Antioxidant activity of some *Salvinia natans* L. extracts and a study of their efficacy against isolated bacteria from diabetic foot ulcers

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Abstract

Salvinia : is an annual plant floating on the surface of water(aquatic plant).There is only one species in Iraq, is *Salvinia natans* L., and it spreads in a high density in the southern part of Iraq Floating freely on the water surface in the Iraqi marshes , *S.. natans L*. is an introduced species . Several tests were used to identify the presence of active chemical compounds in *S. natans* L. and we note that the *S. natans* is rich in active substances. showed phytochemical results were found for the extract ethanol 70% contain carbohydrates, flavonoids, phenols, alkaloid, glycosides, and saponin , and negative detection for both Tannin and triterpenoid. Evaluation of antioxidant activity for *S. natans* extracts by inhibiting the (1,1- diphenyl-2- picrylhydrazyl) radical scavenging activity, , it study results showed that all extracts have antioxidant activity by (1,1-diphenyl-2-picrylhydrazyl) radical scavenging with different concentrations, showed the superiority of the polyphenol 85.03 % inhibition ratio at a concentration of (1000 µg/ml) , flavonoid 80.5% at a concentration of (1000 µg/) ,and hexane extract 74.08 % at a concentration of (1000 µg/ml) Compared to ascorbic acid, which recorded 81.17% at a concentration of 1000 µg/ml. Whereas, the hexane extract recorded the lowest inhibition rate at concentration of (50 µg/ml) . efficacy showed of separated extracts from *S. natans* as antibacterial activity using well diffusion plate method. The SF extract was the highest activity against all isolated pathogenic bacteria from foot ulcers of diabetic patients , while the other extracts showed varying activity against different bacterial species at P. value < 0.01. The results of the current study illustrated that SF extract was the high Zone rate of inhibition/mm2(28.3 ± 1.5) against P. aeruginosa, while not any inhibition/mm2 (0.0 ± 0.0) for hexane extract against same of isolated bacteria. HPLC analysis indicated the presence of flavonoid compounds namely Catechine , Rutin ,Kaempferol and Apigenin with specific retention times. The detected compounds possess antioxidant and antibacterial activities. This results suggested that separated extracts from *S. natans* have high antioxidant and antibacterial activities.In the future, *S. natans* L.extracts could become one of the important alternatives in treating infections of the throat, skin, urinary tract, and digestive system, even if in a limited way due to the spread of types of microbes that are resistant to antibiotic drugs.

Keywords— Antioxidant Activity , HPLC , *Salvinia natans,* Antibacterial Activity.

I. Introduction:

densities in the tropics (Kosowski *etal*.,2020).*Salvinia molesta; S . oblongifolia; S. herzogii; S.auriculata; S. biloba; S. cucullata; S. minima; S.nympheulla; S. hastate Salvinia* originated on the African continent, from which it spread to the other continents, and this genus contains approximately 10 species that live on all continents and in high

