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Research Article

Total Phenolic Profile and Antiviral Activity of *Senna Alexandrina*

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ABSTRACT

Medicinal plants contain various phytochemicals and therefor have a significant influence in treating viral diseases. Humans and animals are both at risk from viral illnesses. Herbal treatments for numerous viral infections are gaining popularity these days due to their broad spectrum of action and lack of adverse effects. In current study, we mainly focused on the isolation of phytochemicals from the *Senna alexandrina* for this purpose different extraction methods were used. Their phenolic profile was determined by FTIR and high-performance liquid chromatography. Current results revealed that the medicinal plant *S. alexandrina* contained large amount of important phenols and flavonoids due to which antioxidant, antimicrobial and antiviral activities were observed. So, it was concluded that the plant body of *S. alexandrina* may have natural compounds and could be used in pharmaceutical industries to boost the body's endogenous antioxidant system, to treat microbial diseases and would be helpful in future for development of drugs against viral diseases.

Keywords: *Senna alexandrina*; Phenolic profile; Flavonoid content; Scavenging activity; Antiviral activity

INTRODUCTION

Humans and animals are also at risk from viral illnesses. Because viral diseases have become drug-resistant and have the potential to spread quickly, they are responsible for a large number of deaths worldwide. Efforts are now being done to manage viral infections utilizing currently available antiviral medicines. Many people in the world's poorest countries, such as Africa, depend on medicinal plants for their health [1,2]. The South region of the world, has a wealth of plants used as an alternative medicine throughout history [3,4]. Plants that are used for medicinal purposes are getting increasingly popular. Many plant species have anti-inflammatory, antibacterial, antiviral, anticancer, and antioxidant properties. [5]. The Prophet's Medicine mentions *Senna alexandrina*. However, in Saudi Arabia, *S. Sana makki*, *C. Senna angustifolia Vahl*, and *C. Senna* are some of the other names for this species. In each of these nations, the plant can be found. In the year 2016 this plant is cultivated for its therapeutic benefits. The FDA has approved this herbal laxative as an over-the-counter herbal medication for adults and children [6]. Pods have been shown to be vanquished by leaves in several investigations. Elansary and his colleagues are also treated with typhoid, spleen and anthelmintic infusions. Natural phytochemicals such as flavonoids and phenolic compounds are found in plants, including leaves, fruits, barks, stems, and roots, and can be used to treat a variety of maladies, including viral disorders [7,8]. Antioxidant effects of flavonoids, particularly phenolic compounds, have been studied and proven to be efficient [9]. The antioxidant activities of these bioactive compounds may be attributable to the activation of antioxidant enzymes, chelating metal catalysts, and scavenging free radicals to combat oxidative stress [10]. The goal of this study was to see if the medicinal plant *Senna alexandrina* might be used as a supplemental and alternative antiviral treatment by analyzing their phenolic profiles. We also wanted to see if the water and NADES extracts had any antioxidant, antibacterial, or antiviral properties. Toxic organic solvents can be replaced by deep eutectic natural solvents (NADES). They are non-toxic, biocompatible, and environmentally friendly. The extraction of components, particularly phytochemicals, from the selected medicinal plants was done with NADES [11]. The extraction efficiency of the water and NADES extracts is assessed by measuring the extracted total phenolic and total flavonoids.

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MATERIALS AND METHODS

Chemical

Various chemicals were employed in this project. Sodium carbonate (Daejum), Gallic acid (AppliChem), methanol (Merck), aluminum chloride (Merck), distilled water, sodium nitrate (RDH), glucose (RDH), and citric acid (AppliChem). folin-ciocalteu reagent (Sigma Aldridge), quercetin (AppliChem), DPPH (Alpha Chemical), sodium carbonate (RDH).

Sample collection and preparation

The leaves of *Senna alexandrina* were collected in July 2019, collected from fresh fields.

Spectrophotometric analysis

The phenolic compounds were extracted using a method described previously, modified slightly [12,13]. Briefly, the plant materials were dried in the shade before being pulverized. Each sample and distilled water was placed into a blender in a 1:5 ratio and blends it. Then sample was poured in the beaker covered with aluminum foil and left for 3 days. Similar approach was utilized for NADES solvent. The sample was filtered with filter paper the next day [14].

Preparation of NADES

NADES were prepared by mixing glucose, citric acid and distilled water in 1:1:1 ratio and placing it on the hot plate at 40-45°C temperature with constant stirring until the solution became clear at room temperature.

