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Evaluation of *Sarcocephalus latifolius* Afzel. ex R.Br. Rubiaceae on Reduction of Creatinine Level and Its Antioxidant In-Vitro

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Abstract

Medicinal herbs are divine gifts from Mother Nature and have been used for centuries as natural remedies. *Sarcocephalus latifolius* has been reported to contain active substances that have therapeutic effects on the kidney. The study aims to investigate the medicinal effects of *Sarcocephalus latifolius* extract on creatinine levels. Phytochemical and antioxidant activity were carried out using standard methods. Vacuum Liquid chromatography and Thin Layer Chromatography were employed to separate constituents of the plant extract, which were then evaluated for their creatinine reduction ability using Jaffe's method. Based on the IC50, which is half the maximal inhibitory concentration value, *Sarcocephalus latifolius* demonstrates stronger antioxidant activity at 43.34µg/ml compared to the standard ascorbic acid at 40.59µg/ml. The Two-way ANOVA Statistical analysis revealed significant differences between the patients' urine (rows) as the P-value = 6.17×10-16 is less than 0.05 significant level, and the dose treatments (columns) as the P-value = 1.71×10-12 is less than 0.05 significant level. Fractions of *Sarcocephalus latifolius* at various concentrations exhibit varied effects on creatinine levels, which could be attributed to the bioactive compounds in the fractions. *Sarcocephalus latifolius* showed potential for creatinine reduction, especially at low doses.

Keywords: *Sarcocephalus latifolius*, *Nauclea latifolia*, Creatinine, Reno-protective, Antioxidant, VLC fractionation

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INTRODUCTION

Creatinine, also known as 2-amino-1-methyl-2-imidazoline-4-one, is a non-protein nitrogenous waste product produced during muscle metabolism.[1,2] Its elimination primarily occurs through renal processes, and a high buildup of creatinine is an indicator of renal dysfunction.[2] The kidneys play an important role in normal physiological processes, such as plasma filtration, regulation of plasma volume, hormone secretion, and acid-base balance. Alterations in these functions can contribute to a wide array of renal disorders with life-threatening implications.[3] Total cessation of renal function often results in death within a week, while partial loss of function leads to varying degrees of deviation from normal, depending on the remaining functional tissue.[4] The pivotal role of the kidney in life is a multifaceted functionality, not limited

to inadequate waste excretion due to diminished glomerular filtration but also excessive losses stemming from tubular dysfunction in reclaiming filtrate.[5] Creatinine is eliminated from the bloodstream by the kidneys via glomerular filtration and proximal tubular secretion.[6] Glomerular filtration rate (GFR) serves as a measure of kidney function and is determined by the urinary or plasma clearance of an ideal filtration marker. Urinary clearance of endogenous filtration markers, such as creatinine, may be compiled and calculated from a timed urine collection or spot urine sample introduced due to the complexities and inconveniences associated with 24-hour urine collection.[7]

Medicinal plants are divine gifts from Mother Nature to maintain and augment health in order to fight disease with possibly no side effects. They have also contributed to human health when the concept of synthetic medicines and surgery didn't exist. It is by far the most used species for pharmaceuticals, healthcare products, and modern drug discovery.[8,9] Several plant species used by rural communities as traditional medicines have a diverse effect on the kidney, with some species (e.g., *Sclerocarya birrea* (Marula), *Persea americana* (Avacado pear), *Ficus thonningii* (wild fig)) being reno-protective and some (e.g., *Hypoxis hemerocallidea* (African potato)) are pernicious.[4]

