

# Antibacterial efficacy of leaf extracts of *Paederia foetida* Linnaeus

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## ABSTRACT

**Aim:** This study was aimed to determine the antibacterial efficacy of the ethyl alcohol and ethyl acetate leaf extracts of *Paederia foetida* that can inhibit the growth of microorganisms causing disease and to identify the presence of alkaloid, flavonoids, saponins and tannins as secondary metabolites.

**Method and Materials:** Determination of antimicrobial effect was done using the paper-disc method and Kirby-Bauer antibacterial sensitivity test against *Staphylococcus aureus* and *Escherichia coli*. Result showed that leaf extracts of *P. foetida* showed antibacterial activity against bacteria *S. aureus* and *E. coli*. Mean zone of inhibition was numerically higher in plates extract obtained using ethyl alcohol (22.70 - 21mm), compared to extract obtained using ethyl acetate as solvent.

**Results:** Results of phytochemical screening showed that extract of the leaves of *P. foetida* contains alkaloids, saponins and tannins but not with the flavonoids.

**Conclusion:** It was concluded that leaf of *Paederia foetida* could be a potent antimicrobial agent for use in the field of pharmaceutical.

**Keywords:** antibacterial, extract, *Escherichia coli*, *Paederia foetida* and *Staphylococcus aureus*.

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## Introduction

*Paederia foetida* is a plant species and known by several local names. In Northern Samar, it is locally known as kantutai or otot-bagtik because of its fetid aroma or sulphurous odor when leaves or stems are bruised or have been crushed (Chanda et al, 2013). The smell is caused by the sulphur compounds that present in the oils of the stem and leaves. Dimethyl disulfide is one such compound that is found in high amount in the leaves. Taxonomically, this plant belongs to the plant family Rubiaceae being herbaceous, hairy or smooth slender vines that climb over shrubs and trees. It can grow high into canopy of trees in a variety of habitats (Langerland and Burks, 2000). Characteristically, leaves of the plant are rich in carotene and vitamin C and also contain high protein content, consisting of arginine, histidine, lysine, tyrosine, tryptophan, phenylalanine, cystine, methionine and valine. Aerial parts also contain a crystalline keto alcohol paederolone, a keto compound, paederone,  $\beta$ - and  $\delta$ -sitosterols and two volatile alkaloids, paederine and paederenine (Duke, 2002 & Wong and Tan, 1994).

Leaves of the plant have a variety of uses. It is used as a remedy in diarrhea, dysentery, herpes infection and to relieve distension due to flatulence. It is also used in treating various intestinal problems like cramps, colic dysentery, and abdominal pain. It can also be applied externally to help treat bruises and swelling. Leaves can also be mashed up and use for nose ulcerations, earache, swollen eyes and swollen belly and it can even be applied in bath water to be used as an anti-rheumatism. Decoction of leaves is used for retention of urine and for urinary bladder stones. For fever, the decocted leaves are given internally or applied externally on the head using cloths that is soaked in the decoction (Tavera 2000).

The roots, on the other hand, have been used as an emetic, useful in piles and beneficial for relieving spleen inflammation as well as pain in the chest and liver. Fruits of the plant are used to relieve tooth pain and for whitening blackened teeth (Ghani 2003). The bark of the plant is known to help relieve constipation, asthma, urethral calculi, and assist in the expulsion of the placenta after a miscarriage.

*Paederia foetida* has been reported to have wide-ranging therapeutic properties such as antioxidant activity (Osman et al. 2009; Dasgupta and De 2007); antinociceptive (Thompson et al., 2005),

