

Full Length Research Paper

Effect of Microbial Spoilage on Antimicrobial Potential and Phytochemical Composition of *Ipecae* Root Extract

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ABSTRACT

The effect of microbial spoilage on phytochemical composition and antimicrobial potential of *Ipecae* root extract was studied. Preliminary phytochemical screening of the neat, undegraded extract showed presence of glycosides and saponins, which were not dictated in the extract after microbial degradation; instead sugars, free phenols and tannins (both hydrolysable and condensed) became prominent. Therefore it was concluded that the microorganisms, responsible for the spoilage, broke down glycosidic linkages and produced simple sugars which formed their food nutrients. In the process, they altered phytochemical composition of the extract, produced phenolic substances and probably hindered the growth of other microorganisms by formation of more potent antimicrobial drugs. It was concluded that acidic metabolite isolated from the microbial degraded extract could be good antibiotic drug (or source of one such drug) in that it inhibited the growth of all test microorganisms and showed inhibition zone diameters (IZD) equal to or greater than 30mm against *Coliform bacilli*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*.

Keywords: Phytochemistry, Antimicrobial potential, Microbial spoilage, *Ipecae* root extract.

INTRODUCTION

Microorganisms use our foods as sources of nutrients for their own growth. They utilize our food ingredients, produce enzymatic and chemical changes in the food materials 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 types and proportions of nutrients in the foods are very essential in determining which microorganisms can grow in them. Thus peptides, amino acids, ammonia, urea and other simple nitrogenous substances may be available to some microorganism and not to others, even in the same medium (Heldman, 1974). The carbohydrates, especially sugars, are commonly used by microorganisms as sources of energy while proteins, peptides and amino acids serve as energy foods for proteolytic organisms (Broughall and Brown, 1984). The effect of food acidity also differs for the microorganisms and each microorganism has an optimal pH for its growth. This is because the pH of food material signifi-

cantly affects microbial cells since they do not have any mechanism to adjust their internal pH. In general, most microorganisms grow best in pH values near neutrality (Corlett and Brown, 1980).

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). Ejele (2010) reported the effect of ethanol extracts of some plants on the microbial spoilage of *Cajanus cajan* and showed that the extracts of *Aloe vera*, *Garcinia kola* (bitter kola), *Ocimum gratissimum* (scent leaf) and *Zingiber officinale* (ginger) were effective against the microbial spoilage of *C. cajan* caused by *Aspergillus oryzae* and *Saccharmyces spp.* and inhibited the growth of these microorganisms to various extents. Ejele and Akujobi (2011) reported the effects of

secondary metabolites of *G. kola* on the microbial spoilage of *C. cajan* extract and found that the acidic metabolite completely inhibited the microbial spoilage.

In the course of our study of plant extracts and their secondary metabolites, we observed the ease of spoilage of some plant extracts (especially the edible plants) and basic metabolites by microorganisms of the air. In an effort to track and identify the food nutrients consumed by these microorganisms and phytochemical changes they caused, we have investigated the phytochemical composition of the plant extracts and/or the secondary metabolites before and after the microbial spoilage. In this paper, we report on the effect of microbial spoilage on antimicrobial potential and phytochemical composition of *Ipecaea* root extract and its acidic, basic and neutral metabolites, with a view to identify which phytochemical compounds were affected by the microbial spoilage and what new products were formed.

MATERIAL AND METHODS

Sample Collection and Preparation of extract

The root of *Ipecaea* tree were collected from Abakiliki in Ebonyi State, Nigeria and authenticated at the Department of Plant Science and Technology, Federal University of Technology, Owerri as *Ipecaea* root, belonging to the family of Rubiaceae. The leaves were sun-dried and ground to produce a semi powdered sample. 30g of the semi-powdered 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 and antimicrobial screening while the other was left in the open laboratory and observed for microbial spoilage, after which the phytochemical and antimicrobial experiments were repeated on the spoilt extract. The results are presented in Tables 1, 2 and 3 respectively.

