Investigation of Essential Oil Extracts from Four Native Jamaican Species of *Bursera* for Antibacterial Activity

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ABSTRACT

Objective: *Bacterial infection with organisms resistant to antibiotics have increased during the last few decades worldwide. Because of this increase, the authors decided to subject the essential oils from the stem, leaves and fruits of the four native Jamaica species of* Bursera *to microbial studies.*
Methods: *Steam distillate extracts from different parts of four native Jamaican* spp *of* Bursera simaruba (Red Birch), Bursera lunanii (Black Birch), Bursera hollickii *and* Bursera aromatica (Siboney) *were tested for their antibacterial activity against six common pathogens:* Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylocococcus aureus, *methicillin resistant* Staphylococcus aureus (MRSA) *and beta-haemolytic* Streptococcus *group A (BHSA) using a disk diffusion assay.*
Results: *The investigation revealed that extracts from two of the four plants tested were active against all the pathogens. These were extracts from the fruits and stems of* B simaruba *and those from the fruit of* B lunanii.
Conclusion: *This study gives credence to the ongoing search for locally available plants whose extracts possess significant antimicrobial activity. This may be useful in the development of naturally derived pharmaceuticals.*

Investigación de los Extractos de Aceites Esenciales a Partir de Cuatro Especies de *Bursera,* Endémicas de Jamaica, Para la Determinación de su Actividad Antibacteriana

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RESUMEN

Objetivo: *La infección bacteriana con organismos resistentes a los antibióticos ha aumentado a nivel mundial durante las últimas décadas. Debido a este aumento, los autores decidieron someter a estudios microbianos los aceites esenciales de cortezas, hojas y frutos de cuatro especies de Bursera endémicas de Jamaica.*
Métodos: *Los extractos destilados al vapor, de diferentes partes de cuatro spp endémicas de Jamaica, a saber, Bursera simaruba (abedul rojo), Bursera lunanii (abedul negro), Bursera hollickii y Bursera aromatica (Siboney), fueron analizados mediante un ensayo de difusión en disco, a fin de determinar su actividad antimicrobiana frente a seis patógenos comunes: Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylocococcus aureus, Staphylococcus aureus resistente a la meticilina (MRSA) y el Streptococcus betahemolítico del grupo A (EBHA).*
Resultados: *La investigación reveló que los extractos de dos de las cuatro plantas sometidas a examen eran activas contra todos los patógenos. Estos fueron los extractos de frutos y ramas de B simaruba y los de frutos de B lunanii.*
Conclusión: *Este estudio da crédito a la investigación que se lleva a cabo con el propósito de determinar qué extractos de plantas disponibles localmente poseen una actividad antimicrobiana significativa. Esto puede resultar útil para el desarrollo de productos farmacéuticos derivados naturalmente.*

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INTRODUCTION

Several of the species of the genus *Bursera* have reported ethnobotanical and ethnopharmacological uses. The aromatic resins of several species of *Bursera* has been burned ritually as incense since pre-historic times in Mexico and the fumes of the incense are used as a cure for various illnesses (1). The resins are also used medicinally in ointments for the treatment of scorpion stings and to relieve various ailments (2–5).

The aromatic wood of *B glabrifolia* is used extensively for the carving of painted figurines called alebrijes which are sold as handicraft. The wood is also used to make household utensils (cups and pots), furniture (stool) and tools (hammers), toys and fencing (6–8). Industrially, many species of *Burseraceae* owe their economical value to the essential oils that these plants produce (9). The oils are highly valued perfumery material and are used in a number of perfumes, cosmetics and in the scenting of some soaps. They are also used in flavouring food and beverages (8).

The family, *Burseraceae*, consists of approximately twenty genera with six hundred species which are widely distributed at low elevation in the sub-tropical regions (10–11). They exist as trees or shrubs with resinous stems, several of which are aromatic species. *Burseraceae* is one of the most important essential oil-yielding plants and for this purpose was introduced into India from Mexico in 1920 (8). Two of the traditional incenses, frankincense and myrrh, are both extracted from species of this family, *Boswellia spp* and *Commiphora spp* respectively (12). There are four native species of *Bursera* in Jamaica, *Bursera simaruba, lunanii, hollickii* and *aromatica,* the last three named being indigenous to the island.

