

## Comparative Evaluation of the Phytoconstituents, Antimicrobial and Functional Group of Stem and Root Extracts of *Breynia Nivosus* Plant

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### ABSTRACT

The study on *Breynia Nivosus* stem and root was to validate and compare the presence of Phytochemicals, their possible use in ethno-medicine and identification of their active compound. The phytochemical screening revealed the presence of flavonoids, saponins, tannins, steroids, terpenoids, alkaloids, phenols, resins, cardiac glycosides, carbohydrates and proteins in both the stem and root of *Breynia Nivosus*. 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 root extract showed no antimicrobial effect on *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. The TLC analysis of the stem extract showed two spots for chloroform-methanol extracts and three spots for chloroform extracts while the root extract revealed one spot each for chloroform and chloroform methanol extracts. The Fourier Transform Infrared and Ultraviolet spectra analysis of the stem extract suggested that the active compound might be 1, 2, 3-trisubstituted aromatic compound with OH, N-H, C=N and N=N groups attached. While that of the root extract suggested that the active compound might be 1, 2, 3-trisubstituted aromatic compound with OH, N-H, C=N and C=O groups attached. The presence of these secondary metabolites proves that *Breynia Nivosus* stem and root exhibit some medicinal values and could be used for ethno-medicinal purpose.

Key words:

*Breynia nivosus*, Phytoconstituents, Anti-microbial and functional groups

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### INTRODUCTION

*Breynia nivosus* belong to the family of *Euphorbiaceae* with its common names as ice plant or snow bush or snow-on-the-

mountain or sweetpea bush and “ogwu eze” as the local name. It is not a tree but a shrub that posses self supporting

stems. It is also among the many plants and herbs used in Nigeria and other countries to cure ailment. It is grown for its attractive red zigzag stem and variegated foliage, found in the tropical region and typically grows 2-4m tall. It has papery leaves which are like rose, white, green and bright red. The slender, angular branches of this ornamental shrub are densely clothed in colourful green leaves marbled with white. Its pink, white and green leaves have a flower like appearance hence the plant other common name of leaf flower. The actual flowers which are insignificant and greenish are sometimes produced,[1]. An interesting feature is the plant ability to change colour. In sunny locations the colour of the new leaves starts out green, then maroon, then pink and finally to pure white. If placed in shade, the leaves will turn to green. They also turn green with age, maintain colour year round by placing it in a sunny area. Snowbush is a rounded shrub that is used primarily for its attractive foliage.

Medicinal plant represents a rich source of powerful anti microbial agent. A wide range of medicinal plant parts (roots, stems, flowers, fruits etc) have their extracts used as raw drugs and they posed varied medicinal properties as a result of their shown activities against most dreaded pathogenic organisms like bacteria, fungi etc[2]. The use of medicinal plants as traditional medicine is well known in rural areas of many developing countries including our

country Nigeria for the treatment of common infections. They are believed to be of an important source of new chemical substance with potential therapeutic effects,[3]. Many of these medicinal plants are used as spices and are sometimes added to food meant for pregnant and nursing mothers for medicinal purposes,[4]. They are generally used in traditional medicine for the treatment of many ailment,[5]. There has been a considerable growth in the field of herbal medicine due to its natural origin, availability and cheapness. In the developed countries 25% of the medical drugs are based on plants and their derivatives,[6]. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on the human body,[7]. Phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. They are formed during the plants normal metabolic processes,[8]. The increasing failure of chemotherapeutic and antibiotics resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity. There is paucity of information in the available literature on the antimicrobial activities of root and stem extracts of *Breynia nivosus*. This present study therefore investigates the phytochemical, functional groups and antimicrobial activities of the root and stem extracts of *Breynia nivosus* to validate its therapeutic effects.

## MATERIALS AND METHODS

### Plant collection and preparation

Fresh roots and stem of the matured plant were respectively collected from Uruagu community in Ummongali village in Oba, Anambra State Nigeria in the month of January 2014. The plant specimen were identified by Prof. J.C. Okafor of Fame

Agricultural Center, No. 3 Kingsway, Enugu, Nigeria. The samples 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. Then, 250 cm<sup>3</sup> of the absolute ethanol was added to 20g of the powdered samples respectively in a conical flask. The mixture was separately stirred, covered and 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],[ 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],[ 9].

## RESULTS AND DISCUSSION

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

Class of Phtochemical compounds	Stem Inference	Root Inference
Alkaloids	++	+++
Flavonoids	+++	++
Resin	+++	++
Tannin	++	++
Saponin	+++	+
Steroids	+	+
Cardiac glycosides	++	++
Carbohydrate	++	++
Protein	+	++
Terpenoids	++	++

Key +++ (highly present)

++ (moderately present)

+ (slightly or sparingly present)

The Phytochemical screening of the stem and root parts of *Breynia nivosus* revealed the presence of all the tested phytocompounds. 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 samples showed equal concentration of some phytocompounds

which include tannins, steroids, cardiac glycosides, carbohydrates and terpenoids. The root and stem samples showed high and moderate concentration of alkaloid respectively which revealed the pharmacological effects when used as recreational drugs such as local anesthesia, stimulant cocaine, caffeine and nicotine,[4].The root and stem also depicted moderate and high concentration of flavonoids respectively 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 moderate concentration of tannin in the tested samples proved their 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 high concentration of Saponins in the stem 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[10].

The moderate concentration of cardiac glycosides in the samples 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 maintenance of blood volume, arterial blood pressure and the electrolyte balance in the body[11].