Analysis of total phenolic content

The quantity of phenols in the extract was obtained by using an assay folin-ciocalteu, [15], with little modification. Briefly, as a standard, a 1% solution of gallic acid was utilized. We dissolved the gallic acid (0.2 g) in 20 mL of distilled water for this experiment. In methanol, the dilutions of 1 µl/ml, 5 µl/ml, 10 µl/ml, 15 µl/ml, 20 µl/ml, 25 µl/ml, 50 µl/ml, 80 µl/ml, 100 µl/ml, and 125 µl/ml were produced. 100 µl of dilution were measured one at a time, after that we measured 500 µl of H_2O and 100 µl of reagent (Folin.c) and added into the test tube. After 6 minutes 1 mL of 7% Na_2CO_3 (3.85 g of Na_2CO_3 dissolve in 50 mL H_2O) and 500 µl H_2O were added. After 90 minutes, at 760 nm readings were taken, a straight curve was plotted, and the same approach was used to determine the phenolic content of samples. The solutions were made in triplicate.

Analysis of total flavonoid content

Flavonoid in plant extracts has been calculated by complex forming assay of aluminium chloride. Modified by some researchers. Quercetin (0.2 g) was dissolved in 20 mL of water. Then, in methanol, dilutions of 1 µl/ml, 5 µl/ml, 10 µl/ml, 15 µl/ml, 20 µl/ml, 25 µl/ml, 50 µl/ml, 80 µl/ml, 100 µl/ml, and 125 µl/ml were produced. Using a micropipette, measure 100 µl of dilution and added into the test tube, add 500 µl H_2O and 100 µl of 5% $NaNO_3$ (0.5 g of $NaNO_3$ in 10 ml H_2O) after 6 min, then add 10% $AlCl_3$ (1 g of $AlCl_3$ dissolved in 10 ml H_2O) 150 µl . After 6 minutes, 1 M NaOH (200 µl) was added (dissolve 0.4 g in 10 ml water). A UV–Vis Spectrophotometer set to 510 nm was used to measure the absorbance of the blank solution and the mixture. The standard solution was Quercetin. The solutions were made in triplicate. The results were presented as mg quercetin equivalent QE g^{-1} of dry weight DW.

Scavenging Activity

The water and NADES extract's ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl stable free radicals was assessed using the DPPH test [16-20]. The antioxidant is added into the methanol and freshly prepared DPPH solution (200 µM), and the antioxidant's ability to scavenge the free radical of stable DPPH is measured at 517 nm. Extracts at various amounts were added to a methanol DPPH solution. After 30 minutes, absorbance was measured at 517 nm at room temperature in the dark. The IC_{50} value is the sample concentration necessary to scavenge 50% of DPPH free radicals.

$$ABTS\ redical\ scavenging\ effect\ \% = \frac{[(Acontrol) - (Asample)]}{(Acontrol)} * 100$$

FTIR analysis

The characterization of plant materials was done using this method. The sample contained the active functional groups were discovered in the 400-4000 nm wavelength range using this approach.

HPLC analysis

It is used for assessment of the amount of Phenols, Flavonoids, and antioxidants present in the extracts. The phenolic components were isolated, described, and measured using this amazing chromatography. Each plant extract test sample was dissolved in 24 mL methanol (and homogenized) were used to perform HPLC. After 16 mL of distilled water, 10 mL of 6 M HCl was added. The combination was then thermostated at 95°C for 2 hours. Prior to HPLC analysis, the final solution was filtered using a 0.45 µm nylon membrane filter. The Shim-pack CLC-ODS (C18), 25 cm × 4.5 mm, 5 µm column was used to separate plant samples using gradient HPLC. The chromatographic separation was carried out with the following mobile phase gradients: A (H_2O:Acetic Acid-94:6, pH=2.27), B (Acetonitrile 100%) 0-15 min=15% B, 15-30 min=45% B, 30-45 min=100% B With 1 mL/min flow rate and UV-VIS detector at 280 nm wavelength at room temperature. Each compound's identity was determined by comparing the retention period, UV-VIS spectra, and peaks with those previously obtained by injecting standards. External calibration of the standards was used to perform the quantification.

Antiviral assay

Viral inoculum preparation: Poured the 10 ml secondary culture media in flacon tubes and placed in centrifuge at 14000 rpm for 10 min at room temperature. Supernatant discarded and added remaining 10 ml culture repeat it 2 times. Added activation media (20 ml $MgCl_2$ and 1.5 µl acetosyringone) in pellet and gently mix it. After that placed the tube in dark for 3 h and then ready to inoculate in host plant.

Administration to the local host plant: Subsequently the Cotton curl Multan virus was injected on *Nicotiana benthamiana* plants that had been cultivated. Water and NADES extract of *Senna alexandrina* were infiltrated to the 10 host plants of local lesion for one day in a row. Distilled water was used as a control. Plants were selected for homogeneity and cultivated in a growth room at 28°C, 16:8 h light/dark cycle with all treatments repeated three times.

