Comparative phytochemical and antioxidant studies of the hexane and ethyl acetate fractions of the methanol extracts of the stem bark of Allophylus africanus

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Abstract

This research is aimed at comparing the phytoconstituents and antioxidant activities of the methanol extract, hexane and ethyl acetate fractions of the stem bark of *Allophylus africanus* Beauv. for development into possible phytopharmaceuticals. About 500 g dry weight of the stem bark sample was macerated for a total of 48 h using 5 L methanol to obtain the methanol extract. The extract was fractionated into hexane and ethyl acetate and each fraction monitored using Thin Layer Chromatography (TLC). The extract and fractions were also subjected to phytochemical screening. Using the DPPH radical scavenging model, the antioxidant studies of the extract and fractions were carried out at concentrations between 1 mg and 0.00625 mg/mL. Alkaloids, carbohydrates and terpenoids were found present in all three samples while anthraquinone, tannins, phenols and steroids were not detected in all three samples of the stem bark. The crude methanol extracts and the hexane fractions of the stem bark of *Allophylus africanus* showed no antioxidant activity when compared to Vitamin C using the DPPH free radical scavenging activity model. However, at concentrations between 1 mg/mL and 0.0625 mg/mL a significant and dose-dependent antioxidant activity was observed with the ethyl acetate fraction of the stem bark. The enrichment of the ethyl acetate fraction with alkaloids, terpenoids and saponins, which possess anti-oxidant activity may be a plausible explanation for the observed anti-oxidant activity. The ethyl acetate fraction of the stem bark can thus be further investigated as a possible antioxidant phytopharmaceutical for the management of oxidative stress.

Introduction

Metabolic processes in the human body have been associated with the generation of free radicals such as reactive oxygen species and

reactive nitrogen species, which can cause damage to the DNA, proteins and lipids at high levels resulting in oxidative stress. Free radicals from the environment are also threats to man (Engwa, 2018). The ability of antioxidants to mop up free radicals in biological systems thereby reducing oxidative stress has well been established (Kaurinovic and Vastag, 2019; Jamova et al., 2023).

Antioxidants delay or inhibit the oxidation of molecules through free radical scavenging, quenching of singlet oxygen, reduction of peroxides, enzyme inhibition or even regeneration of other antioxidants (Nimse and Pal, 2015). Antioxidants play a major role in the management of oxidative stress and inflammation-related cases such as cataract (Kaur et al.; 2012), liver damages (Zhang et al.; 2007), Rheumatoid arthritis (Yadav et al.; 2023), cancers such as breast cancer (Coughlin, 2018) as well as cardiovascular diseases (D'Oria et al.; 2020, Chaudhary et al.; 2023) etc. Although various synthetic antioxidants are in use, there has been a growing global concern about their safety (Uzombah, 2021). This has promoted the preference and hence search and use of more natural antioxidants, especially from plants, in the prevention and management of diseases associated with oxidative stress. These natural antioxidants work through diverse mechanisms and are associated with very minimal or no side effects. A remarkable number of plant-based natural antioxidants have long been identified, and these include polyphenols, carotenoids, vitamins C and E.

Allophylus africanus, also known as the African false currant is a shrub-like tree, which may grow up to 10 m tall. Its flowers could be white, cream, yellow or green in colour, producing red edible fruits. It is often found in the rich and well drained forests of Africa. In different tribes of Nigeria, *Allophylus africanus* is known as 'ebe/ukpe' (Esan), 'akanro'/'akaraesu' (Yoruba) (Sofidiya et al.; 2012), 'akaito' (Igbo) and 'karki' (Hausa) (Mohammed et al, 2020)

The stem bark of *Allophylus africanus* have been used in various parts of Africa as antimalarial, vermicidal, antiarthritic and antidiarrheal agents (Ferreres, 2018, Ribeiro et al.; 2023). Its crude methanol extract in previous reports showed very good anti-bacterial (Sofidiya et al.; 2012), analgesic and anti-inflammatory activities. Using already established *in vivo* models, the analgesic and anti-inflammatory activities gave results which were comparable to that of Ibuprofen (Mohammed et al.; 2020; Ferreres et al.; 2018). The stem bark was also shown to exhibit some anticancer activity against certain human stomach cancer cells (Ribeiro

et al.; 2023) and possible anti-inflammatory activities.

