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Preliminary investigation on the In-vitro Antimicrobial, Antioxidant and Antidiabetic Activities of *Sorindeia warnecke* Extracts

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Abstract

Phytochemicals that are naturally occurring in plants are known to be potentially bioactive, having anti-oxidative, antimicrobial and enzyme inhibiting potentials. This study investigated the antimicrobial, antioxidant and antidiabetic activities of the methanol and dichloromethane extracts of the leaf and stem *Sorindeia warnecke*. The plant extract showed the presence of saponins, flavonoids, terpenoids, tannins, glycosides, coumarins, steroids, alkaloids, anthraquinones and reducing sugars. The antioxidant potential was evaluated using DPPH radical scavenging assay while the antidiabetic activity was evaluated using an in-vitro α -amylase inhibitory assay. The DPPH free radical scavenging activity was expressed in % inhibition with L Ascorbic acid as standard showing IC₅₀ 0.69 μ g/ml with methanol extract while dichloromethane leaf extract having the highest activity at 0.45 μ g/ml. The α -amylase assay showed that the extracts exhibited potent alpha amylase inhibitory activity in a dose dependent manner with the leaf methanol extract having an IC₅₀ of 2.33 μ g/L. The antimicrobial screening using the agar diffusion and agar dilution methods against the bacteria isolates *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Streptococcus mutans* and the fungal isolates *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus oligosporus* showed a good positive result. Preliminary investigation reveals that *Sorindeia warnecke*, contain bioactive compounds that possess promising pharmaceutical potentials that could be exploited.

Keywords: *Sorindeia warnecke*, alpha-amylase, anti-oxidant, antimicrobial, antidiabetic



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1.0 Introduction

Plants contains naturally occurring and biologically active phytochemicals that have potential disease prevention capabilities. They are present in plants as saponins, alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids and phenolics. Antioxidants from plant source are known to reduce oxidative stress and protect the body against free radical-mediated toxicities. (Blokchina et al., 2003) Plant species evaluated for antioxidant activity are known to also exhibit antidiabetic effect. Indeed, it is estimated that more than 400 herbal or plant-derived products are used for the management of type 2 diabetes across the globe (Bailey & Day, 1989). The hypoglycemic effect of some plant extracts has been confirmed in human and animal models of type 2 diabetes (Modak et al., 2007). In addition, antimicrobial resistance has become a major public health concern globally and the emergence of multidrug resistant pathogenic strains and adverse effects of synthetic antibiotics have led to rapid search for new antimicrobials. Development of new, effective, and safe antimicrobial agents is one of the ways by which antimicrobial resistance burden can be reduced. (Jamshidi-Kia et al., 2017)

Sorindeia warneckei Engl, (Family Anacardiaceae), is a west tropical Africa shrub plant which exists in Eastern and Western part of Nigeria. It is commonly called Umu-Ahia amongst the Igbos and Bùjé weere amongst the Yorubas. *Sorindeia warneckei* is a plant whose fruit is used as food and the stem is used as chewing stick. Its sap has general medicinal healing and the plant is used in medicinal baths. The plant also find uses in dyes, stains, inks and tattoos. (*Sorindeia warneckei* in *Global Plants on JSTOR*, n.d.) The in vitro activities of the extracts *S. warneckei* was investigated alongside the extracts of other plants commonly used as chewing sticks, where the extracts of *S. warneckei* showed the greatest inhibitory activities against *A. Bacteroides* gingivitis and *B. mefoninogenicus*. (Rotimi et al., 1988)

Despite the use of *Sorindeia warneckei* sap in treating different diseases and infections by local trado-medicinal practioners, there is insufficient information on the scientific justification for its use and there is dearth of information on the chemical constituents present in the plant. This study was therefore designed to investigate the antioxidant, anti-diabetic and antimicrobial activities of the methanol and dichloromethane extracts of *Sorindeia warneckei*.

2.0 Experimental

2.1 Plant Collection

The *S. warneckei* leaf and stem used for this research were obtained from Ikire in Osun State, Nigeria and the plant sample was identified and authenticated at the Forestry Research Institute of Nigeria (Voucher No: FHI-112541). The leaves and stem were air-dried for two weeks, pulverized and stored in a clean container at room temperature for further analysis.

2.2 Phytochemical Extraction of Plant Leaf and Stem

The pulverized leaf and stem samples were extracted by cold maceration using with methanol and dichloromethane. The plant materials were soaked separately in each solvent for a period of 72 hours with stirring at regular intervals. The resulting extracts were filtered and concentrated using a rotatory evaporator, under reduced pressure at 45 °C and stored at room temperature for future analysis.

