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In vitro evaluation of antibacterial activity of chloroform extract *Andrographis paniculata* leaves and roots, *Durio zibethinus* wood bark and *Psidium guajava* leaves against selected bacterial strains

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Abstract

The study was designed to evaluate the antibacterial potential of three plants which are *Andrographis paniculata*, *Durio zibethinus* and *Psidium guajava*. *Andrographis paniculata* leaves (30mg/ml) and roots (30 mg/ml), *Durio zibethinus* wood bark (10mg/ml), and *Psidium guajava* leaves (15mg/ml) extract was obtained through the process called maceration, filtration, evaporation and the paste form was freshly reconstitute in dimethyl sulfoxide (DMSO) and tested against *Staphylococcus aureus* for *Andrographis paniculata*, *Psidium guajava*. *Streptococcus agalactiae* for *Durio zibethinus* and *Psidium guajava* and *Escherichia coli* for *Durio zibethinus* using Kirby Baur technique and the plates were incubated at 37 ºC. The zone of inhibition was measured after 24 hours and recorded in millimeters. The combination study was conducted using extract in combination with Penicillin G (6.25 µg/ ml) and erythromycin (15 µg/ ml; *Andrographis paniculata*) with the propotion of 1:1 in homogenous condition and incubated at 37 ºC for 24 hours. The zone of inhibition was measured and recorded. Mean and standard deviation was calculated. *Andrographis paniculata* do possesses some antibacterial activity against *Staphylococcus aureus*. Leaves (17.33 mm), roots (10.67 mm), erythromycin (24.00 mm), leaves and erythromycin (20.67 mm), roots and erythromycin (21.67 mm), leaves and roots (17.33 mm). Wood bark against *Streptococcus agalactiae* (14.67 mm), Penicillin G (14.00 mm), and combination (16.67 mm). *Durio zibethinus* showed antibacterial activity against *Escherichia coli* (11.00mm) and Penicillin G (13.33 mm). *Psidium guajava* leaves extract were having slightly higher activity than Penicillin G and in combination activity, leaves were having a slightly higher activity than Penicillin G.

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Introduction

Medicinal herbs are widely used with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It has been recorded that 80% of the world's population has fidelity in traditional medicine, particularly plant based drugs for their primary healthcare (Alireza *et al*., 2011).

Germany and France, together represent 39% of the $14 billion global retail market. In India, about 80% of the rural population depends on medicinal herbs and or indigenous systems of medicine (Mitra and Kannan, 2007).

Andrographis paniculata is a medicinal herb from the family Acanthaceae (Alireza *et al*., 2011). The leaves and roots of *Andrographis paniculata* have been trationally used over the centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as an herbal supplement for health promotion. The Indian pharmacopoeia narrates that *Andrographis paniculata* is a predominant constituent of at least twenty six Ayurvedic formulation (Panneerselvam *et al*., 2011).

Andrographis paniculata (AP) is one of the medicinal plants that seem promising found throughout Southeast Asia. Basically, the taste of andrographis is very bitter. This bitterness is related with its various pharmacological properties (Jegathambigai *et al*., 2010). *Andrographis paniculata* is one among the prioritized medicinal plants in India and this herb is being used mainly for treating fever, liver disease, diabetes, snake bite (Patidar *et al*., 2011).

It is also used as antibiotic, antiviral, antimicrobial, anti inflammatory, anticancer, anti-HIV, anti-allergic (Jegathambigai *et al*., 2010). It is also utilised for common cold, hepatoprotective activity, antimalarial, antidiarrheal and intestinal effect, cardiovascular activity, antifertility activity, pain reduction (Jarukamjorn and Nemoto, 2008). It is also possess antifungal activity, cholerectic activity and in the Unani system of medicine, it is considered aperient, emollient, astringent, diuretic, emmenagogue, gastric tonic, carminative (Akbar, 2011). It is also having potential to be used as herbicidal and it is used as antiarthritis (Alireza *et al*., 2011).

Durian (*Durio zibethinus Murr*) is a popular and expensive tropical fruit widely grown in South-East Asia. Durian is entitled "King of Tropical Fruit" due to the superlative flesh, which is highly nutritional and its appearance which resembles the thorny thrones of Asian kings (Norjana and Noor, 2011). Among exotic fruits durian is less known (Leontowicz *et al*., 2008).

Durian is rich in carbohydrate, protein, fat, phosphorus, iron and vitamin A. Durian is usually used for fresh consumption. The edible portion (aril) of durian has a very strong odor (Norjana and Noor, 2011). Most of the photochemicals are an integral part of the durian fruit and also being used in medicinal formulations. A number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial, and other biological possessions. Currently durian fruit is popular in daily utilization because of their first-class flavor and health-promoting compounds, such as flavonoids, phenolics and carotenoides contents (Ashraf *et al*., 2010). Durian flesh, is said to serve as a medication to eliminate parasitic worms. Moreover, in Malaya, decoction of durian leaves and fruits are applied to swellings and skin diseases while the ash of the burned rind is taken after childbirth (Duazo *et al*., 2012).