Natural antioxidants from herbal resources are currently gaining popularity. Epidemiological and in vitro studies on medicinal plants strongly supported the idea that phytochemicals with antioxidant activity can protect against oxidative stress (Souria *et al.*, 2008).Previous studies on the genus *Salvinia* that demonstrate its chemical compounds and their importance as antioxidants.(Choudhary *etal*.,2008) isolated two glycosides for the first time from the fresh water fern *S. molesta* D.S. Mitch. These compounds demonstrated potent antioxidant radical scavenging activity in a non-physiological assay. The extracts were screened for phytochemical content using standard methods. Out of the eighteen extracts, the chloroform extracts of *S. molesta* had the highest concentration of phytoconstituents, followed by ethanol, acetone, benzene, aqueous, and petroleum ether of bioactive compounds (phenolics, tannin, carbohydrates , Steroid , saponin , xanthoprotein , flavonoid , protein and Carboxylic acid) (Mithraja *etal*.,2011) ,which are highly responsible for antioxidant activity of the extract (Lee and Shin, 2011) .Devi *etal*.,(2015) demonstrated the presence of Alkaloids, Flavonoids, Phenols, Tannins, and Saponins in *S. auriculata* Aubl extracts at various concentrations and percentages. The GC MS analysis of the acetone extract from *S. moletsa* confirmed the presence of bioactive components such as apiol, hexadeconoic acid, pentadecanoic acid, and octadecatriene, among others, which are highly responsible for the extract's antibacterial and antioxidant activity (Nithya *etal*.,2015). Extensive research on the biological potential of ferns has revealed that the majority of ferns have enormous antioxidant potential (Sessa and Der,2016). The results of a quantitative phytochemical screening of *S. molesta* extract revealed (Gaya *etal*.,2016). Total carbohydrates, total soluble protein, tannin, total carotinoids, alkaloids, falavonoids, terpinoids, saponin, and phenol were all studied as phytochemical components. Carbohydrates are the most *and S. natans.S. natans L.* that belongs to the Salviniaceae family (Mirosawa *et al*., 2018). (also known as floating fern, floating watermoss, eared watermoss, floating moss, True Ferns, or water butterfly wings) is an aquatic plant with large bladed leaves (Megaphyllous).In Iraq, there is only one species, *S. natans* L., which is widely distributed in the southern part of the country. *S. natans* L. is an introduced species that floats freely on the surface of the water in Iraqi marshes (Al Saadi and Al-Mayah, 1983: Al-Makrami , 2012 and Mouhamad *et al*. , 2020).Oxidative stress is among the main causative factors in the induction of many chronic and degenerative diseases, such as cancer, diabetes, Alzheimer's disease, stroke, viral infections, neurodegenerative processes, infarction, brain edema, and ageing, (Incent *etal*.,2017). Antioxidants are ions, radicals, or compounds that, when present, can delay or prevent the oxidation of other molecules (Dibacto *et al.,* 2021). Antioxidants protect the body by combating free radicals generated by oxidative stress and maintaining a balance between oxidants and antioxidants, which is triggered by stray oxygen atoms. Antioxidants are either hydrogen donors or free radical sensors (Parcheta *et al*., 2021) . When hydrogen atoms and radicals combine, they form a stable molecule (Khadim and Al-Fartusie, 2021). They protect us by either directly reducing free radical production or preventing their spread (Aziz et al., 2019). Antioxidants can be found in vegetables, fruits, cereals, most medicinal herbs, nuts, some meats, poultry, and fish (Nemzer *et al*., 2019; Jideani *et al.,* 2021). Antioxidants are found naturally in foods and improve human health without causing harm, as opposed to synthetic antioxidants (Loizzo and Silva, 2021). It is the most effective method of protecting the body from free radical damage in an emergency. They are self-defense systems that protect the cell from oxidative stress and play an important role in its function (Aziz *et al*., 2019).

information about *S. natans* extracts in Iraq is still little. Only one study on bacterial activity of *S. natans*, taken from the Shatt al-Arab River near the city of Qurna against E. coli, Vibrio sp. and S. aureus isolated from some infectious sources of patients (Al-Maliki *etal*.,2017). In Iraq, most of these studies have been attempted to evaluate the antioxidant ctivity of some wild plant extracts (Alzayadi , 2021; Farhoud and Hussein2021).

II. MATERIALS AND METHODS

A. Samples Collection

- **The plant collection:**

S. natans whole plant collected in March 2020 from Al-Chibayish marsh- south Iraq ,were collected using sterilized disposable plastic containers, then labeled and transported to the laboratories for diagnosed, The *S. natans* samples was cleaned, washed by distilled water, dried at room temperature at (25 c) for two weeks. Were collected and crushed, then were kept in dark glass containers for further use.

- **Bacteria pathogenic collection**:

A total of 126 swabs were collected from diabetic patients suffering from foot ulcers at the Diabetes Center / Thi-Qar during the period from September to November 2020, the patients' ages ranged from 23 to 74 years of both sexes, and the swabs were placed in sterile tubes with transport media, They were then taken to the laboratory immediately for inoculation on blood agar, MacConkey agar, Nutrient Agar.

