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EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *PERSICARIA CHINENSIS* LEAVES

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ABSTRACT

The scientific basis of medicinal plants often claimed that the valuable ethnobotanical medicinal-related information was true. Hence, researchers always develop deep interest in finding natural resources like medicinal plants to combat infectious diseases instead of using modern pharmaceutical drugs that can lead to vast antibiotic resistance issues. In this present study, various leaf extracts of *Persicaria chinensis* (L.) H. Gross were investigated for their antibacterial and antifungal activities against various Gram-positive and Gram-negative bacteria and fungi by disc diffusion method. The aqueous, chloroform, methanol, and petroleum ether extracts of *P. chinensis* leaves were prepared by cold maceration technique and preliminary phyto-constituent investigation was carried out on the extracts. The antibacterial and antifungal activities were assessed by measuring the diameter of the zone of inhibition, and the results were compared with standard antibiotics, amoxicillin and fluconazole respectively. All leaf extracts possessed antibacterial and antifungal activity against the selected pathogenic bacteria and fungi. Nevertheless, methanol and aqueous extracts and reference drugs (amoxicillin and fluconazole) showed comparable efficacy in inhibiting certain microbial growths. The zone of inhibition signified antibacterial and antifungal potencies of *P. chinensis* leaf extracts and it might be due to the presence of secondary metabolites.

INTRODUCTION: Medicinal plants have been used to sustain human health and to treat various maladies including infectious diseases since antiquity [1]. Infectious diseases, caused by bacteria, viruses, fungi, or parasites have led to significant morbidity and mortality to population worldwide [2, 3].

Synthetic antimicrobial agents such as antibiotics are widely used to cure infections, but their indiscriminate use causes antimicrobial drug resistance, necessitating the use of medicinal plants as the alternative therapeutic agents. Medicinal plants and plant-derived products are cost-effective and easily obtainable [4].

Medicinal plants have promising efficacy to treat intractable infectious diseases, and thus they may be useful in eradicating new emerging microbial strains. In addition, they have profound safety profile because they cause fewer side effects such as hypersensitivity, allergic reactions, and immunosuppression compared to commercial antimicrobials [1].

Persicaria chinensis (L.) H. Gross is an herb belonging to the family Polygonaceae. It is commonly known as 'huo tan mu' in Chinese, Chinese knotweed or red bush in English, 'tsuru-soba' in Japanese, 'liane rouge' in French [5], and 'kukurthotne' in Nepali [6].

P. chinensis grows in wet valley, mixed forests, thickets, or on grassy or mountain slopes [7]. It is available in China, Taiwan, India, Indonesia, Japan, Korea, Malaysia, Singapore, Thailand, Myanmar, Vietnam, Philippines, Nepal, Bhutan, Sri Lanka, Pakistan, New Zealand, Hawaiian Islands, and Papua New Guinea [5, 8, 9].

P. chinensis reported to possess moderate activities against *Helicobacter pylori* infections [10]. Although enormous medicinal plants have claimed to be used as traditional medicines to curb many illnesses, their effectiveness is not scientifically supported. More recently, the ethanol extract of the whole plant of *P. chinensis* reported the presence of bioactive components such as coumarin compound and triterpenes having antimicrobial properties by referring to the ethnobotanical databases [11]. As a result, the inspiration to exploit the use of medicinal plants for treating infectious diseases on a scientific manner was indeed convincing.

Hence, the present study was undertaken to evaluate antibacterial and antifungal activities of leaf extracts of *P. chinensis* to prove its traditional claim scientifically.

MATERIALS AND METHODS:

Plant Material: *Persicaria chinensis* (L.) H. Gross leaves were collected in Kepong, Malaysia in the month of November 2011. The plant was authenticated by Dr. Sugumaran a/l Manickam, Institute of Biological Sciences, Faculty of Science, University of Malaya. A voucher specimen (KUL47704) was deposited at herbarium of University of Malaya, Malaysia.

Preparation of Extract: Fresh leaves of *P. chinensis* were washed, shade-dried, and pulverized into coarse powder. Coarse powdered leaves were equally divided into four portions, and they were extracted with petroleum ether, chloroform, methanol, and distilled water separately by cold maceration technique for 7 days. The extracts were filtered with Whatman filter paper separately, and the excessive solvent was evaporated and concentrated using Rotary Vacuum Evaporator under reduced pressure [12, 13, 14]. The color, consistency, and the percentage yield of various leaf extracts were recorded in **Table 1**.

