Phytochemical Screening, Proximate Composition, and Anti-Inflammatory Activity of American Skullcap (*Scutellaria lateriflora* L.) in Response to Mineral Fertilizer Application

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***Abstract*—** American Skullcap (*Scutellaria lateriflora* L). have an array of beneficial compounds that contributes to the therapeutic values. The availability of the material is mostly from the region of its origin with less attention on the domestication process in other region of the world. In a pilot study of a factorial pot experiment on American skullcap, plant samples were harvested to investigate the effect of mineral fertilizer treatments on the leaves. At maturity the leaves were harvested for phytochemical screening, proximate analysis, and anti-inflammatory activity. A more positive test (+) result was obtained for flavonoids, tannins, saponins, phenols, alkaloids, terpenoids and steroids. However, a negative (–) results were recorded on glycosides across the different treatments. Proximate compositions and anti-inflammatory activity of leaf samples were significant ($p<0.05$) among fertilizer treatments. Overal, treatments with less supplementary Phosphorous out performed those with moresupplementary Phosphorous. The anti-inflammatory activities of the extracts were found to be effective in their percentage inhibitions with the lowest IC_{50} value (352.8 µg/ml) recorded by T_4 for aquous extract comparable to that of the standards drug diclofenac with IC_{50} value of 498.6 µg/ml in this study

***Keywords*—** *Scutellaria lateriflora*, leaves, phytochemicals, proximate composition, anti-inflammatory

I. INTRODUCTION

Herbal products are increasingly used to address human needs in the recent years [1-3]. This continue to grow over time, particularly for plant species with nutritional and medicinal potential [4, 5]. This scenario is mostly observed in developing countries, where it is estimated that about 80% of the rural and urban population depend on and prefer the use of plant-based products for their livelihood [6, 7], causing pressure in their natural habit [8, 9].

American skullcap (S. lateriflora L.), one of the many medicinal plant species used for millennia throughout the American continent to treat anxiety, nervous tension, convulsions, and as a mild sedative [10]. The leaf component is used for the treatment of cholera, epilepsy, diarrhea, and indigestion [11]. Also, it is used as a herbal tea in the different regions of the world where it is sold as herbal tea in health food stores. However, there are fewer occurrences of horticultural production and field domestication operations on this species.

The growing of this species will produce standardized plant materials that have minimal variance in their biological activities as well as a steady concentration of the bioactive chemicals. However, the cultivation with the use of mineral fertilizer is known to have an influence on growth, yield, quality and quantity of phytochemicals. In this regard, the study was undertaking to look into how various fertilizer treatments impacted the output of phytochemical constituents and the biological activities of cultivated American Skullcap leaves in the Winelands region of the Western Cape Province of South Africa.

II. METHODOLOGY

A. Research Design and Site

The leaves were harvested from a pilot study conducted at the Teaching and Research farm at the Department of Agriculture Wellington Campus, Cape Penninsula University of Technology, with coordinates (S33° 37' E19° 37'). The study was a factorial pot experiment of eight treatments replicated five times. Fertilizer does ($T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha). Application of fertilizer was split in two equal doses at seedling transplant and at four weeks after transplant with all protocol diligently observed throughout the experiment.

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B. Phytochemical screening of American skullcap leaves

The harvested leaves samples obtained from the different treatments were screened for the presence of phenol, flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and glucosides [12, 13] using the following methods; foam test was used for saponins; ferric chloride for tannins; NaOH with dilute hydrochloric acid for flavonoids; ferric chloride solution for phenol; Dragendoff`s and Meyer`s reagent for alkaloids; chloroform and acetic acid anhydride with the addition of concentrated sulphuric acid for steroids; glacial acetic acid with drops of FeCl3 and concentrated sulphuric acid for glycosides; chloroform with acetic acid anhydride and concentrated sulphuric acid for terpenoids [12, 13].

C. Proximate analysis of American skullcap leaves.

Proximate analysis was done on dry ground leaf samples from the different treatments. Sulphuric acid and sodium hydroxide was used for crude fibre, Diethyl ether for crude lipid and incineration at 550ºC for ash content using the method of [14]. Protein was analysed using the laboratory Equipment Corporation (LECO nitrogen and protein analyser model 630-100-200). Made in USA (St. Joseph M.I 49085-2396 LECO cooperation 300 Lakeview Awe). The analysis was replicated five times and the carbohydrate content was determined using estimation by difference [by subtracting the total ash content, crude lipid, crude protein and crude fibre from 100] [14].

