

UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E FISIOLOGIA

**Ácidos graxos e óleo essencial de sementes de *Syagrus coronata* (Mart) Becc.
(Arecaceae): composição química e atividade anti-*Staphylococcus aureus***

RODRIGO SANTANA DO NASCIMENTO

RECIFE
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Dissertação apresentada para o
cumprimento parcial das exigências
para obtenção do título de Mestre
em Bioquímica e Fisiologia pela
Universidade Federal de
Pernambuco

Orientador (a): Profa. Dra. Maria Tereza dos Santos Correia.

Coorientador (a): Profa. Dra. Marcia Vanusa da Silva

RECIFE
2014

Catálogo na Fonte:
Bibliotecário Bruno Márcio Gouveia, CRB-4/1788

Nascimento, Rodrigo Santana do

"Ácidos graxos e óleo essencial de sementes de *Syagrus coronata* (Mart.) Becc. (Arecaceae): composição química e atividade anti-*Staphylococcus aureus*" / Rodrigo Santana do Nascimento. – Recife: O Autor, 2014.

69 folhas: il.

Orientadora: Maria Tereza dos Santos Correia

Coorientadora: Marcia Vanusa da Silva

Dissertação (mestrado) – Universidade Federal de Pernambuco. Centro de Ciências Biológicas. Programa de Pós-graduação em Bioquímica e Fisiologia, 2013.

Inclui bibliografia e anexos

- 1. Plantas da caatinga 2. Ácidos graxos 3. Bactérias I. Correia, Maria Tereza dos Santos (orient.) II. Silva, Marcia Vanuza da (coorient.) III. Título.**

634.90981

CDD (22.ed.)

UFPE/CCB-2014-112

Rodrigo Santana do Nascimento

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Dedico aos meus Pais, Professores,
e principalmente a Deus por essa etapa concluída.

AGRADECIMENTOS

Aos meus pais, Antonio José do Nascimento e Lenilda Maria de Santana pelo amor incondicional que fruto é de uma completa doação.

A Prof^a Dra. Maria Tereza dos Santos Correia e a Profa. Dra. Márcia Vanusa da Silva, meus orientadores, que acreditaram no meu potencial para a execução desse projeto, além de darem incentivos e não pouparem esforços para conclusão desse etapa essencial na minha carreira profissional.

Aos Doutorandos Alexandre Gomes Silva e Cibele Maria, pela imensurável contribuição durante a parte experimental desse trabalho, como também na elaboração do artigo. Estarei sempre grato por tão grande contribuição.

Ao Prof. Dr. Thiago Henrique Napoleão e o Pós-doutorado Emmanuel Viana Pontual do laboratório de Glicoproteínas, Departamento de Bioquímica – UFPE, pelos ensinamentos e instrução na parte prática dessa pesquisa.

A minha Noiva Joicy Carla, pelas palavras sábias e por estar sempre presente dando apoio nos momentos de dificuldades, fazendo com que a tristeza momentânea decorrente de experiências sem êxito, desse lugar a uma visão mais otimista.

Aos meus irmãos Ricardo, Robson, Renato, Vitória, pela presença contínua e união.

A todos meus amigos que frutos de um relacionamento verdadeiro

Nada do que é feito por amor é pequeno.

Chiara Lubich.

RESUMO

A Caatinga é uma formação vegetacional localizada na região semiárida do Nordeste brasileiro, que apresenta clima predominantemente seco com uma vegetação xerófila decorrente de longos períodos de seca e temperaturas altas. Devido a sua grande biodiversidade essa região tem sido alvo de estudos, destacando a busca de novos compostos que inibam o crescimento de microrganismos, em vista das altas taxas de resistência destes aos antibióticos tradicionais. A presente pesquisa determinou a composição química e atividade anti-*Staphylococcus aureus* dos ácidos graxos e óleos essenciais extraídos das sementes de *Syagrus coronata* (Mart.) Becc. (Arecaceae), conhecido popularmente por ouricuri, uma planta endêmica da Caatinga. Os óleos essenciais das sementes foram extraídos através da técnica de hidrodestilação. Os ácidos graxos foram extraídos com hexano a frio. Os óleos essenciais e ácidos graxos foram diluídos em água e Tween 80. A atividade antimicrobiana foi realizada através da técnica de microdiluição seriada para determinação da CMB e CMI de óleos essenciais e ácidos graxos de *S. coronata* frente a *S. aureus*. Os óleos essenciais foram identificados através de cromatografia gasosa acoplada a um espectrômetro de massas (GC/MS). Os ácidos graxos foram submetidos a uma reação de esterificação para posterior identificação através do GC/MS. Através de análises cromatográficas foram encontrados 55 compostos no óleo essencial de *S. coronata* sendo o α - Felandreno (26,26%), trans- Cariofileno (18,01%) e β - Felandreno (12,93%) os compostos majoritários. Os principais ácidos graxos identificados ambos saturados foram ácido dodecanóico e ácido tetradecanóico com 41,58 e 9,68%, respectivamente, seguido pelo ácido 9-octadecenóico (ácido graxo monoinsaturado) com 23,81%. Apenas um ácidos graxo poli-insaturado foi identificado, o ácido 9,12-octadecadienóico com 3,59%. O óleo essencial apresentou valores de CIM variando de 0,02 a 0,04 $\mu\text{g/mL}$ e valores de CMB de 0,04 a 0,32 $\mu\text{g/mL}$ para as linhagens de *S. aureus* avaliadas. Os ácidos graxos apresentaram uma concentração inibitória mínima de 0,39 a 1,52 $\mu\text{L/mL}$ e concentração bactericida mínima de 0,32 a 3,04 $\mu\text{L/mL}$ para os isolados avaliados. Os resultados encontrados demonstram que o óleo essencial e os ácidos graxos extraídos de sementes de *S. coronata* possuem um excelente potencial antimicrobiano, podendo ser uma alternativa no tratamento de infecções causada por bactérias multirresistentes.

Palavras-Chave: Caatinga, *Syagrus coronata*, resistência microbiana, óleo essencial e ácidos graxos.

ABSTRACT

The Caatinga is a biome located in the semiarid region of Northeast Brazil, which has predominantly dry climate with a xerophytic vegetation due to long periods of drought and high temperatures. Due to its great biodiversity this region has been investigated, emphasizing the search for new compounds that inhibit the growth of microorganisms, in view of the high rates of bacterial resistance to available drugs for the treatment of infections. This research determined the chemical composition and anti-*Staphylococcus aureus* activity of fatty acids and essential oils extracted from the seeds of *Syagrus coronata* (Mart.) Becc. (Arecaceae), popularly known by ouricuri, a plant endemic to the Caatinga. The essential oils of the seeds were extracted by hydrodistillation technique. The fatty acids were extracted with cold hexane. The essential oils and fatty acids were diluted in Tween 80 and water. The antimicrobial activity was performed by serial microdilution technique. The essential oils were identified by gas chromatography coupled to a mass spectrometer (GC/MS). The fatty acids were subjected to an esterification reaction for identification by GC/MS. GC analysis found 55 compounds in the essential oil of *S. coronata* being the α -phellandrene (26.26 %), trans-caryophyllene (18.01%) and β -phellandrene (12.93%) were the major compounds. The major compounds identified in the fatty acids are saturated fatty acids, the dodecanoic acid and tetradecanoic acid with 41.58 and 9.6%, respectively. Followed by 9-octadecenoic acid, monounsaturated fatty acid, with 23.8 %. Only one polyunsaturated fatty acid was identified, 9,12 - octadecadienoic acid with 3.59%. The essential oils showed a minimum inhibitory concentration from 0.02 to 0.04 $\mu\text{g/mL}$ and minimum bactericidal concentration from 0.02 to 0.312 $\mu\text{g/mL}$ for the strains evaluated. The fatty acids showed a minimum inhibitory concentration from 0.04 to 0.625 $\mu\text{L/mL}$ and minimum bactericidal concentration from 0.04 to 1.25 $\mu\text{L/mL}$ for the strains evaluated. The results demonstrate that the essential oil and the fatty acids extracted from seeds of *S. coronata* have excellent antimicrobial activity and may be an alternative in the treatment of infections caused by multiresistant bacteria.

Keywords: Caatinga, *Syagrus coronata*, microbial resistance, essential oil and fatty acids.

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LISTA DE ABREVIATURAS

OMS	Organização Mundial de Saúde
ANVISA	Agência Nacional de Vigilância Sanitária
UTI	Unidade de Tratamento Intensivo
MRSA	<i>Staphylococcus aureus</i> Resistente a Meticilina
HIV	Vírus da Imunodeficiência Humana
DNA	Ácido desoxirribonucleico
PBP	Proteínas ligadoras de Penicilina
GlcNac	N-acetilglicosamina
AG	Ácidos Graxos

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1- INTRODUÇÃO

O conhecimento sobre as propriedades e características terapêuticas das plantas utilizadas na medicina popular vem sendo acumulado durante os séculos, porém o uso dessas técnicas tradicionais representa na grande maioria das vezes o único recurso terapêutico de várias comunidades. O emprego e a eficácia do tratamento com plantas medicinais são em parte atribuídas a observações vindas da população, que colaboram de maneira significativa para a divulgação das características terapêuticas dos vegetais, mas apesar dos efeitos medicinais relatados, muitas vezes os constituintes químicos não são conhecidos (MACIEL et al., 2002).

Apesar do amplo desenvolvimento de drogas sintéticas, a utilização de plantas medicinais permanece como uma opção no tratamento de doenças, observando que nas últimas décadas há uma valorização no consumo de preparações à base de plantas com fins terapêuticos. Diversos fatores têm contribuído para o aumento do uso das plantas como recurso medicinal, entre eles: o difícil acesso da população à assistência médica e o alto custo dos medicamentos industrializados (BADKEL et al., 2012).

O aproveitamento de plantas com finalidade medicinal está baseado em estudos etnofarmacológicos, porém ainda há poucas pesquisas objetivando a validação de plantas e muitas dessas continuam sendo utilizadas com base no conhecimento popular. Essas informações advindas da população são importantes na descoberta de espécies vegetais com potencial para desenvolvimento de novas drogas, no entanto o uso de plantas com fins terapêuticos sem os respectivos estudos de validação apresenta risco de toxicidade (SCOPEL, 2005, DUTRA 2009, ALMEIDA et al., 2012).

Partes da planta como raiz, caule, folha podem fornecer substâncias ativas para serem empregadas na obtenção de medicamentos (ROSA *et al.* 2012). Conquanto, apesar da importância conferida às plantas, o potencial relativo a essas ainda é pouco explorado. Segundo estimativas, a quantidade de espécies de vegetais superiores pode chegar a 500.000, porém entre 15 a 17% dessas foram investigadas quanto a sua potencialidade medicinal (BARROS, 2008).

Dentre as atividades farmacológicas apresentadas pelos compostos encontrados nos vegetais, a antimicrobiana tem sido amplamente estudada (OLIVEIRA et al., 2006).

As infecções associadas à assistência à saúde representam uma das principais causas de morbidade e mortalidade aos usuários do sistema de saúde, sendo cada vez mais receadas,

devido à dificuldade de tratamento e pelas altas taxas de resistência aos antimicrobianos, consequentemente, apresentam poucas opções terapêuticas (GIAMARELLOU, 2010; NÓBREGA et al., 2013), desse modo é necessário a busca de novos agentes capazes de inibir o crescimento bacteriano.