Sarcocephalus latifolius (Sm.) E. A. Bruce, previously called Nauclea latifolia (Sm.), is classified under Kingdom: Plantae, Phylum: Tracheophyta, Class: Magnoliopsida, Order: Gentianales, Family: Rubiaceae, Genus: Sarcocephalus, and Species: *Sarcocephalus latifolius*.[10,11] It is commonly called Pin cushion tree or African peach in English, scille maritime in French; in Igbo, "*Ubulu inu*"; in Hausa, "*Tafashiya*" or "*Marga*" or "*tabashiya*" or "*tuwonbiri*"; in Yoruba "*Egbesi*," in Ibibio "*Mbom-ibong*'' and "Itu" in Itsekiri, *mahyann* in Fali language.[12] *Sarcocephalus latifolius* has been used traditionally for treating fever, pain, dental caries, septic mouth, and diseases of the central nervous system such as epilepsy [13], malaria [14], gastrointestinal tract disorders, prolonged menstrual flow, sleeping sickness, laxative activities, diabetes mellitus [15,16] and hypertension.[17] Phytochemical investigations of the bark and wood of *Sarcocephalus latifolius* have reported the presence of naucleamides A-E, a group of new monoterpene indole alkaloid compounds from *Sarcocephalus latifolius*.[18] Phytochemical analysis of the plant suggests the presence of Tannins, flavonoids, alkaloids, saponins, cardiac glycosides, terpenoids, steroids, carbohydrates, glycoalkaloids, glycosides, anthraquinones, hydrocyanic acid, phenols, resins, polyphenols, carotenoids, limonoids, xanthonoids, balsam, phlobatannins, phytates, isoflavonoid (Indicanine B and C), cardenolides, cyanogenic glycosides, phosphate, anthraquinones, coumarins, monoterpenes, fatty acid esters, indolo-quinolizidine (anguistine, angustoline), terpenes, indole alkaloids.[12,10] Sarcocephalus latifolius has been reported to have several pharmacological properties. The antidiabetic property of the aqueous extract of the leaves of *Sarcocephalus latifolius* has been reported on both streptozotocin-induced diabetic and normal rats.[19] Previous works have shown the analgesic property of the aqueous freeze-dried extract of the bark of *Sarcocephalus latifolius* by decreasing both the acute and delayed phases of formalin-induced pain in rats and also caused a significant reduction in both yeast-induced pyrexia and egg albumin-induced oedema in rats, it attenuated writhing episodes induced by acetic acid and

increased the threshold for pain perception in the hot-plate test in mice.[20] Anticonvulsant, anxiolytic, and sedative properties of *Sarcocephalus latifolius* root decoction have been reported.[21] Taïwe et al.[22] reported that *Sarcocephalus latifolius* induced hypothermia, had antipyretic effects in mice and produced significant antinociceptive activity in all analgesia animal models used, and that it also protected mice against bicuculline-induced behavioural excitation. Balogun et al.[23] reported the anti-ulcer activity of aqueous leaf extract of *Sarcocephalus latifolius* at 340 and 510mg/kg doses against indomethacin-induced gastric ulcers in rats than Cimetidine (100mg/kg). Orole et al.[24] studied the anti-ulcerogenic potential of ethanolic leaf extracts of *Kigelia africana*, *Sarcocephalus latifolius*, and *Staudtia stipitata* on aspirin-induced ulcer in albino rats, extract of *Sarcocephalus latifolius* at a concentration of 450 mg/kg body weight gave the best results with a significant decrease in ulcer index on aspirin-induced ulcerogenic animals compared to Cimetidine at 300mg/kg. The anti-ulcerogenic effects of aqueous stem bark extract of *Sarcocephalus latifolius* using ethanol/HCl and indomethacin as the ulcerogens was conducted by Balogun et al.[25] and he concluded that *Sarcocephalus latifolius* stem bark extract possessed significant anti-ulcer effects which might be due to its ability to increase gastric mucous secretion. *Sarcocephalus latifolius* possesses broad spectrum antimicrobial activities[12], and the hot aqueous and ethanolic extracts were confirmed to exhibit strong anti-bacterial properties.[26] Ettebong et al.[27] reported significant and dose-dependent antiplasmodial activity in the suppressive, repository, and curative tests of the ethanolic extract and its fractions of the stem bark in plasmodium berghei infected mice with aqueous fraction had the highest percentage chemo suppressive effect. Owolabi et al.[28] reported the anti-diarrheal activity of ethanolic root bark extract of *Sarcocephalus latifolius* by investigating castor oil-induced diarrhea and small intestinal motility in mice. The extract produced a significant dose-dependent reduction in the frequency and severity of diarrhea induced by castor oil. *Sarcocephalus latifolius* had strong antioxidant potentials, with the leaves demonstrating higher in vitro antioxidant activities than the fruits, and this could be responsible for the medicinal properties of this plant.[14] Odimegwu and Esimone.[29] reported the In-vitro antiviral activity of *Sarcocephalus latifolius* root bark extract against the Respiratory Syncytial Virus and also suggested that *Sarcocephalus latifolius* may interfere with several viral targets. Ogugua et al.[30] study on the Hepatoprotective and healthy kidney-promoting potentials of methanol extract of *Sarcocephalus latifolius* in alloxan-induced diabetic male Wister albino rats showed a reduction in creatinine level.

