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Phytochemical and antibacterial activity of Mangrove leaf extract from Lasbella District Baluchistan

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Abstract

Mangrove plants are widely distributed all around the globe. These are being used efficiently now a day, as a low and cost effective solution of obtaining variety of new drugs which are active against various deadliest pathogens. The present study includes the following objectives; phytochemical analysis, antibacterial activity, antioxidant assay, separation of compounds and heavy metal absorption on stem and leave of Conocarpus Erectus mangrove plant. For phytochemical analysis both qualitative and quantitative tests were performed. The results of the qualitative analysis showed that, C. Erectus plant exhibits carbohydrates, aminoacids, alkaloids, falvanoids, glycosides, steroids, phenols and tannis, terpenoids, and phytosterols which are the pivotal compounds against different diseases. For quantitative analysis Folin–Ciocalteu method was performed to determine the total phenolic content. The results of the study showed that the percentage of total phenolic content in methanol is 78.6% whereas, it 84% in hexane extract. Moreover, antibacterial activity was performed by agar well diffusion method. The results showed that non-polar stem extract showed the inhibitory zone of 17mm, 24mm and 10mm. Whereas, the polar leaves extract showed 12mm 15mm and 0mm of inihibtion zone against E.coli, B. Subtilis and S. Typhi respectively. Antioxidant

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analysis was performed by DPPH analysis. The results of the study showed 78.02% inhibition of DPPH for non-polar stem extract and 86.28% of inhibition for polar leaves extract.

Keywords: phytochemicals, Conocarpus Erectus, phenolic content.

INTRODUCTION

Health care and disease prevention are amongst international objectives. These objectives are derived by a country's financial status and an urge to control unpredictable epidemics. Out of the available statistics of death rate, about 56 million deaths are recorded worldwide in 2017. The deaths recorded by non-communicable diseases (NCDs) accounts for about 73.4% deaths worldwide. Furthermore, for communicable diseases the total equates to 18.57 % for injuries it is 8.02 % (Ritchie& Roser 2020).

The chemicals that protect plants from environmental exposures such as pollution, drought, stress, pathogenic attack and radiations are known as phytochemicals. Phytochemicals are biologically active naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients. They play its role in providing colour, aroma and flavour in the plants as well as protect them from disease, cuts and damage. More than 4,500 phytochemicals have been reported and are classified on the basis of their protective functions, and physical and chemical characteristics. These biological components are the active constituents and have marked pharmacological activities. These may include flavanoids, phenols, terpenoids, saponins etc. Studies have shown that these secondary metabolites are not only effective against microbes, but also exhibit antioxidant, antipyretic, analgesic, anti-inflammatory (Vaishnav & Demain 2011) and anti-corrosive activities (Ouache *et al*., 2019).

The major classifications on the basis of structure and elements are carbohydrate, proteins, lipids, alkaloids and phenolic acids. Figure 1 is compiled to show the classification of phytochemicals.

