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Antioxidant And α-Glucosidase Inhibition Potential of the Extracts from the Stem Bark of *Sclerocarya Birrea*

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ABSTRACT

Sclerocarya birrea is a medicinal plant used in herbal medicine for the treatment of various ailments, notably fever, boils and diarrhoea. Bark decoction, when mixed with other medicinal plants, treats various infections such as malaria, syphilis, dysentery and rheumatism. This work investigated the antioxidant and α-glucosidase potential of *Sclerocarya birrea* Stem bark. Thus, the powdered stem bark was macerated with ethanol and crude extract subsequently fractionated to afford various fractions. Evaluation of the fractions for antioxidant activity was conducted using 2,2-diphenyl-1- picryl hydrazil (DPPH) radical and α-glucosidase. The antioxidant activity of the ethyl acetate fraction gave IC_{50} value of 105.41 ±14µg/ml and αglucosidase inhibition with IC50 values 58.27± 5.56µg/ml. While chloroform and aqueous fractions demonstrated lower α-glucosidase inhibition of 134.03, 97.4 and 72.33 µg/ml respectively. This suggest that *Sclerocarya birrea* stem bark may offer compounds with non-excessive α-glucosidase inhibition activity.

Keywords: Antioxidant activity, α-glucosidase, DPPH, *S. birrea*

INTRODUCTION

Sclerocarya birrea, a medicinal plant, almost all its parts has been used in herbal medicine for various treatments, for example, the bark is used to treat a variety of ailments, notably fever, boils and diarrhea (Kgopa *et al*., 2020). Bark decoction, when mixed with other medicinal plants used to treats various infections such as malaria, syphilis, leprosy, hydropsy, dysentery, hepatitis and rheumatism, and is a laxative (John *et al*., 2010). It is also used internally and externally as a prophylactic against gangrenous rectitis. Leaves, bark and roots are used externally for snakebite, and internally for toothache, malaria fevers, diarrhea, dysentery, stomach ailments and headaches (Orwa *et al*. 2009, Hall *et al*., 2002). Dried seeds and nuts are widely consumed by local populations in Africa (Garba *et al*., 2018) (Tanih and Ndip., 2013). The plant parts are used to treat sore eyes, toothache, backache and body pains, infertility, schistosomiasis, it is also used to treat constipation, abdominal cramps and some other unspecified gastrointestinal problems, toothaches, swollen or infected gums, cough, hypertension, arthritis, proctitis, epilepsy, diabetes mellitus, sores, boils, carbuncles, abscesses and other bacterial infections (Mariod and Abdelwahab, 2012).

Previous phytochemical studies on *S. birrea* reveals the presence of Quercetin-3-O-arabinoside, Kaempferol-3-O-pentoside, where isolated from S. *birrea*. (Russo.*et al*., 2013). Similarly, Dihydroxy benzoic acid pentoside, α-muurolene, β-selinene and valencene were isolated from the plant (Dossou., 2011). Also, gallocatechin, catechin, epicatechin gallate, undecanedioic acid procyanidin B2-3,3"-di-O-gallate, and epicatechin gallate were reported by Shoko., 2018.

The isolated compounds have shown ranges of bioactivities including epicatechin gallate which showed good collagenase inhibition activity of 81.93% ± 0.40 (Shoko., 2018, Elmezughi *et al*., 2013 and Yanda *et al*., 2022). A purified compound from the plant have shown a good α-glucosidase and α-amsalyse inhibition and a good antioxidant property against 2,2-Diphenyl-1-picryl hydrazyl radical and water (Sithembiso *et al*., 2021). The research aim was to investigate the antioxidant and α-glucosidase inhibition property of the *S. birrea* stem bark extract.

MATERIALS AND METHODS

Collection of the Plant Material

The stem bark of *S. birrea* was collected from Zangon Danborno Sabon Gari local government Kaduna State. The sample was identified with voucher specimen number 1079 by herbarium curator in the Biological Science Department, Ahmadu Bello University Zaria, Kaduna state Nigeria. The samples were washed in water to remove soil debris, cut into smaller pieces

and air-dried. The dried samples were ground to powder (Thiantongin., 2014).