Preparation of basic metabolite

This metabolite was prepared as earlier stated (Ejele and Alinnor, 2010). The filtrate of ethanol extract was treated with dilute HCl and extracted with chloroform in a separatory funnel. The lower chloroform layer was removed (and reserved for the preparation of neutral metabolite). The HCl layer was treated with dilute NaOH solution until the mixture became basic. Then the resultant solution (with or without precipitate) was allowed to evaporate completely at room temperature to form a gel, which was dissolved in 95% ethanol and filtered. The filtrate was used for antimicrobial experiments without

further purification. A similar experiment was performed with the spoilt extract.

Preparation of neutral metabolite

The chloroform layer obtained above was placed in a separatory funnel and treated with dilute NaOH solution. After equilibrating, the aqueous NaOH layer was removed and reserved for preparation of acidic metabolite. The chloroform layer was removed and allowed to evaporate completely at room temperature to produce a gel, which was dissolved in chloroform and filtered. The filtrate was used without further purification for the antimicrobial experiments. The same experiment was repeated with the spoilt extract.

Preparation of acidic metabolite

The aqueous alkaline layer obtained above was treated with dilute HCl until the solution became acidic. The mixture (with or without precipitate) was allowed to evaporate completely at room temperature to produce gel, which was dissolved in ethanol and filtered. The filtrate was used for antimicrobial experiments without further purification (Ejele and Alinnor, 2010). A similar experiment was carried out with the spoilt extract.

Preliminary Phytochemical Screening of Crude Extract and Metabolites

These tests were carried out on the root extracts and metabolites (neat or spoilt) using standard methods (Evans, 2009) with a view to identify pharmacologically active constituents. Each sample (neat or spoilt) and resultant metabolites (acidic, basic and neutral from both neat and spoilt extract) were divided into two portions. One portion was used for preliminary phytochemical screening of the neat metabolite while the other was allowed to stand in the laboratory for at least 2 weeks to allow for microbial spoilage, after which preliminary phytochemical screening was repeated on the microbial degraded metabolite. The results are presented in Table 1.

Identification of Microbes

The microbes were identified in the Department of Microbiology, Federal University of Technology, Owerri, Nigeria. The media used for the identification were:

i) Nutrient agar, ii) MacConkey agar and iii) Sabourand dextrose agar. Each medium was weighed, dissolved in distilled water, autoclaved and poured into sterilized Petri dishes and allowed to solidify. The microbial samples were taken with sterile wire loop, streaked into the appropriate medium and incubated at 37°C for 24–48h for the MacConkey and nutrient agar. In the case of Sabourand dextrose agar, it was incubated for a period 3–7 days at 30°C.

Antimicrobial Tests

The experiments were carried out in Microbiology Department, Federal Medical Centre, Owerri, Imo State of Nigeria. Test microorganisms used were *C. albicans*, *E. coli*, *S. typhi*, *Strep. spp.*, *S. aureus* and *C. bacilli*. The agar disc diffusion method was used for these tests. An inoculating loop was touched to three isolated colonies of each pathogen placed on an agar plate and used to inoculate a tube of culture broth incubated at 35–37°C until it became slightly turbid and was then diluted to match the turbidity standard. Then a sterile cotton swab was dipped into the standardized bacterial test suspension and used to inoculate the entire surface of the agar plate. After 5min, appropriate antibiotic test disks were placed on the agar plate with a multiple applicator device and incubated at 35-37°C for 16-18 hours. At the end of the experiments, inhibition zones diameters (areas showing no microbial growth) were measured to the nearest mm (Garred and O-Graddy, 1983; Harbarth and Samore, 2005).

RESULTS AND DISCUSSION

Preliminary Phytochemical Tests

Preliminary phytochemical screening of the neat undegraded extract revealed presence of alkaloids, amino acids, cardio-active glycosides, saponins and tannins whereas the microbial degraded extract showed the absence of cardio-active glycosides and saponins, which were originally present in the extract before microbial spoilage. On the other hand, the flavonoids, tannins and sugars (which were initially absent in the extract before microbial attack) became prominent after the microbial degradation of extract (Table 1).