Plantas nativas da região semiárida têm sido utilizadas durante séculos, através da extração seletiva. Algumas populações do Nordeste do Brasil são muito pobres e dependem do uso direto dos recursos naturais para sua subsistência, e a utilização de produtos derivados da vegetação nativa, especialmente plantas medicinais, contribui significativamente para a manutenção da qualidade de vida dessas pessoas (LUCENA et al., 2007).

A Caatinga, região de grande biodiversidade localizada no Nordeste do Brasil, configura-se como um habitat específico para plantas medicinais e aromáticas não encontradas em outras regiões do mundo (MAIA, 2004). Diante desse enorme potencial botânico e da necessidade de se encontrar novos compostos capazes de controlar a ação de microrganismos, buscou-se realizar um trabalho que viabilize um maior conhecimento da espécie *Syagrus coronata* (Mart.) Becc., endêmica dessa região.

Desta forma, este trabalho teve como objetivo analisar a composição química e avaliar a atividade antibacteriana do óleo essencial e ácidos graxos de *S. coronata*.

2 – REFERENCIAL TEÓRICO

2.1- INFECÇÃO HOSPITALAR

As infecções relacionadas à assistência em saúde (IRAS) representam um crescente problema em ambientes hospitalares, devido a consequências na mortalidade de pacientes, como também pelos altos custos no tratamento a estes enfermos. Atualmente, o *Staphylococcus aureus* é considerado no cenário mundial um dos microrganismos mais importantes no que diz respeito às IRAS (CORREAL et al., 2013)

As infecções podem ocorrer quando há lesão no tecido cutâneo ou na mucosa oral, permitindo a entrada de microrganismos nos tecidos subjacentes ou na corrente sanguínea. O risco de se obter uma infecção é maior em pacientes hospitalizados com queimaduras, como também, com o uso de procedimentos invasivos (LOWY, 1998; MUTHAIYAN et al., 2012). Além disso, o desequilíbrio dos mecanismos de defesa do paciente e o contato com a microbiota presente no ambiente hospitalar (ROSSINI et al., 2009) são fatores que contribuem para o desencadeamento da doença.

No Brasil, cerca de 5 a 15% dos pacientes internados em centros médicos adquirem infecções (CATÃO et al., 2013).

As infecções em Unidades de Terapia Intensiva (UTI) são mais facilmente observadas, devido a aspectos como: a gravidade do estado de saúde do paciente, utilização de procedimentos invasivos, hospitalização por um longo período de tempo, ventilação mecânica, uso de imunossupressores, colonização da microbiota normal por microrganismos resistentes e uso de antibióticos inadequados que favorecem a seleção natural de bactérias resistentes (GUSMAO et al., 2004; ALLEN, 2005; COLPAN et al., 2005; WAGENLEHNER et al., 2006).

O *Staphylococcus aureus* é um importante agente etiológico envolvido com infecções hospitalares, sendo considerado um dos principais patógenos humanos causador de um amplo espectro de enfermidades em indivíduos imunologicamente saudáveis e em imunocomprometidos, além de ter fácil disseminação e apresentar resistência aos antimicrobianos (MARK et al., 2002; MENEGOTTO et al., 2007).

Essa bactéria tem a capacidade de invadir e causar doenças em diversos tecidos (GORDON et al., 2008; LOWY, 2008), mas as manifestações ocorrem comumente no tecido

cutâneo, sistema respiratório, tecidos moles, ossos, articulação e infecções endovasculares (BOUCHER et al., 2010).

2.2 – *STAPHYLOCOCCUS AUREUS*

Staphylococcus é um gênero de bactérias que pertence à família Micrococcaceae, apresentando-se sob a forma de cocos Gram-positivos, catalase-positivo, imóveis, não esporulado, geralmente não capsulado com diâmetro médio entre 0,5 e 1,5µm. Morfoloficamente exibem diversas formas: isolados, aos pares, em cadeias curtas ou agrupados irregularmente com aspecto semelhante a cachos de uva. (HARVEY et al., 1988; TORTORA et al., 2003; CASSETARI, 2005).

Atualmente, o gênero *Staphylococcus* possui 33 espécies, sendo que 17 delas já foram isoladas em amostras biológicas humanas. A espécie mais importante do gênero é o *Staphylococcus aureus*, essa denominação é em decorrência da pigmentação amarelada de suas colônias, onde aureus significa dourado (SANTOS et al., 2007), afora isso, tem a capacidade de coagulação do plasma, provocar hemólise e produzir enzimas e toxinas (SANTOS et al., 2007; BASTOS et al., 2013).

É uma bactéria comensal considerada um dos mais importantes microrganismos envolvidos na microbiota humana, geralmente encontrada colonizando as fossas nasais, pele, axilas, vagina, faringe e intestino de pessoas saudáveis (MENEGOTTO et al., 2007; BOUCHER et al., 2010; MARQUES et al., 2012).

2.3 – DOENÇAS OCASIONADAS POR *S. AUREUS*

Apesar de ser encontrado na microbiota humana colonizando principalmente a pele e as cavidades nasais, *S. aureus* pode provocar doenças que vão desde uma simples infecção (espinhas e furúnculos) até enfermidades mais graves, como: pneumonia, meningite, endocardite, síndrome do choque tóxico, septicemia, abscessos e lesões necróticas graves (SANTOS et al., 2007, FERREIRA et al., 2013).

As infecções geralmente ocorrem quando há a entrada do microrganismo em sítios previamente estéreis (tecidos adjacentes e corrente sanguínea) mediante lesões na pele ou na

mucosa e em procedimentos cirúrgicos, tendo o risco aumentado com o uso de cateteres (MENEGOTTO et al., 2007; BOUCHER et al., 2010).

O *S. aureus* apresenta uma alta capacidade adaptativa e quando infecta o organismo humano pode provocar uma ampla variedade de manifestações em sítios como a pele, tecidos moles, ossos, articulações, sistema respiratório, gerando quadros de septicemia (BOUCHER et al., 2010, MUTHAIYAN et al., 2012; MARQUES et al., 2013,), além de doenças invasivas e sistêmicas com significativas taxas de morbidade e mortalidade (GALBUSERA et al., 2011).

Entre as doenças causadas por *S. aureus* a piomiosite é uma infecção muscular subaguda profunda que pode originar abscessos intramusculares únicos ou múltiplos, caracterizada clinicamente por febre, dor muscular localizada, rigidez e edema. Essa enfermidade está associada a infecções sistêmicas, diabetes *mellitus*, terapia imunossupressora, AIDS e mieloma múltiplo (REZENDE et al., 2012).

A espondilodiscite séptica consiste numa infecção bacteriana do espaço intervertebral, originária por disseminação bacteriana via hematogênica, apresentando altas taxas morbimortalidade, diagnóstico tardio e associação à comorbidades, como: imunodepressão, diabetes *mellitus* e alcoolismo. Geralmente, é uma infecção monobacteriana, sendo o *Staphylococcus aureus* o micro-organismo mais prevalente (MORELLI et al., 2001; DORNELES, et al., 2011).

As espécies do gênero *Staphylococcus* são capazes de desencadear infecções cutâneas e nas mucosas, além de infecções septicêmicas, viscerais e ósseas. Em ambientes hospitalares e em profissionais da área da saúde esse microrganismo tem sido isolado com elevada frequência e a sua transmissão para os pacientes ocorre através do contato direto ou indireto com esses trabalhadores, tornando assim, um fator de risco para o desenvolvimento de infecções oportunistas. (WACLAVICEK, et al., 2009; DIAS et al., 2012).

Essa bactéria ainda pode gerar surtos de intoxicação alimentar, mas esses podem ser controlados recorrendo a medidas educativas direcionadas a profissionais que trabalham na manipulação de alimentos (SOUSA et al., 2008).

2.4 – FATORES DE VIRULÊNCIA DE *S. AUREUS*

Entre os fatores de virulência presentes em *S. aureus* destacamos: Polissacarídeos capsulares, peptoglicano, ácidos teicóicos, proteína A, Enzimas, Hemolizinas, toxinas e superantígenos (WINN et al., 2010).

A produção do exopolissacarídeo capsular por *S. aureus* pode impedir a fagocitose do microrganismo por células polimorfonucleares do hospedeiro, esse efeito é conhecido como antifagocítico. Estudos apontam que o polissacarídeo capsular tipo 5 e o tipo 8 estão associados com a produção da toxina da síndrome do choque tóxico (SCHAECHTER et al., 2009; WINN et al., 2010).

A parede celular de *S. aureus* é caracterizada por conter moléculas de peptideoglicano e ácidos teicóicos, que possuem propriedades que contribuem com a virulência bacteriana desencadeando respostas no sistema imune do paciente, gerando: aumento da quimiotaxia das células polimorfonucleares, ativação do complemento, estimulação de anticorpos opsonicos e indução da produção de interleucina-1(WINN et al., 2010).

A proteína A é encontrada na parede celular bacteriana e tem a capacidade de se ligar a região Fc das subclasses da imunoglobulina G humana, exceto a IgG de classe 3, interferindo na opsonização e na ingestão do microrganismo pelas células polimorfonucleares (SCHAECHTER et al., 2009; WINN et al., 2010).

A produção de enzimas sintetizadas por *S. aureus*, como a catalase, atua inativando o peróxido de hidrogênio e os radicais livres tóxicos formados pelo sistema mieloperoxidase no interior das células fagocíticas, após a ingestão do microrganismo. Outra enzima produzida por *S. aureus* é a coagulase, essa enzima se liga à protrombina tornando-a ativa, que atua catalisando a conversão de fibrinogênio em fibrina. O produto dessa reação cobre a superfície bacteriana deixando-a mais resistente à opsonização e à fagocitose (SCHAECHTER et al., 2009; WINN et al., 2010).

2.5 – RESISTÊNCIA BACTERIANA AOS ANTIBIÓTICOS

A microbiota normal é caracterizada por estar presente em sítios corpóreos do hospedeiro, que em condições normais do organismo não são patogênicas. A relação entre o indivíduo com os microrganismos da microbiota normal da membrana de revestimento, das mucosas e da pele admite que eles sobrevivam em associação sem efeitos negativos sobre os ciclos de vida de ambos (MIMS et al., 2002; BONATO et al., 2007).

O diagnóstico das infecções bacterianas é realizado através de técnicas que permitem a visualização da morfologia bacteriana, identificação de seus antígenos, isolamento bacteriano, dosagem de anticorpos séricos, pesquisa da hipersensibilidade tardia e detecção de outras substâncias bacterianas contidas nas secreções e fluidos do organismo. Os métodos rápidos de diagnóstico utilizam de forma direta a amostra clínica do paciente para a identificação bacteriana e seus antígenos (TRABULSI, 2011).

Os termos antimicrobiano, antibiótico e anti-infeccioso compreendem uma ampla variedade de agentes farmacêuticos que incluem: drogas antivirais, antifúngicas, antiparasitárias e antibacterianas. Destes, o último é o mais frequentemente utilizado e, portanto, é o objetivo de vários trabalhos científicos (LEEKHA et al., 2011).

Em 1942, Selman A. Waksman, definiu antibiótico como qualquer produto microbiano capaz de ser antagônico ao crescimento de microrganismos (HUGHES et al., 2010), porém atualmente os agentes bacterianos são classificados em bactericidas e bacteriostáticos. Os bactericidas provocam a morte e a degradação celular, compreendendo principalmente as drogas que atuam sobre a parede celular, membrana ou DNA bacteriano. Já os bacteriostáticos inibem a replicação celular sem matar o microrganismo, sendo geralmente drogas que atuam na síntese proteica. Essa diferenciação entre bacteriostáticos e bactericidas não é absoluta, pois alguns antimicrobianos são bactericidas para determinados microrganismos e mostram-se bacteriostáticos para outros e assim vice-versa. Em infecções graves como endocardite e meningite as drogas bactericidas são as preferidas (LEEKHA et al., 2011).

