The Journal of Phytopharmacology 2021; 10(4):249-255
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(4): 249-255
July- August
Received: 19-05-2021
Accepted: 13-06-2021

doi: 10.31254/phyto.2021.10406

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Black Velvet Tamarind: Phytochemical Analysis, Antiradical and Antimicrobial Properties of the Seed Extract for Human Therapeutic and Health Benefits

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ABSTRACT

Black Velvet Tamarind (BVT) is a famous edible fruit with multiple therapeutic properties. The aim of this study was to determine the secondary metabolites and evaluate the medicinal activities of the seed extract of the plant in order to scientifically report its possible medicinal applications in food and pharmaceutical industries. The pulverized seed was extracted with methanol/ethylacetate (2:1) and the concentrated extract was analyzed using gas chromatography-mass spectrometry (GC-MS), the antioxidant capacity was evaluated using galvinoxyl and 2,2′-diphenyl-1-picryl-hydrazyl (DPPH) assays while the antibacterial activity was determined by agar-well diffusion method. From the GC-MS analysis, Twenty (20) organic compounds were identified in the seed extract, accounting for 99.3% of the identifiable components in the seed extract, and the main constituents were:4-O-methylmannose (40.46%), 9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione (12.30%), palmitic acid (10.00%), nitroisobutylglycerol (8.60%), simiarenol (4.77%) and methyl-*α*-d-mannofuranoside (4.70%). The extract also contained some notable therapeutically active phenolic compounds such as dihydrochavicol (3.60%), *p*-chloro-m-cresol (0.67%) and *p*-vinylguaiacol (0.1%). The seed extract possesses significant free radical scavenging and antioxidant (IC_{50} and AAI) properties; for galvinoxyl assay (30.00-34.00%), 5.0 and 8.4 and DPPH assay (86.78-90.57%), 6.0 and AAI of 6.7, respectively. The result showed that the antioxidant properties of the seed extract of BVT increased in dose-concentration manner due to the synergetic activity of secondary metabolites present in the seed extract. This study showed that the seed of BVT possesses antioxidant and antimicrobial potential and it might be useful against ROS and RNS induced disorders. The seed of BVT can be used as an easily accessible source of natural antioxidant. The extract has high inhibitory effects at different concentrations (1000-250 μgml^{-1}) on *Enterococcus faecalis* (30 mm) and *Serratia marcescens* (15 mm) isolated from clinical samples. The susceptibility of Gram positive and negative bacterial strains to the seed extract was due to the synergic activities of the secondary metabolites in the seed extract, most especially the phenolic compound and the terpenoids. This study showed that the seed extract of BVT has medicinally bioactive phytochemicals that may be useful in the formulation of food preservatives or drug supplements and treatment of bacterial infections.

Keywords: Black Velvet Tamarind, Seed extract, GC-MS, Secondary metabolites, Galvinoxyl, DPPH, Antioxidant, Antibacterial.

INTRODUCTION

Natural products have many pharmacological properties, and there is no doubt that natural products of plant origin can be used as alternative medicine for the treatment of many common diseases among human and animals [1-3]. Natural products from both plants and animals have been screened for several medicinally active compounds that are very useful in the pharmaceutical and food industries [4-6]. Natural products have played serious role globally in treatment and prevention of human and animals' diseases such as viral, bacterial, parasitic diseases and diseases that are linked to reactive oxygen/nitrogen species such as cancer, aging, chronic inflammatory *diseases*, diabetes and neurodegenerative *diseases* [7]. Majority of the active drugs in the pharmaceutical shelves and in clinical use for treatment of various ailments originated from natural products [7-10]. Researches on phytochemicals from natural products provide comprehensive evidence-based alternative medicines which forms the basis of herbal drug industry and discovery of drug targets in the food and pharmaceutical industries. Plant-based drugs are noted for many medicinal activities such as reduction of blood pressure, prevention of cardiovascular diseases. In addition, reducing the risk of cancer is also due to their antioxidant properties. Secondary metabolites are considered as rich resources of ingredients which can be used in drug development either pharmacopoeia, non- pharmacopoeia or synthetic drugs [9, 11-13]. Plants that are rich in phenolic compounds have been associated with the potential of reducing risk of oxidative stress disorders [14-18]. Oxidative stress is a pathological condition in which there is an imbalance between reactive oxygen/nitrogen species (ROS/RNS) and the antioxidant defense system;

