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Full Length Research Paper

Antibacterial activity of extracts of twelve common medicinal plants from the Philippines

Gerard L. Penecilla* and Celia P. Magno

Biological Sciences Department, West Visayas State University, Luna Street, La Paz, Iloilo City 5000, Philippines.

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The antibacterial activity of the n-hexane, acetone/dichloromethane, ethanol and aqueous extracts of twelve common medicinal plants from the Philippines obtained through pounding and solvent extraction was evaluated using disc Agar diffusion. The microorganisms tested were: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa.* Susceptibility test results showed that different extracts (1000 mcg/disc) from ten plants inhibited growth with a mean zone of inhibition range of 15 to 30 mm against *S. aureus*, *E. coli, and P. aeruginosa*. The common medicinal plants which showed highly positive activity were *Psidium guajava (*guava), *Eucalyptus globulus*, *Mangifera indica* (Indian mango), *Nasturtium officinale* (Watercress), *Pterygospermum oleiferum* (Moringa), *Carmona retusa (*Wild tea*), Citrus aurantifolia* (Lemon)*, Citrus sinensis* (Orange)*, Allium sativum* (garlic), and *Allium cepa* (onion). Preliminary phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, glucosides, saponins and steroids/terpenes. The results suggest that the different plant extracts contain bioactive constituent(s) particularly tannins, flavonoids, terpenoids and other glycosides with very strong antibacterial activity and validates the ethno-medical use in the treatment of bacterial skin diseases and other forms of bacterial infections. However, many reputed plant antibacterials have very variable activities depending on certain conditions.

Key words: Antibacterial, medicinal plants from the Philippines, n-hexane, acetone/dichloromethane, ethanol, aqueous extracts, *Psidium guajava*, preliminary phytochemical screening.

INTRODUCTION

In the Philippines, there are only ten (10) medicinal plants approved and recommended for use by the government's Department of Health in spite of the many works done by Guerrero (1921), Quisumbing (1978), Masilungan et al. (1955), Santos et al. (1981), de Padua et al. (1977, 1978, 1981, 1983), Manalo and Genetiano (1998) among others. During the last century or so, many studies on medicinal plants were ably supported by the Department of Science and Technology-Philippine Council for Health Research and Development and the National Research Council of the Philippines and other major academic Institutions such as University of the Philippines and the University of Santo Tomas. However, only one or two plants recommended by the Philippine government are antibacterials, namely guava and akapulco. Yet, the role/importance of medicinal plants/traditional medicine cannot be underestimated in the Philippine setting because many medicinal plants are being utilized ever since as topical/external ointments, liniments, poultices that is for wounds, skin diseases, muscle pains, massage and aromatherapies. Thus, *Psidium guajava* and *Pterygospermum oleiferum* are used for fresh wounds, *Mangifera* as poultices, garlic and onion for dog bite; ginger for viral infections, *Citrus* and *Eucalyptus* for sore throat, and *Carmona retusa* for stomach ache Literature review on the medicinal plants in this study revealed that in the Philippines, the most accessible medicinal plant with antibacterial activity is guava or *P. guajava (*Family Myrtaceae of Class Magnoliopsida*)*. It has achieved a very long history of traditional use for a wide range of diseases (Adeyemi et al., 2009; Elekwa et al., 2009; Nwinyi et al., 2008). In the Philippines, guava fruit is freely eaten for its good taste and nutritional benefits. Many medicinal uses have been validated by scientific researches (Kamath et al., 2008; Adeyemi et al. 2009). Chemicals isolated from guava leaves like quercetin, guaijaverin, several flavonoids and

*Corresponding author Email: gpenecilla@yahoo.com. Tel: 63-33-3200870. Fax: 63-33-3200879.

galactose-specific lecithins have shown promising activity in many human clinical trials (Abdelrahim et al., 2002; Adeyemi et al. 2009). Guava is one of the few medicinal plants which has been very extensively studied in terms of pharma-cological activity of its major components (Adeyemi et al., 2009), and the results indicate great potent antimicrobial/antibacterial activities (Gnan and Demello, 1999; Iwu, 1993; Chah et al., 2006, Nwinyi et al., 2008). Flavonoids extracted from guava leaves including quercetin and were reported to have strong antibacterial action (Rattanachaikunsopon and Phumkhachorn, 2007; Arima and Danno, 2002).