Os antimicrobianos são divididos em várias classes compreendendo os β -lactâmicos, aminoglicosídeos, glicopeptídeos, macrolídeos, tetraciclinas, rifamicinas, quinolonas, lincosamidas, sulfonamidas, estreptograminas, oxazolidinonas, gramicidinas e outros. Cada classe tem suas especificidades, incluindo inibição da replicação de DNA, da transcrição, do metabolismo do ácido fólico, da síntese de proteínas e síntese/ integridade da parede celular. (MARQUES et al., 2007).

Os mecanismos de resistência aos antimicrobianos podem ser intrínsecos ao microrganismo, adquiridos por transmissão de material genético ou, através de mutação (BAPTISTA, 2013).

2.5.1 – Aspectos genéticos da resistência aos antibióticos

A resistência bacteriana aos antimicrobianos ocorre através de dois principais mecanismos: mutação no *loci* do cromossomo ou por meio da transferência horizontal de genes (DZIDIC et al., 2008). As mutações podem ser classificadas em dois grandes grupos: induzidas e espontâneas (VERMELHO et al., 2008).

As mutações espontâneas são as que ocorrem sem a participação de um elemento externo e são comumente decorrentes de erros causados pelas DNA-polimerases, enzimas que sintetizam DNA durante a duplicação, como também por danos espontâneos sofridos pelo DNA a partir de desaminação e hidrólise (VERMELHO et al., 2008).

As mutações induzidas ocorrem devido a agentes externos, classificados em físicos (radiação UV ou ionizante) ou químicos (hidroxilamina, nitrosoguanidina, óxido nitroso, etil-metano-sulfonato, entre outros), que são capazes de introduzir danos ou alterações nos ácidos nucleicos (VERMELHO et al., 2008, BAPTISTA et al., 2013).

A transferência horizontal de genes é um processo de aquisição de material genético entre bactérias da mesma espécie ou espécies diferentes. Há três mecanismos de transferência: transformação, transdução ou conjugação (BAPTISTA, 2013).

A transformação consiste na troca de material genético entre bactérias através do DNA presente no meio envolvente. A porção de DNA incorporada ao material genético da bactéria receptora deve ter ao menos 500 nucleótidos para que possa integrar ao DNA, esse processo resulta na morte ou lise da bactéria doadora (SCHAECHTER et al., 2009, BAPTISTA, 2013).

A transdução envolve a presença de bacteriófagos ou fagos, que funcionam como vetores, transferindo DNA da célula doadora para a receptora. Cada fago transporta uma pequena porção de DNA da bactéria destruída, protegendo a integridade do DNA das nucleases existentes no meio (BAPTISTA, 2013).

A conjugação é a terceira forma de absorção de DNA exógeno, esse processo pode ocorrer entre células bacterianas da mesma espécie ou diferentes, que ao entrarem em contato direto trocam pequenas porções de material genético, tais como os plasmídeos (SCHAECHTER et al., 2009, BAPTISTA, 2013).

A resistência natural ocorre devido a características intrínsecas presentes em determinada espécie bacteriana, acontecendo mesmo sem a exposição prévia do antimicrobiano. A ausência de um processo metabólico influenciável pelo antibiótico, existência de enzimas capazes de inativar a droga e presença de características específicas à morfologia bacteriana são fatores responsáveis pela resistência natural (BAPTISTA, 2013).

Há quatro tipos principais mecanismos de resistência de forma adquirida aos antibióticos: síntese de bombas de efluxo, alteração do sítio alvo, alteração da permeabilidade celular e mecanismos enzimáticos que alteram a estrutura química do antibiótico, figura 1.

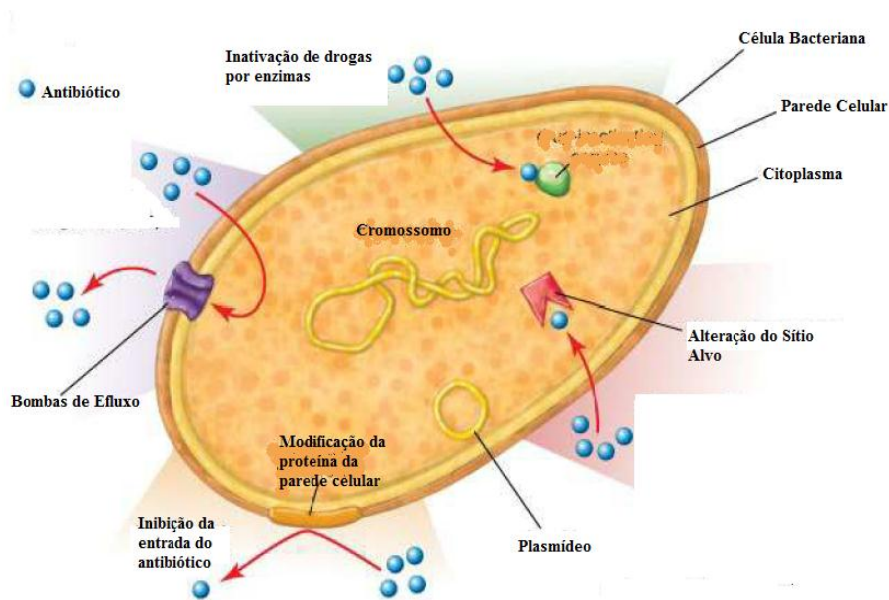


Figura 1 – Representação dos mecanismos de resistência adquirida.
FONTE: BAPTISTA, 2013 MODIFICADO

2.5.2 – Resistência do *S. aureus* à Meticilina

Antes da descoberta dos antibióticos a taxa de mortalidade dos pacientes acometidos com infecções causadas por *S. aureus* era superior a 75%. Em 1942 a penicilina foi introduzida no mercado com o objetivo de tratar pacientes acometidos com infecções originárias de *S. aureus*, porém em 1943 foram encontradas cepas resistentes à penicilina, PRSA, em vista disso em 1959 foi posto em comercialização a Meticilina para tratamento de pacientes infectados com PRSA, sendo que em 1961 nos Estados Unidos já se tinham

descritos microrganismos resistentes a esse medicamento (IPPOLITO et al., 2010; ASKARI et al., 2012).

As infecções hospitalares são consideradas um dos fatores responsáveis pela elevação nas taxas de morbidade e mortalidade em pacientes internados, além de gerar um aumento significativo no custo da internação hospitalar e prolongação do período com a terapia antibiótica (JARVIS, 1987; DHAND et al., 2012).

Outro fator agravante em infecções relacionadas à MRSA é que esses isolados mostram-se resistentes a outras classes de antimicrobianos (NOSTRO et al., 2009), dificultando assim o tratamento, devido a diminuição das opções terapêuticas para o tratamento da doença.

A síntese de peptideoglicano, principal constituinte da parede celular bacteriana, envolve várias etapas iniciando com a conversão da L-Alanina em D-Alanina, através de uma racamase. A próxima fase é junção de dois aminoácidos D-Alanina formando o dipeptídeo, D-Ala-D-Ala, que se liga tripeptídeo Uracil difosfato-N-acetilmurâmico formando o pentapeptídeo Uracil difosfato-N-acetilmurâmico que fica ligado ao transportador lipídico Udecaprenol. Após essas etapas, a adição da N-acetilglicosamina (GlcNac) do Uracil difosfato-GlcNac permite a translocação dos precursores para a superfície externa da membrana citoplasmática, onde o pentapeptídeo N-acetilmurâmico será adicionado ao peptideoglicano por transglicosilação, formando ligações cruzadas por transpeptidação (REYNOLDS, 1989; COURVALIN, 2006), figura 2.

Os antibióticos β -lactâmicos atuam na parede celular bacteriana ligando-se a proteínas ligadoras de Penicilina (PBP) que são responsáveis pela ligação cruzada no estágio final de formação da parede celular, inibindo assim sua síntese, devido ao impedimento da ligação entre as cadeias de peptideoglicano, depois dessa fase é ativado autolisinas que degradam a parede celular (MIMS et al., 2005; MURRAY et al., 2006).

A resistência dos isolados de *S. aureus* a Meticilina está associada à mudança da Proteína Ligadora de Penicilina (PBP), por PBP2' ou PBP2a, que apresenta baixa afinidade de ligação aos antibióticos β -lactâmicos. Essa proteína é codificada pelo gene *mecA*, que é carregado em um elemento móvel chamado Staphylococcal cassette Chromosome (SCCmec), onde em infecções hospitalares resistentes a meticilina predominam os subtipos de SCCmec I, II, III e na comunidade o SCCmec tipo IV (NOSTRO et al., 2004; IPPOLITO et al., 2010).

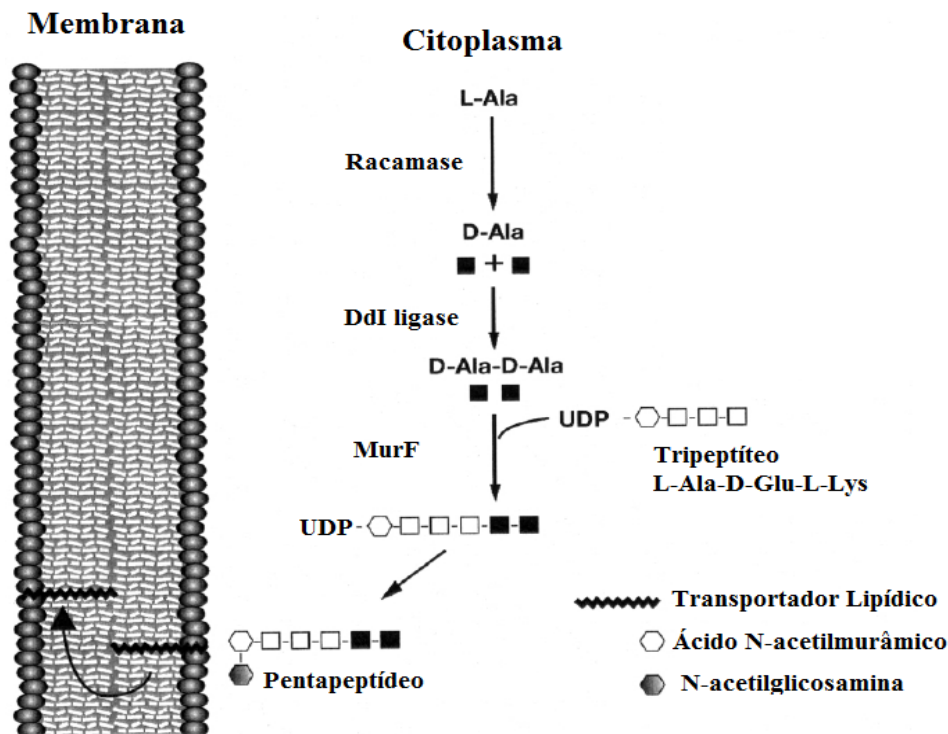


Figura 2 – Síntese de peptidoglicano
FONTE: COURVALIN, 2006 MODIFICADO

2.6 – A CAATINGA

A Caatinga é uma formação vegetacional exclusivamente brasileira, localizada na região semiárida do Nordeste brasileiro, que apresenta clima predominantemente seco com uma vegetação xerófila decorrente de longos períodos de seca e altas temperaturas, composta por árvores arbustivas, vegetação espinhosa, florestas secas (sazonal), cerrados (savana) e com algumas áreas de florestas tropicais. Apesar da ampla diversidade vegetal há poucos estudos relacionados metabólitos de plantas originárias dessa região (BASSO et al., 2005; MORAIS et al., 2006; TRENTIN et al., 2011; FRASSON et al., 2012).

