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Nutritional status, antimicrobial and anti-biofilm activity of *Potamogeton nodosus* Poir

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ABSTRACT

Macrophytes have been approved as a natural resource for nutritional components as well as antibacterial and anti-biofilms agents. In this context the nutritive status and the antimicrobial activity of the Egyptian macrophytes species *Potamogeton nodosus* have been studied. *P. nodosus* have approached a high nutritional value which was reflected in lower ash (11.93%) and considerably high organic nutrient level (88.07%). In addition, this plant contains high protein, carbohydrates, potassium and nitrogen contents with very low concentrations of micro-elements (Iron, Manganese, Zinc and Copper) which make it a valuable source for fishes and animals diets. The extracts also revealed a high phenolic and flavonoids contents (19.313 mg/g gallic acid equivalent and 17.885 mg/g rutin respectively). Both antibacterial, and anti-biofilm activity of three solvents, chloroform, methanol and petroleum ether extracts of *P. nodosus* Poir were tested against *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922.The methanol extract showed the highest antimicrobial and anti-biofilm activity. The findings of this investigation suggested the potential of *Potamogeton nodosus* as a new supplementary food resource. In addition to the antimicrobial activity of its extracts especially methanol extract, which would have promising applications in this field.

INTRODUCTION

The shortage in green summer fodder in addition to increasing cost of imported feed ingredients is become one of the serious problems that limit animal production in many countries like Egypt (Shaltout *et al.* 2010). In addition, bacterial antibiotics resistance has been become one of the vital issues which received the priority concern of public health (Byarugaba 2004, Okeke *et al.* 2005, Joana *et al.* 2014). This of course is due to the antibiotics misapplication in many fields such as disease treatments, food and feed industry, crop production and disinfectants in farms, hospital and households (Bloomfield 2002, McEwen and Fedorka-Cray 2002, Vidaver 2002, Wise and Soulsby 2002). By default, not only bacterial mutations and/or antibiotic resistance have emerged but also there is an increase in their ability to form biofilms. Whilst free floating (planktonic) cells are already resistant to many antimicrobials, unfortunately in biofilms their resistance increase more than 1000 times. The bacterial biofilms may be found just about anywhere, such as water distribution lines, water storage tanks, living tissues, contact lenses, medical devices, where cause a wide range of human infections (Del Pozo *et al.* 2009, Kokare *et al.*

2009). Thus, it has now become very much important to figure out a newer natural food resources with antibiotic properties.

Natural products driven from plants were considered as essential source of many valuable phytochemicals, which had been reconnoitered as an effective antibiotic source (Daboor and Haroon 2012, Abreu *et al.* 2013). These, phytochemicals revealed effective different modes of action not only impairing cell wall synthesis, damaging microbial membrane structures, but also modifying both bacterial membrane surface hydrophobicity and quorum- sensing (Rasooli *et al*. 2008, Jelena *et al.* 2014).

Among the numerous plant secondary metabolites phenolic products constitute one of the most important and widely distributed groups, commonly found in diverse dietary products, particularly vegetables, fruit, chocolate, and beverages (Soobrattee *et al*. 2005). As recorded by Vaquero *et.al.* (2010 and 2011) these phenolic products were subdivided into three groups: phenolic acids, flavonoids, and tannins, which can induce a wide range of biological effects (e.g. antibacterial, antifungal, antiviral, anti-inflammatory, antiallergic, and vasodilatory action).

Potamogeton nodosus Poir, known as Longleaf pondweed is one of floating aquatic macrophytes, belonging to the genus *Potamogeton* and family *Potamogetonaceae.* It is widely distributed in continental and subtropical zones of both hemispheres (Africa, Asia, Europe and North America) Josifovic (1975). *Potamogeton* contain many active compounds such as; alkaloids, tannins, steroids, flavonoids, and a labdane diterpenoid with antimicrobial activity (Haroon 2006, Fareed *et al*. 2008, Durdevic *et a*l. 2014, Haroon and Abdel-Aal 2016). Another point of view deals with the nutritional components of *Potamogeton* species, however no clear findings were for the nutritional value and antimicrobial activity of the Egyptian macrophyte species *P. nodosus* were approved. Herein, we assessed the nutritional value as well as the antimicrobial and antibiofilm efficacy of *Potamogeton nodosus* different extracts against two Gram negative biofilm producers *Escherichia coli ATCC 35218* and *Pseudomonas aeruginosa ATCC 27853*. In addition, the total phenolic and flavonoids contents of the different plant extracts were detected by using HPLC technique using Gallic acid, Tannic acid and Rutin as calibration standards.