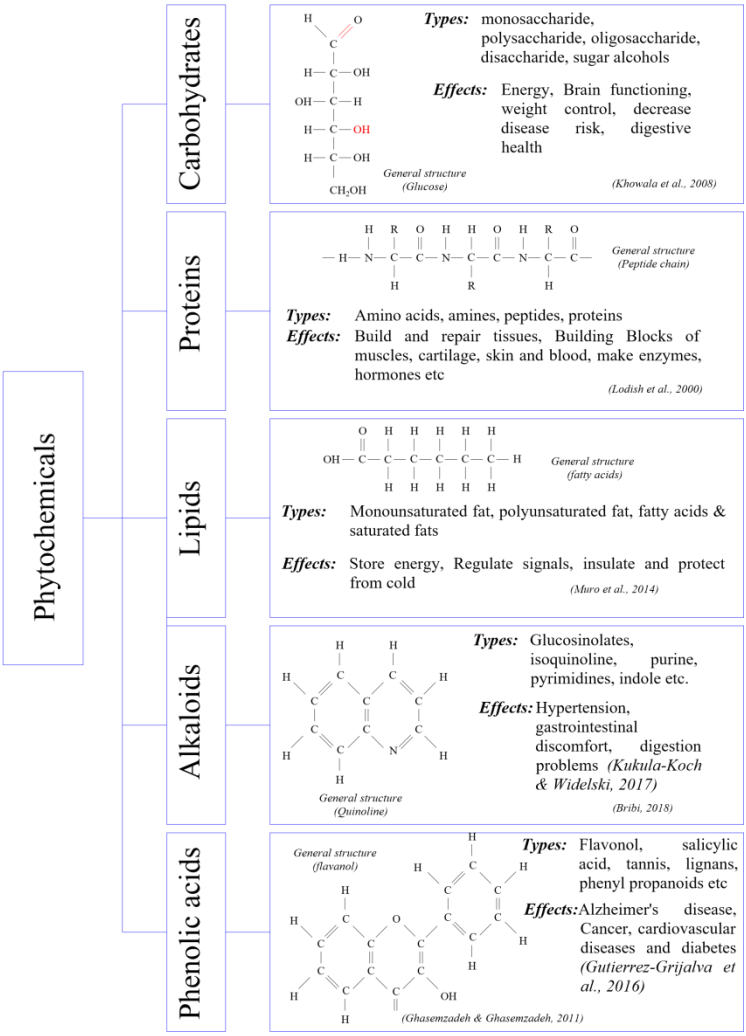

Figure 1: classification of phytochemicals

At present, microbial resistance is one of the major threats to human beings. It is defined as such situation which arise in the body when these microorganism changes in a way that there will be no effect of medicines on these microbes. According to the WHO report there is an urgency of new drugs as of increasing microbial resistance. It is reported that tuberculosis (TB) is amongst the top 10 reason of deaths worldwide. In 2017, 6 million deaths were caused by TB, out of which

95% were in lower middle income countries. According to the data there were 0.6 million cases of TB reported (Shrivastava *et al*., 2018). Another bacterium that is S. aureus is becoming resistant to antibiotic methicillin. Hence, it is becoming difficult to treat the diseases caused by the same. Therefore, it is necessary to look forward for new development of drugs, which can fight against these pathogens.

Prolong exposure to environmental stress such as ultraviolet radiations and other produces free radicals. Free radicals are defined as those molecules or compound having one or more unpaired electrons in its outer shell. These free radicals are produced because of oxidation of chemical compound and metabolism of food in living system. Free radicals are also produced as a result of drugs, cigarette smoking, pesticides etc. They have a significant role in degradation of chemical materials and food spoilage. The presence of unpaired electrons causes the highly reactive compounds to attack, and bind with the molecules in the surrounding areas to stabilize themselves (H., Zhang & Wang T 2014).

Mangroves have been a source of key interest for the existence of unique and natural ingredients. These compounds are biologically actives against vast majority of bacterial, fungal and viral species (Saad *et al*., 2012).

The objective of this research is to assess the Conocarpus Erectus Mangrove plant for different compounds. Sspecifically the following area shall be assessed.1) To detect the presence and quantity of phytochemicals by performing qualitative and quantitative tests. 2) To examine the antibacterial activity through bacterial culture of Conocarpus Erectus plant against various pathogens. 3) To detect the presence of antioxidants.

MATERIAL AND METHODS

Collection and storage

The coastal area of Pakistan is abundant with Conocarpus Erectus Mangrove plant. The plants usually occur in warm temperature and surge the high wind swirls. The plant was collected in fresh state from outskirts of Karachi and was stored in 40 kg bags. The plant was brought at the place of testing and sorting was done by manual

means. The leaf and stem were separated and washed with distilled water. The distil water was prepared in the laboratory by water distiller machine (Model no.W400, Made in England). The leaves and stem were sorted separated and were kept in covered environment for 10 days to shade dry. Dried leaves were grinded with disk mill automatic grinder (Model no. FFC 15, speed 8800rpm, power required 1.1kilowatt, Made in China) to make a granular powder. The powder obtained from leave and stem were separately stored in ½ kg plastic strip bags. About 50gms of sample is stored in each plastic bag. The powdered sample of leaf and stem were weighed on weight machine (Model no. 440 Mettler PC delta range).

