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IN VITRO ANTIOXIDANT ACTIVITIES OF CRUDE EXTRACTS AND TANNINS ISOLATEDFROM THE LEAVES *DALBERGIA HANCIE* BENTH

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Abstract: The potency of some species in *Dalbergia* family are yet to be documented due to lack of scientific evidence. This research investigated the antioxidant activities of crude extracts as well as the isolated tannins fromthe leaves of *Dalbergia hancie*. Hexane, ethyl acetate and methanol were used for the extraction and phytochemical analysis carried out on the crude extracts. Isolation of tannins was conducted by methods explained by Hagerman, 2002. The antioxidant analysis was conducted at different concentration of 500 µg, 250 µg, 125 µg, 62.5 µg and 31.5 µg using DPPH(2,2-diphenyl-1-picryhydrazyl).The phytochemical tests showed the presence of alkaloids, steroids, flavonoids tannins and saponins in all the solvents except in hexane which had no presence of alkaloids in the extract. The DPPH antioxidant analysis showed that the crudeisolated tannins had the highest scavenging activity of 62.26 % at 500 µg, 46.95 % at 250 µg, 37. 00% at 125 µg, 42.79 % at 62.5 µg and 33.97 % at 31.25 µg; followed by the methanol crude extract with 56.61 % at 500 µg, 39.52% at 250 µg, 38.93 % at 125 µg, 37.30 % at 62.5 µg and 30. 02 % at 31.25 µg.The ethyl acetate crude extract had 51.41 %

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scavenging activity at 500 µg, 44. 73 % at 250 µg, 37.59 % at 125 µg, 35. 66 % at 61.5 µg, 19.91 % at 31.25 µg; the n - hexane crude extract showed 41.75 % scavenging activity at 500 µg, 33.73 % at 250 µg, 29.27 % at 125 µg, 24.81 % at 61.5 µg and 2.53 % at 31. 25 µg. These results have shown that Dalbergia *hancie* leaves could be a natural source of antioxidants and could possess biomolecules that could be used to prevent oxidative degradation.

Key words: Phytochemical, anti - oxidant, Dalbergia *hancie*, DPPH, Scavenge

Introduction: *Dalbergia hancie*, locally called *Mgbehuhu* by some parts of South Eastern Nigeria, is a hedge shrub mostly used in fencing of barns and yards for the demarcation of boundaries by the people of Enugu State, South Eastern Nigeria. They are also used as rafters and poles in construction sites, walking sticks for the elderly and the wounded, drumsticks, arrowheads, pestles, traditional cups and plates owing to their high tensile strengths. (Lemmens, 2008).

Dalbergia hancie belongs to the family of Legumes which include a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fibre, fuel, fertilizers, timber, medicines, chemicals, and horticultural varieties (Lewis *et al*., 2005).

Oxidants are produced in biological systems and also encountered exogenously, these free radicals cause different degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing (Singh and Singh 2008). Antioxidants are the compounds, which fight the free radicals by prevailing at any one of the three major steps of the free radical mediated oxidative process; initiation, propagation and termination (Cui *et al*. 2004). These antioxidants are also produced by biological system and occur naturally in many foods.The balance between oxidants and antioxidants decides the health and vigour of an individual (Halliwell, 1996).

The DPPH procedure was designed by Blois (1958) with the aim to determine the antioxidant activity in a like manner by using a stable free radical α, α-diphenyl-β- picrylhydrazyl (DPPH; C18H12N5O6, M=394.33). The experiment measures scavenging capacity of antioxidants towards the free radical. The

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odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Sagar *et al*., 2010).

DPPH is regarded as a stable free radical because of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, like most other free radicals. The delocalisation of electron is responsible for the deep violet colour, with an absorption in ethanol solution at around 520 nm. When DPPH solution is mixed with a substance that can donate a hydrogen atom, it will give its reduced form with the loss of violet colour. Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Where ZH is the reduced form and A• is free radical produced in the first step. The latter radical will then undergo further reactions which control the overall stoichiometry (Sagar *et al*., 2010).

The use of DPPH is a rapid, simple, inexpensive and widely used method to measure the capacity of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It can also be used to quantify antioxidants in complex biological systems, for solid or liquid samples. This method is easy and applies to measure the overall antioxidant capacity (Prakash, 2001) and the free radical scavenging activity of fruit and vegetable juices (Sendra *et al*.; 2006). This assay has been successfully utilized for investigating antioxidant properties of wheat grain and bran, vegetables, conjugated linoleic acids, herbs, edible seed oils, and flours in several different solvent systems including ethanol, aqueous acetone, methanol, aqueous alcohol and benzene (Yull, 2001; Parry *et al*. 2005).