MATERIALS AND METHODS

Macrophytes collection and preparation.

Potamogeton nodosus Poir was collected in summer 2017 (when *Potamogeton nodosus* had the maximum biomass production) from the region of Etay El- Barood (33° 54`38.06"Latitude & 30° 29`.52"Longitude), located in the northern part of El-Rayah El-Nassery River Nile, Egypt. Identification and classification of the plant material was performed according to (Tackholm 1974). The collected plant material was cleaned up from epiphytes and non-living matter, rinsed several times with fresh water, dried at 50^{o}C till constant weight, mechanically grinded into a coarse powder and stored in paper bags until used.

Chemical analysis

Ash percentage was determined after combustion for four hours at 550°C using Muffle Furance (Egyptian Pharmacopoeia 1953). Plant elements and heavy metals were extracted using sulphuric acid and perchloric acid according to the method described by (Burgski 1968). Total nitrogen was assessed by Kejeldahl method (Black 1965). Total phosphorus was measured calorimetrically using the

hydroquinone method described by (Snel and Snel 1967). Total K was photometrically estimated as described by (Brown and Lilland 1946) using a flame photometer (CORNING M410); Fe, Mn, Cu and Zn using atomic absorption (Shimadzu AA-6200), the content of lipid was estimated according to (Blight and Dyer 1959). The total protein content was calculated by multiplying the nitrogen concentration by 6.25 (Ölberg 1956). Nitrogen free extracts (NFE) comprising the sugars, starches and a large part of the material classed as hemi-cellulose was determined according to the equation applied by (Pádua *et al.* 2004):

NFE (in %DW) = 100 – (CP +EE +Ash); where CP = Crude Protein, EE = Ether Extract (total lipids) and ash.

Calculated parameters.

Digestible crude protein (DCP) was estimated according to the equation of (Demarquilly and Weiss 1970): DCP (in % DW) = 0.929 CP (in %DW) – 3.52.

Protein to energy ratio (P/E) was calculated as mg crude protein / K cal EV

Metabolized Energy (ME) was estimated according to **(**Pantha 1982), using the values of 3.4, 8.1 and 4.2 Kcal /100 g DW for carbohydrate, fat and protein respectively.

The energy contents (EV) were determined on the basis of the biochemical composition using the standard conversion factors, for lipids 9.45, carbohydrates 4.10 and protein 5.65 (Brody 1945) and expressed as Kcal /100 g DW where, cal = Calorie; DW= Dry Weight.

Extracts preparation

The dried, ground plant material was extracted by soxhlet apparatus using different organic solvents (petroleum ether 40-60 °C, chloroform 61.15 C and methanol 64.7 °C) and the extraction process was done according to the method adopted by (Sadasivam and Manickam 1996). After complete extraction, extract of each organic solvent was collected separately, dried, weighted and the percentage of each extract was determined following (Abdel-Aal *et al*. 2015). The residues were stored in the dark at 4°C to prevent degradation until used.

Determination of total phenolic and flavonoids contents

The total phenolic and flavonoid contents were separated and determined using high performance liquid chromatograph (HPLC) Shimadzu instrument. The extracted samples were analysed against tannic acid (TA) and gallic acid (GA) as a standards for phenolic compound, while rutin (RU) was used as flavonoid standard. Analyses were conducted at constant temperature of 25 °C using a flow rate 1 mL/min and a sample injection volume of 10 L. Detector wavelengths of 280 nm for gallic acid, tannic acid and 265 for rutin were used.

Bacterial strains and Bioactivity assay using agar diffusion:

Pseudomonas aeruginosa ATCC 27853, and *Escherichia coli* ATCC 25922 were thawed and grown on Trypticase soy agar (TSA) for overnight incubation (18-20 h) at 37°C, one single colony was inoculated into 2 ml Trypticase soy broth (TSB) and incubated for 24 h, at 37 °C and 200 rmp. After, the bacterial culture was adjusted to OD 0.5 at 600 nm, Mueller-Hinton agar plates were covered with 100 µL of each bacterial strain, 5 mm pores were filled with 100 µL each extract at 10 mg/ml. The plates were incubated for 24 h at 37°C, the zones diameters of inhibitions were measured in mm.