2.3 Phytochemical Screening

The crude extracts obtained from the plants parts were subjected to qualitative analysis to test for the presence of phytoconstituents using standard methods described by Evans (2006) (Trease & Evans, 2006) and these were identified by the characteristic colour changes.

2.4 In vitro method DPPH radical scavenging assay

The free radical scavenging activity is measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Shimada et al., 1992). One ml of 0.1 mM DPPH solution in methanol was added to 1 ml of each sample in methanol of 12.5, 25, 50, 75 and 100 µg/ml concentrations respectively, and the mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resultant solution was measured at 517 nm. The DPPH radical-scavenging activity was calculated as follows:

$$\text{scavenging activity (\%)} = \left[\left(\frac{A_0 - A}{A_0} \right) \right] \times 100\%$$

Where A_0 is the absorbance of DPPH without extracts and A is the absorbance of DPPH with the extracts.

2.5 In vitro α -amylase inhibitory assay

The α -amylase inhibitory assay of *S. warneckei* leaf and stem was carried out according the method described by Suhda *et al.* (2011). Solutions of the extract (12.5 to 400 mg/ml) were prepared in 250 µL of DMSO and 250 µL of 0.02 M sodium phosphate buffer containing alpha-amylase solution (1U/ml) was added to each solution. They were thereafter pre-incubated for 10 minutes at 25°C. After pre-incubation, 250 µL of 1% starch solution (prepared by dissolving 1.0 g of potato starch in 100 ml of 0.02 M sodium phosphate buffer (pH 6.9)) was added to each of the solutions and the mixtures were brought to a gentle boil to dissolve. The solutions was cooled and the volume was brought to 100 ml with 0.02 M sodium phosphate buffer to each tube at 5 sec intervals. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with 500 µL of dinitrosalicylic acid colour reagent. The solution was diluted to a final volume of 100 ml with distilled water. (Suthindhiran et al., 2009) The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and absorbance was measured at 540 nm (Suthindhiran et al., 2009). All tests were performed in triplicate. The control represent 100% enzyme activity and was conducted in the same way. The percentage enzyme activity and percentage inhibition was calculated using the following equation (Sudha et al., 2011)

$$\begin{aligned} \text{\% Inhibition in an amylase activity} \\ = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \end{aligned}$$

2.6 Antimicrobial Assay

The antimicrobial zone of inhibition and minimum inhibition concentration of *S. warneckei* was investigated using agar diffusion and agar dilution methods. The test organisms used for the assay were supplied by the Department of Microbiology, Covenant University, Ogun State. The test bacterial isolates used were *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Streptococcus mutans*. The fungal isolates used were *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus oligosporus*. Ketoconazole for antifungal and Ofloxacin for antibacterial at 10 mg/ml were selected

as positive control, while methanol was used as negative control. Sterilized nutrient agar was distributed into plates at the volume of 20 ml per plate and the agar was allowed to solidify. The test organisms were sub-cultured and incubated at 37 °C for 24 hours and standardized using 0.5 M Macfarland standard solution. The standardized organisms were then inoculated on prepared solidified Mueller Hinton agar with aid of swab stick. A cork borer of a 4 mm was used to perforate the solidified agar to form a well and using syringe of 0.2 ml of the extracts was introduced into the bored wells. The agar was allowed to absorb the samples and was then incubated at 37°C for 18-24 hours. Similarly the fungi isolates were inoculated on Saboroud Dextrose Agar and incubated at room temperature for 18-24 hours. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition and the activity was calculated. The analysis was carried out in triplicate and GraphPad Prism Software was used in the statistical analysis of the results obtained from the assay.

3.0 Results and Discussion

The percent yield obtained by using methanol to extract the sample (10.53%) is higher than that obtained via dichloromethane (2.70 %). This is in agreement with the result from *Anacardium occidentale* (Cashew), a similar member of the Anacardaceae family, in which the methanol extract gave the highest yield (15.72%) (Liangpanth & Tongdeesontorn, 2018). This could be attributed to the solvent

