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Preliminary phytochemical screening on locally made energy drink; monkey tail (Mockite) in Nigeria

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Abstract
Energy drinks are beverages designed primarily to increase the consumer's physical performance with claims of therapeutic or nutritional purposes, depending on the chemical constituents present. This study evaluates the preliminary qualitative and quantitative phytochemicals indexes in locally consumed energy drink in Nigeria. Standard procedure were used for the qualitative and quantitative phytochemical screening. Results from the qualitative and quantitative phytochemical study showed the presence of tannins (0.8 mg/ml), steroids, terpenoids (0.2 mg/ml), saponins (0.14 mg/ml), phenol (0.3 mg/ml) and flavonoids (0.5 mg/ml) at varying concentration. In conclusion, the outcome of this study validated the forklore uses of this local energy drink with the phytochicals responsible for its vast therapeutic properties.

Keywords: phytochemical analysis, energy drink, monkey-tail, mockite

Introduction

Energy drink is a type of drink containing sugar and stimulant compounds; usually, caffeine, in most cases, sold as providing mental and physical stimulation (marketed as "energy" but distinct from food energy) [1]. They may or may not be carbonated and contain other sweeteners, herbal extracts, taurine, and amino acids. There are three main subsets of energy drinks: caffeinated drinks, herbal bitters, and un-refined local cocktails [2]. On the other hand, locally brewed energy drinks are derivatives of energy drinks that contain natural stimulants majorly from plant sources and consumed for centuries with claims of treating various medical illnesses. Both wild and cultivated derivatives have found wide acceptance in the past and present among the teeming populace across all socio-economic strata [1]. Monkey tail, also referred to as Mockite, is made from either soaking marijuana leaves, stems, and roots in local gin for few days depending on Units of Intoxication (UOI) intended for the customers. Boiling/extracting water from marijuana leaves, stems, seeds or roots and mixing with local gin can also produce mockite. Depending on who is taking it and the UOI desired, the drink is usually mixed with sweeter elements and taken in shots. Mostly taken by men with claims that this locally brewed energy drink helps maintain healthy blood sugar levels, balance appetite, replenish energy and improve libido. Also, consumers have reported its ability to calm an upset stomach, increase absorption of fat-soluble vitamins A, D, E and K and nausea [1].

In Nigeria, marijuana has been an officially illegal but unofficially legal drug for the longest time. However, contrary to this fact, marijuana-infused brews still come as a novel idea to most common average Nigerians. Skilled herbal mixologists commonly sell it; usually, females have "*Agbo* stations", meaning herb stations by the roadside or are conveniently mobile. Global energy-drink consumption increased by 14% (1.5 billion litres higher) between 2007 and 2011 and had grown by a mean of 10% yearly from 2007 to 2011 with more than half of young adults consuming a minimum of one can of energy drink monthly and about 6% use energy drinks daily [3]. Nigeria ranks 4th in soft drink consumption worldwide, with reports of a lifetime prevalence rate of energy drinks consumption of 55.4% in a cross-sectional descriptive study in Northern Nigeria [4]. Another cross-sectional descriptive study in south-south Nigeria revealed an 87.8% prevalence of energy drink consumption [5]. Although much research on both quantitative and qualitative phytochemical analysis of *Cannabis Sativa* which is an active ingredient of this local energy drink, has been done [6]; phytochemical studies of this mix and, more specifically, locally brewed energy drinks have been inadequate as the focuses of most research are on leaves and fruits of the plants used in making them [7]. Some of these herbal energy drinks contain phytochemicals locally extracted using alcohol or in aqueous form by traditional drug peddlers, as in monkey-tail, showing that they have undocumented medicinal values. The medicinal value of a plant lies in the phytochemical (bioactive) constituents of the plant, which shows various physiological effects on the human body [8]. Therefore, through phytochemical screening, one could detect the various important compounds used as the bases of modern drugs for curing various diseases [8]. The human body under stress condition produce less enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and nonenzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E) [9] but more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide) [10]. An imbalance could result from the above, causing damage to the body cell and other health challenges [11]. Preventive medicine has immensely enhanced the use of these natural plant antioxidants. Plants contain lots of free radical scavenging molecules, including alkaloids, amines, betalains, vitamins, terpenoids, phenolic acids, lignins, stilbenes, and tannin, as well as other secondary metabolites

with a high level of antioxidant activity [10]. Most phytochemicals are antioxidant agents that are essential in reducing the damages caused in tissue during physiological processes. Due to Monkey-tail's increased consumption and attributed medicinal value, this research aimed to determine the qualitative and quantitative phytochemical components in Monkey-tail as indices of producing secondary plant metabolites with medicinal values and application in industries [8].

Materials and methods

Sample collection

We used a freshly prepared (48hr soaked) monkey-tail energy drink sourced from a local vendor for this study.