Assessment of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Both MIC and MBC were determined for the three extracts: methanol (Me) petroleum ether (Pe) and chloroform (Ch) that showed antimicrobial activity, by a

broth micro-dilution method proposed by (Novy *et al.* 2015), with minor modifications. Briefly, plant extracts were dissolved in 10% DMSO and prepared with Mueller-Hinton Broth at a total volume of 100 µL per well in the 96-well plates. The final concentrations of extracts ranged from 20 and 0.04 mg/ml. The bacterial suspension OD 0.5 at 600 nm was diluted 1:100 (≈ 10^6 CFU/ml), non-treated bacterial suspension served as control while medium broth only considered as blank. After overnight incubation, relative humidity 40-50 and 37ºC, absorbance was detected at 600nm using a microtiter plate reader (Expert Plus UV; ASYS Hitech GmbH Eugendorf, Austria). MIC was defined as the lowest concentration of the tested extract that restricted the visible growth of tested strains compared with the blank (Briceland *et al*. 1987). MBC values were detecting by plating 5 µL of each well which had no visible bacterial cells, only concentrations which produced less than 45 colonies per plate were considered bactericidal as defined by inhibition of 99.9% of the starting population (Schoenknecht *et al*. 1985). All experiments were performed in triplicate and the average values were reported as MBC.

Time kill assay

P. nodosus methanol extract was examined against the two bacterial strains using a time kill assay. Both bacterial strains were grown overnight in TSB under appropriate conditions, 100µl cultures (0.1 at 600 nm) of each representative strain, were added to TSB medium mixed with different concentrations of *P. nodosus* methanol extract (1.25, 2.5, and 5.0 mg/ml were tested against *P. aeruginosa* and 0.6, 1.2 and 2.4 mg/ml were used against *E. coli*). After different time points 0, 30 min, 1, 2, 4, 8, 12 and 24 h aspirated samples were serially diluted, plated on TSA plates and incubated for 24 h at 37ºC. The viable counts were determined expressed as CFU/ml (Teanpaisan et al. 2014).

Biofilm formation assay and quantification

The biofilms were assayed as described by (O'Toole and Kolter 1998). In sterile 96-well microtiter plates each well contain 180 µL TSB broth and 20 µL of bacterial suspension OD 0. 5 at 600 nm. After 48 h incubation at appropriate conditions all the planktonic cells were removed, the biofilms were gently washed twice with phosphate buffer saline (PBS) to remove free-floating bacteria. The formed biofilms cells in each well was stained with 200 µL crystal violet (0.1% w/v) and incubated at the room temperature (28 ºC) for 10 min. The stain was removed and washes with distilled water for 30-60 seconds. After 5 min. air drying, the biofilms were solubilized by 200 µL of 98% ethanol, then the optical densities of stained adherent biofilms were measured at 600 nm using microplate reader. (Stepanović *et al*. 2000).

Eradication of biofilm formation

The antibiofilm activity of *P. nodosus* extracts were also examined using the minimum biofilm eradication concentration (MBEC) assay. As mentioned above each representative strain was inoculated into each well of the 96-well microtiter plate, *P. nodosus* extract were added to each well to cover the concentration 20 to 0.6 mg/ml and incubated for 24 h at 37°C. The non -adherent bacterial cells were removed and washed with sterile PBS and the biofilms were assayed using the crystal violet staining assay as described above (Stepanović *et al*. 2000). The percentage of biofilm inhibition was calculated using the following formula:

[(OD negative control – OD extract) / OD negative control] × 100.

The biofilm inhibition concentration (BIC50) was defined as the lowest concentration of extract that showed 50% inhibition on the biofilm formation (Chaieb *et al.* 2011). Solvent control test was performed in order to study an effect of 10% DMSO on the growth of tested strains, no inhibition activity was observed. The

MBEC value was defined as the concentrations that showed at least 50 % reduction of biofilm formed on wells.

Statistical analysis

Standard division and statistical analysis were done suing statistical package program SPSS for Windows, Version 25.0. (Armonk, NY: IBM Corp.)

