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Research article

Antioxidant and anti-inflammatory properties of "Limolanii" grass and perceptions of locals on its survival in the era of changing climate

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ABSTRACT

The aromatic Spear grass *Hyparrhena rufa* (locally called "Limolanii") found in the Saboba District of the Northern Region of Ghana is ingeniously used for dietary, medicinal, and other purposes. Focus group discussions were conducted in communities where "Limolanii" grows to assess the local perception of the importance of the grass and changing climate impacts on its continued existence. Findings indicated that the plant is of immense importance to the locals but has not been validated scientifically and reported for its current use. The communities are also hopeful of future socio-economic benefits of "Limolanii" but expressed concern about the lack of remediation practices to address issues of climate change, increased use of agrochemicals, urbanization, etc., which can lead to the extinction of the grass. "Limolanii" was therefore evaluated for its nutritional and anti-nutritional content by proximate analysis and ethanolic extract evaluated for some medicinal properties. The extract was assessed for its anti-inflammatory property using the carrageenan-induced oedema in chicks' model while antioxidant property evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, Phosphomolybdenum (Total Antioxidant Capacity), Total Phenol (Folin-Ciocalteu), and Total Flavonoid Content (Aluminium-chloride colorimetric) assays. The plant showed good nutritional content, extract exhibited a dose-dependent inhibition of oedema with maximal percentage inhibition of 41.05% at 300 mg/kg body weight and noticeable antioxidant activities. Flavonoids, coumarins, and other phytochemicals detected in the plant could be responsible for these activities, however, at certain levels, these phytochemicals could act as anti-nutrients. "Limolanii" exhibits medicinal properties backing its use traditionally as food supplements and herbs, hence the need to explore its possible commercial cultivation and embark on community sensitization to encourage people to protect and expand its production.

1. Introduction

It is on record that inflammation and oxidative stress is associated with almost all diseases [1]. Conventional anti-inflammatory agents encompassing steroidal and non-steroidal anti-inflammatory drugs, which have shown clinical efficacy for treating inflammatory diseases, have unwanted side effects such as gastrointestinal perforations, bleeding, and Cushing's syndrome [2]. This issue often limits their use and affects patient compliance. Also, foods containing synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) have been reported to have mutagenic and carcinogenic effects when ingested [3]. These health challenges posed by these synthetic antioxidants has created the need to explore safer and healthier alternatives including natural products with antioxidant and anti-inflammation properties [4]. A great deal of medicinal flora and fauna used in folklore medicine presents an enviable repository of untapped drug leads.

One such plant is *Hyparrhena rufa* (spear grass), of the family Poaceae, locally known as 'Limolanii'. "Limolanii" is a grass predominant in Saboba District in the Northern region of Ghana. The grass has an aromatic flavor when fresh and a minty smell when dry. It is used by the local people as a

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spice for cooking meat, treating fever and snakebites and for preserving corpses. Plants used for foods have the recognized potential in preventing malnutrition and diet-related disorders and are also known to be a good source of essential nutrients (micro and macro) for the growth of an individual. Natural products are reported to have therapeutic outcomes: antimicrobial, antioxidant, anti-inflammatory, and analgesic effects which are attributed to the diverse biologically active substances (phytochemicals) they synthesize and contain [5, 6]. However, some biologically active substances (anti-nutrients) could be potentially harmful since they slow down the growth and healing process by hindering nutrient availability to the body, especially, if present in elevated levels. Anti-nutrients like alkaloids, oxalates, tannins, phytates, saponins, etc. could have detrimental impact on health by obstructing protein digestion, and iron and zinc absorption [7, 8]. Scientific report on the nutritional content, anti-nutrients, antioxidant, and anti-inflammatory properties of this indigenous grass of some communities in the northern part of Ghana is not widespread.

"Limolanii" is a perennial grass but grows as an annual grass because of the unimodal rainfall pattern in the study area. The grass commonly grows at the fringes of communities and therefore affected by human activities, thus has disappeared in some of the communities where it used to exist. Beside its use as forage for livestock just as other grass species, "Limolanii" is widely used traditionally for a range of other purposes including food supplements and herbs compared with other grass species in the study area. However, studies on the grasses in the area have mainly focused on their forage used by livestock other than human use [9].

There are reports on the nutritive and anti-nutritive potentials of *cymbopopgon citratus* (lemon grass; family - Poaceae) leaves being effective in the management of fever and infections, headaches, and rheumatic pain, and its ability to act as a sedative, antispasmodic, analgesic, antioxidant, anti-inflammatory, and antihypertensive agent [10]. Plants belonging to the Poaceae family are reported to be under-utilized worldwide for medicinal purposes mainly due to their alkaloid-poor content, however, there are reports of use of *Bambusa vulgaris*, *Sorghum bicolor* and *Saccharum officinarium* for medicinal purposes based on an ethnobotanical survey [10]. This study therefore aimed at investigating the nutritional and anti-nutritional content of "Limolanii" grass (*Hyparrhena rufa)* and to exploring its antioxidant and anti-inflammatory potential as suggested by folklore medicine. The study further sought the perceptions of local users on the effects of changing climate on the existence of the plant.

2. Materials and methods

2.1. Overview of the study area

The Saboba District is one of the sixteen (16) districts in the north-eastern corridor of the Northern Region of Ghana. It lies between Latitudes 24° and 25° North, Longitudes 27° and 13° East, covering an area of 1,751.2 km^2 [11] (Figure 1).

The district is bordered by Chereponi to the north, Tatale Sanguli to the south, Yendi Municipal and Gushegu Municipal to the west and Ghana-Togo international boundary to the east where River Oti serves as the international boundary between the two countries (Ghana and the Republic of Togo). Saboba is the district administrative capital. The district is characterised by two distinct seasons - the dry season between November and April/May and the uni-modal rainfall season between April/May and October/November. The annual rainfall in the district ranges between 750 mm and 1050 mm [12]. Temperatures are generally high throughout the year in the district and range between 21 °C and 41 °C [13].