ROS/RNS submerge antiradical defenses of the organism, causing the inability of human body system to detoxify the reactive intermediates, resulting in oxidative alteration of biomolecules (such as lipid, protein, DNA), tissue injury, and accelerated cellular death as the causes of some chronic diseases [19-22]. Research into natural products continues to provide a novel template for preservatives and drug discovery of lead compounds for the food and pharmaceutical industry [23, 24]. Currently, natural products are key sources of innovative pharmaceutical therapeutic agents, because drug produced from synthetic drug sources exert fewer therapeutic effects with adverse side effects [9, 25-28].

Black velvet tamarind (BVT) is a tall, tropical tree which belongs to the *Fabaceae* family and produces an important edible fruit. It has small, typically grape-sized edible fruits with brown hard inedible shells. It is about 30 m high, with a densely leafy crown, but often shrubby [29, 30]. The leaves are finely hairy, broadly elliptic, blunt at the apex, leathery and have a sunken midrib. Its flowers appear whitish and the branches are horizontally spread. The fruit pulp which is red, with a sweet-sour, astringent flavour which has multiple advantages to human and animals. BVT is essentially a good source of nutrients for humans and animals. It is a good food for human consumption containing several vitamins, trace elements, such as iron which is able to boost red blood production that is needed to prevent anaemia. It also has the potential to reduce micro-nutrient deficiency and lowers body temperature when consumed as raw juice. Pharmacologically, it is a good diuretic, it is able to bind the toxins and optimize urine excretion in the kidney, therefore preventing the kidney stones disease. It is also able to treat ulcer, eye diseases, jaundice, respiratory and inflammatory disorders *such as asthma*, allergy, bronchitis etc [31-35]. It is used locally to prevent abortion of pregnancy. Therefore, it is usually recommended traditionally for women at the early stage of pregnancy, as it strengthens the pregnancy due to the presence of abundant medicinally beneficial vitamin A in the fruit [36-40].

To the best of our knowledge, there is insufficient scientific information on the chemical composition and medicinal properties of the seed of BVT grown in Nigeria so far. Therefore, the present research was undertaken to screen the seed of BVT grown in Nigeria for its secondary metabolites, free radical scavenging, antioxidant and antibacterial potential.

MATERIALS AND METHODS

Collection of Plant Sample

The seeds were collected in Ota, Ogun State, Nigeria and were identified by Dr. O.O. Ovuakporie-Uvo of Department of Biological Sciences, University of Medical Sciences, Ondo, Nigeria as black velvet tamarind (BVT) (*Fabaceae*).

Extraction of the Plant Sample

Air-dried and pulverized seed of BVT was extracted using a mixture of methanol/ethylacetate (2:1). The mixture was left for at least three days. The filtrate was concentrated using a water bath at 40 ºC. The concentrated extract was preserved ina vial and stored in a refrigerator to prevent contamination pending subsequent analysis [41, 42].

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The phytochemical compositional analysis of the concentrated extract was carried out by using GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) system at the Shimadzu Training Centre for Analytical Instruments (STC) Lagos, Nigeria. The analytical specifications of the GC-MS were evaluated as described in an earlier study [16]. Compounds were identified by comparing their mass spectra of some standards and commercial mass spectral databases NIST Mass Spectral Library.

