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Profiling Antinutritional and Antioxidant Activities of Bitter Leaf (*Vernonia amygdalina*) Flower

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Abstract
The antinutritional and antioxidant profiling of the floral parts of the bitter leaf was carried out. The collected floral parts of the bitter leaf were air-dried, pulverized and macerated using absolute ethanol. The concentrated form of the extract (EE_F) was subjected to both qualitative and quantitative anti-nutritional profiling using standard procedures. The anti-oxidant profiling of EE_F was established using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion-reducing antioxidant potential (FRAP) methods. The result of the qualitative analysis indicated the presence of alkaloids, flavonoids, phenols, steroids (terpenoids), quinones, coumarins, anthocyanins, tannins and saponins while quantitatively, alkaloids (1.56±0.02 mg of atropine equivalent, AE), flavonoids (25.278±0.555 mg of quercetin equivalent, QE), total phenolics (14.457±0.231 mg of gallic acid equivalent, GAE), tannins (5.585±0.026 mg of GAE) and saponins (1.27±0.03) % in the floral part of bitter leaf. The antioxidant activity of EE_F increases as its concentration increases with a corresponding increase in concentration from 25 µg/ml to 400 µg/ml. The ferric ion reducing potential of EE_F was observed at 400 µg to have 198.257±0.002 mg ascorbic acid, AAE. The floral part of the bitter leaf is rich in anti-nutrients and anti-oxidant activity.

Keywords: Floral part, Bitter leaf, Antinutrient, Antioxidant, Profiling

Introduction

The treatment of various diseases is over-stretched by existing therapeutical substances. There is a need to explore complementary or alternative phytomedicines with the plant kingdom providing buckets of anti-nutrients. Anti-nutrients have played a significant role in phytomedicines from the isolated morphine from *Papaver somniferum*, an alkaloid, used as an analgesic (Reiss et al., 2022); thymoquinone, a quinone, used as an anti-malarial, anti-inflammation and anti-proliferative agent (Patel et al., 2021); paclitaxel, a terpenoid used as an anti-neoplastic agent (Zhu & Chen, 2019) to the rich dietary classes of flavonoids usually sourced from fruits, nuts, seeds and vegetables, notable for series of activities such as anti-cancer, anti-microbial, anti-inflammation and cardio-protective (Chen et al., 2018; Devi et al., 2015).These anti-nutrients can be sourced from the aerial and floral parts of plants (Yadav & Agarwala, 2011). Anti-nutrient constituents such as alkaloids, steroids, tannins, flavonoids, and polyphenolics are essential for the general well-being of mankind. Thus, there is a need to further analyse plant tissues to establish their therapeutic efficacies in the light of over-stretched existing therapeutical substances and also given the challenging effect of multidrug resistance (MDR) being faced in the clinical world.

Plants such as guava (*Psidium guajava*) have been employed in the treatment of toothache, cough, sore throat and inflamed gums utilizing the antimicrobial activity of its leaves (Adamu, 2021) and Neem (*Azadirachta indica*) leaves in the treatment of skin infections, dental pains and fever (Altayb et al., 2022). Bitter leaf (*Vernonia amygdalina*) plant has been used as alternative therapy in the treatment of cancer, helminthic, protozoal and bacterial-related infections. The therapeutic activities of these plants are enhanced by the presence of phytochemicals such as saponins, alkaloids,

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terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones and edotiles (Farombi & Owoeye, 2011). Thus, the present study is centred on the anti-nutrient of the floral part of bitter leaf.

Materials and Methods

The floral parts of bitter leaf were collected from the Atai Obio Offor community in Uyo Local Government Area of Akwa Ibom State with the complete parts intact. The floral material was air-dried at room temperature, ground and then subjected to extraction using maceration. Ground-dried floral parts (500 g) were macerated in an air-tight container using absolute ethanol as the extracting solvent and then concentrated under pressure using a rotary evaporator (R-250 Pro). The concentrated crude ethanolic extract (EE_F) was stored under vacuum with the aid of a vacuum desiccator.

EE_F was subjected to phytochemical screening to establish the various classes of anti-nutrients present in the floral part of the bitter leaf. The methods described by Gul et al. (2017) were adopted for anti-nutrient profiling. To 2 ml EE_F was added a few drops of Hager's reagent (saturated solution of picric acid), a yellow colouration was an indication of alkaloids. To 1 ml EE_F was added 10% lead acetate ($Pb(OAc)_4$, a yellow colouration was an indication of flavonoids.5 drops of iron (iii) chloride were added to EE_F a yellow-green precipitate was an indicator of phenols. To 5 ml EE_F was added 2 ml $CHCl_3$ and 3 ml concentrated H_2SO_4, appearance of a reddish-brown ring was an indication of steroids (terpenoids). 1 ml EE_F was added to 1 ml concentrated H_2SO_4, formation of a red colouration was an indication of quinones.

