









Research Article

Chemical Constituents, Antioxidant and Anti-inflammatory Properties of De-chlorophyllized Extracts of *Salvia officinalis* Leaf

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Abstract

Background: *Salvia officinalis* is a medicinal plant used for the treatment of various disorders such as inflammation, rheumatism, ulcers, dizziness, high blood pressure and high blood sugar. Studies have associated its medicinal properties to its strong chemical constituents and various pharmacological effects. **Objectives:** The work was to evaluate the total contents of flavonoid, phenolic, saponins and tannin along with the antioxidant and anti-inflammatory properties of dechlorophyllized ethanol extract of *Salvia officinalis* leaf. **Method:** The *Salvia officinalis* leaves were macerated in ethanol and liquid-liquid extraction to obtain complete removal of the chlorophyll content. While the phytochemical constituents and the antioxidant, anti-inflammatory activities of dechlorophyllized extract of *Salvia officinalis* leaf were estimated by standard methods. **Results:** The flavonoid, phenolic, saponins and tannin contents of the *Salvia officinalis* dechlorophyllized leaves were 64.517mgQE/g, 91.433mgGAE/g, 185.666mgSE/g and 47.333mg/TAE/g extract respectively. The dechlorophyllized extract had high antioxidant activity: ferric reducing antioxidant power (FRAP) of 14.157mg/l QE and IC₅₀ of 16.28ppm with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. It had potent anti-inflammatory activity with IC₅₀ value of 115.201ppm. **Conclusion:** The results demonstrated that *Salvia officinalis* leaf is a source of antioxidant and anti-inflammatory pharmacologic agents.

Keywords

Dechlorophyllation, *Salvia officinalis* Leaf, Pharmacologic Agents

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1. Introduction

Chemical compounds in plants are mostly products of primary than secondary metabolic processes. Though secondary metabolites do not actively participate in important processes in plants, but are of unalloyed pharmaceutical and pharmacological benefits to man [1, 2]. Medicinal values of plants lie in the chemical compounds or substrates that produce definitive physiological actions in humans, hence their role in modern medicine is indisputable [3]. An example of such medicinal plants is *Salvia officinalis* (Common Sage) whose leaves extract have been utilized in various therapeutic issues. The plant belongs to the Lamiaceae family and its leaves have a unique aromatic odour or smell. The leaves are commonly used as flavor species and herbal medicine [4, 5]. Several researches did show that *Salvia officinalis* leaves have biologic potentials that include hypoglycaemic, antimicrobial, anti-inflammatory, anti-oxidant, anti-viral, hypotensive, anti-insecticide and can protect the liver in parasitaemic hepatotoxic injury [6-11]. Several studies have also shown that *Salvia officinalis* leaves contain phytochemical constituents such as phenols, flavonoids, tannins saponins with antioxidant potentials [6, 12-15]. Abidin et al., [1] joined the assertion and observed that the initial stage in phytochemical studies is the ability to determine the best extraction method for plant samples and that it contributes in determining the type and amount of the chemical substance extracted. They further stated that such extraction reveals a mixture of complex chemical substances [3, 16]. Also such crude extracts contain chlorophyll-pigments, which alter the quality of the obtained extract but that this can be removed through dechlorophyllization method [17]. The same authors observed that dechlorophyllization method decreases the chlorophyll content of the leaves and reveals the active compounds, hence making phytochemical substances better concentrated and more active biologically. Therefore, the present work, investigated the phytochemical compounds, and pharmacological activity (such as the antioxidant and anti-inflammatory activities) of *Salvia officinalis* (Common Sage) leaf dechlorophyllized in ethanol.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used for this work were of analytical grades.

2.2. Identification of the Plant

Salvia officinalis plant leaves was obtained from Vom-Jos, Plateau State and identified / authenticated by a Botanist (Dr Michael, Ozioma Emmanuel) with a voucher number *DELSU #134* for future reference at Delta State University, Abraka-Nigeria. The green and fresh sage leaves were cleaned, and dried under shade.

2.3. Plant Leaf Extraction

The dried leaves were pulverized and about 100g of the pulverized leaves were extracted with 200ml of 95% ethanol via maceration at room temperature for 72hr. It was filtered and re-macerated several times until complete extraction. The ethanol extract was then subjected to rotary evaporation at 40°C with a reduced pressure to obtain a crude ethanol extract.

2.4. Dechlorophyllation of the Crude (*Salvia officinalis*) Ethanol Extract

This was done by liquid-liquid extraction method, by the addition of 4g of the crude extract into 20ml of methanol, partitioned with 20ml of n-hexane in 250ml separating funnel. This was repeated severally until the hexane layer was cleared. It was later subjected to rotary evaporator. The dried extract was weighed, labeled and stored in an air tight container till needed [18].