The district falls within the Guinea Savanna agro-ecological zone [14], characterised by the Guinea Savannah vegetation that has degraded

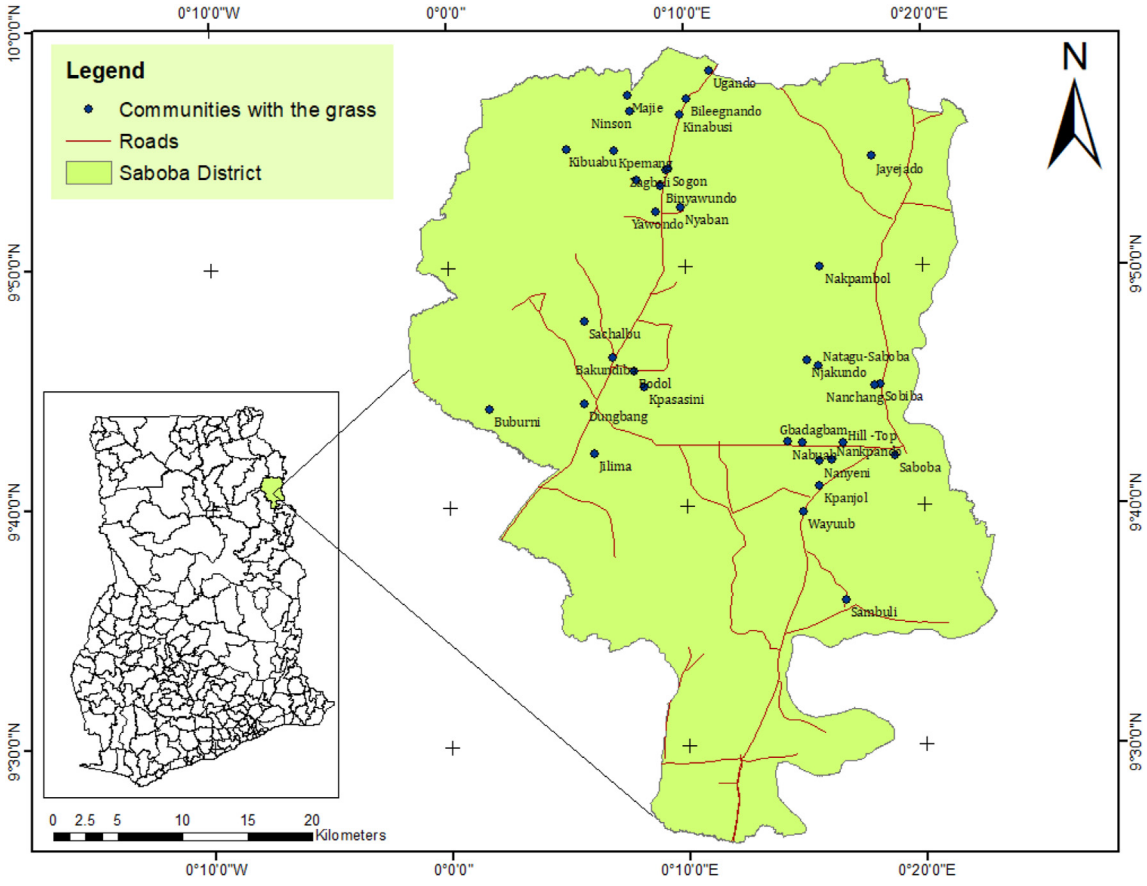

Figure 1. Map of the study area showing communities where the grass exists.

in several areas owing to intensive agricultural activities. The area now consists mainly of grassland and sparsely populated drought-resistant trees such as "dawadawa" and shea trees [15]. During the long dry season, the grassland becomes very dry and gets burnt by bushfires, and many trees lose their leaves. Rampant bush fires and bad farming practices have led to considerable soil erosion and degradation in some parts of the districts [13].

The district's population is 65,706 (49.2% males and 50.8% females) and about 70% of the working population are into agriculture, the primary economic activity in the district. Christianity, Islam, and Traditional Religion are the dominant religious groupings in the district where about 55.6% of the inhabitants are Christians, 28.6% are traditionalists, 9.4% practice Islam and about 6.0% with no religion [11].

The inhabitants, especially the native tribe (Konkombas) have a long historical background of using the native grass locally known as "Limolanii" (Figure 2a, b) for several medicinal, nutritional, and other socio-cultural purposes, even though there are no scientific evidence supporting these claims. Given the vital role of the grass in the lives of the people and the high growing patronage, there is a dire need to validate the nutritional and medicinal value of the grass. Besides, climate variation and anthropogenic activities like farming and bush fires could have considerable implications for the continuous existence of the grass, thus, the need for empirical evidence of such implications.

2.2. Focus group discussions

The study assessed local perceptions of the importance of the plant and the impacts of changing climate on its existence. This was done through focus group discussions (FGDs) in communities where the plant is found.

Prior to the FGDs, a thorough reconnaissance field survey in all the 254 communities in the Saboba District was conducted between June and July 2018 to map out the communities in which the plant, known in the local language as "Limolanii", exists. The reconnaissance survey was in the form of field observations and interactions with community members and was conducted by three (3) teams made of three (3) persons each. A team was assigned to each of the three (3) area councils of the district to visit all the communities within the respective area council. Each team was provided with the district (field) map, list of communities in the respective area council, field notebook, pens, digital camera, and GPS (Global Positioning System) (some used phones). On each visit to the communities, each team carried a sample of the grass ("Limolanii") for situations where a community may have adopted a different local name and may not be familiar with the common local name of the grass.

At each of the communities, the team met the community leader (referred to as elder or chief in the area) to introduce themselves and explain their mission as part of the community entry. With the local name of the grass and the sample, the team then interacted with community members by asking them about (i) whether or not they know the grass; (ii) if they know the grass, do they know it by the local name "Limolanii" and where did they first get to know it; (iii) whether or not the grass exists in their community, and (iv) whether or not they have spotted the grass elsewhere outside of their community – in any other community/town. In the communities where the grass was said to exist, the team was led by community members to the spot to observe for confirmation of the existence of the grass in the community. On the other hand, in all communities where the grass was said not to exist, the team, led by community members, conducted Spot Checks in and around the community surroundings to confirm the non-existence of the grass in the community. In all communities where the grass was found (confirmed) to exist, GPS coordinates of the locations were taken by the team.