In vitro Free Radical Scavenging (FRS) and Antioxidant Potential

The free radical scavenging (FRS) and antioxidant potential of the seed extract was evaluated using two different standard assays as described below**:**

(i) *In vitro* Galvinoxyl Free Radical Scavenging (FRS) Assay

Free radical scavenging activity of BVT extract was evaluated using galvinoxyl according to the method previously used by Dose *et al*. [43] with slight modifications. Briefly, fresh methanolic solution of galvinoxyl was prepared, 2.0 ml reaction mixture containing 1.0 ml galvinoxyl solution and 1.0 ml of the different concentrations of the seed extract (1000, 500, 250, 125 and 100 µgml^{-1}) were added and stirred for a few seconds in separate test tubes. Ascorbic acid was used as reference standard. The absorbance of the reaction mixture was recorded at 429 nm against the blank after 30 min of incubation in the dark at room temperature using SM 7504 UV Spectrophotometer. Measurements were performed in triplicate. The control contained a preparation of galvinoxyl and methanol without the seed extract. The percentage of the radical inhibition activity was evaluated based on the following expression:

$$I\%_{\text{Galvinoxyl}} = \frac{A_{cont} - A_{ext}}{A_{cont}} X\ 100$$

A_{cont} and A_{ext} are the absorbance value for the control and extract solution, respectively. The dose-response curve was plotted and IC_{50} value for the extract and the standard were calculated.

(ii) *In vitro* DPPH (2,2′-Diphenyl-1-picryl-hydrazyl) Assay

Radical scavenging activity of BVT extract was also evaluated by measuring the change in 2,2′-diphenyl-1-picryhydrazyl (purple colour) to diphenylpicrylhydrazine (yellow colour) according to the method previously used by Adebiyi *et al*. [44]. Briefly, a 2.0 ml reaction mixture containing 1.0 ml DPPH (in methanol) and 1.0 ml of the different concentrations of the seed extract (1000, 500, 250, 125 and 100µgml^{-1}) were added and stirred for a few seconds in separate test tubes. As reference standard, ascorbic acid was used. After 30 min incubation in the dark at room temperature the absorbance of reaction mixture was recorded at 517 nm against the blank using SM 7504 UV Spectrophotometer. Measurements were performed in triplicate. The control contained a preparation of DPPH and methanol without the extract. The percentage of the radical inhibition activity was evaluated based on the following expression:

$$I\%_{DPPH^{\bullet}} = \frac{A_{blank} - A_{ext}}{A_{blank}} X\ 100$$

Where: $A_{blank}$and A_{ext} are the absorbance value for the control and extract solution, respectively. The dose-response curve was plotted and IC_{50} value for the extract and the standard were calculated.

Antioxidant Activity Index (AAI): The AAI was calculated as:

$$AAI = \frac{Galvinoxyl\ or\ DPPH^{\bullet} Initial\ Concentration}{IC_{50}}$$

AAI was classified as weak when AAI < 0.5; moderate when AAI ranged between 0.5-1.0; strong when AAI ranged between 1.0-2.0; and very strong when AAI > 2.0.

***In vitro* Screening of Antimicrobial Potential**

The anti-microbial assay was carried out using agar well diffusion technique to investigate the bacteria, susceptibility of seed extract at different concentrations on sterilized Mueller Hinton Agar (MHA) using streak plate method according to the method previously used by Ololade *et al*. [41] and Sewify *et al*. [45]. Two strains of clinically isolated bacteria were used; Gram-positive *Enterococcus faecalis* and Gram-negative *Serratia marcescens*.Pure culture of the isolates were subcultured into sterile nutrient broth and incubated for 18-24 hours at 37 °C. A sterile cotton swab was soaked in the broth containing the bacterial samples and this was swabbed on the surface of sterile Mueller Hinton plates under aseptic conditions. Wells were created by using sterile cork borer of 6 mm diameter at equidistance points. The respective wells were supplied with 1000, 500 and 250 µgml^{-1}solutions of the extract solution. The petri plates were then kept in refrigerator for diffusion and then transferred to biological incubator and plates were incubated at 37°C for 24 hr. Augmentin (AUG) 30 µg/disc was used as positive control to compare the antibacterial potential. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in millimetre (mm) using transparent ruler.

