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Antibacterial Effect of Cashew (*Anacardium occidentale* L.) Varieties during Different Maturity Stages of Kernel

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ABSTRACT

Cashew (*Anacardium occidantae* L.) is a valuable plant with food, medicinal and industrial values. Cashew has become a cash crop in Sri Lanka having improved varieties with high yielding, high quality nut and kernel characters introduced recently. Cashew kernel is commonly used as food with 21% protein, 46% fat, and 25% carbohydrates. Kernel extract do have antimicrobial activity against a reasonable range of pathogenic microorganisms. Although kernel has antibacterial properties, change of antibacterial properties with the different maturity stages of kernel is unknown. The present study was conducted to investigate the antibacterial properties change with different kernel maturity stages in six improved varieties released recently. Three maturity stages of kernel were studied as mature kernel, fully filled kernel and half-filled kernel. Normal hexane was used as a solvent to extract cashew kernel oil. Two gram positive bacteria; *Staphylococcus aureus* and *Bacillus cereus* and two gram negative bacteria; *Escherichia coli* and *Vibrio cholerae* were used for antibacterial susceptible test. This study depicted that antibacterial properties change with the maturity stages and the highest antibacterial properties were found with mature stage of kernel. Further gram positive bacteria become more susceptible than the gram negative bacteria for cashew kernel extraction. Further study confirmed that cashew kernel extraction with 95% hexane is suitable for antibacterial studies.

KEYWORDS: Antibacterial capacity, Cashew kernel, Gram positive and gram negative bacteria

INTRODUCTION

Cashew (*Anacardium occidentale* L.) is a tropical tree, belongs to family Anacardiaceae. It is native to Brazil and introduced to Sri Lanka by the Portuguese. Cashew tree grows up to 15m in height with thick tortuous trunk. It prefers dry weather and poor sandy soil. In Sri Lanka, total extent of cashew is about 30,000 ha and it is distributed throughout the dry zone. The annual raw cashew production is about 12,000 MT and average annual kernel production is about 2,400 MT. Cashew nut is exported as row cashew nuts, bulk cashew kernels, value added cashew kernels (Anon, 2011).

The kernel is considered to be of high nutritive quality and growing conditions or the variety of cashew may have an influence on kernel composition. Overall composition of the kernel is 21% protein, 46% fat, and 25% carbohydrates (Ohler, 1979).

Antibacterial compounds are substances, which kill or retard growth of bacteria. Some photochemical produced by plants have effect as antioxidants; effect to immune system, cellular repair and diseases caused by bacteria, fungus and virus. The nut shell oil is used medicinally and for industrial applications in the plastic and resin industries for its phenol content (Duke, 2001).The main chemicals found in cashew are alanine, alpha-catechin, alpha-linolenic acid, anacardic acid, anacardol, antimony, arabinose, and caprylic acid (Anon, 2011). Cashew leaves have antibacterial compounds as polyphenol, anacardic acid and other compounds, tatrols and tanins (Agedah *et al.*, 2010). *Anacardium occidentale* apple also have antibacterial properties (Aiswarya *et al.*, 2011). Cashew nut extract has antimicrobial activity against a reasonable range of pathogenic microorganisms (Oronsaye and Oseodio, 2008).

At present, cashew is becoming an important cash crop for farmers in Sri Lanka. There is a great potential for increased production for the local and export market. Sri Lankan cashew has assumed greater importance due to its special qualities, consumer preferences and recognized as the "World Tastiest Cashew" in the global market. The main destinations of cashew are Middle-East countries, Canada, USA, Israel, and Japan (Surendra, 2008).

In addition to traditional varieties, improved cashew varieties developed by Cashew Corporation of Sri Lanka jointly with Wayamba University of Sri Lanka are available in the country now. Improved varieties (WUCC 05, WUCC 08, WUCC 09, WUCC 13, WUCC 19 and WUCC 21) were identified as better performers over other varieties and showed high yielding ability at early stages with high quality nut and kernel characters (Jayasekera *et al.*, 2009). Even though cashew shell has highest antibacterial properties, widely used and consumed cashew is its kernel. Antibacterial properties may be varied with the maturity stages of the kernel. In oil extracted from the kernel of *Sclerocarya birrea* total phenolic and flavonoid content increased continuously through the different harvesting dates (Mariod *et al.*, 2010). There appears to be a relationship between the chemical structure of compounds in the cashew bark oil and antibacterial effect (Agedah *et al.*, 2010). But no literature evidence on antibacterial activity change with the maturity stages of cashew kernel could be found. Therefore it was decided to investigate the variation of antibacterial effect with the maturity stages of the kernel of six different newly improved varieties.

MATERIALS AND METHODS

The experiment was carried out in the microbiology laboratory, Department of Plantation Management, Wayamba University of Sri Lanka, from January to April 2013.

Sample Collection

Seeds were collected from Wanathawilluwa seed garden managed by Sri Lanka Cashew Corporation. Six varieties; WUCC 05, WUCC 08, WUCC 09, WUCC 13, WUCC 19, and WUCC 21 were used. Three maturity stages of kernel used for this study were half -filled (21days after pollination), fully filled (35 days after pollination), mature nuts (48 days after pollination).