The plant was visibly found to exist in thirty-five (35) communities in the district during the reconnaissance field survey. The FGDs were then conducted in each of the thirty-five (35) identified communities using an interview guide questionnaire (See Supplementary Material File – interview guide questionnaire used for Focus Group Discussions) thoughtfully designed to facilitate the acquisition of appropriate information for the achievement of the study objective. In each of the community, one focus group discussion (FGD) was conducted consisting of twenty (20) community members. The participants were randomly selected from different houses in the community and consisted of ten (10) each of adult males and females aged 50 + years with at least 30 years' experience of climatic conditions in the area. The age group of the participants was thoughtfully selected, and the discussions conducted in the local language ("Likpakpaln") spoken by the people for reliable responses on the grass and climatic information in that part of Ghana as recommended [16]. Nonetheless, in some communities where additional persons expressed interest in joining the discussions, a maximum of five such persons were allowed to join giving the group the maximum of twenty-five (25) participants in those communities. The additional five persons that consisted of different age groups below 50 + years were not given the opportunity to answer questions on climatic information, except on the importance (usages) of the grass.

2.3. Plant material collection and processing

"Limolanii" grass obtained from Saboba in the Northern region was identified and authenticated by an indigene of the community and a regular user of the plant. A sample of the plant was submitted to the Department of Herbal Medicine's herbarium at the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (Kumasi, Ghana) with specimen number KNUST/HM1/2020/L007. The plant material was cleared of any extraneous matter, air-dried, and milled into coarse powder. A measured quantity of the milled plant sample was extracted with 70% ethanol with the cold maceration method. After 72 h, the mixture was filtered, evaporated to dryness in an oven for 48 h at 40 °C to obtain a dark gummy extract and stored for further analysis.

2.4. Drugs and chemicals used for the experiments

Solvents, drugs, and chemicals used for the analysis were of analytical grade. They comprised Folin-Ciocalteu (FC) reagent,

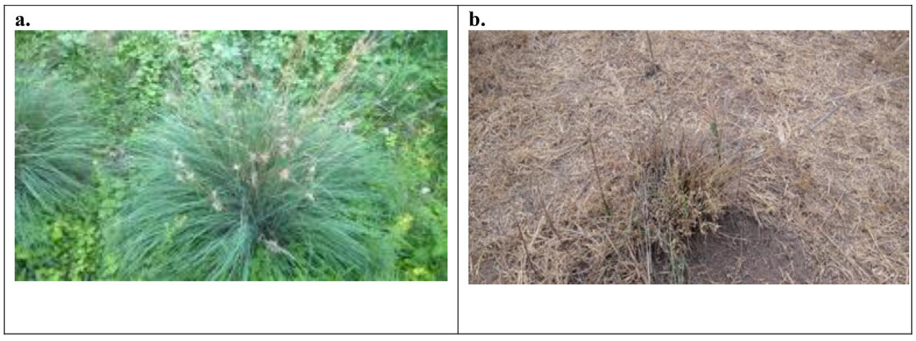

Figure 2. Seasonal view of grass during rainy (a) and dry seasons (b).

carrageenan, ascorbic acid (99.7%), DPPH (2,2-Diphenyl-1-picrylhydrazyl), and gallic acid (≥98%) purchased from Sigma-Aldrich, United Kingdom (UK); ethanol (95%), hydrochloric acid (37%) and sodium carbonate (99%) from SCP, England; lead acetate and ferric chloride (98%) from LOBA CHEM, India; ammonia solution, bismuth nitrate (98%), sulphuric acid (97%), sodium hydroxide (99%), copper sulphate (100%), acetic anhydride (99%), chloroform (99%), diethyl ether (99%), acetic acid (99%) and picric acid (1.2%) from VWR, UK; Fehling's solution B and ammonium molybdate (99%) from (FINEKEM, India); disodium hydrogen phosphate (above 99%) from ROMIL; potassium hydroxide (85%) from KEM LIGHT Laboratories, India; toluene (above 99.5%) from DAEJUNG, Korea and n-butanol and sodium chloride (99%) from QUALIKEMS, India. They were sources through local country agents. Diclofenac injection (75 mg/ml) was sourced from a local Pharmacy in Kumasi.

2.5. Phytochemical screening

The milled plant sample was screened for the presence of phytochemicals using the methods described by [4, 17, 18, 19] with few modifications. It comprised:

2.5.1. Test for alkaloids

The powdered plant material (1 g) was heated with 20 ml dilute sulphuric acid in a water bath for 5 min. The mixture was filtered, and 3 ml of the filtrate was taken. Few drops of dragendorff's reagent were added. The absence of formation of orange-red precipitate showed the absence of alkaloids [4].

2.5.2. Triterpenoids test (Liebermann- Burchard test)

Powdered plant material was screen for triterpenoids using the Liebermann-Burchard test with slight modifications where 1g of the sample was taken and 15 ml chloroform was added. The mixture was heated over the water bath for 5mins, filtered and cooled. Concentrated sulphuric acid was added along the walls of the test tubes containing the chloroform extract. The formation of a brown ring indicates the presence of triterpenoids [4].

2.5.3. Test for phytosterols (Salkowski test)

Acetic anhydride (1 ml) was added to about 1 ml of the chloroform extract of the powdered plant materials. Concentrated sulphuric acid was added along the walls of test tubes. Formation of blue or purple coloration indicates the presence of phytosterols [4].

2.5.4. Test for saponins

The powdered plant (1g) material was taken into a test tube and shaken 10 ml of distilled water. The formation and persistence of froth when left for 10mins indicated the presence of saponins [4].

