PHYTOCHEMICALS, ANTIOXIDANT ASSAY AND CYTOTOXICITY OF THE METHANOLIC LEAF EXTRACT OF AFRICAN FRANKINCENSE TREE, *Boswellia dalzielii*

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J. S. Ezekiel[1]*, Kadam Tadzabia[2] and Nuhu Gambo[1]
[1]Department of Chemistry, Umar Suleiman College of Education, Gashua, Yobe State, Nigeria
[2]Department of Integrated Science, Umar Suleiman College of Education, Gashua, Yobe State, Nigeria
*Corresponding author: nehemiaheze@gmail.com

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Abstract: *Boswellia dalzielii* Hutch (Burseraceae) is a tree species of the genus Boswellia found in Africa commonly called "frankincense tree". The use of the leaf extract by traditional healers for the management of both skin and breast cancer was strongly supported by traditional healers in northern Nigeria. Phytochemical screening, antioxidant assay and cytotoxicity of the methanolic leaf extract was conducted and results showed the presence of terpenoids, flavonoids, cardiac glycosides, tannins, carbohydrates and saponins. Antioxidant assay showed that the extract even at concentration as low as 62.5 µg/ml inhibited the activity of DPPH for 43.84%. Similarly, cytotoxity of the extract against Brine shrimp larvae was so acute that even at 1.0 µg/ml, mortality rate was 68.42%. These findings suggest that the crude methanolic extract of *B. dalzielii* is good for the isolation or preparation of anticancer drug and, this could be the reason why the plant has earlier been reported to be used traditionally in northern Nigeria for the treatment of skin and breast cancer among other ailments.

Keywords: Anticancer, antioxidant, cytotoxicity, frankincense, phytochemicals

Introduction

The search for novel chemicals from bioactive secondary metabolites having pharmacological and physiological properties of plant origin is on the increase. It is in this regard that *B. dalzielii* is considered for this research. *Boswellia dalzielii* Hutch (Burseraceae) is a tree species of the genus Boswellia found in Africa commonly called "frankincense tree" (Mbiantcha *et al*., 2017). Frankincense is a resin obtained from Boswellia species which has a history of use as medicine and of religious significance. There are reports that the same plant has been used traditionally for the management of diseases including cancer (Kafuti *et al*., 2018; Kafuti *et al*., 2019).

Several reports were made on *B. dalzielii* with regard to its chemical constituents and medicinal properties (Aliyu *et al*., 2007; Onariose, 2012; Mbiantcha *et al*., 2017; De Carlo *et al*., 2019). Leaf extracts have been reported to be highly lytic against red blood cells (Amlabu *et al*., 2018). Also, Aliyu *et al*. (2007) worked on the aqueous stem bark extract of the same plant on rat liver and it suggested that it has no damaging effect to hepatocytes as a result of the exposure to the plant extract. Extracts of the leaf, root and stem bark has also shown parasitemic suppression against tripanosoma brucei (Atawodi *et al*., 2011). In another study, the antioxidant activity of *B. dalzielii* showed similar potency with quercetol, a standard antioxidant drug and, the extracts also exhibited antibacterial activities against some tested bacteria (Anago *et al*., 2011)

Antioxidant capacity of *B. dalzielii* has been assayed and concluded that its use in ethno-medicine as anticancer was justified because of its strong antioxidant and anti-proliferative activities (Kafuti *et al*., 2019). Anti-cancer activities of medicinal plants were confirmed in some researches when both the anti-oxidant and cytotoxicity of a particular plant extract yielded positive (Bhatt, *et al*., 2016; Kohoude *et al*., 2016), hence, the anti-oxidant and cytotoxic assay of *B. dalzielii* is necessary if it's ethno-medicinal claims for being an anti-cancer is to be certain.

Materials and Methods

The following standard procedures were used for the analysis of *B. dalzielii* for the phytochemicals, antioxidant capacity and cytotoxicity, the plant parts were collected, identified and processed for the purpose of isolation of bioactive constituents.

Sample collection and extraction

The leaves of *B. dalzielii* were collected in May, 2017 in Gashua town of Yobe state, Nigeria and conveyed to the North east Arid Zone Development Programme (NEAZDP), Gashua where identification was duly made by a Ahmed Abdullahi. The leaves of the plant was collected, dried under shade for seven days and pulverised to coarse powder after which it was macerated in 80% methanol. The filtrate was evaporated in rotavap at 40^{o}C.

Phytochemical screening

Phytochemical screening was performed on the crude methanolic leaf extract using standard methods of Sofowora, 2008, Evans, 2009 and Tiwari, 2011 for the qualitative profiling of the extract.