The World Health Organisation does not just encourage the use of traditional medicines but also recommends scientific evaluation of the medicinal properties of plant extracts.[4,9] The safety and efficacy of the traditional medicinal use of *Sarcocephalus latifolius* in kidney health cannot be confidently guaranteed. Therefore, this study aims to evaluate the use of *Sarcocephalus latifolius* in the reduction of creatinine levels in normal, hypertensive, and diabetic volunteers. By investigating *Sarcocephalus latifolius* impact on creatinine levels, this research builds on previous findings [30] showing creatinine reduction in rats to explore its potential as a natural therapeutic agent for human renal health. Jaffe's principle has been employed in this study to evaluate *Sarcocephalus latifolius*, which is used to

reduce creatinine levels in spot urine with and without the direct addition of *Sarcocephalus latifolius* extracts. The study was done anticipating a significant decrease in creatinine levels among the participants, indicating an improvement in kidney function, which could potentially pave the way for the development of new, plant-based treatments for kidney-related disorders. This offers a safer and more effective alternative to conventional therapies. However, further research and clinical trials are essentially necessary.

METHOD

Chemicals and Reagents

Distilled water, Petroleum ether (Merck), Chloroform (Merck), Mayer's reagent, reagent, Hager's reagent, Wagner's reagent, Sodium hydroxide (Sigma-Aldrich), acetic anhydride, H_2S_04(Sigma-Aldrich), Lead acetate solution, glacial acetic acid, Methanol (Merck), dilute Hydrochloric acid, Ascorbic acid (Reference standard), acetic acid, phosphate buffer, Iron chloride solution, DPPH, Ethanol (Honeywell Riedel-de-Haen), Tannic acid, hydrochloric acid, n-Hexane (Merck), Ethyl acetate (Merck), silica gel (Sigma-Aldrich) and Agappe creatinine 4x50ml kit (creatinine standard, creatinine base, creatinine dye).

Apparatus Used

Test tubes, Conical flasks, Beakers, Crucibles, T80 U V/ VIS Spectrometer, Themoline Hot plate, Equitron Oven, Muslin cloth, capillary tube, Glass funnels, Filter paper, Measuring cylinders, Pipette, Cotton wool, Vacuum pump, Test tube racks, Sample bottles, Universal bottles, Analytical balance, Top load balance, Desiccator, Pasteur pipette, Analytical weighing balance, laboratory milling machine, funnel, pyrex test tubes, burette 250ml beakers, spatula, vacuum flask, sintered funnel (VLC column) and volumetric flask, TLC pre-coated plates.

Plant Collection and Preparation

Sarcocephalus latifolius was collected from the wild in Ibadan, Nigeria, on January 21, 2020. A voucher specimen was deposited in Forest Herbarium, Ibadan, Nigeria, with voucher number FHI 113026. The leaves of *Sarcocephalus latifolius* were air-dried indoors for 106 hours, then pulverised using a Christy and Norris 8" laboratory milling machine. The powdered material was weighed and stored in a cool, dry location for extraction.