D. Anti-inflammatory Assay in vitro of American Skullcap leaves

The anti-inflammatory study was carried out using the method of Chandra, Chatterjee [15] in which 0.2 ml of the egg albumin from the fresh egg was mixed with 2.8 ml of phosphate buffer saline (PBS, pH 6.4) and 2 ml of the test samples or standard drug. The mixture was incubated at 37ºC for 15 minutes after which it was boiled in a water bath at 70ºc for 5 minutes. The mixture was allowed to cool for 15 minutes. Thereafter, 250 uL of the mixture was pipetted in a 96-welled microplate and absorbance measured at 655 nm using a microplate reader model 680-BIORAD, made in USA, against the reference drug (Diclofenac) at a graded concentration (0.031mg/ml -1.0mg/ml) and distilled water as the control. The experiment was carried out in triplicates. The percentage inhibition of protein denaturation was calculated using the formula as follows:

$$\% \text{ Inhibition} = [V_{test} / C_{ontrol} - 1] \times 100$$

Where, V_{test} = the absorbance of the test sample, $V_{control}$ = absorbance of the control. The extract concentration for 50% inhibition (IC_{50}) was determined by the dose-response curve.

E. Statistical analysis

Statistical analysis was performed using SPSS software package (version 22). Results were presented as means and Standard deviation (SD). Data were compared using one way analysis of variance (ANOVA) at $p<0.05$. Means separation was done using the Duncan Multiple Range Test (DMRT).

III. RESULTS AND DISCUSSION

A. Phytochemical screening of Aamerican skullcap leaves

The result of phytochemical constituents of aqueous leaf extract for cultivated American skullcap in response to fertilizer treatments is presented in (Table 1). A more positive (++) result was obtained for flavonoids among the treatments while a positive (+) result was obtained for tannins, saponins, phenols, alkaloids, terpenoids and steroids. However, negative (–) result was recorded on glycosides across the treatment combinations. The medicinal potential of American skullcap is due to the presence of bioactive compounds synthesis in the tissues. The results of the phytochemical analysis of aqueous leaf extracts of American skullcap were positive except for the glycoside which was negative. This indicates that fertilizer treatments had less influence on the quality of secondary metabolites of the plants regardless of the cultural practice. Although there is limited information on the phytochemical properties of this plant species, the results obtained in this study are in accordance with those of Delange, Rico [16] who reported the presence and absence of the phytochemicals in three different extracts of American skullcap. The collective presence of the different phytochemicals that were investigated may be linked to the therapeutic values of the plant [17], most especially in the treatment of nervous disorders [18] and their anxiolytic property [19].

TABLE I: Phytochemical screening of aqueous leaf extracts of *Scutellaria lateriflora* L. in response to treatments.

Trts	Tan	Sap	Flav	Terp	Gly	Alk	Phen	Ster
T_1	+	+	++	+	-	+	+	+
T_2	+	+	++	+	-	+	+	+
T_3	+	+	++	+	-	+	+	+
T_4	+	+	++	+	-	+	+	+
T_5	+	+	++	+	-	+	+	+
T_6	+	+	++	+	-	+	+	+
T_7	+	+	++	+	-	+	+	+
T_8	+	+	++	+	-	+	+	+

Legend (++) Highly present, (+) present (–) absent, Trts= treatments, Tan=Tannins, Sap= Saponins, Terp=Terpenoids, Gly= Glycosides, Alk= Alkaloids, Phen= Phenols and Ster= Steroids$T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$, $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha.

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B. Proximate composition of American Skullcap leaves as affected by fertilizer treatments

Overall, fertilizer treatment combinations had a significant influence ($P<0.05$) on the nutritional constituents of *S. lateriflora* leaves (Table 2). Considering the Ash content T_3 recorded the highest yield (10.6%) with a 3.9 % increase compared to the control (T_1) with the second highest yield. Additionally, for crude lipid, the control (T_1) had the highest yield (10.2 %) followed by T_3 with a 13.7 % reduction from that of the control. It was observed that treatments with the least supplementary phosphorous had a higher yield response than those with more supplementary phosphorous for ash and

crude lipid content. However, this was not the case with fibre and protein content. For crude fibre content, T_6 had the highest yield (30.32 %) with a 21.09 % increase compared to the control (T_1) which had the least yield (25.04 %). Additionally, for protein content T_1 had the highest yield (14.44%) with higher protein content recorded by treatments with lower phosphorous levels than those with higher phosphorous levels among treatment combinations. Furthermore, for carbohydrate content T_8 recorded the highest yield with a 27.17 % increase compared to the control (T_1), followed by T_4 (49.02%) with a 22.18 % increase compared to the control (T1) (Table 2).