The extracts were kept separately in the refrigerator until further studies.

TABLE 1: COLOR, CONSISTENCY AND % YIELD OF VARIOUS *PERSICARIA CHINENSIS* LEAF EXTRACTS

Extracts	Color	Consistency	% yield
Petroleum ether	Dark brown	Solid	4.62
Chloroform	Dark brown	Solid	6.90
Methanol	Greenish brown	Sticky semisolid	9.50
Aqueous	Brown	Sticky semisolid	13.70

Preliminary Phytochemical Analysis: The extracts of *P. chinensis* were subjected to preliminary phytochemical screening by the standard methods to identify the presence of plant secondary metabolites such as alkaloids, amino acids, carbohydrates and glycosides, fixed oils and fats, flavones and flavanones, gums and mucilage, phenolic compounds and tannins, proteins, saponins, sterols, and triterpenoids [12]. The results of preliminary phytoconstituent studies of *P. chinensis* leaf extracts were tabulated in **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL STUDIES OF LEAF EXTRACTS OF *P. CHINENSIS*

Chemical Constituents	Pet. Ether extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	-	-	-	-
Carbohydrates and glycosides	-	-	-	-
Proteins and amino acids	-	-	-	-
Sterols	-	-	-	-
Fixed oils and fats	-	-	-	-
Phenolic compounds and tannins	+	+	+	+
Triterpenoids	+	+	+	+
Saponins	-	-	+	+
Gums and mucilage	-	-	+	+
Flavones and flavanones	+	+	+	+

+ denotes the presence; - denotes the absence

Antimicrobial Activity:

1. **Collection of microorganisms:** Thirteen bacterial strains and four fungal strains were obtained from microbiology laboratory, Masterskill University College of Health Sciences and used to investigate antimicrobial activity of various *P. chinensis* leaf extracts. Six Gram-positive bacteria such as *Bacillus cereus* (ATCC 13061), *Corynebacterium diphtheriae* (ATCC 27010), *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 49461), and *Streptococcus viridans* (ATCC 10551) and seven Gram-negative bacteria such as *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Proteus hauseri* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 14901), and *Vibrio cholerae* (ATCC14033) were used for antibacterial screening tests. The four fungal strains such as *Candida albicans* (ATCC 14053), *Candida krusei* (ATCC 34135), *Candida tropicalis* (ATCC 13803), and *Mucor racemosus* (ATCC 7935) were used for antifungal screening tests.

2. **Antibacterial activity:** Agar disc diffusion Kirby-Bauer method was applied to investigate antibacterial activity of leaf extracts of *Persicaria chinensis* (L.) H. Gross [15]. Whatman filter paper discs of 6mm in diameter were prepared and sterilized in hot air oven at 160^{o}C for an hour [16]. The paper discs were impregnated with 10µL of various leaf extracts containing concentration of 100mg/mL [17]. Bacterial cultures were streaked uniformly in two directions at 90^{o} by using sterile swabs on the surface of Muller Hinton agar and blood agar in accordance with the suitability of medium for growth of the particular bacterial strains [18].

   Then, the agar plates were left to dry before placing the loaded discs with extracts on the agar surface. For each bacterial strain, a standard antibacterial agent, amoxicillin (10µg/disc) was placed on the seeded agar plate. The extracts in the discs were allowed to diffuse for five minutes before incubation, and the plates were kept in the incubator at 37^{o}C for 24h. After incubation, the presence or absence of bacterial growth was assessed by measuring the diameter of the zone of inhibition in mm [15]. The experiment of each extract or standard drug against each bacterium was carried out in triplicate, and the data were expressed as mean ± standard error of mean (SEM) [12, 19]. The results of bacterial sensitivity testing on leaf extracts of *P. chinensis* were compared with reference standard and noted in **Table 3**.