Another medicinal plant of importance to bacterial infections in the Philippines is *Eucalyptus globulus* (also of Family Myrtaceae under Class Magnoliopsida*)* which is a large and very common tree in forest parks and even in urban centers in the Philippines. The essential oils *Eucalyptus* had been well (Batista-Pereira et al., 2006; Ghalem and Mohamed, 2008). *Eucalyptus* species produce numerous volatile compounds(Ghalem and Mohamed, 2008) in large amounts, especially terpenoids, which are accumulated in glands abundantly distributed throughout the leaves and bark (Rakotonirainy and Lavédrine, 2005; Moleyar and Narasimham, 1986). Eucalyptol or the major essential oil of *Eucalyptus* is incorporated in vapor rubs and some menthol candies (Ghalem and Mohamed, 2008).

Garlic (*Allium sativum* Linn. of Family Liliaceae, Class Liliopsida) is one of those plants that had been heavily investigated quite a time (Jabar and Al-Mossawi, 2007; Cavallito and Bailey, 1994) and one that has already reached the market together with ginger. It has been medicinally used for centuries to fight infections (Onyeagba et al., 2006; Abubakar, 2009a). According to Abubakar (2009a) Jabar and Al-Mossawi (2007), the early Egyptians used it to treat diarrhea, the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, Abubakar (2009a) and Jabar and Al-Mossawi (2007) added that garlic is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Ankri and Mirelman, 1999; Jabar and Al-Mossawi, 2007). The phytochemical constituents of garlic have already been established by several workers (Farbman et al., 1993; Cavallito and Bailey, 1994; Ankri and Mirelman, 1999; Prados-Rosales et al., 2003). The antimicrobial properties of garlic were first described by Pasteur in 1858, and since then, research had demonstrated its effectiveness against bacteria, protozoa, fungi and some viruses (Jabar and Al-Mossawi, 2007; Abubakar (2009a). Lemon (*Citrus aurantifolia* Linn.) and other citrus fruits of Family Rutaceae (Class Magnoliopsida) are also considered in this study. Lemon pericarp contains terpenoids whose main components are citral, limonene, B pinene and fenchone (Belewu et al., 2009). *Mangifera indica* Linn. belongs to the family Anacardiaceae (Class Magnoliopsida). It is widely used as a source of food, medicines and timber but a local strain called “Indian mango” was specifically used in this study has been cultivated everywhere nowadays in the Philippines. Among the compounds isolated from *M. indica* extract are terpenoidal saponins, several triterpenoid and 2-hyroxymangiferonic acid (Abubakar, 2009b).

P. oleiferum (Family Moringaceae, Class Magnoliopsida) leaves, a very common vegetable in the Philippines also contain alkaloids which commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Kasolo et al., 2010). On the other hand, they are also reported to modify tumorigenesis (Ueno et al., 2009), inhibit carbohydrate-mediated tumor growth (Nangia-Makker et al., 2002), induc a stress response and apoptosis in human breast cancer cells (Kasolo et al., 2010).

It is very noteworthy that most of the antibacterial medicinal plants considered in this study had been investigated by many authors from different countries. The present study would therefore provide a better comparison of activities in the light of a local situation in the Philippines.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

The plant samples were harvested in the early morning from various places in Iloilo City and Province, Philippines during the period June to September 2010. The mean annual rainfall of the area is about 1600 mm and temperature range of 20 to 35°C. It was authenticated by the senior researcher at the herbarium section of the Biological Sciences Department, West Visayas State University, where voucher specimens were deposited for future reference. The plant parts were removed from the 20 different plant samples, oven-dried for 4 h (WTC BINDER, 7200 Tuttlingen, Germany) for not more than 40°C, pounded using mortar and pestle and immediately proceeded for extraction.

Extraction

Pounded plant parts (50 g) were extracted using mechanical shaker (Shaker Bath BT-25 Yamato Scientific Co., LTD. Japan) with n-hexane and then (in succession) with acetone or dichloromethane (DCM), ethanol, and water for 48 h each; the n-hexane extract, acetone/DCM, ethanol/methanol and aqueous extracts were filtered (WHATMANN’S No. 1) and the filtrates dried in rotary evaporator at 40°C, 120 rpm (Heidolph WB2000).. All the extracts were kept in refrigerator prior to use.

Test organisms

The microorganisms used were obtained from the University of the Philippines Institute of Biology, Los Baños, Laguna, Philippines. All bacterial cultures were checked for purity and maintained in a nutrient agar slants. The organisms tested include *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas*

Table 1. Comparative phytochemical tests on the ethanol extracts of 20 common Philippine medicinal plants.