Preparation of Extract

The leaf sample was weighed 531gm and solvent having pure methanol 1000mL was added to make a solution. The extract was kept for 10 days in glass bottle. After 10 days the solution was stained with nylon cloth and again stored in glass bottle. Subsequently, the total weight of stem sample was 213.894. The extract was made with different sets of weight and solvents having hexane and 70% methanol (30mL distilled water + 70mL methanol). The solvent was added with a ¾ proportionate ratio. The solutions were stored in air tight glass bottles for 3 days. Shake the bottles regularly. After the wait of three days the solutions were stained with the nylon cloth as shown in figure 2. The extracts of both leaf and stem were again put into fresh solvent to get another set of extract. As per standard procedure the process was repeated three times. The stem extracts were also prepared by standard soxhlet method. The apparatus used to perform the study was washed with methanol solvent and dried to prevent impurities. The weightings for stem and solvent are summarized and tabulated as under: After obtaining the extracts rotary evaporator (Model no. PFR 1000 E4ELA Tokyo RIKAKIKAI Co LTD, Japan) was used to separate solvent from extract. The pure extract was stored in plastic bottles with open caps so that leftover solvent is evaporated. The entire process was carried out in standard temperature and pressure conditions. The prepared extract was analyzed for the presence of phytochemicals, for both qualitative and quantitative tests.

Table 1 - shows the weightings for stem and leaves

Methods	Stem sample (gm)	Solvent Pure hexane (ml)	Solvent 70% methanol (ml)	Leaf sample (gm)	Solvent pure methanol (ml)	Time / days
Soxhlet	21gm	150mL	--	--	--	8 hours
	25 gm	200mL	--	--	--	7 hours
From stainer	50 gm each	300mL	300Ml	513gm	1000mL	3 and 10 days for former and later

QUALITATIVE TESTS

Test for Carbohydrates.

Carbohydrate tests for the qualitative screening of Conocarpus Erectus plant are performed according to the standard procedures. For the presence of carbohydrates three tests were performed namely; Molisch, Benedict and Fehling's test according to the standard procedures reported by Thirunavukkarasu *et al*., 2017.

Test for proteins and amino acids

The presence of proteins in Conocarpus Erectus mangrove plants was confirmed by performing three tests likewise; Biuret and Xanthotropic test whereas, for presence of amino acids ninhydrin test was performed as suggested by (Ali, *et al*., 2018).

Tests for Alkaloids

Alkaloids in Conocarpus Erectus plant was detected by four tests such as; Mayer's test Hager's test Dragendroff's test Wagner's test accoding to the standard procedure given by (Behlil et al., 2019).

Tests for Flavanoids

The identification of flavonoids in Conocarpus erectus mangrove plant species was confirmed by performing three tests correspondingly;

Shinoda test, Alkaline reagent test and Lead acetate test reported by AbuQaoud et al., (2018).

Test for Phytosterols

For the identification of phytosterols in Conocarpus erectus mangrove plant two test was performed reported by Thirunavukkarasu et al., (2017).

Test for Glycosides

The stem and leaf extract of mangrove plant were tested for the presence of glycosides, three tests were performed namely; Liebermann's test, Salkowski's test and Keller-killani test reported in the study performed by Gul et al., 2017.

Tests for steroids

The presence of steroids in the mangrove plant was confirmed by performing a test reported in the study, Thirunavukkarasu et al., 2017.

Test for Phenols and Tanins

For the detection of phenols and tannins a test was performed, given by Thirunavukkarasu et al., 2017.