Some species of the *Bursera* genus have been reported to possess bioactive metabolites and thus exhibit medicinal properties. In a quest to find bioactive metabolites, one of the plants from the Yucantecan, *Bursera simaruba,* was investigated. The bioactive metabolite was extracted from the resin of the plant and identified as picropolgamin (8–9). This polar compound is hypothesized to contribute to the anti-inflammatory activity of *Bursera simaruba* reported by Abad (13) and Sosa *et al* (14). In the Abad study, a hexane leaf extract of *Bursera simaruba* was shown to possess potent anti-inflammatory activity against both acute and chronic phases of adjuvant-carrageenan-induced inflammation (ACII).

The practice of traditional medicine, based on the use of plants to alleviate common ailments, has not desisted totally (15). Over the past two decades, there has been an increase in the investigation of natural materials aimed at finding new and better drugs (16). Hundreds ($2.5–7.5 \times 10^5$) of plants have been evaluated for bioactive metabolites and a significant number (35 000) of bioactive metabolites have been obtained. In the late 19th century, there were approximately 120 drug preparations that were derived from traditional medicine (17) and this figure is increasing as consumers show a preference for naturally derived pharmaceuticals and as bacteria continue to grow increasingly resistant to antibiotics (18). Because of this increase in the resistance of bacteria to antibiotics, the search for new and improved antibiotics has taken a forward surge.

In the continued search for natural products with potential antibacterial activity, it was decided to subject the essential oils from the stems, leaves and fruits of the four native Jamaica species of *Bursera* to microbial studies. The essential oils of the plants (Table 1) were screened against six micro-organisms utilizing a disk diffusion method (20–22).

MATERIAL AND METHODS

Plant material

The plant materials (stems, leaves and fruits) were collected in Jamaica from the Long Mountain region of St Andrew and in the Cockpit Country of Trelawny and identified at the herbarium in the Department of Life Sciences, The University of The West Indies. Samples of *Bursera simaruba, B lunanii, B hollickii* and *B aromatica* were deposited at the herbarium and assigned accession numbers: 35034, 35035, 35221 and 35225 respectively.

Extraction

The plant materials were collected and separated into leaves, stems and fruits. They were then milled, weighed and hydrodistilled for three-hour periods using a Clevenger-type apparatus. When the distillate cooled, the water and essential oils were separated and the oils dried over anhydrous sodium sulfate for use in further investigations.

Antibacterial assay

The disk diffusion method is extensively used at The University Hospital of the West Indies (UHWI) clinical laboratories for determining antimicrobial activities. This method was employed to investigate the antimicrobial activity of the essential oils extracted from the different *Bursera* species being examined. In the experiment, 10 µL of the undiluted and the diluted (1/10 oil in hexane) extracts were injected onto a 6 mm diameter disc. Isolates of *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylocococcus aureus,* methicillin-resistant *Staphylococcus aureus* (*MRSA*) and beta-haemolytic S*treptococcus* group A (BHSA were obtained and standardized culture suspensions of 1.5×10^8 colony-forming units were used for each inoculum (23–24). The plates were then incubated at 35 to 37°C for 18–24 hours. After this period, the zone of inhibition around the disks was measured with a micrometer. The diameters of the zone of inhibition were recorded as resistant (10 mm or less), intermediate (11–13 mm), susceptible (14–17 mm) and significantly susceptible (18 mm or more) and were assigned the following symbols –, +, ++, +++ respectively. Amoxicillin/clavulanate (20/10 µg) was used as the standard for control of the zone of inhibition.

RESULTS

All extracts were mobile, pale yellow oils except those from the leaves of *B simaruba* which were clear deep yellow viscous oils. The yields of the oils were low ranging from 0.03% to 1.12% (Table 1). Screening the oils for antibacterial activity revealed that extracts from only two species: *B simaruba* and *B lunanii* were active at the concentration examined (Table 2). The undiluted extracts were subjected to a one in ten (1/10) dilution and both undiluted and diluted extracts were tested for antibacterial activity.

Table 1: Percentage yields (w/w) of essential oils extracted from the plant parts of *B aromatica*, *hollickii*, *lunanii* and *simaruba*.