Plants/Species	Alkaloids	Flavonoids	Glycosides	Tannins	Terpenoids	Saponins
Allium cepa (Onion bulb)	+	++	++	++	+++	+++
Allium sativum (Garlic cloves)	+	++	++	++	+++	+++
Carmona retusa (Wild Tea)	+	+++	++	+++	+	+++
Citrus aurantiacum (Lemon Rind)	+	++	++	+	++	+
Citrus sinensis (Orange Peel)	+	++	++	+	++	+
Eucalyptus globulus (shoots)	-	++	++	+++	+++	+++
Mangifera indica (Indian Mango shoots)	-	+++	++	+++	+	++
Musa sapientum (Shoots)	+	+	++	+++	++	+++
Nasturtium officinale (Water cress)	+	++	++	-	++	++
Piper nigrum (Seeds)	+	+	+	+	+	+
Psidium guajava (Guava Shoots)	-	+++	+++	++	++	++
Pterygospermum oleiferum (Mallunggay shoots)	+	++	++	++	-	++

aeruginosa. The microorganisms were inoculated into nutrient broth and incubated for 24 h at 37°C. An 8-fold serial dilution was prepared from the stock cultures.. The inoculum used was 0.2 ml of 10^6 cfu/ml for all the organisms except for *B. subtilis* where the inoculum of 0.2 ml of 10^4 cfu/ml was used. Each inoculum was mixed with 20 ml of nutrient agar after sterilization and cooling to 55°C.

Bioassay studies

The disc Agar diffusion method (Sardari et al., 1998; Abdullahi et al., 2010) was used for the test. Filter paper disc (6 mm in diameter) impregnated with sample solutions were placed on nutrient agar plates which have been inoculated with test organisms. The extracts dissolved in their respective extraction solvents (solvent: water 1:1) were tested at a concentration of 1000 µg/disc. The plates were incubated at 37°C for 24 h, after which the diameters of the inhibition zones were measured and recorded in millimeters using a transparent ruler. Filter paper disc containing extraction solvents without any test extract served as control and no inhibition was observed because the discs were allowed to dry for a few minutes before introducing into the agar plates. The reference antibacterial drug Cloxacillin (5 mg/disc) together with Imepenem, Streptomycin, Isoniazid, Rifampicin, Ethambutol, and Pyrazinamide were used as a positive controls for *S. aureus*, Chloramphenicol was used for *P. aeruginosa and E. coli*.

Phytochemical screening

The phytochemical analyses of the ethanolic extracts of the 12 medicinal plants were carried out following the methods of Sofowora (1986) in Adebayo and Ishola (2009), Trease and Evans (1983), Wallis (1967), Rai and Obayemi (1973) and Elujoba et al. (1986) and modified according to Laboratory Manual for the UNESCO (1986) utilizing the 3 point scale (+, ++, or +++) with relative scoring based on the Handbook on Philippine Medicinal Plants (de Padua et al.,1981) and the Plants Database at West Visayas State University (Penecilla, 2004). The various phytochemical tests were the following: Alkaloids (Sofowora, 1986), flavonoids (Wallis, 1967), glycosides, tannins, (Sofowora, 1986), terpenoids (Trease and Evans, 1983), and Saponins (Sofowora, 1986). n-hexane extracts of the common medicinal plants in this study usually have no activity except for the *Allium spp,, Citrus spp*. and *Nasturtium officinale*. For aqueous extracts, it was only *C. retus*a, *M. indica*, *P. guajava*, and *Piper nigrum* which showed good activity. Acetone extracts usually have similar or lower activity compared with Ethanolic extracts. Thus, there was no need to perform phytochemical screening on these particular extracts.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical tests in Table 1 showed the presence of ubiquitous secondary natural products such as flavonoids, glycosides and saponins in all 12 plants and also tannins in almost (except in *N. officinale)* all 12 plants. Terpenoids are also common (except in *P. oleiferum*) while alkaloids are found in most plants. except in *E. globulus*, *M. indica*, and *P. guajava*. Thus, many extracts from Philippine medicinal plants can possibly be used as antibacterials. It is of high probability that this potential can be developed into antimicrobial drugs if specific compounds can be isolated and purified or at least if these plant extracts can be standardized using HPLC as to its contents and origin.