TABLE II. Proximate Analysis For Leaf Samples Of American Skullcap Of Different Treatments. Values Are Means At (P=0.05)

TRT	% Ash	% Lip	% Fib	% Pro	%Carb
T_1	10.2 ± 2.3^{ab}	10.2 ± 6.7^{a}	25.1 ± 4.8^{b}	14.4 ± 0.2^{a}	40.1 ± 5.8^{c}
T_2	9.0 ± 1.9^{ab}	6.7 ± 1.6^{ab}	26.7 ± 3.7^{ab}	13.6 ± 0.3^{b}	44.2 ± 2.5^{c}
T_3	10.6 ± 1.6^{a}	8.8 ± 2.4^{ab}	28.2 ± 3.4^{ab}	12.8 ± 0.4^{bc}	40.4 ± 4.2^{c}
T_4	6.4 ± 0.8^{c}	8.4 ± 1.9^{ab}	26.9 ± 1.9^{ab}	10.8 ± 0.5^{d}	49.0 ± 5.4^{ab}
T_5	$9.8\pm 0.9^{a\ b}$	8.5 ± 3.1^{ab}	26.7 ± 3.6^{ab}	13.2 ± 0.9^{bc}	41.1 ± 6.7^{c}
T_6	8.5 ± 1.9^{ab}	5.3 ± 0.9^{b}	30.3 ± 3.2^{a}	12.5 ± 0.7^{c}	43.4 ± 2.9^{bc}
T_7	8.1 ± 0.7^{bc}	6.5 ± 1.3^{ab}	29.6 ± 2.5^{ab}	11.5 ± 1.1^{d}	44.3 ± 2.6^{bc}
T_8	8.9 ± 0.4^{ab}	7.2 ± 2.3^{ab}	27.6 ± 1.7^{ab}	6.7 ± 0.1^{e}	51.0 ± 2.2^{a}
LSD	0.056	0.106	0.062	0.080	0.069

Means±SD , Difference letters showed significant difference using Duncan multiple range test (DMRT) at p<0.05 level.$T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$,. TRT=Treatment, ,Ash= Ash, Lip= Lipid, Fib= Fibre, Pro= Protein, Carb=Carbohydrate.

The significant difference in nutritional components of cultivated American Skullcap in pots may be attributed to the different fertilizer levels used which might have influenced the yield and the nutritional quality of the leaf samples [20]. This is in agreement with Wang, Li [21] who reported that fertilizer application in plants generally influences crop yield as well as the nutritional quality of plants. The high fibre content recorded among the different plant samples as affected by fertilizer treatments suggested that leaves can be a potential source of dietary fibre complementing the anti-inflammatory and antitumor properties of this species [17, 22]. Also, the high carbohydrate content of the different samples with more supplementary phosphorous treatment has high carbohydrate content than those with less supplementary phosphorous with increased nitrogen levels. This may be attributed to the fact that nitrogen is a core nutrient element that influences the process of photosynthesis, protein, and carbohydrate metabolism in plant cells [23]. Furthermore, adult's dietary lipid intake is between 20-35% of the total calories from food. However, the lower crude lipid values of 10.2% and below recorded in this study indicate this species may be beneficial for people with cholesterol related problems [24].

C. Influence of fertilizer treatments on anti-inflammatory activity of aqueous and methanolic dried leaf extracts

The anti-inflammatory effect of the aqueous and methanol dried leaf extract of American Skullcap as affected by fertilizer treatment combination was evaluated using fresh egg albumin in phosphate buffer saline (pH 6.4) on the standard drug Diclofenac 500g. Significant (p<0.05) anti-inflammatory activity was recorded on leaf extracts among treatment combinations on aqueous and methanolic extracts. T_4 recorded the best anti-inflammatory with an IC_{50} value of 352.8 µg/ml for aqueous leaf extract. However, for methanolic extract T_7 demonstrated the most ideal with the lowest IC_{50} value of 834.1 µg/ml among treatment combinations indicating the best anti-inflammatory activity. Overall, aqueous extract recorded a more favourable anti-inflammatory response compared to methanolic extract that demonstrated a lower IC_{50} value among the corresponding aqueous treatments (Table 3).

