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PHYTOCHEMICAL SCREENING, ANALYTICAL PROFILE AND GREEN SYNTHESIS OF SILVER NANOPARTICLES OF SENNA ALEXANDRINA MILL

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ABSTRACT
Phytochemical screening of *Senna alexandrina* Mill leaves extract exhibited the presence of carbohydrates, saponins, alkaloids, glycosides, proteins, flavonoids, tannins and steroids. The percentage of studied elements (w/w) was as follows; sodium 0.199, potassium 1.076, calcium 1.250 and barium 0.250. *Senna alexandrina* leaf extract was used as the reducing agent to synthesize silver nanoparticles (Ag-NPs). During synthesis, the color was changed after addition of aqueous extract indicating the formation of silver nanoparticles. UV-Vis spectrophotometer confirmed the formation of Ag-NPs complex by appearance of absorption peak in visible region owing to localized surface Plasmon resonance (LSPR). Quantity of the aqueous extract and heating time of bioreduction reaction effects on the formation of Ag-NPs were evaluated. FTIR analysis showed characteristic common functional groups of hydroxyl, amino, carbonyl, and protein which involved in the synthesis and stabilizing the covering layer of Ag-NPs in aqueous medium.

KEY WORDS: Phytochemical, *Senna alexandrina*, Green synthesis, FTIR, Sliver nanoparticles.

INTRODUCTION
Medicinal plants contain several natural substances which produce specific physiological action on the human body and these bioactive compounds include alkaloids, carbohydrates, glycosides, phytosterols, phenols, tannins, flavonoids, terpenoids, steroids and quinones.[1,2] The *Sennas*, is a large genus of flowering plants in the legume family *Fabaceae*, and the sub-family *Caesalpiniaceae*. This family consist about 260 to 350 species, about 50 species of *Senna* are known in farming.[3,4] This type of medicinal plants a known locally in Libya and middle east countries as Sanna Makki, and it has been widely used in folk medicinal in these countries. The medicinal activities of *Senna* leaves can be attributed mainly to the anthraquinone glycosides, especially sennoside A and B.[5,6] The *Senna alexandrina* plant is indigenous to tropical Africa, and grows wildly near the River Nile from Aswan in south Egypt to Kordofan in the west of Sudan; also it's naturally spread in Somalia, Pakistan, and India.[6] The *Senna* plants are small shrubs, up to 1.5 m high and grow in subtropical climate over sandy loam soil after autumn season and can be maintained as a perennial crop for 2 to 3 years.[6,7] The *Senna alexandrina* Mill leaves are alternate, par pinnate, having 5 to10.5 cm long, with 5to 9 pairs of lance late leaflets, with the whole edge, with acute tip, long about 1.2 to 4.5 cm and 3.5 to 10 mm wide, glabrous hairy on both sides, of pale green color, and contain glycosides, sennoside A, B, C and D.[8]

The biosynthesis of metal nanoparticles using medicinal plants extract have been studied expansively because of their unique physicochemical characteristics including antibacterial properties, electronic properties, catalytic activity, optical properties, and magnetic properties, the previous effects are related to their extremely small size and large surface to volume ratio.[9,10] Silver and Gold nanoparticles are the most important nanoparticles and their biomedical applications are growing everyday.[11] The aim of this research is to investigate the phytochemical constituents of *Senna alexandrina* Mill leaves extracts and evaluation the level of essential elements in *Senna alexandrina* Mill leaves using flame photometer. The study also aims to perform a green synthesis Ag-NPs using *Senna* leaf extract as the reducing agent.

MATERIALS and METHODS
Plant Material
The leaves of *Senna* (Figure 1) was collected from the local market, the dried leaves were milled to a fine powder (Figure 1) using lab Blender (Conair Waring, model 7011HS), stored in the dark at room temperature in closed containers until required.

Fig. 1: (A) *Senna alexandrina*, (B) leaves powder.

3.0 g of dried leaf powder of *Senna alexandrina* was macerated separately in 100 mL of, distilled water, 95% ethanol, methanol, ethyl acetate, chloroform, and diethyl ether at ambient temperature for two days with occasional shaking and then filtered off. The solvent was evaporated in vacuum to give a crude product.[12]

The pH of the five extracts of *Senna* leaf was determined using calibrated pH meter, model 3510 from Jenway company. 1.0 g of the different solvent extracts of *Senna* leaf was dissolved in 100 mL of distilled water, and then the pH values of the crude extracts were recorded.