RESULTS AND DISCUSSION

Biochemical composition and elemental analysis of *Potamogeton nodosus.*

Results in Tables 1 showed that, *P. nodosus* contains (as % DW) 88.21% of water, 88.07 % of organic matter, 11.93 % of ash, 70.35% of NFE, 13.13% of total protein and a very low content of lipid 4.60 %. In addition to a high value of digestible crude protein 8.67% and suitable value of energy value 4.06 K Cal/ g DWand metabolized energy 3.32 K Cal /g DW. The elemental analysis of macro and micro-elements (Table, 1) showed that *P. nodosus* contains (in % DW) 3.40 K, 2.10 N and $1.93*10^{-2}$ % of P. The plant was also containing (in µg/ g DW) 1570.75 of Fe, 164.25 of Mn and very low concentrations of Zn and Cu.

Table 1: Mean values ± standard deviation of organic and inorganic constituents of *Potamogeton nodosus*.

Biochemical composition (% DW)	
Water content	88.21± 0.11
Ash	11.93±0.00
Organic matter	88.07± 0.11
NFE	70.35±0.02
Protein	13.13±0.02
Lipid	4.60±0.10
Calculated parameters	
DCP %	8.67±0.02
EV (K Cal/g DW)	4.06±0.03
ME (K Cal/ g DW)	3.32±0.04
P/L %	2.85±0.03
P/E %	3.23±0.02
P/NFE %	0.19±0.05
Macro-elements (% DW)	
K	3.40±0.001
N	2.10±0.003
$P*10^{-2}$ %	1.93±0.00
Micro-elements (µg/gDW)	
Fe	1570.75±0.01
Mn	164.25±0.07
Zn	17.25±0.01
Cu	15.00±0.02

Where: DW= Dry Weight, Digestible crude protein (DCP), Energy value (EV), Metabolized energy (ME), Protein/Lipid (P/L), Protein/ Energy (P/E) and Protein/Nitrogen Free Extracts (P/NFE).

Comparing the results of this species with the previously recorded results for *Potamogeton pectinatus*, *Ceratophyllum demersum*, *Eichhornia crassipes*, *Echinochloa stagnina*, *Polygonum tomentosum*, *Pistia stratiotes*, *Nymphaea lotus*, Azolla filiculoides and *Potamogeton crispus* (Haroon 2008, 2009 and 2010, Sharshar and Haroon 2009, Shaltout *et al*. 2010, 2012 and 2016) which also grow in the water courses of Nile Delta, it is clear that NFE and lipid contents were higher in *P. nodosus* than in other species, while ash and micro-elements were lower than that reported for other species, in addition the plant was characterized by high organic matter contents and a suitable amount of protein which was higher than that reported

for *Echinochloa stagnina* L. and *Polygonum tomentosum* L., nearly similar to that reported for *Eichhornia crassipes* and *Pistia stratiotes* but lower than other species.

These results indicated that *P. nodous* has the ability to absorb nutrients and very small concentrations of heavy metals from water and can act as fodder but not as a bio- filter.

Total phenolic and flavonoid contents.

In this investigation the total extraction yield, total phenolic and total flavonoid contents of three different organic solvent (petroleum ether, chloroform and methanol) of *P. nodosus* were evaluated and the results are presented in Table (2).

Table 2: Variation in % crude extracts, total phenolic and flavonoid contents of *Potamogeton nodosus* with different organic solvents.

Solvent	Extracts yield (g100/g DW)	Total phenolic content		Flavonoid content mg RU/g of extract
		mg GA/g of extract	mg TA/g of extract	
petroleum ether	1.03	2.271	406.438	4.524
Chloroform	1.94	1.837	131.647	13.358
Methanol	5.34	19.313	ND	17.885

The results showed that, the solvent type had a significant effect on the extraction yield, where methanol maintained the highest percentage of extraction yield (5.34 % g DW) followed by chloroform (1.94 % g DW) and petroleum ether (1.03% g DW). The TPC and TFC in the three different crude extracts of *P. nodosus* were analysed using HPLC technique and the chromatograms of different crude extracts are shown in Figs. 1and 2.

