

## The Phytochemical, Functional Group and Anti-Microbial Assay of the Chloroform Methanol and Chloroform Stem Extracts of *Breynia nivosus* Plant

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**Abstract:** Phytochemical activities on *Breynia nivosus* stem also known as snowbush plants, is a study of organic compounds present in *Breynia nivosus* as a medicinal plant (herb). The screening of *Breynia Nivosus* stem revealed the presence of flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, phenols, resins, cardiac glycosides, carbohydrates and proteins. The anti-microbial screening of the crude extract of *Breynia nivosus* stem showed marked anti-microbial activities on *candida albicans*, hence they are fungicidal. The TLC analysis showed two spots for chloroform-methanol extracts and three spots for chloroform extracts. The infrared and ultraviolet spectra of the isolated components from the chloroform-methanol fractions revealed the presence of some functional groups such as OH, N-H, C=N, C=O and C-H. The chloroform extracts contained mainly; N-H, C=N, C=C, C=O, C-O and C-H groups attached to conjugated 1, 2, 3, trisubstituted amine or amide. The presence of these secondary metabolites justifies the use of the plant stem to cure tooth defects of any kind.

**Key words:** *Breynia nivosus* • Phytochemical • Anti-microbial and Functional Groups

### INTRODUCTION

*Breynia nivosus* stem is among the many plants and herbs used in Nigeria and other countries to cure ailment, it is used in treating dental defects. These plants and herbs are well known as medicinal plants which are products of nature. They exert all their curative and preventive power when used in with other health improving natural products such as the sun, water, fresh air, healthy food and mental balance. They should not be used only when looking for a curative action as we do with pharmaceutical products when ailment appears [1].

*Breynia nivosus* is a rounded shrub that is used primarily for its attractive foliage. This 5- to 8- foot-tall, vase-shaped rounded plant has variegated leaves with white, green and red coloration. The plants appear to differ in foliage coloration with some showing whitish new growth and others with a white and green variegation. It is a round, tropical perennial plant with its fruits as red berries that are 3/8 inch wide [2]. The plant stems are also vibrant in color, often portraying a bright red hue that can add depth to garden arrangements. When placed in

different locations, the plant can also change color. To avoid brown leaves, *Breynia Nivosus* plants are kept safely at temperatures higher than 60° Fahrenheit (16°C). Other names for the shrub include snowbush, snow-on-the-mountain, ice plant or sweetpea bush. They are native to Australia and Asia and members of the *phyllanthaceae* family. Found in warm climates and normally cannot survive harsh winters in colder areas. There is paucity of information in the available Nivosus, claimed to possess some ethnomedicinal uses. Therefore, the aim of the present study was to investigate the phytochemical, functional groups and antimicrobial activities of the extracts of *Breynia Nivosus* stem in order to validate the uses of the plant in the treatment of microbial diseases.

### MATERIALS AND METHODS

**Plant Collection:** Fresh stems of the matured plant were collected from Uruagu community in Ummongali village in Oba, Anambra State Nigeria in the month of January 2014. The plant specimen was identified by Prof. J.C. Okafor of Fame Agricultural Center, No. 3 Kingsway, Enugu, Nigeria.

**Preparation of Materials:** The stems of *Breynia Nivosus* plant were chopped to smaller sizes, washed and air dried under room temperature 25°C for several days. It was pulverized using a manual blender and stored dry in an air tight container until needed for analysis.

**Extraction:** 250 cm<sup>3</sup> of the absolute ethanol was added to 20g of the powdered stem in a conical flask. The mixture was stirred, covered and was allowed to stand for 24 h and filtered using sterile Whatman No. 1 filter paper.

**Phytochemical Screening:** The preliminary screening of the extract was carried out for flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, phenols, resins, cardiac glycosides, carbohydrates and proteins using standard laboratory procedures [3] and [4]. The chloroform and chloroform-methanol extracts were monitored using TLC, Fourier Transform Infrared and Ultraviolet Spectroscopic methods.