Statistical analysis

Three tests were conducted, with the findings displayed as a ± standard derivation. Pearson's correlation coefficient and other statistics were calculated using Excel 2007. An ANOVA test was run on Windows using the SPSS 16.0 program to determine the statistical significance of the various treatments. The statistical significance level was set at 0.05, the lowest achievable value.

RESULTS

The water and NADES extracts of *Senna alexandrina* were collected and submitted to various analyses in the current investigation. Their reported characteristics and findings are detailed and discussed here;

Analysis of total phenolic content

Phytochemicals like phenols mostly present in plant extracts contain hydroxyl groups that provide antioxidant activity [21]. By using the standard method of the Folin-Ciocalteu reagent total phenolic content was calculated. For this purpose, as a standard 1% solution of Gallic acid was used as shown in Table 1 and Figure 1. *Senna alexandrina* the water extract showed more phenolic content of 318.2 mg GAE/g as compared to NADES extract of 296.8 mg GAE/g as shown in Figure 2.

Table 1: Regression equation and R^2 values of Gallic acid (GA) and Quercetin (Q) standards.

Standard	Regression equation	R^2
GA	y=0.0118 x +0.0086	0.9986
Q	y=0.0406 x +0.0097	0.9996

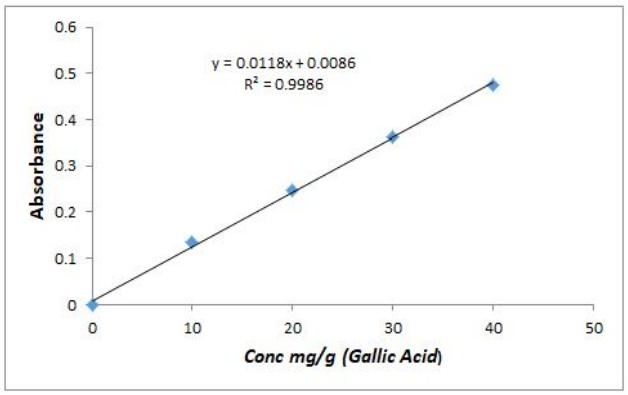

Figure 1: Final standard graph of TPC.

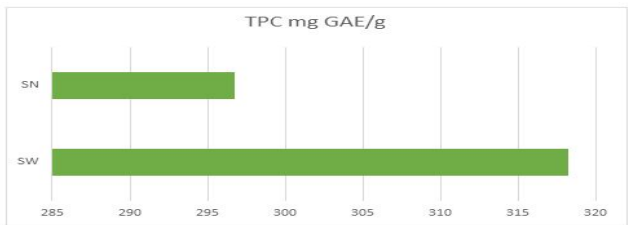

Figure 2: TPC mg GAE g-1 of water extract of *Senna alexandrina* (SW) and NADES extract of *Senna alexandrina* (SN).

Analysis of total flavonoid content

The flavonoids in extracts were calculated using a spectrophotometric method. 1% solution of quercetin was used as a standard solution, its standard curve is shown in Figure 3 value is given in Table 1. Water extracts of *Senna alexandrina* showed more TFC as compared to the NADES extracts. The value of TPC of all samples is shown in graphs Figure 4.

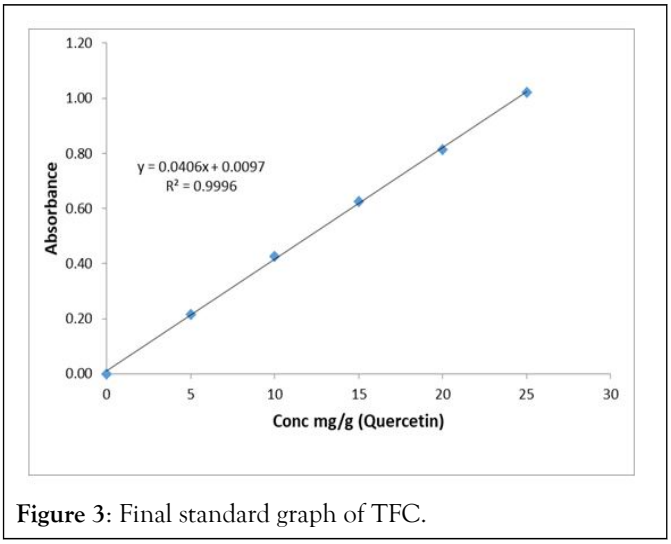

Figure 3: Final standard graph of TFC.