2.5. Percentage Chlorophyll (*Salvia officinalis* Leaf) Removal Determination

This was done utilizing UV-spectrophotometer as described by Supatak et al., [19]. The absorbance of the crude extract and the dechlorophyllized extract were measured. Then the percentage (%) chlorophyll removed was determined using the equation below:

$$\% \text{ Chlorophyll removed} = \frac{\text{Abs C} - \text{Abs D}}{\text{Abs C} \times 100\%}$$

Where Abs C = absorbance of crude ethanol extract, Abs D = absorbance of the dechlorophyllized extract.

2.6. Total Flavonoid Content of the Dechlorophyllized Extract

This was determined by spectrophotometric method as described by Kusuma et al., [20]. One (1ml) of the 500mg/l of the extract in methanol was mixed with 1ml of 2% aluminum chloride plus 1ml of 120mmol of potassium acetone. The thoroughly mixed tube was kept at room temperature for 30min. The absorbance was read in triplicate in spectrophotometer. Quercetin standard calibration curve was prepared from which the total flavonoid content was determined and results expressed as mg Quercetin equivalent per gram extract (mgQE/g extract).

2.7. Phenol Content of the Dechlorophyllized *Salvia officinalis* Extract

This was also determined by the modified Folin-Ciocalteu method as described by Mapoung et al., [21]. One (1) ml of 300mg/l, of the extract in methanol plus 1ml of 10% Folin – Ciocalteu reagent. The tube mixture was incubated at room

temperature for 5min after which 1ml of 7% sodium carbonate was added into it, mixed and incubated for further. The absorbance was read at 754nm wavelength. A standard calibration curve of Gallic acid at 8, 12, 16, 20 and 24mg/l concentrations were prepared. The spectrophotometric measurements, was done in triplicate. The phenolic content of the extract was expressed as mg gallic acid equivalent per gram of the extract (mgGAE/g extract).

2.8. Saponins Content of the Dechlorophyllized *Salvia officinalis* Extract

The vanillin acetic acid method of Cheok et al., [22] was used. An aliquot (0.6ml) of 500mg/l extract in ethanol was evaporated to dryness, into which 0.2ml of freshly prepared 5% vanillic acetic acid was added plus 0.8ml of perchloric acid. It was well mixed and incubated at 60°C for 15min. It was then allowed to cool in an ice bath for 20s with the addition of 5ml of glacial acetic acid. Later the absorbance of the mixture was read in triplicate at 466nm wavelength. A standard calibration curve of sapogenin at 60, 80, 100, 120 and 140mg/l concentrations was prepared from which the total saponins content was calculated as mg saponin equivalent per gram extract (mgSE/g extract).

2.9. Tannin Content of the Dechlorophyllized *Salvia officinalis* Extract

This was also done according to the method of Ojiha et al., [23]. An aliquot (0.5ml) of 500mg/l of the dechlorophyllized extract in methanol was added to 8ml of distilled water plus 0.5ml of 0.1M ferric chloride and 0.5ml of 8mM potassium ferricyanide. The tube was mixed and incubated at room temperature for 10min. The absorbance was read in triplicate at 756nm spectrophotometrically. A concentration curve of tannic acid at concentrations of 15, 20, 25, 30 and 35mg/l was used as standard. The total tannin content was determined as mg tannin acid equivalent/ gram extract (mg TAE/gram extract).

2.10. Antioxidant Activity of the Dechlorophyllized *Salvia officinalis* Extract

2.10.1. Reducing Power Assay

The ferric reducing antioxidant power (FRAP) method of Ouriagli et al., [24] also adapted by Abidin et al., [1] was used. One (1ml) of 1000mg/l of the extract in ethanol was mixed with 1ml of 0.2M phosphate buffer pH 6.6. One ml of 1% potassium ferricyanide was added and incubated at 50°C for 20min. Then the reaction was stopped by the addition of 1ml of 10% trichloroacetic acid. It was centrifuged at 3000rpm for 10min. Later 1ml of the supernatant was mixed with 1ml of

distilled water plus 0.5ml of 1% ferric chloride. The tube was incubated at room temperature for 5min and the absorbance measured in triplicate spectrophotometrically at 720nm. Standard calibration curve of quercetin, at 10, 15, 20, 25 and 30mg/l concentrations was prepared. Then the antioxidant activity in terms of reducing power was established as mg/l of quercetin equivalent (mg/LQE). Increasing the absorbance of the mixture indicates increasing reducing power.

