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Phytochemical Analysis and Preliminary *in Vitro* Non Mutagenic Activity of *Caulis bambusae* Stem Extract

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Abstract: **In this study, the phytochemical composition and mutagenic potential of *Caulis bambusae* stem extract were investigated following standard procedures, with a view to ascertain its health implications and possible deleterious effects on body cells. Results of the phytochemistry showed that *Caulis bambusae* stem extract contains flavonoids (0.09%), alkaloids (0.05%), tannins (2.10mg/dl), steroids (1.40mg/dl), carbohydrates (22.30%), anthraquinone (0.03%), glycosides (0.14%), and saponins (1.75mg/dl), while the mutagenicity study on characterized *E. coli* strain showed no significant increase (P>0.05) in induced tryptophan revertant colonies and a mutagenic index of 1.00±0.12µg/plate when compared to the positive control, indicating absence of mutagenic potential. The significant presence of these phytochemical agents in *Caulis bambusae* suggests that the plant is highly enriched with bioactive substances which can be harnessed into safe and potent medicines for the treatment of diseases and also gives credence to the use of the plant in ethno medicine for the treatment of various ailments.**

***Keywords: Caulis bambusae*, Ethno medicine, Mutagenecity, Phytochemical, Plants.**

1. INTRODUCTION

As the search for more effective ways of managing diseases continues, several plants and plant based materials have come under serious scientific evaluation and will continue to be studied for the identification of their bioactive components. These scientific investigations most times are based on the ethno medicinal values of such plants. This is why orthodox medicine appears to be strongly anchored on traditional medicine, since the results of these scientific researches when carefully managed may provide new sources of healing in addition to providing templates for the development of new orthodox synthetic drugs (Uchendu, 1999; Ijioma *et al.*, 2014). Therefore the primary aim of sourcing for plants drug through any of the known strategies is mainly to detect the active ingredients in plants that exert definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery (Ojieh *et al.*, 2013).

Phytochemicals are chemical compounds that occur naturally in plants which are usually responsible for color, flavor, odor and other organoleptic properties of the plant. Although only a small fraction of phytochemicals have been studied closely, over 4000 have indeed been identified. Current researches strongly suggest that consuming foods rich in phytochemicals provides protective health benefits (Densie, 2013). While it is evident that plants phytochemicals could act as protective agents against human carcinogenesis by acting against the initiation, promotion or progression of the progression stages of the disease (Horn *et al*., 2002), mutagenicity and cytotoxicity have also been reported in association with the use of these phytochemical agents (Horn *et al*., 2002), thus, calling for serious investigation of the toxicity levels

of the agents before use. This when done will help to eliminate possible deleterious effect arising from the use of these plant based products via careful identification, isolation and removal of the toxic component(s) without altering the required pharmacological effect. *Caulis bambusae* is one of plants that are currently being evaluated for the presence of these bioactive substances.

Caulis bambusae (bamboo) is a plant belonging to family *bambusoideae* that is commonly found in the tropical and subtropical area of Asia, where the plants is reported to have widespread ecological, environmental and social benefits (Chung, 2011). In Eastern Asia extracts from the plant are used to treat coughs and asthma. Recent scientific examination of *Caulis bambusae* stem extract revealed anti-inflammatory, anti-allergic, immune regulating and anti-oxidative capacities (Lu *et al*., 2006). The extract has also being used to treat diseases such as haemorrhoids (Corcoran *et al.*, 1994), scabies, eczema and dermatitis (Qiu *et al.*, 1992).

This study was designed to screen the stem extract of *Caulis bambusea* for the presence and quantity of phytochemicals with a view assess its possible health benefits and providing additional literature for further studies.

2. MATERIALS AND METHODS

2.1 Collection of Plant material and preparation stem Extract:

Fresh stems of *Caulis bambusea* were collected from Agbo, Ika Local Government Area of Delta State, Nigeria. The stems were separated from other plants parts, cut into smaller pieces and dried at a temperature of 33-34^{0}C. The dried stems were pulverized in a mill (Pyecan, England) to obtain a powdered sample. Fifty (50) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 70^{0}C for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 13.20g and represented a yield of 26.40%.

2.2 Qualitative Phytochemical Screening:

Preliminary Phytochemical studies was carried out using the methods of Trease and Evans, (1989), Harborne, (1973) and Sofowora, (1993) as reported by Deka and Kalita, (2012).