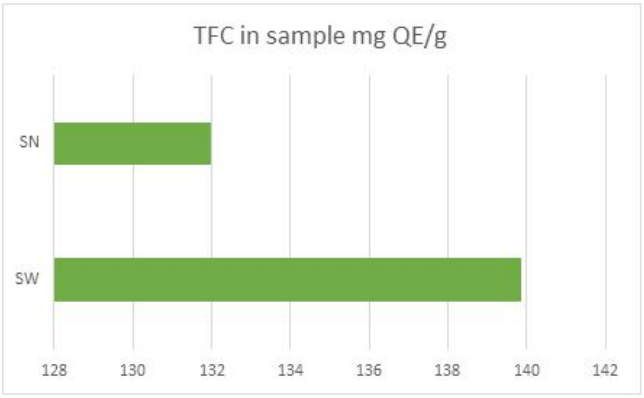

Figure 4: TFC mg GAE g^{-1} of water extract of *Senna alexandrina* (SW) and NADES extract of *Senna alexandrina* (SN).

Antioxidant assay

Antioxidant-rich plant species have been used to treat various ailments. The conventional scavenging method of free radicals was employed to calculate the antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl. When it comes to antioxidant activity, UV radiations were employed to analyze water and NADES extracts of *Senna alexandrina*. UV radiation was absorbed differently by each sample. SW and SN samples absorb 1.052 and 1.325 cm^{-1}, respectively. Samples SW and SN, on the other hand, show percent inhibition of 47.6 and 34, respectively. The graph below shows the comparison of the antioxidant activity of all samples (Figure 5).

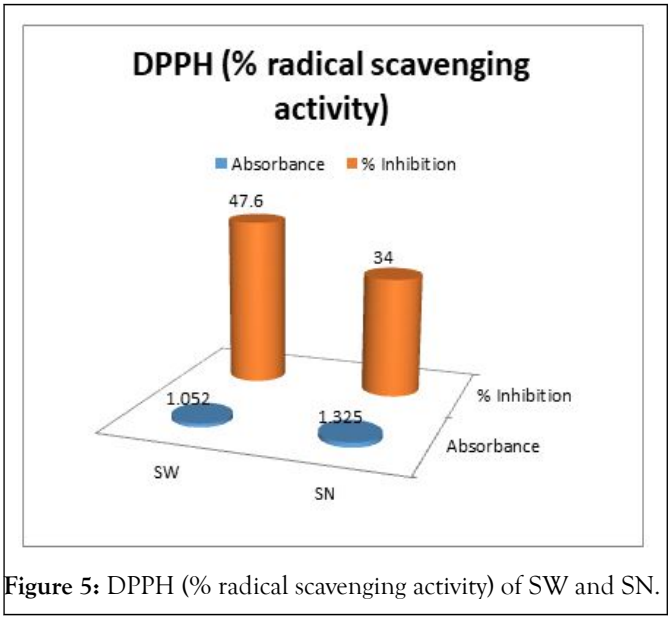

Figure 5: DPPH (% radical scavenging activity) of SW and SN.

HPLC results

The phenolic components were isolated, described, and quantified using this chromatography. In the Water extract of *Senna alexandrina* nine phenolic acids and one flavonoid (Quercetin) were extracted as shown in Table 2. In NADES extract of *Senna alexandrina* six phenolic acids including Gallic Acid and one flavonoid (Quercetin) were extracted as shown in Table 3.

Table 2: Water extract of *Senna alexandrina*.

Compound name	Retention time in ppm
Quercetin	2.953
Gallic acid	4.607
Vanillic acid	13.013
Benzoic acid	14.58
Syringic acid	16.28
p-coumaric acid	17.673
m-coumaric acid	20.233
Ferulic acid	22.653
Cinnamic acid	24.653
Sinapic acid	26.06

Table 3: NADES extract *Senna alexandrinea*.

Quercetin	3.08
Gallic acid	4.94

Caffeic acid	12
Vanillic acid	13.147
Benzoic acid	14.273
Chlorogenic acid	15.007
Syringic acid	16.893

FT-IR results

Infrared spectroscopy, often known as FTIR, which uses infrared light to shake bonds between the molecules inside the sample that absorbs it. Because the majority of the samples have different subatomic bonds or different subatomic bond configurations, FTIR can be used to provide compound data on particles inside the specimen. Based on the maximum value in the region of IR, the FTIR spectroscopy was utilized so that the functional group could be determined. The presence of numerous compounds in SW and SN has been revealed by FTIR spectroscopy analyses, with various peaks values according to stretching frequency in the water and NADES extracts of *Senna alexandrina*.

Water extracts of *Senna alexandrina*: The existence of numerous compounds in the Aqueous extracts of *Senna alexandrina* is confirmed by FT-IR spectroscopy as shown in Table 4 and Figure 6.