Materials and Methods

Collection of Materials

The fresh leaves of *Dalbergia hancie* were collected from a known herbalist, authenticated by the Botany Department and Deposited in the Herbarium, with voucher number 201A. The leaves were washed and dried under shade to prevent the loss or destruction of active components. The dried leaves were pulverised and stored in a glass container.

Extraction of the plant material

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The extraction of secondary metabolites from the leaves of *Dalbergia hencie* Benth was conducted using Soxhlet extraction. Three solvents with different degree of polarity namely, n-hexane, ethyl acetate and methanol were used.

Phytochemical Screening

Phytochemical identification was conducted for all the extracts using standard procedure. The phytoconstituents tested are alkaloids, steroids, flavonoid, saponins, tannins, glycoside, anthracene and resin.

Extraction of tanning crude:

The ground plant - leaves material, 224 g was macerated in 600 cm^3 of ethanol containing 10 mM ascorbic acid. It was stirred for 45 minutes and the extract containing lower molecular weight phenol was discarded. To the residue, 300 cm^3 of methanol containing 10 mM ascorbic acid was poured in and the solution was stirred for 45 minutes, and the crude containing tannin supernatant was saved. This was repeated three times, increasing the tannin supernatant. The phenolics crude extract was concentrate and filtered. A solution of 300 cm^3 acetic acid 0.05 M was added to the extract, yielding a cloudy orange solution. The methanol was completely removed by evaporation. The phenolic containing solution was extracted with 300 cm^3 of thioacetate in a separating funnel after shaken, the lower layer (aqueous layer) was saved (Hagerman, 2002).

Preparation of standard solution

Ascorbic acid 10 mM was prepared by dissolving 1.76 g ascorbic acid in 1000 cm^3 of absolute ethanol. The ascorbic, 10 mM was prepared by dissolving 1.76 g ascorbic acid in methanol. Acetic acid 0.05 M was prepared by mixing 2.85 cm^3 of glacial acetic acid with 800 cm^3 of distilled water, the pH was adjusted 4.00 by adding a solution of sodium hydroxide and the final volume was brought to 1000 cm^3.

ANTIOXIDANT ANALYSIS

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The free radical scavenging activity (antioxidant activity) was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) procedure. The extract 0.5 ml of different concentrations were pipette into a test tube in duplicate/ triplicate. Then 0.5 ml of the prepared DPPH was added. Then 4 ml of methanol was added to the mixture to bring the volume to 5 ml. Absorbance was read at 517 nm using spectrophotometerBarros *et al.* (2013). The extent of scavenging ability of DPPH radicals obtained by the extracts were compared to that of ascorbic acid.

The percentage DPPH scavenging activity was calculated using the formula:

$$\frac{Absorbanceblank - absorbancesample}{Absorbanceblank} \text{ X } \frac{100}{1}$$

Results and Discussion

Phytochemical analysis of the crude extracts of*Dalbergia hancie* Benth

Table 1: Phytochemical results for crude extract of *Dalbergia hancie* Benth

	n- hexane	Ethyl acetate	Methanol
Saponin	+	+	+
Glycoside	-	-	-
Tannins	+	+	+
Flavonoid:	+	+	+
Anthracene	-	-	-
Steroids:	-	+	+
Alkaloids:	-	+	+
Resin	-	-	-

+ = present, - = absent

Table 2 showed the result of the phytochemical studies conducted on the crude extra phytochemical analysis of n- hexane crude extract showed that saponin, tannin, flavonoi present. In the ethyl-acetate crude extract, saponin, tannins, flavonoid, steroid and alkaloi crude extract showed the presence of saponin, tannins, flavonoids, steroids and alkaloids.

The species of Dalbergia are known to possess phytoconstituents that are potent in treating

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flavonoids, saponins, reducing sugars, terpenoids, glycosides, and proteins, (Faiza *et al*.; 20 contains phenol, and flavonoids, and has been confirmed to possess antioxidant activity. T contains is flavonoids and other phenolics (Jairo *et al*.; 2020). Sagar and Upadhyaya conduc of D. sissoo. The methanolic extract showed the presence of Alkaloids, Carbohydrates, sa (Cardiac glycosides, anthraquinone glycoside and saponin glycosides) and steroids (S Phytochemical investigation of the ethanolic extract of D. sissoo indicated the presence of acids, phenolic compounds, and paranoids (Mohammad and Arun, 2011).