Test for Terpenoids

The existence of terpenoids in Conocarpus erectus mangrove plant a test was performed reported by Doss 2009.

QUANTITATIVE TEST

Total phenolic content

The total phenol content of the plant stem and leaves extract were determined by using the method Folin–Ciocalteu with slight modifications. 500 mL of plant extract in methanol and hexane was mixed with 2 mL of reagent, and it was diluted nearly 10 times with distilled water. After waiting for 5 minutes, 2.5 mL of sodium

carbonate solution (Na_2CO_3) was added. Consequently, the mixture was allowed to stand for 90 minutes with irregular shaking. The absorbance was measured at 760nm of the resulting solution. The total phenolic content was determined by using gallic acid as a standard at concentration ranging between 0-600mg/L.

Antibacterial activity

The prepared extract of methanol in leaves and hexane in stem were examined for antibacterial assay. Furthermore, the samples were tested against pathogenic Escherichia Coli and Bacillus Subtilis. The antibacterial activity was determined by agar well diffusion method. The bacterial culture was prepared by weighing the nutrient agar, 28 grams of agar was weighed and dissolved in 1000ml of distil water. In addition, nutrient agar was poured in petri plates, with yellow and blue tips. Moreover, holes were created in agar medium with borer and were autoclave at 37 ºC for 20 minutes. Afterwards, the media was cooled and the selected bacterium was poured along the petri plates. 100µL of the sample was poured in the plates well at equal distance. The plates were again incubated in incubator for 24 hours at 37 ºC. However, antibacterial activity was measured in diameter, and was determined by the zone produced around the well. For the positive control Ampicillin was used to examine the antibacterial activity of mucus of fish. Subsequently, for negative control sodium chloride was used to examine the antibacterial activity (Balouiri et al., 2016).

Antioxidant Activity Assay

For analyses of antioxidant activity of Conocarpus Erectus mangrove plant, stem and leaves take 1mL of 0.004% DPPH (Diphenyl-1-picrylhydrazyl) in methanol solution (use freshly prepared solution), and add to 3mL of plant extracts of stem in hexane, and leaves in methanol at different concentrations. Afterwards, the mixture solution was kept for 30 minutes in dark, and the absorbance was noted at 517 nm. Furthermore, high radical scavenging activity was measured at low absorbance of reaction mixture. The standards used in the study were BHT and ascorbic acid. These were also analyzed. However, all the tests were performed thrice. The solution without

plant extract was used as control. The analysis of antioxidant activity was performed by the method of Yen and Chen (1995) with minor changes made in the study. The percentage inhibition of DPPH radical scavenging activity of the samples was calculated by the given formula.

$$\text{DPPH inhibition (\%)} = \frac{\text{Absorbance of blank - Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

RESULTS AND DISCUSSION

Qualitative analysis of mangrove plant

Tests for carbohydrates

The presence of carbohydrate was confirmed through Molisch test with the appearance of Violet ring at the interface. The Carbohydrate is dehydrated by the sulphuric acid which produces an aldehyde. The aldehyde reacts with α - Naphthol resulting in appearance of this violet ring. The Table 2 shows the compilation of the results of the tests for carbohydrates.

The brick red precipitates that are appeared in the Fehling's test are due to the oxidation of aldehydes by the Fehling's reagents. This test is negative for sugars containing ketone as functional group. The reducing sugars in alkaline conditions produce enediols. These enediols are powerful reducing agents. The Cupric ions are reduced to Cuprous ions. Red cuprous oxide is gradually deduced from the heating process from Cuprous hydroxide.