3. **Antifungal activity:** Agar disc diffusion Kirby-Bauer method was used to determine the antifungal efficacy of various *P. chinensis* leaf extracts [15]. Whatman filter paper discs of 6mm in diameter were prepared and sterilized in hot air oven at 160^{o}C for an hour [16]. The paper discs were impregnated with 10µL of leaf extracts containing concentration of 100mg/ml [17]. Fungal cultures were inoculated and streaked uniformly in two directions at 90^{o} by using sterile swabs on the surface of Sabouraud dextrose agar [18]. Then, the agar plates were left to dry before placing the loaded discs with extracts on the agar surface. For each fungal strain, a standard antifungal agent, fluconazole (30µg/disc) was placed on the agar plate.

   The extracts in the discs were allowed to diffuse for five minutes before incubation. All the plates were incubated at 28^{o}C for 48h. After incubation, the presence or absence of fungal growth was assessed by measuring the diameter of the zone of inhibition in mm [15, 20]. The experiment of each extract or standard drug against each fungus was conducted in triplicate, and the data were expressed as mean ± standard error of mean (SEM) [19]. The results of fungal susceptibility testing on various leaf extracts were compared with reference standard and recorded in **Table 3**.

Statistical Analysis: Dunnett's One-Way ANOVA test was performed by using SPSS software version 20 where the reference standard was stated as the control group and all extracts were compared to it. P values were enumerated by showing the significant differences between the extracts and the reference standard at the statistical significant levels of 0.05, 0.01 and 0.001 [19, 21].

TABLE 3: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *PERSICARIA CHINENSIS* LEAF EXTRACTS

Test organisms	Zone of inhibition in diameter (mm)				
	Pet. Ether Extract	Chloroform Extract	Methanol Extract	Aqueous Extract	Reference Standard
B. cereus	9.33 ± 0.7***	9.67 ± 0.9***	10.33 ± 0.9***	12.67 ± 0.7***	26.67 ± 0.9 (a)
C. diphtheriae	8.67 ± 0.3***	9.33 ± 0.3***	18.67 ± 1.2 NS	16.33 ± 0.3 NS	16.67 ± 0.3 (a)
M. luteus	9.67 ± 0.9***	10.00 ± 0.6***	22.33 ± 0.3***	18.00 ± 0.6***	30.67 ± 1.2 (a)
S. aureus	9.00 ± 0.6***	9.67 ± 1.5***	14.00 ± 1.2***	16.33 ± 0.3***	26.33 ± 0.3 (a)
S. epidermidis	8.00 ± 0.6***	7.33 ± 0.7***	8.67 ± 0.7***	14.00 ± 0.6*	17.33 ± 0.9 (a)
S. viridans	9.33 ± 0.9***	8.67 ± 0.3***	15.67 ± 0.3 NS	18.33 ± 0.9 NS	16.67 ± 0.3 (a)
E. aerogenes	8.33 ± 0.9***	9.00 ± 0.6***	14.67 ± 0.3*	16.67 ± 0.7 NS	18.00 ± 0.6 (a)
E. coli	9.33 ± 0.3***	7.33 ± 0.9***	14.33 ± 0.3***	16.67 ± 1.2**	22.67 ± 0.7 (a)
K. pneumoniae	10.00 ± 1.0***	8.67 ± 0.9***	18.67 ± 0.7***	16.33 ± 0.3***	0.00 ± 0.0 (a)
P. hauseri	8.33 ± 0.9***	9.00 ± 1.0***	20.67 ± 0.7***	18.00 ± 1.2***	30.33 ± 0.3 (a)
P. aeruginosa	7.67 ± 0.7**	8.67 ± 0.3**	10.33 ± 0.9 NS	14.33 ± 0.9 NS	13.00 ± 0.6 (a)
S. typhi	9.33 ± 1.5***	10.33 ± 0.9***	16.00 ± 0.6***	12.67 ± 0.9***	30.67 ± 0.9 (a)
V. cholerae	8.67 ± 0.9***	9.67 ± 1.2***	12.33 ± 0.9***	18.00 ± 1.0**	25.00 ± 0.6 (a)
C. albicans	7.33 ± 0.9***	9.00 ± 1.0***	18.67 ± 0.9 NS	16.00 ± 1.0*	20.33 ± 1.3 (b)
C. krusei	9.33 ± 0.3***	7.00 ± 0.6***	18.67 ± 0.7 NS	16.67 ± 0.3*	19.67 ± 1.2 (b)
C. tropicalis	9.33 ± 0.9***	8.33 ± 0.7***	16.33 ± 0.9***	12.33 ± 0.9***	30.33 ± 0.3 (b)
M. racemosus	8.67 ± 0.9***	8.67 ± 0.7***	22.00 ± 0.6*	16.67 ± 0.9***	26.0 0.6 (b)