B. Extraction of phytochemicals from *S. natans*:

Preparation of ethanol extract, 20 g of *S. natans* were powdered and soaked in solution (200 ml of 70% ethanol and distilled water). The process of secondary metabolites extraction from *S. natans* by ultrasonic for (48 hr., 45°C). Next day, all the extracts were

abundant of the studied components, while phenol is the least abundant.The findings of Gini and Jothi (2018) indicate the possibility of using *S. molesta* as a source for a plausible antioxidant agent that could be isolated and used as a lead candidate for the development of antioxidant drugs that help stop or limit free radical damage and counteract oxidative stress, thereby preventing a variety of chronic and degenerative diseases. When the novel *S. molesta* isolate was tested for antioxidant activity (using DPPH, superoxide anion radical scavenging, oxidative burst, and Fe+2 chelation assays), it showed promising antioxidant potential with IC 50 values of 48.2 0.3, 60.3 0.6, and 42.1 1.8 M, respectively (Naheed,*etal*.,2021). According to the findings of the study (Attallah *etal*.,2022), *S. auriculata* methanol extract has the potential to be a potent hepatoprotective therapy for the treatment of oxidative stress-mediated liver damage.

There have been very few studies of phytochemicals and their biological activity as antioxidants of *S. natans* L. study demonstrated for (Srilaxmi,*etal*.,2010) the protective effect of natansnin from Salvinia natanse against CCl 4 induced oxidative stress and cellular degeneration in rat liver tissue. Narasimhulu *etal.* (2010) reported an anti-oxidant dibenzoyl glycoside, natansnin, from *S. natans* L. FTIR spectroscopy results revealed that many regional plants, including *S. natans*, contain a variety of important phytochemical functional groups (Mohamad *et al*.,2020). *S. natans* (L.)All. is used to treat general exhaustion, fever, eczema, skin diseases, as an antimicrobial, tonic and fortifying agent, and as an antioxidant (Na *etal* ., 2003; Mohamed and Egorov 2011; Ya ,2015). *S. natans* extract and constituents have been shown to have antioxidant, antimicrobial, antipyretic, anti-inflammatory, and analgesic properties (Bolotova, 2015; Narasimhulu *et al.*, 2010). Many authors have reported the medical and antioxidant activity of *S. natans* extracts collected worldwide, While the

The extraction of phenolic compounds from *S. natans* by ultrasound was carried out using 500 mL ethanol (70%) (a water–ethanol mixture (30:70)ml) with (50 g) of *S. natans* L , at temperature (45°C), and the extraction time (48 hours) (Altemimi *et al.,* 2015) .

2 Isolation of flavonoids from *S. natans* L:.

Powder of the whole *S. natans* plant (50gm) was treated with 250ml of (80%) methanol by using stirring at room temperature for 24 hours. (Yadav & Kumar, 2012).

filtered using titron cloth three times(Sani *et al.,* 2013 ,Al-Kunani,2015).Qualitative phytochemical tests were performed as per standard methods. (Harborne, 1998).

Prepare hexane extract following the method (Bobby et al., 2012), in which 20 g of *S. natans* were placed in a thimble and then placed in Soxhlet extractor using 200 ml of hexane for 24 h.

C. Extraction and fractionation of different active constituent.

1 Isolation of Polyphenols from *S. natans* L. by ultrasound-assisted extraction (UAE):

D. Percentages of *S. natans* extracts:

Extraction Percentages was calculated from equation as:

$$\text{Extraction percentage (\%)} = \frac{\text{Weight of extracts (g)}}{\text{Powder Weight of plant (g)}} * 100$$

powder weight of plant (g)=20g

was expressed as the percentage of free radicals inhibited by the sample (Xiao *etal*.,2020).

F. Investigation of studied extracts activity as antibacterial:

investigation of studied extracts activity of *S natans* as antibacterial using well diffusion plate method, that the bacterial suspension was spread on the culture medium (Muller Hinton Agar for bacteria), then the suspension was spread by means of a sterile cotton swab on the plates and left for two minutes to dry. studied extracts were dissolved with the organic solvent DMSO to obtain concentration (100 µg/ml), Then added to each hole 50 µl of the studied extracts Using a micropipette, and incubated a at a temperature of 37 °C,for 24 hours (Dubey *et al.,* 2014; Khalif and Al-Waheeb, 2020) .