The Extraction Method

The extraction of the active ingredients of cashew kernel was carried out using the method described by Harbone (1994). Normal hexane was used as extraction solvent.

Extraction was performed using 30 g of chopped kernel from each variety in 300 ml of 95% n-hexane using soxh-let extractor. The extraction was carried out through three hours and hexane was separated by evaporation using rotary evaporator.

Test Organisms

Four bacteria species were used for experiment as test organisms, obtained from Microbial culture collection Centre, University of Kelaniya. Two gram positive and two gram negative bacteria types were used for testing (Table 1).

One milliliter from each broth culture was pipetted out and transferred to culture bottles that contain ten milliliter of Nutrient broth medium and was kept overnight in incubator at 37 ^{0}C (Ranathunge *et al.*, 2010).

Table 1. Bacteria species

Bacteria species	Gram reaction	Shape
Staphylococcus aureus	+	Coccus
Bacillus cereus	+	Rod
Vibrio cholerae	-	Comma
Escherichia coli	-	Rod

Antibacterial Susceptibility Test

The antibacterial tests for the extracts were carried out using Kirby Bauer paper disc inhibition method (Bauer *et al.*, 1966).

In this method, overnight broth culture of 0.1 ml was transferred and spread on the 14 cm diameter petri dish containing Meuller-Hinton agar (Meuller and Hinton, 1941). Six millimeter paper discs were soaked in filtrate for 24 hrs and were placed on agar surface. The plates were incubated for 24 hrs at 37 ^{0}C (Bauer *et al.*, 1966).

Antibacterial effect of extract against the test bacteria was measured by the diameter of the inhibition zone (IZ). The three replicates were used for each bacterial species. Three types of standard antibiotic discs such as Tetracycline, Ampicillin, and Penicillin discs were also used for comparison purposes.

Statistical Analysis

ANOVA General linear model was applied to determine antibacterial effect of maturity stages of kernel, varieties and bacterial species. Data was analyzed using SAS statistical package, 2002 by SAS institution Inc., Cary, NC, USA, Version 9.2.

RESULTS AND DISCUSSION

Antibacterial Susceptible Test

Analytical data for antibacterial susceptible test is given in Table 2. These results confirmed the presence of antibacterial properties of Cashew kernel as recorded by Srinath and Weerakkody (2011). Inhibition zone for kernels in different maturity stages and bacteria species were significantly different ($P<0.0001$) but antibacterial properties were not significantly different among varieties ($P <0.3879$).

When total effects described by maturity of kernel, varieties and bacteria species are considered, the highest effect was recorded from maturity stages of kernel (Table 2). Among all three stages, mature cashew kernel indicated the highest inhibition zone as measured by its diameter (12.51±6.11 mm) while half-filled cashew kernel showed the lowest inhibition as 7.99±1.02 mm (Table 3). Antibacterial properties were gradually decreased in the order of mature kernel stage >

fully filled kernel stage > half-filled kernel stage.

Table 2. ANOVA general linear model analytical data

Component	DF	F value	Pr>F
Model	10	13.37	0.0010
Cashew Variety	05	01.05	0.3879
Maturity stages of kernel	02	28.11	<.0001
Bacteria Sp.	04	24.08	<.0001

Significant at 0.05 confident level

Table 3. Antibacterial effect in different maturity stages of kernel

Maturity stage of kernel	IZ mean value (mm)	SD value
Mature nut kernel	12.51[a]	± 6.11
Fully filled kernel	11.18[b]	± 4.05
Half-filled kernel	07.99[c]	± 1.02

Means with the same letter are not significantly different

Being significantly different, three stages of cashew kernel clearly revealed that antibacterial properties of cashew kernel vary with the maturity stages. Cashew kernel consist several antimicrobial compounds as triterpenoids, phenolic, volatile oils, flavonoids, xanthoprotein and carbohydrates (Kannan *et al.*, 2009). However Gordon *et al* (2010) reported that bioactive compounds and poly phenolic content change with the ripening stages of cashew apple. In other investigation it was reported that oil extracted from the kernel of *Sclerocarya birrea* total phenolic and flavonoid content increased continuously through the different harvesting dates (Mariod*et al.*, 2010). Agedah *et al.* (2010) reported that relationship between the chemical structure of compounds in the cashew bark oil and antimicrobial activity. These suggest that chemical structure of compounds in cashew kernel may also be changed with the maturity stages and thereby change the antimicrobial properties. Even though it is reported to decrease antibacterial properties with maturity of cashew apple (Gordon *et al.*, 2010), present study showed an increase of antibacterial effect with matured cashew kernel.

Generally cashew kernel is consumed as raw nuts or processed nuts. Cashew nuts are mostly harvested at mature stage where antibacterial activity is at high level which is good for human health. Further studies are necessary to reveal the antibacterial properties of matured kernel during processing and value addition.