Phytochemical Screening

Freshly prepared extracts were subjected to standard phytochemical analysis to investigate the presence of phytochemical constituents such as carbohydrates, saponins, alkaloids, glycosides, proteins, flavonoids, phenols, sesquiterpenes, tannins, and phytosterols according to the standard methods.[1,13]

i) Test for alkaloids

1.0 g of the crude extract was dissolved in 3.0 mL of slightly warm solution of 1% Hydrochloric acid, the mixture was filtered and then added 2.0 mL of:

a) Mayer's reagent ($HgCl_2$ + KI), the formation of yellow precipitate, confirmed the presence of alkaloids.
b) Wagner's reagent (KI / I_2), the yellow precipitate was taken as indicator for the presence of alkaloids.
c) Dragendorff reagen ($BiNO_3$ + KI) t, the brown to red precipitate was taken as indicator for the presence of alkaloids.
d) Hager's (picric acid conc.) reagent, the formation of yellow precipitate, confirmed the presence of alkaloids.[14]

ii) Tests for carbohydrates: 5.0 mL of extract solutions were added to:

a) Molisch's reagent (α-Naphathol in alcohol), a violet ring at the liquids interphase indicates the presence of carbohydrate.
b) Benedict's test, extracted solution was mixed with 3.0 mL of Benedict's reagent and boiled. A reddish brown precipitate indicates the presence of reduced sugars.
c) Fehling's test, 3.0 mL of extracted solution was mixed with 4.0 mL of equal volumes of Fehling A and Fehling B solutions and boiled. Formation of pink precipitate indicates the presence of reduced sugars.[14]

iii) Tests for Glycosides

1.0 g of the crude solvents extract was dissolved in 2.0 mL of 1% HCl, the mixture was filtered and then added 2.0 mL of:

a) Borntrager's (modified) test, the acidic extract was mixed with 2.0 mL of ferric chloride (1:1) and shaken in water bath for 5 minutes, 4.0 mL of benzene added to the cold mixture, filtered and 5.0 mL of 10% ammonia solution was added to the filtrate. The mixture was stirred; the presence of a pink color in the lower layer indicates the presence of anthrol glycosides.
b) Legal's test (sodium nitropruside in pyridine + NaOH), the pink to red color was taken as indicator for the presence of cardiac glycosides.[14]
c) Keller Kiliani test: 2.0 mL of solvent extract was mixed with 2.0 ml of glacial acetic acid containing drops of 2% ferric chloride solution. The mixture was then poured into a test tube containing 1.0 mL of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of cardiac glycosides.[15]

iv) Tests for Phytosterols

a) Salkowski's test, 2.0 mL of each extract was dissolved in 2.0 mL of chloroform and filtered, then the filtrate was treated with few drops of concentrated Sulphuric acid then shaked and left to stabilize. Formation of a golden yellow color indicated the presence of phytosterols.
b) Liebermann's test, 4.0 mL of each extract was mixed with 4.0 mL of chloroform and filtered, then 4.0 mL of acetic acid was added and the mixture cooled well in ice. Drops of Sulphuric acid was added carefully. A violet ring at the interphase indicates the presence of a Phytosterols.[14]

v) **Tests for Saponins:** Foam test, 10 mL of solvent extract was mixed with 10 mL of distilled water. The mixture was shaken vigorously. Formation of 1.0 cm foam layer indicated for the presence of Saponins.[14]

vi) **Tests for Tannins (phenols):** 1.0 g of crude extract was dissolved in 2.0 mL of distilled water, then the extracted solution was mixed with few drops of 10% ferric chloride solution. A blue, green or black color indicate the presence of tannins and phenols.[13]

vii) **Test for flavonoids**

a) Shinoda's test, 4.0 mL of each extract was added to magnesium metal, then drops of conc. Hydrochloric acid was added carefully. The formation of a yellow precipitate after a few minutes was taken as a positive result for flavonoids.[15]

viii) **MTests for Proteins and Amino acids**

a) Millon test, 2.0 mL of each extract was mixed with 2.0 mL of distilled water, then drops of Millon reagent was added and shaken gently. A white precipitate was taken as a positive result for protein.[15]

b) Biuret test, 2.0 mL of each extract was mixed with drops of 2 % copper sulphate, then 1.0 mL 95% ethanol and pieces of sodium metal was added. Formation of pink color in ethanol layer indicated for the presence of proteins.[16]

c) Xanthoprotic test, 2.0 mL of crude extract was mixed with 1.0 mL of conc. Nitric acid, then boiled and cooled. Formation of yellow color confirmed the presence of proteins.

d) Ninhydrin test, 2.0 mL of each crude extract was mixed with 1.0 mL of 25% Ninhydrin, then boiled for two minutes and cooled. Formation of violet to black color confirmed the presence of amino acids.[14]

ix) **Test for Terpenoids:** 1.0 g of crude extract was shaken with 2.0 mL of chloroform and evaporated to dryness. 2.0 mL of conc. sulphuric acid was added, and then heated for two minutes. Formation a greyish color confirmed the presence of terpenoids.

x) **Test for Quinone:** 1.0 mL of solvent extract mixed with 1.0 mL of concentrated sulphuric acid**.** The formation of a red precipitate was taken as a positive result for quinone.

