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Antimicrobial Activities of Selected Plant Extracts from the Guyana's Flora

R.C. Jagessar* and Nazrana Mohamed

Department of Chemistry, University of Guyana, South America.

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Antimicrobial activities of leaves of selected plants: sweet broom (*Scoparia dulcis*), sandbox tree (*Hura crepitans*), orange (*Citrus sinensis*), papaya (*Carica papaya*) and soursop (*Annona muricata*) were investigated using the pour plate method. The dried leaves of the above mentioned plants were exhaustively extracted in dried hexane, dichloromethane and methanol. Solvents were removed in *vacuo* to yield paste and viscous oil. Each solvent type extract was subjected to antimicrobial activity tests using the pour plate method. Using the number of non existence colonies as an indication of the antibacterial and antifungal properties of the extract, it was found that the methanol extract was the most antibacterial (zero colonies survive) followed by dichloromethane extract and hexane extract. Controls for the bacteria/ fungus were tested against the solvents and it was found that the solvent had no antiseptic effect on the microorganisms. Future work involves the isolation and purification of natural products and an investigation of their individual antimicrobial activity.

Key words: Antimicrobial, selected plants, pour plate, antibacterial, antifungal, antiseptic, microorganisms, natural products.

This paper serves to indicate the antimicrobial properties of leaves of four plants from the coast plain of Guyana and their use as possible herbal medicines. Guyana has a rich diverse flora whose crude extracts, both organic and aqueous can be investigated for their antimicrobial activity. Also, the specified plants parts of the same species be screened for natural products whose antimicrobial activity can also be correlated with that of each solvent type extract.Following this, clinical trials can lead to the formulation of a plant herbal medicine and herbal tablets. Modern synthetic drug discovery owe their success to a mimic of structures of antimicrobial drugs or natural products isolated from plants[10-12] rather than to the total imagination and creativity of contemporary organic chemist. Research in herbal medicine and isolated drug discovery need to be continued, considering the threat of new emerging disease such as SARS, ebiola virus, bird flu not to mention AIDS and the recent swine flu, H1N1 virus . Plants are a good source of herbal medicine and natural products[1-9]. Five plants species: *Annona muricata*, *Carica papaya*, *Citrus sinensis* and *Scoparia dulcis* were collected from the coastal plain of Guyana and their organic extract investigated for antimicrobial activities. Organic extracts: CH_2Cl_2, hexane and CH_3OH were tested against two

* To whom all correspondence should be addressed.
E-mail: raymondjagessar@yahoo.com

bacteria: *E. coli*, *Staphylococcus aureus* and one fungi, *Candida albicans*.

E. coli can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia. *Staphylococcus aureus* can cause furuncles (boils), carbuncles (a collection of furuncles) [13]. In infants, *Staphylococcus aureus* can cause a severe disease[14]. Staphylococcal scalded skin syndrome (SSSS). Staphylococcal endocarditis (infection of the heart valves) and pneumonia may be fatal. *Candida albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans[15]. *C. albicans* is amongst the gut flora, the many organisms that live in the human mouth and gastrointestinal tract. Overgrowth of *Candida albicans* results in candidiasis.

Annona muricata, Carica papaya, *Citrus sinensis* and *Scoparia dulcis* are native plants of the Guyana flora. *Annona muricata* commonly called soursop belongs to the family annonaceae(annona family) which comprises about 150 species. It's a small tree of tropical South America, no more than 20 feet tall. The leaves are leathery, very dark and shiny green.Traditionally, a tea of the leaves is used against edgy nerves, hypertension with nervousness. It is also used against flu and fevers. Fresh leaves are used against sleeplessness (insomnia) [16].

Carica papaya, common name papaya or paw paw belongs to the family caricaceae. It's a tropical tree growing up to 21' tall. It has an erect, branchless trunk with scars from old leaf stems.Its widely grown in Guyana for its edible fruit known as papaya. In Guyana's traditional medicine, the boiled green leaves of papaya are used against malaria and as an anthelminthic. The seeds as a vermifuge and tea of the fallen leaves used against hypertension [17].