E. Estimation of antioxidant activity for *S. natans* L. extracts by non-enzymatic method

Free radical scavenging assay (DPPH),It is a fast method to show the radical scavenging activity

: 1ml DPPH solution (A freshly formulated (0.004 w/v) by dissolving 0.004 gm in 100ml methanol : DMSO (75:25) , was mixed with 1 ml at various concentrations (50,100, 250, 500, 1000 µg/ml) of the (hexane extract, Polyphenols and flavonoids from *S. natans*) ,solvent was methanol : DMSO (75:25). Then the samples were kept in drak at r.t (25C°) for 30 minutes .then The absorbance was measured at a wavelength of 517 nm with a UV-Vis Spectrophotometer ,using ascorbic acid at the same concentrations as a standard solution, . The following formula was used to measure radical scavenging behavior, which

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HPLC Conditions
• Mobile phase: solution A(0.5 ml trifluoro acetic acid in one liter of water)
+ solution B (0.5 ml trifluoroacetic acid in one liter of acetonitrile)
Solution A: solution B (20:80 %)
• Column type C-18
• Column temperature: 22 c0
• Flow rate: 1.0 ml / min
• Injection volume: 5 µL
• Detection: UV Detector at λ330 nm
extracts because that ethanol is a good solvent for the extraction of alkaloid, saponins, tannins, phenols, glycosides, steroids, flavonoids and carbohydrates and some medium polar compounds (Fayaz *et al.*, 2017).

G. High Performance Liquid Chromatography (HPLC) Analysis for detection of Active Compounds .

An HPLC analysis was performed for the detection and estimation of High Performance Liquid Chromatography (HPLC) Analysis of active compounds in Separated flavonoid from *S. natans* .Analysis was carried out by HPLC. Reversed-phase HPLC processing was used to quantify individual phenolic compounds, using a SYKAMN HPLC chromatographic device fitted with a UV detector, Chemstation, and a Zorbax Eclipse

III. RESULTS AND DISCUSSION

Figure (1) indicates the results of percentages of *S. natans* extracts. It is noted that the ethanolic 70% extract has the highest percentage of extraction from other general

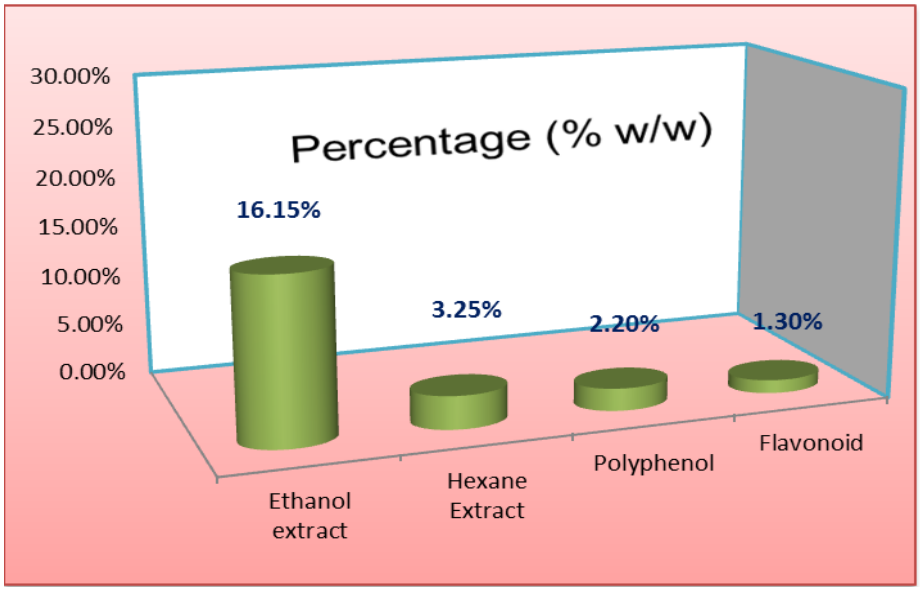


Figure (1) : Percentages of *S. natans* extracts.

these factors are responsible for the overall amount extracted (Chisté *et al.*, 2014). Several tests were used to identify the presence of active chemical compounds in *S. natans* and phytochemical results were found for the extract ethanol 70% contain carbohydrates, flavonoids, phenols, alkaloid, glycosides, and saponin , and negative detection for both Tannin and triterpenoid.