Determination of the Antibacterial Activity Index (AI)

The AI of the tested seed extract with respect to the positive control was evaluated according to the method previously described by Sridhar *et al*. [46].

Activity index for the extract was calculated using the following formula:

$$Antibacterial\ Activity\ (AI) = \frac{Inhibition\ Zone\ of\ the\ Extract}{Inhibition\ Zone\ of\ the\ Standard}$$

RESULTS AND DISCUSSION

The result of phytochemical screening of the extract of BVT is using GC-MS shown in table 1, whereas the results of free-radical scavenging, antioxidant and antimicrobial potential are depicted in figures2 and 3, respectively.

Chemical Constituent of the Seed Extract

The Gas Chromatography-Mass spectrometry (GC-MS) analysis of the methanol/ethyl acetate seed extract reveals twenty-five (25) compounds, amounting to 99.49% of the secondary metabolites in the extract (Table 1). Taking into consideration the chemical nature of the compounds in the extract; varieties of secondary metabolites were present in the extract. The main constituents identified were:4-O-methylmannose (40.46%), 9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione (12.30%), palmitic acid (10.00%), nitroisobutylglycerol (8.60%), simiarenol (4.77%) and methyl-*α*-d-mannofuranoside (4.70%). The seed extract of BVT also contained some notable therapeutically active phenolic compounds such as dihydrochavicol (3.60%), *p*-chloro-m-cresol (0.67%) and *p*-vinylguaiacol (0.1%).The diversity of secondary metabolites in the seed extract justifies the therapeutic potential observed in this study. Previous studies on the GC-MS phytochemical screening of leaf extract of a related species such as *Tamarindus indica* from Cuba showed the presence of the following as the most abundant component in its solvent extract; limonene (9.05%), methyl 15-tricosenoate (8.39%), longifolene (7.51%), 2,6-di-tert-butyl-4-methylphenol (7.24%), methylhexadecanoate (6.41%), caryophyllene (5.56%), diphenyl ether (5.47%), cryptopinone (5.28%) [47] showing that there is no similarity between the extract of BVT investigated in this study and the extract of *T. indica* from Cuba.

Table 1: Chemical Composition of the Seed Extract of BVT

Compound	Retention Index	Percentage Composition
N-methyl-N-nitroso-2-propanamine	813	0.28
alletone	1022	0.26
thymine	1118	0.30
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	1173	0.37
p-chloro-m-cresol (phenolic)	1194	0.67
nitroisobutylglycerol	1444	8.60
dihydrochavicol (phenolic)	1213	3.60
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1269	2.55
p-vinylguaiacol (phenolic)	1293	0.10
methyl-3,6-anhydro-*α*-D-mannopyranoside,	1353	0.26
1*α*,2*β*,3*α*,5*β*-cyclohexanetetrol	1472	1.50
9,9-dimethoxybicyclo[3.3.1]nona-2,4-dione	1610	12.30
methyl-*α*-d-mannofuranoside	1667	4.70
4-O-methylmannose	1714	40.46
i-propyl-9-tetradecenoate	1822	1.20
α-monocaprin	1886	0.60
palmitic acid, methyl ester	1878	0.57
palmitic acid	1968	10.00
cyclobarbital	2022	2.00
oleic acid, methyl ester	2085	0.40
17-octadecynoicacid	2165	1.70
cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	2266	2.00
1-acetoxynonadecane	2276	0.20
2-methyl-9-*β*-d-ribofuranosylhypoxanthine	2749	0.10
simiarenol	2827	4.77
Percentage Total		**99.49**

Evaluation of Free Radical Scavenging and Antioxidant Potential

For the screening of the antioxidant capacity of the seed extract, two different assays were used to obtain valid results; this is due to the fact that antioxidant compounds present different mechanisms of reactions with the possibility of having synergistic interactions depending on the type of assay used. In this study, the antioxidant potential of the seed extract was evaluated using the galvinoxyl and DPPH assays (Figure1).