*** Test for Carbohydrate:** 1ml each of Fehling A and Fehling B was added to diluted extract and heated for 30 minutes and observed for the formation of brick red colour which indicated the presence of carbohydrate.

*** Test for Tannins:** 5ml of 45% ethanol was added to 2g of the extract and boiled for 5 minutes. The mixture was then cooled and filtered. 3 drops of lead sub acetate solution was added to 1ml of the filtrate. A gelatinous precipitate indicated the presence of tannins. Another 1ml of the filtrate was added to 0.5ml of bromine water. A pale brown precipitate confirmed the presence of tannins.

***Test for Saponins:** 0.5g of the extract was added to 5ml of distilled water in a test tube. The solution was then shaken vigorously and observed for a persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously. The formation of an emulsion indicated the presence of saponins.

*** Test for Flavonoids:** 0.5g of the extract was introduced into 10ml of ethyl acetate and heated in boiling water for 1 minute and filtered. 4ml of the filtrate was shaken with 1ml of 1% aluminum chloride solution and kept. The formation of a yellow colour in the presence of 1ml dilute ammonia solution indicated the presence of flavonoids.

*** Test for Alkaloids:** 5g of ground plant material was extracted with 10ml of Ammonical chloroform and 5ml of chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5M H_2SO_4. The observation of creemish white precipitate indicated the presence of alkaloids.

*** Test for Steroids:** 2ml of acetic anhydride was added to 0.5g of the extract and 2ml of sulphuric acid was added by the sides of the test tube. A violet or blue-green colouration indicated the presence of steroids.

*** Test for Phenols:** a little quantity of the extract was mixed with distilled water in a test tube and warmed followed by the addition of 2ml of Ferric chloride solution. The formation of green or blue colour indicated the presence of phenols.

*** Test for Glycosides:** about 0.5ml of the extract was added to 1ml of glacial acetic acid containing traces of Ferric chloride in a test tube. To this solution 1ml of conc. sulphuric acid was added and observed for the formation of reddish

brown colour at the junction of the two layers with the upper layer turning bluish green, indicating the presence of glycosides.

2.3 Quantitative Phytochemical Analysis:

Caulis bambusae ethanol extract was examined qualitatively and quantitatively for the presence of the following phytochemical agents: flavonoids, alkaloids, tannins, steroids, carbohydrate and anthraquinone using standard procedures as outlined by Trease and Evans (1989) and Harbone, (1973) and reported by Wisdom *et al.*, (2011).

2.4 Mutagenicity study:

Mutagenicity of *Caulis bambusae* stem extract was determined using the plate incorporation technique, modified by preincubation of *Escherichia coli* strain in the presence of metabolic activation, as reported by Maron and Ames, (1983). 100µl of an overnight grown culture, 100µl the extract and buffer were put together and preincubated at 37^0C for 30 minutes. 2ml of nutrient agar was then added and the mixture was vortexed and poured onto a plate. For each assay positive and negative controls were included. Negative control was assayed with same volume of distilled water while positive control constituted of 1µg/plate of Sodium azide. Plates were incubated at 37^0C for 72 hours before the revertant tryptophan bacterial colonies were counted. The mutagenic index (M.I) was calculated as the ratio of the number of tryptophan inductants per plate of test sample to spontaneous revertants of the negative control.

3. RESULTS

3.1 Qualitative Phytochemical composition of *Caulis bambusae* stem extract:

Qualitative phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, steroids, carbohydrates, anthraquinone, glycosides and saponins (Table 1)

Table 1: Quantitative Phytochemical composition of *Caulis bambusae* stem extract

S/N	Phytochemical	Result
1.	Flavonoids	+
2.	Alkaloids	+
3.	Tannins	+
4.	Steroids	+
5.	Carbohydrate	+
6.	Anthraquinone	+
7.	Glycosides	+
8.	Saponins	+

KEY: + = Present

3.2 Quantitative Phytochemical composition of *Caulis bambusae* stem extract:

The phytochemicals so tested were found to be present in varying quantities with carbohydrate being the highest (23.20%) and alkaloids the least (0.056%). Details are as contained in table 2.