Citrus sinensis commonly called sweet orange belongs to the family called Rutaceae-Rue family. It's a small pyramidal tree. Leaves are oblong, evergreen, smooth and shiny.Medicinally, its reported to have anti-depressant, anti inflammatory,antiseptic and antispasmodic, bacterial, digestive, astringent, fungicidal and sedative properties[18].

Scoparia dulcis L. common name broomweed, bitterbroom belongs to the family scrophulariaceae(figworth family). It's a common annual herb in Guyana growing up to 2' in height. It has serrated leaves and many small flowers. In Guyana's traditional medicine, a mixture of *Scopria dulcis* is used against filariasis, dysentery and also as an aphrodisiac [19].

The Sandbox tree, *Hura crepitans*, also known as Possumwood and Jabillo, is an evergreen tree of the spurge family (Euphorbiaceae), native to tropical regions of North and South America in Amazon Rainforest. It is characterised by the many dark, pointed spines and smooth brown bark.

Sandbox trees can grow to 100 ft, and the large ovate leaves grow to two feet wide. They are monoecious. The red flowers have no petals. The fruit is a large capsule with explosive dehiscence.Fishermen have used the milky, caustic sap from this tree to poison fish. The Caribs made arrow poison from its sap. *Hura crepitans* is an additive to some forms of the hallucinogenic drink Ayahuasca. The wood is used for furniture [20].

MATERIAL AND METHODS

Collection of Plant Materials

The leaves of the above four mentioned plants were collected off the coastal plain of Guyana. The detached plant leaves were subjected to aerial drying for two weeks and crushed into very small pieces. This increased the surface area for extraction.

Preparation of various Plant extracts

The dried, crushed leaves were placed into appropriate extraction flasks for extraction using the recommended solvents. Extraction was done thrice using solvents of increasing polarity: hexane, dichloromethane and methanol respectively. For each solvent, three extractions were done. Extracts were dried over $MgSO_4$, filtered and solvents removed in *vacuo* to yield paste and viscous oil. The viscous oils or paste was further dried to remove all traces of solvents and water.

Source of Microorganisms

Plant extracts were tested for antibacterial and antifungal activity using the

pour plate method. Bacteria used were: *Staphylococcus aureus*, *Escherichia coli*. Fungi used was *Candida albicans* in nature. These were obtained from Georgetown Public Hospital and were stored in the refrigerator. 3-5 colonies from an overnight plate was transferred to a tube containing 10 ml of distilled water and mixed. The solution was compared with the 1.0 Mc Farland Standard and the density was adjusted either by adding more colonies or adding more distilled water to the tube. The choice of the agar used for bacterial and fungal growth was nutrient agar which accommodates non fastiduos growth of microbes.

Screening for antimicrobial activities

23g of the agar powder was suspended in 1L of distilled water. It was boiled for 1 minute so that the powder was completely dissolved. Autoclaving was done for 15 minutes at 121°C. The plant extract and the microorganism were mixed in a 1:1 proportion and placed in a sterilized vial.The nutrient agar after autoclaving was left to semi cool (45°C) and then poured into sterilized glass plates (90 mm diameter; depth: 4 mm = 25ml per plate). At the almost cool state, the mixture of the microorganism and the plant extract was vigorously shaken and inoculated into the agar media using a sterilized rod. The agar