(i) *In vitro* Galvinoxyl Free Radical and Antioxidant Potential

Galvinoxyl is a stable phenoxyl radical that exhibits characteristic UV absorption at 429 nm in methanol solution. The radical has strong absorption in the visible region, while its absorption decreases proportionally upon receiving an electron or hydrogen from the antioxidants. The free radical scavenging potential of the phytochemicals in seed extract was obtained based on the absorption change. The result of galvinoxyl radical scavenging assay of the seed extract of BVT grown in Nigeria is shown in figure 1. The extract was evaluated at the concentrations of 1000, 500, 250, 125 and 100 µgml^{-1} and with percentage free radical scavenging of 34, 32, 31, 30 and 30%, respectively. The seed exhibited low inhibition concentration (IC_{50}) of 5.0 µgml^{-1} and antioxidant activity index (AAI) of 8.4. The radical scavenging ability of galvinoxyl was more than that of ascorbic acid (the reference compound), which had IC_{50} and AAI values of 15.0 µgml^{-1} and 2.8. The galvinoxyl antioxidant potential of the seed investigated in this study is higher than that obtained in a previous study on the seed methanolic extract of *Annona cinera* from Nigeria with galvinoxyl IC_{50} and AAI values of 100.0 µgml^{-1} and 0.4, respectively [48]. Moreover, the result of this study showed that the seed extract evaluated in this study has a more intense antiradical capability than ascorbigen which showed no activity on the DPPH and galvinoxyl radicals [49]. The reaction between extract and galvinoxyl proceeded slowly and continuously for a longer incubation time than DPPH.

(ii) *In vitro* DPPH Free Radical Scavenging and Antioxidant Potentials

The results of DPPH radical scavenging assay of seed of BVT grown in Nigeria are shown in figure 1. The seed extract showed concentration-dependent increases in radical scavenging potential. The extract was evaluated at concentrations of 1000, 500, 250, 125 and 100 µgml^{-1} and with percentage free radical scavenging of 90.6, 88.9, 87.7, 87.2 and 86.8%, respectively. The seed exhibited low inhibition concentration (IC_{50}) of 6.0 µgml^{-1} and antioxidant activity index (AAI) of 6.7. The IC_{50} values represent the concentration at which 50% of DPPH was reduced. A low IC_{50} value indicates a potent antioxidant activity. Ascorbic acid showed the inhibition concentration of IC_{50} to be 8.0 µgml^{-1}. The extract showed a similar antioxidant property compared to the reference drug. The seed extract investigated in this study gave more promising free radical scavenging and antioxidant activity than the ethanolic and ethyl acetate extracts of *Tamarindus indica* leaves from Dakar (Senegal) with DPPH IC_{50} of60.53 and 453.33 µgml^{-1}, respectively [50]. Generally, phytochemicals and natural products from plants act as electron donors or hydrogen atom transfer due to their high content of phenolic compounds. This justifies the radical scavenging potential observed in the seed extract of BVT investigated. This result corroborates a previous study which demonstrated that antioxidant potential of secondary metabolites increase in dose-concentration manner [51, 52]. Generally, the extract investigated has been shown to be a good antioxidant agent even at very low concentrations. The percentage radical scavenging activity was very low in galvinoxyl assay compared to DPPH assay, this due to the steric hindrance among adjacent bulky groups within the galvinoxyl molecule limited the ability of the extract in scavenging galvinoxyl radicals effectively compared to DPPH in which the extract showed a powerful capacity for scavenging free radicals [48, 53, 54]. Natural antioxidants from plants help to maintain an adequate antioxidant status in human body. Antioxidants decrease the oxidative damage directly via reacting with free radicals or indirectly by preventing the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes [55, 56]. Chain-breaking antioxidants exert their action through either hydrogen atom (H•) and electron (e−) donation or both (i.e., proton-coupled electron transfer), such methods are commonly classified as HAT- and SET-based assays [53]. On the basis of mechanism, antioxidants can be classified into two groups, namely, hydrogen atom transfer (HAT) and single electron transfer (SET) assays. The HAT-based assays measure the potential of antioxidants to quench free radicals by H-atom donation [57-59]. The SET mechanism measures the ability of antioxidant to transfer an electron to reduce free radical [58, 60-61]. ROS and RNS health disorders such as multiple sclerosis is one of the complex diseases of the central nervous system (brain and spinal cord) affecting many people globally [62-69]. Natural products, mainly fresh fruits and vegetables derived from plants with protective antioxidant phytochemicals such as dietary supplements (e.g., melatonin, omega-3 PUFAs), herbal supplements (e.g., curcumin, cannabinoids), polyphenol compounds, and vitamins (e.g., vitamin A and vitamin D3) are beneficial in treatment of ROS/RNS diseases conditions such as multiple sclerosis and other chronic kidney and cardiovascular diseases [70, 71]. Studies showed that *alpha*-lipoic acid, a naturally protective antioxidant that reduces brain atrophy (reduction in brain volume due to the loss of neurons) among patients with secondary progressive multiple sclerosisin [15, 72-75]. Naturally grown foods, vegetables and plants that contain *α*-lipoic acid include spinach, broccoli, yams, green pea, potatoes, yeast, tomatoes, Brussels sprouts, carrots, beets, rice bran, bovine (cow) spleen, brain, kidney and heart. Natural *α*-lipoic acid has major dual advantages among others; being fat and water soluble; ability to cross brain-blood barrier unlike some other antioxidant [76, 77].