2.5.5. Test for tannins

The powdered plant sample (2g) was taken into a test tubes and 20 mL of distilled water was added. It was boiled over the water bath, cooled, and then filtered. A general test for tannins was carried out by taking 1 mL of the filtrate and 3 ml of water was added. About 1 mL of lead acetate was added. The formation of precipitate indicates the presence of general tannins [4].

2.5.6. Test for glycosides

The powdered plant sample (1 g) was taken; 20 ml dilute sulphuric acid was added and heated over the water bath for about 5 min. The mixture was filtered and few drops of 20% sodium hydroxide were added. Equal amounts of Fehling's solutions A and B were added and heated over the water bath for some minutes. The formation of a brick-red precipitate indicates the presence of general glycosides [18].

2.5.7. Test for flavonoids

Chips of magnesium ribbon were added to 1ml of aqueous extract of the plant. Drops of conc. hydrochloric acid was added. The presence of red coloration indicated the presence of flavonoids [19].

2.5.8. Test for coumarins

A small amount of the powdered plant material was taken, and chloroform was added. It was heated over the water bath, filtered and about 5 ml of the extracts were evaporated to dryness in a Petri dish. The residue was dissolved in hot distilled water, cooled, and divided into two portions, A and B. 0.5 ml of 10% ammonia solution was added to extract A in the test tube. They were observed under UV light. The observation of an intense bluish fluorescence indicated the presence of coumarins [17].

2.6. Proximate analysis

The powdered plant sample was evaluated for moisture content, protein, crude fat, crude fiber, carbohydrate, and ash as described previously by [20].

2.7. In-vitro antioxidant assays

2.7.1. DPPH free radical scavenging assay

DPPH free radical scavenging activity was evaluated using the method described previously [21]. The extract solutions of different concentrations, 1 mL each was added to 3 mL of 20 mg/L DPPH solution in labeled test tubes. The reaction mixture was incubated in the dark for 30 min at room temperature (25–27 °C). The reaction was repeated for ascorbic acid of different concentrations (200–3.125 μg/mL). Absorbances of residual DPPH was measured at 517 nm using the Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Kocherwaldstr. 34, D-74177 Bad Friedrichshall, Germany). The DPPH radical scavenging activity was estimated as:

$$\%\text{DPPH radical scavenging activity} = 1 - \left(\frac{\text{Abs of sample}}{\text{Abs of control}}\right) \text{x } 100\%$$

The concentration of the extract required to scavenge 50% of DPPH was expressed as IC_{50}.

2.7.2. Total antioxidant capacity assay

The Folin-Ciocalteau's method was adopted with slight modifications to measure the plant extract's total phenol content spectrophotometrically. In this method, 0.5 mL of each of the various solutions (1000–31.25 μg/mL) of the plant extract were measured into labelled test tubes and 2.5 mL of the Folin-Ciocalteau reagent was added to each. Two milliliters of the aqueous sodium carbonate solution (75 mg/ml) were added to each and kept in the oven at 50 °C for 10 min and the absorbances read at 760 nm using the Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek Instruments. The gallic acid solutions (100–0.781 μg/mL) were taken through the same procedure and used to plot a calibration curve. The phenolic content was expressed as gallic acid equivalents (mg GAE/g extract) [22].

2.7.3. Total flavonoid content assay

The total flavonoid content was determined by the aluminium chloride colorimetric assay using a method based on the formation of a complex flavonoid-aluminium chloride. About 0.3 mL of the different concentrations (1000–31.25 μg/mL) of the extract were measured into test tubes and 3.4 mL of 30% methanol, 0.15 mL of sodium nitrite (0.5M) and 0.15 mL of aluminium chloride (0.3 M) were added. One milliliter of sodium hydroxide solution was added after 5 min. The absorbances were measured at 506 nm in the Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek Instruments. Quercetin solution (100–0.781 μg/mL),

used as reference flavonoid, were taken through the same procedure. The standard curve with reference quercetin solution was made and the flavonoid content was expressed in milligram of quercetin equivalent per gram of extract (mg QE/g extract) [23] with modifications.

2.8. In-vivo anti-inflammatory activity

2.8.1. Test animals

A-day-old post hatch chicks (*Gallus gallus*) obtained from Akati Farms, Kumasi, Ghana were kept in stainless steel cages (34 × 57 × 40cm3) at a population density of 12–13 chicks per cage. Room temperature was maintained at (29 ± 10 °C). Feed (Chick Mash) and water were made available. An incandescent illumination was maintained overhead on a 12-hour light-dark cycle. Daily maintenance of the chicks was done till the seventh day when the experiment was conducted. Throughout the study period, the animals were treated as humanely as possible. The Ethics Committee of the Department of Pharmacology, KNUST gave approval and clearance of the animal studies carried out.

2.8.2. Anti-inflammatory activity

On day 7, the chicks were weighed and divided into treatment and control groups randomly to be used in the evaluation of the anti-inflammatory activity. The carrageenan-induced foot oedema model as described by [21] with slight modifications was employed. Group sample sizes of 5 were used throughout the studies. Footpad volumes were measured with a digital caliper -Powerfix profi, model no. Z22855 (OWIM GmbH & Co. KG, Stiftsbergstraße 1, D-74167 Neckarsulm, Germany) before inducing the oedema in the right foot pads of the chicks by sub-plantar injection of 10 μL of 2% w/v solution of carrageenan in saline. The foot volumes were measured 1 h after injection and the extracts dosed at 30, 100 and 300 mg/kg weight were given to the chicks orally. Diclofenac (10, 30 and 100 mg/kg) and dexamethasone (0.3, 1 and 3 mg/kg), the standard drugs were given intraperitoneally with the control animals receiving only the vehicle. Hourly readings of the foot volumes were taken till the 5th hour. The oedema was evaluated by measuring the foot volume differences at time t = 0 (before the carrageenan injection) and at the subsequent time intervals. The percentage inhibition of the oedema components of the inflammation for the treated groups were determined using the following equation:

$$\% \text{ Inhibition} = \left(\frac{\text{AUC control} - \text{AUC treatment}}{\text{AUC control}}\right) \text{x } 100\%$$

2.9. Statistical evaluations

Graph pad prism (version 5 for windows, San Diego, USA) was used to carry out the statistical and graphical analysis. The differences in AUCs (Area under the curve) were analysed by One-way Analysis of Variance (ANOVA) followed by Students-Newman-Keul's *post hoc* test.