2.10.2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity of *Salvia officinalis*

The DPPH method as described by Faisal and Handayani [25] and modified by Masri et al., [26] was adapted. To 1ml of the extract (in various concentrations: 5, 10, 15, 20 and 25mg/l) was added 3ml of 35mg/l of DPPH solution. It was incubated in the dark for 30min. The absorbance of the concentration was read at 517nm. Later antioxidant activity was determined as percentage inhibition of free radical using the equation below: -

$$\% \text{ Inhibition} = \frac{\text{Abs(Control)} - \text{Abs(Extract)}}{\text{Abs(Control)}} \times 100\%$$

Where Abs Control = the absorbance of DPPH solution free of the extract.

Abs Extract = the absorbance of the extract in DPPH solution.

The half maximal inhibitory concentration (IC₅₀) was obtained from the regression analysis. The lower the IC₅₀ value the higher the antioxidant activity.

2.11. Anti-inflammatory Activity of Dechlorophyllized *Salvia officinalis* Extract

The protein albumin denaturation assay: - The method of Almira et al., [27] and as adapted by Abidin et al., [1] was used. Into 10µl of the *Salvia officinalis* extract (at various concentrations: 50, 150, 250 and 350mg/l) was added 5ml of bovine serum albumin (BSA) at pH 6.2. It was mixed and incubated at room temperature for 25min and subsequently subjected to 90°C for 5min. The mixture was allowed to cool and measured spectrophotometrically at 660nm wavelength. 0.2% BSA in ethanol was used to zero the spectrophotometer. The anti-inflammatory activity was determined using the equation below:

$$\% \text{ Inhibition} = \frac{\text{Abs Control} - \text{Abs Extract}}{\text{Abs Control}} \times 100\%$$

Where the Abs Control = the Abs of negative control.

Abs Extract = the Abs of Extract in BSA. Note that the percentage inhibition greater than 20% depicts anti-inflammatory activity.

3. Results

3.1. Extraction /Yield of Crude and Dechlorophyllized Extract of Leaves

The yields are as shown in Table 1. There was a moderate

yield of 8.23% for the crude extract and greater high yield of 35.75% for the dechlorophyllized extract. The result of the percentage chlorophyll removed from the dechlorophyllized is also shown in Table 2. A higher absorbance reading of the crude extract was observed in comparison to dechlorophyllized *Salvia officinalis leaf extract*. Also the percentage of chlorophyll removal was 51.630.

3.2. Phytochemical Contents of Dechlorophyllized Extract of *Salvia officinalis* Leaf

Table 1. Yield of Crude and Dechlorophyllized extract of *Salvia officinalis* leaf.

Sample	Weight of leaves (g)-	Weight of Crude extract (g) -	Extraction yield (g)-	Percentage yield (%)
Crude Extract	-100.06 -	-	8.23 -	8.23
Dechlorophyllized Extract	-	4.00	1.43	35.75

Table 2. Percentage Chlorophyll removed from the Dechlorophyllization method of *Salvia officinalis* leaf.

Riplicate	ABS. C	ABS. D	Percentage Chlorophyll Removed (%)	Average Chlorophyll Removed (%)
1	0.501	0.216	56.67	51.630 ± 7.76
2	0.346	0.191	44.76	
3	0.432	0.201	53.47	

Table 3. Total Flavonoid, Phenolic, Saponin and Tanin Contents of Dechlorophyllized extract of *Salvia officinalis* leaf.

Riplicate	Absorbance	Total Flavonoid (mgQE/g extract)	Average (mgQE/g extract)
1	0.54	58.602	64.519 ± 8.55
2	0.548	60.543	
3	0.586	74.414	
Riplicate	Absorbance	Total Phenolic (mgGAG/g extract)	Average (mgGAG/g extract)
1	0.336	76.67	91.433 ± 5.62
2	0.391	94.160	
3	0.401	103.47	
Riplicate	Absorbance	Total Saponin (mgSE/g extract)	Average (mgSE/g extract)

Riplicate	Absorbance	Total Flavonoid (mgQE/g extract)	Average (mgQE/g extract)
1	0.421	178.517	
2	0.425	180.618	185.666 ± 13.07
3	0.468	197.014	
Riplicate	Absorbance	Total Tanin (mgTAE/g extract)	Average (mgTAE/g extract)
1.	0.504	46.124	
2.	0.508	47.348	47.333 ± 3.31
3.	0.612	48.487	

Table 4. Antioxidant Activity of Dechlorophyllized Extract of *Salvia officinalis* leaf.