Elements analysis

About 1.0 g of *Senna* leaves dry powder was digested using 20.0 mL of mixture of nitric acid and perchloric acid (1:1). Digested sample was filtered and volume is made up to 50.0 mL with distilled water and analyzed by flam photometer (BWB Technologies, UK).

Synthesis of Silver Nanoparticles (Ag-NPs)

About 1.0 g of *Senna alexandrina* leaves were boiled for 10 minutes in 50.0 mL of distilled water. The extract was centrifuged at 600 rpm for 20 minutes, then the supernatant layer was filtrated with Whatman filter paper. The final filtrate was stored in refrigerator at 4 ^{o}C for further use. Optimization studies were performed to find out the best conditions for the synthesis, plant extract to silver salt solution ratio and heating times. Different quantities of *Senna* extract was added to 1.0 mM of $AgNO_3$ in glass tube to get different mixture ratios (0.50:10, 1.0:10, 1.50:10, 2.0:10 and 3.0:10). The mixed solutions were heated for 50 minutes at 60 ^{o}C the optimum ratio (2.0:10) which showed more pronounced peak with high intensity related to absorption band of LSPR. Similarly, the mixture with 2.0:10 ratio was heated at optimum temperature (60 ^{o}C) for different times, from 5 to 160 minutes, after 5 minutes, the color was changed indicating the formation of Ag-NPs.

IR and UV Measurements

IR Spectra were recorded on Perkin Elmer Paragon RX I FTIR spectrophotometer accompanied with ATR accessory. 0.01 g of Ag-NPs was mixed with 0.15 g KBr and pressed to form transparent disc, then the infrared spectrum was recorded.

RESULTS and DISCUSSION

The moisture and Ash content of *Senna alexandrina leaf* were found to be 5.360 and 10.84 % respectively. The results of metals composition analysis of *Senna alexandrina* leaf (Table 1) showed that the leaf sample is rich with calcium and potassium. Trace amounts of sodium and barium were also detected, mineral metals compositions are calculated based on the dry leaf.

Calcium is the main constituent of the skeleton, in addition, calcium has some physiological activities such as blood coagulation and convulsion and excitation of muscles. Potassium ions play important role in the maintenance of the cardiac rhythm and in constipation.[17,18]

Table 1: Elemental percentage in *Senna* leaves (w/w%).

The element	Na	K	Ca	Ba
Percentage	0.199	1.076	1.250	0.250

Table 2 shows that all extracts were acidic and pH falls within the range of 5.6 to 6.1, similar pH range was recorded in previous study.[19] Different solvents were used to extract the different chemical components, the extraction percentage was 50%, 48%, 43%, 36%, 35, and 27% for distilled water, ethanol, methanol, ethyl acetate, chloroform, and diethyl ether respectively, then crude extracts were subjected to several phytochemical screening tests to detect the active constituents present in each solvents extract.

Table 2: pH and phytochemical screening of *Senna alexandrina* Mill leaves extracts.

Phytochemical constituents	Extract	Water	Ethanol	Methanol	Ethyl acetate	Chloroform	Ether
	pH	**5.8**	**5.7**	**5.6**	**5.6**	**5.9**	**6.1**
Alkaloids	Mayer's	+	+	_	_	+	_
	Wagner's	+	+	+	+	+	_
	Dragendorff 's	_	_	+	+	+	+
	Hager's	+	_	_	+	+	_
Carbohydrates	Molisch's	+	_	+	_	_	_
	Benedict's	_	_	+	_	_	_
	Fehling's	+	+	+	_	+	+
Glycosides	Borntrager's	_	+	+	+	+	_
	Legal's	+	_	+	+	_	+
	Keller-Killiani	_	+	+	+	_	_
Phytosterols	Salkowski's	_	_	+	+	_	_
	Liebermann's	+	_	+	+	_	_
Saponins		+	_	+	_	_	_
Phenols	$FeCl_3$ test	+	+	+	_	_	_
Tannins		+	+	+	+	-	-
Flavonoids	Alkali test	_	_	+	_	+	+
	Lead acetate	+	_	+	_	+	+
	Shinoda test	_	+	+	+	_	_
Proteins and Amino acids	Millon's	+	_	+	+	_	+
	Biuret	_	_	_	+	_	_
	Xanthoproteic	+	_	_	+	_	_
	Ninhydrin	_	_	_	_	_	_
Sesquiterpenes		+	+	+	+	+	+
Terpenoids		-	-	-	-	-	-
Quinones		-	-	-	-	-	-