The variance in the percentages of extracting the active substances in different solvents is due to several reasons, including type of plant used ,the solubility intensity of each specific structure in the solvents used and the polarity of the solvent. Solvent extraction is one of the basic methods for separating active ingredients that may be affected by various factors such as sample quantity and quality, time of collection , solvent, solvent to substance ratio. All of

(DPPH•) + HO-R-OH→ (DPPH): H + HO-R-O•

Antioxidants cause a decrease in optical absorbance of 517nm, which determines the DPPH radical's return strength.

The DPPH assay is fast test for determining the antioxidant activity of *S.natans* plant extracts. the effects of DPPH radical scavenging operation appear to improve With an increased concentration ratio of extracts, , also the study results shown in the table (1) and figure (1), compared to standard ascorbic acid, showed the superiority of the polyphenol 85.03 % inhibition ratio at a concentration of 1000µg/ml , flavonoid isolated 80.5% at a concentration of 1000 µg/ml ,and hexane extract 74.08 % at a concentration of 1000 µg/ml Compared to ascorbic acid, which recorded 81.17% at a concentration of 1000 µg/ml. Whereas, the hexane extract recorded the lowest inhibition rate at concentration of 50 µg/ml.

Through the results obtained from the phytochemical study, we note that the *S. natans* is rich in active substances, and this is expected because previous studies found many active compounds present in the form of chemical hosts in the *Salvinia* plant such as tannin, flavonoid, alkaloid, saponin, and phenolic compound (Muzaini and Pa'ee,2021) .

The DPPH assay is a commonly used test to determine the antioxidant function of plant extracts. It is a more stable and well-known nitrogen-centered free radical dependent on the reduction of accepting hydrogen or electron donor. A stable free radical with a dark purple color is used in this research, which changes color to yellow as antioxidant compounds are returned by giving it an electron or a hydrogen root (Hu *et al.,* 2011) to become a stable molecule as

follows:

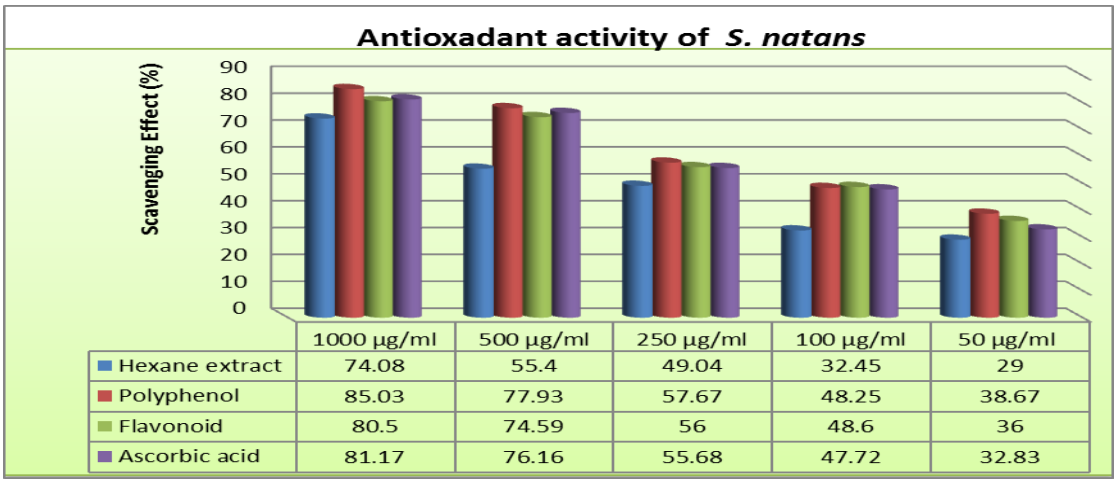


Figure (2) : DPPH radical scavenging activity of separated *S. natans* extracts with five different concentrations when compared to the standard (ascorbic acid).

Table 1: Antioxadant activity of separated extracts of *S. natans* by DPPH method

Extract type	Scavenging Effect (%)				
	50 µg/ml	100	250	500	1000

		µg/ml	µg/m	µg/m	µg/m
Hexane extract (SH)	29	32.45	49.04	55.4	74.08
Polyphenol (SP)	38.67	48.25	57.67	77.93	85.03
Flavonoid (SF)	36	48.6	56	74.59	80.5
Ascorbic acid (SC)	32.83	47.72	55.68	76.16	81.17

flavonoids present in this extract, as several studies have shown a connection between flavonoids' chemical structure and their displacement effect (Seyoum *et al.,* 2006). Antioxidant phytoconstituents can neutralize (DPPH) radicals either by transferring hydrogen atoms or by transferring an electron (Taia, 2006).