Caracterizada pela baixa umidade e com média de temperatura anual de 27,5°C (a estação de seca tem duração de sete meses ou mais), a caatinga tem média de precipitação anual de 250-500 mm, extensão territorial de aproximadamente 734.478 km², exibindo solos férteis, drenados e oxigenados. Essa região abrange grande parte dos estados da região Nordeste incluindo Paraíba, Pernambuco, Alagoas, Piauí, Ceará, Rio Grande do Norte,

Sergipe, Bahia, além de parte do Nordeste de Minas Gerais (BASSO et al., 2005; TRENTIN et al., 2011; FRASSON et al., 2012)

2.7 – PRODUTOS NATURAIS

O metabolismo das plantas é dividido em dois tipos: o primário e o secundário. O metabolismo primário é responsável por produzir as substâncias essenciais para a manutenção da vida da célula, compreendendo a síntese de aminoácidos (FILHO, 2010), proteínas, lipídios e outras moléculas responsáveis pela manutenção das reações vitais da célula.

Os metabólitos secundários (MS) têm a função de participar das interações extra e intercelular da própria planta ou com outros organismos, ocorrendo por meio de MS quimioatraentes, como os monoterpenos em processos de polinização (WINK, 2003; FILHO, 2010).

Os Metabólitos secundários estão distribuídos em todas as plantas superiores abrangendo uma diversidade de produtos. A figura 3 apresenta os principais metabólitos encontrados.

Em vista de serem organismos que não tem a capacidade de se mover, nem terem um sistema imunológico, os metabólitos secundários (alcaloides, cianogênicos, glicosídeos, terpenos, taninos, saponinas, glicosinolatos, antraquinonas, poliacetilenos) auxiliam na defesa contra pestes e outras doenças (WINK, 2003; FILHO, 2010).

A concentração desses metabólitos na planta irá depender de variáveis como o solo, temperatura, pluviosidade, sazonalidade, radiação UV, composição dos gases atmosféricos, altitude, nutrientes, água, patógenos, ataque de predadores e estágio evolutivo da planta (GOBBO-NETO et al., 2007; MACÍAS et al., 2007).

Produtos Naturais

Nitrogenados

1. Alcalóides
2. Aminoácidos (Não Proteico)
3. Aminas
4. Glicosídeos Cianogênicos
5. Glicosinolatos

Não - Nitrogenados

6. Monoterpenos
7. Sesquiterpenos
8. Diterpenos
9. Triterpenos, Saponinas, Esteróides
10. Fenilpropanóides, Cumarinas, Flavanóides
11. Poliacetilenos, ácidos graxos
12. Policetídeos

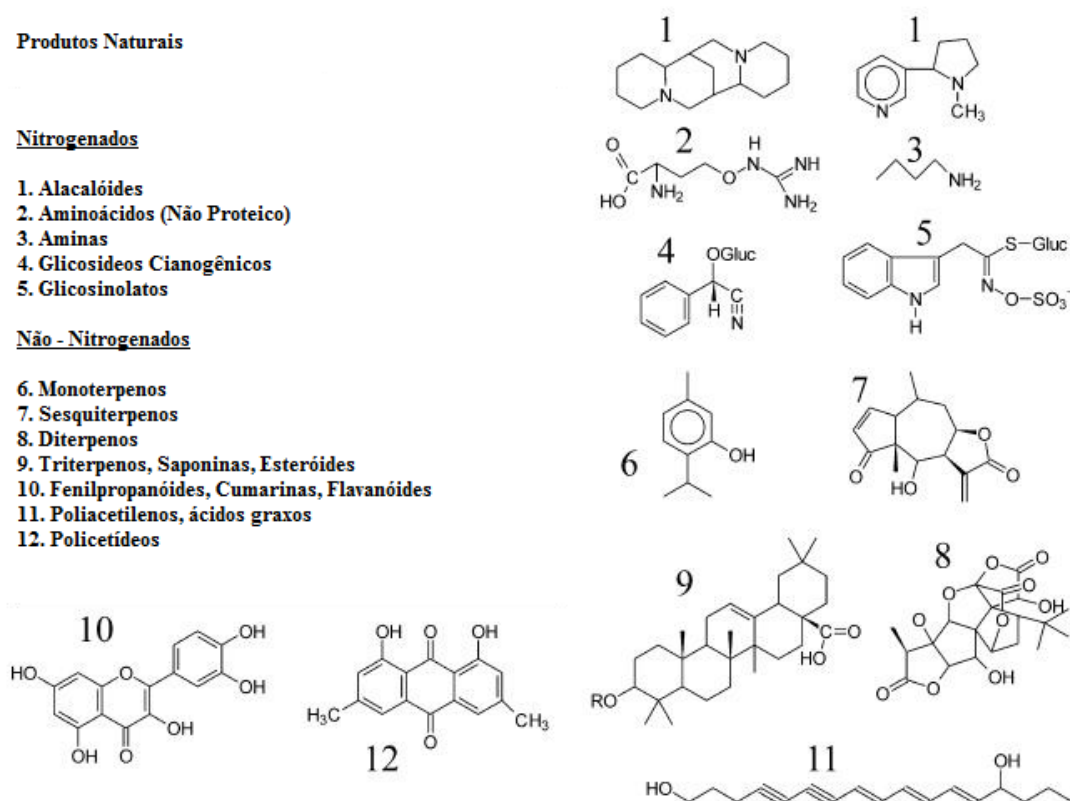


Figura 3 – Estrutura e diversidade dos metabólitos secundários em plantas.

Fonte: WINK, 2003 MODIFICADO.

2.7.1 – Ácidos Graxos

Os ácidos graxos (AG) são considerados como ácidos monocarboxílicos alifáticos, amplamente distribuídos em vegetais, onde geralmente estão ligados a açúcares, glicerol e grupos fosfatos para formar lipídios (DESBOIS et al., 2010; RUIZ-RODRIGUEZ et al., 2010; LIMA et al., 2011).

Os AG comumente estão ligados a lipídios e para sua remoção é necessário o uso de enzimas. A catálise dessas moléculas gera os ácidos graxos livres, que são cadeias de carbono ligadas a átomos de hidrogênio. O número de átomos de carbonos dos AG variam entre 4 e 28, tendo em uma de suas extremidades um grupo carboxila e na outra o grupo metila. (DESBOIS et al., 2010; RUIZ-RODRIGUEZ et al., 2010), Figura 4.

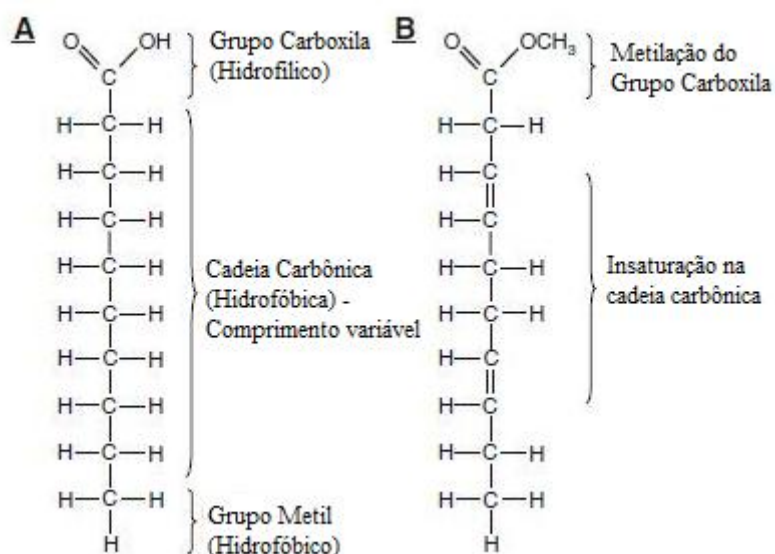


Figura 4 – Estrutura de um ácido graxo livre. a) Ácido graxo livre saturado, ácido cáprico, com 10 átomos de carbono. O comprimento da cadeia pode variar mas em uma extremidade há um grupamento carboxila (-COOH) enquanto que na outra extremidade há um grupamento metil (-CH₃). O grupamento carboxila é hidrofílico e ionizado quando solubilizado em água, enquanto que o grupamento metil é hidrofóbico, tornando a molécula inteira anfipática. b) Ácido graxo livre insaturado, tem uma ou mais duplas ligações (C=C) e, aqui, o ácido graxo está metilado, grupamento carboxílico tem um grupamento adicional - CH₂. Este ácido graxo tem 10 átomos de carbono, duas duplas ligações das quais a primeira dessas está localizada no terceiro carbono (DESBOIS et al., 2010).

Os ácidos graxos são classificados de acordo com o tipo de ligações entre átomos de carbonos, quando possuem apenas ligações simples são referidos como saturados, já se tiverem ligações duplas são designados de acordo com o número destas, sendo monoinsaturados (uma ligação dupla em toda a molécula) e poli-insaturados, quando apresentam mais de uma ligação dupla (DESBOIS et al., 2010; RUIZ-RODRIGUEZ et al., 2010).

Outra classificação é baseada de acordo com o número de átomos de carbono na estrutura dos ácidos graxos, onde cadeias com número inferior a oito carbonos são referidos como ácidos graxos de cadeia curta e acima de 16 carbonos são designados ácidos graxos de cadeia longa (DESBOIS et al., 2010).

Os ácidos graxos apresentam várias atividades biológicas, incluindo antimicrobiana, citotóxica, antioxidante e sinalização (DESBOIS et al., 2010; MEZNI et al., 2012).

A atividade antibacteriana de cada ácido graxo é influenciada por sua estrutura e forma, onde o comprimento da cadeia e a presença, o número, posição e orientação das ligações duplas interferem na atividade (DESBOIS et al., 2010).

2.7.2 – Óleos Essenciais

Tem-se verificado um grande avanço científico envolvendo os estudos químicos e farmacológicos de plantas, visando obter novos compostos com propriedades terapêuticas (FILHO et al., 1997). Neste sentido, dentre os agentes terapêuticos provenientes de plantas destacam-se os óleos essenciais, também denominados de óleos voláteis ou óleos etéreos.

Os óleos essenciais do ponto de vista químico são misturas complexas de substâncias voláteis, lipofílicas, geralmente odoríferas e líquidas. São extraídos de diversas partes das plantas (flores, inflorescências, sementes, folhas, gravetos, cascas, frutos e raízes) por processos específicos. São dotados de aroma geralmente agradável, ligeiramente amarelado de aparência oleosa e incolores quando recentemente extraídos. Tem como característica principal a volatilidade, que os difere dos óleos fixos que são misturas de substâncias lipídicas obtidas geralmente de sementes (SIMÕES et al., 2007).

As substâncias químicas encontradas nos óleos essenciais são formadas por ésteres de ácidos graxos, terpenóides, fenilpropanonas, alcoóis, aldeídos e em alguns casos, por hidrocarbonetos alifáticos. No entanto, óleos essenciais são constituídos principalmente de terpenos, sesquiterpenos, ésteres, alcoóis, fenóis, aldeídos, cetonas e ácidos orgânicos (ROCHA & SANTOS, 2007). Os compostos terpênicos mais frequentes nos óleos são os monoterpenos (cerca de 90%) e os sesquiterpenos, podendo também estar presente os diterpenos (SIMÕES et al., 2007).

A composição e a concentração das substâncias que constituem os óleos essenciais podem sofrer influência de fatores como a radiação, temperatura, precipitação, ventos fortes, altitude, solo, época de coleta, entre outros (GOUINGUENÉ et al., 2002).