Extraction and Filtration

Five hundred grams of finely powdered *Sarcocephalus latifolius* leaves were macerated in absolute ethanol for 11 days. Maceration involves soaking the plant material in airtight amber glass bottles and intermittently shaking them. The resulting macerate was filtered through muslin cloth to obtain the extract, which was then concentrated using a rotary evaporator. The concentrated extract was dried in a water bath, transferred into McCartney bottles, and stored in a refrigerator.

Collection of Urine

Urine samples were obtained from three female adults representing three categories:

1. Normal volunteers;
2. Diabetic volunteers;
3. Hypertensive volunteers.

Samples were collected into labeled bottles, placed in cellophane bags, and stored in a refrigerator for preservation until analysis.

Ethical approval and consent to participate

All information was handled with uttermost confidentiality. Privacy was implemented in line with the data privacy policy and protection. Written informed consent was obtained from the subjects, and the research subjects reserve the right to privacy and non-disclosure of their details. The research received ethical

approval from the College of Medicine, University of Lagos health research ethics committee with the CMULHREC number CMUL/HREC/02/20/717 on February 28, 2020.

Phytochemical Screening

The ethanolic leaf extract underwent screening for various compounds using methods described in relevant literature.[31,32] The filtrates were prepared by diluting the crude extracts with distilled water and boiled in a 100˚C water bath for 10 minutes, cooled, and filtered using filter paper.

Alkaloids

a. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-coloured precipitate indicates the presence of alkaloids.
b. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.
c. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.

Carbohydrate

Molisch's Test: 1ml of concentrated sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates.

Cardiac glycoside

Into 2ml filtrate, 1ml of glacial acetic acid, 1ml ferric chloride, and 1ml concentrated sulphuric acid were added. The presence of a green-blue colouration indicated cardiac glycoside.

Courmarins

3 ml NaOH was added into 2 ml filtrate, and a yellow colouration indicated the presence of courmarins.

Flavonoids

Treat the filtrate with dilute NaOH, followed by the addition of dilute HCl. A yellow coloration is observed with NaOH, which turns colourless with the addition of dilute HCl.

Glycosides

2ml chloroform and 2ml acetic acid were added to 2ml of filtrate and observed for a blue to green coloration.

Phenols

Two drops of lead acetate were added to 2 ml of filtrate. The formation of a white substance at the bottom of the test tube indicates the presence of phenolic compounds.

Phlobatanins

2ml dilute hydrochloric acid was added to 2ml filtrate and heated for 5 minutes. The formation of a red precipitate is an indication of phlobatannins.

Protein

Milion's Test: To 3ml filtrate, 5ml of million's reagent was added and heated. The appearance of a white precipitate, which changed to brick red on heating, indicates the presence of proteins.

Quinones

1 ml of concentrated sulfuric acid was added to 2 ml of filtrate. The colour change to red indicates the presence of quinones.

Reducing sugar

Fehling's Test: 1ml each of Fehling's A and Fehling's B solutions were added to 3ml of filtrates and shaken vigorously, then heated in a boiling water bath for 10 min, with an appearance of emerald green, which became darker and then formed a brick red solution when boiled indicates the presence of reducing sugars.

Saponins

Frothing Test: 2ml of the filtrate was shaken in a test tube for 20 minutes and left to rest for 5 minutes. The formation of foam that remains stable after 5 minutes of rest indicates the presence of saponins.

Steroids

In 1 ml filtrate, 2 ml of acetic anhydride and 2 ml of H_2S_{04} were added and then observed for a blue or green coloration.

Tannins

Ferric chloride test: 2 drops of ferric chloride were added to 2 ml filtrate and observed for brownish green-black or a blue-black coloration.

Terpenoids

5 ml filtrated was treated with 2 ml of chloroform and 3 ml of concentrated sulphuric acid. It was then observed for a reddish-brown colour of interface to indicate terpenoids.

Volatile oils

A few drops of sodium hydroxide and hydrochloric acid were added to 2ml of filtrates and shaken. Observation of a white precipitate indicated the presence of volatile oils.

DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of the extracts was evaluated using the stable DPPH 2,2-Diphenyl-1-picrylhydrazyl free radical. Methanol was used as blank and control, while ascorbic acid was used as the standard for positive control. 0.06g of extracts were mixed in methanol to make a stock solution of concentration 1000µg/ml. From this stock solution, different concentrations of the extract in the range 20, 40, 60, 80, and 100µg/ml were prepared. 2ml of reagent solution from 0.004g of DPPH in 300ml methanol, mixed with 3ml of the concentrations of extracts prepared, was put into triplicate test tubes. The test tubes were shaken and incubated at room temperature in a dark cupboard. There was a notable color change after 30 minutes, and the absorbance at 517nm was read. The DPPH scavenging activity was calculated with the following formula:

$$\% \text{ inhibition} = \left(\frac{\text{A blank} - \text{A sample}}{\text{A blank}}\right) \times 100\%$$

[31]

Chromatography

The extract was absorbed with silica gel. The column is packed by dry method with 500g of dry silica gel of 70-230 mesh and allowed to settle under gravity with gentle tapping of the column. A vacuum is applied to press the silica further for a uniform and tightly packed column. A filter paper is placed on the surface of the silica gel, and then the fine extract/silica mixture is added in a uniform spread to cover the entire surface area. Cotton wool is placed over it. The column is run twice using solvents n-hexane, ethyl acetate, and methanol in gradient ratios of 100%, 75%:25%, 50%:50%, and 25%:75% to obtain nine fractions labeled A to I representing different solvent gradients respectively: 100% n-hexane, 75% n-hexane: 25% ethyl acetate, 50% n-hexane: 50% ethyl acetate, 25% n-hexane: 75% ethyl acetate, 100% ethyl acetate, 75% ethyl acetate: 25% methanol, 50% ethyl acetate: 50% methanol, 25%ethyl acetate: 75% methanol, 100% methanol. These were collected in beakers and left to evaporate.[31]

Thin Layer Chromatography of the methanol solution of the fractions was done on silica gel-coated glass plates using chloroform: methanol (0.5:9.5) as the solvent front. The plate was visualised under UV radiation, and the fractions components' Rf values were calculated.[31]

Creatinine Test

For the Creatinine Test, two packs of 4x50ml Agappe creatinine kits were utilised, each containing creatinine standard, creatinine base, and creatinine dye. In a test tube, a creatinine standard solution was prepared using 0.03ml of the creatinine standard diluted with distilled

water to make up 3ml. 1ml of both creatinine base and creatinine dye was added to the test tube, which was shaken and allowed to stand for 2 minutes. The standard UV spectrophotometer reading was taken at a wavelength of 517nm.[31]

Determination of Creatinine Concentration

The urine sample was diluted with distilled water using a 1:100 dilution factor. Crude extracts and fractions at concentrations of 1%, 5%, and 10% were prepared. The diluted urine was separated into 3 ml in test tubes and was combined with 1 ml of each extract and fraction in each tube containing urine samples, followed by 1 ml each of creatinine base and creatinine dye. The content was shaken and left to stand for 2 minutes to allow colour change in the mixture, and the absorbance reading was taken at a wavelength of 517nm using a UV spectrophotometer. The control contained only diluted urine. This procedure was repeated for all urine samples collected at different concentrations of the extracts and fractions, with readings taken in triplicate. The creatinine concentration in g/l was determined using the formula:

Creatinine concentration=

$$\frac{Absorbance\ of\ test \times standard\ concentration \times dilution\ factor}{Absorbance\ of\ standard \times 100}$$

Where 100 is the conversion factor from mg/dl to g/l.[31]

RESULTS AND DISCUSSION

In this study, *Sarcocephalus latifolius* was examined in vitro for its potential to reduce creatinine levels and exhibit antioxidant activity. Since creatinine excretion can be affected by drugs, diet, and gender[33], the three female participants were interviewed regarding their medications and dietary habits to ensure accuracy, but only the hypertensive and diabetic volunteers were on medications and special diets.