Studied extracts activity of *S natans* as antibacterial:

The SF extract exhibited the highest activity against all isolated pathogenic bacteria from foot ulcers of diabetic patients , while the other extracts showed varying activity against different bacterial species at P. value < 0.01 as demonstrated in Table 2 and Figure 3.

Table 2 : Activity of separated extracts of *S. natans* against isolated pathogenic bacteria from foot ulcers of diabetic patients.

As we note the hexane extract and the isolated groups have high inhibition results compared to ascorbic acid, and this indicates that *S.natans* plant has a high activity as an antioxidant. also the current results showed were apparent that the polyphenol outperforms the other extracts, The presence of hydroxyl groups in some compounds, such as phenolic compounds or flavonoids, may influence their ability to scavenge free radicals (Bhakya *etal.,* 2016). Because of the relationship between the concentrations of the compounds, the high contents of total phenolic and total flavonoid compounds can lead to essential antioxidants. This finding suggests that the extracts have a part of the oxidative action, and this role has a synergistic effect on the antioxidant effect of the DPPH radical. The potency of antioxidants and the phenolic removed, indicating that these substances are mainly responsible for It acts as an antioxidant and has a polar appearance so it is an effective antioxidant (Ubando-Rivera *et al.,* 2005), This influence may also be traced back to the

pathogenic bacteria	Inhibition Zone/ mm2 Mean + SD		
	SH	SP	SF
P. aeruginosa	0.0 ± 0.0	18.3 ± 1.5	28.3 ± 1.5
p. mirabilis	15.3 ± 1.5	13.3 ± 1.5	26.7 ± 1.2
Enterococcus	14.2 ± 1.3	15.0 ± 0.6	25.5 ± 0.5
K. pneumoniae	18.5 ± 1.2	14.6 ± 0.6	26.3 ± 1.2
S. aureus	13.3 ± 1.2	10.5 ± 1.1	26.7 ± 0.8
P. Value	< 0.01		

Hexane extract (SH), Polyphenol (SP), Flavonoid (SF)