Test for Proteins and Amino acids

Three tests were performed for detection of proteins in both stem and leaf extract. The acquired results for both the extracts show negative results for presence of protein. Subsequently, the plant extract for stem and leaf shows positive results for presence of amino acids, which is in agreement with the results reported by (Ali, *et al*., 2018). Table 2 shows the assemblage of the results for presence of proteins.
The absence of proteins in Conocarpus Erectus plant for both the extracts was may be due to its very low concentration in the sample which may be not detected by the Benedict reagent used in the test performed. The results were also negative for Xanthotropic test for proteins in both the extracts that confirms the sure absence of

proteins in the same. The blue coloration for presence of amino acids is due to the reaction of amino acids with Ninhydrin reagent which yields ammonia (NH_3), Hydrindantin (reduced form of Ninhydrin) and carbon dioxide (CO_2) upon oxidative Deamination. The ammonia produced during the process reacts with Ninhydrin molecule to give Diketohydrin called as Ruhemann's complex, which is accountable for the development of deep blue colour.

Test for Alkaloids

For the identification of alkaloids in Conocarpus erectus mangrove plant four tests were performed, all the tests shows positive results for the presence of alkaolids as tabulated in table 2. These results were in accordance with the results reported by (Behlil et al., 2019).

The presence of alkaloids is confirmed by the turbidity of the solution through Mayer's test, which is due to the reaction of potassium ion of dipotassium tetraiodomercurat ($K_2(HgI_4)$), with the electrons of nitrogen atom. Thus, the solution gets turbid indicating the presence of alkaloids. The appearance of yellow precipitates in Hager's test is due to the reaction of reagent with the nitrogen of an alkaloid; hence a complex is obtained which form precipitates of yellow colour in solution. However, in Drangendroff's test for alkaloids orange coloured precipitates are appeared due to the reaction of nitrogen commonly present as tertiary amines in alkaloids with the heavy metals of the reagent, as a result of which an ion pair is formed which produces an orange coloured complex. Subsequently, reddish brown precipitates in Wagner's test are formed as a result of binding of the nitrogen of alkaloids with potassium ion of the reagent, via a coordinate covalent bond. In addition a complex of potassium alkaloid is formed which give reddish brown precipitates.

Test for Flavonoids

The identification of flavonoids in Conocarpus erectus mangrove plant species was confirmed by performing three tests which gives positive results for the presence of flavonoids in the plant. The obtained results were in accordance with the results reported by AbuQaoud et al., (2018) as shown in the table 2.

For the presence of flavonoids Shinoda test was performed, which give positive result because of reductive elimination reaction which yields stable anthocyanidin product. However, conjugation in the compounds of flavonoids gives yellow colour to the solution. Whereas, extended conjugation in anthocynaidin product moves the colour towards the redish pink region of the spectrum. This reaction is similar to the Clemmmensens reduction reaction. The appearance yellow colour in alkaline reagent test was due to the addition NaOH which deprotonates the polyphenolic molecules present in flavonoids. Furthermore, this sodium hydroxide turns the phenolic compounds in respective phenoxides, which are more soluble in water as compared to the phenols. The above solution decolourizes upon of addition of small amount of hydrochloric acid (HCL), which therefore prevents the development of many coloured phenolic complexes and all enols. Hence, confirming the presence of flavonoids.

Test for Phytosterols

For the identification of phytosterols in Conocarpus erectus mangrove plant two test was performed. The obtained results were in accordance with the results reported by Thirunavukkarasu et al., (2017) as given in table 2.

In the above test colour change was observed which is due to the addition of concentrated sulphuric acid which removes water molecule from the third carbon atom of cholesterol molecule and hence oxidizes to give 3,5-cholestadiene. This compound gets converted to a polymer named chromophore which gives colour to the solution, confirming the presence of phytosterols.

The appearance of steroidal ring in salkowski test is on account of the formation of di-sulphonic acid and di-cholestadiene. This later compound is formed in a reaction when concentrated sulphuric acid removes two water molecules from cholesterol and di-cholestadiene is formed. Subsequently, sulphuric acid sulphonates this compound at 7,7 location of aromatic ring resulting in the formation of di-sulphonic acid of di-cholestadiene which is responsible for the development of red steroidal ring.