Phytochemical Analysis

The preliminary phytochemical screening of *Sarcocephalus latifolius* shows they contain important phytochemicals, alkaloids, carbohydrates, courmarins, flavonoids, glycosides, phenols, phlobatanins, protein, quinones, reducing sugar, saponins, tannins, terpenoids, and volatile oil, which are capable of bestowing health benefits.[30] Albeit similar result of photochemical content, the absence of cardiac glycosides and the presence of coumarins and glycosides observed in our study contradicted the previous findings of Iheagwam et al.[34], which showed the presence of cardiac glycoside and absence of glycosides and courmarins.

PHYTOCHEMICAL	***SARCOCEPHALUS LATIFOLIUS***
Alkaloids	+
Carbohydrate	+
Cardiac glycoside	-
Courmarins	+
Flavonoids	+
Glycosides	+
Phenols	+
Phlobatanins	+
Protein	+
Quinones	+
Reducing sugar	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+
Volatile oil	+

+ means positive/present - means negative/absent

Antioxidant Activity

Table 2. DPPH scavenging activity with IC50 values

Concentration (µg/ml)		20	40	60	80	100	IC50
% Inhibition	**Ascorbic Acid**	67.50	90.40	90.70	92.90	92.60	40.59
	S. latifolius	75.90	98.14	82.04	83.60	83.30	43.34

The DPPH scavenging activity of the methanolic extract, tested at concentrations ranging from 20 to 100µg/ml, demonstrated strong antioxidant activity with an IC50 value of 43.34 µg/ml, surpassing that of the ascorbic acid standard of 40.59 µg/ml. This suggests significant DPPH radical scavenging activity of *Sarcocephalus latifolius* in relation to that of ascorbic acid used as the standard, in alignment with Ayeleso et al.[14], who found that the leaves have strong DPPH radical scavenging activity in their study of leaves and fruits of *Sarcocephalus latifolius*. The plant's ethanolic crude extract can act as an antioxidant agent to neutralise the free radical traits of DPPH, which is attributed to the structural conformation of its bioactive compounds. Dennis and Witting[35] affirm that antioxidants are reno-protective and ameliorative. In the same light, they posit polyphenols and flavonoids, which include curcumin contained in *Sarcocephalus latifolius* to be effective on rhabdomyolysis and ischemic renal injury.

Chromatography

Vacuum Liquid Chromatography Fractionation

Table 3 represents the VLC fractions obtained from the crude extract using the corresponding solvent systems and their characteristics colours. The plant extract had more affinity to polar solvents. The first two solvent systems with the lowest polarity seemed blank.

Thin Layer Chromatography Analysis

Figure 1 represents the TLC profile of the fractions from Table 3, showing that the plant contains polar and semi-polar compounds as well as fluorescence compounds that absorb UV light at 366mm. More information about the specific compounds spotted on the plate requires further studies.

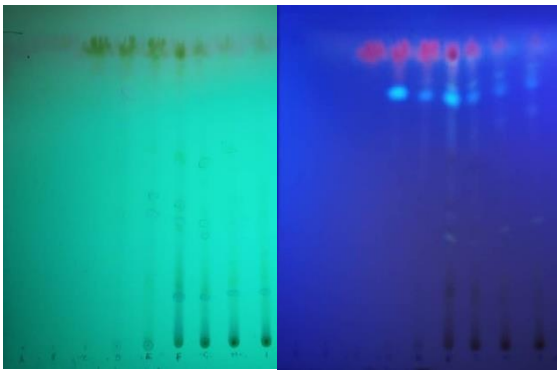

Figure 1: Profile of VLC fractions of *Sarcocephalus latifolius* developed on TLC plate as seen under UV radiation 254nm (A) and UV radiation 366nm (B)

Table 3. VLC fractionation of *Sarcocephalus latifolius*

Fractions	solvent gradient	colour
A	100% n-hexane	colourless
B	75% n-hexane: 25% ethyl acetate	colourless
C	50% n-hexane: 50% ethyl acetate	lemon tint
D	25% n-hexane: 75% ethyl acetate	light green
E	100% ethyl acetate	green
F	75% ethyl acetate: 25% methanol	sage
G	50% ethyl acetate: 50% methanol	dark sage
H	25% ethyl acetate: 75% methanol	brownish green
I	100% methanol	lemon