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

Sample	Number of spots		Rf Values	
	Stem	Root	Stem	Root
Chloroform methanol extract	2	1	0.6 & 0.8	0.6
Chloroform extract	3	1	0.3, 0.5 & 0.8	0.8

The thin layer chromatography of the stem extract showed two components with  $R_f$  values of 0.6 and 0.8 when  $\text{CHCl}_3$ -MeOH extract was spotted, that of  $\text{CHCl}_3$  extract showed three spots with  $R_f$  values of 0.3, 0.5 and 0.8 respectively. While the

root extract showed one component each when  $\text{CHCl}_3$ -MeOH and  $\text{CHCl}_3$  extracts were spotted with  $R_f$  values of 0.6 and 0.8 respectively. The extracts TLC results confirmed the presence of some components and their high purity.

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

Wave number ( $\text{cm}^{-1}$ )	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 $\lambda$ 213, 256 & 366nm	Indicating highly conjugated trisubstituted aromatic compound

**Table 4: Results of Fourier Transformed Infrared and Ultraviolet spectra of Chloroform stem 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 $\lambda$ 21,235,244,255, 265,270,305,397,400 & 659nm	Indicating highly conjugated 1,2,3 trisubstituted aromatic compound.

**Table 5: Results of Fourier Transformed Infrared and Ultraviolet spectra of Chloroform root extract**

Wave number (cm <sup>-1</sup> )	Suspected chromophores
3421.83	O-H stretch of alcohols, phenols and carboxylic acid
2983.98	C-H stretch of alkanes and aromatics
2852.81	C-H stretch of alkanes
2123.70	C=N stretch of nitriles.
1647.26	C=O stretch of ketones, acid amides and esters
1458.23	C=C stretch of alkenes and aromatics
1021.34	C-H deformation bonds for alkyl groups.
UV $\lambda$ 721 & 657	Indicating highly conjugated 1,2,3 trisubstituted aromatic compound with C=O & C=N

**Table 6: Results of Fourier Transformed Infrared and Ultraviolet spectra of Chloroform-methanol root extract**

Wave number (cm <sup>-1</sup> )	Suspected chromophores
3398.69	N-H stretch of amines and amides
2951.19	C-H stretch of alkanes and aromatics
2841.24	C-H stretch of alkanes
2142.99	C=N stretch of nitriles
1652.09	C=O stretch of ketones, acid amides, esters and carboxylic acids
1438.94	C=C stretch of alkenes and aromatics
1110.07	C-O stretch of alcohols, esters & acids
1023.27	C-H deformation bonds for alkyl groups
UV $\lambda$ 745 & 495nm	Indicating highly conjugated trisubstituted aromatic compound with C=O, C=N & C=C

Table 3 represents the FTIR chloroform methanol stem extract results with strong absorption at 3442.09cm<sup>-1</sup>, 3432.44cm<sup>-1</sup> and 3424.73cm<sup>-1</sup> which

indicated the presence of alcohols, phenols and amines. The absorption at 2104.41cm<sup>-1</sup>, 1644.37cm<sup>-1</sup> and 1018.45cm<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 depicted in table 4 for keto attached to benzene ring, alkenes and alcohols were shown by absorption at  $1654.01\text{cm}^{-1}$ ,  $1452.45\text{cm}^{-1}$  and  $1108.14\text{cm}^{-1}$  respectively. The absorption in the ultraviolet visible spectra and FTIR spectra of the stem extract 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 the chloroform root extract of FTIR (table 5) showed strong absorption at  $3421.83\text{cm}^{-1}$ ,  $2983.98\text{cm}^{-1}$  and  $2852.81\text{cm}^{-1}$  which indicated the presence of alcohols, phenols, carboxylic acids, alkanes and

aromatics. The absorption at  $2123.70\text{cm}^{-1}$ ,  $1647.26\text{cm}^{-1}$  and  $1021.34\text{cm}^{-1}$  showed the presence of nitriles, ketones, acid amides and C-H deformation bonds for alkyl groups. The presence of C=O, C=C and C-O represented in table 6 for keto attached to benzene ring, alkenes and alcohols were shown by absorption at  $1652.09\text{cm}^{-1}$ ,  $1438.94\text{cm}^{-1}$  and  $1110.1407\text{cm}^{-1}$  respectively. The absorption in the ultraviolet visible spectra and FTIR spectra of the root extract suggested that the active compound might be 1, 2, 3-trisubstituted aromatic compound with OH, N-H, C=N and C=O groups attached.

**Table 7: Anti-microbial Screening of Methanol extracts of *Breynia nivosus* stem and roots**

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

The results of antimicrobial sensitivity tests 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. Though, the root sample showed marked negative antimicrobial effect on the tested microorganisms.

### CONCLUSION/RECOMMENDATION

The results of the phytochemical screening with all the secondary metabolites present showed that both the stem and root extracts of *Breynia nivosus* possess pharmacological activities, responsible for the use of plant parts in traditional phytomedicine to treat diseases caused by pathogenic microorganisms. There depict a higher concentration of alkaloids and protein in the root extract while flavonoids, resins

and saponin were of higher concentration in the stem extract. As a result, *Breynia nivosus* stem and root could be used in the cure and management of various diseases such as toothache, gum inflammation and enamel infection. TLC result showed high purity of crude fractions and the stem 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 and root should be encouraged. However, there is every need for more research on the plant for more

antimicrobial effect, structural determination of the active components, toxicological effect and dosage.

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