extracting ability of methanol. The phytochemical screening of the leaves and stem methanol extracts showed the presence of saponins, flavonoids, terpenoids, tannins, glycosides, coumarins and reducing sugars. Steroids, alkaloids and anthraquinone were not detected in the methanolic leaves extracts, but were present in the methanolic stem extract. Steroids, coumarins and anthraquinones were detected in both of the dichloromethane extracts, but flavonoids and reducing sugars were not. Tannins and glycosides were present in the stem dichloromethane extract, while alkaloids were present in the leaves. The antioxidant activities of *Sorindeia warneckei* of the leaf and stem crude extracts using 2, 2-diphenyl-1-picrylhydrazide (DPPH) peroxide radicals in a peroxy-generating system reveals according to table 3, at a minimum concentration of 12.5 µg/mL, the standard ascorbic acid which shows the activity of 52.05% while the SWLD extract show activity of 36.26% and at a maximum concentration of 100 µg/mL, the standard showed an activity of 70.05 % while the SWLD extract show activity of 46.49 %. The radical scavenging of the DPPH is was observed to be in a dose dependent manner. Thus, antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule 2,2-diphenyl-1-hydrazine is formed. Studies show that presence of flavonoids, coumarins, and tannin contribute significantly to the antioxidant activity of medicinal plants (Ningsih et al., 2016) which could have been the reason for the observation. Table 4 show that the extracts

Table 1: Yield of *Sorindeia warneckei* crude extracts

Extracts	Colour	Wt of Plant (kg)	Wt of extract (g)	% Yield
SWLD	Dark greenish	1.920	51.89	2.70
SWLM	Dark green	1.920	202.18	10.53
SWSD	Dark green	1.430	41.05	2.87
SWSM	Dark brownish	1.430	115.13	8.05

Key: SWLD - *Sorindeia warneckei* leaf dichloromethane extract, SWLM - *Sorindeia warneckei* leaf methanol extract, SWSD - *Sorindeia warneckei* stem dichloromethane extract, SWSM - *Sorindeia warneckei* stem methanol extract.

Table 2: The phytochemical constituents of *Sorindeia wanecke* leaves and stem

Metabolites	SWLM	SWLD	SWSM	SWSD
Saponins	+	-	+	+
Flavonoids	+	-	+	-
Terpenoids	+	+	+	+
Steroids	-	+	+	+
Tannins	+	-	+	+
Glycoside	+	-	+	+
Coumarin	+	+	+	+
Alkaloids	-	+	+	-
Anthraquinone	-	+	+	+
Reducing Sugar	+	-	+	-

Table 3: Percentage inhibition of DPPH antioxidant activity of *Sorindeia warneckei* extracts

Extracts	12.5 µg/ml	25µg/ml	50µg/ml	75µg/ml	100µg/ml	IC ₅₀
SWLM	53.8	55.84	68.71	73.68	75.15	0.45
SWLD	36.26	37.71	38.89	45.32	46.49	6.23
SWSM	51.96	60.89	74.12	82.12	88.64	0.72
SWSD	49.16	58.1	63.13	67.97	70.2	0.74
Ascorbic Acid	52.05	55.85	68.13	72.51	76.02	0.69

Table 4: Percentage inhibition of α -amylase assay *Sorindeia warneckei* leaf and stem extracts

Extracts	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	IC ₅₀ mg
SWLD	36.66	36.75	54.5	64.67	73.68	90.9	2.6
SWLM	35.95	38.62	56.64	87.15	71.36	90.5	2.33
SWSD	30.33	36.4	50.04	64.05	71.9	90.37	2.9
SWSM	30.95	37.28	55.93	71.28	80.82	90.09	2.62
Acarbose	20.42	26.32	38.26	50.76	80.73	88.49	3.4

Table 5: Results of antimicrobial zone of inhibition measured bacterial isolates of *Sorindeia warneckei* leaf and stem extracts.

Extracts	<i>S. aureus</i>	<i>E. coli</i>	<i>S. mutans</i>	<i>S. typhi</i>
SWLM	30	30	30	40
SWLD	R	R	R	R
SWSM	30	18	30	28
SWSD	R	16	28	23
Ofloxacin	30	38	30	28

Table 6: Results of antimicrobial zone of inhibition measured fungal isolates of *Sorindeia warneckei* leaf and stem extracts.