Table 4. Rf values from TLC of *Sarcocephalus latifolius*

Fractions	Rf values
A	No movement observed
B	No movement observed
C	0.92
D	0.79, 0.92
E	0.75, 0.92
F	0.17, 0.33, 0.38, 0.58, 0.78, 0.80, 0.91
G	0.17, 0.57, 0.78, 0.82, 0.94
H	0.36, 0.70, 0.85, 0.94
I	0.39, 0.74, 0.88, 0.94

Creatinine Reduction activities

Table 5. Creatinine concentration at different treatments using *Sarcocephalus latifolius*

SARCOCEPHALUS LATIFOLIUS							
Sample	**Dose**	**Normal urine (g/L)**	**Hypertensive urine (g/L)**	**Diabetic urine (g/L)**	**Sum**	**Average**	**Variance**
Control		4.75±0.038	0.49±0.020	2.01±0.016	7.25	2.42	4.66
Crude Extract	10	3.12±0.012	0.76±0.010	2.89±0.017	6.76	2.25	1.69
	50	2.41±0.084	0.81±0.001	2.60±0.039	5.81	1.94	0.96
	100	2.38±0.004	0.88±0.003	2.83±0.028	6.10	2.03	1.04
A	10	2.36±0.010	0.65±0.014	2.84±0.021	5.85	1.95	1.32
	50	2.67±0.051	0.69±0.001	3.06±0.014	6.42	2.14	1.62
	100	3.84±0.006	0.94±0.002	3.26±0.001	8.05	2.68	2.36
B	10	3.41±0.053	0.76±0.001	2.98±0.041	7.15	2.38	2.02
	50	5.68±0.001	2.40±0.087	4.73±0.031	12.81	4.27	2.84
	100	5.37±0.087	2.75±0.055	6.16±0.153	14.28	4.76	3.19
C	10	3.11±0.034	0.80±0.015	3.10±0.040	7.01	2.34	1.77
	50	3.52±0.020	0.81±0.011	2.97±0.008	7.30	2.43	2.06
	100	7.35±0.329	3.47±0.008	6.84±0.087	17.66	5.89	4.46
D	10	2.95±0.014	1.09±0.012	3.15±0.014	7.20	2.40	1.29
	50	3.65±0.058	2.62±0.032	3.38±0.016	9.64	3.21	0.29
	100	8.24±0.075	12.71±0.009	7.81±0.111	28.77	9.59	7.36
E	10	2.80±0.033	0.87±0.006	3.00±0.014	6.66	2.22	1.38
	50	3.66±0.013	1.11±0.003	3.07±0.016	7.84	2.61	1.79
	100	7.31±0.046	3.40±0.019	6.04±0.127	16.75	5.58	3.98
F	10	3.20±0.015	1.09±0.002	3.07±0.010	7.36	2.45	1.39
	50	4.60±0.002	2.49±0.024	4.64±0.026	11.73	3.91	1.52
	100	6.39±0.014	5.05±0.005	8.22±0.105	19.67	6.56	2.53
G	10	2.91±0.010	0.96±0.012	3.07±0.007	6.94	2.31	1.38
	50	4.10±0.053	1.43±0.004	3.18±0.014	8.72	2.91	1.84
	100	5.95±0.097	5.27±0.017	8.01±0.137	19.23	6.41	2.04
H	10	3.54±0.023	0.85±0.004	2.75±0.022	7.13	2.38	1.90
	50	4.20±0.004	1.84±0.016	3.29±0.006	9.33	3.11	1.42
	100	6.14±0.033	5.67±0.080	7.53±0.107	19.34	6.45	0.94
I	10	3.48±0.014	0.83±0.002	2.73±0.013	7.03	2.34	1.87
	50	3.47±0.005	1.82±0.004	3.49±0.011	8.78	2.93	0.92
	100	5.16±0.009	5.20±0.013	8.20±0.125	18.56	6.19	3.05
Sum		131.71	70.49	130.90			
Average		4.25	2.27	4.22			
Variance		2.55	6.17	3.95			