Preliminary qualitative phytochemical screening

Phytochemical screening on Monkey tail (Mockite) was performed via standard methods by Sofowora [12]; Evans [13]. Phytochemically screening for alkaloids, flavonoids, tannins, Phlobatannins, Anthraquinone, terpenoids, saponins, phenolic compounds, glycosides, steroid.

Tests for Saponins

Frothing test: One (1) ml Monkey tail dissolved in 10 ml of distilled water (DW) with thorough shaking. The presence of saponin established via constant frothing (1 cm layer).

Fehling's test: Ten (10) ml of Monkey tail added to 5 ml of dilute H_2SO_4. Each mixture is adequately mixed and boiled for about 15 min, filtered and allowed to cool. A 2.5 ml volume of the filtrate was prepared into alkaline using 20 % NaOH solution and further boiled using 0.1 ml Fehling solutions A and B for 120 seconds. Colour modification on heating showed that saponins were present.

Test for Flavonoids

Three (3) mls of Monkey tail was defatted using acetone, filtered, and the residue extracted via water in a water bath. It was further filtered. After subsequent filtration, 10 % Lead acetate solution at 5 ml solution was added to filtrate while 5 ml solution of 10 % NaOH was involved in equal filtrate volume. The presence of yellow colouration showed that flavonoid was present.

Shinoda test: Monkey tail at 0.5 g was dissolved each in 1 ml solution of 50 % methanol. Approximately 5 drops of concentrated HCl added. The reddish or orange colour indicated flavonoid aglycone was present [14].

Test for Phenolic Compounds

Ferric chloride test: Two (2) ml of Monkey tail was introduced separately into 5 ml distilled water accompanied by 2 drops of 5 % ferric chloride solution. A blank test, conducted by adding 2 drops of ferric chloride solution to 5ml distilled water. The development of intense colouration in the test sample indicated that phenolic compounds were present.

Test for Tannins

The addition of Lead acetate to 3 drops of Monkey tail with a Red colour precipitate showed tannins.

Ferric Chloride Test: A 0.5 ml of Monkey tail was diluted in 10ml water and was filtered. Three drops of ferric chloride solution added into the filtrate that produced a blackish blue precipitate indicating a hydrolysable tannin with greenish precipitate indicated the presence of tannin.

Test for Alkaloids

Two (2) ml of Monkey tail was added to Dragendorff's reagent at 2 drops and produced a reddish-brown precipitate that indicated the presence of alkaloids.

Two (2) ml of Monkey tail product was added into Wagner's reagent in 2 drops and produced a brownish precipitate, which indicated the presence of alkaloids.

Two (2) ml of Monkey tail was added to 2 drops of Hager's reagent and formed a yellow precipitate that indicated alkaloid presence.

Two (2) ml of Monkey tail added 2 drops of Mayer reagent to form a milky precipitate indicating that alkaloids were present.

Test for Glycosides

Five millilitres of Concentrated sulphuric acids were added to 2g Monkey tail, boiled for 15min and allowed to be neutralised and cooled with 20% KOH and further divided into the dua segment. Another fraction of leaves and root extracts dissolved into distilled water; this served as control, without acid hydrolysis.

Fehlings solution test: Fehling solution A and B of 5 ml of Monkey tail to section and boiled for some minutes, reddish precipitate showed glycone fraction due to hydrolysis.

Ferric chloride test: Solution of Ferric chloride (3 drops) added into Monkey tail. Green to blackish precipitate showed phenolic aglycone as an outcome of glycoside hydrolysis [15].

Test for Terpenes and Steroids

Terpenes and Steroids (Burchard test) were tested by adding 1 ml of anhydrous acetic acid to 2 ml of petroleum ether crude leaf extract of monkey tail in a test tube. Concentrated tetraoxosulphate (V1) acid (H2SO4) was carefully added down the side of the test tube and observed for a reddish colour change and interphase formation

Quantitative Phytochemical Screening

Quantitative phytochemicals screening found in Monkey tail was evaluated and quantified via standard procedures.

Phenolic Content Determination

Monkey tail content evaluated using Folin-Ciocalteu reagent (FCR) as illustrated by. The standard used was garlic acid. Graded garlic acid concentration was 1 mg/ml of Monkey tail. One millilitre solution of Monkey tail and the standard added, 1 ml solution of Folin-Ciocalteu reagents (10 dilutions with water) and allowed to stand for 3min. Similarly, 1ml of 10 % sodium carbonate and the mixture mixed adequately in the dark at room temperature. Absorbance was recorded at 760 nm as the blank accommodate every reagent, excluding the extracts and standard. Standard Garlic acid calibrated curve assembled. The tests carried out threefold, and total phenolic content anticipated with gallic acid correspondent (mgGAE/ g dry sample) [16]. The values articulated as Mean and Standard Error of Mean.