3. Results and discussion

3.1. Focus group discussions

Of the 254 communities in the Saboba District in which the reconnaissance field survey was conducted the plant was visibly found to exist in thirty-five (35) communities. In seven (7) of the remaining 219 communities, reconnaissance field survey revealed that the plant existed there in years past but has disappeared in averagely a decade ago. Results from the focus group discussions conducted in the thirty-five (35) communities are as summarized below.

3.1.1. The origin of the plant

In all the focus group discussions (FGDs), respondents noted that they did not know the origin of the plant, known in the local language as "Limolanii." *"From centuries it has been there. We do not know the actual origin,"* all of them said. The respondents could not also tell how old the plant has been in their respective communities – *"we cannot tell how old it has been in our community because we grew up to meet it,"* they unanimously said. It was revealed during the FGDs that the plant was of two types and described locally as female and male types - the one being investigated is referred to as the female and most useful type.

The known places (communities/towns) where the plant is most found currently were identified to include: Saboba Township and its surrounding communities, Nankpando, Sobiba, Gbadagban, Nabuah and Sambuli (Figure 1). Some respondents during the FGDs indicated that the plant could also be found in some communities in Chereponi District and Yendi Municipality as well as in some areas in Northern Togo.

3.2. Uses of the plant

In all the FGDs, respondents mentioned the following as the common uses of the plant:

- Treating fever - the leaves are boiled and the affected person bathes and massages his/her body with the resulting water.
- Snakebites – the leaves are used with other herbs to remove venom of snakebites from affected persons.
- Spice/flavour in cooking dog meat, which is a delicacy among some tribes in Northern Ghana. Besides dog meat, it is also used in some communities as spice to cook or parboil any other fresh meat.
- Hatching of chickens as it prevents poultry mites from attacking the hen during brooding or incubation.

Some other uses that were revealed in some communities include:

- Used to drive away evil spirits.
- Preservation of corpses – it is boiled, and the resulting water is traditionally used to bathe the corpse to preserve it.
- Weaving zana mats – in the rural areas of Northern Ghana, grain stores (silo) are built of woven tough grass including the plant under study.

3.3. Socio-cultural and socio-economic role of the plant

Socio-culturally, the communities value the plant as playing an invaluable role in traditional medicine for treatment of many diseases. They believe that the traditional use of the plant as medicine could potentially develop to become one of the most effective ways of treating common diseases such as malaria in their communities. This, they believe could bring enormous socio-economic benefits to their communities in the future, because the scientific development of such herbal medicine in the area will provide sources of livelihoods for many inhabitants and reduce the burden of common diseases like malaria in the area. Nonetheless, respondents believe, that hope of future socio-economic benefits of the plant can only be realised if the present threats on its existence are eliminated or reduced to the barest minimum.

3.4. Perceived existential threats to the plant

The key threats on the existence of the *plant species* as identified by the respondents are namely: changing weather pattern over the years, increased use of strong agro chemicals (weedicides), bush fires, urbanisation, and the use of tractor in ploughing farm fields. The respondents stressed that the threat by change in climate, bush fires and increased use of strong agro chemicals were particularly severe and related. According to the respondents, the three threats are related because the frequent bushfires are due to rising temperatures and the increased use of agro chemicals was mainly due to frequent pests and weeds invasion caused by the rising temperatures.

The respondents' view on the effects of changing climate on the plant was almost unanimous. In all the interviews, respondents asserted that

there had been a sharp decline in the land cover of the plant over the past three decades and changes in the climate was the key contributing factor. According to them, apart from increasing bushfires due to rising temperatures, the impact of climate change realized included erratic and decreased rainy season as well as poor seasonal rainfall distribution in the past three decades. These, they perceived were responsible for the extinction of some plant species and are threats to the diversity of "Limolanii".

The respondents contended that the "Limolanii" species faces existential threats if their perceived threats of changing climate persist for the next three decades without any effective intervention. They however, admitted that communities had so far failed to make any attempt of taking any measures to protect the "Limolanii" from the threats of climate change and other aforementioned factors. The failure to initiate any adaptation measures were blamed on communities' general attitude of indifference and compounded by other factors such as poverty, frequent water shortages and fear of snake bites as the plant could attract snakes due to its shrubby basement.

Majority of the respondents suggested that, going forward; members of the communities could explore the use of backyard gardens for the "Limolanii" as a key step to save the plant from possible extinction eventually. Their reason was that it was almost impossible to stop bushfires and the use of agro-chemical products which were key threats to the existence of the plant. Others proposed community enforcement of selective-use of agro-chemical products or complete ban on the use of such products all together, as a way of protecting the plant from possible extinction.

Although, these local perceptions by the communities could not be compared with observed local climatic conditions of the study area due to data deficiency, we believe they can be situated in the context of climate change analysis by previous studies over the wider area within which Saboba district falls, particularly studies over Oti River Basin (ORB) and the Northern Ghana. This is because, most of these previous studies have observed similar climatic conditions over these areas mainly due to the fact that they fall largely within the same agro-climatic zone.

A brief review of these previous studies conducted over the area has shown that they lend credence to the perceptions held by the communities with regards to climate change in the present study. For instance, it is reported that the mean annual temperature across Ghana increased between 1961 to 2000 by 1.0 °C, with the maximum rate of increase experienced in the north–eastern part of the country where Saboba district is [24]. It is again projected that temperatures will increase over northern Ghana, including Saboba district, by 0.5 °C–2.5 °C toward the year 2080 with implications on environment and farming activities. In terms of climate extremes, droughts are frequent in the area [25].