Extracts	<i>C. albicans</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>R. oligosporus</i>
SWLM	0	0	0	30
SWLD	0	0	0	0
SWSM	0	0	30	24
SWSD	0	0	28	22
Ketoconazole	28	32	32	25

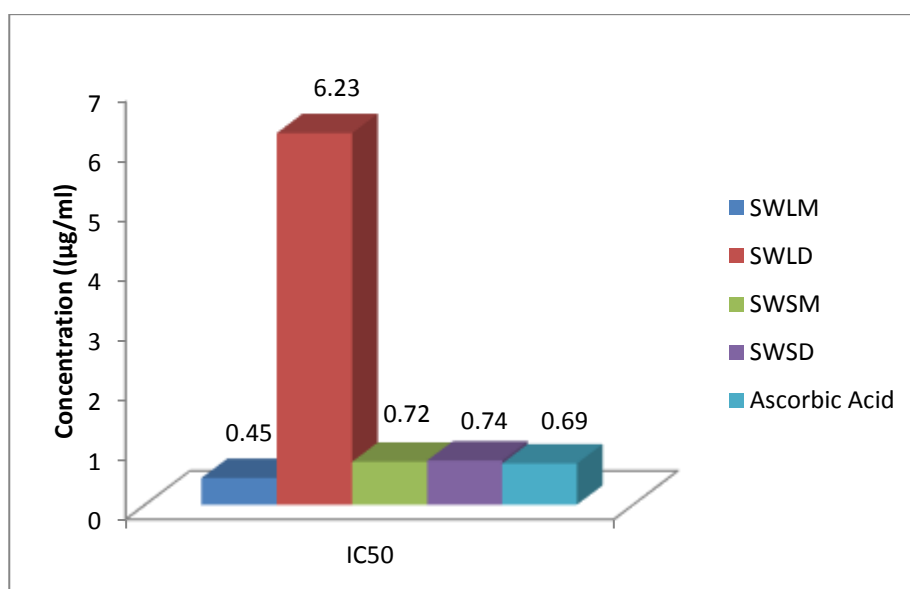
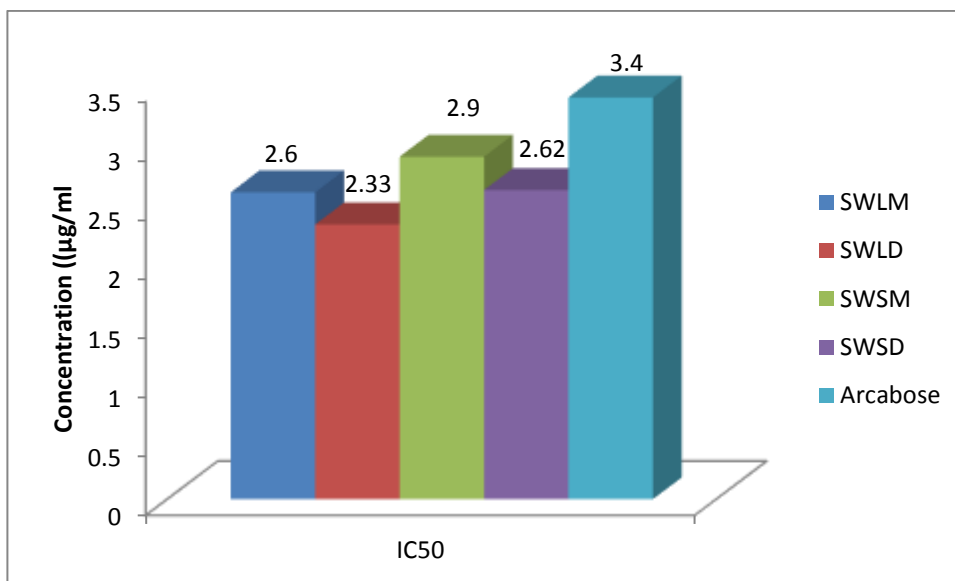
**Figure 1:** In vitro antioxidant assay of *Sorindeia warneckei* leaf and stem extract

Table 7: Results of minimum inhibition concentration of *Sorindeia warnecke* leaf and Stem extracts (mg/ml)

Extracts	<i>R.oligosporus</i>	<i>S.aureus</i>	<i>E.coli</i>	<i>S. mutans</i>	<i>S.typhi</i>	<i>A. flavus</i>
SWLM	25	7.81	156.2	125	31.25	-
SWLD	-	-	-	-	-	-
SWSM	31.25	15.62	-	62.5	62.5	62.5
SWSD	62.5	-	-	125	125	125

**Figure 2:** In vitro alpha amylase assay of *Sorindeia warnecke* leaf and stem extracts.**Figure 3:** Zone of inhibition of bacterial and fungi isolates of *Sorindeia warnecke* stem extracts