Replicate Reading	Absorbance	Antioxidant Activity (mg/LQE)	Average Antioxidant Activity (mg/LQE)
1.	0.574	13.138	
2.	0.585	13.918	14.157 ± 1.02
3.	0.601	15.101	

*Ferric reducing antioxidant power
Continuation*

Concentration (mg/L)	Absorbance Extract	Absorbance DPPH	Percentage (%) Inhibition	IC50 (mg/L)
5	0.523	0.716	26.955	
10	0.460	0.716	35.754	
15	0.407	0.716	43.156	16.28
20	0.328	0.716	54.166	
25	0.268	0.716	62.570	

DPPH radical scavenging activity

Table 5. Anti-Inflammatory Activity of Dechlorophyllized Extract of *Salvia Officinalis* leaf.

Concentration (mg/L)	Absorbance	% Inhibition	IC50 (ppm)
Negative Control	1.340	-	
50	0.731	45.69	
150	0.629	53.27	115.20
250	0.528	60.77	
350	0.412	69.39	

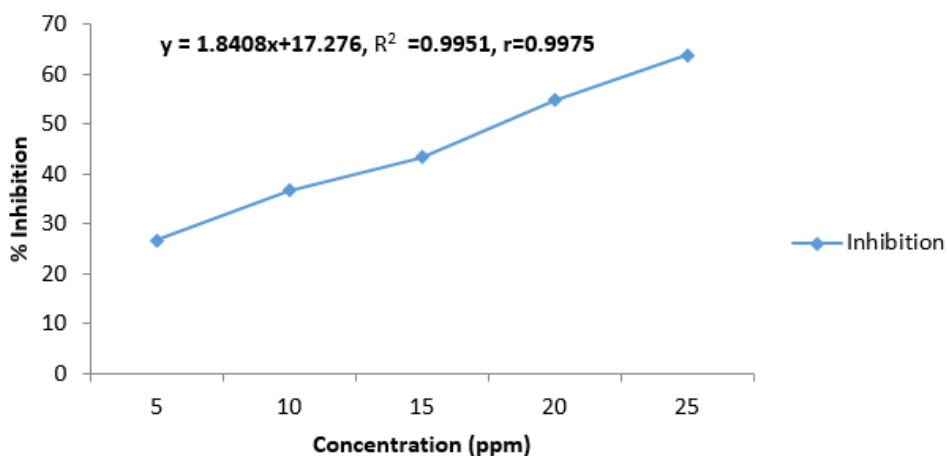


Figure 1. Concentration response curve of DPPH radical scavenging activity of Dechlorophyllized extract of *Salvia officinalis* leaf.

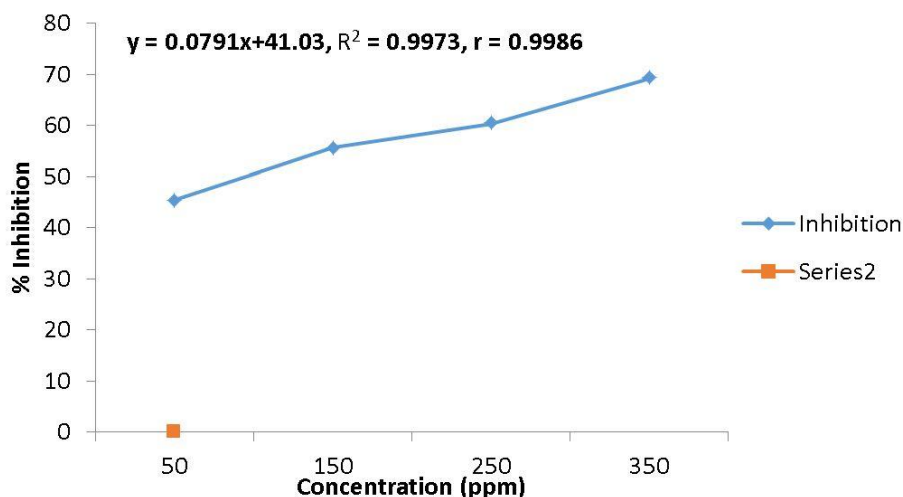


Figure 2. Concentration response curve of anti-inflammatory activity of dechlorophyllized extract of *Salvia officinalis* leaf.