Test for Glycosides

The stem and leaf extract of mangrove plant were tested for the presence of glycosides. The result of the tests shows the presence of glycosides in Conocarpus erectus plant extracts for both stem and leaves. The results of the tests were in compliance with the results obtained in the study performed by Gul et al., 2017. The table 2 summarized the results of the tests for glycosides.

Steroidal ring is appeared in salkowski test due to the formation of disulphonic acid and dicholestadiene. This compound gives red steroidal ring in the solution. The formation brown ring in keller killani test is the result of the acid hydrolysis of deoxy sugars that is digitoxin which later transformed to digitoxigenin and digitoxose. This molecule eventually transformed to cymarose which is responsible for the colour formation.

Test for Steroids

The presence of steroids in the mangrove plant was confirmed by performing a test. The results were positive for the existence of steroids which were in conformity with the results reported in the study, Thirunavukkarasu et al., 2017 as shown in table 2. The red colour is appeared because of the development of disulphonic acid of dicholestadiene during the reaction which is responsible for the colour formation and confirms the presence of steroids.

Test for Phenols and Tannins.

For the detection of phenols and tannins a test was performed, the results obtained from the test as shown in table 2 were in accordance with the results reported by Thirunavukkarasu et al., 2017.

Phenols and tannins both were present in Conocarpus erectus plant. The black colour appear due to the reaction of both of these compounds with Fe^{+3} ions from iron (III) chloride, thus resulting in the formation of a complex black coloured compound.

Test for Terpenoids

The existence of terpenoids in Conocarpus erectus mangrove plant a test was performed, which shows positive results for both stem and leaf extract. The obtained results were in accordance with the results reported by Doss 2009 as shown in table 2. The emergence of reddish brown colour in the extracts for terpenoids is because of the formation of bisulphonic acid of bicholestadiene the end product of the reaction.

Test for Saponins

For occurrence of saponins in Conocarpus Erectus mangrove plant a test was performed according to the study as shown in the table 2. The result for the presence of saponins in leaves indicates positive outcomes as per the results given in (Bankole et al., 2016). However, the stem extract of Conocarpus erectus gives negative results. The negative results may be due to the lower quantity of glycols and steroids in stem which may not be detected in the plant.

The stable form for saponins in the above test is appeared because it contains glycosyls as polar groups and steroids etc as non-polar group. Both these groups that are polar and non-polar present in the compounds are surface active. When shaken thoroughly with water it forms miscellanea. In the formation of these miscellanea the non-polar groups are directed outside while polar groups are directed inside. Hence, this phenomenon is responsible for the formation of stable foam.

Table 2: shows compilation of Phytochemical tests

Phytochemical compound*	**Methanolic extract 70%**	**Pure Methanolic extract**	**Results/Remarks**	**Reference**
	Stem	**Leaves**		
Test for Carbohydrates				
Molisch test	+	+	Violet ring	
Fehling's test	+	+	Brick red precipitate	***Thirunavukkarasu et al., 2017***
Benedict test	+	+	Reddish brown precipitate	
Test for proteins				
Biuret test	-	-	No violet colour.	
Ninhydrin test.	+	+	Blue colour.	***Ali et al ., 2018***
Xanthotropic test	-	-	No yellow colour.	
Test for Alkaloids				
Mayer's test	+	+	Solution gets Turbid.	
Hager's test	+	+	Yellow precipitates.	***Behlil et al., 2019***
Dragendroff's test	+	+	Orange precipitates.	
Wagner's test	+	+	Reddish brown precipitates are formed.	
Test for Flavonoids				
Shinoda test	-	-	No pink colour.	
Alkaline reagent test.	+	+	Intense yellow turned colourless on addition of few drops of acid.	***AbuQaoud et al.,2018***
Lead acetate test	+	+	Yellow colour.	
Test for Phytosterol				
Liberman-Burchard's test.	+	+	Colour change.	***Thirunavukkarasu et al., 2017***
Salkowski's test	+	+	Brown ring.	
Test for Glycosides				
Liebermann's test	+	+	Violet to green.	
Salkowski's test.	+	+	Steroidal ring.	***Gul et al., 2017***
Keller-killani test.	+	+	Brown ring.	
Test for Steroids				
Steroids	+	+	Red colour.	***Thirunavukkarasu et al., 2017***
Test for Phenols and				