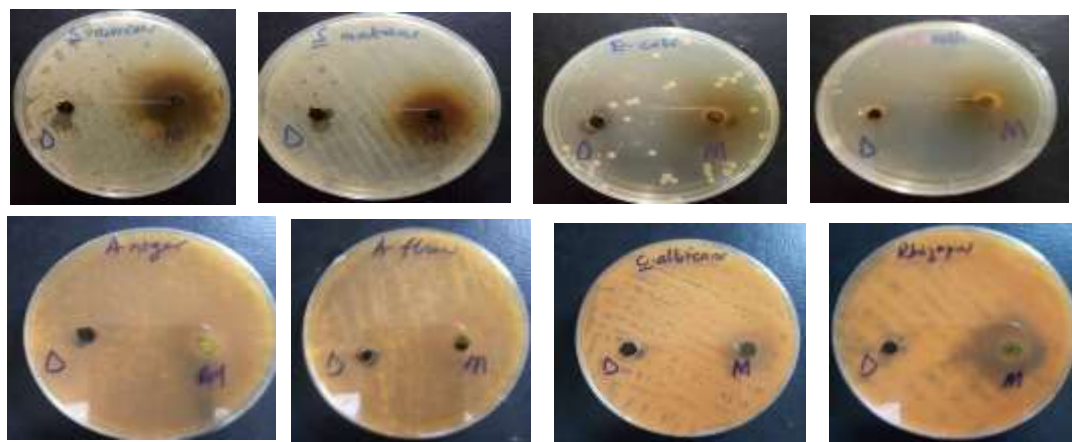


Figure 4: Zone of inhibition of bacterial and fungi isolates of *Sorindeia warnecke* leaf extracts

SWLD, SWLM, SWSD and SWSM concentrations exhibited potent alpha amylase inhibitory activity in a dose dependent manner with the percentage inhibition ranging from (36.66 - 90.90%), (35.95 - 90.5%), (30.95 - 90.09%) and (30.33 - 90.37%) against acarbose (20.42 - 88.49%) and the concentration of extracts that could inhibit 50% of alpha-amylase enzyme activity IC_{50} was used to determine the anti-diabetic capacity of the plant (fig. 2). The methanol extract of *S. warnecke* showed maximum α -amylase inhibitory activity IC_{50} of 2.33 mg. This could be attributed to the presence of polyphenols and flavonoids which inhibit carbohydrate hydrolyzing enzymes because of their ability to bind with proteins (Banerjee et al., 2017). The plant extracts could find use as starch blockers since it prevents or slows the absorption of starch into the body mainly by blocking or inhibiting the hydrolysis of 1, 4-glycosidic linkages of starch and other oligosaccharides into maltose and other simple sugars.

The methanol leaf extract of *S. warnecke* showed zone of inhibition in *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans* at 30 mm had wide zone of inhibition in *Salmonella typhimurium* at 40 mm higher than Ofloxacin of 28 mm. While the stem methanol extract had zone of inhibition in *Streptococcus mutans* and *Staphylococcus aureus* at 30 mm and stem dichloromethane extract had lesser inhibition than Ofloxacin. The leaf methanol extract which equally had wider zone of inhibition in *Rhizopus oligosporus* at 30 mm against Ketoconazole at 25 mm. Meanwhile, the (SWSM) and (SWSD) extracts had lesser zone of inhibition in *Rhizopus oligosporus* and *Aspergillus flavus* than Ketoconazole at 32 mm and 25 mm respectively. The minimum concentration of the extracts that could inhibit microbial growth was used to determine the antimicrobial properties of *S. warnecke* leaf and stem in Table 4.5.3. SWLM extracts with lowest minimum inhibition concentration of 7.81 mg against *Staphylococcus aureus* and SWSM extracts with low MIC of 31.25 mg against *R. oligosporus*. The SWLM and SWSM displayed good antimicrobial potential which could be attributed to the presence of saponin, flavonoids, terpenoids, coumarins and tannins in the plant extracts. (Falana and Nurudeen, 2022)

4.0 Conclusion

The result revealed that extracts of leaf and stem of *Sorindeia warnecke* have free radical scavenging properties and inhibitory activity against α -amylase. Antimicrobial assay likewise showed that methanol leaf and stem extracts possessed some antimicrobial properties. The results indicate that *Sorindeia warnecke* leaf and stem extracts possesses promising therapeutic efficacy.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conception: Osamudiamen P.M., Aiyelaagbe O.O.
Design: Osamudiamen P.M., Aiyelaagbe O.O.
Execution: Durodola B., Nwude D.O., Osamudiamen F.T.
Interpretation: Osamudiamen P.M., Durodola M., Aiyelaagbe O.O., Osamudiamen F.T., Nwude D.O.
Writing the paper: Osamudiamen F.T., Durodola B., Osamudiamen P.M.

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