Fig. 1. Dried plants parts been solvent extracted

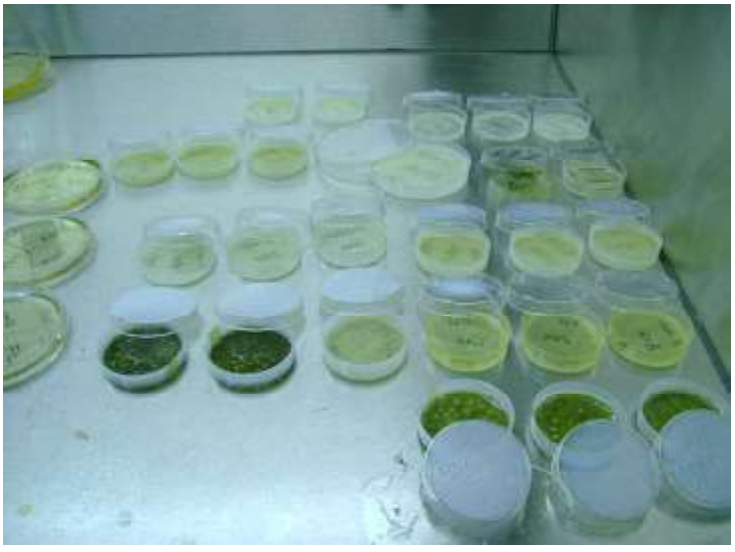


Fig. 2: Pour Plate Method

Table 1. Hexane extracts vs. Microorganisms

Plant Extract	Microorganism (No. of Colonies) *S. aureus*	Microorganism (No. of Colonies) *E. coli*	Microorganism (No. of Colonies) *C. albicans*
Annona muricata (1)	4.67 ± 1.53	0 ± 0	TNTC
Citrus sinensis (ii)	0 ± 0	0 ± 0	0 ± 0
Carica papaya (iii)	0 ± 0	12.67± 4.04	0 ± 0
Scoparia dulcis (iv)	0 ± 0	0± 0	0 ± 0
Hura crepitans L (v)	TNTC	30.00 ± 6.08	TNTC
Reference	0± 0	0 ± 0	0 ± 0
Control	TNTC	TNTC	TNTC

Table 2. Dichloromethane extracts vs. Microorganisms:

Plant Extract	Microorganism (No. of Colonies) *S. aureus*	Microorganism (No. of Colonies) *E. coli*	Microorganism (No. of Colonies) *C. albicans*
Annona muricata	0 ± 0	0 ± 0	TNTC
Citrus sinensis	0 ± 0	0 ± 0	0 ± 0
Carica papaya	0 ± 0	0 ± 0	0 ± 0
Scoparia dulcis	0 ± 0	0 ± 0	0 ± 0
Hura crepitans L.	40.67 ± 5.67	0 ± 0	TNTC
Reference:	0 ± 0	0 ± 0	0 ± 0
Control	TNTC	TNTC	TNTC

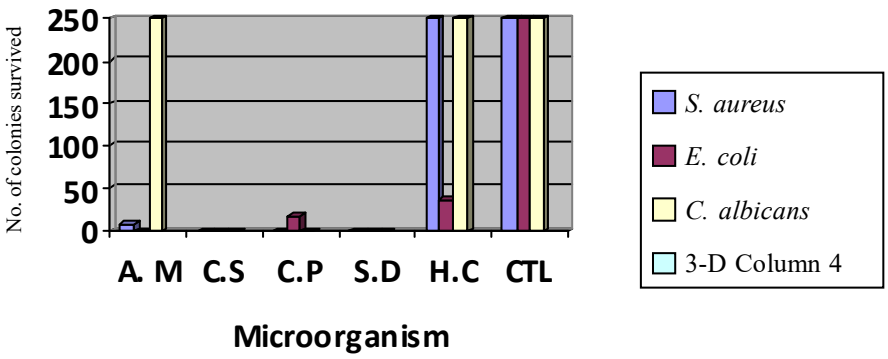
Table 3. Methanol extracts vs. Microorganisms:

Plant Extract	Microorganism (No. of Colonies) *S. aureus*	Microorganism (No. of Colonies) *E. coli*	Microorganism (No. of Colonies) *C. albicans*
Annona muricata	0 ± 0	0 ± 0	TNTC
Citrus sinensis	0 ± 0	0 ± 0	0 ± 0
Carica papaya	0 ± 0	0 ± 0	0 ± 0
Scoparia dulcis	0 ± 0	0 ± 0	0 ± 0
Hura crepitans L.	40.67 ± 5.67	0 ± 0	TNTC
Reference:	0 ± 0	0 ± 0	0 ± 0
Control	TNTC	TNTC	TNTC