Phytochemical screening of the extracts revealed the presence of carbohydrates, saponins, alkaloids, glycosides, proteins, flavonoids, phenols, sesquiterpenes, tannins, and phytosterols. Terpenoids and Quinones testsfor all extracts were negative. The screening also exhibited that, the aqueous extract was rich in all phytochemicals constituents followed by methanol extract. The presence of several phytochemicals in *Senna* extracts has also been revealed by many researchers.[1,7,20] The phenolic compounds such tannins and flavonoids act as primary antioxidants or free radical scavenger and used as antibacterial.[1] Alkaloids are present in all extracts, alkaloids are have wide range of pharmacological activities including anticancer and antimalarial.[13] The leaves extracts of *Senna alexandrina* plant contains also anthraquinone glycosides (Sennosides) that have a significant laxative effect.[8]

The first indication of the formation of Silver nanoparticles (Ag-NPs) was accomplished during the reaction, the clear change of color solution as a result of surface Plasmon vibrations.[21,22] As the different volume of *Senna alexandrina* leaf extract were added to 10 mL of 1 mM of $AgNO_3$ solution, the color of the solution changed from yellowish to brownish and finally to dark brown indicating reduction of silver and Ag-NPs complex formation (Figure 2).

Fig. 2: Color change during bioreduction synthesis of Ag-NPs solution over several volume ratio.

Figure 3 shows the absorption spectra for silver nitrate mixed with different quantity of *Senna alexandrina* leaf extract heated for 50 minutes at 60 ^oC. The Plasmonic peak of silver nanoparticles formed in the reaction media remained almost at about the same wave length with a slight shift of λ_{max} from 405 to 415 nm due to surface Plasmon resonance of Ag-NPs.The absorbance intensity of this peak was increased with increasing the ratio between *Senna* extract and silver nitrate solution until the ratio reached 2.0:10 for saturated leaf extract and 1mM $AgNO_3$ respectively, and then the absorbance intensity was decreased when the ratio was further increased.

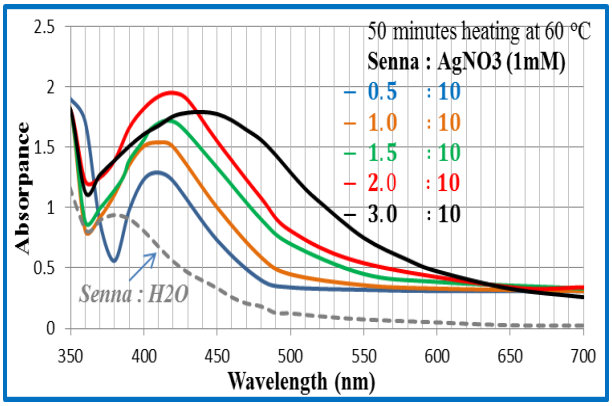

Fig. 3: UV-Vis spectrum of Ag-NPs produced using different concentration of *Senna* leaf extract.

The optimum heating time of bioreduction reaction between *Senna* extract and $AgNO_3$ in 2.0:10 ratio at 60 ^oC was estimated by measurement of the absorbance of the reaction mixture at 415 nm in different time intervals. Fig. 4 shows that, only 5 minutes was required for Ag-NPs color intensity development and the intensity of brown color was stabled after 80 minutes.

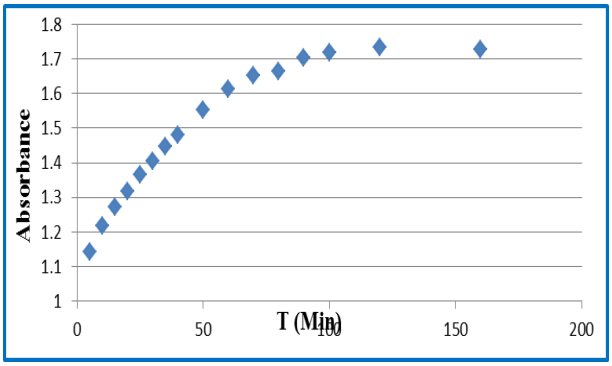

Fig. 4: Absorbance *versus* Time / min of bioreduction reaction at 60^{o}C.