The total flavonoid content of the dechlorophyllized extract of *Salvia officinalis* leaf measured spectrophotometrically at 431nm wavelength was 64.519mgQE/g extract (Table 3). The curve was observed to be linear at correlation coefficient (r) of 0.9979 and a $y = 0.0315x - 0.5138$ as regression equation. Also the total phenolic content gave a linear correlation coefficient (r) of 0.9985 at a regression equation of $y = 0.0203x - 0.0445$. The total phenolic content was 91.433mgGAE/g extract (Table 3). Equally, the total saponins content obtained via estimation from a standard calibration curve of sapoginin, at a linear correlation coefficient (r) of 0.9946, at a regression equation of $y = 0.005x - 0.0533$. The saponin content of the dechlorophyllized *Salvia officinalis* leaf extract was 185.666mgSE/g extract. While the total tannin content also obtained through estimation from a standard calibration curve of solution of tannic acid. The calibration curve was also linear at a correlation coefficient (r) 0.0213x - 0.018. The total tannin content of the dechlorophyllized extract of *Salvia officinalis* leaf was 47.333mgSE/mg extract (Table 3).

3.3. Pharmacological Activity of Dechlorophyllized Ethanol Extract of *Salvia officinalis* Leaf

3.3.1. Antioxidant Activity

(i). Ferric Reducing Antioxidant Power (FRAP)

The determination was gotten through a standard calibration curve of quercetin. There was a linear relationship at correlation coefficient (r) of 0.9987 and a regression equation of $y = 0.118x - 0.4213$. The ferric reducing antioxidant power of dechlorophyllized extract of *Salvia officinalis* leaf was 14.157mg/LQE (Table 4).

(ii). DPPH Radical Scavenging Activity

The results of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of dechlorophyllized extract of

Salvia officinalis leaf are shown in Table 4 and Figure 1. The dechlorophyllized extract of *Salvia officinalis* leaf has a linear regression equation at the concentration response curve of DPPH scavenging activity as $y = 1.8407x + 17.276$ with a correlation coefficient (r^2) of 0.995. With the IC_{50} value of 16.28mg/l (< 50mg/l), the dechlorophyllized extract of *Salvia officinalis* leaf was observed as possessing very strong antioxidant activity.

3.3.2. Anti-inflammatory Activity of the Dechlorophyllized Extract of *Salvia officinalis* Leaf

This was estimated from the calibration curve of the sample solution based on the absorbance value measured spectrophotometrically at 660nm wavelength. There was a linear relationship with correlation coefficient (r) of 0.9971 and a regression equation $y = 0.0811x - 41.01$ in the calibration curve. The dechlorophyllized extract had the anti-inflammatory activity as the IC_{50} value which was 75.20ppm as shown in Table 5 and Figure 2.

4. Discussion

The crude extract of *Salvia officinalis* exhibited a moderate yield of 8.23% unlike the dechlorophyllized extract which exhibited a higher yield of 35.75%. This finding is in line with other studies which stated that there are more phytochemical constituents in the leaves of plants than in other parts [1, 28, 29]. The percentage of chlorophyll removal measured spectrophotometrically at 660nm wavelength and depicted by increased absorbance of the crude ethanol extract of *Salvia officinalis* in comparison to the dechlorophyllized extract showed that, chlorophyll was removed. This observation is in agreement with other findings [1, 3, 30, 31] which state that complete dechlorophyllization is achieved in ethanol crude extract in the presence of n-hexane as non-polar solvent in liquid-liquid extraction method without alteration in the biological activity of the extract. The phytochemical constituents: - flavonoids, phenolic, saponin and tannin contents of dechlorophyllized ethanol extract of *Salvia officinalis* leaf are high and they are all higher than their respective total contents in ethanol crude extract as reported in other studies [1, 3, 9, 30]. The antioxidant activity of dechlorophyllized extract of *Salvia officinalis* leaf utilizing ferric reducing antioxidant power (FRAP) and DPPH revealed strong antioxidant activity. While the anti-inflammatory activity of dechlorophyllized extract of *Salvia officinalis* leaf showed an IC_{50} value of 115.20ppm with the percentage of protein inhibition lying between 45.69 to 69.39%. This observation is also in agreement with other findings [1, 27, 32] which states that compounds, that inhibit protein denaturation are potentials for anti-inflammatory drugs.

5. Conclusion

The findings from this study showed that there are increased concentrations of total flavonoids, phenols, saponins

and tannins along with strong antioxidant as well as potent anti-inflammatory activity in dechlorophyllized ethanol extract of *Salvia officinalis* leaf.

Abbreviations

ABC	Absorbance
BSA	Bovine Serum Albumin
DPPH	2, 2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Oxidation Power

Author Contributions

Azukaego Thomas Hughs Mokogwu: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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Benson O Eyenubo: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing

Godwin O Awwioro: Data curation, Formal Analysis, Funding acquisition, Resources, Software, Supervision, Visualization, Writing – review & editing

Data Availability Statement

All relevant data are within the manuscript and its supporting information files. Additional data will be available on request according to the journal policy.

Conflicts of Interest

The authors declare no conflicts of interest.

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