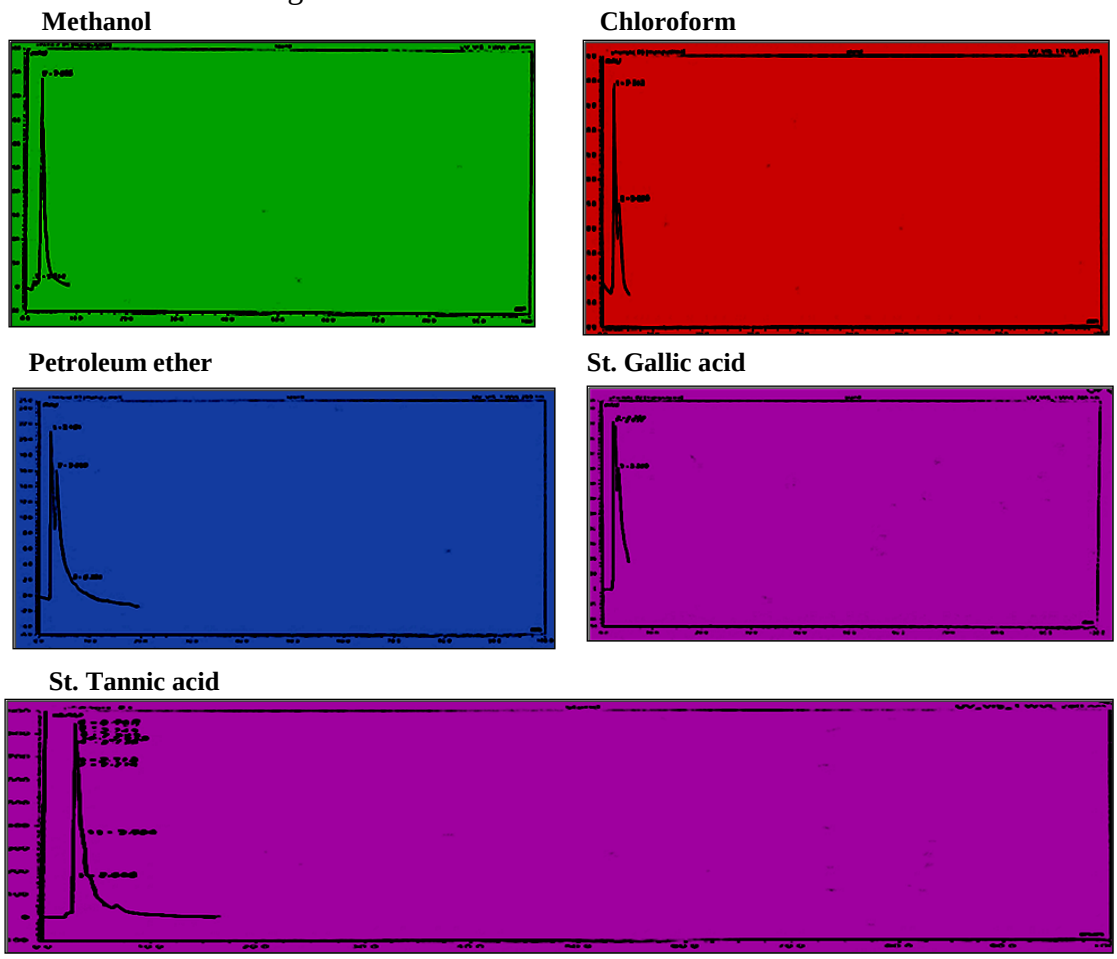

Fig. 1: High performance liquid chromatography (HPLC) chromatograms of total phenolic content detected in *P. nodosus* different extracts using gallic acid and tannic acid as a standard.