(a) - Amoxicillin (10µg/disc), (b) - Fluconazole (30µg/disc); Values of triplicates were shown as mean ± SEM. The mean of the zone of inhibitions of the extracts against each bacterium or fungus was compared to that of the reference drug (amoxicillin and fluconazole). *P<0.05 - less significant, **P<0.01 - significant, and ***P<0.001 - highly significant, and NSP>0.05 - not significant

RESULTS AND DISCUSSION: The color, consistency, and percentage yield of *Persicaria chinensis* (L.) H. Gross leaf extracts were tabulated in Table 1. The percentage extractive values of various leaf extracts were ranged from 4.62 to 13.70. Aqueous and methanol extracts of *P. chinensis* had the highest percentage yield because of the presence of more secondary metabolites which may be soluble in high polarity solvents [19].

Medicinal plants possess enormous ethnomedicinal values, for instance, antimicrobial activity, and thus they have beneficial effect in treating microbial infectious diseases. The presence of certain phytoconstituents contributes to their efficacy in eradicating pathogenic microbial strains [13]. According to the results of preliminary phytochemical screening (Table 2), most *P. chinensis* leaf extracts reported the presence of plant secondary metabolites such as flavones, flavanones, gums, phenolic compounds, saponins, triterpenoids, and tannins. Saponins are the chemical constituents that may protect ones from gastrointestinal infections [22, 23].

Therefore, ethanol extract of root of *P. chinensis* was reported with established efficacy in obliterating *Helicobacter pylori* in the stomach [10].

Besides, plants containing flavonoids and tannin compounds exhibit satisfactory antimicrobial activity because tannins are responsible for the inactivation of adhesions, enzymes, and cell-enveloped proteins of microbes, in conjunction with the ability to bind to the extracellular and soluble proteins and cell wall of microbes by flavonoids consisting of flavones and flavanones [22, 23]. Triterpenoids such as α-amyrin, betulinic acid, oleanolic acid, and ursolic acid were also reported for their antimicrobial potencies [24].

In the present study, various leaf extracts of *P. chinensis* displayed prominent antibacterial and antifungal properties against the selected human pathogens. Gram-positive and Gram-negative bacteria and fungi used in this study were vulnerable to all four leaf extracts of *P. chinensis*. These extracts were comparably active against Gram-positive and Gram-negative bacteria. Aqueous extract showed moderate antibacterial and antifungal activity against all fungi and most bacteria except *M. luteus*, *P. hauseri*, *S. viridans*, and *V. cholerae*. Methanol extract displayed variable antimicrobial activity against the selected pathogens, but it possessed potent antibacterial and antifungal activity against *M. luteus* and *M. racemosus* respectively.

Based on the results in Table 3, methanol extract had greater antifungal activity than aqueous extract which may be due to higher percentage yield of bioactive components in methanol extract. Methanol and aqueous extracts exhibited comparable antibacterial potency with amoxicillin against *C. diphtheriae*, *S. viridans*, *E. aerogenes*, and *P. aeruginosa*, in conjunction with remarkable antifungal efficacy as fluconazole against *C. albicans* and *C. krusei* which were proven by less significant difference ($P<0.05$) or non-significant difference ($P>0.05$). *K. pneumoniae* was greatly inhibited ($P<0.001$) by all leaf extracts because amoxicillin (10µg/disc) did not exhibit any antibacterial activity against it.

CONCLUSION: From these findings, the results concluded that the traditional use of *P. chinensis* for treating infectious diseases was scientifically supported. The presence of zone of inhibition of various leaf extracts of *P. chinensis* indicated their potential to function as a probable source of newer antibiotics.

All leaf extracts reported to possess promising antibacterial and antifungal activity against the selected pathogenic microorganisms because of the presence of phenolic compounds, tannins, triterpenoids, and flavonoids. Isolation of the respective phyto-constituents for antibacterial and antifungal properties of *P. chinensis* and investigation of their possible mechanism of action may become a useful approach to develop natural bioactive products and new semisynthetic agents with great anti-infective efficacy.

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