These results suggest that the microorganisms responsible for the microbial spoilage probably destroyed the glycosides and saponins to produce aglycons and simple sugars that formed their food nutrients. This would involve the splitting of glycosidic linkages and release of the sugars. The presence of flavonoids and increased levels of tannins and free phenols in the degraded extract confirmed that polyphenolic glycosides were probably involved. On the other hand, the lower concentrations of amino acids in the degraded extract probably suggested that the microorganisms probably fed on them, since proteins, peptides and amino acids are known to serve as energy foods for proteolytic microorganisms (Frazier and Westhoff, 1995)). A similar observation had earlier been made by Ejele *et al* (2012).

Comparison of Antimicrobial Potential of Extracts

A comparison of antimicrobial activity of the degraded and undegraded extracts on some human pathogens was

presented in Table 2, from which it may be seen that the extracts were active against the test microorganisms: *C. albicans*, *E. coli*, *S. typhi*, *Strep. spp.*, *S. aureus* and *C. bacilli* to various extents, except that the neat undegraded extract showed no activity against *C. albicans* and *S. aureus*, although the activity of degraded extract against *C. albicans* and *S. typhi* was not determined. The data showed that the undegraded extract exhibited its greatest activity against *E. coli* and *Strep. spp.* with IZD of 20mm whereas the least activity was against *C. bacilli* with IZD of 12mm.

Similarly the microbial degraded extract exhibited its greatest activity against *Strep. spp.* with IZD of 20mm and its least activity against *E. coli* with IZD of 5mm. Generally speaking, the microbial degradation of the extract did not enhance its antimicrobial potential, except that the spoilage produced better activity against *S. aureus* with IZD of 12mm and weaker effect against *E. coli*.

Comparison of Antimicrobial Potential of Acidic Metabolites

A comparison of antimicrobial activity of acidic metabolite of both degraded and undegraded extracts on human pathogenic organisms was shown in Table 3, from which it was seen that both metabolites showed strong activity against the test microorganisms to various extents, although acidic metabolite of the neat, undegraded extract showed no activity against *C. albicans* and *S. aureus*, whereas the metabolite of degraded extract was active against these microorganisms with IZD of 25 and 30mm respectively.

The results showed that acidic metabolite of degraded extract exhibited its greatest activity against *S. typhi* with IZD of 31mm and least activity against *C. albicans* and *Strep. spp.* with IZD of 25mm. It was concluded that acidic metabolite of the microbial degraded extract could be a good antibiotic drug (or the source of one such drug) since it gave inhibition zone diameter equal to or greater than 30mm against *E. coli*, *S. typhi*, *S. aureus* and *C. bacilli*.

Comparison of Antimicrobial Potential of Basic Metabolites

A comparison of antimicrobial activity of basic metabolite from degraded and undegraded extracts on these pathogenic organisms was shown in Table 4, from which it was seen that the basic metabolites from both neat and degraded extracts were generally ineffective against the test organisms and their activities were low.

The data showed that the basic metabolite of neat extract showed strong activity against *E. coli* with IZD of 30mm; was weak against *C. bacilli* with IZD of 10mm and no activity at all against *C. albicans*, *S. typhi*, *Strep. spp.* and *S. aureus*. Similarly the basic metabolite of degraded

Table 1. Preliminary phytochemical screening on extracts

Phyto-compounds tested	Crude extract	Degraded extract
Alkaloids	++	++
Amino acids	++	+
Aldehydes / Ketones	-	++
Cardio-active glycosides		
a) Libermann's test	++	-
b) Salkowski test	++	-
Flavonoids	+	+++
Phenols	+	+++
Saponins	+++	-
Steroids/triterpenes	-	+
Sugars	-	+++
Tannins	+	++
+++ Prominent; ++ Moderate; + Small; - Negative		