As far as phytochemical screening is concerned, the plants with the most abundant phytochemical constituents were as follows: *P. guajava* abundant for flavonoids and glycosides as well as terpenoids; *C. retusa* for flavonoids, tannins, and saponins*; Eucalyptus* for tannins, terpenoids and saponins; garlic and onions for terpenoids and saponins and *M. indica* for tannins and flavonoids.

This comparative study of 12 common medicinal plants enabled us to have an instant grasp on the antibacterial activities of these common medicinal plants in the Philippines. In Table 2, it can be gleamed that *P. guajava* at 15 mm to 25 mm zones of inhibition in all microorganisms studied was still the most active and had the broadest spectrum of antibacterial activity utilized by

Table 2. Susceptibility test of different extracts (1000 mcg/disc) of 12 common Philippine medicinal plants against the test organisms (mm).

Plant Species/Solvents	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
***Allium cepa* (Onion bulb)**				
n- Hexane	16	15	(-)	15
Acetone	10	10	8	8
Ethanol	10	9	(-)	10
Aqueous	(-)	(-)	(-)	(-)
***Allium sativum*(Garlic cloves)**				
n-Hexane	9	10	7	30
Acetone	(-)	(-)	(-)	15
Ethanol	20	18	(7)	23
Aqueous	(-)	(-)	(-)	(-)
***Carmona retusa* (Wild Tea)**				
n-Hexane	9	10	(-)	(-)
Acetone	(-)	(-)	(-)	12
Ethanol	10	10	(-)	12
Aqueous	15	15	(-)	13
***Citrus aurantifolia* (Lemon Rind)**				
n- Hexane	15	13	(-)	13
Acetone	9	9	(-)	9
Ethanol	14	14	(-)	15
Aqueous	(-)	(-)	(-)	13
***Citrus sinensis* (Orange Peel)**				
n- Hexane	(-)	(-)	(-)	15
Acetone	9	10	(-)	8
Ethanol	10	10	(-)	12
Aqueous	(-)	(-)	(-)	(-)
***Eucalyptus globulus* (shoots)**				
n-Hexane	(-)	(-)	(-)	10
Acetone	23	22	7	19
Ethanol	23	22	8	18
Aqueous	(-)	(-)	(-)	(-)
***Mangifera indica* (Indian Mango shoots)**				
n-Hexane	9	10	(-)	10
Acetone	15	16	(-)	13
Ethanol	15	15	(-)	16
Aqueous	23	22	(-)	25
***Musa sapientum* (Banana Shoots)**				
n-Hexane	10	10	(-)	10
Acetone	9	9	(-)	(-)
Ethanol	9	9	(-)	(-)
Aqueous	9	9	(-)	(-)

Table 2. Contd.

***Nasturtium officinale* (Water cress)**				
n-Hexane	22	20	(-)	15
Acetone	(-)	(-)	(-)	8
Ethanol	9	10	(-)	10
Aqueous	12	11	(-)	9
***Piper nigrum* (Black Pepper Seeds)**				
n-Hexane	(-)	(-)	(-)	(-)
Acetone	9	9	(-)	(-)
Ethanol	11	10	(-)	(-)
Aqueous	10	9	(-)	13
***Psidium guajava* (Guava Shoots)**				
n-Hexane	(-)	(-)	(-)	(-)
DCM	24	20	15	19
Ethanol	21	22	17	20
Aqueous	25	25	15	22
***Pterygospermum oleiferum* (Mallunggay shoots)**				
n-Hexane	(-)	(-)	(-)	8
Acetone	15	12	(-)	8
Ethanol	8	10	(-)	9
Aqueous	(-)	(-)	(-)	9

common folks in the Philippines and closely followed by garlic at 18 mm to 23 mm zones of inhibition in all microorganims studied except in E. coli and *E. globules* at 18 mm to 22 mm zones of inhibition in all microorganims studied except in E. coli *M. indica* aqueous extract and *Allium sativum* n-hexane extract at 25 mm and 30 mm zones of inhibition respectively was the best for *P. aeruginosa* but not for *E. coli*. Onion and garlic showed significant activities against gram positive and gram negative bacteria.

Meanwhile *P. oleiferum (Moringa*) at 15 mm zone of inhibition often used by local folks on wounds was just active against *S. aureus*. *Citrus* species at 9 mm to 15 mm zones of inhibition were also active against gram-positive and gram negative bacteria except *E.coli*.

Traditionally, medicinal herbs have been considered to be nontoxic and have been used for treating various problems by the general public "and/or" traditional medicine doctors worldwide (Oduola et al., 2007).