Antioxidant (DPPH) activity of crude and pure samples of tannins and saponins of *D. hancie* Benth.

Table 2: Antioxidant (DPPH) activity results of crude and pure samples of tannins and saponins of *D. hancie* Benth.

Sample	500µg	250 µg	125 µg	62.5 µg	31.25 µg
CP	0. 254±0.001	0.357±0.001	0.424±0.001	0.385±0.052	0.459±0.001
CH	0.392±0.002	0.446±0.034	0.473±0.003	0.506±0.002	0.656±0.001
CE	0.327±0.001	0.372±0.002	0.420±0.001	0.4330.003	0.539±0.001
CM	0.292±0.002	0.407±0.001	0.411±0.001	0.422±0.003	0.471±0.003
Ascorbic acid	0.128±0.002	0.158±0.005	0.201±0.005	0.215±0.005	0.321±0.004

Description of samples: CP = crude phenol extract, CH = crude hexane extract, CE = crude ethyl acetate extract, CM = crude methanol extract.

In table 2 above, the extracts showed increase in the absorbance values and the concentration of the extracts decreases. The crude phenolic extract showed the least absorbance for among other extract at all concentration apart from 125 µg where its concentration is above that of crude ethyl acetate and methanol extracts. The absorbance of crude phenol and crude methanol increased from 500 µg to 250 µg and 125 µg, decreased at 61.5 µg and then increased again. the absorbance of crude n - hexane and ethyl acetate increase all through as concentration decreases. Ascorbic acid (the standard) has the least absorbance at all concentration.The DPPH has a nitrogen atom with a pair of electrons which is delocalised and it is responsible for the oxidative property of DPPH.

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Antioxidants releases protons which consume these electrons and quenches the damaging effect of the compound.

Table 3: The percentage antioxidant (DPPH) scavenging activity result of crude and pure samples of tannins and saponins of*Dalbergia hancie* Benth.

Sample	500 µg	250 µg	125 µg	62.5 µg	31.25 µg
CP	62.26	46.95	37.00	42.79	33.97
CH	41.75	33.73	29.72	24.81	2.53
CE	51.41	44.73	37.59	35.66	19.91
CM	56.61	39.52	38.93	37.30	30.02
Ascorbic acid	80.98	76.52	70.13	69.54	53.79

Theresult of Antioxidant DPPH analysis has shown that samples of *Dalbergia hancie* have the ability to scavenge free radicals. The crude phenolic has a very high scavenging activity compared to other crude extracts and a moderate activity when equated to the standard used (ascorbic acid).Crude phenolics,ethyl acetate and crude methanolic extractsshowed scavenging activity above 50 % at 500 µg. The polar solvents had shown be good in extracting phytochemicals which can supress the activities of free radical. these phytochemicals include; flavonoids, is flavonoids, tannins, saponins, and steroids. The crude n - hexane extract showed scavenging activity above 40 % at 500 µ. The Dalbergia species are known for their medicinal power in local medicine. Bark of Dalbergia sissoo can be used as anti- inflammatory and as anti- oxidant. (Kumari and Kakkar, 2008).In DPPH free radical searching movement was carried on distinctive stem concentrate of *D. sissoo* like petroleum ether, chloroform, and methanol. Out of all different stem concentrate chloroform concentrate having astonishing result in all models in different measurements where as petroleum ether and methanol concentrate having moderate activity (Kaur, 2011). Jairo and his co - workers proved that the leaves of *D. ecastaphyllum*had better antioxidant capacity in the tests by the DPPH method and β-carotene bleaching. There were 49 chemical compounds, of which 38 belonged to the class of flavonoids. The results they got indicate that stems and leaves of D. *ecastaphyllum* have biological properties. Leaves particularly are better for functional food formulation and as natural antioxidant (Jairo*et a*l., 2020). Hajare and co - workers reported that D.

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sissoo has a better anti-oxidant activity than regularly used anti-oxidants like Selenium and vitamin E (Hajare *et al.;* 2001).

Conclusion: The phytochemicals present in *Dalbergia hancie* include, saponin, saponin glycoside, alkaloids, flavonoids, tannins, hydrolysable tannins and steroids. The treatment from antioxidant analysishas shown that the phenolic crudeis effective in combating free radical. Phenols has been shown to be present in other Dalbergia species and that they are responsible for the medicinal activities of the family.

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