Plant Parts	B aromatica	B hollickii	B lunanii	B simaruba
Stem oil (%)	0.09	0.17	0.05	0.03
Fruit oil (%)	1.12	–	0.60	0.28
Leaf oil (%)	0.03	0.15	0.17	0.07

Table 2: *Bursera* species and their antibacterial activities using 10 µL of the diluted and undiluted essential oil extracts

Botanical name (Common name)	Plant part	Dilution 1/10	neat	Pathogens E coli	S aureus	P mirabilis	P aeru	BHSA	MRSA
	leaf		/	–	–	–	–	–	–
Bursera simaruba	stem		/	+++	+++	++	++	+	+++
(red birch)	stem	/		–	+	–	–	–	+
	fruit		/	+++	+++	+	–	++	+++
	fruit	/		–	+	–	–	–	+
	stem		/	–	–	–	–	–	–
Bursera lunanii	stem		/	–	–	–	–	–	–
(black birch)	fruit		/	+++	+++	+++	++	+	+++
	fruit	/		+++	+	–	–	–	+
	leaf	/	–	–	–	–	–	–	–
Bursera hollickii	stem	/	–	–	–	–	–	–	–
	fruit	/	–	–	–	–	–	–	–
	leaf	/	–	–	–	–	–	–	–
Bursera aromatica	stem	/	–	–	–	–	–	–	–
(siboney)	fruit	/	–	–	–	–	–	–	–

P aeru = *P aeruginosa;* BSHA = beta-haemolytic S*treptococcus* group A; +++, organism significantly susceptible; ++, susceptible; +, intermediate; –, resistant.

Undiluted extract

Oils from the stems of *B simaruba* and fruits of *B lunanii* were active against all of the six pathogens screened and showed similar activity in respect to each pathogen. Oils from the fruits of *B simaruba* were active against five of the six pathogens; *P aeruginosa* was the exception.

Diluted extract

B simaruba fruit and bark oils were only weakly active against two of the pathogens screened, *S aureus* and methicillin resistant *S aureus.* The result was similar for *B lunanii* except for its effect on *E coli* which was extremely sensitive to the extract.

DISCUSSION

Of the four plant essential oils investigated, two (*B simaruba* and *B lunanii*) showed antibacterial activity. The active oils were present in the fruits and stems of the extracts from *B simaruba* and in the fruits of *B lunanii*. These oils were active against all the pathogens tested including MRSA. An exception was the extract of *B simaruba* which was not active against *P aeruginosa*.

The activity of the extracts were independent of the group that the bacteria fell in: Gram-positive (*S aureus, MRSA* and BHSA) or Gram-negative (*E coli, P mirabilis, P aeruginosa*). *S aureus,* MRSA and *E coli* were extremely sensitive to the oils. However, BHSA was the most resistant of all the pathogens followed by *P aeruginosa,* then *P mirabilis.*

The essential oils from the leaves and fruits of *B simaruba* and fruits of *B lunanii* had excellent activity against MRSA. Methicillin-resistant *Staphylococcus aureus* is difficult to treat and can progress to life-threatening infection. It has been recognized as a problem in the healthcare setting for over 20 years. The resistance of MRSA is due to the presence of the *mec* gene which alters the site at which methicillin binds to kill the organism. Thus, methicillin is unable to bind to the bacteria. Although MRSA is resistant to many drugs, it is susceptible to the antibiotics vancomycin and teicoplanin. Both drugs must be administered via injection as there is no orally available treatment for infections caused by *MRSA* as yet. These oils therefore possess tremendous potential in the quest to obtain effective agents against this pathogen.

In a study by Camporese *et al* (25), extracts from the bark of *B simaruba* was reported to show anti-bacterial activity against gram-negative test stains of *E coli* and *P aeruginosa*. However, an earlier study (26) of an ethanol extract (50% v/v) of *B simaruba* showed no activity against *E coli*, *Salmonella enteritidis, Salmonella typhi, Shigella dysenteriae* and *Shigella flexneri.*

This study gives credence to the ongoing search for locally available plants whose extracts possess significant antimicrobial activity which may be useful in the development of naturally derived pharmaceuticals (27).

This antibacterial property could explain the ethnomedical use of *B simaruba* as a treatment for wounds, bruises, abscesses, ulcers and sores in the Caribbean islands and in Central America. (2, 3, 5).

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