Determination of Total Flavonoids

Total flavonoid for Monkey tail evaluated by aluminium chloride intricate assay reported by Mervat *et al*. [17]. Utilisation of Quercetin as standard. Graded quercetin concentration synthesised in methanol. One millilitre of standard and Monkey tail added to 0.1 ml solution of 10 % aluminium chloride solution and a 0.1 ml solution of 1M potassium acetate prepared in methanol. The mixture was

mixed meticulously and kept for 30min in the dark. Absorbance read at wavelength 420nm, and analysis performed three times. Total flavonoid of monkey tail projected in quercetin milligram in correspondent (QE) per gram of dry sample (mgQE/g dry sample) [18]. Standards analysed as Mean±Standard Error of Mean.

Determination of total alkaloids

According to Harborne [19]; Zhang *et al*. [20] method, alkaloids content evaluated on the Monkey tail.

Procedure

Five gram of Monkey tail weighed in a 250ml beaker followed by a 200ml solution of 10 % acetic acid prepared with ethanol, enclosed and left for 4hr. It was adequately filtered and concentrated using a water bath of the one-quarter main volume. The concentrated ammonium hydroxide added in drops of Monkey tail pending precipitation. The entire solution was settling and precipitate washed using diluted ammonium hydroxide then filtered. The residue was alkaloids which were dried and weighed.

Determination of Total Tannins

Tannins concentration was resolute through the standard technique described by AOAC [21]; Zhang *et al*. [20] 2 grams of Monkey tail boiled using 300ml distilled water and diluted in a calibrated volumetric flask filtered with a ball of non-permeable cotton wool along a volume of 25ml in 2000ml porcelain dish. Titration using 0.1N potassium permanganate originally standardised with 0.1N oxalic acid awaiting turns a blue solution into a green solution. Few drops of 0.1N potassium permanganate added drop wisely till the solution turned golden yellow.

Determination of Total Saponins

Two grams of Monkey tail weighed in a thimble then placed in soxhlet extractor using condenser fixed top. Extraction performed using acetone in a 250ml round bottom flask for 3hr; another weighed 250ml round bottom flask with methanol fixed to the same extractor, and extraction persists for a further 3hr. In the latter part of the second extraction, methanol was regained via distillation while the flask was oven-dried to evaporate the solvent present in the flask. The flask was left to cool inside a desiccator, and the resultant residue weighed.

Formular:

% Saponins= A- B/ W× 100

A= Weight of flask and extract (saponins)

B= weight of empty flask

W= weight of the sample

Results and Discusion

Test results of the phytochemical screening of Monkey tail showed that Dragendoff's reagent test did not produce orange colouration confirming the absence of alkaloids. There was a visible change in the Monkey tail's appearance after conducting tests for the presence of saponins, tannins, and phenolics, implying that these phytochemicals are present in the Monkey tail. The presence of light yellow, reddish-brown, violet, reddish-brown colour at the interphase between the acetic acid anhydride and sulphuric acid layers confirmed the presence of flavonoids resins terpenes and steroids, respectively, in the Monkey tail (Table 1).

Table 1: Qualitative phytochemical screening of Monkey tail

Phytochemical	Quality	Colour	Test
Alkaloid	-	Nil	Dangendroff" s test
Saponin	+	Dark brown	Frothing test
Flavonoids	++	Light yellow	Lead acetate
Tannins	+++	Yellow	Aluminium chloride
Cardiac Glycosides	-	Nil	Keller-Killiani test
Phenols	++	Reddish Brown	Folin-Ciocalteu reagent
Terpenes& Steroids	+	Red	Burchard test

+++High presence; ++ moderate presence; + scantily present; – Absence

The proximate quantitative chemical composition of the monkey tail in this study revealed no quantity of alkaloid and glycoside. The saponins, terpenes and steroids content gave the most negligible yield of 0.14 mg/ml and 0.2mg/ml, respectively, while flavonoids and phenols seen to be moderately present ±with 0.5mg/ml and 0.3mg/ml, respectively. Tannins were present in the highest concentration with a total of 0.8mg/ml (Table 2).

Phytochemicals described as non-essential nutrients found in the plant; non-essential means they are not *required* to sustain life. However, a growing body of evidence suggests phytochemicals are nonetheless *beneficial* to health. Although evidence still gathered, research suggests that phytochemicals can protect us from various diseases. Phytochemical screening of monkey tail (Mockite) revealed saponin, tannins, phenols, flavonoids, terpenes and steroids presence. Saponins are a class of chemical compound found in particular abundance in various plant species. They are amphipathic glycosides grouped phenomenologically by the soap-like foam they produce. Structurally, they have one or more hydrophilic glycone moieties or steroid derivative [6]. The amphipathic nature of saponins gives them activity as surfactants with the potential ability to interact with cell membrane components, such as cholesterol and phospholipids, possibly making saponins useful for developing cosmetics and drugs [22]. A minute quantity of saponin (0.14mg/ml) observed in this study's monkey tail. Saponins protect against microbial attack; it is also helpful in treating yeast and fungal infections [7]. According to Sodipo *et al.* [12] most phytochemicals serve as natural antibiotics, which assist the body in fighting microbial invasion and infections. Monkey tail can be helpful for its saponin content.