Table 6. Calculated ANOVA result table from table 5 (based on 0.05 significant level)

Source of Variation	*Sum of Squares SS*	*Degree of Freedom Df*	*Mean square MS*	*F-statistics*	*P-value*	*F-critical*
Rows (urine)	325.7451	30	10.85817	12.01622	6.17E-16	1.649141
Columns (treatment)	79.54672	2	39.77336	44.01528	1.71E-12	3.150411
Error	54.21757	60	0.903626			
Total	459.5094	92				

Creatinine levels of both hypertensive and diabetic controls fall within the stipulated normal range by Agappe creatinine test kit 0.2 – 2.75 g/l, but normal control shows a higher level of 4.75 g/l. In normal patients, treatments with *Sarcocephalus latifolius* resulted in a decrease in creatinine concentration compared to controls, although treatments B50 (5.68), B100 (5.37), C100 (7.35), D100 (8.24), E100 (7.31), F100 (6.39), G100 (5.95), H100 (6.14), I100 (5.16) showed notable increases above the control levels. Hypertensive patients generally exhibited increased creatinine levels with *Sarcocephalus latifolius* treatments, particularly at D100 (12.71), showing a peaked increase. The results from the diabetic volunteer also showed increased creatinine levels with treatments of *Sarcocephalus latifolius*, especially at B100 (6.16), C100 (6.84), D100 (7.81), E100 (6.04), F100 (8.22), G100 (8.01), H100 (7.53) and I100 (8.20) with a marked increase reported. Table 6, showing the ANOVA result, shows that the row p-value = 6.17×10-16 is smaller than the 0.05 level of significance, and the row F-statistics is greater than row F-critical, indicating significant differences within the rows. The column p-value = 1.71×10-12 is smaller than the 0.05 level of significance, and the column F-statistics is greater than the column F-critical, indicating significant differences within the columns. This means that both the urine types and dose treatments of extracts and fractions have a significant effect on the result, which could be either negative or positive.

The extracts and fractions of *Sarcocephalus latifolius* at varying doses exhibit mixed effects on urine sample creatinine concentration, with the greatest reduction observed in the lowest dose of fraction A in normal volunteer samples. While controls generally fall within the normal range, exceptions such as high concentrations in normal patients could be attributed to factors like diet or medication intake, such as diuretics.[33,36] The result in this study presents that the *Sarcocephalus latifolius* treatment on diabetic patient's urine increased creatinine levels or had no significant effect, which opposes Ogugua et al.[30] in-vivo study with methanolic extract of *Sarcocephalus latifolius* that reported the reduction in creatinine level of male albino rats with diabetes induced by alloxan. The varying effects of *Sarcocephalus latifolius* extract and fractions may be due to the presence or absence of bioactive compounds it contains, and given that increased oxidative stress is implicated in the mechanisms of various forms of renal injury[37], it is plausible that the plant's antioxidant properties contribute to its ability to reduce creatinine levels.

CONCLUSION

Creatinine reduction properties of the extract were demonstrated, indicating the prospect of the plant improving a compromised kidney, despite irreversible kidney damage, its progression of the damage from the very early stage (Stage one) to the final stage of kidney Stage Five) damage can be slowed down drastically by exploiting available medicinal plants in the environment. The plant extracts also exhibit significant antioxidant activity, which may help prevent further oxidative damage and slow or halt the progression of kidney disease. *Sarcocephalus latifolius* gave significant creatinine reduction in low doses, which may effectively reduce creatinine levels in normal patients. However, caution is warranted in hypertensive and diabetic patients, as it may lead to increased creatinine levels, potentially precipitating renal failure.

CONFLICT OF INTEREST

Authors declare no conflict of interest

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