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RESEARCH PAPER

Phytochemical and Physicochemical Studies of Fermented Liquid of *Citrulus lanatus* (Egusi) Pod as a Potential Herbicide

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Abstract

The fermented liquid of *Citrulus lanatus* (Egusi) pod was observed to have a herbicidal behavior after discarding it on grasses in the process of removing the seed from the pod which includes washing and filteration. Preliminary studies like phytochemical screening, physicochemical analysis (colour, pH, chloride, phosphate, phosphorus, total alkalinity, odour) and heavy metals (Zn and Cu) determination were carried out on the fermented Egusi pod liquid. The phytochemical screening showed that steroids, triterpenoids, saponins, terpenoids and triterpenes were present, while phenol, alkaloids, glycosides, anthracenes and flavonoids were absent. The physical analysis showed that the fermented liquid colour is creamy, turbid in appearance, a pH of 4.59, with an objectionable odour. The chemical analysis showed the absence of chloride, total alkalinity and total hardness. The total amount of phosphate and phosphorus present were 217.8 mg/L (4.35%) and 71.03 mg/L (1.42%) respectively, while the amount of Zn and Cu were 0.4426 mg/L and 4.4352 mg/L respectively. The herbicidal behavior could be attributed to its acidic nature and the high amount of phosphate.

Keywords: *Citrilus lanatus*; Fermentation; Phytochemicals; Physicochemicals; Phosphate

INTRODUCTION

Citrulus lanatus (Egusi) is indigenous to the West African region; although, it is the progenitor of the watermelon it was domesticated only for its seeds in West Africa and the Two major seed types of egusi melon (*Citrullus lanatus*) "Bara" and "Serewe" are common in Nigeria (Blench, 1997). Egusi is the name for the fat- and protein-rich seeds of certain cucurbitaceous plants (squash, melon, gourd), which after being dried and ground are used as a major ingredient in West African cuisine (Rachel, 2011). The origin of melon is Africa and Asia and areas where it is widely cultivated include the Caribbean, Indonesia and Africa. In Nigeria, the existence of melon dates back to the 17th century. Egusi melon is a popular fruit in Nigeria because of the edible seeds which are commonly used in the preparation of local soup or stew and snacks such as fried melon seed ball known as "Robo" in South - Western Nigeria (Douglas and Glenn, 1982). Major egusi-growing nations include Mali, Burkina Faso, Togo, Ghana, Côte d'Ivoire, Benin, Nigeria, and Cameroon (National Research Council, 2006).

In West Africa, the name Egusi is applied to members of the gourd family having seeds of high oil content. The Egusi Melons described here are a subspecies of the watermelon species. Both Egusi Ibara and the watermelon are of tropical African origin (ECHOcommunity.org.htm). In the East, the seeds are sometimes boiled and eaten as snacks too. The seeds are rich in oil (30 – 50 percent) which is comparable to other oil plants and the oil contains a high level of saturated fatty acids. Egusi melon is also an important component of the traditional cropping system usually interplanted with such staple crops as cassava, maize, sorghum, etc. (Adeniran and Wilson, 1981; Omidiji, 1997).

It is a succulent fleshy whitish pendam with a relatively hard but smooth and greenish epicarp. Though some varieties have their green colour streaked with white (Figure 1). The majority of the fruits are nearly spherical in shape but some are ellipsoids having slightly elongated head-tail axial dimensions (Omafuvb, 1998; Chen et. al., 1996). Egusi fruit is composed of a thick and tough outer coat, the epicarp, a softer fleshy part, the mesocarp, a segmented endocarp and the seeds. The epicarp is strongly attached to the much softer mesocarp to form what is jointly referred to as the rind. The segmented endocarp is separated from each other by the septum and within the segments are the seeds. However, on the inside, egusi fruit is neither red, nor luscious, nor sweet. Indeed, it is white and dry and bitter enough to be repulsive (Nwosu, 1988). Generally, the traditional method of removing egusi seed from the fruits involves manual cracking of the fruit with wooden clubs or cutting off the head or tail portion of the fruit with a knife, all done in order to create access for microorganisms to enter and cause the decomposition of the fleshy mesocarp and endocarp (fermentation). The fruit so treated is left for about 7 days to decompose. Then the seeds are removed by washing in water (Nwakuba, 2016).

Figure 1. (a) Matured Egusi fruit ready for harvest. (b) Horizontal view of the fruit showing seeds.

Processing of melon involves depodding, fermentation, washing drying, cleaning and shelling (Kushwaha et. al., 2005). After depodding, the pods are left on the field to rot and ferment for three to four days; after the pods are rotten and soft, the washing stage is then initiated. Fermentation is usually carried out to make the removal of the seeds from the pod easy (Jackson et. al., 2013). Fermentation is usually carried out in a moist solid state involving contact with appropriate inocula of assorted microorganism. The desired state of fermentation is indicated by the formation of mucilage and over tones of ammonia produced as a result of the breakdown of amino acids during fermentation (Omidiji, 1997). Research have been carried out on the melon pod fermentation and its effects on physicochemical characteristics of melon seeds (Jackson et. al., 2013). After removing the seeds from the fermented pod, the fermented liquid is discarded and it was observed to have herbicidal properties. Therefore the aim of this work is to carry out phytochemical and physicochemical studies on the fermented liquid as a potential herbicide.