The IR spectrum of silver NPs (Fig. 5) showed seven peaks, with a broad one at about 3309.81 cm^{-1} most likely for hydroxyl groups O-H stretching vibration and H-bonded of phenols and alcohols. Weak peak at 2927 cm^{-1} probably for aliphatic C-H symmetry stretching vibration, and two peaks also at 1714.7 and 1604.2 cm^{-1} probably for carbonyl groups C=O and N-H bend of primary amines respectively, this N-H groups are responsible for silver NPs stability as reported in the recent studies.[23] Strong peak occurred at 1324.13 cm^{-1} probably for -CH_3 bend vibration, and the remaining peaks at 1071.2 and 1025.38 cm^{-1} were assigned to C–C bond. The weak peak at 822.6 cm^{-1} was characterization of out of the plane deformation vibration of C-H bond in aromatic components.[24]

The phytochemical analysis was carried out to confirm the presence of flavonoids, and proteins molecules consists a carbonyl bonded to nitrogen has strong binding ability with metal. The flavonoids present in the *Senna* leaf extract are powerful reducing agents which may be responsible for the bioreduction reaction with silver nitrate, while the carboxylate group present in proteins can act as surfactant to attach on the surface of silver nanoparticles and it stabilizes silver - NPs through electrostatic stabilization. Thus it is found that *Senna* leaf extract has the ability to perform dual functions of reduction and stabilization of silver NPs.

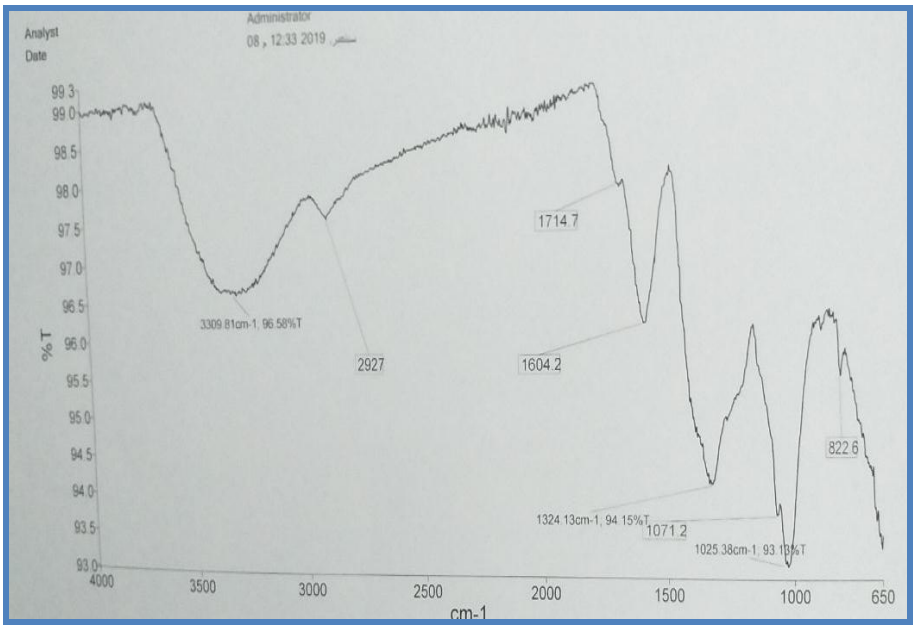

Fig. 5: FTIR spectrum of synthesized silver NPs using *Senna alexandrina* leaf extract.

CONCLUSION

The study revealed that *Senna alexandrina* leaves contain carbohydrates, saponins, alkaloids, glycosides, proteins, flavonoids, phenols, sesquiterpenes, tannins, and phytosterols. Therefore, the *Senna* leaves could be a good source for pharmaceuticals. Also in this work, Ag-NPs were synthesized by the Turkevich chemical method using *Senna alexandrina* leaves extract as the reducing agent. The prepared samples were characterized using UV-visible absorption spectroscopy. The change in color during synthesis processes and the presence of pronounced peak in the visible region in absorption spectra confirms the formation of Ag-NPs. The effect of *Senna* extract quantity and heating time on the formation of Ag-NPs was investigated. The obtained results showed that the quantity of *Senna* has significant effect only on the reaction rate and the optimum ratio was found to be 2.0:10 for *Senna alexandrina* extract to silver nitrate. In addition, it is also found that increasing of heating time at 60 ^{o}C was required for the maximum color intensity development and the brown color was stabled after 80 minutes. FTIR measurements were carried out and confirmed the presence of multiple function groups.

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