Varietal Antibacterial Effect against Different Bacterial Species

Effect for bacterial species by each variety is given in Table 4 and 5. Although antibacterial properties of varieties were not significantly different in the model, varieties were significantly different within the bacterial species. Inhibition zone of *Staphylococcus aureus* for WUCC 21 (09.00 mm) was the lowest and it was significantly different from WUCC 05, WUCC 08, WUCC 09 varieties. *Staphylococcus aureus* was highly susceptible for WUCC 09 variety with the inhibition zone mean diameter of 14.06 mm (Table 4).

Table 4. Varietal difference on *Staphylococcus aureus*

Variety	IZ mean diameter (mm)
WUCC 05	12.67[a]
WUCC 08	14.00[a]
WUCC 09	14.06[a]
WUCC 13	13.11[a]
WUCC 19	11.72[ab]
WUCC 21	09.00[b]

Means with the same letter are not significantly different

Table 5. Varietal difference on *Vibrio cholerae*

Variety	IZ mean diameter (mm)
WUCC 05	10.72[a]
WUCC 08	08.22[bc]
WUCC 09	09.17[b]
WUCC 13	07.33[c]
WUCC 19	08.00[bc]
WUCC 21	07.61[c]

Means with the same letter are not significantly different

When *Vibrio cholerae* is considered WUCC 05, WUCC 09, WUCC 13 and WUCC 21 were significantly different among varieties while WUCC13 and WUCC21 were not. Lowest mean diameter was indicated by cashew variety WUCC13 (7.33 mm) while WUCC 05 showed the highest (10.72 mm) as shown in Table 5. Varietal differences were not found either with *Escherichia coli* or *Bacillus cereus.*

Effect of Bacterial Species

Inhibition zones of each bacterial species used is given in Table 6. The highest inhibition zone was recorded by *Bacillus cereus* (12.43±1.90 mm) and the lowest by *Escherichia coli* (8.33±4.81 mm). *Staphylococcus aureus* and *Bacillus cereus* depicted the highest inhibition zones for cashew kernel extraction with no significant difference. Further, *Vibrio cholerae* and

Escherichia coli recorded the lowest inhibition zones with no significant difference. However the bacteria that depicted the lowest and the highest inhibition zones were significantly different.

Table 6. Effect of bacteria species

Bacteria species	Means (mm)	SD value	Gram reaction
Staphylococcus aureus	12.43[a]	±1.90	+
Bacillus cereus	12.98[a]	±6.21	+
Escherichia coli	08.33[b]	±4.81	-
Vibrio cholerae	08.51[b]	±2.03	-

Means with the same letters are not significantly different

Antibacterial effect of cashew kernel on bacteria was significantly different from each other. *Staphylococcus aureus* and *Bacillus cereus* were significantly different from *Vibrio cholerae* and *Escherichia coli* (Table 6). Literature revealed that gram positive bacteria are more susceptible to antibacterial properties than gram negative bacteria. A gram positive organism, without an outer membrane in its cell wall was more sensitive than gram negative organism, which possesses an outer membrane in its cell wall (Agedah *et al.*, 2010). Even though *Staphylococcus aureus* and *Bacillus cereus* were not significantly different, *Bacillus cereus* showed the highest antibacterial activity (12.98 ±6.21 mm). Similar situation was documented in in-vitro antimicrobial activity and phytochemical analysis in some Indian medicinal plants (Parekh and Chanda, 2006). Similar to past records the lowest effect was depicted by *Escherichia coli.* It suggests that *staphylococcus aureu* sand *Bacillus cereus* were more susceptible for Cashew kernel while *Escherichia coli* were the least susceptible. Further it is possible to confirm that antibacterial properties of cashew kernel tend to inhibit gram positive bacteria than gram negative bacteria.

Antibacterial effects of cashew varieties were not significantly different from antibiotics; Ampicillin, Penicillin. But antibacterial effects of varieties were significantly different from Tetracycline. Therefore antibacterial effect of cashew kernel extraction was similar to Ampicillin and Penicillin antibacterial effect.

In the present study cashew kernel extraction was performed using 95% normal hexane which was successful in extraction of substances responsible for antibacterial properties. Similar results were recorded by Srinath and Weerakkody (2011). Therefore it can be confirmed that cashew kernel extractions with 95% hexane is suitable for antimicrobial studies.

CONCLUSIONS

This study revealed that antibacterial effect of cashew kernel changed with the maturity stages and the highest antibacterial effect was shown by mature stage of kernel. Further studies are required to find the reason for variation of antibacterial effect with maturity stages. Although antibacterial effects of varieties were not significantly different, with in the bacterial species varietal effect could be seen for *Staphylococcus aureus* and *Vibrio cholerae.* In *Staphylococcus aureus* WUCC 09 showed the highest inhibition zone and WUCC 21 showed the lowest inhibition zone. *Vibrio cholerae* showed the highest inhibition zone for WUCC 05 and lowest for WUCC 13. Further gram positive bacteria were more susceptible than the gram negative bacteria for cashew kernel extraction. Cashew kernel extraction was discovered similar antibacterial effect to the commercially available antibiotics such as ampicillin and penicillin. This study also confirmed 95% of normal hexane is suitable to extract the antibacterial properties from cashew kernel.

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