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antiviral (Afroz et al, 2006), anti-tussive (DE et al, 1994), anti-cancer activity (Nandkoni, 2002), anti-diarrheal activity (Chopra et al. 1969; Chopra et al. 1956; Barua et al. 2007; Borah et al. 2006; Kar and Borthukar 2008a, b; Kalita and Phukan 2010; Das 1997; Chanda et al. 2011; Srivastava and Singh 2010); and anti-arthritis activity (Rajashekhara et al., 2009); Methanolic leaves extract of *P. foetida* showed significant results in the investigation of analgesic activity (Hossain et al. 2006), anti-helminthic activities (Dey and Pal, 2011), anti-inflammatory activity (Prakash et al. 2009; Ravi et al. 2009), and hepatoprotective activity (De et al., 1993b; De et al., 1996; Yang et al. 1987). Other reported applications of *P. foetida* are in treatment of some aspects of male vitality. Research shows that it plays a vital role in increasing testosterone level in men (Raghunathan and Mitra 1982). It also showed significant amelioration of experimentally induced colitis, which may be attributed to its anti-inflammatory and antioxidant property (Das et al, 2013), acute shigellosis (Haider et al. 1991), and tripsy Chemolithotripsy (WI 2001). Therefore, the study was planned to determine the antibacterial efficacy of the ethyl alcohol and ethyl acetate leaf extracts of *Paederia foetida* that can inhibit the growth of microorganisms causing disease and to identify the presence of alkaloid, flavonoids, saponins and tannins as secondary metabolites.

## Materials and Methods

### Preparation of Extracts

Fresh leaves of *P. foetida* were collected from Palapag, Northern Samar and taxonomically authenticated at the Botany Division of Natural History Museum, Philippines. About 150 grams of freshly collected *Paederia foetida* (kantutai) were segregated and thoroughly cleansed with distilled water, dried and extracted using a manual juice. The dried plant part was mixed with a solvent, ethyl alcohol and ethyl acetate and was placed for 24 hours in an oven where it has the standard room temperature (32°C). The ratio of the grinded kantutai against the solvent is 1:2 or for every 1 gram of kantutai, its counterpart was 2 mL of the solvent.

After 24 hours of soaking, they were filtered to separate the solid from the extract using a cheese cloth and then filtered using Whatman no 41mm filter paper. Filtered extract was subjected to distillation procedure to separate the solvent from the crude extract. After distillation, the extract was

placed in a sterilized bottle and was labelled for easy identification.

### Detection of Secondary Metabolites

**Test for Alkaloids:** The plant extract was tested for the presence of alkaloids using Dragendorff's and the Mayer's reagents (Guevara 2005). For every 2mL of the extract, 1mL hydrochloric acid and six drops of Mayer's and Dragendorff's reagents were added. A positive result manifested as the formation of orange precipitate in Dragendorff's reagent and a white precipitate in Mayer's reagent indicated presence of alkaloids.

**Test for Saponins:** The capillary tube test was used to determine the presence of saponins. If the level of the plant samples in the capillary tube is half or less than in the other test tube containing water, the presence of the saponins will be inferred (Guevara 2005). Load a capillary tube with a plant extract by immersing the tube to the height of ten mm in the plant samples.

**Test for Flavonoids:** Test tube screening method was used to determine the presence of flavonoids. About 0.5 of plant samples were taken and was dissolved in ethanol, warm and then filtered. Three pieces of magnesium chips was added to the filtrate followed by a few drops of concentrated hydrochloric acid (Guevara 2005). A Yellowish color is the evidence for the presence of flavonoids.

**Test for Tannin:** The test tube method was used to determine the presence of tannins (Guevara 2005). Ten grams of the plant samples was centrifuge about 20 minutes to allow the solid particles of the extract to settle down at the bottom of the test tube, then the clear supernatant liquid was decanted and tested for the presence of tannin. About 3ml of supernatant extract was added to a few drops of 1% lead acetate, the yellowish precipitate indicated the presence of tannins.

### Preparation of culture media and sensitivity discs

Culture media for bacteria was prepared following by dissolving 19g Nutrient Agar (NA) in 500mL distilled water. Potato dextrose agar (PDA) (9.5g) was also dissolved in 250mL distilled water as culture medium for fungi. The mixtures were agitated vigorously to completely dissolve the media and were pressure sterilized at 121°C at 15psi pressure. Then allowed to cool down to about 50°C, and aseptically dispensed into individual petridishes and allowed to solidify at room temperature.

Sensitivity discs were prepared using filter paper (WhatmanNo.41) formed into round discs

using a puncher. Each disc was placed in petridish, pressure sterilized at 121°C at 15psi pressure, and soaked in the *P. foetida* crude extracts.