According to Rattanachaikunsopon and Phumkhachorn (2010), regardless of types of guava leaves used for isolation, quercetin and morin-3-O-arabinoside were the most and the least abundant flavonoids, respectively. Quercetin has been the most frequently studied flavonoid which is considered to be the major antibacterial compound in guava leaves. Thus, *P. guajava* leaves and shoot have long been recognized for their antibacterial activity. They were shown to inhibit both Gram-positive and Gram-negative bacteria such as *S. aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus* vulgaris, *Shigella* dysenteriae and *E. coli* as reported by Cowen (1999) and and Rattanachaikunsopon and Phumkhachorn (2010).

Medicinal plants have recently attrac-ted the attention of the biological scientific communities (Das et al., 2010). another driving factor for the renewed interest in plant antimicrobials has been the rapid rate of plant species extinction (Lewis and Elvin-Lewis, 1995) as there is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, 1996) particularly in the Philippines where slash and burn activities are still practiced (Das et al., 2010). *E. globulus* ethanol and acetone extracts follows guava as the best antibacterial agent in this study particularly for gram-positive bacteria and also for the gram-negative *P. aeruginosa* but not *E. coli.* Just recently Abubakar (2010) reported the antibacterial activity of the crude leaf extracts of *Eucalyptus camaldulensis* using also the agar well diffusion method against clinical isolates of *E. coli*, *S.aureus, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae*. Earlier Ghalem and Mohamed (2008) was able to determine the antibacterial activities of essential oils from leaves of two *Eucalyptus* species

Table 3. Positive controls

Rank	Control Drugs(5 mg/ml)	Zone of Inhibition (mm) (Against *S. aureus)*
1	Imepenem + Cilastatin	25
2	Rifampicin	30
3	Streptomycin	31
4	Cloxacillin	31
4	Isoniazid	30
5	Ethambutol	13
	Pyrazinamide	(-)
	Chloramphenicol	31 (*P. aeruginosa*)

(globulus and *camaldulensis)* against *S. aureus* Gram (+) and *E. coli* Gram (-) bacteria. This would further substantiate the potential usefulness of *Eucalyptus* species as a microbiostatic antiseptic or as disinfectant agent.

The effect of some terpenes on microorganisms has already been studied (Zwenger and Basu, 2008; Andrews et al., 1980). Terpenes, have shown increasing promise *in vivo*, inhibiting multiple species of bacteria (Zwenger and Basu, 2008) as shown in this study on the four most active medicinal plant species: *P. guajava, M. indica*, *A. sativum*, and *Eucalyptus globulus*. The result of the phytochemical screening also revealed that tannins, flavonoids and other glycosides were present in the ethanolic extracts of *E. globulus* (shoots) which could be responsible for its antibacterial properties. Pamploma-Roger (1999) earlier reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections(Egwaikhide et al., 2009).

A. sativum or garlic n-hexane and acetone extracts are very effective against *P. aeruginosa* as well as *S. aureus* while all extracts *of M. indica* are also very effective against *S. aureus*, *B.subtilis*, and *P.aeruginosa* except *E.coli*. Only the acetone extract of *P. oleiferum* was effective against *S. aureus*.

The two common medicinal plants with the least antibacterial activity were *Piper nigrum* and *Musa sapientum* with slight activity on the gram positive bacteria *S.aureus* and *B. subtilis* only. *Allium cepa* (onion) may also be included as one of the least if not for the n-hexane extract with good activity on *S. aureus*, *B. subtilis*, and *P. aeruginosa* (Table 3). All *Allium* spp. aqueous extracts showed no activity against all bacterial specimens studied n-Hexane extract of *N. officinale* wasvery effective against *S. aureus*. *B. subtilis* and *P. aeruginosa* but other extracts such as ethanol, water and acetone were not effective. *Citrus* species, (lemon and orange peelings) showed good actvitiy also in *S. aureus*, *B. subtilis* and *P. aeruginosa* but not their aqueous extracts except for lemon with a good activity in n-hexane on gram positive bacteria and its aqueous except on *P. aeruginosa.*

Indeed, *P. guajava, E. globulus, A. sativum, M. indica* were the four most active antibacterial medicinal plants species in the Philippines today.

Conclusion

This study has shown that many plants used in traditional medicine in the Philippines have very potent antibacterial activity but bioactive components of the plant may vary. The characterization of the active components of these plants may lead to full utilization by the local folks particularly for *P. guajava*, *E. globulus*, *A. sativum, M. indica*, *C. retusa*, and *Citrus* spp..

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