Table 2. Antimicrobial potential of crude extracts

Test microorganisms	Concentration = 100mg/ml	
	Neat extract	Degraded extract
<i>C. albicans</i>	-	ND
<i>E. coli</i>	20±4mm	5mm
<i>S. typhi</i>	15±3mm	ND
<i>Strep. spp</i>	20±5mm	20mm
<i>S. aureus</i>	-	12mm
<i>C. bacilli</i>	12±2mm	18mm
	→ ND Not Determined;	→ - abs

Table 3. Antimicrobial potential of acidic metabolites

Test microorganisms	Concentration = 100mg/ml	
	Acidic (from neat extract)	Acidic (from degraded extract)
<i>C. albicans</i>	-	25±5mm
<i>E. coli</i>	20±3mm	30±6mm
<i>S. typhi</i>	20±5mm	31±3mm
<i>Strep. spp</i>	25±4mm	25±5mm
<i>S. aureus</i>	-	30±3mm
<i>C. bacilli</i>	20±3mm	30±4mm

Table 4. Antimicrobial potential of basic metabolite

Test microorganisms	Concentration = 100mg/ml	
	Basic (from neat extract)	Basic (from degraded extract)
<i>C. albicans</i>	-	-
<i>E. coli</i>	30±5mm	10±3mm
<i>S. typhi</i>	-	25±5mm
<i>Strep. spp</i>	-	-
<i>S. aureus</i>	-	-
<i>C. bacilli</i>	10±2mm	10±4mm

extract showed moderate activity against *S. typhi* with IZD of 25mm; was weak against *E. coli* and *C. bacilli* with

IZD of 10mm and no activity at all against *C. albicans*, *Strep. spp.* and *S. aureus*.

Table 5. Antimicrobial potential of neutral metabolites

Test microorganisms	Concentration = 100mg/ml	
	Neutral (from neat extract)	Neutral (from degraded extract)
<i>C. albicans</i>	-	10±2mm
<i>E. coli</i>	32±4mm	10±4mm
<i>S. typhi</i>	20±3mm	12±3mm
<i>Strep. spp</i>	15±3mm	5±2mm
<i>S. aureus</i>	-	5±3mm
<i>C. bacilli</i>	30±5mm	10±3mm

Table 6. Antimicrobial potential of control drugs

Test microorganisms	Ciprofloxacin 100mg/ml	Lerofloxacin 200mg/ml	Gentamycin 100mg/ml	Augmentin 300mg/ml	Acidic (Degraded) 100mg/ml
<i>C. albicans</i>	15±3mm	18±3mm	18±5mm	20±5mm	25±5mm
<i>E. coli</i>	22±5mm	23±5mm	12±4mm	18±5mm	30±6mm
<i>S. typhi</i>	20±4mm	25±3mm	15±2mm	-	31±3mm
<i>Strep. spp</i>	18±3mm	22±4mm	10±3mm	-	25±5mm
<i>S. aureus</i>	20±4mm	15±3mm	20±5mm	18±5mm	30±3mm

Comparison of Antimicrobial Potential of Neutral Metabolites

A comparison of antimicrobial activity of neutral metabolite of both degraded and undegraded extracts on the human pathogens was shown in Table 5.

The results showed that the neutral metabolite from neat, undegraded extract exhibited strong antimicrobial activity against the test microorganisms to various extents, especially against *E. coli* and *C. bacilli* with IZD of 32 and 30mm respectively; was weak against *S. typhi* and *Strep. spp.* with IZD of 20 and 15mm respectively and no activity at all against *C. albicans* and *S. aureus*. On the contrary the metabolite from degraded and spoil extract was weak against *S. typhi*, *C. albicans*, *E. coli*, *C. bacilli*, *Strep. spp.* and *S. aureus* with IZD ranging from 12 to 5mm. It was concluded that the neutral metabolite from the neat undegraded extract was better antibiotic drug (against *E. coli* and *C. bacilli*) and the microbial spoilage significantly reduced antibiotic efficacy of neutral metabolite.