Table 4: FT-IR results of aqueous extracts of *Senna alexandrina*.

S.No	Wave number cm^{-1} (*Senna alexandrina* aqueous extract)	Wave number cm^{-1} (reference article)	Functional group assignment	Expected phytcompounds identified
1	3629.37	>3000	sp^2 C-H stretch	Alkenes, Aromatic compounds
2	3492.45	3600-3200 cm^{-1} (strong and broad) 3500-3350 cm^{-1}, 3600-2500 cm^{-2}	O-H stretch, N-H stretch, O-H of $-CO_2H$	Alcohols, Amines, Carboxylic acids
3	3409.53	3600-3200 cm^{-1} (strong and broad) 3500-3350 cm^{-1}, 3600-2500 cm^{-2}	O-H stretch, N-H stretch, O-H of $-CO_2H$	Alcohols, Amines, Carboxylic acids
4	3315.03	3600-3200 cm^{-1} (strong and broad) 3500-3350 cm^{-1}, 3600-2500 cm^{-1} 3333-3267 cm^{-1}	O-H stretch, N-H stretch, O-H of $-CO_2H$, sp C-H stretch	Alcohols, Amines, Carboxylic acids, Alkynes
5	1625.7	1680-1620 cm^{-1} (often weak)	C=C stretch	Alkenes
6	723.18	~ 900-700 cm^{-1}	Aromatic out-of-plane ring bends	Aromatic compounds
7	825.38	~ 900-700 cm^{-1}	Aromatic out-of-plane ring bends	Aromatic compounds

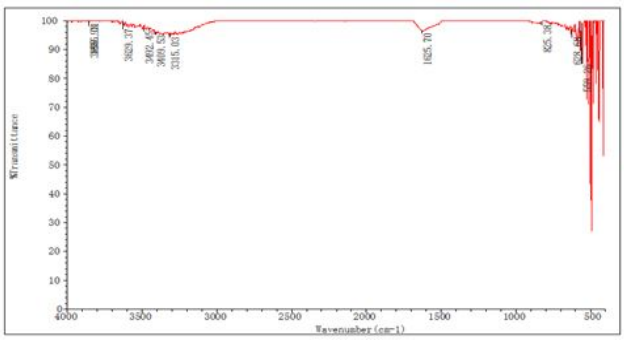

Figure 6: FTIR results of SW.

NADES extracts of *Senna alexandrina*: FTIR spectra of SN extract confirmed that many other functional groups also detected as shown in Table 5 and Figure 7.

Table 5: Results of FT-IR spectroscopy of *Senna alexandrina* NADES extract.

S.No	Wave number cm^{-1}(*Senna alexandrina* NADES extract)	Wave number cm^{-1} (reference article)	Functional group assignment	Expected phytcompounds identified
1	1720.19	1740-1720 cm^{-1}, 2000-1667 cm^{-1}		Aldehydes, Ketones, Carboxylic acids, Esters, Aromatic compounds
2	1222.65	1300-1000 cm^{-1},1342-1266 cm^{-1} (aromatic)	C-O stretch, C-N stretch (often weak and hard to find)	Esters, Amines
3	1108.87	1300-1000 cm^{-1},1342-1266 cm^{-1} (aromatic) 1150-1050 cm^{-1}	C-O stretch, C-N stretch (often weak and hard to find, C-O stretch	Esters, Amines, Alcohols
4	1074.16	1300-1000 cm-1,1342-1266 cm^{-1} (aromatic) 1150-1050 cm^{-1}	C-O stretch, C-N stretch (often weak and hard to find, C-O stretch	Esters, Amines, Alcohols
5	1025.94	1300-1000 cm^{-1},1342-1266 cm^{-1} (aromatic)	C-O stretch, C-N stretch (often weak and hard to find	Esters, Amines
6	896.74	~ 900-700 cm^{-1}	Aromatic out-of-plane ring bends	Aromatic compounds
7	723.18	~ 900-700 cm^{-1}	Aromatic out-of-plane ring bends	Aromatic compounds

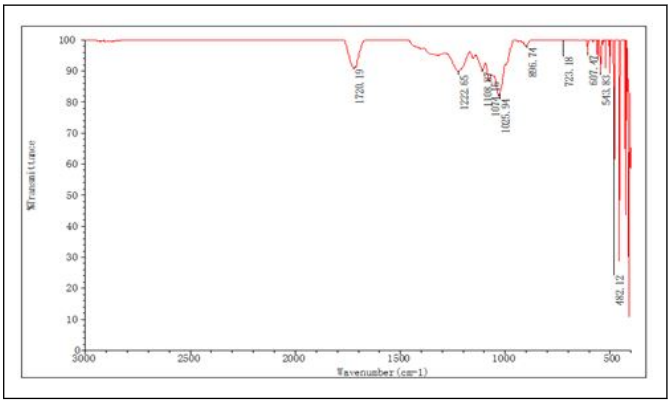

Figure 7: FTIR Spectrum analysis of NADES extract of *Senna alexandrina*.