Extraction and Fractionation

The finely powdered air-dried stem bark of *S. birrea (*917g) was extracted with 2.5 litters of ethanol. The extract was decanted filtered and concentrated using a rotary evaporator (Buchi Rotavapor II, Buchi, Germany) at 40°C under reduced pressure (Mudi *et al*, 2010). Part of the crude extract 5 g was dissolved in water and partitioned sequentially with n-hexane, $CHCl_3$ and EtOAc in the ratio of (2:1, v/v), each to give aqueous, n-hexane, $CHCl_4$ and EtOAc fractions.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical scavenging activity

The antioxidant activity of the plant extract was determined using DPPH, in a 96 well plate according to the published procedure (Ionita., 2003; Mariko., et al 2016). In this method 50µl of various concentrations 1000 µg/ml, 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml of the crude extract and its fractions were added in to the plate well in triplicate, followed each by (195 µl) of DPPH solution (0.1 mM) added in to each well respectively. Exactly 50 µl of the ascorbic acid added 50µl of DPPH each form the positive control. Wells containing only ethanol others only DPPH serve as blank and control. Absorbance was read at 517nm. Each test was in triplicate and the average absorbance value was use to calculated its DPPH free radical scavenging percentage, according to this formula:

The capability to scavenge the DPPH radicals was calculated using equation 1, DPPH free radical scavenging (%);

$$\text{DPPH (\%)} = \frac{(AO - A1)}{AO} X\ 100 \text{ ……..... (1)}$$

A0 = absorbance of blank sample
A1= absorbance of sample
In order to obtain IC_{50}, a graph of inhibition rate against the sample concentration was plotted. (Jain *et al*., 2012).

α-Glucosidase Inhibition Assay

The effect of the plant extracts on α-glucosidase was determined according to the method described by Kazeem *et al*., (2013), using α-glucosidase from *Saccharomyces cerevisiae* and p-nitropheynyl-α-D- glucopyranoside (pNPG) (3.0 mM) as substrate. In this method 50 µL of various concentrations of the extract and its fractions (30, 60, 120 and 240 µg/mL) each was pre- incubated with 100 µL of α-glucosidase (1.0 U/ml) for 10 mins. Then 50 µL of 3.0 mM (pNPG) as a substrate dissolved in 20 mM phosphate buffer (pH 6. The reaction mixture was incubated at 37 ºC for 20 mins and stopped by adding 2 ml of 0.1M Na_2CO_3. The experiments were carried out in three replicates. The α-glucosidase activity was determined by measuring the yellow-coloured para-nitrophenol released from pNPG at 405 nm. The results (% Inhibition) are expressed as percentage of the blank (control) as in Equation 2.

$$\%\ \text{Inhibition} = \frac{(\text{Ac} - \text{Ae})}{\text{Ac}} X\ 100 \text{ ……..... (2)}$$

where Ac and Ae are the absorbance of the control and extract, respectively. The concentration of extract resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel.

RESULTS AND DISCUSSION

The free radical scavenging activity of the ethanol, chloroform, ethyl acetate and aqueous fractions alongside the positive control ascorbic acid were assessed using DPPH at (50,100,250,500 and 1000 µg/m). It was found that the ethanol, aqueous, ethyl acetate, chloroform and ascorbic acid afforded IC_{50} value of 5.49 ± 5.35, 75.67 ± 12.50, 105.41 ± 14, 276.81 ± 53.42 and 44.59 ±9.15 µg/m respectively. The ethanol fraction gave the highest DPPH scavenging activity of 5.49 µg/ml compared to the rest of the fractions (Table 1). Percentage inhibition potential of the fractions at various concentrations gave free radical percentage inhibitions range of 40% - 72% (Figure 1). Investigations of the fractions for α-glucosidase inhibition gave IC_{50} range 5.49 ± 5.35 - 276.81 ± 53.42 µg/ml. The ethyl acetate fraction gave lower IC_{50} value 58.27 than the positive control (Table 2). Percentage inhibition potential of the fractions at various concentrations gave α-glucosidase percentage inhibitions range of 38% - 89% (Figure 2)

The concentration of extract resulting in 50 % inhibition of enzyme activity (IC_{50}) was determined graphically using Microsoft Excel.