Other studies like [26] conducted spatiotemporal analysis of rainfall over the ORB within which Saboba district falls and reports an expected continual decrease over the area in the near future (2021–2050). Another study which examined the rainfall onset, cessation, and length of rainy season (LRS) over ORB predicted a likely increase and decrease in rainfall onset and cessation dates, respectively, from the current 8 May to 24 May and from the current 29 October to 7 October, respectively [27]. The study also projected that LRS would decline from the current 173 days–136 days over the area (ibid). Similarly, a study conducted an analysis of extreme rainfall over ORB using a long term observed daily data from 1921 to 2018 reported that the area was experiencing a downward trend in extreme rainfall while the dry spells were on the rise [28].

In examining changes in climate variables over Northern Ghana, a study compared the local perceptions with observed meteorological data for the area [29]. The study reported that local perceptions about climate in the area, including erratic and decreased rainfall trends were consistent with observe data, indicating the potential accuracy in communities' perspectives on climate change and its impacts on their environment; and the need to incorporate such indigenous knowledge in climate policy decisions and actions (ibid). Generally, the local perceptions about climate change across Northern Ghana have been consistent and mostly corroborate with observed climate data. For instance, the communities' perspectives about climate change in the present study corroborate with the findings by [16] that showed perceptions of increased temperature, decreased rainfall (amount, intensity, duration, and rainy days) in the Vea catchment of Northern Ghana. The similarity in these findings is not surprising because the two study areas fall within the same agro-ecological and climatic zones.

In support of the recommendations and strategies proffered by the communities, government and its relevant institutions and agencies, particularly the district assembly, agriculture and forestry departments could initiate interventions to protect the natural habitats (in situ) of such species like the "Limolanii" for its long-term survival, conservation, and sustainable use [30]. Such interventions could be in the form of an extension programme backed by a public policy to achieve its maximum benefits [31] and improved conservation through participatory and inclusive planning [30]. This will include sensitisation and awareness creation and adoption of both *in situ* and *ex situ* conservation of the plant with a blend "of *a well-designed and well-managed protected*" natural habitats (in situ) and *ex situ* conservation structures [32].

3.5. Phytochemical screening

Phytochemical analysis of the plant material afforded some secondary metabolites (see Table 1) such as triterpenoids, steroids, tannins, glycosides, flavonoids and coumarins. Alkaloids and saponins were however absent. Some of the secondary metabolites, such as flavonoids, tannins have been reported to have antioxidant and anti-inflammation properties. Phytosterols and coumarins play the role of detoxifying agents whereas terpenoids are also known for their antioxidant and neuropharmacological activities [33, 34] . Detection of these phytochemicals in "Limolanii" supports its ethnomedicinal use by the indigenes (Table 1).

3.6. Proximate analysis

Nutritional foods are known for the role they play in good health, disease treatment and prevention [6]. Functional foods and nutraceuticals have become one of the leading food categories where research and development efforts are concentrated due to the natural bioactive components, they contain that have numerous health benefits to the body [35]. It is important to investigate the nutritional components of the plant under study mainly because of its use by the locals. The present study sought to evaluate the nutrients and anti-nutrients present in this underutilized plant to guide it dietary and medicinal uses. 'Limolanii' was found to have considerable amount of fat, fibre, carbohydrate, and proteins (Table 2).

Phytates are reported to bind with minerals in the digestive tract hence preventing mineral absorption and utilization in the body. According to the classification of high/low phytate plant, "Limolanii" can be classified as a low phytate plant [36] and safe for consumption since it had low phytate content. It also did not form persisting froth/foam when shaken with water which is a characteristic of saponin-containing plants. Saponins are also known for their bitter taste at high

Table 1. Results of the phytochemical screening of "Limolanii".

Phytochemical	Detected (+)/Not Detected (-)
Alkaloids	-
Triterpenoids	+
Steroids	+
Saponins	-
Tannins	+
Glycosides	+
Flavonoids	+
Coumarins	+

Table 2. Nutritive and anti-nutritive content of "Limolanii".

Nutritive Factors	"Limolanii" grass mg/g
Fat	46.47 ± 3.33
Crude fibre	382.77 ± 3.02
Total ash	89.77 ± 3.81
Moisture	71.67 ± 2.88
Protein	52.23 ± 0.12
Carbohydrate	357.10 ± 0.90
Anti-nutritive Factors	
Phytates	12.67 ± 7.64
Saponins	0.000 ± 0.000

concentrations. They are reported to inhibit nutrient uptake by inhibiting enzymes, binding with minerals like zinc and also affect protein digestion in the gut [8].

3.7. Antioxidant activity

Studies have revealed that phytochemicals function as antioxidants via several mechanisms including hydrogen donors, reducing agents, metal chelators or singlet oxygen quenchers. Several *in-vitro* assays are employed in evaluating this activity and due to the limitations of these investigative assays, a multi-assay strategy is usually adopted to assess the antioxidant activity based on the several mechanisms of action of the phytochemicals [37, 38].

3.7.1. DPPH free radical scavenging activity

The extract's ability to scavenge free radicals through electron or hydrogen donation to the DPPH free radicals was investigated DPPH free radical scavenging assay was employed because of its simplicity, accuracy, sensitivity, and popularity [37, 39]. The extract and reference compound ascorbic acid exhibited a concentration-dependent radical scavenging activity with IC50 values of 465.7 μg/mL and 11.63 μg/mL respectively.

3.7.2. Total antioxidant capacity

In the total antioxidant capacity assay of the plant, it was observed that every gram of the plant extract had equivalent activity (i.e., reduction of molybdenum, Mo^{6+} radicals to form phosphate-molybdate (Mo^{5+}) complex) as 68.72 mg of ascorbic acid (Table 3). The Ascorbic Acid Equivalent (AAE) was obtained from the calibration curve with

Table 3. Total phenolic content, total flavonoid content, and total antioxidant capacity of the extracts.