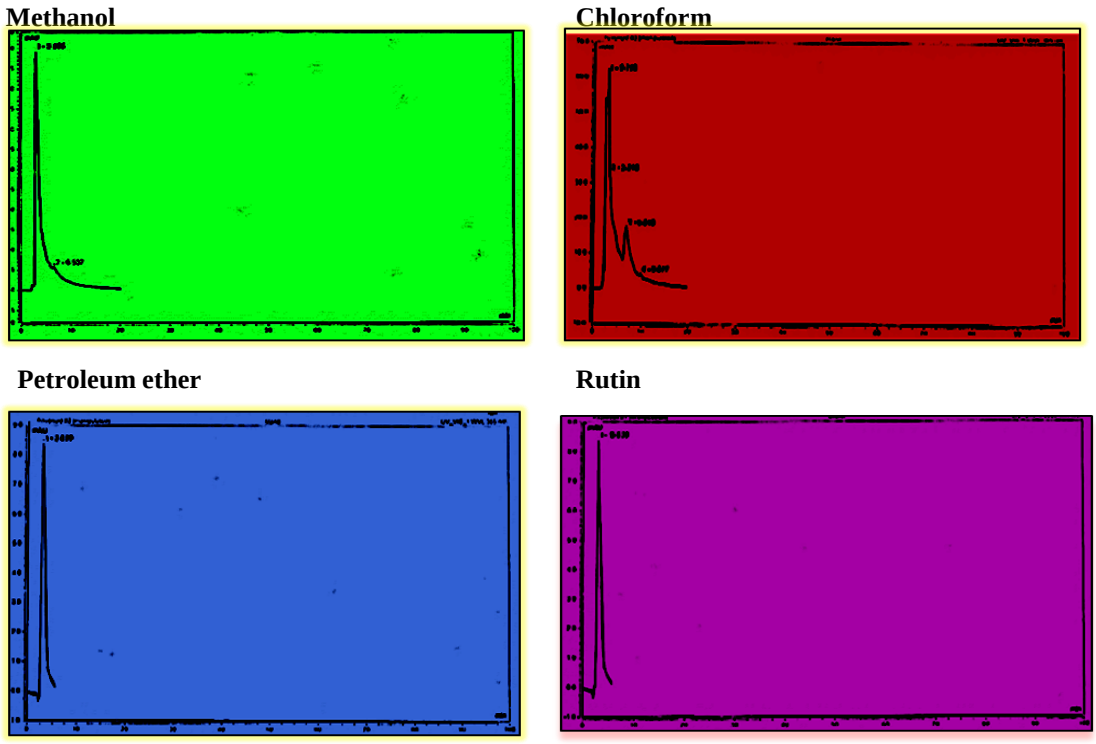

Fig. 2: High performance liquid chromatography (HPLC) chromatograms of total flavonoids detected in *P. nodosus* different extracts using rtuin as a standard material.

The highest total phenol content was found in the methanol extract (19.313 mg GA/g) while the lowest content was in the chloroform extract (2.271 mg GA/g) and the similar case was with the total flavonoid content, up to 17.885 mg RU/g for the methanol extract, while the lowest content was detected in petroleum ether extract (4.525 mg RU/g). TPC measured as tannic acid were higher than that measured as gallic acid ranged from 406.438 mg TA/g in petroleum ether extract to 131.647 mg TA/g in chloroform, with no appearance in methanol extract.

It was realized that within different extracts differences in TPC and TFC existed with the use of different solvents, this could be attributed to the polarity of the solvents. In this context Hemalatha *et al*. (2013) recorded ethanol and acetone as the most effective for extraction of phenolic compounds as compared to the other solvent like hexane. In addition, Jelena *et al*. (2014) recorded the effect of extraction solvent in extraction yield as well as in total phenolic and flavonoids detected in *P. nodosus* from western Serbia. Where the highest values of TPC and TFC were detected in ethyl acetate extract (28.45 mg GA/g and 102.09 mg RU/g respectively) and the lowest in ethanol extract. These results were higher than that recorded in this study and this could be related to many factors such as, the effect of the extraction solvent, plant growth form, time of samples collection and the environmental conditions at the area of samples collection.

Antimicrobial and anti-biofilm tests of the various plant extracts

As shown in results, the data in Table (3) revealed that, the used solvents liberated some bioactive materials that inhibited both tested bacterial strains. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with variable degrees. Regarding to the solvent type the methanol extract was much effective than chloroform and petroleum ether, the largest inhibition zoon recorded for *Escherichia coli* was 25.3±1.5 mm. The lowest concentration for MIC and MBC

were 0.6 mg/ml and 2.5 mg/ml, respectively, meanwhile *P. aeruginosa* show less susceptibility against antimicrobial agents extracted by petroleum ether, where 13.6 ±2.0 mm zoon of inhibition was recorded and no detected action was observed with the maximum concentration 20 mg/ml.

Table 3: Inhibition zoon, MIC and MBC values of *P. nodosus* extracts against *E. coli* ATCC 25922 (EC) and *P. aeruginosa* ATCC 27853 (PA) compared to gentamicin.

Bacterial strains	Methanol			Chloroform			Petroleum ether			Gentamycin		
	IZ mm	MIC mg/ml	MBC mg/ml	IZ mm	MIC mg/ml	MBC mg/ml	IZ mm	MIC mg/ml	MBC mg/ml	IZ mm	MIC µg/ml	MBC µg/ml
PA	21.3 ± 1.5	1.25	5	16.6 ± 1.5	5	10	13.6 ±2.0	2.5	ND	47.3 ±2.5	0.8	1.6
EC	25.3 ±1.5	0.6	2.5	17.3 ± 2.0	2.5	10	17.0 ±1.0	1.25	ND	35.0	1.6	3.13

Inhibition zone of 10 mg/ml *P. nodosus* extracts and 1µg/ml gentamycin, results are presented as means ± standard deviation, SD for three replicates.