Table 1: Antioxidant effect of *S. birrea* stem bark fractions

Conc (µg/ml)	IC_{50} (µg/ml)
CHF	276.81 ± 53.42
EAF	105.41 ± 14
ETF	5.49 ± 5.35
AQF	75.67 ± 12.50
AA	44.59 ± 9.15

Table 2: α-glucosidase Inhibition Activity of the *S. birrea* stem bark fractions

Extract	IC_{50} (µg/ml)
CHF	97.4
EAF	58.27
ETF	134.03
AQF	72.33
AC	64.13

Key: CHF= Chloroform fraction; EAF= Ethyl acetate fraction; ETF= Ethanol fraction; AQF= Aqueous fraction; AA=Ascorbic acid; AC=Acarbose

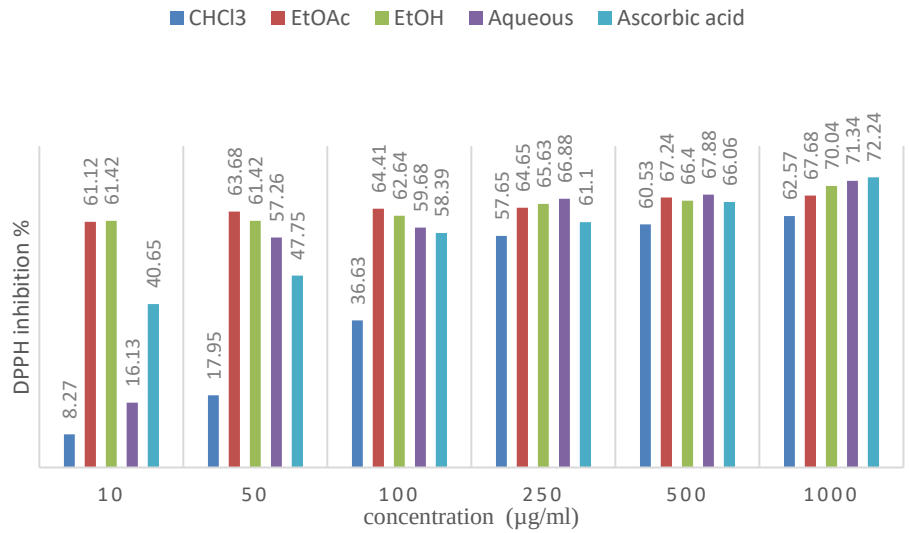

Figure 1: DPPH radical scavenging activity (%) of stem bark extracts of *S. birrea*

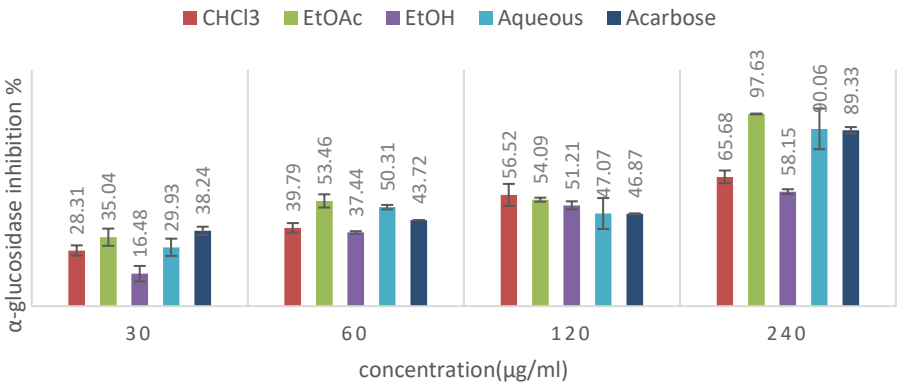

Figure 2: α-glucosidase inhibitory activity (%) of stem bark extracts of *S. birrea.*

