Characterization and anti-staphylococcal activity of the essential oil from *Turnera subulata* Sm.

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RESUMO: O presente estudo é pioneiro em analisar a composição química dos óleos essenciais das partes aéreas de *Turnera subulata* Sm. e sua atividade antibacteriana frente à *Staphylococcus aure*us resistentes a antibióticos. As partes aéreas da planta foram secas em estufa, pulverizadas em moinho mecânico e submetidas à hidrodestilaçao em aparato tipo Clevenger. A composição dos óleos essenciais foi analisada por cromatografia gasosa acoplada a espectrometria de massas (CG-EM), e a identificação realizada por comparação dos espectros de massas com a biblioteca do CG-EM e índices de retenção. A atividade antimicrobiana do óleo essencial foi avaliada usando o método de diluição em caldo, em concentrações que variaram de 3.125µg/mL to 3200µg/mL. Foi possível identificar 45 substâncias (92,1%) do óleo essencial, sendo os componentes majoritários: *trans*-cariofileno (6,7%), citronelol (5,6%), espatulenol (5,3%), α-cadinol (4,3%), n-tricosano (4,3%), geraniol (4,1%) e *trans*-geranilacetona (3,7%), n-pentacosano (3,5%), globulol (3,4%) e óxido de cariofileno (3,2%). O óleo essencial de *T. subulata* Sm. mostrou atividade antibacteriana eficaz para as várias cepas de *S. aureus* testadas, com valores de CIM entre 25 µg/mL e 1600 µg/mL. O estudo do óleo essencial de *Turnera subulata* Sm. evidenciou sua complexa mistura, contendo várias classes de substâncias, tendo os sesquiterpenos como constituintes majoritários seguido dos monoterpenos, e mostrou significativa atividade antibacteriana frente as cepas testadas.

Palavras-chave: *Turnera subulata*, CG-EM, *Turneraceae,* óleo essencial, atividade antiestafilocócica

ABSTRACT: Characterization and anti-staphylococcal activity of the essential oil from *Turnera subulata* Sm. The present study is pioneer in analyzing the chemical composition of the essential oil from aerial parts of *Turnera subulata* Sm. and evaluates their antibacterial activity against a panel of drug-resistant strains of *Staphylococcus aure*us. The aerial parts were kiln-dried and then powdered in mechanical mill, and was subjected to hydrodistillation in a Clevenger-type apparatus. The components were analyzed using a mass spectrometrycoupled gas chromatography (GC-MS), and the identification of substances was performed by comparison of the mass spectra obtained with the mass spectra of the database of the GC-MS and retention indices . The essential oil was evaluated using the method of broth dilution at concentrations ranging from 3.125µg/mL to 3200µg/mL. It was possible to identify 45 substances (92.1%) of the essential oils, and the major components were *trans*-caryophyllene (6.7%), citronellol (5.6%), sphatulenol (5.3%), α-cadinol (4.3%), n-tricosano (4.3%), geraniol (4.1%) and *trans*-geranilacetone (3.7%), n-pentacosano (3.5%), globulol (3.4%), caryophyllene oxide (3.2%). The essential oil of *T. subulata* Sm. showed effective antibacterial activity for the various strains of *S. aureus* tested, with the MIC values between 25 µg/mL and 1600 µg/mL. The study of the essential oil of *Turnera subulata* Sm. showed that it consists of a complex mixture of several classes of compounds having the sesquiterpenes as major constituents followed by monoterpenes, and showed an antibacterial activity significant front the strains tested.

Keywords: *Turnera subulata*, GC-MS, *Turneraceae,* essential oils, antistaphylococcal activity

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INTRODUCTION

Essential oils are volatile elements contained in many vegetable organs, and are related to several functions necessary for the plant survival, also has great importance in the defense against pathogens (Siqui et al., 2000). Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Essawi, 2000).