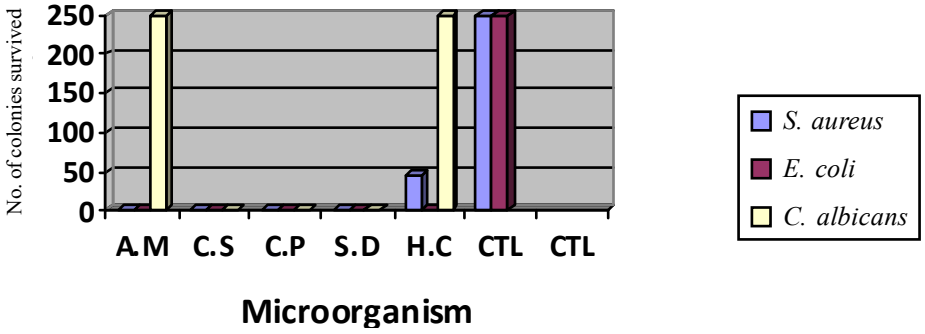
TNTC: Too Numerous colonies to count, > 250.
TLC analyses: TLC RESULTS: HEXANE EXTRACT

Table 4. R_f values

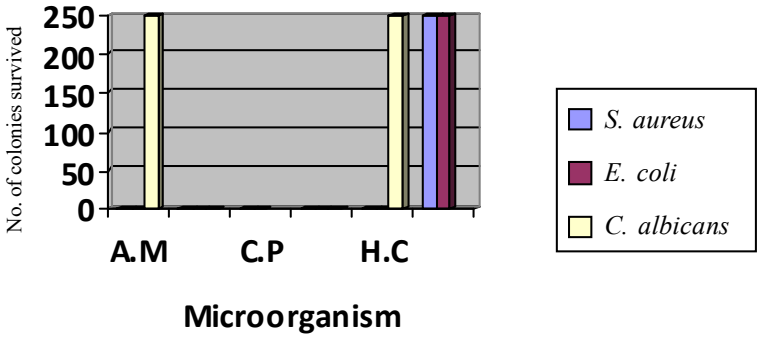
Plant Extracts	Number of Spots	R_f values $_F$
Annona muricata (I)	7	0.08, 0.31, 0.56, 0.61, 0.72, 0.89, 0.98
Citrus sinensis (II)	5	0.11, 0.19, 0.64, 0.69, 0.94
Carica papaya (III)	7	0.05, 0.31, 0.36, 0.44, 0.58, 0.72, 0.94
Scoparia dulcis (IV)	6	0.03, 0.11, 0.25, 0.47, 0.58, 0.97
Hura crepitans L. (V)	5	0.06, 0.33, 0.5, 0.61, 0.97



No. of colonies survived vs microorganism for Hexane extract



No. of colonies survived vs microorganism for dichloromethane extract



No. of colonies survived vs microorganism for methanol extract

Key to graphs:

A.M = *Annona Muricata*
C.A = *Carica Papaya*
H.C = *Hura crepitans*
C.A = *Candida albicans*
E.C = *Eschericia Coli*
C.S = *Citrus sinensis*
S.D = *Scoparia dulcis*
CTL = Control
S.A = *Staphylococcus aureus*

Graph 1. A plot of the number of colonies survived versus microorganism for specific plant species

was left to harden for about 30 minutes under aseptic conditions. The glass plates were covered, flipped upside down and placed into the incubator at 37°C. Incubation lasted 24 hrs and 48 hrs for bacteria and fungal species respectively. After the incubation period was over, the number of colonies was counted using a colony counter so as to determine how effective this plant extract was against bacteria or fungus

Reference and Control

For each type of microorganism, one reference was used. Gentamicin was used for all bacterial species: *E. coli* and *S. aureus* whereas Nyastatin was the reference for the fungus, *Candida. albicans.* The Control consists of a plate of solidifying agar onto which was inoculated pure solvents (hexane, dichlormethane and methanol) with microorganism mixed in a 1:1 portion [23].