In this study, S. *birrea* stem bark was extracted using ethanol, the filtered concentrated extract was fractionated using solvent -solvent fractionation with n-hexane, chloroform, and ethyl acetate. The crude extract and its fractions were screened for antioxidant and α-glucosidase inhibitory activity. The antioxidant assay using (DPPH) of the extracts in this study was performed according to the method described by (Sabah *et al*., 2016). The hyperglycaemia and oxidative stress need to be addressed by compounds with α-glucosidase inhibition and or antioxidant property to deal with diabetes-induced oxidative stress and preventing diabetic complications. The oxidative stress is greatly increased in prolonged hyperglycaemia due to increase in free radical generation flow out from auto-oxidation of glucose. The free radicals can cause damage to β-cells and oxidation homeostasis imbalance resulted in developing insulin resistance which is a risk factor for diabetes type 2 (Coulidiaty *et al.*, 2021). Dealing with diabetes-induced oxidative stress may

be a necessary approach towards preventing diabetic complications (Coulidiaty *et al*., 2021).

In this study, the ethanol fraction afforded the highest DPPH scavenging activities of 5.49 ± 5.35 µg/ml compared to other solvent fractions, chloroform, aqueous and ethyl acetate table1. Nuno *et al*., 2013. reported the methanol and aqueous extract from *S. birrea* exhibited DPPH scavenging activity of 9.8 and 19.6 µg/ml, the aqueous fraction gave lower scavenging ability compared to the methanol, which agree with present research findings with reference to ethanol and aqueous fractions antioxidant ability table1. Niang *et al.*, 2021 reported the S. *birrea* stem bark aqueous extract afforded DPPH inhibition percentage of 91.04 ±0.001 % higher compared to ascorbic acid which afforded 94.86 ± 0.008%. This agreed well with findings of present study Fig. 1. In another related study Mousinho *et al*., (2013) reported *S. birrea* extracts displayed DPPH radicals scavenging ability in a concentration dependent manner, this agreed well with findings of present study Fig. 1.

S. *birrea* stem bark methanol extract demonstrated lower α-glucosidase inhibition than the standard acarbose with IC_{50} 0.032 ± 0.08, 0.029 ± 0.10 and 0.026 ± 0.04 mg/ml respectively (Mogale *et al*., 2011), the higher polar solvents comparable to methanol in the present study afforded lower α-glucosidase inhibition than the standard Table. 2. In the present study, the ethyl acetate fractions afforded higher α-glucosidase inhibition than acarbose, followed by the aqueous and chloroform fraction that demonstrated a moderate inhibition while the ethanol fraction afforded the lowest inhibition Table. 2.

The *S. birrea* extracts inhibited α-glucosidase in a concentration dependent manner, producing comparable results with acarbose (Coulidiaty *et al.,* 2021), this is in support of this study findings. Fig. 2.

The antioxidant property afforded by the ethanol and aqueous fractions in this study proves them potential target for antioxidant compounds for diabetes- induced oxidative stress therapy in coupled with the aqueous fraction moderate α-glucosidase inhibition compare with the positive standard.

The chloroform and ethanol fractions demonstrated mild α-glucosidase inhibition. This mild inhibition can serve to prevent side effect experience with synthetic drugs. The range of α-glucosidase inhibition values from the findings of the present research suggested the plants stem bark extracts as potential source of antidiabetic compounds.

CONCLUSION

The problem of excessive α-glucosidase inhibition and the resulting side effects of synthetic antidiabetic drugs could be overcome by antidiabetic compounds with low to moderate α-glucosidase inhibition from natural products. The aqueous, chloroform and ethanol fractions prevails the presence of phytochemicals with promising non excessive α-glucosidase inhibition and with moderate antioxidant capacity to overcome induced oxidative stress.

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