Antioxidant assay

The presence of antioxidant phytochemicals in the leaf extracts of *B. dalzielii* was determined by their ability to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) stable free radical. The capacity of the extracts to scavenge DPPH was described by Marinova and Batchvarov (2011) and Soni and Sheetal (2013).

2 ml of 1 mg/ml solution of the extract in 95% methanol was mixed with 2 ml 0f 0.1 Mmol methanolic solution of DPPH. The test solution of 1.0 mg/ML (1000 µg/ml) was serially diluted to 500, 250, 125, 62.5 µg/ml. The mixtures were vortexed thoroughly for one minute at room temperature and incubated for 30 minutes in the dark. Finally, the absorbance of each of the mixtures was read at 517 nm on a spectrophotometer. The same experiment was conducted with a negative control or blank constituted of 0.1 Mmol DPPH in 95% methanol and also with a positive control constituted of 1 mg/ml of L-(+)-Ascorbic acid in 95% methanol. The antioxidant capacity of each sample was expressed in terms of percentage inhibition and was calculated as:

$$\%\ inhibition = \frac{Ab - Ac}{Ac}$$

Where: Ab is absorbance of blank or negative control; **Ac** is absorbance of extract or positive control

Cytotoxicity

In this technique, the Brine shrimp lethality assay (BSLA) was employed for the cytotoxicity test of the extract. Eggs of Brine shrimp were added to a hatching chamber containing sea water and, the chamber was kept under inflorescent lamp

for 48 hours for the eggs to be hatched into shrimp larvae; that is *nauplii* according to Tawaha (2006) and Olowa and Nuneza (2013). 20 mg of the methanolic extracts of *B. dalzielii* was dissolved in 2 ml of methanol to serve as stock solution 1. From the stock solution 1, 10-fold serial dilutions were prepared. All of the sample vials containing 0.5 ml of various sample concentrations were allowed to completely evaporate. Each sample was prepared in triplicate. To each of the evaporated sample vial, 4.5 ml sea water was introduced from pipette followed by a drop of DMSO to aid in solubilisation. Approximately ten free swimming *nauplii* were introduced into each vial from Pasteur pipette and the total volume of each vial was made 5 ml by adding drops of sea water. Each test sample had 4 serial concentrations of 1000, 100, 10 and 1 µg/ml. A control (blank) solution was made by taking some sea water and a drop of DMSO in a sample vial to make 5 ml followed by the addition of ten free swimming *nauplii*. All of these solutions containing *nauplii* were allowed to stand for 24 h after which the number of free swimming *nauplii* were counted and the percentage mortality were computed to give the final cytotoxicity of the test extracts.

Results and Discussion

Result of the phytochemical analysis, antioxidant assay and brine shrimp lethality assay of *B. dalzielii* was presented in tabular forms and in figure as seen in Tables 1, 2 and Fig. 1.

Hytochemical screening of the methanolic leaf extract of *B. dalzielii* Hutch revealed the presence of carbohydrates, tannins, cardiac glycosides, flavonoids, terpenoids and saponins. These are common plant phytochemicals that have shown different physiological and pharmacological properties in several researches. Mbiantcha *et al*. (2017) in a similar research reported the presence of all of the phytochemicals listed above including alkaloid which was not detected in the current work even though *B. dalzielii* leaf and similar procedures were employed.

Bioactive phytochemicals that were detected is presented in Table 1. The presence of flavonoids is a pointer that the extract could possess health benefits because they are implicated with biological activities such as antibacterial, anti-inflammatory, antioxidant, antihypertensive, antiviral, hepatoprotective, antimutagenic, (Chang and Kinghorn, 2006). Similarly, C. glycosides was detected and they are important natural product drugs whose actions include both beneficial and toxic. Glycosides have been used as poisons as well as heart tonic for at least since 1500 BC (Cardiac glycosides, 2015). Tannins was also detected and literature classified them into two categories: Condensed (phlobatannins) and hydrolysable tannins. Hydrolysable tannins inhibit the absorption of irons which may lead to anaemia, but phlobatannins do not inhibit iron absorption (Clinton, 2009).