Table 6. TLC analyses: methanol extract

Plant Extracts	Number of Spots	R_f values $_F$
Annona muricata (I)	4	
Citrus sinensis (II)	5	0.13, 0.29, 0.39, 0.87,
Carica papaya (III)	5	0.19, 0.32, 0.35, 0.84, 0.97
Scoparia dulcis (IV)	8	0.13, 0.32, 0.61, 0.97, 0.99
Hura crepitans L. (V)	5	0.19, 0.26, 0.29, 0.32, 0.35, 0.45, 0.71, 0.97

Table 5. TLC Results: dichloromethane extract

Plant Extracts	Number of Spots	R_f values $_F$
Annona muricata (I)	7	0.08, 0.11, 0.31, 0.56, 0.64, 0.83, 0.97
Citrus sinensis (II)	6	0.11, 0.17, 0.28, 0.33, 0.5, 0.58
Carica papaya (III)	8	0.06, 0.14, 0.22, 0.33, 0.58, 0.72, 0.86, *0.94*
Scoparia dulcis (IV)	6	0.11, 0.17, 0.28, 0.33, 0.5, 0.58
Hura crepitans L. (V)	4	0.03, 0.14, 0.53, 0.96

Aseptic conditions

The asceptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for one hour.

Retention factor, a chromatography index: R_f = Distance moved by spot/Distance moved by solvent Front. In general, the most polar compound has the lowest R_f value.

Turbidity (opacity) standard[23]

This is the barium chloride standard against which the turbidity of the test and control innocula can be compared. When matched with the standard, the inocula should give semiconfluent growth. The turbidity of the standard is equivalent to an overnight broth culture.

Preparation of Turbidity Standard (0.5 Mc Farland)[23]

1% v/v sulphuric acid: 1 ml of concentrated sulphuric acid was added to 99 ml of distilled water. 1.175% w/v of barium chloride solution: 2.35g of barium chloride, $BaCl_2.2H_2O$ was dissolved in 200ml of distilled water. To make the turbidity standard, 0.5 ml of the barium chloride solution was added to 99.5 ml of the sulphuric acid solution and thoroughly mixed. The standard solution was dispersed into screw cap tubes as the same type as those for the preparation of the test and control innocula. It was stored in the dark at room temperature.

DISCUSSION

The pour plate method was successful in determining all four plants antimicrobial properties against selected microbial strains: *S. aureus, E. coli and C. albicans.* Any plant extract that is antimicrobial will inhibit the growth of bacteria/fungi colonies and vice versa. It was noticeable that inhibition of microorganism growth was solvent dependent. For the hexane extract, *Scoparia dulcis* and *Citrus sinensis* completely inhibited the growth of all three microorganisms (zero colonies were observed). However, for *Carica papaya*, a selectivity of antimicrobial activity was observed whereby *Carica papaya* inhibited the growth of *S. aureus* and *C. albicans* but promoted the growth of *E. Coli* to some extent. *Hura crepitans* on the other hand, induced the growth of all three microorganisms.

For the CH_2Cl_2 extract, all plant species with the exception of *Hura crepitans* and *Annona muricata* to some extent inhibited the growth of all three microorganisms with the exception of *C. albicans*. With *C.albicans* too numerous colonies were observed for *Hura crepitans* and *Annona muricata.*

However, the methanol extract of all five plant species with the exception of *Annona muricata* and *Hura crepitans* completely inhibited the growth of all three microorganisms. The methanol extract has polar antimicrobial compounds (natural products) that inhibited microbial growth. Thin Layer Chromatography, TLC analyses revealed that the number of compounds for the five plants: (*Annona muricata*)-(*Hura crepitans*) range from (5)-(7), (4)-(8) and (4)-(8) for the hexane, dichloromethane and methanol extracts respectively. These components with their respective R_f values are given in table 4.0, 5.0 and 6.0. The solvent system used for the development of the TLC plate was CH_2Cl_2/ Hexane. The most polar antimicrobial natural products have the smallest R_f values. It 's not only plant extracts that have shown to be selective antimicrobial agents against the above pathogens but synthesized compounds also[24-26].

CONCLUSION

The pour plate method was successful in determining all five plants antimicrobial activities. All five plants methanolic extracts were found to be antimicrobial (zero colonies survive), whereas with the exception of two plants, the hexane extract was least effective. Thus, the methanolic extracts of these plants can be used as a possible herbal medicine against *E. coli* and *S. aureus* and *C. albicans*. Future work involves the isolation of bioactive components from each solvent type plant extract via preparative TLC chromatograpy and an investigation of the antimicrobial activity of isolated plant components. Also, a comparison with that of the crude plant extract. Clinical trials should lead to the formulation of an antimicrobial cream.

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