The concentration used to reduce the biofilm activity for both tested strains was much higher than that required to inhibit the bacterial growth (planktonic cells), Figure (3a and b) represented the formed biomass after treatment with *P. nodosus* extracts. The data indicate that *P. nodosus* extracts significantly and effectively disrupt the biofilms formed by *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. However, the used solvent for extraction did not significantly (P> 0.05, n=8) influence the bacterial sensitivity, thence the inhibitory activity of each solvent towards *P. aeruginosa* ATCC 27853 and *E. coli* ATCC was in the same level, meanwhile the methanol was significantly more effective than the petroleum ether and chloroform. Obviously, the remained biofilm was reduced compared to the initial non-treated biofilm biomass (Figure 3a), where the biofilms treated with methanol extracted recorded the lowest biofilm biomass as confirmed by crystal violet staining. Even with 18 mg/ml *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 biofilms were almost completely eliminated by 76.0 and 88.0 % respectively (Figure 3b).

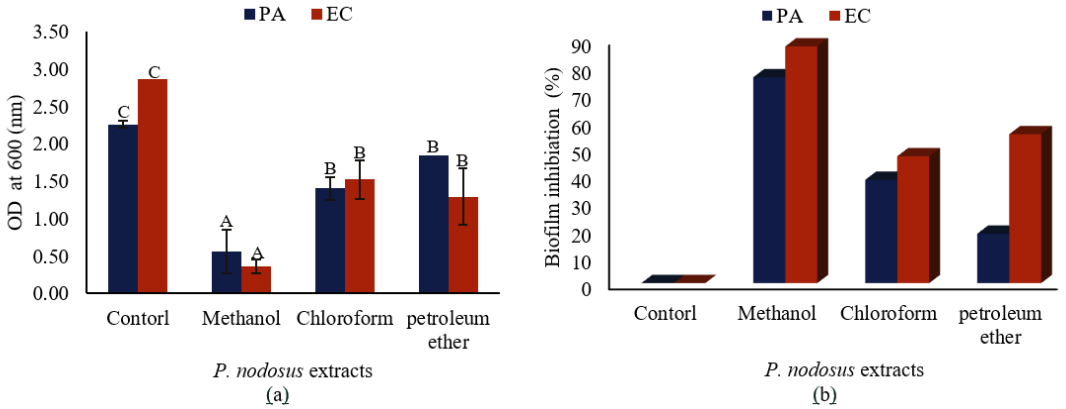

Fig. 3: *P. nodosus* extracts reduced the biofilms biomass (a) of *P. aeruginosa* ATCC 27853 (PA) and *E. coli* ATCC 25922 (EC), and inhibited the formed biofilm (b). Error bars represent SD of three replicates.

To verify the effectiveness of methanol extract against tested bacteria time-kill curves were established, data in figure 3a and b show that both concentration and contact time significantly (P<0.05, n= 3) influenced the killing activity. Generally, at zero time all the three tested concentrations were at the same level, no obvious killing rate was observed. After 2 h incubation the concentration of 1.25 mg/ml significantly reduced the bacterial numbers (log 10) 6.50 ±0.03. Meanwhile, both 2.5 mg/ml and

5.0 mg/ml concentrations were at the same activity level at 2 h, and 4 h, whoever after 24 h the cells (log10) number were significantly dropped, as 5.0 mg/ml concentration eliminated 99% of *P. aeruginosa* strains after 24 h incubation (Figure 4 a). For *E. coli* there was no significant differences (P>0.05, n=3) between both 0.6 mg/ml and 1.2 mg/ml, during the first four hours. Both concentrations were running the same killing rate activity (Figure 4b). It was clear that, as the concentration and incubation time increased the killing rate was increased, concentration of 2.4 mg/ml, after 24 h incubation dramatically reduced the living rate to almost zero with 99% elimination value.