Os óleos essenciais demonstram uma imensa variedade de ações farmacológicas, tornando-os potenciais fontes para o desenvolvimento de novas drogas. Dentre estas ações estão à antiparasitária, antimicrobiana, analgésica, diurético, antimalárico, antihemorroidário, mio relaxante, anti-inflamatório, anticonvulsivante e gastroprotetora (OLIVEIRA et al., 2001; ABDON et al., 2002), também tendo grande aplicação na indústria de perfumaria, cosmética, alimentos e como coadjuvantes em medicamentos (BIZZO et al., 2009).

Estudos apontam que existem aproximadamente 3000 óleos essenciais, desses cerca de 10% tem importância comercial (NERIO et al., 2010). O Brasil ocupa grande influência no mercado internacional na produção de óleos essenciais, tendo como produtos principais os óleos de laranja, limão e lima, além de outros gerados de cítricos (BIZZO et al., 2009).

2.8 – GÊNERO *SYAGRUS*

As espécies pertencentes à família Arecaceae (Palmae) são comumente chamadas de palmeiras, sendo muito interessantes em vista das suas características químicas e farmacológicas. Essas palmeiras estão largamente difundidas nas zonas temperadas, em especial em regiões onde o índice pluviométrico é alto (CRUZ, 1965; ZOFEMLER, 1994).

Do ponto de vista químico, as plantas dessa família são comumente não cianogênicas. Os alcaloides (ocasionalmente pirimidínicos) e proantocianidinas podem estar presentes nessas espécies. Os flavonoides são raramente encontrados, mas quando presentes são derivados do tricina, kaempferol, luteolina e quercetina. Saponinas e sapogeninas estão ocasionalmente presentes. Éteres metílicos de triterpenos já foram isolados dos frutos de algumas espécies de palmeiras (HEIN DE BALSAC et al., 1931; SHIMOKOMAKI et al., 1975; HARBORNE et al., 1994; LUBRANO et al., 1994; BROTONS et al., 1995; GARCIA et al., 1995; LUBRANO, 1997; ROBIN, 1997; LEWIS, 2000; ZONA, 2000).

Syagrus é um gênero muito variável morfologicamente, quase exclusivo da América do Sul (exceto por *Syagrus amara* (Jacq.) Mart., que ocorre no Caribe), representado até o momento por 53 espécies, das quais 47 ocorrem no Brasil (NOBLICK, 2010; LEITMAN et al., 2013). É composto por palmeiras monoicas, polícarpas, de pequeno ou grande porte, solitárias ou entouceiradas, com estipe subterrâneo ou elevado, raramente estolonífero (*Syagrus campylospatha* Barb. Rodr.), liso ou coberto pelos remanescentes das bainhas das folhas já caídas (DRANSFIELD et al., 2008; NOBLICK, 2010). A maioria das espécies acaulescentes e de pequeno porte está confinada às áreas semiáridas ou de cerrado, enquanto um menor número de espécies de porte arbóreo é comumente encontrado nas áreas tropicais ou subtropicais úmidas, sendo componentes bastante conspícuos em várias formações vegetacionais do Brasil (BARBOSA RODRIGUES, 1903; BONDAR, 1964; DRANSFIELD et al., 2008).

Algumas espécies do gênero são muito valorizadas localmente, em vista dos produtos extraídos como: palmito, amêndoas, polpa dos frutos e folhas para o artesanato; é o caso da

guariroba (*Syagrus oleracea* (Mart.) Becc.), do ouricuri (*Syagrus coronata* (Mart.) Becc.) e do gerivá (*Syagrus romanzoffiana* (Cham.) Glassman) (BONDAR, 1964; NOBLICK, 2010); outras espécies vêm sendo recentemente introduzidas com sucesso no paisagismo (NOBLICK, 2010). No entanto, a maioria das espécies encontra-se bastante ameaçada pela expansão da agricultura, especialmente as de pequeno porte, comuns nos cerrados e caatingas.

2.8.1 – *Syagrus coronata*

Syagrus coronata (Mart.) Becc. (Figura 5) é uma palmeira pertencente à família Arecaceae, subfamília Arecoideae, tribo Cocoeae, subtribo Butineae (CREPALDI et al., 2001; SALLES et al., 2010; BELVISO et al., 2013). Com 115 gêneros e 1500 espécies a subfamília Arecoideae é a maior entre a família Arecaceae (CREPALDI et al., 2001).

S. coronata é popularmente conhecido por: licuri, ouricuri, aricuri, coqueiro cabeçudo, licurizeiro, nicuri, urucuri e coqueiro dicori (DRUMOND, 2007; SALLES et al., 2010; BELVISO et al., 2013). Essa palmeira é comumente encontrada em regiões secas e áridas da Caatinga ocupando a parte oriental e central da Bahia até o Sul de Pernambuco, o norte de Minas Gerais e áreas de Alagoas e Sergipe (CREPALDI et al., 2001; BELVISO et al., 2013).

O ouricuri se propaga de forma exclusivamente sexuada, desenvolvendo-se em solos férteis e profundos, mesmo em afloramentos rochosos, porém não se adapta a solos alagados e permanentemente úmidos (DRUMOND, 2007).

O tamanho da palmeira é cerca de 6 a 10 metros, com folhas grandes que chegam a medir de 2 a 3 metros de comprimento, distribuídas em espiral ao longo do fuste (DRUMOND, 2007, CREPALDI et al., 2001).

As flores do ouricuri são pequenas, amarelas e reunidas em cachos. Os frutos quando verdes possuem o endosperma líquido e a medida que atingem o processo de amadurecimento ficam sólidos originando as amêndoas. As tonalidades dos frutos maduros alternam de Amarelo Claro ao Laranja, dependendo do estágio de maturação, com cachos apresentando aproximadamente 1450 frutos. A média do comprimento e diâmetro dos frutos ficam em torno de 1,4 cm e 2 cm (CREPALDI 2001; BELVISO et al., 2013, DRUMOND, 2007), figura 5.



Figura 5 – Detalhe da infrutescência do ouricuri.

Fonte: BELVISO et al., 2013.

Os frutos são produzidos anualmente, no entanto, há um maior rendimento nos meses de março, junho e julho (CREPALDI et al., 2001; SALLES et al., 2010).

O ouricuri tem grande importância social e econômica para a região, já que envolve a participação da comunidade local na colheita dos frutos e processamento das sementes, que pode ser consumida crua, cozida ou torrada ou utilizada para a obtenção de óleo que é consumido pela culinária local (BELVISO et al., 2013). As folhas servem para a alimentação de gados, aves e animais silvestres (DRUMOND, 2007).

Além dos pontos acima mencionados, a semente de ouricuri tem um grande valor nutricional (49.2% de lipídios, 9.7% de Carboidratos, 11.5% de proteínas; 2.6×10^6 J/100g) (CREPALDI et al., 2001; BELVISO et al., 2013;).

2.8.2 – Constituintes químicos e atividades biológicas de *Syagrus* spp.

Alguns estudos já foram conduzidos à cerca dos constituintes químicos de *S. coronata*, porém todos no sertão baiano e mineiro (CREPALDI et al., 2001, SEGALL et al., 2004, BELVISO et al., 2013).

Os lipídios, proteínas, carboidratos, nitrogênio e vitaminas são componentes químicos encontrados em amêndoas e frutos de *S. coronata*. As amêndoas contêm maior teor de lipídios e proteínas, já na polpa há maior presença de carboidratos.

Estudo realizado para analisar a composição fenólica de sementes de *S. coronata* constatou a presença de 13 compostos fenólicos, entre eles Procianidina B1, Catequinas, Procianidina B2, Epicatequinas, Quercetina-3-O-glicosídeo, Rutinas, Miricetinas, Quercetina-3-O-raminosídeo (BELVISO et al., 2013).

Em relação à composição dos ácidos graxos do óleo extraído das sementes de *S. coronata*, estudo comprovou a presença de ácido láurico, mirístico, palmítico, esteárico e linoleico (SEGALL et al., 2004).

BELVISO e colaboradores (2009) relataram que o óleo de *S. coronata* apresenta capacidade antioxidante.

Há escassos trabalhos na literatura que abordam os óleos essenciais da família Arecaceae. Apenas um único trabalho estudou os compostos voláteis dos frutos de *S. coronata* (BELVISO et al. 2013).

As atividades biológicas registradas para as espécies de *Syagrus* são escassas, sendo *S. coronata* e *S. oleracea* as espécies mais estudadas (SILVEIRA et al., 2005; HUGHES et al., 2013).

Silveira et al. (2005) avaliaram a atividade antimicrobiana do extrato etanólico (e suas partições em hexano e acetato de etila) do epicarpo/mesocarpo dos frutos e do extrato hexânico das amêndoas (endosperma) frente às bactérias *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* e *Escherichia coli* através da técnica de microdiluição seriada.

Observou-se que para o epicarpo/mesocarpo de *S. oleracea*, de uma maneira geral, a atividade antimicrobiana aumenta com a diminuição da polaridade dos extratos, ocorrendo uma exceção apenas no teste realizado com *S. aureus*, onde o extrato etanólico obteve percentual de inibição microbiana superior à partição em acetato de etila. Estes resultados sugerem que as substâncias presentes nos extratos hexânicos provavelmente são as principais responsáveis pela atividade antimicrobiana de *S. oleracea*. Os ácidos graxos presentes,

principalmente nos extratos lipofílicos, podem ser os responsáveis pela atividade antimicrobiana.

Hughes et al. (2013) avaliaram a atividade antimicrobiana dos extratos aquoso e metanólico (e suas frações) das folhas, inflorescências, endosperma líquido e endosperma sólido frente às bactérias *Bacillus cereus*, *Escherichia coli*, *E. coli* sensível à trimetoprima e resistente à sulfonamida, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Staphylococcus aureus*, *S. aureus* resistente à estreptomicina e diidroestreptomicina, *S. aureus* resistente à novobiocina e contra os fungos *Candida albicans*, *C. albicans* resistente a amfotericina B e fluconazol e *Malassezia furfur*. A técnica utilizada foi o teste em disco. O extrato aquoso da inflorescência foi o único entre os extratos aquosos que apresentou atividade antimicrobiana, particularmente contra *B. cereus* e *S. aureus*.

Bora e Moreira (2003) analisaram composição química dos ácidos graxos dos frutos de guariroba (*S. oleraceae*). Os autores identificaram 15 e 19 ácidos graxos na polpa e na amêndoa, respectivamente. Esses óleos contêm 48,9 e 73,2% de ácidos graxos saturados, respectivamente. O ácido oleico foi o principal ácido graxo monoinsaturado em ambos os óleos.

Coimbra e Jorge (2011) também analisaram os ácidos graxos dos frutos de guariroba e do jerivá (*S. romanzoffiana*). Foram registrados 16 ácidos graxos na polpa, sendo o ácido palmítico predominante. Para as amêndoas foram encontrados nove ácidos graxos para guariroba e 10 ácidos graxos para o jerivá. Os principais ácidos graxos foram o láurico, mirístico e oleico, os quais representaram cerca de 70% dos ácidos graxos.

3 – OBJETIVOS

3.1 – OBJETIVO GERAL

Analisar a composição química e avaliar a atividade antibacteriana do óleo essencial e de ácidos graxos extraídos das sementes de uma palmeira endêmica, *S. coronata*, coletadas em uma área prioritária para conservação da Caatinga.

