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Full Length Research Paper

Effect of microbial spoilage on phytochemistry, antisickling and antimicrobial potential of *Newbouldia laevis* leaf extract

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The effect of microbial spoilage on phytochemistry, antisickling and antimicrobial potential of *Newbouldia laevis* leaf extract has been reviewed. Preliminary phytochemical screening of the neat, undegraded extract showed presence of glycosides and saponins, which were not observed after microbial spoilage, instead sugars, free phenols and tannins became prominent. The antimicrobial activities of both good and spoilt samples showed they were active against the three human pathogens tested (*Coliform bacilli, Staphylococcus aureus* and *Salmonella typhi*). However, the microbial spoilage produced a better antibiotic drug against *Coliform bacilli* but weakened the drug activity against *Salmonella typhi* and *Staphylococcus aureus*. It was concluded that the spoilage microorganisms broke down glycosidic linkages and produced simple sugars that formed their food nutrients. In the process, they altered the phytochemistry of the extract, produced acidic substances, reduced the pH and probably hindered the growth of other microorganisms.

Keywords: Antimicrobial, Antisickling, Phytochemistry, Microbial spoilage.

INTRODUCTION

Medicinal plants have received huge attention both in the developed and developing nations. Their economic importance has drawn attention of various world bodies mostly; the World Health Organization (WHO) which released a special document concerning collection practices for medicinal plants (WHO, 1973; 2003). The plant, *Newbouldia laevis* (also called the “Tree of life” or “Ogirishi” in Igbo language) of the family of *Bignoniaceae*, is commonly grown as a live fence and may be found around groves and shrines. It is easily recognized by its short branches, coarsely toothed leaflets and purple and white flowers (Anibijuwon *et al.*, 2010). The leaves of *Newbouldia laevis* are used among the Igbo of South Eastern Nigeria for the treatment of conjunctivitis, ear-ache, dysentery, cough, hernia and stomach ache. The plant is used to stop vaginal bleeding in threatened abortion. The leaves and roots (mixed together and boiled) are used to treat fever, convulsion and epilepsy. The root alone is used as round worm vermifuge and treatment for migraine. The stem bark is used in the treatment of impotence, infertility and various skin infections. One thing remarkable about this plant is that it hardly dies hence it is used to indicate boundary marks among the Igbo people of South Eastern Nigeria.

The bactericidal effects of plant extracts have been reported and several attempts made to destroy various bacteria and their spores by the application of these extracts (Jussi-Pekka *et al.*, 2000; Smith-Palmer *et al.*, 2001; Kotzekidou *et al.*, 2008; Bakkali *et al.*, 2008), yet several plant extracts and their metabolites are destroyed by attack of microorganisms of the air (Ejele, 2010; Akpan, 2011). Microorganisms use our food materials as sources of nutrients for their own growth. They utilize food ingredients, produce enzymatic and chemical changes in the food materials and contribute off-flavours by the breakdown of carbohydrates, fats, proteins and other food products. They may even synthesize new products resulting in deterioration and spoilage of the food (Larkin, 1973; Frazier and Westhoff, 1995). The carbohydrates, especially sugars, are commonly used by

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Table 1. Preliminary phytochemical screening on extracts

Test	Good	Spoilt
Tannins	+	++
Saponins	+++	-
Flavonoids	+	++
Steroids	-	+
Cardio-active glycosides		
a- Hiberman test	++	-
b- Salkowski test	++	-
Sugars	-	+++
Amino acids	++	+
Alkaloids	++	++
Phenols	+	+++

+++ → Prominent ++ → Moderate + → Low – → Negative

microorganisms as sources of energy while proteins, peptides and amino acids serve as energy foods for proteolytic organisms (Broughall and Brown, 1984).

In the course of our study of plant extracts, we have observed the ease of spoilage of some plant extracts by microorganisms of the air. In an effort to identify the food nutrients these microorganisms fed on and changes produced in these extracts, we have studied the change in phytochemistry of these plant extracts before and after the microbial attack. In this paper, we report on the effect of microbial spoilage on phytochemistry, antimicrobial and antisickling potential of *Newbouldia laevis* leaf extract.