**Test Microorganisms:** Pure isolates of *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* were obtained from FEZ Laboratory Onitsha, Anambra State, Nigeria.

**Antimicrobial Activity:** The antimicrobial activity of different concentrations of the methanol extracts were determined by modified agar-well diffusion method [5] and [6]. Briefly, solutions of varying concentrations, ranging from 1.5 - 20 mg/ml were prepared for the extract. Nutrient agar was prepared, sterilized and used as the growth medium for the pathogenic microorganisms. Normal saline was used to prepare a turbid suspension of the microorganisms and the solution was diluted to the McFarland scale ( $1.5 \times 10^8$  cfu/ml). The medium was seeded with the test microorganisms and the inocula were spread evenly over the surface of the medium on the plate using sterile swab. A standard cork borer of 8-mm diameter was used to cut the wells on the surface of the medium and the extracts were introduced into the wells. The plates were incubated at 37°C for 24 h and observed for zones of inhibition of growth, produced by the minimum inhibition concentration (MIC). The diameters of the zone of inhibition were measured and recorded in millimeters. The minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) test was carried out<sup>7</sup> in order to determine whether the extract induced the death or only inhibited the growth of the microorganisms. Briefly, nutrient agar plates were

prepared according to the manufacturer's instructions and sterilized at 121°C for 15 min. Thereafter, the medium was poured into plates, cooled and allowed to solidify. The contents of the MIC test-tubes in the serial dilution were sub-cultured into each labeled blood agar plate, using sterile wire loop and spread on the blood agar. The plates were incubated at 37°C for 24 h, after which they were observed for growth. The MBC was the value obtained in the plate with the lowest concentration of the extract without growth. The MBC values were determined and recorded. A control experiment was also set up using pure dimethyl sulphoxide for each of the test microorganisms.

## RESULTS AND DISCUSSION

The Phytochemical screening of the stem part of *Breynia nivosus* revealed the presence of alkaloids, flavonoids, resins, saponins, carbohydrates, tannins, cardiac glycosides, steroids and protein (Table 1). The secondary metabolites have been found to possess pharmacological activities, responsible for the use of plants in traditional phytomedicine to treat diseases caused by pathogenic microorganisms. All the secondary metabolites present proved the high efficiency of the plant in herbal medicine. The sample tested revealed high concentration of flavonoids which indicated that the plant is a good source of antioxidants which mops up free radicals in the system and helps the immune system to function properly. The concentration of tannin in the tested sample proved the authentic ability to act as an antidote to poisoning by an alkaloid and its ability to heal fresh injury. Tannin is used as parasite expeller from the body due to its antiseptic properties. Also the concentration of alkaloid in the crude extract of *Breynia nivosus* stem revealed the pharmacological effects as used as recreational drugs such as local anesthesia, stimulant cocaine, caffeine and nicotine [4]. Saponins possess a unique property to effect hydrolysis of red-blood cells (RBC) even in high dilutions, therefore, have been used in the treatment of a number of cardiovascular disorders, facilitate and ease the process of digestion [7] and [8].

The presence of cardiac glycosides showed that the extract can invariably be employed to slow the heart rate in atrial fibrillation and also be administered in congestive heart failure (CHF). Highly presence of rennin confirms the possibility of the extract potency in cardiovascular disorders. This is because, the role of rennin in the cardiovascular disorders is extremely vital and critical by virtue of the fact that it exclusively is responsible for the

Table 1: Phytochemical screening of *Breynia nivosus* stem extract.

Class of Phtochemical compounds	Inference
Flavonoids	+++
Resin	+++
Tannin	++
Saponin	+++
Steroids	+
Cardiac glycosides	++
Carbohydrate	++
Protein	+

Key +++ (highly present)  
 ++ (moderately present)  
 + (slightly or sparingly present)

Table 2: Thin layer chromatographic characteristics extracts of *Breynia nivosus*

Sample	Number of spots	Rf Value
Chloroform/Methanol extract	2	0.6 & 0.8
Chloroform extract	3	0.3, 0.5 & 0.8

Table 3: Results of Fourier Transformed Infrared and Ultraviolet spectra of Chloroform-methanol extract