3.2 – OBJETIVOS ESPECÍFICOS

- Extrair e caracterizar óleos essenciais das sementes de *S. coronata*;
- Extrair e caracterizar os ácidos graxos das sementes de *S. coronata*;
- Avaliar a atividade antimicrobiana contra isolados clínicos de *Staphylococcus aureus* resistentes a antibióticos, dos óleos essenciais e ácidos graxos de *S. coronata*.

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4 - ARTIGO CIENTÍFICO

ARTIGO SUBMETIDO AO PERIÓDICO FOOD CHEMISTRY

Essential oils and fatty acids from *Syagrus coronata* (Mart.) Becc. (Areaceae): chemical composition and anti-*Staphylococcus aureus* action

Essential oils and fatty acids from *Syagrus coronata* (Mart.) Becc. (Areaceae): chemical composition action anti-*Staphylococcus aureus*

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ABSTRACT

The aim of this study was to investigate the chemical composition and anti-*Staphylococcus aureus* effect of the seeds essential oils and fatty acids of *Syagrus coronata* in experimental models. The chemical composition of the essential oils and fatty acids were analysed by GC/MS. *In vitro* anti-microbial activity of the essential oils and fatty acids were determined by broth microdilution method. The major compounds identified in the essential oils were α -phelandreno (26.26%), *trans*-Caryophyllene (18.01%), and β -phelandreno (12.93%). The major compounds identified in the fatty acids were the saturated fatty acids, dodecanoic acid and tetradecanoic acid with 41.58 and 9.68%, respectively. Following by 9-octadecenoic acid, a monounsaturated fatty acid, with 23.81%. The only polyunsaturated fatty acid presentes in the chemical composition of the fatty acids was 9,12-octadecadienoic acid with 3.59%. The essential oil was dominated by sesquiterpene constituents and fatty acids were dominated by saturated fatty acids and have some interesting antimicrobial activity. Essential oil shows a

very strong activity against the standard *S. aureus* strain. The values of MIC against clinical *S. aureus* strains ranged from 0.002 $\mu\text{L/mL}$ to 0.09 $\mu\text{L/mL}$.

Keywords

Syagrus coronata, Arecaceae, Essential oil, Fatty acids, Chemical composition, Antimicrobial activity

1. Introduction

The rapid development of antimicrobial drug-resistant of pathogens and their spread around the world are amongst the most serious threats to public health and to successful antibacterial treatment. In recent years, the emergence of bacterial resistance against multiple antibiotics has accelerated dramatically (Grundmann et al 2011; Lai et al. 2011). Community and hospital acquired pathogens, including *Staphylococcus aureus*, *Salmonella*, *Shigella*, coagulase-negative *Staphylococcus*, *Enterococcus* sp., *Escherichia coli*, and *Pseudomonas aeruginosa*, are some of the main multi-drug-resistant bacteria. In particular, *S. aureus* has the capability to express a variety of virulence factors that are considered medically relevant when encountered in clinical specimens. Although antibiotic vancomycin has been used as the drug of last resort for MRSA infections, the emergence of vancomycin resistant strains have been reported worldwide (Tenover et al., 2004; Sibanda et al., 2010). Thus, there is a growing interest for search of new agents against resistant strains of *S. aureus*, in particular from medicinal plants (Da Silva et al., 2012; Silva et al. 2013).

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Cox 1990; Cassady et al. 1999). Interest in plants with antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics (Potterat & Hamburger 2008; Chaudhary et al. 2010; Vieira 2010; Silva et al. 2013).

Brazil is fifth largest country in the world but it is the most megadiverse. There is a great diversity of biomes and a particularly stands out for being exclusively distributed in Brazil: the Caatinga. This region is marked by an accentuated dryness (rainfall is usually less than 900 mm/year). The Caatinga is also named as Semi-arid region and occupies a large portion of Brazil's Northeast, comprising several plant species (Albuquerque et al., 2012). As a result of the environmental conditions to which they are exposed, the Caatinga plants have developed interesting chemical features and they have been described as excellent weapons

against microorganisms (Almeida et al., 2012; Silva et al., 2012; Da Silva et al., 2012; Tretin et al., 2013).

Among the alternative therapeutic arsenal, the essential oils and fatty acids could be an interesting choice against this pathogen; the antiseptic properties essential oils and fatty acids have been demonstrated, at least *in vitro* (more than 4000 publications about antimicrobial activity of EOs referenced in PubMed since 2002).

Fatty acids are ubiquitous molecules typically found bound to other compounds such as glycerol, sugars or phosphate headgroups to form lipids. Lipids are integral components of cell structures, e.g. membranes, which are made up of phospholipids, and energy stores that are often composed of triglycerides. Fatty acids can be released from lipids, typically by enzyme action, to become free fatty acids, which have diverse and potent biological activities. Free fatty acids consist of a chain of carbon atoms attached to hydrogen atoms. The number of carbon atoms varies, but those in biological systems usually have an even number between 10 and 28. At one end of the carbon chain is a carboxyl group ($-\text{COOH}$) and, at the other end, is a methyl group ($-\text{CH}_3$). The carboxyl group is hydrophilic and ionised when solubilised in water, whereas the carbon chain is hydrophobic, making the entire molecule amphipathic. FAs with <8 carbon atoms are considered short chain, whereas those with >16 carbon atoms are regarded as long chain. Unsaturated FAs have one or more $\text{C}=\text{C}$ double bonds in the carbon chain, while the carbon atoms in saturated fatty acids are all joined by $\text{C}-\text{C}$ single bonds.

Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. They are liquid, volatile, limpid and rarely coloured, lipid soluble and soluble in organic solvents with a generally lower density than that of water. They can be synthesized by all plant organs, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. Essential oils are very complex natural mixtures which can contain about 20–60 components at different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetic origin (Croteau et al., 2000; Betts, 2001; Bowles, 2003; Pichersky et al., 2006). The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight.

The aim of this study was to examine the content and composition of the essential oil and fatty acid obtained from seeds of *S. coronata*, a endemic palm to the Caatinga and evaluate the antimicrobial activity against different *Staphylococcus aureus* strains isolated from clinical samples, which presented different profiles of resistance to various antibiotics conventionally applied in clinical therapy.

2. Material and methods

2.1. Plant material

The samples (Fig. 1) of fruits were collected at Catimbau National Park (Pernambuco, Northeastern Brazil) in mature fruit stage, during the month of March, 2013, identified by Agronomic Institute of Pernambuco and a voucher specimens/(IPA 86950) was deposited. The region was characterized by annual precipitation of 600-900 mm/annual and mean annual temperature of 23.5°C in which the highest (36.2°C) being in January and the lowest (18.3°C) in June. The seeds were removed from matured fruits and dried (33°C) at open area with active ventilation until attained constant weight (three weeks). The seed kernels were removed from the seed and then grind to the small pieces using domestic grinder.

2.2. Essential oil extraction

Samples of seeds (250 g) of *S. coronata* were submitted to hydrodistillation for 4 h, in a Clevenger-type apparatus. The oils were dried over anhydrous Na₂SO₄ and their percentage contents were calculated on the basis of the dry weight of plant material. The oils were stored at 4°C until further analysis.

2.3. Fatty Acids extraction

Samples of seeds (200g) of *S. coronata* were dried at room temperature for 5 days and the powder transferred to a 1-liter round bottom flask. To the flask was added sufficient hexane (Acros Organics) to bring the volume of the mixture to ca. 500 mL. The flask was sealed, transferred to a rotary shaker (Thermolyne AROS 160) and the contents mixed for 72 hours. The mixture was then allowed to settle for several hours and then twice vacuum filtered through Fisher Scientific P5 (Atlanta, GA) filter paper. The filtrates were concentrated by rotary evaporation, yielding light yellow oils.

2.4. Microorganisms

S. aureus strains used as test microorganisms were isolated from clinical material (Table 1) by standard procedures (Murray, 1999; Konneman, 2001). The most strains presented resistance profile to some antibiotics applied in clinical therapy (Table 2). Antibiotic resistance study was carried out according to NCCLS (2011). Stock cultures were maintained on Muller-

Hinton agar slants at 7°C ($\pm 1^\circ\text{C}$). Overnight cultures inoculated in Muller-Hinton agar slants at 37 °C were used to prepare the bacterial inoculum used in the antimicrobial assays. The inoculum was of $1,5 \times 10^8$ colony forming units per mL (CFU/mL) prepared in sterile saline solution (0.85%) and standardized according to the turbidity of McFarland scale 0.5 tube and adjusted for presenting the wished microbial population. Essential oil solutions and fatty acids solutions used in antimicrobial assays were obtained according to the following procedure: 400 μL of the essential oil or fatty acid, 40 μL of Tween 80 and q.s.f. 5 mL of sterile water were added in sterile assay tube and shaken for 5 minutes using Vortex (Fanem), obtaining a solution with final essential oil concentration of 80 $\mu\text{L/mL}$. Following serial dilution technique, it was obtained solutions with concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.08, 0.04, 0.02, 0.01, 0.005 and 0.002 $\mu\text{L/mL}$ (Allegrine et al. 1973 with adaptation).

2.5. Determination of minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

MIC was determined by the microdilution method (CLSI, 2011). A twofold serial dilution of the solution containing essential oil/fatty acids was prepared in Mueller Hinton Broth (MHB) and 100 μL (approximately 1.5×10^8 CFU/ml) of bacteria suspension was added. The samples were incubated for 24 h at 37°C. Resazurin solution (0.01%) was used as an indicator by color change visualization: any color changes from purple to pink were recorded as bacterial growth. The lowest concentration at which no color change occurred was taken as the MIC. Afterwards, cultures were seeded in MHA medium and incubated for 24 h at 37°C to determine the MBC which corresponds to the minimum concentration of extract/fractions that eliminated the bacteria.

2.6. Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized with two parameters such as Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). According to the ratio MBC/MIC, we appreciated antibacterial activity. If the ratio $\text{MBC/MIC} = 1$ or 2, the effect was considered as bactericidal but if the ratio $\text{MBC/MIC} = 4, 8, 16$ or 32, the effect was defined as bacteriostatic (Berche et al. 1988, Oliveira et al., 2012).

2.7. Analysis of fatty acids

The fatty acids composition of oils was determined by converting into fatty acids methyl esters followed by gas chromatography (GC). Samples of 50 mg oil were accurately weighed in thick-walled 15 ml glass tubes. The tubes were prepared in advance with an accurately

determined amount of the saturated fatty acids, nonadecanoic acid, 19:0 as internal standard. This was added to the tubes by pipetting 50.0 μl of a solution of 19:0 in chloroform into the tubes, and then allowing the chloroform to evaporate. This pipetting was carried out with the handystep electronic, motorized repetitive pipette. Anhydrous methanol, 750 μl , containing hydrogen chloride in a concentration of 2 mol/L, was added, the tubes were securely closed with teflon-lined screw caps and placed in an oven at 90°C for 2 h. The lipids were methanolysed, leaving all fatty acids as fatty acids methyl esters. After cooling to room temperature, approximately half the methanol was evaporated under a stream of nitrogen after which 0.5 mL distilled water was added.

The fatty acids methyl esters were extracted 2 times from the methanol/water phase with 1 ml hexane by vigorous shaking by hand for 1 min each time, followed by centrifugation at 3000 rpm. Analysis was carried out with a Hewlett-Packard 5890A gas chromatograph and Hewlett-Packard 7673A auto-sampler. A fused silica capillary column with polyethylene glycol as the stationary phase with a thickness of 0.2 μm (CP-WAX 52CB from Chrompac, 25m $\times$ 0.25mm) was used. The carrier gas was helium at a flow rate of 1.7ml/min at 40°C. One microliter of the combined fatty acids methyl esters extracts was automatically injected splitless (the split was opened after 4 min), on a capillary column. The temperature program was 90°C for 4 min, 90 to 165°C with 30°C/min, 165 to 225°C with 3°C/min, 225°C for 10 min, total run time 43 min, cooling included. Injector and detector temperatures were 260 and 330°C, respectively.