Tannis				
Phenols and Tannis	+	+	Black colour.	***Thirunavukkarasu et al., 2017***
Test for Terpenoids				
Terpenoids	+	+	Red brown colour.	***Doss 2009***
Test for Saponins				
Saponins	-	+	Stable foam.	***Bankole et al., 2016***

'+' shows presence of carbohydrates, '–' shows absence of carbohydrates

Quantitative analysis.

Total phenolic content

Table 3 - shows total phenolic content

Plant extract	Absorbance		Results in %	
Methanol	0.158	0.393	78.6	78.6%
Heaxane	0.491	0.42	84	84%

Table 3 shows the absorbance and results of the total phenolic content. The percentage of total phenolic content in methanol is 78.6% whereas, it 84% in hexane extract. The greater quantity of phenol in hexane extract shows that it is best suited solvent for phenol absorption in comparison to methanol extract.

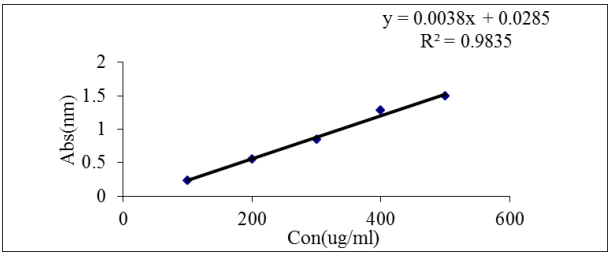

Figure 2: shows the standard curve

The standard curve was constructed by using gallic acid as a standard solution ranging between 0-600mg/L as shown in figure 2.

Antibacterial activity

The mangrove plant stem and leaves were tested for antibacterial activity. The non-polar stem extract in n-hexane has shown the inhibitory diameter of 17mm against E. Coli. Conversely, the polar leaves extract in methanol showed the inhibitory zone of 12mm. However, B. Subtilis showed the inhibitory zone of 24mm against the

non-polar extract. On the other hand, the polar leaves extract showed the inhibition of 15mm, as shown in table 4.

Table 4 - Antibacterial activity against the selected pathogens, and diameter of zone of inhibition in (mm) by agar well diffusion method.

Bacteria type	**Pathogenic strains**	**Zone of inhibition (mm)**	
		Non- polar extract	Polar extract
		n-Hexane (stem) 100µL	Methanol (leaves) 100µL
Gram negative	Escherichia Coli	17mm	12mm
Gram positive	Bacillus Subtilis	24mm	15mm
Gram negative	Salmonella typhi	10mm	0mm

Ampicillin used as positive control; sodium chloride used as negative control

E. Coli is known to cause traveller's diarrhea, pneumonia, bacteremia and neonatal meningitis Bhimba *et al* (2010). B. Subtilis is known to cause endocarditis, bacteremia, pneumonia and septicaemia. Salmonella typhi is the leading agent responsible for the development of lethal disease known as typhoid. At present, antibiotic resistance is increasing rapidly. The present study provides a hope to overcome antibiotic resistance, by the formation of new drugs against infectious pathogens. Previous studies have shown that mangrove plants exhibit a huge quantum of biologically active compounds. These include; phenols, saponins, flavonoids, terpenoids, alkaloids and glycosides etc. Studies have shown that these natural compounds are the active molecules responsible for inhibiting the growth of bacterial species. The non-polar hexane extract of stem showed maximum zone of inhibition against E. Coli. Similarly, non-polar hexane extract of stem extract has shown significant reduction in the growth the bacteria against B. Subtilis. Whereas, the non-polar stem extract against salmonella typhi showed zero inhibition. The obtained results from the study were corroborated by the findings of Ramasubburayan *et al*., (2015). However, the polar methanolic extract of leaves has shown notable activity against E. Coli. Whereas, the methanolic extract of leaves has shown considerable activity against B. Subtilis. Moreover, the polar extract showed efficient inhibition against salmonella typhi. The reported results were in accordance with the result reported by Naikpatil & Rathod (2011). Consequently, it was concluded that stem extract in hexane of Conocarpus Erectus plants is more active in