**Antimicrobial Screening:** The antibacterial activity of *P. foetida* extract was determined through the modified Kirby Bauer antibacterial sensitivity test (Bauer et al. 1996) against the test microorganisms *S. aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria), which were provided by the Centro Escolar University (CEU) Microbiological Laboratory. Chloramphenicol was used as positive control for *S. aureus* and Tetracycline for *E. coli*. The culture media plates were aseptically inoculated with bacteria, and the filter paper discs soaked in *P. foetida* extracts were carefully and aseptically placed at the center of each agar plate to maximize the space for bacterial growth and to facilitate measurement of the zone of inhibition. The plates were incubated at 37°C for 24h for bacteria. The zones of inhibition around the discs, which were measured using a vernier caliper, indicated that growth of the microorganism had been inhibited by diffused into the agar from discs. Absence of zone of inhibition indicated the resistance of the microorganisms to *P. foetida* extract.

## Results and discussion

### Phytochemical screening

Tannins, alkaloids and saponins were detected in extracts from leaves of *P. foetida* as shown in Table 1. Flavonoids were not detected from extracts regardless of solvent used. Saponin and alkaloid was only present in extracts obtained using ethyl alcohol as solvent.

Table 1. Chemical Presence of the Extract from the Leaf of *Paederia foetida*

Chemical properties	Leaf Extract	
	Ethyl alcohol	Ethyl acetate
Alkaloids	+	-
Flavonoids	-	-
Saponins	+	-
Tannins	+	+

Legend: (+) = present; (-) = not detected

### Antimicrobial Property

Zones of inhibitions in plates inoculated with *S. aureus* and *E. coli* were numerically higher in extracts produced using ethyl alcohol as solvent as shown in Table 2. Inhibitory activity was observed only against bacteria. There is no significant difference of the leaf extracts as to their antibacterial activity on *E. coli* and *S. aureus*.

Table 2. Zone of inhibition (mm) formed in Nutrient Agar with extracts from leaves of *P. foetida*.

Leaf Extract	Test microorganism	
	<i>S. aureus</i>	<i>E. coli</i>
Ethyl alcohol	22.70 mm	21.00 mm
Ethyl acetate	13.95 mm	14.20 mm
Control	21.00* mm	19.70**mm

\*chloramphenicol / \*\*tetracycline

There is no significant difference between the solvents used in the study as to their antimicrobial resistance and activity. Both of them showed great antimicrobial activity. Either of the two can be used as a source of substances capable to inhibit the growth of or kill microorganisms.

The result of the phytochemical screening of the leaf extracts of *P. foetida* showed that alkaloids, saponins and tannins are all present in the ethyl alcohol leaf extracts. Whereas, only tannins were present in the ethyl acetate extracts as presented (Table 1). Flavonoids were not detected from extracts regardless of solvent used. Saponins and alkaloids were only present in extracts obtained using ethyl alcohol as solvent. Afroz et al (2006) also reported similar findings. However, Ravi et al (2009) reported anti-inflammatory effects of medicinal plants.

In terms of the antibacterial activity of the ethyl alcohol and ethyl acetate leaf extracts, *P. foetida* were screened for antibacterial activity against Gram positive and Gram negative bacteria. Both the extracts showed a great antibacterial resistance and activity. However, ethyl alcohol leaf extract showed a higher zone of inhibition of 22.70 mm for *S. aureus* and 21mm for *E. coli* than the ethyl acetate leaf extract with a zone of inhibition of 13.95 mm for *S. aureus* and 14.20 mm for *E. coli*. Osman et al (2009) also observed similar findings of *P. foetida*. Thus, the leaf extracts can be used as a good source of substance capable of inhibiting the growth, or even kill microorganisms.

## Conclusion

It was concluded that growth of *E. coli* and *S. aureus* can be inhibited by the ethyl alcohol and ethyl acetate leaf extracts of *P. foetida*. It is confirmed that the plant has a great potential as a good source of organic antibacterial effective against gram-positive and Gram-negative bacteria. It can be recommended for further research to discover the activity of isolated compounds against other microorganisms.

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