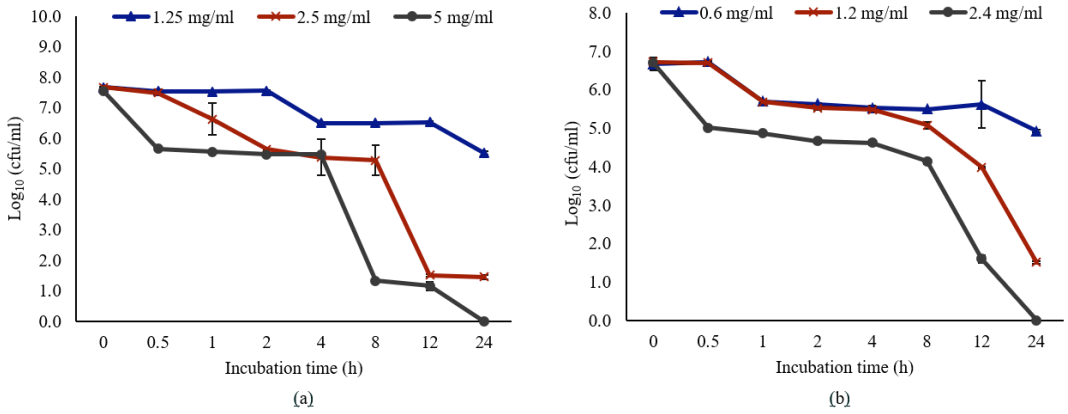

Fig. 4: Time-kill of *P. nodosus* methanol extraction with different MICs against a) *P. aeruginosa* ATCC 27853 and b) *E. coli* ATCC 25922. Error bars represent SD of three replicates.

Biofilms formed by many pathogenic bacteria as one of their life styles is considered one of the important virulence factors during the infection progression process. Unfortunately, the conventional antibiotics not yet effective. The bacterial cells in biofilms are extremely resistant to treatment with antibiotics (Hentzer *et al*. 2003), thence, screening of different compounds that could eradicate and/or disturb biofilms have been considered as an urgent task (Plyuta *et al.* 2013). Natural compounds such as phenolic compounds demonstrated an antibacterial, antibiofilm, anti-inflammatory, and antitumoral drugs, their medicinal aspects are due to antioxidant activity (Croft 1998, Fresco *et al*. 2006, Li *et al.* 2011, Oueslatia *et al*. 2012).Therefor, we studied the effect of chloroform, methanol and petroleum ether to extract active compounds from *P. nodosus* against the biofilms formed by *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922.

The methanol extract demonstrated antibacterial activity against both *P. aeruginosa* and *E. coli*, it reduced and weak the bacterial biomass after 24 h incubation, of course this weakly growth prohibited the biofilm formation. Till now, the exact mechanisms responsible for the effects of antibiotics on biofilm formation remain not exactly clear, however we assumed that, the high concentration of phenolic and flavonoid compounds within the methanol extract is a supportive rationale to interpret the antibiofilm activity that was observed with this extract. A positive relationship was reported between antibiofilm potential activity and the total phenolic compounds, where these compounds reduced the production of pyocyanin and biofilm formation of *Pseudomonas aeruginosa* (Ugurlu *et al*. 2016). Meanwhile, Jelena *et al*. 2014 reported both ethanol and ethyl acetate extracts as the best effective extracts against biofilm formed by *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa*, however the highest total phenol and flavonoids contents were found in the ethyl acetate extract and the lowest content in the ethanol extract. Not surprisingly, as reported by several researchers, the phenolics probably interacted

with *P. aeruginosa* and *E. coli* metabolism, and supressed the virulence factors (pyocyanin, elastase, and protease) in *P. aeruginosa* PAO1 and reduced attachment of the bacteria to polypropylene surfaces (Walker *et al*. 2004, Rudrappa and Bais 2008). Intriguingly, the flavonoid improved the proteolysis of *E. coli* biosensor signal receptor TraR protein which significantly reduce the biofilm formation (Zeng *et al*. 2008)

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CONCLUSION

The present study suggests that the Egyptian macrophyte species *P. nodosus* could be as an excellent supplementary feed source for animals and fishes, because of it is high level of nitrogen free extracts, proteins, digestible crude protein in addition to the heavy metals which is not above the nutritional threshold. HPLC analysis of different plant extracts revealed the presence of polyphenols and flavonoids with different concentration varied with the extraction solvent, with the highest in methanol extract. Among the used solvent methanol was recorded as the best extraction solvent and methanol extract was the best effective extract against both *P. aeruginosa* and *E. coli*, but more studies are still needed to assign which polyphenol structural was responsible for antimicrobial and biofilm disturbance activity.

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