Extracts	TPC (mg/g GAE)	TFC (mg/g QE)	TAC (mg/g AAE)
Ethanol/water extract	23.80 ± 8.21	123.0 ± 4. 79	68.72 ± 9.47

Note: All data were recorded as mean and their standard deviation. TPC – total phenolic content; GAE - gallic acid equivalent; TFC – total flavonoid content; QE - quercetin equivalent TAC – total antioxidant content; AAE - ascorbic acid equivalent.

equation of the line: $y = 0.0026x - 0.0633$ where y is the absorbance and x are the concentration in μg/mL and r^2 value of 0.9963.

3.7.3. Total phenol and flavonoid content

Phenolic compounds, generally grouped into flavonoids and non-flavonoid compounds, have been considered as a natural alternative to treating inflammation-related diseases based on their benefits to the health and food industry. They have been reported to play a key role in curbing chronic diseases attributed to their antioxidant properties and redox properties which helps them act as hydrogen donors, reducing agents, and singlet oxygen quenchers [5, 40]. For this reason, phenols and flavonoids were quantified.

Using Gallic acid as the standard, a calibration curve was plotted, and the phenolic content assessed from the equation of the line: $y = 0.00258x - 0.0643$ with r^2 value 0.9994. Every gram of "Limolanii" extract showed the same activity as 23.80 mg of gallic acid (Table 3). The assay used is based on the ability of phenols present to transfer of electrons in alkaline medium to form a blue coloured phosphotungstic/phosphomolybdenum complex with the maximum absorption depending on the concentration of the phenols [41].

The total flavonoid content was expressed as quercetin equivalent in mg/g weight of the extract. "Limolanii" extract had a total flavonoid content of 123.0 mg/g QE (Table 3). This was calculated from the Quercetin standard curve with the equation of the line; $y = 0.00022x - 0.0429$ and r^2 value of 0.9966. The principle of action of this assay is based on the ability of carbonyls groups (C4, C5) and hydroxyl groups (C3 and ortho position of B ring) of flavonoid compounds to form stable complex or labile acid complexes with aluminium chloride [42].

3.8. Anti-inflammatory activity

Over the years, chicks have widely and successfully been used as animal models for chronic inflammation research. Several inflammation inducers such as turpentine, carrageenan etc., were used for the studies,

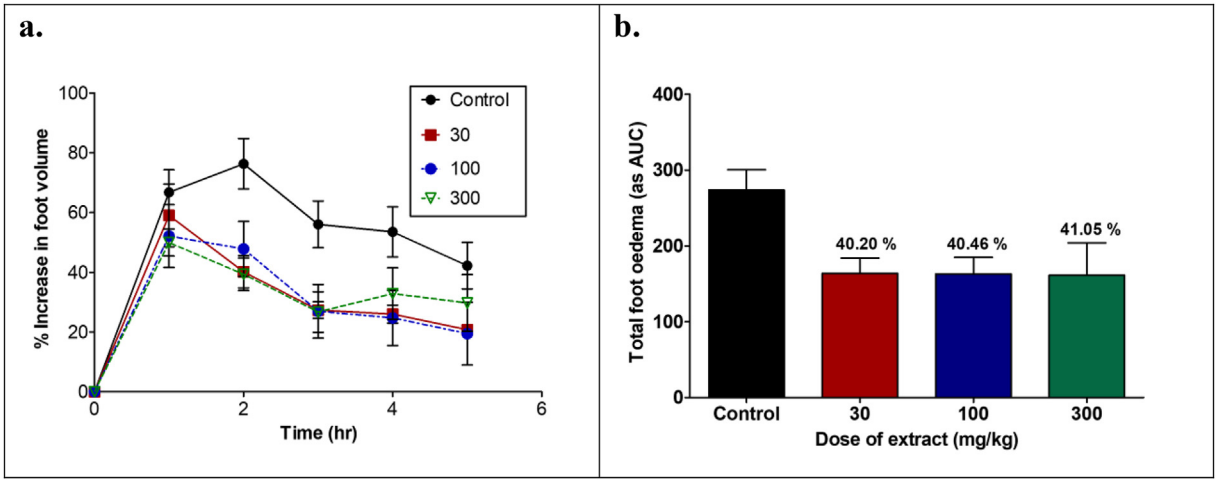

Figure 3. Effect of ethanolic extract of "Limolanii" (30–300 mg/kg, oral) on time course curve (a) and total oedema response expressed as AUC (Area Under the Curve) (b) in carrageenan induced foot oedema in chicks.

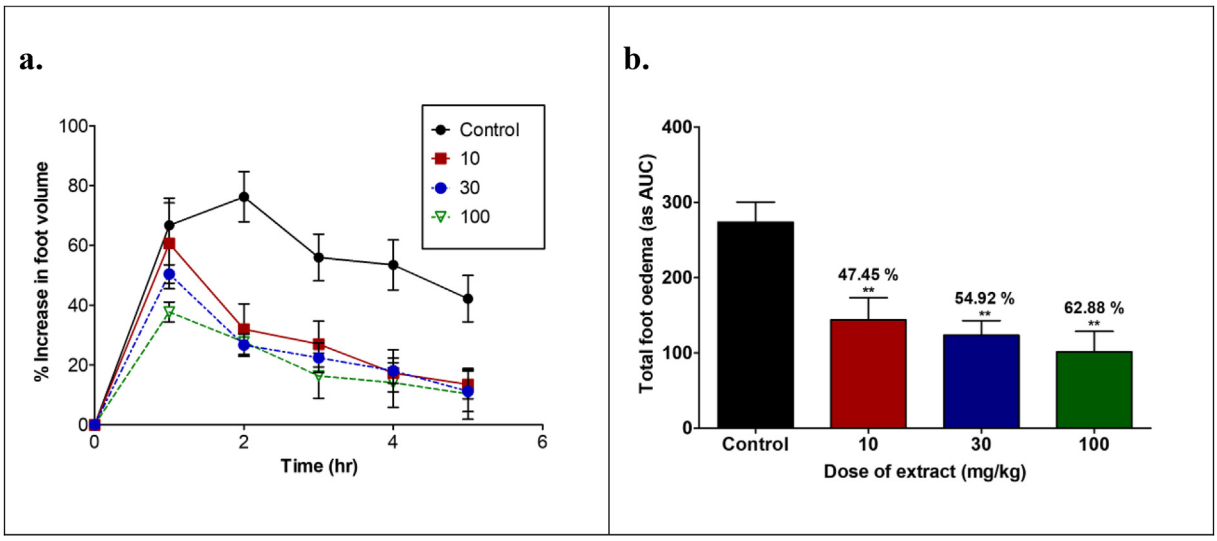

Figure 4. Effect of Diclofenac (10–100 mg/kg, i.p.) on time course curve (a) and total oedema response expressed as AUC (b) in carrageenan induced foot oedema in chicks.