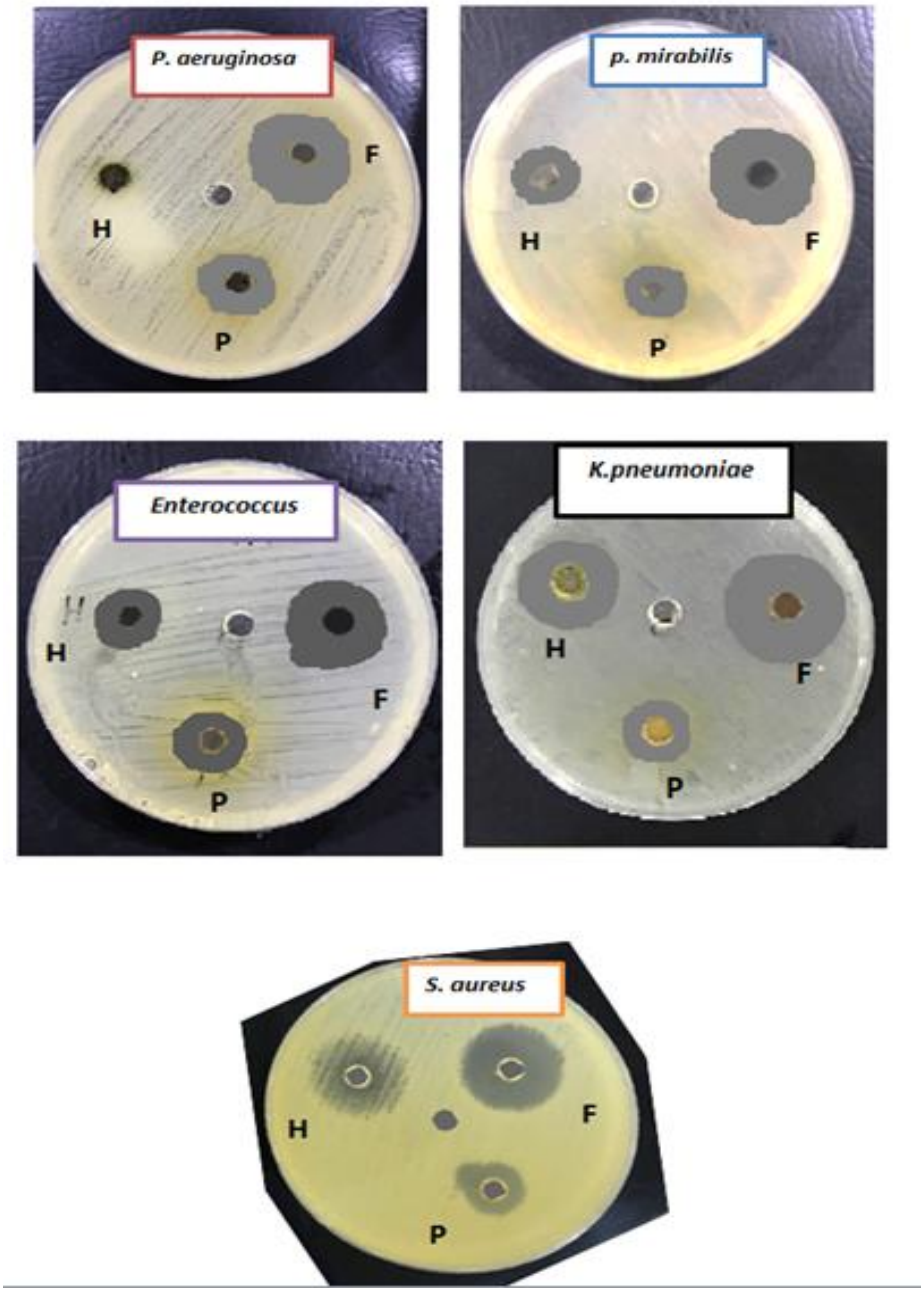


Figure (3) : Activity of separated extracts of *V. sessilis* and *S. natans* against isolated pathogenic bacteria (A= *P. aeruginosa* ,B= *p. mirabilis* ,C= *Enterococcus* , D= *K. pneumoniae* ,E= *S. aureus*)

according to results of the present study, *S. natans* flavonoid extract (SF) of had highly activity against pathogenic bacteria.

The high efficacy of the Flavonoid extract (SF) separated from *S. natans* was indicated

analysis indicated the presence of flavonoid compounds

carried out to evaluate the antibacterial activity of two water plants *Nymphaea alba* and *Salvinia natans* leaves against pathogenic bacteria (*E. coli, Vibrio sp. and S. aureus*) ,they found that *S. natans* ethanol extract showed higher inhibition zones of 10.3 mm against *Vibrio sp* .The difference in the level of effectiveness of extracts on pathogenic bacteria isolated, may be due to the extract's content of the active compounds, or it may be due to the genetic variation between the isolated microbes or the lipid content in the cell wall of gram negative bacteria that works to impede the entry of the active compound into the germ cell (Elshouny *et al.*, 2017).

by their containment of effective compounds ,as in Figure (4) and Tables (3,4), HPLC namely Catechine , Rutin,Kaempferol and Apigenin with specific retention times. The detected compounds possess antioxidant and antibacterial activities., and this is consistent with the findings of researchers (Pagliarulo *et al.*, 2016 ; Rempe *et al.*, 2017 and Chibane *et al*., 2019).