Psidium Guajava is Called guayaba in Spanish speaking countries and goiaba in Brazil, guava is a common shade tree or shrub in door yard gardens in

the tropics. It belongs to family Myrtaceae, genus: Psidium ,species: guajava and common names of the plant are Guava, goiaba, guayaba. Plant parts which are used are fruits, leaves and barks. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100 g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin a dietary fiber. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity. Quercetin is thought to contribute to the antidiarrhea effect of guava, it is able to relax intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes in guava leaves show antispasmodic activity (Vyas *et al*., 2010).
Microorganisms have created resistance to various antibiotics and this had developed immense clinical difficulty in the curing of contagious illness. The enlarge in resistance of microbes due to indiscriminate utilize of commercial antimicrobial medicines supported scientists to investigate for modern antimicrobial substances from several sources including medicinal plants (Alagesaboopathi and Kalaiselvi, 2012)

Thus this study was conducted to evaluate the antibacterial potentials of *Andrographis paniculata* leaves, *Durio zibethinus* wood bark and *Psidium guajava* leaves against selected bacterial strains.

Material and method

Collection of plant materials

Andrographis paniculata leaves and roots, *Durio zibethinus* wood bark and *Psidium guajava* leaves were collected from small village in Raub, Pahang, Malaysia. The plant materials then wash thoroughly under running tap water and dried under shade. They are then finely ground to a powder in an electric blender and stored separately (Soma *et al*., 2009).

Bacterial strains

In current research, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae* were used. The pure strains were obtain from ASIA Metropolitan University's laboratory. The organisms were maintained on nutrient agar media at 4°C and sub cultured for 24 hours before use (Alagesaboopathi and Kalaiselvi, 2012).

Preparation of plant extracts for Andrographis paniculata and Psidium guajava

The solvents used for the extraction procedure in the present study was chloroform. About 25 g of dried *Andrographis paniculata* leaves and roots and *Psidium guajava* leaves powder were extracted using 250 ml of the extraction solvents separately for 48 hours (Soma *et al*., 2009). The filtrates is concentrated using a rota vapour, at 40°C and then in water bath until the paste is form (Rusmiati 2010). The percentage of yield was 11.2% for leaves of *Andrographis paniculata*, 5.8% for roots of *Andrograhis paniculata* and 10.9% for *Psidium guajava* leaves. The paste was then kept in air tied container separately and refrigerated at 4°C.

Preparation of plant extract of Durio zibethinus

The solvents used for the extraction procedure in the present study was chloroform. About 18 g of dried *Durio zibethinus* wood bark powder were extracted using 180 ml of the extraction solvents for 48 hours (Soma *et al*., 2009). The filtrates is concentrated using a rota vapour, at 40°C and then in water bath until the paste is form (Rusmiati 2010). The percentage of yield was 0.6 %. The paste was then kept in air tied container and refrigerated at 4°C.

Antibacterial assay for Andrographis paniculata

The media used were Muller Hinton agar (Al-Haddad, 2005). 30 mg of extracts of leaves and roots were

freshly reconstituted separately with dimethyl sulphoxide (DMSO). Antibacterial activity was determined by the well diffusion method. Wells (8 mm diameter) were cut into the agar. 200µl of the plant extracts were tested in a concentration of 30 mg/ ml (leaves and roots) and 200µl of Erythromycin (positive control) were tested in a concentration of 15µg/ ml separately. The agar were seeded with 24hours culture of the *Staphylococcus aureus*. Incubation was performed at 37ºC for 24 hours for bacterial strain. Bacterial growth was determined by measuring the diameter of zone of inhibition in millimeters (Kataky and Handique, 2010). The work was done in triplicate (Alagesaboopathi and Kalaiselvi, 2012).

Combination activity of Andrographis paniculata leaves and roots with Erythromycin

The combination activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform plant extract of *Andrographis paniculata* (leaves and roots) 30 mg/ ml was used in combination with Erythromycin 15 µg/ ml in propotion of 1:1 (100 µl : 100 µl) separately against *Staphylococcus aureus* . The combination were in homogenous condition. The plates were then incubated at the standard conditions for 24 hours at 37°C and the zone diameters was measured in the second day. The work was done in triplicate.