MATERIAL AND METHODS

Sample collection and extraction

The leaves of *Newbouldia laevis* were collected from Obowu in Imo State, Nigeria and authenticated at the Department of Plant Science and Technology, Federal University of Technology, Owerri as *Newbouldia laevis*, belonging to the family of *Bignoniaceae*. The leaves were sun-dried and ground to produce a semi powdered sample. 30g of this sample was extracted with 250ml of ethanol for 12h in a soxhlet extractor equipped with a reflux condenser. The ethanol extract was allowed to evaporate at room temperature to give a gel-like solid, which was dissolved in ethanol/water mixture (4:1) and filtered. The filtrate was divided into two equal portions. One portion was used for preliminary phytochemical, antimicrobial and antisickling screening while the other was left open in the laboratory and observed for microbial spoilage, after which the phytochemical, antimicrobial and antisickling experiments were repeated on the spoilt extract as earlier reported (Ejele and Njoku, 2008; Ejele and Akujobi, 2011). The results are presented in Tables 1, 2 and 3 respectively.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the undegraded plant extract revealed the presence of alkaloids, amino acids, cardio-active glycosides, saponins and tannins whereas the extract attacked by microorganism showed the absence of cardio active glycosides and saponins which were originally present before microbial spoilage (Table 1). However, some phytochemicals, such as flavonoids, tannins and sugars, which were initially absent before the microbial attack, became prominent in the spoilt extract. These results suggest that the microorganisms responsible for the spoilage (*Bacillus spp, Aspergillus spp, Staphylococcus spp and Mucor spp*) probably destroyed the saponins and other glycosides to produce simple sugars and aglycons. This would involve the splitting of glycosidic linkages. The presence of flavonoids and increased levels of tannins and phenols in the microbial degraded extract confirmed that polyphenolic glycosides were involved.

On the other hand, the reduced concentrations of amino acids probably suggests that the microorganisms may have fed on them, since proteins, peptides and amino acids are known to serve as energy foods for proteolytic organisms (Broughall and Brown, 1984).

The antimicrobial activity of the undegraded ethanol extract on some human pathogens was shown in Table 2, from which it may be seen that extract was active against the three microrganisms tested; namely: *Coliform bacilli, Salmonella typhi* and *Staphylococcus aureus*. Table 2 showed that the MIC of ethanolic extract against *Coliform bacilli* was 0.5mg/ml while that of the spoilt extract against this organism was 0.25mg (Table 3). The MIC for the spoilt and unspoilt extracts against *Salmonella typhi* was 0.125mg The MIC for the good ethanolic extract against *Staphylococcus aureus* was 0.0625mg/ml while that of the spoilt extract was 0.125mg/ml.

The zone of inhibition at 1.0mg/ml concentration of the

Table 2. Antimicrobial activity / MIC of Good (Unspoilt) Extract

Microorganism	Concentration				
	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
Coliform bacilli	12mm	8.3mm	-	-	-
Salmonella typhi	25mm	18mm	12mm	8.5mm	-
Staphyl. aureus	28mm	20mm	15mm	10.5mm	7mm

Table 3. Antimicrobial activity and MIC of Spoilt Extract

Microorganism	Concentration				
	1.0mg/ml	0.5mg/ml	0.25mg/ml	0.125mg/ml	0.0625mg/ml
Coliform bacilli	24 mm	16 mm	10 mm	-	-
Salmonella typhi	22 mm	18 mm	14 mm	8 mm	-
Staphyl. aureus	25 mm	15 mm	10 mm	8 mm	-

Table 4. Antisickling potential of the Unspoilt extract RBC Count ($X10^6$/ne)

Sample number	Volume of extracts (drops)	Number of sickled cells	Number of unsickled cells	Gelation time (min)
Control I HbAA	-	-	500	-
Control II HbSS	-	500	-	4
Slide D_1	2	50	450	25
D_2	4	110	390	21
D_3	6	130	370	18
D_4	8	150	350	13
D_5	10	200	300	9

good (unspoilt) extract were 12, 25 and 28mm against *Coliform bacilli, Salmonella typhi* and *Staphylococcus aureus* respectively whereas those of the microbial degraded extract were 24, 22 and 25mm respectively, showing that microbial spoilage produced a better antibiotic drug against *Coliform bacilli* but produced a slightly weaker drug against *Salmonella typhi* and *Staphylococcus aureus*.

Antisickling potential of the samples

The antisickling potential of the undegraded extract is shown in Table 4, which indicated that at all concentrations the extract inhibited the sickling of HbSS erythrocytes.

It was observed from the Table that the number of sickled RBCs counted increased with increasing extract concentration from 50 to 200 (x 10^6/ne) while that of unsickled RBCs dropped from 450 to 300 (x 10^6/ne) as the extract concentration increased from 2 to 10 drops. A similar trend was also seen in Table 5, which showed the antisickling properties of the spoilt, microbial degraded extract. These results showed that both the good and bad samples were good inhibitors of the sickling phenomenon but the ability to reverse the sickled erythrocytes to their normal morphology decreased with increasing extract concentration. The gelation time also decreased in like manner (with increasing concentration of extract) suggesting that the presence of “unfriendly components” in both samples. A similar observation had earlier been made concerning the antisickling and reversal potential of *Aloe vera* extract (Ejele and Njoku, 2008) and the basic metabolites of *Cajanus cajan* extract (Ejele, 2010).