Wave number (cm <sup>-1</sup> )	Suspected chromophores
3442.09	O-H stretch of alcohols and phenols
3432.44	N-H stretch of amines
3424.73	N-H stretch of amides
2104.41	C=N stretch of nitriles
1018.45	C-H deformation bonds for alkyl groups
UV <sub>max</sub> 213,256&366nm	Indicating highly conjugated 1,2,3 trisubstituted aromatic compound

Table 4: Results of Fourier Transformed Infrared and Ultraviolet spectra of Chloroform-methanol extract

Wave number (cm <sup>-1</sup> )	Suspected chromophores
3423.76	N-H stretch of amines and amides
2329.12	C=N stretch of nitriles
2115.02	C=C stretch of alkenes
1654.01	C=O stretch of ketones, carboxylic acids and esters.
1452.45	C=C stretch of alkenes and aromatics
1108.14	C-O stretch of alcohols, carboxylic acids and esters
1023.27	C-H deformation for alkyl groups.
UV <sub>max</sub> 221,235,244,255, 265,270,305,397,400 &659nm	Indicating highly conjugated 1, 2, 3 trisubstituted aromatic compound.

Table 5: Results of Anti-microbial Screening of Methanol extracts of *Breynia nivosus* stem

Test organism	Zone of Inhibition (mm)
<i>Candida albicans</i>	12
<i>Staphylococcus aureus</i>	-
<i>Escherichia coli</i> ,	-

maintenance of blood volume, arterial blood pressure and the electrolyte balance in the body [8]. The thin layer chromatography of the stem extract (Table 2) showed two

components with R<sub>f</sub> values of 0.6 and 0.8 when CHCl<sub>3</sub>-MeOH extract was spotted, that of CHCl<sub>3</sub> extract showed three spots with R<sub>f</sub> values of 0.3, 0.5 and 0.8 respectively. The extracts TLC results confirmed the presence of some components and its high purity. The results of the FTIR (Table 3) showed strong absorption at 3442.09 cm<sup>-1</sup>, 3432.44 cm<sup>-1</sup> and 3424.73 cm<sup>-1</sup> which indicated the presence of alcohols, phenols and amines. The absorption at 2104.41 cm<sup>-1</sup>, 1644.37 cm<sup>-1</sup> and 1018.45 cm<sup>-1</sup> showed the presence of nitriles, ketones and C-H deformation for alkyl groups. The presence of C=O, C=C and C-O (Table 4) for keto attached to benzene ring, alkenes and alcohols were shown by absorption at 1654.01 cm<sup>-1</sup>, 1452.45 cm<sup>-1</sup> and 1108.14 cm<sup>-1</sup> respectively. The absorption in the ultraviolet visible spectra and FTIR spectra suggested that the active compound might be 1, 2, 3-trisubstituted aromatic compound with OH, N-H, C=N and N=N groups attached. The results of antimicrobial sensitivity tests (Table 5) confirmed that the stems of *Breynia nivosus* exert a marked antifungal screening activity on *Candida albicans* while for *Staphylococcus aureus* and *Escherichia coli*, the antimicrobial screening activity marked negative. These findings support the reasons *Breynia nivosus* has position among medicinal plants used for the treatment microbial infections.

**Conclusion/Recommendation:** The results of the phytochemical screening and antimicrobial activity of *Breynia nivosus* agree with the previous findings by Amadi *et al.* [2] and [10-15]. The presence of cardiac glycosides and resin in high concentration of the stem extract of *Breynia nivosus* can be used for the treatment of cardiovascular disorder. *Breynia nivosus* stem could be used in the cure and management of other various diseases such as toothache, gum inflammation and enamel infection due to the phytochemical properties present in the plant. TLC result showed high purity of crude fractions and the extract have been shown to be effective against *Candida albicans*. The FTIR and UV spectra showed that it contained some bioactive compounds. The medicinal use of the stem should be encouraged. However, there is every need for more research on the plant for structural determination of the active components, toxicological effect and dosage.

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