Combination activity of Andrographis paniculata leaves and roots

The combination activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform plant extract of *Andrographis paniculata* leaves and roots 30 mg/ ml was used in combination with in propotion of 1:1 (100 µl : 100 µl) against *Staphylococcus aureus*. The combination were in homogenous condition. The plates were then incubated at the standard conditions for 24 hours at 37°C and the zone diameters was measured in the second day. The work was done in triplicate.

Antibacterial assay for Durio zibethinus

The media used were Muller Hinton agar (Al-Haddad, 2005). 10 mg of extracts of wood bark freshly reconstituted with dimethyl sulphoxide (DMSO). Antibacterial activity was determined by the well diffusion method. Wells (8 mm diameter) were cut into the agar. 200µl of the plant extracts were tested in a concentration of 10 mg/ ml (leaves and roots) and 200µl of Penicillin G (positive control) were tested in a concentration of 6.25 µg/ ml separately. The agar were seeded with 24h culture of the *Streptococcus agalactiae* and *Escherichia coli*. Incubation was performed at 37ºC for 24 hours for bacterial strain. Bacterial growth was determined by measuring the diameter of zone of inhibition in millimeters (Kataky and Handique, 2010). The work was done in triplicate (Alagesaboopathi and Kalaiselvi, 2012).

Combination activity of Durio zibethinus with Penicillin G

The combination activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform plant extract of *Durio zibethinus* wood bark of 10 mg/ ml was used in combination with Penicillin G 6.25 µg/ ml in propotion of 1:1 (100 µl : 100 µl) separately against *Streptococcus agalactiae*. The combinations were in homogenous condition. The plates were then incubated at the standard conditions for 24 hours at 37°C and the zone diameters was measured in the second day. The work was done in triplicate.

Antibacterial assay for Psidium guajava

The media used were Muller Hinton agar (Al-Haddad, 2005). 10 mg of extracts of leaves freshly reconstituted with dimethyl sulphoxide (DMSO). Antibacterial activity was determined by the well diffusion method. Wells (8 mm diameter) were cut into the agar. 200µl of the plant extracts were tested in a concentration of 15 mg/ ml (leaves) and 200µl of Penicillin G (positive control) were tested in a concentration of 6.25 µg/ ml separately. The agar were seeded with 24h culture of

the *Streptococcus agalactiae* and *Staphylococcus aureus*. Incubation was performed at 37°C for 24 hours for bacterial strain. Bacterial growth was determined by measuring the diameter of zone of inhibition in millimeters (Kataky and Handique, 2010). The work was done in triplicate (Alagesaboopathi and Kalaiselvi, 2012).

Combination activity of Psidium guajava with Penicillin G

The combination activity study was calculated by means of cup plate method (Kirby and Bauer technique). Chloroform plant extract of *Psidium guajava* leaves of 15 mg/ ml was used in combination with Penicillin G 6.25 µg/ ml in propotion of 1:1 (100 µl : 100 µl) separately against *Streptococcus agalactiae* and *Staphylococcus aureus*. The combination were in homogenous condition. The plates were then incubated at the standard conditions for 24 hours at 37°C and the zone diameters was measured in the second day. The work was done in triplicate.

Statistical analysis

The mean and standard deviation of zone of inhibition was calculated.

Result and discussion

From the Table 1, *Andrographis paniculata* do possesses some antibacterial activity against *Staphylococcus aureus*. Leaves (17.33 mm), roots (10.67 mm), erythromycin (24.00 mm), leaves and erythromycin (20.67 mm), roots and erythromycin (21.67 mm), leaves and roots (17.33 mm). From the previous studies mentioned that andrographolide from *Andrographis paniculata*'s plant was playing vital role in antimicrobial properties of the plant (Chowdhury *et al.,* 2012**).** Four xanthones such as 1,8-di-hydroxy-3,7-dimethoxy-xanthone; 4,8-dihydroxy-2,7-dimethoxy-xanthones; 1,2-dihydroxy-6,8-dimethoxy-xanthones and 3,7,8-trimethoxy-1-hydroxy xanthone were isolated from Andrographis *paniculata*'s root (Chowdhury *et al.,* 2012). Phytochemistry studies need to be conducted with these four xanthones to identify which compound out of these four compounds are having the antibacterial activity against *Staphylococcus aureus*.

Table 1. Antibacterial activity of leaves and roots of *Andrographis paniculata* against *Staphylococcus aureus*.

	Zone of inhibition (in mm)
Leaves	17.33 ± 0.47
Roots	10.67 ± 0.47
Erythromycin	24.00 ± 0.82
Leaves + Erythromycin	20.67 ± 0.47
Roots + Erythromycin	21.67 ± 0.47
Leaves + Roots	17.33 ± 0.47
DMSO	00.00 ± 0.00

Table 2. Antibacterial activity of wood bark of *Durio zibethinus* against *Streptococcus agalactiae*.