It was expected that as the concentration of the extracts increased, the number of unsickled RBCs as well as the gelation times should increase, while the number of sickled RBCs should decrease, since this would show there was reversion of sickled cells, but these were not observed. The ability of an agent or compound to increase the gelation time of human HbSS blood sample could be taken as a measure of the antisickling potential

Table 5. Antisickling potential of Microbial degraded extract RBC Count (X 10^6/ne)

Sample number	Volume of extracts (drops)	Number of sickled cells	Number of unsickled cells	Gelation time
Control I HbAA	-	-	500	-
Control II HbSS	-	500	-	4
Slide E_1	2	-	500	-
E_2	4	100	400	31
E_3	6	130	370	25
E_4	8	150	350	18
E_5	10	160	340	15

of the compound and determines the ability of the agent to retard the aggregation of erythrocyte cells in blood vessels. Such reduction in aggregation rate is related to gelation inhibition. This notwithstanding, both samples could be very effective and useful anti-sickling agents if used at low concentrations. For example, on the addition of two drops of the microbial degraded extract, 100% reversal of the sickling phenomenon was indicated and no sickling was observed in the presence of sodium metabisulphite, a strong reducing agent (Table 5). Similarly, the unspoilt extract showed 90% reversal under the same conditions (Table 4), suggesting that both samples possessed antisickling and reversal potential.

Thus, this study supports the already known fact that hydrolysis of the glycosides produce simple sugars and aglycons; and the aglycon in this case may probably be a simple phenol, flavonoid or tannin, which could be a useful antimicrobial and/or antisickling agent. Hence, it might be concluded that the microorganisms involved in the spoilage (*Bacillus spp, Aspergillus spp, Staphylococcus spp and Mucor spp*) opened glycosidic bonds to release simple sugars (such as glucose or fructose) upon which they fed, altered the phytochemistry of the extract and produced free phenolic aglycon.

CONCLUSION

The effect of microbial spoilage on phytochemistry, antisickling and antimicrobial properties of *Newbouldia* leaf extract was studied. Preliminary phytochemical screening of the extract showed presence of glycosides and saponins, which .were not observed after microbial spoilage, instead sugars, free phenols and tannins became prominent. The antimicrobial potential of both good and microbial degraded samples showed significant bioactivity against three human pathogens; *Coliform bacilli, Salmonella typhi* and *Staphylococcus aureus*. However, the microbial spoilage produced a better antibiotic drug against *Coliform bacilli* but reduced activity of the extract against *Salmonella typhi* and *Staphylococcus aureus*. It was concluded that the spoilage microorganisms broke down glycosidic linkages and produced simple sugars that formed their food nutrients. In the process, they altered the phytochemistry of the extract, produced acidic substances, reduced the pH and probably hindered the growth of other microorganism.

REFERENCES

Akpan IO (2011). "Evaluation of Antimicrobial, Antisickling and Antimalarial Potentials of Plant Extracts". M.Sc. Thesis. Federal University of Technology, Owerri. Imo State, Nigeria.

Anibijuwon IL, Duyilemi OP, Onifade AK (2010). "Antimicrobial Activity of Leaf of *Aspila Africana* on Some Pathogenic Organisms of Clinical Origin". Niger. J. Microbiol. 24(1): 2048 – 2055.

Bakkali F, Averback S, Averback D, Idaomar M (2008). "Biological effects of essential oils – A review". Food and Chemical Toxicology: 46, 446 – 475.

Broughall JM, C Brown (1984). "Hazard analysis applied to microbal growth in foods: development and application of three – dimensional models to predict microbial growth." Food Microbiology: 1, 13 – 22.

Ejele AE, Njoku PC (2008). Anti-sickling potential of Aleo vera extract. J. Sci. Food and Agric. 88, 1482 – 1485.

Ejele AE (2010). "Effect of some Plants Extracts on the Microbial Spoilage of *Cajanus cajan*". International J. Tropical Agriculture and Food Systems: 4(1), 46 -49.

Ejele AE, CO Akujobi (2011). "Effects of Secondary Metabolites of *Garcinia kola* on the Microbial Spoilage of *Cajanus cajan* Extract". Int. J. Tropical Agric. Food Systems: 5(1): 8 – 14.

Frazier WC, DC Westhoff (1995). Food Microbiology. 4th ed. Tata McGraw – Hill Publisging Company Limited, New Delhi.

Jussi-Pekka Rauha, Susanna Remes, Marina Heinonen, Anu Hopia, Marja Kahkonen, Tytti Kujala, Kalevi Pihiaja, Heikki and Pia Vuorela (2000). "Antimicrobial effects of Finnish plant extracts containing flavonoid and other phenolic compounds". Int. J. Food Microbiol. 58, 3 – 12.

Kotzekidou P, Giannakidis P, Boulamatsis A (2008). "Antimicrobial Activity of some plant extracts and essential oils against foodbourne pathogens *in vitro* and on the face of inoculated pathogens in chocolate". LWT – Food Science and Technology: 41, 119 – 127.

Larkin EP (1973). The public health significance of viral infections of food animals. *In* B.C. Hoobs and J.H.B. Christian (eds). The microbial safety of Foods. Academic Press, Inc., London.

Smith-Palmer A, Stewart J, Fyfe L (2001). "Potential application of plant essential oils as natural food preservatives in soft cheese". Food Microbiology: 18, 463 – 470.

WHO (1978). "The promotion and Development of Traditional Medicine". Technical Report Series. p 622.

WHO (2003). "WHO guidelines on Good Agricultural and collection practices (GACP) for Medicinal plants". World Health Organization, Geneva.