To 2 ml EE_F, was added 3 ml 10% sodium hydroxide, and yellow precipitation was an indication of the presence of coumarins.To 2 ml EE_F added 2 ml of 2M HCl, a pinkish-red to bluish-violet colouration was an indicator of the presence of anthocyanins.To EE_F was added 10 ml bromine water. The decolourization of bromine water was an indication of the presence of tannins.To 200 mg EE_F was added 5.0 ml of distilled water in a test tube. The mixture was mixed thoroughly and then a few drops of olive oil were shaken further. The appearance of foam showed the presence of saponins.Quantitative anti-nutritional analysis was carried out on selected vital antinutrients. The anti-nutrients include: alkaloids (mg/g AE), flavonoids (mg/g QE) phenolics (mg/g GAE), tannins (mg/g GAE) and saponins (%) using the methods(Mythili et al., 2014; Harborne, 1973; Obadoni & Ochuko, 2001).

The anti-oxidant profiling of EE_F was done by exploring the 2, 2-diphenyl-1-picrylhydrazyl, (DPPH) and ferric-reducing antioxidant potential (FRAP) standard procedures. The antioxidant profiling of EE_F using the DPPH method described by Odokwo and Salawu (2021), and Uzoekwe and Odokwo (2023) in a dose range concentration between 25.00 µg/ml to 400.00 µg/ml, using ascorbic acid as standard and read at 517 nm (UV-Visible spectrophotometer (Labomed spectro UV-2505). The percentage inhibition, *PI* was calculated using equation 1:

$$PI = \frac{A_{BLANK} - A_{\mathrm{EE_F}}}{A_{BLANK}} \qquad (1)$$

Where: A_{BLANK} – absorbance of the blank,
$A_{\mathrm{EE_F}}$ – absorbance of EE_F

Fe-reducing antioxidant potential of EE_F was assessed by the method described by Gohari et al. (2011). The absorbance was read at 700 nm and the antioxidant potential was calculated and expressed as mg of ascorbic acid equivalent using equation 2:

$$\text{Fe Reducing potential} = \frac{A_{\mathrm{EE_F}} \times C_{std}}{A_{std} \times EE_{F_{stk}}} \qquad (2)$$

Where: $A_{\mathrm{EE_F}}$- absorbance of EE_F
C_{std}- concentration of standard
A_{std}- absorbance of standard
$EE_{F_{stk}}$- sample stock

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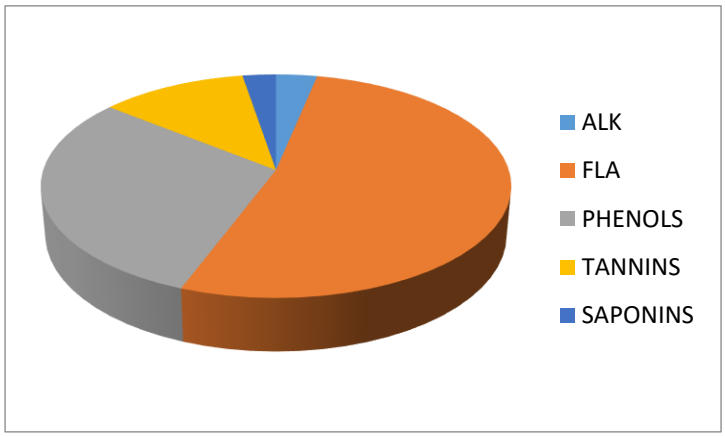
Data obtained were expressed as mean of triplicates determinations ± standard deviation (SD). The Statistical Package for Social Scientists (SPSS version 20.0) was used for all data analysis.

Results

The results of the qualitative and quantitative distribution of the antinutrient profiling are presented in Table 1 and figure 1 respectively. The antioxidant profiling was presented in Figure 2 and table 2.