	Zone of inhibition (in mm)
Wood bark	14.67 ± 0.47
Penicillin G	14.00 ± 0.00
Wood bark + Penicillin G	16.67 ± 0.47
DMSO	00.00 ± 0.00

Table 3. Antibacterial activity of wood bark of *Durio zibethinus* against *Escherichia coli*.

	Zone of inhibition (in mm)
Wood bark	11.00 ± 0.82
Penicillin G	13.33 ± 1.24
DMSO	00.00 ± 0.00

Erythromycin which was used as a positive control against *Staphylococcus aureus* showed a largest inhibition zone. Erythromycin belongs to the macrolides group of antibiotics, which mostly produced from *Streptomyces*. Erythromycin is a 14 membered ring macrolide shows a broad spectrum

antimicrobial activities against gram positive and gram negative bacteria (Hawkyard and Koerner, 2007). The macrolides mainly achieve inhibition of protein synthesis by binding in the exit tunnel of the ribosome where the evolving peptide is primarily formed by 23S rRNA (Hawkyard and Koerner, 2007). This type of action by erythromycin causes the bacteria to be killed due to the protein synthesis inhibition within the bacteria. Combination of leaves and erythromycin (20.67 mm), roots and erythromycin (21.67 mm) however do not show synergistic or additive activity but the combination showed the antagonism effect where the zone of inhibition was less than 24mm that had been achieved by the erythromycin. Combination of leaves and roots does not produce any significant effect where it was just representing the value of the leaves's inhibition value.

Table 4. Antibacterial activity of *Psidium guajava* against *Staphylococcus aureus* and *Streptococcus agalactiae*.

	Zone of inhibition (in mm)	
	Staphylococcus aureus	*Streptococcus agalactiae*
Leaves	17.33 ± 0.47	14.67 ± 0.47
Penicillin G	12.67 ± 0.47	14.33 ± 0.47
Leaves + Penicillin G	17.67 ± 0.47	15.00 ± 0.00
DMSO	00.00 ± 0.00	00.00 ± 0.00

From the table 2, *Durio zibethinus* wood bark showed some antibacterial activity against *Streptococcus agalactiae*. The wood bark (14.67 mm), Penicillin G (14.00 mm), and combination of these both showed 16.67 mm. In wood bark extract of *Durio zibethinus*, two new triterpenoids, namely, methyl 27-O-trans-caffeoylcylicodiscate and methyl 27-O-cis-caffeoylcylicodiscate, a new phenolic, 1,2-diarylpropane-3-ol, and seven known compounds, fraxidin, eucryphin, boehmenan, threo-carolignan E, (–)-(3R,4 S)-4-hydroxymellein, methyl protocatechuate, and (+)-(R)-de-O-methyllasiodiplodin contents were present (Lim, 2012). These compounds need to be included in further studies to look at the antibacterial potential because to the best knowledge the identification of compound which is having the antibacterial activity has been not conducted. Definitely 1 or more compound do possesses antibacterial properties since the chloroform wood bark extract inhibit the bacterial growth on the Muller Hinton agar plate.

Penicillin G act on cell wall. Pencillin G, inhibits the third and final stage which is involved in the synthesis of peptidoglycan, which is a heteropolymeric component of the cell wall, where it provides a rigid mechanical stability by virtue of its highly cross-linked lattice work structure. This cross linking is accomplished by a transpeptidation reaction that occurs outside the cell membrane (Esimone *et al.*, 2006). Combination of both wood bark and penicillin G showed 16.67 mm of zone of inhibition. This double attack of the both agent (penicillin G on the cell wall and wood bark need to do further studies) caused the bacterial cell to die. Penicillin G is a preferred drug for curing disease related to *Streptococcus agalactiae* but it is reported that allergy reaction due to penicillin in some patients forced the physician to prescribed erythromycin as a second line drug (Beitune *et al.*, 2005). From the table 3, wood bark extract of *Durio zibethinus* showed antibacterial activity against *Escherichia coli* (11.00mm) and Penicillin G (13.33 mm).

From the table 4, *Psidium guajava* leaves extract showed antibacterial activity against *Staphylococcus aureus* and *Streptococcus agalactiae*. In both bacteria, leaves were having slightly higher activity than Penicillin G and in combination activity also the leaves were having a slightly higher activity than Penicillin G. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is

attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity (Vyas *et al*., 2010). Most Staphylococci isolated from individuals outside the hospital are resistant to Penicillin G due to beta lactamases, which inactivate the drug (Esimone *et al*., 2006). In current world, the bacterial resistance towards the antibiotics has created big problems in healthcare industry. Basically the bacterial resistances can occurs in three ways that are preventing the drug from reaching its target, altering the target and inactivating the antibiotic (Soares *et al*., 2012).

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