Table 2: Quantitative phytochemical screening of Monkey tail (Mockite)

Phytochemical	Mean±SEMQuantity (mg/ml)
Alkaloid	0.00±0.00
Cardiac Glycosides	0.00±0.00
Saponin	0.14±0.01
Terpenes & Steroids	0.20±0.00
Flavonoids	0.50±0.02
Phenols	0.30±0.01
Tannins	0.80±0.03

Tannins are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins. They also precipitate other organic compounds, including amino acids and

alkaloids. Usually found in a large proportion, as revealed in our study. However, not surprising, as principal human dietary sources of tannins are in tea and coffee [1]. Most wines aged in charred oak barrels possess tannins absorbed from the wood. This concentration gives the wine its signature astringency, experienced using monkey tail as reported by consumers [7]. Coffee pulp has earlier found to contain low to trace amounts of tannins [4]. In addition to the alpha acids extracted from hops to provide bitterness in beer, condensed tannins are also present.Tannins have traditionally been considered anti-nutritional but known these properties depend upon their chemical structure and dosage. The new technologies used to analyse molecular and chemical structures have shown that a division into condensed and hydrolysable tannins is too simplistic [12]. Condensed tannins inhibit herbivore digestion by binding to consume plant proteins, making them more difficult for animals to digest and interfering with protein absorption and digestive enzymes. Many tannin-consuming animals secrete a tannin-binding protein (mucin) in their saliva. The tannin-binding capacity of salivary mucin is directly related to its proline content. Salivary proline-rich proteins (PRPs) sometimes used to inactivate tannins. One reason is that they inactivate tannins to a greater extent than dietary proteins resulting in reduced faecal nitrogen losses [1].

Phenolic compounds are either simple phenols or polyphenols based on the number of phenol units in the molecule. Phenols are synthesised industrially and produced by plants and microorganisms, with variation between and within species. Phenols are antioxidants in human and plants [15].

Phenolic compounds regulate the various metabolic functions, including structure and growth, pigmentation and are resistant to different pathogens in plants [18]. Phenolics derived from various natural sources are helpful as an antioxidant with anti-inflammatory properties. They can also be anti-allergic, anti-carcinogenic, and antihypertensive. There are also reports of cardioprotective, anti-arthritic and antimicrobial activities in phenolic compounds. Our study revealed that the Monkey tail contained a 0.3mg/ml amount of phenols, making it the third-highest phytochemical constituent. Flavonoids are known to have antioxidant effects and have shown to inhibit the initiation, promotion, and progression of tumours through cell signalling pathways and antioxidant effects [17]. Also, the reduction of coronary heart disease associated with flavonoid intake [19].

This research has shown that the monkey tail contains an appreciable quantity of flavonoids (0.5mg/ml). Apart from the antioxidant properties of flavonoid, other biological functions, it possesses protection against platelet aggregation, microorganisms, hepato-toxins, viruses, tumours, ulcers, free radicals, inflammation and allergies [23]. Steroids contain the backbone of cyclopentanoperhydrophenanthrene with four hydrocarbon rings in their structures. They widely exist in tissues of animals and plants and have vital significance in living activities. Steroids include sterol and its derivatives, such as Zymosterol, zoosterol, and phytosterol. Phytosterol is involved in the metabolism of plants. Cholesterol, one of the zoosterols, plays a vital role in sustaining the physical state of biomembrane. Besides, it is involved in atherosclerosis. Bile acid and vitamin D are the commonly seen sterol derivations, both of which are important to growth and development [15].

Terpenes are linear or cyclic hydrocarbons that are chemically related to steroids: they are often precursors of steroids in biosynthesis. Although terpenes are in small quantities in a cell, it has pivotal biology functions in cell signalling, metabolism, and biosynthesis. Besides, terpenes are also beneficial active ingredients in agricultural pesticides [15]. Monkey tail contains 0.2mg/ml of terpenes and steroid and can be helpful in the above-highlighted qualities.

Conclusion

This study, for the first time, has provided a qualitative and quantitative analysis of Monkey tail. Previous and present study, showed the need to exploit these metabolites' numerous health benefits. However, there will be a need to analyse further other compounds that can be obtained through a proximate analysis using more advanced molecular separation techniques for proteins, nutrients, heavy metals, and possible amino acid profiling.

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