Table 1: Results of the phytochemical screening of *B. dalzielii*

Phytochemicals									
Saponins	**Alkaloids**	**Carbohydrates**	**Anthraquinones**	**Phlobatannins**	**Tannins**	**Cardiac glycosides**	**Flavonoids**	**Steroids**	**Terpenoids**
+	-	+	-	-	+	+	+	-	+

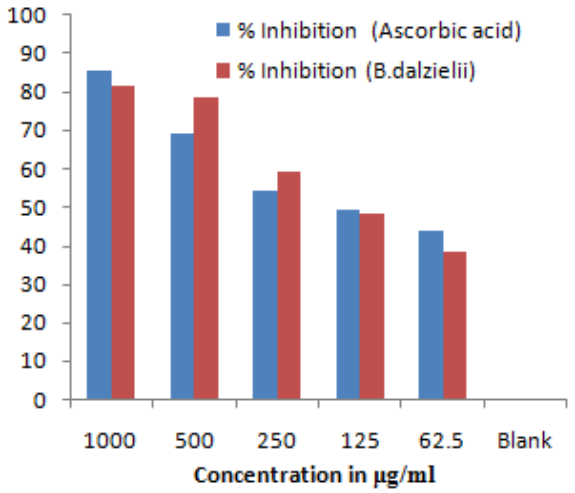

Fig. 1: Antioxidant assay of *B. dalzielii* and Ascorbic acid expressed as % inhibition

The antioxidant assay of *B. dalzielii* with DPPH (Fig. 1) revealed that it contains potential antioxidant compounds. At highest concentration of 1000 µg/ml, the percentage inhibition was 82.02% which compares with that of control drug, ascorbic acid (85.75%). When the experiment was conducted at lowest concentration (62.5 µg/ml), inhibition due to *B. dalzielii* extract was 43.84% whereas that of ascorbic acid was 38.38%. This finding shows that antioxidant activity of the test plant is higher at lesser concentration than that of the standard antioxidant, ascorbic acid. It also revealed that antioxidant capacity of the methanolic leaf extract increases with increase in concentration.

It could be extrapolated from Fig. 1 that the IC_{50} of *B. dalzielii* and that of the control drug, ascorbic acid is both less than one (IC_{50} ‹ 1.0 mg/ml). This result revealed that *B. dalzielii* crude methanolic leaf strongly inhibit the activity of DPPH stable free radical even at low concentration which is comparable to the activity of pure ascorbic acid. This finding is in agreement with that of Anago *et al*. (2011).

Strong antioxidant activity of plant extracts has been associated with anticancer candidacy (Pisoschi and Negulescu, 2011, Kafuti *et al*., 2018 and Kafuti *et al*., 2019). Anticancer agents have often been isolated from plant extracts which showed antioxidant properties by the inhibition of DPPH stable free radical. Therefore *B. dalzielii* has shown to contain antioxidant compounds and it justifies the Bade people's claim of the potency of the leaf aqueous extracts used as an anticancer of skin and breast (personal communication).

In the same vein, the cytotoxicity of the crude methanolic extract was determined by conducting the Brine shrimp lethality assay (BSLA) which showed a dose dependent activity. At the highest concentration of 1000 µg/ml, there was a 94.44% mortality whereas at the lowest concentration (1 µg/ml) it gave a percentage mortality of 68.42% (Table 2). The blank test also gave a percentage mortality of 12.50% which could have been as a result of the toxicity of the solvent (methanol) used.

Table 2: Brine shrimp lethality assay (BSLA) of *B. dalzielii*

Conc. of *B. dalzielii* extracts (µg/ml)	Number of *Nauplii* used	Number of Survived *Nauplii*	Total survivors	% Mortality
1000	7	1	1	94.44
1000	5	0		
1000	6	0		
100	6	0	5	75.00
100	7	1		
100	7	4		
10	5	3	5	72.22
10	6	0		
10	7	2		
1	7	2	6	68.42
1	6	2		
1	6	2		
0	6	6	21	12.50
0	10	8		
0	8	7		

Hence, the crude methanolic leaf extract of *B. dalzielii* is a good candidate for more research to isolate an anticancer agent. It is commonly reported that anticancer agents were isolated from plant extracts that exhibited both antioxidant against DPPH and cytotoxic to Brine shrimp larvae, *nauplii* (Ezekiel *et al*., 2016).

Conclusion

Six bioactive secondary metabolites were detected in the methanolic leaf extract of *Boswellia dalzielii* but, alkaloids were not detected as reported in some literature. The crude extract exhibited strong antioxidant activity on DPPH free radical as well as strong cytotoxicity on Brine shrimp larvae, *nauplii.* This finding suggests that the crude methanolic extract of *B. dalzielii* is a good candidate for the isolation or preparation of anticancer drug and, this could be the reason why the plant has earlier been reported to be used traditionally in northern Nigeria for the treatment of skin and breast cancer among other ailments.

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Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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