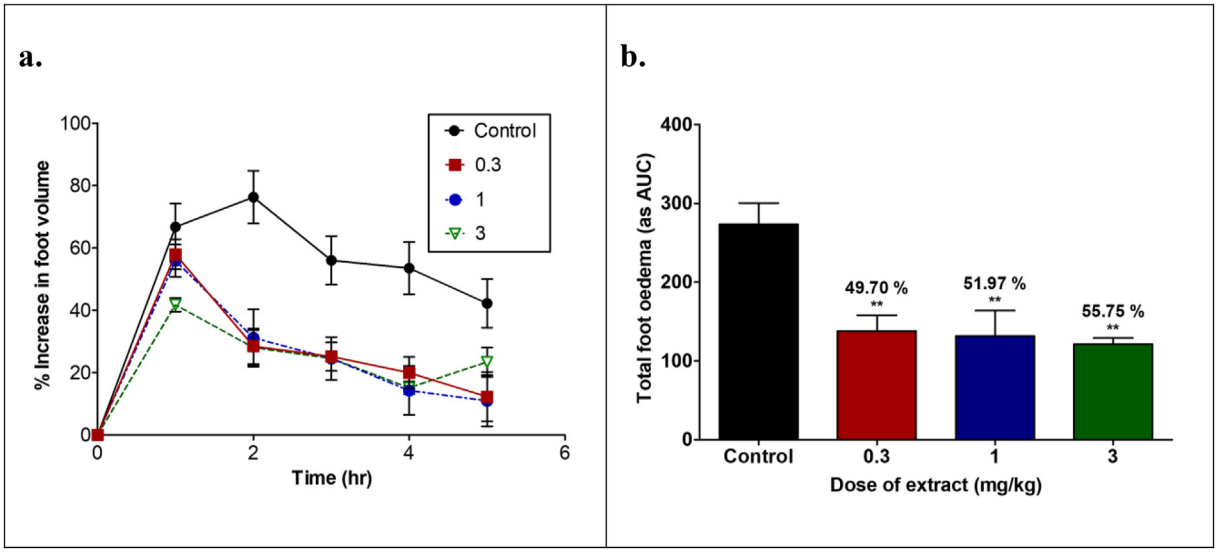

Figure 5. Effect of Dexamethasone (0.3–3 mg/kg, i.p.) on time-course curve (a) and total oedema response expressed as AUC (b) in carrageenan-induced foot oedema in chicks.

and in comparison, to rodent models, the oedema response was found to be similar in both models [43]. Many similarities have been found between mammal and chick pain-related neurophysiology and their responses to multiple classes of anesthetic agents are also reported to be akin to mammals. Their nociceptive and inflammatory symptoms are reduced by usual opiates, NSAIDs, steroidal anti-inflammatory drugs, and colchicine [44]. Based on the suitability of the chick model for the investigation of pain mechanisms and pharmacological action, its inexpensiveness, and availability, chicks were used instead of rodents for the studies.

The extract was given to the chicks orally at doses: 30 mg/kg, 100 mg/kg, and 300 mg/kg 1 h after the induction of oedema. Dexamethasone and Diclofenac were used as the standard drugs. There was a prominent increase in foot thickness of the control chicks 1 h after oedema induction, reached the peak of inflammation in the second hour and slowly declined for the next 3 h this can been seen in Figure 3a. The plant extract though was able to reduce the carrageenan-induced inflammation in the footpad of the chicks to an extent (Figure 3a), the inhibition was an insignificant ($P < 0.05$) when subjected to the Neumann-Keuls Multiple comparison test. The standard drugs showed significant effect in reducing the total oedema with time and it was dose dependent (Figures 4a and 5a) showing the workability of the method used in investigating the anti-inflammation properties of the plant under study.

Comparing the percentage inhibition of the plant extract with Diclofenac and Dexamethasone, which are well-known anti-inflammatory agents, the highest concentration of the extract showed a percentage inhibition of 41.05% (Figure 3b) while the highest concentrations of Diclofenac and Dexamethasone recorded 62.88% (Figure 4b) and 55.75% (Figure 5b). The results obtained scientifically supports the usage of the plant for treating inflammation-related conditions such as snake bites and fever.

4. Conclusions

This study revealed "Limolanii" to contain high content of carbohydrate, crude fiber, protein, crude fat, and ash. Thus, its nutritive potential is not in doubt and can significantly impact human health requirements without posing deleterious health risk due to its low anti-nutritional

content. The work has also shown that the extract of "Limolanii" grass exhibits considerable antioxidant activity and some anti-inflammatory property backing its use traditionally with other herbs for medicinal purposes.

Despite the significant role of the plant species as food supplements, herbs, and socio-cultural use as well as its future potential socio-economic value, the plant could be facing a possible risk of diminishing population and existential threat associable to climate change and urbanization. In view of the socio-cultural importance and the commercial potential of "Limolanii", there is the need to pay appropriate attention to its conservation and sustainable use. Further study on the plant drought vulnerability assessment would be essential to understand the level of "Limolanii" sensitivity to climate variables and inform appropriate climate adaptation strategies.

Declarations

Author contribution statement

Andrew M. Limantol; Vivian Etsiapa Boamah, PhD: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Blessed Agbemade: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mercy Badu; Kingsley I. Amponsah: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Firdaws C. N. Adam: Performed the experiments.

Rahmatu B. Mohammed: Performed the experiments; Analyzed and interpreted the data.

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Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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