Samples were chromatographed in random order with a standard solution, GLC 68D containing 20 FAMES, for each 8th sample. The quantitatively most important FAs were identified in the samples by way of the standard mixture and by using previous experience (Grahl-Nielsen et al., 2000) of relative retention times of fatty acids methyl esters and mass spectrometry.

The response factors for the fatty acids methyl esters for which there were no standards, were estimated by comparing with the standard fatty acids methyl esters which resembled each of those most closely in terms of chain length and number of double bonds. The relative amount of each fatty acids in a sample was expressed as a percentage of the sum of all fatty acids in the sample. Three replicate analyses were carried out for all samples. Analysis of variance (ANOVA) was performed for fatty acids composition of seeds from all plant species using GenStat Statistical Computer Package (GenStat Release 7.1, 2003) and the means were separated using LSD ($P \leq 0.05$).

2.8. GC/MS analysis of essential oil

The GC/MS analyses were performed in EI mode on a Hewlett Packard-6890 GC system with a fused capillary column (30 m x 0.25 mm x 0.25 μ m, HP-5MS, Crossbond 5% phenyl-95% dimethylpolysiloxane) directly coupled to on Hewlett Packard 5973 selective mass detector. The conditions of injection were the same as described above. The mass spectrometer was operated at 70 eV. The constituents of the essential oils were identified by comparison of their mass spectral pattern and retention indices (RI) with those given in the literature (Adams 2007). The retention indices (RI) were calculated according to van den Dool and Kratz (1963).

3. Results and Discussion

The fatty acid composition from *S. coronata* seeds kernel oils is shown in Table 3. Nineteen fatty acids were detected including 7 in trace concentrations. The distribution of fatty acids was: 72.3% saturated, 23.9% monounsaturated and 3.6% polyunsaturated fatty acids. Normally, odd carbon numbered fatty acids are not reported in oils, but kernel oil showed the presence of C7, C9, C11, C13 and C15 fatty acids, though at trace concentrations. Among saturated fatty acids, C12 (lauric acid) as its principal acid (41.58%), while C14 (9.68%), C16 (7.19%), C8 (5.32%), C10 (4.54%) and C18 (3.54%) were also present in appreciable concentrations. Oleic acid (C18:1) was the dominant monounsaturated fatty acid constituting about 84.9% of mono-unsaturated fatty acids with concentration of 16.9% of total fatty acids in kernel oils.

Lauric acid was detected by Coimbra and Jorge (2011) in the three kernel oils of Arecaceae plants (*S. oleraceae*, *S. romanzoffiana* and *Acrocomia aculeata*) as the major saturated fatty acid, in contents ranging from 325.8 to 424.3 g kg⁻¹. The predominance of saturated fatty acids with medium chain length, such as lauric acid, is also a characteristic of other oils from the Palmae family species, such as coconut (Laureles et al. 2002) and palm tree kernel (Bora et al. 2003).

Research indicates that in some products the presence of solid fat is essential for maintaining the texture and consistency, the replacement of hydrogenated vegetable fat, with high *trans* fatty acids levels, by saturated fat, with high lauric acid content, seems be an interesting alternative, since this type of fat results in a more favorable blood lipid profile than a solid fat rich in *trans* fatty acids (Mensink et al. 2003; Ros et al. 2001). Furthermore, some oils rich in lauric acid have antibacterial activity, inhibit protozoa, reduce methane production and ammonia concentration, and thus are successfully used in the enrichment of diets rich in maize grains (Yabuuchi et al. 2006).

The fatty acid profile presented by the Chilean palm (*Jubaea chilensis*) was very similar to the ouricuri kernel. The saturated fatty acids of Chilean palm (Masson et al. 2008) totaled 84.78% and the majority of fatty acids were lauric, caprylic and oleic acids, with levels of 428.2, 130.1 and 121.5 g kg⁻¹, respectively; these values are very close to those presented by ouricuri kernel and others palms of *Syagrus*.

The fatty acids obtained from *S. coronata* show a very strong activity against the standard *S. aureus* strain (UFPEDA 02) and also against the examined *S. aureus* strains obtained from the clinical materials. The values of MIC against clinical *S. aureus* strains ranged from 0,156 µL/mL to 2,5 µL/mL. The growth of the standard *S. aureus* strain, UFPEDA 02, was inhibited by 1,56 µL/mL of the tested oil. The majority of *S. aureus* strains studied: eight out of 16, were sensitive to the oil concentrations of up to 0,625µL/mL or lower and eight were sensitive to the oil concentrations of up to 1,25 µL/mL (Table 5). These strains were isolated from blood, oropharynx nasal, wound secretion and eye discharge (Table 1). The MBC values to fatty acids were ranged from 0.156 to 2.5 µL/mL. The bactericidal and bacteriostatic effect of the fatty acids was determined using the ratio MBC/MIC (Table 5).

The data analysis indicates that the tested fatty acids showed the significant results when compared with the control. This may be due to the fact that the bioactive constituents such as saturated fatty acids and monounsaturated fatty acids compounds were responsible for the antimicrobial activity. In effect, some previous studies showed that saturated fatty acids and monounsaturated fatty acids compounds cause inhibition of a wide range of microorganisms.

From the chromatographic and spectrometric analyzes is possible to observe the presence of 55 compounds in the formation of the essential oil from the leaves of *S. coronata* of which 46 were identified. The major constituent of the essential oil of *S. coronata* is α -phellandrene, about 26% of the oil but can also evidence the presence of trans-caryophyllene (18.01%) and β -phellandrene (12.93%) in concentrations prominent. Among the compounds lower proportion we highlight the germacrene D (5.99%) and α -Humulene (5,46). About 11 constituents are present in proportions between 1 and 3% of the oil content; and 39 less than 1%. The whole chemical composition of the tested oil is shown in Table 4.

The compounds are distributed between six classes, the dominant class sesquiterpenes 41.8% of the oil, followed by monoterpenes (18.2%), unidentified compounds (16.3%), oxygenated sesquiterpenes (14.5 %) and oxygenated monoterpenes (7.3%). Ester compounds are lesser extent (1.8%). Commonly derivatives terpenoids are the most essential oil

compounds, with the monoterpenes and sesquiterpenes most common classes (Simoes and Spitzer, 1999).

No bibliographic data have been reported on essential oils compounds of ouricuri seeds and others neotropical palms. Only one study on the volatile fraction of *S. coronata* is available (Belviso et al. 2013). The authors evaluated the volatile fraction of raw and roasted seeds. A total of 59 volatile compounds were identified in licuri (34 in raw and 55 in roasted) belonging to 8 chemical classes. Among these, 30 compounds were found in both raw and roasted licuri. Studies on the volatile fraction and essential oil of other palm fruits such as coconut (*Cocos nucifera* L.) and date palm *Phoenix dactilifera* L.) belonging to the family Arecaceae (Jayalekshmy, Narayanan, & Mathew, 1991; Lin & Wilkens, 1970; Prades et al., 2012; Santos et al., 2011; Demirci et al. 2013) are available too. These researches high lighted that δ -lactones, aldehydes, alcohols, methyl ketones and fatty acids are the most importante compounds for volatile profile in coconut oil (Santos et al., 2011) and in coconut water (Prades et al., 2012).

The essential oil obtained from *S. coronata* shows a very strong activity against the standard *S. aureus* strain (UFPEDA 02) and also against the recently isolated *S. aureus* strains. The values of MIC against clinical *S. aureus* strains ranged from 0.002 $\mu\text{L/mL}$ to 0.08 $\mu\text{L/mL}$. The growth of the standard *S. aureus* strain, DEPA 02, was inhibited by 0.002 $\mu\text{L/mL}$ of the tested oil. The majority of *S. aureus* strains studied: eleven out of 16, were sensitive to the oil concentrations of 0.01 $\mu\text{L/mL}$ or lower and five were sensitive to the oil concentrations of up to 0.01 $\mu\text{L/mL}$ (Table 5).

The MBC values to essential oils were ranged from 0.002 to 0.312 $\mu\text{L/mL}$. The bactericidal and bacteriostatic effect of the fatty acids was determined using the ratio MBC/MIC (Table 5).

The data analysis indicates that the tested essential oil showed the significant results when compared with the control. This may be due to the fact that the bioactive constituents such as α -phellandrene, trans-cariophyllene, and β -phellandrene compounds were responsible for the antimicrobial activity. In effect, some previous studies showed that saturated fatty acids and monounsaturated fatty acids compounds cause inhibition of a wide range of microorganisms. These strains were isolated from blood, oropharynx nasal, wound secretion and eye discharge (Table 1).

In the last years there has been a great scientific interest in chemical and pharmacological investigations regarding the biological properties of medicinal plants (Referências recentes). It is known that medicinal plants have been source of many drugs

applied in clinical procedures (e.g morphine, emetine, rutine). Essential oils and fatty acids are involved in many important actions related to the plant survival, playing prominent role in its defense against microorganisms. The use of essential oils and fatty acids as antimicrobial agents presents two principal characteristics: i) their natural origin meaning more safety for users and environment; ii) there is low risk of rising microbial resistance to their action because essential oils/fatty acids are mixtures of several compounds that, apparently, presents different antimicrobial action making more difficult the microbial adaptability (Daferera et al., 2003).

Aligiannis et al. (2001) proposed a classification about the antimicrobial potential of plant products, based on MIC results: strong inhibitors – MIC of up to 0.5 $\mu\text{L/mL}$; moderate inhibitors – MIC between 0.6 and 1.5 $\mu\text{L/mL}$; weak inhibitors – MIC above 1.6 $\mu\text{L/mL}$. Regarding the MIC values found for all assayed *S. aureus* strains, the classification criteria above cited confirms the strong anti-staphylococcal property of *S. coronata* essential oil and moderate inhibitors of fatty acids. Still, this intense anti-*S. aureus* activity becomes more important when regarded that the most assayed strains showed resistance to at least three clinically used antibiotics (Table 2).

The antibacterial activity of lauric acid and their monoglycerides was reported. This compounds could be used to control to growth of some bacterial pathogens. The attention was focused on gram-negative bacteria (*Salmonella* spp., *E. coli* O157:H7 and *Y. enterocolitica*). The effectiveness of fatty acids, seemed to be pH dependente (Skrinaková et al., 2005); some authors, in fact, suggested that the fatty acids could enter the cell in the undissociated form, dissociate in the interior and cause the dissipation of trans-membrane H^+ gradient. Hydrophobic groups of saturated fatty acids have the greatest influence on antibacterial activity (Branen & Davidson, 1980; Altieri et al., 2005) and the increase of the hydrophobicity with the length of the chain could reduce their solubility in aqueous systems. Thus, hydrophobic groups may be prevented from reaching sufficient concentration to interact with hydrophobic proteins or lipids on the bacterial cell surface (Wang & Johnson, 1992); otherwise, the antimicrobial activity of monoglycerides is proposed to act as non-ionic surfactants that penetrate and become incorporated into bacterial plasma membrane, thereby altering membrane permeability (Bergsson et al., 1998; Altieri et al., 2005).

To our knowledge, literature reported that the fatty acids and their monoglycerides seemed less effective against gram-negative bacteria, because the outer membrane could act as an hurdle and inhibit their diffusion in the cytoplasm (Ouattara et al., 1997).

