products Chemistry*, 35, 423–444. https://www.sciencedirect.com

(12)Mangrio, A. M., Rafiq, M., Naqvi, S. H. A., Junejo, S. A., Mangrio, S. M., & Rind, N. A. (2016). Evaluation of Phytochemical Constituents and Antibacterial Potential of *Avicennia marina* and *Rhizophora mucronata*. *Pakistan Journal of Biotechnology*, 13(4), 259–265. http://pjbt.org

(13)Mohamed, B. F. (1984). Ecological observations on mangroves of the Red Sea shores of the Sudan. Department of Botany, Faculty of Science, University of Khartoum, Sudan.Hyrobiologia

(14)Mouafi, E. F., Abdel-Aziz, M. S., Bashir, A. A., & Fyiad, A. A. (2014). Phytochemical analysis and antimicrobial activity of mangrove leaves (*Avicennia marina* and *Rhizophora stylosa*) against some pathogens. *World Applied Sciences Journal*, 29(4), 547–554. https://www.idosi.org

(15)Okla, M. K., Alamri, S. A., Alatar, A. A., Hegazy, A. K., Al-Ghamdi, A. A., et al. (2020). Antioxidant, hypoglycemic, and neurobehavioral effects of a leaf extract of *Avicennia marina* on autoimmune diabetic mice. *Evidence-Based Complementary and Alternative Medicine*. https://www.ncbi.nlm.nih.gov/pmc

(16)Parekh, J. and S. Chanda, (2007): In vitro antimicrobial activ-

ity of Trapa natans L. fruit rind extracted in different solvents. Afr. J. Biotechnol.6:766-770.

(17)Raja Keerthi, R., Kavya, Baskar, & Mavishna, M. (2021). Antibacterial and antioxidant activity of mangrove plant (*Avicennia marina*) extract against some specific oral pathogens. *International Journal of Health Sciences*. https://www.sciencescholar.us

(18)Ramanathan, D. (2012). Phytochemical Characterization and Antimicrobial Efficiency of Mangrove Plants *Avicennia marina* and *Avicennia officinalis*. *International Journal of Pharmaceutical & Biological Archive*, 3(2). http://ijpba.info

(19)Thatoi, H. N., Patra, J. K., & Das, S. K. (2014). Free radical scavenging and antioxidant potential of mangrove plants: A review. *Acta Physiologiae Plantarum*, 36, 561–579. https://link.springer.com

(20)Vasanthakumar, K., Dineshkumar, G., & Jayaseelan, K. (2019). Phytochemical Screening, GC-MS Analysis and Antibacterial Evaluation of Ethanolic Leaves Extract of *Avicennia marina*. *Journal of Drug Delivery and Therapeutics*, 9(4-A), 145–150. http://jddtonline.info

(21)Wu, et al. (2008). Isolation of antimicrobial phytoconstituents naphthoquinones and avicequinone from *Avicennia marina* leaves, stems, and roots. *Brazilian Journal of Biology*. https://www.scielo.br