Table 2: Quantitative phytochemical composition of *Caulis bambusae* stem extract

S/N	Parameter	Specifications
1.	Flavonoids	0.09%
2.	Alkaloids	0.05%
3.	Tannins	2.10mg/dl
4.	Steroids	1.40mg/dl
5.	Carbohydrate	22.30%
6.	Anthraquinone	0.03%
7.	Glycosides	0.14%
8.	Saponins	1.75mg/dl

3.2 Mutagenicity activity of *Caulis bambusae* stem extract:

Caulis bambusae stem extract showed no mutagenic potential. The tryptophan revertant colonies of the test was less than 2.0 and was significantly different ($P<0.05$) when compared to the number of revertant colonies in the positive control group which had a mutagenic index of 128 (Table 3).

Table 3: Mutagenicity effect of *Caulis bambusae* stem extract

Assay	Amount per plate	Mutagenic index
Test	1	1.00 ± 0.16
Positive control	1	128 ± 0.11
Negative control	1	1.00 ± 0.13

4. DISCUSSION

The phytochemical properties of *Caulis bambusae* stem extract were determined and quantified. The identified pharmacological active principles identified includes flavonoids, alkaloids, tannins, steroids, carbohydrates and anthraquinones and suggest that the stem extract of *Caulis bambusae* is enriched with significant amounts of phytochemicals and could therefore be relevant in the continuous search for potent plant based medicinal ingredients. These results tends to agree with the report of Zang *et al*., (2006). The nutritional value of *Caulis bambusae* is revealed by the presence of significant amount of carbohydrates, secondary metabolites which serve as competitive weapons against micro organisms and metal transporting agents and as agent of symbiosis between microbes and plants (Demain and Fang, 2000).

The presence of flavonoids in *Caulis bambusae* stem extract gives credence to its medicinal value. Flavonoids are a major part of phytophenolic compounds and are common dietary components present in many beverages and foods. Flavonoids have indeed been reported to have antioxidant activity, free radical scavenging capacity and have been used in the prevention of coronary heart disease and management of cancer (Yao *et al*., 2004). Flavonoids have been implicated in wound healing, cellular regeneration and cytoprotection (Lewis *et al.*, 1999; Kumar *et al*., 2013) and as such may be of benefit in ulcer management. The anti-malarial potential of flavonoids has also been reported and acts by inhibiting the fatty acid biosynthesis of the parasite and the influx of L- glutamine and myoinositol into infected erythrocytes (Ntie-kang *et al*., 2014). In this study, the percentage concentration of alkaloids present in *Caulis bambusae* is 0.056% and suggests that the extract may be useful in cancer prevention and management. Alkaloids are reported to contain large group of nitrogenous compounds which are widely used as cancer chemotherapeutic agents (Jioa *et al*., 2007; Jin-Jan *et al*., 2012). The anticancer potentials of *Caulis bambusae* is further supported by the presence of both tannins and anthraquinones in the stem extracts. Many tannin components and tea polyphenols have been reported to be anticarcinogenic, reduce mutagenic activity of a number of mutagens which may be due to their antioxidative property which is important in protecting cellular oxidative damage including lipid peroxidation. Tannins also inhibit the generation of superoxide radicals with strong antimicrobial activities (Chung *et al*., 1998). Huang *et al*., (2007) had reported the anticancer effects of anthraquinones. The ability of tannins to react with protein to provide a typical tannin effect which is important for the treatment of inflammatory or ulcerated tissues has also been reported (Parekh *et al.*, 2005) and most plants that contain tannin as their main component are astringent in nature and are used for treating intestinal disorders such as diarhoea and dysentery (Wang, 2003). Steroids, which are part of the phytochemical composition of *Caulis bambusae* is reported to increase protein synthesis, promote growth of muscles and bones and shows some level of antiviral activities (Neuman *et al*., 2005). Saponins and glycosides are reportedly been used to alleviate cardiac problems associated with hypertension (Trease and Evans, 1985). Saponins have also been found to be useful in the management of hypercholesterolaemia in humans as it binds to cholesterol in the body to inhibit the reabsorption of the later thereby facilitating its excretion from the body. The non mutagenic and non cytotoxic potential of *Caulis bambusae* stem extract attests to the high margin of safety associated with its use for treatment purposes. This non mutagenic effect of the extract may be due to the presence of flavonoids which have been implicated in antimutagenecity (Horn *et al*., 2002).

The significant presence of these phytochemical agents in *Caulis bambusae* suggests that the plant is highly enriched with bioactive substances and can be harnessed into potent medicines to help meet the numerous health challenges of man. The use of the plant in ethno medicine is hereby justified and credence is given to its medicinal potentials.

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