Antiviral activity

Simultaneous infiltration of inhibitor (Water extract of *Senna alexandrina*, NADES extract of *Senna alexandrina*, and cotton leaf virus was done on Nicotiana bathaminia that was grown in a standard condition required for best growth mentioned above.

Nades extract of *Senna Alexandrina* as an Inhibitor

Plants that were infiltrated with NADES extract of *Senna alexandrina* inhibitor and cotton leaf Multan virus could not survive showed in Figure 8 of 2 weeks analysis under optimum condition.

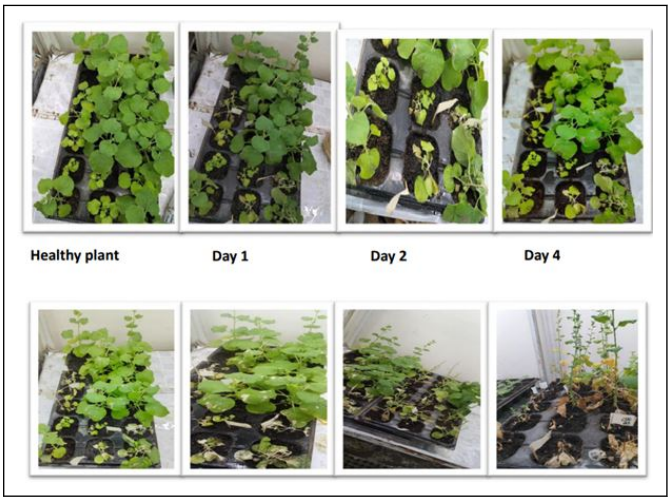

Figure 8: SN as an inhibitor

Water extract of *Senna Alexandrina*

Plants that were infiltrated with Water extract of *Senna alexandrina* inhibitor and cotton leaf Multan virus survived as shown in the following Figure 9 of 2 weeks analysis under optimum condition.

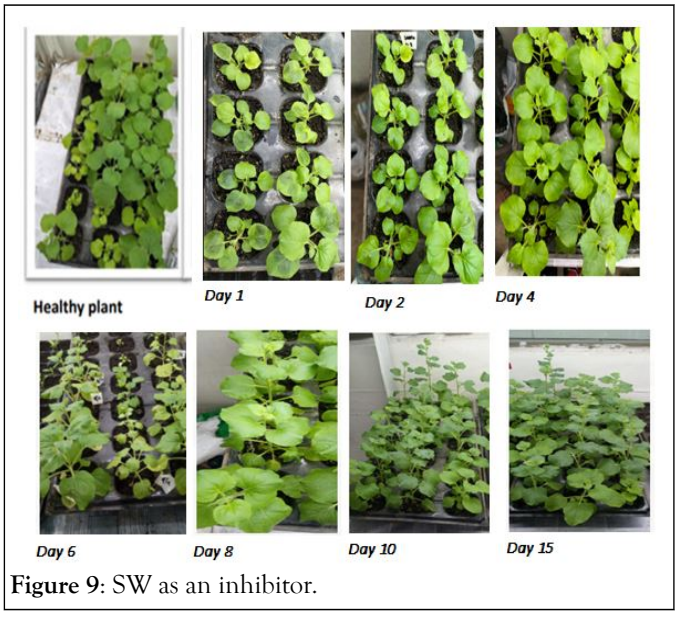

Figure 9: SW as an inhibitor.

DISCUSSION

Senna alexandrina the water extract showed more phenolic content of 318.2 mg GAE/g as compared to NADES extract of 296.8 mg GAE/g as shown in Figure 2. Plant species exhibit a wide range of phenolic compounds, which are diverse secondary metabolites [22,23]. In this study, the phenol content was different from what was reported previously. *Senna alexandrina* showed TPC of 318.2 mg GAE g^{-1} DW, which was lower than that reported (3761 mg GAE g^{-1} DW) [24]. It is possible that these medicinal plants could differ in their phenolic compounds and phytochemicals depending on the phytogeography area and season during which they were harvested caused variation [25,26]. Different extraction techniques and solvent polarities could have caused the variations as well. Among other factors, plant maturity and genotype may have contributed to the variation in phenolic compound concentrations.