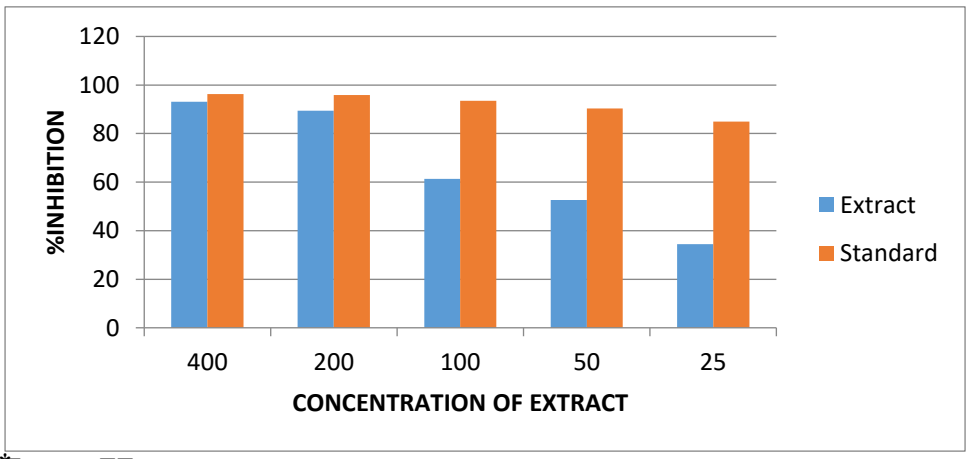
Table 1. Qualitative antinutritional profiling of EE_F

S/N.	Antinutrient	Present	Absent
	Alkaloids	√	
	Flavonoids	√	
	Phenols	√	
	Steriods/Terpenoids	√	
	Quinones	√	
	Coumarins	√	
	Anthocyanins		√
	Tanins	√	
	Saponins	√	



*ALK-alkaloids, FLA-flavonoids

Fig. 1. Quantitative distribution of antinutrients in EE_F



*Extract-EE_F

Fig. 2. DPPH antioxidant profiling of EE_F

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Table 2. FRAP (mg AAE/µg)

Sample	1	2	3	Mean	SD	Mean±SD
EE_F	198.252	198.256	198.254	198.254	0.002	198.257±0.002

Discussion

The qualitative profiling has revealed the presence of vital antinutrients in the floral part of the bitter leaf plant. Classes of antinutrients such as the alkaloids, flavonoids, phenols, steroids, terpenoids, coumarins, tannins and saponins have been reported to be present in the leaves (Farombi & Owoeye, 2011). However, quinones have been identified in the present study to be present in the floral part of bitter leaf. Anthocyanins were not detected in the ethanolic extract of the floral part under the present study. Antinutrients such as the alkaloids, tannins, saponins and glycosides are responsible for the bitter taste of bitter leaf (Bonsi et al., 1995), which implies the presence of these antinutrients could be responsible for any bitter taste associated with the floral part. This could also serve as alternative to hop in beer production. Medicinally, the floral part is also efficacy due to the presence of these sets of antinutrients. Alkaloids are known therapeutical substances with amino chemical characteristics and have been widely used in the pharmaceutical industry. Substances such as quinine (anti-malarial), morphine (analgesic), emetine (amebiasis, SARS-COV-2) and piperine (anti-cancer) are all important alkaloids pharmacological relevance. The floral part of the bitter leaf was richer in both flavonoids and phenols quantitatively. This makes the floral part an excellent tissue for antioxidant activity. Flavonoids are known polyhydroxylated polyphenols with unique free radical scavenging efficacy. The presence of flavonoids is a basis for the possible exploration of the floral part of the bitter leaf in the treatment and management of health-related issues such as diabetes, inflammation, cancer and cardiac arrest. The synergistic effect of the various antinutrients present in the floral part should be worth considering in alternative phytomedicines.

The antioxidant profiling of the floral part of bitter leaf has shown that the floral part has high antioxidant activity. The higher the concentration of the ethanolic extract the higher its antioxidant activity. This is further justified by the presence of higher amount of flavonoids and phenols in the floral part of the bitter leaf. The ferric ion reducing the potential of the floral part of bitter leaf was observed at 400 µg to have 198.257±0.002 mg AAE. This shows that the floral part of bitter leaf can reduce the oxidative effect of ferric ions by donating electrons in an electron transferred reaction.

Conclusion

The floral part of bitter leaf is rich in antinutrients that are of medicinal and pharmaceutical importance. It also has an excellent antioxidant profiling which is a basis for any therapeutical application.

Recommendations

1. Toxicological studies should be carried out so as to ascertain the safety limit of the floral part of bitter leaf.
2. Isolation characterization of individual antinutrient should as well be examined for further studies.

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