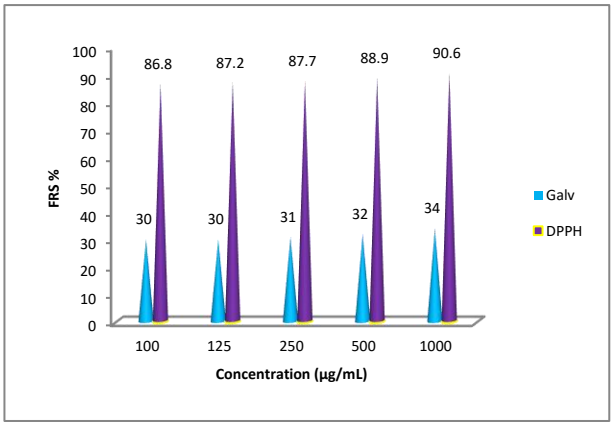

Figure 1: Antioxidant Potential of the BVT Seed Extract

Antibacterial Potential

Agar well diffusion method was used to evaluate the antimicrobial potential of the seed extract. Maximum zone of inhibition value was observed for Gram positive bacteria (*E. faecalis*) compared to Gram negative bacteria (*S. marcescens*). As depicted in figure2, the extract had a zone of inhibition of 30 mm on *E. faecalis* which indicated that *E. faecalis* was highly susceptible compared to *S. marcescens* with ZI of 15 mm within the concentration of 1000 µgml^{-1} of seed extract of methanol/ethyl acetate of BVT in this study. It is noteworthy that for the Gram positive bacteria (*E. faecalis*), there was no difference in the ZI and the activity of the extract at lower concentrations of 500 and 250 µgml^{-1} when compared to 1000 µgml^{-1} of the extract. At the concentration of 1000 µgml^{-1} of the seed extract, *S. marcescens*(15