Phytochemical studies on the whole *Allophylus africanus* plant led to isolation of four new compounds: alloeudesmenol, hanocokinoside, allotaraxerolide and alloaminoacetaldeyde as well as two known compounds: stigmastane-3β,4β-diol, a phytosterol and pinitol, a cyclic polyol. (Oladosu et al.; 2015). Stigmastane sterols often display good antioxidant, anti-inflammatory and anticancer activities (Okoye et al.; 2010, Dube et al., 2023). HPLC-DAD profiling of the hydroalcoholic extract of *Allophylus africanus* has also led to the detection of some anticancer apigenin glycosides viz: vicenin-2 and apigenin-6-C-hexoside-8-C-pentoside; apigenin-7-O-hexiside-8-C-hexoside; apigenin-8-C-(2-rhamnosyl)hexoside and apigenin-6-C-(2-rhamnosyl) hexoside (Ribeiro, et al.; 2023).

In this preliminary research report, we aim at comparing the phytoconstituents and antioxidant activities of the methanol extracts as well as the hexane and ethyl acetate fractions of the stem bark of *Allophylus africanus* Beauv. in order to optimize the phytoconstituents for use as possible development into phytopharmaceuticals. Phytopharmaceuticals refer to plant derived compounds that have pharmacological activities (Mtewa et al., 2021). They are often a group of phytochemicals such as solvent fractions containing a few compounds but which show some biological activities.

Materials and methods

Sample collection and preparation

Healthy stem bark samples of *Allophylus africanus* were collected from Mbaukwu in Anambra State of Nigeria. The samples were identified by a taxonomist and compared with a sample deposited in the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka with voucher number (PCG/UNN/0112). The sample was rinsed with distilled water, air-dried under a shade after which it was pulverized into fine powder. The dried and pulverized sample was stored in a cool place in dry sample containers until when needed.

Extraction and fractionation

About 500 g of the dry weight of the stem bark sample was macerated for 24 h using methanol after which the extract was decanted and subsequently filtered off using Whatman No. 1 filter paper. This process was repeated for another 24 h using a total of 5 L methanol and thereby giving rise to the methanol extract (ME). Using hexane, and subsequently,

ethyl acetate, the methanol extract was fractionated to obtain the corresponding fractions. Each fraction was monitored with Thin Layer Chromatography (TLC) using precoated aluminum plates.

Phytochemical screening

The phytochemical screening of the crude methanol extract as well as the hexane and ethyl acetate fractions of the stem bark were conducted as previously reported (Shaikh and Patil, 2020).

Antioxidant screening

The antioxidant screening of the methanol extract as well as the hexane and ethyl acetate fractions were carried out using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging model as previously described (Okoye and Okoye, 2016a; 2016b). Different concentrations (1 mg/mL, 0.5 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.00625 mg/mL) each, of the methanol extracts and the hexane and ethyl acetate fractions of the stem bark of *Allophylus africanus* were prepared. One mL of 0.1 mM DPPH solution in ethanol was added to 2.5 mL of the extract and allowed to incubate in the dark for 30 min at room temperature after which a UV absorbance reading was taken at 517 nm. The same treatment was given to vitamin C. The antioxidant activity was thereafter calculated using the following formula:

$$\%DPPH\ Radical\ scavenging\ activity = \frac{Abs0 - Abs1}{Abs0}\ x\ 100$$

where Abs_0 = absorbance of the control (DPPH + methanol); Abs_1 = absorbance of the extract/fractions/Vit. C solutions)

Results and discussion

The results of the phytochemical screening in Table 1 below, show the presence of alkaloids, carbohydrates and terpenoids in the crude methanol extract and similarly in the hexane and ethyl acetate fractions.

Table 1. Phytochemical composition of the crude methanol extract, hexane and ethyl acetate fractions of *Allophylus africanus* stem bark

S/N	Secondary metabolite	Crude methanol extract	Hexane fraction	Ethyl acetate fraction
1	Alkaloids	++	+	+
2	Anthraquinones	-	-	-
3	Terpenoids	++	++	++
4	Steroids	-	-	-
5	Carbohydrates	++	+	+
6	Saponins	++	-	+
7	Tannins	-	-	-

8	Flavonoids	++	-	-
9	Glycosides	+	-	-
10	Proteins	-	-	+++
11	Phenols	-	-	-

According to reports, certain alkaloids such as isoquinolines etc. display significant anti-inflammatory activities by inhibiting the production of pro-inflammatory mediators such as cytokines, prostaglandins and leukotrienes (Souto et al., 2011) while terpene compounds can act as direct antioxidants by scavenging free radicals and as indirect antioxidants by enhancing the human body's own antioxidant defences. In so doing, they contribute significantly to the management of oxidative stress (Gonzalez-Burgos and Gomez-Serranillos, 2012).