In plants there are many functions of flavonoids, they have been suggested to be secondary antioxidant defenses in plants in response to various stresses [27]. Water extracts of *Senna alexandrina* showed more TFC as compared to the NADES extracts. The value of TPC of all samples is shown in graphs (Figure 4). This study's results differ from those reported in the literature [28]. There may be similar factors responsible for the variations in TPC as well.

Antioxidant activity results of *Senna alexandrina* are similar to those reported in the literature. SW and SN samples absorb 1.052 and 1.325 cm^{-1}, respectively. Samples SW and SN, on the other hand, show percent inhibition of 47.6 and 34, respectively. The sample SW absorbed the least amount of UV light but shows a maximum of % inhibition its mean that the anti-oxidant activity of sample SW is at its peak. Basically, a lower IC_{50} value was connected with a stronger DPPH radical-scavenging activity. It has been postulated that samples with an IC_{50} of less than and equal to 50-100 g/mL are strong antioxidants, samples with an IC_{50} of 101-150 µg/mL are moderate antioxidants, and samples with an IC_{50} of greater than 150 µg/mL are weak antioxidants. The DPPH radical is a preferred substrate for measuring antioxidant activity fast due to its stability in the radical state and simplicity of assay. According to recent research, phenolic chemicals extracted from plants may be responsible for the antioxidant action, particularly flavonoids, due to -OH groups, which possess redox properties [29].

The phenolic components were isolated, described, and quantified using HPLC. In the Water extract of *Senna alexandrina* nine phenolic acids and one flavonoid (Quercetin) were extracted as shown in Table 2. In NADES extract of *Senna alexandrina* six phenolic acids including Gallic Acid and one flavonoid (quercetin) were extracted as shown in table 3. There are differences in chemistry between them. The presence of these compounds is also reported in the literature [30].

The existence of numerous compounds in the water extracts of *Senna alexandrina* is confirmed by FT-IR spectroscopy as shown in Figure 6. The existence of six functional groups in *Senna alexandrina* aqueous extracts is demonstrated. The strong absorption at 3492.45 cm^{-1} indicates the O-H, N-H, and O-H of $-CO_2H$ are present. This indicates that carbonyl compounds were present in the aqueous extract. Other groups are absorbed at a slower rate as shown in Table 4. In FTIR of SN the prominent band at 1720.19 cm^{-1} stretching frequency indicate carbonyl (C=O) group which is associated with conjugated aromatic compounds. The band at 1222.65 cm^{-1}, 1108.87 cm^{-1}, 1074.16 cm^{-1} and 1025.94 cm^{-1} was due to the presence of C-O and C-N (normally weak and difficult to find) stretch groups, respectively. The intense bands at 1222.65 cm^{-1} and 1108.87 cm^{-1} stretching suggest that the esters and secondary alcohols are present in the extract. These results are according to those reported in the literature [31,32].

CONCLUSION

Plants that were infiltrated with water extract of *Senna alexandrina* inhibitor and cotton leaf Multan virus survived in 2 weeks analysis under optimum condition. While plants that were infiltrated with NADES extract of *Senna alexandrina* could not survive. SW extract contains a large amount of phenolic acid along with quercetin that inhibits the virus to attack and as result plants survive. Quercetin and p-coumaroyl show antiviral activity by inducing autophagy and inhibiting viral mRNA synthesis. So, the results are following the literature. [33,34]. The *Senna alexandrina* presented antiviral and higher levels of antioxidant activity due to their phenolic compounds. This means that these herbs can be used in pharmaceuticals to boost the body's endogenous antioxidant system and reduce free radical levels. To validate their use in the management of viral diseases, further experiments and clinical studies are recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