TABLE III. Influence Of Fertilizer Treatments On Anti-Inflammatory Activities For Dried Leaf Extract Of American Skullcap. Values Are Means At (P-0.05)

Treatments	ME root(µg/ml)	A E (µg/ml)
T1	1345.47 ± 88.27^{a}	775.68 ± 24.52^{a}
T2	937.67 ± 41.94^{c}	455.15 ± 04.25^{cd}
T3	1225.07 ± 16.46^{b}	473.37 ± 20.29^{bc}
T4	1340.09 ± 08.24^{a}	352.76 ± 12.17^{e}
T5	963.77 ± 25.28^{c}	489.33 ± 15.76^{b}
T6	956.50 ± 55.08^{c}	494.52 ± 03.47^{b}
T7	834.09 ± 18.83^{d}	430.06 ± 19.16^{d}
T8	920.97 ± 28.53^{c}	464.29 ± 18.02^{bc}
Diclofenac	498.55±50.55	498.55±50.55

Means ± SD, Difference letters showed significant difference using Duncan multiple range test (DMRT) at p<0.05 level Values are means of IC50 (µg/ml) at (P-0.05). $T_1=N_{350}P_{213}K_{213}$, $T_2=N_{350}P_{320}K_{213}$, $T_3=N_{525}P_{213}K_{213}$, $T_4=N_{525}P_{320}K_{213}$ $T_5=N_{700}P_{213}K_{213}$, $T_6=N_{700}P_{320}K_{213}$, $T_7=N_{800}P_{213}K_{213}$, $T_8=N_{800}P_{230}K_{213}$ Kg/ha, ME= methanol extract, AE=aqueous extract.

Inflammation is a complex biological process that is associated with an increase in protein denaturation that causes pain in vascular tissues and membrane alteration [25]. Several inflammatory effects usually result in organ injury [26]. The use of plant products with anti-inflammatory activity such as American Skullcap to target inflammatory response can be beneficial for the treatment of inflammation as well as many other chronic diseases. In this study significant differences were observed among the extracts of different treatment combinations. The anti-inflammatory activities of the extracts were found to be effective in their percentage inhibitions with the lowest IC_{50} value (352.8µg/ml) recorded by T_4 for aqueous extract and 834.1µg/ml recorded by T_7 for methanolic extract comparable to that of the standards drug diclofenac with IC_{50} value of 498.6µg/ml in this study. The differences in activity may be attributed to the different treatment effects which may have resulted in the differences in the concentrations of the bioactive compounds that may be responsible for the anti-inflammatory activity of this species [27].

IV. CONCLUSION

Phytochemical constituents of the different extracts of American Skullcap (*Scutellaria lateriflora* L.) were greatly impacted to a varying degree by fertilizer treatment combinations. A positive test result was observed for all phytochemicals investigated except for glucosides. This complements the diverse pharmacological activities of this species like the anti-inflammatory activity investigated.

Nutritionally, the high fibre and carbohydrate contents of this species indicate this species can be recommended as a potential source of dietary fibre and calorie intake for body functioning. Likewise, the ideal amount of protein content and low lipid content also indicate that this species can be beneficial for people with related cholesterol problems, complementing the therapeutic values. Methanol solvent demonstrated a more ideal solvent than aqueous solvent for extract preparation of this species due to its polarity. This preliminary investigation indicates that *S. lateriflora* cultivated in the Winelands region of the Western Cape Province of South Africa can still retain its secondary metabolites of bioactivity and therapeutic values. Nevertheless, more investigation needs to be done using other propagation techniques and, in the field, to potentially validate these findings.

V. Acknowledment

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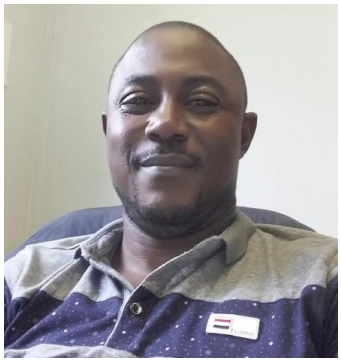

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