MATERIALS AND METHODS

The fermented liquid was collected immediately after washing process, filtered with muslin cloth and stored in the refrigerator. Phytochemical and physicochemical analysis was carried out using standard methods. Atomic Absorption Spectrometer (AAS) was used for the metal analysis. All chemicals used were of analar grade.

Phytochemical Analysis of the Samples

The phytochemical analyses of the oil samples were determined as described in (Emmanuel et. al., 2014). The presence or absence of the following plant secondary metabolite was determined as follows:

Phenols

Equal volumes of each extract and ferric chloride solution (which is prepared by dissolving 135.2 g of $FeCl_3.6H_2O$ in distilled water containing 20 ml of concentrated HCl dilute to 1 L) are added together. A deep bluish green precipitate indicates the presence of phenol.

Alkaloids

Each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with (2 g of iodine in 6 g of KI in 100 mL of distilled water). Formation brown or reddish brown precipitate indicates presence of alkaloids.

Steroids

Each extract was added to 2 mL acetic anhydride and 2 mL H_2SO_4. Colour change from violet to blue or green indicates the presence of steroids.

Terpenes

Each extract was added to 0.5 mL acetic anhydride and few drops of concentrated H_2SO_4. A bluish green precipitate indicates the presence of terpenes.

Flavonoids

Ammonium solution 5 mL was added to aqueous filtrate of each extract and then few drops of concentrated H_2SO_4. Yellow coloration indicates the presence of flavonoids.

Saponins

Each extract (1 g) was boiled with 5 mL distilled water and filtered. An exact amount (3 mL) of distilled water was added to the filtrate and shaken vigorously for 5 min. Persistent frothing on warming indicates the presence of Saponins.

Triterpenoids
Crude extract was mixed with chloroform and few drops of concentrated H_2SO_4 was added, shaken and allowed to stand for some time. The formation of a yellow coloured layer indicates the presence of triterpenoids.

Glycosides
5 mL H_2SO_4 was added to each of the test extract in a boiling tube. The mixture was heated in boiling water for 15 min. Fehling's solution A and B was added and the resulting mixture was heated to boiling. A brick red precipitates indicates the presence of glycosides.

Balsam
3 drops of alcoholic $FeCl_3$ was added to 4 mL of extract which was warmed. A dark green colouration indicates the presence of balsams.

Physicochemical analysis of the samples
The physicochemical parameters such as pH, chloride, total alkaline, total hardness and phosphate (PO_4^{3-}) was determined using methods described by Bande et. al., (2013). While the minerals; zinc, lead and copper was determined using the AAS.

RESULTS AND DISCUSSION

The phytochemical results of the fermented egusi pod liquid were presented in Table 1. The results showed the presence of steroids, triterpenoids, saponins, terpenoids and triterpenes. Phenol, alkaloids, glycosides, anthracenes and flavonoids were absent. The presence of Saponins with soapy characteristic is an indication that the fermented liquid possesses bioactive agents (Abdullahi, 2012). The physicochemical properties of the fermented egusi pod liqiud is shown in Table 2. The colour of the fermented liquid is creamy, turbid in appearance with objectionable odour. It has a pH of 4.59 indicating the acidic nature of the liquid. The liquid contains no chloride. The amount of phosphate and phosphorus present in the liquid were 217.8 mg/L (4.35%) and 71.03 mg/L (1.42%), respectively. The herbicidal behavior of the fermented liquid could be attributed to the presence of the phosphate and its acidic nature. The liquid also contain Zn (0.4426) mg/L; Pb (6.9352) mg/L and Cu (4.4352) mg/L.

Table 1. Phytochemical results of the fermented Egusi pod liquid.

Phytochemicals	Test
Steroids	+
Phenol	-
Alkaloids	-
Triterpenoids	+
Saponins	+
Glycosides	-
Anthraquinones	-
Terpenoids	+
Anthracenes	-

Flavonoids	-
Triterpenes	+

Table 2. Physicochemical properties of the fermented Egusi pod liquid.

Parameters	**Test**
Appearance	Turbid
Odour	Objectionable
Colour	Creamy
pH	4.59
Total alkalinity	NIL
Total chloride	NIL
Total hardness	NIL
Phosphate	217.8 mg/L
Phosphorus	71.03 mg/L
Zinc	0.4426 mg/L
Pb	6.9352 mg/L
Copper	4.4352 mg/L

CONCLUSION

At the of this work, the phytochemical screening of the fermented liquid of egusi pod showed the presence of bioactive metabolites like steroids, triterpenoids, saponins, terpenoids and triterpenes. The physicochemical analysis showed that there is high amount of phosphate in the acidic fermented liquid which could be the reason why the fermented liquid was herbicidal in nature.

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