mm) ZI was very high, meanwhile the ZI of the seed extract at the concentrations of 500 and 250 µgml^{-1}for *S. marcescens* showed ZI values of 10 mm, respectively. These were significantly lower when compared to the activity at 1000 µgml^{-1}. The susceptibility of both Gram positive and negative bacterial strains to the seed extract was due to the synergic activities of the secondary metabolites in the seed extract, most especially the phenolic compound and the terpenoids. Generally, the antibacterial index (AI) ranged between 0.8-3.0. The maximum antibacterial activity index (AI) values was observed against *E. faecalis* (3.0) compared to *S. marcescens*with AI value of 0.8. The AI values indicated the bacteriostatic nature of the extract and also having good correlation with the zone of inhibition values. The AI values are very useful in evaluating the antibacterial potential quantitatively compared to the standards used in the study. The seed extract at all the concentrations showed significant antibacterial potential compared to the reference drug (augmentin) in dose dependent manner. Therefore, we can infer that the seed extract has good antibacterial potential against both Gram positive and Gram-negative bacteria compared to the standard augmentin. Comparatively, the antibacterial activity of the seed extract investigated in this study have higher antibacterial potential compared to the leaf extract of *Tamarindus indica* from India which was investigated for its *invitro* antibacterial activity against *Clostridium botulinum*, *Salmonella enterica*, *Pseudomona sputida* and *Klebsiela granulomatis* with the zones of inhibition of 5, 4, 3 and 3.8 mm, respectively [78]. The antibacterial potential of the seed extract was linked with some well-defined mechanisms such as: firstly, adhesion of the extract onto the surface of cell wall and membrane. Secondly, the ability of the extract to penetrate into the cell damaging the intracellular structures (mitochondria, vacuoles, ribosomes) and biomolecules (protein, lipids, and DNA). Thirdly, the extract induced cellular toxicity and oxidative stress caused by generation of reactive oxygen species (ROS) and free radicals, and lastly, modulation of signal transduction pathways. Besides these well-recognized mechanisms, the extract also modulated the immune system of the human cells by orchestrating inflammatory response, which further aid in inhibition of microorganisms [79, 80]. The lower ZI values of Gram negative bacteria may be due to its resistant cell wall composition. From the results we can infer that the seed extract shows antibacterial potential even at lower concentrations [81-83].

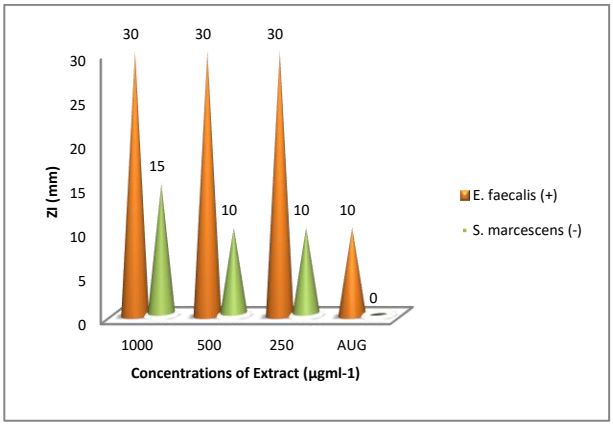

Figure 2: Antibacterial Potential of the BVT Seed Extract

CONCLUSION

This study revealed that the extract of BVT caused a concentration-dependent inhibition of free radicals and bacterial isolates. These activities may be due to the presence of noticeable amount of different secondary metabolites such as phenolic compounds and terpenoids in the seed extract of BVT which play key roles in inhibiting oxidative reactions and microbial infections. The seed of BVT can be used as an easily accessible source of natural antioxidant. However, further studies are required to isolate the active components. The activity guided isolation of different phytochemicals will be purposeful to establish their antioxidant potential and other disease therapeutic potential in different preclinical mechanisms. Moreover, *in vivo* pharmacological studies and clinical trial are required to elucidate the mechanism of action of the seed extract in the management of human diseases resulting from oxidative stress. Moreover, as an easily accessible and cheap natural antioxidant, its applications in pharmaceutical and food industries as food and drug preservatives will be very useful to human and animals.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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HOW TO CITE THIS ARTICLE
Zacchaeus SO, Iyadunni AA, Johnson AA, Daubotei IU. Black Velvet Tamarind: Phytochemical Analysis, Antiradical and Antimicrobial Properties of the Seed Extract for Human Therapeutic and Health Benefits. J Phytopharmacol 2021; 10(4):249-255. doi: 10.31254/phyto.2021.10406