The bacterial resistance to multiple antibiotics has increased throughout the decades, making conventional antibiotics impotent against the bacterial resistance, such as *Staphylococcus aureus* (lowy, 2003), which culminates in the necessity for new drugs discovery. The essential oils have been used in folk medicine for decades as antimicrobial (Fisher and Phillips, 2008). Thus, natural products of plant origin are a rich source of new antimicrobials (Lewis & Ausubel, 2006).

Turnera subulata Sm, popularly known as "chanana" or "flor-do-Guarujá", is a member of the family *Turneraceae* (Gonzalez and Arbo, 2005), which consists of 10 genera and about 200 species that have distribution from North to South America, and in Africa, including Madagascar and the Mascarene Islands (Arbo, 2007).

In Brazil the family *Turneraceae* is represented by two genera, *Piriqueta* and *Turnera,* being this the most representative in the country having about 140 species (Arbo, 2008). According to Arbo (2007), Brazil holds the top number of species, the highest number of endemics being found in the mountains of the states of Bahia, Goiás and Minas Gerais. The genus is characterized by the presence of terpenoids, like mono and sesquiterpenes, flavonoids, steroids, benzenoids, alkaloids and lipids (Barbosa et al., 2007).

The roots of *Turnera chamaedrifolia* Cambess. and *Turnera subulata* Sm. are sold in street markets of the State of Paraíba and used in folk medicine (in the form of tea) in the treatment of amenorrhea, dysmenorrhea and as an abortifacient (Barbosa et al., 2007).

Previous studies have defined the many constituents of the essential oil of several species of *Turnera*, including *T. diffusa, T. aphrodisiaca* and *T. brasiliensis* (Kumar et al., 2006; Maia et al., 2007).

It was observed that the phytochemical studies have mainly focused on the characterization of the extracts, with few reports on the composition of essential oils. The present study is pioneer in analyzing the chemical composition of the essential oil from aerial parts of *Turnera subulata* Sm. The essential oil was also evaluated for antibacterial activity against a panel of drug-resistant strains of *Staphylococcus aure*us.

Experimental

Plant material: The aerial parts of *Turnera subulata* Sm. was collected in the University campus, in João Pessoa, the coastal region of Paraíba, Brazil, in August 2008, and indentified by one of the authors (M.F.A.). A voucher specimen (Agra & Barbosa 6273) was deposited at the herbarium Prof. Lauro Pires Xavier (JPB), Federal University of Paraíba.

The aerial parts were kiln-dried, with air circulating system, at 40°C for a period of 48 hours, and then powdered in mechanical mill. 427.7 g of plant material was subjected to hydrodistillation in a Clevenger-type apparatus for 3 hours. For the analysis, 1µL of the essential oil was diluted in 1mL of ethyl acetate (chromatographic degree).

Analysis of the essential oils: The components of the essential oil were analyzed using a gas chromatograph coupled to mass spectroscopy (GC-MS, Shimadzu QP-5000, Japão) equipped with a capillary column OV-5 (Ohio Valley Specialty Chemical, Inc.; 30.0m x 0.25mm x 0.25m). The column temperature was programmed from 60°C to 280°C, at a rate of 3°C/min. The temperatures of the injector and detector were 220°C and 230°C, respectively. Helium was used as the carrier gas, at a flow rate of 1mL/min. The injection of the oil was manually, 1µL the solution, with split of 1:10. The mass spectra were obtained with electron impact (EI) of 70 eV.

Identification and quantification the components of the oil were identified by comparison of their retention indices (determined in relation to an homologous series of n-alkanes) with those of pure standards or as reported in the literature (Adams, 1995). Comparision of fragmentation patterns in the MS with those stored on the CG-MS databases (NIST 62) was also performed. The percentages of each component were reported as raw percentages without standardization.