REFERENCES

1. Hostettmann K, Marston A, Ndjoko K, Wolfender JL. The potential of African plants as a source of drugs. Curr Org Chem. 2000;4(10):973-1010.
2. Alves RRN, Rosa IL, Albuquerque UP, Cunningham AB. Medicine from the wild: an overview of the use and trade of animal products in traditional medicines. Anim Tra Fol Med. 2013:25-42.
3. Saxena PK. Development of plant-based medicines: conservation, efficacy and safety. Springer. 2001;251–255.
4. Elansary HO, Mahmoud EA, Shokralla S, Yessoufou K. Diversity of plants, traditional knowledge, and practices in local cosmetics: A case study from Alexandria, Egypt. Econ Bot. 2015;69(2):114-126.
5. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74(17):2157-84.
6. Illamola SM, Amaeze OU, Krepkova LV, Birnbaum AK, Karanam A, Job KM, et al. Use of herbal medicine by pregnant women: What physicians need to know. Front Pharmacol. 2020; 10:1483.
7. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep. 2009;26(8): 1001-1043.
8. Qurishi Y, Hamid A, Zargar M, Singh SK, Saxena AK. Potential role of natural molecules in health and disease: Importance of boswellic acid. J Med Plants Res. 2010;4(25):2778-2786.
9. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013.
10. Fang SC, Hsu CL, Lin HT, Yen GC. Anticancer effects of flavonoid derivatives isolated from Millettia reticulata Benth in SK-Hep-1 human hepatocellular carcinoma cells. J Agric Food Chem. 2010;58(2): 814-820.
11. Grozdanova T, Trusheva B, Alipieva K, Popova M, Dimitrova L, Najdenski H, et al. Extracts of medicinal plants with natural deep eutectic solvents: enhanced antimicrobial activity and low genotoxicity. BMC Chem. 2020;14(1):1-9.
12. Surveswaran S, Cai Y-Z, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. 2007;102(3):938-953.
13. Tembo DT, Holmes MJ, Marshall LJ. Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (*Adansonia digitata*) pulp from Malawi. J Food Compos Anal. 2017;58:40-51.
14. Alamgir A. Herbal drugs: their collection, preservation, and preparation; evaluation, quality control, and standardization of herbal drugs. Therapeutic Use of Medicinal Plants and Their Extracts. Volume 1. Springer, Cham. 2017.
15. Vernon LS, Rudolf O, Rosa M, Lamuela-Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999; 299:152-178.
16. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc. 2007;2(4):875-877.
17. Devendra K, Kiran D, Ritesh V, Satyendra B, Abhishek K. To evaluation of Total Phenolics and Flavonoids in different Plant of Chhattisgarh. J Pharmacognosy Phytochem. 2013;2(4):116-118.
18. Chen HY, Lin YC, Hsieh CL. Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem. 2007;104(4):1418-1424.
19. Kremer D, Bolarić S, Ballian D, Bogunić F, Stešević D, Karlović K, et al. Morphological, genetic and phytochemical variation of the endemic Teucrium arduini L. (*Lamiaceae*). Phytochem. 2015;116:111-119.
20. Ueda M, Nakamura Y. Plant phenolic compounds controlling leaf movement. Recent Adv Polyphenol Res. 2010;2:226-237.
21. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: chemical properties, biological activities, and synthesis. Angew Chem Int Ed. 2011;50(3):586-621.
22. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 1999;66(4):401-436.
23. Sangwan R, Chaurasiya N, Misra L, Lal P, Uniyal G, Sharma R, et al. Phytochemical variability in commercial herbal products and preparations of *Withania somnifera* (Ashwagandha). Current Sci. 2004:461-465.
24. Alsiede MMSA, Abddrahman MA, Saeed AEM. Phytochemical screening, total phenolics content and antioxidants activity of Cassia Singueana. J Med Plants Stud. 2015;3(5):160-165.
25. Mpofu A, Sapirstein HD, Beta T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. J Agric Food Chem. 2006;54(4):1265-1270.
26. Soni U, Brar S, Gauttam VK. Effect of seasonal variation on secondary metabolites of medicinal plants. Int J Pharm Sci Res. 2015; 6(9):3654-3662.
27. Panche A, Diwan A, Chandra S. Flavonoids: an overview. J Nutr Sci. 2016;5:1-15.
28. Mwamatope B, Tembo D, Chikowe I, Kampira E, Nyirenda C. Total phenolic contents and antioxidant activity of Senna singueana, Melia azedarach, Moringa oleifera uand Lannea discolor herbal plants. Scientific African. 2020;9:00481.
29. Borkataky M, Kakoti B, Saikia L. Influence of total phenolic content and total flavonoid content on the DPPH radical scavenging activity of

Costus speciosus (Koen Ex. Retz.) Sm. South Asian J Exp Biol. 2015; 4(5):261-266.

30. Elansary HO, Szopa A, Kubica P, Ekiert H, Ali HM, Elshikh MS, et al. Bioactivities of traditional medicinal plants in Alexandria. J Evidence-Based Complementary Altern Med.
31. Yadav A, Kaushik A, Joshi A. Green synthesis of silver nanoparticles using *Ocimum sanctum* L. and *Ocimum americanum* L. for their antibacterial potential Int J Life Sci Pharma Res. 2018;8(1):42-49.
32. Najah Z, Awad M, Alshawish M. Phytochemical screening, analytical profile and green synthesis of silver nanoparticles of *senna alexandrina* mill. Eur J Biomed Pharm Sci. 2019; 6(12):47-53.
33. Solnier J, Fladerer JP. Flavonoids: A complementary approach to conventional therapy of COVID-19? Phytochem. Rev. 2021; 20(4): 773-779.
34. Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, et al. The ORF3a protein of SARS-CoV-2 induces apoptosis in cells. Cell Mol Immunol. 2020;17(8):881-88.