Furthermore, saponins which were present in the crude methanol sample were found appearing in the ethyl acetate but not in the hexane fraction, while both flavonoids and glycosides which was found in the crude methanol extract were absent in both the hexane and ethyl acetate fractions suggesting that a more polar fraction like the butanol fraction may contain the flavonoid glycosides. This is still in sync with previous reports of apigenin diglycosides from the stem bark extracts of *A. africanus* (Ferreres et al., 2018, Ribeiro et al.; 2023). In a similar independent work, apigenins have been confirmed to possess significant antioxidant activity among other pharmacological activities (Chen et al., 2023). Both saponins and flavonoid glycosides have also been widely reported to show antioxidant, anti-inflammatory, antibacterial, neuroprotective and anticancer activities etc. (Kumar and Pandey, 2013; Okoye and Okoye, 2016a; Ullah et al.; 2020, Dias et al., 2021, Zhong et al., 2022). Additionally, certain saponins are linked to inflammatory diseases and cancer due to their ability to act directly on proinflammatory cytokines (eg. Tumor necrosis factor alpha (TNF-α), inducible nitrogen oxide synthase (iNOS) and interleukin-6 (IL-6), (Khan et al.; 2022). Ullah et al, (2020) earlier suggested a link between saponins and flavonoid glycosides; and the prevention as well as the treatment of certain degenerative diseases such as diabetes mellitus, cancer and arthritis, possibly due to its ability to scavenge free radicals in biological systems.

Other phytochemicals which were not detectable in the methanol extracts, hexane and ethyl acetate fractions of *A. africanus* include anthraquinone, tannins, phenols and

steroids. They may, however, be present in the stem bark, but in very low concentrations.

The results of the antioxidant activity of the crude methanol extract, hexane and ethyl acetate fractions of the stem bark of *Allophylus africanus* are shown in Table 2 below.

Table 2. DPPH radical scavenging test results of the crude methanol extract, hexane and ethyl acetate fractions of *Allophylus africanus* stem bark

S/N	Concentration	Crude Methanol extract	Hexane fraction	Ethyl acetate fraction	Vit C
1	1 mg/mL	1.97	-13.06	71.4	61.00
2	0.5 mg/mL	5.99	-7.07	64.24	67.00
3	0.25 mg/mL	-0.63	-1.52	44.23	79.34
4	0.125 mg/mL	-8.41	-2.50	37.38	79.70
5	0.0625 mg/mL	4.651	-0.09	28.26	88.19

The methanol extract showed very poor antioxidant activity while the hexane fraction did not show any antioxidant activity at concentrations between 1 mg/mL and 0.0625 mg/mL when compared to Vitamin C using the DPPH free radical scavenging activity model. However, at these same concentrations, a significant and dose-dependent antioxidant activity was observed with the ethyl acetate fraction of the methanol extract of the stem bark. It is important to note that although the crude methanol extract showed very poor antioxidant activity, the ethyl acetate fraction showed a dose dependent antioxidant activity. The plausible explanation is the possible enrichment of this fraction with phytochemicals that have been shown to exhibit strong antioxidant activity. This projects the ethyl acetate fraction of the stem bark as the antioxidant fraction, a potential active ingredient for herbal based antioxidant products.

While it is noble to trace down pharmacological activities to pure compounds which may serve as lead drug molecules, the incidences of certain detected compounds in plant extracts losing their numerous associated pharmacological activities when isolated and re-tested is becoming common (Newman 2021, Nasim et al.; 2022). This notion reinforces the argument for phytopharmaceuticals. Herbal-based phytopharmaceuticals from different solvent fractions of *Allophylus africanus* stem bark could be achieved from solvent fractions with established phytochemical profile linked to pharmacological activities.

In general, the observed antioxidant activity in the ethyl acetate fraction of the stem bark may be attributed to the presence of alkaloids, terpenoids and saponins which were found present in the samples.

Conclusion

The methanol extract, the hexane and the ethyl acetate fractions of the stem bark of *Allophylus africanus* have all been found to contain alkaloids and terpenoids which often show antioxidant and anti-inflammatory activities. The Comparative research on the pharmacological and antioxidant activities of fractions of *Allophylus africanus* stem bark has not been previously compiled and this could assist in the optimization of phytopharmaceuticals for the management of various ailments. The results so far, could also form a basis for some targeted isolation of secondary metabolites from the fractions where necessary. These preliminary results presented in this report have not only corroborated the use of *Allophylus africanus* stem bark in herbal medicine for the managements of various degenerative diseases but has also positioned the ethyl acetate fraction of the stem bark to the frontline for further investigation as a possible antioxidant phytopharmaceutical.

Conflict of Interest

The authors declare no conflict of interest.

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