Bacterial strains: Three strains possessing efflux pumps for resistance to fluoroquinolones, macrolides and tetracycline, *vis*.SA-1199B, RN4224 (pUL5054) and IS-58, respectively (Gibbons et al., 2003; Falcão-Silva et al., 2009), and five strains resistant to methicillin and to aminoglycosides (Freitas et al., 1999). The strain ATCC 25923 was used as reference control. The strains, were maintained in blood Agar base (Laboratórios Difco Ltda., Brazil) slants, and prior to use, the cells were grown overnight at 37ºC in brain heart infusion broth (BHI – Laboratórios Difco Ltda., Brazil).

Antibacterial assay: The stock solution of the essential oil was prepared in DMSO which

at its highest final concentration after dilution in the broth (4%) caused no inhibition of bacterial growth. The minimum inhibitory concentration (MIC) of the essential oil was determined in BHI by the microdilution assay (CSLI, 2005) using a suspension of *ca*. 10^5 cfu/mL and an essential oil concentration range of 3200 – 3.125 µg/mL (two-fold serial dilutions). The MIC, determined in duplicate, is defined as the lowest concentration at which no growth is observed.

RESULTS AND DISCUSSION

Through the analysis of the essential oil of *Turnera subulata* in GC-MS it was possible to identify 45 substances , representing 92.1% of the total essential oil (Table 1).

It was observed that main component of the essential oil of *T. subulata trans*-caryophyllene representing about 6.7% of the total chemical composition, followed by citronellol (5.6%), spathulenol (5.2%), α-cadinol (4.3%), n-tricosane (4,3%), geraniol (4.1%), *trans*-geranilacetone (3.7%), n-pentacosane (3,5%), globulol (3.4%) and caryophyllene oxide (3.2%) (Table 1).

It can be verified that this essential oil is composed of a complex mixture of several kinds of substances, comprising an alcohol, five monoterpenes, twenty-three sesquiterpenes, eight hydrocarbons, four fatty acids, one ester, two ketones and one aldehyde.

It is important to stand out that, in the same way as reported for *Turnera brasiliensis* (Maia et al., 2007), this study with the essential oil of *Turnera subulata* Sm, showed the presence of (*E*)-caryophyllene (6.7%), α-humulene (1.3%), and α-selinene (2.0%). On the other hand, when compared the studies of the essential oil extracted from the leaves of *T. diffusa* and *T. aphrodisiaca* (Kumar et al., 2006; Maia et al., 2007), with *T. subulata* (Table 1**)**, this studies did not exhibited any results in common**.** The essential oil extracted from the leaves of *T. diffusa* showed the presence of monoterpenes 1,8-cineole, p-cymene, α-pinene, β-pinene (Antônio, 1996). The oil from *T. aphrodisiaca* showed the presence of α-pinene, β-pinene, p-cymene, 1,8-cineole and β-sitosterol (Kumar et al., 2006).

Studies with two samples of essential oils of *Turnera brasiliensis* predominantly showed (*E*)-caryophyllene (23.8% and 3.7%, respectively), α-humulene (22.0% and traces), alloaromadendrene (1.3% and 42.4%), β-selinene (5.8% and 12.2%) and α-selinene (15.2% and 24.4%) (Maia et al., 2007).

A study with *Turnera ulmifolia* showed that its essential oil had excellent antifungal activity against dermatophyte species, inhibiting 100% of

Table 1. Chemical composition (%) of the essential oil of *Turnera subulata* Sm.