The present study results were similar to Mofanato and Vasantha studed (2013), showed that the methanolic extract of *S. natan* had the best antibacterial activity , the most sensitive Gram-positive bacteria was *S. aureus* , while the most sensitive Gram negative bacteria was *K. pneumoniae* .while it were not similar to Al-Maliki *et al.* study (2017),

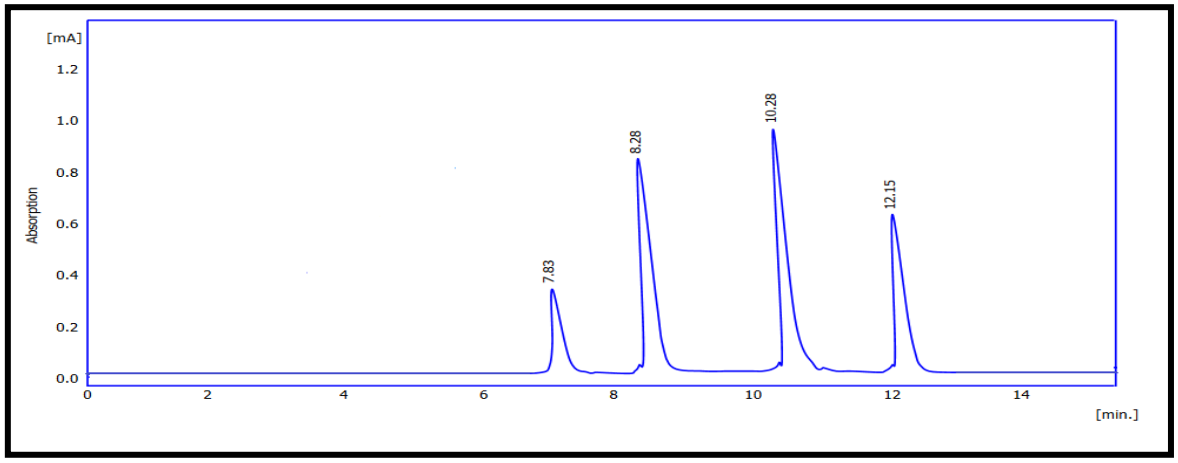


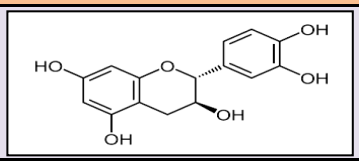
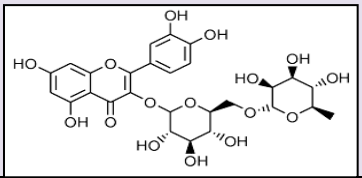
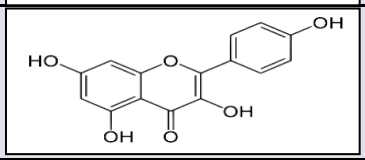
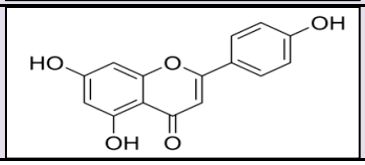
Table (3) : Retention time of sample separated Flavonoid compounds of *S. natans* extract.

No.	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Conc. compounds (ppm)	Compound Name
1	7.83	3956.08	390.55	17.70	14.87	0.10	22.45	Catechine
2	8.28	6544.18	784.24	29.28	29.87	0.20	18.97	Rutin

3	10.25	7195.41	860.33	32.19	32.76	0.22	25.24	Kaempferol
4	12.15	4654.98	590.78	20.83	22.5	0.15	16.88	Apigenin
	Total	22350.65	2625.9	100.0	100.0			

Figure (4) : HPLC study of sample separated Flavonoid compounds of *S. natans* extract.

Table(4): Retention time of standard Flavonoid compounds

No .	Compound Name	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Chemical Structure
1.	catechine	7.80	1324.70	792.55	100.00	100.00	0.25	
2.	Rutin	8.20	968.77	794.25	100.00	100.00	0.25	
3.	Kaempferol	10.36	874.15	750.44	100.00	100.00	0.25	
4.	Apigenin	12.19	1844.08	960.12	100.00	100.00	0.25	

Hexane extract and two isolated groups of *S.natans* possess an antioxidant activity by DPPH that increases with increasing the concentration of the extract as compared with the antioxidants (ascorbic acid) and was the highest Polyphenol (SP) activity Whereas, the hexane extract recorded the lowest activity,and these flavonoid extract (SF) may also exhibit

IV. CONCLUSION

Results of the present study showed the *S.natans* extracts have the presence of most of the phytochemicals (carbohydrates, alkaloid, flavonoids, polyphenols, glycosides, steroids, and saponins).

2022, 15, 549. https://doi.org/10.3390/ph15050549

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strong antibacterial action in addition to their well-known antioxidant activity.

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