Comparison of Antimicrobial Potential of Control Drugs

A comparison of antimicrobial activity of some control drugs and acidic metabolite from degraded extract on the human pathogens was shown in Table 6.

The results showed that all control drugs were active against the test microorganisms except Augmentin (at 300mg/ml concentration), which was active against *E.*

coli, *S. typhi* and *C. bacilli* only. In general, the acidic metabolite from degraded extract (at 100mg/ml concentration) exhibited the best antimicrobial activity against the test microorganisms compared with the control drugs.

Phytochemical Screening of Various Metabolites

Phytochemical screening of various metabolites before microbial degradation was presented in Table 7. Results showed that acidic metabolite was composed principally of flavonoids and free phenols plus small amounts of amino acids and tannins.

The flavonoids are polyphenolic compounds of plant origin and their presence in the acidic metabolite makes it a useful drug because numerous physiological activities have been attributed to them. Thus, small quantities of flavones may act as cardiac stimulants; some flavones appear to strengthen weak capillary blood vessels while hydroxylated analogues act as diuretics, anti-oxidants and anti-inflammatory agents. The flavonoids can also act as vasodilators, which make them useful in the management of hypertension by decreasing oxidative stress in liver and kidney (Ikan, 1991). They are also used for treatment of capillary permeability, venous insufficiency and hemorrhoids. A deficiency of flavonoids in diet has been linked with abnormal capillary leakiness and pain in the extremities, resulting to aches, weakness and leg cramps (Yang *et al*, 2012)..

Plant phenolics are currently of growing interest due to their functional properties in promoting good human health. Jussi-Pekka *et al* (2000) carried out the antimicro-

Table 7. Phytochemical Screening Tests of Various Metabolites

Constituents	Acidic	Basic	Neutral
Alkaloids	-	++	-
Amino acids	+	++	-
Amides	-	-	+
Esters	-	-	+
Flavonoids	++	-	-
Phenols	++	-	-
Saponins	-	++	-
Steroids / Triterpenes	-	-	++
Tannins	+	+	-

+++ → Prominent; ++ → Moderate; + → Weak;
 - → Negative

bial screening of 13 phenolic substances and 29 plant extracts against selected microbes and found that some phenolic compounds such as flavone, quercetin and naringenin and some plant extracts inhibited growth of the microorganisms to different extents. Although the mechanism of action of plant extracts and metabolites are not known with certainty, *in vitro* physicochemical assays characterize most of them as antioxidants, which possess ability to limit oxidation and inhibit microbial growth.

SUMMARY AND CONCLUSION

The effect of microbial spoilage on antimicrobial potential and phytochemical composition of *Ipaeca* root extract and its metabolites was studied. Preliminary phytochemical screening of extract showed (among others) the presence of glycosides and saponins, which were not observed in the extract after microbial spoilage, instead flavonoids, free phenols and sugars became prominent. Thus, this study supports the fact that the biodegradation of glycosides produce simple sugars and aglycons, which could be flavonoids, simple phenols or tannins that possess antimicrobial potential. The antimicrobial potential of acidic metabolite of the microbial degraded extract showed significant antimicrobial activity against the human pathogens tested (*C. albicans*, *E. coli*, *S. typhi*, *Strep. spp.*, *S. aureus* and *C. bacilli*). Hence, it was concluded that microorganisms involved in the spoilage (*Bacillus spp.* and *Aspergillus spp.*) probably broke down glycosidic linkages and produced simple sugars upon which they fed. In the process they altered the phytochemical composition of the extract and probably hindered the growth of other microorganisms by production of more potent antimicrobial drugs. It was concluded that the acidic metabolite isolated from the microbial degraded extract could be good antibiotic drug in view of the fact that it inhibited growth of all test microorganisms and showed

inhibition zone diameters (IZD) equal to or greater than 30mm against *C. bacilli*, *E. coli*, *S. typhi*, and *S. aureus*.

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