SUBSTANCES	%	IR exp	IR lit
ALCOHOLS			
1-octen-3-ol	0.6	976	978
Total	0.6	-	-
MONOTERPENES			
linalool	2.1	1096	1098
citronellol	5.6	1224	1228
geraniol	4.1	1250	1255
trans-mirtanol	1.1	1254	1259
trans-geranilacetone	3.7	1447	1453
Total	16.6		
SESQUITERPENES			
α-copaene	0.8	1374	1376
β-elemene	1.3	1390	1391
trans-caryophyllene	6.7	1417	1418
α-humulene	1.3	1451	1454
trans-β-farnesene	0.8	1455	1458
γ-muurolene	0.6	1474	1477
germacrene - D	1.0	1478	1480
α-selinene	2.0	1494	1494
α-muurolene	0.6	1498	1499
δ-cadinene	1.3	1521	1524
occidentalol	0.5	1545	1548
trans-nerolidol	2.3	1560	1564
spathulenol	5.2	1574	1576
Caryophyllene oxide	3.2	1579	1581
globulol	3.4	1580	1583
1,10-di-epi-cubenol	1.4	1611	1614
1-epi-cubenol	0.9	1624	1627
epi-α-cadinol	1.2	1637	1640
α-muurolol	1.0	1642	1645
β - eudesmol	0.7	1645	1649
α-cadinol	4.3	1650	1653
14-hydroxy-9-epi-β-caryophyllene	0.6	1663	1664
Unidentified sesquiterpenes	9.5	-	-
Total	50.6	-	-
HIDROCARBONS			
n-hexadecane	1.1	1630	1600
n-tetradecane	0.6	1402	1399
n-heneicosane	1.5	2102	-
n-tricosane	4.3	2302	2300
n-tetracosane	0.6	2401	2400

continua...

Table 1. Chemical composition (%) of the essential oil of *Turnera subulata* Sm.

		continuação...	
n-pentacosane	3.5	2502	2500
n-hexacosane	1.3	2602	-
n-heptacosane	0.5	-	-
Total	11.8	-	-
FATTY ACIDS			
Palmitoleic acid	2.2	1964	-
Palmitic acid	1.4	1964	-
Linoleic acid	0.9	2157	-
Linolenic acid	0.8	2163	-
Total	5.3	-	-
ESTERS			
Ethyl palmitate	0.9	1992	-
Total	0.9	-	-
ALDEHYDES			
octadecanal	2.0	1710	-
Total	2.0	-	-
KETONES			
2-pentadecanone	0.5	1695	-
2-octadecanone	1.7	1842	-
Total	2.3	-	-
OTHERS COMPONENTS			
β -*trans*-ionone	2.1	1480	1488
TOTAL	**92,1**		

IR: Retention Indices

the strains tested (Lima et al., 2000).

MIC values of the essential oil of *Turnera subulata* Sm against strains of S aureus are presented in Table 2. Essential oils are potential sources of new antimicrobial compounds especially against bacterial pathogens (Prabuseenivasan et al., 2006).

An important characteristic of the essential oil is its hydrophobicity, which allows them to change the lipids in the cell membrane of bacteria, destructuring and making them more permeable to ions and other molecules that may lead to cell death (Sikkema et al., 1994).

CONCLUSION

The essential oil of leaves of *T. subulata* is constituted of complex mixture of different kinds of substances: alcohols (0,6%), monoterpenes (16,6%), sesquiterpenes (50,6%), hydrocarbons (11,8%), fatty acids (5,3%), esters (0,9%), aldehydes (2,0) and ketones (2,3%). The sesquiterpene *trans*-caryophyllene (6,7%) predominate in essential oil.

Table 2. Anti-staphylococcal activity of the essential oil of *Turnera subulata* Sm

Strains	MIC
ATCC 25923	200µg/mL
IS-58*	800µg/mL
RN-4220*	800µg/mL
SA-1199B*	1600µg/mL
05H**	200µg/mL
09c**	25µg/mL
02H**	1600 µg/mL
015**	1600µg/mL
171c**	1600µg/mL

* effluxing strains

**strains MARSA (*methicillin and aminoglycoside resistant Staphylococcus aureus*)

This essential oil of *Turnera subulata* Sm showed effective antibacterial activity for the various strains of *S. aureus* tested, with the MIC values between 25 µg/mL and 1600 µg/mL (Table 2). Really, van Vuuren (2008) has stated that essential oils with a MIC value of 2mg/ml or lower could be considered noteworthy.

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