The results obtained in this study showed the strong anti-staphylococcal property of *S. coronata* essential oil and fatty acids noted by small MIC value. The MIC value found for all assayed strains was prominently smaller than other MIC values found by many researches emphasizing the antimicrobial potential of medicinal plants on *S. aureus* strains. These data are promising and could encourage further researches on phytochemical, toxicological and pharmacological aspects of *S. coronata* by-products in order to support their possible rational use in the antimicrobial therapy, particularly, in anti-*S. aureus* therapy.

Various constituents of essential oils and fatty acids from *S. coronata* had been investigated by GC–MS. This is the first time the fatty acids and essential oils of *S. coronata* are evaluated against *S. aureus*. The fatty acids and essential oils could be made the *S. coronata* oils important for a variety of healthy applications such as pharmaceutical, cosmetic and perfume industries. In addition, essential oils and fatty acids are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils.

Conflict of interest

The authors have declared that there is no conflict of interest.

Acknowledgements

The authors would like to acknowledge MCT/CNPq and NANOBIOTEC-Brasil from CAPES for financial support. To the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for research Grants. We are very grateful to the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for authorizing collections in PARNA do Catimbau (Sisbio 16.806).

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Figure 1 - The samples of fruit (left) and seeds (right) of *Syagrus coronata*.

Table 1. Origin of *Staphylococcus aureus* strains used in the antimicrobial assays.

Strains	Origin
<i>S. aureus</i> 02	UFPEDA Collection
<i>S. aureus</i> 1	Oropharynx
<i>S. aureus</i> 2	Eye discharge
<i>S. aureus</i> 3	Blood
<i>S. aureus</i> 4	Wound secretion
<i>S. aureus</i> 5	Oropharynx
<i>S. aureus</i> 6	Blood
<i>S. aureus</i> 7	Wound secretion
<i>S. aureus</i> 8	Wound secretion
<i>S. aureus</i> 9	Blood
<i>S. aureus</i> 10	Blood
<i>S. aureus</i> 11	Blood
<i>S. aureus</i> 12	Wound secretion
<i>S. aureus</i> 13	Blood
<i>S. aureus</i> 14	Blood
<i>S. aureus</i> 15	Blood
<i>S. aureus</i> 16	Blood

Table 2. Resistance profile of *Staphylococcus aureus* strains to clinically used antibiotics.

Strains	Antibiotics								
	Erythromycin	Clindamycin	Oxacilin	Penicilin	Linezolid	Tetracycline	Vancomycin	Chloramphenicol	Gentamicin
<i>S. aureus 1</i>	R	S	S	R	S	S	S	S	S
<i>S. aureus 2</i>	S	S	S	R	S	I	S	S	S
<i>S. aureus 3</i>	R	R	R	R	S	S	S	S	S
<i>S. aureus 4</i>	R	R	R	R	S	S	S	S	S
<i>S. aureus 5</i>	S	S	S	R	S	I	S	S	S
<i>S. aureus 6</i>	S	S	S	R	S	I	S	S	S
<i>S. aureus 7</i>	I	S	S	R	S	S	S	S	S
<i>S. aureus 8</i>	S	S	S	R	S	S	S	S	S
<i>S. aureus 9</i>	R	R	R	R	S	S	S	I	R
<i>S. aureus 10</i>	R	R	S	R	S	R	S	S	S
<i>S. aureus 11</i>	R	R	R	R	S	S	S	S	S
<i>S. aureus 12</i>	R	S	S	R	S	S	S	S	S
<i>S. aureus 13</i>	S	S	S	R	S	S	S	S	S
<i>S. aureus 14</i>	R	R	R	R	S	R	S	I	R
<i>S. aureus 15</i>	R	R	R	R	S	R	S	I	R
<i>S. aureus 16</i>	R	R	R	R	S	R	S	I	R

R: resistant; S: sensitive; I: intermediate

Tabela 3 - Fatty acid composition (Mean $\pm$ SD) of *S. coronata* kernel oils.

Fatty Acid		% of the total fatty acids
Saturated fatty acids:		72.35
Hexanoic acid	C _{6:0}	Tr
Heptanoic acid	C _{7:0}	Tr
Octadecanoic acid	C _{8:0}	5.32 $\pm$ 0.02
Nonanoic acid	C _{9:0}	Tr
Decanoic acid	C _{10:0}	4.54 $\pm$ 0.11
Undecanoic acid	C _{11:0}	Tr
Dodecanoic acid	C _{12:0}	41,58 $\pm$ 0.90
Tridecanoic acid	C _{13:0}	Tr
Tetradecanoic acid	C _{14:0}	9.68 $\pm$ 0.06
Pentadecanoic acid	C _{15:0}	Tr
Hexadecanoic acid	C _{16:0}	7.19 $\pm$ 0.12
Heptadecanoic acid	C _{17:0}	Tr
Octadecanoic acid	C _{18:0}	3.54 $\pm$ 0.11
Eicosanoic acid	C _{20:0}	0.21 $\pm$ 0.002
Docosanoic acid	C _{22:0}	0.22 $\pm$ 0.01
Tetracosanoic acid	C _{24:0}	0.07 $\pm$ 0.02
Monounsaturated fatty acids		23.90
9-octadecenoic acid	C _{18:1}	23.81 $\pm$ 0.72
11-eicosenoic acid	C _{20:1}	0.09 $\pm$ 0.01
Polyunsaturated fatty acids		3.59
9,12-octadecadienoic acid	C _{18:2}	3.59 $\pm$ 0.10

Tabela 4 - Chemical composition of the seeds essential oil of *S. coronata*.

Peaks	Compounds	Retention Indices		%
		Calculated ^a	Literature ^b	
1	α -Pinenol	932	932	1.41
2	β -Pinenol	975	974	0.13
3	β -Mirceno	991	988	0.38
4	α -Felandreno	1003	1002	26.26
5	(Z)-3-Hexenil acetate	1008	1004	0.16
6	α -Terpineno	1016	1014	0.28
7	o-Cimeno	1024	1022	1.36
8	β -Felandreno	1028	1025	12.93
9	Eucaliptol	1030	1026	0.59
10	trans-Ocimeno	1049	1044	0.23
11	γ -Terpineno	1058	1054	0.20
12	α -Terpinoleno	1088	1086	0.18
13	Linalol	1100	1095	0.41
14	Terpine-4-ol	1177	1174	0.14
15	α -Terpineol	1190	1186	0.24
16	δ -Elemeno	1337	1335	0.14
17	α -Copaeno	1378	1374	0.18
18	Unidentified coumpound	1387		0.06
19	β -Elemeno	1394	1389	1.60
20	trans -Cariophyleno	1423	1417	18.01
21	β -Copaeno	1432	1432	0.29
22	Aromadendreno	1442	1439	0.18
23	Unidentified coumpound	1444		0.14
24	trans-Muurolo-3,5-dieno	1455	1451	0.16
25	α -humuleno	1458	1452	5.46
26	Cariofileno <9-epi-(E)->	1465	1464	2.26
27	γ -Muurolo	1481	1478	1.72
28	Germacreno D	1486	1480	5.99
29	β -Selineno	1491	1489	1.14
30	cis-beta- Guaiene	1493	1489	0.15
31	δ -Selineno	1496	1492	0.45
32	α -Selineno	1500	1498	2.74
33	β -Alaskene	1501	1498	2.74
34	α -Muurolo	1505	1500	0.89
35	Germacreno A	1510	1508	1.51
36	γ -Cadineno	1519	1513	0.43
37	δ -Cadineno	1528	1522	2.33
38	Unidentified coumpound	1530		0.15
39	Cadina-1,4-dieno	1537	1533	0.13
40	α -Cadineno	1542	1537	0.10
41	Germacreno B	1561	1559	0.07
42	Palustrol	1571	1567	0.19
43	Unidentified coumpound	1587		0.65
44	Unidentified coumpound	1595		0.44

Peaks	Compounds	Retention Indices		%
		Caculated ^a	Literature ^b	
45	Cubenol-11-ol	1597	1595	0.40
46	Rosifoliol	1605	1600	0.27
47	Unidentified coumpound	1616		0.25
48	Junenol	1622	1618	0.13
49	Unidentified coumpound	1626		0.19
50	Unidentified coumpound	1628		0.19
51	Cubenol <1-epi->	1631	1627	0.13
52	Unidentified coumpound	1635		0.10
53	Muurolol <epi-alpha->	1645	1640	0.86
54	α -Muurolol	1649	1644	0.25
55	α -Cadinol	1657	1652	1.74
Total				99.79

^a Calculated on DB-5MS column according to Van Den Dool and Kratz (1963), based on a homologous series of normal alkanes (C9-C19); ^b According to Adams (2009).

Table 5. Inhibitory activity of *S. coronata* fatty acids and essential oil on *Staphylococcus aureus* strains isolated from clinical material.

Strain	Essential Oil			Fatty Acids			Control		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
UFPEDA 02	0.002	0.002	1	0.156	0.156	1	0.04	0.04	1
1	0.01	0.01	1	0.156	1.25	8	0.04	0.625	16
2	0.01	0.04	4	0.156	2.5	16	0.04	0.625	16
3	0.01	0.01	1	0.156	1.25	8	0.04	0.625	16
4	0.005	0.02	4	1.25	2.5	2	0.312	10	32
5	0.002	0.004	2	0.156	0.625	4	0.04	1.25	32
6	0.01	0.01	1	0.625	2.5	4	0.04	0.625	16
7	0.01	0.02	2	0.156	1.25	8	0.04	0.625	16
8	0.01	0.02	2	0.156	0.156	1	0.04	0.625	16
9	0.04	0.04	1	1.56	2.5	2	0.08	1.25	16
10	0.04	0.04	1	1.56	2.5	2	0.08	1.25	16
11	0.04	0.156	4	0.625	0.625	1	0.04	0.08	2
12	0.08	0.312	4	2.5	2.5	1	0.08	0.625	8
13	0.02	0.02	1	2.5	2.5	1	0.04	0.08	2
14	0.01	0.02	2	1.25	2.5	2	0.625	10	16
15	0.01	0.02	2	2.5	5	2	0.312	5	16
16	0.01	0.02	2	2.5	5	2	0.312	10	32

6. CONCLUSÕES

Este trabalho é pioneiro no estudo de óleos essenciais de espécies de palmeiras neotropicais. Os poucos trabalhos investigaram apenas frações voláteis de extratos de endosperma líquido e sólido. Os trabalhos disponíveis com espécies de palmeiras são restritos a palmeiras da região da península arábica, especificamente com o gênero *Phoenix*, exclusivo daquela região e a cosmopolita *Cocos nucifera*.

Estudos que abordam a ação dos ácidos graxos e óleos essenciais de palmeiras são também escassos. Em nosso estudo, os ácidos graxos e óleos essenciais extraídos das sementes de *Syagrus coronata* mostraram forte atividade contra isolados de *Staphylococcus aureus* isolados de material clínico. Os valores obtidos através da concentração mínima inibitória variaram entre 0.002 a 1.25 µL/mL.

Apesar ácidos graxos e óleos essenciais mostrarem forte atividade anti-*Staphylococcus aureus* estudos complementares são necessários, como por exemplo, a atividade anti-*Staphylococcus aureus* dos principais compostos majoritários dos ácidos graxos e óleos essenciais. Também se faz necessário investigar qual o mecanismo de ação que esses compostos, de natureza lipídica, exercem no microrganismo avaliado.

Embora a maioria dos pesquisadores considerarem que concentração mínima inibitória dessa magnitude são indicadores de ação antibacteriana outros estudos necessitam ser complementados afim de determinar uma potencial utilização clínica para os óleos essenciais e ácidos graxos de *S. coronata*.

ANEXOS



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