inhibiting the growth of these infectious microbes likewise; E. coli and B. subtilis in comparison to the polar extract. On the contrary, it showed zero inhibitions against S. typhi which means that the stem sample is not efficient in overcoming the deadliest effects of the same. Moreover, the polar extract in leaves showed maximum zone of inhibition against S. typhi which means this part of the plant is capable to fight against the deadlier virus. Hence, mangrove plant shall be used as a source of production of new drugs to control the uprising bacterial resistance.

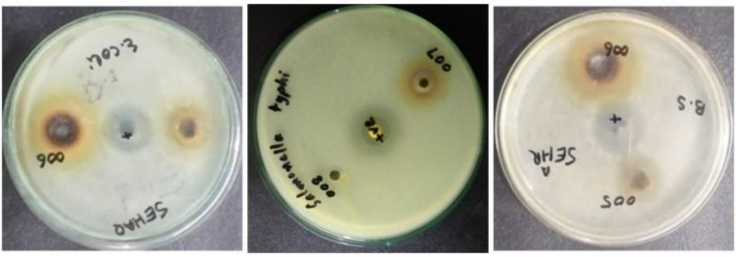

Figure 3: Antibacterial activity

Following figure 3 shows the antibacterial activity of the three selected pathogens (a) E.Coli (b) S. Typhi (c) B. Subtilis.

Antioxidant activity.

Table 5- shows percentage inhibition of DPPH

Sample	Solvents	Blank absorbance	Sample absorbance	% inihibition of DPPH
Stem	Hexane	2.56	0.564	78.02%
Leaves	Pure methanol	2.567	0.352	86.28%

The table 5 shows the compilation of percentage of inhibition of DPPH. The results of the study showed that stem sample in hexane gave the absorbance at 0.564, with percentage inihibition of 78.02%. Whereas, leaves sample in methanol showed the absorbance at 0.352, with percentage inhibition of 86.28%, which is in corroboration of the results reported by (Arulkumar *et al*., 2020)

Antioxidants are capable of scavenging activity against the free radicals which are produced as a result of various biological processes. The obtained results suggested that the leaves sample in methanol showed greater percentage of DPPH inhibition, than the

stem sample in hexane. This could be due to the presence of flavanoids and phenols containing the –OH group which are able to scavenge free radicals particularly hydrogen peroxide. Moreover, the hexane extract also showed magnificent antioxidant activity. Consequently, it has been shown that Conocarpus erectus plant is able to denote hydrogen ions to stabilize the free radicals; hence, mangrove plants shall be used as a source of antioxidant activity.

CONCLUSION

Conocarpus Erectus mangrove plant possesses huge quantum of phytochemicals which are the active ingredients in curbing the microbial infections. Is also encompasses a huge proportion of antioxidants which are the effectively working to overcome the free radicals. Moreover, Concocarpus Erectus has tremendous ability to absorb heavy metals thus protect the aquatic and terrestrial animals and environment. This plant has numerous compounds which are easily separated by thin layer chromatography. All in all this plant has outstanding properties and compounds which enable it to use for the formation of new drugs and as an agent to overcome the environmental aquatic stress.

FUTURE RECOMMENDATIONS

Study needs to be conducted to evaluate this plant for the detection of compounds separated by thin layer chromatography. Study needs to be conducted to evaluate the functions of the compounds present in the plant. Study needs to be conducted to determine the total flavanoid content of Concocarpus Erectus of Pakistan.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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